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Effect of using Algae (*Nannochloropsis Oculata*) in Grey Mullet (*Liza Ramada*) Larval Diets on Growth Performance and Feed Utilization

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ABSTRACT

This study was carried out in the Faculty of Agriculture (Saba- Basha), Alexandria University to study the effect of five dietary Green microalgae *Nannochloropsis oculata* levels (0, 7, 14, 21, and 28%) on growth performance and feed utilization for grey mullet (*Liza ramada* Risso 1829) fry with initial body weight of 0.153 g. Each treatment was three replicate aquaria and lasted for eight weeks. Fish were given feed twice daily at a feeding rate of 12% of fish biomass seven days / week. The results showed that Weight gain, final body weight (FBW), specific growth rate (SGR), energy retention ER% and protein productive value PPV% of grey mullet (*Liza ramada*) fry increased significantly with increasing dietary algae up to levels 21%. Feed conversion ratio (FCR), Protein efficiency ratio (PER) and the fish body protein% improved with increasing dietary algae level up to 21% and it did not show clear trend. There were No significant differences in the body lipid % of grey mullet (*Liza ramada*) fry with increasing dietary algae levels. A negative trend was shown between (T2 and T5). It could be conclude that using green microalgae (Nannochloropsis oculata) at 21% level for grey mullet fry the best in term of growth performance and feed utilization..

Keywords:

INTRODUCTION

Introduction Mullet have been recognized as very desirable species for pond culture in China, Egypt, Hawai, Italy, Japan, Philippine, Taiwan and other parts of the world, where they are mainly grown in polyculture with carps, tilapia, and milkfish (Perlmulter *et al*., 1957; Blanco and Acosta, 1958; Yashouv, 1966; Lin, 1968 and Bardach *et al*., 1972).

Mullets (*Mugil cephalus* and *Liza ramada*, Family Mugilidae) are considered highly esteemed in Egypt .They are also the most important marine fish, used for culture either in brackish or fresh water (Eissawy *et al*., 1974 and Sarig 1981). Because of their great productivity for aquaculture, there is a worldwide interest in mullet culture. They are recognized as highly wanted fish for pond culture in Italy, Japan, Philippines and other parts of the world (Yashouv, 1966; Yashouv

and Ben-Shachar 1968, Bardach *et al*.,1972, Oren,1975, Benetti and Fugundo Netto, 1991a,b and El-Dahhar, 2000).

The poor survival rates of the wild mullet larvae is a limiting factor in mullet production and many investigations were made to determine environmental and nutrition requirements (Brusle, 1981; Alexis and Papaparaskeva- Papoutsoglou, 1986; Benetti and Fagundes Netto, $1991_{a,b}$ and El-Dahhar, 1999). The poor digestion in their larvae stages according to the primitive digestive system leads to the importance of exogenous additives in the diet to improve survival and growth of mullet larvae (Person Le Ruyet *et al*., 1993 and El-Dahhar, 1999). Treating the diet by heat and pressure improve the diet quality and make the best nutritional use of the raw materials. It can sterilize the diet and give the opportunity to use raw materials with different quality (Botting, 1991 and De-Silva and Anderson, 1995).

After establishing some constituents of mullet acclimatization needs, e.g the use of exogenous zymogen in mullet feed (El-Dahhar, 1999), descending salinity acclimatization (El-Dahhar *et al*., 2000), determination of some vitamin requirements for mullet larvae (El-Dahhar, 2000), survival rate improved from zero to over 90% under the laboratory conditions. Thus, it is possible now to determine the nutritional requirements and establish diets for mullet larvae during the period of acclimatization.

In Egypt, high mortality percentage was recorded for *Mugil cephalus* and *Liza ramada* during transportation and rearing. Generally, the survival of the stocked fry was found to range from 12 to 25% at end of rearing period (El- Zarka and Fahmy 1966 and Eissawy *et al*., 1974). Salama *et al*.,(1984) found that mortality of mullet fry was less in aquaria filled with sea water mixed with brackish than with tap water, as the differenece was found to be significant. The authors attributed that to the existence of the natural food in the brackish water. Gosline and Brook (1965) reported that *Mugil cephalus* prefers brackish water areas, while (Pillay 1975) showed that the mullet can be cultured in both brackish and freshwater farms. (Sivalingam 1975) reported that it is possible to acclimatize *M.cephalus* to fresh water within 36 hrs by gradual dilution and can be reared with common carps in fresh water pond. The importance of fry nutrition on growth and mortality has been investigated by (Salama 1989 and 1990). He found that combined feeding on natural and artificial feed achieved by far the best growth and survival.

