Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(3): 433 – 457 (2022)
www.ejabf.journals.ekb.eg

Impacts of dietary replacement of soybean meal with dried *Ulva lactuca* meal on growth performance, feed efficiency, and physiological responses of *Oreochromis niloticus*

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ARTICLE INFO

Article History:
Received: April 13, 2022
Accepted: May 14, 2022
Online: June 7, 2022

Keywords:
Green seaweeds,
Ulva lactuca,
Growth,
Feed efficiency,
Physiological responses

ABSTRACT

Seaweeds have gained focus as a sustainable aqua feed ingredient due to their restricted land-based resources requirement and their highest nutritional value. The present study was designed to investigate the effect of a partial replacement of soybean meal (SBM) with dried seaweeds *Ulva lactuca* meal (ULM) on growth performance, feed efficiency, body composition, and physiological responses of the monosex Nile tilapia (*Oreochromis niloticus*) fry for 84 days. A total of 150 *O. niloticus* fry individuals, with an average initial body weight (0.81± 0.02g), were randomly distributed into 5 treatments with triplicate groups in 15 glass aquaria. Five isonitrogenous (30% crude protein) and isocaloric (440 kcal/100 g) diets were formulated to contain ULM as SBM replacers at levels 0.0 (as a control diet, D_1), 5.0 (D_2), 15.0 (D_3), 25.0 (D_4) and 35.0% (D_5) substitution. The fish fed different levels of ULM, especially level 15% (D_3), showed an improvement in the rearing water quality parameters, and significantly increased growth performance, condition factor, and feed utilization, besides a recorded improvement in the hepatosomatic index, and physiological responses of *O. niloticus* compared to those fed the control diet (D_1). In addition, the negative effects of high levels of dietary ULM were detected on the afore-mentioned parameters. Thus, it could be concluded that the beneficial use of ULM instead of SBM in *O. niloticus* fry diet, without reaching 15%, may have an economic efficiency and environmental friendly effects on fish farms.

INTRODUCTION

Globally, the production of aquaculture plays an increasing role in meeting the demand for fish and other fishery products. Aquaculture is the fastest-growing food-producing sector, where aquaculture industry accounts over 50 percent of the global fish production **(Galappaththi *et al*., 2020)**. In the developing countries such as Egypt, there is a severe shortage of animal protein sources, which resulted in a dramatic increase in their prices. The total annual fish production in Egypt was estimated at 2.0 million tons

(GAFRD, 2019). Generally, the Nile tilapia (*Oreochromis niloticus*) is an ideal fish species for culture due to its high growth rates, adaptability to a wide range of environmental conditions, ability to grow, reproduce in captivity, and feed at low trophic levels **(El-Sayed, 2006)**. Tilapia can be grown on plant-based diets including algae, aquatic plants and a variety of feeds from diverse origins due to its nutritional requirements **(Watanabe *et al*., 2002)** and thus it is relatively inexpensive. Tilapia undergoes a diet and feeding mode shift from carnivorous to omnivorous feeding at a standard length of 2–3cm and then becomes phytoplanktivorous at 6–7cm, with algae accounting for a significant part of their diet during the adult stage **(Beveridge & Baird, 2000)**. In addition, tilapia feed at low trophic levels, and their feed costs are lower than carnivorous fishes, and genus *Oreochromis* are primarily microphagous, feeding mainly on phytoplankton, periphyton and detritus **(El-Sayed, 2006)** and can efficiently ingest aquatic plants, small invertebrates, benthic fauna, bacterial films (**FAO, 2012**), and even other fish and fish eggs.

For the increased prices of aqua feeds, the identification of less expensive and locally available feed sources have become necessary to assure the proposed aquaculture development, particularly in rural areas, where the use of non-conventional feed sources is an important key to reduce feed costs in fish cultivation **(Velásquez, 2016)**. Aqua feed accounts about 50-80% of aquaculture production cost; therefore, its use has to be carefully considered. Nutritionally balanced fish diets contain common feed ingredients such as fishmeal, soybean meal, forming the major sources of protein **(Saleh, 2020)**. However, food security of these ingredients is a growing concern due to the limited agricultural resources of arable land and fresh water **(Pretty, 2008)**, the declining wild fish stocks **(Tacon & Metian, 2008)**, the increasing demand to supply livestock protein **(Von Braun, 2008)**, the increasing world population **(Godfray *et al*., 2010)** and the increasing cost **(Saleh, 2020)**. Consequently, there is a critical important role for alternative crops in securing the future supply of protein and sources of fish feed ingredients, especially for the aquaculture sector **(Boland *et al*., 2013; Saleh, 2020)**. The utilization of soybean has rapidly increased in aquatic feeds.Despite its global fluctuating price, its cost remains a burden on fish farmers **(Chan *et al*., 2019)**. In this respect, scientific research exerted great efforts to search for low-cost ingredients' alternatives for soybean, and the attention turned to seaweeds. For marine macro algae (seaweeds), they are simple plant-like organisms found worldwide growing in both marine and freshwater ecosystems **(Wang *et al*., 2015)**. Whereas, seaweeds are often proposed as an alternative protein crop used in compound diets for animals since they have high crop productivities per unit area **(Nielsen *et al.*, 2012; Mata *et al*., 2016)**. In addition, they are highly considered for their high protein value, essential amino acid content, vitamins and trace metals in fish feeding **(Patel *et al*., 2018)**.

Aquatic plants are important nutritional sources for herbivorous-omnivorous fish, and they have been frequently reported as suitable alternative animal feed **(Kalita *et al*., 2007)**, and in many cases, they could replace up to 25% of the formulated diets and up to 50% of commercial feeds (35% crude protein), without adverse effects on fish growth and body composition **(Velásquez, 2016)**. Aquatic plants have been widely used as a nutrient source in diets for tropical freshwater fish, mainly tilapia and carp **(El-Sayed, 2003; Diler *et al*., 2007)**. Green seaweeds have been reported as a "super food", with significant positive effects on fish performance, higher nutritive values, source of protein and better digestibility **(Ismail *et al*., 2017)**. In addition, green seaweeds represent a source of dimethyl sulphonyl propionate, which maintains the integrity of cells **(Butawan *et al*., 2017)**, and they act as a source of amino acids **(Cruz-Suárez *et al*., 2009)**, pigments **(Parisenti *et al*., 2011)**, vitamins and minerals **(Christaki *et al*., 2012)**, polyunsaturated fatty acids **(Guedes *et al*., 2015**) and vitamin C **(Nielsen *et al*., 2021)**. They improve the immune system **(Ghaeni & Roomiani, 2016)** and are used to obtain the oxidative stability of fish products **(Undeland, 2016)**. Moreover, they are antiviral and antibacterial besides improving the gut function **(Michiels *et al*., 2012)** and stress resistance **(Sheikhzadeh *et al*., 2012)**. Furthermore, green seaweeds promote lipid mobilization and metabolism, and improve the absorption and assimilation efficiency in fish **(Güroy *et al*., 2011)**.

