Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Impact of *Spirulina platensis* as a Dietary Supplement on Growth Performance, Blood Biochemical Parameters, and Expression of Growth-Related Genes in Nile Tilapia (*Oreochromis niloticus*)

Hesham Abozaid[1*], Ali S. M. Elnady[1], Dalia M. Aboelhassan[2], Hayam Mansour[2], Abd Elmeged Abedo[1], Hashem M. Abdelrahman[1], Inas S. Ghaly[2], Hasnaa A. Radwan[2], Wafaa T. Abbas[3], Ibrahim M. Farag[2]

[1]*Department of Animal Production, Agricultural and Biology Institute. National Research Centre, Dokki, Giza, 12622, Egypt.*
[2]*Department of Cell Biology, Biotechnology Research Institute, National Research Centre, Giza, Dokki, Giza, 12622, Egypt.*
[3]*Department of Hydrobiology, Veterinary Research Institute, National Research Centre, Dokki, Giza, 12622, Egypt.*

THE present investigation was designed to verify the effect of using *Spirulina platensis (Sp)* on growth performance, blood biochemical parameters, and expressions of growth hormone (GH), growth hormone receptor (GHR), insulin-like growth factor1 (IGF-1), insulin-like growth factor 2 (IGF-2), and myostatin (MSTN) genes in Nile tilapia. A total of 96 fingerlings (12 ± 0.2 g) were randomly allocated into eight groups, each containing 12 fish, and fed diets containing 0%, 1% (10 g), 2% (20 g), and 3% (30 g) SP/kg for 8 weeks. Significant improvements in growth were observed in the 1% SP group, with the best feed conversion rate at 1.09. Biochemical parameters, including serum protein, globulin, liver enzymes (ALT and AST), cholesterol, and glucose concentrations, were analyzed using a spectrophotometer for calorimetric analysis. Higher serum protein and globulin levels were observed in the 1% SP group compared to the control. RT-PCR analysis indicated elevated GH expression in the brain and muscles, along with increased IGF-1 and IGF-2 levels observed in the liver and muscle tissues of the 1% SP group compared to the control and other treatments. However, there was a positive correlation between the 1% SP diet and enhanced MSTN mRNA levels in muscle tissues. Except for higher ether extract (EE) in the 2% SP treatment, there were no significant changes in fish survival or body composition. Overall, this study recommends utilizing 1% SP as a dietary supplement to improve growth, feed utilization, biochemical parameters, and growth-related gene expressions in Nile tilapia.

Keywords: Spirulina, Nile tilapia, growth performance, blood biochemical parameters, growth-related gene expressions.

Introduction

The increasing demand for aquaculture as a primary source of animal protein required for human sustenance [1, 2] has intensified due to the limited biological capacities of natural fish resources and the escalating challenges associated with environmental stressors [3]. Tilapia aquaculture has witnessed exceptional growth in the last few decades [4], with an annual production increase of approximately 13.5% [5]. The rapid growth in production necessitates higher costly inputs, with feeding accounting for about 70% of the total production cost [6]. Consequently, the quest for innovative functional feed supplements remains a significant challenge in tilapia production.

Spirulina platensis (Sp), a photosynthetic filamentous blue-green microalga, harbors diverse biological activities and possesses highly valuable protein content, essential amino acids, vitamins, beta-

*Corresponding author: Hesham Abozaid, E-mail: g_hesham@yahoo.com. Tel.:00201153046771
(Received 23/11/2023, accepted 17/12/2023)
DOI: 10.21608/EJVS.2023.250660.1678

carotene, and various pigment substances [7]. While fish meal and fish oil are known to be nutritious and easily digestible feed sources for farmed fish, their elevated prices and costs make them less favorable [8]. Hence, the search for natural and alternative sources of nutrition to replace expensive animal-derived proteins in fish diets is imperative.

Spirulina has emerged as a significant plant protein source capable of replacing animal proteins in fish feed [9]. It boasts high-quality protein content containing all essential amino acids and is rich in bioactive components such as carotenoids, vitamins, essential fatty acids, minerals, phycocyanin, and antioxidant pigments, displaying anti-inflammatory and antioxidant properties [10, 11].

Additionally, supplementing fish diets with Sp may stimulate the immune system by increasing lysozyme, phagocyte, and skin mucus bactericidal activities, white blood cell count, and regulating cytokine gene expression crucial for the immune system in tilapia leukocytes, acting as signaling molecules [12, 13]. Sp is also recognized for its antimicrobial properties, with its ethanol and methanol extracts being effective against various fish pathogens [14, 15]. Furthermore, the inclusion of Spirulina as a feed additive in fish diets has shown improvements in growth rate, carcass quality, and physiological responses to diseases and stress across various fish species [16, 17].

Various studies have highlighted the positive effects of Sp on fish production, notably influenced by the quantity used as feed additives in aquaculture. Some studies have demonstrated significant improvements in growth performance even with small amounts of Sp in diets [18]. This impact is attributed to the high protein content (about 70%) and lipids (7-16%) in its dry weight [19]. Considering its nutritional value, Sp is considered a potential food source for both animals [20] and humans [21], making it an excellent nutrient and energy source for a wide range of animals [22, 23].

Consequently, this study was conducted to assess the impact of different concentrations of dietary Spirulina on Nile tilapia (*O. niloticus*) fingerlings, focusing on growth performance, feed utilization, body composition, blood biochemical parameters, and the expression of growth-related genes, including GH, GHR, IGF-1, IGF-2, and MSTN."

Material and Methods

Fish and experimental protocol

Ninety-six mono-sex fingerlings (12 ± 0.15 g) were randomly distributed into four treatments, each with two replicates. The subgroups were housed in separate 50 L aquariums within a closed water recirculation system. Daily monitoring of water parameters was conducted after feeding [24]. The experimental design included four diets formulated with varying levels of SP: control T1 (0% SP), T2 (1% SP), T3 (2% SP), and T4 (3% SP), as detailed in Table 1. The proximate composition of the diets and the chemical composition of SP are presented in Table 2. Ingredients were ground, blended, pelleted through a 2 mm die, and stored at -30°C. During the initial 7 days, fish were acclimated to the control diet. Subsequently, they were fed the experimental diets by hand twice daily for 56 days at a rate of 3% of their body weight. Fish weights and feed intake were recorded biweekly. At the end of the 56-day period, measurements were taken for live weight gain, feed efficiency, growth performance parameters, and feed nutrient utilization.

Growth performance and feed utilization

The study evaluated various growth performance metrics and feed utilization parameters. Weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR) were calculated using established formulas [26, 27]. WG was determined by subtracting the initial weight (WI) from the final weight (WT). SGR% was calculated using the natural log of WT and WI divided by the experimental period. Feed intake (FI) was maintained at 3% of body weight daily, and FCR was computed as dry matter intake divided by total gain. Protein efficiency ratio (PER) was determined by dividing total gain by protein intake, following Stuart and Hung's methodology [28]. Protein retention efficiency (PRE) was calculated according to Zehra and Khan [29], expressed as Protein gain divided by Protein intake multiplied by 100.

For further analysis, three fish per treatment were randomly selected and anesthetized using a clove-essence solution. Blood samples were collected from the caudal vein using heparinized syringes and centrifuged to obtain serum, which was stored at -20°C for subsequent chemical analysis.

