ISSN 1110-0354
Bull. Nat. Inst. of Oceanogr. & Fish., A.R.E., Vol. (27) 2001: 347 - 371

EFFECT OF DIFFERENT ARTIFICIAL DIETS ON THE MAJOR FLESH BIOCHEMICAL CONTENTS OF FISH RABDOSARGUS HAFFARA REARED IN TANKS

BY

MOHAMED ALAA A. OMRAN*; MESSADA M. EL-GHARABAWY AND MAGDY MOSTAFA O. EL-HALFAWY

*Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt.
e-mail: maomran@ismailia.ie-eg.com

Keywords: Artificial food, Biochemical parameters, *Rhabdosargus Haffara*

ABSTRACT

Rhabdosargus haffara is one of the most important commercial fish from family Sparidae in the Suez Gulf. This fish has been cultured in Suez fish farms some years ago, but fish farmer till know not used artificial diets in their farms. The main aim of this study is to evaluate the effect of using different artificial diets varying in their protein sources, such as animal source (fish & blood) and plant source (soybean), on the flesh major biochemical contents of fish *Rhabdosargus haffara* reared in tanks.

Changes in flesh protein content, lipid, DNA, RNA and amino acids components in muscles of *R. haffara* fish fed on different meals for 7 months were measured. Also, Isoelectricfocusing (IEF) techniques were done to identify the different muscle protein bands of reared fish.

The data showed that fish fed on fish meal gave better biochemical parameters than fish fed on other protein sources, even the control ones. Flesh of *R. haffara*, fed on this diet has the highest protein content, DNA and RNA values. Elevation of protein levels correlated

well with the recorded high values of RNA. This was evident from the results obtained by using IEF technique.

Economically, this study recommends the use of combined diet between fish, soybean and blood sources in calculated percentage of proteins where it may give an excellent biochemical composition for this important species in the Suez Gulf.

INTRODUCTION

Although the fish culture in Egypt is potentially high, the contribution to fish production of this sector is surprisingly low. Its yield is about 16.9 % of the total fish production (EL-Shahat, 1998). Thus, in order to meet the continuous increasing demand for fish, the stress should be on the extent of utilization and proper management of natural fishery resources and using different culture methods specially by using artificial diets.

Generally most fish species require high protein diets. Sufficient level of dietary protein, feeding frequency and methods are also needed for rapid growth (Magouz, 1990; El-Ebiary, 1994; Essa and EL-Ebiary, 1995). Information on nutritional and diet development work was only carried out on cold water species in advanced countries (FAO, 1983).

Most nutritional studies on fish have been confined to those species of the greatest commercial importance. The value of any protein to a fish is a function of the energy it contains and the amino acids that compose it. The absolute amount of protein in a diet will vary with the water. Most fish feeds contain 20 % to 40 % proteins (EL-Shahat 1998).

Lipid in fish feeds must be digestible, which means that hard fats from animal sources should be avoided and replaced by oils. The level of lipid in most fish diets is about 5 %. Little is known about the carbohydrate requirements of fish, although they do not appear to be as important as they are in mammals. Carbohydrates generally are kept at diets such as 40 % starch. Fishes require many vitamins and the commercial dry diets usually contain vitamins as mixture. In contrast, little is known about the mineral requirements

of fish. Vitamins and minerals are present in traces not exceeding 1 % of the food content.

Family Sparidae is one of the most important families in the Red Sea. They inhabit tropical and temperate coastal waters. They are demersal inhabitants of both the continental shelf and slope. In the Gulf of Suez, the sparid *Rhabdosargus haffara* is considered as one of the most important commercial fish. It seems to be endemic to the Red Sea and especially common in the North.

El-Boray (1997) compared between the average protein content in muscle of *R. haffara* reared in two different fish farms which having different natural food types. He noticed that flesh protein content was nearly equal from May to August. While from September to January (end of rearing period), the fish in the first farm has a lower protein content than in the second farm (with extra animal protein source). This difference referred to the variation of food source in each farm.

In view of the international need to increase food production, in particular fish proteins, attention has been directed to the fish aquaculture. In Suez City, *R. haffara* has been cultured during the last six years. In spite of its economic importance, little work has been done in this respect.

The main aim of this study is to evaluate the effect of using different artificial diets varying in their protein sources, such as animal source (fish & blood) and plant source (soybean), on the flesh major biochemical contents of *R. haffara* fish reared in tanks. Assessment of the results of these parameters will provide some valuable information about the best diet that can be used for rearing this economically important fish in fish farms.

MATERIALS AND METHODS

Rhabdosargus haffara fries were captured randomly from North Suez Bay (average weight 0.27g ± 0.23). Fries were collected and transported in an aerated tank to the aquatic laboratory of National Institute of Oceanography and Fisheries (NIOF) Suez and Aqaba Gulfs branch at Suez City, Egypt.

The experiment was performed in 10 aerated fibreglass tanks (2000L volume each) supplied with filtered sea water that pumped directly from the Suez Bay, with salinity 41 ppt. and pH of about 8.3. The collected fries were divided into 5 groups (50 fries/tank). The first three months of the experiment emphasized the adoption of fish to the conditions present throughout the experiment, which continued for 7 months.

Before beginning the experiment, the fish were left without food in all tanks for 24 hours. Later, four diet formulations were used. These meals differ in their protein sources (fishmeal, fish and Soybean meal, soybean meal and blood meal), in addition to yellow corn, starch, oil, vitamins and mineral (Table 1). The diets were prepared in the form of pellets as described by Bryant *et al.* (1980). One group was left to feed on crushed shrimp and minced fresh fish (control). All tanks were exposed to the natural light and were frequently cleaned of wastes and algal growths every week, then filled with water up to the third of the tank volume.

Table (1): Composition of the four different artificial diets used in the rearing experiments of fish *Rhabdosargus haffara*

Ingredients	Diet 1 Fish meal	Diet 2 Fish + Soybean meal	Diet 3 Soybean meal	Diet 4 Blood meal
Fish powder	40%	--	--	--
Fish and Soybean	--	40%	--	--
Soybean	--	--	40%	--
Blood	--	--	--	40%
Yellow corn	35%	35%	35%	35%
Starch	21%	21%	21%	21%
Oil	2%	2%	2%	2%
Vitamines and minerals (1:1)	2%	2%	2%	2%

Feeding regime: Fish groups were fed on dry pelleted diets three times every day. The amounts of given food, throughout the experiment, were adjusted according to the fish body weight (3 %) and it was distributed equally over the tank. Food was offered in small successive portions, noting the complete consumption of all the pellets after each feed offered. The experiment lasted seven months and fish samples were collected from tanks twice monthly after starvation for 24 hr. Samples from the white muscle of *Rhabdosargus haffara* were taken in closed vials then stored at -20 °C for the biochemical analysis.

