

Postprint: Optimal Phosphorus Requirement and Relative Bioavailability of Highly Water-Soluble Monocalcium Phosphate in Largemouth Bass (*Micropterus salmoides*)

Authors: Shi Bo, Yu Huanhuan, Zheng Yin Hua, Wu Xiufeng, Chen Pei, Liang Xufang(13, Xue Min

Date: 2018-12-24T00:00:00+00:00

Abstract

This experiment was conducted to determine the optimal phosphorus requirement of largemouth bass (*Micropterus salmoides*) and the relative bioavailability of highly water-soluble monocalcium phosphate (MDCP) compared to monocalcium phosphate (MCP) by supplementing a low-phosphorus basal diet with different levels of MCP and highly water-soluble MDCP and investigating their effects on growth performance, nitrogen and phosphorus digestibility and retention rates, whole-body and vertebral phosphorus content, and whole-body crude ash content. A low-phosphorus basal diet was first formulated and then supplemented with 8.86, 11.08, 13.29, and 15.51 g/kg MCP, as well as 9.34, 11.67, 14.01, and 16.34 g/kg highly water-soluble MDCP, respectively, so that the phosphorus provided by both phosphate sources was 2.0, 2.5, 3.0, and 3.5 g/kg, respectively. The nine experimental diets were designated as P0, P2, P2.5, P3, P3.5, DP2, DP2.5, DP3, and DP3.5. Each diet was fed to four replicates of largemouth bass with an initial body weight of (8.5±0.1) g, with 40 fish per replicate, for a culture period of 8 weeks. The results showed that dietary supplementation with different levels of MCP and highly water-soluble MDCP significantly improved weight gain rate, phosphorus digestibility and retention rate, and whole-body crude ash content of largemouth bass ($P < 0.05$), with no significant difference between the two phosphorus sources in their effects on these parameters ($P > 0.05$). With increasing MCP supplementation levels, the weight gain rate and whole-body crude ash content of largemouth bass exhibited a trend of initial increase followed by stabilization, while phosphorus retention rate showed a trend of initial increase followed by decrease, with the P0 group being the lowest for all these parameters. With increasing levels of highly water-soluble MDCP supplementation, the weight gain rate and phos-

phorus digestibility of largemouth bass showed a continuous increasing trend, while phosphorus retention rate exhibited a trend of initial increase followed by decrease, with the P0 group having the lowest phosphorus retention rate and the DP3 group having the highest. Using weight gain rate and phosphorus retention rate as indicators, the relative bioavailability of highly water-soluble MDCP compared to MCP was calculated to be 100% and 118%, respectively, by the slope ratio method. When MCP was used as the phosphorus source, weight gain rate and phosphorus retention rate reached their maximum at a dietary digestible phosphorus content of 0.51%. When highly water-soluble MDCP was used as the phosphorus source, phosphorus retention rate reached its maximum at a dietary digestible phosphorus content of 0.52%.

Full Text

Study on Optimum Phosphorus Requirement and Relative Biological Availability of High Water-Soluble Monodicalcium Phosphate for Largemouth Bass (*Micropterus salmoides*)

SHI Bo¹, YU Huanhuan¹, ZHENG Yinhua¹, WU Xiufeng¹, CHEN Pei¹, LIANG Xuefang³, XUE Min^{1, 2*}

¹National Aquafeed Safety Assessment Station, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China

²Key Laboratory of Feed Biotechnology of Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China

