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# SUPPLEMENTING NILE TILAPIA (*OREOCHROMIS NILOTICUS*) (LINNAEUS, 1758) LARVAE WITH DIETARY BETA-GLUCAN COULD IMPROVE THEIR GROWTH, SURVIVAL, IMMUNE FUNCTION, INTESTINAL AND LIVER HISTOMORPHOLOGY

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**Abstract**

**β-glucan exerts a positive impact and is considered as a functional feed additive to enhance fish growth, immunity, control disease outbreaks and pathogen infections by increasing functional, immune and decreasing harmful responses. The present study aimed to determine the most effective dietary level of beta-glucan (βG) to improve Nile tilapia *Oreochromis niloticus* (L.) fry growth performance, feed utilization, and hematological indicators. Seven isonitrogenous (32% crude protein) and isocaloric diets (18 MJ/kg) were formulated. All diets were identical except for the variation in βG levels. The basal experimental diet (control diet) had no βG added. Diets 2–7 each contained βG at levels of 250, 500, 1000, 2000, 4000, and 8000 mg/kg diet, respectively. A total of 840 fish with an average body weight of 0.2±0.01 g were allocated into the seven experimental treatments (in triplicate). The trial lasted for 120 days, Nile tilapia fry growth performance and feed utilization were significantly (P≤0.05) higher in all treatments receiving βG diets than in the control diet. The fish fed with $ßG_{0.1}$ diet showed the highest significant growth indices and the best FCR values. The survival (S%) of the fish also showed a significant increase in the βG diets up to $ßG_{0.8}$ levels, when compared to the other experimental diet groups. All hematological parameters increased (P≤0.05) in fish fed with a diet supplemented up to $ßG_{0.8}$ compared to the control group. Histological examination of the intestine and liver in the control group revealed histopathological alterations in the villi. On the other hand, the groups treated with βG had longer and structurally normal villi. The most well-preserved intestinal tissue and the tallest villi were observed in $ßG_{0.1}$, followed by $ßG_{0.050}$ and $ßG_{0.8}$ groups. In the liver, the control group exhibited fatty degeneration, necrosis and pyknosis whereas the $ßG_{0.1}$ group displayed the most preserved hepatic tissue, followed by $ßG_{0.8}$ and $ßG_{0.2}$ groups. The results indicate that according to FBW-based broken-line model analysis, the optimal dietary level of βG for Nile tilapia fry to exhibit superior growth and diet utilization efficiency associated with the best FCR for Nile tilapia should be 0.12% βG/kg diet. To increase the immunity of fish and improve the properties of hematological and histopathological indices, the dose can be increased to 0.8% βG without any adverse effects.**

**Key words:** ***Oreochromis niloticus*****, feed intake, FCR, lymphocytes, lysosomes**

Aquaculture is a vital food source worldwide, however, it encounters environmental pressures that impede its progress and development. The industry's rapid growth has led to several challenges, particularly concerning waste and pollution control (Alprol et al., 2021). Additionally, unstable environmental conditions have created favorable conditions for the spread of pathogenic factors that can infect aquatic animals. Aquatic animals are vulnerable to the spread of diseases and infection, often caused by unstable water environmental conditions (Duan et al., 2017; Mahmoud et al., 2019). To ensure the sustainable development of aquaculture and minimize biodiversity impacts, it is crucial to find viable solutions to the pressures present in the aquaculture environment (Awad and Awaad, 2017; Goda et al., 2023). Feed can make up 50–60% of the total costs of aquaculture production. Therefore, aquaculture feed management has a vital role because fish feed that meets fish feed standards, promotes growth, and appropriate feed diversion rations are directly linked to economic and environmental sustainability (Abdelrhman et al., 2022; Aboseif et al., 2022; Mabrouk et al., 2022).

Nile tilapia is a highly important freshwater fish that is cultivated in various regions worldwide, adapting to a wide range of geographical, climatic, and technological conditions (Van Doan et al., 2019). However, due to its widespread prevalence, this species is vulnerable to numerous stressors that can result in diseases, posing a significant threat to the health of the fish (Abdel-Daim et al., 2019; Apino et al., 2022). Nile tilapia is the most commonly cultured species in Egypt, but it is susceptible to a variety of bacterial diseases that are prevalent in freshwater environments, especially during times of stress. Semi-intensive Nile tilapia production has been

experiencing acute mortality, particularly during the summer months (Elsheshtawy et al., 2019). According to Abbas et al. (2022), the summer mortality of Nile tilapia is caused by a combination of factors. These include poor water quality, bacterial pathogens, and weakened immune systems in the fish, which together contribute to the onset of mortality. Specialized feed composition is an effective and preferred strategy to reduce the harmful effects of diseases, pathogen infections and control disease outbreaks of aquatic animals (Awad and Awaad, 2017; Goda et al., 2023, Doan et al., 2024). Using bioactive compounds derived from medicinal plants is a promising protocol acting as growth promoters and immunostimulants to boost immune system response and cope with the deleterious effects of climate change (Doan et al., 2024). Beta-glucan (βG), a yeast cell wall component and glucose polymer, is a popular bioactive compound supplement for fish as it stimulates both innate and adaptive immune systems in most aquatic animals (Pilarski et al., 2017; Abd El Hakim et al., 2019). Furthermore, βG can reduce intestinal inflammation by enhancing local immunity, which improves resistance to infectious diseases and toxicity (Petit and Wiegertjes, 2016; Abd El Hakim et al., 2019). βG's benefits include overall antioxidant, anti-inflammatory, immune-stimulating, antimicrobial, and microbiota-regulating effects (Ji et al., 2017). Also, beta-glucan has been shown to enhance the immune system and welfare of Nile tilapia when exposed to fipronil toxicity (Abd El Hakim et al., 2019). Furthermore, Kamilya et al. (2006) found that βG supplementation can boost carp, *Catla catla* antibody production following an *Aeromonas hydrophila* challenge. Recently, Elmowalid et al. (2023) and Rodrigues et al. (2020) have shown that incorporating βG into the diet of *Labeo rohita* fingerlings can enhance their feed conversion ratio, growth, survival, and immunity against infection in several ways, including increasing resistance to infections and environmental stress. Moreover, high incorporation of βG have been shown to harm the gut bacteria population in some fish species. βG concentration correlates negatively with the quantity of *Vibrio* sp. in pompano fish, *Trachinotus ovatus* (L. 1758) (Do Huu et al., 2016). For tinfoil barb, *Barbonymus schwanenfeldii* (Bleeker, 1854) inclusion of βG in the diet at 1% resulted in reduced bacterial diversity in the gut compared with those fed at 0.1% (Jung-Schroers et al., 2019). In contrast, βG did not impact the species diversity of microbiota populations in the gut of Nile tilapia, *Oreochromis niloticus* (Ran et al., 2015). Thus, βG represents a mechanism capable of both directly and indirectly affecting fish health and survival by modulation of the immune response and gut bacterial population. Further deep research is necessary to investigate the effects of beta glucan on physiological and histological changes in tilapia fry during the summer months. This study aims to determine the impact of various multiple dietary βG levels (0, 250, 500, 1000, 2000, 4000, and 8000 mg/kg diet) on growth performance, feed utilization, innate immunity, and histological structure of the liver and intestine of Nile tilapia fry during the summer season, as limited information is currently available.