Biochemical analysis: The total protein of flesh was determined by Biuret method according to Gronall (1949). Samples were homogenized in saline solution then centrifuged at 6000 rpm and the supernatant was used for determination of total proteins using spectrophotometer at 540 nm against blank. Total lipid of the flesh of *R. haffara* was determined according to the method mentioned by Knight *et al.* (1972).

Nucleic acids (DNA and RNA) were extracted by digestion of the flesh three times with 5% trichloroacetic acid for 15 min at 90°C followed by cooling and centrifugation at 3000 rpm. The combined supernatants were used for nucleic acids determination. DNA concentration and RNA content were measured by using coloremetric method described by Dische and Schwarz (1954).

Amino acid content in the flesh was measured following the techniques of Moore and Stein (1958). Standard mixture of the common 17 amino acid was prepared in a similar way of fish sample extracts then injected into the amino-acid analyzer for qualitative and quantitative determination. Data obtained from the amino-acid analysis were calculated and all amino acids were expressed as gram amino acid/100 gram fresh weight of each sample.

The soluble protein in *R. haffara* was studied by using isoelectric focusing method (phast system apparatus, pharmacia LKB, S 75/82, 1987). Electrofocusing is using high-resolution technique for separating proteins and peptides according to their isoelectric point. Resolution as high as 0.01-0.02 pH units can be obtained. Briefly, a polyacrylamide gel is prepared to include ampholine carrier ampholytes, a specially prepared mixture of small amphteric molecules. When the current is applied to the gel, the ampholytes form a linear pH gradient and lose their charge completely where the pH of the gradient is

equal to the isoelectric point (PI) of the component. At this point, movement stops and each component is concentrated into a narrow zone.

Fresh muscle (0.5 g) of *R. haffara* fish fed on the different food type was homogenized with 5 ml of tris-HCl buffer pH 8.6. Homogenate solutions were centrifuged at 6000 rpm for 10 min. The clear supernatant liquid were pipetted into vials and kept frozen at -20 °C until used. The frozen extracted samples were thawed at room temperature then applied onto the gel plates (50 x 43 mm diameter and 0.34 mm thick) at pH from 3-9. Eight samples were applied to each gel plate including two standard proteins and protein bands were silver stained. Gels were dried, photographed and scanned using Hoefer scientific instrument (GS 300 with GS 365 w Electrophoresis data system, version 3.01). Isoelectric points (PI) of proteins were measured using Pharmacia PI calibration Kit (Pharmacia LKB).

RESULTS

Changes in flesh protein content, lipid, DNA, RNA and amino acids components in muscles of *R. haffara* fish fed on different meals for 7 months were measured. Also, Isoelectricfocusing techniques were done to identify the different muscle protein bands of reared fish during the experimental time.

Protein content:

Table (2) shows the alteration in flesh protein content of *R. haffara*, which reared and fed on different meals varying in their protein source. It is very important to mention that the control group was fed on minced fish and shrimp during the whole experiment and this type of food is similar to the natural food of this species. The protein content of *R. haffara* fed on the fishmeal was (5.8 ± 0.12 g/100g) at the beginning of the experiment. This value increased gradually during the feeding period and the maximum level (17.7 ± 0.19 g/100g) was recorded at the end of the experiment (210 days). The highest percentage difference to the control fish (30.9 %) was recorded after 150 days. The same patterns were obtained after feeding the fishes on diet number 2 (Fish and Soybean meal) and diet number 4 (Blood meal) but the highest protein level (16.7 ± 0.27) was reached (+38 %) in fish and soybean meal and (+18.2 %) in fish fed on the blood meal after 180 days. Although Soybean meal induced an increase in the protein content, these values were not in the

same magnitude like fish reared on the other diets especially after 6 and 7 months of the experiment.

Lipid content:

Changes in flesh lipid content of *R. haffara* that recorded during feeding the fish on different artificial diets are shown in Table (3). The lipid content was 1.1 ± 0.23 g/100g at starting of the experiment. All fishes fed on artificial diets recorded their maximum values of lipid content at the same time of experiment (60 days). Fishes fed on meal 2 (Fish and Soybean) showed the highest lipid content (2.3 ± 0.36) and the percentage of difference to control fish was (+142 %). On the other hand the lowest lipid content (-29.5 %) was obtained after 150 days in case of fish fed on meal 1 (fishmeal).

DNA content:-

The flesh DNA content of fish offered meal 1 (fishmeal) recorded its maximum value (0.97 ± 0.04 g/100g) after 90 days of feeding (Table 4). The percentage difference was +107.9 % compared to control fish. The elevation of DNA levels were observed in fish fed on Fish and Soybean meal and also Soybean meal (meal number 3) and the highest DNA level was reached after 150 days (+51.1 %) for meal 2 and after 120 days (+15.8 %) on meal 3. Blood meal induced a decrease in DNA content during the whole time of the experiment (210 days) and the lowest value was recorded after 30 days.

RNA content:-

Table (5) demonstrate the variation in the RNA content of *R. haffara* reared and fed on miscellaneous protein diets. The mean value of RNA content of fish fed on fish diet (meal 1) increased gradually during the first 4 months, then decreased after 5 & 6 months followed by another elevation after 7 months where reached its maximum level (8.98 ± 0.56 g/100g) at the end of experiment. The percentage difference was +59.8 % comparing to control. RNA levels were decreased following feeding *R. haffara* fish on the other diets (meal 1, 2 & 3) after 2 months from the rearing period till the end of the experiment. The lowest levels were 36.8 % after 180 days, 28.3 % after 150 days and 36.7 % after 90 days following feeding on meal 2, 3 & 4 respectively.

Table (2): Flesh protein contents (g/100g) of fish *Rhabdosargus haffara* during the rearing period and feeding on different artificial diets.

