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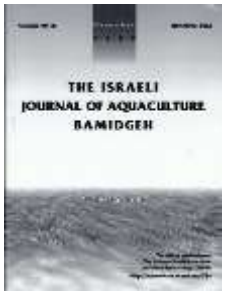

Dietary Phosphorus Requirements of Juvenile Hybrid Tilapia (*Oreochromis niloticus*♀× *O. Aureus*♂) Fed Fishmeal-free Practical Diets

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Abstract

A growth trial was conducted to estimate the optimum levels of dietary phosphorus (P) for juvenile hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) fed fishmeal-free practical diets. Hybrid tilapia (1.68 ± 0.08 g) were fed diets containing various levels (0.0%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0%) of additional inorganic phosphorus for 10 weeks using two different sources of phosphorus : calcium dihydrogen phosphate (MCP), and sodium dihydrogen phosphate (MSP). Hybrid tilapia fed the P-supplemented diets showed significantly higher weight gain (WG) and mineral deposition than those fed the unsupplemented diet. Based on weight gain and vertebral phosphorus content, the available phosphorus requirements of hybrid tilapia were estimated as 1% and 1.31% (0.6% and 0.9% based on additional phosphorus content) respectively, when MSP was used as a phosphorus source. When MCP was the phosphorus source, the requirement estimated for weight gain was 0.95% (0.61% based on additional phosphorus content). In addition, MCP contributed to increased growth and a higher mineral deposition rate than did MSP, in freshwater-reared hybrid tilapia.

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Introduction

Phosphorus (P) is one of the most important nutrients for fish because it is essential to the formation of adenosine triphosphate (ATP), nucleic acids, phospholipids, cell membranes and coenzymes. It also plays a major role in many metabolic processes involving carbohydrates, lipids, and nitrogen (Yang et al., 2006). As the concentration of P in water is very low, fish in aquaculture must obtain most of their P from their diet (Shao et al., 2008).

In the past, formulated fish diets typically contained high levels of fishmeal or other animal feedstuffs (Sugiura et al., 2004). These sources which are high in animal-protein often contribute an adequate proportion of total P to fish diets (Pimentel-Rodrigues and Oliva-Teles, 2007). However, due to declining fish stocks, plant protein sources are being increasingly used as alternatives to fishmeal in fish diets. These plant protein sources, however, have lower P levels than fishmeal, and their P is present primarily in the form of phytic acid, which has low bioavailability for fish (Viola et al., 1986). Inorganic phosphates are therefore added as P supplements in practical feed. The two main P sources that are currently used as feed additives are calcium dihydrogen phosphate (MCP) and sodium dihydrogen phosphate (MSP).

Previous fish studies have indicated that P deficiency can cause reduced growth, anorexia, skeletal deformity, decreased feed efficiency, and high body lipid content (Roy and Lall, 2003). However, excess dietary P can contribute to algal growth or eutrophication, and inferior water quality (Liang et al., 2012). Therefore, since there are both economic and environmental reasons to identify the optimal dietary P levels of feed, it is important to ascertain the dietary P requirements in cultured aquatic animals.

Tilapia is one of the most important, and extensively cultured, fish species in the world. In China, the hybrid tilapia (*Oreochromis niloticus* ♂ × *O. aureus* ♀) is the most widely cultured type of tilapia because of its rapid growth and high resistance to disease. The P requirements of tilapia have been studied (Furuya et al., 2008; Watanabe et al., 1980) however the range of criteria used to determine the P requirements has grown. The levels of P in plasma or vertebrae, the expression of genes involved in the absorption of P, and common production parameters such as weight gain (WG), and biomass gain (BG), all provide useful information regarding the optimal P level in fish diets (Antony Jesu Prabhu et al., 2013). However, little is known about the P requirements in tilapia fed fishmeal-free practical diets, which are widely used in hybrid tilapia culture.

Our study had two objectives: 1) to estimate the dietary P requirements of hybrid tilapia using fishmeal-free practical diets, and 2) to evaluate and compare the effects of two different phosphorus sources (MSP and MCP).

Materials and Methods

Experimental design, fish rearing system, and feeding. Juvenile tilapias with an initial average weight of 1.68 ± 0.08 g were obtained from the PanYu Tilapia Improved Variety Company. Tilapia (n = 1,800) were randomly distributed into 36 fiberglass tanks (98 cm × 48 cm × 42 cm) at densities of 30 fish/tank, and each type of diet was assigned to 3 parallel tanks. Dechlorinated freshwater was supplied to each tank via a circulating system. Fish were housed under a 12:12 hour light/dark photoperiod. Dissolved oxygen level and water temperature were monitored each day, averaging 8.13 ± 0.60 mg/L and 26.5 ± 1.7 ℃, respectively. The concentration of dissolved ammonia in water was 0.33 ± 0.05 mg/L, and pH was 7.43 ± 0.21. For 3 weeks prior to the experiment, the subject fish were acclimated to the experimental conditions, and then their initial weight was measured. During the experiment, fish were fed twice daily (at 10:00 and 15:00) for 10 weeks. Individual daily food consumption until satiation was approximately 3-4 % of fish body weight. The total body weight of fish in each tank was measured once every two weeks, and from this the daily food consumption per tank was calculated. Feces collection started at the 8th week. One hour after feeding, the tanks were cleaned, and the feces were collected by siphon. Feces with complete membranes were dried at 105℃ and preserved at -20℃ for analysis.

Diet preparation. A basal diet was formulated using plant protein sources from soybean meal, rapeseed meal, peanut meal, corn, and distiller's dried grains with solubles (DDGS). (see Table 1).