Chemical body composition analysis

Chemical body composition analysis, including assessment of moisture, protein, lipid, ash, carbohydrate, etc., in fish, was conducted following the guidelines outlined in AOAC [30].

TABLE 1. Feed formulation and proximate chemical analysis of the experimental diets

Ingredients	Control	1% SP	2% SP	3% SP
Protein concentrate	17	16	15	14
Soybean meal	40	40	40	40
Corn	28	28	28	28
Wheat bran	10	10	10	10
SP* (powder)	0	1	2	3
Oil	3	3	3	3
Vit. & Min.**	2	2	2	2
Chemical composition,% on dry matter basis				
OM	87.53	87.71	87.71	87.88
CP	27.42	28.22	28.24	28.50
CF	11.45	11.03	11.59	11.63
EE	3.94	3.90	3.93	3.96
NFE***	44.72	44.56	43.95	43.79
Ash	12.47	12.29	12.29	12.12
GE (kcal/100g)****	415.46	417.25	417.44	418.7

*SP: Spirulina platensis (powder)
**Vit. & min. mixture/kg premix: Vitamin D3, 0.8 million IU; A, 4.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g, riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; pantothenic acid, 4 g; Nicotinic acid, 8 g; folic acid, 0.4 g biotin,20 mg , Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4g; I, 0.4 g, selenium, 0.4 g and Co, 4.8 mg.
***Nitrogen free extract (NFE) =100-(CP+EE+CF+ash).
****GE (Gross energy value) was calculated from their chemical composition, using the factors 5.6, 9.45, 4.00 and 4.00 (k cal/g) for protein, fat, fiber and NFE, respectively [25].

TABLE 2. Chemical composition of *Spirulina platensis*

Component	Content (%)
Moisture	9.00
Component, % on DM basis	
OM	85.35
CP	50.21
EE	2.24
CF)	10.92
NFE	21.98
Ash	14.65

Biochemical analysis

Blood samples were obtained from the fish's caudal vein using a 3 ml syringe after fish anesthesia with clove oil (0.5 ml L−1). The collected samples were transferred into clean, dry centrifuge tubes and left at room temperature until clotting occurred. Subsequently, the tubes underwent centrifugation at 3000 rpm for 15 minutes to separate the serum, which was stored at -80°C until further biochemical analysis.

Biochemical analyses encompassed the estimation of serum total proteins [31], albumin [32], globulin (calculated by subtracting albumin from total protein concentrations), alanine aminotransferase (ALT), aspartate aminotransferase (AST) [33], cholesterol [34], and glucose concentrations [35]. Commercial biochemical kits (Spectrum-diagnostics, Egypt) were used for these assays. Each biochemical parameter was analyzed calorimetrically based on the manufacturer's instructions using an Agilent Cary UV-Vis spectrophotometer (100/300 Series).

Gene expression analysis

RNA extraction

Total RNA extraction was performed from muscle, liver, and brain tissues following the manufacturer's protocol outlined in the TRIzol® reagent kit (Invitrogen, Germany). Initially, 50 mg of tissue was individually homogenized in 750 µL of TRIzol® reagent and incubated at room temperature for 5 minutes to facilitate the dissociation of nucleoprotein complexes within the TRIzol solution. Subsequently, 140 µL of chloroform was added to the homogenized samples, vigorously shaken, and then centrifuged at 12,000 xg for 15 minutes at 4°C.

The resulting RNA present in the aqueous phase was carefully separated, precipitated by adding 600 µL of 100% isopropyl alcohol, and centrifuged at 12,000 xg for 10 minutes at 4°C. The RNA pellet obtained was washed with 70% DEPC ethanol and dissolved in RNase-free water at 60°C. RNA concentration and purity were determined using a nanodrop-1000 spectrophotometer (Thermo Scientific, Rockford, IL, USA) with an absorbance ratio (260/280 nm) ranging between 1.8 and 2.1 for all samples.

To eliminate any potential DNA contamination, all RNA samples underwent DNase I treatment (Invitrogen) before storage at -80°C.

Complementary DNA synthesis

The isolated RNA from the tissues was utilized for the synthesis of complementary DNA (cDNA) using the oligo (dT)15 primer provided in the maxime RT PreMix Kit (iNtRON Biotechnology, Korea, Cat. No. 25081/96 tubes). The reaction volume was set at 20 µL as per the kit instructions. The reverse transcription (RT) reaction took place at 45°C for 60 minutes, followed by RTase inactivation at 95°C for 5 minutes. The synthesized cDNA was stored at -20°C until required for DNA amplification [36, 37]

Determination of mRNA expression using Real-Time PCR

To determine the mRNA expression levels of GH, GHR, IGF-1, IGF-2, and MSTN genes, Real-Time PCR (RT-PCR) was performed using Fast SYBR Green Master Mix (Topreal TM PCR 2X pre Mix, SYBR green with low ROX, enzynomics Korea). The final primer concentration was 500 pmol, and the reaction volume comprised 20 µL [2 µL of cDNA, 1 µL of each Forward and Reverse primer for the aforementioned genes (Table 3), 10 µL of SYBR green master mix, and 7 µL of water]. The Step One Plus instrument (Agilent stratagene mx3000p) was employed, and the enzyme was activated at 95°C for 15 min. The amplification protocol involved denaturation at 95°C for 10s, annealing/extension at 60°C for 15s, and elongation at 72°C for 30s, spanning forty amplification cycles. The β-actin housekeeping gene served for Ct value normalization. Relative quantification of the candidate genes in comparison to the reference (β-actin) gene was calculated using $2^{-\Delta\Delta Ct}$ method [43].

Ethical approval

This study was approved by the Ethics of Medical Research Committee of the National Research Centre, Al Buhouth St., Dokki, Cairo, Egypt (Approval Date: 6.7.2023 / Approval No: 074130723).

Statistical analysis

Statistical analyses were performed, presenting data as mean ± SE. One-way ANOVA was utilized to assess the effects of SP on chemical body composition, gene expression, and biochemical parameters, utilizing SAS [44] (version 17). Duncan's multiple range test was employed to compare mean differences (at a significance level of 5%) [45].

Results

Growth Performance and Feed Utilization

Spirulina platensis supplementation at 1% (T2) significantly influenced the growth of Nile tilapia compared to control (0% SP), 2%, and 3% SP (Table 4). A substantial increase ($P < 0.05$) in body weight gain (BWG) occurred in T2 (2.96±0.56 g) throughout the experimental period, contrasting the decrease observed in T1, T3, and T4 groups (1.25±0.15 g,

2.4±0.06 g, and 1.53±0.02 g, respectively; P < 0.05). T2 also exhibited the highest protein efficiency ratio (PER) at 36.5 compared to T1, T3, and T4 (24.95, 22.00, and 21.90, respectively; Table 4).

A significant increase in feed conversion ratio (FCR) and specific growth rate (SGR) was observed in T2, recording the highest values of 1.09±0.01 and 1.63±0.01, respectively (P < 0.05). Conversely, the lowest FCR value was noted in T4. No significant differences were observed in survival rates (SR) among treatments. Importantly, no statistically significant variations were observed in fish body composition except for ether extract (EE) content in the T3 (2% SP) (Table 5).

