Annals of Agricultural Science, Moshtohor (ASSJM)
https://assjm.journals.ekb.eg/
www.fagr.bu.edu.eg
© 2023, Faculty of Agriculture, Benha University, Egypt. ISSN:1110-0419
Original Article Vol. 61(3) (2023), 631 – 638 DOI: 10.21608/ASSJM.2024.252055.1258

Effect of Using Dried Microalgae as Growth Promoters for Nile Tilapia: Growth, Hematology and Related Genes Expression

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Abstract

In Nile tilapia, *Oreochromis niloticus*, dietary dry *Golenkinia longispicula* supplementation (0.0, 5, 10 and, 15 g kg^{-1} diets) was utilized to improve growth, feed efficiency, and hematologically related gene expression. Fingerlings weighing 3.29 ± 0.16 g at hatching were randomized and placed in triplicate into twelve fiberglass tanks measuring 0.5 m3 and holding 250 L of water for each treatment. After 90 days of the feeding trial, the results demonstrated final body weight (FBW), weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR) were significantly ($P < 0.05$) improved by a diet contained 15 g kg^{-1} *G. longispicula*. The highest hematocrit (Hct), hemoglobin (Hb), and red blood cells (RBCs) levels were detected in the fish-fed diet that had 15 g of *G. longispicula* kg^{-1}. The values of growth hormone (GH) and growth hormone receptor were upregulated fish fed a diet that included 15 g kg^{-1} diet of dry *G. longispicula*.

Keywords: Nile tilapia, Microalgae, Growth, Gene expression

Introduction

Since the output from catch fisheries has been stagnating for the past few decades, aquaculture will be the primary means of providing humans with an acceptable amount of animal protein (FAO, 2020). In order to attain the projected growth in aquaculture, the sector needs to assess current challenges in addition to prospective demands arising from worldwide issues (e.g., the worldwide spread of antibiotic resistance) and the system's intensification (ChJawahar et al., 2016; Burgos-Aceves et al., 2018). Long-term and short-term climate changes, such as sea level rise, storms, droughts, and high temperatures, will also likely have a negative impact on water quality by changing salinity, lowering water oxygen levels, and introducing pollutants into aquaculture systems. These factors will put more stress on animals raised for food and raise the possibility of disease outbreaks (Harikrishnan and Balasundaram, 2005; Boyd and Tucker, 2012; El Megid et al., 2020).

The use of feed additives with immunostimulant qualities has been more and more important in the recent few decades (Yılmaz et al., 2015; Acar et al., 2015; Baba et al., 2016; Soltan et al., 2016; Hassaan et al., 2019; Ali et al., 2023; Mohammady et al., 2022). Microalgae as natural antioxidants, protein sources, and immunostimulant feed additives for aquaculture are currently hot topics of research in the industry. Microalgae are rich in minerals, vitamins, amino acids, carbohydrates, polyunsaturated fatty acids, and pigments (carotenoids, phycobiliproteins, and chlorophylls), among other beneficial substances (Raja and Hemaiswarya, 2010; Huerlimann et al., 2010; Koen et al., 2012; Ishaq et al., 2016; Han et al., 2019).
Ten species of unicellular organisms with spherical cells with spiny projections and a single cup-shaped chloroplast with a single pyrenoid are described in the genus Golenkinia (Komárek and Fott, 1983). One species of chlorophytes belonging to the Golenkiniaceae family is found within *Golenkinia longispicula*. Given this, the goal of the current study was to evaluate how dietary *G. longispicula* affected the expression of related genes, performance, hematology and antoantioxidant, and innate immune markers in Nile tilapia fingerlings.