Days After Stocking	Control	Meal (1) Fish meal		Meal (2) Fish + Soybean meal		Meal (3) Soybean meal		Meal (4) Blood meal	
	Mean ± SE.	Mean ± SE.	% *	Mean ± SE.	% *	Mean ± SE.	%*	Mean ± SE.	%*
Start	5.83 ± 0.12	5.83 ± 0.12	0	5.83 ± 0.12	0	5.83 ± 0.12	0	5.83 ± 0.12	0
30	6.94 ± 0.23	6.2 ± 0.15	-10.6	6.34 ± 0.12	-8.7	6.71 ± 0.22	-3.31	7.54 ± 0.12	8.6
60	8.72 ± 0.84	7.4 ± 0.34	-15.1	6.93 ± 0.23	-2.5	7.12 ± 0.24	-18.3	8.91 ± 0.77	2.2
90	9.4 ± 1.13	10.6 ± 0.34	12.8	10.1 ± 0.42	7.5	11.5 ± 0.41	22.3	10.81 ± 0.60	15
120	11.8 ± 0.66	13.9 ± 0.26	17.8	13.2 ± 0.27	11.8	13.0 ± 0.32	10.21	11.5 ± 0.34	-2.5
150	11.0 ± 0.58	14.4 ± 0.38	30.9	14.9 ± 0.38	35.5	12.0 ± 0.40	9.1	12.5 ± 0.87	13.6
180	12.1 ± 0.72	15.6 ± 0.36	28.9	16.7 ± 0.27	38.0	13.9 ± 0.49	14.8	14.3 ± 1.11	18.2
210	14.2 ± 1.27	17.7 ± 0.19	24.6	16.0 ± 0.72	12.7	13.2 ± 0.52	-7.0	16.5 ± 0.57	16.2

SE = Standard error.

* Represents percentage of change compared to control values.

Table (3): Flesh lipid contents (g/100g) of fish *Rhabdosargus haffara* during the rearing period and feeding on different artificial diets.

Days After Stocking	Control	Meal (1) Fish meal		Meal (2) Fish + Soybean meal		Meal (3) Soybean meal		Meal (4) Blood meal	
	Mean ± SE.	Mean ± SE.	% *	Mean ± SE.	% *	Mean ± SE.	%*	Mean ± SE.	%*
Start	1.1 ± 0.23	1.1 ± 0.23	0	1.1 ± 0.23	0	1.1 ± 0.23	0	1.1 ± 0.23	0
30	0.97 ± 0.25	1.2 ± 0.21	23.7	1.8 ± 0.35	85.5	1.3 ± 0.24	44	1.62 ± 0.34	-67
60	0.95 ± 0.16	1.4 ± 0.35	47.4	2.3 ± 0.96	142	1.9 ± 0.43	100	1.79 ± 0.34	88
90	0.87 ± 0.17	1.1 ± 0.26	26.4	1.67 ± 0.14	91	1.65 ± 0.05	89.6	1.34 ± 0.31	54.7
120	0.76 ± 0.07	0.84 ± 0.26	10.5	1.15 ± 0.01	51.3	1.10 ± 0.35	44.7	1.07 ± 0.13	40.8
150	0.71 ± 0.11	0.60 ± 0.52	-29.5	0.79 ± 0.23	11.3	0.38 ± 0.07	23.7	0.78 ± 0.16	9.8
180	0.64 ± 0.05	0.58 ± 0.09	-9.4	0.61 ± 0.13	-4.7	0.67 ± 0.10	4.73	0.59 ± 0.09	-7.8
210	0.49 ± 0.05	0.50 ± 0.64	2.0	0.58 ± 0.24	18.4	0.48 ± 0.05	-2.0	0.43 ± 0.07	-12.2

SE = Standard error.

*Represents percentage of change compared to control values.

Table (4): Flesh DNA contents (g/100g) of fish *Rhabdosargus haffara* during the rearing period and feeding on different artificial diets.

Days After Stocking	Control	Meal (1) Fish meal		Meal (2) Fish + Soybean Meal		Meal (3) Soybean meal		Meal (4) Blood meal	
	Mean ± SE.	Mean ± SE.	% *	Mean ± SE.	% *	Mean ± SE.	%*	Mean ± SE.	%*
Start	0.32 ± 0.03	0.32 ± 0.03	0	0.32 ± 0.03	0	0.32 ± 0.03	0	0.32 ± 0.03	0
30	0.41± 0.03	0.71 ± 0.04	73	0.43 ± 0.03	4.9	0.42 ± 0.03	2.4	0.25 ± 0.02	-39
60	0.41 ± 0.01	0.85 ± 0.03	107.3	0.40 ± 0.02	-2.4	0.35 ± 0.02	-14.6	0.26 ± 0.03	-36.6
90	0.38 ± 0.05	0.97 ± 0.04	107.9	0.44 ± 0.07	15.8	0.37 ± 0.05	-2.6	0.25 ± 0.01	-34.2
120	0.38 ± 0.05	0.83 ± 0.05	118	0.49 ± 0.06	28.9	0.44 ± 0.06	15.8	0.26 ± 0.01	-31.5
150	0.45 ± 0.06	0.78 ± 0.06	73	0.68 ± 0.05	51.1	0.48 ± 0.012	6.7	0.37 ± 0.03	-17.8
180	0.57 ± 0.07	0.51 ± 0.06	-10.5	0.44 ± 0.06	-22.8	0.61 ± 0.06	7	0.39 ± 0.04	-31.5
210	0.56 ± 0.05	0.76 ± 0.07	35.7	0.52 ± 0.06	-7.1	0.64 ± 0.04	14.3	0.50 ± 0.05	-10.7

SE = Standard error.

* Represents percentage of change compared to control values.

Table (5): Flesh RNA content (g/100g) of fish *Rhabdosargus haffara* during the rearing period and feeding on different artificial diets.

Days After stocking	Control	Meal (1) Fish meal		Meal (2) Fish + Soybean meal		Meal (3) Soybean meal		Meal (4) Blood meal	
	Mean ± SE.	Mean ± SE.	% *	Mean ± SE.	% *	Mean ± SE.	%*	Mean ± SE.	%*
Start	2.31 ± 0.32	2.31 ± 0.32	0	2.31 ± 0.32	0	2.31 ± 0.32	0	2.31 ± 0.32	0
30	2.40 ± 0.32	2.46 ± 0.27	2.5	2.72 ± 0.23	13.7	2.60 ± 0.25	8.3	2.10 ± 0.22	-12.5
60	3.13 ± 0.23	3.19 ± 0.32	1.9	2.17 ± 0.24	-30.7	2.30 ± 0.31	-26.5	2.03 ± 0.31	-35.1
90	3.24 ± 0.22	3.67 ± 0.22	13.3	3.18 ± 0.22	-1.9	2.80 ± 0.23	-13.5	2.05 ± 0.32	-36.7
120	3.32 ± 0.32	4.16 ± 0.22	25.3	3.20 ± 0.23	-3.6	3.31 ± 0.34	-0.3	2.20 ± 0.22	-33.7
150	4.42 ± 0.33	4.29 ± 0.35	-2.9	4.46 ± 0.46	-1.4	3.17 ± 0.33	-28.3	3.23 ± 0.34	-26.9
180	5.43 ± 0.53	4.35 ± 0.45	-19.9	3.43 ± 0.35	-36.8	4.56 ± 0.46	-16.0	3.46 ± 0.23	-36.2
210	5.62 ± 0.64	8.98 ± 0.56	59.8	5.50 ± 0.56	-2.1	5.58 ± 0.67	-0.7	4.64 ± 0.55	-17.4

SE = Standard error.