TABLE 3. Primer sequences used for gene expression analysis of genes related to growth

Target gene	Accession No	Primer Sequences 5′ to 3′	Reference
GH	M26916	F - CTGTCTGTCTGTCTGTCAGTCGT R- AGAGGAGACGCCCAAACAC	Rentier-Delrue *et al.* [38]
GHR	EF052862	F - CGACCCAGAACCATCACC R - GTCTCCTGACTGAGGGCAAG	Ma *et al*. [39]
IGF-1	EU272149	F - CCCGAACTTCCTCGACTTGA R - CCTCAGCCAGACAAGACAAAAA	Wang *et al*. [40]
IGF-2	EU272150	F - CCCCTGATCAGCCTTCCTA R - GACAAAGTTGTCCGTGGTGA	Wang *et al*. [40]
MSTN	AF197193	F - ACCAGCCCCACCTGAACT R - ATCTGGGACGTGGCTCTCT	Rodgers *et al*. [41]
*β-actin	EU887951	F - ACCCACACAGTGCCCATC R - CAGGTCCAGACGCAGGAT	Monteiro *et al*. [42]

TABLE 4. Mean growth performance and feed utilization ± SE of Nile tilapia in different treatments at the end of 56 days experiment

Treatment	Control	1% SP	2% SP	3% SP
n	12	12	12	12
IW	12.0±0.5^{a}	12.0±1.8^{a}	12.0±1.3^{a}	12.0±0.2^{a}
FW	17.83±8.0^{ab}	25.83±29.5^{c}	19.16±3.0^{b}	16.97±14.7^{a}
WG/day	1.25±0.15^{b}	2.96±0.56^{a}	2.41±0.06^{b}	1.53±0.02^{c}
FCR	1.26±0.02^{b}	1.09±0.01^{a}	1.30±0.01^{b}	1.40 ±0.02^{c}
SGR	1.28±0.01^{b}	1.63±0.01^{a}	1.38±0.01^{b}	1.38 ±0.0^{b}
PER	24.95±2.9^{b}	36.50±10.8^{a}	22.00 ±1.1^{b}	21.90 ±5.5^{b}
SR	88.9±5.6^{a}	87.7±4.8^{a}	88.9±2.8^{a}	90.7±4.8^{a}

TABLE 5. Body composition of Nile tilapia (*O. niloticus*) in different treatments

Treatment	Dry matter	Component, % on DM basis		
		Crude protein	Ether extract	Ash
Control	29.5±0.3^{a}	52.1±2.6^{a}	24.5±2.6^{b}	12.9±0.5^{a}
SP (1%)	28.8±1.3^{a}	50.0±2.5^{a}	26.6±4.9^{b}	11.8±1.6^{a}
SP (2%)	26.8±1.9^{a}	49.2± 0.3^{a}	29.5±0.1^{a}	11.5±1.5^{a}
SP (3%)	28.3±0.3^{a}	52.2±2.3^{a}	26.5±2.0^{b}	11.4±0.8^{a}

Biochemical analyses

The T2 group (1% SP) exhibited a significant (P < 0.05) increase in serum protein and globulin levels compared to the control. Conversely, the T3 (2% SP) and T4 (3% SP) groups showed significantly decreased levels of these parameters compared to the control. Albumin concentration remained consistent across all groups (P > 0.05). In terms of liver function (AST and ALT), the highest values were observed in the T3 group (P < 0.05), followed by the T2 group, compared to the control. Serum glucose concentration significantly increased in the T3 group compared to both the control and the other treatment groups (P < 0.05). However, cholesterol concentration remained unchanged across all treated groups (P > 0.05) (Table 6).

TABLE 6. Blood biochemical parameters of Nile tilapia (O. niloticus) fed different levels of Spirulina

Parameters	Control	1% SP	2% SP	3% SP
Total protein (g/dl)	5.38±0.05^{b}	7.03±0.04^{a}	3.52±0.05^{c}	2.73±0.28^{d}
Albumin (g/dl)	1.63±0.30^{a}	1.86±0.19^{a}	1.79±0.03^{a}	1.40±0.13^{a}
Globulin (g/dl)	3.75±0.26^{b}	5.17±0.19^{a}	1.73±0.06^{c}	1.33±0.20^{c}
Glucose (mg/dl)	55.21±6.45^{b}	78.86±2.74^{b}	80.55±13.9^{b}	140.7±9.13^{a}
Cholesterol (mg/dl)	164.2±10.5^{a}	142.0±11.46^{a}	143.8±14.62^{a}	150.0±10.75^{a}
AST (Unit/l)	131.8±4.25^{bc}	114.3±7.04^{c}	142.1±5.01^{b}	182.6±11.13^{a}
ALT (Unit/l)	34.16± 0.78^{b}	36.36±0.90^{b}	44.08±1.86^{a}	45.30±1.40^{a}

Gene expression analysis

Gene expression in brain tissues

The expression of the GH gene in brain tissues significantly increased in fish fed a diet containing 1% Spirulina compared to the control group (0% SP) and those fed with 2% SP ($P < 0.05$) and 3% SP ($P < 0.01$). Moreover, a notable rise in GH gene expression ($P < 0.05$) was also evident in the 2% Spirulina group when compared to both the control and 3% SP groups (Figure 1).

Gene expression in liver tissues

The expressions of the IGF-1 and IGF-2 genes in liver tissues were significantly higher ($P < 0.01$) in fish fed 1% Spirulina compared to the control, 2% SP ($P < 0.05$), and 3% SP ($P < 0.01$) groups. Additionally, both IGF-1 and IGF-2 mRNA levels exhibited a significant increase ($P < 0.05$) in the group fed with 2% SP in comparison to the control and 3% SP groups (Figure 2).

Gene expression in muscle tissues

In muscle tissues, the expressions of GH, GHR, IGF-1, IGF-2, and MSTN genes were evaluated (Figure 3). The expression of the GH gene significantly increased ($P < 0.05$) in fish fed a diet containing 1% SP compared to both the control group (0% SP) and other Spirulina treatments (2% and 3% SP). Similarly, the level of GHR mRNA showed a significant ($P < 0.01$) increase in the 1% SP group in comparison to the control, 2% SP ($P < 0.05$), and 3% SP ($P < 0.01$) groups. Notably, the 2% SP group displayed a significant ($P < 0.05$) increase in GHR gene expression compared to the control and 3% SP group.

Regarding the expression of the IGF-1 gene, significant elevation ($P < 0.001$) was observed due to the 1% SP treatment compared to the control, 2% SP ($P < 0.05$), and 3% SP ($P < 0.01$) treatments. Additionally, up-regulation ($P < 0.01$) of IGF-1 was detected in the 2% SP group compared to the control and 3% SP ($P < 0.05$) groups.

The expression level of the IGF-2 gene significantly increased ($P < 0.01$) in the 1% SP group compared to the control, 2% SP ($P < 0.05$), and 3% SP ($P < 0.01$) groups. Likewise, the 2% SP group showed a significant ($P < 0.05$) increase in the IGF-2 mRNA level compared to the control and 3% SP groups.

Regarding mRNA expression for the MSTN gene, the highest expression level (4.70) was observed in fish fed 3% SP, while the lowest expression level (0.93) was found in the 1% SP group. Statistical analysis revealed significant differences between the 3% SP treatment and each of the control ($P < 0.01$), 1% SP ($P < 0.01$), and 2% SP ($P < 0.05$) treatments. Furthermore, treatment with 2% SP led to a significant up-regulation ($P < 0.05$) of MSTN compared to the control and 1% SP treatments.

