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Effects of microalgae inoculated diet on growth performance and blood parameters of Nile tilapia (*Oreochromis niloticus)*

KAFIA ISLAM AMIRA, MOHAMMAD REDWANUR RAHMAN, HELENA KHATOON*, SUCHANDAN SIKDER[1], SKM AZIZUL ISLAM[2], JINAT AFRUJ, FOUJIA JAMAL[3] AND MOHAMMAD EKRAMUL HAQUE

Department of Aquaculture, [1]Department of Medicine and Surgery,
[2]Department of Physiology, Biochemistry and Pharmacology,
Chattogram Veterinary and Animal Sciences University, Chattogram-4225
[3]Department of Zoology, Chittagong University, Chattogram-4331, Bangladesh
*Corresponding author's Email: helena@cvasu.ac.bd

Abstract. This study was intended to evaluate growth performance, physicochemical and blood parameters of *Oreochromis niloticus* for comparing the effects of five test diets, prepared by replacing fish meal by 25%, 50% with microalgae *Tetraselmis* sp. and *Nannochloropsis* sp. with feed without replacement. Two hundred seventy Nile tilapia (mean individual weight 0.023 ± 0.0001 g) were randomly allocated into five treatments in triplicate. The fry was raised in 15 rectangular glass aquarium (18 fish/tank within 18 L water) encompassing water holding capacity up to 30L (each). The results revealed that both the supplemented microalgae alone could not significantly enhance the growth performance in compare to control diet ($p > 0.05$). Low palatability and antinutritional factors may be responsible for the poor growth performance. However, significantly higher survival, better TAN, NO_2-N and SRP was noticed in T25 than control ($p < 0.05$). The blood parameters exhibited significant increase of certain parameters within reference range, nevertheless significant decrease in some blood parameters was also noticed ($p < 0.05$). Considering the beneficial impacts on water quality, survivality and blood parameters inclusion of microalgae in fish diet can be a better option for fish cultivation.

Keywords: Nile tilapia, Microalgae, Water quality, Blood parameter

Introduction

Microalgae are a diverse group of aquatic organisms that can be found in both marine and freshwater environments. *Nannochloropsis* sp. are unicellular small green algae which has the potentiality to enhance the nutritional quality of the human diet (Gbadamosi and Lupatsch 2018). *Tetraselmis* sp. is a large green flagellate widely used for feeding juveniles, bivalve molluscs, penaeid shrimp larvae and rotifers. This genus has rapid growth rate, good sources of vitamin E, large spectrum of antimicrobial activity and probiotic properties, and also can withstand with any broad range of temperature and pH (Brown *et al.* 1999, Khatoon *et al.* 2014). Diet composed of two or more different algal species provides better result in the growth and nutrition of animal than a diet composed of single algal species (Spolaore *et al.* 2006). Gbadamosi and Lupatsch (2018) concluded that *Nannochloropsis salina* can replace fish meal and soyabean meal up to 100% in tilapia feeds without showing any negative results in the growth, survival and health of the fish. Tulli *et al.* (2012) showed that, dried *Tetraselmis suecica* is able to replace up to 20% of fish meal without hampering the growth performance of sea bass and has the potential to become an alternative dietary ingredient to be used in organic feed production. High level of ammonia, nitrogen toxicity, low level of dissolved oxygen, high level of pH, and CO_2 etc. can cause a great loss in the growth and health status of fish. Microalgae is a potential source to stabilize quality of water in an expected outcome (Yang *et al.* 2021). In the

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field of aquaculture, blood parameters are considered as most convenient indicators to determine the health status of farmed and uncultured fish species as these are capable of providing reliable information on possible exposure to mutagens, metabolic stress, deficiencies and chronic stress status before clinical symptoms appear (Bahmani *et al.* 2001). Hassaan *et al.* (2020) depicted that dietary supplementation with extracted bioactive compounds β-carotene or phycocyanin from *Spirulina* showed a significantly better result in the enhancement of the total serum protein, albumin, and globulin.