Thus to increase the production of mullet in Egyptian fish farms we need further research on mullet nutrition. Salama (1994) found that the fish growth, survival, condition factor, water quality and profitably were achieved by low stocking rate (15.000 fish / h) and supplied with artificial feed.

The present work aimed for studying the effect of different levels of microalgae *Nannochloropsis oculata*, on growth performance, feed utilization and survival rate of *Liza ramada*, Family *Mugilidae*).

MATERIALS AND METHODS

Materials This study was carried out in the Marine Fish Laboratory (MFL), Faculty of Agriculture Saba Basha Alexandria

University, Egypt. This experiment was conducted to study the effect of dietary marine algae inclusion rate at (0, 7, 14, 21and 28%) in grey mullet (*Liza ramada*) larval feed on growth performance, feed utilization and survival rate of mullet larvae.

PRODUCTION OF MARINE ALGAE

Green microalgae *Nannochloropsis oculata* produced in the MFL is a marine green microalgae produced as feed for rotifer and water conditioner for larvae rearing to improve water quality and sustain rotifer alive. *N. oculata* is selected for its high productivity, the relative ease of its culture, suitability for rotifer culture and its tolerance of a wide range of salinities and temperature. *N.oculata* is high in nutritional value containing Eicosapentanoic acid (EPA) and Decosahexanoic acid (DHA). It has been reported that these highly unsaturated fatty acids (HUFAs) are essential for the growth and development of finfish larvae (Tamaru *et al*., 1991). *Nannochloropsis oculata* is produced at the MFL, Faculty of Agriculture (Saba Basha) using a technique explanted as follows:

SEA WATER SUPPLY SYSTEM

The sea water supply system consists of three components, the seawater supply facilities, the sedimentation and the water storage tanks.

The Seawater Supply Facilities

Sea water is transferred from the sea to the lab by car prepared for this purpose. The tank over the car is filled by means of a pump and the water transferred to the lab by a pipe line to a ground storage tank beside the lab. The reserved water then pumped to the tanks over the Lab and the salinity was adjusted in each of them, by adding fresh water.

Sedimentation, Storage and Aseptic Facilities

At the ground storage tank, all the suspended particles in the transferred water are precipitated. Water then pumped to the reservoir tanks over the Lab. Four tanks over the MFL are used to reserve the water at the desired salinities.

ALGAE PRODUCTION

Algae ***(Nannochloropsis oculata)*** culture starts with laboratory flask culture and up scaled gradually to outdoor mass culture. The outdoor culture is conducted in glass aquaria with a fiber glass cylindrical tank prepared to provide the algae culture aquaria with continuous algal flow. In the facilities of MFL, algae production consists of different stages as follows:

Indoor algae culture

Maintenance and stock culture in 250 ml flasks and

Starter culture: In 2 L bottles and 10 L polyethylene bags

Outdoor algae culture

Intermediate culture: In a fiber glass cylindrical tank and

Mass culture: In 90 L glass aquaria

Culture procedure

Indoor culture

In an air conditioned laboratory Fig (1) indoor cultures take place in maintenance and stock cultures using 250ml flasks and tike wise to starter culture using 2 l plastic bottles and 10 l polyethylene bags. The indoor algae room is kept aseptic during the whorle stages of the algal production. Before use, filtered sea water is enriched with the algae medium

modified by Allen and Nelson, 1910 and sterilized by means of an autoclave or by boiling technique. One liter stock solution is made and used to enrich the indoor algae medium as given in (Table 1). The medium is then provided with trace metals (Table 2) using the Hawaiian Oceanic Institute mixture (Liu and Kelley, 2001). Each sterilized flask is filled with 150 ml of the sterilized prepared medium. Flasks then inoculated with algae using healthy stock among maintenance culture. Three ml of *N.oculata* is added to each 150 ml prepared medium to give an initial density of 6-8 $X1o^4$ cell/ml. For maintenance culture, 0.15 ml alga is added to 150 ml medium. The flasks are closed using aluminum foil stopper after inculation.

