

Effects of Vitamin D Supplementation Level on Growth Performance, Body Composition, and Serum Biochemical Indices of GIFT Tilapia in Mid-growing Stage (Postprint)

Authors: Yu Lijuan, Wenhua, Zhou Shuhua, Tian Juan, Jiang Ming, Wu Fan, Liu Wei, Yang Changgeng, Lu Xing

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Abstract

A total of 270 GIFT tilapia with initial body weight of (78.58 ± 1.93) g were randomly divided into 6 groups (3 replicates per group, 15 fish per replicate) and fed six diets with vitamin D levels of 0 (control group), 200, 400, 800, 1,600, and 3,200 IU/kg (crude protein and crude lipid contents were 30% and 7.6%, respectively). After 12 weeks of feeding, growth performance, body composition, and serum biochemical indices were measured to determine the optimal dietary vitamin D supplementation level for mid-growing GIFT tilapia. The results showed that the weight gain rate of GIFT tilapia in all vitamin D supplementation groups was significantly higher than that of the control group ($P < 0.05$), and the feed efficiency of the 200, 400, 800, and 1,600 IU/kg groups was significantly higher than that of the control group ($P < 0.05$). No significant differences were observed in whole-body moisture and crude protein contents among all groups ($P > 0.05$). With increasing dietary vitamin D supplementation levels, whole-body crude lipid content first increased and then decreased, reaching the maximum in the 200 IU/kg group, which was significantly higher than the other five groups ($P < 0.05$). Whole-body crude ash content in all vitamin D supplementation groups was significantly higher than that of the control group ($P < 0.05$), but no significant differences were found among the vitamin D supplementation groups ($P > 0.05$). With increasing dietary vitamin D supplementation levels, serum alkaline phosphatase activity first increased and then decreased, with the 200, 400, and 800 IU/kg groups being significantly higher than the control group ($P < 0.05$). Serum total cholesterol, total protein, and albumin contents all reached their maximum values in the 200 IU/kg group and were significantly higher than those of the control group ($P < 0.05$). It was concluded that appropriate dietary vitamin D supplementation could promote

the growth of mid-growing GIFT tilapia, while excessive vitamin D supplementation might cause liver damage and affect lipid metabolism. Using weight gain rate as the evaluation index, regression analysis determined that the optimal dietary vitamin D supplementation level for mid-growing GIFT tilapia was 259.8 IU/kg.

Full Text

Effects of Dietary Vitamin D Supplementation Level on Growth Performance, Body Composition, and Serum Biochemical Indices of Genetically Improved Farmed Tilapia (GIFT, *Oreochromis niloticus*) in the Mid-Growth Stage

YU Lijuan, WEN Hua*, ZHOU Suhua, TIAN Juan, JIANG Ming, WU Fan, LIU Wei, YANG Changgeng, LU Xing

Key Laboratory of Freshwater Biodiversity Conservation, Ministry of Agriculture, Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Wuhan 430223, China

Abstract

A total of 270 genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*) with an average initial body weight of (78.58 ± 1.93) g were randomly distributed into six groups with three replicates each (15 fish per replicate). The fish were fed six experimental diets containing vitamin D at levels of 0 (control), 200, 400, 800, 1,600, or 3,200 IU/kg (with crude protein and crude lipid contents of 30% and 7.6%, respectively) for 12 weeks. Growth performance, body composition, and serum biochemical indices were measured to determine the optimal dietary vitamin D supplementation level for GIFT in the mid-growth stage. The results showed that the weight gain rate of fish in all vitamin D-supplemented groups was significantly higher than that of the control group ($P < 0.05$), and feed efficiency in the 200, 400, 800, and 1,600 IU/kg groups was significantly higher than that of the control group ($P < 0.05$). No significant differences were observed in whole-body moisture or crude protein content among groups ($P > 0.05$). Whole-body crude lipid content increased initially and then decreased with increasing dietary vitamin D levels, reaching its maximum in the 200 IU/kg group, which was significantly higher than the other five groups ($P < 0.05$). Whole-body ash content in all vitamin D-supplemented groups was significantly higher than that of the control group ($P < 0.05$), though no significant differences existed among the supplemented groups ($P > 0.05$). Serum alkaline phosphatase activity increased initially and then decreased with rising dietary vitamin D levels, with the 200, 400, and 800 IU/kg groups showing significantly higher values than the control group ($P < 0.05$). Serum total cholesterol, total protein, and albumin contents all reached their maximum values in the 200 IU/kg group, which were significantly higher than those of the control group ($P < 0.05$). These find-