In this study, Nile tilapia was used as experimental fish because tilapia is one of the most important groups of cultured fish species. They are widely cultivated because of their high commercial value, eat wide range of natural food organisms, can withstand with poor water quality, grow rapidly at warm temperatures and low cost of maintenance. But there is limited information on the blood parameters of tilapia. Hence the main objectives of this work are to assess the growth, water quality and blood parameters of tilapia using different percentage of *Nannochloropsis* sp. and *Tetraselmis* sp. microalgae inoculated within diets.

Materials and Methods

***Experimental site and microalgae collection*:** The feeding trial experiment on tilapia was done at the Wet laboratory of Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University with maintaining proper precautions. The duration of tilapia fry feeding trial was 8 weeks. Two species of marine microalgae, *Tetraselmis* sp. and *Nannochloropsis* sp. were used in this experiment. They were in the live feed research corner of Department of Aquaculture, Chattogram Veterinary and Animal Sciences University following Tompkins *et al.* (1995) and Islam *et al.* (2021).

***Experimental diets*:** Five diets were prepared using different levels of algal biomass (0%, T25%, T50%, N25%, N50%). During diet formulation 25% and 50% fish meal were replaced by the dried biomass of *Nannochloropsis* sp. and *Tetraselmis* sp. and there was no replacement in control diets (Table I). All feeds were formulated with commonly used ingredients.

Table I. Diet formulation for *Oreochromis niloticus* diet (% dry weight basis)

Constituents	CF	T25	T50	N25	N50
Commercial fishmeal	67.55	50.67	33.77	50.67	33.77
Nannochloropsis sp.	_	_	_	16.88	33.78
Tetraselmis sp.	_	16.88	33.78	_	_
Corn flour	9.65	9.65	9.65	9.65	9.65
Wheat flour	9.65	9.65	9.65	9.65	9.65
Rice bran	9.65	9.65	9.65	9.65	9.65
Vitamin mixture	1	1	1	1	1
Mineral mixture	1	1	1	1	1
Dicalcium phosphate	1	1	1	1	1

Collection of fish and experimental design: Tilapia fry were collected from a commercial hatchery named "Niribili Tilapia Hatchery", situated at Cox's Bazar. Fourteen days old 270 Nile tilapia fry (mean individual weight 0.023 ± 0.0001 g) were used to conduct this feeding

trial. All the fries were distributed randomly into 15 clear glass aquaria (45×30×30 cm) encompassing water holding capacity up to 30L. Fish were acclimatized in a large tank at laboratory condition for 2 days prior to the stocking. Continuous aeration was provided to maintain the sufficient oxygen level in tank and commercial starter feed was given as feed to the fish at the time of conditioning. The stocking density was 18 fish/tank within 18L water. Five treatments were designed namely CF, T25, T50, N25, N50 in triplicate to conduct this experiment and tilapia fry were fed with diet containing *Tetraselmis* sp. and *Nannochloropsis* sp. along with a control feed with no microalgae. All the five categories of feed were grinded and provided 4 times in a day (at 8 AM, 11 AM, 2 PM and 5 PM) at a rate of 15% of the total body weight of the fish. Excreta and leftover feeds were removed from the bottom of each aquarium through siphoning on a regular basis. One third volume of culture water was exchanged daily from each experimental tank.

***Analysis of proximate composition*:** Crude protein and lipid of formulated feed was analyzed using micro Kjeldhal method (Kirk and Sawyer 1991) and AOAC (2005) procedure, respectively. To determine carbohydrate content of formulated feed and microalgae the method of Dubois *et al.* (1956) was followed. Method provided by Lowry *et al.* (1951) was followed to determine the protein content of microalgae. The reading of absorbance for the prepared solution for protein and carbohydrate was obtained by using spectrophotometer (T80 UV/VIS Spectrophotometer, UK) at 750 nm and 488 nm wavelength respectively. Method provided by Bligh and Dyer (1959) was followed to determine the lipid content of microalgae. Proximate composition of experimental diets and microalgae are shown in Table II.

