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Original Article

Available phosphorus requirement of sex-reversed red tilapia fed all-plant diets

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Abstract

A feeding trial was conducted to estimate the optimum requirement of dietary phoshporus (P) for sex-reversed red tilapia in glass aquaria (50x100x47cm). Six practical diets were formulated to contain graded levels (0.58, 0.66, 0.72, 0.75 and 0.82%) of available P from all-plant raw ingredients and dicalcium phosphate (DCP). Each diet was randomly assigned to triplicate groups of fish, and each group was stocked with 20 fish (initial body weight, 25.16g 0.13). Fish were fed twice daily (08:00 and 16:00) *ad libitum* for 8 weeks. Average body weight and weight gain significantly increased with increasing available P (P<0.05). The whole body composition analysis showed that lipid and protein as well as P contents in whole body, vertebrae ash and vertebrae p, were significantly affected by available P (P<0.05). The blood biochemistry analysis showed that serum P and serum alkaline phosphatase activity increased with the increase of dietary available P levels (P<0.05). Data for weight gain, FCR, whole body P, vertebrae ash, vertebrae P, muscle protein, muscle fat and visceral fat were subjected to regression analysis to determine effects of the dietary levels of available P on these responses. Employing quadratic non-linear regression model of the relationship between available dietary P and P in vertebrae and whole body to study the P requirement, it was found that available dietary P requirement for sex-reversed red tilapia from the current study were of 0.76 and 0.79%, respectively. Increasing the dietary available P to higher concentration appears to reduce muscle fat while muscle protein increases.

Keywords: Phosphorus, sex-reversed tilapia, tilapia, dicalcium phosphate, alkalinephosphatase

1. Introduction

P is one of the most important minerals required by fish (Lall, 2002). It is a major constituent of skeletal tissue and involved in a variety of metabolic processes including energy transformations, permeability of cellular membranes, and genetic coding (Lovell, 1989). About 85-90% of total P in fish is contained in vertebrae and scales (Lovell, 1988) while the remaining 10-15% constitutes blood and tissues, present in the form of organic phosphates i.e. adenosine triphosphate, phospholipids, deoxyribonucleic acid and co-emzyme. Inorganic phosphates function mainly as buffer to maintain pH of intra and extracellular fluid (Halver *et al*., 2002). Due to low concentration of P in natural waters (Boyd, 1971) and low absorption rate of P from the water (Phillips *et al*., 1958), fish must obtain most of P from their diets. The optimal amount of P supplementation in commercial feeds is not only important economically, but also for environmental reasons. The P excreted from cultured animals into water contributes to algal growth, and results in deteriorating water quality (Beveridge, 1984; Auer *et al*., 1986). P metabolism in cultured aquatic species has become a popular research subject, due to rising concerns about P discharged into aquaculture environment (Wiesmann *et al*., 1988; Ketola and Harland, 1993). Therefore, there has been a trend towards the reduction of dietary P to levels that satisfy, but do not exceed P requirements to produce maximum growth of fish and protect water quality (Lall, 1991;

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Oliva-Teles *et al*., 1998; Bureau and Cho, 1999). Available dietary P requirements ranging from 0.5 to 0.9% have been reported for rainbow trout (Ogino and Takeda, 1978), Atlantic salmon (Ketola, 1975; Lall and Bishop, 1977; Asgard and Shearer, 1997; Vielma and Lall, 1998), chum salmon (Watanabe *et al*., 1980b); carp (Ogino and Takeda, 1976) and red sea bream (Sakamoto and Yone, 1978). The P requirement of catfish and Japanese eel is approximately 0.45% and 0.3% available P, respectively. The objective of the following study was designed to determine the requirement of dietary available P in sex-reversed red tilapia fed practical diets under controled conditions in glass aquaria.

2. Materials and Methods

2.1 Experimental diets

Six experimental diets were formulated from practical ingredients and dicalcium phosphate (DCP) to contain graded levels (0.91%, 1.06, 1.16, 1.27, 1.34 and 1.46%) of total P (Table 2). Total P was determined by the molybdovanadate method (AOAC, 1990). Available P was calculated from determined apparent digestibility coefficient for P in the diets from the present study. Thus feed formulae 1 to 6 contained available P 0.46%, 0.58, 0.66, 0.72, 0.75 and 0.82%, respectively. Tested feeds were moist pellet with all plant materials. Raw materials were analyzed for nutritional value (Table 1) and the contents of protein, lipid and digestible energy in all diets were designed to be about 30%, 7% and 3,300 kcal /kg feed, which have been shown to be sufficient to support optimal growth of sex-reversed red tilapia (Phromkunthong and Gabaudan, 2006). Raw materials were seived through 30- m mesh and ingredients were ground into fine powder. All the ingredients were thoroughly mixed for 10 min then fish oil and water was added to produce a stiff dough. The dough was then pelleted by Hobart Mixer Model A 200T with 3 mm diameter. The feed were dried at 60 C for 24 hours, packed in plastic bags and stored at 4 C (Phromkunthong *et al*., 1997). A portion of each six experimental diets was removed for chemical analyses including protein, lipid, ash and P content (Table 3).

2.2 Experimental procedure

The feeding trial was conducted at the Department of Aquatic Science, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Thailand. Sex-reversed red tilapia were obtained from a commercial farm in Phathalung Province, Thailand. The fish were stocked into a 1 m^3 fiber glass tank and fed diet 1 (low P basal diet) twice daily for three weeks to acclimate to the experimental diets and conditions. Before the commencement of the feeding study, fish were fasted for 24 h, and then weighed after being anesthesized with quinaldine (50 ppm) (Fluka, Switzerland). The fish with similar size (25.16g 0.13) were distributed to 18 glass aquaria (50x100x47cm) at density of 20 fish per aquarium. Each diet was randomly assigned to triplicate aquaria. Fish were hand-fed experimental diets to apparent satiation twice daily (08:00 and 16:30) for 8 weeks.

