Effects of Partial Replacement of Monocalcium Phosphate with Neutral Phytase on Growth Performance, Body Composition, and Phosphorus Utilization in Allogynogenetic Gibel Carp: Postprint

Authors: Zeng Xiao'ai, Xu Shude, Chen Qinghua, Xuan Yanjun, Zhao Qinghai

Date: 2017-10-23T00:00:00+00:00

Abstract

This study aimed to investigate the effects of partially replacing calcium dihydrogen phosphate with neutral phytase on growth performance, body composition, serum biochemical indices, and phosphorus utilization in gibel carp (Carassius auratus gibelio). Seven experimental groups were established: D1 (positive control group, feed supplemented with 1.5% calcium dihydrogen phosphate), D2 (negative control 1, feed supplemented with 1.0% calcium dihydrogen phosphate), D3 (feed supplemented with 1.0% calcium dihydrogen phosphate + 400 U/kg neutral phytase), D4 (feed supplemented with 1.0% calcium dihydrogen phosphate + 800 U/kg neutral phytase), D5 (negative control 2, feed supplemented with 0.5% calcium dihydrogen phosphate), D6 (feed supplemented with 0.5% calcium dihydrogen phosphate + 400 U/kg neutral phytase), and D7 (feed supplemented with 0.5% calcium dihydrogen phosphate + 800 U/kg neutral phytase), with 3 replicates per group and 25 fish per replicate [initial average weight (23.39±0.10) g], and an 8-week growth trial was conducted. The results showed that after gibel carp were fed the experimental diets for 8 weeks, the D5 group exhibited the poorest growth performance, with weight gain rate, specific growth rate, and feed intake significantly lower than those of all other groups ($P<0.05$), while feed conversion ratio was significantly higher than that of all other groups ($P<0.05$). The weight gain rate and specific growth rate of D2 and D6 groups were significantly lower than those of D1 group ($P<0.05$), while feed conversion ratio was significantly higher than that of D1 group ($P<0.05$). The growth performance indices of D3, D4, and D7 groups showed no significant differences from those of D1 group ($P>0.05$). Whole-body moisture, crude protein, crude ash, and phosphorus contents, as well as serum cholesterol content and alkaline phosphatase activity of gibel carp showed no significant differences among

all groups (P>0.05). Except for D5 group, serum phosphorus and triglyceride contents showed no significant differences among the other groups (P>0.05). At the same calcium dihydrogen phosphate supplementation level, the phosphorus retention rate and apparent phosphorus digestibility of groups supplemented with neutral phytase were significantly higher than those of groups without neutral phytase supplementation (P<0.05), while phosphorus discharge showed the opposite trend. Therefore, supplementation of 400 and 800 U/kg neutral phytase in feed can reduce calcium dihydrogen phosphate supplementation by 0.5% and 1.0%, respectively, without compromising growth performance, body composition, and serum biochemical indices of gibel carp. In gibel carp feed, partial replacement of calcium dihydrogen phosphate with neutral phytase can improve feed phosphorus utilization and reduce phosphorus discharge, thereby conferring both economic and ecological benefits.

Full Text

Effects of Partially Replacing Monocalcium Phosphate with Neutral Phytase on Growth Performance, Body Composition and Phosphorus Utilization of Gibel Carp (Carassius auratus gibelio)

ZENG Xiaoai[1],[2], XU Shude[2], CHEN Qinghua[1]*, XUAN Yanjun[2], ZHAO Qinghai[2]

(1. College of Animal Science and Technology, Hunan Agricultural University, Changsha 410128, China;
2. Guangdong VTR Co., Ltd., Zhuhai 519060, China)

Abstract

An 8-week feeding trial was conducted to investigate the effects of partially replacing monocalcium phosphate (MCP) with neutral phytase on growth performance, body composition, serum biochemical parameters, and phosphorus utilization in gibel carp (Carassius auratus gibelio). Seven experimental groups were established: D1 (positive control, 1.5% MCP), D2 (negative control 1, 1.0% MCP), D3 (1.0% MCP + 400 U/kg neutral phytase), D4 (1.0% MCP + 800 U/kg neutral phytase), D5 (negative control 2, 0.5% MCP), D6 (0.5% MCP + 400 U/kg neutral phytase), and D7 (0.5% MCP + 800 U/kg neutral phytase). Each group comprised three replicates of 25 fish with an initial average body weight of (23.39±0.10) g.

