STUDIES ON GROWTH PERFORMANCE AND SURVIVAL OF *OREOCHROMIS NILOTICUS* AND *OREOCHROMIS AUREUS* FRY REARED IN AQUARIUM AND FED ON DIFFERENT PROTEIN LEVELS SUPPLEMENTED WITH NATURAL FOOD

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Abstract

The present experiment was conducted at the Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharkia, Egypt, to compare the optimum growth performance and survival rate of *Oreochromis niloticus* and *Oreochromis aureus* fry reared in glass aquaria as affected with different levels of dietary protein in association with phytoplankton as a natural food.

The experiment was done using 24 glass aquaria (80×40×50 cm, each), each aquarium was stocked with 100 fry where 12 aquaria (group 1) represented *O. niloticus* and the other 12 aquaria (group 2) contained *O. aureus*. Each group subdivided into 4 subgroups (triplicates each). The fry of subgroups 1-4 of each species were fed on diets contained 25, 35, 45 and 55 % dietary protein respectively along with live phytoplankton. *Chlorella* and *Scenedesmus* spp. were added for all aquaria at a density of 50-300 x 10^4 cells ml^{-1} starting at day 1 for 9 weeks.

The protein levels tested released no significant effects on growth performance of both tested species.

Analysis of variance showed no significant differences between *Oreochromis niloticus* and *Oreochromis aureus* fry reared in glass aquarium at different protein levels ($P< 0.05$). This study revealed that no significant effects for the growth performance of *Oreochromis niloticus* and *Oreochromis aureus* fry reared in glass aquarium for 9 weeks. The diet containing low protein (25%) with green algae (50-300 x 10^4 cells ml^{-1}) is recommended for good growth performance and more economical rearing of *Oreochromis niloticus* and *Oreochromis aureus* fry.

Key words: *Oreochromis*, Protein, Natural food.

INTRODUCTION

Rapid growth rates, high tolerance to low water quality, efficient feed conversion, ease of spawning, resistance to disease and good consumer acceptance make tilapia a suitable fish for culture. (El-Sayed, 1999). The diet of fishes must be balanced and contain the primary or basic food components - proteins, carbohydrates and lipids (fats) - in requisite though differing amounts for different species of fishes. Vitamins and minerals are also required for growth, sustenance and replacement of tissues as well as for normal metabolism (Villegas, 1975).

Protein is the single most expensive ingredient in fish diets. The fact that high levels of dietary protein may lead to the consumption of protein for energy purposes,

has led to the investigation of the use of non-protein energy sources in fish diets (De Silva *et al.*, 1991) and (Erfanullah Jafri, 1995). Protein is the main constituent of the fish body thus sufficient dietary supply is needed for optimum growth. Protein is the most expensive macronutrient in fish diet (Pillay, 1990). So, the amount of protein in the diet should be just enough for fish growth where the excess protein in fish diets may be wasteful and cause diets for unnecessarily expensive (Ahmad, 2000).

Unicellular algae are widely used as food in the hatchery production of commercially valuable fish. Of the many species of algae, only a few can be cultured, and of these only a handful are routinely used for their "nutritive" quality in hatcheries. *Chlorella* and *Scenedesmus* are unicellular phytoplankton genus belonging to the phylum Chlorophyta. There are many species of *Chlorella* and *Scenedesmus* in both fresh water and sea water. Certain freshwater *Chlorella* and *Scenedesmus* are cultured as health foods for humans and animals because of the proteins, vitamins, minerals and other substances they contain. (Suwapepan 1984, Hill and Nakagawa 1981).

Natural foods play an important role in green water culture systems using supplementary diets. Under this management system, some natural food is available to the fry at all times, providing essential vitamins and minerals which may be deficient in the supplementary diets. (Suwapepan 1984).

Protein is of primary importance for fish growth, providing the basic materials for tissue-building and energy. To sustain the high metabolic rates of the rapidly growing fry, protein requirements are very high. The quantity of protein that is ultimately included in the diet will depend on the age of the fry and the source and quality of protein. (El-Sayed and Teshima. 1991)

For tilapias the protein requirement declines with age. For rapidly growing tilapia fry, protein levels ranging between 30 and 50% have given good growth. For fry up to 0.5 g, 50% protein is required if fed 3–4 times /day at 10–15% of body weight/day. Lower protein levels may be adequate if feeding frequency is increased to eight times/day and ration increased to 20–25% of body weight/day. Diets containing protein levels of 30–35% have proved adequate when fed at 10–15% body weight/day in semi-intensive systems where natural food supplements the artificial diet. (Balarin and Haller, 1982)

The present study aimed to compare the optimum growth performance and survival rate of *Oreochromis niloticus* and *Oreochromis aureus* fry reared in glass aquaria after using different levels of dietary protein in association with phytoplankton as a natural food.