Table 1. Ingredient composition of the experimental diets (%)

Ingredients	Ctrl 1	Ctrl 2	MSP02	MSP04	MSP06	MSP08	MSP10	MCP02	MCP04	MCP06	MSP08	MSP10
Wheat flour	23	19.73	21.99	20.99	19.98	18.97	17.97	22.19	21.37	20.56	19.75	18.94
Soybean meal	30	30	30	30	30	30	30	30	30	30	30	30
Rapeseed meal	20	20	20	20	20	20	20	20	20	20	20	20
DDGS	7	7	7	7	7	7	7	7	7	7	7	7
Corn	5	5	5	5	5	5	5	5	5	5	5	5
Peanut meal	10	10	10	10	10	10	10	10	10	10	10	10
Corn oil	2	2	2	2	2	2	2	2	2	2	2	2
$Ca(H_2PO_4)^2 \cdot H_2O$	0	0	0	0	0	0	0	0.81	1.63	2.44	3.25	4.06
$NaH_2PO_4 \cdot 2H_2O$	0	0	1.01	2.01	3.02	4.03	5.03	0	0	0	0	0
Soybean Phospholipid	1	1	1	1	1	1	1	1	1	1	1	1
Mineral premix[1]	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Vitamin mixture[2]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Choline	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
VC-Phosphateester	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Calcium lactate		3.27										
Chromium	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

[1] Mineral premix (mg/g of mixture): MgSO4, 255; NaCl, 120; KCl, 100; FeSO4•7H2O, 62.05; ZnSO4,7.6; MnSO4•H2O, 3.85; CuSO4, 1.57; CoSO4, 0.96; KIO3, 0.13; All ingredients were diluted with cellulose to 1 g.
[2] Vitamin mixture (mg/g of mixture): thiamin hydrochloride, 2.5; riboflavin, 10; calcium pantothenate, 25; nicotinic acid, 37.5; pyridoxine hydrochloride, 2.5; folic acid, 0.75; inositol, 100; menadione, 2; alpha tocopheryl acetate, 20; retinol acetate, 1; cholecalciferol, 0.0025; biotin, 0.25; vitamin B12, 0.05. All ingredients were diluted with cellulose to 1 g (Lin and Shiau, 2003).

Each experimental diet was individually supplemented with 0.2%, 0.4%, 0.6%, 0.8%, or 1.0% inorganic phosphorus using two kinds of phosphorus sources : MCP and MSP. Vitamin and mineral elements were added to the main ingredient mixture. All ingredients were weighed and mixed for 15 min. Deionized water (250 ml/kg dry ingredient mixture) was added, and the diet was mixed for an additional 15 min. The wet dough was placed in a mono-screw extruder (Institute of Chemical Engineering, South China University of Technology, Guangzhou, P.R. China) and extruded through a 2.5-mm die. The pellets were air dried and stored at −20 °C until use. The ingredient analysis of the diet is presented in Table 2.

Table 2. Analytical composition of the experimental diets[1]

Components	*Control 1*	*Control 2*	*MSP 0.2*	*MSP 0.4*	*MSP 0.6*	*MSP 0.8*	*MSP 1.0*	*MCP 0.2*	*MCP 0.4*	*MCP 0.6*	*MCP 0.8*	*MCP 1.0*
Protein (%)	35.93	35.7	36.3	36.55	36.29	37.7	36.01	36.7	36.6	36.5	36.45	35.52
Energy (KJ/g)	20.64	19.56	19.8	19.97	19.79	19.6	19.2	20.3	19.9	19.9	19.63	19.06
Energy Protein (KJ/g)	57.44	54.8	54.7	54.64	54.53	52	53.32	55.3	54.5	54.4	53.87	53.66
Ca (%)	0.34	0.81	0.31	0.3	0.29	0.3	0.31	0.44	0.57	0.77	0.85	0.97
Total P (%)	0.66	0.66	0.89	1.1	1.31	1.51	1.8	0.91	1.11	1.32	1.54	1.75
Available P^2 (%)	0.32	0.32	0.52	0.76	1	1.15	1.43	0.58	0.71	0.95	1.15	1.36

[1] Analysis results are expressed on a dry matter basis.
[2] The values were calculated based on the digestibility of phosphorus of the experimental diets

Data collection. At the beginning of the feeding trial, 6 fish were randomly sampled for the initial analysis of whole body composition. At the end of the 70-day experiment, the total weight of fish in each tank was recorded. Ten fish from each tank were sampled at random: 2 for analysis of whole body composition, and 8 for measurements of individual body weight, body length, viscera weight, liver weight, and mesenteric fat weight; the latter were anesthetized in advance with MS-222.

Fish carcasses were boiled in water for 6 min, and the surrounding tissues were removed from the vertebrae. The vertebrae were rinsed with deionized water, dried, and ground for mineral analyses. The mineral contents of the feed, vertebrae, and feces,

were determined by inductively coupled plasma-atomic emission spectrophotometry (ICP, model IRIS Advantage (HR), Thermo Jarrel Ash Corporation, Boston, U.S.A.) after wet digestion with nitric acid and perchloric acid. Crude protein, crude lipid, and gross energy (GE) were all determined using standard methods (AOAC, 1995). Moisture content was determined by drying in an oven at 105 °C for 24 h. Crude protein was analyzed by the Kjeldahl method after acid digestion (1030-Auto-analyzer, Tecator, Höganäs, Sweden). Crude fat was determined by the ether extraction method as described by Soxtec System HT (Soxtec System HT 6, Tecator, Sweden). Gross energy was determined using an adiabatic bomb calorimeter.

