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Optimal Incorporation Level of Dietary Alternative Phosphate ($MgHPO_4$) and Requirement for Phosphorus in Juvenile Far Eastern Catfish (*Silurus asotus*)

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ABSTRACT: A growth trial was conducted to determine the optimal incorporation level of dietary magnesium hydrogen phosphate (MHP, $MgHPO_4$), which was manufactured from swine manure and phosphorus (P), required by juvenile far eastern catfish (*Silurus asotus*). Graded MHP of 0.5%, 1.0%, 1.5%, and 2.0%, and 2.0% monocalcium phosphate (MCP) each was added to the basal diet (control) in lieu of cellulose to become the range of available P (AP) from 0.4% to 0.8% of which diets were designated as control, MHP0.5, MHP1.0, MHP1.5, MHP2.0, and MCP, respectively. Control diet contained fish meal (20%), soybean meal (40%), wheat flour (27%), corn gluten meal (5%), fish oil (2%) and soy oil (2%) as main ingredients. Following a 24 h fasting, 540 fish with a mean body weight of 11.8 g were randomly allotted to 6 groups in triplicate, whereby 18 tanks (0.4×0.6×0.36 cm, water volume of 66 L) were prepared. The feeding experiment lasted for 8 weeks. Fish group fed the control diet showed the lowest weight gain (WG) and feed efficiency (FE) among treatments. The WG was, however, not significantly different ($p>0.05$) from that of fish group fed MHP0.5. Fish group fed MHP2.0 showed the highest WG and FE of which values were not significantly different from those of fish groups fed diets MHP1.0 and MHP1.5 as well as MCP ($p>0.05$) except fish groups fed control and MHP0.5. Aspartate aminotransferase was significantly decreased with an increase in available P, while alanine aminotransferase did not show a significant difference among treatment. The highest inorganic P in plasma was observed in fish fed MHP2.0. From the present results, a second-order regression analysis revealed that the optimal dietary MHP level and the AP requirement were found to be 1.62% and 0.7%, respectively. (**Key Words:** *Silurus asotus*, Magnesium Hydrogen Phosphate [MHP], Weight Gain, Feed Efficiency, Available P Requirement)

INTRODUCTION

Phosphorus (P) is one of the essential nutrients for normal life processes including growth, reproduction and health for plants and animals. In the form of phosphate, P plays a pivotal role in all fundamental biochemical reactions of respiration, photosynthesis, muscular contraction, cell division, transmission of genetic information and fermentation (Lall, 1991). Although fish and other aquatic organisms are able to absorb minerals from water, P should be provided through the food due to its low concentration from 0.02 to 0.6 mg/L in both fresh water and seawater and its low absorption rate (Boyd, 1971). On the other hand, voided P through fish excreta and wasted feed may cause the enrichment of the aquatic environment and the growth of algae (Auer et al., 1986). Therefore, P level in fish feed is recommended to meet but not exceed the fish's requirements. The requirement of P for optimal growth of cultured fish species was reported to range from 0.3% to 0.9% of dry diet (NRC, 2011). In spite of one of major freshwater culture species in Korea, the P requirement of far eastern catfish (*Silurus asotus*) has not been quantified to date.

Because P availability in animal and plant feedstuffs is low, inorganic P (Pi) additives like monocalcium phosphate (MCP), dicalcium phosphate (DCP) and tricalcium phosphate are generally being supplemented to the diet of

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fish and land animals to meet the requirement of P for maximum growth. However, global reserves of mined P sources are being gradually depleted (Shu et al., 2006). One resource to retrieve P could be swine manure which contains a high level of P 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 alternative P source could be effectively employed in domesticated animal feed including aquaculture, it would not only substitute for import of several P additives but also protect our environment through recycling of the waste source. Our previous study (Yoon et al., 2014) revealed that magnesium hydrogen phosphate (MHP, $MgHPO_4$) obtained from swine manure could replace MCP as an alternative P source with respect to weight gain (WG) and feed efficiency (FE) as well as P availability. The present study was, therefore, carried out to investigate the optimal dietary MHP level and available P (AP) requirement for maximal growth of far eastern catfish. In addition to growth performance, hematological and serological parameters, and whole body composition of the fish were analyzed.