* Represents percentage of change compared to control values.

Table (6): Flesh RNA/DNA ratio of fish *Rhabdosargus haffara* during the rearing period and feeding on different artificial diets.

Day after stocking	Control	Fish meal	Fish + soybean meal	Soybean meal	Blood meal
	Average ± SE.	Average ± SE.	Average ± SE.	Average ± SE.	Average ± SE.
Start	3.73 ± 0.30	3.73 ± 0.30	3.73 ± 0.30	3.73 ± 0.30	3.73 ± 0.30
30	5.85 ± 0.28	3.46 ± 0.31	6.35 ± 0.45	6.19 ± 0.22	8.40 ± 0.37
60	7.63 ± 0.26	3.75 ± 0.34	5.43 ± 0.32	6.57 ± 0.53	7.81 ± 0.31
90	8.53 ± 0.47	3.78 ± 0.46	7.23 ± 0.33	7.57 ± 0.61	8.20 ± 0.29
120	8.74 ± 0.65	5.01 ± 0.55	6.53 ± 0.45	7.52 ± 0.56	8.46 ± 0.48
150	9.82 ± 0.38	5.50 ± 0.35	6.41 ± 0.56	6.60 ± 0.68	8.73 ± 0.58
180	9.53 ± 0.58	8.53 ± 0.37	7.80 ± 0.41	7.4 ± 0.39	8.87 ± 0.49
210	10.04 ± 0.61	11.82 ± 0.39	10.6 ± 0.61	8.72 ± 0.49	9.28 ± 0.31

SE = Standard error

RNA/DNA ratio:

Transformation in the RNA/DNA ratio, which occurred in *R. haffara* reared and fed on various meals, was illustrated in Table 6. The recorded ratio was 3.73 at the beginning of the experiment then it was increased gradually after feeding on the fishmeal and reached 11.82 at the end of rearing period. The RNA/DNA ratio of fish fed on meals 2, 3 & 4 were elevated over the control values after 30 days and the highest value (8.40) was recorded following feeding on the blood meal. On the other hand, this ratio was lower than the control levels after 60 days up to 210 days except fish fed on meal 2.

Amino acid content:

Seventeen amino acids were detected and measured in the flesh of *R. haffara* fish at the end of rearing period (Figure 1). Concentration of seven amino acids (Aspartic, Glutamic, Alanine, Valine, Isoleucine, Leucine and Lysine) were higher than the others in all diets but with different percentage differences compared to control. The amino acid Glutamic recorded the highest concentrations (2.45, 2.04, 2.19 & 2.8) in all groups. Although, Systein amino acid showed the lowest concentrations all over the different diets, it recorded the highest percentage differences compared to the control fish. On the other hand, Proline and Methionine content were decreased after feeding *R. haffara* fish on the different diets compared to the control with percentage differences (-24.8, -30.9, -20.7 & -11.2) and (-34. -31.2, -28.5 & -32.5) respectively.

Isoelectricfocusing:

Muscle protein of reared *R. haffara* fish in relation to feeding on different diets is shown in Table 7 and Figure 2. Phast Gel IEF of reared *R. haffara* showed some differences in electrophoretic pattern. These bands were varied from diet to another at the end of rearing period according to the food type.

At the beginning of the experiment, the muscle protein bands of fingerlings *R. haffara* was separated to give a number of 13 bands. They are varying in their concentration. Data represented in Table (7) showed that there are three specific bands at PI's 4.4, 7.2 & 7.7 with percentage concentrations of 15.0, 9.1 & 4.6 were recorded respectively. Seventeen protein bands were separated from muscle of *R. haffara*, which fed on fishmeal. They have three specific bands separated at PI's of 5.0, 6.0, & 8.3 with 4.4, 4.7 &12.0 percentage concentration respectively. There are 13 protein bands separated from muscle of *R. haffara* that fed on fish & Soybean meal with only one specific band demonstrated at PI values of 4.2 with concentration of 7%.

The muscle of *R. haffara* fed on Soybean meal gave the lowest number of PI's (12 band) compared to other diets. There was four specific bands characterized at PI's of 3.70, 3.90, 5.10 & 9.1 with concentrations equal to 1.8, 14.5, 7.0 & 1.2 respectively. Fifteen protein bands were separated from the muscle of *R. haffara* that ate blood meal. There were two specific bands at PI s 7.1 & 7.6 with percentage concentrations 7.1 & 2.8 respectively.