2.3 Sample collection and analysis

At the beginning of the feeding trial, 10 fish from each aquarium were randomly sampled for carcass composition analysis (AOAC, 1990). Furthermore, at the beginning of the experiment and at 2-week intervals during the trial, fish were counted and bulk-weighed after a 24-h fast. During the experiment, behavior of fish in all treatments was observed, e.g., swimming, feed acceptance and external feature such as body color, hemorrhage, fin and vertebrae deformity and lesion on fin, skin and other external organs.

To determine the P digestibility, 0.5% Cr_2O_3 was used as indicator in the feed. Feces was collected after the week 8th of the experimental period by siphoning. This was made in the evening about 1 h after feeding then feces was stored at -20 C until analysis of P content.

At the end of feeding trial, total numbers and mean body weight of fish in each aquarium were determined. Two fish from each aquarium were randomly sampled for carcass composition. Blood samples were obtained from 4-5 anesthetized quinaldine (50 ppm) fish of each aquarium with 1-ml syringe by puncture of the caudal vein. The blood was centrifused (3,000 g, 4 C, 10 min) and serum was separated and analyzed for alkaline phosphatase by the method of Sigma Chemical Company (1960), using p-nitrophenol phosphate as the substrate. Serum P concentration was determined by the colorimetric method of Chen *et al* (1956). Five sampled fish from replicate were used for determining the P concentrations in vertebra. The vertebrae were collected from each fish and frozen at -20 C until utilized. The vertebrae were thawed, boiled in distilled water for 10 min and adhering flesh was removed. The vertebraes were dried for

Table 1. Proximate analysis of feed ingredients (% as fed basis)[1]

Feed ingredients	Moisture	Protein	Fat	Ash	Crude fiber	P	NFE
Soybean meal	9.08	39.85	2.87	6.54	6.25	0.75	35.40
Rice bran	8.52	12.69	16.70	9.00	6.37	1.99	46.71
Broken rice	9.93	7.39	2.18	3.58	0.25	0.18	76.75
DCP	-	-	-	-	-	16.34	-

NFE: nitrogen free-extract

Table 2. Composition of experimental diets

Ingredients (g/100 g feed)	diet formulae					
	1	2	3	4	5	6
Soybean meal	67	67	67	67	67	67
Broken rice	10	10	10	10	10	10
Rice bran	14	14	14	14	14	14
Fish oil	1	1	1	1	1	1
Vitamin mixtures[1]	1	1	1	1	1	1
Choline chloride	0.6	0.6	0.6	0.6	0.6	0.6
Mineral mixtures[2]	3	3	3	3	3	3
Methionine	0.7	0.7	0.7	0.7	0.7	0.7
Dicalcium phosphate (DCP)	0	0.58	1.16	1.74	2.32	2.90
Chromic oxide (Cr_2O_3)	1	1	1	1	1	1
Rice hull	1.7	1.12	0.54	0	0	0
P content (%)						
Total P[3]	0.91	1.06	1.16	1.27	1.34	1.46
Available P (AvP)[4]	0.46	0.58	0.66	0.72	0.75	0.82

[1] Vitamin mixture (g/kg feed): Thiamine(B_1) 10 mg; Riboflavin(B_2) 20 mg; Pyridoxine (B_6) 10 mg; Cobalamine (B_{12}) 2 mg; Retinal (A) 4,000 IU; Cholecaciferol (D_3) 2,000 IU; Menadione sodium bisulfite (K_3) 80 mg; Folic acid 5 mg; Calcium pantothenate 40 mg; Inositol 400 mg; Niacin 150 mg; Tocopherol (E) 50 mg ; Biotin 1 mg; Ascorbic acid (C) 500 mg

[2] Mineral mixture (g/kg feed): Na 3.278 g.; Mg 25.25 g.; K 76.612 g.; Ca 49.096 g.; Fe 4.821 g.; Zn 0.667 g.; Mn 0.433 g.; Cu 0.069 g.; CO 0.00198 g.; I 0.01g

[3] datas from analysis

[4] calculated from apparent digestibility coefficient for P in each feed formular

Table 3. Proximate analysis of experimental diets (% on dry matter basis)

Experimental group	AvP (%)	Composition (%)						
		Moisture	Protein	Fat	Ash	Crude fiber	P	NFE
1	0.46	1.77	31.58	5.52	9.30	4.55	0.91	47.21
2	0.58	1.94	31.96	5.54	9.41	5.05	1.06	46.46
3	0.66	2.17	30.96	6.47	9.64	5.39	1.16	46.06
4	0.72	2.80	30.21	5.65	10.17	4.79	1.27	46.38
5	0.75	2.71	30.21	5.81	10.82	4.88	1.34	45.58
6	0.82	2.47	31.24	6.26	10.66	3.22	1.46	46.15

NFE: nitrogen free extract

2 h at 100 C, ether extracted in a Soxlet apparatus for 12 h (AOAC, 1990) to remove fat, dried again, ground and ashed in a muffle furnance for 12 h at 600 C. The ash was weighed and subsequently analyzed for P by the molybdovanadate method (AOAC, 1990). Each fish was filleted and the fillets were sealed in a plastic bag and stored frozen at -20 C until analyzed for protein, fat and moisture. Crude protein was determined by the macro-Kjeldahl method, moisture content was determined by oven drying and crude fat by Bligh and Dyer (1959).

