

## Postprint: Dietary Vitamin B2 Requirement for GIFT Tilapia

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### Abstract

This experiment aimed to determine the dietary vitamin B2 requirement of GIFT tilapia.

### Full Text

#### Dietary Vitamin B2 Requirement of Genetically Improved Farmed Tilapia (*Oreochromis niloticus*)

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### Abstract

This study was conducted to determine the dietary vitamin B2 requirement of genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*). Six experimental diets were formulated using casein, gelatin, and dextrin as primary ingredients to contain graded levels of vitamin B2 at 0.5, 2.6, 5.4, 10.8, 20.4, and 39.7 mg/kg. Each diet was fed to three replicate groups of 15 GIFT with an initial body weight of (59.5±\$0.9) g in an indoor recirculating aquaculture system for 10 weeks. Growth performance, liver vitamin B2 content, and selected serum biochemical parameters were measured after the feeding trial.

The results showed that the weight gain rate of GIFT increased linearly with dietary vitamin B2 content and plateaued at 10.8 mg/kg, while feed efficiency followed a similar trend. The viscerosomatic index in the 10.8, 20.4, and 39.7 mg/kg groups was significantly higher than in the 0.4 and 2.6 mg/kg groups

( $P < 0.05$ ), and the hepatosomatic index in the 10.8, 20.4, and 39.7 mg/kg groups was significantly higher than in the 0.4 mg/kg group ( $P < 0.05$ ). Dietary vitamin B2 content had no significant effect on condition factor ( $P > 0.05$ ).

Whole-body crude lipid content initially increased then decreased with rising dietary vitamin B2 levels, with the 5.4 mg/kg group showing significantly higher values than other groups ( $P < 0.05$ ). Whole-body moisture content was lowest in the 5.4 mg/kg group, being significantly lower than all groups except the 39.7 mg/kg group ( $P < 0.05$ ). Whole-body crude protein content was highest in the 39.7 mg/kg group, significantly higher than all groups except the 5.4 mg/kg group ( $P < 0.05$ ). No significant differences were observed in whole-body ash content among groups ( $P > 0.05$ ). Dietary vitamin B2 content did not significantly affect moisture, crude protein, or crude lipid contents in muscle or liver ( $P > 0.05$ ).

Liver vitamin B2 content increased linearly with dietary vitamin B2 content and stabilized at 20.4 mg/kg. Serum total cholesterol, triglyceride contents, and alanine aminotransferase and aspartate aminotransferase activities in the 0.4 mg/kg group were significantly higher than in other groups ( $P < 0.05$ ). Broken-line model analysis indicated that the dietary vitamin B2 requirement of GIFT was 10.2 mg/kg for optimal growth and 19.3 mg/kg for maximum liver vitamin B2 accumulation.

**Keywords:** GIFT; riboflavin; requirement; growth

## Introduction

Vitamin B2 (riboflavin) is a component of the coenzymes flavin mononucleotide and flavin adenine dinucleotide, which serve as prosthetic groups for oxidoreductases involved in the metabolism of keto acids, fatty acids, and amino acids [1]. Low vitamin B2 intake in animals leads to poor growth, anemia, neurological dysfunction, and increased risk of certain cancers. Since vitamin B2 cannot be synthesized in vivo, it must be obtained from the diet [2]. Dietary vitamin B2 deficiency in fish causes physiological dysfunction, manifesting as one or more deficiency symptoms including anorexia, stunted growth, short-body syndrome, locomotor incoordination, dark skin coloration, eye hemorrhage, and cataracts [1,3]. Therefore, vitamin B2 is an essential water-soluble vitamin for cultured fish, and research on its nutritional requirements in major aquaculture species is necessary.

Current literature reports vitamin B2 requirements for several cultured fish species: 5.54-7.90 mg/kg for grass carp (*Ctenopharyngodon idella*) [4-5], 5.21 mg/kg for blunt snout bream (*Megalobrama amblycephala*) [6], 3.76 mg/kg for gibel carp (*Carassius auratus gibelio*) [7], 5.0 mg/kg for Jian carp (*Cyprinus carpio* var. Jian) [8], 6-9 mg/kg for channel catfish (*Ictalurus punctatus*) [9-10], and 3-15 mg/kg for rainbow trout (*Oncorhynchus mykiss*) [11].

