ORIGINAL ARTICLE

Used of microbial phytase to replace inorganic phosphorus in sex-reversed red tilapia: 1 dose response

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Abstract

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Sex-reversed red tilapia of average initial body weight 5.5 g were fed seven practical diets containing 0, 500, 1,000, 2,000 and 4,000 units of microbial phytase/kg and two diets containing 0.2 and 0.3% feed grade dicalcium phosphate (DCP) (but no microbial phytase), respectively. The experiment was carried out in 235-l glass aquaria filled with 180 l water and attached with a closed-recirculating water system with 0.8 l/min flow rate. The experimental period was 10 weeks. All experimental diets were formulated with plant-based protein of 30% and 6% fat. Results indicated an improvement in apparent digestibility coefficient of phosphorus (ADCP) in fish given phytase supplemented feed. There was no difference in ADCP when 1,000 unit phytase/kg diet or higher phytase levels (2,000 and 4,000 unit phytase/kg diet) or 0.2 and 0.3% DCP were supplemented. A significant increase was noted for hemoglobin in tilapia that received 1,000 unit phytase/kg diet or higher levels compared to the control. Serum phosphorus markedly increased when the fish were given feeds with 1,000 unit phytase/kg diet and over, while the supplementation of 500 unit phytase/kg diet

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and over increased serum zinc level. Higher levels of phosphorus were retained in bone whereas lower levels of phosphorus presented in the feces of tilapia fed feeds supplemented with phytase. Growth performance was markedly influenced when the fish were given feed with 4,000 unit phytase/kg diet.

Key words : phytase, inorganic phosphate, phosphorus, apparent digestibility coefficient, sex-reversed red tilapia, *Oreochromis niloticus*

บทคัดย่อ

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การใช้ไฟเตสทดแทนอนินทรีย์ฟอสฟอรัสในปลานิลแดงแปลงเพศ 1: dose response

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ทดลองเลี้ยงปลานิลแดงแปลงเพศที่มีน้ำหนักเฉลี่ยเริ่มต้นตัวละ 5.5 กรัม ด้วยอาหารทดลอง 7 สูตร โดยการเสริมเอนไซม์ไฟเตส 0, 500, 1,000, 2,000 และ 4,000 ยูนิต/อาหาร 1 กก. ส่วนอาหารอีกสองสูตรเสริมไดแคลเซียมฟอสเฟต 0.2 และ 0.3% ตามลำดับ การทดลองทำในตู้กระจกความจุน้ำ 235 ลิตร โดยเติมน้ำในตู้ทดลอง 180 ลิตร ระบบทดลองเป็นระบบกรองแบบปิด มีอัตราการไหลของน้ำ 0.8 ลิตร/นาที ระยะเวลาการทดลอง 10 สัปดาห์ อาหารทดลองมีส่วนประกอบเป็นวัตถุดิบพืชทั้งหมด โดยมีโปรตีน 30% และไขมัน 6% จากผลการทดลองพบว่าปลาที่ได้รับอาหารเสริมไฟเตสที่ระดับตั้งแต่ 1,000 ยูนิต/อาหาร 1 กก. ขึ้นไป และปลาที่ได้รับอาหารเสริมไดแคลเซียมฟอสเฟต 0.2 และ 0.3% ทำให้ค่าสัมประสิทธิ์การย่อยฟอสฟอรัสสูงขึ้นอย่างมีนัยสำคัญ ($p<0.05$) และปริมาณฮีโมโกลบินสูงขึ้นอย่างมีนัยสำคัญ ขณะที่ปริมาณสังกะสีในเลือดสูงขึ้นอย่างมีนัยสำคัญ เมื่อปลาได้รับอาหารเสริมไฟเตสที่ระดับ 500 ยูนิต/อาหาร 1 กก. ขึ้นไป ปริมาณฟอสฟอรัสในกระดูกสูงขึ้นขณะที่ฟอสฟอรัสในมูลปลามีค่าลดลงเมื่อปลาได้รับอาหารที่เสริมไฟเตส การเจริญเติบโตของปลาเพิ่มสูงขึ้นอย่างมีนัยสำคัญ เมื่อปลาได้รับอาหารที่เสริมไฟเตส 4,000 ยูนิต/ อาหาร 1 กก.