## Material and methods

### Culture technique

An experimental grow-out was conducted for 120 days at the National Institute of Oceanography and Fisheries (NIOF) Experimental Farm in Cairo, Egypt.

### Experimental fish and spawning

Nile tilapia brooders were carefully spawned at the National Institute of Oceanography and Fisheries (Qanater Khaireya, Egypt) (Helal et al., 2020). Subsequently, the fry was collected and acclimated to experimental conditions for one week before the feeding trials. Once the yolk sac was fully absorbed, the fries were randomly placed in a cement pond with a capacity of 8 $m^3$. During a 21-day incubation period, monosex Nile tilapia were fed with commercial diets containing 32% CP and 18 kJ/kg gross energy. The diets were supplemented with 60 mg/kg 17 α-testosterone. The fish were fed 3% of their body weight for 7 days a week, with the daily ration divided into five equal amounts and offered five times a day (09:00, 11:00, 13:00, 15:00, and 17:00 h). After 21 days, random samples of the fish from the cement pond were weighed to determine the initial body weight (IBW) for the study.

A total of 840 Nile tilapia fry, with an average weight of 0.2±0.01 g, were stocked into 21 fiberglass tanks, with each tank containing 40 fish and a water capacity of 80 liters. Three replicate tanks were randomly assigned to each treatment. Prior to the start of the experiment, the fish were given a week to acclimate to the experimental conditions, and during this period, they were fed a control diet at a rate of 5% of their body weight. The fish were then given one of the seven experimental diets and were fed this for 120 days. Throughout the experiment, the fish were fed their respective diets at a rate of 3% of their body weight, for six days a week. The daily ration was split into three equal amounts and provided three times a day (at 09:00, 12:00, and 15:00 h). Biweekly, random samples of at least 75% of the fish from each replicate tank were weighed, and the amount of daily allowance was adjusted accordingly. The tanks were supplied with fresh water from a well water source. The water in each tank was replaced by 20% every day and fish were maintained under a natural light cycle (12:12 h light: dark schedule). Water quality was monitored throughout the trial by measuring water temperature, dissolved oxygen, pH, and ammonia and maintaining them within the optimum range for Nile tilapia. Water temperature was recorded daily at 13:00 h using a mercury thermometer suspended at a depth of 30 cm. Dissolved oxygen (DO) was measured at 05:00 h using YSI model 56 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA) and pH at 09:00 h by using a pH meter (Orion pH meter, Abilene, Texas, USA). Ammonia and alkalinity were measured three times a week according to APHA (1998).

**Experimental diets and feeding protocols**

Seven isonitrogenous (30% crude protein) and isocaloric (19.62 Mj/kg diet) diets were formulated (Table 1). Fish were fed the experimental diets at a level of 3% of body weight. The daily diet was divided into three equal amounts and offered three times a day (08:.00, 11:00, and 14:00 h), six days a week throughout the experimental period. Fish were weighed bi-weekly and the amount of daily allowance was adjusted accordingly. All diets were identical except for the variation in βG levels. The basal experimental diet (control diet) had no βG added. Diets 2–7 each contained βG at levels of 0, 250, 500, 1000, 2000, 4000, and 8000 mg/kg diet, respectively (as stated previously by Abd El Hakim et al., 2019). The proximate chemical composition of the basal experimental diet is presented in Table 1. The diets were processed by blending the dry ingredients in a homogenous mixture. A laboratory pellet mill was used to produce 2 mm pellets. The pellets were air-dried for 4 hours (approximately 10% moisture content). Before usage, all diets were wrapped in cellophane bags and cooled to 4°C.

**Proximate composition**

To determine the initial approximate whole-body composition, a pooled random sample of 50 fish was collected, anesthetized with t-amyl alcohol, and sacrificed at the start of the study. Five fish were selected at random from each tank at the end of the feeding trial, anesthetized with t-amyl alcohol, sacrificed, and homogenized in a blender to calculate the final estimated whole-body composition. For each tank, the fish were pooled, oven-dried, ground, and stored at –20°C for analysis afterward. The chemical composition of fish body and diet samples was evaluated using AOAC (2003) procedures. After drying the samples in an oven (105°C) for 24 hours, dry matter was determined. For 12 hours, ash was burnt at 550°C. The crude protein content was measured using the micro-Kjeldahl method, N% 6.25 (using a Kjeltech autoanalyzer, Model 1030, Tecator, Höganäs, Sweden), and the crude fat content was determined using Bligh and Dyer (1959) methodology. Total dietary gross energy (GE) was determined using protein, lipid, and carbohydrate conversion coefficients of 23.7, 39.5, and 17.2 kJ/g, respectively (Brett, 1973).

Table 1. Formulation and proximate composition of the experimental diets (g/kg)

| | Control | ßG$_{0.025}$ | ßG$_{0.05}$ | ßG$_{0.1}$ | ßG$_{0.2}$ | ßG$_{0.4}$ | ßG$_{0.8}$ |
|---|---|---|---|---|---|---|---|
| Fishmeal 60% | 80 | 80 | 80 | 80 | 80 | 80 | 80 |
| Soybean meal 44% | 354 | 354 | 354 | 354 | 354 | 354 | 354 |
| Corn gluten 60% | 80 | 80 | 80 | 80 | 80 | 80 | 80 |
| Yellow corn | 155 | 154.75 | 154.5 | 154 | 153 | 151 | 147 |
| Wheat bran | 280 | 280 | 280 | 280 | 280 | 280 | 280 |
| Soybean oil | 40 | 40 | 40 | 40 | 40 | 40 | 40 |
| β-glucan (g/kg) | 0 | 0.25 | 0.5 | 1.0 | 2.0 | 4.0 | 8.0 |
| NaCl | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Lysine | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Methionine | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Premix* | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | | | **Proximate composition** | | | | |
| Dry matter | 89.71 | 89.71 | 89.71 | 89.71 | 89.72 | 89.72 | 89.74 |
| Crude protein | 30.34 | 30.35 | 30.36 | 30.34 | 30.38 | 30.45 | 30.50 |
| Crude lipid | 6.66 | 6.67 | 6.68 | 6.68 | 6.68 | 6.68 | 6.70 |
| Crude fiber | 5.56 | 5.56 | 5.56 | 5.56 | 5.56 | 5.56 | 5.57 |
| Nitrogen free extract (NFE)** | 51.69 | 51.66 | 51.64 | 51.66 | 51.61 | 51.51 | 51.37 |
| Ash | 5.75 | 5.76 | 5.76 | 5.76 | 5.77 | 5.80 | 5.86 |
| GE KJ /kg*** | 19.62 | 19.62 | 19.62 | 19.62 | 19.62 | 19.62 | 19.62 |

*Premix: each kg of fish premix contained: $MnSO_4$, 40 mg; MgO, 10 mg; $K_2SO_4$, 40 mg; $ZnCO_3$, 60 mg; KI, 0.4 mg; $CuSO_4$, 12 mg; ferric citrate, 250 mg; $Na_2SeO_3$, 0.24 mg; Co, 0.2 mg; retinol, 40,000 IU; cholecalciferol, 4,000 IU; α-tocopherolacetate, 400 mg; menadione, 12 mg; thiamine, 30 mg; riboflavin, 40 mg; pyridoxine, 30 mg; cyanocobalamin, 80 mcg; nicotinic acid, 300 mg; folic acid, 10 mg; biotin, 3 mg; pantothenic acid, 100 mg; inositol, 500 mg; ascorbic acid, 500 mg.