ings indicate that appropriate dietary vitamin D supplementation promotes the growth of mid-stage GIFT, while excessive vitamin D may cause liver damage and affect lipid metabolism. Based on regression analysis using weight gain rate as the evaluation index, the optimal dietary vitamin D supplementation level for GIFT in the mid-growth stage was determined to be 259.8 IU/kg.

Keywords: Genetically improved farmed tilapia (GIFT); vitamin D; requirement; growth; serum biochemical indices

Introduction

Vitamin D is a steroid derivative, with vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol) being its most important members. As a fat-soluble vitamin, vitamin D plays a crucial role in material metabolism and is essential for maintaining normal life activities in aquatic animals, though it cannot be synthesized in animal bodies. Its primary physiological functions involve regulating calcium and phosphorus metabolism and promoting bone growth and calcification. Vitamin D deficiency in aquatic animals manifests in various deficiency symptoms. For instance, rainbow trout fed vitamin D-deficient diets exhibit reduced growth, increased liver lipids, impaired calcium balance, muscle spasms, and altered ultrastructure of axial white muscle [1]. Vitamin D-deficient rainbow trout also show symptoms such as vertebral lordosis and muscle tissue weakness [2]. Channel catfish (*Ictalurus punctatus*) fed vitamin D-deficient diets for 16 weeks display growth retardation and reduced whole-body calcium, phosphorus, and ash contents [3]. Hybrid tilapia [4] deficient in vitamin D show growth inhibition, decreased feed efficiency, and reduced hepatosomatic index, along with declining plasma alkaline phosphatase activity and hemoglobin content. In *Haliotis discus hannai*, vitamin D deficiency affects the foot muscle fiber structure [5].

Current research on vitamin D requirements in fish feeds includes studies showing that rainbow trout require 1,600-2,400 IU/kg [2], chinook salmon require 2,400 IU/kg [6], hybrid tilapia require 374.8 IU/kg [4], juvenile sea bass require 431.0 IU/kg [7], and channel catfish require 250-500 IU/kg [8]. These findings demonstrate significant variation in vitamin D requirements among different aquaculture species.

Genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*) is a genetically improved strain of Nile tilapia characterized by rapid growth, high fillet yield, low disease incidence, and stable genetic traits. It has been widely cultured in southern China, particularly in Guangdong, Guangxi, and Hainan provinces [9]. Current research on vitamin D requirements has primarily focused on hybrid tilapia weighing approximately 1 g [4], while no studies have reported the vitamin D requirement for GIFT in the mid-growth stage. Since vitamin D requirements vary among fish species and across different growth stages within the same species, this study investigated the effects of graded dietary vitamin D

levels on growth performance, body composition, and serum biochemical indices of mid-stage GIFT [initial weight (78.58 ± 1.93) g] to determine the optimal supplementation level, enrich nutritional requirement data for tilapia at different growth stages, and provide a theoretical basis for vitamin D supplementation in aquafeeds.

1.1 Experimental Diets

A purified basal diet was formulated using casein and gelatin as protein sources, dextrin as carbohydrate source, and corn oil and soybean oil as lipid sources. The composition and nutrient levels of the basal diet are shown in Table 1. Six experimental diets were prepared by supplementing the basal diet with vitamin D at different levels (0, 200, 400, 800, 1,600, or 3,200 IU/kg). The vitamin D source was a vitamin D micro-particle premix containing 476,000 IU/g. All ingredients were ground to pass through a 0.3 mm mesh screen, thoroughly mixed, and then moistened with appropriate water before being processed into 2.0 mm diameter strips using a meat grinder (Model 62, Fu'an Yongte Motor Co., Ltd.). The strips were dried in a ventilated area using electric fans, then crushed into cylindrical pellets approximately 4.0 mm in length using a grinder (Model 280, Zhengzhou Huicheng Machinery Equipment Co., Ltd.) and stored at -20 °C until use.

