# Optimal Dietary Monocalcium Phosphate Supplementation Level for Xiangyun Crucian Carp Feed: Postprint

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## Abstract

This experiment used juvenile Xiangyun crucian carp with an initial body weight of (40.00±0.34) g as research subjects, which were randomly divided into 6 groups (3 replicates per group, 20 fish per replicate) and fed six isonitrogenous and isoenergetic practical diets supplemented with monocalcium phosphate (MCP) at levels of 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, and 3.5% for an 8-week culture trial, aiming to investigate the optimal supplementation level of MCP in the diet of Xiangyun crucian carp. The results showed that with increasing dietary MCP supplementation, the final mean weight and weight gain rate (WGR) of Xiangyun crucian carp exhibited a trend of initially increasing and then decreasing, while feed conversion ratio (FCR) showed a trend of initially decreasing and then increasing; the final mean weight and WGR reached their maximum values at 2.5% MCP supplementation, and FCR reached its minimum value at 3.0% MCP supplementation. With increasing dietary MCP supplementation, the crude lipid content in whole fish, muscle, and liver of Xiangyun crucian carp showed a trend of initially decreasing and then increasing, reaching the minimum value at 2.5% supplementation; whereas the crude protein content in whole fish and the phosphorus content in whole fish and vertebrae reached their maximum values at this supplementation level, and the moisture content in whole fish showed no significant difference among groups (P>0.05). At 2.5% MCP supplementation, the activities of intestinal amylase, lipase, and trypsin, as well as serum superoxide dismutase and alkaline phosphatase activities, reached their maximum values, while serum triglyceride content and hepatic malondialdehyde content reached their minimum values. Serum cholesterol content showed no significant difference among groups (P>0.05). Serum phosphorus content exhibited a trend of initially increasing and then stabilizing with increasing dietary MCP supplementation, while serum calcium content showed no significant difference among groups (P>0.05). Using weight gain rate, feed conversion ratio, and phosphorus content in whole fish and vertebrae as observation indicators, analysis through quadratic curve model and broken-line model revealed that the optimal supplementation level of MCP in the diet of Xiangyun crucian carp was 2.55%-2.71%.

## Full Text

# Optimal Supplemental Level of Monocalcium Phosphate in Diets for Triploid Crucian Carp

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### Abstract

This experiment investigated the optimal supplemental level of monocalcium phosphate (MCP) in diets for triploid crucian carp. Juvenile triploid crucian carp with an initial body weight of (40.00±0.34) g were randomly divided into six groups (three replicates per group, 20 fish per replicate) and fed six isonitrogenous and isoenergetic practical diets containing 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, and 3.5% MCP for eight weeks. The results showed that with increasing dietary MCP levels, final average body weight and weight gain rate (WGR) initially increased then decreased, while feed conversion ratio (FCR) showed the opposite trend. The highest final average body weight and WGR occurred at 2.5% MCP supplementation, whereas the lowest FCR was observed at 3.0% MCP. Whole-body, muscle, and liver crude lipid content decreased initially then increased, reaching minimum values at 2.5% MCP supplementation. Conversely, whole-body crude protein content and phosphorus content in whole-body and vertebrae peaked at 2.5% MCP. No significant differences were observed in whole-body moisture content among groups ($P>0.05$). At 2.5% MCP supplementation, intestinal amylase, lipase, and trypsin activities, as well as serum superoxide dismutase (SOD) and alkaline phosphatase (AKP) activities, reached their maximum values, while serum triglyceride content and liver malondialdehyde content reached their minimum values. Serum cholesterol content did not differ significantly among groups ($P>0.05$). Serum phosphorus content increased initially then plateaued with increasing MCP supplementation, whereas serum calcium content showed no significant differences among groups ($P>0.05$). Based on quadratic and broken-line model analyses using WGR, FCR, and whole-body and vertebrae phosphorus content as evaluation indices, the optimal MCP supplemental level in diets for triploid crucian carp was determined to be 2.55%-2.71%.