³College of Fisheries, Huazhong Agricultural University, Wuhan 430070, China

Abstract

This experiment investigated the optimum dietary phosphorus requirement and relative biological availability of high water-soluble monodicalcium phosphate (MDCP) compared to monocalcium phosphate (MCP) in largemouth bass (*Micropterus salmoides*). Various levels of MCP and high water-soluble MDCP were supplemented to a low-phosphorus basal diet to evaluate their effects on growth performance, nutrient digestibility and retention, whole-body and vertebral phosphorus content, and whole-body ash content. A low-phosphorus basal diet was formulated and supplemented with 8.86, 11.08, 13.29, and 15.51 g/kg MCP, or 9.34, 11.67, 14.01, and 16.34 g/kg high water-soluble MDCP, providing equivalent phosphorus levels of 2.0, 2.5, 3.0, and 3.5 g/kg from each phosphate source. The nine experimental diets were designated as P0, P2, P2.5, P3, P3.5, DP2, DP2.5, DP3, and DP3.5. Each diet was fed to four replicate groups of 40 largemouth bass (initial weight 8.5±0.1 g) for 8 weeks.

Results showed that dietary supplementation with either MCP or high water-soluble MDCP significantly improved weight gain rate, phosphorus digestibility

and retention, and whole-body ash content ($P < 0.05$), with no significant differences between the two phosphorus sources ($P > 0.05$). As MCP supplementation increased, weight gain rate and whole-body ash content initially rose then plateaued, while phosphorus retention rate peaked then declined, with the P0 group showing the lowest values. With increasing high water-soluble MDCP levels, weight gain rate and phosphorus digestibility showed continuous improvement, while phosphorus retention rate peaked then declined, reaching its maximum in the DP3 group. Using the slope ratio method, the relative biological availability of high water-soluble MDCP compared to MCP was 100% based on weight gain rate and 118% based on phosphorus retention rate. When MCP served as the phosphorus source, maximum weight gain rate and phosphorus retention rate were achieved at a dietary digestible phosphorus content of 0.51%. With high water-soluble MDCP, maximum phosphorus retention rate occurred at 0.52% dietary digestible phosphorus.

Keywords: largemouth bass (*Micropterus salmoides*); monocalcium phosphate; high water-soluble monocalcium phosphate; phosphorus requirement; relative biological availability

Introduction

Largemouth bass (*Micropterus salmoides*), also known as California bass, belongs to Perciformes, Centrarchidae, *Micropterus*. As a carnivorous warm-water fish, largemouth bass has become an important aquaculture species in China due to its rapid growth, strong disease resistance, wide temperature tolerance, and delicious meat quality [1].

Phosphorus is an essential mineral element that plays crucial roles in animal nutrition. It constitutes a major component of bones and scales, forms ATP and phosphocreatine for energy metabolism, and facilitates lipid and fat-soluble vitamin absorption as phospholipids. Phosphate groups participate in carbohydrate, lipid, and protein metabolism, while blood phosphates serve as important buffering agents for acid-base balance [2]. However, phosphorus is also a primary contributor to water eutrophication. In aquaculture, different phosphate sources vary significantly in efficacy, and excess or poorly utilized phosphorus is discharged into aquatic environments, causing eutrophication, algal blooms, oxygen depletion, fish kills, and rendering water unsuitable for human use [3-4]. Therefore, determining optimal phosphorus requirements and identifying highly efficient phosphate sources can meet fish growth and health needs while reducing environmental phosphorus pollution. This study investigated optimal phosphorus requirements and the relative biological availability of high water-soluble MDCP versus MCP in largemouth bass by supplementing graded levels of these phosphates to a low-phosphorus basal diet and evaluating growth performance, nitrogen and phosphorus digestibility and retention, whole-body and vertebral phosphorus content, and whole-body ash content.

Materials and Methods

1.1 Experimental Fish Largemouth bass were obtained from Foshan Sanshui Platinum Aquatic Seedling Co., Ltd. in May 2016. Prior to the experiment, fish were acclimated in the culture system for 4 weeks and fed a commercial diet during acclimation.

