

Effects of Dietary Neutral Phytase Supplementation in Feeds with Different Calcium Dihydrogen Phosphate Levels on Growth, Nutrient Apparent Digestibility, Deposition Rate, and Plasma Biochemical Indices of Allogynogenetic Silver Crucian Carp (Postprint)

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Abstract

This experiment investigated the effects of dietary neutral phytase supplementation on growth performance, nutrient apparent digestibility, deposition rate, and plasma biochemical indices of allogynogenetic silver crucian carp fed diets with varying calcium dihydrogen phosphate levels. A positive control diet containing 1.5% calcium dihydrogen phosphate and negative control diets containing 1.0% and 0.5% calcium dihydrogen phosphate were formulated. Neutral phytase was supplemented at 400 and 800 IU/kg to the diet containing 1.0% calcium dihydrogen phosphate, and at 400 IU/kg to the diet containing 0.5% calcium dihydrogen phosphate, resulting in six experimental diets that were fed to allogynogenetic silver crucian carp with an initial body weight of (39.0 ± 3.0) g for 10 weeks. Each diet was assigned to three replicates, with 15 fish per replicate. The results showed that in the absence of neutral phytase, increasing dietary calcium dihydrogen phosphate levels significantly increased weight gain rate, apparent digestibility of dry matter and phosphorus, protein and ash deposition rates, and whole-body phosphorus content ($P < 0.05$), while significantly decreasing plasma alkaline phosphatase (ALP) activity ($P < 0.05$). Supplementation of 800 IU/kg neutral phytase in the diet containing 1.0% calcium dihydrogen phosphate significantly improved weight gain rate ($P < 0.05$), and concurrently increased protein, ash, and phosphorus deposition rates, apparent digestibility of dry matter and phosphorus, whole-body phosphorus content, vertebral calcium content, and plasma triglyceride (TG) content, while significantly reducing plasma ALP activity ($P < 0.05$). Supplementation of 400 IU/kg neutral phytase in diets containing 0.5% and 1.0% calcium dihydrogen phosphate

both significantly enhanced apparent phosphorus digestibility and phosphorus deposition rate ($P < 0.05$). Dietary neutral phytase supplementation across different calcium dihydrogen phosphate levels had no significant effects on plasma total protein (TP), albumin (ALB), calcium, or phosphorus contents ($P > 0.05$). These findings indicate that supplementation of 800 IU/kg neutral phytase in a diet containing 1.0% calcium dihydrogen phosphate promotes growth and improves apparent digestibility of dry matter and phosphorus in allogynogenetic silver crucian carp, and that 800 IU/kg neutral phytase can replace 0.5% calcium dihydrogen phosphate supplementation under adequate substrate conditions.

Full Text

Effects of Supplementation of Neutral Phytase in Diets with Various Monocalcium Phosphate Contents on Growth, Apparent Digestibility and Deposition Rates of Nutrients, and Plasma Biochemical Indices of Allogynogenetics Silver Crucian Carp (*Carassius auratus gibelio*)

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Abstract: This study investigated the effects of supplementing neutral phytase in diets with varying monocalcium phosphate (MCP) contents on growth performance, apparent digestibility and deposition rates of nutrients, and plasma biochemical indices of allogynogenetics silver crucian carp (*Carassius auratus gibelio*). Six experimental diets were formulated: a positive control diet with 1.5% MCP, negative control diets with 1.0% and 0.5% MCP, and three phytase-supplemented diets (1.0% MCP + 400 IU/kg neutral phytase, 1.0% MCP + 800 IU/kg neutral phytase, and 0.5% MCP + 400 IU/kg neutral phytase). Fish with an initial body weight of (39.0±3.0) g were fed the experimental diets for 10 weeks. Each diet was assigned to three replicates with 15 fish per replicate. The results showed that in diets without phytase supplementation, increasing MCP content significantly increased weight gain rate (WGR), apparent digestibility of dry matter (DM) and phosphorus (P), deposition rates of protein and ash, and whole-body P content ($P < 0.05$), while significantly decreasing plasma alkaline phosphatase (ALP) activity ($P < 0.05$). Supplementation of 800 IU/kg neutral phytase in the 1.0% MCP diet significantly improved WGR ($P < 0.05$) to a level comparable to the 1.5% MCP group, and significantly increased deposition rates