After the 8-week feeding period, the D5 group exhibited the poorest growth performance, with significantly lower weight gain rate, specific growth rate, and feed intake compared to all other groups (P<0.05), while showing a significantly higher feed conversion ratio (P<0.05). The D2 and D6 groups also displayed significantly lower weight gain and specific growth rates, and significantly higher feed conversion ratios compared to the D1 group (P<0.05). In contrast, the D3,

D4, and D7 groups showed no significant differences in any growth performance indices compared to D1 (P>0.05).

Whole-body moisture, crude protein, ash, and phosphorus contents, as well as serum cholesterol levels and alkaline phosphatase activity, did not differ significantly among all groups (P>0.05). Serum phosphorus and triglyceride concentrations were also similar across groups, except for D5, which showed significantly lower serum phosphorus and significantly higher triglyceride levels (P<0.05). At equivalent MCP supplementation levels, groups receiving neutral phytase demonstrated significantly higher phosphorus retention rates and apparent digestibility compared to their non-phytase counterparts (P<0.05), while phosphorus discharge showed the opposite trend.

These results indicate that dietary supplementation with 400 and 800 U/kg neutral phytase can reduce MCP inclusion by 0.5% and 1.0%, respectively, without compromising growth performance, body composition, or serum biochemical parameters in gibel carp. Partial replacement of MCP with neutral phytase in gibel carp diets enhances phosphorus utilization efficiency and reduces phosphorus discharge, thereby offering both economic and ecological benefits.

Keywords: gibel carp; neutral phytase; monocalcium phosphate; growth performance; body composition; phosphorus utilization

Introduction

Fish meal is a resource-limited ingredient, and its price has surged in recent years due to constrained catch quotas. Consequently, minimizing fish meal usage has become a primary strategy for feed manufacturers to reduce costs. A successful approach involves maximizing the utilization of plant protein sources to replace portions of fish meal, thereby increasing the proportion of plant-based ingredients in aquafeeds. Aquatic animals have substantial phosphorus requirements, which are typically met by adding considerable amounts of monocalcium phosphate to compound feeds. However, monocalcium phosphate is a mineral phosphorus source that is non-renewable.

Plant protein ingredients contain substantial amounts of phytic acid or phytates, which represent the primary form of phosphorus. Since aquatic animals lack endogenous phytase in their digestive tracts, they cannot utilize phytate-bound phosphorus [1-2]. Unabsorbed phytate phosphorus excreted into the environment can cause water eutrophication and aquatic pollution. Phytase catalyzes the hydrolysis of phytate phosphorus into inorganic phosphorus while simultaneously releasing nutrients bound to phytic acid, thereby enhancing the potential nutritional value of feeds [3]. Therefore, supplementing aquafeeds with exogenous phytase can reduce monocalcium phosphate usage, achieving both cost savings and environmental protection. Numerous studies have demonstrated that dietary phytase supplementation effectively improves phosphorus utiliza-

tion and reduces phosphorus pollution in aquatic animals [4-8], making it a promising trend for future aquafeed industries.

Gibel carp, developed through artificial gynogenesis using triploid Fangzheng crucian carp (Carassius auratus gibelio) as the maternal parent and diploid Xingguo red carp (Cyprinus carpio var. singuonensis) as the paternal parent, exhibits rapid growth, large size, and strong stress resistance. These traits demonstrate excellent economic performance in aquaculture production. The species also offers superior meat quality and nutritional value, making it the primary crucian carp variety in Chinese aquaculture and a popular product in domestic and international markets, including Korea, Japan, and Southeast Asia [9-10].