MATERIALS AND METHODS

Preparation of two P sources

Magnesium hydrogen phosphate and commercially available MCP (BIOFOS, Plymouth, MN, USA) were employed as P additives in the experimental diets. The MHP was manufactured from swine manure using a pilot scale reactor in Kangwon National University. The struvite forming process was performed with hydraulic retention time of 3 h and pH 8-9 was maintained by CO_2-stripping (aeration rate of 33 L/m^3 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. The 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 phosphate additive.

Preparation of diets

The graded MHP of 0.5%, 1.0%, 1.5%, and 2.0%, and 2.0% MCP as a positive control were incorporated to diet without P source (control) in lieu of cellulose and the diets were designated as MHP0.5, MHP1.0, MHP1.5, MHP2.0, and MCP, respectively. Fish meal (20%), soybean meal (40%), wheat flour (27%), corn gluten meal (5%), fish oil (2%) and soy oil (2%) were employed as major ingredients to formulate the control diet containing 42.8% protein and 6.8% lipid (Table 1). Prior to diet formulation, chemical composition of fish meal, soybean meal, wheat flour, corn gluten meal and P additives 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 mm and 3.5 mm, respectively for feeding during first and second 4 week growth trials, respectively. Extrusion conditions were same as previously described by Yoon et al. (2014). After extrusion, an aliquot of 10 kg of the control diet were fully ground and mixed with 1.0% chromic oxide for P digestibility measurement. Then, 20% distilled water was added to the diet mixture and the mixture was pelletized using a meat chopper and dried for 6 hours in a ventilated oven at 60℃. The diet was stored in a freezer at –20℃ for P digestibility measurement following the growth trial.

Growth trial

Juvenile far eastern catfish with approximate body weight (BW) of 6 g were purchased from a private hatchery and acclimated to the experimental conditions for 3 weeks prior to the feeding trial. During this period, they were fed a control diet. Following a 24 h fasting, 6 groups (three replicates/group) of 540 fish with a mean BW of 11.8 g were randomly allotted to each of 18 tanks (0.4×0.6 ×0.36 cm, water volume 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 4% of BW for 6 days in every 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 during the 1st and 2nd feeding for 4 weeks, respectively. Fish were bulk-weighed at the beginning of the experiment and every 4 weeks. Mortality was daily recorded and correction for 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. BW/d), WG (%), FE (%), protein efficiency ratio (PER), SGR (%) and survival rate (SR, %) were calculated as follows:

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

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

Table 1. Ingredient and chemical composition of the experimental diets[1]

Ingredient (%)	Control	MHP				MCP
		0.5%	1.0%	1.5%	2.0%	
Fish meal	20.00	20.00	20.00	20.00	20.00	20.00
Soybean meal	40.00	40.00	40.00	40.00	40.00	40.00
Corn gluten M	5.00	5.00	5.00	5.00	5.00	5.00
Wheat flour	27.18	27.18	27.18	27.18	27.18	27.18
Soya oil	2.00	2.00	2.00	2.00	2.00	2.00
Fish oil	2.00	2.00	2.00	2.00	2.00	2.00
Vit. mix[1]	0.70	0.70	0.70	0.70	0.70	0.70
Min. mix[2]	0.30	0.30	0.30	0.30	0.30	0.30
Lysine-HCl	0.30	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.20	0.20	0.20	0.20	0.20	0.20
Choline-HCl	0.30	0.30	0.30	0.30	0.30	0.30
Antioxidant	0.02	0.02	0.02	0.02	0.02	0.02
Cellulose	2.00	1.50	1.00	0.50	-	-
MHP	-	0.50	1.00	1.50	2.00	-
MCP	-	-	-	-	-	2.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Composition (%, DM)						
Crude protein	42.78	42.87	42.80	42.56	42.49	43.04
Crude lipid	6.77	6.59	6.53	6.45	6.57	6.57
Crude fiber	4.29	3.76	3.10	2.80	2.32	2.21
Crude ash	7.45	8.33	8.67	8.97	9.34	8.58
Ca	1.40	1.45	1.51	1.55	1.59	1.65
P	1.08	1.16	1.24	1.32	1.42	1.49
AP[5]	0.42	0.50	0.57	0.65	0.73	0.79

MHP, magnesium hydrogen phosphate; MCP, monocalcium phosphate; DM, dry matter; AP, available P.

[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] Calculated based on P availabilities of control diet and P sources.