1.2 Experimental Diets MCP (analytical grade, colorless flakes, 15.1% calcium, 22.6% phosphorus, water solubility 1.99 g at 20°C) was purchased from Sinopharm Chemical Reagent Co., Ltd. High water-soluble MDCP was produced by direct reaction of concentrated phosphoric acid with calcium carbonate, generating a crystalline mixture of dicalcium phosphate and MCP. The test MDCP contained 14.7% calcium and 21.4% phosphorus with water solubility of 0.37 g at 20°C, provided by Yuntianhua Co., Ltd. Water-soluble phosphorus content (as percentage of total phosphorus) was 92.0% for MCP and 84.5% for high water-soluble MDCP, substantially exceeding the national standard (HG/T 3776-2005) requiring 40% water-soluble phosphorus for feed-grade MDCP.

The low-phosphorus basal diet (P0) contained no exogenous phosphorus with a background phosphorus content of 6.6 g/kg. Graded levels of 8.86, 11.08, 13.29, and 15.51 g/kg MCP or 9.34, 11.67, 14.01, and 16.34 g/kg high water-soluble MDCP were added to provide equivalent phosphorus levels of 2.0, 2.5, 3.0, and 3.5 g/kg from each source. The basal diet was designated P0, MCP-supplemented diets as P2, P2.5, P3, and P3.5, and MDCP-supplemented diets as DP2, DP2.5, DP3, and DP3.5. Yttrium oxide (Y O) was added at 0.1% as an indigestible marker. Ingredients were ultrafine ground, mixed thoroughly, extruded using a twin-screw extruder (Yang Gong Machinery TSE65), air-dried, and stored at -20°C. Dietary composition and nutrient levels are presented in Table 1 .

1.3 Experimental Design and Management The trial was conducted at the National Aquafeed Safety Assessment Station (Nankou, Beijing) using an indoor recirculating aquaculture system. Healthy, uniform largemouth bass (average initial weight 8.53 ± 0.01 g) were randomly stocked into 0.26 m³ conical tanks. The experiment comprised 9 dietary treatments with 4 replicates per treatment, each containing 40 fish, for 8 weeks. Fish were hand-fed to apparent satiation twice daily at 08:00 and 16:00.

Water quality parameters were monitored regularly: dissolved oxygen >7.0 mg/L, total ammonia nitrogen <0.3 mg/L, pH 7.5-8.5, and temperature $23 \pm 1^\circ\text{C}$. During week 2, feces collection began daily during peak defecation periods (determined from week 1 observations). Intact, membrane-enclosed feces were collected by siphoning for determination of phosphorus and nitrogen digestibility, then freeze-dried at -20°C pending analysis.

After the 8-week growth trial, fish were fasted for 24 h, then weighed and counted for growth calculations. Four fish per tank were sampled for morphological measurements (body length, weight, viscera weight, liver weight). Six fish per tank were anesthetized with chlorobutanol, and blood was collected from the caudal vein using sodium fluoride-potassium oxalate anticoagulant. Plasma was separated by centrifugation (4°C, 4000 r/min, 10 min) and stored at -80°C.

1.4.1 Growth and Morphological Indices Formulas for calculated indices were:

Feeding rate (FR, %) = $100 \times \text{total feed intake} / \{[(\text{final weight} + \text{initial weight} + \text{dead fish weight})/2] \times \text{days}\}$

Survival rate (SR, %) = $100 \times \text{final fish number} / \text{initial fish number}$

Weight gain rate (WGR, %) = $100 \times \text{weight gain} / \text{initial weight}$

Specific growth rate (SGR, %/d) = $100 \times (\ln \text{ final mean weight} - \ln \text{ initial mean weight}) / \text{days}$

Feed conversion ratio (FCR) = $\text{feed intake} / (\text{final weight} + \text{dead weight} - \text{initial weight})$

Condition factor (CF, g/cm³) = $\text{average body weight} / \text{average body length}^3$

Hepatosomatic index (HSI, %) = $100 \times \text{liver weight} / \text{body weight}$

Viscerasomatic index (VSI, %) = $100 \times \text{viscera weight} / \text{body weight}$

1.4.3 Phosphorus and Nitrogen Digestibility Phosphorus and nitrogen digestibility were calculated as:

ADCd (%) = $100 \times [1 - (\text{Md} \times \text{Nf}) / (\text{Mf} \times \text{Nd})]$

Where ADCd represents apparent digestibility coefficient of nutrient (phosphorus or nitrogen); Md is marker (Y O) percentage in diet; Mf is marker percentage in feces; Nd is nutrient percentage in diet; Nf is nutrient percentage in feces.