Previous research on phytase application in aquafeeds has primarily focused on acid phytase, which is suitable for gastric species with acidic stomach pH such as salmonids, perch, and groupers, but not for agastric freshwater cyprinids with neutral digestive tracts. Qiu et al. [11] investigated the effects of neutral phytase on gibel carp growth and its distribution of enzymatic activity in the intestine, but did not examine MCP replacement. This study utilized neutral phytase adapted to the intestinal environment of aquatic animals to evaluate its feasibility for replacing monocalcium phosphate in gibel carp diets, focusing on growth performance, body composition, feed nutrient utilization, particularly phosphorus utilization, to provide reference for the broader application of neutral phytase in aquaculture.

1.1 Experimental Diets

The experimental diet formulation was designed based on commercial crucian carp feed formulas. Seven experimental diets were prepared: D1 served as the positive control with 1.5% MCP and no neutral phytase; D2 was negative control 1 with 1.0% MCP and no phytase; D3 contained 1.0% MCP + 400 U/kg neutral phytase; D4 contained 1.0% MCP + 800 U/kg neutral phytase; D5 was negative control 2 with 0.5% MCP and no phytase; D6 contained 0.5% MCP + 400 U/kg neutral phytase; and D7 contained 0.5% MCP + 800 U/kg neutral phytase.

All feed ingredients were ground and passed through a 60-mesh sieve before being accurately weighed according to the formulation table (Table 1). Ingredients were mixed in a feed mixer for 30 minutes, after which soybean oil and water (40% of feed dry weight) were slowly added during continuous mixing. The mixture was then processed into 1.5 mm hard pellets using a hard pellet extruder (produced by Shanghai Fisheries Machinery Research Institute). The pellets were air-dried to 8-10% moisture content, sealed in plastic bags, and stored at -20°C until use. The neutral phytase (trade name Weitelin) was produced by Guangdong VTR Co., Ltd. through liquid fermentation of a naturally selected thermotolerant strain followed by spray drying, with an activity of 2,000 U/g at pH 6.5 and 25°C. Enzyme activity remained essentially unchanged after feed pelleting.

1.2 Experimental Fish and Feeding Trial

Gibel carp were purchased from a hatchery in Hunan Province and acclimated to experimental conditions in indoor aquaria for two weeks prior to the trial. At the start of the experiment, fish were fasted for 24 hours, then anesthetized with 74 mg/L MS-222 and individually weighed. Healthy fish with uniform body weight were stocked at 25 fish per aquarium (200 L capacity) with an initial average weight of (23.39±0.10) g. Each dietary treatment had three replicates (aquaria). Fish were fed the corresponding experimental diets during the trial.

A micro-flow-through system was used with water temperature maintained at (23±2)°C and pH around 7.5. Continuous aeration ensured dissolved oxygen concentration 5 mg/L. Feces were removed each morning by siphoning, and ammonia nitrogen concentration was maintained below 0.01 mg/L. Fish were hand-fed to satiation twice daily (08:30 and 16:30), with feed consumption recorded. The feeding trial ran from May 15 to July 8, 2015, lasting 8 weeks.

1.3 Sample Collection and Analysis

At the end of the trial, fish were fasted for 24 hours and anesthetized with MS-222 before individual weighing. Three fish were randomly selected from each aquarium (9 fish per group), weighed, cooked, dried, and ground into powder for whole-body proximate composition analysis. The powdered samples were stored at -20°C. Another three fish per aquarium were randomly selected for blood collection (0.5 mL per fish) from the caudal vein using 1 mL disposable syringes. Blood samples were placed on ice for over 4 hours, then centrifuged at 4,000 r/min for 10 minutes at 4°C. The collected serum was stored at -20°C for biochemical analysis.