Table II. Proximate composition (%) of diet and microalgae

Nutrients	Formulated Diets					*Nannochloropsis* sp.	*Tetraselmis* sp.
Carbohydrate	20.43	23.19	26.22	22.33	23.18	17	22
Protein	40.00	38.00	36.90	36.03	33.45	49	57
Lipid	11.21	12.29	13.11	14.37	16.22	25	19

Analysis of physicochemical parameters: Digital glass thermometer (SARAAN SCIENTIFIC INDUSTRIES), pH meter (pHep-HI98107, HANNA, India), and DO meter (Lutron A20 DO5509 Dissolve Oxygen Meter, Taiwan, China) were used to measure the daily readings of water temperature, pH, and DO in the culture tanks. Weekly determination of total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N), and soluble reactive phosphorous (SRP) were done using the chemical method of Parsons *et al.* (1984).

***Determination of growth parameters*:** Growth parameters like, weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and survival of each treatment of the Nile tilapia was determined by the following formulas:

A. Weight gain (g) = final weight – initial weight
B. Specific growth rate(%/day) = (Ln (W_t)-Ln (W_i))/t × 100
   Where, t is the time in days. Ln(w_i) is the natural logarithm of the initial weight and Ln(w_t) is the natural logarithm of the final weight at time t.
C. Feed conversion ratio = amount of dry food intake (g)/fresh weight gain in fish (g)

D. Survival rate (%) = (Number of fishes at the end of the experiment / Number of fishes at the beginning of the experiment) × 100

Analysis of blood parameters: Blood hematology parameters such as: red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hct), white blood cell count (WBC), lymphocyte (LYM) and platelet (PLT) were determined using hematology analyzer (NIHON KOHDEN, India). Meanwhile, using biochemistry analyzer (Humalyzer 3000, Germany) biochemical parameters of blood serum like: total serum protein, albumin, globulin, triglyceride, cholesterol, blood glucose, urea and blood urea nitrogen (BUN) of fish was determined.

Statistical analysis: Data obtained from the current research is presented as mean±SE (standard error). IBM SPSS software (v.26) tool was used to analyze all data. ANOVA (one way analysis of variance) test was conducted to confirm the significancy ($p<0.05$) and means of different treatments were compared through multiple range test of Duncan.

Results

Growth performance: The inclusion of *Nannochloropsis* sp. and *Tetraselmis* sp. microalgae in diet did not significantly ($p>0.05$) increase the WG, FCR and SGR of *Oreochromis niloticus* fry compared to reference group CF but significantly ($p<0.05$) increased the survival rate than the control. Among all the treatment group T25 showed the highest survivality (92.59±1.85%) and the lowest survival was recorded in control group (61.11±3.20%) (Table III).

Table III. Effect on growth performance of *Oreochromis niloticus* fry

Parameters	Treatment				
	CF	N25	N50	T25	T50
Initial body weight (g)	0.023±0.00^{a}	0.023±0.00^{a}	0.0233±0.00^{a}	0.022±0.00^{a}	0.022±0.00^{a}
Final body weight(g)	2.12±0.00^{a}	1.33±0.00^{d}	1.004±0.00^{e}	1.87±0.00^{b}	1.53±0.00^{c}
Weight gain (g)	2.10±0.00^{a}	1.31±0.00^{d}	0.98±0.00^{e}	1.85±0.00^{b}	1.50±0.00^{c}
Specific growth rate (%)	8.05±0.01^{a}	7.24±0.01^{d}	6.72±0.01^{e}	7.87±0.03^{b}	7.52±0.03^{c}
Feed conversion ratio	2.4±0.01^{e}	3.4±0.00^{b}	4.3±0.01^{a}	2.7±0.01^{d}	3.1±0.01^{c}
Survival (%)	61.11±3.20^{c}	87.03±1.85^{ab}	85.18±1.85^{b}	92.59±1.85^{a}	90.74±1.85^{ab}

CF-control feed with no replacement of fish meal with microalgae; T25-25% replacement of fish meal with *Tetraselmis* sp.; T50-50% replacement of fish meal with *Tetraselmis* sp.; N25-25% replacement of fish meal with *Nannochloropsis* sp.; N50-50% replacement of fish meal with *Nannochloropsis* sp. Mean±SE with different letters within each column are statistically significant.