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Phosphorus and its compounds are the substances that aquatic animals require in large proportions to build up the bones and scales in combination with calcium. This mineral is a vital component of organic phosphates such as adenosine triphosphate, phospholipids, coenzymes and DNA, which have major roles in the metabolism of carbohydrates, fats and amino acids (Lovell, 1989; Davis and Gatlin, 1991; NRC, 1993). Minimal quantities of phosphorus are available to aquatic species as their concentrations in the water are lower than 0.1 ppm. As a result, the soluble phosphorus available to aquatic species is less than 1 percent of that provided by the feeds (NRC, 1983). The dietary phosphorus is supplied by plant as well as animal raw materials used in the feed preparation and also by the inorganic phosphate, phosphate compounds in particular. Several feed raw materials contain high levels of phosphorus; however, only small proportions are utilizable by aquatic species. Hydroxyapatite and tricalcium phosphate, components in the fish bones and scales, are major forms of phosphorus in the fish meal (Jobling, 1994). In plant raw material, about two-thirds of the total phosphorus content are in the form of phytic acid or myo-inositol pentakisphosphate, which are normally present in association with phytin, the compounds of calcium, magnesium and potassium (Dey and Harborne, 1990). Phytate is a compound of phytic acid, with inositol and phosphate (Uhlig, 1998). Hendricks and Bailey (1989) recognized the phytic acid as a

plant born toxin and this form of phosphorus is unutilizable by monogastric animals and fishes. Therefore, other forms of phosphorus like dicalcium phosphate may be supplemented in the feeds (Eya and Lovell, 1997). Such supplementation increases the feed cost and can lead to the water pollution as the unutilizable phosphorus is excreted as feces which can accumulate in the water and result in the unbalanced proliferation of zooplankton and phytoplankton communities.

Phytase is an enzyme categorized in phosphatase group. Phytase acts in the acceleration of hydrolysis to eliminate the phosphates from the phytate molecules and become utilizable to the fishes. This reduces the amount of phosphorus accumulated in the water and prevents the water pollution. Riche and Brown (1996) reported a 46.2-76.8% apparent phosphorus availability in rainbow trout given the phytase-supplemented feed with plant raw materials. Similar result was reported by Lanari *et al*. (1998), who reported that the supplementation of 1000 unit phytase/kg feed increased the apparent phosphorus availability by 58.6-68.1% in rainbow trout. Jackson *et al*. (1996) experimented with the supplementation of 0, 500, 1000, 2000 and 4000 unit phytase/kg feed with major components from plant and given to channel catfish. The feeds with the supplementation of phytase improved the weight gain, accumulation of ash and phosphorus in the bones and feed efficiency. The best weight gain, feed efficiency and feed conversion ratio were noted in the fish given the feed with 1000 unit phytase/kg feed. There is minimal information regarding the effects of phytase in the fish species of economic significance in the Indo-Pacific region. This study was conducted to evaluate the efficacy of phytase for improving phosphorus bioavailability in plant protein base diets as well as to compare the efficacies of supplemented microbial phytase and inorganic phosphorus for sex-reversed red tilapia.

Materials and Methods

Experimental diet

All raw materials used in feed preparation were analysed for proximate composition prior to feed formulation (Table 1). The all-plant basal diet was formulated to contain 30% protein, 6% lipid and 3.5 kcal digestible energy/g diet (Table 2). The microbial phytase (Phytase L) derived from *Aspergillus niger* was supplied by Roche Aquaculture Centre Asia Pacific (Bangkok, Thailand). Microbial phytase was added (sprayed) to the basal diet at 0, 500, 1,000, 2,000 and 4,000 unit/kg diet, respectively (0, 0.1, 0.2, 0.4, 0.8 g phytase in 40 ml distilled water) (Diets 1 to 5). Diets 6 and 7 were the same as Diet 1 except that 1.13 and 1.69% feed grade dicalcium phosphate was added (diets 6 and 7 composed of 0.2 and 0.3% phosphorus from DCP, respectively). Ronozyme P provided an activity of 5,000 unit/g phytase. The diets (sinking pellet) were prepared according to procedures described previously (Phromkunthong *et al*., 2004). The proper amount of phytase was dissolved in 40 ml of distilled water and sprayed onto 1 kg of the basal diet as needed. Diets 1, 6 and 7 were sprayed with 40 ml distilled water to maintain an equal level of moisture. The nutritional values of the test diets were analyzed by the method of AOAC (1990) and are presented in Table 3. Total phytate phosphorus in feed

Table 1. Proximate analysis of raw materials (% as fed)[1]

Raw materials	Moisture	Protein	Fat	Ash	Fiber	P	Phytate P	Zn	NFE
Soybean meal	7.58±0.34	41.03±0.24	4.39±0.28	7.39±0.36	6.31±0.18	0.70±0.01	0.403±0.05	0.0047±0.00	33.29±0.40
Broken rice	4.53±0.40	8.09±0.24	1.45±0.03	8.37±0.23	0.13±0.04	0.11±0.01	0.099±0.01	0.0064±0.00	71.26±0.53
Rice bran	5.75±0.20	12.22±0.34	20.80±0.14	15.70±0.21	6.91±0.03	2.02±0.03	1.292±0.02	0.0015±0.00	39.23±0.40
DCP	-	-	-	-	-	17.25±0.18		-	-