In aquaculture, seaweeds meal has received a great attention as suitable feed ingredients sources for farmed fish since their protein content ranged from 8%- 44% **(Xuan *et al*., 2013)**. In recent years, green seaweeds have become important as a dietary ingredient for a wide range of fish species **(El-Meinshawy, 2018; Kotit, 2018)**. Several studies recommended a 15% of seaweeds as an optimal level in fish diets to improve growth performance and feed utilization of the red tilapia **(El-Tawil, 2010)**, the black sea bream **(Xuan *et al*., 2013)**, *Cachama blanca* juveniles **(Velásquez, 2016)** and the Nile tilapia **(El-Meinshawy, 2018)**.

Consequently, the general objective of the present study was to investigate the nutritional potential of aquatic plants available in rural Egypt such as *Ulva lactuca* meal, (ULM), through replacement at different levels (0, 5, 15, 25 and 35%) instead of soybean meal (SBM) for 84 days and evaluate their effects on the growth performance, condition factor, feed and nutrients utilization, carcass composition, and the physiological responses of the Nile tilapia, *Oreochromis niloticus* fry.

MATERIALS AND METHODS

This study was carried out at the Fish Nutrition Laboratory, Animal and Fish Production Department, Faculty of Agriculture, Alexandria University, Alexandria Governorate, Egypt in summer 2019. The present experiment was designed to investigate the effect of the replacement of SBM with different levels of dried ULM, besides evaluating the effect of these treatments on the growth performance, feed utilization, carcass composition, and the physiological criteria of *O. niloticus* fry.

1. Collection and preparation of the green seaweeds

Fresh green seaweeds, *Ulva lactuca* were collected (picked by hand) from Mariut Lake, Alexandria Governorate, Egypt. *U. lactuca* samples were taken to the laboratory in plastic bags. The samples were well rinsed in freshwater and cleaned using soft brush to eliminate the epiphytes and salts present on their surfaces, and then they were air-dried in the dark at room temperature (25 to 30°C) on absorbent paper. The dried samples of *U. lactuca* were grinded to super fine powder (<30 µm) and stored at –20°C until use for analysis in the laboratory. The dry matter and relative humidity, ash, protein, fat and fiber were determined, and carbohydrates and total energy in plant were estimated as well. The collected aquatic plant was identified following the methods of **Jha *et al*. (2009)**, and confirmed using algae base website **(Guiry & Guiry, 2011)**. While, the tested aquatic macrophytes were identified according to **Littler and Littler (2000)**.

2. Experimental diets

Five practical experimental diets (D_1, D_2, D_3, D_4 and D_5) were formulated to partially replace 0 (as a control diet), 5, 15, 25 and 35% of SBM with dried ULM on an equivalent protein basis. The experimental diets were formulated using the feed ingredients as shown in Table (1).

Table 1. Feed ingredients of the experimental diets

Item	Experimental diets*				
	D_1	D_2	D_3	D_4	D_5
Feed ingredient (g 100g^{-1} dry matter)					
Fishmeal	23.00	23.00	23.00	23.00	23.00
Soybean meal (SBM)	30.00	28.50	25.50	22.50	19.50
Ulva lactuca meal (ULM)	0.00	3.13	9.38	15.63	21.89
Wheat bran	12.00	12.00	12.00	12.00	12.00
Yellow corn	30.00	28.37	25.12	21.87	18.61
Sunflower oil	3.00	3.00	3.00	3.00	3.00
Vit. & Min. mix.**	2.00	2.00	2.00	2.00	2.00
Total	100.00	100.00	100.00	100.00	100.00

* D_1 (as a control diet 0.0% ULM); diets 2, 3, 4, and 5 contained 5, 15, 25, and 35% of ULM instead of SBM.

** The composition of vitamins and minerals mixture of each 1 kg contains: Vitamin A - 50,00,000 IU; Vitamin D_3- 10,00,000 IU; Vitamin B_2 , 2.0g; Vitamin E , 750 units; Vitamin K , 1.0g; Calcium pantothenate 2.5g; Nicotinamide , 10.0g; Vitamin B_{12} , 6.0g; Choline Chloride , 150.0g; Calcium , 750.0g; Manganese , 27.5g; Iodine, 1.0g; Ion, 7.5g; Zinc , 15.0g; Copper , 2.0g; Cobalt , 0.45g.

3. Chemical analysis of the experimental diets and fish

The experimental diets, ULM, and SBM were chemically analyzed to determine the dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), and ash contents according to the methods of **AOAC (2000)** as shown in Table (2). Nitrogen free extract (NFE) was calculated by differences through deducting the sum of the percentages of moisture, CP, EE, CF and ash from 100. Gross energy (GE) contents of the experimental diets, ULM, and SBM (Table 2) and the energy content of the fish body were calculated using the factors of 5.64, 9.44 and 4.12 kcal g^{-1} of protein, lipid and carbohydrates, respectively **(NRC, 2011)**. The routine chemical analysis of the experimental fish was also done at the beginning and at the end of the experiment according to the methods described by **AOAC (2000)**.

4. Macro and trace elements determination

Samples of ULM and SBM were subjected to wet ashing **(AOAC, 2000)** method. Then, the digested material was diluted and transferred to a volumetric 200 mL flask. Samples were injected in the atomic absorption Spectrophotometer (England made, 2002) to determine four macro elements (calcium, magnesium, potassium and sodium) and four micro elements (copper, ferric, manganese and zinc) as shown in Table (3).

5. Determination of essential amino acids and nonessential amino acids

Essential amino acids and nonessential amino acids profiles **(**g 100g^{-1} protein) of ULM, and SBM were analyzed according to **Csomós *et al*. (2002**) and shown in Table (4). Dried and defatted powder sample (about 0.2g) was hydrolyzed with 6 N HCL (10 mL) in sealed tube, heated in oven at 100ºC for 24 hours. Resulting solution was completed to 25mL with de-ionized water. After filtration, five mL of hydrolysate was evaporated until being free from HCL vapor, then the residue was dissolved in diluted buffer (0.2 MNa, pH 2.2), and a sample was injected to amino acids analyzer.