**Keywords:** triploid crucian carp; monocalcium phosphate; growth performance; lipid metabolism

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Phosphorus is an essential macro-mineral element for normal growth and skeletal mineralization in fish. It constitutes a primary structural component of bone tissue and scales, and serves as a crucial constituent of cell membranes, nucleic acids, ATP, and phosphocreatine, participating in all intracellular energy-producing reactions. Although fish can absorb phosphorus directly from water, both water phosphorus concentrations and the utilization efficiency of phosphorus from feed ingredients are typically low. Consequently, phosphorus supplementation in formulated diets is necessary to meet fish requirements.

Numerous studies have reported phosphorus requirements for various fish species, which vary considerably due to differences in species, size, and feed formulation. The phosphorus requirement for major freshwater aquaculture species ranges from 0.50% to 1.57%, with MCP supplemental levels of 1.4%-3.6% in feeds. Triploid crucian carp represents an important freshwater aquaculture species in China. Recent advances in biotechnology have continuously improved its breeding stock, yielding notable advantages including rapid growth, strong stress resistance, tolerance to low temperatures and hypoxia, and broad feeding habits, leading to widespread cultivation across the country. As growth performance improves, nutrient requirements change accordingly. However, comprehensive data on available phosphorus from protein sources, energy sources, and inorganic phosphorus supplements in practical feed formulations remain incomplete, resulting in unscientific and uncertain phosphorus supplementation in triploid crucian carp feeds. For agastric cyprinid fish, MCP represents the most efficient and economical phosphorus source. Therefore, this study examined the effects of dietary MCP supplementation at 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, and 3.5% on growth, digestion, antioxidant capacity, and lipid metabolism in triploid crucian carp to determine the optimal supplemental level and provide a basis for rational feed formulation.

### 1.1 Experimental Diets

A basal diet was formulated using fish meal, soybean meal, rapeseed meal, and cottonseed meal as protein sources, and tilapia oil as the lipid source. A single-factor experimental design was employed with MCP as the phosphorus source. Six experimental diets were prepared by supplementing the basal diet with 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, and 3.5% MCP, with microcrystalline cellulose used to adjust the formulation. The composition and nutrient levels of the experimental diets are presented in . All feed ingredients were passed through a 40-mesh sieve, mixed, extruded into pellets with a diameter of 2.0 mm, air-dried, packaged, and stored at -20 °C until use.

### 1.2 Experimental Fish and Culture Management

Experimental fish were purchased from Chongqing Beibei Xiema Fish Farm. After disinfection with 3% saline solution, they were temporarily reared in indoor recirculating aquaria (392 L) and acclimated with commercial feed for 10 days. Prior to the formal experiment, 360 healthy and uniformly sized triploid crucian carp [initial body weight (40.00±0.34) g] were selected and randomly divided into six groups with three replicates per group, each containing 20 fish. Fish were fed three times daily (08:00, 12:00, 17:00) at 3%-5% of body weight. Feeding rates were adjusted every 10 days based on weight gain. Water was completely exchanged once daily, two hours after the final feeding. The experiment lasted eight weeks. During the culture period, water temperature was maintained at 22-28 °C, dissolved oxygen concentration >6.0 mg/L, ammonia nitrogen <0.10 mg/L, pH 6.6-7.0, and nitrite concentration <0.10 mg/L.

### 1.3 Sample Collection and Preparation

At the end of the experiment, fish were fasted for 24 hours. Eight fish were randomly selected from each replicate, anesthetized with MS-222, weighed, and measured for body length and height. Three of these fish were selected, their tails dried with gauze, and blood collected from the caudal vein using a 1 mL sterile syringe. Blood samples were left at room temperature for three hours, then centrifuged at 4,000 r/min (4 °C) for 10 minutes. Serum was collected and stored at -80 °C for biochemical analysis. After blood collection, the visceral mass was obtained for crude lipid determination, and the remaining body was microwave-heated until cooked. The vertebral column was separated, dried, and used for vertebrae phosphorus determination. The remaining five fish were dissected on ice to obtain dorsal white muscle for muscle crude lipid determination and visceral mass for liver and intestine separation. Surface moisture was absorbed with filter paper. All samples were snap-frozen in liquid nitrogen and stored at -80 °C.