Starter culture begins in 2 liter plastic bottle and is then up scaled to 10 liters bags which is the stock for the cylindrical fiber glass tank, the intermediate outdoor culture. To each 2 and 10 liters starter culture the sterilized prepared medium is added. Laboratory stock culture is used to inoculate the 2 L bottle. One flask is needed for each bottle. The initial density is 3 – 4 X 10^5 cells/ml. To inoculate the 10 L starter culture, one 2 L bottle is used. The initial density is 3–4 $X10^6$ cells/ml.

Table (1): ***Indoor algae nutrient stock solutions (modified by Allen and Nelson, 1910).***

STOCK SOLUTION	Add1L Distilled water g/L	Usage Add to 1L Medium
A- Nitrate Stock Solu		
Potassium nitrate	202.0	2
B- Phosphate stock s		
Sodium phosphate (basic)	36.9	
HCL (concentrated)	28.0	0.5
Calcium chloride	50.6	
C- Iron Stock Solutic		
Fe cl_2	5	0.5
Na EDTA	7	0.5
D- $CoCl_2$	0.14g/25ml	0.25
E- Mn Cl_2	8.5g/25ml	0.25

Table (2): ***Trace metals mixture (modified from Liu and Kelley, 2001).***

Compound	Weight (g)
H_3BO	0.88 g
$CuSO_4 + 5H_2O$	0.1 g
Na - EDTA	36.0 g
Mg $SO_4 + 7H_2O$	13.6 g
Na Mo O_4	3.6 g
Zn $SO_4 + 7 H_2O$	3.1 g
Total	56.28

Outdoor culture

Intermediate culture is conducted outdoor in a fiber glass cylindrical tank prepared to provide the glass aquaria with continuously algae flow. It is sterilized by thoroughly mopping with a towel soaked with a 10% formalin solution and allowed to dry before use. Nutrients mixture of inorganic fertilizers (Table 3) is added to the water flow using a bronchial tub with a conductor mixer to mix the sea water with fertilizers while the water flow entering the intermediate culture. Two bags of 10 L (starter culture are put in the 180 L fiber glass cylindrical tank then sterilized sea water with the fertilizers flow to the cylindrical tank from a 85 L glass aquaria over it (Fig 2) . The flow rate of the water with the fertilizers is 500 ml / minute.

Mass culture is started after the intermediate culture is completed. The 85L glass aquarium over the tank is filled daily by

Table (3): ***Agricultural grade nutrients used for outdoor culture***

Nutrients	Medium Concentration $5m^3$ mg / L	Storage Concentration g/L	Usage ml/L
$NH_4 SO_4$	100	100	1
Super Phosphate	70	70	1
Urea	10	10	1
Fe Cl_2	5	5	1
Na – EDTA	7	7	1

sea water at salinity of 25‰ and sterilized with Huwa. San ® 25 %. The over flow of the algae produced at the cylindrical tank is transferred to a group of glass aquaria by means of a PVC tubes branched to the aquaria each with a valve to distribute the produced algae to each one of the glass aquaria to complete mass culture in.

Culture conditions

Flasks are kept under constant illumination using four 34-watt fluorescent lamps suspended behind the flasks. Aeration did not provide at this stage to minimize the risk of contamination. Flasks are shaken several times daily to prevent the cells clumping at the flask bottom. The peak density 3 - 4 $X10^6$ cells/ml of *N. oculata* could be obtained five to six days after inoculation. After that the cultures are ready for use as stock for starter culture (Table 4).

The method of drying algae

The algae Nannochloropsis oculata was dried by two ways:

1-By settling for one day, so algae settled at the bottem of the bottle or sac. The sediment was taken in a low quantity of water then fresh water added and shaked to eliminate any salts from it. The remaining algae were taken in Petri dishs at in the low quantity of water, and dryed at temperature (60 – 70 ^{0}C) to avoid any losses of its nutritional components.

2-Algae in a 50 million cells/ml were centrifuged at 10.000 rpm. The sedimented algae were taken, washed by fresh water and dried at a temperature of (60 – 70 ^{0}C). The dried algae was ground in grinder mixer and stored in plastic sacs or glass bottles until adding to the diets. One L of dried algae medium gives about 1.3 g dry algae

Table (4): ***Generalized set of condition for culturing micro-algae (modified from Anonymous, 1991).***

Parameters	Range	Optima
Temperature (C)	16 - 27	18-24
Salinity (g^{-1})	12 – 40	20-24
Light intensity (lux)	1.000-10.000 (depends on volume and density)	2500 - 5000
Photoperiod (light dark. Hours)		16:8minimum 24:0 maximum
PH	7- 9	8.2-8.7