[1] Mean ± Standard deviation of three replications
NFE: Nitrogen free extract

Table 2. Composition of experimental feeds

Ingredient (g/kg feed)	Feed formulae						
	1	2	3	4	5	6	7
Soybean meal	670	670	670	670	670	670	670
Broken rice	100	100	100	100	100	100	100
Rice bran	140	140	140	140	140	140	140
Fish oil	10	10	10	10	10	10	10
Vitamin mixtures[1]	10	10	10	10	10	10	10
Choline choride	6	6	6	6	6	6	6
Mineral mixtures[2]	1.96	1.96	1.96	1.96	1.96	1.96	1.96
Methionine	7	7	7	7	7	7	7
Phytase[3]	0	0.11	0.22	0.44	0.88	0	0
Di-calcium	0	0	0	0	0	11.3	16.9
Cr_2O_3	10	10	10	10	10	10	10
Rice hull	45.04	44.93	44.82	44.60	44.16	33.74	28.14

[1] Vitamin mixtures contains the following vitamins per kg feed: Thiamine (B_1) 10 mg; Riboflavin (B_2) 20 mg; Pyridoxine (B_6) 10 mg; Cobalamin (B_{12}) 0.05 mg; Retinal (A) 4 mg (7,000 IU); Cholecalciferol (D_3) 0.1 mg (4,000); Phylloquinone (K_1) 80 mg; Folic acid 5 mg; Calcium pantothenate 40 mg; Inositol 400 mg; Niacin 150 mg; Tocopherol (E) 60 mg (66 IU); Ascorbic acid (C) 500 mg; Biotin 3 mg

Vitamin A (vitamin A-palmitate)	**1,750 IU/mg**
Vitamin D (vitamin D3; cholecalciferol)	**40,000 IU/mg**
Vitamin E (vitamin E; DL-α-tocopherol)	**1.1 IU/mg**

[2] Mineral mixtures contains the following minerals per kg feed: NaCl 12.7897 mg; $MgCO_3$ 78.3053 mg; KH_2PO_4 1178.3493 mg; $CaHPO_4.2H_2O$ 656.4706 mg; $FeSO_4.H_2O$ 27.3641 mg; Ca $(CH_3CH(OH)COO)_2$ 8.5167 mg; $ZnSO_4$ 2.5883 mg; $MnSO_4..H_2O$ 1.0060 mg; $CuSO_4.5H_2O$ 0.1813 mg; $CoSO_4$ 0.0064 mg; KI 0.0749 mg

[3] c.c./kg

Table 3. Proximate analysis of experimental feeds (% as fed)[1]

Treatment	Phytase	Moisture (unit/kg diet)	Protein	Fat	Ash	Fiber	P	Zn	NFE
1	0	4.62±0.02	30.06±0.26	6.33±0.47	7.48±0.04	7.30±0.13	0.89±0.01	0.0052±0.00	44.21±0.72
2	500	3.69±0.04	30.11±0.28	6.37±0.40	7.42±0.10	7.24±0.10	0.90±0.01	0.0050±0.00	45.18±0.16
3	1,000	3.42±0.06	30.52±0.46	6.36±0.44	7.41±0.06	7.43±0.18	0.89±0.08	0.0052±0.00	44.87±0.92
4	2,000	7.56±0.045	29.64±0.31	6.36±0.25	7.39±0.11	7.19±0.04	0.92±0. 05	0.0050±0.00	41.86±0.16
5	4,000	5.04±0.12	29.68±0.27	6.25±0.46	7.36±0.03	7.22±0.06	0.89±0.01	0.0050±0.00	44.45±0.71
6	0.2% DCP	5.96±0.05	29.63±0.45	6.28±0.19	7.54±0.04	6.64±0.01	1.05±0.01	0.0051±0.00	43.95±0.60
7	0.3% DCP	5.71±0.11	29.79±0.28	6.22±0.33	7.90±0.09	6.56±0.04	1.14±0.02	0.0050±0.00	43.82±0.75

[1] Mean ± standard deviation of three replications

ingredients and experimental diets was analysed by Animal Nutrition Health research, Village-Neuf, France (Tables 1 and 4, respectively). Available phosphorus of experimental diets are presented in Table 4. The phytase content was analysed by Biopract GmbH, Germany and results was presented in Table 5. The diets were kept at 4ºC until used. Each of the seven diets was assigned randomly to seven aquaria.

