# Dietary Vitamin B6 Requirement of GIFT Tilapia (Postprint)

**Authors:** Wu Fan, Ren Chun, Wenhua, Jiang Ming, Liu Wei, Tian Juan, Yang Changgeng, Yu Lijuan, Lu Xing

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## Abstract

This experiment was conducted to investigate the effects of dietary vitamin B6 levels on growth performance, whole-body and muscle nutritional composition, and serum and liver biochemical indices of GIFT tilapia, in order to determine the dietary vitamin B6 requirement of GIFT tilapia. A total of 360 GIFT tilapia with an initial average body weight of (56.3±1.8) g were randomly divided into 6 groups, with 3 replicates per group and 20 fish per replicate. Each group was fed one of six purified diets containing vitamin B6 levels of 0.3 (control group), 2.7, 5.4, 10.1, 20.5, and 39.8 mg/kg for a 10-week feeding trial. The results showed: 1) With increasing dietary vitamin B6 levels (0.3~10.1 mg/kg), the weight gain rate of GIFT tilapia increased significantly ($P<0.05$) and plateaued when dietary vitamin B6 level reached 10.1 mg/kg; the feed conversion ratio of the 5.4, 10.1, 20.5, and 39.8 mg/kg groups was significantly lower than that of the control and 2.7 mg/kg groups ($P<0.05$); the hepatosomatic index of the 5.4 and 20.5 mg/kg groups was significantly higher than that of the control group ($P<0.05$). 2) The whole-body crude protein and crude ash contents of the 2.7, 5.4, 10.1, 20.5, and 39.8 mg/kg groups were significantly higher than those of the control group ($P<0.05$); the whole-body crude fat content was lowest in the 10.1 mg/kg group, which was significantly lower than that of the control and 2.7, 5.4, 39.8 mg/kg groups ($P<0.05$); the muscle crude protein content of the 5.4, 10.1, and 20.5 mg/kg groups was significantly higher than that of the control group ($P<0.05$). 3) There were no significant differences in serum glucose, total protein content, and liver aspartate aminotransferase activity among all groups ($P>0.05$). Serum alkaline phosphatase activity of the 20.5 and 39.8 mg/kg groups was significantly higher than that of the control group ($P<0.05$); serum urea nitrogen content of the 10.1 and 39.8 mg/kg groups was significantly higher than that of the control group ($P<0.05$); liver alanine aminotransferase activity of the 10.1, 20.5, and 39.8 mg/kg groups was significantly higher than that of the control and 2.7, 5.4 mg/kg groups ($P<0.05$). In summary, using weight

gain rate and liver alanine aminotransferase activity as evaluation indices, the dietary vitamin B6 requirements for GIFT tilapia with an initial body weight of (56.3±1.8) g were determined to be 9.22 and 10.11 mg/kg, respectively, through broken-line regression analysis.

## Full Text

# Dietary Vitamin B6 Requirement of Genetically Improved Farmed Tilapia (GIFT, *Oreochromis niloticus*)

**WU Fan**[1], **REN Chun**[2], **WEN Hua**[1]*, **JIANG Ming**[1], **LIU Wei**[1], **TIAN Juan**[1], **YANG Changgeng**[1], **YU Lijuan**[1], **LU Xing**[1]

[1]Fish Nutrition and Feed Division, Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Wuhan 430223, China
[2]College of Fisheries, Huazhong Agricultural University, Wuhan 430070, China