Experimental procedure

Grey mullet (*Liza ramada*) larvae were obtained from Rosita fry collection center and Maadia fishing port. Fish were transported to the MFL in the faculty of Agriculture Saba Basha and acclimated in salinity the same as that from which the fish were transferred (10 ppt), the fish acclimatization take place in glass aquaria for (15) days on the experimental diet and environmental conditions before the start of experiment. Aquaria of dimensions (100 x 30 x 40 cm) were supplemented with continuous aeration and water changed daily by stocked tap water to flush out wastes. In this experiment, water temperature was maintained constant at (20^0C ± 0.2) by thermostatically heaters, one in each glass aquarium. Before the experiment, aquaria were rinsed with chlorinated water for 24h and then diluted sea water (10ppt) was applied to each aquarium.

Aquaria were cleaned before each feeding. All fish in each aquarium were weighed at the beginning of the experiment and on weekly basis. Thirty fish of grey mullet were killed at the beginning of the experiment and kept frozen for further chemical analysis. At the end of the experiment, (15) fish were taken randomly from each aquarium, killed and dried at 70^0C for about 48 hours for final chemical analysis.

Diets formulation and preparation

Diets were formulated from commercial ingredients of fish meal (FM), wheat flour, fish oil, milk, eggs, (Algae), Ascorbic acid, vit and mineral mixture and carboxy methyl cellulose (CMC) in the experiments. Diets composition and its chemical analysis are shown in Table (5):

Dry ingredients were passed through a sieve (0.6 mm diameter hole) before mixing into the diets. Oil was emulsified with equal amount of water using 0.7 % phosphatedyl choline (lecithin) according to El-Dahhar and El-Shazly (1993). Mixtures were homogenized in a food mixer model SNFGA (Kitchen aid St. Joseph, M 149085 USA). Boiling water then added to the mixtures at the rate of 50 % for pelleting. An autoclave was used to heat the diets for 20 min after adding water at a maximum pressure of 1.2 kg / cm^2 G.Vitamins, minerals mixture, Ascorbic acid and exogenous zymogen were added to diets after the heat treatment

Aquaria management, heat treating of the diet and exogenous zymogen addition were made according to El-Dahhar (1999). The diets were pelleted using meat grinder of kitchen aid with a 1.5 mm diameter and kept frozen in a deep freezer until they were used.

The Experimental Design

This experiment was designed to evaluate the effect of algae (AL) additions at the levels of (0,7,14,21 and 28% of the diets) on Growth Performance , feed utilization and

Table (5): Composition and chemical analysis of the five feeding mixtures used in the experiment.

Ingredients \ (AL) level	Control (0%)	(7%)	(14%)	(21%)	(28%)
Wheat flour	50.7	42.2	33.7	25.2	16.7
Fish meal	15.0	15.0	15.0	15.0	15.0
Fish oil	1.0	1.5	2.0	2.5	3.0
Milk	12.0	13.0	14.0	15.0	16.0
Egg	17.0	17.0	17.0	17.0	17.0
AL[1]	0.0	7.0	14.0	21.0	28.0
CMC[2]	3.0	3.0	3.0	3.0	3.0
Vit.&Min.Mix[3]	0.9	0.9	0.9	0.9	0.9
Ascorbic acid	0.4	0.4	0.4	0.4	0.4
Proximate analysis (%)					
Moisture	12	11.53	11.09	12.19	11.42
Crude protein	27.6	27.5	27.4	27.3	27.2
Crude lipid	8.76	8.78	8.33	8.5	8.63
Crude fiber	0.0	0.0	0.0	0.0	0.0
Carbohydrate (NFE)[4]	44.94	43.23	40.27	35.27	32.26
Ash	6.7	8.96	12.91	16.74	20.49
Gross Energy (Kcal/g)	4.26	4.19	4.02	3.83	3.71

1- (AL) is Algae.
2- (CMC) is carboxy methyl cellulose.
3- Vitamin and mineral/ Kg premix: vitamin A, 4.8 million IU, D3, 0.8 million IU; E, 4g; K, 0.8g, B1 0.4g riboflavin, 1.6g; B6, 0.6g, B12, 4mg; Pantothenic acid, 4g, Nicotinic acid, 8g, Folic acid, 0.4g; Biotin, 20mg; choline chloride, 200g; CU, 4g; 1, 0.4g; Iron, 12g; Mn, 22g; Zn 22g, Selenium, 0.4g.
4- (NFE) is nitrogen free extract.