Experimental fish

Juvenile sex-reversed red tilapia were

Table 4. Available phosphorus of experimental diets

Treatment	Phytase (unit/kg feed)	Total P (%)	Total Phytate P[1] (%)	Available P[2] (%)
1	0	0.89	0.46	0.43
2	500	0.90	0.46	0.44
3	1,000	0.89	0.46	0.43
4	2,000	0.92	0.46	0.46
5	4,000	0.89	0.46	0.43
6	0.2% DCP	1.05	0.46	0.59
7	0.3% DCP	1.14	0.46	0.68

[1] Total phytate P from ingredients
[2] Available P(%) = Total P(%) - Total phytate P(%)

Table 5. Phytase content in seven feeds formulae (determined by analysis)[1]

Experimental group	Declaration (unit/kg diet)	Analysed (mg phytase equiv./kg diet)
1	0	124.0±44.02
2	500	770.5±170.34
3	1,000	1,763.5±134.89
4	2,000	2,916.25±138.38
5	4,000	4,687.75±692.89
6	0.2% DCP	NA
7	0.3% DCP	NA

[1]Mean ± standard deviation of four replicates.
NA = not analysed

obtained from a private farm in Phattalung Province. Twenty fish averaging 5.5 g/fish were stocked into each of 40 235-L flow-through aquaria. The aquaria were supplied with recirculated water of flow rate 0.8 L/min and continuous aeration. Water parameters i.e. temperature, DO, pH, alkalinity, hardness, NH_3 were determined every week by the method of Boyd and Tucker (1992) to maintain water quality which is suitable for fish (temperature: 26ºC; DO: 6.4 ppm; pH: 8.3; alkalinity: 62 ppm; hardness: 73 ppm and NH_3: 0.15 ppm).

Prior to initiation of the experiment, the fish underwent a 1-wk conditioning period during which they readily adjusted to the basal diet and experimental conditions. Fish were fed twice daily (0800 and 1600 h) and the aquaria were cleaned every week. Fish in each aquarium were counted and weighed collectively every 2 wk. The percentage weight gain, feed conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization (ANPU) were calculated using the methods of Robinson and Wilson (1985).

Digestibility measurements

Feces were collected once daily in the morning by siphoning from the 6th week to the 10th week of rearing period. Five replicate groups of fish were fed the diets containing chromic oxide as an indicator of visual satiety twice a day 9.00 h and 17.00 h. Two hours after feeding in the morning the rearing tank were brushed to remove

uneaten feed and fecal residues. Feces were frozen and subsequently pooled and oven dried at 60ºC for 48 hrs.

Proximate analysis of feces was done for protein, fat, ash, fiber, phosphorus and zinc using the method of AOAC (1990) while Cr_2O_3 concentration in the feed and feces was determined by the method of Furukawa and Tsukahara (1966).

Chemical analysis

Thirty fish at the beginning from each tank were used for protein analysis by the method of AOAC (1990) to determine ANPU.

Phosphorus and zinc in bone of experimented fish in each treatment were determined by the method of AOAC (1990).

At the completion of the experiment, two fish from each tank were anesthetized with quinaldine. A blood sample was drawn from caudal peduncle of two individuals in each replicate using heparin as an anticoagulant. Hematocrit was determined by the modified method of Blaxhall and Daisley (1973), while hemoglobin was determined as Cyanmet-haemoglobin (Larsen and Snieszko, 1961).

Phosphorus in blood serum was determined by the modified method of Henry (1974), while zinc was analysed by the method of AOAC (1990).

Data analysis

Data were analyzed using ANOVA (CRD) and the differences among averages compared using Duncan's Multiple Range Test (Duncan,1955). SPSS program was used for statistical analysis.

Results

Digestibility coefficient

Digestibility coefficient of dry matter in fish given the feeds with 1,000 and 2,000 unit phytase/kg diet were higher than in those given the feed without phytase and with 500 unit phytase/kg diet ($p<0.05$), whereas there was no significant difference among 1,000 and 2,000 unit phytase/kg diet in comparison with fish given the feeds with 4,000 unit phytase/kg diet and 0.2 and 0.3% DCP ($p>0.05$) (Table 6).

Digestibility coefficients of protein in fish given the feed with 1,000 unit phytase/kg diet was higher than in those given the feeds without phytase and with 500 unit phytase/kg diet ($p<0.05$). The supplementation of 1,000 unit phytase/kg diet made no difference in digestibility coefficient of protein in comparison to other treatments ($p>0.05$) (Table 6).

Digestibility coefficients of fat in fish given the feed with 1,000 and 2,000 unit phytase/kg diet were higher than in those given the feeds without phytase and with 500 unit phytase/kg diet ($p<0.05$), whereas there was no significant difference among these two treatments in comparison to other treatments ($p>0.05$) (Table 6).