MATERIALS AND METHODS

The present experiment was carried out at the Central Laboratory of Aquaculture Research, Abbassa. Fry were obtained from Abbassa fish hatchery, General Authority for Fish Resources Development, to compare the optimum growth performance and survival rate of *Oreochromis niloticus* and *Oreochromis aureus* fry reared in glass aquaria as affected with different levels of protein in association with phytoplankton as a natural food.

The experimental system consisted of 24 experimental glass aquaria (80×40×50 cm, each), each aquarium stocked with 100 fry where the first 12 aquaria (group 1) contained *O. niloticus* with initial weight of 0.013 g and initial length of 8.0 mm and second 12 aquaria (group 2) contained *O. aureus* with initial weight of 0.012 g and initial length of 8.0 mm. Each group was subdivided into 4 subgroups (triplicates each). The fry of subgroups 1-4 were fed on diets contained 25, 35, 45 and 55 % dietary protein respectively along with live phytoplankton. *Chlorella* and *Scenedesmus* were added for all aquaria at a density of 50-300 x 10^4 cells ml^{-1} starting at day 1 for 9 weeks.

Each aquarium was supplied with compressed air via air-stones from air pumps (Boss 9500, Germany).Well-aerated water supply was provided from a storage fiberglass tank. Water level in glass aquarium was kept at 40 cm depth.

All aquaria were drained and cleaned every day during experimental period. Water temperature and dissolved oxygen were measured by using YSI model 58 oxygen meter (Yellow Springs Instrument, Yellow Springs, OH, USA). Total ammonia and nitrite were measured once weekly using a DREL, 2000 spectrophotometer (Hach, Loveland, CO, USA). Total alkalinity and chloride were monitored once a week using the titration method, and pH was monitored once a week using an electronic pH meter (pH pen, Fisher Scientific, Cincinnati, OH, USA). Average of water quality parameters were: water temperature 27.8 c, dissolved oxygen 5.1 mg L^{-1}, total ammonia 0.2 mg L^{-1}, nitrite 0.05 mg L^{-1}, total alkalinity 182 mg L^{-1}, chlorides 550 mg L^{-1} and pH 7.6.

Isolates of *Chlorella.* and *Scenedesmus* spp. were obtained from Nile water samples according to Pascher (1915). The microalgae were subcultured in a solid Blood's basal medium (BBM) (Bischoff and Bold, 1963). The cultures were allowed to grow in the algae culture room at 25 °C and 14/10 light-dark cycle (5000 lux). Stock cultures of *Chlorella* and *Scenedesmus* were prepared at plankton unit of Central Lab. for Aquaculture Research in two litters capacity flasks in the laboratory for 5-6 days, then inoculated in carboy cultures at a density of 1 x 10^5 cells ml^{-1}. The carboy cultures were used as inoculate for two different phases of production in indoor and

outdoor in glass aquarium. The transfer of the algal cells to fish aquarium was achieved at a density of 5×10^6 cells ml^{-1}.

Fry were fed according to Buddle, (1984) who fed fry four times daily; 7 day $week^{-1}$ for 28 days at 20 % of body weight. Initially the experimental fry were fed 20 % of body weight per day until they attained an average length of 16 mm, after which the feeding rate was decreased by 1 % of body weight per day until a minimum rate of 12% per day was reached for 9 weeks. Dead fish were removed and recorded daily. The fry were fed on diets contained 25, 35, 45 and 55 % of dietary protein with live phytoplankton. Composition of the experimental diets (Table 1) and their proximate analysis in (Table 2). *Chlorella* and *Scenedesmus* were added for all aquaria at a density of 50-300 $\times 10^4$ cells mL^{-1} starting at day 1 for 9 weeks.

The following formula was used to compute for the required volume of green algae to be added into the aquarium (Tendencia *et al.*, 2005).

Volume to be added

$$= \frac{\text{(desired density-existing density) x volume of water in aquarium}}{\text{Density of stock culture}}$$

Table 1. Composition of the experimental diets for production of Nile and Blue tilapia reared in glass aquaria

Ingredients (%)	Protein levels %			
	25	35	45	55
Fish meal	15.6	20.3	31.0	20.0
Soybean meal	20.0	40.0	50.0	60.0
Wheat bran	5.0	5.0	5.0	5.0
Ground corn	52.63	28.42	9.44	9.44
Fish oil + Corn oil (1:1)	2.0	2.0	2.0	2.0
Vitamins and minerals premix	1.5	1.5	1.5	1.5
Ascorbic acid	0.06	0.06	0.06	0.06
Starch	2.21	1.72	0.0	0.0
Carboxymethyl cellulose	1.0	1.0	1.0	1.0
Total	100	100	100	100

Table 2. Proximate analysis of experimental diets.