of protein, ash, and P, apparent digestibility of DM and P, whole-body P content, vertebral calcium (Ca) content, and plasma triglyceride (TG) content, while significantly reducing plasma ALP activity ($P < 0.05$). Supplementation of 400 IU/kg neutral phytase in both 0.5% and 1.0% MCP diets significantly improved P apparent digestibility and P deposition rate ($P < 0.05$). No significant differences were observed in plasma total protein (TP), albumin (ALB), Ca, or P contents among all groups ($P > 0.05$). These results indicate that supplementation of 800 IU/kg neutral phytase in a diet containing 1.0% MCP can promote growth and improve apparent digestibility of DM and P in allogeneetics silver crucian carp, and that 800 IU/kg neutral phytase can replace 0.5% MCP supplementation under sufficient substrate conditions.

Keywords: neutral phytase; allogeneetics silver crucian carp (*Carassius auratus gibelio*); phosphorus apparent digestibility; growth

Introduction

Phosphorus is an essential mineral element for fish. Dietary phosphorus deficiency can affect intermediary metabolism, leading to reduced growth and feed conversion efficiency, skeletal deformities, and fat accumulation [1-2]. Since phosphorus content in water is typically low, adequate phosphorus must be supplied through the diet. Although plant-based ingredients are rich in phosphorus, most exists as phytate phosphorus, which is poorly utilized by animals [3-5]. Consequently, inorganic phosphates are commonly added to meet the phosphorus requirements of aquatic animals. However, this practice not only increases feed costs but, more importantly, results in substantial excretion of unutilized phytate phosphorus and undigested phosphates into the water, causing severe environmental pollution.

Phytic acid, or inositol hexaphosphate, is the primary storage form of phosphorus in plants. It possesses strong chelating capacity, forming insoluble complexes with minerals such as calcium, magnesium, potassium, sodium, zinc, iron, copper, and manganese, as well as complexing with proteins to inhibit digestive enzyme activity. Research has shown that phytic acid can reduce the utilization of dietary minerals [6-7] and impair protein digestion and utilization [8].

Phytase catalyzes the degradation of phytate (salts) into inositol and inorganic phosphorus, simultaneously releasing other nutrients bound to phytate (salts), thereby improving nutrient utilization and reducing the need for inorganic phosphate supplementation. Phytases have multiple optimal pH ranges and are classified as either acidic or neutral phytases based on their pH optima. Acidic phytases generally have an optimal pH range of 2.0-7.0, while neutral phytases have an optimal pH around 7.0. In recent years, acidic phytases have been extensively studied and applied in livestock such as chickens [9-10], ducks [11], and pigs [12-14], and in aquatic species including channel catfish (*Ictalurus punctatus*) [15], Japanese flounder (*Paralichthys olivaceus*) [16], and grass carp

(*Ctenopharyngodon idellus*) [17]. Neutral phytases are primarily applied in feeds for stomachless freshwater cyprinid fish whose foregut pH is neutral. Allogeny silver crucian carp (*Carassius auratus gibelio*), bred by artificial insemination using naturally gynogenetic Fangzheng crucian carp as the maternal parent and Xingguo red carp as the paternal parent, is characterized by rapid growth and strong stress resistance, and has become the main cultured crucian carp variety in China. This study primarily investigated the effects of supplementing different levels of neutral phytase as a replacement for monocalcium phosphate on growth, body composition, nutrient digestibility and deposition rates, and plasma biochemical indices of allogeny silver crucian carp, providing a basis for the rational application of neutral phytase in aquafeeds.