### Abstract

This experiment was conducted to investigate the effects of dietary vitamin B6 content on growth performance, whole-body and muscle nutritional composition, and serum and liver biochemical indices of genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*), and to determine the dietary vitamin B6 requirement of GIFT. A total of 360 GIFT with an average initial body weight of (56.3±1.8) g were randomly divided into 6 groups with 3 replicates per group and 20 fish per replicate. The fish were fed six purified diets containing 0.3 (control group), 2.7, 5.4, 10.1, 20.5, and 39.8 mg/kg vitamin B6, respectively, for 10 weeks. The results showed that: (1) With increasing dietary vitamin B6 content (0.3–10.1 mg/kg), the weight gain rate of GIFT increased significantly ($P<0.05$) and plateaued when dietary vitamin B6 content reached 10.1 mg/kg. The feed conversion ratio in the 5.4, 10.1, 20.5, and 39.8 mg/kg groups was significantly lower than that in the control and 2.7 mg/kg groups ($P<0.05$). The hepatosomatic index in the 5.4 and 20.5 mg/kg groups was significantly higher than that in the control group ($P<0.05$). (2) The whole-body crude protein and ash contents in the 2.7, 5.4, 10.1, 20.5, and 39.8 mg/kg groups were significantly higher than those in the control group ($P<0.05$). The 10.1 mg/kg group had the lowest whole-body crude lipid content, which was significantly lower than that in the control, 2.7, 5.4, and 39.8 mg/kg groups ($P<0.05$). The muscle crude protein content in the 5.4, 10.1, and 20.5 mg/kg groups was significantly higher than that in the control group ($P<0.05$). (3) There were no significant differences in serum glucose, total protein, or liver aspartate aminotransferase activity among all groups ($P>0.05$). Serum alkaline phosphatase activity in the 20.5 and 39.8 mg/kg groups was significantly higher than that in the control group ($P<0.05$). Serum urea nitrogen content in the 10.1 and 39.8 mg/kg groups was significantly higher than that in the control group ($P<0.05$). Liver alanine aminotransferase activity in the 10.1, 20.5, and 39.8 mg/kg groups was significantly higher than

that in the control, 2.7, and 5.4 mg/kg groups ($P<0.05$). In summary, broken-line regression analysis indicated that using weight gain rate and liver alanine aminotransferase activity as response criteria, the optimal dietary vitamin B6 requirements for GIFT [initial body weight (56.3±1.8) g] were 9.22 and 10.11 mg/kg, respectively.

**Keywords:** GIFT; vitamin B6; growth performance; biochemical indices; requirement

## Introduction

Vitamin B6 is a water-soluble B-complex vitamin that includes pyridoxine, pyridoxal, and pyridoxamine, and is therefore also known as pyridoxine. In the body, vitamin B6 exists in phosphate ester forms and serves as a cofactor for more than 140 enzymes, participating in the biosynthesis and catabolism of amino acids as well as carbohydrate and lipid metabolism, thus playing crucial physiological roles. Vitamin B6 deficiency in aquatic animals leads to symptoms such as anorexia, motor incoordination, growth retardation, and other neurological dysfunctions [1-2]. Previous studies have reported dietary vitamin B6 requirements for various aquatic species, including channel catfish (*Ictalurus punctatus*) at 3 mg/kg [3], Atlantic salmon (*Salmo salar*) at 5 mg/kg [4], rainbow trout (*Oncorhynchus mykiss*) at 2 mg/kg [5], Indian catfish (*Heteropneustes fossilis*) at 3.21 mg/kg [6], Jian carp (*Cyprinus carpio* var. Jian) at 6.07 mg/kg [7], gibel carp (*Carassius auratus gibelio*) at 0.95 mg/kg [8], and Indian major carp (*Cirrhinus mrigala*) at 5.63–8.61 mg/kg [9].

Genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*) is a genetically enhanced strain of tilapia characterized by rapid growth, strong disease resistance, and high adaptability, making it an important aquaculture species in China. Previous international studies have reported vitamin B6 requirements for tilapia. For hybrid tilapia (*Oreochromis niloticus* × *O. aureus*), dietary vitamin B6 requirements varied with dietary protein levels, ranging from 1.7–9.5 mg/kg (28% protein) to 15.0–16.5 mg/kg (36% protein) [10]. Red hybrid tilapia (*Oreochromis mossambicus* × *O. niloticus*) required 3 mg/kg dietary vitamin B6 [11]. Since GIFT exhibits faster growth rates than other strains, and different growth rates can cause physiological and metabolic differences, their nutrient requirements may also differ. Therefore, it is necessary to evaluate the dietary vitamin B6 requirement of GIFT. This study investigated the effects of dietary vitamin B6 content on growth performance, whole-body and muscle nutritional composition, and serum and liver biochemical indices of GIFT to determine their dietary vitamin B6 requirement.

