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Effects of Dietary Supplementation of Magnesium Hydrogen Phosphate ($MgHPO_4$) as an Alternative Phosphorus Source on Growth and Feed Utilization of Juvenile Far Eastern Catfish (*Silurus asotus*)

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ABSTRACT: The present study was conducted to investigate a supplemental effect of magnesium hydrogen phosphate (MHP, $MgHPO_4$) as an alternative phosphorus (P) source on growth and feed utilization of juvenile far eastern catfish (*Silurus asotus*) in comparison with three conventional P additives (monocalcium phosphate (MCP), dicalcium phosphate (DCP) and tricalcium phosphate [TCP]) as positive controls. A basal diet as a negative control was prepared without P supplementation and four supplemental P sources were added at the level of 2%. Five groups of 450 fish having mean body weight of 11.3 g following 24 h fasting after three week adaptation period were randomly distributed into each of 15 tanks (30 fish/tank). Fish were hand-fed to apparent satiety twice a day for 8 weeks. Fish fed MHP had weight gain (WG), protein efficiency ratio and specific growth rate comparable to those fed MCP. Fish fed MHP and MCP had feed efficiency (FE) significantly higher ($p<0.05$) than those fed DCP. Fish groups fed control and TCP showed the lower FE than the other groups which was significantly different ($p<0.05$) from those of fish fed the other diets. Survival rate was not significantly different ($p>0.05$) among treatments. Fish fed control had the lowest hematocrit, which was significantly different ($p<0.05$) from that of fish fed MHP. Fish fed MCP and MHP had plasma P higher ($p<0.05$) than fish fed the other diets. Relative efficiencies of MCP, DCP and TCP to MHP were found to be 100.5 and 101.3%, 92.0 and 91.6%, and 79.1 and 80.9% for WG and FE, respectively. P availability was determined to be 88.1%, 75.2%, 8.7%, and 90.9% for MCP, DCP, TCP, and MHP, respectively. Consequently, MHP recovered from wastewater stream showed that as an alternative P source its performance was comparative with MCP on growth and feed utilization of juvenile far eastern catfish. (**Key Words:** *Silurus asotus*, Weight Gain, Feed Efficiency, Phosphorus Availability, Alternative Phosphorus, Plasma Phosphorus)

INTRODUCTION

Like other animals, fish have the dietary requirement of phosphorus (P) for growth which was reported to range from 0.3% to 0.6% for channel catfish, *Ictalurus punctatus* (Wilson et al., 1982), rainbow trout, *Oncorhynchus mykiss* (Rodehutscord and Pfeffer, 1995), striped bass, *Morone saxatilis* (Brown et al., 1993; Dougall et al., 1996) and white fish, *Coregonnus lavaretus* (Vilema et al., 2002). However, somewhat higher requirement value of 0.7% to 0.9% was estimated for haddock, *Melanogrammus aeglefinus* (Roy and Lall, 2003), red tilapia, *Tilapia rendalli* (Phromkunthong and Udom, 2008), African catfish, *Clarias gariepinus* (Nwanna et al., 2009), red drum, *Sciaenops ocellatus* (Davis and Robinson, 1987) and yellow croaker, *Pseudosciaena crocea* (Ma et al., 2006). On the other hand, discharged P into water is known to play a vital role in promoting algal growth causing eutrophication (Beveridge, 1984; Auer et al., 1986). Theoretically diet for fish to achieve both maximal growth and minimum P discharge should contain all essential nutrients including P above the needs and maintain total P levels as low as its available requirements. However, the practical diet generally contains an excessive P originating from animal and plant sources. Such sources have low P availability by stomachless species like carp and even by fish with stomach in the early stage of

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growth due to a lack of gastric juice. Therefore, soluble P sources like monocalcium phosphate (MCP) and dicalcium phosphate (DCP) are being supplemented to the diet to meet the requirement of P for maximum growth. Based on this point of views, some significant discharge of P is inevitable from fish farming (Kim and Ahn, 1993). Kim et al. (1998) reported dietary available P of 0.7% with 2% MCP exerted both maximal growth and minimum P loss in juvenile carp. An adequate combination of low-P protein meals supplemented with 0.5% MCP significantly reduced the P loading from rainbow trout without compromising the growth (Satoh et al., 2003; Hernandez et al., 2004; 2005).

Phosphorus, one of non-renewable elements in the nature, is mostly produced from phosphate rock of which production in the world has constantly increased from 198 million metric tons in 2011 to 210 million metric tons in 2012 according to US Geological Survey (USGS, 2013). Shu et al. (2006) expected all reserves of phosphate rock would be depleted by 2090, assuming an annual increase of 1.5% in its demand. Considering this estimation, it is necessary to recover phosphorous from diverse downstream of agricultural and industrial fields. One of resources to retrieve phosphorous could be swine manure which contains high levels of phosphorous and nitrogen. Swine manure becomes a source of pollution on surface waters and induces eutrophication near the site when it is under improper treatment. Thus, the control of wastewater stream must be achieved and struvite precipitation could be an effective way to control P from wastewater stream with the addition of magnesium (Liu et al., 2011). If this magnesium hydrogen phosphate recovered from swine manure could be effectively employed as an available P source for animals including fish, it would not only substitute for import of several phosphates but also protect our environment through recycling of the waste source.

In Korea, far eastern catfish (*Silurus asotus*) is one of major culture species in freshwater and its production reached 4,300 metric tons in 2010 (KOSTAT, 2012). Nevertheless, any nutritional study has not been conducted to investigate P availability and the supplemental effects of various P additives on growth, feed utilization, and hematological and serological parameters of the fish. Furthermore, the potential use of dietary magnesium hydrogen phosphate as an available P source for growth of the fish was not examined until now. This study was therefore carried out to investigate the supplemental effect of various P sources and magnesium hydrogen phosphate (MHP) on growth, feed utilization, hematological and serological parameters of juvenile catfish.