Calculations and statistical analyses. Parameters were determined as follows:

(1) Apparent digestibility coefficients (ADC) of nutrients (%) = 100 × [1-(F/D × DCr/FCr)], where F is the percentage of nutrient in feces, D is the percentage of nutrient in diet, DCr is the percentage of chromic oxide in diet and FCr is the percentage of chromic oxide in feces (Cho & Kaushik 1990).

(2) Available P content in diet (%) = total phosphorus content in diet (%) ×ADC of phosphorus (%).

(3) Biomass gain (BG) (g) = final biomass−initial biomass.

(4) Specific growth rate (SGR) (%/day) = [ln(final body weight)− ln(initial body weight)] × (100/days of the experiment).

(5) WG (%) = 100 × (final body weight−initial body weight) / (initial body weight).

(6) Survival (%) = 100 × (final fish number)/(initial fish number).

(7) VSI (viscera somatic index) (%) = 100 × viscera weight (g)/body weight (g).

(8) HSI (hepato-somatic index) (%) = 100 × liver weight (g)/body weight (g).

(9) MFI (mesenteric fat index) (%) = 100 × mesenteric fat weight (g)/body weight (g).

(10) Condition factor = 100 × final body weight (g)/body length^3 (cm).

(11) Total P retention (%) = 100 × fish P gain (g)/total P intake (g).

(12) Available P retention (%) = 100 × fish P gain (g)/available P intake (g).

All data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test (Duncan, 1955) at a significance level of $P<0.05$. Two-way ANOVA were used to test for significant interactions between factors (P sources and P levels) within the experimental groups. All statistical analyses were carried out using SPSS version 18.0 (SPSS, IL, USA). Curve estimation (SPSS 18.0) was employed to select the best model for the estimation of the dietary P requirements and to describe the regression models.

A broken-line model (Robbins et al., 1979) was used to estimate the dietary P requirements based on WG, and BG, using MCP as the P source. The model equation was $Y = L - U \times (R - X)$; where Y is the value of the parameter, L is the maximum value of the parameter, U is the slope, X is the level of dietary P, and R is the required value. By definition, $U = 0$ when $X > R$.

The relationship between vertebral P content and dietary P levels was investigated using quadratic and cubic curvilinear regression models (Shao et al., 2008). The equations for MCP and MSP groups were (1) $Y = a + bX + cX^2$ and (2) $Y = a + bX + cX^2 + dX^3$, respectively; where Y is the vertebral P content, X is the dietary phosphorus level, and a, b, c, and d are the model parameters.

Results

Growth and survival. Growth data of the hybrid tilapia are shown in Table 3.

Table 3. Growth performance of hybrid tilapia fed experimental diets for 8 weeks[1]

Treatments	*FBW (g)*	*WG (%)*	*SGR(%/day)*	*BG (g)*	*Survival (%)*
Control 1	11.44±0.43^{ab}	550.7±28.2^{a}	3.40±0.13^{a}	279.00±14.88^{a}	96.67±3.33
Control 2	9.79±0.22^{a}	509.3±14.7^{a}	3.28±0.09^{a}	229.00±10.79^{a}	94.44±5.09
MSP0.2	11.13±0.26^{ab}	595.2±16.0^{ab}	3.52±0.08^{a}	263.37±7.85^{a}	93.33±3.33
MSP0.4	13.81±0.87^{bc}	760.9±47.3^{bc}	3.91±0.22^{b}	334.40±18.63^{abc}	92.22±3.85
MSP0.6	17.50±1.96^{d}	992.3±82.4^{def}	4.32±0.36^{cde}	437.23±32.27^{c}	92.22±1.92
MSP0.8	15.85±0.61^{cd}	883.3±26.5^{cde}	4.15±0.12^{bcd}	401.47±15.09^{bc}	94.44±3.85
MSP1.0	18.93±1.48^{d}	1080.7±55.4^{ef}	4.48±0.22^{de}	440.40±21.02^{c}	95.56±7.7
MCP0.2	12.30±0.72^{ab}	597.2±30.1^{ab}	3.53±0.18^{a}	304.60±17.61^{ab}	96.67±3.33
MCP0.4	16.34±0.07^{cd}	831.1±2.6^{cd}	4.06±0.01^{bc}	421.17±9.36^{c}	96.67±3.33
MCP0.6	22.39±1.73^{e}	1177.6±59.0^{f}	4.62±0.23^{e}	595.70±25.93^{d}	96.67±3.33
MCP0.8	22.23±1.90^{e}	1169.8±73.6^{f}	4.61±0.30^{e}	594.10±34.41^{d}	96.67±3.33
MCP1.0	22.59±0.21^{e}	1185.7±6.4^{f}	4.64±0.02^{e}	602.33±5.46^{d}	96.67±0.00
Two-way ANOVA: P-values[2]					
P sources	<0.001	0.01	0.01	<0.001	0.05
P levels	<0.001	<0.001	<0.001	<0.001	0.93
P sources × P levels	0.26	0.34	0.41	0.26	0.93

[1] Mean±S.E.M. of three replicates. Values with different superscripts within the same column are significantly different (P <0.05).
[2] P values of the effects of phosphorus source, phosphorus level and their interaction are presented in corresponding columns. Significance was evaluated at P<0.05.

Final body weight (FBW), weight gain (WG), Biomass gain (BG), and Specific growth rate (SGR), increased linearly with the addition of supplemental P up to 0.6% and then stabilized thereafter (P<0.05), with a significantly higher plateau phase in the MCP group than in the MSP group. FBW, WG, BG and SGR were significantly higher in groups fed 1.0% additional P than in those fed 0.2% additional P, 0.4% additional P, and control diets (P<0.05), regardless of P source (MCP or MSP). P source and P level both significantly impacted growth performances. However, there was no significant difference in survival among the groups.