**NFE = nitrogen free extract = 100 – (crude protein + lipid + ash + fiber content).

***Gross energy (GE KJ /kg) was calculated using gross calorific values of 23.63, 39.52, and 17.15 KJ $g^{-1}$ for protein, fat, and carbohydrate, respectively according to Brett (1973).

**Blood samples and hematological analysis**

At the end of the experiment, blood samples were collected. The hematological parameters were analyzed using the method described by AbouShabana et al. (2018). Blood samples were collected using ten fish/replicates. A Neubauer hemocytometer (Marienfeld Superior, Lauda Königshofen, Germany) was used to measure hematocrit (Hct), total red blood corpuscle counts (RBC), and total white blood corpuscle counts (WBC). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined using the method established by Reitman and Frankel (1957). The total glutathione content (GSH) was determined using a slightly modified method developed by Cuesta et al. (2007). The peroxidase activity (unit/ml serum) was calculated by defining one unit of peroxidase as one OD (optical density) change in absorbance. Heparinized as well as EDTA-treated blood (50 µl) was taken in micro-hematocrit capillaries and centrifuged at 12,000 rpm for 5 min to obtain hematocrit value.

**Immune parameters**

The total amount of immunoglobulin-M in the serum was measured using a mouse anti-tilapia IgM monoclonal antibody (Aquatic Diagnostics Ltd., Scotland, UK), according to Swain et al. (2023) protocol. Leukocyte phagocytic function as phagocytic activity was measured using the method described by Cai et al. (2004), and leukocyte respiratory burst was determined using the nitroblue tetrazolium (NBT) assay, with some modifications to the protocol of Glasser and Fiederlein (1990). The lysozyme activity and level in blood serum were determined according to Anderson and Siwicki (1995), by a turbidimetric assay using hen egg white lysozyme (Sigma, St. Louis, MO, USA) in phosphate-buffered saline (PBS) as a standard. The results were presented as lysozyme unit $ml^{-1}$, as reported by Demers and Bayne (1997). The alternative complement activity (ACH50) was evaluated by the method described by Yano (1992) using rabbit red blood cells (RaRBC). The ACH50 units $ml^{-1}$ for each fish were obtained by determining the volume of serum producing 50% hemolysis (ACH50) and plotting the percentage of hemolysis against the volume of serum added, as reported by Asadi et al. (2012).

**Growth indices**

Mean final body weight (FBW) of each experimental treatment, weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV), fat retention (FR) and energy retention (ER) were calculated using the following equations as described previously by Abdelhamid et al. (2020):

WG = final body weight (g) – initial body weight (g); SGR (%/day) = (ln FBW – ln IBW)/t ×100; where: FBW is final body weight (g); IBW is initial body weight (g); ln = natural logarithmic; t = time in days; FCR = feed intake (g)/weight gain (g); PER = weight gain (g)/protein intake (g); PPV = (protein gain (g)/protein intake (g)) ×100; FR = (fat gain (g)/fat intake (g)) ×100 and ER = (energy gain (kJ)/energy intake (kJ)) ×100.

**Histopathology**

Specimens from the intestine and liver were collected and immediately fixed in a 10% buffered neutral formalin solution for 48 hours. The specimens were then dehydrated in ascending grades of ethanol (70–100%), cleared in xylene, and finally embedded in paraffin. Paraffin sections were prepared and then stained with hematoxylin and eosin (H&E) dyes, according to Suvarna et al. (2018). A light microscope was used to examine the histological section and photographs were taken by a digital camera connected to a light microscope.

**Statistical analysis**

The data were subjected to a one-way analysis of variance ANOVA using SPSS 18 (Chicago, IL, USA). Duncan's multiple range test was used to compare differences between treatment means when significant F values were observed (Duncan, 1955), at P£0.05 level. All percentage data were arc-sin transformed before analysis (Zar, 1984). However, data are presented untransformed to facilitate comparisons. Polynomial regression analyses were applied to determine the best regression model between dietary β-glucan levels and the FBW (g). The broken line model reported by Robbins et al. (1979) was used to predict the breakpoints for dietary β-glucan supplementation levels.

## Results

**Growth performance**

Tables 2 and 3 illustrate the data for the growth performance and feed efficiency indices. The data show that supplementing βG to Nile tilapia diets indicates an improvement in fish growth relative to the control group. The final body weight, weight gain, and specific growth rate of Nile tilapia increased as the βG diet was added compared to the control group. The highest protein efficiency ratio, protein productive value, crude protein gain, and gross energy gain values all followed the same pattern (Table 3). The feed conversion ratio values improved significantly ($P \leq 0.05$) in fish fed βG-supplemented diets compared to the control group. According to FBW-based broken-line model analysis (Figure 1), the optimal dietary level of βG associated with the best FCR for Nile tilapia should be 0.12% kg diet. When compared to the control diet group, the addition of βG to tilapia diets had a positive impact on S% values. The $\beta G_{0.8}$ diet recorded significantly lower survival (%) values as compared to other experimental groups.

Table 2. Growth performance and survival (%) of Nile tilapia reared for 120-day experimental treatments

| | Control | $\beta G_{0.025}$ | $\beta G_{0.05}$ | $\beta G_{0.1}$ | $\beta G_{0.2}$ | $\beta G_{0.4}$ | $\beta G_{0.8}$ |
|---|---|---|---|---|---|---|---|
| Initial weight (g) | 0.2±0.01 a | 0.2±0.01 a | 0.2±0.01 a | 0.2±0.01 a | 0.2±0.01 a | 0.2±0.01 a | 0.2±0.01 a |
| Final weight (g) | 31.08±0.27 e | 34.48±0.58 cd | 38.03±0.33 b | 40.37±1.11 a | 35.43±1.14 c | 34.64±0.45 cd | 34.11±0.62 d |
| Gain (g) | 31.06±0.27 e | 34.46±0.59 cd | 38.02±0.32 b | 40.36±1.11 a | 35.42±1.14 c | 34.62±0.44 cd | 34.09±0.63 d |
| SGR | 4.21±0.01 d | 4.29±0.01 cd | 4.37±0.33 ab | 4.42±0.02 a | 4.31±0.31 bc | 4.30±0.33 cd | 4.28±0.35 cd |
| Feed intake (g) | 48.64±0.79 e | 50.37±0.31 c | 50.94±0.08 b | 52.00±0.11 a | 50.21±0.06 c | 51.59±0.34 a | 49.53±0.60 d |
| FCR | 1.58±0.01 a | 1.47±0.02 b | 1.35±0.01 d | 1.29±0.04 e | 1.42±0.03 c | 1.46±0.02 b | 1.46±0.04 b |
| Survival (%) S | 96.50±1.50 c | 94.50±1.50 d | 97.50±1.50 bc | 99.50±0.25 a | 98.50±2.50 a | 99.50±0.25 a | 93.50±1.25 e |

Values represented as mean ± SD (n = 3). Values in the same row with different letters are significantly different (P<0.05).