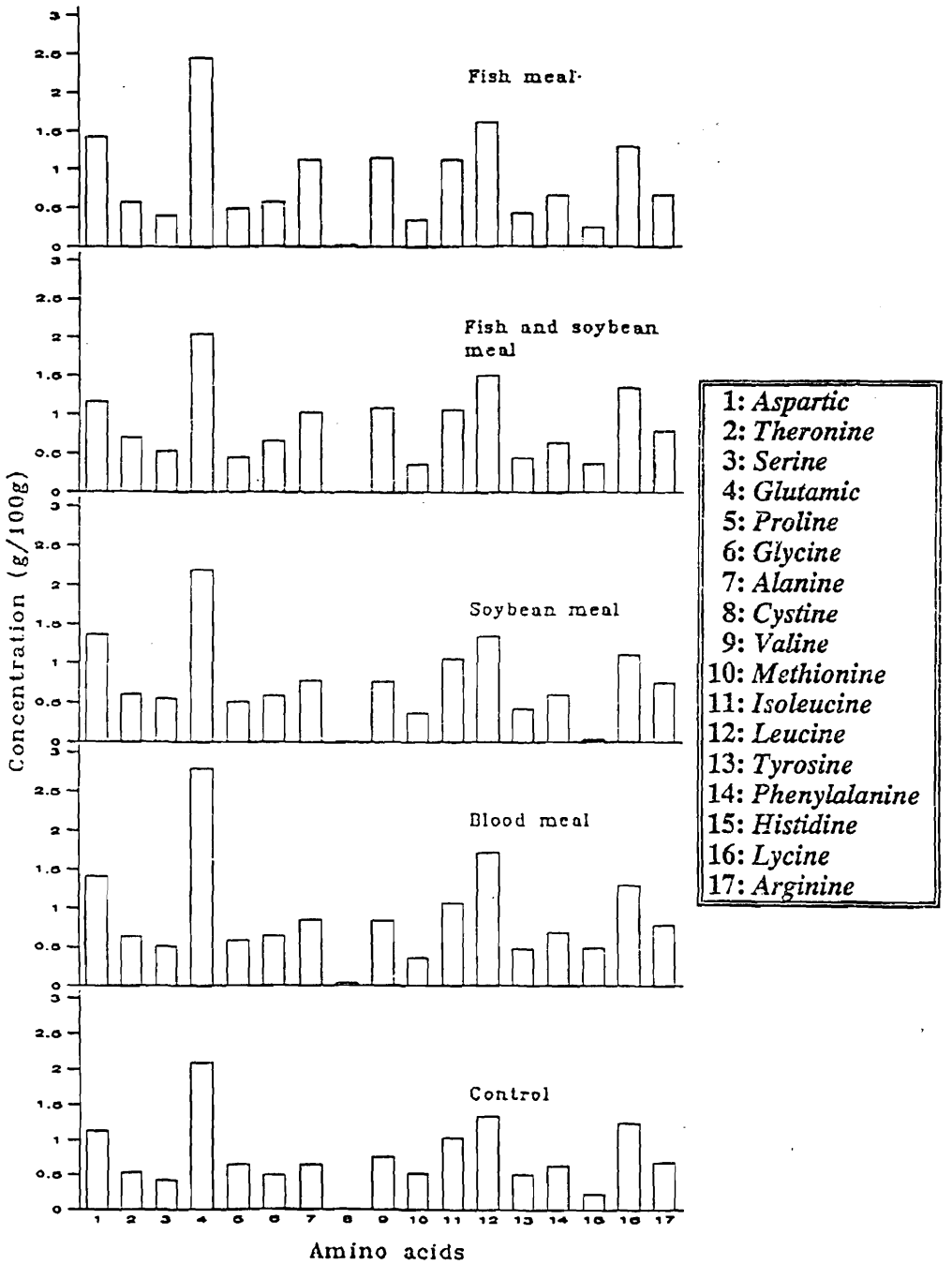

Figure (1): Total amino acids content (g/100g) in the muscle of fish *Rhabdosargus haffara* fed on different artificial meals.

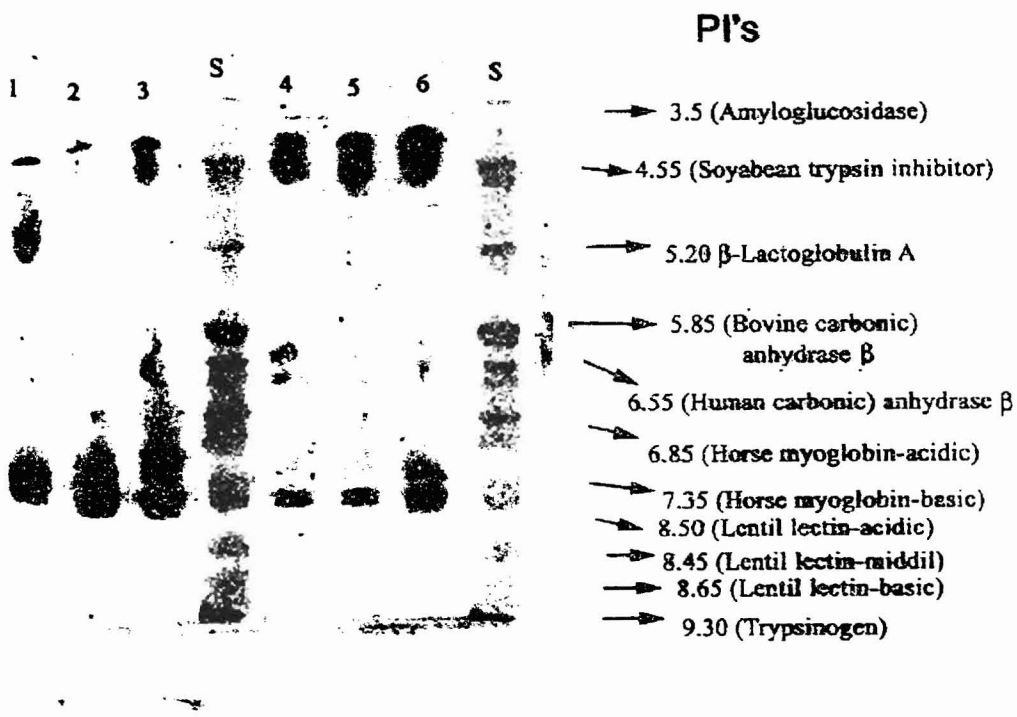

Figure (2): Isoelectric focusing shows muscle protein bands of fish *Rhabdosargus haffara* fed on different artificial meals.

1- Start
2- Fishmeal
3- Fish and Soya meal
4- Soya meal
5- Blood meal
6- Control
S: Standard protein

Table (7): The isoelectric point (PI's) values and the corresponding percentage (%) of quantitative values of scanning pattern in muscle protein of fish *Rhabdosargus haffara* fed on different artificial diets.