Physicochemical parameters: No significant ($p>0.05$) differences were observed in the results of DO, pH, temperature (Table IV) but significantly ($p<0.05$) higher TAN, NO_2-N and SRP concentration were obtained in CF tanks. In contrast, significantly ($p<0.05$) lower TAN value was recorded in T25 (0.46±0.00 mg/L) treatment, NO_2-N reported in T25 (0.42±0.01 mg/L), T50 (0.42±0.01 mg/L) and SRP reported in N25 (0.10±0.00 mg/L), T25 (0.10±0.00 mg/L), treatment respectively (Table IV).

Table IV. Results of physicochemical parameters tested during feeding experiment

Treatment	Physical parameters			Chemical parameters		
	DO (mg/L)	pH	Temperature (^{0}C)	TAN (mg/L)	NO_2-N (mg/L)	SRP (mg/L)
CF	6.60±0.03^a	8.54±0.05^a	27.64±0.07^a	0.66±0.01^a	0.51±0.01^a	0.15±0.00^a
N25	6.58±0.06^a	8.50±0.05^a	27.65±0.09^a	0.54±0.00^c	0.44±0.00^c	0.10±0.00^d
N50	6.55±0.05^a	8.44±0.07^a	27.65±0.09^a	0.59±0.00^b	0.47±0.00^b	0.13±0.00^b
T25	6.55±0.03^a	8.42±0.05^a	27.80±0.09^a	0.46±0.00^d	0.42±0.01^d	0.10±0.00^d
T50	6.55±0.04^a	8.42±0.06^a	27.81±0.12^a	0.54±0.00^c	0.42±0.01^d	0.11±0.00^c

Mean±SE (standard error) with same letters within each column are not statistically significant ($p>0.05$) but different letters within each column defining significance level ($p<0.05$).

***Blood hematology*:** Significantly($p<0.05$) higher values of RBC, Hb, Hct and WBC were recorded in T25 (1.74±0.021×$10^6/\mu$l), (9.6±0.11 g/dl), (32.6±0.14%) and (26.3±0.27 ×$10^3/\mu$l) respectively, compared to the control (Table V). Significant ($p<0.05$) lower values of these parameters were recorded in CF which are correspondingly, (1.46±0.014 ×$10^6/\mu$l), (6.8±0.12 g/dl), (23.3±0.17%) and (18.4±0.29 ×$10^3/\mu$L) (Table V). Significantly ($p<0.05$) higher level of LYM and PLT was recorded in CF (58.6±0.31%) and (66.3±0.05×$10^3/\mu$L). However, lowest LYM and PLT was found accordingly, in T25 (47.3±0.2%) and N50 (28.6±0.12 ×$10^3/\mu$L), followed by T50 (37.4±0.14 ×$10^3/\mu$L) treatment (Table V).

Table V. Effect on hematological parameters of *Oreochromis niloticus* fry

Parameters	Unit	Treatment				
		CF	N25	N50	T25	T50
RBC	×$10^6/\mu$L	1.46±0.01^d	1.67±0.01^b	1.61±0.01^c	1.74±0.02^a	1.72±0.01^a
Hb	g/dl	6.8±0.12^e	8.3 ± 0.15^c	7.4 ± 0.12^d	9.6 ± 0.11^a	8.7 ± 0.11^b
Hct	%	23.3±0.17^e	28.2±0.14^c	27.2±0.14^d	32.6±0.14^a	30.5±0.14^b
WBC	×$10^3/\mu$L	18.4±0.29^e	21.4±0.24^c	19.3±0.2^d	26.3±0.27^a	23.4±0.23^b
LYM	%	58.6±0.31^a	52.1±0.18^c	56.4±0.26^b	47.3±0.2^e	48.2±0.25^d
PLT	×$10^3/\mu$L	66.3±0.05^a	45.5±0.08^b	28.6±0.12^e	43.1±0.08^c	37.4±0.14^d

Mean ± SE (standard error) with different letters within each column are statistically significant ($p<0.05$).