Materials and Methods

1.1 Experimental Materials

The heat-resistant neutral phytase was provided by Guangdong VTR Bio-Tech Co., Ltd., with a suitable pH range of 4.5-8.0 (the pH in the digestive tract of stomachless fish is 6.5-8.4) and an activity of 2,000 IU/g.

1.1 Experimental Design

Six experimental diets were formulated: a positive control diet containing 1.5% monocalcium phosphate (MCP) and negative control diets containing 1.0% and 0.5% MCP, plus three phytase-supplemented diets (1.0% MCP + 400 IU/kg neutral phytase, 1.0% MCP + 800 IU/kg neutral phytase, and 0.5% MCP + 400 IU/kg neutral phytase). The phytase supplementation levels were reduced appropriately based on reference [18]. Yttrium oxide (Y₂O₃) was added at 0.05% as an internal marker for determining nutrient digestibility. All ingredients were passed through a 40-mesh sieve, mixed sequentially, and processed into 2-mm sinking pellets using a single-screw extruder (SLP-45, developed by Fishery Machinery and Instrument Research Institute, Chinese Academy of Fishery Sciences) at a pelleting temperature of (85±5)°C. The diets were air-dried in a ventilated, shaded area and stored at 4°C until use. The composition and nutrient levels of the experimental diets are presented in Table 1.

1.2 Experimental Fish and Feeding Management

Allogeny silver crucian carp were purchased from a hatchery in Jinhua, Zhejiang Province. Prior to the formal experiment, fish were fed a diet containing 0.5% MCP for two weeks to gradually adapt to the experimental diets. A total of 270 healthy fish with uniform body weight [(39.0±3.0) g] were selected and randomly divided into six groups with three replicates (tanks) per group, each containing 15 fish. An indoor aerated recirculating aquaculture system was used, with each experimental tank measuring 0.60 m × 0.50 m × 0.50 m (135 L). The tanks were siphoned daily to maintain water quality, and one-tenth of the water volume was replaced. Fish were hand-fed three times daily (08:30,

11:30, and 16:30) for approximately 20 minutes per feeding at a feeding rate of 2.5%-3.0% of body weight, adjusted according to appetite to achieve apparent satiation without residual feed. All tanks received essentially the same feed amount. During the experimental period, water temperature was maintained at $(26.0 \pm 2.0)^\circ\text{C}$, pH at 6.5-7.3, and dissolved oxygen concentration at >4 mg/L.

1.3.1 Growth Indices and Body Indices

At the end of the feeding trial, fish were fasted for 24 h. The number and weight of fish in each tank were recorded to calculate weight gain rate (WGR), feed conversion ratio (FCR), and survival rate (SR). Three fish from each tank were sampled to measure body length and weight. Viscera and hepatopancreas were dissected and weighed to calculate hepatosomatic index (HSI), viscera-somatic index (VSI), and condition factor (CF).

The formulas were as follows: - Weight gain rate (%) = $100 \times [\text{final weight (g)} - \text{initial weight (g)}] / \text{initial weight (g)}$ - Feed conversion ratio = $\text{feed intake (g)} / \text{weight gain (g)}$ - Survival rate (%) = $100 \times \text{final fish number} / \text{initial fish number}$ - Hepatosomatic index (%) = $100 \times \text{liver weight (g)} / \text{body weight (g)}$ - Viscera-somatic index (%) = $100 \times \text{viscera weight (g)} / \text{body weight (g)}$ - Condition factor (%) = $100 \times \text{body weight (g)} / \text{body length (cm)}^3$