Materials and methods

2.1. Design of experiments, methods for raising fish and diets

Four isonitrogenous and isoenergetic practical diets were formulated (Table 1) to meet the nutritional requirements of Nile tilapia (NRC, 2011). The first is the control diet without addition. Diets 2-4, contain 5 g kg^{-1}, 10 g kg^{-1}, and 15 g kg^{-1} *Golenkinia longispicula,* respectively. After properly combining all the ingredients with the *G. longispicula*, the mixture was shaped into 2 mm diameter pellets using a local pelleting hand noodle maker. Following a period of drying at ambient temperature, the pellets were stored at 4°C for duration of three days. Table 3 displays the chemical composition of the ingredients and diet as evaluated by AOAC (2012) and gross energy as reported by Brett and Groves (1979). Purchased from a private farm called Elsahaba, hatchery Kafer Elsheekh Government, Egypt, Nile tilapia was moved to the intensive fish farm of Benha University's Faculty of Agriculture and were raised for 15 days in a 4 x 2 x 1.25 m concrete pond housed in a greenhouse. Fish were fed a commercial feed at a rate of 5% of their body mass, which contained 30% crude protein and 6% fat. After acclimatization, twelve fiberglass tanks (0.5 m^3, 250 L) containing healthy Nile tilapia (average beginning weight: 3.29±0.16 g/fish) were randomly assigned to three treatments.

Table 1. Formulation and proximate composition of the experimental diets (g kg^{-1} diet, dry matter)

Items	*G. longispicula*			
	0.0	5	10	15
Ingredients				
Fish meal	100	100	100	100
Corn gluten	50	50	50	50
Soybean meal	470	465	460	455
Yellow Corn	220	220	220	220
Wheat bran	90	90	90	90
Soy oil	50	50	50	50
Premix[1]	20	20	20	20
Golenkinia longispicula	0	5	10	15
Proximate chemical analysis (%)				
Crud protein	32.63	32.66	32.69	32.77
Crude lipids	67.8	68	68.1	68.2
Crude fiber	7.5	7.4	7.2	7.3
Ash content	42.3	42.1	42.5	42.4
Nitrogen free extract (NFE)[2]	50.23	50.16	50.49	50.67
Gross energy (MJ kg^{-1} diet)[3]	17.85	17.88	17.87	17.89

[1]Vitamin and mineral mixture kg^{-1} of mixture contains: 4800 I.U. Vit A, 2400 IU cholecalciferol (vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit B12, 4.0 g Vit B2, 6 g Vit B6, 4.0 g, Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 g Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Zinc, 0.04 g Selenium. folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg; ferrous sulfate (FeSO4.7H2O, 20% Fe), 65 mg; manganese sulfate (MnSO4, 36% Mn), 89 mg; zinc sulfate (ZnSO4.7H2O, 40% Zn), 150 mg; copper sulfate (CuSO4.5H2O, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I).
[2]NFE =100 - (crude protein + crude lipid + ash content +crude fiber).
[3]Gross energy calculated using gross calorific values of 23.63, 39.52 and 17.15 kjg^{-1} for protein, fat, and carbohydrate, respectively according to Brett and Groves (1979).

2.2. Hematology

Each treatment involved the collection of blood from the caudal vein using five fish, which was subsequently divided using sterile 1-milliliter syringes. The first half was collected using the procedure of Rawling et al. (2009), which involved estimating hematological parameters with ethylenediaminetetraacetic acid (EDTA; 10%). The method of Blaxhall and Daisley (1973) was employed to determine the WBC differential counts.

2.3. Gene expression

Following a 90-day feeding trial, the livers of five fish selected at random from each treatment group were removed, and the Tissue Lyser LT device (QIAGEN GmbH, QIAGEN Strasse 1, Hilden, Nordrhein-Westfalen-40724, Germany) was used to grinned the fish instantly. Using the RNeasy® Mini kit (QIAGEN, Cat. No. 74104), ribonucleic acid (RNA) was extracted from the tissues according to the

manufacturer's instructions. After synthesizing cDNA from 1000 ng of total RNA using the high capacity high-capacity cDNA Reverse Transcription Kit (Applied Biosystems, MA, USA, Cat# no.4368813), the cDNA was stored at 80 °C for further molecular investigations. Using real-time PCR (qRT-PCR) techniques, primers were used to amplify the genes producing growth hormone and growth hormone receptor to measure the target genes' expression. Growth hormone (GH); F: TCGACAAACACGAGACGCA, R: CCCAGGACTCAACCAGTCCA, Growth hormone receptor (GHr), F: CAGACTTCTAGGCTCAGGTC, R: CTGGATTCTGAGTTGCTGTC. For quantitative PCR, the following materials were used: 2.5 µg/l cDNA, 12.5 µl SYBR Green PCR Master Mix (QuantiTect SYBR Green PCR Kit, QIAGEN), 0.3 µM forward and reverse primers, and a final volume of 25 µl sterile double distilled water. With an Applied Biosystems 7500 Real-time PCR Detection system (Applied Biosystems), the reaction was run for 10 minutes at 95°C, followed by 45 cycles of 20 seconds at 95°C, 20 seconds at 60°C, and 20 seconds at 72°C. All modifications to the gene expression under investigation that are done experimentally are expressed as n-fold deviations from the control.