1.2 Experimental Fish and Culture Management

Experimental GIFT were obtained from the Guangxi National Tilapia Breeding Farm. Upon arrival at the Yangtze River Fisheries Research Institute, fish were disinfected with povidone iodine and acclimated in 500 L indoor recirculating aquaculture tanks. They were fed the basal diet for two weeks to adapt to the experimental environment and deplete stored vitamin D reserves. Prior to the formal experiment, fish were fasted for 24 hours. A total of 270 healthy, uniformly sized GIFT with an initial average weight of (78.58 ± 1.93) g were selected and randomly stocked into 18 tanks (15 fish per tank). The 18 tanks were randomly assigned to six groups (three replicates per group), each receiving one of the six experimental diets. Fish were hand-fed to apparent satiation three times daily (08:00, 12:00, and 16:00). One-quarter of the water volume was exchanged each morning. Fish were weighed every two weeks to adjust feeding rates. The experiment lasted for 12 weeks. Water temperature, feeding behavior, and mortality were recorded daily. During the culture period, water temperature ranged from 27 to 33 °C, pH was 7.2–7.5, dissolved oxygen concentration was above 6.0 mg/L, and ammonia nitrogen concentration was below 0.03 mg/L.

1.3 Sample Collection

After 12 weeks of culture, fish were fasted for 24 hours. The total weight and number of fish in each tank were recorded to calculate final average weight, weight gain rate (WGR), and survival rate (SR). Total feed consumption per

replicate was recorded to calculate feed efficiency (FE). Three fish were randomly selected from each tank for determination of whole-body crude protein, crude lipid, moisture, and ash contents. Another three fish from each tank were anesthetized with MS-222, and their body length and weight were measured to calculate condition factor (CF). Blood was collected from the caudal vein, allowed to clot at 4 °C for 2 hours, then centrifuged at 3,000 r/min for 10 minutes to obtain serum for biochemical analysis. Fish were then dissected to separate viscera and liver, which were weighed to calculate hepatosomatic index (HSI) and viscerosomatic index (VSI). Liver samples were preserved for subsequent analysis. Both serum and liver samples were stored at -80 °C.

1.4.1 Proximate Composition Analysis

Moisture content in diets and whole fish was determined by oven drying at 105 °C to constant weight (GB/T 5009.3–2016). Crude protein content was measured by the Kjeldahl method (GB/T 5009.5–2016). Crude lipid content was determined by Soxhlet extraction (GB/T 5009.6–2016). Ash content was measured by incineration in a muffle furnace (GB/T 5009.4–2016).

1.4.2 Growth Performance Indices

Growth performance parameters were calculated using the following formulas:

- Weight gain rate (%) = $100 \times (\text{final average weight} - \text{initial average weight}) / \text{initial average weight}$
- Feed efficiency = $(\text{final total weight} - \text{initial total weight}) / \text{total feed consumption}$
- Survival rate (%) = $100 \times \text{final fish number} / \text{initial fish number}$
- Hepatosomatic index (%) = $100 \times \text{liver weight} / \text{body weight}$
- Viscerosomatic index (%) = $100 \times \text{viscera weight} / \text{body weight}$
- Condition factor (g/cm^3) = $100 \times \text{body weight} / \text{body length}^3$

1.4.3 Serum Biochemical Indices

Serum alkaline phosphatase (ALP) activity and total protein (TP), total cholesterol (T-CHO), and albumin (ALB) concentrations were measured using a Sysmex automatic biochemical analyzer (CHEMIX-800) with commercial reagents purchased from Sysmex Corporation.

1.5 Data Processing

Experimental data were analyzed using one-way ANOVA in SPSS 18.0 statistical software. Duncan's multiple range test was used for post-hoc comparisons when significant differences were detected. Results are expressed as "mean \pm standard deviation." Differences were considered significant at $P < 0.05$.

