



Influence of several dietary protein and lipid levels on nutritional parameters and liver functions of *Solea aegyptiaca* juveniles

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ABSTRACT

A feeding trial was designed to assess the effects of dietary protein and lipid content on growth, feed utilization efficiency, and muscle proximate composition of *Solea aegyptiaca* juveniles. Eight diets were formulated (4 protein levels; 40, 45, 50 & 55% at 2 lipid levels; 8, 12%). The results showed that growth performance in terms of weight gain, specific growth rate, condition factor, feed conversion ratio, protein efficiency ratio and net protein utilization were much higher for fish group fed on a diet contained 45% protein at 12% lipid than the rest of experimental groups. The feed intake of experimental fish tended to reduce with rising dietary lipid levels. No significant differences in *Solea aegyptiaca* for digestibility coefficient and body chemical composition were observed. The body content of lipid and liver enzymes (alanine aminotransferase and aspartate aminotransferase) activities were positively correlated with dietary lipid levels. The present results pointed to the diet containing 45% protein and 12% lipid with 22.5 MJ/kg⁻¹ had a protein-sparing effect and was optimal for *Solea aegyptiaca* juveniles.

INTRODUCTION

Protein is considered the highest cost component in fish meals and plays a critical role in fish growth performance. The researchers pointed out more benefits of protein (as the main content in fish diets) like using it in metabolic energy, utilizing the synthesis of muscle tissue, etc. (Williams *et al.*, 2003; Ozorio *et al.*, 2006). Protein sparing by lipid has been recorded only in the salmonids fish (Azevedo *et al.*, 2004), and by non-protein energy sources in a large scale of species (Thoman *et al.*, 1999; Azevedo *et al.*, 2004; Kim and Lee, 2005).

The precise data on the protein requirement for fish is very essential for any new aquaculture activity in light of the high cost of protein ingredients (Ng *et al.*, 2008). The usage of dietary protein is identified with both protein levels and accessibility of non-protein energy sources (Ai *et al.*, 2004). Ideally, dietary lipids or carbohydrates ought to be expanded in fish feeds to save dietary protein from being utilized for energy (Deng *et al.*, 2011). Lessening the dietary protein content by expanding fat as well as edible carbohydrate content, with no negative impact on the growth performance is known as "the protein-sparing effect" (Yamamoto *et al.*, 2005).

A recent study on the manufacture of aqua feed show that elevated levels of lipid concentrations in the diet and improving fish performance considers an important target. Dietary lipids play a conspicuous role in fish nutrition as a primary energy source and to provide essential fatty acids to maintain biological structures and normal functions of cell membranes (Sargent *et al.*, 1999). Increasing dietary lipid levels improves diet efficiency by minimizing protein degradation (Peres and Oli'va-Teles, 1999).

The large attention to fish oil was reported done in many human health studies due to its high content of unsaturated fatty acids (HUFA). Also, fish oil is the main source of lipid which used in the formulation of the diet for many marine fish species (Nasopoulou and Zabetakis, 2012). On the other hand, the continuous increasing of world vegetable oil production, elevated the interest to use vegetable oils for fish feed to partial replacement and reducing the dependency on fish oil (Montero *et al.*, 2008; Xu *et al.*, 2015).

The successful extending of fish culture required to consider the cost and refined fish species for new aquaculture. Individuals from the genus *Solea sp.*, are reported among the most vital and significant commercial flatfishes in Egypt (Gabr *et al.*, 2003). In Egypt, the first hatching of common sole (*Solea solea*) was observed in 1986 (Zaki and Hamza, 1986, 1988; Assem *et al.*, 2012). Senegalese sole is a common flatfish in the Mediterranean and considered to be one of an attractive species for mariculture expansion in this area (Imsland *et al.* 2003; Canãvate *et al.*, 2006), which has the similar growth of that other aquaculture fish species such as Gilthead sea bream or Sea bass (Dinis *et al.*, 1999; Imsland *et al.*, 2003). In Egyptian Qaroun Lake, the total Egyptian sole catch was represented from 35 to 50% of total lake catch production, according to different seasons (Kariman, 2009).