Phosphorus and nitrogen retention rates were calculated as:

Phosphorus retention rate (%) = $100 \times (\text{Wt} \times \text{Wtph} - \text{W} \times \text{W ph}) / (\text{Wf} \times \text{Wfph})$

Nitrogen retention rate (%) = $100 \times (\text{Wt} \times \text{Wtp} - \text{W} \times \text{W p}) / (\text{Wf} \times \text{Wfp})$

Where W = initial weight (g); Wt = final weight (g); Wtph = final whole-body phosphorus content (%); W ph = initial whole-body phosphorus content (%); Wtp = final whole-body nitrogen content (%); W p = initial whole-body nitrogen content (%); Wf = feed intake (%); Wfph = dietary phosphorus content (%); Wfp = dietary nitrogen content (%).

1.5 Statistical Analysis All data are expressed as mean±SE. SPSS 20.0 was used for one-way and two-way ANOVA, with Duncan's multiple comparison test for significant differences ($P < 0.05$). Broken-line and binary regression models were used to estimate optimal phosphorus requirements based on regression coefficients. Digestible phosphorus was calculated as (total phosphorus × phosphorus digestibility). MCP biological availability was set at 100%, and slope ratio method was used to calculate MDCP relative biological availability:

Relative biological availability of MDCP (%) = $100 \times \text{slope of test substance regression (bt)} / \text{slope of standard substance regression (bs)}$

Results

2.1 Effects of Dietary MCP and High Water-Soluble MDCP Levels on Growth Performance Final survival rates exceeded 99% across all groups. Effects of phosphorus source and level on growth performance are shown in Table 2. One-way ANOVA revealed that dietary MCP and high water-soluble MDCP significantly improved WGR and SGR ($P < 0.05$) while reducing FCR and CF ($P < 0.05$). WGR increased initially then plateaued with rising MCP levels, with P0 showing the lowest value ($P < 0.05$). WGR increased continuously with MDCP supplementation, remaining lowest in P0 ($P < 0.05$). Two-way ANOVA indicated no significant effects of phosphorus source on growth parameters ($P > 0.05$) and no significant source × level interactions ($P > 0.05$). Since WGR did not reach a plateau with MDCP supplementation, broken-line analysis of MCP groups identified 0.51% dietary digestible phosphorus as optimal for maximum WGR (Figure 1 [Figure 1: see original paper]).

2.2 Effects on Nitrogen and Phosphorus Digestibility and Retention Effects on digestibility and retention are presented in Table 3. MCP and MDCP supplementation significantly improved phosphorus digestibility and retention ($P < 0.05$). Phosphorus digestibility increased with MCP level, lowest in P0 ($P < 0.05$), while retention peaked then declined, with P2.5 showing the highest value ($P < 0.05$). MDCP supplementation similarly increased phosphorus digestibility (lowest in P0, highest in DP3.5, $P < 0.05$) and retention (peaking in DP3, $P < 0.05$). Nitrogen digestibility and retention showed no consistent patterns. Two-way ANOVA revealed no significant phosphorus source effects or source × level interactions for nitrogen or phosphorus digestibility and retention ($P > 0.05$). Binary regression analysis of MCP groups indicated 0.51% dietary digestible phosphorus maximized phosphorus retention, while broken-line analysis of MDCP groups indicated 0.52% dietary digestible phosphorus was optimal (Figures 2-3 [Figure 2: see original paper][Figure 3: see original paper]).