## Materials and Methods

**1.1 Experimental Diets** Casein and gelatin served as protein sources, corn oil and soybean oil as lipid sources, and dextrin as carbohydrate source to formu-

late the basal diet, whose composition and nutrient levels are shown in Table 1 . Six purified diets were formulated with designed vitamin B6 levels of 0 (control), 2.5, 5.0, 10.0, 20.0, and 40.0 mg/kg. Vitamin B6 was first mixed with cellulose to prepare a vitamin B6 premix at a specific concentration, which was then added to the diets at different levels according to the design, with cellulose adjusted to balance the differences in premix addition among groups. During diet preparation, dry ingredients were ground and passed through a 60-mesh sieve, weighed accurately according to the formulation, and mixed stepwise. Corn oil and soybean oil were added and mixed thoroughly, followed by the addition of appropriate water to produce 2.0 mm diameter strips using a pelletizer. After air-drying, the strips were broken into cylindrical pellets approximately 4.0 mm in length and stored at -20 °C until use.

Using a microbiological assay (test kit from R-Biopharm AG, Germany), the actual measured vitamin B6 contents in the six purified diets were 0.3, 2.7, 5.4, 10.1, 20.5, and 39.8 mg/kg.

**1.2 Experimental Design and Culture Management** Experimental fish were obtained from the Guangxi National Tilapia Breeding Farm and temporarily reared in net cages at the Shandong Township aquaculture base in Jiangxia District, Wuhan. During the acclimation period, fish were fed the basal diet without vitamin B6 supplementation. After two weeks, 360 healthy GIFT with uniform size and an average initial body weight of (56.3±1.8) g were selected and randomly divided into 6 groups with 3 replicates per group and 20 fish per replicate. Each replicate was randomly assigned to one of 18 net cages (1.0 m × 1.0 m × 1.5 m) containing 20 fish each. The six groups were fed the six purified diets containing 0.3 (control), 2.7, 5.4, 10.1, 20.5, and 39.8 mg/kg vitamin B6, respectively, for 10 weeks.

Fish were fed twice daily (09:00 and 16:00) to apparent satiation. Body weight was measured every two weeks. The experiment lasted for 10 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 >5.0 mg/L, and total ammonia nitrogen concentration was <0.05 mg/L.

**1.3 Sample Collection** After 10 weeks of culture, fish were fasted for 24 h, and the total weight and number of fish in each cage were recorded. Three fish were randomly selected from each cage for measurement of body length and weight. Blood was collected from the caudal vein, followed by dissection to rapidly separate viscera and liver, which were weighed to calculate viscerosomatic index (VSI) and hepatosomatic index (HSI). Liver samples were retained for biochemical analysis. Blood-filled centrifuge tubes were placed in a 4 °C refrigerator for 2 h, then centrifuged at 3,000 r/min for 10 min to obtain serum for biochemical analysis. An additional three fish from each cage were collected for determination of whole-body crude protein, crude lipid, moisture, and ash

contents. Liver, serum, and whole-body samples were stored at -40 °C until analysis.

### 1.4 Analytical Methods

**1.4.1 Growth Performance** Growth performance indices were calculated using the following formulas:

- Survival rate (SR, %) = 100 × Nt/N0
- Weight gain rate (WGR, %) = 100 × (Mt -M0)/M0
- Feed conversion ratio (FCR) = Wf/(Wt -W0)
- Viscerosomatic index (VSI, %) = 100 × Mv/Mw
- Hepatosomatic index (HSI, %) = 100 × Mh/Mw
- Condition factor (CF, %) = 100 × Mw/$L^3$

Where N0 and Nt are the initial and final number of fish; M0 and Mt are the initial and final mean body weight (g); Wf is the total feed intake (g); W0 and Wt are the initial and final total body weight (g); Mv is the viscera weight (g); Mh is the liver weight (g); Mw and L are the body weight (g) and body length (cm) of sampled fish, respectively.

**1.4.2 Whole-Body and Muscle Nutritional Composition** Whole-body and muscle nutritional composition were analyzed according to AOAC (2000) [12] methods. Moisture content was determined by oven-drying at 105 °C to constant weight, crude protein content by the Kjeldahl method, crude lipid content by Soxhlet extraction, and ash content by muffle furnace incineration.