Table 6. Digestibility coefficient of sex-reversed tilapia fed 7 experimental feeds for a 10-week period[1]

Treatment	Phytase (unit/kg diet)	Digestibility (%)				Available P (%) (Calculation)
		Dry matter	Protein	Fat	P	
1	0	22.40±2.53^{a}	88.01±0.30^{a}	74.73±2.96^{a}	45.88±4.58^{a}	0.41
2	500	31.04±5.53^{b}	87.88±1.15^{a}	76.65±3.58^{ab}	51.23±4.49^{a}	0.46
3	1,000	39.75±2.75^{cd}	90.02±1.07^{bc}	81.04±2.20^{cd}	61.58±3.63^{b}	0.55
4	2,000	39.86±2.39^{cd}	89.55±1.00^{abc}	84.08±1.25^{cd}	63.94±2.86^{b}	0.59
5	4,000	33.82±4.51^{bc}	89.17±0.81^{abc}	85.01±0.47^{d}	61.48±2.57^{b}	0.55
6	0.2% DCP	46.26±2.42^{d}	90.64±0.43^{c}	84.02±1.13^{cd}	61.80±3.05^{b}	0.65
7	0.3% DCP	43.87±5.69^{d}	88.46±1.28^{ab}	80.20±1.71^{bc}	64.12±3.50^{b}	0.73

[1] **Mean ± standard deviation of three replications. Means within each column not sharing a common superscript are statistically different ($p<0.05$)**

Non-significant difference for phosphorus digestibility coefficient was shown in fish given the feed with 1,000-4,000 unit phytase/kg diet and 0.2 and 0.3% DCP ($p>0.05$), which were higher than in those given the feeds without and with 500 unit phytase/kg diet ($p<0.05$) (Table 6).

Blood parameter

No difference was noted for hematocrit in any of the fish groups ($p>0.05$). However, fish given the feed with 1,000-4,000 unit phytase/kg diet showed a tendency to higher hematocrit values than fish fed the diets without and with 500 unit phytase/kg diet (Table 7). The supplementations of 500-4,000 unit phytase/kg diet resulted in higher hemoglobin levels than did as phytase supplementation ($p<0.05$). No difference in hemoglobin level was noted between the two levels of DCP-fed groups ($p>0.05$) (Table 7).

Phosphorus and zinc in blood serum

The supplementations of 1,000-4,000 unit phytase/kg diet resulted in higher phosphorus in serum than fish given feeds without phytase supplementation and with 0.2% DCP ($p<0.05$). There was a significantly higher serum phosphorus in fish given the feeds with phytase supplementations at 4,000 unit/kg diet in comparison with fish given feeds with 0.2 and 0.3% DCP ($p<0.05$) (Table 8).

The supplementation of 500-4,000 unit phytase/kg diet resulted in no difference of zinc in serum among treatments ($p>0.05$), but they were higher than in fish given the feed without phytase supplementation and in those with 0.2 and 0.3% DCP ($p<0.05$) (Table 8).

Phosphorus and zinc in bones

No difference was noted for bone phosphorus in the fish given the feeds without phytase supplementation in comparison to the treatments which were supplemented with phytase 500-2,000 unit phytase/kg diet ($p>0.05$) (Table 9). The supplementation of 4,000 unit phytase/kg diet resulted in higher bone phosphorus than did the feed without or with 500-1,000 unit phytase/kg diet ($p<0.05$) (Table 9). Comparing the DCP levels, higher bone phosphorus levels were noted in the fish groups with 0.3% DCP in feed, than in those with 0.2% DCP and higher than other treatments, which were supplemented with phytase ($p<0.05$). Highest bone Zn levels were noted in the fish with supplementation of 500 and 1,000 unit phytase/kg diet, followed by fish given feeds with 4,000 and 2,000 unit phytase/kg diet ($p<0.05$) and the lowest bone Zn level was found in those without phytase supplementation in the feed ($p<0.05$). No difference was noted for bone Zn in those given the feeds

Table 7. Haematrocrit and haemoglobin of sex-reversed tilapia fed 7 experimental feeds for a 10-week period[1]

Treatment	Phytase (units/kg)	Haematrocrit (%)	Haemoglobin (g/dl)
1	0	19.92±8.68[a]	1.71±0.43[a]
2	500	18.33±8.78[a]	3.37±2.16[ab]
3	1,000	23.17±11.43[a]	4.94±0.76[b]
4	2,000	24.17±5.35[a]	4.07±0.71[b]
5	4,000	24.00±9.26[a]	4.13±1.53[b]
6	0.2% DCP	13.33±1.53[a]	2.90±1.21[ab]
7	0.3% DCP	19.58±3.50[a]	2.75±0.72[ab]

[1] **Mean ± standard deviation of three replicates. Means within each column not sharing a common superscript are statistically different ($p<0.05$)**

Table 8. Phosphorus and zinc in blood serum of sex-reversed tilapia fed 7 experimental feeds for a 10-week period[1]