Table 2: Proximate chemical analysis (% on DM basis) of the experimental diets, *Ulva lactuca* meal (ULM), and soybean meal (SBM)

Item	Experimental diets*					ULM	SBM
	D_1	D_2	D_3	D_4	D_5		
Dry matter	95.1	94.4	94.87	94.7	94.31	90.09	89.12
Crude protein	30.7	30.6	30.96	30	30.1	20.15	42.00
Ether extract	8.5	8.63	8.77	9.16	9.37	8.13	4.10
Ash	5.95	6.38	7.16	8.15	8.89	18.13	4.35
Crude fiber	3.57	3.6	4.14	3.73	4.27	5.74	7.30
Nitrogen free extract	51.3	50.8	48.97	48.9	47.37	47.85	42.25
GE (Kcal 100 g^{-1})**	465.0	464.00	459.60	458.00	453.80	387.53	449.65
P/E ratio ***	66.00	65.90	67.37	65.6	66.33	51.99	93.40

* D_1 (as a control diet 0.0% ULM); diets 2, 3, 4, and 5 contained 5, 15, 25, and 35% of ULM instead of SBM.

GE (Kcal 100g^{-1} DM) = Gross energy was calculated using factors 5.64, 9.44 and 4.12 kcal per gram of protein, lipid and carbohydrate, respectively **(NRC, 2011).

***P/E ratio = Protein to energy ratio mg crude protein Kcal^{-1} gross energy.

6. Fish and feeding regime

Monosex *O. niloticus* fry with an average initial body weight (0.82 ± 0.02) were purchased from a commercial fish farm in Kafr El-sheikh Governorate, Egypt. Early in summer 2019, fish were acclimatized in fiberglass tanks for two weeks. Fish were randomly stocked in triplicates, in 15 glass aquaria (70 × 40 × 30 cm allowing 70L safe water volume capacity of each). Fish stocked at a density of 10 fish per aquarium. Each aquarium was supplied with two air stones and dechlorinated tap water, which was stored in tanks for 24 hours and aerated by air pump (Model-Rina 301) during all the experimental period. The water level was maintained to a fixed level by the addition of new well-aerated fresh water. Fish were manually fed the experimental diets twice daily, six days a week at a rate of 10% of fish biomass weight for the first 30 days, after that the rate was reduced to 6% for another 30 days, followed by a rate of 3% until the end of experiment (84 days). To insure sufficiency of the feed quantity fish weight was measured and recorded biweekly.

7. Experimental parameters

7.1. Water quality parameters

In the present study, water quality parameters were determined according to the methods of **APHA (1992**). Water temperature (ºC) and dissolved oxygen (DO, mg L^{-1}) were measured at 9:00 a.m. The pH-value and total ammonia-nitrogen (TAN, mg L^{-1})

were measured by a Nilebot-Water Quality Monitoring system. Nilebot is a real-time water quality monitoring and alarming system for aquaculture powered by Conative Labs and it's based on Internet of Things for fish farms.

7.2. Fish growth performance parameters

At the end of the experiment, the growth performance parameters of the Nile tilapia fry were calculated regarding all treatments using the following equations;

1- Total weight gain (TWG, g fish^{-1}) = [final body weight – initial body weight];
2- Average daily gain, (ADG, g fish^{-1} day^{-1}) = [TWG/time (days)];
3- Specific growth rate (SGR, % day^{-1})= [ln final weight-ln initial weight] ×100/time (days)

7.3. Condition factor (K, %)

At the end of the experiment, final fish body weight (g) and their final standard length (cm) were individually measured to calculate the condition factor (K) using the following equation of **Froese (2006)**:

$$\mathbf{K}\,(\%) = \frac{\textbf{Fish body weight (g)}}{\textbf{Standard length }(\mathbf{cm^3})} \times \mathbf{100}$$

7.4. Feed and nutrients utilization criteria

At the end of the experiment, the feed and nutrients utilization criteria of the Nile tilapia fry were measured considering all treatments using the following equations:

1- Feed conversion ratio (FCR)= feed intake (g)/body weight gain (g);
2- Protein efficiency ratio (PER)= gain in weight (g)/protein intake in feed (g);
3- Protein productive value (PPV,%)= 100 [protein gain in fish (g)/protein intake in feed (g)];
4- Energy utilization (EU, %) = 100 [energy gain/energy intake in feed].

7.5. Fish carcass composition

At the end of the experiment, fish of each treatment were killed and dried, and then whole-body carcass composition was carried out as dry matter (DM,%), crude protein (CP,%), ether extract (EE,%), ash (%) and energy content (EC, Kcal 100g^{-1} DM).

7.6. Hepatosomatic index (HSI, %)

The fish body and the liver were individually weighed using GF.3000 digital balance, 3100 ×0.01g to calculate of hepatosomatic index (HSI) according to **Akombo *et al*. (2013**) using the following formula:

$$\mathbf{HSI}\,(\%) = \frac{\textbf{Liver weight (g)}}{\textbf{Fish body weight (g)}} \times \mathbf{100}$$

7.7. Plasma biochemical parameters

At the end of experiment, five fish from each aquarium in all treatments were anaesthetized by putting them in a small plastic aquaria containing 5L of water supplemented with 3mL of pure clove oil (solved in 10 mL absolute ethanol) as a natural anesthetic material. Blood samples were collected from the fish caudal peduncle by a plastic syringe (3mL), which contained trisodium citrate (4%) as an anticoagulant to avoid clotting of blood sample during the collecting process before transferring it to dried small plastic vials for the determination of plasma glucose (mg dL^{-1}) according to **Henry (1964)**, total protein (g dL^{-1}), albumin (g dL^{-1}) according to **Gornall *et al*. (1949)**, and globulin (g dL^{-1}) according to **Doumas and Biggs (1972)**; addingly, albumin/globulin (AL/GL) ratio was calculated.