Table 3. Feed efficiency of Nile tilapia reared for 120-day experimental treatments

| | Control | $\beta G_{0.025}$ | $\beta G_{0.05}$ | $\beta G_{0.1}$ | $\beta G_{0.2}$ | $\beta G_{0.4}$ | $\beta G_{0.8}$ |
|---|---|---|---|---|---|---|---|
| Protein efficiency ratio | 2.33±0.02 d | 2.50±0.03 cd | 2.73±0.02 b | 2.84±0.07 a | 2.59±0.07 c | 2.52±0.04 cd | 2.50±0.07 cd |
| Protein productive value | 31.98±1.07 | 36.09±1.14 b | 38.62±1.23 a | 38.57±1.21 a | 35.18±1.11 b | 33.79±0.49 c | 36.72±1.00 b |
| Energy retention | 22.55±0.54 c | 24.39±0.65 b | 25.29±1.04 a | 24.78±0.85 b | 23.20±0.63 b | 22.19±0.19 c | 24.01±0.44 b |
| Fat retention | 69.29±1.32 b | 70.80±1.02 a | 70.14±1.30 a | 66.70±0.93 | 64.94±2.00 c | 61.77±1.51 d | 66.10±1.21 c |
| Crude protein gain | 4.23±0.08 d | 4.95±0.11 b | 5.36±0.07 a | 5.46±0.10 a | 4.79±0.12 b | 4.62±0.07 c | 4.98±0.08 b |
| Fat gain | 2.01± 0.10 b | 2.13±0.05 a | 2.14±0.19 a | 2.08±0.11 b | 1.95±0.11 c | 1.86±0.08 d | 1.97±0.07 c |
| Gross energy gain | 179.58±1.24 d | 201.24± 4.33 b | 211.01± 2.16 a | 211.21±8.24 a | 190.17±10.04 c | 182.58±8.11 cd | 195.36 ±4.25 b |

Values represented as mean ± SD (n = 3). Values in the same row with different letters are significantly different (P<0.05).

Table 4. The whole-body composition (% on dry matter basis, DM) of Nile tilapia reared for 120 days

| | Control | $\beta G_{0.025}$ | $\beta G_{0.05}$ | $\beta G_{0.1}$ | $\beta G_{0.2}$ | $\beta G_{0.4}$ | $\beta G_{0.8}$ |
|---|---|---|---|---|---|---|---|
| Moisture (%) | 75.83±0.94 a | 75.75±1.33 a | 75.14±1.26 a | 76.13±1.20 a | 76.87±0.88 a | 75.15±1.65 a | 75.51±0.94 a |
| Protein (CP, %) | 55.35±0.63 d | 56.54±2.30 cd | 58.08±1.04 abc | 59.33±1.58 a | 58.81±1.47 ab | 57.65±0.51 abc | 56.98±0.80 bcd |
| Ether extract | 26.89±1.13 a | 25.04±1.44 b | 23.67±0.25 b | 22.37±0.58 c | 22.23±0.24 c | 22.04±0.66 c | 21.66±0.49 d |
| Ash (%) | 17.76±0.62 c | 18.42±0.27 b | 18.25±0.61 b | 18.30±0.19 b | 18.96±0.60 b | 20.31±0.34 a | 21.36±0.12 a |
| Gross energy (KJ/100 g)** | 237.09±2.12 a | 232.56±6.45 b | 230.79±4.45 b | 228.60±6.67 b | 226.82±3.28 c | 223.33±2.75 c | 220.24±7.11 d |

*Each value represents mean ± SD (n = 3). Values in the same rows with different letters are significantly different (P<0.05).

**Gross energy was calculated using gross calorific values of 23.63, 39.52, and 17.15 KJ $g^{-1}$ for protein, fat, and carbohydrate, respectively according to Brett (1973).

Table 5. The hematological parameters of Nile tilapia reared for 120 days

| Treatments | Control | $ßG_{0.025}$ | $ßG_{0.05}$ | $ßG_{0.1}$ | $ßG_{0.2}$ | $ßG_{0.4}$ | $ßG_{0.8}$ |
|---|---|---|---|---|---|---|---|
| RBCs | 1.35±0.07 c | 1.63±0.15 bc | 1.77±0.23 ab | 1.63±0.15 bc | 1.70±0.17 ab | 1.73±0.15 ab | 1.97±0.05 a |
| WBCs | 42.90±0.70 c | 47.76±1.31 c | 48.80±2.54 c | 64.0±2.00 b | 69.33±1.72 ab | 75.00±1.00 a | 74.00±2.00 a |
| Hemoglobin | 10.60±0.00 c | 12.37±0.98 ab | 11.73±1.28 bc | 13.73±0.36 a | 12.46±0.25 ab | 12.73±0.23 ab | 12.76±0.32 ab |
| Hematocrit | 15.85±0.77 c | 16.83±2.38 c | 20.90±2.85 b | 25.90±0.30 a | 24.86±0.49 a | 26.56±0.41 a | 27.03±0.15 a |
| Lymphocytes | 72.00±1.41 c | 79.66±1.52 bc | 77.33±4.72 bc | 85.00±2.00 b | 85.33±1.85 b | 97.00±1.00 a | 95.00±1.00 a |
| Platelet count | 60.00±5.65 d | 116.33±1.02 cd | 128.00±1.31 cd | 202.67±8.65 bc | 267.33±9.88 ab | 327.00±2.65 a | 343.00±4.35 a |

Values represented as mean ± SD (n = 3). Values in the same rows with different letters are significantly different ($P<0.05$).

Table 6. Biochemical parameters, concentration of alanine aminotransferase (ALT), aspartate aminotransferase (AST) (U $l^{-1}$), and glutathione (GSH) in control and treated fingerlings of Nile tilapia after 120 days of experiment

| Treatments | Control | $ßG_{0.025}$ | $ßG_{0.05}$ | $ßG_{0.1}$ | $ßG_{0.2}$ | $ßG_{0.4}$ | $ßG_{0.8}$ |
|---|---|---|---|---|---|---|---|
| ALT | 42.60±2.08 a | 41.20±0.34 ab | 40.50±0.70 b | 36.95±0.07 c | 42.17±0.28 ab | 41.17±0.28 ab | 42.23±0.32 ab |
| AST | 35.63±2.56 a | 35.13±0.15 a | 33.60±0.20 a | 29.95±0.07 b | 34.26±0.02 a | 34.10±0.10 a | 34.26±0.25 a |
| GSH | 15.41±0.24 d | 15.36±010 d | 15.13±0.03 d | 15.33±0.06 d | 17.95±0.06 c | 19.48±0.27 b | 20.48±0.31 a |

Values represented as mean ± SD (n = 3). Values in the same rows with different letters are significantly different ($P<0.05$).
ALT = alanine transferase, AST = aspartate transferase, GSH = glutathione.