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

PER = wet WG (g)/protein intake

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

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

Digestibility trial

At the end of the growth trial, a digestibility measurement was conducted to calculate the AP of the control diet. Following a 24 h fasting, 150 fish (mean BW, 55.4 g) were randomly distributed into each of three 130 L capacity tanks (50 fish/tank) with a fecal collection column. Following one week of feeding, fecal collections were made for 5 days. Each diet was fed by hand to apparent satiety twice a day (08:30 and 16:30). After one hour from the 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 diet was calculated according to the following equation:

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

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.

Available P in diets containing P additive was calculated based on P availability of MHP (90.9%) and MCP (88.1%) previously determined (Yoon et al., 2014) with the addition of that determined in the control diet according to the following equation:

AP (%) = *APCD*+*APAD*

where *APCD* indicates % AP determined in the control diet and *APAD* is % AP calculated by the P addition level in other experimental diets.

Sample collection and analysis

At the end of the 8 week feeding trial, 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 of 5 mL volume with a 22G (1-1/4″) needle from two fish of each tank after the fish were starved for 24 h and anesthetized with AQUI-S. At the beginning (30 fish) and the end (10 fish per each tank) of the feeding trial, fish were collected for the whole body analysis. The initial and final fish samples were finely ground, mixed well and stored at –80℃ until further analysis where necessary. Feces collected in the same bottle from each tank for 5 days a week were used as one replicate for the treatment. After collection of three replicate samples during 1 week, fecal samples were lyophilized, finely ground and frozen at –20℃ until the analysis.

The chemical analyses of feed ingredients, diets, whole body of fish 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 diet and fecal samples for P digestibility measurement was analyzed using a spectrophotometer (UV-120-12, Shimadzu, Japan) at a wavelength of 440 nm after perchloric acid digestion (Bolin et al., 1952). The P in diets, P additives, feces and whole body 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. The Hb standard prepared from human blood (Sigma Chemical, St. Louis, MO, USA) was employed. Blood plasma was obtained after blood centrifugation (3,500×*g*, 4℃ for 5 min) and stored at –80℃ until AST (aspartate aminotransferase), ALT (alanine aminotransferase), TP (total protein), ALB (albumin), TCHO (total cholesterol) and Pi 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 from the growth trial (DFI, WG, FE, PER, SGR, and SR), hematological and serological parameters and the whole body composition were analyzed using one-way analysis of variance and significant differences among treatment means were compared using Duncan's multiple range test (Duncan, 1955). The second-order polynomial regression analysis (Zeitoun et al., 1976) was introduced to the optimum dietary MHP level and P requirement of juvenile cat fish. All statistical analyses were carried out using the SPSS Version 10 (SPSS Inc., Chicago, IL, USA). The statistical significance of the differences was determined by a significant level of 5% ($p<0.05$).

RESULTS

Growth performance

Available P level was determined to be 0.42% for control and calculated to range from 0.5% for MHP to 0.79% for MCP (Table 1). At the end of the 8-week growth trial, DFI (%) per averaged weight ranged from 2.49 (MHP2.0) to 2.85% (control). Fish group fed MHP2.0 showed the highest WG of 446.7%, which was not significantly different ($p>0.05$) from those of fish groups MHP1.0, MHP1.5, and MCP, while fish groups fed control and MHP0.5 showed lower WG than the other groups ($p<0.05$). The highest FE was found in fish group fed MHP2.0, while fish group fed control showed the lowest FE among treatments. However, there was no significant difference ($p>0.05$) in FE among fish groups except control. The PER of fish groups fed diets with above 1.0% MHP and 2.0% MCP ranged from 2.33 to 2.47, which were not significantly different ($p>0.05$), while fish fed control (2.15) and MHP0.5 (2.14) showed lower PER than the other groups ($p<0.05$). The SGR of fish group fed control was the lowest (2.78%), while those of other fish groups did not significantly vary from 2.92% to 3.02% ($p>0.05$). Fish group fed MHP0.5 showed SR significantly lower than other groups (Table 2). Mortality of fish was caused by leaping from the tanks only during the 1st 4 week of the growth trial and any of dead fish did not show pathogenic symptoms.