Table 3. Macro and trace elements of *Ulva lactuca* meal (ULM), and soybean meal (SBM)

Element	**ULM**	**SBM**
Macro elements(g kg^{-1})		
Calcium, Ca	4.42	0.43
Magnesium, Mg	4.94	0.42
Potassium, K	4.56	2.50
Sodium, Na	4.25	0.09
Trace elements (mg kg^{-1})		
Copper, Cu	9.12	17.25
Ferric, Fe	298.12	225.24
Manganese, Mn	49.14	50.68
Zinc, Zn	20.35	55.49

Table 4. Essential amino acid and nonessential amino acid profile (g $100g^{-1}$ protein) of *Ulva lactuca* meal (ULM) and soybean meal (SBM)

Amino acid profile	ULM	SBM
Essential amino acid (EAA):		
Arginine	1.69	2.31
Histidine	0.29	1.25
Isoleucine	0.80	2.14
Leucine	1.57	3.42
Lysine	1.13	2.73
Methionine	0.42	0.82
Phenylalanine	1.26	2.41
Threonine	1.19	1.80
Tryptophan	1.41	1.71
Valine	1.42	1.97
Nonessential amino acid (NEAA):		
Alanine	1.89	2.45
Aspartic Acid	2.44	4.94
Glutamine	5.87	7.53
Glycine	1.20	1.91
Proline	1.03	2.32
Serine	1.07	2.34
Tyrosine	0.90	1.95

8. Statistical analysis of data

All data were statistically analyzed using ANOVA, F-test, and least significant difference (L.S.D) procedures available in SAS® software package, version 9.1 **(SAS, 2004)**, where the following model was used;

$$\mathbf{Y_{ij} = \mu + \alpha_i + e_{ij}}$$

Where, Y_{ij} = the observation of the fish with eaten diet; μ = overall mean; α_i = effect of j the effect of diet. e_{ij} = random error assumed to be independently and randomly distributed. Data normality was tested according to Kolmogorov-Smirnov **(Frank & Massey, 1951)**. Analyses were run by Duncan's test at 5% significance in the software SAS®.

RESULTS

1. Water quality parameters

Data of water quality parameters (temperature, pH, TAN, and DO) are shown in Table (5). Water temperature throughout the experiment was within the average of 28.48 °C, pH value was 7.82, DO average was 6.90 mg L^{-1}, and TAN value was 0.002 mg L^{-1}.

Table 5. Effect of partially replacement of soybean meal (SBM) with different levels 0, 5, 15, 25 and 35% of *Ulva lactuca* meal (ULM) on rearing water quality parameters of the Nile tilapia fry

Diet*	Temperature (°C)	pH	DO (mg L^{-1})	TAN (mg L^{-1})
D_1	28.40±0.25	7.60±0.10^c	6.60±0.11^c	0.001±0.0001^c
D_2	28.50±0.10	7.90±0.11^b	6.90±0.10^b	0.002±0.0001^b
D_3	28.60±0.15	8.10±0.10^a	7.40±0.12^a	0.003±0.0001^a
D_4	28.40±0.10	7.90±0.14^b	6.90±0.11^b	0.002±0.0001^b
D_5	28.40±0.12	7.60±0.10^c	6.70±0.14^c	0.001±0.0001^c
Overall mean	28.48	7.82	6.90	0.002
C.V.**	0.55	2.15	6.20	0.0001
L.S.D (P < 0.05)***	0.53	0.50	0.41	0.0001

* D_1 (as a control diet 0.0% ULM); diets 2, 3, 4, and 5 contained 5, 15, 25, and 35% of ULM instead of SBM.
** Coffvar
*** Mean followed by different letters in the same column significantly differs at P < 0.05.
DO= Dissolved oxygen; TAN= Total ammonia-nitrogen.

2. Fish growth performance

Growth performance parameters as final weight (FW), TWG, ADG, and SGR of *O. niloticus* fed different levels (0, 5, 15, 25 and 35%) of ULM instead of SBM are shown in Table (6). The highest values of the growth performance parameters (FW, TWG, ADG, and SGR) were recorded in fish fed diet containing 15% ULM instead of SBM (D_3), followed by those fed diets No. 4, 2, 5 and 1, respectively, with significant differences (P<0.05) between all treatments in all the above parameters. Meanwhile, the lowest values for FW, TWG, ADG, and SGR were recorded in fish fed the control diet (D_1), without significant differences (P > 0.05) with fish fed D_5.

Table 6. Effect of partially replacement of soybean meal (SBM) with different levels 0, 5, 15, 25 and 35% of *Ulva lactuca* meal (ULM) on growth performance of the Nile tilapia fry

Diet*	Body weight		TWG[1] (g $fish^{-1}$)	ADG[2] (g $fish^{-1}day^{-1}$)	SGR[3] (% day^{-1})
	Initial (g $fish^{-1}$)	Final (g $fish^{-1}$)			
D_1	0.82 ±0.01	14.16^{d}±0.05	13.34^{d}±0.05	0.16^{d}±0.001	3.38^{d}±0.01
D_2	0.82 ±0.01	16.61^{c}±0.18	15.78^{c}±0.18	0.19^{c}±0.001	3.57^{c}±0.03
D_3	0.82 ±0.01	22.73^{a}±0.09	21.92^{a}±0.09	0.26^{a}±0.001	3.96^{a}±0.01
D_4	0.83 ±0.01	17.28^{b}±0.06	16.45^{b}±0.06	0.20^{b}±0.001	3.62^{b}±0.01
D_5	0.83 ±0.02	14.36^{d}±0.06	13.53^{d}±0.06	0.16^{d}±0.001	3.89^{d}±0.01
Overall mean	0.82	17.03	16.20	0.19	3.59
C.V.**	0.02	0.37	0.37	0.001	0.05
L.S.D (P<0.05)***	1.02	0.84	0.89	0.001	0.59

* D_1 (as a control diet 0.0% ULM); diets 2, 3, 4, and 5 contained 5, 15, 25, and 35% of ULM instead of SBM.
** Coffvar
*** Mean followed by different letters in the same column significantly differs at P < 0.05.
[1]TWG = total weight gain; [2]ADG = Average daily gain; [3]SGR = Specific growth rate.

3. Condition factor

Data of Fulton's condition factor (K) of the Nile tilapia fry are shown in Fig. (1). The highest value of K-factor (1.71%) was recorded in fish fed D_3, followed by those fed diets 4, 2, 1, and 5, with significant differences between all treatments (P<0.05) except fish fed D_2 and D_4. The lowest K-factor (1.11%) was obtained for the Nile tilapia fed diet containing the high level of ULM (35%) instead of SBM (D_5).

4. Feed and nutrients utilization

Feed and nutrients utilization as feed intake (FI), FCR, PER, PPV, and EU of the Nile tilapia fry are shown in Table (7). The highest values of FI, PER, PPV, and EU and the best FCR were registered in fish fed D_3, containing 15% of ULM instead of SBM. No significant differences (P > 0.05) were observed among all treatments of FI. Meanwhile, fish fed the control diet showed the lowest values of PER (1.16), PPV (18.53%), and EU (10.99%) and the worst FCR (2.86), while those fed D_2 achieved the lowest FI (33.62g $fish^{-1}$).