2.4 Calculation for P requirement

P requirement is calculated using linear and different types of nonlinear regression models. For nonlinear models, quadratic and cubic model were used. For linear regressions, simple and piecewise linear (fitting two different regression functions to the same data) equations were used. Dietary P requirement of sex-reversed red tilapia was estimated by using a nonlinear quadratic regression model of vertebrae P and whole body P of fish sampled after 8 weeks of feeding various experimental diets, against dietary P concentration. The quadratic equation used in the model was as follows:

Table 4. Average body weight of sex-reversed red tilapia fed the experimental diets for 8 weeks[1]

Experimental Group	AvP (%)	Rearing period (week)				
		0	2	4	6	8
1	0.46	25.15±0.06	35.52±2.61	47.24±4.41[ab]	62.58±6.56[ab]	75.11±9.24[ab]
2	0.58	25.23±0.12	34.47±0.95	43.49±0.85[a]	55.54±1.67[a]	66.99±3.74[a]
3	0.66	25.09±0.11	33.85±0.88	44.33±2.72[a]	60.54±3.47[ab]	75.37±3.52[ab]
4	0.72	25.28±0.20	35.39±0.96	47.03±1.97[ab]	63.66±4.81[bc]	81.68±9.27[bc]
5	0.75	25.02±0.02	37.12±1.79	52.05±1.45[c]	71.27±3.29[c]	92.08±4.12[c]
6	0.82	25.17±0.14	36.65±1.71	49.76±2.34[bc]	70.47±3.25[c]	90.53±6.46[c]
ANOVA *P*		NS	NS	0.012	0.004	0.003
Regression Effect		-	-	Quadratic[a]	Quadratic[b]	Quadratic[c]
R^2 values		-	-	0.387	0.547	0.576
P		NS	NS	0.026	0.003	0.002

[1]Mean ± standard deviation of three replications
Mean within each column not sharing a common superscript are significantly different (*P*<0.05)
[a]Y = 121.319X² - 141.04X + 85.7725
[b]Y = 227.956X² - 258.50X + 132.135
[c]Y = 289.856X² - 310.68X + 154.678

$$Y = a + bx + cx^2$$

Where Y = measure ash content; a = intercept; b = coefficient terms; c = coefficient of the quadratic terms; s = dietary P content (Roy and Lall, 2003)

Data from each treatment were subjected to one-way analysis of variance using SPSS 11.5 for windows. Statistical significance was chosen at $P<0.05$ and the results are presented as mean ± S.D. (standard deviation). When overall differences were significant, Duncan's test was used to compare the means between individual treatments.

3. Results

3.1 Fish behavior and external features

There were no body deformity or external feature with normal behavior and healthy for all treatments throughout the feeding period.

3.2 Growth performance

1) Average body weight

Average body weight of fish with initial weight range 25.02 ± 0.02 - 25.28 ± 0.20 g. Average body weight increased with the feeding duration. Statistical analysis showed marked differences from week 4, the fish with 0.75 and 0.82% AvP showed higher average body weight than other treatments ($P<0.05$), though not different from those with 0.72% AvP ($P>0.05$). During week 6-8, the same trend was observed as in week 4; fish in treatments 1-3 (0.46-0.66% AvP) showed low average body weight while those in treatments 4-6 (0.72-0.82% AvP) showed higher average body weight ($P<0.05$, Table 4).

2) Weight gain, feed conversion ratio (FCR) and survival

Weight gain, FCR, rate of feed intake and survival of fish for all 6 treatments are presented in Table 5. Weight gain showed similar trend as the average body weight. Fish given feed with 0.75% AvP (Formula 5) and with 0.82% AvP (Formula 6) showed the highest weight gain ($P<0.05$) although not different from those with 0.72% AvP in their feed ($P>0.05$, Table 5). There was no significant difference in FCR or survival with the addition of supplemental DCP to the basal diet ($P<0.05$) (Table 5).

3) Apparent digestibility coefficients of P (ADCP)

ADCP differed significantly among treatments ($P < 0.05$) ranging 50.46± 0.33 - 57.18 ±0.85 %. Highest ADCP were provided by formulae 3 and 4 feeds, followed by formulae 5, 6, 2 and 1, respectively (Table 6).

4) Whole body P, vertebrae P and vertebrae ash

Whole body P, vertebrae P and vertebrae ash showed linear increase with P supplementation (Table 7). Whole body P and vertebrae P content data were subjected to non-linear regression analysis to determine optimum requirement for sex-reversed red tilapia. The mean corrected R^2 values for whole body P were 0.735, 0.814 and 0.816 for simple linear, quadratic and cubic relation equation, respectively. Based on the measured R^2, we chose the quadratic with the simpler description of the data. The quadratic analysis indi-

Table 5. Weight gain, FCR and survival of sex-reversed red tilapia fed the experimental diets[1]

Experimental group	AvP (%)	Weight gain (%)	FCR	Survival (%)
1	0.46	194.24 ±43.45^{ab}	1.39±0.16	98.33± 0.03^{a}
2	0.58	165.52±15.28^{a}	1.34±0.07	100±0.00^{a}
3	0.66	200.38 ±13.36^{ab}	1.27±0.06	100±0.00^{a}
4	0.72	223.16 ±36.49^{bc}	1.20±0.09	100±0.00^{a}
5	0.75	268.05±16.43^{c}	1.18±0.02	100±0.00^{a}
6	0.82	259.71±27.55^{c}	1.18±0.05	100±0.00^{a}
ANOVA *P*		0.005	NS	NS
Regression Effect		Quadratic^{a}	Quadratic^{b}	-
R^2 values		0.563	0.503	-
P		0.002	0.005	NS

[1] Mean ± standard deviation of three replications
Mean within each column not sharing a common superscript are significantly different (*P*<0.05)
[a] $Y = 1084.92X^2 - 1137.2X + 479.846$
[b] $Y = 0.363X^2 – 1.1006X + 1.828$

cated that the available dietary P requirement of sex-reversed red tilapia is 0.76% available P. However, the dietary requirement based on vertebrae P was estimated at 0.79% available P.