Body composition and morphometric indices. As shown in Tables 4 and 5, the added levels of dietary P caused significant changes in body composition and morphometric indices of tilapia relative to the control diets. Body protein content initially increased, then decreased with the increase in added dietary P. However, there were no significant differences among most experimental groups.

A significantly higher liver lipid level was found in fish fed lower P diets. In the MSP and MCP groups, the lipid contents of the 0.2% groups were significantly higher than those of both the 0.8% and 1.0% groups.

Whole body crude ash content ranged from 4.22-5.42% and showed a significant linear increase with dietary P supplementation in the MSP groups using linear regression (R=0.9026, P<0.05). Moreover, similar correlations were found between vertebral crude ash content and dietary P levels within the MCP groups by linear regression (R=0.9825, P<0.05).

HIS, VSI and MFI decreased significantly with increasing P levels in the MSP groups. In addition, control 2 group had the highest levels of the morphometric indices of all groups.

Table 4. Whole body, liver and vertebrae composition[1] of hybrid tilapia fed experimental diets for 8 weeks[2]

Treatments	*Whole body*			*Liver*	*Vertebrae*
	Protein (%)	*Lipid (%)*	*Ash (%)*	*Lipid (%)*	*Ash (%)*
Control 1	16.74±0.18^{ab}	6.67±1.57^{ab}	4.22±0.10^{a}	13.09±1.54^{bcde}	53.03±1.03^{a}
Control 2	16.84±0.21^{ab}	8.54±0.31^{b}	4.44±0.09^{ab}	15.02±0.21^{e}	53.86±0.55^{a}
MSP0.2	16.11±0.17^{a}	7.81±0.54^{ab}	4.44±0.19^{ab}	14.15±0.16^{de}	53.51±1.56^{a}
MSP0.4	16.32±0.07^{ab}	6.17±0.75^{a}	4.81±0.16^{abc}	9.65±0.88^{abc}	54.30±2.20^{a}
MSP0.6	16.80±0.12^{ab}	6.53±0.31^{ab}	5.30±0.36^{c}	10.22±0.90^{abcd}	54.51±0.61^{a}
MSP0.8	16.54±0.25^{ab}	5.90±0.29^{a}	5.11±0.73^{c}	9.15±1.96^{ab}	55.46±0.65^{ab}
MSP1.0	16.34±0.28^{ab}	6.43±1.22^{ab}	5.42±0.29^{c}	7.86±1.34^{a}	54.65±1.41^{a}
MCP0.2	15.96±0.12^{a}	6.89±0.44^{ab}	4.80±0.50^{abc}	13.35±1.70^{cde}	54.14±1.76^{a}
MCP0.4	16.26±0.34^{ab}	6.70±0.51^{ab}	4.90±0.29^{bc}	12.21±1.44^{bcde}	55.76±2.08^{ab}
MCP0.6	17.45±0.97^{b}	6.54±0.31^{ab}	5.02±0.75^{bc}	10.32±0.98^{abcd}	57.91±1.99^{bc}
MCP0.8	16.84±0.45^{ab}	6.97±0.33^{ab}	4.92±0.32^{bc}	8.06±0.93^{a}	59.27±1.92^{c}
MCP1.0	16.71±0.18^{ab}	6.37±0.12^{ab}	5.00±0.16^{bc}	7.32±1.53^{a}	59.84±0.58^{c}
Two-way ANOVA: P-values[3]					
P sources	0.37	0.73	0.08	0.95	<0.001
P levels	0.09	0.42	0.01	0.01	0.01
P sources × P levels	0.84	0.51	0.05	0.63	0.13

[1] The composition results of whole body and liver are expressed on a wet matter basis. Vertebrae ash results are expressed on a dry matter basis.
[2] Means±S.E.M. of three replicates, and values with different superscripts within the same column are significantly different (P <0.05).
[3] P values of the effects of phosphorus source, phosphorus level and their interaction are presented in corresponding columns. Significance was evaluated at P<0.05.

Table 5. Morphological indices of hybrid tilapia fed experimental diets for 8 weeks[1]

Treatments	*HSI(%)*	*VSI (%)*	*Condition factor*	*MFI (%)*
Control 1	1.82±0.66^{bcd}	11.91±1.24^{ef}	3.58±0.07^{bc}	2.22±1.15^{cd}
Control 2	2.18±0.44^{d}	12.09±1.78^{f}	3.77±0.04^{c}	2.38±1.24^{d}
MSP0.2	1.95±0.56^{cd}	11.63±1.21^{def}	3.75±0.07^{c}	2.14±0.18^{cd}
MSP0.4	1.89±0.52^{cd}	10.12±2.60^{bc}	3.76±0.05^{c}	1.48±0.65^{ab}
MSP0.6	1.93±0.80^{cd}	10.49±3.64^{cd}	3.63±0.13^{bc}	1.73±0.64^{bc}
MSP0.8	1.64±0.40^{abc}	9.41±1.79^{abc}	3.64±0.06^{bc}	1.23±0.72^{ab}
MSP1.0	1.39±0.54^{a}	8.61±1.84^{a}	3.67±0.05^{bc}	1.19±0.77^{ab}
MCP0.2	1.74±0.52^{abc}	11.84±1.62^{ef}	3.68±0.04^{bc}	1.42±0.98^{ab}
MCP0.4	1.72±0.44^{abc}	10.66±1.23^{cde}	3.60±0.06^{bc}	1.58±0.65^{ab}
MCP0.6	1.75±0.40^{abc}	9.00±2.31^{ab}	3.48±0.06^{ab}	1.73±0.80^{bc}
MCP0.8	1.78±0.42^{abc}	9.82±1.06^{abc}	3.37±0.08^{a}	1.25±0.93^{ab}
MCP1.0	1.44±0.41^{ab}	8.87±0.95^{ab}	3.51±0.06^{ab}	0.99±0.15^{a}
Two-way ANOVA: P-values[2]				
P sources	0.35	0.96	0.01	0.20
P levels	0.01	<0.001	0.02	<0.001
P sources × P levels	0.49	0.17	0.23	0.16