5. Carcass composition

Carcass composition of the Nile tilapia fry as DM, CP, EE, ash, and EC are shown in Table (8). No significant difference (P>0.05) was detected in DM, EE, and EC among all treatments. However, significant differences (P<0.05) were observed between treatments in CP and ash contents. DM recorded the highest value in fish fed D_3 (28.67%), while the lowest value was recorded in fish fed D_5 (27.87%). The highest CP content (58.89%) was noted in the Nile tilapia fry fed diet D_3, while the lowest value of CP content (57.22%) was recorded in fish fed the control diet (D_1). No significant differences of CP content were recorded among fish fed D_2, D_3, and D_4 (58.78, 58.89, and 58.64, respectively). On the other hand, the highest value of EE (23.88%) was recorded in fish fed D_5, while the lowest value was determined in fish fed the control diet (D_1).

Regarding the ash content, fish fed the control diet (D_1) recorded the highest value (19.35%), while the lowest value (17.49%) was recorded in fish fed D_2. The highest EC (556.36 Kcal/100g) was shown in fish fed D_2, while the lowest value was recorded in fish fed the control diet (D_1).

6. Hepatosomatic index and physiological responses

Data of fish HSI, plasma glucose, total protein, albumin (AL), globulin (GL), and Al/GL ratio are shown in Table (9). Fish fed the control diet (D_1) achieved the highest value of HSI (2.15%), while fish fed D_3 registered the lowest value (1.76%). The same trend was observed in plasma glucose (mg dL^{-1}), where fish fed the control diet (D_1) achieved the highest value (176.70 mg dL^{-1}), while those fed D_3 realized the lowest value (141.11 mg dL^{-1}). Differences were significant ($P < 0.05$) in HSI between treatments, except for fish fed D_2 (1.98%) and D_5 (1.92%). On the contrary, differences were not significant ($P > 0.05$) in glucose between all treatments, except for fish fed D_3. On the other hand, fish fed D_3 achieved the highest value of plasma total protein, albumin, and globulin, while the lowest values recorded of fish fed the control diet (D_1), where the differences were significant ($P < 0.05$) between all treatments of above parameters. Regarding to Al/GL ratio, fish fed D_5 recorded the highest value (1.58), while those fed D_2 recorded the lowest value (1.27). Yet, differences between all treatments of Al/GL ratio were significantly different ($P < 0.05$).

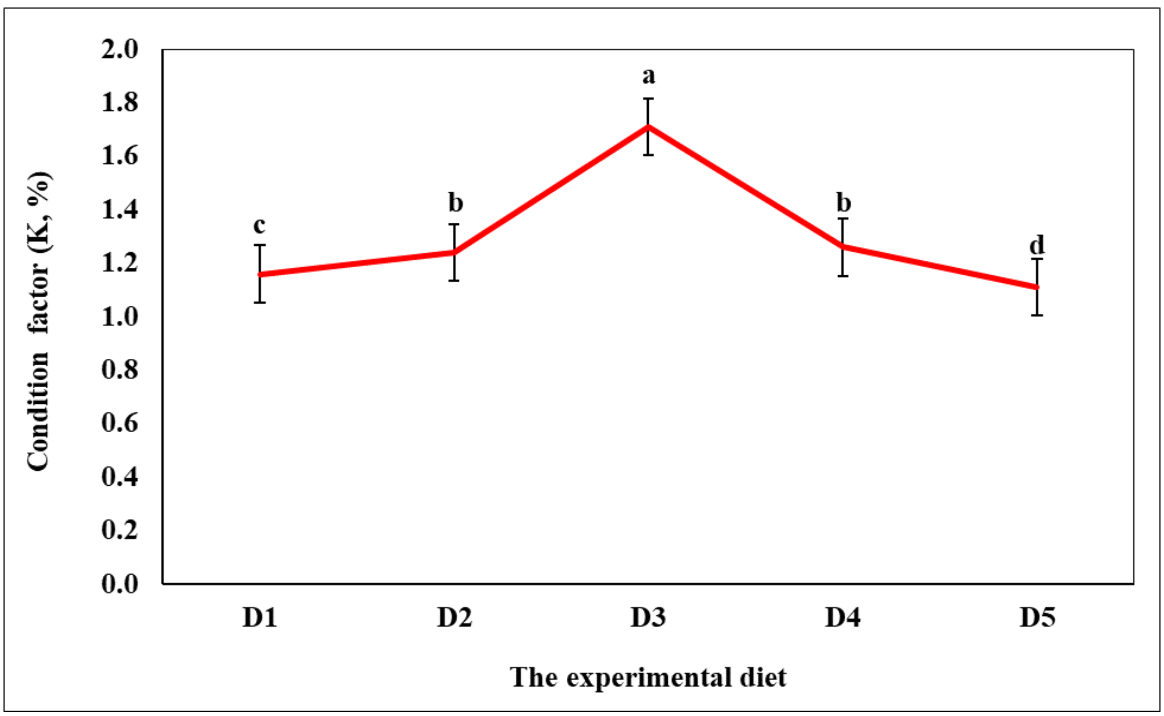

Fig. 1. Effect of partially replacement of soybean meal (SBM) with different levels 0, 5, 15, 25, and 35% of *Ulva lactuca* meal (ULM) on Fulton's condition factor (K) of the Nile tilapia fry

Table 7. The effect of partially replacement of soybean meal (SBM) with different levels 0, 5, 15, 25 and 35% of *Ulva lactuca* meal (ULM) on feed and nutrients utilization of the Nile tilapia fry

Diet*	Feed intake (g fish^{-1})	Feed conversion ratio	Protein utilization		Energy utilization (%)
			Protein Efficiency ratio	Protein productive value (%)	
D_1	38.14^a±0.13	2.86^a±0.12	1.16^b±0.05	18.53^c±1.50	10.99^c±0.86
D_2	33.62^a±0.13	2.13^c±0.02	1.44^{ab}±0.09	25.78^b±0.33	15.11^b±0.24
D_3	40.98^a±0.28	1.87^c±0.02	1.61^a±0.14	29.99^a±0.28	17.44^a±0.17
D_4	39.98^a±1.63	2.43^b±0.09	1.48^{ab}±0.07	22.94^b±1.09	13.38^b±0.62
D_5	38.15^a±1.15	2.82^a±0.09	1.34^{ab}±0.11	19.01^c±0.80	11.23^c±0.45
Overall mean	38.17	2.42	1.40	23.24	13.63
C.V.**	4.05	0.30	0.36	3.37	1.95
L.S.D (P<0.05)***	4.12	4.77	9.99	5.65	5.55

* D_1 (as a control diet 0.0% ULM); diets 2, 3, 4, and 5 contained 5, 15, 25, and 35% of ULM instead of SBM.
** Coffvar
*** Mean followed by different letters in the same column significantly differs at P < 0.05.