Ingredients (%)	Protein levels %			
	25	35	45	55
Moisture	9.3	9.5	9.3	9.3
Dry Matter	90.7	90.5	90.7	90.7
Crude Protein	26.1	35.0	44.6	54.7
Crude Fat	9.0	9.8	11.2	11.5
Ash	15.2	14.4	13.4	13.4

Growth response was calculated as a follows:

Specific growth rate (SGR) (% day $^{-1}$) = 100 (Ln final weight – Ln initial weight) / days,
Gain in weight (g fish $^{-1}$) = mean final body weight – mean initial body weight
Condition factor (k) =100 (Wt/L^3), where Wt is fish body weight (g), L is total length (cm) According to Hengsawat and Jaruratjamorn (1997).

RESULTS AND DISCUSSION

Growth performance of *Oreochromis niloticus* fry:

Averages of final body weight were not affected by protein level of Nile tilapia fry as illustrated in Table (3). Analysis of variance showed no significant differences between all treatments for average of final body weight of *Oreochromis niloticus* fry.

Generally, fish diets tend to be very high in protein. Foods for fry and fingerlings frequently exceed 50% crude protein. As growth rate decreases and fish age, protein levels in diets are decreased accordingly. Protein levels on grow-out diets often approach or exceed 40% crude protein, while maintenance diets may contain as little as 25-35%. In addition to decreasing the protein content of the food as fish grow, the particle size must also be changed. Many fish require live food when they are hatched because their mouth parts are so small (Winfree, 1992).

Results of the same table show that protein levels tested had insignificant effects on daily gain in weight, specific growth rate, condition factor and mortality rates of *Oreochromis niloticus* fry when these diets are supplemented with live algae.

Analysis of variance showed no significant differences between 25%, 35%, 45%, and 55% dietary protein in diet with natural food) at ($P< 0.05$). So, it can use 25% dietary protein in the presence of live algae for fry feeding. These results are in disagreement with (Tacon, 1987) who found that dietary protein level varies from 42% for fry and 35% for growing adult and with (Al-Hafedh *et al.* 1999) who found that the better growth of Nile tilapia was obtained at high dietary protein levels 40-45 % rather than 25-35 % protein in the absent of live algae. While, under semi-intensive conditions where green water systems are used and feeding is supplemented with low protein diets (ab. 25%), lower fry densities must be used. Under these conditions and tanks should be stocked with 100–200 early-fry/m^2 (Balarin and Haller 1982).

The focal point of nutrients in these microalgae is the concentrations of omega-3 unsaturated fatty acids (HUFAs). Numerous studies have shown that marine fish are

unable to synthesize sufficient quantities of two essential HUFAs, Eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) [Kanazawa, 1979.] These two fatty acids are essential in the growth and development of fish. In general terms, the higher level of HUFAs, the more nutritious the phytoplankton are to fish.

The condition factor (K) showed that there were no significant differences between 25%, 35%, 45% and 55% protein) of *Oreochromis niloticus* fry. The survival rate % was very high throughout the experimental groups as presented in Table (3) and the same trend there were no significant differences between all treatments at ($P< 0.05$). The results agreed with those of (Ahmad *et al.*, 2004).

Table 3. The effect of protein level on growth performance of Nile tilapia (*Oreochromis niloticus*) fry reared in glass aquarium (Means ±SE).

Variables Parameters	Treatments (protein level %)			
	25 %	35 %	45 %	55 %
Final body weight(gm)	0.98±0.127 a	1.003±0.083 a	1.25±0.209 a	1.27±0.088 a
Final body length(cm)	3.75±0.159ab	3.52±0.161 b	4.18±0.240a	4.20±0.179 a
Condition factor (K)	1.83±0.076 a	2.40±0.518 a	1.67±0.008 a	1.72±0.105 a
Weight gain (gm)	0.97±0.127 a	0.99±0.084 a	1.24±0.205a	1.25±0.085 a
Daily weight gain(gm)	0.016±0.003a	0.016±0.003 a	0.02±0.005 a	0.02±0.005 a
Specific growth rate %	6.44±0.129 a	6.48±0.080 a	6.67±0.179 a	6.72±0.071 a
Survival rate %	99.00±0.58 a	100±0.000 a	100±0.000 a	100±0.000 a

Means with the same letter in the same row are not significant differences ($P < 0.05$).