**1.4.3 Serum and Liver Biochemical Indices** Approximately 1 g of liver sample (accurate to 0.01 g) was homogenized mechanically on ice with 9 volumes (mL) of physiological saline, then centrifuged at 3,000 r/min for 10 min. The supernatant was used for liver biochemical analysis. Serum alkaline phosphatase (ALP) activity and glucose (GLU), total protein (TP), and urea nitrogen (UN) contents, as well as liver aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, were measured using a Sysmex Chemix-800 automatic biochemical analyzer. Liver homogenate protein concentration was determined using a Coomassie brilliant blue assay kit (Nanjing Jiancheng Bioengineering Institute). Liver AST and ALT activities were calculated as:

Liver AST or ALT activity (U/g prot) = AST or ALT activity measured by biochemical analyzer (U/L) / Liver homogenate protein concentration (g/L prot)

**1.5 Statistical Analysis** Data were analyzed using one-way ANOVA and Duncan's multiple comparison tests with SPSS 19.0 statistical software. Results are expressed as means ± standard deviation, with significance set at $P < 0.05$. Dietary vitamin B6 requirements for GIFT were estimated using broken-line regression analysis [13].

## Results

**2.1 Effects of Dietary Vitamin B6 Content on Growth Performance of GIFT** As shown in Table 2 , the weight gain rate of GIFT increased significantly ($P<0.05$) with increasing dietary vitamin B6 content and plateaued thereafter. The 2.7, 5.4, 10.1, 20.5, and 39.8 mg/kg groups were significantly higher than the control group ($P<0.05$), while the 10.1, 20.5, and 39.8 mg/kg groups were significantly higher than all other groups ($P<0.05$) but did not differ significantly among themselves ($P>0.05$). Feed conversion ratio decreased with increasing dietary vitamin B6 content, with the 5.4, 10.1, 20.5, and 39.8 mg/kg groups being significantly lower than the control and 2.7 mg/kg groups ($P<0.05$). The hepatosomatic index in the 5.4 and 20.5 mg/kg groups was significantly higher than that in the control group ($P<0.05$), with no significant differences among other groups ($P>0.05$). No significant differences in condition factor were observed among the 0.3, 2.7, 5.4, 10.1, and 20.5 mg/kg groups ($P>0.05$); the 39.8 mg/kg group had the highest condition factor, which was significantly higher than the 2.7 and 5.4 mg/kg groups ($P<0.05$). No significant differences in viscerosomatic index were detected among all groups ($P<0.05$).

Broken-line regression analysis (Figure 1 [Figure 1: see original paper]) indicated that the dietary vitamin B6 requirement for maximum weight gain rate of GIFT was 9.22 mg/kg.

**2.2 Effects of Dietary Vitamin B6 Content on Whole-Body and Muscle Nutritional Composition of GIFT** As shown in Table 3 , whole-body crude protein and ash contents in the 2.7, 5.4, 10.1, 20.5, and 39.8 mg/kg groups were significantly higher than those in the control group ($P<0.05$), with no significant differences among these five groups ($P>0.05$). The 2.7 mg/kg group had the lowest whole-body moisture content, which was significantly lower than the control and 10.1, 20.5 mg/kg groups ($P<0.05$). The 10.1 mg/kg group showed the lowest whole-body crude lipid content, significantly lower than all groups except the 20.5 mg/kg group ($P<0.05$). No significant differences in muscle moisture or crude lipid contents were observed among groups ($P<0.05$), but muscle crude protein content in the 5.4, 10.1, and 20.5 mg/kg groups was significantly higher than that in the control group ($P<0.05$).

**2.3 Effects of Dietary Vitamin B6 Content on Serum and Liver Biochemical Indices of GIFT** As shown in Table 4 , serum alkaline phosphatase activity and urea nitrogen content increased with dietary vitamin B6 content. Serum alkaline phosphatase activity in the 20.5 and 39.8 mg/kg groups was significantly higher than that in the control group ($P<0.05$), with no significant difference between these two groups ($P>0.05$). Serum urea nitrogen content in the 10.1 and 39.8 mg/kg groups was significantly higher than that in the control group ($P<0.05$), with no significant differences among other groups ($P<0.05$). No significant differences in serum total protein or glucose contents were observed among all groups ($P>0.05$). No significant differences in liver aspartate

aminotransferase activity were detected among groups (P>0.05). Liver alanine aminotransferase activity in the 10.1, 20.5, and 39.8 mg/kg groups was significantly higher than that in the control, 2.7, and 5.4 mg/kg groups (P<0.05), and the 5.4 mg/kg group was significantly higher than the control and 2.7 mg/kg groups (P<0.05).