Treatment (units/kg)	Phytase (mg/l)	Phosphorus in serum (mg/l)	Zinc in serum
1	0	14.40±6.22[a]	13.80±2.83[a]
2	500	18.25±1.63[ab]	23.20±2.26[b]
3	1,000	24.60±0.90[bc]	24.50±6.36[b]
4	2,000	26.75±2.33[bc]	19.80±1.70[b]
5	4,000	31.80±0.14[c]	23.40±0.28[b]
6	0.2% DCP	14.45±4.17[a]	17.40±0.28[a]
7	0.3% DCP	16.80±1.41[ab]	13.40±3.96[a]

[1] Mean ± standard deviation of two replications. Means within each column not sharing a common superscript are statistically different ($p<0.05$)

Table 9. Phosphorus and zinc in bone of sex-reversed tilapia fed 7 experimental feeds for a 10-week period[1]

Treatment	Phytase (units/kg)	Bone phosphorus (%)	Bone zinc (%)
1	0	3.20±0.10[a]	0.0044±0.00[a]
2	500	3.18±0.01[a]	0.0058±0.00[c]
3	1,000	3.19±0.07[a]	0.0059±0.00[c]
4	2,000	3.30±0.04[ab]	0.0049±0.00[ab]
5	4,000	3.38±0.09[b]	0.0053±0.00[c]
6	0.2% DCP	3.75±0.07[c]	0.0048±0.00[ab]
7	0.3% DCP	3.96±0.03[d]	0.0049±0.00[ab]

[1] Mean ± standard deviation of three replicates. Means within each column not sharing a common superscript are statistically different ($p<0.05$)

Table 10. Phosphorus and zinc in fecal of sex-reversed tilapia fed 8 experimental feeds for a 10-week period1

Treatment	Phytase (units/kg)	Fecal phosphorus (%)	Fecal zinc (%)
1	0	0.62±0.03[b]	0.0057±0.00[bc]
2	500	0.63±0.03[b]	0.0059±0.00[c]
3	1,000	0.56±0.03[a]	0.0059±0.00[c]
4	2,000	0.55±0.02[a]	0.0050±0.00[ab]
5	4,000	0.52±0.01[a]	0.0049±0.00[a]
6	0.2% DCP	0.74±0.03[c]	0.0060±0.00[c]
7	0.3% DCP	0.73±0.01[c]	0.0058±0.00[c]

[1]Mean ± standard deviation of three replicates. Means within each column not sharing a common superscript are statistically different ($p<0.05$)

with supplementations of two levels of DCP ($p>0.05$) (Table 9).

Phosphorus and zinc in feces

The supplementations of 1,000-4,000 unit phytase/kg diet resulted in lower fecal phosphorus contents than did feeds without and with 500 phytase unit/kg diet ($p<0.05$), whereas the supplementation of 0.2 and 0.3% DCP resulted in highest fecal phosphorus content ($p<0.05$). The fecal Zn levels were lower in the fish with supplementations of 2,000 and 4,000 phytase unit /kg diet than in other treatments ($p<0.05$) (Table 10).

Average body weight

The differences in average body weight (Table 11) were noted from week 2. The fish given the feeds with both supplemented DCP levels showed higher average body weight than those given phytase-supplemented feeds at all levels ($p<0.05$). Between week 4 to 6, the supplementation of the 4,000 unit phytase/kg diet resulted in highest average body weight which was significantly higher ($p<0.05$) than in those given the feeds with other levels of supplemented phytase, except in those given 2,000 unit phytase/kg diet. At week 10, the 4,000 unit phytase/kg diet resulted significant difference in average body weight which was higher that of fish given the feeds with other levels of supplemented phytase. However, there were no differences in average body weight among the fish given 4,000 unit phytase/kg feed and 0.2% DCP ($p>0.05$), which were lower than that of fish given 0.3% DCP ($p<0.05$).

Weight gain, specific growth rate, rate of feed intake and survival rate

Weight gain, specific growth rate, rate of feed intake and survival rate are demonstrated in Table 12. Similar trends were noted for weight gain and specific growth rate while rates of feed intake and survival showed significantly different among treatments ($p<0.05$).

Feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilization (ANPU)

Feed conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization (ANPU) are shown in Table 13. Poor FCRs were noted for the fish given the feeds without and with 500 unit phytase/kg diet. Better FCR resulted from the supplementation of 1000 phytase unit./kg diet. No differences were noted for FCR in the fish given the feeds with 1,000-4,000 phytase unit/kg diet and with supplemented DCP ($p>0.05$).