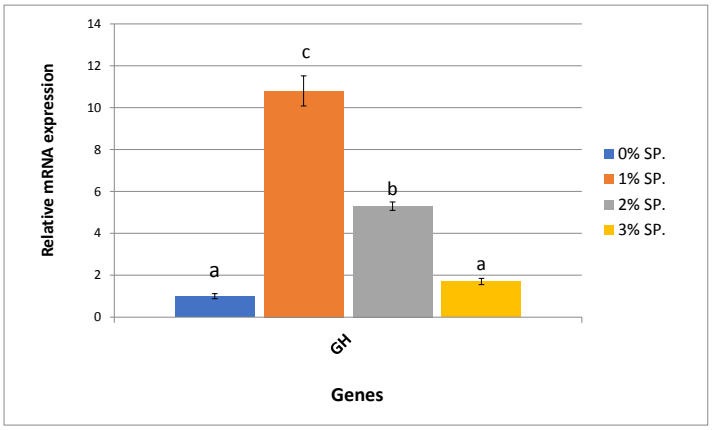

Fig. 1. Relative gene expression levels of GH gene were determined by real-time PCR in brain tissues of fish fed with four Spirulina diets (0%, 1%, 2%, and 3%).

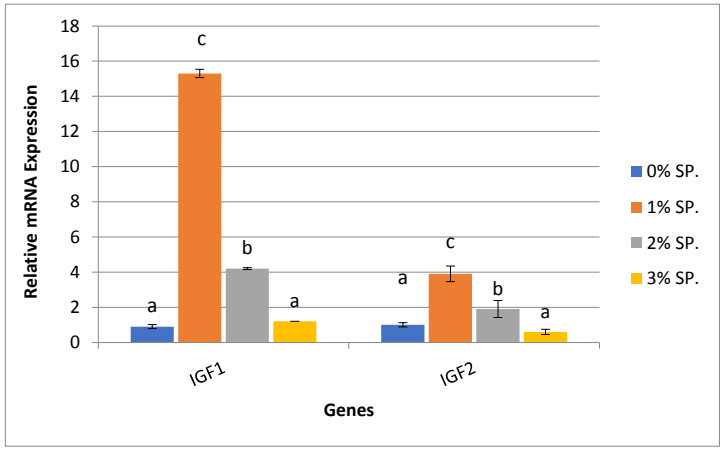

Fig. 2. Relative gene expression levels of IGF-1 and IGF-2 genes were determined by real-time PCR in liver tissues of fish fed with four Spirulina diets (0%, 1%, 2%, and 3%).

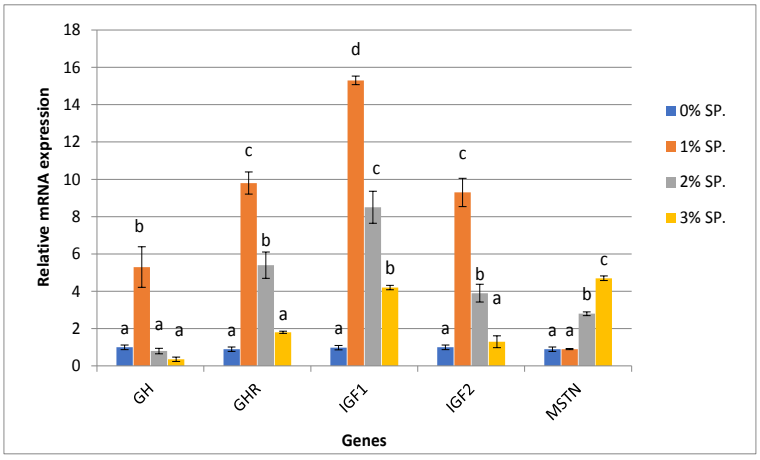

Fig. 3. Relative gene expression levels of GH, GHR, IGF-1, IGF-2, and MSTN were determined by real-time PCR in muscle tissues of fish fed with four Spirulina diets (0%, 1%, 2%, and 3%).

Discussion

Nile tilapia (*O. niloticus*) represents an adaptable and rapidly growing species in various aquaculture systems, with profound implications for human nutrition and economic welfare [12, 46]. The utilization of SP in tilapia diets has been a subject of interest in aquaculture due to its potential as a feed additive or protein source. This study investigated the effects of SP on the growth performance, biochemical parameters, and gene expression in Nile tilapia.

Effects of Spirulina on growth performance

In this study, the highest body weight gain was observed in T2 (1% SP) compared to the control group and the other groups. This result is consistent with Amer [47], who reported that 1% SP was the best level among four levels (0.0, 0.5%, 1%, and 1.5%) in Nile tilapia. Belal *et al.* [48] also found that adding 1% SP to tilapia diets led to significant improvement of average weight gain (AWG), specific growth rate (SGR), and feed conversion ratio (FCR) compared to the control. Güroy *et al.* [49] observed that adding 2.5% SP to yellow tail cichlid fish diet increased the total egg production significantly ($P<0.05$) compared to the control diet. Ramakrishnan *et al.* [50] reported enhancement of SGR, FCR, and AWG by using Spirulina levels ranging from 1 to 3% in common carp diet.

However, other investigations used SP as a protein source and found different results. Velasquez *et al.* [51] found improved growth performance of Nile tilapia fed diets containing 30% Spirulina, and reported that up to 60% of the alga did not induce any adverse effects on growth performance. El-Sheekh *et al.* [52] observed enhanced overall growth performance in hybrid red tilapia (O. niloticus X O. mossambicus) fed Spirulina up to 75% in fish diet. Adel *et al.* [17] showed increased SGR and AWG in great sturgeon (Husohuso) fed 10% Spirulina-enriched feed.

On the other hand, some studies reported no positive effects or even negative effects of Spirulina on growth performance. Olvera-Novoa *et al.* [53] demonstrated that tilapia fed diet supplemented with Spirulina ranging from 20 to 100% of protein source did not show any improvement in growth performance. Ungsethaphand *et al.* [18] showed no growth enhancement in hybrid red tilapia fed Spirulina ranging from 5 to 20%. Adel *et al.* [17] detected no increases in SGR and AWG in Huso huso fish at 2.5% of Spirulina in fish diet. El-Sayed [54] found no significant differences for growth performance between silver sea bream fed 50% Spirulina-enriched diet and those fed control diet, and reported adverse effect on growth performance when the Spirulina level was 75%.

Effects of Spirulina on Biochemical Parameters

Biochemical analyses are important tools to evaluate the physiological status of fish, especially after adding food additives [55]. Liver enzymes (ALT and AST) are biomarkers of hepatic damage and liver function. Total serum protein, albumin, and globulin play essential roles in immunological and nutritional aspects [56]. Moreover, blood glucose level serves as stress-sensitive indicator. Fish exposed to external stressors release hormones such as adrenaline and cortisol, triggering glycogenesis and glycogenolysis pathways, consequently elevating glucose production [57]. In our experimental results, significant alterations were observed in the levels of ALT, AST, total serum protein, and blood glucose across different Spirulina supplementation levels. These changes indicate a potential impact of Spirulina on the liver function, immune system, and stress response of Nile tilapia, shedding light on the physiological changes induced by Spirulina-based diets in aquaculture settings.