MATERIALS AND METHODS

Preparation of various phosphorus additives

Commercially available P additives, MCP (BIOFOS, Plymouth, MN, USA), DCP (SICHUAN MIANJHUSANJIA FEED Co, Sichuan, China) and tricalcium phosphate (TCP, FOODCHEM, Shandong, China) were obtained from fish feed companies in Korea. The magnesium hydrogen phosphate ($MgHPO_4$) was manufactured from swine manure using a pilot scale reactor with the effective volume of 0.4 m^3 in Kangwon National University. The struvite forming process was performed with hydraulic retention time of 3 h and pH 8 to 9 was maintained by CO_2-stripping (aeration rate of 33 $L/m^3 \cdot min$). Magnesium chloride was added to meet Mg to P ratio of approximately 1.0. Collected precipitate from the reactor was dried and analyzed using X-ray diffractometer (Rigaku, Model D/Max-2500V, Tokyo, Japan) to confirm the formation of struvite. MHP was obtained by removing ammonium-N through incineration at 550℃ for 30 min of the recovered struvite. It was finely ground to use as a P additive.

Preparation of diets

The P sources were incorporated to diet without P source (control) at the level of 2% in lieu of cellulose and the diets were designated as MCP, DCP, TCP, and MHP. Fish meal (25%), soybean meal (40%), wheat flour (27%), fish oil (2%) and soy oil (2%) were employed as major ingredients to formulate control diet containing 42.5% protein and 6.5% lipid (Table 1). Prior to diet formulation, chemical composition of fish meal, soybean meal, wheat flour and 4 different P sources were determined. To make a mixture of 500 kg per diet, weighed ingredients following the formula were ground to 100 mesh size by a hammer mill and thoroughly mixed for 10 min using a V-mixer (Hangjin co., Gwangju, Korea). Then, the mixture was transferred to a twin extruder (Model ATX-2, Fesco Precision Co., Daegu, Korea) and manufactured to the sinking pellets with two sizes of 1.5 and 3.5 mm. Extrusion conditions were as follows: feeder speed, 16 to 18 rpm; conditioner temperature, 80℃ to 90℃; main screw speed, 250 to 320 rpm; temperature of the 2nd and the 3rd barrel compartment, 105℃ to 135℃; steam heater pressure, 4 to 6 kgf/cm^2 and temperature of the 4th barrel compartment, 80℃ to 90℃. Extruded pellets were oven-dried at 60℃ for 6 h to maintain the moderate moisture content of 5% to 8%.

An aliquot of 10 kg of each extruded diet were fully ground and mixed with 1.0% chromic oxide for P digestibility measurement. Then, 20% distilled water were added to each diet mixture and the mixture was pelletized using a meat chopper and dried for 6 hours in a ventilated oven at 60℃. The diets were stored in a freezer at –20℃ for P digestibility measurement following the growth trial.

Growth trial

Far eastern catfish fry of 5,000 with around body weight

Table 1. Ingredient and chemical composition of the experimental diets

Diet	Control	MCP	DCP	TCP	MHP
Ingredient (%)					
Fish meal	25.00	25.00	25.00	25.00	25.00
Soybean meal	40.00	40.00	40.00	40.00	40.00
Wheat flour	27.18	27.18	27.18	27.18	27.18
Soya oil	2.00	2.00	2.00	2.00	2.00
Fish oil	2.00	2.00	2.00	2.00	2.00
Vitamin mix[1]	0.70	0.70	0.70	0.70	0.70
Mineral mix[2]	0.30	0.30	0.30	0.30	0.30
Lysine-HCl	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.20	0.20	0.20	0.20	0.20
Choline-HCl	0.30	0.30	0.30	0.30	0.30
Antioxidant	0.02	0.02	0.02	0.02	0.02
P source	-	2.00	2.00	2.00	2.00
Cellulose	2.00	-	-	-	-
Total	100.00	100.00	100.00	100.00	100.00
Composition (%, DM)[3]					
Crude protein	42.27	42.85	42.20	42.70	42.52
Crude lipid	6.90	6.67	6.62	6.65	6.65
Crude ash	8.41	9.38	9.77	10.33	9.63
Ca	1.74	1.93	2.16	2.30	1.75
P	1.20	1.57	1.53	1.51	1.53
Available P[4]	0.46	0.78	0.70	0.48	0.76

MCP, monocalcium phosphate; DCP, dicalcium phosphate; TCP, tricalcium phosphate; MHP, magnesium hydrogen phosphate; DM, dry matter; BHT, butylated hydroxytoluene; BHA, butylated hydroxyanisol.

[1] Vitamin added to supply the following (per kg diet): vitamin A, 4,000 IU; vitamin D_3, 800 IU; vitamin E, 150 IU; vitamin K_3, 20 mg; thiamine HCl, 25 mg; riboflavin, 50 mg; D-Ca pantothenate, 100 mg; biotin, 1 mg; folic acid, 20 mg; vitamin B_{12}, 0.2 mg; niacin, 200 mg; pyridoxine HCl, 20 mg; ascorbic acid, 500 mg; inositol, 200 mg; BHT, 15 mg; BHA, 15 mg.

[2] Mineral added to supply the following (per kg diet): copper sulfate (25.4% Cu), 30.5 mg; zinc sulfate (22.7% Zn), 230 mg; manganous sulfate (32.5% Mn), 100 mg; cobalt chloride (24.8% Co), 20 mg; potassium iodide (76.4% I), 6.5 mg; sodium selenite (45.6% Se), 2.2 mg; sodium fluoride (45.2% F), 8 mg.

[3] Values are means of 2 determinations.