[1] Means±S.E.M. of three replicates. Values with different superscripts within the same column are significantly different (P <0.05).
[2] P values of the effects of phosphorus source, phosphorus level and their interaction are presented in corresponding columns. Significance was evaluated at P<0.05.

Body and vertebral mineral concentrations. The body and vertebral mineral concentrations increased significantly (P<0.05) with dietary P levels (Table 6). Both body P (R=0.8668, P<0.05) and calcium (R=0.8946, P<0.05) contents were significantly higher in groups fed 1.0% additional P, compared to those fed control diets. However, no such changes were observed in the MCP groups. Body zinc content in control group 1 was significantly higher than in all other groups.

Vertebral P and Ca content increased significantly with increased P concentration up to 0.8% in the MSP groups; no further significant increase was observed at 1.0% additional P. Vertebral P content in MCP groups showed ascending quadratic responses to increasing dietary P concentration, with no stabilization or peak. In the different MCP treatments (R=0.999, P<0.05) zinc content in the MSP groups tended to decrease with an increase in P.

Table 6 Mineral concentrations in body and vertebrae[1] of hybrid tilapia fed experimental diets for 8 weeks[2]

Treatments	*Body*			*Vertebrae*		
	Ca (%)	*P (%)*	*Zn (%)*	*Ca (%)*	*P (%)*	*Zn (%)*
Control 1	2.64± 0.20^{a}	1.56± 0.10^{a}	0.017± 0.010^{b}	18.68± 0.13^{ab}	9.34± 0.06^{abc}	0.024± 0.001^{de}
Control 2	2.66± 0.15^{a}	1.60± 0.11^{a}	0.011± 0.002^{ab}	16.10± 1.30^{a}	8.28± 0.64^{ab}	0.027± 0.003^{e}
MSP0.2	2.56± 0.24^{a}	1.55± 0.19^{a}	0.013± 0.004^{ab}	16.62± 3.11^{ab}	8.24± 1.31^{ab}	0.025± 0.001^{de}
MSP0.4	2.82± 0.33^{ab}	1.66± 0.19^{a}	0.008± 0.001^{a}	15.83± 2.55^{a}	7.92± 1.46^{a}	0.023± 0.003^{de}
MSP0.6	3.23± 0.39^{abc}	1.88± 0.22^{abc}	0.009± 0.002^{a}	17.53± 2.62^{ab}	8.95± 1.59^{abc}	0.021± 0.003^{bcd}
MSP0.8	3.37± 1.21^{abc}	1.74± 0.38^{ab}	0.007± 0.003^{a}	19.10± 1.36^{abc}	9.76± 0.58^{bc}	0.022± 0.003^{cd}
MSP1.0	5.10± 0.52^{d}	2.38± 0.52^{c}	0.008± 0.001^{a}	19.08± 0.46^{abc}	9.82± 0.31^{bc}	0.019± 0.004^{abc}
MCP0.2	4.10± 0.48^{bcd}	1.79± 0.12^{ab}	0.009± 0.002^{a}	16.08± 1.13^{a}	8.20± 0.72^{ab}	0.018± 0.001^{ab}
MCP0.4	3.85± 0.68^{c}	1.93± 0.11^{abc}	0.007± 0.001^{a}	16.20± 2.58^{a}	8.45± 1.03^{ab}	0.017± 0.001^{a}
MCP0.6	4.23± 0.56^{cd}	2.22± 0.16^{bc}	0.008± 0.001^{a}	17.71± 1.29^{ab}	9.13± 0.86^{abc}	0.017± 0.001^{a}
MCP0.8	4.09± 0.55^{cd}	1.96± 0.26^{abc}	0.008± 0.002^{a}	19.98± 1.56^{bc}	10.28± 0.75^{cd}	0.017± 0.001^{a}
MCP1.0	3.90± 0.66^{bc}	2.23± 0.42^{bc}	0.007± 0.002^{a}	22.30± 1.11^{c}	11.52± 0.53^{d}	0.018± 0.001^{ab}
Two-way ANOVA: P-values[3]						
P sources	0.02	0.11	0.06	0.26	0.12	<0.001
P levels	0.03	0.01	0.04	0.01	0.01	0.13
P sources × P levels	0.02	0.64	0.34	0.52	0.59	0.17

[1] The body and vertebrae mineral concentration are expressed on a dry matter basis.
[2] Means±S.E.M. of three replicates. Values with different superscripts within the same column are significantly different (P <0.05).
[3] P values of the effects of phosphorus source, phosphorus level and their interaction are presented in corresponding columns. Significance was evaluated at P<0.05.

Apparent digestibility of P, and P retention. Significant correlations were found between the various P utilization efficiency parameters (ADC of P, and P retention) and dietary P supplementation (Table 7). P digestibility significantly increased with dietary P addition (P<0.05), and the highest values were observed in fish fed 1.0% additional P in both the MSP and MCP groups.