2.1 Effects of Vitamin D Supplementation Level on Growth Performance of Mid-Stage GIFT

The effects of dietary vitamin D level on growth performance are presented in Table 2 . After 12 weeks of indoor culture, survival rate was 100% in all groups. Dietary vitamin D supplementation promoted growth in GIFT, with weight gain rates in all supplemented groups significantly higher than the control group ($P < 0.05$). The 400 IU/kg group showed significantly higher weight gain than the 1,600 and 3,200 IU/kg groups ($P < 0.05$), but did not differ significantly from the 200 and 800 IU/kg groups ($P > 0.05$). Feed efficiency increased initially and then decreased with rising dietary vitamin D levels, peaking in the 400 IU/kg group. The 200, 400, 800, and 1,600 IU/kg groups exhibited significantly higher feed efficiency than the control group ($P < 0.05$). Hepatosomatic index, viscerosomatic index, and condition factor showed no significant changes across dietary vitamin D levels ($P > 0.05$).

Broken-line regression analysis of weight gain rate (y) against dietary vitamin D level (x) yielded the equation: $y = 0.153x + 237.4$, $r^2 = 0.808$ (Figure 1 [Figure 1: see original paper]). Based on this regression equation, the dietary vitamin D supplementation level for maximum growth in GIFT was determined to be 259.8 IU/kg.

2.2 Effects of Vitamin D Supplementation Level on Body Composition of Mid-Stage GIFT

The effects of dietary vitamin D level on body composition are shown in Table 3 . Whole-body crude lipid content increased initially and then decreased with rising dietary vitamin D levels, reaching its maximum in the 200 IU/kg group, which was significantly higher than the other five groups ($P < 0.05$). No significant differences were observed in whole-body moisture or crude protein content among groups ($P > 0.05$). Whole-body ash content in all vitamin D-supplemented groups was significantly higher than that of the control group ($P < 0.05$), though no significant differences existed among the supplemented groups ($P > 0.05$).

2.3 Effects of Vitamin D Supplementation Level on Serum Biochemical Indices of Mid-Stage GIFT

The effects of dietary vitamin D level on serum biochemical indices are presented in Table 4 . Dietary vitamin D supplementation increased serum alkaline phosphatase activity, with the 200, 400, and 800 IU/kg groups showing significantly higher values than the control group ($P < 0.05$). Serum total cholesterol, total protein, and albumin contents exhibited fluctuating changes with increasing dietary vitamin D levels, reaching maximum values in the 200 IU/kg group, which were significantly higher than those of the control group ($P < 0.05$).

3.1 Effects of Vitamin D Supplementation Level on Growth Performance

The effects of vitamin D on fish growth vary depending on species, age, culture period, and environmental conditions. Lovell et al. [3] reported that channel catfish fed vitamin D-deficient diets for 16 weeks showed significantly reduced weight gain. Shiau et al. [4] observed that vitamin D-deficient hybrid tilapia exhibited poor growth and reduced feed efficiency. O'Connell et al. [11] found that dietary vitamin D levels significantly affected blue tilapia growth during the first 12 weeks but not during the subsequent 12 weeks. He et al. [12] demonstrated that vitamin D-deficient Pacific white shrimp exhibited reduced appetite, poor growth, and incomplete shell mineralization. In the present study, dietary vitamin D supplementation significantly improved weight gain and specific growth rate in GIFT, while the control group did not show obvious deficiency symptoms. This may be attributed to the relatively large size of the experimental fish, which had accumulated substantial vitamin D reserves in tissues that were insufficient for maximum growth but adequate to prevent deficiency symptoms. Additionally, the 12-week culture period may have been insufficient for deficiency effects on growth performance to become apparent. Furthermore, high dietary vitamin D levels did not inhibit GIFT growth, possibly due to the larger fish size and higher tolerance to elevated vitamin D levels. Similar findings were reported in juvenile Atlantic salmon [13] and channel catfish [8], where high dietary vitamin D levels did not suppress growth, although few studies have reported growth inhibition from excessive vitamin D [14].

In this study, dietary vitamin D supplementation did not significantly affect survival rate in GIFT, consistent with results from studies on Chinese shrimp [15] and juvenile sea bass [7]. However, Shiau et al. [16] reported significantly lower mortality in vitamin D-supplemented hybrid tilapia compared to controls, a discrepancy likely resulting from differences in species, age, culture period, and environmental conditions.