[4] Available P calculated based on apparent digestibility coefficient values.

of 6 g were purchased from a private hatchery and acclimated to the experimental conditions for 3 weeks. During this period, they were fed a control diet. Following a 24 h fasting, 5 groups (three replicates/group) of 450 fish of a mean body weight of 11.3 g were randomly allotted to each of 15 tanks (0.4×0.6×0.36 cm, water level of 66 L). The feeding experiment lasted 8 weeks during which each diet was hand-fed to apparent satiety twice a day (08:30 and 16:30) at the 4% of body weight during 6 days per week. A recirculation freshwater system, where water temperature and dissolved oxygen were maintained at 26±1.2℃ and 5.5 to 6.4 mg O_2/L, respectively, was employed. The flow rate was held at 5 L/min. The extruded pellet of 1.5 and 3.5 mm sizes were fed for 1st and 2nd feeding of 4 weeks, respectively. Fish were bulk-weighed at the beginning of the experiment and every 4 weeks. Mortality was daily recorded and correction of dead fish was made based on the specific growth rate (SGR) using the equation described by Hardy and Barrows (2002). Daily feed intake (DFI, %/av. body weight/d)), weight gain (WG, %), feed efficiency (FE, %), protein efficiency ratio (PER), SGR (%), survival rate (SR, %) and relative efficiencies (RE, %) of MCP, DCP and TCP to MHP in terms of WG and FE were calculated as follows:

DFI (%/av. body wt/d)
= feed intake (g, dry matter)
/((initial wt+final wt)/2)/experimental days×100

WG (%) = (final weight (g) initial weight (g))
× 100/initial weight (g)

FE (%) = wet weight gain (g)×100/feed intake (g, DM)

PER = wet weight gain (g)/protein intake

SGR (%) = (Ln final weight (g)–Ln initial weight (g))
/experimental days×100

SR (%) = final fish number/initial fish number×100

RE (%) = WG or FE of fish groups fed MCP, DCP, or TCP containing diets/WG or FE of fish group fed MHP containing diet×100

Digestibility trial

At the end of the growth trial, digestibility measurement was conducted to calculate the available P of the experimental diets. Following a 24 h fasting, 250 fish (mean body weight, 52.2 g) were randomly distributed into each of five 130 L capacity tanks (50 fish/tank) with a fecal collection column. Following one week of feeding, fecal collections were made for 3 consecutive weeks as described by Kim et al. (2006). Each diet was fed by hand to apparent satiety twice a day (08:30 and 16:30). One hour after final feeding of the day, the drain pipes and fecal collection columns were thoroughly cleaned with a brush to remove feed residues and feces from the system. The settled feces and surrounding water were carefully collected into 250 mL centrifuge bottles each morning (08:00). Apparent digestibility coefficient (ADC) of P in the experimental diets was calculated according to the following equation (Maynard and Loosli, 1969):

$$\text{ADC}(\%) = \left(1 - \frac{ID \times PF}{IF \times PD}\right) \times 100$$

where *ID* is % indicator in the diet, *PF* represents % P in the feces, *IF* indicates % indicator in the feces, and *PD* is % P in the diet.

P availability of the phosphorus additives was calculated according to the following equation:

$$\text{P availability } (\%) = \frac{APDP - APCD}{TPDP - TPCD} \times 100$$

where APDP indicates % available P in the diet containing P source, APCD is % available P in control diet, TPDP shows % total P in the diet containing P source, and TPCD is % total P in control diet.

Sample collection and analysis

At the end of the experimental period, fish were anesthetized with AQUI-S (New Zealand Ltd., Lower Hutt, NZ) and bulk-weighed and counted for calculation of WG, FE, SGR, PER, and SR. Blood samples were obtained from the caudal vessels with a heparinized syringe from two fish of each tank after fish were starved for 24 h and anesthetized with AQUI-S. Feces collected in the same bottle from each tank for 6 days a week were used as one replicate for the treatment. After collection of three replicate samples of each diet during 3 weeks, fecal samples were lyophilized, finely ground and frozen at –20℃ until analysis.

Chemical analyses of feed ingredient, diets and feces were performed by the standard procedure of AOAC (1990) for moisture, crude protein, crude fat and crude ash. Moisture content was obtained after drying in an oven at 105℃ for 24 h. Crude protein (N×6.25) was determined by Kjeldahl method after acid digestion. Crude fat was determined by the soxhlet extraction method by using Soxtec system 1046 (Foss, Hoganas, Sweden) and crude ash from incineration in a muffle furnace at 550℃ for 12 h. Chromium in diets and feces for P digestibility measurement was analyzed using a spectrophotometer (Shimadzu, UV-120-12) at a wavelength of 440 nm after perchloric acid digestion (Bolin et al., 1952). Ca and P were measured using inductively coupled plasma mass spectrometer (ICP-MS) (Perkin-Elmer, NexION 300D, Waltham, MA, USA) after the pretreatment of test materials following the method from US Environmental protection agency (USEPA, 1996). Hematocrit (PCV, %) and hemoglobin (Hb, g/dL) were measured with the same fish by the microhematocrit method (Brown, 1980) and the cyan-methemoglobin procedure using Drabkins solution, respectively. Hb standard prepared from human blood (Sigma Chemical, St. Louis, MO, USA) was employed. Blood plasma was obtained after blood centrifugation (3,500×g, 5 min, 4℃) and stored at –80℃ until AST (aspartate aminotransferase), ALT (alanine aminotransferase), TP (total protein), ALB (albumin), GLU (glucose), TCHO (total cholesterol) and inorganic P were analyzed. The plasma parameters were measured using a blood chemical analyzer (HITACHI 7600-210, Hitachi High-Technologies co. Ltd., Tokyo, Japan) with commercial clinical investigation reagent (Pureauto S AST, Pureauto S ALT, Clinimate TP, Clinimate ALB, Pureauto S GLU, Pureauto S CHO-N and Clinimate IP, Sekisui medical co. Ltd., Tokyo, Japan).

Statistical analysis

Data of growth trial (DFI, WG, FE, PER, SGR, SR, and RE) and hematological and serological parameters were analyzed using one-way analysis of variance (ANOVA) and significant differences among treatment means were compared using Duncan's multiple range test (Duncan, 1955). Prior to the analysis, homogeneity of variance of all data was verified using Cochran's test (Sokal and Rohlf, 1994). All statistical analyses were carried out using the SPSS Version 10 (SPSS, 1999). Statistical significance of the differences was determined by a significant level of 5% ($p<0.05$).