Table 8: Effect of partially replacement of soybean meal (SBM) with different levels 0, 5, 15, 25 and 35% of *Ulva lactuca* meal (ULM) on whole body composition of the Nile tilapia fry

Diet*	Dry matter (%)	% On dry matter basis			Energy content (Kcal 100g^{-1})
		Crude protein	Ether extract	Ash	
D_1	27.88±0.73	57.22^c±0.41	23.43±0.12	19.35^a±0.26	544.13±3.51
D_2	28.15±0.06	58.78^a±0.34	23.73±0.33	17.49^b±0.67	556.36±5.04
D_3	28.67±0.03	58.89^a±0.01	23.50±0.01	17.61^{ab}±0.02	554.82±0.24
D_4	28.50±0.24	58.64^a±0.12	23.63±0.05	17.73^{ab}±0.06	554.62±0.21
D_5	27.87±0.15	57.65^b±0.07	23.88±0.14	18.47^{ab}±0.04	551.39±0.73
Overall mean	28.17	58.28	23.73	17.98	553.56
C.V.**	1.61	0.67	0.59	1.12	8.34
L.S.D (P<0.05)***	2.22	0.45	0.96	2.42	0.59

* D_1 (as a control diet 0.0% ULM); diets 2, 3, 4, and 5 contained 5, 15, 25, and 35% of ULM instead of SBM.
** Coffvar
*** Mean followed by different letters in the same column significantly differs at P < 0.05.

DISCUSSION

Water quality requirements are essential to a healthy, balanced, and functioning aquaculture system **(Bhatnagar & Devi, 2013)**. Thus, in the present study, the quality parameters of the Nile tilapia rearing water, including temperature, pH, TAN, and DO were routinely measured, where all these parameters were within the acceptable ranges for rearing the Nile tilapia fry as cited in **Boyd (1998)**, without harmful effects on fish during all the experimental period. Generally, the best-preferred temperature range for optimum growth of tilapia is 25 to 27°C, while the perfect pH ranges between 6 and 9 **(Ekubo & Abowei, 2011)**. In addition, the tested water quality parameters are not adversely affected by the dietary replacement of SBM by different levels of ULM.

Table 9. The effect of partially replacement of soybean meal (SBM) with different levels 0, 5, 15, 25 and 35% of *Ulva lactuca* meal (ULM) on hepatosomatic index, plasma glucose, total protein, albumin, globulin, and albumin/globulin ratio of the Nile tilapia fry

Diet*	HSI (%)	Glucose (mg dL^{-1})	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Globulin (g dL^{-1})	AL/GL ratio
D_1	2.15^a±0.01	176.70^a±1.01	4.62^e±0.01	1.35^e±0.01	0.87^e±0.02	1.55^a±0.02
D_2	1.98^b±0.03	164.30^a±1.10	5.23^c±0.01	1.72^c±0.01	1.35^c±0.02	1.27^c±0.02
D_3	1.76^d±0.01	141.11^b±1.12	6.38^a±0.01	2.64^a±0.01	1.75^a±0.02	1.51^a±0.02
D_4	1.82^c±0.03	157.10^a±1.15	5.43^b±0.01	2.10^b±0.01	1.42^b±0.02	1.48^b±0.02
D_5	1.92^b±0.12	168.40^a±1.11	4.75^d±0.09	1.42^d±0.01	0.92^d±0.02	1.58^a±0.02
Overall mean	1.5	161.52	5.285	1.84	1.26	1.48
C.V.**	0.43	3.25	0.18	0.72	0.18	0.40
L.S.D (P<0.05)***	10.19	11.75	0.93	12.25	8.37	9.85

* D_1 (as a control diet 0.0% ULM); diets 2, 3, 4, and 5 contained 5, 15, 25, and 35% of ULM instead of SBM.
** Coffvar
*** Mean followed by different letters in the same column significantly differs at P < 0.05.
HSI (%) = Hepatosomatic index; AL/GL = Albumin/Globulin ratio.

Fish growth performance depends on a wide range of biotic and abiotic factors **(Mahavadiya *et al*., 2018)**. In the present study, the Nile tilapia fingerlings fed diet containing 15% ULM instead of SBM (D_3) recorded the highest significant growth performance parameters among other dietary replacement levels and the control diet as well. These positive effects on fish growth performance may be due to the abundance of vitamins, minerals, and bioactive compounds in seaweeds, which may promote lipid mobilization, improve absorption, and assimilate the efficiency of treated fish **(Xuan *et al*., 2013)**. In aquaculture respect, green seaweeds, *U. fasciata* and *Enteromorpha flaxusa*, exhibited a positive effect on the growth performance of rabbit fish fry and reduced the feed cost as added in half with artificial feed **(Abdel Aziz & Ragab, 2017)**. Thus, the use of moderate algal inclusion in fish feed has been recommended since it improves growth performance, feed utilization, carcass quality, higher survival rate, and assures better immune responses (**Hasan and Chakrabarti, 2009**).

In harmony with the obtained results, **El-Tawil (2010)** reported that green seaweeds (*Ulva sp.*) could be supplemented to the red tilapia (*Oreochromis sp.*) diet at an optimum level of 15% to improve growth performance without any adverse effect on feed efficiency or survival rate. In this respect, **Hussein (2017**) suggested that low algae inclusion (20% or less) in *O. niloticus* feed improves growth and digestive efficiency of feed than 30% inclusions and higher levels. Similarly, **Younis *et al*. (2018)** indicated that dietary incorporation of red algae, *Gracilaria arcuate* less than 20% could be practicable to improve growth performance of *O. niloticus*, compared to the higher replacer levels of fishmeal. The same positive effects on growth performance of *O. niloticus* were reported in **Arori *et al*. (2019)**. Some studies showed that the superiority of fish fed low levels of macroalgae might be attributed to its gross energy content, which affects fish appetite and, in turn, gastric evacuation time, and feed gastric evacuation rate, that decreases with the decreasing energy content in feed **(Hlophe & Moyo, 2011)**. Meanwhile, other studies attributed the decrease of fish growth and feed utilization when the diet contained the higher levels of seaweeds to the presence of anti-nutritional factors, viz. saponin, tannins and gossypols that occur in several plants **(Francis *et al*., 2001)**, which interfere with digestion and absorption of food, thus depressing the growth of fish **(Hajra *et al*., 2013)**. Inversely with the current findings, no significant effect was detected on the growth performance and feed utilization of *Sparus aurata* and *Dicentrarchus labrax* when fed green seaweed, *Ulva ohnoi* meal up to 5% **(Martínez-Antequera *et al*., 2021)**. Impaired growth rates and feed efficiency were noted when *U. ohnoi* was included in feeds for *Solea senegalensis* **(Vizcaíno *et al*., 2019)**. Similar observations were noted in the case of African catfish, *Clarias gariepinus* fed dried marine seaweed, *Gracilaria arcuate* as a dietary replacer of fishmeal **(Al-Asgah *et al*., 2016)**. In the same trend, **Guerreiro *et al*. (2019)** found that no significant (P > 0.05) effect was recorded on the growth performance of *S. aurata* juveniles fed 5% *U. lactuca*, or 5% *Chondrus crispus* seaweeds meal. This conflicting trend between different studies is mainly due to the seaweed type, its levels, experimental fish species, age, and feeding habits of fish as well.