Moisture, crude protein, crude lipid, ash, and total phosphorus contents in feed and whole-body samples were determined according to GB 6435-1986, GB 6432-1994, GB 6433-1994, GB/T 6438-1992, and GB/T 6437-2002, respectively. Phytate phosphorus content was measured using the method described by Carlsson et al. [12]. Serum phosphorus, triglyceride, cholesterol concentrations, and alkaline phosphatase activity were determined using assay kits from Nanjing Jiancheng Bioengineering Institute.

During the final week of the trial, 1% chromium(III) oxide (Cr O) was added to the experimental diets to estimate apparent phosphorus digestibility. Feces were collected after feeding, and Cr O content in feed and feces was determined by atomic absorption spectrophotometry [13].

1.4 Calculation Methods

The following formulas were used for calculations:

- Weight gain rate (%) = 100 × [final average weight (g) - initial average weight (g)] / initial average weight (g)

- Specific growth rate (%/d) = 100 × [ln(final average weight (g)) - ln(initial average weight (g))] / feeding days (d)
- Feed intake (g/fish) = total feed consumption (g) / number of fish
- Feed conversion ratio = total feed consumption (dry weight, g) / weight gain (wet weight, g)
- Protein efficiency (%) = 100 × weight gain (g) / protein intake (g)
- Survival rate (%) = 100 × final number of fish / initial number of fish
- Hepato-somatic index (%) = 100 × liver weight (g) / body weight (g)
- Viscero-somatic index (%) = 100 × viscera weight (g) / body weight (g)
- Phosphorus intake (g) = feed intake (g) × dietary total phosphorus content (%)
- Phosphorus retention (g) = final body phosphorus content (g) - initial body phosphorus content (g)
- Phosphorus retention rate (%) = 100 × phosphorus retention (g) / phosphorus intake (g)
- Phosphorus discharge (g/kg weight gain) = [phosphorus intake (g) - phosphorus retention (g)] / weight gain (kg)
- Apparent phosphorus digestibility (%) = 100 × {1 - [fecal total phosphorus content (%) / feed total phosphorus content (%)] × [feed Cr O content (%) / fecal Cr O content (%)]}
- Available phosphorus content (%) = apparent phosphorus digestibility (%) × dietary total phosphorus content (%)

1.5 Statistical Analysis

Raw data were processed using Microsoft Excel 2000, followed by one-way ANOVA and Tukey' s multiple comparison tests using Origin 9.0 software. Differences were considered significant at $P<0.05$. Results are presented as mean ± standard error (mean±SE, n=3).

Results

2.1 Growth Performance

As shown in Table 2 , after 8 weeks of feeding, the D5 group exhibited the poorest growth performance. Weight gain rate, specific growth rate, and feed intake were significantly lower than all other groups ($P<0.05$), while feed conversion ratio was significantly higher ($P<0.05$). Protein efficiency and viscero-somatic index in D5 were significantly lower and higher, respectively, compared to D1, D3, and D4 ($P<0.05$), and this group also showed the lowest survival rate.

The D2 and D6 groups displayed significantly lower weight gain and specific growth rates, and significantly higher feed conversion ratios compared to D1 ($P<0.05$). In contrast, D3, D4, and D7 showed no significant differences in any growth performance indices compared to D1 ($P>0.05$). No significant differences in hepato-somatic index were observed among all groups ($P>0.05$). These results indicate that reducing dietary MCP levels impairs fish growth performance,

while supplementation with 400 and 800 U/kg neutral phytase can compensate for 0.5% and 1.0% MCP reduction, respectively, without negatively affecting growth.