Broken-line regression analysis (Figure 2 [Figure 2: see original paper]) indicated that the dietary vitamin B6 requirement based on liver alanine aminotransferase activity was 10.11 mg/kg.

## Discussion

**3.1 Effects of Dietary Vitamin B6 Content on Growth Performance of GIFT** Vitamin B6 participates in the synthesis and metabolism of neurotransmitters, glycogen, and heme in animals as a coenzyme or prosthetic group, primarily in amino acid metabolism-related reactions. Therefore, vitamin B6 deficiency affects normal growth and various physiological and biochemical processes in aquatic animals, ultimately leading to deficiency symptoms. Gibel carp fed vitamin B6-deficient diets exhibit anorexia, lethargy, erratic swimming, and increased mortality [8]. Vitamin B6 deficiency in grouper (*Epinephelus coioides*) results in loss of appetite, low survival, convulsions, and spiral swimming [14]. Hybrid tilapia fed vitamin B6-deficient diets developed anorexia and motor incoordination within three weeks, with increased mortality and edema in dead fish [10]. In this study, fish in the control group showed only slow growth without other obvious deficiency signs. This may be because the experimental fish (initial weight ~50 g) had accumulated some vitamin B6 during their early growth stage, preventing the appearance of deficiency symptoms typically observed in juvenile fish within the experimental period. Another reason could be that the net cage culture environment contained microorganisms that may have facilitated partial vitamin B6 synthesis by GIFT. The weight gain rate of GIFT increased with dietary vitamin B6 content up to 10.1 mg/kg and then plateaued, demonstrating that vitamin B6 is essential for GIFT growth. This trend was also observed in hybrid tilapia [10] and grass shrimp (*Penaeus monodon*) [15], indicating that vitamin B6 can support growth within a certain range, but excess amounts are not utilized for growth and may be diverted to other physiological activities.

Viscerosomatic index, hepatosomatic index, and condition factor reflect the nutritional status of fish. Gibel carp fed diets without vitamin B6 supplementation had significantly lower hepatosomatic indices than other vitamin B6-supplemented groups [8]. In this study, hepatosomatic index increased with dietary vitamin B6 content, and condition factor peaked in the 39.8 mg/kg group. Although no other visible deficiency signs were observed in the control group, the reduced hepatosomatic index suggests that vitamin B6 deficiency may cause malnutrition and adverse physiological effects in GIFT.

**3.2 Effects of Dietary Vitamin B6 Content on Whole-Body and Muscle Nutritional Composition of GIFT** In this study, whole-body crude protein content in vitamin B6-supplemented groups was significantly higher than in the control group, and muscle crude protein content also showed an increasing trend, indicating that vitamin B6 promoted protein utilization and synthesis. Previous studies have shown that gibel carp fed vitamin B6-supplemented diets had higher protein content than the control group [8], with similar results reported for red hybrid tilapia [11]. The coenzyme forms of vitamin B6, pyridoxal phosphate and pyridoxamine phosphate, participate in amino acid metabolic reactions including transamination, deamination, and desulfuration, which are essential for amino acid absorption and synthesis and beneficial for protein synthesis [16]. As a coenzyme, vitamin B6 also participates in unsaturated fatty acid metabolism. In rats, vitamin B6 deficiency reduces hepatic microsomal enzyme activity, blocks essential fatty acid metabolism, and increases accumulation of metabolic precursors [17]. In this study, 10.1 mg/kg dietary vitamin B6 reduced whole-body crude lipid content in tilapia, consistent with findings in snakehead (*Channa argus*) [18]. Studies on grass carp (*Ctenopharyngodon idella*) also demonstrated that vitamin B6 facilitates lipid metabolism and prevents tissue deposition [19].