Survival rate of grey mullet (*Liza ramada*) fry in glass aquaria using brackish water (10ppt) for 8 weeks . The five treatments were evaluated in a block design with three replicates for each treatment.

Grey mullet fry of initial body weight (IBW) ± SE (0.153 ± 0.0) were stocked in each glass aquarium at the rate of 25 fish per aquarium. Fish were fed twice daily at 9.00 a.m and 15.00 p.m using the rate of 12% of body weight which was adjusted weekly in response to weight gain. Water temperature was maintained constant at (20^0C ± 0.2) by thermostatically heaters.

RESULTS

Survival and growth

It was found a significant ($P < 0.05$) difference in survival rate among experimental groups (Table 6). The highest survival rates 61.33% were obtained for group of mullet fry. Fed 21% algae followed by group of mullet fry in diets 5, 1, 2 and 3 respectively. A significant difference ($P < 0.05$) was observed between FBW of grey mullet fed (T4) and that fed the control diet (T1), (T2) and (T3). However, there are no significant differences between control, T2 and T3.

The highest ADG was recorded at the fry fed 21% AL diet (T4) having a value of (0.00535± 0.0002g/day) followed by that of the fry fed 28% AL diet (T5) 0.00448±0.0005g/day, control diet (T1) 0.00376±0.0003g/day, 14% AL diet (T3) 0.00364±0.0003g/day and 7% AL diet (T2) 0.00339±0.0004g/day. A significant difference ($P < 0.05$) was observed in ADG of grey mullet between (T4) and control (T1), (T2), and (T3). Nevertheless, the latter treatments are not significantly different.

Likewise, (SGR) showed the highest value in (T4) 1.936± 0.049 followed by (T5) 1.729±0.12, (T1) 1.543±0.086, (T3) 1.511±0.074 and (T2) 1.439±0.117. Significant difference ($P < 0.05$) was observed in SGR of grey mullet between (T4) and control (T1), (T2) and (T3). Again, (T1), (T2) and (T3) did not differed significantly.

The survival rate coincides with algae inclusion level in the diet up till 21% (T4) 69.33± 1.885% followed by (T5) 58.67±1.188%, (T1) 48±11.314%, (T2) 45.33±14.73% and (T3) 40±5.657%. A significant difference ($P < 0.05$) was observed in survival of grey mullet between (T4) and (T3).

Weight gain (g/fish), offered feed (g/fish) and feed conversion ratio (FCR) of grey mullet in the experiment are shown in Table (7). The results showed that the highest weight gain was recorded with the larvae fed (T4) 0.299± 0.0127g/fish followed by (T5) 0.251±0.0258g/fish, (T1) 0.210±0.0174g/fish, (T3) 0.204±0.0146g/fish and (T2) 0.190±0.0229g/fish. A significant difference ($P < 0.05$) in weight gain of grey mullet between (T4) and control (T1), (T2) and (T3). The latter treatments (T1, T2 and T3) did not differ significantly.

The result concerning offered feed indicated that the highest offered feed was recorded with the larvae fed (T4) with the value of 0.691± 0.0302g/fish followed by (T5) 0.619±0.0093g/fish (T1) 0.601±0.0843g/fish, (T2) 0.572±0.0743g/fish and (T3) 0.548±0.0072g/fish, without any significant difference ($P > 0.05$) between them.

The best FCR was recorded with the fish fed (T4) having the value of 2.311±0.140 followed by (T5) 2.493±0.235, (T3) 2.702±0.209, (T1) 2.841±0.175 and (T2) 3.089± 0.706, without any significant difference ($P > 0.05$) between them.

Table (6). ***Means ± SE of final body weight (g/fish), average daily gain (ADG, g/fish/day), specific growth rate (SGR, %/day) and survival rate (%) of grey mullet fry (0.153g Initial BW) fed the five dietary algae (AL) inclusion levels (0, 7, 14, 21 and 28%) of the diet in the experiment.***

Treatments / AL levels	Final BW	ADG	SGR	Survival %
T1 (0%) Control	0.363 ± 0.0174[b]	0.00376 ± 0.0003[b]	1.543 ± 0.086[b]	48 ± 11.314[ab]
T2 (7%)	0.343 ± 0.0229[b]	0.00339 ± 0.0004[b]	1.439 ± 0.117[b]	45.33 ± 14.73[ab]
T3 (14%)	0.357 ± 0.0146[b]	0.00364 ± 0.0003[b]	١.511 ± 0.074[b]	40 ± 5.657[b]
T4 (21%)	0.453 ± 0.0127[a]	0.00535 ± 0.0002[a]	1.936 ± 0.049[a]	69.33 ± 1.885[a]
T5 (28%)	0.404 ± 0.0257[ab]	0.00448 ±0.0005[ab]	1.729 ± 0.12[ab]	58.67 ± 1.188[ab]