2.3 Effects on Whole-Body and Vertebral Phosphorus and Whole-Body Ash Content Effects on phosphorus and ash content are shown in Table 4. MCP and MDCP supplementation significantly increased vertebral

phosphorus and whole-body ash content ($P < 0.05$). Whole-body ash increased initially then stabilized with MCP, and increased continuously with MDCP, with P0 showing the lowest values ($P < 0.05$). Two-way ANOVA indicated no significant phosphorus source effects or source \times level interactions for these parameters ($P > 0.05$).

2.4 Relative Biological Availability of High Water-Soluble MDCP

Based on WGR and phosphorus retention rate, the relative biological availability of high water-soluble MDCP compared to MCP was 100% and 118%, respectively (Table 5).

Discussion

3.1 Effects on Growth Performance The results demonstrate that increasing MCP levels enhanced weight gain rate without affecting feed intake, indicating that elevated phosphorus within a certain range promotes growth performance in largemouth bass. This aligns with findings in catla (*Catla catla*) [5], Chinese sucker (*Myxocyprinus asiaticus*) [6], black seabream [7], Chinese sturgeon (*Acipenser sinensis*) [8], and European whitefish (*Alburnus alburnus*) [10], confirming that phosphorus supplementation improves growth and weight gain.

The significantly lower specific growth rate and higher feed conversion ratio in P0 indicate that phosphorus deficiency impairs growth and feed utilization, consistent with studies on GIFT tilapia (*Oreochromis niloticus*) [11], Siberian sturgeon (*Acipenser baeri*) [12], Japanese flounder (*Paralichthys olivaceus*), and turbot (*Scophthalmus maximus*) [13]. Under phosphorus-limiting conditions, most available phosphorus is diverted to essential metabolic processes, leaving insufficient phosphorus for growth. Young animals are more sensitive to nutrient deficiencies than later developmental stages due to more efficient nutrient utilization during rapid growth phases [13].

The significantly higher condition factor in P0 may result from combined effects of phosphorus-calcium synergy on bone development and phosphorus's influence on lipid metabolism. Phosphorus supplementation reduces body fat content [8], potentially explaining the lower condition factor in phosphorus-supplemented groups.

3.2 Effects on Nutrient Digestibility and Retention Nutrient digestibility measures digestive efficiency, while retention measures deposition efficiency. MCP and MDCP supplementation did not significantly affect nitrogen digestibility (~96% across groups), but improved nitrogen retention, with P2.5 showing significantly higher values than P0. This suggests that elevated phosphorus levels within a certain range can increase body protein content. Phosphorus deficiency reduces both lipid deposition and protein content [8], potentially because

phosphorus limitation inhibits fatty acid -oxidation, forcing protein catabolism for energy production [8]. Phosphorus digestibility directly reflects phosphorus availability [16], and the significantly lower values in P0 confirm that phosphorus deficiency impairs both digestion and deposition, similar to findings in Jian carp (*Cyprinus carpio* var. Jian) [17].

3.3 Effects on Whole-Body and Vertebral Phosphorus and Ash Content Whole-body phosphorus content is a primary criterion for determining phosphorus requirements [18-19], while vertebral phosphorus content serves as an additional reference since most phosphorus resides in bone [8,20]. In this study, dietary phosphorus level did not significantly affect whole-body phosphorus content, consistent with findings in gilthead seabream (*Sparus aurata*) [21] and American cichlid (*Cichlasoma urophthalmus*) [22]. This suggests that during phosphorus deficiency, body phosphorus is not substantially lost. Bone matrix consists primarily of inorganic minerals and collagen, mineralizing as calcium hydroxyapatite [$\text{Ca}(\text{PO})_3(\text{OH})$] with a 5:3 calcium:phosphorus ratio [8]. Only small phosphorus amounts deposit in bone, while most is stored as calcium phosphate for cartilage growth. During deficiency, bone phosphorus is mobilized first, causing calcium loss rather than whole-body phosphorus depletion [15,24].

Vertebral phosphorus content was significantly lower in P0 than in phosphate-supplemented groups, indicating phosphorus deficiency reduces vertebral deposition, consistent with black seabream studies [23]. However, no significant differences among supplemented groups may be attributed to measurement error from residual phospholipids in non-defatted vertebrae samples.