2.2 Body Composition

Table 3 presents the whole-body composition of gibel carp after 8 weeks. No significant differences were observed among groups in moisture, crude protein, ash, or phosphorus contents ($P>0.05$). However, crude lipid content in D5 was significantly higher than in D1, D3, D4, and D7 ($P<0.05$), though not significantly different from D2 and D6 ($P>0.05$). These findings suggest that phosphorus deficiency induces lipid accumulation, while replacing 0.5% and 1.0% MCP with 400 and 800 U/kg neutral phytase, respectively, does not affect body composition.

2.3 Serum Biochemical Parameters

Serum biochemical parameters are summarized in Table 4 . Serum cholesterol and alkaline phosphatase activity showed no significant differences among groups ($P>0.05$). Serum phosphorus and triglyceride concentrations were also similar across groups, except for D5, which exhibited significantly lower phosphorus and significantly higher triglyceride levels compared to all other groups ($P<0.05$). These results demonstrate that reducing MCP levels decreases serum phosphorus while increasing triglycerides, but these effects are mitigated when neutral phytase is added at 400 or 800 U/kg to replace 0.5% or 1.0% MCP, respectively.

2.4 Phosphorus Utilization in Experimental Diets

Phosphorus utilization data are presented in Table 5 . Phosphorus retention rate showed no significant differences among D1, D2, and D5 ($P>0.05$), nor between D3, D6, and D1 ($P>0.05$). However, D2 and D5 were significantly lower than D3, D4, D6, and D7 ($P<0.05$), while D4 and D7 were significantly higher than D1 ($P<0.05$). Phosphorus discharge was significantly higher in D2 and D5 compared to all other groups ($P<0.05$), and significantly lower in D4 and D7 ($P<0.05$). Apparent phosphorus digestibility was lowest in D5 and second lowest in D2, both significantly lower than other groups ($P<0.05$), while D4 and D7 were significantly higher than all others ($P<0.05$). No significant differences were observed among D3, D6, and D1 ($P>0.05$).

These results indicate that 400 and 800 U/kg neutral phytase provide available phosphorus equivalent to 0.5% and 1.0% MCP, respectively. Partial replacement of MCP with neutral phytase improves phosphorus utilization efficiency and reduces phosphorus discharge.

Discussion

3.1 Effects of Partial MCP Replacement on Growth Performance

Phosphorus is an essential mineral nutrient for fish growth, playing crucial roles in skeletal systems, energy metabolism, and cellular processes [1]. Due to high phosphorus requirements and low utilization of plant-derived phosphorus, aquafeeds typically contain substantial MCP supplementation [14]. Phytase catalyzes phytate phosphorus hydrolysis into inorganic phosphorus, thereby increasing available phosphorus content and allowing reduced MCP inclusion [15].

Yu et al. [16] reported that pretreating soybean meal with 500 and 1,000 U/kg phytase to replace 1.6% MCP (with a control MCP level of 2.5%) did not significantly affect growth performance or feed utilization in gibel carp. In the current study, reducing MCP by 0.5% and 1.0% significantly decreased weight gain and specific growth rates, indicating that MCP reduction led to insufficient available phosphorus, impairing normal growth. The significantly increased viscero-somatic index in the 1.0% MCP reduction group reflects visceral fat accumulation, a known symptom of phosphorus deficiency in cyprinids [17].

However, simultaneous supplementation with 400 and 800 U/kg neutral phytase at 0.5% and 1.0% MCP reduction levels, respectively, did not significantly affect weight gain, specific growth rate, protein efficiency, or feed conversion ratio. This suggests that phytase-released available phosphorus compensated for the reduction in MCP-derived phosphorus. Nevertheless, 400 U/kg neutral phytase replacing 1.0% MCP resulted in significantly lower weight gain and specific growth rate compared to the positive control, indicating insufficient phosphorus liberation to fully replace 1.0% MCP. This aligns with calculated available phosphorus contents based on apparent digestibility. Therefore, 400 and 800 U/kg neutral phytase can replace 0.5% and 1.0% MCP, respectively. Further research is needed to determine whether higher phytase doses could completely replace MCP in gibel carp diets.