5) P and alkaline phosphatase in serum

Dietary treatment had a significant effect on serum P and serum alkaline phosphatase activity (Table 8). Serum P increased from 1.89 to 2.48 mg/l with the increase of dietary available P supplementation from 0.46% to 0.75% (*P*<0.05), and leveled off at 0.82% dietary available P. Serum alkaline phosphatase activity in fish did not show statistic difference among fish fed diets with the addition of supplemental DCP to the basal diet (*P*>0.05) (Table 8).

6) Muscle fat, muscle protein, muscle moisture and visceral fat

The muscle fat and visceral fat content decreased from 5.42% to 3.57% (*P*<0.05) with the increase in dietary available P (Table 9). However, the protein content showed an increase from 84.28% to 85.80%, and no significant differences were observed among the other dietary treatments.

4. Discussion

Datas from this study showed that the growth response of sex-reversed red tilapia was significantly affected by the supplementation of dietary available P, and a positive relationship was found between the growth and dietary available P levels. Weight gain was lower in fish fed the basal diet due

Table 6. Apparent digestibility coefficients of P (ADCP) from experimental diets for sex-reversed red tilapia

Experimental group	AvP (%)	ADC P
1	0.46	50.46±0.33^{a}
2	0.58	54.47±1.73^{b}
3	0.66	57.18±0.85^{c}
4	0.72	56.67±0.53^{c}
5	0.75	56.02 ±0.87^{bc}
6	0.82	55.94 ±0.22^{bc}
ANOVA *P*		<0.001
Regression Effect		Quadratic^{a}
R^2 values		0.863
P		<0.001

[1] Mean ± standard deviation of three replications
Mean within each column not sharing a common superscript are significantly different (*P*<0.05)
[a] $Y = (-96.715X^2) + 138.507X + 7.1615$

to insufficient P being available for growth after being allocated for utilization in other physiological processes (Brown *et al*., 1992). Reduced growth was the main P deficiency sign observed in most fish species. Lower growth and high FCR due to dietary P deficiency also have been observed in haddock (Roy and Lall, 2003), rainbow trout (Ketola and Richmond, 1994), sunshine bass (Brown *et al*., 1992), channel catfish (Wilson *et al*., 1982), common carp (Ogino and Takeda, 1978) and red sea bream (Sakamoto and Yone,

Table 7. Whole body P, vertebrae ash, vertebrae P and fecal P of sex-reversed red tilapia fed the experimental diets for 8 weeks (%)[1]

Experimental group	AvP (%)	Whole body P	Vertebrae P	Vertebrae ash
1	0.46	2.22 ± 0.16[a]	9.55 ± 0.74[a]	60.02 ± 0.29[a]
2	0.58	2.56 ± 0.02[b]	11.43 ± 0.22[b]	59.77 ± 0.20[a]
3	0.66	2.79 ± 0.02[bc]	11.60 ± 0.26[bc]	62.47 ± 0.59[b]
4	0.72	2.83 ± 0.16[c]	12.25 ± 0.27[cd]	63.92 ± 0.26[c]
5	0.75	2.94 ± 0.10[c]	12.41 ± 0.51[d]	64.28 ± 0.20[c]
6	0.82	2.87 ± 0.22[c]	12.06 ± 0.17[bcd]	64.96 ± 0.15[d]
ANOVA *P*		<0.001	<0.001	<0.001
Regression Effect		Cubic[a]	Quadratic[b]	Quadratic[c]
R^2 values		0.814	0.862	0.887
P		<0.001	<0.001	<0.001

[1] Mean ± standard deviation of three replications: Mean within each column not sharing a common superscript are significantly different (*P*<0.05)

[a] $Y = (-3.0117X^3) + 5.6917X - 0.1160$ (Xmax = 0.79)

[b] $Y = (-28.413X^2) + 43.2070X - 4.2672$ (Xmax =0.76)

[c] $Y = 22.174X^2 + 12.069X + 60.48$ (Xmax = 0.79)

Table 8. Serum P content and serum alkaline phosphatase activity of sex-reversed red tilapia fed the experimental diets for 8 weeks [1]

Experimental group	AvP (%)	Serum P (mg/l)	Serum alkaline phosphatase activity (IU/l)
1	0.46	1.89 ± 1.26[a]	19.33 ± 1.15
2	0.58	2.13 ± 1.49[b]	20.00 ± 1.00
3	0.66	2.42 ± 1.06[c]	21.50 ± 1.53
4	0.72	2.47 ± 1.77[c]	22.33 ± 1.15
5	0.75	2.48 ± 2.47[c]	23.33 ± 2.08
6	0.82	2.18 ± 0.65[b]	20.79 ± 4.35
ANOVA *P*		<0.001	NS
Regression Effect		Quadratic[a]	Quadratic[b]
R^2 values		0.816	0.423
P		<0.001	0.016

[1] Mean ± standard deviation of three replications
Mean within each column not sharing a common superscript are significantly different (*P*<0.05)