RESULTS

Growth performance and P digestibility

At the end of the 8-week growth trial, DFI (%) ranged

Table 2. Growth performance of catfish fed diets containing various phosphorus sources for 8 weeks[1]

Parameters	Diet				
	Control	MCP	DCP	TCP	MHP
DFI[2]	2.89±0.08[a]	2.38±0.02[c]	2.60±0.03[b]	2.78±0.06[a]	2.38±0.06[c]
WG[3]	376.77±28.40[b]	484.39±12.09[a]	443.37±13.56[a]	381.09±31.72[b]	481.96±16.98[a]
FE[4]	82.38±4.45[c]	107.42±0.69[a]	97.14±1.87[b]	85.72±4.20[c]	106.02±3.61[a]
PER[5]	2.06±0.11[b]	2.62±0.02[a]	2.45±0.05[a]	2.12±0.10[b]	2.59±0.09[a]
SGR[6]	2.79±0.10[b]	3.15±0.04[a]	3.02±0.04[a]	2.80±0.12[b]	3.13±0.05[a]
SR[7]	97.78±3.14 (2)[ns]	96.67±2.72 (3)	90.00±4.71 (9)	94.44±5.67 (5)	98.89±1.57 (1)

MCP, monocalcium phosphate; DCP, dicalcium phosphate; TCP, tricalcium phosphate; MHP, magnesium hydrogen phosphate; ns, nonsignificant; SE, standard error; DM, dry matter.
[1] Values (means±SE of triplicates) with different superscripts in the same row are significantly different ($p<0.05$).
[2] Daily feed intake (%/av. wt/d) = dry feed intake (g/fish)/((initial wt+final wt)/2)/experimental days×100.
[3] Weight gain (%) = (final weight (g)– initial weight (g))×100/initial weight (g).
[4] Feed efficiency (%) = wet weight gain (g)×100/feed intake (g, DM).
[5] Protein efficiency ratio = wet weight gain (g)/protein intake.
[6] Specific growth rate (%) = (Ln final weight (g)–Ln initial weight (g))/experimental days×100.
[7] Survival rate (%) = final fish number/initial fish number×100; total numbers of dead fish in each fish group are shown in parenthesis.

from 2.38% (MCP and MHP) to 2.89% (control). Fish fed MCP showed the highest WG, which was not significantly different ($p>0.05$) from those of fish fed DCP and MHP, while fish fed control and TCP showed lower WG than the other groups ($p<0.05$). The FE ranged from 107.4% (MCP) to 82.4% (control). The PER of fish fed MCP, DCP, and MHP were not significantly different ($p>0.05$), while fish fed control (2.06) and TCP (2.12) showed lower PER than the other groups ($p<0.05$). The SGR of fish fed MCP was the highest (3.15%), while that of fish fed control was the lowest (2.79%). The SR were not significantly different ($p>0.05$) among fish groups. Mortality of fish was caused by leaping from the tanks only during 1st 4 week growth trial and any of dead fish did not show pathogenic symptoms. Relative efficiencies of MCP, DCP, and TCP to MHP were found to be 100.5% and 101.35, 92.0% and 91.6%, and 79.1% and 80.9% for WG and FE, respectively (Table 2). Apparent availability of P in the experimental diets varied from 32.0% to 49.8% for control and MCP, respectively. The values of P were found to be 88.1%, 75.2%, 8.7%, and 90.9% for MCP, DCP, TCP, and MHP, respectively (Table 3).

Hematological and serological characteristics

Hematological and serological characteristics of fish fed the experimental diets are shown in Table 3. Hematocrit (%) of fish fed control (33.3) showed a significant difference ($p<0.05$) from that of fish fed MHP (39.8). Hb (g/dL) of fish ranged from 9.4 (MCP) to 10.3 (TCP and MHP), which were not significantly different among fish groups ($p>0.05$). The highest AST (IU/L) were obtained in fish fed TCP (115.5), which were not significantly different ($p>0.05$) from that of fish fed control (110.3). However, fish fed MCP (72.7), DCP (79.8), and MHP (83.2) showed relatively lower AST than the two groups. Alanine aminotransferase (IU/L) of fish ranged from 7.3 (DCP) to 11.5 (TCP), the latter was not significantly different ($p>0.05$) from those of fish fed control (10.0) and MCP (9.8). The total protein (g/dL) was lowest in fish fed MCP (3.4), which was significantly different ($p>0.05$) from those (3.7) of fish fed control and MHP. Albumin (g/dL) ranged from 1.0 to 1.2, which did not show significant differences ($p>0.05$) among fish groups. Glucose (mg/dL) of fish fed MCP showed the highest (143.3), while the lowest (103.8) was found in fish fed TCP. Total cholesterol (mg/dL) ranged from 115.0 (MCP) to 143.3 (control), which were not significantly different ($p>0.05$). Fish fed MCP (15.4) and MHP (15.8) showed a significantly higher P (mg/dL) in plasma among fish groups, while the P level in fish fed the other diets was kept at 13.2 (DCP) to 13.9 (TCP) (Table 4).

DISCUSSION

Magnesium hydrogen phosphate (MHP) was newly developed to recycle P from swine manure. It is first attempt to investigate the effect of the MHP as dietary P

Table 3. Apparent availability (%) of phosphorus in diets and various phosphorus sources[1]

	P availability				
	Control	MCP	DCP	TCP	MHP
Diet	38.0±1.6[b]	49.8±1.8[a]	46.0±4.8[a]	32.0±3.8[c]	49.4±2.7[a]
Phosphorus sources		88.1±3.7[a]	75.2±4.9[b]	8.7±2.5[c]	90.9±3.3[a]

MCP, monocalcium phosphate; DCP, dicalcium phosphate; TCP, tricalcium phosphate; MHP, magnesium hydrogen phosphate; SD, standard deviation.
[1] Means±SD of three replicates of each group with different superscript letter in the same row are significantly different ($p<0.05$).