2.4. Data of Growth

Each tank's population of fish was counted both prior to and after the feeding trial. The footnote of Table 2 contains a list of all the formulas used to calculate the growth parameters and feed utilization efficiency.

Table 2. Growth performance and feed utilization of Nile tilapia fed the experimental diets for 90 days

Items	Experimental Diets				±MS	P Value
	Control	5 g kg^{-1} diet	10 g kg^{-1} diet	15 g kg^{-1} diet		
Initial body weight (g/ fish)	3.35	3.25	3.2	3.45	0.094	0.06
Final body weight (g/fish)	19.73^{b}	23.85^{a}	25.15^{a}	25.45^{a}	1.012	0.021
Weight gain (g/fish)	16.38^{b}	20.60^{a}	21.95^{a}	22.00^{a}	1.06	0.001
Specific growth rate	2.36^{b}	2.65^{ab}	2.75^{a}	2.66^{a}	0.087	0.024
Feed conversion ratio	1.91^{a}	1.41^{b}	1.32^{c}	1.19^{c}	0.15	0.001
Protein efficiency ratio	1.69^{c}	2.21^{bc}	2.38^{b}	2.65^{a}	0.158	0.001

The values are presented as means ± standard error of triplicate groups (n=3).
Means followed by different letters in the same row are significantly different ($P < 0.05$).
Weight gain (WG) = final weight (g)−initial weight (g); Specific growth rate (SGR) = LnW2−LnW1/t (days), Where, Ln = the natural log; W1 = initial fish weight, W2 = the final fish weight in grams and t = Period in days; Feed conversion ratio (FCR) was calculated according to by the equation: FCR = Feed intake (g)/weight gain (g); Protein efficiency ratio (PER) = Weight gain (g) / protein ingested (g)

2.5. Statistical analysis

Homogeneity and normality tests were run prior to data analysis. The SAS ANOVA program was then used to analyze the data using one-way analysis of variance, and Duncan's multiple range test was used to assess mean differences (SAS, version 6.03, Soft Inc., Tusla, OK, USA, SAS, 1996). Standard errors of the mean (±SEM) and means are used to represent the data.

Results

2.6. Growth

According to Table 2, FBW, WG, SGR were significantly higher in fish fed diet supplemented with 5g, 10 g, and 15 g kg^{-1} *Golenkinia longispicula* than the control diets. No significant differences were found in final body weight (FBW), weight gain (WG), specific growth rate (SGR) in fish group fed diet 10 and 15 g kg^{-1} *G. longispicula*. The higher growth performance and feed utilization was observed in fish group 15 g kg^{-1} *G. longispicula*

2.7. Hematology

Values of hemoglobin (Hb), hematocrit (Hct), red blood cells (RBCs) and white blood cells count (WBCs) were noted in Table 3. The highest hematocrit (Hct), hemoglobin (Hb), red blood cells (RBCs) and WBCs levels were detected in the fish-fed diet that had 15 g of *G. longispicula* kg^{-1}.