Higher PER was noted in the fish given 4,000 unit phytase/kg diet than in those given the feeds with phytase supplementation at all levels or without phytase supplementation ($p<0.05$). Non-significant difference was demonstrated among

Table 11. Average body weight of sex-reversed tilapia fed 7 experimental feeds for a 10-week period[1]

Treatment	Phytase (units/kg)	0	2	4	6	8	10
1	0	5.48±0.10	9.50±0.41^{a}	15.51±0.51^{a}	22.84±0.67^{a}	29.92±0.89^{a}	38.26±1.12^{a}
2	500	5.48±0.09	9.48±0.22^{a}	15.60±0.65^{a}	22.94±1.67^{ab}	29.44±2.56^{a}	38.01±3.03^{a}
3	1,000	5.47±0.07	9.47±0.34^{a}	15.67±0.64^{a}	22.28±0.92^{a}	29.97±0.67^{a}	37.96±1.06^{a}
4	2,000	5.49±0.06	9.60±0.31^{a}	15.93±0.93^{ab}	23.81±1.23^{ab}	31.84±3.33^{ab}	39.00±2.72^{a}
5	4,000	5.48±0.09	9.77±0.37^{a}	16.78±0.62^{b}	24.98±1.82^{b}	32.86±2.32^{bc}	43.33±3.32^{b}
6	0.2% DCP	5.49±0.11	10.36±0.42^{bc}	18.56±0.83^{c}	28.55±1.34^{c}	36.20±2.08^{cd}	46.21±3.07^{bc}
7	0.3% DCP	5.49±0.09	10.51±0.29^{c}	19.46±0.85^{c}	30.10±1.64^{c}	38.76±1.68^{d}	49.86±3.95^{d}

[1]Mean ± standard deviation of three replicates. Means within each column not sharing a common superscript are statistically different ($p<0.05$)

Table 12. Weight gain, specific growth rate, rate of feed intake and survival rate of sex-reversed tilapia fed 7 experimental feeds for a 10-week period[1]

Treatment	Phytase (units/kg)	Weight gain (%)	Specific growth rate (% day)	Rate of feed intake (%/fish/day)	Survival rate (%)
1	0	595.59±16.36^{a}	2.28±0.03^{a}	3.10±0.09^{bc}	81.25±8.54^{a}
2	500	594.31±64.66^{a}	2.28±0.10^{a}	3.11±0.06^{bc}	96.00±4.18^{b}
3	1,000	593.56±23.33^{a}	2.28±0.04^{a}	3.13±0.15^{c}	86.00±4.18^{ab}
4	2,000	610.03±48.01^{a}	2.30±0.08^{a}	3.08±0.11^{bc}	94.00±4.18^{b}
5	4,000	681.75±52.32^{b}	2.42±0.08^{b}	2.93±0.23^{bc}	91.67±10.41^{ab}
6	0.2% DCP	740.99±55.61^{b}	2.50±0.08^{bc}	2.88±0.25^{b}	89.00±10.25^{ab}
7	0.3% DCP	812.27±69.87^{c}	2.60±0.09^{c}	2.71±0.15^{a}	83.75±8.54^{ab}

[1] **Mean ± standard deviation of three replicates. Means within each column not sharing a common superscript are statistically different (p<0.05)**

Table 13. FCR, PER, ANPU of sex-reversed tilapia fed 7 experimental feeds for a 10-week period[1]

Treatment	Phytase (units/kg diet)	FCR	PER	ANPU (%)
1	0	2.27±0.65^{c}	2.34±0.07^{a}	31.00±0.90^{ab}
2	500	2.13±0.41^{bc}	2.33±0.11^{a}	29.21±1.38^{a}
3	1,000	1.72±0.13^{ab}	2.41±0.12^{a}	31.79±1.58^{ab}
4	2,000	1.91±0.29^{abc}	2.43±0.08^{a}	29.67±1.02^{a}
5	4,000	1.84±0.21^{abc}	2.66±0.18^{b}	33.47±2.36^{bc}
6	0.2% DCP	1.67±0.22^{ab}	2.80±0.20^{b}	35.27±2.52^{c}
7	0.3% DCP	1.46±0.21^{a}	3.10±0.26^{c}	39.26±3.26^{d}

[1] **Mean ± standard deviation of three replicates. Means within each column not sharing a common superscript are statistically different (p<0.05)**

fish given 4,000 unit phytase/kg diet and fish with 0.2% DCP in feed (p>0.05), whereas fish given the feeds with 0.3% DCP provides the highest PER (p<0.05). There was no significant differences in ANPU (p>0.05) among fish groups given the feeds without and with varying phytase levels in their feeds (Table 13). The feeds with 0.3% DCP resulted in higher ANPU than in fish groups given feeds of other treatments (p<0.05).