Tilapia is one of the world's major aquaculture species. Current reports on

dietary vitamin B2 requirements for tilapia include 6.0 mg/kg for blue tilapia (*Oreochromis aureus*) [12] and 5.0 mg/kg for red tilapia (*Oreochromis mossambicus* × *O. niloticus*) [13]. Genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*) is a genetically enhanced strain of Nile tilapia and one of the main tilapia culture strains in China, with a growth rate 5-30% faster than other strains [14]. Its vitamin B2 requirement may differ from that of blue and red tilapia. Previous studies in our laboratory have also found that GIFT exhibits different requirements for vitamin C [15], choline [16], and niacin [17] compared with other tilapia strains. Therefore, it is necessary to re-evaluate the dietary vitamin B2 requirement of GIFT. This study investigated the effects of dietary vitamin B2 content on growth performance, body composition, selected serum biochemical indices, and liver vitamin B2 content to determine the vitamin B2 requirement of GIFT.

## Materials and Methods

### 1.1 Experimental Diets

A semi-purified basal diet was formulated using casein and gelatin as protein sources, corn oil and soybean oil as lipid sources, and dextrin as the carbohydrate source. Graded levels of vitamin B2 (98% purity, Sigma R4500) were supplemented to the basal diet at 0, 2.5, 5.0, 10.0, 20.0, and 40.0 mg/kg, producing six experimental diets. The analyzed vitamin B2 contents of the six diets, determined by high-performance liquid chromatography according to GB/T 14701-2002, were 0.5, 2.6, 5.4, 10.8, 20.4, and 39.7 mg/kg.

Feed ingredients (except corn oil and soybean oil) were ground to pass through a 0.20 mm sieve and mixed according to the proportions shown in Table 1. Minor components were incorporated using the progressive enlargement method. After thorough mixing of dry ingredients, corn oil and soybean oil were added and mixed until no oily particles remained. Approximately 20% water was then added, and the mixture was kneaded and extruded through a meat grinder to produce 2.00 mm diameter strands. The strands were dried in the dark at room temperature with an electric fan to moisture content <10%, broken into approximately 4.0 mm cylindrical pellets, and sieved through a 0.90 mm sieve to remove fines. The final pellets were stored at -20 °C until use.

### 1.2 Experimental Fish and Culture Management

Experimental GIFT were obtained from the Guangxi National Tilapia Breeding Farm. After transportation, fish were disinfected and acclimated for two weeks, during which they were fed the basal diet to adapt to the experimental environment and feed. Prior to the formal experiment, fish were fasted for 24 h, and 270 healthy juveniles with uniform size [average initial weight (59.5±\$0.9) g] were randomly distributed into 18 tanks with three replicates per dietary treatment and 15 fish per replicate. The experiment was conducted in an indoor recirculating aquaculture system at the Yangtze River Fisheries Research Institute.

The system consisted of 18 cylindrical fiberglass tanks (500 L volume, 400 L effective water volume) equipped with water treatment and aeration facilities.

The sand filter was backwashed daily at 11:00, and feces were removed from each tank. Approximately one-third of the total water volume was replaced daily with aerated municipal tap water. Fish were fed their respective experimental diets by hand to apparent satiation twice daily (09:00 and 16:00) for approximately 30 minutes each feeding. The feeding trial lasted 10 weeks. During the culture period, water temperature was maintained at 27–33 °C, pH at 7.2–7.5, dissolved oxygen concentration >5 mg/L, and total ammonia nitrogen concentration <0.5 mg/L.

### 1.3 Sample Collection, Measurement, and Calculation

After 10 weeks of feeding, fish were fasted for 24 h. Total weight and number of fish in each tank were recorded to calculate final body weight, weight gain rate (WGR), and survival rate (SR). Feed consumption per tank was recorded to calculate feed efficiency (FE). Six fish were randomly sampled from each tank: three for whole-body proximate composition analysis and three for measurement of body length and weight to calculate condition factor (CF). Blood was collected from the caudal vein of the latter three fish, after which they were dissected to rapidly separate viscera and liver for weighing to calculate viscerosomatic index (VSI) and hepatosomatic index (HSI). Liver samples were retained for vitamin B2 content and antioxidant enzyme activity determination, while dorsal muscle samples were collected for proximate composition analysis. Blood samples were allowed to clot at 4 °C for 2 h, then centrifuged at 3,000 r/min for 10 min to obtain serum for biochemical analysis. Liver, muscle, and whole-body samples were stored at -40 °C until analysis. Formulas for calculating WGR, FE, SR, CF, HSI, and VSI followed those described in reference [18].

Moisture content was determined by oven drying at 105 °C to constant weight. Crude protein content was measured by the Kjeldahl method. Crude lipid content was analyzed by Soxhlet extraction. Ash content was determined by muffle furnace incineration. Gross energy was measured using an oxygen bomb calorimeter (Parr-6200). Liver vitamin B2 content was determined by high-performance liquid chromatography according to GB/T 9695.28-2008. Serum triglyceride and total cholesterol concentrations and aspartate aminotransferase and alanine aminotransferase activities were measured using a Sysmex automatic biochemical analyzer (Chemix-800).