3.2 Effects of Partial MCP Replacement on Body Composition

No significant differences were observed among groups in whole-body moisture, crude protein, ash, or phosphorus contents. Previous studies have similarly reported that dietary phosphorus levels and phytase supplementation do not affect these parameters [18-19]. However, other research has shown that low available phosphorus significantly reduces ash and phosphorus contents in Atlantic salmon (Salmo salar) juveniles [20] and ash content in grouper (Epinephelus coioides) juveniles [21]. These discrepancies may relate to species differences, fish size, culture environment, or trial duration.

Increased crude lipid content with reduced MCP supplementation likely results from suppressed fatty acid -oxidation under phosphorus deficiency conditions [22]. The absence of significant effects on crude lipid content when 400 or 800 U/kg neutral phytase was added alongside MCP reduction confirms that

phytase-liberated phosphorus compensates for the reduced MCP phosphorus.

3.3 Effects of Partial MCP Replacement on Serum Biochemical Parameters

Phosphorus deficiency in aquatic animals typically causes anorexia, growth retardation, reduced feed efficiency, increased visceral fat deposition, and altered blood biochemical parameters including phosphorus, triglycerides, cholesterol, and alkaline phosphatase [23]. Studies have reported significantly lower serum phosphorus in fish fed phosphorus-deficient diets [24-25]. In this study, serum phosphorus decreased with MCP reduction, with the 1.0% reduction group significantly lower than the positive control. However, simultaneous neutral phytase supplementation prevented these reductions, confirming that dietary phosphorus deficiency lowers serum phosphorus.

No significant differences in alkaline phosphatase activity were observed among groups, consistent with Sugiura et al. [26] who reported no relationship between plasma alkaline phosphatase activity and dietary phosphorus in rainbow trout (Oncorhynchus mykiss), but contrasting with Zheng et al. [23] who found significantly higher activity in phosphorus-deficient Nile tilapia (Oreochromis niloticus × O. aureus).

3.4 Effects of Partial MCP Replacement on Phosphorus Utilization

Studies have demonstrated that phytase supplementation in plant-based diets improves phosphorus utilization in fish [18,27]. The current study confirms that neutral phytase increased apparent phosphorus digestibility in gibel carp. In fishmeal-free or low-fishmeal diets, phytate phosphorus constitutes a large proportion of total phosphorus. Since fish lack endogenous phytase, they cannot utilize phytate phosphorus, but exogenous phytase catalyzes its hydrolysis to release inorganic phosphorus [15], thereby improving utilization efficiency.

In this study, replacing 0.5% and 1.0% MCP with 400 and 800 U/kg neutral phytase increased phosphorus retention rates by 3% and 10%, and apparent digestibility by 8% and 17%, respectively, with higher phytase doses yielding greater digestibility. Xu et al. [28] reported a 7% improvement in apparent phosphorus digestibility in black porgy (Acanthopagrus schlegelii) when replacing 1.0% MCP with 400 U/kg neutral phytase. Yang et al. [29] found that 1,500 and 3,000 U/kg phytase improved apparent phosphorus digestibility in common carp (Cyprinus carpio) by 9% and 11%, respectively. These differences may stem from variations in diet formulation and fish species. Yu et al. [15] reported a 6% increase in phosphorus retention rate when using phytase-pretreated soybean meal to replace 1.6% MCP in gibel carp diets.

As phosphorus represents a major pollutant in aquaculture, reducing its discharge is critical for minimizing environmental impact. Phytase replacement of MCP not only reduces total dietary phosphorus but also improves utilization efficiency, substantially decreasing phosphorus output. This study found that

replacing 0.5% and 1.0% MCP with 400 and 800 U/kg neutral phytase reduced phosphorus discharge by 13% and 33%, respectively, consistent with previous findings [30-32].