2. Growth performance of *Oreochromis aureus* fry:

The average growth performance of *Oreochromis aureus* fry reared in glass aquarium was not affected by protein levels 25%, 35%, 45% and 55% in the presence of natural food as tested in Table (4). The results showed no significant differences between all treatments in averages final body weight, weight gain, daily weight gain and specific growth rate of *Oreochromis aureus* fry at ($P< 0.05$). Results reveal that acceptable the growth performance of *Oreochromis aureus* fry can be obtained with 25% dietary protein level with addition of live green algae. These results are in disagreement with Ahmad *et al.*, (2004) who found that optimal dietary protein level was 45% for fry and 35% for growing adult without natural food.

Chlorella and *Scenedesmus* spp. are single-cell algae and are perfect food. These algae contain 50-60% protein, much vitamin C and more vitamin B-12, minerals and essential amino acids (Halama 1990).

In this study *Chlorella* and *Scenedesmus* were added for all aquaria at a density of 50--300 $\times 10^4$ cells ml^{-1} starting at day 1 for 9 weeks. These algal densities are in

accordance with Liao, (1979) who reported that *Chlorella* was added at a density of 50–350 × 10^4 cells ml^{-1} starting at day 1 to day 21 to maintain water quality for Larval rearing of milkfish, *Chanos chanos* Forskal.

Among the problems associated with the use of algal cells as fish feed is that the low digestibility of the algal cells makes the algal biomass unsuitable for rearing fishes. Moreover mixed diets containing several species of microalgae have been reported to give better results for some organisms (Hu 1990).

The condition factor (K) of *Oreochromis aureus* fry had ranged from 1.57 to 2.21 (Table 4). The results agreed with those of (Osman, 1991) who reported that K values in general, for fish ranged between 2.20 and 2.33. Statistical analysis showed that there were no significant differences in K values as affected with protein levels (25%, 35%, 45% and 55% protein) at (P< 0.05). Survival rate as presented in Table (4) showed that no significant differences between 35% 45% and 55% protein throughout the experiment with protein different level at (P< 0.05). These results are in accordance with (Ahmad *et al.*, 2004).

Table 4. Effect protein levels on growth performance of Blue tilapia (*Oreochromis aureus*) fry reared in glass aquarium (Means ±SE).

	Treatments (protein level %)			
Variables Parameters	25 %	35 %	45 %	55 %
Final body weight(gm)	0.87±0.124 a	0.903±0.083 a	1.16±0.209 a	1.17±0.088 a
Final body length(cm)	3.68±0.188ab	3.49±0.133 b	4.15±0.218 a	4.16±0.156 a
Condition factor (K)	1.73±0.091 a	2.21±0.461 a	1.57±0.066a	1.62±0.066 a
Weight gain (gm)	0.86±0.124 a	0.89±0.083 a	1.14±0.205a	1.15±0.085 a
Daily weight gain(gm)	0.013±0.003a	0.013±0.003 a	0.016±0.003a	0.02±0.000 a
Specific growth rate %	6.44±0.144 a	6.49±0.088 a	6.71±0.194 a	6.75±0.074 a
Survival rate %	98.66±0.333b	99.33±0.333 ab	100±0.000 a	100±0.000 a

Means with the same letter in the same row are not significant differences (P < 0.05).

3. Growth performance of *Oreochromis niloticus and Oreochromis aureus* fry

Analysis of variance showed no significant differences between *Oreochromis niloticus* and *Oreochromis aureus* fry in growth performance at protein different levels (P< 0.05).

Growth performance parameters as affected by tilapia species with protein levels tested are shown in table (5). Results revealed that differences in growth performance parameters within each protein levels tested (final body weight, condition factor, weight gain, daily weight gain, specific growth rate and Survival rate) were almost insignificant, however these parameters tended to increase with increasing the dietary protein level. These results indicate that both *O. niloticus* and *O. aureus* fry

perform similar in growth at all protein levels tested when the diet was supplemented with green algae.

Many culturists engaged in the mass production of brackish water and marine fish larvae believe that the presence of phytoplankton in the rearing tanks is beneficial to the whole rearing procedure. Apart from serving as direct feed for fish larvae and zooplankton, phytoplankton probably plays a role in stabilizing the rearing environment through the removal of metabolites or the supplementation of necessary vitamins or amino acids in solution. Phytoplankton apparently acts as the "conditioner" of the rearing system.