### 1.4 Statistical Analysis

Experimental data were analyzed by one-way ANOVA and Duncan's multiple comparison using SPSS 19.0 statistical software. Results are expressed as mean±SD. Differences were considered significant at  $P < 0.05$ . The dietary vitamin B2 requirement of GIFT was determined using broken-line regression analysis [19].

## Results

At the end of the 10-week feeding trial, fish in the 0.4 mg/kg (5 fish) and 2.6 mg/kg (3 fish) groups exhibited short-body syndrome, while no other visible deficiency symptoms were observed.

### 2.1 Growth Performance, Feed Efficiency, and Body Indices

Table 2 presents the growth performance, feed efficiency, and body indices of GIFT fed diets with different vitamin B2 contents. Final body weight and weight gain rate increased linearly with dietary vitamin B2 content and plateaued at 10.8 mg/kg, with the 0.4 mg/kg group showing significantly lower values than other groups ( $P < 0.05$ ). Feed efficiency showed a similar trend to weight gain rate. Viscerosomatic index in the 10.8, 20.4, and 39.7 mg/kg groups was significantly higher than in the 0.4 and 2.6 mg/kg groups ( $P < 0.05$ ). Hepatosomatic index in the 10.8, 20.4, and 39.7 mg/kg groups was significantly higher than in the 0.4 mg/kg group ( $P < 0.05$ ). No significant differences in condition factor were observed among groups ( $P > 0.05$ ). Survival rate was 100% across all groups with no mortality during the trial. Broken-line model analysis of the relationship between dietary vitamin B2 content and weight gain rate (Figure 1 [Figure 1: see original paper]) indicated that the optimal dietary vitamin B2 content for maximum growth of GIFT was 10.2 mg/kg.

### 2.2 Whole-Body, Muscle, and Liver Proximate Composition and Liver Vitamin B2 Content

As shown in Table 3, whole-body crude lipid content initially increased then decreased with rising dietary vitamin B2 levels, with the 5.4 mg/kg group being significantly higher than other groups ( $P < 0.05$ ). Whole-body moisture content was lowest in the 5.4 mg/kg group, which was not significantly different from the 39.7 mg/kg group ( $P > 0.05$ ) but significantly lower than other groups ( $P < 0.05$ ). Whole-body crude protein content was highest in the 39.7 mg/kg group, which was not significantly different from the 5.4 mg/kg group ( $P > 0.05$ ) but significantly higher than other groups ( $P < 0.05$ ). No significant differences in whole-body ash content were observed among groups ( $P > 0.05$ ). Dietary vitamin B2 content had no significant effects on moisture, crude protein, or crude lipid contents in muscle or liver ( $P > 0.05$ ).

Liver vitamin B2 content increased linearly with dietary vitamin B2 content and plateaued at 20.4 mg/kg. The 5.4 mg/kg group had significantly lower liver vitamin B2 content than other groups ( $P < 0.05$ ), while the 2.6, 5.4, and 10.8 mg/kg groups had significantly lower values than the 20.4 and 39.7 mg/kg groups ( $P < 0.05$ ). Broken-line model analysis of the relationship between dietary vitamin B2 content and liver vitamin B2 content (Figure 2 [Figure 2: see original paper]) indicated that the dietary vitamin B2 requirement for maximum vitamin B2 accumulation in liver was 19.3 mg/kg.

### 2.3 Serum Biochemical Indices

Table 4 shows the serum biochemical indices of GIFT fed diets with different vitamin B2 contents. Serum total cholesterol concentration in the 0.4 mg/kg group was significantly higher than in other groups ( $P < 0.05$ ), with no significant differences among the 5.4–39.7 mg/kg groups ( $P > 0.05$ ). Serum triglyceride concentration and alanine aminotransferase and aspartate aminotransferase activities in the 0.4 mg/kg group were significantly higher than in other groups ( $P < 0.05$ ), with no significant differences among the other groups ( $P > 0.05$ ).

### Discussion

Vitamin B2 deficiency in fish typically causes one or more deficiency symptoms including cataracts, short-body syndrome, anorexia, stunted growth, locomotor incoordination, dark skin coloration, and eye hemorrhage [3]. In this study, fish in the 0.4 mg/kg group exhibited stunted growth and short-body syndrome, but no other obvious vitamin B2 deficiency signs. These results are consistent with findings in channel catfish [9–10], blue tilapia [12], and gibel carp [7]. Vitamin B2 deficiency affects the photoreceptive process, causing cataracts in salmonids [3] and hybrid striped bass [20]; however, no cataracts were observed in this study. This may be because the experimental fish had relatively large initial body size with more mature eye development, and the basal diet contained trace amounts of vitamin B2 sufficient to maintain normal eye development.