1.3.2 Body Composition and Nutrient Deposition Rates

Prior to the feeding trial, six fish were sampled for initial whole-body proximate composition analysis. At the end of the trial, three fish from each tank (nine per group) were sampled, stored at -20°C for whole-body proximate composition analysis. The vertebral column was obtained by microwaving eviscerated fish (with lateral muscle removed) for 5 min, removing the skull, and cleaning the vertebrae. The vertebrae were dried at 105°C and stored at -20°C for calcium and phosphorus content determination. Proximate composition of diets, whole-body, and vertebrae was determined as follows: moisture by oven drying at 105°C ; crude protein by Kjeldahl method (2300 Automatic Analyzer, Switzerland); crude lipid by Soxhlet extraction; crude ash by incineration at 550°C (SXL-1008 Muffle Furnace, Shanghai Jinghong Experimental Equipment Co., Ltd.); calcium and phosphorus contents by colorimetric assay kits (calcium methyl thymol blue and phosphomolybdic acid methods, respectively) purchased from Nanjing Jiancheng Bioengineering Institute. Based on these data, protein, lipid, ash, and phosphorus deposition rates were calculated using the following formulas:

- Protein deposition rate (%) = $100 \times (\text{Wt} \times \text{Wtp} - \text{W} \times \text{Wp}) / (\text{Wf} \times \text{Wfp})$
- Lipid deposition rate (%) = $100 \times (\text{Wt} \times \text{Wtl} - \text{W} \times \text{Wl}) / (\text{Wf} \times \text{Wfl})$
- Ash deposition rate (%) = $100 \times (\text{Wt} \times \text{Wta} - \text{W} \times \text{Wa}) / (\text{Wf} \times \text{Wfa})$
- Phosphorus deposition rate (%) = $100 \times (\text{Wt} \times \text{Wtph} - \text{W} \times \text{Wph}) / (\text{Wf} \times \text{Wfph})$

Where: W = initial weight (g); W_t = final weight (g); W_{tp} = final whole-body crude protein content; W_{tl} = final whole-body crude lipid content; W_{ta} = final whole-body crude ash content; W_{tph} = final whole-body phosphorus content; W_p = initial whole-body crude protein content; W_l = initial whole-body crude lipid content; W_a = initial whole-body crude ash content; W_{ph} = initial whole-body phosphorus content; W_f = total feed intake; W_{fp} = dietary crude protein content; W_{fl} = dietary crude lipid content; W_{fa} = dietary crude ash content; W_{fph} = dietary phosphorus content.

1.3.3 Nutrient Apparent Digestibility

During the last two weeks of the feeding trial, feces were collected by siphoning (starting 1 h after feeding, twice daily). Only feces with intact membranes were collected, dried at 60°C, and stored at -20°C until analysis. Dietary and fecal contents of dry matter, crude protein, phosphorus, and Y O were determined to calculate apparent digestibility of dry matter, protein, and phosphorus. Yttrium was analyzed by inductively coupled plasma (ICP) spectroscopy, while other indices were determined as described in section 1.3.2.

The formulas were: - Dry matter apparent digestibility (%) = $100 \times (a - b) / a$ - Protein apparent digestibility (%) = $100 \times (a \times c - b \times d) / (a \times c)$ - Phosphorus apparent digestibility (%) = $100 \times (a \times e - b \times f) / (a \times e)$

Where: a = indicator content in feces; b = indicator content in diet; c = crude protein content in diet; d = crude protein content in feces; e = phosphorus content in diet; f = phosphorus content in feces.

1.3.4 Plasma Biochemical Indices

At the end of the feeding trial, after 24 h of fasting, five fish from each tank were anesthetized and blood was collected from the caudal vein using heparinized syringes. Plasma was separated by centrifugation at 3,000 r/min for 10 min at 4°C and stored at -80°C until analysis. Plasma biochemical indices were determined using an automatic biochemical analyzer (Mindray BS-200, Shenzhen), including alkaline phosphatase (ALP) activity (AMP buffer method) and contents of triglycerides (TG) (oxidase method), total protein (TP) (biuret method), albumin (ALB) (bromocresol green method), phosphorus (phosphomolybdic acid method), and calcium (arsenazo III method).

1.4 Statistical Analysis

Results are expressed as means \pm standard deviation. One-way ANOVA was performed using SPSS 17.0. When significant differences were detected, Duncan's multiple range test was used for post-hoc comparisons. The significance level was set at $P < 0.05$.