In spite of the successful progress in hatching, rearing larvae and weaning stage for Sole fish was investigated, the shortage information about this species still standing (Dinis *et al.*, 2000; Conceic,aõ *et al.*, 2007). Thus, extensive efforts was achieved in studying the nutrient requirements of Sole, but some essential aspects of nutrition and physiology of this kind of fish are still missing. The fundamental assessment shows that Sole juveniles require 530g kg⁻¹ dietary protein for the highest growth rate (Rema *et al.*, 2008) and for *Solea aegyptiaca* 550g kg⁻¹ (Yones and Abdel-Hakim, 2011). Otherwise, Guerreiro *et al.* (2012) reasoned that dietary protein could be diminished from 550g kg⁻¹ to 450g kg⁻¹, without influencing on fish performance by rising dietary lipid or carbohydrate level. Protein represents as a major factor of diet cost and optimizing protein utilization is essential for the sustainability of fish production. Dias *et al.* (2004) reported that Senegalese sole growth was identical with 110g or 210g kg⁻¹ of lipids. Furthermore, Borges *et al.* (2009) concluded that Senegalese sole juveniles have low lipid tolerance and recommended that maximum dietary lipid inclusion should be limited to 80g kg⁻¹ for the best nutrient utilization and optimal growth. Guerreiro *et al.* (2012) studied the effect of dietary levels of protein (450g kg⁻¹ and 550g kg⁻¹) and lipid (80g kg⁻¹ and 160g kg⁻¹) on performance of Sole juveniles and revealed the best feed utilization with the diet including 550g kg⁻¹ protein and 80g kg⁻¹ lipid. However, the growth was identical with the diets of 450 g kg⁻¹ protein and 160 g kg⁻¹ lipid, this suggesting that the protein spared by dietary lipids. Hence, it is vital to decide the correct dietary protein, energy levels and/or their ratio for boosting growth rate, feed utilization and lowering the environmental impact on fish farms. In flatfishes, a protein sparing by dietary lipids was beforehand recorded in Atlantic halibut (Helland and Grisdale-Helland, 1998) and Turbot fishes (Cho *et al.*, 2005). In other studies in flatfishes were

not able to demonstrate a protein sparing by dietary lipid (Berge and Storebakken, 1991), whilst the drooping in growth was observed at high levels of dietary lipids (Lee *et al.*, 2000; Regost *et al.*, 2001). These conflicting consequences might be linked to the protein/lipid ratio of the experimental diets. For example, Andersen and Alsted (1993) observed that protein contents in Turbot have a huge impact in dietary lipid.

The present study was conducted to evaluate the effects of dietary protein and lipid levels as practical feeds on growth performance, feed utilization, digestibility coefficient and body composition of *Solea aegyptiaca* fish.

MATERIALS AND METHODS

Fish and Experimental Design

Sole, *Solea aegyptiaca* juveniles were obtained from Qaroun Lake to the experimental tanks of the Fayoum research station, National institute of oceanography and fishers, Egypt. The rearing tanks were supplied with water from the Qaroun Lake by two water pumps, each pump was drawing the water to the water storage tanks after it passes on upstream sandy filter units, then water forced through polyvinyl chloride (PVC) tubes into the fish rearing tanks with a flow rate of 3L/min, approximately. The continual aeration in each rearing tank was maintained throughout the experimental rearing period. Prior to starting the experiment, the juveniles of Sole fish were stocked and reared in circular fiberglass tanks for 2 weeks in order to acclimatize it in the experimental conditions. Through this period, the Sole fishes were fed twice daily on a control diet. At the beginning of the experiment, the fish were fasted for 24h before weighing. The fishes of similar sizes (initial weight of 10.62 ± 0.28 g) were distributed into twenty-four fiberglass tanks (water volume for each tank was 1M^3) with 30 juveniles per tank. Each treatment of this experiment was randomly specified to triplicate tanks. The fishes were hand-fed to apparent satiation twice (10:00 a.m. and 4:00 p.m.) daily and weighing every two weeks to adjust the amount of feed consumption. The trial was conducted for a period of 120 days (from May to August 2016). Physicochemical characteristics of water for every tank were analyzed weekly according to (APHA, 1992).