There are several dietary factors affecting the growth performance of fish; these include protein, energy, lipid and fiber content, as well as the presence of anti-nutritional factors **(Hlophe & Moyo, 2011)**. Further studies attributed retrogressive values in growth performance parameters to higher fiber content. In the present study, declining values in the growth performance parameters were detected in fish fed diet included increasing replacement level up to 15% of ULM, which may be due to higher fiber content compared to other experimental diets; despite that the fiber contents were lower than the levels recommended in **De Silva and Anderson (1995)** (80-90 g/kg) and **Al-Ogaily (1996)** (90-120 g/kg). In agreement with the current findings, **Ulloa and Verreth (2002)** reported that high levels of dietary fiber reduce growth performance, feed and protein utilization of *O. aureus* fingerlings. In addition, **Majed *et al*. (2006)** reported that higher crude fiber content in the diets reduce the digestibility of protein, the bioavailability of nutrients, which consequently led to reduce the growth performance of fish. In that respect, **Azaza *et al*. (2008)** found that a possible reason for diminished growth at higher level of ULM (30%) could be due to the high fiber content and its possible effects digestibility of protein and dry matter.

The condition factor (a length-weight relationship) is an essential tool in biology, physiology, ecology and stock assessment of fishes (**Bolognini *et al*., 2013**). The condition factor might be influenced by sex, age, species type, maturity and environmental conditions (**Anyanwu *et al*., 2007**). The obtained data herein revealed that fish fed diet inclusion of 15% ULM instead of SBM significantly increased of condition factor among other experimental diets. The highest value of condition factor recorded for fish fed D_3 is in harmony with the obtained results regarding the positive effects of D_3 on growth performance parameters among other experimental diets as well, which also indicated of the good health condition during the experiment and it is indicating an isometric growth, which is the desirable for fish farm. In that respect, **Migiro *et al*.**

(2014) suggested that fish were provided a favourable condition and were in good health. As in the current findings, **Ighwela *et al*. (2011)** also found that a significant correlation between length and weight, while the condition factor computed for *O. niloticus*. In concurrence with our study, **Shahabuddin *et al.* (2015)** reported that gradually incorporation of red alga, *Pyropia pheroplasts* in *O. niloticus* juvenile's diet had a positive effect on the condition factor. In addition, **Younis *et al.* (2018)** recorded a constant condition factor of 1.6 for Nile tilapia fed diets containing red algae, *Gracilaria arcuate.*

It is remarkable that the algae ULM showed superiority, especially at 15% level instead of SBM of *O. niloticus* diet, regarding the feed and nutrients utilization parameters than other experimental diets. Many researchers have used many types of seaweeds as feed for other fish species (**Kotit, 2018; Vizcaíno *et al*., 2019**). Most of these studies found a progressive result of using seaweeds in fish feeding, regarding to improve growth performance, feed utilization, nutrients retention, survival, carcass quality, immune responses, stress and disease tolerance (**Magnoni *et al*., 2017**), in association with a strengthening of antioxidant systems (**Wu *et al*., 2017**). In this manner, **El-Tawil (2010)** stated that the highest value of protein efficiency ratio (PER) and apparent net protein utilization (NPU) obtained with red tilapia fed diet containing *Ulva* meal. Yet, **Kamunde *et al*. (2019)** suggested that dietary addition of 3% AquaArom (brown seaweeds, *Laminaria* sp.) significantly increased of PER, and tended to improve FCR of *Salmo salar*. As in the present study, laterally studies also found that the inclusion of different seaweeds (*Cystoseira barbata*, *Ulva lactuca*, *U. rigida*, and *G. cornea*) at high levels (20% and 30%) leads to a decline in all growth performance and feed utilization parameters (**Abdel-Warith *et al*., 2016**). Whereas, high levels of crude fiber content cause more rapid passage of food through the gastrointestinal tract, thus decreasing significantly the time available for digestion, consequently led to adversely affecting nutrients absorption of the diet (**Shiau, 1997**). In addition, **Azaza *et al.* (2008)** stated that the apparent protein digestibility was lowest (69.91%) when Nile tilapia fed a diet containing 30% *U. rigida* meal, the authors confirmed that this could be due to existence of some of anti-nutritional factors such as saponins and phytates. Moreover, **Francis *et al*. (2001)** reported that saponin could be caused by an effect on biological membrane by the permeability of the intestinal mucosal cells is increased, decreasing the activity of nutrients transport. In the same direction, **Martínez-Antequera *et al*. (2021)** recently demonstrated that the incorporation of *Ulva* or *Gracilaria* species in the diet of *S. aurata* did not significantly affect the growth performance or feed utilization.

Proximate body composition is reflected as a good indicator of physiological and health statues of fish (**Saliu *et al*., 2007**). The inclusion of a small amount of algal meal to the fish diet can have significant effects on carcass quality (**Valente *et al*., 2006**). Protein content of seaweeds varies not only among species, but also among habitats, and the quality of seaweed protein is acceptable compared to other diet vegetables, mainly due to its high content of essential amino acids (**Stedt *et al*., 2022**). Regarding to the fish carcass composition, no significant difference (P>0.05) in DM, EE, and EC among all the experimental diets. While, *O. niloticus* fry fed replacer diet with 15% ULM instead of SBM (D_3) followed by D_2, and D_4 significantly increased CP content compared to D_1 and D_5. In addition, fish in D_3 showed the highest DM content among other experimental diets. Meanwhile, high inclusion level of ULM (35%, D_5) showed that significantly decreased of CP, but increased EE contents among other diets. However, Nile tilapia fed 5% ULM (D_2) gave the highest value for EC and the lowest value for EE. Generally, it could be noted that there are unclear trend of the most carcass composition parameters among all dietary treatments. Similarly, with the current findings, **Natify *et al*. (2015)** concluded that *U. lactuca* could be considered as a potential fish feed additive, where the incorporation of *U. lactuca* up to 5% allows a slight increase of fish body composition. The current findings agree with those obtained by **Azaza *et al*., (2008)** who pointed out a reduction in body lipid and an improvement in protein content in the carcass when *Ulva* meal was added to Nile tilapia diets. Benefits of inclusion of *Ulva* meal in practical diets may be interpreted by the presence of vitamin C, which affects lipid metabolism, especially lipolysis (**Ortiz *et al*., 2006**). In this respect, **Ji *et al*. (2003)** reported that ascorbic acid in diet stimulates lipolysis and depresses lipogenesis. In addition, **Brinker (2009)** stated that green seaweed contains soluble non-starch polysaccharides, which bind water or mineral exchange to absorb organic compounds. **Cruz-Suárez *et al*. (2008)** also illustrated that seaweeds contain dimethyl sulphonyl propionate and amino acids as an attractant for fish, increases food consumption which resulted in improvement of PER, and FCR. On the other hand, **MacArtain *et al*. (2007)** reported that the contribution of seaweed to energy requirements of animals is rather low because of the high fiber content and low digestibility of polysaccharides and the relatively low fat content.