Discussion

Results indicated an improvement in digestibility coefficient (dry matter, protein, fat and phosphorus) in the fish given phytase-supplemented feed. Liener (1994) suggested that in addition to combining with protein, phytate also inhibits pepsin, trypsin and alpha-amylase functioning. Similar finding was reported by NRC (1993); fish given low-phosphorus feed or the feed with phytate form showed reduced mineral utilization and protein digestibility. The supplementation of phytase in fish feed, therefore, improves the functioning enzymes in digesting protein, fat and carbohydrate. A congruent result was reported by Cheng and Hardy (2003) who studied the apparent digestibility coefficients in 170 g rainbow trout (*Oncorhynchus mykiss*) and phytase supplementation in extruded soybeans improved apparent digestibility coefficient (ADC)

of magnesium, total phosphorus, phytate phosphorus and manganese. Phytase supplementation in expelled soybean improved ADC of crude protein, sulfur, total phosphorus and phytate phosphorus as compared to that when soybean alone was used. The process in the raw material production affects the functioning of phytase. Furthermore, the results of the current experiment showed no difference in phosphorus digestibility coefficient when 1,000 unit phytase/kg diet, or high phytase levels (2,000 and 4,000 unit/kg diet), or 0.2 and 0.3% DCP were supplemented. Similar result was reported by Li and Robinson (1997) who reported the replacement of 1% DCP by 250 units phytase/kg diet in channel catfish feed while maintaining the same growth performance and feed utilization. Schafer *et al*. (1995) reported a 50% increase in digestibility of phytate phosphorus in common carp when 500 unit phytase/kg diet was supplemented. The supplementation of 1,000 unit phytase/kg diet provides 0.55% available phosphorus which is insufficient for tilapia growth. The best weight gain of tilapia is achieved when 0.3% DCP-supplemented feed (0.73% available phosphorus) is used. Result of experiment also showed that the available phosphorus was higher than 0.55% (a minimum of 0.73% is required) and supplementation of higher than 1,000 unit phytase/kg diet did not affect tilapia growth. It indicated that available phosphorus was insufficient with increased phytase supplementation, fish growth is not always improved as the substrate (phytate phosphorus in the feed) in insufficient for the enzyme to react with (Kornegay, 2001). The supplementation of only 1,000 unit phytase/kg diet results in best phosphorus digestibility coefficient which is sufficient for tilapia feed. A congruent result was reported by Eya and Lovell (1997) who found 31.2% phosphorus net absorption in channel catfish given basal feed with plant raw materials. Similar results were reported 25% soybean as the main feed component for rainbow trout (*Oncorhynchus mykiss*) (Rodehutscord and Pfeffer, 1995), 29% for channel catfish (*Ictalurus punctatus*) (Wilson *et al*., 1982); 32% for common carp (*Cyprinus carpio*) (Schafer *et al*., 1995) with 1,000 or 3,000 unit supplemented phytase/kg diet in feed with all plant raw materials, Eya and Lovell (1997) reported an increase from 76 to 100% net absorption of plant phosphorus, indicating the effect of phytase in hydrolyzing myoinositol hexaphosphoric acid into orthophosphate which is utilizable by channel catfish. Rodenutscord and Pfeffer (1995) reported that supplementation of 1,000 units phytase/kg diet increased the digestibility of plant phosphorus by 75% in rainbow trout. Schafer *et al*. (1995) reported a 50% increase in the net absorption of phytase phosphorus in common carp fed 1,000 units of phytase/kg diet. Jackson *et al*. (1996) noted that 500 units phytase/kg diet was sufficient for maximum weight gain and bone phosphorus deposition in channel catfish fingerlings. Consequently, the supplementation of phytase as well as the adjustment of available phosphorus must be taken into account in feed formulation so that maximum amounts of plant materials can be used by the fish.

A trend of increase was noted for hematocrit and hemoglobin in tilapia given diet supplemented with phytase at the level more than 1,000 unit. Similar result was reported by Li and Robinson (1997) who experimented with channel catfish. All blood parameters were in the range typical for tilapia (Dabrowska *et al*., 1989; Boonyaratpalin and Phromkhunthong, 2000; Phromkunthong *et al*., 2004). It is conceivable that phytase acts in the release of Fe^{2+} for hemoglobin formation (Ensminger *et al*., 1994; Vielma *et al*., 2002). Several experiments reported similar results of better releases of phosphorus and minerals with phytase supplementation in trout and other fishes feeds (Cain and Garling, 1995; Lanari *et al*., 1998; Oliva-Teles *et al*., 1998; Vielma *et al*., 2000; Cheng and Hardy, 2003). Increase in hematocrit and hemoglobin enhance fish resistance to withstand the environment. In addition, serum phosphorus markedly increases when the fish are given feeds with 1,000 unit phytase/kg diet and over while the supplementation of 500 unit phytase/kg diet and over increases the serum Zn. The role of phytase has been recognized in degrading phytates and hence the release of cations which is in congruity with Lei *et al*. (1993) who reported that supple-

mentation of phytase to animal feeds may improve bioavailability of protein and trace minerals such as zinc which might lead to improvement in weight gain.

Higher levels of phosphorus retained in the bone with the lower level of phosphorus present in the feces of tilapia fed feeds supplemented with phytase indicated that less phosphorus being excreted into the pond water. This may result in less environmental pollution by means of reducing phytoplankton diversity and improved water quality.

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