Table 7. Immunological parameters of Nile tilapia fingerlings after 120 days of experiment

| Treatments | Control | $ßG_{0.025}$ | $ßG_{0.05}$ | $ßG_{0.1}$ | $ßG_{0.2}$ | $ßG_{0.4}$ | $ßG_{0.8}$ |
|---|---|---|---|---|---|---|---|
| Lysosomes | 182.50±0.70 c | 199.60±1.53 c | 237.00±2.00 b | 240.00±6.24 b | 266.67±7.85 b | 366.63±4.5 a | 380.67±1.00 a |
| Respiratory burst | 14.50±0.70 d | 16.13±1.03 cd | 18.60±3.53 cd | 19.83±4.56 c | 25.23±0.32 b | 34.56±0.49 a | 33.66±3.21 a |
| ACH50 | 25.00±0.00 d | 28.53±0.47 d | 35.33±0.30 c | 49.27±6.88 b | 53.83±7.87 b | 62.46±4.73 a | 65.33±0.49 a |
| Lysozyme | 24.50±0.70 c | 39.40±0.53 b | 41.33±0.41 b | 70.17±5.70 a | 72.93±1.38 a | 80.36±0.32 a | 75.23±0.20 a |
| IgM | 3.37±0.15 d | 3.46±0.40 d | 3.60±0.10 cd | 3.66±0.15 cd | 3.70±0.10 cd | 3.93±0.15 bc | 4.25±0.70 a |

Values represented as mean ± SD (n = 3). Values in the same rows with different letters are significantly different ($P<0.05$).

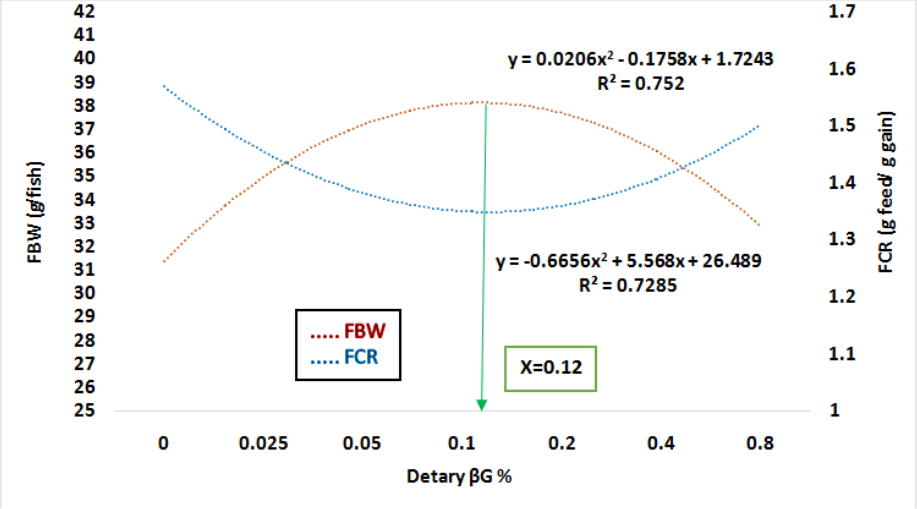


Figure 1. Broken line analysis of dietary beta-glucan supplementation level (g/kg diet) in Nile tilapia fed graded levels of dietary beta-glucan. Values of the X-axis are the beta-glucan % in the experimental diets. Values are means ± SD of 3 replicates

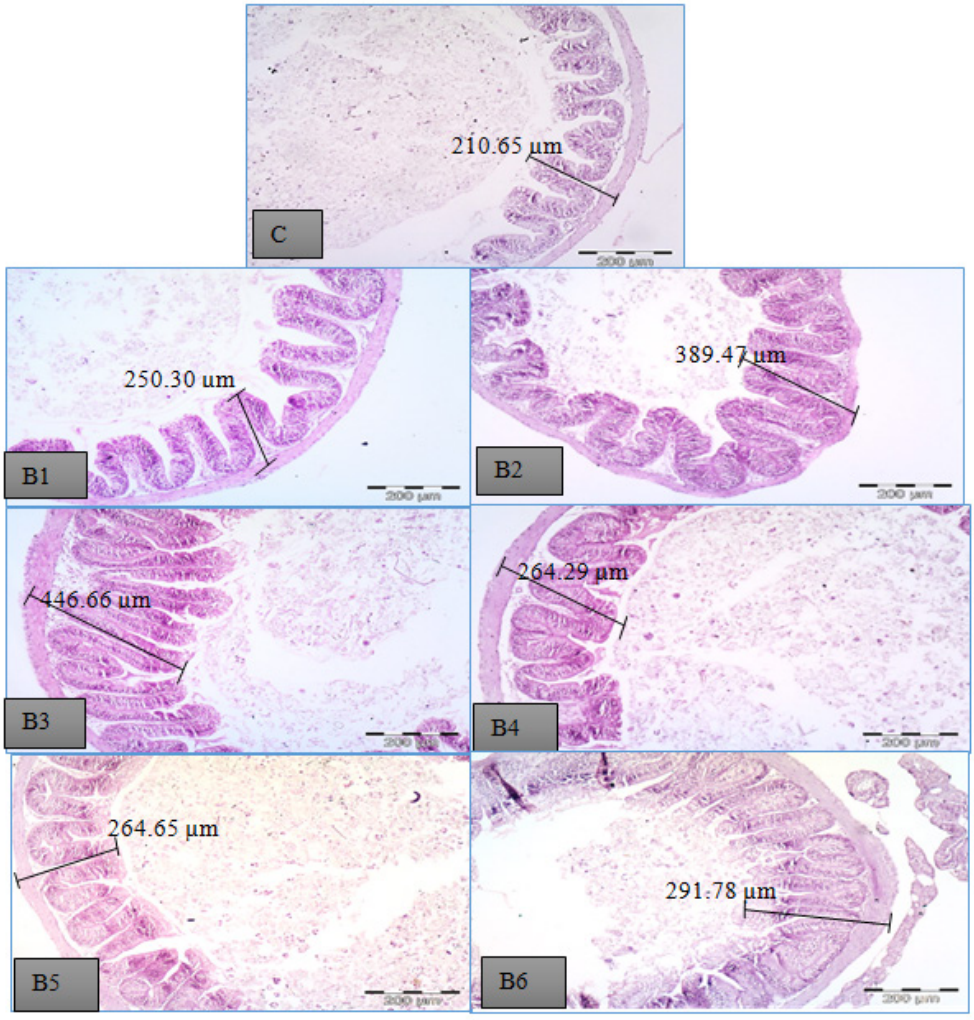


Figure 2. The histological examination of the intestinal villi (10X) revealed height of villi in the examined groups, (C) control group, BG treated groups (B1, B2, B3, B4, B5, and B6). The longest villus height was recorded in B3 followed by B2 and B6, respectively