[a] $Y = (-4.2222X^2) + 7.2178X - 0.5578$

[b] $Y = 10.1716X^2 + 0.5718X + 11.7577$

1978). In contrast, no significant changes in growth of red snapper (Pongmaneerat *et al*., 2006), seabass (Chaimongkol and Boonyaratpalin, 2001) and Atlantic salmon (Vielma and Lall, 1998) fed low P diets have been observed. The requirement of essential elements affects feed efficiency (Shearer, 1995). Several factors including age, stage of development, diet composition, duration of experiment, health and rearing condition may affect growth. Generally, young animals are more sensitive to nutrient deficiency than those at later stage of development because during the rapid growth period dietary energy is utilized more efficiently. Redlip mullet fry (initial weight, 3.8 g) showed a clearer growth response to dietary P supplementation than large fish (initial weight 26.5 g) under the same experimental conditions (El-Zibdeh *et al*., 1995). Small fish were also more sensitive to P deficiency.

Dietary P levels had a significant effect on sex-reversed red tilapia whole body P, vertebrae ash and vertebrae P content. Due to the function of P in the vertebrae structure, the vertebrae P concentration is responsive to dietary P intake (Ogino and Takeda, 1978; Sakamoto and Yone, 1978; Chavez-Sanchez *et al*., 2000). Thus vertebrae P and whole body P including vertebrae ash contents are considered to be the most sensitive criterium for P utilization in freshwater fish (Ketola, 1975; Watanabe *et al*., 1980a; Ketola and Richmond, 1994; Rodehutscord, 1996, Jahan *et al*., 2001) and marine fish (Sakamoto and Yone, 1978; Dougall *et al*., 1996; Borlongan and Satoh, 2001). A close correlation was observed between dietary P and vertebrae ash content, which indicates that vertebrae ash values could be used to determine the efficiency of dietary P utilization and to estimate the P requirement of sex-reversed red tilapia. On the basis of vertebrae P and whole body P content, the estimated dietary P requirement of sex-reversed red tilapia was 0.76% and 0.79% of available P, respectively. The estimated P requirement value was slightly lower than reported values for tilapia and Atlantic salmon determined by Watanabe *et al*. (1980) and Asgard and Shearer (1997), respectively. Roy and Lall (2003) suggested that tilapia has acellular vertebrae, whereas other fish species i.e. salmonids and cyprinids have cellular vertebrae. Some differences in the estimated P requirement of various fish species may be due to the following factors: 1) species differences and variation in intestinal P absorption rate (Riche and Brown, 1999; Avila *et al*., 2000), 2) differences in the availability of various inorganic and organic P sources (Lall and Vielma, 2001; Satoh *et al*., 2002), 3) fish size, condition factor and the stage of development (Shearer, 1984; El-Zibdeh *et al*., 1995; Ronsholdt, 1995), and dietary energy content and feed efficiency (Shearer, 1995) differences in experimental design (Shearer, 2000).

In this study, P in serum was found with the increase of dietary available P. In most fish, the skeleton represents a reservoir of Ca, P and other ions that are in a state of continual exchange with electrolytes found in blood and extracellular fluids (Lall, 2002). The result of this study agrees with the experiments reported in other fish species i.e. channel catfish (Eya and Lovell, 1997), haddock (Roy and Lall, 2003) and Japanese seabass (Zhang *et al*., 2006). Roy and Lall (2003) suggested that the concentration of plasma or plasma phosphate is known to be influenced by several dietary and physiological factors (Cross *et al*., 1990). Rodehutscord (1996) observed a wide range in reported plasma phosphate concentration due to the different range of time lapsed after feeding when blood samples were collected. Thus establishment of reasonable time for blood sample collection after feeding fish may produce consistent results (Roy and Lall, 2003). In this study, the first plateau of serum P appeared when dietary available P increased from 0.58-0.75% indicating that the first plateau emerged before dietary available P reached the minimum level for optimum growth. Hence, the first plateau was probably the result of the ion buffering effects of skeleton and other tissues of fish. When dietary available P level increased from 0.58-0.75%, the P levels in serum, whole body and vertebrae increased significantly, and then leveled off. This suggests that serum P concentration has been beyond the buffering capacity of skeleton and other tissues of fish at 0.75% dietary available P. Some previous studies showed that blood P concentrations were influenced by several dietary, physio-

Table 9. Means of crude fat, crude protein and moisture contents of muscle and visceral fat content of sex-reversed red tilapia fed the experimental diets containing different concentration of dietary available P[1]

Experimental group	AvP (%)	Composition (%)			
		Muscle fat	Muscle protein	Muscle moisture	Visceral fat
1	0.46	5.42 ± 0.12[c]	84.28 ± 0.64	78.17 ± 0.43	33.48 ± 1.27[c]
2	0.58	4.43 ± 0.02[b]	84.78 ± 0.40	78.93 ± 0.54	29.48 ± 0.69[b]
3	0.66	4.67 ± 0.16[b]	85.31 ± 0.30	78.75 ± 0.15	27.90 ± 2.79[b]
4	0.72	4.49 ± 0.20[b]	85.80 ± 0.08	79.20 ± 0.31	23.86 ± 1.07[b]
5	0.75	3.57 ± 0.30[a]	84.59 ± 0.08	78.88 ± 0.42	21.82 ± 0.66[b]
6	0.82	3.58 ± 0.07[a]	85.50 ± 1.13	78.91 ± 0.62	19.54 ± 0.82[a]
ANOVA *P*		<0.001	NS	NS	<0.001
Regression Effect		Linear[a]	-	-	Quadratic[b]
R^2 values		0.723	-	-	0.825
P		<0.001	NS	NS	<0.001