Table 3. Hematology of Nile tilapia fed the experimental diets for 90 days

Items	Experimental Diets				±MS	P Value
	Control	5 g kg^{-1} diet	10 g kg^{-1} diet	15 g kg^{-1} diet		
Hb (g dL^{-1})	3.35	3.25	3.2	3.45	0.094	0.06
HCT (%)	19.73^{b}	23.85^{a}	25.15^{a}	25.45^{a}	1.012	0.021
RBCs ($\times 10^{3}$cells/µL)	16.38^{b}	20.60^{a}	21.95^{a}	22.00^{a}	1.06	0.001
WBCs ($\times 10^{3}$ ells/µL)	2.36^{b}	2.65^{ab}	2.75^{a}	2.66^{a}	0.087	0.024

The values are presented as means ± standard error of triplicate groups (n=3).
Means followed by different letters in the same row are significantly different ($P < 0.05$).
Hematocrit (HCT), Hemoglobin (Hb), Red blood cells (RBC), White blood cells (WBCs)

2.8.Gene expression

Transcription of GH and GHR were showen in Fig. 1 and Fig. 2. The values of growth hormone (GH) and growth hormone receptor (GHR) were upregulated fish fed a diet that included 15 g kg^{-1} *G. longispicula* kg^{-1} diet.

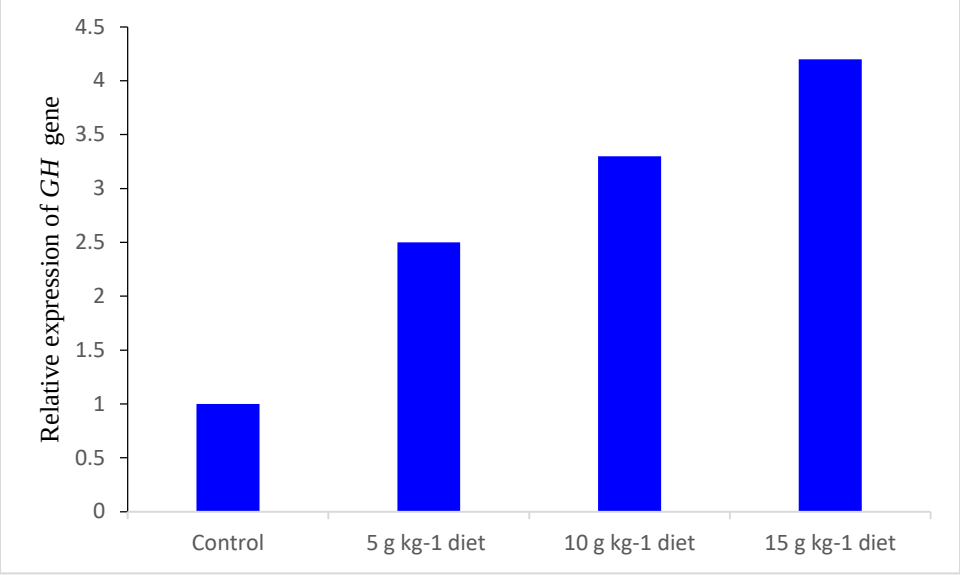

Fig. 1. Relative expression of GH gene / 18s rRNA of Nile tilapia after feeding experimental diets.