For liver and intestine samples, the livers and intestines from three fish were minced and mixed, then homogenized with physiological saline (4 °C) at a 1:9 ratio (mass/volume, g/mL) in an ice bath. The homogenate was centrifuged at 3,500 r/min (4 °C) for 10 minutes, and the supernatant was collected as crude enzyme extract for liver and intestine index determination. Livers from the remaining two fish were minced and mixed for liver crude lipid determination.

### 1.4 Index Determination

Growth performance indices were calculated as follows:
Weight gain rate (WGR, %) = 100 × (Wt - W0) / W0;
Feed conversion ratio (FCR) = F / (Wt - W0);

where W0 is initial average body weight (g), Wt is final average body weight (g), and F is average feed intake (g).

Routine nutrient composition determination: Moisture content was determined by constant temperature drying (105 °C), crude lipid by Soxhlet extraction, crude protein by Kjeldahl semi-micro distillation, crude ash by high-temperature incineration (550 °C), and phosphorus content by molybdenum yellow colorimetry (GB/T 6437-2002).

Serum biochemical indices: Intestinal amylase, lipase, and trypsin activities, liver malondialdehyde (MDA) content, serum superoxide dismutase (SOD) and alkaline phosphatase (AKP) activities, and serum triglyceride (TG), cholesterol (CHO), calcium, and phosphorus contents were determined using assay kits provided by Nanjing Jiancheng Bioengineering Institute.

### 1.5 Data Processing and Analysis

Experimental data were analyzed using one-way ANOVA with SPSS 22.0. Duncan's multiple comparison test was used to analyze significant differences among groups ($P<0.05$). Quadratic and broken-line models were used for regression analysis of WGR, FCR, whole-body phosphorus content, and vertebrae phosphorus content against dietary MCP supplemental level. Data are expressed as mean ± standard deviation (mean±SD).

### 2.1 Effects of Dietary MCP Supplemental Level on Growth Performance of Triploid Crucian Carp

As shown in , with increasing dietary MCP supplemental level, final average body weight and WGR of triploid crucian carp initially increased then decreased, peaking at 2.5% MCP supplementation. Compared with the 1.0% group, the 2.5% group showed 15.62% and 29.90% increases in final average body weight and WGR, respectively. Conversely, FCR initially decreased then increased, reaching its minimum at 3.0% MCP supplementation, which represented a 16.05% reduction compared with the 1.0% group.

Using dietary MCP supplemental level (X) as the independent variable and WGR (Y1) and FCR (Y2) as dependent variables, regression equations were obtained: $Y1 = -12.246X^2 + 62.479X + 57.164$ ($R^2 = 0.9710$, extremum at 2.55%), and $Y2 = 0.0936X^2 - 0.4845X + 1.9999$ ($R^2 = 0.9257$, extremum at 2.59%). Extremum analysis indicated that the optimal MCP supplemental level for maximum growth performance was 2.55%–2.59%.

### 2.2 Effects of Dietary MCP Supplemental Level on Routine Nutritional Components of Triploid Crucian Carp

As shown in , whole-body crude protein content initially increased then decreased with increasing dietary MCP supplemental level, reaching its maximum in the 2.5% group, which was significantly higher than other groups

(P<0.05). Whole-body crude lipid content showed the opposite trend, reaching its minimum in the 2.5% group, which was significantly lower than other groups (P<0.05), representing a 15.67% decrease compared with the 1.0% group. Whole-body and vertebrae phosphorus content increased initially then plateaued, with no significant differences between the 2.5%, 3.0%, and 3.5% groups (P>0.05). Muscle, viscera, and liver crude lipid content initially decreased then increased, reaching minimum values in the 2.5% group, which were significantly lower than other groups (P<0.05), decreasing by 39.10%, 25.98%, and 22.86%, respectively, compared with the 1.0% group. Muscle and liver crude lipid content reached maximum values in the 3.5% group, increasing by 14.6% and 15.6%, respectively, compared with the 1.0% group.