Whole-body ash content is another indicator of phosphorus status [24]. The positive correlation between ash content and dietary phosphorus level aligns with previous studies in Siberian sturgeon [27], black seabream [28], and topmouth culter (*Erythroculter ilishaeformis*) [29]. In this study, the correlation was strong when total dietary phosphorus was below 0.98% but weakened above this level.

3.4 Relative Biological Availability of High Water-Soluble MDCP MCP exhibits the highest biological availability among phosphate sources for aquatic animals and is most commonly used in aquafeed production. Studies in juvenile hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) reported relative biological values of 100%, 99%, 97%, and 75% for MCP, dicalcium phosphate, monopotassium phosphate, and monosodium phosphate, respectively [30]. In spotted steed (*Hemibarbus maculatus*), relative biological availabilities based on phosphorus digestibility were 100.00%, 76.84%, and 53.77% for MCP, dicalcium phosphate, and calcium phosphate [31]. Common carp (*Cyprinus carpio*) showed phosphorus digestibility values of 44.56%, 32.14%, 22.04%, 33.49%, and 10.59% for MCP, dicalcium phosphate, calcium phosphate, dicalcium-MCP mixture, and bone meal, respectively [32].

This study is the first to evaluate high water-soluble MDCP in aquatic ani-

mals. The test MDCP contained 84.5% water-soluble phosphorus, substantially exceeding the national standard (40%). Water-soluble phosphorus content directly affects utilization efficiency [33], and the high MCP proportion in the crystalline mixture contributed to superior availability [30-31]. The relative biological availability of 100% (based on weight gain) and 118% (based on phosphorus retention) indicates that high water-soluble MDCP can replace MCP in aquafeeds, offering cost advantages. The continuous increase in weight gain with MDCP supplementation, without reaching a plateau, suggests greater growth potential compared to MCP.

Previous phosphorus requirement studies in fish have primarily used highly available monophosphates. For example, using MCP, juvenile Amur sturgeon (*Acipenser schrencki*) required 0.88-1.00% phosphorus [34], Siberian sturgeon required 0.69% [28], and cobia (*Rachycentron canadum*) required 0.91-0.95% [16]. Using monopotassium phosphate, adult GIFT tilapia required 0.88-1.23% [35] and spotted steed required 0.91-1.17% [31]. Using monosodium phosphate, juvenile Japanese seabass (*Lateolabrax maculatus*) required 1.71% [36]. This study is the first to determine phosphorus requirements using high water-soluble MDCP, providing more accurate estimates by comparing two phosphate sources and using consistent phosphorus retention criteria.

Conclusion

With MCP as the phosphorus source, maximum weight gain rate and phosphorus retention rate were achieved at 0.51% dietary digestible phosphorus. With high water-soluble MDCP, maximum phosphorus retention rate occurred at 0.52% dietary digestible phosphorus. The relative biological availability of high water-soluble MDCP compared to MCP was 100% based on weight gain rate and 118% based on phosphorus retention rate.

References

- [1] Li EC, Chen LQ. Research progress on nutritional requirements of largemouth bass (*Micropterus salmoides*) [J]. *Modern Agricultural Science and Technology*, 2011(21): 312-316, 318.
- [2] Sun HJ, Liu B, Qu Q. Important effects of calcium and phosphorus on animals [J]. *Animal Husbandry and Veterinary Science and Technology Information*, 2009(9): 18.
- [3] Xiao PZ, Zhang BT, Tang J, et al. Research progress on phosphorus nutritional requirements and raw material digestibility in fish [J]. *China Feed*, 2009(2): 34-37.
- [4] Sugiura SH, Marchant DD, Kelsey K, et al. Effluent profile of commercially