PI's values	Start %	Fish meal %	Fish and soybean meal %	Soybean meal %	Blood meal %	Control %
3.70	---	---	---	1.8*	---	---
3.80	5.8	2.2	---	---	---	---
3.90	---	---	---	14.5*	---	---
4.00	5.4	---	0.6	---	---	---
4.20	---	---	7.0*	---	---	---
4.30	---	---	---	---	---	3.2*
4.40	15.0*	---	---	---	---	---
4.50	---	---	3.9	---	1.3	---
4.60	---	---	16.	6.7	---	---
4.70	---	4.4	---	---	---	0.6
4.80	---	2.3	---	---	8.2	---
4.90	4.3	---	4.4	---	---	---
5.00	---	4.4*	---	---	---	---
5.10	---	---	---	7.*	---	---
5.20	---	0.9	---	---	4.1	---
5.30	4.7	---	9.8	---	---	20.9
5.40	---	6.6	---	7.6	3.6	---
5.60	3.9	1.5	---	---	---	---
5.70	---	19.3	---	---	10.3	7.3
5.80	---	7.0	3.0	---	---	---
8.90	---	---	---	5.1	11.2	---
6.00	---	4.7*	---	---	---	---
6.10	---	---	---	---	---	3.7*
6.20	---	4.0	7.5	---	---	---
6.30	---	---	---	2.7	7.9	---
6.40	---	3.8	4.4	6.5	---	3.5
6.70	3.9	---	---	---	---	3.4
7.10	---	---	---	---	7.1*	---
7.20	9.1*	---	---	---	---	---
7.50	.2	---	1.2	---	3.2	5.
7.60	---	---	---	---	2.8*	---
7.70	4.6*	----	---	---	---	---
7.80	---	10.9	---	---	---	5.8
7.90	---	5.3	---	10.0	---	---
8.10	---	---	5.6	---	---	11.5
8.20	25.6	---	---	6.2	3.3	---
8.30	---	12.0*	---	---	---	---
8.50	9.8	---	---	---	1.8	---
8.60	---	1.6	---	---	10.5	3.0
8.70	---	---	---	---	---	---
8.80	---	3.1	8.7	---	---	20.1
8.0	1.7	---	2.1	---	3.7	---
9.10	---	---	---	1.2*	---	---
9.30	---	---	---	21.8	5.9	4.6

* Specific band

The protein pattern of *R. haffara* reared on minced flesh of fish (as control for the experiment) was characterized by two specific bands separated at PI's 4.3 & 6.1 with percentage concentrations equal to 3.2 & 3.7 respectively. It is important to mention that there are thirteen protein bands separated from the muscle of the control fish

DISCUSSION

The biochemical constituent of fishes, which reared and fed on artificial food, is an important and interesting aspect. There was a correlation between the changes of biochemical constituents and the type of nutrition.

In the present study the protein content in the flesh of *R. haffara* showing different patterns of change according to the type of artificial foods. The protein make-up of fishes fed on fish & Soybean meal and Soybean meal reached to its highest level (16.7 and 13.9 g/100g, respectively) after 180 days of rearing period. While the flesh protein content of *R. haffara* fed on fishmeal, blood meal and control increased gradually with increasing the period of feeding till recorded the highest average values at the end of feeding period (17.7, 16.5 & 14.2 g/100g, respectively). At the mean time, the highest percentage difference of the protein content (38.0 % & 30.9 %) were recorded in fishes fed on meal 2 after 180 days and meal 1 after 150 days, respectively. Wassef and Abu-El-wafaa (1985) reared gilthead bream *Sparus aurata* on three types of artificial food protein and reported that fish fed on Soybean meal and fishmeal contained more protein than fish raised on diet of poultry meal. Boonyaratpalin (1997) found that fish like other species utilize protein of animal origin better than plant proteins. El-Boray (1997) stated that the variation in the average protein content in muscle of *R. haffara* reared in two different fish farms was due to the differences in the food source in each farm.

Generally, it was obvious that a continuous increase in average protein content during the period of rearing from the start related to feeding activity of the fish, where *R. haffara* in this period build up their bodies The gradual rise of protein level of *R. haffara* was attributed to the high feeding activity and the natural growth of fish (Medford, 1978). Mohmoud (1997) found that *Mugil seheli* reared in fish farm attained high values of protein during the rearing time and that may be attributed to the feeding and growth activities. Shakweer *et al*

(1998) indicated that the percentage of protein content in the muscles of *Mugil cephalus* were usually affected by several factors such as type of food, fish size and stage of maturity. Also, they found that the major biochemical constituent of muscle including protein differs significantly from one fishing area to another. Such variations could be related to the biota and the biotic conditions, which are prevailing in these areas. Our results showed that the protein levels of *R. haffara* fed on the different diets were lower than the control fish during the first 60 days of rearing. This might be explained on the basis of acclimatization of the fish on the artificial diets. These results agree with Papoutsoglou and Papaparakeva (1978) who found that protein content of rainbow trout fed on three types of artificial food decreased in the beginning followed by a slight increase.

The present experiment indicated that the lipid content in the muscle of *R. haffara* recorded high values at the beginning of rearing period. Its content increased with increasing time of stocking up to 60 days. This may be related to the tendency of the fish to store more lipids as additional source of energy during the acclimatization period. It is important to mention that the glycogen content could not be detected during this study because they were very low in concentration. So, it can be postulated that this fish species accumulated lipids as main energy source instead of glycogen at the start of rearing period and adaptation on the artificial food. At the end of rearing period the flesh lipid content recorded low values than at start where the feeding activity of the fish increased during the growing period.

The lowest lipid content (0.43 g/100g) was recorded at the end of rearing period in case of feeding the fish on the blood meal, which might be related to the maturation of gonads at this time (El-Halfawy, 2001). Love (1970) and Wassef (1978) have mentioned that lipid content was the most variable component in the fish. Mikhail *et al* (1982) pointed out that the fat content in the muscles of the young fish is higher than that in the adults of two species of Serranid fishes *Epinephalus aeneus* and *E. alexandrinus*. The same result obtained by Mahmoud (1997) in *Mugil seheli*. The lower values of lipids of adults which may be coincides with the beginning of spawning peak (Wassef and Abo-El-Wafaa 1985) probably due to the consumption of such materials for gonadal development (Wassef & Shehata, 1989). Also, our results agree with El-Boray (1997) who found that the decrease in the average lipid content in the muscle of *R. haffara* reared in two different fish farms can be attributed to the

feeding activity and the growing of the gonads towards ripening. This explain the lowest value of lipid content recorded in blood meal group than other diets where the gonads reached to the highest maturation stage during rearing period (El-Halfawy, 2001). Masurekar and Pai (1979) and Sivakami (1981) found that fat content in the muscle of the common carp in both sexes reached the lowest level in the spent stage, indicating its utilization for spawning activity

Bulow (1987) reported that in normal cells, deoxy ribonucleic acid (DNA) is found in the nucleus in association with chromosomal material. Small amounts of DNA is found in the cytoplasm (Shalaby, 1998). The total quantity of DNA per cell is remarkably constant in all normal somatic tissue within a given species and this amount is not altered by starvation or other kind of stress (Mirsky and Ris, 1951; Davidson and Leslie, 1950a; Hawk *et al.*, 1954). Davidson and Leslie (1950 a & b) suggested that the constant DNA concentration could be used to determine the total number of cells in a given tissue. Therefore, DNA became a standard reference for revealing changes in cell number and composition (Lesile 1955).