Blood serum parameters: Significant ($p<0.05$) variation in all the values of total protein (Fig. 1A), albumin (Fig. 1B), globulin (Fig. 1C) and A/G (albumin/globulin) ratio (Fig. 1D) of blood serum was noticed in comparison to control. N50 showed the lowest level of total protein (3.9±0.02 g/dl), albumin (1.9±0.01 g/dl) and globulin (2.0±0.02 g/dl). In contrast, CF achieved the highest level of total protein (6.09±0.03 g/dl), albumin (2.47±0.02 g/dl) and globulin (3.62±0.02 g/dl).

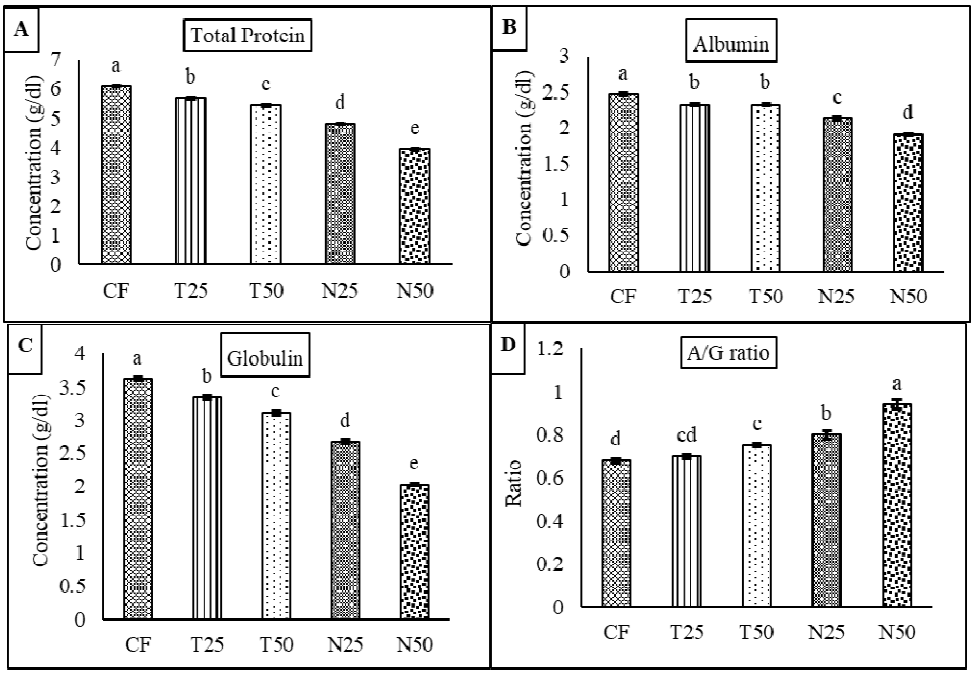

Fig. 1. Total protein (A), albumin (B), globulin (C) and A/G ratio (D) of *Oreochromis niloticus* at level of 5% significance.

Significantly ($p<0.05$) higher level of triglyceride (213.6±0.27 mg/dl) and cholesterol (250.3±0.2 mg/dl) was recorded in CF but in contrast, N50 showed the lowest triglyceride (194.1±0.27 mg/dl) and cholesterol (204.5±0.35 mg/dl) (Fig. 2A and 2B) concentration in blood serum.