Experimental Diets

All experimental diets were consisted of various commercial ingredients by using fish meal as a protein source, fish oil, sunflower oil as a lipid source and wheat bran as a carbohydrate source. The ingredients were mixed and grounded into a fine powder image through a 175- μm mesh. Hence, the diets were pelleted by California pelleting machine with 2mm diameter and stored in a refrigerator at 5°C until used. Eight diets were formulated (4 protein levels; 40, 45, 50 and 55% at 2 lipid levels; 8, 12%) with different ratios of gross energy ranging from 20.87 to 23.51 MJ kg⁻¹ diet (Table, 1). Chromic oxide (Cr₂O₃) as an inert marker was added in diets to assess the apparent nutrient digestibility.

Table 1: Formulation and proximate composition of experimental diets (% D.M basis).

Ingredients	Diets (protein/lipid %)							
	(40/8%)	(45/8%)	(50/8%)	(55/8%)	(40/12%)	(45/12%)	(50/12%)	(55/12%)
Fish meal	20.0	30.0	40.0	55.0	20.0	30.0	40.0	60.0
Poultry-by product meal	20.0	20.0	25.0	20.0	24.0	20.0	25.0	18.0
Soybean meal	24.0	22.0	15.0	10.0	20.0	23.0	15.0	5.0
Wheat bran	26.0	18.0	10.0	5.0	22.0	13.0	6.0	3.0
Fish oil	4.0	4.0	4.0	4.0	6.0	6.0	6.0	6.0
Sunflower oil	4.0	4.0	4.0	4.0	6.0	6.0	6.0	6.0
Vitamin Min. Mix ¹	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Proximate analysis (%D.M.)								
Dry matter	92.8	92.6	92.5	92.8	92.6	92.5	92.7	92.6
Crude Protein	40.36	45.28	50.6	55.2	40.12	45.06	50.04	55.0
Crude lipid	14.85	16.25	17.07	18.16	19.64	20.11	21.46	22.63
N.F.E	30.26	24.11	17.52	11.45	26.67	21.29	13.97	7.84
Crude fiber	4.47	4.05	3.15	2.38	4.43	3.63	2.78	1.91
Ash	10.06	10.31	11.66	12.81	9.14	9.91	11.75	12.62
GE/ MJ kg ⁻¹ diet ²	20.87	21.52	21.96	22.42	22.08	22.5	22.94	23.51
ME/MJ kg ⁻¹ diet ³	17.36	17.89	18.22	18.75	18.51	18.8	19.16	19.61

1-Vitamin mineral premix (mg or IU/kg of diet): thiamin, 24.5mg; riboflavin, 36mg; pyridoxine HCl, 19.8mg; vitamin B12, 0.1mg; vitamin K3, 5.1 mg; inositol, 784mg; pantothenic acid, 58.8mg; niacin acid, 198 mg; folic acid, 19.6mg; biotin, 1.20 mg; retinol acetate, 16,000 IU; cholecalciferol, 2500 IU; alpha-tocopherol, 200 mg; choline chloride (50%),2500 mg; ascorbic acid (35%),1000 mg; mold inhibitor, 1000mg and ethoxyquin, 500mg; MgSO₄·7H₂O, 1200mg; CuSO₄·5H₂O, 10mg; ZnSO₄·H₂O, 50mg; FeSO₄·H

2O, 80mg; MnSO₄·H₂O, 45mg; COCl (1%), 50mg; Na₂SeO₃ (1%), 20; Ca (IO₃)₂ (1%), 60mg; Zeolite, 85mg; Ca (H₂PO₃)₂· H₂O, 5000mg.

2-Gross energy (MJ Kg⁻¹ diet) was calculated by using the following calorific values: 23.9, 39.8 and 17.6 KJ g⁻¹ diet for protein, ether extract and nitrogen free extract, respectively (Lozano, 2007).

3-The metabolizable energy(MJ Kg⁻¹ diet) of the experimental diets were calculated as 18.9, 35.7 and 14.7 KJ g⁻¹ diet for protein, lipid and nitrogen free extract, respectively (Jobling,1994).