[1] Mean ± standard deviation of three replications

Mean within each column not sharing a common superscript are significantly different (*P*<0.05)

[a] $Y = 7.5009 - 4.7265X$

[b] $Y = 280.825X^2 - 351.64X + 135.337$

logical and environmental factors (Phillips, 1962; Hille, 1982; Cross *et al*., 1990). Thus, plasma P or serum P level will be not suitable as an indicator for P utilization or requirement until the mechanism of P homeostasis is clarified (Eya and Lovell, 1997; Skoberg *et al*., 1997; Roy and Lall, 2003). Alkaline phosphatase is closely associated with the metabolism of calcium and P and takes part in chondrogenic and osteoblastic activities in birds (Vinuela *et al*., 1991). An elevation of serum alkaline phosphatase activity has been reported to occur with an increase in osteoblastic activity (Kaplan, 1972). In the present study, although there was no statistical difference among tilapia fed diets with the addition of supplemental DCP to the basal diet, the activity of serum alkaline phosphatase increased with the increase of available dietary P from 0.58% to 0.75%. Similar result has been found in rainbow trout (Skoberg *et al*., 1997). This may be explained by the higher mineralization of vertebrae that resulted in a raise of alkaline phosphatase activity. However, Sakamoto and Yone (1980) found a low P intake by red sea bream increased plasma alkaline phosphatase activity. Shearer and Hardy (1987) reported plasma alkaline phosphatase activity in rainbow trout was not significantly affected by feeding P sufficient and deficient diets. The difference is probably due to many factors influencing plasma alkaline phosphatase activity, including water chemistry (Bowser *et al.*, 1989), feed intake (Sauer and Haider, 1979), temperature (Sauer and Haider, 1977; Sakaguchi and Hamaguchi, 1979; Lie *et al*., 1988), and life stage (Johnston *et al*., 1994). The decreased muscle lipid and increased muscle protein with increasing dietary available P in the present study was consistent with some previous studies, including red seabream (Sakamoto and Yone, 1978), common carp (Takeuchi and Nakazoe, 1981), channel catfish (Eya and Lovell, 1997), rainbow trout (Skoberg *et al*., 1997), haddock (Roy and Lall, 2003). Higher visceral fat seen in sex-reversed red tilapia that received low dietary available P agrees with results obtained with red seabream (Sakamoto and Yone, 1978). These studies suggest impaired oxidative phosphorylation because of P deficiency leads to inhibition of the TCA cycle and accumulation of acetyl-CoA. In this study, the protein content of muscle of sex-reversed red tilapia showed a decrease with the increase in muscle lipid content. Lower protein in the muscle of fish fed low P diet may explain that inhibition of β-oxidation of fatty acid resulted in a lower utilization of lipid as an energy source, then fish utilize protein for energy purposes as an alternative to lipid (Roy and Lall, 2003).

5. Conclusion

It is obvious from results of this study that P is essential for growth, efficient feed utilization and vertebrae mineralization of sex-reversed red tilapia. Excess P caused excessive excretion of this element in environment and it has a negative effect on vertebrae mineralization. According to the results, 0.76-0.79% dietary available P was required for sex-reversed red tilapia. These values can be used as references to produce environment friendly aquatic feeds for sex-reversed red tilapia to minimize excess P discharge to the environment and therefore protect the aquatic environment.

References

AOAC. (Association of Official Analytical Chemists). 1990. Official Methods of Analysis. Washington, DC: AOAC.

Asgard, T. and Shearer, K.D. 1997. The dietary P requirement of juvenile Atlantic salmon (*Salmo salar*) and its relationship to the P requirements reported for other fishes. Aquaculture Nutrition 3,17-23.

Avila, A.M., Basantes, H.T. and Ferraris, R.P., 2000. Dietary phosphate regulates intestinal transport and plasma concentrations of phosphate in rainbow trout. Journal of Comparative Physiolology B 170, 201-209.

Auer, M.T., Kisser, M.S. and Canale, R.P. 1986. Identification of critical nutrient levels through field verification of models for phosphorus and phytoplankton growth. Canadian Journal of Fisheries and Aquatic Science 43, 379-388.

Beveridge, M.C.M. 1984. Cage and pen fish farming: carrying capacity models and environmental impact. FAO Fisheries Technical-Paper, pp. 255

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Fisheries and Aquatic Science 37, 911-917.

Borlongan, I.G. and Satoh, S., 2001. Dietary P requirement of juvenile milkfish, *Chanos chanos* (Forsskal). Aquaculture Research 32 (Suppl. 1), 26-32.

Bowser, P.R., Wooster, G.A., Aluisio, A.L. and Blue, J.T. 1989. Plasma chemistries of nitrite stressed Atlantic salmon *Salmo salar*. Journal World Aquaculture Society 20, 173-180.

Boyd, C.E. 1971. Phosphorus dymamic in ponds. Proceeding of the Annual Conference of the Southeast Associated, Game Fish Comm. 25, 418-426.

Brown, M.L., Jaramillo, F. and Gatlin III, D.M. 1992. Dietary phosphorus requirements of juvenile sunshine bass at different salinities. Progessive Fish-Culturist 54, 148-156.

Bureau, D.P. and Cho, C.Y. 1999. Phosphorus utilization by rainbow trout (*Oncorhynchus mykiss*): Estimation of dissolved phosphorus waste output. Aquaculture 179, 127-140.