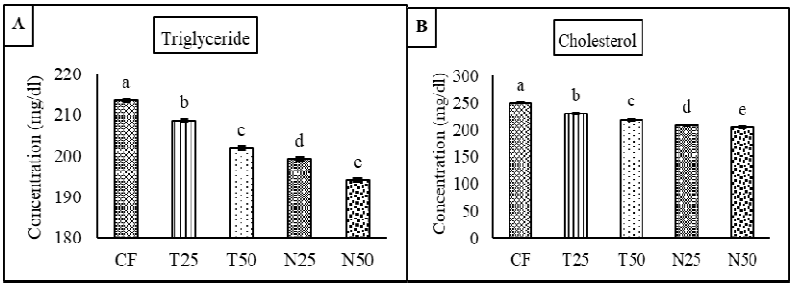

Fig. 2. Triglyceride (A) and cholesterol (B) concentration of *Oreochromis niloticus* at level of 5% significance.

In response to stress indication significantly ($p<0.05$) the highest concentration (79.2±0.28 mg/dl) of blood glucose was recorded in CF and the lowest concentration (67.5±0.08 mg/dl) was obtained by T25 treatment (Fig. 3).

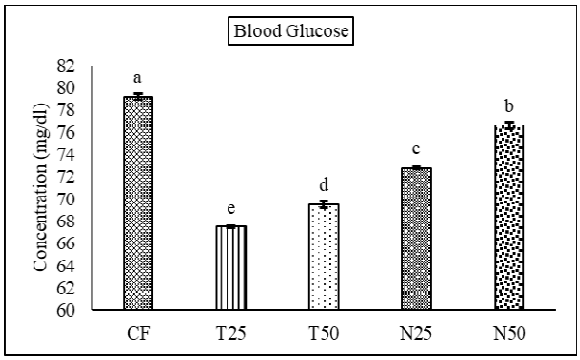

Fig. 3. Blood glucose concentration of *Oreochromis niloticus* at level of 5% significance.

Significantly ($p<0.05$) lower concentration of urea (23.8±0.18 mg/dl) and BUN (11.1±0.08 mg/dl) was noted in N50 besides higher concentration of urea (30.9±0.47 mg/dl) and BUN (14.4±0.26 mg/dl) was obtained by CF treatment (Fig. 4A and 4B).

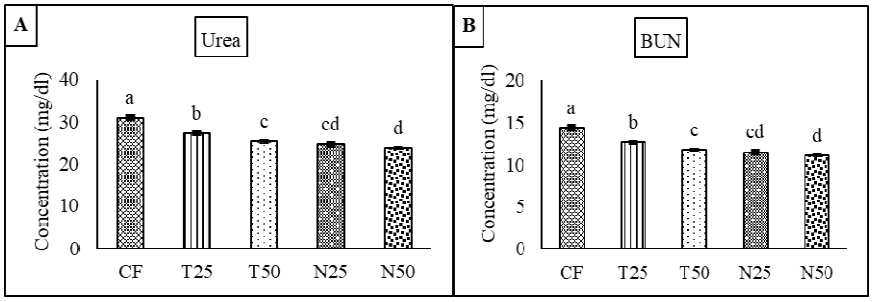

Fig. 4. Urea (A) and BUN (B) concentration of *Oreochromis niloticus* at level of 5% significance.

Discussion

Fish meal alone is a high source of protein in diet for *Oreochromis niloticus*. Expected outcomes were not obtained in case of growth study in this research. *Nannochloropsis* sp. and *Tetraselmis* sp. microalgae inoculated diet exhibited poor growth, feed utilization performance compared to control treatment (Table III). Although microalgae are rich in vitamins, minerals, carotenoids, essential amino acids and fatty acids (PUFA), higher proportion of microalgae also contain more fibre content which is not easily digestible (Sarker *et al.* 2018) in fish body and responsible to create palatability problem. In this sense, the dietary quality of microalgae protein might be less high than that of fish meal. Walker and Berlinsky (2011) elucidated that feed consumption and growth rate of Atlantic cod, *Gadus morhua* declined when feed was formulated replacing fish meal with 15% and 30% (dry weight basis) *Isochrysis* sp. and *Nannochloropsis* sp. microalgae. Sarker *et al.* (2018) also found similar result and stated that, with the increasing percentage of microalgae *Nannochloropsis oculata* up to 33%, 66% and 100% in fish diet could reduce the growth performance of *Oreochromis niloticus* compared to control diet. The result of