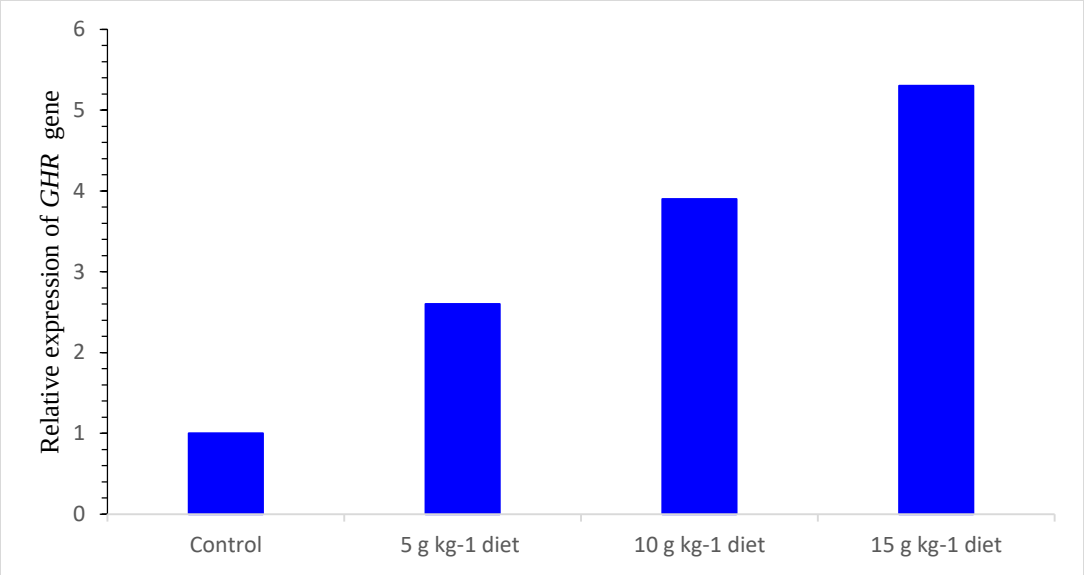

Fig. 2. Relative expression of GHR gene / 18s rRNA of Nile tilapia after feeding experimental diets.

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Discussion

The findings of this study showed how *G. longispacula* enhanced the Nile tilapia's growth performance and feed utilization. In this paper, we present an innovative approach to protect aquatic animals from the negative effects of intensive farming practices by employing functional nutritional supplements. It's interesting to note that adding *G. longispacula* to the diet at a level of 10 g or 15 g kg-1 enhanced the growth performance and reduced the fishery control ratio (FCR) in Nile tilapia. It's possible that *G. longispacula* enhanced fish performance in the current study because of its high flavonoid and phenolic component concentration. This improvement demonstrates how consuming *G. longispacula* can increase the efficacy of feed. In this regard, Lizárraga-Velázquez et al. (2019) reported that zebrafish (Danio rerio) fed a diet supplemented with phenolic compounds displayed higher growth. Additionally, the growth performance and feed utilization of *Hypophthalmichthys molitrix*, the silver carp, were enhanced by the addition of alginic and glycyrrhizic acid to their diet (Harikrishnan et al., 2022). According to several studies, feeding silver carp glycyrrhizic acid (Harikrishnan et al., 2021a), striped snakehead (*Channa striatus*) cinnamondehyde (Harikrishnan et al., 2021b), chrysophanic acid (*Catla, Catla catla*) (Harikrishnan et al., 2021c), and cassic acid (*Claritias gariepinus*)—airbreathing catfish (Clarikrishnan et al., 2020).

According to Burgos-Aceves et al. (2019), haematological traits are essential markers for evaluating the nutritional status and overall health of fish, as well as their response to different environmental stressors. The haematological indices of fish in the current study that received feed supplemented with varying concentrations of *G. longispacula* were higher than those of fish that did not receive supplementation. According to Mahmoud and El-Hais (2017), increased Nile tilapia stocking density has a deleterious impact on haematological markers.

G. longispacula supplementation showed positive results on the haematological indicators. Therefore, the current study discovered that supplementing fish raised the erythrocytes' capability to contain high Hb levels in high-density fish, increasing the Nile tilapia's ability to carry oxygen in their blood. The immunostimulatory and anti-stress effects of *G. longispacula* are demonstrated by the rise in RBC count herin after the drug's administration. Furthermore, *G. longispacula* may collaborate with immune cells such as neutrophils, monocytes, and lymphocytes to enhance innate immunity and promote non-specific immunity, which is indicated by an increase in the number of white blood cells (WBCs) (Nayak, 2010).

The improved biochemical and antioxidant status of the fish in this study was consistent with their increased development and immunity. This illustrates

how additional feeding improves fish health. The antioxidant and immunological response of Nile tilapia were enhanced by dietary quercetin (Zhai and Liu 2014). Furthermore, quercetin-supplemented diets for snout bream and grass carp improved the antioxidant and immunity indices, according to studies by Jia et al. (2019) and Xu et al. (2019). Additionally, caffeic acid enhanced Nile tilapia's antioxidant and immunological responses (Yilmaz, 2020). diet include cinnamaldehyde in striped snakehead, Channa striatus (Harikrishnan et al., 2021b), alginic acid and glycyrrhizic acid in silver carp (Harikrishnan et al., 2022; Harikrishnan et al., 2021a).

Conclusion

It has been discovered that feeding dried *G. longispicula* to Nile tilapia improves the fish's immune system. Additionally, *G. longispicula* at 15 g kg^{-1} diet could improve fish development and related hormone function.

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