Growth Performance and Feed Utilization

The parameters for growth and feed utilization were calculated as follows:

Weight gain, WG (gm/fish) = final weight – initial weight.

Average daily gain, ADG (gm/fish/day) = total weight gain (gm) /period (day).

Condition Factor (K) = fish weight, grams / (fish length, cm)³ × 100.

Specific growth rate, SGR = LnW₂ – LnW₁ / T × 100.

Where: Ln = the natural log.

W₂ = final weight at certain period (gm).

W₁ = Initial weight in the same period (gm).

T = period of experiment (day).

Survival rate, SR (%) = No. of surviving fishes/total No. of fishes at the beginning×100.

Feed conversion ratio (FCR) = feed intake (gm)/ weight gain (gm).

Protein efficiency ratio (PER) = Weight gain (gm)/Crude protein intake (gm).

Net protein Utilization (NPU %) = 100 (Final body protein, gm - initial body protein, gm /protein intake, gm).

Fish Samples and Chemical Analysis

At the experimental start, nine fishes were randomly collected and divided into three pooled groups to serve as an initial fish sample and stored frozen at -20 °C for doing the carcass composition analysis according to the procedures described by Standard Methods (AOAC, 2006). At the experimental end, all fishes fasted for 24 h before harvest and the same step for collected fish samples was made for each treatment (9fishes/replicate). Also, the proximate analysis of experimental diet composition was occurred according to the same procedures mentioned above.

Digestibility trail

After the sample collection described above, the remaining fish were reared and fed with the similar diets after adding 0.5% Chromic oxide (Cr_2O_3 , as an inert marker) to define the apparent digestibility coefficients (ADCs) as an indicator for nutrient digestibility. The fish feces were collected after passing the time of fish feeding from 5-6 hours. Fishes from each treatment were anesthetized with MS-222 (50mg/L) and manually stripped of feces by applying gentle pressure in the anal area according to the method described by Borges *et al.* (2013). The amounts of feces were collected and stored at -20°C until a sufficient amount of dried feces had been covered for analysis. The fish feces and treated diets with Chromic oxide additive were determined using an inductively coupled plasma-atomic emission spectrophotometer (IRIS Advantage [HR], Thermo Jarrell Ash, Woburn, MA, USA) after Perchloric acid digestion, triplicate analysis have been made for each sample of each treatment. ADCs % of nutrient was calculated according to the following equation:

ADC of nutrients (%) = $100 - (100 \times \text{nutrient in feces \%} / \text{nutrient in diet \%} \times \text{Cr}_2\text{O}_3 \% \text{ in diet} / \text{Cr}_2\text{O}_3 \% \text{ in feces})$.

Liver functions

Hepatosomatic index (HSI) was defined for each liver by weighed it after excised from nine collected fishes per treatments, then frozen in liquid nitrogen and stored at -80°C to define whole lipid, glycogen, alanine aminotransferase, ALT and aspartate aminotransferase, AST. The whole lipid of liver samples was defined according to the followed method of Folch *et al.* (1957), whereas liver glycogen was defined by a colorimetric enzymatic method using commercial kits (supplied by Nanjing Jiancheng Bio-Tech Co., Nanjing, China). The followed method for assaying protein-metabolism enzyme activities from Liver crude extract was obtained by homogenization of frozen tissue in ice-cold 0.7% saltwater and following by centrifugation stage (3200 rpm, for 20 min at 4°C). Activities of liver enzymes ALT and AST were measured using specific analytical procedures and commercially available kits (supplied by Nanjing Jiancheng Bio-Tech Co., Najing, China). Lipase activity was defined by the method of Pan and Wang (1997). HSI was calculated as a percentage of the whole fish weight as follows:

Hepatosomatic index (HSI) = $100 [\text{liver weight (g)} / \text{fish body weight (g)}]$.

Statistical Analysis

The resulted data of different levels and their interactions were performed using statistical SPSS software version 18 (IBM Corp., New York, USA). The homogeneity test of variances was done for trialed data. Duncan post-hoc test with multiple ranges (Duncan, 1955) was obtained to compare differences among means at 5% significance level (Differences were regarded as significant when $P < 0.05$).