Results

2.1 Growth Performance and Body Indices

As shown in Table 2 , in diets without neutral phytase, increasing MCP content from 0.5% to 1.5% significantly increased WGR and significantly decreased FCR, HSI, and VSI of allogenyetics silver crucian carp ($P < 0.05$), while CF showed no significant change ($P > 0.05$). Supplementation of 400 IU/kg neutral phytase in the 1.0% MCP diet increased WGR by 9.2% ($P > 0.05$), whereas supplementation of 800 IU/kg neutral phytase increased WGR by 11.8% ($P < 0.05$), reaching a level comparable to the 1.5% MCP group, with a trend toward reduced FCR ($P < 0.10$). Supplementation of 400 IU/kg neutral phytase in the 0.5% MCP diet showed a trend toward improved WGR ($P < 0.10$), reaching a level similar to the 1.0% MCP group. Survival rate was 100% in all groups at the end of the experiment.

2.2 Body Composition and Nutrient Deposition Rates

As shown in Table 3 , no significant differences were observed in whole-body moisture, crude protein, or crude ash content among all groups ($P > 0.05$). In diets without neutral phytase, increasing MCP content from 0.5% to 1.5% significantly decreased whole-body crude lipid content ($P < 0.05$). Supplementation of 800 IU/kg neutral phytase in the 1.0% MCP diet significantly decreased whole-body crude lipid content ($P < 0.05$), while supplementation of 400 IU/kg neutral phytase in the 0.5% MCP diet showed a trend toward reduced crude lipid content ($P < 0.10$).

Regarding nutrient deposition rates, in diets without neutral phytase, increasing MCP content from 0.5% to 1.5% significantly increased protein and ash deposition rates ($P < 0.05$), while lipid deposition rate remained unchanged ($P > 0.05$). Supplementation of 800 IU/kg neutral phytase in the 1.0% MCP diet significantly increased protein and ash deposition rates ($P < 0.05$) to levels comparable to the 1.5% MCP group. In diets without phytase, the 1.5% MCP group showed significantly higher phosphorus deposition rate than the 0.5% and 1.0% MCP groups ($P < 0.05$), though no significant difference was observed between the latter two groups ($P > 0.05$). Supplementation of 400 and 800 IU/kg neutral phytase in the 1.0% MCP diet and 400 IU/kg neutral phytase in the 0.5% MCP diet all significantly increased phosphorus deposition rate ($P < 0.05$).

2.3 Apparent Digestibility of Dry Matter, Protein, and Phosphorus and Vertebral Calcium, Phosphorus, and Ash Contents

As shown in Table 4 , no significant differences were observed in protein apparent digestibility among groups ($P > 0.05$). In diets without neutral phytase, increasing MCP content from 0.5% to 1.5% significantly increased apparent digestibility of dry matter and phosphorus ($P < 0.05$). Supplementation of 400 IU/kg neutral phytase in the 1.0% MCP diet significantly increased phosphorus apparent digestibility ($P < 0.05$), while supplementation of 800 IU/kg neutral phytase

significantly increased both dry matter and phosphorus apparent digestibility ($P < 0.05$). Supplementation of 400 IU/kg neutral phytase in the 0.5% MCP diet significantly increased phosphorus apparent digestibility ($P < 0.05$) and showed a trend toward improved dry matter apparent digestibility ($P < 0.10$).

In diets without neutral phytase, increasing MCP content from 0.5% to 1.5% significantly increased vertebral calcium and phosphorus contents ($P < 0.05$), while vertebral ash content remained unchanged ($P > 0.05$). Supplementation of 800 IU/kg neutral phytase in the 1.0% MCP diet significantly increased vertebral calcium content ($P < 0.05$), though vertebral phosphorus content showed no significant change ($P > 0.05$). Supplementation of 400 IU/kg neutral phytase in the 0.5% MCP diet numerically increased both vertebral calcium and phosphorus contents ($P > 0.05$).