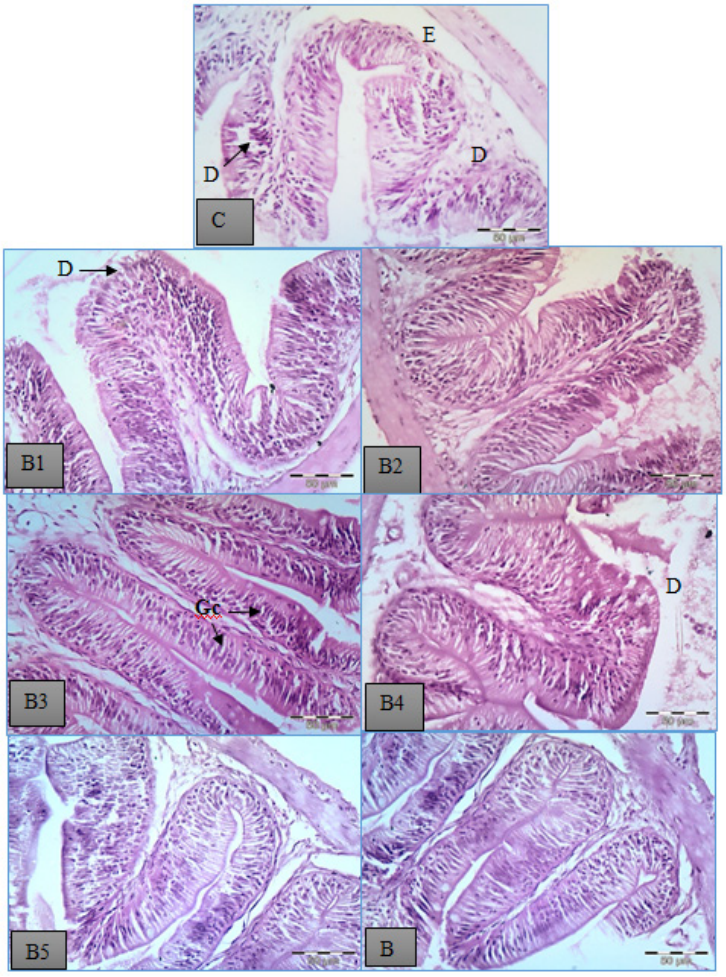


Figure 3. The histological examination of the intestinal villi (40X) revealed (c) the control group showed degeneration (D) in lamina propria and superficial epithelium and edema (E) in between the lamina propria and tunica muscularie. On the other hand the BG treated groups showed normal structure, whereas (B3) BG3 group showed the most intact walls and villi with normal lining epithelium and increased number of goblet cells (Gc)

**Proximate body composition**

Table 4 shows the whole-body composition of the different experimental fish. Increasing β-glucan supplementation levels resulted in significantly higher crude protein content. Nile tilapia fry fed a $\beta G_{0.1}$ diet recorded the highest whole-body crude protein content (59.33%), which was significantly ($P \leq 0.05$) higher than the rest of the treatments. Dietary $\beta G$ significantly reduced lipid content in the whole body of tilapia in comparison to those fed a control diet. A highly positive correlation ($R^2 = 0.99$) with an inverse relationship was observed between the different levels of βG in the diets and the body percentage of ether extract, ash ($R^2 = 0.97$), and gross energy contents (kj/kg fish, $R^2 = 0.99$).

**Hematological parameters**

All hematological parameters increased ($P \leq 0.05$) in all experimental treatments as compared to the control group. Total RBC count and hematocrit increased in all treated groups, and the highest results were reported in $\beta G_{0.8}$, $\beta G_{0.05,}$ and $\beta G_{0.4}$, respectively. Similarly, total WBC counts and the lymphocyte count were significantly higher in $\beta G_{0.4}$ and $\beta G_{0.8}$ groups than in all other groups; however, platelets were higher in treated groups than in controls, with the highest levels in $\beta G_{0.8}$, $\beta G_{0.4}$, and $\beta G_{0.2}$, respectively (Table 5).

**Serum biochemical and immune parameters**

All βG-treated groups had lower significant ($P \leq 0.05$) ALT levels than the control group. The same pattern was observed in AST levels. In the diet $\beta G_{0.1}$ group, alanine transaminase (ALT) and aspartate aminotransferase (AST) showed a low significant difference ($P \leq 0.05$). In comparison to the control group, the antioxidant activity generated by the content of glutathione (GSH) shows a highly significant difference ($P \leq 0.05$) in the group fed the diet $\beta G_{0.8}$, followed in decreasing order by $\beta G_{0.4}$ and $\beta G_{0.2}$ groups (Table 6). The total lysosome count and IgM levels were significantly higher in the βG-treated groups than in the control group, with the highest levels of total lysosomes and lysozymes recorded in $\beta G_{0.8}$ and $\beta G_{0.4}$ groups. The highest level of IgM was reported in the $\beta G_{0.1}$ group. The

results for respiratory burst and ACH50 were substantially equal, with the highest significant values obtained in $\beta G_{0.4}$ and $\beta G_{0.8}$ groups, followed by other treated groups that were higher than the control group (Table 7).

**Histological examination**

The intestines and liver of the seven groups were examined to identify if there were any histopathological alterations. The histological examination of the intestinal villi revealed that the control group showed degeneration (D) in lamina propria and superficial epithelium and edema (E) in between the lamina propria and tunica muscularis. The $\beta$G-treated groups, on the other hand, had longer lengths and normal structure, whereas the $\beta G_{0.1}$ group had the most intact walls and villi with normal lining columnar epithelium, and the intestinal villi appeared long and crowded, with an increase in the number of goblet cells (Figures 2 and 3). The villus height revealed that all βG-treated groups exhibited higher villus height than the control group, with $\beta G_{0.1}$, $\beta G_{0.05}$, and $\beta G_{0.8}$ (446.95, 383.26, and 272 µm, respectively) as shown in Table 8. Regarding histological analysis of the liver, the control group had fatty degeneration (Fd) of hepatic cells and pyknosis (P), while the $\beta G_{0.025}$ group showed degeneration (D) and necrosis (N) of the hepatic cell wall. $\beta G_{0.05}$ group showed degeneration (D) and hemorrhage (Hr) with erythrocyte infiltration, while $\beta G_{0.1}$ showed more intact hepatic tissue with slight erythrocyte infiltration (e), and $\beta G_{0.2}$ showed intact tissue with slight degeneration (D) and hemolysis (Hl) in blood sinusoids. $\beta G_{0.4}$ group exhibited some congested (c) blood sinusoids as well as erythrocyte infiltration (e), while $\beta G_{0.8}$ showed intact tissue with little deterioration (Figure 4).

Table 8. Villus height of the intestine of Nile tilapia after 120 days of experiment

| Treatment | Villus height (µm) |
|---|---|
| Control | 210.65±6.99 e |
| $\beta G_{0.025}$ | 250.30±0.67 d |
| $\beta G_{0.05}$ | 389.47±8.77 b |
| $\beta G_{0.1}$ | 446.66±0.40 a |
| $\beta G_{0.2}$ | 264.29±0.51 c |
| $\beta G_{0.4}$ | 264.65±0.47 c |
| $\beta G_{0.8}$ | 291.78±0.06 c |

Values represented as mean ± SD (n = 3). Values in the same rows with different letters are significantly different (P<0.05).