RESULTS

The analysis of variance achieved to determine whether there were differences between treated groups, and a possible interaction between protein and lipids.

The resulted data in this nutritional trail were found that, a clear correlation between protein and lipid levels in feeding *Solea aegyptiaca* juveniles. The data in Table (1) showed that there is a clear variation in the diets constitutes from protein and lipid levels. Also, sunflower oil and fish oil were incorporated in all experimental diets as a percent of 1:1 in order to spare 50% of high price fish oil.

From Table (2), the results reported that the water physicochemical characteristics such as temperature, salinity, pH, dissolved oxygen and unionized ammonia are in the optimum rates for Sole farming according to Fonds (1976). The resulted data showed constant physicochemical results in all fish tanks.

Table 2: The mean of water physicochemical characteristics features during the trial interval.

Parameters	Diets (protein/lipid %)							
	(40/8%)	(45/8%)	(50/8%)	(55/8%)	(40/12%)	(45/12%)	(50/12%)	(55/12%)
Temperature (°C)	28.2±0.2	28.5±0.3	28.3±0.2	28.4±0.4	28.4±0.3	28.4±0.2	28.2±0.2	28.2±0.2
Salinity (g/l)	33.2±1.2	33.1±1.2	33.2±1.2	33.1±1.2	33.1±1.1	33.1±1.2	33.1±1.1	33.1±1.2
pH	7.6±0.3	7.8±0.2	7.5±0.2	7.8±0.2	7.6±0.4	7.5±0.3	7.8±0.2	7.6±0.2
Dis. Oxy ¹ (mg/l)	6.2±0.1	6.1±0.2	6.2±0.4	6.1±0.2	6.0±0.2	6.2±0.1	6.1±0.2	6.2±0.2
Uni. am. ² (mg/l)	0.03±0.01	0.03±0.01	0.02±0.01	0.03±0.01	0.02±0.01	0.03±0.01	0.03±0.01	0.04±0.01

1-Dissolved oxygen. 2-Unionized ammonia

The recorded averages of initial weights of Egyptian sole (*Solea aegyptica*) ranged between 10.4 to 11.0 g/fish with no significant differences among the treatments, which referred that the random distribution of the trial fish among dietary groups as in Table (3) and Figure (1). Interestingly, the data in Table (3) explained that the survival ratio of fishes in all dietary groups during the experiment was 98%. This showed that the used diets increased the survival rates of Sole fish. On other hand, the deceased fish ratios were resulted of the external factors during the samples collection every two weeks to adjust the amounts of feed intake. The data in Table (3) showed that the dietary protein and lipid levels effect significantly ($P < 0.05$) on growth performance, feed utilization, and there interactions between them. From Table 3, the data indicated that the diet (45/12%, protein/lipid) had significant increasing in weight gain and specific growth rate ($P < 0.05$) higher than other dietary groups. However, fish fed by the diet of (40/8, 45/8 and 40/12%, protein/lipid) showed a decrease in growth performance and feed utilization.

Table 3: Effect of various diets on the growth performance of Egyptian sole (*Solea aegyptica*) fishes.