FCR in this study is in line with the study of Olvera-Novoa *et al.* (1998). Dietary microalgae have also been seen to have some negative impact on fish health, reported by other researchers. For instance, in comparison with the reference diet of fish which was based on fish meal, 10 percent (dry-weight basis diet) freeze-dried meals derived from *Tetraselmis chuii* and *Phaeodactylum tricornutum,* separately or in addition with *Bacillus subtilis* (10^7 CFU/g) probiotic, in *Sparus aurata*, decreased variety of bacteria and disturbed bowel morphologies (Cerezuela *et al.* 2012); at the same time insignificant inflammation of the digestive area was caused by 10 percent inclusion of freeze-dried *Navicula* sp. in dry weight diet. Buentello *et al.* (2015), indicated that these was may be due to some meals of microalgae which contains antinutritional components for example, protease inhibitors and oligosaccharides present in soybean meal like basal source of protein, it prompted related responses in different fish species, may explain the evidence of disturbed fish digestive area and decreased palatability. Conversely, contradictory results were found in some earlier studies and evidence were also reported by different researchers (Kiron *et al.* 2012, Patterson and Gatlin 2013). The result of survivality (Table III) of this research accomplished the expected outcome confirming that microalgae can be a potential source for increasing the survivality of cultured species. Similarly high survivality obtained in the study of Patterson and Gatlin (2013). High survivality was achieved in this research might be due to presence of some bioactive compounds in *Tetraselmis* sp. and *Nannochloropsis* sp. such as, carotenoids, vitamin E, terpenes and phenolic complexes comprising anthelmintic, cytostatic, antioxidant, antifungal, antiviral and antibacterial actions which was absent in fish meal and thus, helped to boost the immunity (Brown *et al.* 1999, Newman *et al.* 2003) of *Oreochromis niloticus*.

The environment in which fish lives need to be well suited for fish health. In this sense optimum water quality is required for fish culture. In this study the values obtained for physical parameters such as DO (DeLong *et al.* 2009), temperature (DeLong *et al.* 2009), pH (DeLong *et al.* 2009) and chemical parameters like TAN (Caldini *et al.* 2015), NO_2-N (DeLong *et al.* 2009), SRP (Boyd 1998) was within the optimum level (Table IV).

The nutritional condition and health status of fish can be reflected by its hematology. Blood of fish is therefore extremely necessary for the precise assessment of fish health. Microalgae inclusion in diet has positively influenced the RBC, Hb, Hct, WBC compared to control. The values of RBC, Hb, Hct, WBC, LYM and PLT of *Oreochromis niloticus* are within reference range (Table V) with the values provided by Hrubec *et al.* (2000) and Mauel *et al.* (2007) for tilapia fish. RBC is related to the oxygen carrying capacity of teleost fish species. Decreasing level of RBC in CF is indicating that, less oxygen binding molecule Hb is present in blood and restricted fish capacity to deliver the tissues with enough oxygen, resulted in physical activity decline. However, Hb loss is dangerous for oxygen transfer and any form of blood dyscrasia and erythrocyte degradation in fish exposed to toxicants can be identified as pathological disease (Javed *et al.* 2016). It was found that microalgae *Nannochloropsis* sp. and *Tetraselmis* sp. are rich in essential vitamins like, ascorbate, β-carotene, α-tocopherol, thiamine, riboflavin, folates, pyridoxine, cobalamin, biotin and the presence of retinol was only identified in *Tetraselmis* sp. (Brown *et al.* 1999). Presence of vitamins in microalgae has acted like immunostimulant in *Oreochromis niloticus* and it led to the increase of WBCs (Table V) in tilapia to create quick recovery of its body against stress factors. The results of LYMs and PLTs were in line with the study of Souza *et al.* (2020). *Tetraselmis* sp. and *Nannochloropsis* sp. microalgae contains high amount of essential n-3 fatty acids, PUFA (Servel *et al.* 1994). The dietary administration of microalgae containing PUFA is the probable reason of low level of lymphocytes in T25