Conclusions

1. Dietary supplementation with 400 and 800 U/kg neutral phytase can reduce monocalcium phosphate inclusion by 0.5% and 1.0%, respectively, without adversely affecting growth performance, body composition, or serum biochemical parameters in gibel carp.
2. Partial replacement of monocalcium phosphate with neutral phytase in gibel carp diets maintains growth performance while improving phosphorus utilization efficiency and reducing phosphorus discharge, thereby providing both economic and ecological benefits.

References

[1] NRC. Nutrient requirements of fish[S]. Washington, D.C.: National Academy Press, 1993.
[2] JACKSON L S, LI M H, ROBINSON E H. Use of microbial phytase in channel catfish Ictalurus punctatus diets to improve utilization of phytate phosphorus[J]. Journal of the World Aquaculture Society, 1996, 27(3): 309-313.
[3] KUMAR V, SINHA A K, MAKKAR H P S, et al. Phytate and phytase in fish nutrition[J]. Animal Physiology and Animal Nutrition, 2012, 96(3): 335-364.
[4] LIEBERT F, PORTZ L. Nutrient utilization of Nile tilapia Oreochromis niloticus fed plant based low phosphorus diets supplemented with graded levels of different sources of microbial phytase[J]. Aquaculture, 2005, 248(1/2/3/4): 111-119.
[5] LI M H, ROBINSON E H. Microbial phytase can replace inorganic phosphorus supplements in channel catfish Ictalurus punctatus diets[J]. Journal of the World Aquaculture Society, 1997, 28(4): 402-406.
[6] ROBINSON E H, LI M H, MANNING B B. Comparison of microbial phytase and dicalcium phosphate for growth and bone mineralization of pond-raised channel catfish, Ictalurus punctatus[J]. Journal of Applied Aquaculture, 2002, 12(3): 81-88.
[7] PORTZ L, LIEBERT F. Growth, nutrient utilization and parameters of mineral metabolism in Nile tilapia Oreochromis niloticus (Linneaus, 1758) fed plant-based diets with graded levels of microbial phytase[J]. Journal of Animal Physiology and Animal Nutrition, 2004, 88(9/10): 311-320.
[8] PHROMKUNTHONG W, GABAUDAN J. Used of microbial phytase to replace inorganic phosphorus in sex-reversed red tilapia: 1 dose response[J]. Songklanakarin Journal of Science and Technology, 2006, 28(4): 731-743.
[9] XUE M, CUI Y B. Effect of several feeding stimulants on diet preference