Parameters	Diets (protein/lipid %)							
	(40/8%)	(45/8%)	(50/8%)	(55/8%)	(40/12%)	(45/12%)	(50/12%)	(55/12%)
Initial weight (g/fish)	10.5 ±0.3	10.4 ±0.3	11.0 ±0.3	10.6 ±0.3	10.2 ±0.3	11.0 ±0.3	10.5 ±0.3	10.8 ±0.3
Final weight (g/fish)	35.4 ^c ±1.4	36.2 ^c ±1.2	38.4 ^b ±1.5	38.4 ^b ±1.2	36.5 ^c ±1.1	42.8 ^a ±1.8	38.6 ^b ±1.4	39.1 ^b ±1.2
Total weight gain (g/fish)	24.9 ^c ±1.1	25.8 ^c ±1.4	27.4 ^b ±1.2	27.8 ^b ±1.1	26.3 ^c ±1.4	31.8 ^a ±1.1	28.1 ^b ±1.2	28.3 ^b ±1.4
Average weight gain (g/fish/d)	0.2 ^b ±0.05	0.21 ^b ±0.04	0.23 ^b ±0.05	0.23 ^b ±0.02	0.22 ^b ±0.04	0.35 ^a ±0.02	0.23 ^b ±0.02	0.23 ^b ±0.04
Specific growth rate	1.01 ^b ±0.1	1.04 ^b ±0.1	1.05 ^b ±0.1	1.07 ^b ±0.1	1.06 ^b ±0.1	1.13 ^a ±0.1	1.08 ^b ±0.1	1.07 ^b ±0.1
Condition factor (g/cm ³)	2.04 ±0.4	2.04 ±0.2	2.05 ±0.4	2.05 ±0.2	2.04 ±0.4	2.08 ±0.4	2.05 ±0.2	2.06 ±0.2
Survival rate %	98	98	98	98	98	98	98	98
Feed consumed (g/fish)*	35.0	38.0	35.0	35.0	38.0	36.0	35.0	33.0
Feed conversion ratio	1.41 ^c ±0.2	1.47 ^c ±0.1	1.28 ^b ±0.1	1.26 ^b ±0.1	1.44 ^b ±0.2	1.13 ^a ±0.1	1.25 ^b ±0.2	1.17 ^b ±0.2
Protein efficiency ratio	1.76 ^{ab} ±0.1	1.50 ^c ±0.2	1.55 ^c ±0.1	1.44 ^c ±0.1	1.72 ^{ab} ±0.1	1.96 ^a ±0.2	1.60 ^b ±0.2	1.56 ^c ±0.1
Net protein Utilization (%)	33.84 ^b ±1.1	28.56 ^c ±1.4	29.36 ^c ±1.1	28.11 ^c ±1.2	32.75 ^b ±1.0	37.50 ^a ±1.1	36.26 ^b ±1.2	29.80 ^c ±1.4
Hepatosomatic index (%)	1.10 ±0.1	1.20 ±0.2	1.11 ±0.1	1.10 ±0.1	1.10 ±0.2	1.20 ±0.1	1.10 ±0.2	1.20 ±0.1

Means in the same row with different superscript letters are significantly different ($P < 0.05$).

*Non-consumed portion of feed was collected, dried and deducted from total given ration.

The results in Table (3) and Figs. (1 & 2), indicated that the fish fed diet (45/12%, protein/lipid) recorded the best value (low feed consumed with high gain), lower feed conversion ratio (FCR) compared to other experimental dietary groups. There were a significant differences ($P < 0.05$) in protein efficiency ratio (PER) and net protein utilization (NPU) among experimental groups with increasing dietary protein and lipid levels. However, the lower value was recorded in the fish fed diet with (55/8%, protein/lipid). All dietary protein or lipid levels had no effect on condition factor (CF) and hepatosomatic index (HSI).

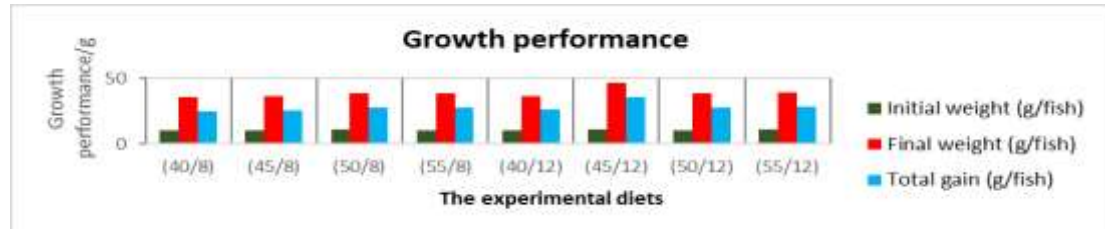


Fig. 1: Growth performance of Egyptian sole fed the experimental diet during 120 days.