3.2 Effects of Vitamin D Supplementation Level on Body Composition

Vitamin D deficiency has been shown to increase body lipid content and decrease skeletal ash content in rainbow trout [1] and abalone [5]. In this study, the control group without vitamin D supplementation exhibited significantly lower whole-body ash content than all supplemented groups, while no significant differences were observed among supplemented groups, indicating that dietary vitamin D promotes mineral deposition. However, excessively high vitamin D levels tended to reduce whole-body ash content, suggesting that vitamin D overdose may inhibit osteoblast formation and reduce calcium and phosphorus absorption. Studies on livestock have shown that dietary vitamin D levels 4-10 times the normal requirement can cause toxicity when fed long-term [17]. In this study, the highest vitamin D supplementation group still maintained relatively high ash content, possibly due to the larger fish size, stronger tolerance, and relatively short feeding duration.

Whole-body crude lipid content was significantly affected by dietary vitamin D level, showing an initial increase followed by a decrease. This indicates that vitamin D supplementation influences lipid metabolism in GIFT, though the specific metabolic pathways require further investigation. Zhang et al. [7] found that whole-body crude lipid content in juvenile sea bass increased with dietary vitamin D levels, with the highest liver lipid content corresponding to the lowest whole-body lipid content. They speculated that excessive vitamin D accumulation in the liver may impair phospholipid or apolipoprotein synthesis, preventing triglyceride transport from the liver and causing hepatic lipid accumulation and pathological changes. Consequently, when liver lipid content becomes abnormally elevated, whole-body lipid content decreases significantly. This suggests that the reduced whole-body crude lipid content observed at high vitamin D supplementation levels in GIFT may result from liver damage. Meanwhile, the lack of significant effects on whole-body moisture and crude protein content indicates that these parameters are insensitive indicators of vitamin D status.

3.3 Effects of Vitamin D Supplementation Level on Serum Biochemical Indices

Alkaline phosphatase is an important metabolic regulatory enzyme closely associated with phosphate group transfer and calcium-phosphorus metabolism. Present in various tissues including liver, intestine, and bone, alkaline phosphatase serves as a key indicator of bone metabolism. When bone cell metabolism is active, alkaline phosphatase secretion increases and readily enters the bloodstream, elevating serum alkaline phosphatase activity [7]. In this study, dietary vitamin D supplementation significantly increased serum alkaline phosphatase activity, though excessively high supplementation levels tended to decrease it. Similar findings have been reported in abalone [5], grass carp [18-19], and tiger shrimp [16], possibly because vitamin D exerts bidirectional effects on bone cell metabolism. At appropriate dietary levels, vitamin D promotes bone cell metabolism, with some bone-derived alkaline phosphatase entering the bloodstream and increasing serum activity. However, at excessive levels, vitamin D becomes toxic and inhibitory. Therefore, the decreased serum alkaline phosphatase activity observed at high supplementation levels in this study may result from suppressed bone cell metabolism due to vitamin D toxicity.

Serum total protein and albumin contents reflect protein absorption and metabolism status, with albumin and globulin together constituting total protein. Serum albumin maintains plasma colloidal osmotic pressure and participates in tissue repair; liver damage reduces serum albumin content [20]. In this study, dietary vitamin D significantly affected serum total protein content, with appropriate supplementation (200 IU/kg) significantly increasing serum total protein. This mechanism may involve vitamin D promoting protein digestion and absorption, thereby increasing serum total protein content [21]. However, further elevation of vitamin D supplementation reduced serum

total protein and albumin contents, possibly due to liver damage caused by excessive vitamin D. Serum total cholesterol content reflects lipid metabolism status and body fat accumulation, with approximately 70–80% of blood cholesterol originating from the liver and a small portion from the digestive tract. Liver damage alters serum cholesterol content [22]. In this study, serum total cholesterol showed an initial increase followed by a decrease, possibly because appropriate vitamin D levels promoted lipid metabolism in the liver, accelerating fat decomposition and increasing saturated fatty acid production, thereby elevating cholesterol content. However, excessive vitamin D may have caused liver damage, impairing lipid metabolism and reducing cholesterol release into the bloodstream.

4 Conclusion

Appropriate dietary vitamin D supplementation promotes growth in mid-stage GIFT, while excessive levels may cause liver damage and affect lipid metabolism. Based on regression analysis using weight gain rate as the evaluation index, the optimal dietary vitamin D supplementation level for GIFT in the mid-growth stage is 259.8 IU/kg.

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