Means within column followed by different letter are significantly different (P < 0.05)

Table(7*). Means ± SE of weight gain (g/fish), offered feed (g/fish) and feed conversion ratio (FCR) of grey mullet fry fed the five dietary algae (AL) inclusion levels (0,7,14,21 and 28%) of the diet in the experiment.*

Treatments / AL levels	Weight gain	Offered feed	FCR
T1 (0%) Control	0.210±0.0174[b]	0.601±0.0843	2.841±0.175
T2 (7%)	0.190±0.0229[b]	0.572±0.0743	3.089±0.706
T3 (14%)	0.204±0.0146[b]	0.548±0.0072	2.702±0.209
T4 (21%)	0.299±0.0127[a]	0.691±0.0302	2.311±0.140
T5 (28%)	0.251±0.0258[ab]	0.619±0.0093	2.493±0.235

Means within column followed by different letter are significantly different (P < 0.05)

Body composition

Moisture %, protein % and lipid % of grey mullet larval body after eight weeks feeding on the five diets in the experiment are shown in Table (8). Results concerning Moisture %, it is evident that the highest fish body Moisture % was recorded with the fish fed 21% AL (T4) having the value of 70.48± 0.432% followed by (T5) 70.32±1.369%, (T2) 70.22±2.154%, (T1) 70.18±1.299% and (T3) 69.27±2.047%, without any significant difference (P > 0.05) was observed between treatments. The highest fish body protein % was recorded at the highest inclusion level of (AL) 21% (T4) with the value of 14.421± 1.806% followed by (T5) 13.322±1.161%, (T2) 12.726±0.537%, (T3) 12.612±1.222% and (T1) 12.329±0.3081%, without any significant difference (P > 0.05) between them.

The results concerning the fish body lipid % showed the highest lipid % was recorded with the fish fed the control diet (T1) 12.625± 1.618% followed by (T3) 12.591±1.461%, (T5) 11.844±0.233%, (T4) 11.630±0.349% and (T2) 11.296±0.356%, without any significant difference (P > 0.05) was observed between treatment.

Protein and energy utilization

Protein efficiency ratio (PER), energy retention (ER %) and protein productive value (PPV %) of grey mullet larvae fed the five diets in the experiment are shown in Table (9). The results concerning Protein efficiency ratio (PER) indicated that the highest Protein efficiency ratio (PER) was recorded with the fish fed 21% AL (T4) having a value of 1.889± 0.112 followed by (T3) 1.699±0.126, (T5) 1.673±0.149, (T2) 1.469±0.363 and (T1) 1.396±0.089, without any significant difference (P > 0.05) between them.

Table (8). Means ± SE of moisture (%), protein (%) and lipid content (%) in the carcass of grey mullet fry fed the five dietary algae (AL) inclusion levels (0,7,14,21 and 28%) of the diet in the experiment (fresh weight basics).

Treatments AL levels	Moisture %	Protein %	Lipid %
T1 (0%) Control	70.18±1.299	12.329±0.308	12.625±1.618
T2 (7%)	70.22±2.154	12.726±0.537	0.356±11.296
T3 (14%)	69.27±2.047	12.612±1.222	12.591±1.461
T4 (21%)	70.48±0.432	14.421±1.806	11.630±0.349
T5 (28%)	70.32±1.369	13.322±1.161	11.844±0.233

Table (9). ***Means ± SE of protein efficiency ratio (PER), energy retention (ER%) and protein productive value (PPV%) of grey mullet fry fed the five dietary algae (AL) inclusion levels (0,7,14,21 and 28%) of the diet in the experiment.***

Treatments AL levels	PER	PPV %	ER %
T1 (0%) Control	1.396 ±0.089	23.412±2.081 ^{b}	20.740 ±2.069^{ab}
T2 (7%)	1.469 ±0.363	25.929±4.105 ^{b}	19.529 ±4.226^{b}
T3 (14%)	1.699 ±0.126	29.453±3.484^{ab}	23.804 ±1.874^{ab}
T4 (21%)	1.889 ±0.112	34.848±3.476 ^{a}	27.994 ±1.515^{a}
T5 (28%)	1.673 ±0.149	29.335±1.262 ^{ab}	27.273 ±0.679^{a}

In regard to (ER %), the highest energy retention (ER %) was recorded with the fish fed 21% AL (T4) 27.994± 1.515% followed by (T5) 27.273±0.679%, (T3) 23.804±1.874%, (T1) 20.740±2.069% and (T2) 19.529±4.226%. A significant difference ($P < 0.05$) was observed between energy retention (ER %) of grey mullet fed (T5, T4) and that fed 7% AL diet (T2).