2.4 Plasma Biochemical Indices

As shown in Table 5, no significant differences were observed in plasma TP, ALB, calcium, or phosphorus contents among all groups ($P > 0.05$). In diets without neutral phytase, plasma ALP activity decreased significantly with increasing MCP content ($P < 0.05$). Supplementation of 400 and 800 IU/kg neutral phytase in the 1.0% MCP diet and 400 IU/kg neutral phytase in the 0.5% MCP diet all significantly reduced plasma ALP activity ($P < 0.05$). Plasma TG content in the 1.0% MCP diet supplemented with 400 and 800 IU/kg neutral phytase was significantly higher than in other groups ($P < 0.05$).

Discussion

The unique structure and physicochemical properties of phytic acid enable strong chelation with numerous mineral elements and some protein-active substances. Phytases catalyze the hydrolysis of phytic acid and phytate salts into inositol and inorganic phosphorus, simultaneously releasing other nutrients bound to phytic acid (salts), thereby improving nutrient utilization, increasing animal weight gain, reducing inorganic phosphate supplementation in feeds, and decreasing phosphorus excretion in feces. Most major cultured cyprinid fish species in China are stomachless with neutral pH in their digestive tracts, making neutral phytase more suitable for these species. In this study, supplementation of 800 IU/kg neutral phytase in the 1.0% MCP diet increased WGR by 11.8% and significantly improved apparent digestibility of dry matter and phosphorus, indicating that dietary neutral phytase released nutrients and phosphorus chelated by phytic acid, making them more available for utilization [19], meeting the phosphorus requirements of allogynetics silver crucian carp, improving phosphorus digestibility, and promoting fish growth. Zhang et al. [20] reported that dietary supplementation of 200 mg/kg acidic phytase increased WGR in Japanese seabass (*Lateolabrax japonicus*) from 859.3% to 947.2%. Similar results have been reported for Nile tilapia (*Oreochromis niloticus*) [21] and rainbow trout (*Oncorhynchus mykiss*) [22]. In Japanese seabass, supplementation of 1,000 and 1,500 FTU/kg neutral phytase increased

total phosphorus digestibility by 5.8%-18.9% and significantly improved dry matter digestibility when MCP supplementation was reduced by 60%-100% [23], consistent with our findings. Significant effects of neutral phytase supplementation on phosphorus apparent digestibility have also been reported in black seabream (*Acanthopagrus schlegelii*) [18], common carp (*Cyprinus carpio* var. Jian) [24], and sutchi catfish (*Pangasius pangasius*) [25]. Additionally, Baruah et al. [26] in rohu (*Labeo rohita*), Vandenberg et al. [27] in rainbow trout, and Zhu et al. [28-29] in yellow catfish (*Pelteobagrus fulvidraco*) reported that dietary acidic phytase supplementation significantly improved dry matter digestibility. In this study, supplementation of 400 and 800 IU/kg neutral phytase in the 1.0% MCP diet significantly increased phosphorus deposition rate, indicating that phytase supplementation improved phosphorus utilization and consequently promoted fish growth. Qin et al. [24] also reported that dietary acidic phytase supplementation improved phosphorus deposition rate in common carp, consistent with our results. In this study, supplementation of 400 IU/kg neutral phytase in diets containing 1.0% and 0.5% MCP increased WGR by 9.2% and 9.4%, respectively, though not significantly, suggesting that the phytase dosage may have been insufficient. This may be related to the short digestive tract of fish and rapid feed passage, where low phytase doses cannot hydrolyze sufficient inorganic phosphorus within the limited time. This result aligns with reports that 500 IU/kg acidic phytase supplementation in Nile tilapia diets did not significantly affect growth, whereas 1,000 IU/kg significantly improved growth [20]. Additionally, 500 IU/kg acidic phytase supplementation showed no significant effects on growth in Japanese seabass [30] and channel catfish [31].