by juvenile gibel carp (Carassius auratus gibelio), fed diets with or without partial replacement of fish meal by meat and bone meal[J]. Aquaculture, 2001, 198(3/4): 281-292.
[10] ZOU Z, CUI Y, GUI J, et al. Growth and feed utilization in two strains of gibel carp, Carassius auratus gibelio: paternal effects in a gynogenetic fish[J]. Journal of Applied Ichthyology, 2001, 17(2): 54-58.
[11] QIU M, WANG A M, LÜ L L, et al. Effects of neutral phytase on growth of gibel carp fingerlings and distribution of enzyme activity in the intestine[J]. Cereal & Feed Industry, 2010(2): 38-41.
[12] CARLSSON N G, BERGMAN E L, SKOGLUND E, et al. Rapid analysis of inositol phosphates[J]. Journal of Agricultural and Food Chemistry, 2001, 49(4): 1695-1701.
[13] AOAC. Official methods of analysis of AOAC international[S]. 16th ed. Arlington, VA: AOAC, 1995.
[14] LAINING A, ISHIKAWA M, KOSHIO S, et al. Dietary inorganic phosphorus or microbial phytase supplementation improves growth, nutrient utilization and phosphorus mineralization of juvenile red sea bream, Pagrus major, fed soybean-based diets[J]. Aquaculture Nutrition, 2012, 18(5): 502-511.
[15] CAO L, WANG W M, YANG C T, et al. Application of microbial phytase in fish feed[J]. Enzyme and Microbial Technology, 2007, 40(4): 497-507.
[16] YU F N, WANG D Z. Effects of phytase on growth and phosphorus utilization in gibel carp[J]. Journal of Fishery Sciences of China, 2000, 7(2): 106-109.
[17] HALVER J E, HARDY R W. Fish nutrition[M]. 3rd ed. Amsterdam: Academic Press, 2002.
[18] YOO G Y, WANG X J, CHOI S, et al. Dietary microbial phytase increased the phosphorus digestibility in juvenile Korean rockfish Sebastes schlegeli fed diets containing soybean meal[J]. Aquaculture, 2005, 243(1/2/3/4): 315-332.
[19] LIU L W, SU J M, LUO Y L. Effect of partial replacement of dietary monocalcium phosphate with neutral phytase on growth performance and phosphorus digestibility in gibel carp, Carassius auratus gibelio (Bloch)[J]. Aquaculture Research, 2012, 43(9): 1404-1413.
[20] BAEVERFJORD G, ASGARD T, SHEARER K D. 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] YE C X, LIU Y J, TIAN L X, et al. Effect of dietary calcium and phosphorus on growth, feed efficiency, mineral content and body composition of juvenile grouper, Epinephelus coioides[J]. Aquaculture, 2006, 255(1/2/3/4): 263-271.
[22] SKONBERG D I, YOGEV L, HARDY R W, et al. Metabolic response to dietary phosphorus intake in rainbow trout (Oncorhynchus mykiss)[J]. Aquaculture, 1997, 157(1/2): 11-24.
[23] WANG X Y, HE Z P, LIU Y F. Application of phytase in aquaculture (review)[J]. Journal of Hebei Normal University of Science & Technology, 2008, 22(2): 71-75.
[24] ZHENG T, PAN Q, LI G F, et al. Effects of dietary phosphorus and

phytase supplementation on growth performance and body composition of GIFT tilapia fed fishmeal-free diets[J]. Journal of Fishery Sciences of China, 2006, 13(1): 112-118.
[25] LI M, WANG G Q. Effects of phytase on calcium and phosphorus metabolism in golden crucian carp[J]. China Feed, 2015(8): 34-38.
[26] SUGIURA S H, RABOY V, YOUNG K A, et al. Availability of phosphorus and trace elements in low-phytate varieties of barley and corn for rainbow trout (Oncorhynchus mykiss)[J]. Aquaculture, 1999, 170(3/4): 285-296.
[27] SAJJADI M, CARTER C G. Effect of phytic acid and phytase on feed intake, growth, digestibility and trypsin activity in Atlantic salmon (Salmo salar, L.)[J]. Aquaculture Nutrition, 2004, 10(2): 135-142.
[28] XU S D, WANG S Q, YOU C H, et al. Effects of replacing monocalcium phosphate with neutral phytase on growth and phosphorus utilization in black porgy[J]. Journal of Fishery Sciences of China, 2014, 21(3): 522-530.
[29] YANG Y H, GUO Q, ZU L C, et al. Effects of phytase on growth and phosphorus utilization in common carp[J]. Freshwater Fisheries, 2006, 36(5): 20-23.
[30] LANARI D, D' AGARO E, TURRI C. Use of nonlinear regression to evaluate the effects of phytase enzyme treatment of plant protein diets for rainbow trout (Oncorhynchus mykiss)[J]. Aquaculture, 1998, 161(1/2/3/4): 345-356.
[31] FORSTER I, HIGGS D A, DOSANJH B S, et al. Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout (Oncorhynchus mykiss) held in 11°C fresh water[J]. Aquaculture, 1999, 179(1/2/3/4): 109-125.
[32] VIELMA J, RUOHONEN K, PEISKER M. Dephytinization of two soy proteins increases phosphorus and protein utilization by rainbow trout, Oncorhynchus mykiss[J]. Aquaculture, 2002, 204(1/2): 145-156.

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