Gene expression analysis

The growth trait in farmed fish is intricately regulated by a network of growth-related genes, including somatotropic axis-related genes like GH, GHR, IGF-1, IGF-2, and MSTN, which play pivotal roles in various physiological processes [46, 58-61]. Notably, our study elucidated the modulation of these genes in Nile tilapia (O. niloticus) in response to dietary Spirulina supplementation .

Recent advancements in molecular genetics have emphasized the importance of identifying optimal gene expression levels that govern growth traits in fish. Our findings are in line with studies demonstrating differential regulation of growth-related genes under varying nutritional conditions. For instance, fish fed a 1% Spirulina diet showed significant improvements in GH, IGF-1, and IGF-2 expressions in brain, liver, and muscle tissues compared to the control and higher Spirulina-fed groups [62-66].

Interestingly, while most genes showed upregulation with Spirulina, the MSTN gene exhibited a downregulation, indicating its role as a negative regulator of skeletal muscle development [64]. Similar observations were reported in European sea bass and rainbow trout, where cytokine gene expressions were notably affected by varied Spirulina levels in the diet [65, 67].

However, our study noted an intriguing increase in MSTN gene expression with escalating Spirulina levels in the diet, contrasting previous reports [65]. Such disparities highlight the complexity of gene regulation influenced by dietary components.

Notably, the nutritional content and bioactive components of Spirulina may pose challenges despite its potential as a growth promoter or protein source. While studies have highlighted the benefits of microalgae in reinforcing the immune system and promoting growth in certain fish species, excessive amounts or specific formulations can hinder growth [68-74].

Our experimental results mirrored previous findings that increasing Spirulina levels beyond a certain threshold, from 1% SP to 2% or 3% SP in diets, negatively affected growth parameters. These observations, evident in other fish species, highlight the intricate balance required when incorporating microalgae into fish diets to avoid growth inhibition [72-74]. These insights underscore the significance of meticulous dietary management to harness the potential benefits of Spirulina while avoiding adverse effects on growth in tilapia and other fish species.

Conclusion

The study demonstrates that incorporating Spirulina platensis at a 1% level in Nile tilapia diets significantly enhances growth performance, gene expression, and biochemical parameters. These findings underscore Spirulina's potential as a beneficial feed additive or protein source for O. niloticus fingerlings in aquaculture. However, exceeding this optimal level resulted in detrimental effects on certain physiological aspects. Thus, careful optimization of Spirulina concentrations in fish diets is crucial to leverage its benefits effectively while avoiding adverse impacts.

Competing Interests

The authors declare that they have no financial interests.

Funding statement:

There's no financial/personal interest or belief that could affect the manuscript objectivity.

Acknowledgement

We acknowledge the National Research Centre-Giza, Egypt, for providing the equipment and laboratories that we used to perform this work.

References

1. FAO (Food and Agriculture Organization). Cultured Aquatic Species Information Programme Oncorhynchus mykiss. Food and Agriculture Organization, Fisheries and Aquaculture Department, FAO Publication series (2018).
2. Dawood, M.A. Nutritional immunity of fish intestines: Important insights for sustainable aquaculture. *Reviews in Aquaculture*, **13**(1), 642-663 (2021). https://doi.org/10.1111/raq.12492.
3. de Punder, K. and Pruimboom, L. Stress induces endotoxemia and low-grade inflammation by increasing barrier permeability. *Frontiers in Immunology*, **6**, 223 (2015). https://doi.org/10.3389/fimmu.2015.00223
4. FAO (Food and Agriculture Organization). Climate change implications for fisheries and aquaculture: Summary of the findings of the intergovernmental panel on climate change fifth assessment report, by A.Seggel, & C.De Young. FAO Fisheries and Aquaculture Circular No. 1122. Rome (2016).
5. FAO (Food and Agriculture Organization). FAOSTAT Database, Food and Agriculture Organization, Rome, Italy (2014). http://faostat.fao.org.
6. El-Sayed, A.F.M. ed. Tilapia culture. CABI publishing (2006). https://doi.org/10.1079/9780851990149.0000
7. Ozdemir, G., Ulku Karabay, N., Dalay, M.C. and Pazarbasi, B. Antibacterial activity of volatile component and various extracts of Spirulina platensis. Phytotherapy Research: An *International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, **18**(9), 754-757 (2004). https://doi.org/10.1002/ptr.1541
8. Mo, J., Yang, Q., Zhang, N., Zhang, W., Zheng, Y. and Zhang, Z. A review on agro-industrial waste (AIW) derived adsorbents for water and wastewater treatment. *Journal of Environmental Management,* **227**, 395-405 (2018). https://doi.org/10.1016/j.jenvman.2018.08.069
9. Velasquez, J., Fernández-Lafuente, R., Rodrigues, R.C., Plata, V., Rosales-Quintero, A., Torrestiana-Sánchez, B. and Tacias-Pascacio, V.G. Production and characterization of biodiesel from oil of fish waste by enzymatic catalysis. *Renewable Energy,* **153**, 1346-1354 (2020). https://doi.org/10.1016/j.renene.2020.02.100
10. Christaki, E., Florou-Paneri, P. and Bonos, E. Microalgae: a novel ingredient in nutrition. *International Journal of Food Sciences and Nutrition,* **62**(8), 794-799 (2011). https://doi.org/10.3109/09637486.2011.582460
11. Wu, Q., Liu, L., Miron, A., Klímová, B., Wan, D. and Kuča, K. The antioxidant, immunomodulatory, and anti-inflammatory activities of Spirulina: an overview. *Archives of Toxicology*, **90** (2016), 1817-1840. https://doi.org/10.1007/s00204-016-1744-5
12. Zhang, F., Man, Y.B., Mo, W.Y. and Wong, M.H. Application of Spirulina in aquaculture: a review on wastewater treatment and fish growth. *Reviews in Aquaculture,* **12**(2), 582-599 (2020). https://doi.org/10.1111/raq.12341
13. Youssef, I.M., Saleh, E.S., Tawfeek, S.S., Abdel-Fadeel, A.A., Abdel-Razik, A.R.H. and Abdel-Daim, A.S. Effect of Spirulina platensis on growth, hematological, biochemical, and immunological parameters of Nile tilapia (Oreochromis niloticus). *Tropical Animal Health and Production,* **55**(4), 275 (2023). https://doi.org/10.1007/s11250-023-03690-5

14. Abdel-Tawwab, M. and Ahmad, M.H., 2009. Live Spirulina (Arthrospira platensis) as a growth and immunity promoter for Nile tilapia, Oreochromis niloticus (L.), challenged with pathogenic Aeromonas hydrophila. *Aquaculture Research,* **40**(9), 1037-1046 (2009). https://doi.org/10.1111/j.1365-2109.2009.02195.x

15. Pradhan, J., Das, B.K., Sahu, S., Marhual, N.P., Swain, A.K., Mishra, B.K. and Eknath, A.E. Traditional antibacterial activity of freshwater microalga S pirulina platensis to aquatic pathogens. *Aquaculture Research,* **43**(9), 1287-1295 (2012). https://doi.org/10.1111/j.1365-2109.2011.02932.x

16. Teimouri, M., Amirkolaie, A.K. and Yeganeh, S. The effects of Spirulina platensis meal as a feed supplement on growth performance and pigmentation of rainbow trout (Oncorhynchus mykiss). *Aquaculture,* **396**, 14-19 (2013).https://doi.org/10.1016/j.aquaculture.2013.02.009

17. Adel, M., Yeganeh, S., Dadar, M., Sakai, M. and Dawood, M.A. Effects of dietary Spirulina platensis on growth performance, humoral and mucosal immune responses and disease resistance in juvenile great sturgeon (Huso huso Linnaeus, 1754). *Fish & Shellfish Immunology,* **56**, 436-444 (2016). https://doi.org/10.1016/j.fsi.2016.08.003

18. Ungsethaphand, T., Peerapornpisal, Y., Whangchai, N. and Sardsud, U. Effect of feeding Spirulina platensis on growth and carcass composition of hybrid red tilapia (Oreochromis mossambicus× O. niloticus). *Maejo International Journal of Science and Technology,* **4**(2), 331-336 (2010).