Table 2. Growth performance of catfish fed the experimental diets for 8 weeks[1]

Parameters	Diet					
	Control	MHP0.5	MHP1.0	MHP1.5	MHP2.0	MCP
Feed intake (%/av. wt/d)	2.85±0.08^{a}	2.69±0.07^{b}	2.56±0.09^{c}	2.61±0.04^{bc}	2.49±0.06^{c}	2.60±0.05^{bc}
WG[2]	374.76±26.62^{b}	414.38±32.58^{b}	441.29±19.78^{a}	443.26±26.95^{a}	446.73±42.80^{a}	442.88±24.62^{a}
FE[3]	87.07±4.47^{c}	93.68±3.38^{b}	100.41±2.49^{ab}	97.69±1.47^{ab}	102.50±5.43^{a}	99.24±2.32^{ab}
PER[4]	2.15±0.10^{b}	2.14±0.06^{b}	2.41±0.10^{a}	2.33±0.03^{a}	2.47±0.10^{a}	2.39±0.05^{a}
SGR[5]	2.78±0.10^{b}	2.92±0.11^{ab}	3.01±0.06^{a}	3.02±0.09^{a}	3.02±0.13^{a}	3.02±0.08^{a}
SR[6]	93.33±3.33 (6)^{a}	78.89±1.92 (19)^{b}	92.22±8.39 (7)^{a}	91.11±5.09 (8)^{a}	94.44±6.93 (5)^{a}	93.33±3.33 (6)^{a}

MHP, magnesium hydrogen phosphate; MCP, monocalcium phosphate; WG, weight gain; FE, feed efficiency; PER, protein efficiency ratio; SGR, specific growth rate; SR, survival rate; SD, standard deviation; DM, dry matter.
[1] Values (means±SD of triplicates) with different superscripts in the same row are significantly different ($p<0.05$).
[2] WG (%) = [final weight (g) – initial weight (g)]×100/initial weight (g).
[3] FE (%) = wet weight gain (g)×100/feed intake (g, DM).
[4] PER = wet weight gain (g)/protein intake.
[5] SGR (%) = [ln final weight (g) – ln initial weight (g)]/experimental days×100.
[6] SR (%) = final fish number/initial fish number×100; total numbers of dead fish in each fish group are shown in parenthesis.

Hematological and serological characteristics

Hematological and serological characteristics of fish fed the experimental diets for 8 weeks are shown in Table 3. The PCV (%) of fish did not show a significant difference ($p>0.05$) among treatments ranging from 35.3% to 37.7%. Also, Hb (g/dL) of fish were not significantly different among fish groups ($p>0.05$). An increase in dietary AP resulted in a decrease in AST (IU/L) from 98.3 (control) to 67.2 (MCP). However, the ALT (IU/L) did not show such a relevant trend and ranged from 6.2 (MHP1.5 and MCP) to 8.0 (MHP0.5), which was not significantly different among fish groups. The TP (g/dL) and ALB (g/dL) were ranged from 3.1 to 3.4 and from 0.9 to 1.0, respectively, which were not significantly different among fish groups. The TCHO (mg/dL) slightly decreased with the addition of P additive in diets, the significant difference ($p<0.05$) was, however, found only between fish groups fed control (152.2) and MHP2.0 (110.5). Significantly higher Pi (mg/dL) in plasma was found in fish groups fed MCP (13.2) and MHP2.0 (13.6), while the level in fish fed the other diets was kept at 11.4 (MHP1.0) to 12.4 (MHP0.5 and MHP1.5).

Whole body composition

Compared to the initial value of 78.4%, the final moisture contents of fish were decreased to 75.5% in fish group fed MCP, while any significant difference was not observed among fish groups fed the experimental diets. On the contrary, fat contents significantly increased in all fish groups fed the experimental diets, compared to that (3.6%) of the initial. However, protein, ash and P contents were not significantly different ($p>0.05$) between the initial and final fish (Table 4).