Dietary nutrients, particularly protein and lipid, typically affect fish body indices [32-33]. Niu et al. [34] reported that acidic phytase supplementation in largemouth bass (*Micropterus salmoides*) diets reduced HSI, possibly because phytase degradation of phytic acid produces inositol, which promotes lipid metabolism in the liver and other tissues, preventing hepatic lipid deposition and thus reducing HSI. In this study, increasing MCP content without phytase supplementation significantly reduced VSI and HSI in allogynogenetics silver crucian carp. Supplementation of 800 IU/kg neutral phytase in the 1.0% MCP diet also significantly reduced VSI and HSI. Ma et al. [17] reported that 1,000 IU/kg acidic phytase supplementation in grass carp diets significantly reduced VSI and HSI, consistent with our results. Yang et al. [35] reported that neutral phytase supplementation did not significantly affect condition factor in common carp (*Cyprinus carpio*) as dietary MCP decreased from 3.0% to 0.5%, similar to our findings.

When dietary phosphorus is deficient, the lipid oxidation process is inhibited, leading to increased lipid deposition [36]. When phosphorus requirements are met through phosphate supplementation or phytase addition, body lipid deposition decreases. In this study, whole-body crude lipid content in the 1.5% MCP group was significantly lower than in the 0.5% MCP group, indicating that phosphorus deficiency resulted in higher body lipid content. Reduced whole-body

crude lipid content has been observed with acidic phytase supplementation in large yellow croaker (*Pseudosciaena crocea*) [37] and neutral phytase supplementation in grass carp [38], as well as in our study with neutral phytase in the 1.0% MCP diet. This study also found that phytase supplementation significantly increased ash deposition rate, consistent with Zhang et al. [37], likely because phytase can break the chelation bonds between phytic acid and minerals, improving mineral (calcium, phosphorus, etc.) absorption and utilization. Research has shown that 1,000 IU/kg acidic phytase supplementation significantly increased vertebral phosphorus content in Nile tilapia [18]. Phytase promotion of skeletal mineralization and improvement of body composition has also been demonstrated in sutchi catfish [25] and rohu [39]. In this study, supplementation of 800 IU/kg neutral phytase in the 1.0% MCP diet significantly increased whole-body phosphorus content and vertebral calcium content, with a trend toward increased vertebral phosphorus content, indicating that phytase not only releases inorganic phosphorus from phytic acid degradation but also liberates previously chelated proteins and minerals, promoting nutrient digestion and absorption, thereby improving body composition and mineral deposition in vertebrae [40].

Alkaline phosphatase (ALP), also known as orthophosphoric monoester phosphohydrolase, is an important indicator of bone metabolism. Plasma ALP plays a crucial role in mineralization and membrane transport in aquatic animals [41]. In this study, plasma ALP activity decreased significantly with increasing MCP content from 0.5% to 1.5%, and also decreased significantly with increasing phytase supplementation in the 1.0% MCP diet. Similar results were reported by Liu et al. [11] in meat ducks, who suggested that higher plasma ALP activity is associated with active bone remodeling. When dietary available phosphorus is deficient, osteoblast activity increases, resulting in higher ALP activity. As phytase supplementation increases dietary available phosphorus, bone phosphorus turnover slows, thereby reducing plasma ALP activity. In this study, supplementation of 400 and 800 IU/kg neutral phytase in the 1.0% MCP diet significantly increased plasma TG content, indicating enhanced lipid metabolism and reduced body lipid deposition. Plasma TP and ALB contents play important roles in immune response, and plasma TP content is an important indicator of nutritional status and health [42]. In this study, no significant differences in plasma TP and ALB contents were observed among groups, indicating that feeding allogenyetics silver crucian carp diets with low MCP content for 8 weeks was insufficient to affect metabolic function. Similarly, plasma calcium and phosphorus contents showed no significant changes, consistent with reports that acidic phytase supplementation did not affect plasma phosphorus content in yellow catfish [28-29].

In conclusion, supplementation of 800 IU/kg neutral phytase in a diet containing 1.0% MCP can significantly improve weight gain rate, protein and phosphorus deposition rates, and apparent digestibility of dry matter and phosphorus in allogenyetics silver crucian carp, achieving effects essentially equivalent to those of a diet containing 1.5% MCP.

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