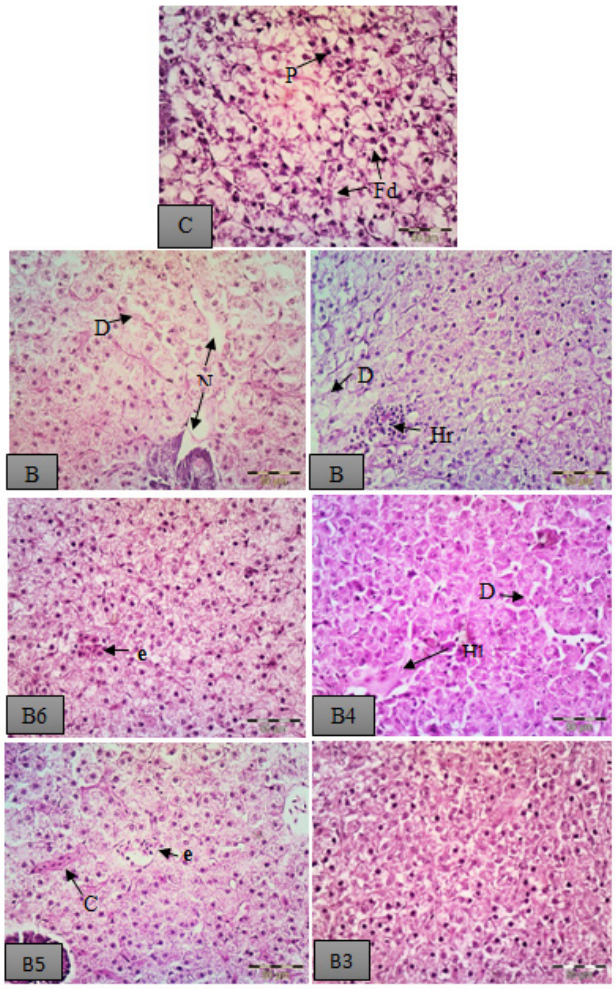


Figure 4. The histological examination of the liver revealed (C) the control group, fatty degeneration (Fd) and pyknosis (P). (B1) BG1 group, degeneration (D) and necrosis (N). (B2) BG2 degeneration (D) and hemorrhage (Hr). (B3) BG3, erythrocyte infiltration (e). (B4) BG4 degeneration (D) and hemolysis (Hl) in blood sinusoids. (B5) BG5 congestion (c) and erythrocyte infiltration (e). (B6) BG6 revealed intact tissue

## Discussion

Efficient feeding is an emerging practice in the aquaculture sector that strives to provide balanced nutrition diets supplemented with feed additives to boost the growth, health and disease resistance of cultured fish (Sharawy et al., 2022; Elmowalid et al., 2023). Recently increasing attention has been focused on the use of functional dietary supplements, such as βG as growth promoters and immunostimulants, in aquaculture. The importance of dietary βG for Nile tilapia (*Oreochromis niloticus*) growth is highlighted in the current study. The present feeding trial findings showed that the growth, feed efficiency, immune status, and survival of Nile tilapia *O. niloticus* fry improved significantly when fed a diet enriched with βG in comparison with the control group ($P \leq 0.05$). The present results are consistent with Dawood et al. (2020 a) and Khanjani et al. (2021) who found that inclusion of βG improved growth performance and feed efficiency of Nile tilapia fed diets supplemented with βG. Furthermore Rodrigues et al. (2020) and Khanjani et al. (2021) reported that inclusion of βG in African catfish diets improved the growth performance, feed utilization efficiency and immune system response toward pathogen infection and disease outbreaks. In addition, the results of the present study were similar to those reported that dietary βG could boost growth and survival rate for several other species, such as koi carp, *Cyprinus carpio koi* (0.2% of diet) (Lin et al., 2011), large yellow croaker, *Pseudosciaena crocea* (Ai et al., 2007) (0–0.18% of diet), rainbow trout, *Oncorhynchus mykiss* (0–0.2% of diet) (Ji et al., 2017), and river prawn *Macrobrachium nipponense* (0–1.0% of diet) (Tian et al., 2023). In addition, Hoseinifar et al. (2015) found that using different levels of dietary *Lactobacillus acidophilus* as feed supplement proved its beneficial effects on mucosal immune parameters, intestinal microbiota, stress resistance and growth parameters of black swordtail and the appropriate inclusion is $6 \times 10^8$ CFU $g^{-1}$. Moreover, Ahmadifar et al. (2015) found that inclusion of *Lactobacillus fermentum* (LF) and/or ferulic acid (FA) in common carp (*Cyprinus carpio*) diets revealed that LF and/or FA can be used as beneficial feed additive to improve growth, immune responses and disease resistance in early stages of common carp culture. Alike, Ahmadifar et al. (2022) reported that using cornelian cherry (*Cornus mas* L.) fruit extract at the rate of 0, 0.25, 0.5 and 1% improved growth performance, feed conversion ratio, immune responses, and disease resistance in common carp (*Cyprinus carpio*) and the appropriate inclusion level is 0.5–1% to enhance growth performance and relieve the impacts of *A. hydrophila* infection in common carp. Following the same patterns, Sarhadi et al. (2020) found that addition of artemisia (*Artemisia annua*) leaves extract (ALE) improved growth and feed conversion ratio and immune parameters as skin mucus total protein, lysozyme and respiratory burst activity. Hence, it can be concluded that ALE has considerable potential as a natural immunostimulant and growth promoter supplement for the common carp. However, the presents results are inconsistent with Whittington et al. (2005), Shelby et al. (2009), Barros et al. (2015), Hong-Bo et al. (2018), Yamamoto et al. (2018), Amphan et al. (2019), Xu et al. (2020), Koch et al. (2021) and Dou et al. (2023) who found that administration of βG had no positive impacts on growth and feed efficiency.

In the present study, regarding broken line analysis of dietary βG supplementation level (mg/kg diet), final body weight increased linearly in response to dietary βG level, and the breakpoint was observed in FBW at 0.12% dietary βG for optimum growth associated with optimum FCR. Based on FBW-broken-line model analysis the βG requirement for Nile tilapia (*O. niloticus*) should be between 100 mg/kg diet (0.1%) and 120 mg/ kg diet (0.12%). In line with the present study, dietary βG requirements of *O. niloticus* have been reported to be 0.1% of diet by Welker et al. (2012), 0.4% of diet by Abd El Hakim et al. (2019), 0.1% of diet by Abdelhamid et al. (2020), 0.01–0.1% of diet by Dawood et al. (2020 b), and 0.01% of diet by Fath El-Bab et al. (2022) based on the growth performance. The wide variation observed in the requirements for βG among fish species may be due to the improvements in the feed intake associated with βG feeding (Welker et al., 2012), the ability of tilapia to prevent infection, and resist diseases (Abd El Hakim et al., 2019), the intestinal digestive functions (Fath El-Bab et al., 2022), the degradation of βG by glucanase to produce energy (Medri et al., 2000) and diet formulation, size and age of fish, genetic differences, feeding practices and rearing conditions. Adequate βG dosage and source are essential for providing maximum benefit to each fish species, resulting in diverse results. To optimize the amount and frequency of βG supplied to specific fish species, extensive research should be undertaken before application (Ching et al., 2021). The findings indicated that the fish fed diets supplemented with βG groups exhibited the best feed conversion ratio and higher protein efficiency ratio, protein productive value, fat gain, and gross energy gain values compared to the control group. The enhanced performance could be attributed to β-glucan's ability to increase feed intake by stimulating feed palatability, resulting in improved feed efficiency. Glucanases can break down βG in the digestive gland, providing energy and enabling increased protein intake for growth and feed utilization, and could be an effective approach to enhance fish performance. Beta-glucanase most likely degraded β-glucan in the digestive gland to give energy, allowing for increased protein consumption for feed utilization and growth (López et al., 2003; Wang, 2011; Pilarski et al., 2017).