19. Vonshak, A. and Tomaselli, L. Arthrospira (Spirulina): systematics and ecophysioIogy. In The ecology of cyanobacteria: their diversity in time and space (p. 505-522). Dordrecht: Springer Netherlands (2000).

20. Cohen, Z. The chemicals of Spirulina. In: A. Vonshak (Ed.), Spirulina platensis (Arthrospira): Physiology, Cell-biology and Biotechnology. London: 175-204 (1997).

21. Qureshi, M.A., Kidd, M.T. and Ali, R.A. Spirulina platensis extract enhances chicken macrophage functions after in vitro exposure. *Journal of Nutritional Immunology,* **3**(4), 35-45 (1996). https://doi.org/10.3109/08923979609052747

22. Glombitza, K.W. and Koh, M. Secondary metabolites of pharmaceutical potentials. In: R.C. Cresswell, T.A.V. Rees and N. Shah (Eds.), Algal and Cyanobacterial Biotechnology. Logman, Harlow, UK: 161-238 (1989).

23. Kim, S.S., Rahimnejad, S., Kim, K.W. and Lee, K.J., 2013. Partial replacement of fish meal with Spirulina pacifica in diets for parrot fish (Oplegnathus fasciatus). *Turkish Journal of Fisheries and Aquatic Sciences,* **13**(2), 197-204 (2013). https://doi.org/10.4194/1303-2712-v132-01.

24. APHA (American Public Health Association), 1998. Standard methods for the examination of water and waste water. Method 4500-ClO2 E, p.473-479.

25. Jobling, M. A short review and critique of methodology used in fish growth and nutrition studies. *Journal of Fish Biology*, **23**(6), 685-703 (1983). https://doi.org/10.1111/j.1095-8649.1983.tb02946.x

26. We, R. Computation and interpretation of biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada,* **191**, 1-382 (1975).

27. Castell, J.D. and Tiews, K. Report of the EIFAC, IUNS and ICES Working Group on Standardization of Methodology in Fish Nutrition Research, Hamburg, Federal Republic of Germany, 21-23 March 1979. Documents Techniques de la CECPI (FAO) (1980).

28. Stuart, J.S. and Hung, S.S. Growth of juvenile white sturgeon (Acipenser transmontanus) fed different proteins. *Aquaculture,* **76**, 303-316 (1989). https://doi.org/10.1016/0044-8486(89)90083-5

29. Zehra, S. and Khan, M.A. Dietary protein requirement for fingerling Channa punctatus (Bloch), based on growth, feed conversion, protein retention and biochemical composition. *Aquaculture International,* **20**, 383-395 (2012).

30. AOAC, 2005. Association of Official Analytical Chemists. Official methods of analysis. Arlington,VA: Association of Official Analytical Chemists.

31. Cannon, D.C., Olitzky, I. and Inkpen, J.A. Proteins. In: Clinical Chemistry, principles and techniques, 2nd ed., R.J. Henery, D.C. Cannon, J.W. Winkelman (eds.), Harper and Row New York, p: 407-421 (1974).

32. Tietz, N.W. Clinical guide to laboratory tests. 2nd ed. Philadelphia, WB Saunders. p: 566 (1990).

33. Reitman, S. and Frankel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology,* **28**(1), 56-63 (1957). https://doi.org/10.1093/ajcp/28.1.56

34. Ellefson, R.D. and Caraway, W.T. Fundamentals of clinical chemistry. Ed Tietz, N.W. Philadelphia, WB Saunders. p: 506 (1976).

35. Caraway, W.T. and Watts, N.B. Carbohydrates In: Fundamentals of clinical chemistry. 3ry ed. Edited by Tietz, N.W. Philadelphia, WB Saunders. p: 422-47 (1987).

36. Chen, J., Ren, X., Li, L., Lu, S., Chen, T., Tan, L., Liu, M., Luo, Q., Liang, S., Nie, Q. and Zhang, X., 2019. Integrative analyses of mRNA expression profile reveal the involvement of IGF2BP1 in chicken adipogenesis. *International Journal of Molecular Sciences,* **20**(12), 2923 (2019). https://doi.org/10.3390/ijms20122923

37. Wang, Y., Luo, W. and Wang, Y. PARP-1 and its associated nucleases in DNA damage response. *DNA Repair,* **81**, 102651 (2019). https://doi.org/10.1016/j.dnarep.2019.102651

38. Rentier-Delrue, F., Swennen, D., Philippart, J.C., L'hoir, C., Lion, M., Benrubi, O. and Martial, J.A. Tilapia growth hormone: molecular cloning of cDNA and expression in Escherichia coli. *DNA,* **8**(4), 271-278 (1989). https://doi.org/10.1089/dna.1.1989.8.271

39. Ma, X., Liu, X., Zhang, Y., Zhu, P., Ye, W. and Lin, H. Two growth hormone receptors in Nile tilapia (Oreochromis niloticus): molecular characterization, tissue distribution and expression profiles in the gonad during the reproductive cycle. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology,* **147**, 325-39 (2007).

40. Wang, B., Xu, Y., Liu, X., Liu, Q., Liu, Y., Zhang, Y. and Shi, B. Molecular characterization and expression profiles of insulin-like growth factors in yellowtail kingfish (Seriola lalandi) during embryonic development. *Fish Physiology and Biochemistry,* **45**, 375-390 (2019).

41. Rodgers, B.D., Weber, G.M., Sullivan, C.V. and Levine, M.A. Isolation and characterization of myostatin complementary deoxyribonucleic acid clones from two commercially important fish: Oreochromis mossambicus and Morone chrysops. *Endocrinology,* **142**(4), 1412-1418 (2001). https://doi.org/10.1210/endo.142.4.8097

42. Monteiro, S.M., dos Santos, N.M., Calejo, M., Fontainhas-Fernandes, A. and Sousa, M. Copper toxicity in gills of the teleost fish, Oreochromis niloticus: effects in apoptosis induction and cell proliferation. *Aquatic Toxicology*, **94**(3), 219-228 (2009).https://doi.org/10.1016/j.aquatox.2009.07.008

43. Livak, K.J. and Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCt method. *Methods*, **25**(4), 402-408 (2001). doi: 10.1006/meth.2001.1262.

44. SAS (1997). SAS/STAT Guide for personal computer. SAS Inst. Cary, N.C.(ISBN: 3-540-65014-8.Statistical Package for the Social Sciences, SPSS, USA.