treatment. It was demonstrated that by reducing the capacity of T-lymphocytes to spread, PUFAs can lower pro-inflammatory reactions. When DHA is administered the number of T lymphocytes suppressor increased then negatively controls other lymphocytes and this is the possible reason of such regulation (Souza *et al.* 2020). Platelets in blood is responsible for the creation of protective barrier. However, n-3 PUFA has the quality to cease the platelet quagulation (Thorngren and Gustafson 1981) and tilapia got PUFA from microalgae which resulted in low amount of platelet in N50 (Table V). In this sense, further study needs to clarify this observation.

The results obtained for total serum protein, albumin, globulin, blood glucose and cholesterol are within the reference range provided by Hrubec *et al.* (2000) for tilapia fish. During the experiment, *Oreochromis niloticus* could not successfully utilize protein nutrition from feed because in certain conditions, the total protein concentration reduces due to decreased synthesis capability, absorption, or protein depletion (Bernet *et al.* 2001) which was reflected into its blood serum protein, albumin, globulin and A/G ratio (Fig. 1). Similar results obtained in the study of Abd El-Ghany *et al.* (2020). Cholesterol and triglyceride are the sources of structural cell membrane component and cellular storage energy for fish health (Patriche *et al.* 2011). An increase in these two parameters in blood serum may reflect high storage of lipid and metabolic syndrome. Microalgae inclusion in diet showed significant lowering impact of cholesterol and triglyceride level and it was highly noticed in N50 treatment (Fig. 2). Servel *et al.* (1994) confirmed that *Nannochloropsis* sp. and *Tetraselmis suecica* are abundant in n-3 fatty acid EPA, respectively 30.1% and 6.2%. High EPA in diet could reduce the cholesterol and triglyceride level because EPA like PUFA can lower the concentration of lipid through affecting hyperlipidemia (Sathasivam *et al.* 2019). Glucose is an instant energy supply that involves heart and muscle activity of the body. Significant increase in serum glucose levels was found in CF (Fig. 3). Serum glucose concentration changes are particularly related to renal damage. Lowest accumulation of stress enzymes can be possible due to addition of microalgal meal in diet (Mukherjee *et al.* 2019). Moreover, it was found that only 1% *Spirulina platensis* addition in diet of *Oreochromis niloticus* significantly lowered blood glucose level than control treatment (Al-Deriny *et al.* 2020). Meanwhile, high antioxidant properties present in *Tetraselmis* sp. suppressed the activity of stress producing factors by accelerating quick recovery of its body and thus, lowered the blood glucose concentration in T25 (Fig. 3). Urea is the main food-protein and tissue-protein metabolite. The liver generates nitrogen as the body metabolizes protein. To form urea nitrogen which generated from liver, binds with other substances derive from liver. After passing through the circulation into the kidney urea drained out of the blood and left in urine (Ajeniyi and Solomon 2014). Incidentally, BUN reflects only the nitrogen content of urea as waste product. The body removes urea from the urine, which increases the BUN level as the activity of the kidneys reduces. Thus, increase in protein rich diet increases blood urea also (Ajeniyi and Solomon 2014). In this study, lower feed intake, disturbance in liver protein metabolism lowered urea and BUN concentration in N50, followed by N25 compared to CF treatment (Fig. 4).

This study indicates that, inclusion of *Tetraselmis* sp. and *Nannochloropsis* sp. microalgae in diet created problem regarding palatability. Thus, lowering the microalgae inclusion percentage could resolve this problem. However, positive impact on water quality, survivality and hematology was noticed. Worthwhile influence on some serum parameters was also recorded with microalgae-based diets. Considering the beneficial impacts on water quality, survivality, hematology and serum chemistry microalgae can be a potential source for

commercial fish cultivation. Further research with other commercial fish species using more treatments and replicates is needed to evaluate more consistently and reliably the negative effect of microalgae on growth.

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