The biochemical composition of fish, such as protein, lipid, carbohydrates, and fatty acids, is frequently used to assess their nutritional value and overall fish health (Abo-Taleb et al., 2020). The present study demonstrated that dietary βG-supplementation significantly affected the body composition of tilapia. Supplying βG to tilapia diets increased protein content as well as decreased total body

fat and gross energy contents compared to the control group. The present results agree with Mills et al. (2003) who suggest that βG-supplemented diets could inhibit lipogenesis by reducing insulin sensitivity in adipocytes, reducing body fat. Furthermore, Huang et al. (2016) found that fish fat decrease leads to enhanced lipolysis through β-receptor activation and β-adrenergic agonist effects on adipose tissue. Following the same patterns, Aoe et al. (2017) found a decrease in body weight and visceral fat area (VFA) with barely high βG. Furthermore, β-glucan lowers white adipose tissue accumulation in obese rats by boosting the activity of hepatic transferase acetyl CoA carboxylase, fatty acid synthase, carnitine palmitoyl, and decreasing the activity of epididymal fat lipoprotein lipase (Lee et al., 2017). These results suggest that βG may thoroughly reduce lipid levels and regulate specific lipid metabolic enzymes to inhibit adipogenesis and reduce fat accumulation. In opposition to the foregoing findings, several investigations found that βG administration did not affect protein and lipid content in rainbow trout, *Oncorhynchus mykiss* (Sealey et al., 2008), turbot, *Scophthalmus maximus* (Fuchs et al., 2015), and Nile tilapia (Selim and Reda, 2015).

Hematological measurements can track fish health, nutritional, and environmental condition that affect striped bass hematology (Fazio et al., 2019). Feed additives are frequently considered to be the main cause of increased or decreased hematological and biochemical variables in fish relative to normal values (Femi-Oloye et al., 2020). The present study found that all groups treated with βG had significantly higher levels of RBC, hemoglobin, WBC platelets count, and lymphocytes. The present results are consistent with Elmowalid et al. (2023) who found that supplementing fish with β-1,3-glucan led to a significant increase in target blood measures, potentially indicating nonspecific immune stimulation. In addition, Nawaz et al. (2018) found that prebiotics have revealed potential as treatment for numerous illnesses in fish via enhanced gut immune system and stimulated beneficial gut microbial population, and production of intermediate metabolites, for example short chain fatty acids (SCFAs) that support immune system. Furthermore, Fath El-Bab et al. (2022) discovered that feeding βG to fish, either individually or in combination, results in considerably higher red blood cell, hemoglobin, WBC, and lymphocyte concentrations. Similarly, monocytes and neutrophils increased in spotted rose snapper, *Lutjanus guttatus* fed 0.05% and 0.1% βG, respectively (Del Rio-Zaragoza et al., 2011). In contrast, some investigations found that β-glucan had no significant effect on the RBC and erythrocyte indices of channel catfish, *Ictalurus punctatus* (Welker et al., 2007), beluga juveniles, *Huso huso* (Hoseinifar et al., 2011), and white fish, *Rutilus frisii kutum* (Rufchaie and Hoseinifar, 2014).

Lysozyme activity is linked to fish immunity against gram-positive and -negative pathogenic bacteria cell wall damage (Saurabh and Sahoo, 2008). The study revealed that the control group had lower lysozyme activity, while those who received dietary βG had higher activity. The present results agree with Fath El-Bab et al. (2022) who reported that βG-supplemented diets, either independently or in combination with other additives, boost cellular immune activity, improve epithelial barrier function, increase intestinal mucosal adherence, or inhibit pathogen adhesion and innate immune system regulation. The study demonstrated that fish fed diets $\beta G_{0.1}$ and $\beta G_{0.8}$ exhibited the highest levels of IgM. Uribe et al. (2011) and Medagoda et al. (2023) noticed that oral administration of βG enhanced immunoglobulin in fish via binding to βG receptors on macrophages, neutrophils, leukocytes, and NK cells. Rodrigues et al. (2020) showed that binding βG to receptors enhances immunological activity, including phagocytosis and immunoglobulin production. According to Fath El-Bab et al. (2022), feeding fish with βG increases total protein and immunoglobulin levels in the bloodstream.

Our study indicated that the $\beta G_{0.4}$ and $\beta G_{0.8}$ groups had the highest respiratory burst and alternative complement activity (ACH50) values. The present results are consistent with Medagoda et al. (2023) who found that inclusion of βG in olive flounder diets enhances respiratory burst and complements activity. Furthermore, AbouShabana et al. (2018) found that using intraperitoneal injection of beta-glucan and mannan oligosaccharides derived from *Saccharomyces cerevisiae* showed a significant increase in leukocyte count and hematocrit percentage and significant enhancement in the immune assay as phagocytic, respiratory burst, and lysozyme activities of sea bream. The high activity of liver enzymes aspartate transaminase (AST) and alanine transaminase (ALT) is mainly caused by damaged or impaired liver tissue (Lu et al., 2013). ALT and AST are used to check the level of proteins secreted by the liver during toxicity (Racicot et al., 1975). In the present study, ALT and AST tended to decrease in the case of fish exposed to highly concentrated beta-glucan compared to control as described earlier in several fish species (Adel et al., 2017).

Histopathological characteristics are widely used as indicators to assess fish health following exposure to toxic substances that produce inflammatory alterations. Water-borne toxins harm intestinal tissue, where they can be absorbed and accumulate in cells, resulting in toxicological effects such as inflammation (Ahmed et al., 2019). The liver is important for detoxifying toxic substances and can be severely harmed, leading to loss of function (Rochman et al., 2013). In the current study, histological examination of intestinal villi and liver tissue showed some histopathological lesions in the control group. However, the groups fed BG had improved tissue structure, with normal and intact tissue, particularly $\beta G_{0.1}$ and $\beta G_{0.8}$, which were the most improved groups. Likewise improves liver tissue and restores normal features of fish fed diets supplemented with beta-glucan versus control diets. The present results are consistent with Dawood et al. (2020 b) who found that βG supplementation can improve intestinal structure by increasing villus height,

folding, and lateral branching. Furthermore, Elmowalid et al. (2023) found that dietary βG supplementation improved normal cell structure, which supports our results. In addition, Kiron et al. (2016) discovered that βG feed additives increased the presence of goblet cells and villus height in fish guts. Following the same patterns, Medagoda et al. (2023) found that βG increased intestinal villi height and goblet cell count in Nile tilapia, promoting mucus release from goblet cells and resulting in optimal intestinal absorption.

## Conclusion

The current study investigated the impact of supplementation of Nile tilapia larval feeds with several doses of βG in order to determine the optimal dose recommended for aquaculture purposes. It was concluded according to FBW-based broken-line model analysis, that the optimal dietary supplementation level of βG for Nile tilapia fry should be 0.12% βG/kg to exhibit superior growth and diet utilization efficiency associated with the best feed conversion ratio. This was in line with better blood biochemical, immunological response, and improvement of intestinal structure by increasing villus height, folding, and lateral branching and displaying healthy liver tissue architecture.

## Acknowledgment

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## Ethical approval

All the experimental protocols were approved by the NIOF Committee for Institutional Care of Aquatic Organisms and Experimental Animals.

## Declaration of competing interest

The authors declare no conflict of interest

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