Table 4. Hematological and serological characteristics of catfish fed diets with various phosphorus sources for 8 weeks[1]

Parameters	Diet				
	Control	MCP	DCP	TCP	MHP
PCV (%)[2]	33.3±5.2[b]	38.0±4.0[ab]	38.3±2.7[ab]	34.8±5.4[ab]	39.8±1.8[a]
Hb (g/dL)3	9.7±0.7[ns]	9.4±0.5	9.8±0.4	10.3±1.6	10.3±1.6
AST (IU/L)[4]	110.3±48.5[a]	72.7±18.4[b]	79.8±16.0[ab]	115.5±34.2[a]	83.2±7.8[ab]
ALT (IU/L)[5]	10.0±2.9[a]	9.8±1.7[ab]	7.3±2.5[b]	11.5±4.2[a]	7.8±1.6[b]
TP (g/dL)[6]	3.7±0.2[a]	3.4±0.2[b]	3.6±0.2[ab]	3.5±0.1[ab]	3.7±0.2[a]
ALB (g/dL)[7]	1.1±0.1[ns]	1.0±0.1	1.1±0.1	1.1±0.1	1.2±0.1
GLU (mg/dL)[8]	108.8±15.2[b]	143.3±37.5[a]	126.7±16.5[ab]	103.8±25.3[b]	112.2±18.3[b]
TCHO (mg/dL)[9]	143.3±32.5[ns]	115.0±7.4	128.2±16.1	140.3±21.3	133.5±24.0
Phosphorus (mg/dL)	13.3±1.3[b]	15.4±0.9[a]	13.2±0.6[b]	13.9±1.8[b]	15.8±0.8[a]

MCP, monocalcium phosphate; DCP, dicalcium phosphate; TCP, tricalcium phosphate; MHP, magnesium hydrogen phosphate; PCV, hematocrit; Hb, hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TP, total protein; ALB, albumin; ns, nonsignificant; GLU, glucose; TCHO, total cholesterol; SD, standard deviation.

[1] Means±SD of each group (n = 6) with different superscript letter in the same row are significantly different (p>0.05).

source on growth and feed utilization of fish. Fish fed MCP showed the best WG at the end of growth trial, although it was not significantly different from those of fish fed MHP and DCP. Same tendencies were found in PER and SGR (Table 2). The result suggests that far eastern catfish could utilize P from MHP and DCP as effectively as MCP. On the other hand, fish group fed DCP had FE lower than fish groups fed MCP and MHP, while the group had FE higher than control and TCP groups. From the results, it was evident that MHP was a good P source competitive with MCP in terms of WG and FE in juvenile far eastern catfish. As shown in Figure 1, RE of MCP, DCP, and TCP to MHP at the level of 2% in each diet were 100.5%, 92.0%, and 79.1% on WG and 101.3%, 91.6%, and 80.9% on FE for MCP, DCP, TCP, respectively. Such relative differences among various P sources might be due to the difference in availability of P, by which the requirement could be met or not. Available P requirement is known to be 0.45% (Lovell, 1978) and 0.8% (Andrews et al., 1973) for channel catfish and 0.67% to 0.82% (Nwanna, 2009) for African catfish. Although the requirement for far eastern catfish has not been evaluated to date, it may be anticipated to be higher than 0.7% based on WG and FE from the present study.

Ogino et al. (1979) extensively studied the P availability of inorganic P sources and various feed ingredients by fish. They reported that the availability of MCP, DCP, and TCP was 94%, 46% and 13%, and 94%, 71% and 64% by carp and rainbow trout, respectively, using egg ALB based diet. Pimentel-Rodrigues and Oliva-Teles (2007) reported the availability of MCP, DCP, and TCP using corn gluten based diet by European sea bass was found to be 65%, 66%, and 42%, respectively at higher inclusion level (1.6%, 2.2%, and 2.0% in each diet) of the phosphate. However, the values were found to be 47%, 71%, and 60%, respectively at lower inclusion level (0.8%, 1.1%, and 1.0% in each diet). Even though the P availabilities were not affected by dietary P levels in their study, the values showed great variation by the inclusion levels as well as the additive sources. Sarker et al. (2009) determined P availability of different P sources by yellowtail using ALB based diet. They incorporated MCP (2.7%), DCP (3.7%) and TCP (3.6%) into the respective diets and obtained P availabilities of 92.4%, 59.2%, and 48.8% for MCP, DCP and TCP, respectively.

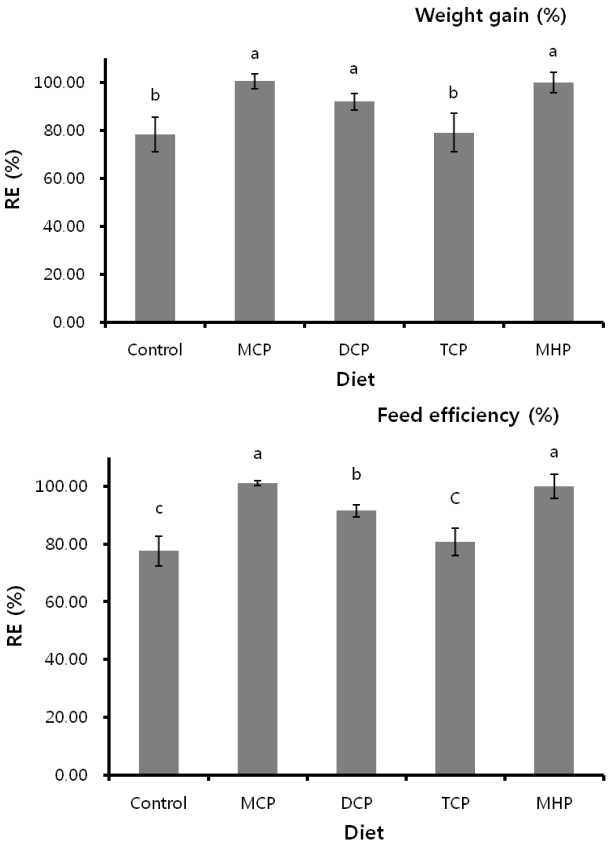

Figure 1. Relative efficiency of various phosphorus sources on weight gain (%) and feed efficiency (%) of juvenile catfish. RE, relative efficiencies; MCP, monocalcium phosphate; DCP, dicalcium phosphate; TCP, tricalcium phosphate; MHP, magnesium hydrogen phosphate.