Chaimongkol, A. and Boonyaratpalin, M. 2001. Effects of ash and inorganic phosphorus in diets on growth and mineral composition of seabass, *Lates calcarifer* (Bloch). Aquaculture Research 32, 53-59

Chavez-Sanchez, C., Martinez-Palacios, C.A., Martinez-Oerez, G. and Ross, L.G. 2000. Phosphorus and calcium requirements in diet of the American cichlid, *Cichlasoma urophalmus* (Gunther). Aquaculture Nutrition 6, 1-9.

Chen, P.S., Toribara, T.Y. and Warner, H. 1956. Microdetermination of P. Analytical Chemistry 28, 1756-1758.

Cross. H.S., Deviec, H. and Peterlic, M. 1990. Mechanism and regulation of intestinal phosphate absorption. Mineral Electrolyte Metabolism 16, 115-124.

Dougall, D.S., Woods III, L.C., Douglass, L.A. and Soares, J.H., 1996. Dietary P requirement of juvenile striped bass, *Morone saxatilis*. Journal of World Aquaculture Society 27, 82-91.

El-Zibdeh, M., Yoshimatsu, T., Matsui, S., Furuichi, M., Kitajima, C. and Azuma, R., 1995. Requirement of redlip mullet for dietary P. Journal of the Faculty of Agricultural, Kyushu University 40 (12), 135-145.

Eya, J.C. and Lovell, R.T. 1997. Available P requirement of food-size channel catfish (*Ictalurus punctatus*) fed practical diets in ponds. Aquaculture 154, 283-291.

Halver, J.E., Hardy, R.W. and Hardy, D.M. 2002. Fish Nutrition. Academic Press, New York.

Hille, S. 1982. A literature review of the blood chemistry of rainbow trout *Salmo gairdneri* Richardson. Journal of Fish Biology 20, 535-569.

Jahan, P., Watanabe, T., Satoh, S. and Kiron, V. 2001. Formulation of low P loading diets for carp (*Cyprinus carpio* L.). Aquaculture Research 32 (Suppl. 1), 361-368.

Johnston, C.E., Homey, B.S., Deluca, S., MacKenzie, A., Eales, J.G. and Angus, R. 1994. Changes in alkaline phosphatase isozyme activity in tissue and plasma of Atlantic salmon (*Salmo salar*) before and during smoltification and gonadal maturation. Fish Physiology and Biochemistry 12: 485-497.

Kaplan, M.M. 1972. Medical intelligence. Current concepts alkaline phosphatase. The New England Journal of Medicine 286: 200-202.

Ketola, H.G. 1975. Requirement of Atlantic salmon for dietary P. Transactions of the American Fisheries Society 104, 548-551.

Ketola, H.G. and Harland, B.F. 1993. Influence of phosphorus in rainbow trout diets on phosphorus discharges in effluent water. Transactions of the American Fisheries Society 122, 1120-1126.

Ketola, H.G. and Richmond, M.E. 1994. Requirement of rainbow trout for dietary P and its relationship to the amount discharged in hatchery effluent. Transactions of the American Fisheries Society 104, 587-594.

Lall, S.P. 1991. Digestibility, metabolism and excretion of dietary P in fish. *In*: Cowey, C.B. and Cho, C.Y. (eds.). Nutritional Strategies and Aquaculture Waste. Proceeding of the First International Symposium on Nutritional Strategies in Management of Aquaculture Waste. University of Guelph, Guelph. ON, Canada.

Lall, S.P. 2002. The minerals. *In*: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition, 3rd ed. Academic Press, San Diego, CA.

Lall, S.P. and Bishop, F.J. 1977. Studies on mineral and protein utilization by Atlantic salmon grown in seawater. Fisheries and Marine Service Technical Report 88, 1-17.

Lall, S.P., and Vielma, J. 2001. P in fish nutrition. *In*: Merican, Z. (Ed.), Inter. Aqua Feed. Issue I, Andrew West. Turret RAI, Middlesex, England, pp. 26-29.

Lie, O., Waagboe, R. and Sandnes, K. 1988. Growth and chemical composition of adult Atlantic salmon (*Salmo salar*) fed dry and silage-based diets. Aquaculture 69, 343-353.

Lovell, R.T. 1988. Nutritional and Feeding of Fish. Auburn, Alabama. Auburn University. 267 pp.

Lovell, R.T. 1989. Nutrition and Feeding of Fish. Van Nostrand Reinhold, New York, NY. 260 pp.

Ogino, C. and Takeda, H. 1976. Mineral requirements in fish: III. Calcium and phosphorus require-ments of carp. Bulletin Japanese Society and Scientific Fisheries 42, 739-799.

Ogino, C., and Takeda, H. 1978. Requirement of rainbow trout for dietary calcium and P in carp and rainbow trout. Bulletin Japanese Society and Scientific Fisheries 45, 1527-1532.

Oliva-Teles, A., Pereira, J.P., Gouveia, A. and Gomes, E. 1998. Utilisation of diets supplemented with microbial phytase by seabass (*Dicentrarchus labrax*) juveniles. Aquatic Living Resources 11, 255-259.

Phillips, A.M., Podoliak, H.A., Brockway, D.R. and Vaughn, R.R. 1958. The nutrition of trout. Cortland Hatch. Report, No. 26, Fish. Res. Bull. No.21, New York Conservation Department, Albany, NY.

Phillips Jr, A.M. 1962. Effect of dietary and water temperature on the blood phosphorus of brook trout. Progressive Fish-Culturist 24, 22-25.

Phromkunhong, W., Boonyaratpalin, M. and Storch, V. 1997. Different concentrations of ascorbyl-2-monophosphate-magnesium as a dietary sources of vitamin C for seabass, *Lates calcarifer* (Bloch). Aquaculture. 151, 225-243.