After 10 weeks of feeding, inadequate dietary vitamin B2 reduced final body weight and feed efficiency in GIFT, while increasing dietary vitamin B2 content significantly improved weight gain rate. This result aligns with studies on grass carp [4], gibel carp [7], and channel catfish [9]. Appropriate dietary vitamin B2 promotes protein, carbohydrate, and lipid metabolism, thereby enhancing fish growth [3]. Using weight gain rate as the criterion, broken-line model analysis indicated a vitamin B2 requirement of 10.2 mg/kg for GIFT, which is higher than values reported for red tilapia [12] and blue tilapia [13]. This may be attributed to the long-term selective breeding of GIFT, which has resulted in a growth rate 5–30% faster than other tilapia strains [14]. Rapid growth requires substantial energy, and vitamin B2 plays a crucial role as a hydrogen carrier in biological oxidation and is closely associated with protein, lipid, and carbohydrate metabolism [1]. Consequently, faster-growing GIFT may have higher vitamin B2 requirements. Similar findings have been reported in genetically improved pigs, which require more vitamin B2 than conventional pigs due to their faster growth rate [21].

Liver vitamin B2 accumulation is commonly used as a criterion for determining vitamin B2 requirements in aquatic animals, typically yielding higher requirement values than growth performance criteria [3]. In this study, liver vitamin B2 content increased linearly with dietary vitamin B2 content and then stabilized. Using liver vitamin B2 accumulation as the criterion, broken-line model analysis indicated a dietary vitamin B2 requirement of 19.3 mg/kg, which is

higher than the 10.2 mg/kg value obtained using weight gain rate. This pattern is consistent with results reported for rainbow trout [11] and grass carp [4-5].

In this study, whole-body crude lipid content in GIFT initially increased then decreased with rising dietary vitamin B2 levels, similar to results observed in grass carp [4] and blunt snout bream [6]. Inadequate vitamin B2 affects the nervous system, causing reduced feed intake [3] and consequently lower body lipid content. Appropriate vitamin B2 levels improve nutrient digestion and absorption, thereby increasing body lipid content, whereas excessive vitamin B2 may enhance lipid catabolism, leading to reduced body lipid content. Studies in pigs have also shown that excess vitamin B2 can reduce carcass fat content [22]. Reports on the effects of vitamin B2 on whole-body crude protein, ash, and moisture contents are inconsistent. Wang et al. [6] reported no significant effects of dietary vitamin B2 on whole-body moisture, crude protein, or ash contents in blunt snout bream. Wang [7] found no significant effects on whole-body moisture or crude protein contents in gibel carp but observed significant effects on ash content. Jiang et al. [5] reported no significant effects on whole-body composition in grass carp. In this study, dietary vitamin B2 had no significant effect on whole-body ash content, but high vitamin B2 levels increased whole-body crude protein content, possibly because protein deposition requires more vitamin B2 [22]. These discrepancies among studies may be attributed to differences in experimental species and conditions.

Hepatosomatic index partially reflects liver health status, but the effects of vitamin B2 on HSI vary among fish species. Studies on grass carp [4], hybrid striped bass [20], and the present work found that HSI was significantly lower in vitamin B2-deficient groups compared with supplemented groups. However, no significant effects of dietary vitamin B2 on HSI were observed in gibel carp [7], while vitamin B2-deficient diets resulted in significantly higher HSI in gilt-head seabream (*Sparus aurata* L.) [23]. These differences may be related to dietary lipid levels [7], although interactive effects between dietary lipid level and vitamin B2 have not been reported in fish nutrition research, warranting further investigation.

Vitamin B2 exhibits anti-lipid peroxidation and lipid-lowering effects, reducing serum triglyceride, low-density lipoprotein cholesterol, and total cholesterol concentrations [24-25]. Appropriate dietary vitamin B2 significantly decreases serum malondialdehyde, total cholesterol, triglyceride, and low-density lipoprotein cholesterol levels while enhancing superoxide dismutase activity [26-28]. This study also found that dietary vitamin B2 supplementation significantly reduced serum triglyceride and total cholesterol concentrations in GIFT, confirming that adequate vitamin B2 promotes lipid metabolism and reduces serum lipid levels. Previous studies have demonstrated that vitamin B2 protects tissues from oxidative damage [28] and reduces serum aspartate aminotransferase activity in mice [29]. The present results are consistent with these findings, suggesting that vitamin B2 may also have hepatoprotective effects in GIFT.

## Conclusion

1. Inadequate dietary vitamin B2 reduces growth rate and feed efficiency in GIFT.
2. Weight gain rate and liver vitamin B2 content in GIFT increased linearly then plateaued with increasing dietary vitamin B2 content. Based on broken-line model analysis using weight gain rate and liver vitamin B2 content as criteria, the dietary vitamin B2 requirements of GIFT are 10.2 and 19.3 mg/kg, respectively.

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