The highest available P retention in the experimental groups occurred in those fed 0.6% additional P. Available P retention in the MCP groups increased significantly from 19.97% in the 0.2% diet to 27.69% in the 0.6% diet, and additional P levels higher than 0.6%, significantly reduced the retention values. However, fish fed the control diets appeared to have higher available P retention than those fed the experimental diets.

Table 7. Apparent digestibility of P and P retention of hybrid tilapia fed experimental diets for 8 weeks[1]

Treatments	*P digestibility (%)*	*Total P retention*	*Available P*
Control 1	48.80±1.92^{a}	15.56±0.23^{ab}	31.94±2.00^{e}
Control 2	49.11±1.70^{a}	15.47±0.99^{ab}	31.52±3.56^{e}
MSP0.2	58.74±2.60^{b}	10.41±1.74^{a}	17.27±3.94^{abc}
MSP0.4	69.65±4.45^{d}	11.15±1.67^{a}	16.01±4.11^{ab}
MSP0.6	75.69±2.31^{efg}	13.93±0.87^{a}	18.44±2.23^{abc}
MSP0.8	76.49±1.49^{fg}	10.03±1.37^{a}	13.08±2.82^{a}
MSP1.0	79.55±1.53^{g}	14.03±2.89^{a}	17.73±6.69^{abc}
MCP0.2	63.97±1.34^{c}	12.77±0.43^{a}	19.97±1.50^{abc}
MCP0.4	64.15±3.17^{c}	15.33±0.57^{ab}	23.90±1.03^{cd}
MCP0.6	72.06±1.39^{de}	19.98±2.36^{b}	27.69±5.47^{de}
MCP0.8	74.78±3.22^{ef}	14.17±2.22^{a}	21.09±3.24^{bcd}
MCP1.0	78.15±2.18^{fg}	14.62±1.87^{a}	16.27±0.49^{ab}
Two-way ANOVA: P-values[2]			
P sources	0.14	0.01	0.01
P levels	<0.001	0.06	0.10
P sources × P	0.02	0.61	0.19

[1] Means±S.E.M. of three replicates, and values with different superscripts within the same column are significantly different (P <0.05).
[2] P values of the effects of phosphorus source, phosphorus level and their interaction are presented in corresponding columns. Significance was evaluated at P<0.05.

Dietary P requirements of juvenile hybrid tilapia. In this study, WG, BG, and vertebral P content, were analyzed using broken-line modeling, cubic regression, and quadratic

regression, in order to determine the optimum total, available, and additional P requirements for juvenile hybrid tilapia (Table 8). Based on the calculated R values, we chose the broken-line model as the best fitting model for WG and BG, whereas the quadratic and cubic regressions were selected as the best fit for the vertebral P content in the MCP and MSP groups, respectively.

Broken-line analysis revealed that, based on WG, the available dietary P requirement for juvenile hybrid tilapia was 1.00% in the MSP groups and 0.95% in the MCP groups (Fig. 1). Based on the vertebrae P content, the requirement of available dietary P using MSP as a P source was 1.31% (Fig. 2). However, the vertebral P content in the MCP groups ranged from 8.20%-11.52%, showing no maximum value.

Table 8. Regression analysis of P requirements of hybrid tilapia based on WG, BG and vertebral P content[1]

Parameters[1]	*MSP (% dry matter)*			*MCP (% dry matter)*		
	Total P	*Available P*	*Additional P*	*Total P*	*Available P*	*Additional P*
WG	1.31	1.00	0.60	1.33	0.95	0.61
BG	1.30	0.92	0.59	1.33	0.95	0.62
Vertebral P content	1.67	1.31	0.90	nm	nm	nm

[1] Broken-line model was used to estimate the requirement of dietary P based on WG and BG. Quadratic and cubic regressions were conducted to estimate the requirement of dietary P on vertebral P content in MCP and MSP groups, respectively.

[2] nm indicates that the vertebral P content results did not reach a maximum value across the different MCP treatments.

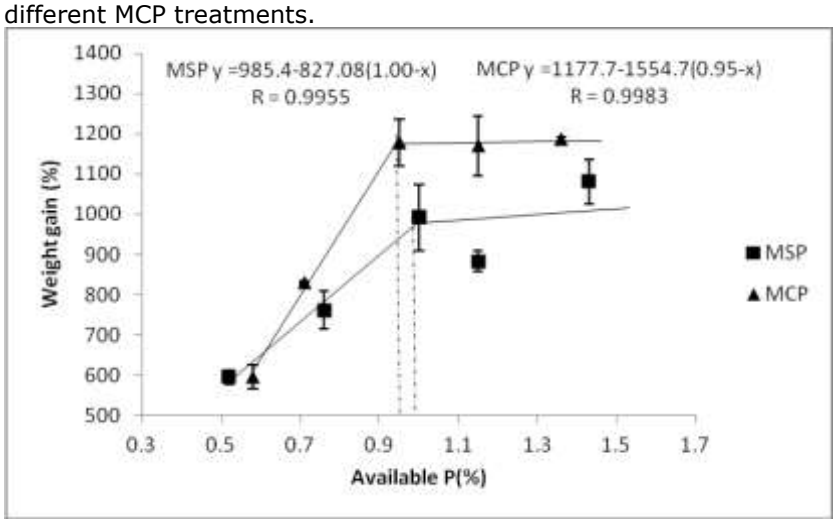

Figure 1 Effect of dietary available P on the WG of hybrid tilapia fed experimental diets for 8 weeks. The breakpoint of the broken line occurs at 1.00% dietary available P using MSP as a P source and 0.95% using MCP as a P source.