Using whole-body and vertebrae phosphorus content (Y3 and Y4) as evaluation indices, broken-line model analysis yielded regression equations: Y3 = 0.254X + 1.173 (X 2.63, $R^2$ = 0.9874), Y3 = 1.84 (X > 2.63), with whole-body phosphorus content reaching its maximum at 2.63% MCP; Y4 = 0.228X + 7.241 (X 2.71, $R^2$ = 0.9528), Y4 = 7.86 (X > 2.71), with vertebrae phosphorus content reaching its maximum at 2.71% MCP. Extremum analysis indicated that optimal MCP supplemental levels for achieving satisfactory whole-body and vertebrae phosphorus content were 2.63%–2.71%.

### 2.3 Effects of Dietary MCP Supplemental Level on Serum Biochemical Indices of Triploid Crucian Carp

As shown in , dietary MCP supplemental level had no significant effect on serum calcium content (P>0.05). Serum AKP activity initially increased then decreased as dietary MCP increased from 1.0% to 3.5%, reaching its maximum in the 2.5% group, which was 48.3% higher than the 1.0% group (P<0.05). Serum phosphorus content increased gradually as dietary MCP increased from 1.0% to 2.5%, with significant differences among groups (P<0.05), but plateaued when MCP increased from 2.5% to 3.5%, with no significant differences among these groups (P>0.05).

### 2.4 Effects of Dietary MCP Supplemental Level on Intestinal Digestive Enzyme Activities and Serum Lipid Metabolism Indices of Triploid Crucian Carp

As shown in , intestinal amylase, lipase, and trypsin activities initially increased then decreased as dietary MCP increased from 1.0% to 3.5%, reaching maximum values in the 2.5% group. Compared with the 1.0% group, amylase, lipase, and trypsin activities increased by 93.33%, 95.44%, and 39.79%, respectively (P<0.05), with amylase and lipase showing greater increases than trypsin. Dietary MCP supplemental level had no significant effect on serum CHO content (P>0.05). Serum TG content initially decreased then increased as dietary MCP increased from 1.0% to 3.5%, reaching its minimum in the 2.5% group, which was 28.1% lower than the 1.0% group (P<0.05).

### 2.5 Effects of Dietary MCP Supplemental Level on Antioxidant Indices of Triploid Crucian Carp

As shown in , serum SOD activity initially increased then decreased as dietary MCP increased from 1.0% to 3.5%, reaching its maximum in the 2.5% group, which was significantly higher than other groups ($P<0.05$) and 23.26% higher than the 1.0% group. Conversely, liver MDA content showed the opposite trend, reaching its minimum in the 2.5% group, which was significantly lower than other groups ($P<0.05$) and 37.40% lower than the 1.0% group.

### 2.6 Correlation Analysis Between Selected Indices and Growth Performance

As shown in , using WGR as an indicator of growth performance, whole-body, viscera, muscle, and liver crude lipid content showed significant quadratic correlations with WGR ($P<0.05$) as dietary MCP supplemental level varied. Serum AKP and SOD activities and intestinal amylase and lipase activities showed significant positive correlations with WGR ($P<0.05$), while liver MDA content and serum TG content showed significant negative correlations with WGR ($P<0.05$).

## 3 Discussion

Phosphorus is an essential element for fish, and appropriate dietary phosphorus supplementation can improve growth and feed utilization. Excessive phosphorus supplementation not only causes metabolic disorders but also increases feed costs and pollutes water bodies, whereas insufficient supplementation reduces growth rate, feed efficiency, and may cause skeletal deformities. The present results indicate that insufficient phosphorus supplementation also manifests as fat accumulation in the body and tissues, reduced digestive capacity, and decreased antioxidant capacity, though no skeletal developmental abnormalities were observed. This suggests that the low supplementation level (1.0% MCP) may have already met the basic phosphorus requirements of triploid crucian carp.