Somatic indices, such as HSI, VSI and GSI, are used to evaluate the nutritional status of fish. HSI is an index of liver, which is more reliable measure of fish condition than simple morphometric indicators and is a good indicator of the energetic condition of fish (**Davidson and Marshall, 2010**). Partially, liver is an important organ for energy storage and is usually the first site for lipid (energy) storage in a number of benthic and demersal fish species (**Rui, 2014**). In the present study, *O. niloticus* fry fed (D_3) showed the lowest value of HSI (%), and plasma glucose (mg dL^{-1}), besides the highest levels of plasma total protien (g dL^{-1}), albumin (g dL^{-1}) and globulin (g dL^{-1}) and the best among all experimental diets. These current findings revealed that diet inclusion of ULM instead of SBM, especially with level not up to 15% (D_3) significantly improved the HSI and physiological responses of experimental fish than those treated with other experimental diets. As the obtained results herein, **Natify *et al*. (2015)** found that reduction in HSI with *O. niloticus* fed *Ulva* supplemented diets and it was explained by the decreasing of fat deposition in liver, which clearly affected its weight. In the same line, **Hussein (2017)** reported that inclusion of seaweed, *Taonia atomaria* in diets for *O. niloticus* led to significantly decreased of HSI compared to the control diet. Likewise, **Xuan *et al*. (2013)** explained that dietary inclusion level of 20% of macro-algae, *Gracilaria lemaneiformis* significantly decrease HSI of the black sea bream. In contrast, **Guerreiro *et al*. (2019)** found that no significant ($P > 0.05$) effect on HSI of *S. aurata* juveniles fed 5% *Chondrus crispus* seaweed meal. On the contrary, with the current findings and previous studies as well, **Nur *et al*. (2020)** recently reported that dietary incorporation of 30% seaweed (*Hypnea musciformis*) as fishmeal replacement significantly increase HSI of *O. niloticus* than those fed the control diet.

Blood biochemical parameters are closely associated to fish health and their immune responses. The results of plasma biochemical parameters as glucose, total protein, albumin, and globulin in the current study are in accordance with those obtained by **Fadl *et al*. (2017)** and **Mahmoud *et al*. (2020)** when tilapia fed *Chlorella vulgaris*, or

Schizochytrium sp. serum glucose was significantly lower, while total protein were significantly higher compared to fish fed the control diet. Similarly, with the current findings, **Natify *et al*. (2015**) reported that the incorporation 5% of *U. lactuca* in *O. niloticus* diets promoted the immune responses parameters. In the same trend, *U. rigida* low-level dietary incorporation has improved growth, feed efficiency, carcass quality, disease resistance, physiological activity and reduced stress response (**Valente *et al*., 2006**). In this respect, **Guerreiro *et al*. (2019)** reported that dietary incorporation of *Ulva* leads to significantly lower of plasma glucose of *Sparus aurata* juveniles. In a recent study, **Jo Rivero (2021)** also suggested dietary supplementation of microalgae (*Chlorella* sp., *Haematococcus* sp., and *Schizochytrium sp.*) at a level of 0.5% of each could be used as immune enhancer agents for juvenile rainbow trout, *Oncorhynchus mykiss*. Additionally, **Kamunde *et al*. (2019)** suggested that dietary addition of AquaArom (brown seaweeds, *Laminaria* sp.) up to 10% significantly improve antioxidant capacity and alleviate the adversely effects of stressors such as temperature of *Salmo salar*. Inversely, **Khalafalla and El-Hais (2015)** reported that no significant effect were obtained for serum total protein, globulin and albumin of *O. niloticus* fingerlings fed red algae (*Pterocladia capillacea*) and green algae (*U. lactuca*). This greatly conflict between the findings of different studies may be due to some variations like algae species, dietary inclusion levels, exposure time, fish species, age, and the experimental management.

Finally, the positive effects of dietary ULM used in the present study as a replacer of SBM, especially at level 15% (D_3) on growth performance, nutrients utilization, physiological, and immune responses of *O. niloticus* fingerlings among other experimental diets or the free-ULM control diet, may be due to its high nutritive values containing equitable amounts of pigments and vitamins (**Parisenti *et al*., 2011** and **Christaki *et al*., 2012**), and contains reasonable amounts of iodine and smart compound such as Dimethyl sulphonyl propionate (**Valente *et al*., 2021**). In addition, these beneficial effects of seaweeds were also recorded when addition or included in aqua feeds for other fish species (**Guerreiro *et al*., 2019; Kamunde *et al*., 2019; Jo Rivero, 2021**) may be due to many secondary metabolites produced by seaweeds included functional proteins (**Cruces *et al*., 2012**), peptides (**Harnedy and FitzGerald, 2011**), amino acids (**Carreto and Carignan, 2011**), fatty acids (**Alamsjah *et al*., 2007**), functional carbohydrates (**Karsten *et al*., 1996**), vitamins (**Pinto *et al*., 2003**), carotenoids, and phenolics (**Dethier *et al*., 2005**), and other secondary metabolites (**Oliveira *et al*., 2013; Svensson *et al*., 2013**).

CONCLUSION

From the obtained results in the present study, dietary replacement of SBM by different levels of ULM are promising to practicable for *O. niloticus* fingerlings, especially at level not up to 15% instead of SBM, regarding significantly increased the growth performance, and feed utilization, besides improved the physiological responses of treated fish. These positive findings may be have an economic efficiency and environmental friendly effects for fish farmers. Meanwhile, further research should be done of this seaweed by using different techniques to improve its components bioavailability to fish, increase their potential in fish feeding, and to reduce the inverse effect of fiber content on fish' growth performance and feed utilization not only for *O. niloticus* fingerlings, but also for other freshwater or marine fish species at different life stages.

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