45. Duncan, D.B. Multiple range and multiple F tests. *Biometrics,* **11**(1), 1-42 (1955). https://doi.org/10.2307/3001478

46. Herkenhoff, M.E., Ribeiro, A.O., Costa, J.M., Oliveira, A.C., Dias, M.A.D., Reis Neto, R.V., Hilsdorf, A.W.S. and Pinhal, D. Expression profiles of growth-related genes in two Nile tilapia strains and their crossbred provide insights into introgressive breeding effects. *Animal Genetics,* **51** (4), 611-616 (2020). https://doi.org/10.1111/age.12944

47. Amer, S.A. Effect of Spirulina platensis as feed supplement on growth performance, immune response and antioxidant status of mono-sex Nile Tilapia (Oreochromis niloticus). *Benha Veterinary Medical Journal,* **30**(1), 1-10 (2016). https://doi.org/10.21608/bvmj.2016.31332

48. Belal, E.B., Khalafalla, M.M.E. and El-Hais, A.M.A. Use of spirulina (Arthrospira fusiformis) for promoting growth of Nile Tilapia fingerlings. *African Journal of Microbiology Research*, **6**(35), 6423-6431 (2012). https://doi.org/10.5897/AJMR12.288

49. Güroy, B., Şahin, İ., Mantoğlu, S. and Kayalı, S. Spirulina as a natural carotenoid source on growth, pigmentation and reproductive performance of yellow tail cichlid Pseudotropheus acei. *Aquaculture International,* **20**, 869-878 (2012). https://doi.org/10.1007/s10499-012-9512-x

50. Ramakrishnan, C.M., Haniffa, M.A., Manohar, M., Dhanaraj, M., Arockiaraj, A.J. and Arunsingh, S.V. Effects of probiotics and Spirulina on survival and growth of juvenile common carp (Cyprinus carpio). *Israeli Journal of Aquaculture - Bamidgeh*, **60**(2),128-133(2008).

51. Velasquez, S.F., Chan, M.A., Abisado, R.G., Traifalgar, R.F.M., Tayamen, M.M., Maliwat, G.C.F. and Ragaza, J.A. Dietary Spirulina (Arthrospira platensis) replacement enhances performance of juvenile Nile tilapia (Oreochromis niloticus). *Journal of Applied Phycology,* **28**, 1023-1030 (2016). https://doi.org/10.1007/s10811-015-0661-y

52. El-Sheekh, M., El-Shourbagy, I., Shalaby, S. and Hosny, S. Effect of feeding Arthrospira platensis (Spirulina) on growth and carcass composition of hybrid red tilapia (Oreochromis niloticus x Oreochromis mossambicus). *Turkish Journal of Fisheries and Aquatic Sciences,* **14**(2), 471-478 (2014). https://doi.org/10.4194/1303-2712-v14_2_18

53. Olvera-Novoa, M.A., Domínguez-Cen, L.J., Olivera-Castillo, L. and Martínez-Palacios, C.A. Effect of the use of the microalga Spirulina maxima as fish meal replacement in diets for tilapia, Oreochromis mossambicus (Peters), fry. *Aquaculture Research,* **29** (10), 709-715 (1998). https://doi.org/10.1046/j.1365-2109.1998.29100709.x

54. El-Sayed, A.F.M. Evaluation of soybean meal, spirulina meal and chicken offal meal as protein sources for silver seabream (Rhabdosargus sarba) fingerlings. *Aquaculture,* **127**(2-3), 169-176 (1994). https://doi.org/10.1016/0044-8486(94)90423-5

55. Rashidian, G., Bahrami Gorji, S., Farsani, M.N., Prokić, M.D. and Faggio, C. The oak (Quercus brantii) acorn as a growth promotor for rainbow trout (Oncorhynchus mykiss): growth performance, body composition, liver enzymes activity and blood biochemical parameters. *Natural Product Research,* **34** (17), 2413-2423 (2020). https://doi.org/10.1080/14786419.2018.1538994

56. Vali, S., Mohammadi, G., Tavabe, K.R., Moghadas, F. and Naserabad, S.S. The effects of silver nanoparticles (Ag-NPs) sublethal concentrations on common carp (Cyprinus carpio): Bioaccumulation, hematology, serum biochemistry and immunology, antioxidant enzymes, and skin mucosal responses. *Ecotoxicology and Environmental Safety,* **194**, 110353 (2020). https://doi.org/10.1016/j.ecoenv.2020.110353

57. Authman, M.M., Abbas, W.T., Abbas, H.H., Kenawy, A.M., Ibrahim, T.B. and Abd El-Hady, O.K. Ameliorative effect of the dietary Egyptian leek (Allium ampeloprasum L. var. Kurrat) on zinc toxicity of the African catfish Clarias gariepinus (Burchell, 1822). *Aquaculture Research,* **52**(11), 5656-5672 (2021).https://doi.org/10.1111/are.15440

58. Segev-Hadar, A., Alupo, G., Tal, K., Nitzan, T. and Biran, J. Identification and characterization of a non-muscular myostatin in the Nile tilapia. *Frontiers in Endocrinology,* **11**, 94 (2020). https://doi.org/ 10.3389/fendo.2020.00094

59. Wardani, W.W., Alimuddin, A., Junior, M.Z., Setiawati, M., Nuryati, S. and Suprayudi, M.A. Growth performance, robustness against stress, serum insulin, IGF-1 and GLUT4 gene expression of red tilapia (Oreochromis sp.) fed diet containing graded levels of creatine. *Aquaculture Nutrition,* **27** (1), 274-286 (2021). https://doi.org/10.1111/anu.13184

60. Zhong, H., Lou, C., Ren, B. and Zhou, Y. Insulin-like growth factor 1 injection changes gene expression related to amino acid transporting, complement and coagulation cascades in the stomach of tilapia revealed by RNA-seq. *Frontiers in Immunology,* **13**, 959717 (2022). https://doi.org/10.3389

61. Yeşilbaş, A. and Oğuz, A.R. Investigation of some hormones affecting osmoregulation of Lake Van fish (Alburnus tarichi) during reproductive migration. *Aquaculture Research,* **53**(3), 1011-1018 (2022). https://doi.org/10.1111/are.15642

62. Zhong, H., Zhang, X., Xu, Q., Yan, J., Han, Z., Zheng, H., Xiao, J., Tang, Z., Wang, F., Luo, Y. and Zhou, Y. Nonadditive and asymmetric allelic expression of growth hormone in hybrid tilapia. *Frontiers in Genetics,* **10**, 961 (2019). https://doi.org/10.3389/fgene.2019.00961

63. Cruz, E.M.V., Brown, C.L., Luckenbach, J.A. Picha, M.E., Bolivar, R.B. and Borski, R.J., Insulin-like growth factor-I cDNA cloning, gene expression and potential use as a growth rate indicator in Nile tilapia, Oreochromis niloticus. *Aquaculture,* **251**(2-4), 585-595(2006). https://doi.org/10.1016/j.aquaculture.2005.06.039

64. Liu, L., Yu, X. and Tong, J. Molecular characterization of myostatin (MSTN) gene and association analysis with growth traits in the bighead carp (Aristichthys nobilis). *Molecular Biology Reports,* **39**(9), 9211-9221 (2012). https://doi.org/10.1007/s11033-012-1794-6