- used low-phosphorus fish feeds [J]. *Environmental Pollution*, 2006, 140(1): 95-101.
- [5] Sukumaran K, Pal AK, Sahu NP, et al. Phosphorus requirement of Catla (*Catla catla* Hamilton) fingerlings based on growth, whole-body phosphorus concentration and non-faecal phosphorus excretion [J]. *Aquaculture Research*, 2009, 40(2): 139-147.
- [6] Yuan YC, Yang HJ, Gong SY, et al. Dietary phosphorus requirement of juvenile Chinese sucker, *Myxocyprinus asiaticus* [J]. *Aquaculture Nutrition*, 2015, 17(2): 159-169.
- [7] Shao QJ, Ma JJ, Xu ZR, et al. Dietary phosphorus requirement of juvenile black seabream, *Sparus macrocephalus* [J]. *Aquaculture*, 2008, 277(1/2): 92-100.
- [8] Roy PK, Lall SP. Dietary phosphorus requirement of juvenile haddock (*Melanogrammus aeglefinus* L.) [J]. *Aquaculture*, 2003, 221(1/2/3/4): 451-468.
- [9] Rodehutsord M, Gregus Z, Pfeffer E. Effect of phosphorus intake on faecal and non-faecal phosphorus excretion in rainbow trout (*Oncorhynchus mykiss*) and the consequences for comparative phosphorus availability studies [J]. *Aquaculture*, 2000, 188(3/4): 383-398.
- [10] Jokinen EI, Vielma J, Aaltonen TM, et al. The effect of dietary phosphorus deficiency on the immune responses of European whitefish (*Coregonus lavaretus* L.) [J]. *Fish & Shellfish Immunology*, 2003, 15(2): 159-168.
- [11] Yao YF. Studies on effective phosphorus and calcium/phosphorus ratio in juvenile and adult GIFT tilapia (*Oreochromis niloticus*) diets [D]. Master's thesis. Wuhan: Huazhong Agricultural University, 2012.
- [12] Zheng QS. Study on phosphorus requirement of juvenile Siberian sturgeon (*Acipenser baeri*) [D]. Master's thesis. Harbin: Northeast Agricultural University, 2009.
- [13] Zhu L. Nutritional physiology studies on inorganic minerals zinc and phosphorus in turbot and Japanese flounder [D]. Master's thesis. Qingdao: Ocean University of China, 2011.
- [14] Sugiura SH, Hardy RW, Roberts RJ. The pathology of phosphorus deficiency in fish—a review [J]. *Journal of Fish Diseases*, 2004, 27(5): 255-265.
- [15] Cheng KM, Hu CQ, Liu YN, et al. Effects of dietary calcium, phosphorus and calcium/phosphorus ratio on the growth and tissue mineralization of *Litopenaeus vannamei* reared in low-salinity water [J]. *Aquaculture*, 2006, 251(2/3/4): 472-483.
- [16] Liu XQ. Studies on suitable phosphorus source, phosphorus level and calcium/phosphorus ratio in juvenile cobia (*Rachycentron canadum*) diets [D]. Master's thesis. Guangzhou: Guangdong Ocean University, 2010.