DISCUSSION

After 8 weeks of the feeding trial, fish group fed control did not show any significant difference from that fed diet with 0.5% MHP in WG, PER, and SGR. However, fish groups fed diets containing above 1.0% of MHP resulted in a significant increase in WG, FE, PER, and SGR, which were comparable to those of fish group fed 2% MCP. As reported in our previous study (Yoon et al., 2014), the present results demonstrated that dietary addition of MHP above 1.0% as P additive could improve growth and feed

Table 3. Hematological characteristics and serological parameters of catfish fed the experimental diets for 8 weeks[1]

Parameters	Diet					
	Control	MHP0.5	MHP1.0	MHP1.5	MHP2.0	MCP
PCV (%)	35.3±2.8^{ns}	37.2±1.9	36.7±5.6	35.7±1.5	37.7±3.9	37.7±1.4
Hb (g/dL)	9.8±0.9 ^{ns}	9.1±1.8	9.2±1.4	9.0±0.5	9.2±1.1	10.4±0.4
AST (IU/L)	98.3±11.8^{a}	93.7±28.0^{ab}	74.3±13.4^{b}	75.8±19.1^{b}	73.0±16.7^{b}	67.2±11.5^{c}
ALT (IU/L)	7.5±1.2^{ns}	8.0±2.2	6.7±1.4	6.2±0.8	7.0±2.0	6.2±0.8
TP (g/dL)	3.4±0.3^{ns}	3.1±0.2	3.2±0.3	3.3±0.2	3.3±0.2	3.4±0.2
ALB (g/dL)	1.0±0.1^{ns}	1.0±0.1	0.9±0.1	1.0±0.1	1.0±0.1	1.0±0.1
TCHO (mg/dL)	152.2±35.6^{a}	124.0±10.5^{ab}	125.0±32.8^{ab}	119.7±20.9^{ab}	110.5±24.9^{b}	120.0±17.7^{ab}
Pi (mg/dL)	11.6±1.4^{c}	12.4±0.7^{abc}	11.4±1.1^{c}	12.4±0.6^{bc}	13.6±0.6^{a}	13.2±0.8^{ab}

MHP, magnesium hydrogen phosphate; MCP, monocalcium phosphate; PCV, hematocrit; Hb, hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TP, total protein; ALB, albumin; TCHO, total cholesterol. Pi, inorganic P; SD, standard deviation.
[1]Values (means±SD [n = 6] of each group) with different superscript letter in the same row are significantly different; ns, nonsignificant ($p>0.05$).

Table 4. Whole body composition of the initial and the final fish fed the experimental diets for 8 weeks[1]

Composition	Diet						
	Initial	Control	MHP0.5	MHP1.0	MHP1.5	MHP2.0	MCP
Moisture	78.38±0.51[a]	75.36±0.46[b]	75.38±0.20[b]	75.35±0.83[b]	75.37±0.44[b]	75.39±0.23[b]	75.46±0.18[b]
Crude protein	14.91±0.11[b]	15.30±0.17[b]	15.36±0.43[b]	15.16±0.21[b]	15.29±0.23[b]	15.96±0.41[a]	15.26±0.44[b]
Crude lipid	3.55±0.50[b]	5.63±0.48[a]	5.86±0.66[a]	5.81±0.51[a]	5.47±0.46[a]	5.08±0.82[a]	5.21±0.56[a]
Ash	2.52±0.33[ns]	2.46±0.24	2.38±0.33	2.41±0.32	2.40±0.20	2.42±0.09	2.47±0.19
Phosphorous	0.53±0.04[ns]	0.42±0.07	0.49±0.10	0.49±0.09	0.49±0.06	0.49±0.07	0.49±0.08

MHP, magnesium hydrogen phosphate; MCP, monocalcium phosphate; SD, standard deviation.
[1] Values (means±SD of triplicate groups) with different superscript letter in the same row are significantly different; ns, nonsignificant ($p>0.05$).

utilization of far eastern catfish. Many researchers have reported the supplemental effects of P additive in practical fish diets, although such an effect has not been reported for far eastern catfish to date. Kim et al. (1998) studied the optimal level of MCP in diets with its graded levels of 0% to 5% for mirror carp, *Cyprinus carpio* and reported that the addition of 2% MCP resulted in the best growth and feed utilization. Hernandez et al. (2005) suggested that 0.5% to 1% MCP should be provided in low fish meal diet for rainbow trout, *Oncorhynchus mykiss* to improve growth performance. Recently, Liu et al. (2012) observed that MCP addition level could be reduced from 2% to 1% with phytase supplementation in diet for gibel carp, *Carassius auratus*. Nwanna et al. (2009) observed the best growth performance of African catfish, *Clarias gariepinus* fed diets with 3% to 4% DCP. Even though the MCP is widely used in a practical fish diet to boost the AP due to its high solubility, it is not only one of the expensive feed ingredients but limited source of P which could be depleted. This is the reason why we tried to recover P from swine manure and apply to dietary P additive for animals. Based on WG from the present study, a polynomial regression analysis revealed the optimal level of MHP of 1.62% (Figure 1).