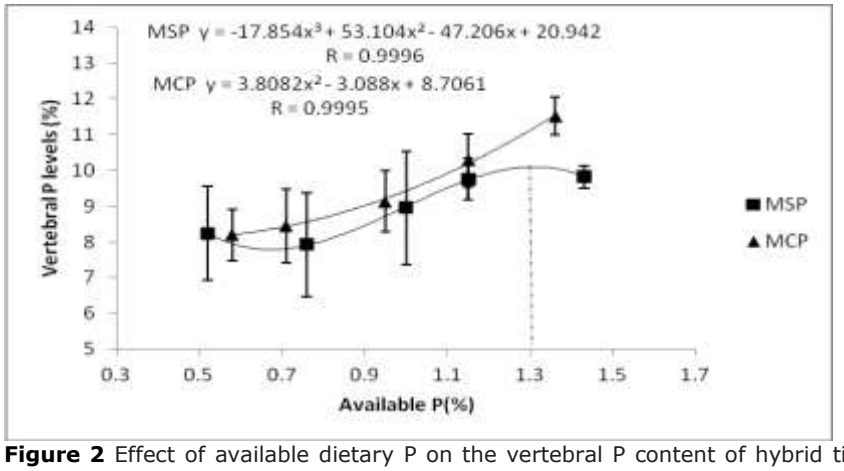

Figure 2 Effect of available dietary P on the vertebral P content of hybrid tilapia fed experimental diets for 8 weeks. The peak of the cubic regression curve occurs at 1.31% available dietary P using MSP as a P source. Vertebral P content did not reach a maxima across the different MCP treatments.

Discussion

The purposes of the present work were to calculate the optimum phosphorus levels for fishmeal-free practical tilapia diets and to compare the efficacy of two different phosphorus sources. In addition, a second control group was fed a diet with high calcium content (0.81%) and the influence of Ca on P absorption was assessed. The basal diet in this experiment contained a wide variety of plant protein sources common to most commercial diets. These included soybean meal, rapeseed meal, peanut meal, and DDGS, which contributed 30%, 20%, 10%, 7%, and 5% to the basal diet, respectively; these proportions are representative of those in commercial fish diets and are proven to be safe for tilapia (Fagbenro and Davies, 2000; Soares et al., 2001; Lim et al., 2007; Shiau et al., 1990).

After 8 weeks of study, FBW, WG, BG, and SGR, of tilapia significantly increased with phosphorus supplementation. Enhanced growth resulting from the addition of P relative to the basal diet has been observed in other species such as grass carp (Liang et al., 2012). Research has shown that stagnation of the growth of fish fed low-P diets is possibly due to P deficiency which impairs metabolic function (Baeverfjord et al., 1998).

In addition to restricted growth, signs of P deficiency in the present experiment included reduced mineral deposition and increased lipid accumulation, these have also been observed in milkfish (Borlongan and Satoh, 2001). However, the symptoms of P deficiency can appear under different conditions. Milkfish (Borlongan and Satoh, 2001) can show signs of P deficiency when dietary P levels are just below the requirement. On the contrary, in the present study, significant signs of P deficiency in the MSP groups appeared in groups with less than 0.76% available P, which is 24% lower than the requirement level predicted from WG. These study differences in the appearance of P deficiency symptoms may be due to variations among studies in experimental cycles, species, experimental conditions, or growth stages (Yang et al., 2006; Roy and Lall, 2003).

In the present study, liver lipid content and MFI decreased significantly as P levels increased. Some researchers have speculated that insufficient inorganic phosphate inhibits the β-oxidation process of fatty acids, which might cause lower utilization and therefore accumulation of lipids (Roy and Lall, 2003). However, body protein was not significantly affected by P level in this research, in contrast to a study on grass carp (Liang et al., 2012) but consistent with observations on groupers *(Epinephelus coioides)* (Ye et al., 2006). No direct relationship between P levels and protein synthesis in fish has yet been established, and more research is needed on the mechanisms underlying P protein-lipid interactions.

Ash, Ca, and P contents of whole body and vertebrae are common indicators of the status of dietary phosphorus in fish nutrition studies (Zhang et al., 2006). In the present experiment, marked increases in crude ash, Ca, and P contents of whole body and vertebrae were observed when additional P levels exceeded 0.8%, indicating that P was necessary for bone mineralization. Interestingly, the different P sources also significantly influenced mineral deposition. In the MSP groups, body ash, Ca, and P contents increased significantly with increasing dietary P; nevertheless, vertebrae ash, and Ca content were not significantly affected. Conversely, in the MCP groups, body ash, Ca, and P contents did not change significantly with increasing MCP levels while vertebrae ash, Ca, and P all increased significantly. These results indicate that MCP promoted vertebral mineral deposition more than MSP.

Conflicting relationships between Zn and supplemental P levels have been observed (Roy and Lall, 2003). Zn can influence bone mineralization either directly by acting on nucleation and mineral accumulation or indirectly as a cofactor of enzymes involved in the process (Gomez et al., 1999). In the present experiment, it is understandable that vertebrae Zn content decreased significantly with increasing P levels in the MSP groups.

The apparent digestibility of P in the basal diet was only 48.8%, whereas it was 79.55% in the 1.0% MSP diet, and 78.15% in the 1.0% MCP diet. There was a similar improvement in the ADC of P following the addition of water-soluble P (Roy and Lall 2003). We also found that P retention increased significantly with increasing dietary MCP

level until the available P levels exceeded 0.95%, significantly reducing the retention values. Extra soluble phosphorus excretion occurs when the available P intake exceeds the levels sufficient for retention (Liang et al., 2012). In the present study, the available retention in the control groups was higher than in all the experimental groups, indicating that additional P excretion should be considered before supplementing with soluble phosphorus.