In this study the DNA content in the muscle of *R. haffara* showed variations from diet to another. These differences in the DNA content varied according to the types of diets and the rate of cell division. The fishmeal recorded highest DNA content while, blood meal recorded lowest values, which may be related to weight of specimens where there is a correlation between increasing weight, number of cells and the value of DNA (Love, 1970).

Ribonucleic acid (RNA) is present in variable quantity in the nucleus and cytoplasm. It is responsible for transfer of the genetic code of nuclear DNA in the cytoplasm and with the actual synthesis of new protein. The quantity of RNA varies directly with the activity of protein synthesis, therefore, it is expected to be more concentrated in tissue undergoing faster growth or protein synthesis (Bulow, 1987). The change in the RNA content in muscle of *R. haffara* reared and fed on different types of diets showed a continuous increase from the beginning of this study and recorded the highest RNA values at the end of feeding period. The type of diets influenced this augmentation of RNA contents in the flesh where the fish build up its body. This increase facilitates the synthesis of more protein, which correlated well with the recorded increase in the protein content during this period. Many authors (Mustafa and Zofair,

1985; El-Boray, 1997) reported that large quantity of RNA is synthesized as a result of food intake.

Analysis of RNA/DNA ratio reflects the growth rate of fish. It helps to evaluate the response of an organism to its environment and the linkage between environmental conditions and recruitment variability (Buckley and Lough, 1987). Individual with high RNA/DNA ratios can be imagined to accumulate protein and grow faster than the ones with low ratios which stunted growth (Bulow, 1970 and Mustafa & Mittal, 1982). Here, RNA/DNA ratio in muscle of *R. haffara* showed an increase from the beginning of feeding till the end of rearing period with all different types of artificial food. The diet contain animal protein sources (fish & blood) showed higher RNA/DNA ratios which confirm the findings that different fish species utilize animal protein better than plant protein (soybean), leading to more growth of the fish.

Amino acids are the structural components of proteins. Most monogastric animals, including fish require the same essential amino acids, which are already included in the different diets used in this study as indicated by the feeding Tables established by National Research Council (NRC, 1984).

Dietary amino acids are required for two purposes, firstly for growth, which mainly consists of protein deposition, and secondary for a number of processes that are conceptually described as maintenance. The relative requirements for protein deposition and maintenance clearly depend on the relative rate of growth (Cowey, 1994). The present study indicated that *R. haffara* requires the same essential amino acids as other fishes and land animals. These are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, therionine and valine. Generally the concentration of these essential amino acids varied according to the type of diet where they arranged as follow: blood meal, fish and Soybean meal, fishmeal, control and Soybean meal. The mean values of the total amino acids content in *R. haffara* fish gave the same patterns of concentrations where the highest values was recorded after the blood meal. This might give evidence that the blood meal contains the right quality and quantity of the required amino acids for the growth of this fish. The constituent of the amino acids in the blood meal is the highest (80-86 % protein) and highly digestible than other artificial diets as reported by NRC (1984).

Based on NRC (1984) requirements, soybean protein is not deficient in any essential amino acid. Although soybean protein has one of the best amino acid profiles of all proteins rich foodstuffs for meeting the essential amino acid requirements of fish, some fish find soybean meal unpalatable. The flesh of *R. haffara* fed on soybean meal showed low amino acids concentration, which might be related to the normal feeding habits of the fish since it is a carnivorous species. On the other hand, control fishes of *R. haffara* showed the lowest concentration of amino acids than all artificial diets. This indicated that artificial diets with supplemented protein increase the concentration of amino acids with variation in protein source than natural source (Schuhmacher *et al.*, 1997).

Electrofocusing is using high-resolution technique for separating proteins and peptides according to their isoelectric point. In the present study, Electrofocusing procedure used for studying the effect of artificial diet on the formation or accumulation of different protein patterns in the flesh of fish *R. haffara* according to the type of diet. Also, it is used to evaluate the efficiency of flesh protein at the start and at the end of rearing and feeding period since there is not previous work done concerning this aspect.

The results of this investigation showed that there are variations in the number of protein patterns and its concentration according to the types of diet. At start of feeding period the number of protein patterns were 13 with three specific bands varying in their concentrations. The protein patterns separated from *R. haffara* fed on fishmeal have the largest number of protein bands (seventeen) with different concentrations. The lowest number of protein bands (12) were separated from fish fed on soybean meal. They have four specific bands with different concentrations. The highest number of protein patterns in flesh of *R. haffara* fed on fishmeal and the lowest levels of protein bands in flesh of fish fed on Soybean meal refer to the nature of feeding of this fish where, it is carnivorous fish. This means that digestion and assimilation of fish diets is better and more effective than Soybean meal.

This study recommends the use of combined diet between fish, soybean and blood sources in calculated percentage of proteins where it may give an excellent biochemical composition for this important species in the Suez Gulf.

ACKNOWLEDGEMENT

The authors thank Prof. Abdel-Fattah A. Ghobashy, Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt and Prof. Hussein K. Badawi, National Institute of Oceanography and Fisheries, Alexandria Branch, Egypt for their great contribution and merit comments.

REFERENCES

Boonyaratpalin, M., 1997. Nutrient requirements of marine food fish cultured in South East Asia. *Aquaculture.* 151: 283-313.

Bryant, P.; K. Jauncey and T. Atack; 1980. Backyard fish farming. Prism press. PP. 170

Buckley, L. J. and R. G. Lough, 1987. Recent growth, biochemical composition and prey field of larval haddock (*Melanogrammus aeglefinus*) and Cod (*Godus morhua*) on Georges Bank. *Can. J. Fish. Aqua. Sci.* 44: 14-25.

Bulow, F. J., 1970. RNA/DNA ratios as indicators of recent growth rates of a fish. *J. Fish. Res. Bd. Can.* 27: 2343-2349.

Bulow, F. J., 1987. RNA/DNA ratios as indicators of growth in fish: Areview In: " The age and growth of fish" Summerfell, R. C. and G. E. Hall (eds.) *Towa State University press, Ames, Towa.* 5D010.

Cowey, C. B., 1994. Review amino acid requirements of fish: a critical appraisal of present values. *Aquaculture.* 124: 1-11.

Davidson, J. N. and I. Leslie, 1950a. A new approach to the biochemistry of growth and development. *Nature.* 156: 49-53.