This result revealed that no significant effects for the growth performance of *Oreochromis niloticus* and *Oreochromis aureus* fry reared in glass aquaria for 9 weeks. It also indicated that the diet containing low protein (25%) with green algae (50-300 x 10^4 cells ml^{-1}) were better for growth performance and more economical for rearing of *Oreochromis niloticus* and *Oreochromis aureus* fry.

Table 5. Effect of tilapia species (*Oreochromis niloticus* and *Oreochromis aureus*) fry on growth performance within each protein level tested.

Protein Level	Species	Growth parameters FBW	K	WG	DWG	SGR	SR
25 %	*O. niloticus*	0.98±0.127a	1.83±0.076a	0.97±0.127a	0.016±0.003a	6.44±0.129a	99.00±0.58a
	O. aureus	0.87±0124 a	1.73±0.092a	0.86±0.124a	0.013±0.003a	6.45±0.144a	98.66±0.33b
35 %	*O. niloticus*	1.003±0.08a	2.40±0.518a	0.99±0.084a	0.016±0.003a	6.48±0.080a	100±0.000 a
	O. aureus	0.903±008a	2.21±0.461a	0.89±0.083a	0.013±0.003a	6.49±0.088a	99.33±0.33a
45 %	*O. niloticus*	1.26±0.209a	1.67±0.008a	1.24±0.205a	0.02±0.005a	6.67±0.179a	100±0.000 a
	O. aureus	1.16±0.209a	1.57±0.066a	1.14±0.205a	0.016±0.003a	6.71±0.194a	100±0.000 a
55 %	*O. niloticus*	1.27±0.088a	1.72±0.105a	1.25±0.085a	0.02±0.000a	6.71±0.071a	100±0.000 a
	O. aureus	1.17±0.088a	1.62±0.066a	1.15±0.085a	0.02±0.000a	6.75±0.074a	100±0.000 a

Means with the same letter in the same column are not significant differences ($P < 0.05$).

FBW = Final body weight (gm), K = Condition factor, WG = Weight gain (gm),

DWG = Daily weight gain (gm), SGR = Specific growth rate % and SR = Survival rate %.

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دراسات على أداء النمو والبقاء لزريعة أسماك البلطي النيلي و الاوريا المرباة في أحواض زجاجية على مستويات مختلفة من البروتين مع الغذاء الطبيعي.

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المعمل المركزي لبحوث الثروة السمكية بالعباسة - مركز البحوث الزراعية - مصر

التجربة الحالية أجريت في المعمل المركزي لبحوث الثروة السمكية بالعباسة - أبو حماد - شرقية - مصر بهدف مقارنة النمو المثلي ونسبة البقاء لزريعة أسماك البلطي النيلي والاوريا المرباة في أحواض زجاجية على مستويات مختلفة من البروتين مع الغذاء الطبيعي.

اشتملت الدراسة على ٢٤ حوض زجاجي مقاس ٨٠×٤٠×٥٠ سم. كل حوض خزن به ١٠٠ زريعة. حيث قسمت هذه الأحواض إلى مجموعتين المجموعة الأولى (١٢ حوض زجاجي لرعاية زريعة أسماك البلطي النيلي) والمجموعة الثانية (١٢ حوض زجاجي لرعاية زريعة أسماك البلطي الاوريا) كل مجموعة قسمت الى ٤ معاملات وكل معاملة ٣ مكررات. زريعة كل معاملة غذيت على العلا ئق التي تحتوي على مستويات مختلفة من البروتين ٢٥، ٣٥، ٤٥ ، ٥٥ % بروتين بالإضافة إلى الغذاء الطبيعي لرعاية زريعة أسماك البلطي النيلي والاوريا.

دلت نتائج معدلات النمو على عدم وجود فروق معنوية عند رعاية زريعة أسماك البلطي النيلي والاوريا بمستويات مختلفة من البروتين مع الغذاء الطبيعي لمدة ٩ أسابيع عند مستوى معنوية ٥ %. وعند عمل مقارنة بين رعاية زريعة أسماك البلطي النيلي والاوريا بالنسبة لأداء النمو لوحظ عدم وجود فروق معنوية عند مستوى ٥ %.

توصي هذه الدراسة: بالرغم من أن النتائج تدل على وجود زيادة في معدلات النمو والإعاشة عند مستوى البروتين ٥٥ % عن باقي المستويات الأخرى إلا إنها غير معنوية مع المعاملات الأخرى.

لذا يفضل المستوى ٢٥ % بروتين مع الغذاء الطبيعي لرعاية زريعة أسماك البلطي النيلي والاوريا حيث تكون أكثر اقتصادية.