With respect to protein productive value (PPV %), the highest protein productive value (PPV %) was recorded with the fish fed 21% AL diet (T4) with value of 34.848± 3.476% followed by T3 (14% AL) 29.453±3.484%, T5 (28% AL) 29.335±1.262%, T2 (7% AL) 25.929±4.105% and diet free of AL (T1) 23.412±2.081%. Significant difference ($P < 0.05$) was observed between protein productive value (PPV %) of grey mullet fed (T1, T2) and that fed 21% AL diet (T4).

DISCUSSION

This study was carried out to investigate the effect of dietary levels of Green microalgae Nannochloropsis oculata on weight gain, feed utilization and body composition of grey mullet (Liza ramada) fry.

Sheeno and Sahu, (2006) indicated that the use of mixed diet (azolla protein concentrate (APC) mixed with dry Spirogyra powder (SP) at 4:1 ratio) to fed Labeo rohita fry as a substitute of fish meal at T1 (0%), T2 (25%), T3 (50%), T4 (75%) and T5 (100%) affected each of growth, WG, SGR, FCR. With increasing APC- SP inclousion rate in the diet from 0 to 50%, WG, SGR, and FCR did not affected significantly ($P < 0.05$) but they decreased significantly ($P < 0.05$) as the APC-SP content increased in the diet beyond 50%. This finding was in agrement with the results of the present work experiment with increasing Algae beyond 21%.

Ekpo and Bender, (1989) reported that Nile tilapia (Tilapia nilotica) and common carp (Hypophthalmichthys molitrix) can digest the microbial mat at the percent of 75% and 81%, respectively. The dried form was significantly less digestible by both species. Fresh microbial mat compared favorably with commercial catfish feed in digestibility by Nile tilapia. These studies illustrate that Nile tilapia, Silver carp and cat fish differ in their digestibility from mullet. In which they digest fresh diet better than dried one, and that may be due to differences in feeding habits.

Different results were obtained when different species of microalgae were incorporable in fish diet. Stanley and Jones, (2003) recorded poor growth and FCR for grass carp when fed on spirogyra sp. Tilapia aurea and big mouth buffalo fed on Spirulina and spirogyra and showed high growth rate of 29g dry weight/ kg for 28 days, 14g/ kg body weight respectively; FCR was 2.0, 10, respectively.

The incorporation of microalgal powder in diets for marine fishes appears more effective, since lower percentages are needed to cover the mineral requirements. Most mineral needs of turbot can be covered with low percentages of marine microalgal powder in the diet: 3.8% of Tetraselmis suecica, 5.7% of Isochrysis galbana, 3.57% of Dunaliella tertiolecta and 3.9% of Chlorella stigmatophora. Mn and Co must, however, be added. Thus, incorporation of small amounts of marine microalgae in diets can replace a mineral mixture (Fabregas and Herrero, 1986). This study investigated the importance of algae addition to marine fishes diet compensate minerals.

The study of Badwy et al., (2005) was designed to investigate the effect of partial replacement of fish meal with dried microalgae (Chlorella spp and Scenedesmus spp) in Nile tilapia (Oreochromis niloticus) diets on fish growth performance, feed efficiency and body composition. Nine isonitrogenous (32% cp), isocaloric (440 kcal/100 g) diets were formulated to contain Chlorella and Scenedesmus as fish meal replacers at zero (control), 10, 25, 50 and 75% substitution, (Chlorella spp 0, 3.43, 8.56, 17.11, 25.66% and Scenedesmus spp 0, 3.13, 7.82, 15,64, 23.46% of the total diet). Results indicated that, growth performance, feed conversion ratio and protein productive value were significantly ($P<0.05$) higher in fish feed diets containing 50% of both Chlorella spp and Scenedesmus spp, whereas fish feed diets containing 75% algae had significance lower performance ($P < 0.05$). This finding was in agrement with the results of the present work.