Lovell (1978) reported that the availability was found to be 94% and 65% for reagent grade MCP and DCP, respectively in channel catfish. On the other hand, Eya and Lovell (1997) determined the net absorption of P from various P sources using all-plant basal diet in channel catfish. They obtained the values of 81.2%, 74.8%, and 54.8% for MCP, DCP, and TCP, respectively.

To our knowledge, this is the first time P availability of feed grade MCP, DCP, and TCP as well as MHP was determined for far eastern catfish. In the present study, P availabilities of both MCP and DCP were found to be high while that of TCP was very low (Table 3). P availability of MCP seems to be lower than those reported by Ogino et al. (1979), Sarker et al. (2009) and Lovell (1978). However, it was comparable to that reported by Kim et al. (1997) and higher than those reported by Pimentel-Rodrigues and Oliva-Teles (2007) and Eya and Lovell (1997). The availability of DCP was comparable to those obtained in rainbow trout (Ogino et al., 1979) and channel catfish (Eya and Lovell, 1997), while it was higher than those obtained in carp (Ogino et al., 1979), European sea bass (Pimentel-Rodrigues and Oliva-Teles, 2007), Yellowtail (Sarker et al., 2009) and channel catfish (Lovell, 1978). On the other hand, reported P availability of TCP by stomach fish ranged from 42% (Pimentel-Rodrigues and Oliva-Teles, 2007) to 64% (Ogino et al., 1979). In contrast, the value from the present study was as low as that obtained by carp, stomachless species (Ogino et al., 1979). Bioavailability of dietary P is influenced by several factors including chemical form, digestibility of diet, particle size, interaction with other nutrients, feed processing and water chemistry (Lall, 1991). It remains to be explained whether such discrepancies in P availability of various P sources, especially TCP, are due to the differences in species and diet composition employed for the digestibility measurement as well as methodological approach in fecal collection (Kim et al., 1996). On the other hand, P availability of MHP was as high as those of MCP obtained from carp and rainbow trout by Ogino et al. (1979) and from yellowtail by Sarker et al. (2009), suggesting that MHP could be a potential P source for fish.

Hematological and serological parameters of blood plasma are useful in monitoring the physiological status of fish and as indicators of the health of the aquatic environment, although they are not routinely used in fish disease diagnosis (El-Sayed et al., 2007). Hematocrit (%) provides an indirect measurement of the body's oxygen carrying ability, while Hb (g/dL) a direct measurement of the oxygen carrying capacity of the blood (McClatchey, 1994). It was reported that hematological parameters could be influenced by nutritional status (Spannhof et al., 1979), infectious disease (Barham et al., 1980; Iwama et al., 1986), environmental changes (Giles et al., 1984) and stress (Ellsaesser and Clem, 1986). The normal ranges of healthy adult Atlantic salmon were reported 44 to 49 and 8.9 to 10.4 for PCV and Hb, respectively (Sandnes and Waagbo, 1988). Somewhat lower values for cichlid fish were reported by Vazquez and Guerrero (2007), which were 22.5 to 39.2 for PCV and 5.2 to 8.3 for Hb. Recently, Rahimnejad and Lee (2013) reported 30.7 to 34.3 for PCV and 4.4 to 5.4 for Hb of red sea bream fed various dietary valine levels. Our findings for the parameters are in good agreement with those obtained from tilapia (Hrubec et al., 2000) and striped bass (Hrubec et al., 2001), although there is no available information on the effect of dietary available P on the parameters of fish. Alanine aminotransferase and AST are two of the most useful measures of liver cell injury, although the AST is less liver specific than the ALT level (Suman and Carey, 2006). In addition to the parameters, TP, ALB, GLU, and TCHO are generally employed as valuable diagnostic means in nutritional studies for fish (Lee et al., 2003; Yan et al., 2007; Cho et al., 2007; Lee et al., 2012; Rahimnejad and Lee, 2013). However, standard ranges of such parameters in fish species as wells as effects of dietary available P on them were not reported to date. Therefore, it remains to be explained why fish fed diet containing low available P (control and TCP) showed higher AST and ALT as well as TP values than fish fed the other diets in the present study. On the other hand, fish fed MCP showed GLU significantly higher than fish fed control and TCP as well as MHP, while ALB and TCHO were not significantly different among fish groups. Due to lack of data related to serological parameters of fish fed diet containing various levels of dietary P, more researches need to be conducted to explain the discrepancies in the present results. An increase in available P in diet resulted in an increase in plasma Pi (Vielma and Lall, 1998; Bureau and Cho, 1999; Avila et al., 2000). The present study clearly showed that plasma P increased in fish fed MCP and MHP with higher available P, resulting in the significant improvement in WG and FE by meeting dietary P need (Table 4).

From the present results, the potential use of MHP recovered from swine wastewater was proven sufficiently to replace MCP as an alternative P source with respect to WG and FE as well as P availability, although it remains to be elucidated the optimal dietary level to maximize growth and feed utilization of far eastern catfish. Further, such a re-use of P from wastewater stream could positively influence on the environmental protection and resource security.

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REFERENCES

Andrews, J. W., T. Murai, and C. Campbell. 1973. Effects of dietary calcium and phosphorus on growth, food conversion, bone ash and hematocrit levels of catfish. J. Nutr. 103:766-771.

AOAC. 1990. Official Methods of Analysis. Association of Official Analytical Chemists, Arlington, VA, USA. 1298 p.

Auer, M. T., M. S. Kiesser, and R. P. Canale. 1986. Identification of critical nutrient levels through field verification of models for phosphorus and phytoplankton growth. Can. J. Fish. Aquat. Sci. 43:379-388.