- [17] Qin WL, Yang Y, Leng XJ, et al. Effects of phytase supplementation in diets with different MCP levels on growth, phosphorus utilization, body composition and digestive enzyme activities of Jian carp (*Cyprinus carpio* var. Jian) [J]. *Journal of Shanghai Ocean University*, 2015, 24(3): 383-390.
- [18] Zhang CX, Mai KS, Ai QH, et al. Dietary phosphorus requirement of juvenile Japanese seabass, *Lateolabrax japonicus* [J]. *Aquaculture*, 2006, 255(1/2/3/4): 201-209.
- [19] Zhang CX, Ai QH, Mai KS, et al. Dietary lysine requirement of large yellow croaker, *Pseudosciaena crocea* R [J]. *Aquaculture*, 2006, 253(1/2/3/4): 123-127.
- [20] Baeverfjord G, Åsgård T, Shearer KD. Development and detection of phosphorus deficiency in Atlantic salmon, *Salmo salar* L., parr and post-smolts [J]. *Aquaculture Nutrition*, 1998, 4(1): 1-11.
- [21] Pimentel-Rodrigues AM, Oliva-Teles A. Phosphorus requirements of gilt-head sea bream (*Sparus aurata* L.) juveniles [J]. *Aquaculture Research*, 2015, 32(S1): 157-161.
- [22] Chavez-Sanchez C, Martinez-Palacios CA, Martinez-Perez G, et al. Phosphorus and calcium requirements in the diet of the American cichlid *Cichlasoma urophthalmus* (Günther) [J]. *Aquaculture Nutrition*, 2015, 6(1): 1-9.
- [23] Xiong W. Effects of phosphorus source and concentration on growth performance of juvenile black seabream [D]. Master's thesis. Hangzhou: Zhejiang University, 2010.
- [24] Skonberg DI, Yogev L, Hardy RW, et al. Metabolic response to dietary phosphorus intake in rainbow trout (*Oncorhynchus mykiss*) [J]. *Aquaculture*, 1997, 157(1/2): 11-24.
- [25] Bureau DP, Cho CY. Phosphorus utilization by rainbow trout (*Oncorhynchus mykiss*): estimation of dissolved phosphorus waste output [J]. *Aquaculture*, 1999, 179(1/2/3/4): 127-140.
- [26] Schäfer A, Koppe WM, Meyer-Burgdorff KH, et al. Effects of a microbial phytase on the utilization of native phosphorus by carp in a diet based on soybean meal [J]. *Water Science and Technology*, 1995, 31(10): 149-155.
- [27] Zheng QS, Zhang WL, Xu QY, et al. Effects of dietary phosphorus levels on plasma biochemical indices of juvenile Siberian sturgeon (*Acipenser baeri*) [J]. *Chinese Journal of Fisheries*, 2009, 22(1): 38-41.
- [28] Hu WL. Effects of dietary phosphorus on growth and tissue biochemical indices of juvenile black seabream [D]. Master's thesis. Hangzhou: Zhejiang University, 2005.
- [29] Chen JM, Ye JY, Pan Q, et al. Phosphorus requirement of topmouth culter (*Erythroculter ilishaeformis*) fingerlings [J]. *Acta Hydrobiologica Sinica*, 2007, 31(1): 99-103.

- [30] Chen B, Pan Q, Zheng WC, et al. Effects of different phosphorus sources on growth performance of juvenile hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) [J]. *Feed Industry*, 2007, 28(10): 26-28.
- [31] Zhao CY, Zhou HQ, Chen JM, et al. Dietary phosphorus requirement of spotted steed (*Hemibarbus maculatus* Bleeker) [J]. *Journal of Fisheries of China*, 2008, 32(4): 614-620.
- [32] Yang YH, Guo Q, Chen SB, et al. Study on biological availability of different phosphorus sources to common carp (*Cyprinus carpio*) [J]. *Journal of Northeast Agricultural University*, 2006, 37(4): 484-488.
- [33] Zhong GY. Effects of different phosphorus sources on growth performance of juvenile black seabream [D]. Master's thesis. Hangzhou: Zhejiang University, 2009.
- [34] Wen H, Yan AS, Gao Q, et al. Dietary phosphorus requirement of juvenile Amur sturgeon (*Acipenser schrencki*) [J]. *Journal of Fisheries of China*, 2008, 32(2): 242-248.
- [35] Jiang M, Yao YF, Wen H, et al. Dietary available phosphorus requirement of adult GIFT tilapia (*Oreochromis niloticus*) [J]. *Journal of Fisheries of China*, 2013, 37(11): 1725-1732.
- [36] Lin XY. Study on dietary phosphorus requirement of juvenile Japanese seabass (*Lateolabrax maculatus*) [D]. Master's thesis. Hangzhou: Zhejiang University, 2007.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv – Machine translation. Verify with original.