Mustafa et al., (1994) indicated that feeding of red sea bream, Pagrus major on both Ascophyllum and Spirulina elevated growth rates and improved feed conversion efficiency, protein efficiency ratio, and muscle protein deposition without negative effects. In our study there is negative effects after 21% the differences in both studies is may be due to differ in species of algae.

CONCLUSION

The conclusion from these results indicated that the best algae level for grey mullet (Liza ramada) 0.153 g IBW is 21% in term of total weight gain and FCR.

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تأثير استخدام الطحالب البحرية فى اعلاف يرقات الطوبارة على اداء النمو والأستفادة من الغذاء

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أجريت هذه الدراسة في كلية الزراعة (سابا باشا) جامعة الإسكندرية ، حيث أجريت التجربة بهدف دراسة تأثير مستويات الاعشاب البحرية علي كفاءة النمو والاستفادة من الغذاء ليرقات أسماك العائلة البورية في مراحل النمو الأولي. تم الحصول علي زريعة الطوبارة من بوغاز المعدية وقد استخدم تصميم القطاعات العشوائية الكاملة في ثلاث مكررات عشوائية.

تم إنتاج الطحالب البحرية في معمل الأسماك البحرية بكلية الزراعة سابا باشا وتم تجفيفها بطريقتين :

أولا: بفصل التهوية عن الطحالب البحرية فتترسب فيؤخذ الراسب في أقل كمية من الماء في طبق بتري، ويتم غسلها بالماء العذب لإزالة ما بها من أملاح وتجفيفها وطحنها وتعبئتها في أكياس لحين الاستخدام.

ثانيا: بعملية الطرد المركزي حيث استخدم جهاز الطرد المركزي علي عشرة الاف لفة لكل دقيقة، فتم فصل السائل عن الطحالب وإزالته وتم غسلها بالماء العذب لازالة ما بها من أملاح، وتجفيفها وطحنها وتعبئتها في أكياس لحين الاستخدام.

الهدف من إجراء هذه التجربة :

الهدف من إجراء هذه التجربة هو دراسة تأثير مستويات مختلفة من الطحالب البحرية علي كفاءة النمو والاستفادة من الغذاء لأسماك الطوبارة في مراحل النمو الأولي في تجربة مكونة من خمس معاملات (٠ ، ٧، ١٤، ٢١، ٢٨%). كررت كل معاملة ثلاث مرات بعد فترة أقلمة استمرت أسبوعين، حيث استخدم ١٥ حوضا زجاجيا بمقاس (١٠٠× ٣٠ × ٤٠ سم) مزودة بمصدر تهوية ، بمعدل تخزين ٢٥ سمكة بكل حوض زجاجي.

وقد استمرت التجربة ٨ أسابيع بوزن ابتدائي (٠.١٥٣ جم) ، ومعدل التغذية ١٢% من وزن الأسماك طوال أيام الأسبوع وتقدم العلائق مرتين يوميا.

وقد أظهرت النتائج ما يلى :

١- أفضل معدلات نمو تم الحصول عليها عند مستوي طحالب بحرية ٢١% .

٢- أن الأسماك التي تم تغذيتها علي ٢١% طحالب بحرية كانت أفضل في معدلات نموها معنويا بمستوي معنوية ٠.٠٥ عن الأسماك التي تم تغذيتها علي باقي المستويات من الطحالب البحرية (٧، ١٤، ٢٨%).

٣- أفضل كفاءة تحويلية تم الوصول اليها بتغذية الأسماك علي مستوي ٢١% من الطحالب البحرية .

٤- لم يكن لمستويات الطحالب البحرية تأثير معنوي علي محتوي أسماك الطوبارة من الرطوبة.

٥- لم يكن لمستويات الطحالب البحرية تأثير معنوي علي محتوي أسماك الطوبارة من الدهون .

٦- محتوي جسم الاسماك من البروتين قد تأثر بزيادة مستوي الطحالب البحرية ولكن لا يوجد فرق معنوي بزيادة مستوي الطحالب البحرية الي ٢٨% عند مستوي ٠.٠٥ .

ومما سبق نستنتج : **أن أفضل مستوي من الطحالب البحرية هو ٢١% وهو المستوي الأمثل الذي حقق أعلي معدل نمو وافضل كفاءة تحويلية للغذاء لأسماك الطوبارة عند وزن (٠.١٥٣ جم) .**