**3.3 Effects of Dietary Vitamin B6 Content on Serum and Liver Biochemical Indices of GIFT** Aminotransferases catalyze amino group transfer, participating in amino acid decomposition and synthesis and playing important roles in growth and metabolism, particularly aspartate aminotransferase and alanine aminotransferase. Vitamin B6 serves as a cofactor for aminotransferases and is closely related to protein metabolism, thus affecting aminotransferase activity. For example, vitamin B6 significantly increased aspartate aminotransferase and alanine aminotransferase activities in viscera and muscle of abalone (*Haliotis discus hannai*) [20]; grass shrimp liver aspartate aminotransferase activity increased initially and then decreased with increasing dietary vitamin B6 content [15]; and Pacific white shrimp (*Litopenaeus vannamei*) fed diets containing 200 mg/kg vitamin B6 showed significantly higher serum aspartate aminotransferase and alanine aminotransferase activities than the control group [21]. In this study, liver alanine aminotransferase activity increased significantly with dietary vitamin B6 content and plateaued at 10.1 mg/kg. Liver aspartate aminotransferase activity showed an increasing trend without significant differences, and serum total protein content showed a similar pattern. Although vitamin B6 can affect aminotransferase activity and regulate protein metabolism, the lack of significant differences among groups may be due to insufficient culture duration. Alternatively, enhanced amino acid metabolism may have increased circulating amino acid levels, primarily affecting whole-body and muscle protein content rather than serum total protein content.

Serum urea nitrogen is a non-protein nitrogenous compound that reflects protein metabolism status in fish. In this study, serum urea nitrogen content in tilapia increased with dietary vitamin B6 content, indicating that vitamin B6

affected protein metabolism. However, some studies suggest that increased urea nitrogen content indicates enhanced protein metabolic burden [22], suggesting that excessively high dietary vitamin B6 levels may increase metabolic stress. Few reports exist on vitamin B6 effects on alkaline phosphatase activity. In this study, increased serum alkaline phosphatase activity was likely influenced by protein metabolism. Alkaline phosphatase is a phosphomonoesterase that regulates metabolism related to proteins and serves as a lysosomal marker enzyme for evaluating immune function. Elevated serum alkaline phosphatase activity suggests that vitamin B6 may enhance immune function, but objective evaluation requires combination with other immune indices and warrants further investigation.

**3.4 Dietary Vitamin B6 Requirement of GIFT** Broken-line regression analysis in this study indicated that the dietary vitamin B6 requirement for maximum weight gain rate in GIFT was 9.22 mg/kg. This value is higher than those reported for rainbow trout (2 mg/kg) [5], channel catfish (3 mg/kg) [3], and Indian catfish (3.21 mg/kg) [6], but lower than that for yellowtail (*Seriola quinqueradiata*) (11.7 mg/kg) [23] and chinook salmon (*Oncorhynchus tshawytscha*) (15–20 mg/kg) [2]. These differences may be attributed to species, initial body weight, and experimental conditions. The dietary vitamin B6 requirement for maximum growth in red hybrid tilapia was 3 mg/kg [11], lower than our result. This may be because long-term selective breeding has increased the growth rate of GIFT by 5–30% compared to other tilapia strains [24], and accelerated metabolism may increase nutrient requirements.

Cowey [25] suggested that biochemical indices such as specific enzyme activities could be used to evaluate nutrient requirements in cultured fish. Since many vitamins function as coenzymes or modified coenzymes, tissue enzyme activities are often used to supplement growth-based assessments of vitamin requirements. Alanine aminotransferase, primarily located in hepatocyte cytoplasm, participates in amino acid decomposition and synthesis and requires pyridoxal phosphate as a cofactor; thus, liver alanine aminotransferase activity is commonly used to evaluate vitamin B6 status in fish. In this study, the dietary vitamin B6 requirement based on liver alanine aminotransferase activity was 10.11 mg/kg, slightly higher than the 9.22 mg/kg estimated from weight gain rate. In hybrid tilapia fed 28% protein diets, the dietary vitamin B6 requirement was 1.7 mg/kg based on growth and 9.5 mg/kg based on liver alanine aminotransferase activity in juveniles (0.73±0.01 g); with 36% protein diets, these values were 16.5 and 15.0 mg/kg, respectively [10]. Since vitamin B6 is closely related to amino acid metabolism, dietary protein level affects vitamin B6 requirements. Whether different tilapia strains have different vitamin B6 requirements should be compared under identical experimental conditions with similar initial body weights and the same dietary formulation.

## Conclusion

Based on the results of this study, broken-line regression analysis using weight gain rate and liver alanine aminotransferase activity as response criteria indicated that the optimal dietary vitamin B6 requirements for GIFT with an initial body weight of (56.3±1.8) g were 9.22 and 10.11 mg/kg, respectively.

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