65. Güroy, B., Güroy, D., Bilen, S., Kenanoğlu, O.N., Şahin, I., Terzi, E., Karadal, O. and Mantoğlu, S. Effect of dietary spirulina (Arthrospira platensis) on the growth performance, immune-related gene expression and resistance to Vibrio anguillarum in European seabass (Dicentrarchus labrax). *Aquaculture Research,* **53**(6), 2263-2274 (2022). https://doi.org/10.1111/are.15745

66. Abu-Elala, N.M., Galal, M.K., Abd-Elsalam, R.M., Mohey-Elsaeed, O. and Ragaa, N.M. Effects of dietary supplementation of Spirulina platensis and garlic on the growth performance and expression levels of immune-related genes in Nile tilapia (Oreochromis niloticus). *Journal of Aquaculture Research and Development,* **7**(7), 433-442 (2016). https://doi.org/10.4172/2155-9546.1000433

67. Teimouri, M., Yeganeh, S., Mianji, G.R., Najafi, M. and Mahjoub, S. The effect of Spirulina platensis meal on antioxidant gene expression, total antioxidant capacity, and lipid peroxidation of rainbow trout (Oncorhynchus mykiss). *Fish Physiology and Biochemistry,* **45**, 977-986 (2019). https://doi.org/10.1007/s10695-019-0608-3

68. Kokou, F. and Fountoulaki, E. Aquaculture waste production associated with antinutrient presence in common fish feed plant ingredients. *Aquaculture,* **495**, 295-310 (2018). https://doi.org/10.1016/j.aquaculture.2018.06.003

69. Schreckenbach, Knösche and Ebert. Nutrient and energy content of freshwater fishes. *Journal of Applied Ichthyology,* **17**(3), 142-144 (2001). https://doi.org/10.1111/j.1439-0426.2001.00295.x

70. Liang, G., Liangliang, H., Lufeng, Z., Xiaoyu, S., Zijun, J., Lilian, D., Weizheng, R., Jian, Z., Jianjun, T. and Xin, C. Coupling rice with fish for sustainable yields and soil fertility in China. *Rice Science*, 27,175-179(2020). https://doi.org/10.1016/j.rsci.2020.04.001

71. Vizcaíno, A.J., Rodiles, A., López, G., Sáez, M.I., Herrera, M., Hachero, I., Martínez, T.F., Cerón-García, M.C. and Alarcón, F.J. Growth performance, body composition, and digestive functionality of Senegalese sole (Solea senegalensis Kaup, 1858) juveniles fed diets including microalgae freeze-dried biomass. *Fish physiology and Biochemistry*, **44**, 661-677 (2018). https://doi.org/10.1007/s10695-018-0462-8

72. Dallaire, V., Lessard, P., Vandenberg, G. and De La Noüe, J. Effect of algal incorporation on growth, survival and carcass composition of rainbow trout (Oncorhynchus mykiss) fry. *Bioresource Technology,* **98**(7),1433-1439 (2007). https://doi.org/10.1016/j.biortech.2006.05.043

73. Gong, Y., Nunes, L.M., Greenfield, B.K., Qin, Z., Yang, Q., Huang, L., Bu, W. and Zhong, H. Bioaccessibility-corrected risk assessment of urban dietary methylmercury exposure via fish and rice consumption in China. *Science of the Total Environment,* **630**, 222-230 (2018). https://doi.org/10.1016/j.scitotenv.2018.02.224

74. Niccolai, A., Venturi, M., Galli, V., Pini, N., Rodolfi, L., Biondi, N., D'Ottavio, M., Batista, A.P., Raymundo, A., Granchi, L. and Tredici, M.R. Development of new microalgae-based sourdough "crostini": Functional effects of Arthrospira platensis (spirulina) addition. *Scientific Reports,* **9**(1), 19433(2019).https://doi.org/10.1038/s41598-019-55840-1

تأثير مستويات مختلفة من الاسبيرولينا كإضافات غذائية على أداء النمو والتحليل البيوكيميائي للدم والتعبيرات الجينية المرتبطة بالنمو في أسماك البلطي النيلي

هشام ابوزيد[1]، على سليمان محمد النادى[1]، داليا محمد ابوالحسن[2]، هيام منصور[2]، عبدالمجيد عبيدو[1]،

هاشم حامد عبد الرحمن[1] ، ايناس صلاح الدين غالى[2]،حسناء احمد رضوان[2] ، وفاء توفيق عباس[3]

و ابراهيم محمد فرج[2]

[1]قسم الانتاج الحيوانى - معهد البحوث الزراعية والبيولوجية - المركز القومي للبحوث - الدقى- الجيزة- 12622- مصر.
[2] قسم بيولوجيا الخلية- معهد بحوث التقنيات الحيوية - المركز القومي للبحوث - الدقى- الجيزة - 12622- مصر.
[3] قسم الأحياء المائية - معهد البحوث البيطرية - المركز القومي للبحوث - الدقى- الجيزة - 12622- مصر.

يعتبر طحالب سبيرولينا بلاتنسيس (.Sp) مصدرًا طبيعيًا ممتازًا للمغذيات البديلة التي يمكن أن تحل محل البروتينات المشتقة من الحيوانات في النظام الغذائي للأسماك. المستوى المناسب من .Sp قد يعزز السمات المرتبطة بالنمو التي تقلل تكاليف الإنتاج. تم تصميم هذا البحث للتحقق من تأثير إستخدام مستويات مختلفة نت الاسبيرولينا .2%, 1%) (3% كمحفز للنمو، ودراسة تأثيرها على معدلات النمو وتركيب الدم والتعبيرات الجينية المرتبطة بالنمو مثل (GH)، (GHR)، (IGF1)، (IGF2)، (MSTN) للبلطي النيلي. تم توزيع الأسماك 96 سمكة وحيدة الجنس بمتوسط وزن (12±02 جم) عشوائياً على ثماني مجموعات بمعدل 24 سمكة لكل معاملة تم تقسيم كل معاملة على حوضين كل حوض يحتوى على 12 سمكة سعة الحوض 50 لتر لمدة 8 أسابيع. لقد تحسن أداء النمو والإستفادة من العلف في المعاملة الثانية (1%) . وكانت أعلى نسبة كفاءة تحويلية هى 1.09 يليها 1.26، 1.40 و1.30 في المعاملات، 2% .Sp و 3% .Sp، على التوالي. وقد سجلت المؤشرات البيوكيميائية نفس الاتجاه، حيث ارتفع مستوى البروتين والجلوبيولين في مصل الدم معنوياً ($P<0.05$) في المعاملة الثانية مقارنة بالمعاملات الأخرى، في حين إنخفض تركيزهما في المعاملة الثالثة والرابعة معنوياً ($P<0.05$) مقارنة. بالإضافة إلى ذلك أظهرت النتائج أن الأسماك التي تم تغذيتها بـ 1% من الاسبيرولينا تمتلك تحسنًا كبيرًا في التعبير الجيني لـ GH في المخ وIGFI وIGF2 في الكبد وGH وGHR وIGFI وIGF2 في أنسجة العضلات مقارنةً بالمعاملات الأخرى بينما إنخفض مستوى MSTN mRNA في الأنسجة العضلية مما يوضح أن هذه المعاملة كانت أفضل من المعاملات الغذائية الأخرى.

الكلمات الدالة: سبيرولينا، البلطي النيلي، أداء النمو، التحاليل البيوكيميائية، التعبيرات الجينية المرتبطة بالنمو.