Even though far eastern catfish is one of major cultured freshwater fish in Korea, dietary P requirement essential for optimal feed formulation has not been established to date. Many studies to estimate P requirement of fish employed purified or semi-purified diets with reagent-grade P sources like Na_2HPO_4 and KH_2PO_4 and most of the requirement was determined based on WG, whole body P, vertebral P, and plasma P as the response criteria (Antony Jesu Prabhu et al., 2013). However, it seems that an extrapolation of such data to practical feed formulation would be misleading unless P availability of the ingredients is known for the target fish. In the present study we tried to analyze growth performance as well as whole body composition and plasma parameters using a practical diet. Only cellulose was used to replace graded levels of MHP and MCP. The AP levels in the experimental diets were maintained at 0.42 (control) to 0.79% (MCP). Fish fed 0.42% P grew as well as those fed 0.50% P (MHP0.5), although FE was significantly lower. Any other difference in the whole body composition and plasma biochemical parameters was not observed between the two fish groups (Tables 3 and 4). This suggests that the control diet met at least the minimum requirement of P to prevent overt deficiency signs including reduced growth (Sugiura et al., 2000). However, fish fed above 0.57% AP

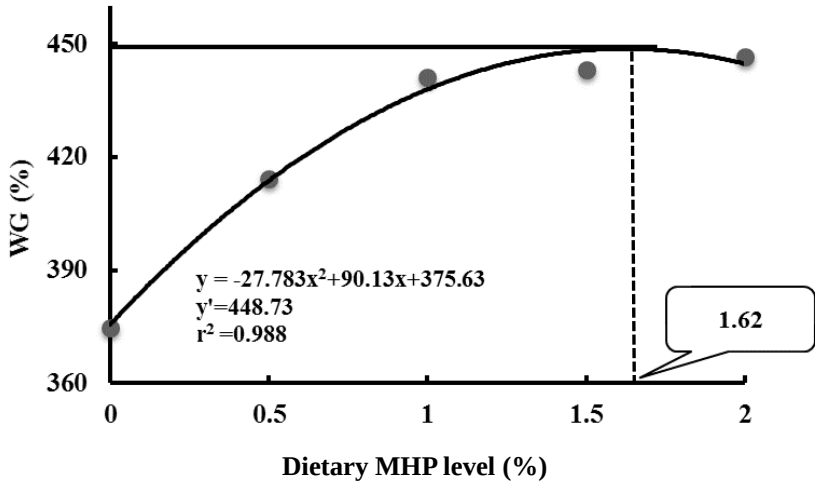

Figure 1. Polynomial regression analysis on weight gain (WG, %) of far eastern catfish to dietary magnesium hydrogen phosphate (MHP) levels.

(MHP1.0) showed a significantly improved WG, FE, PER, and SGR with a peak at 0.73% AP. Plasma Pi level also showed similar tendency (Table 3), whereas the whole body P level was kept constant at 0.49% for fish fed above 0.57% AP (Table 4). A polynomial regression revealed that the AP requirement of the fish was 0.7% of dry diet based on WG and FE (Figure 2). The incorporation level of fish meal in control diet was reduced from 25% in the previous study to 20% with 5% corn gluten meal in the present study. Nevertheless, fish fed control diet in two studies conducted under the same conditions showed an equal WG, although those of fish fed MHP2.0 and MCP in present study decreased more than 40% point. This phenomenon suggests that a replacement of dietary fish meal by plant protein source would alter the essential amino acid profile, which could affect the growth of fish (Gatlin et al., 2007). On the other hand, an increase in dietary AP resulted in a decrease in AST and TCHO. It remains to be clarified whether the parameters could be influenced by P levels in diets.