Avila, E. M., H. Tu, S. Basantes, and R. P. Ferraris. 2000. Dietary phosphate regulates intestinal transport and plasma concentrations of phosphate in rainbow trout. J. Comp. Physiol. 170B:210-209.

Barham, W. T., G. L. Smit, and H. J. Schoonbee. 1980. The haematological assessment of bacterial infection in rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol. 17:275-281.

Beveridge, M. C. M. 1984. Cage and pen fish farming: carrying capacity models and environmental impact. FAO Fish. Tech. Pap. 255, 131.

Bolin, D. W., R. P. King, and E. W. Klosrerman. 1952. A simplified method for the determination of chromic oxide (Cr_2O_3) when used as an inert substance. Science 116(3023):634-635.

Brown, B. A. 1980. Routine hematology procedures. In: Hematology: Principles and Procedures. Lea and Febiger. Philadelphia, PA, USA. pp. 71-112.

Brown, M. L., F. Jaramillo, Jr., and D. M. Gatlin, III. 1993. Dietary phosphorus requirement of juvenile sunshine bass, *Morone chrysops* × *M. saxatilis*. Aquaculture 113:355-363.

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

Cho, S. H., S. M. Lee, B. H. Park, S. C. Ji, C. Y. Choi, J. H. Lee, C. Y. Kim, J. H. Lee, and S. Y. Oh. 2007. Effect of daily feeding ratio on growth and body composition of subadult olive flounder, *Paralichthys olivaceus*, fed an extruded diet during summer season. J. World Aquacult. Soc. 38:68-73.

Davis, D. A. and E. H. Robinson. 1987. Dietary phosphorus requirement of juvenile red drum *Sciaenops ocellatus*. J. World Aqucult. Soc. 18:129-136.

Dougall, D. S., L. C. Wood, III, L. W. Douglass, and J. H. Soares. 1996. Dietary phosphorus requirement of juvenile striped bass *Morone saxatilis*. J. World Aquacult. Soc. 27:82-91.

Duncan, D. B. 1955. Multiple range and multiple 'F'tests. Biometrics 11:1-42.

Ellsaesser, C. F. and L. W. Clem. 1986. Haematological and immunological changes in channel catfish stressed by handling and transport. J. Fish Biol. 28:511-521.

El-Sayed, Y. S., T. T. Saad, and S. M. El-Bahr. 2007. Acute intoxication of deltamethrin in monosex Nile tilapia, *Oreochromis niloticus* with special reference to the clinical, biochemical and haematological effects. Environ. Toxicol. Phamacol. 24:212-217.

Eya, J. C. and R. T. Lovell. 1997. Net absorption of dietary phosphorus from various inorganic sources and effect of fungal phytase on net absorption of plant phosphorus by channel catfish *Ictalurus punctatus*. J. World Aquacult. Soc. 28:386-391.

Giles, M. A., H. S. Majewski, and B. Hobden. 1984. Osmoregulatory and haematological responses of rainbow trout (*Salmo gairdneri*) to extended environmental acidification. Can. J. Fish. Aquat. Sci. 41:1686-1694.

Hardy, R. W. and F. T. Barrows. 2002. Diet formulation and manufacture. In: Fish Nutrition (Eds. J. E. Halver and R. W. Hardy), 3rd ed. Academic Press, London, UK. pp. 505-600.

Hernandez, A., S. Satoh, V. Kiron, and T. Watanabe. 2004. Phosphorus retention efficiency in rainbow trout fed diets with low fish meal and alternative protein ingredients. Fish. Sci. 70:580-586.

Hernandez, A., S. Satoh, and V. Kiron. 2005. Effect of monocalcium phosphate supplementation in a low fish meal diet for rainbow trout based on growth, feed utilization, and total phosphorus loading. Fish. Sci. 71:817-822.

Hrubec, T. C., J. L. Cardinale, and S. A. Smith. 2000. Hematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis* hybrid). Vet. Clin. Pathol. 29:7-12.

Hrubec, T. C., S. A. Smith, and J. L. Robertson. 2001. Age related changes in haematology and chemistry values of hybrid striped bass chrysops *Morone saxatilis*. Vet. Clin. Pathol. 30:8-15.

Iwama, G. K., G. L. Greer, and D. J. Randall. 1986. Changes in selected haematological parameters in juvenile Chinook salmon subjected to a bacterial challenge and a toxicant. J. Fish Biol. 28:563-572.

Kim, J. D. and K. H. Ahn. 1993. Effects of monocalcium phosphate supplementation on phosphorus discharge and growth of carp (*Cyprinus carpio*) grower. Asian Australas. J. Anim. Sci. 6:521-526.

Kim, J. D., K. S. Kim, J. S. Song, J. Y. Lee, and K. S. Jeong. 1998. Optimum level of dietary monocalcium phosphate based on growth and phosphorus excretion of mirror carp, *Cyprinus carpio*. Aquaculture 161:337-344.

Kim, J. D., K. S. Kim, J. S. Song, K. S. Jeong, Y. B. Woo, N. J. Choi, and J. Y. Lee. 1996. Comparison of feces collection methods for determining apparent phosphorus digestibility of feed ingredients in growing mirror carp (*Cyprinus carpio*). Korean J. Anim. Nutr. Feed. 20:201-206.

Kim, J. D., K. S. Kim, S. B. Lee, and K. S. Jeong. 1997. Nutrient and energy digestibilities of various feedstuffs fed to Israeli strain of growing common carp (*Cyprinus carpio*). J. Aquacult. 10:327-334.

Kim, J. D., S. M. Tibbets, J. E. Milley, and S. P. Lall. 2006. Effect of the incorporation level of herring meal into test diet on apparent digestibility coefficients for protein and energy by juvenile haddock, *Melanogrammus aeglefinus* L. Aquaculture 258:479-486.

KOSTAT (Statistics Korea). 2012. Fishery production survey report. http://kostat.go.kr Accessed February 16, 2012.