Various indices can be used to evaluate optimal phosphorus supplementation levels in fish feeds, with different indices yielding different results. Based on experimental conditions, this study selected WGR, whole-body phosphorus content, and vertebrae phosphorus content as parameters to comprehensively determine the optimal MCP supplemental level. Using WGR as the evaluation index, 2.55% MCP supplementation satisfied optimal growth requirements. Using whole-body and vertebrae phosphorus content as evaluation indices, broken-line model analysis indicated that minimum MCP supplemental levels for maximum phosphorus deposition were 2.63% and 2.71%, respectively. Collectively, the optimal MCP supplemental level in diets for triploid crucian carp is 2.55%–2.71%, corresponding to total phosphorus content of 1.40%-1.44%. These results are similar to findings for Nile tilapia (*Oreochromis niloticus*), Amur sturgeon (*Acipenser schrenckii*), and prenant' s schizothoracin (*Sclizothorax prenanti*), but higher than those for crucian carp (*Carassius auratus*), Jian carp (*Cyprinus carpio* var. Jian), and Chinese sucker (*Myxocyprinus asiaticus*). Variations in phosphorus requirements among fish species may be attributed to differences in species, size, growth stage, feed formulation, phosphorus source, and culture environment.

Digestive enzymes play crucial roles in nutrient digestion, and their activities directly reflect digestive capacity. The present results demonstrate that appropriate MCP supplementation significantly increased intestinal amylase, lipase, and trypsin activities in triploid crucian carp. Notably, lipase activity in the 2.5% group increased by 95.44% compared with the 1.0% group, indicating that optimal MCP supplementation significantly enhanced digestive capacity and improved feed utilization efficiency.

Fish growth also depends on nutrient deposition, particularly protein and lipid. Previous studies have shown that appropriate dietary phosphorus supplementation significantly increases whole-body crude protein content while promoting lipid metabolism and reducing whole-body crude lipid content. The present results align with these findings, demonstrating that appropriate MCP supplementation significantly increased whole-body crude protein content while decreasing crude lipid content in whole-body, muscle, and liver. Compared with the optimal phosphorus group (2.5% MCP), liver crude lipid content in the low-phosphorus (1.0% MCP) and high-phosphorus (3.5% MCP) groups increased by 29.6% and 50.0%, respectively. The underlying mechanism may involve enhanced oxidative phosphorylation and citric acid cycle activity with optimal MCP supplementation, accelerating acetyl-CoA utilization and increasing fat utilization as an energy source, while insufficient or excessive phosphorus may inhibit this process. Additionally, 2.5% MCP supplementation significantly reduced serum TG content. In summary, appropriate MCP supplementation reduces hepatic lipid synthesis rate, thereby decreasing crude lipid deposition in fish body and tissues, though the detailed mechanism requires further investigation.

Limited information is available regarding the effects of phosphorus on animal antioxidant capacity. Feng et al. reported that appropriate dietary phosphorus supplementation increased blood SOD activity while decreasing blood MDA content in Jian carp. The present results demonstrate that appropriate MCP supplementation significantly increased serum SOD activity and decreased liver MDA content in triploid crucian carp, consistent with previous findings. Tang et al. also reported that appropriate phosphorus supplementation significantly increased liver SOD activity in catfish. These effects may be related to phosphorus as a major component of cell membrane phospholipids, where appropriate supplementation maintains cell membrane integrity and reduces attack by superoxide radicals. Enhanced antioxidant capacity may also contribute to improved growth performance in triploid crucian carp.

## 4 Conclusion

Based on WGR, FCR, and whole-body and vertebrae phosphorus content as evaluation indices, the optimal MCP supplemental level in diets for triploid crucian carp is 2.55%-2.71%. This supplementation level reduces hepatic lipid synthesis, decreases body fat deposition, enhances digestive and antioxidant capacity, and improves growth performance.

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