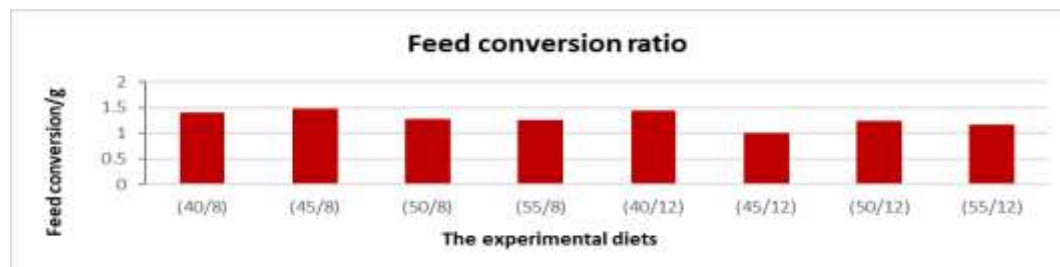


Fig. 2: Feed conversion of Egyptian sole fed the experimental diets during 120 days.

Moreover, no significant different among experimental groups by dietary protein and lipid levels, there was a clear increase of the different digestibility coefficient ratios such as dry matter, crude protein, and crude lipid, and this was evident without significance differences among tested treatments according to ADCs of experimental diets as shown in Table (4). Furthermore, the ADCs of carbohydrate was decreased but not significantly affected by dietary protein and lipid levels.

Data in Table (5) illustrated that constitutes of the Sole juvenile whole body like dry matter, crude protein and ash were not affected by various dietary protein to lipid levels. On the contrary, fish treated with (50/12 and 55/12% protein/lipid) showed a high significance difference ($P < 0.05$) in the crude lipid content in comparison with other tested groups.

Table 4: Apparent digestibility coefficient (ADCs) of the experimental diets (Mean±S.E. n=3).

Parameters	Diets (protein/lipid %)							
	(40/8%)	(45/8%)	(50/8%)	(55/8%)	(40/12%)	(45/12%)	(50/12%)	(55/12%)
Dry mater	92.6±1.2	92.4±1.5	91.8±1.6	92.1±1.3	92.5±1.4	91.5±1.2	91.2±0.5	91.4±1.3
Crude protein	91.2±1.8	91.4±1.4	91.1±1.5	91.5±1.4	91.2±1.2	91.1±1.5	91.6±1.4	91.4±1.5
Crude lipid	93.5±1.4	93.2±1.6	93.4±1.2	93.6±1.4	93.3±1.5	93.1±1.5	93.6±1.2	93.2±1.4
Carbohydrate	55.2±1.2	55.4±1.4	55.2±1.2	55.3±1.4	55.2±1.5	55.5±1.2	55.8±1.4	55.2±1.2

Means in the same row with different superscript letters are significantly different ($P < 0.05$).

Table 5: Proximate analysis of Egyptian sole fed on the experimental diets, Mean±S.E. (% w/w basis).

Parameters	Diets (protein/lipid %)							
	(40/8%)	(45/8%)	(50/8%)	(55/8%)	(40/12%)	(45/12%)	(50/12%)	(55/12%)
Dry mater	28.1 ±1.2	28.2 ±1.4	28.0 ±1.6	28.2 ±1.3	28.8 ±1.4	28.5±1.2	28.7±0.5	28.9 ±1.4
Crude protein	19.2 ±1.4	19.4 ±1.6	19.1 ±1.2	19.5 ±1.1	19.1 ±1.4	19.2±1.2	19.0 ±1.4	19.2 ±1.2
Crude lipid	5.1 ^b ±1.4	5.2 ^b ±1.6	5.1 ^b ±1.2	5.2 ^b ±1.4	6.3 ^a ±1.5	6.1 ^a ±1.5	6.5 ^a ±1.2	6.6 ^a ±1.4
Ash	3.8 ±1.1	3.6 ±1.4	3.8 ±1.2	3.5 ±1.4	3.4 ±1.5	3.2 ±1.2	3.2 ±1.4	3.1 ±1.2

Means in the same row with different superscript letters are significantly different ($P < 0.05$). Initial body composition (% wet weight) was dry matter 27.6%, crude protein 19.4 %, crude fat 4.0 %, and ash 4.2%.