Davidson, J. N. and I. Leslie, 1950b Nucleic acids interrelation to tissue growth: *Review Cancer Res.* 10: 587-594.

Dische, Z and K. Schwartz, 1954. Estimation of nucleic acids in "*Methods of Biochemical analysis*". *Vol. I. Click, D. led, P229, New York, Interscience publisher Inc.*

EL-Boray, K. F , 1997 Reproductive biological studies on *Rhabdosargus haffara* In different water fish farms Ph D Thesis, Faculty of Science Zagazig University, Egypt

El-Ebiary, E. H., 1994. Studies on fish production: Relationship between nutrition and reproduction of *Tilapia sp.* Ph. D. Thesis, Faculty of Agriculture , Alexandria. University, Egypt.

EL-Halfawy, M. M., 2001. Effect of types of food on the development , growth and biochemical composition of the fish *Rhabdosargus haffara* (Farsskal, 2775). (family: Sparidae) in the north Suez Gulf. Ph. D. Thesis, Faculty of Science, Suez Canal University. Egypt.

EL-Shahat. M. I., 1998. Studies on the culture of red Tilapia. *Oreochromis sp.* in net enclosures. M. Sc. Thesis, Faculty of Science. Tanta University. Egypt.

Essa, M. A. and E. H. El-Ebiary. 1995. Protein sparing effects in the fingerlings of Nile Tilapia, *Oreochromis niloticus*, and its hybrids. Delta J. of Science, Faculty of Science, Tanta University, Egypt.

FAO, 1983. Fresh water aquaculture in China. Report of the FAO/UNDP. FAO fisheries Tech. Pap. No. 215.

Gronall, A. G.; C. J. Bardawill and M. M. David; 1949. Cited from spectrophotometry and spectoflurimetry, a practical approach. Ed. Bashford. C. L. And Harris, D. A. (1987) *IRL Press, Oxford, England.*

Hawk, P. B.; B. I. Oser and W. H. Summerson, 1954. Practical physiological Chemistry-13 th ed. Mc Graw-Hill Book *Co., Inc., New York.*

Knight, J. A.; S. Andreson and J. M. Raule; 1972. Chemical basis of the sulfo-phospho-lipids. *Clin. Chem.*, 18: 199-202.

Lesile, I., 1955 The nucleic acid content of tissues and cells. PP. 1-50. In: *E. charagaff* and J. N. Davidson, eds. The nucleic acids. Chemistry and biology *Vol. 2 Academic Press, Inc. New York, N. K.*

Love, R. M., 1970. The chemical biology of fishes. *Acad. Press. Inc. (London)* pp. 547.

Magouz, F. I., 1990. Studies on optimal protein and energy supply for tilapia (*Oreochromis niloticus*) in intensive culture. Ph. D. Dissertation, Faculty of Agriculture., Georg- August- University, Gattingen.

Mahmoud, W. F., 1997. Reproductive biology and physiological characters of *Mugil seheli* in fish farms. M.Sc. Thesis, Faculty of Science, Suez Canal University. Egypt.

Masurekar, V. B. and S. R. P. Pai, 1979. Observations on the fluctuations in protein, fat and water content in *Cyprinus carpio* (Linn.) in relation to the stages of maturity. *Indian J. Fish.* 26: 216-224.

Medford, B. A., 1978. Protein and Lipid content of gonads, liver and muscle of northern pike (*Esox lucius*) in relation to gonad growth. *J. Fish. Res. Bd. Vol.* 35: 213-219.

Mikhail, M. Y.; A. A. Ezzat; M. T. Hashem and W. F. Wadie; 1982. Fluctuations in water and fat contents of two species of Serranid fishes in the Egyptian Medetrranean coast. *Bull. Inst. Ocean. Fish.* 8(1): 123-144.

Mirsky, A. E. and H. Ris, 1951. The deoxyribonucleic acid content of animal cells and its evolutionary significance. *J. Gen. Physiol.*, 34: 451-462.

Moore, S. and H. W. Stein, 1958 Chromatography of amino acids on sulfonated polystyrene resins. *Anal. Chem.* 30: 1185.

Mustafa, S. and S. M. Zofair, 1985. Seasonal variations in protein, RNA and DNA content ratios in major carps; *Calta calta, Labeo rahita* and *Lirrhina mirgala*. *Japanese Journal of Ichthyology*. 32(2): 258-262.

Mustafa, S. and A. Mittal, 1982. Protein, RNA and DNA levels in liver and brain of starved catfish *Clarias batrachus*. *Japanes J. Ichthyology*. 28(A): 396-400.

National Research Council (NRC), 1984. Nutrient requirements of warm water fish. Washington, DC: National Academy of Sciences.

Papoutsoglou, S. E. and E. G. Papaparaskeva, 1978. Comparative studies on body composition of rainbow trout (*Salmo gairdeneri R.*) in relation to type of diet and growth rate. *Aquaculture*. 13: 235-243.

Schuhmacher, A.; C. Wax and J. Gropp, 1997. Plasma amino acids in rainbow trout (*Oncorhynchus mykiss*) fed intact protein or a crystalline amino acid diet. *Aquaculture*. 151: 15-28.

Shakweer, L. M.; E. H. Ebiary and M. A. Zaki; 1998. Comparative study of the major biochemical constituents in muscles of *Mugil cephalus* inhabiting the Mediterranean water, the northern delta lakes and fish farms of Egypt. *Bull. Nat. Inst. of Oceanog. & Fish*. 24: 79-101.

Shalaby, S. M. H., 1998. Studies on fish production Nutrition requirements of Rabbit fish *Siganus rivulatus* fingerlings. Ph. D. Thesis, Faculty Agriculture, Alexandria University, Egypt.

Sivakami, S., 1981. Studies on the cyprinid fishes of the genus Rasbora Bleeker of Kerala. Ph. D. Thesis, Kerala University. Quoted from Sivakami (1986).

Wassef, E. A., 1978. Biological and physiological studies on marine and acclimatized fish *Sparus aurata* (L). Ph. D. Thesis, Faculty of Science, Cairo University, Egypt.

Wassef, E. and M. B. Shehata, 1989. Biochemical composition of gilthead bream *Sparus aurata* L. Third Egyptian British Conference on Animal, Fish and Poultry production Alexandria, 7-10, October

Wassef, E. and M. Abu-EL-Wafaa, 1985. Effect of various dietary proteins on the growth and body composption of Gilthead seabream *Sparus aurata* L. *J. Egypt. Vet. Med. Ass.* 45(1): 41-52.