Phromkunthing, W. and Gabaudan, J. 2006. Used of microbial phytase to replace inorganic phosphorus in sex-reversed red tilapia: 1 dose response. Songklanakarin Journal of Science and Technology 28, 731-734.

Pongmaneerat, J., Chainark, P., Koaean, K., Silakes, S., Yuttayong, D. and Chindamaikul, T. 2006. Optimum phosphorus level in diets for red snapper (*Lutjanus argentimatus*, Forskal). Thai Fisheries Gazette 59, 252-258.

Riche, M. and Brown, P.B.1999. Incorporation of plant protein feedstuffs into fish meal diets for rainbow trout increases P availability. Aquaculture Nutrition 5, 101-105.

Rodehutscord, M. 1996. Response of rainbow trout (*Onchorhynchus mykiss*) growing from 50 to 200g to supplements of dibasic sodium phosphate in a semipurified diet. The Journal of Nutrition 126, 324-331

Rønsholdt, B. 1995. Effect of size/age and feed composition on body composition and P content of rainbow trout, *Oncorhynchus mykiss*. Water Science and Technology

31, 175-183.

Roy, P.K. and Lall, S.P. 2003. Dietary P requirement of juvenile haddock (*Melanogrammus aeglefinus* L.). Aquaculture 221, 451-468.

Sakaguchi, H. and Hamaguchi, A. 1979. Physioligical studies on cultured red sea bream: I. Seasonal variation of chemical constientd in plasma, hepatopancreas and other viscera. Bulletin of the Japanese Society of Scientific Fisheries 45, 443-448.

Sakamoto, S. and Yone, Y. 1978. Effects of dietary P on chemical composition of red sea bream. Bulletin of the Japanese Society of Scientific Fisheries 44, 227-229.

Sakamoto, S. and Yone, Y. 1980. A principal source of deposited lipid in phosphorus deficient red sea bream. Bulletin of the Japanese Society of Scientific Fisheries 46, 1227-1230.

Satoh, S., Takanezwa, M., Akimoto, A., Kiron, V. and Watanabe, T. 2002. Changes of phosphorus absorption from several feed ingredients in rainbow trout during growing stages and effect of extrusion of soybean meal. Fisheries Science 68, 325-331.

Sauer, D.M. and Haider, G. 1977. Enzyme activities in the serum of rainbow trout, *Salma gairdneri* Richardson: the effect of water temperature. Journal of Fish Biology 11, 605-612.

Sauer, D.M. and Haider, G. 1979. Enzyme activities in the plasma of rainbow trout, *Salmo gairdneri* Richardson. The effect of nutritional status and salinity. Journal of Fish Biology 14, 407-412.

Sigma Chemical Company. 1960. The colorimetric determination of P, alkaline, acid and prostatic acid. Technical Bulletin No. 104. Revised edn, St. Louis. MO.

Shearer, K.D. 1984. Changes in elemental composition of hatchery-reared rainbow trout, *Salmo gairdneri*, associated with growth and reproduction. Canadian Journal of Fisheries and Aquatic Science 41, 1592-1600.

Shearer, K.D. and Hardy, R.W. 1987. Phosphorus deficiency in rainbow trout fed a diet containing deboned fillet scarp. The Progressive Fish-Culturist 49, 192-197.

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

Shearer, K.D. 2000. Experimental design, statistical analysis and modeling of dietary nutrient requirement studies for fish: A critical review. Aquaculture Nutrition 6, 91-102.

Skoberg, D.I., Yogev, L., Hardy, R.W. and Dong, F.M. 1997. Metabolic response to dietary phosphorus intake in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 157, 11-24.

Takeuchi, M. and Nakazoe, J. 1981. Effect of dietary phosphorus on lipid content and its composition in carp. Bulletin of the Japanese Society of Scientific Fisheries 47, 347-352.

Vielma, J. and Lall, S.P. 1998. P utilization by Atlantic salmon (*Salmo salar*) reared in freshwater is not influenced by higher dietary calcium intake. Aquaculture 160, 117-128.

Vinuela, J., Ferrer, M. and Recio, F. 1991. Age- related variations in plasma levels of alkaline phosphatase, calcium and inorganic phosphorus in chick of two species of raptors. Comparative Biochemistry and Physiology 99, 49-54.

Watanabe, T., Takeuchi, T., Murakami, A. and Ogino, C. 1980 a. The availability to *Tilapia niloticus* of P in white fishmeal. Bulletin of the Japanese Society of Scientific Fisheries 46 , 897-899.

Watanabe, T.,Takeuchi, T., Murakami, A. Nose, T. and Ogino, C. 1980b. Requirement of chum salmon held in freshwater for dietary P. Bulletin of the Japanese Society of Scientific Fisheries 46, 361-367.

Wiesmann, D., Scheid, H. and Pfeffer, E. 1988. Water pollution with phosphorus of dietary origin by intensively fed rainbow trout (*Salmo gairdneri* Rich.). Aquaculture 69, 263-270.

Wilson, R.P., Robinson, E.H., Gatlin. D.M. and Poe, W.E. 1982. Dietary phosphorus requirement of channel catfish. The Journal of Nutrition 112, 1197-1202.

Zhang, C., Mai, K., Ai, Q., Zhang, W., Duan, Q., Tan, B., Ma, H., Xu, W., Linfu, Z. and Wang, X. 2006. Dietary phosphorus requirement of juvenile Japanese seabass, *Lateolabrax japonicus*. Aquaculture 255, 201-209.