Lall, S. P. 1991. Digestibility, metabolism and excretion of dietary phosphorus in fish. In: Nutritional Strategies and Aquaculture Wastes (Ed. C.B. Cowey and C. Y. Cho). University of Guelph, Ontario, Canada. pp. 21-36.

Lee, D. H., S. R. Lim, C. S. Ra, and J. D. Kim. 2012. Effects of dietary garlic extracts on whole body amino acid and fatty acid composition, muscle free amino acid profiles and blood plasma changes in juvenile sterlet sturgeon, *Acipenser ruthenus*. Asian Australas. J. Anim. Sci. 25:1419-1429.

Lee, S. M., J. H. Lee, and K. D. Kim. 2003. Effect of dietary essential fatty acids on growth, body composition and blood chemistry of juvenile starry flounder (*Platichthys stellatus*). Aquaculture 225:269-281.

Liu, Y. H., S. Kumar, J. H. Kwag, J. H. Kim, J. D. Kim, and C. S. Ra. 2011. Recycle of electronically dissolved struvite as an alternative to enhance phosphate and nitrogen recovery from swine wasterwater. J. Hazard. Mater. 195:175-181.

Lovell, R. T. 1978. Dietary phosphorus requirements of channel catfish. Trans. Am. Fish. Soc. 107:617-621.

Ma, K., C. Zhang, Q. Ai, Q. Duan, W. Xu, L. Zhang, Z. Luifu, and B. Tan. 2006. Dietary phosphorus requirement of large yellow croaker, *Pseudosciaena crocea* R. Aquaculture 251:346-353.

Maynard, L. A. and J. K. Loosli. 1969. Animal Nutrition, 6th ed. McGraw-Hill, New York, USA. 613 p.

McClatchey, K. D. 2002. Clinical Laboratory Medicine. Williams & Wilkins, Baltimore, MD, USA. pp. 833-839.

Nwanna, L. C., I. A. Adebayo, and B. O. Omitoyin. 2009. Phosphorus requirements of African catfish, *Clarias gariepinus*, based on broken-line regression analysis methods. ScienceAsia 35:227-233.

Ogino, C., L. Takeuchi, H. Takeda, and T. Watanabe. 1979. Availability of dietary phosphorus in carp and rainbow trout. Bull. Jap. Soc. Sci. Fish. 45:1527-1532.

Phromkunthong, W. and U. Udom. 2008. Available phosphorus requirement of sex-reversed red tilapia fed all-plant diets. Songklanakarin J. Sci. Technol. 30:7-16.

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

Rahimnejad, S. and K. J. Lee. 2013. Dietary valine requirement of juvenile red sea bream *Pagrus major*. Aquaculture 416-417:212-218.

Rodehutscord, M. and E. Pfeffer. 1995. Requirement for phosphorus in rainbow trout (*Oncorhynchus mykiss*) growing from 50 to 200 g. Water Sci. Technol. 31:137-141.

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

Sandnes, K., O. Lie, and R. Waagbo. 1988. Normal ranges of some blood chemistry parameters in adult farmed Atlantic salmon, *Salmo salar*. J. Fish Biol. 32:129-136.

Sarker, P. K., H. Fukada, and T. Masumoto. 2009. Phosphorus availability from inorganic phosphorus sources in yellowtail (*Seriola quinqueradiata* Termminck and Schlegel). Aquaculture 289:113-117.

Satoh, S., A. Hernandez, T. Tokoro, Y. Morishita, V. Kiron, and T. Watanabe. 2003. Comparison of phosphorus retention efficiency between rainbow trout (*Oncorhynchus mykiss*) fed a commercial diet and a low fish meal based diet. Aquaculture 224:271-282.

Shu, L., P. Schneider, V. Jegatheesan, and J. Johson. 2006. An economical evaluation of phosphorus recovery as struvite from digester supernatant. Bioresour. Technol. 97:2211-2216.

Sokal, R. R. and F. J. Rohlf. 1994. Nested analysis of variance. In: Biometry, 3rd ed. Freeman W. H., New York. pp. 272-342.

Spannhof, L., D. Nasev, and H. L. Kreutzmann. 1979. Early recognition of metabolic disturbances in trout (*Salmo gairdneri* Rich.) stocks. Aquaculture 18:317-323.

SPSS. Inc. 1999. SPSS Base 10.0 for Windows User's Guide. SPSS Inc., Chicago IL, USA.

Suman, A. and W. D. Carey. 2006. Assessing the risk of surgery in patients with liver disease. Cleve. Clin. J. Med. 73:398-404.

USEPA (US Environmental protection agency). 1996. Acid digestion of sediments, sludge, and solids, Method 3050B, Revision 2. http://www.epa.gov/epaoswer/hazwaste/test/3050b.pdf Accessed December, 1996.

USGS (US Geological survey). 2013. Mineral commodity summaries. Digital Data Series. http://minerals.usgs.gov/minerals/pubs/mcs/2013/mcs2013.pdf. Accessed January, 2013.

V´azquez, G. R. and G. A. Guerrero. 2007. Characterization of blood cells and hematological parameters in *Cichlasoma dimerus* (Teleostei, Perciformes). Tissue Cell 39:151-160.

Vilema, J., J. Koskela, and K. Ruohonen. 2002. Growth, bone mineralization, and heat and low oxygen tolerance in European whitefish (*Coregonnus lavaretus* L.) fed with graded levels of phosphorus. Aquaculture 212:321-333.

Vielma, J. and S. P. Lall. 1998. Control ofphosphorus homeostasis of Atlantic salmon (*Salmo sala*) in fresh water. Fish Phosiol. Biochem. 19:83-93.

Wilson, R. P., E. H. Robinson, D. M. Gatlin, III and W. E. Poe. 1982. Dietary phosphorus requirement of channel catfish. J. Nutr. 112:1197-1202.

Yan, Q., S. Xie, X. Zhu, W. Lei, and Y. Yang. 2007. Dietary methionine requirement for juvenile rockfish, *Sebastes schlegeli*. Aquacult. Nutr. 13:163-169.