MSP and MCP are two P sources commonly used to study the requirements and utilization of phosphorus (Antony Jesu Prabhu et al., 2013). Although research has been conducted to identify the different inorganic P sources in fish diets (Pimentel-Rodrigues and Oliva-Teles, 2007), research-based published information on the bioavailability and comparison of MSP and MCP in tilapia is not readily available. The present study showed that MCP improved WG, BG, and vertebral mineral deposition to a greater level than MSP. Moreover, the two-way ANOVA results demonstrated that the P source significantly affected WG, BG, vertebral ash, and body Ca content ($P < 0.05$). In both the MSP and MCP groups of fish fed an additional 0.8% P, the ADC of the total P are statistically similar. However, WG was significantly higher in the 0.8% MCP groups than in 0.8% MSP groups. These results suggest that Ca is responsible for the observed increase in body weight.

Whereas the supplementation of plant-based fish diets with inorganic P is considered necessary (Sugiura et al., 2004), the necessity of dietary Ca supplementation is debatable. Dietary Ca requirements in fish are species specific and depend on the Ca absorption capacity, concentration of environmental Ca, and the bioavailability of Ca from dietary sources (Hossain and Yoshimatsu, 2014). In the present study, the highest vertebral mineral content and greatest growth were found in tilapia fed high-Ca and high-P diets (the 1.0% MCP groups). We hypothesized that the hybrid tilapia in this study might not be able to obtain sufficient Ca due to their low Ca absorption rate and/or the insufficient concentration of Ca in freshwater. This would explain why the MCP groups receiving Ca supplementation exhibited increased growth rate and mineral deposition performance, compared to the MSP groups.

Most fish maintain a constant ratio of Ca:P in bone as well as in plasma; this has been repeatedly proven to be crucial to the bioavailability and retention of P (Hossain and Yoshimatsu, 2014). The Ca:P ratio in MCP groups (0.8) appears to better meet the requirements of tilapia than in MSP groups (0.2-0.6), improving both growth rate and vertebral mineralization. In the current study the high Ca content in control group 2 was found to inhibit growth. This is consistent with the observation that excess dietary Ca, particularly in the presence of an excessive Ca-P ratio (1.24 in control group 2), combines with P to form Ca phosphates, which are not biologically available (Andrews et al., 1973).

WG and P deposition are all reliable measures for estimating the P requirements of fish, provided that the fish are not P deficient (Antony Jesu Prabhu et al., 2013). BG is an objective parameter used to evaluate growth performance based on similar survival rates (Niu et al., 2014). In many fish nutrition studies, whole-body P is commonly used as an indicator of dietary P status (Shao et al., 2008); however, phosphorus deficiencies are associated with skeletal disorders, which are serious problems in fish hatcheries and commercial farms (Lall and Lewis-McCrea, 2007). To provide information relevant to the prevention of skeletal disorders, we chose vertebral P as our indicator of dietary P needed for mineralization. Based on WG, broken-line analysis indicated that 1.00% and 0.95% available P (0.6% and 0.61% additional P) in the diet was adequate for growth in juvenile hybrid tilapia in the MSP and MCP groups, respectively. Further, 1.31% available dietary phosphorus (0.9% additional P) was required for P deposition in vertebrae using MSP as a P source. However, in the MCP groups in the present study, the increase in additional P from 0.8% to 1.0% continued to enhance vertebral P levels; the range of P levels considered here might not have reached the break-point; therefore, further studies are needed. The optimum dietary P levels estimated using vertebral P content as the criterion are approximately 31% higher than those estimated with WG and BG, consistent with many other studies (Zhang et al., 2006; Shao et al., 2008). Requirement results based on other response criteria are provided in Table 8.

Past research has shown inconsistent results for the dietary P requirements of tilapia, with variable values of: 0.55-0.64% (Furuya et al., 2008) and 0.9% (Watanabe et al., 1980). These variations are due mainly to factors such as differences in the availability of

P sources, intestinal P absorption rates, fish size, and the fish developmental stage (Shearer, 1995). However, our requirement estimates were higher than those of most other tilapia studies. Our method of fecal collection (siphoning after 1 hr) may have caused the over estimation since in the hour before fecal collection, the indigestible P from the basal diet was not readily soluble in water, however, the indigestible P in the feces of the supplemented groups was soluble in water and leached into the water resulting in higher digestibility values than were actually absorbed by the fish. The over-estimation of P availability might be the primary reason for the higher estimate of P requirement obtained in this study. Although the P requirement presented as related to the available P may have been over-estimated, results based on additional P content and the comparison of bioavailability between MSP and MCP with practical diets still provide valuable information.

In conclusion, the current study indicated that supplemental dietary phosphorus is essential for maintaining normal physiology, growth, and bone mineralization of juvenile hybrid tilapia (*Oreochromis niloticus* ♀×*O. aureus* ♂) fed a fishmeal-free practical diet. Based on WG and the phosphorus content in vertebrae, the available P requirements of hybrid tilapia were estimated at 1% and 1.31% (0.6% and 0.9% based on additional P content), respectively, using MSP as phosphorus source. While using MCP, the corresponding requirements for WG was 0.95% (0.61% based on additional P content); however, the maximum vertebral P content was not found within the P level range of the present study. Although MCP and MSP were both highly utilizable phosphorus resources, additional Ca supplementation in the MCP groups contributed to enhanced growth and a higher mineral deposition rate than in the MSP groups of the freshwater-reared hybrid tilapia. Further work is required to identify the optimal level of dietary P for vertebral mineral deposition using MCP as P source.

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