The AP requirement of fish varies by species and size and a variation exists among the same species according to the methodological approaches like diet preparation, type of Pi source and response criteria. The requirement of Atlantic salmon, *Salmo sala* was reported to be 0.6 (Ketola, 1975) and 0.9% (Asgard and Shearer, 1997), which were determined based on WG and Whole body P, respectively using either semi-purified or practical diets. For carp, the requirement ranged from 0.5% to 0.7% based on WG (Schaefer et al., 1995; Kim et al., 1998; Nwanna et al., 2010; Xie et al., 2011). The AP requirement with a narrow range of 0.37% to 0.54% based on WG was reported from several researchers for rainbow trout fed semi-purified diets (Ketola and Richmond, 1994; Rodehutscord, 1996; Sugiura et al., 2007). Sugiura et al. (2000) estimated the AP requirement based on nonfecal excretion of Pi and total nitrogen for large rainbow trout, which was 0.66% and 0.55% for 203 g and 400 g fish, respectively. Although the requirement shows a difference from species to species, the

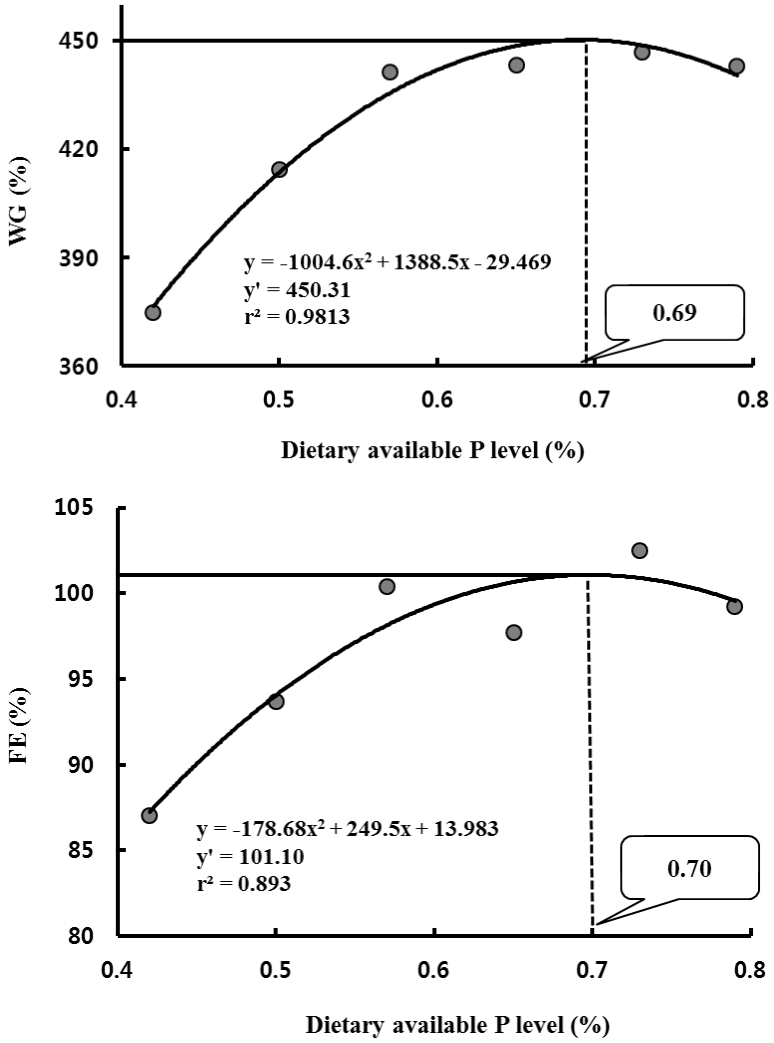

Figure 2. Polynomial regression analysis on weight gain (WG, %) and feed efficiency (FE, %) of far eastern catfish to dietary available p levels.

present result closely corresponds to those determined in carp (Kim et al., 1998) and haddock, *Melanogrammus aeglefinus* (Roy and Lall, 2003). Recently, Antony Jesu Prabhu et al. (2013) recommended that the requirement of P expressed in terms of "g AP intake per kg $BW^{0.8}$ per day" would be more precise, giving 0.062 g/kg $BW^{0.8}$/d. The present result revealed the AP requirement of around 0.045 g/kg $BW^{0.8}$/d based on the best WG and FE of fish group fed diet with 2.0% MHP. In conclusion, it was demonstrated that dietary MHP could be used as a good P additive with the optimal incorporation level of 1.62% and that 0.7% AP in diet was adequate for maximum WG and FE for far eastern catfish.

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