As shown in Table (6), there was a significant ($P < 0.05$) higher lipid content, hepatic glycogen concentrations, and hepatic enzymes related of Juvenile sole fed the different protein/lipid diets. Dietary protein to lipid concentrations had a significant ($P < 0.05$) effect on liver lipid content. The results revealed that hepatic glycogen, liver lipid; AST and ALT concentrations were significantly ($P < 0.05$) increased with the increasing dietary lipid from 8 to 12 %. Further, the results showed that the diets containing 12% lipid, had significant highest ($P < 0.05$) AST and ALT than the rest of experimental groups.

Table 6: Hepatic glycogen, liver lipid, AST and ALT of Egyptian sole fed on the experimental diets, Mean \pm S.E. n=3 (% w/w basis).

Parameters	Diets (protein/lipid %)							
	(40/8%)	(45/8%)	(50/8%)	(55/8%)	(40/12%)	(45/12%)	(50/12%)	(55/12%)
Hepatic glycogen (mg/g)	39.2 ^c \pm 1.4	39.7 ^c \pm 1.8	40.4 ^c \pm 1.6	40.2 ^c \pm 1.4	45.2 ^b \pm 1.2	46.1 ^b \pm 1.8	48.4 ^a \pm 1.5	48.8 ^a \pm 1.4
Lipid of liver (mg/g)	15.4 ^c \pm 1.2	15.2 ^c \pm 1.5	15.5 ^c \pm 1.4	15.1 ^c \pm 1.1	20.2 ^b \pm 1.6	22.4 ^b \pm 1.4	24.5 ^a \pm 1.5	25.0 ^a \pm 1.2
AST (U/mg protein)	6.6 ^c \pm 1.2	6.7 ^c \pm 1.4	6.9 ^c \pm 1.0	6.4 ^c \pm 1.1	14.4 ^b \pm 1.5	14.8 ^b \pm 1.2	17.2 ^a \pm 1.1	17.5 ^a \pm 1.6
ALT (U/mg protein)	7.9 ^c \pm 1.4	7.8 ^c \pm 1.2	7.6 ^c \pm 1.5	8.1 ^c \pm 1.2	16.2 ^b \pm 1.4	17.1 ^b \pm 1.1	20.2 ^a \pm 1.2	20.8 ^a \pm 1.8

Means in the same row with different superscript letters are significantly different ($P < 0.05$).

DISCUSSION

Egyptian sole, *Solea aegyptiaca* juveniles fed the diets containing (45/12%, protein/lipid) showed the highest growth performance values compared with the other feeding groups suggesting a protein sparing by dietary lipid. However, the protein level at 50 and 55% with 12% lipid level, showed a decrease in the performance of Sole fish. The dietary non-protein energy is the main reason for the growth resulting from protein and lipid levels, these results were similar to the results of (McGoogan and Gatlin, 1999; Kim and Lee, 2005; Alam *et al.*, 2008).

Many authors also reported that there is a protein level beyond which further growth is not supported and may even decrease it where energy was speculated to be insufficient (McGoogan and Gatlin, 1999). This might be explained a proportion of protein will be deaminated, and the carbon skeleton used as an energy source at the high dietary protein level. The excreted ammonia nitrogen leads to deterioration of water quality, so elevated levels of a dietary protein that do not enhance production characteristics should be avoided (Azevedo *et al.*, 2002, 2004; El-Sayed and Kawanna, 2008).

The current data were identical with those of (Guerreiro *et al.*, 2012; Borges *et al.*, 2009) in the Senegalese sole, they recoded that the best performance achieved with diets including either 550g kg⁻¹ protein or 450g kg⁻¹ protein, depending on dietary lipid content. In the opposite of these studies Dias *et al.* (2004) who observed no significant effects on growth performance or protein deposition by increasing dietary lipids from 110 g kg⁻¹ to 210 g kg⁻¹ in 510g kg⁻¹ protein diets. A protein

sparing by dietary lipids was also reported in some studies with flatfish (Cho *et al.*, 2005) but not in others (Lee *et al.*, 2000; Martins *et al.*, 2007).

The apparent discrepancy between studies may be related to the dietary protein levels used in many various experiments. The protein requirements may affect by other factors, comprised, fish species, life stage, dietary protein sources, non-protein energy sources and levels, amino acid composition and digestibility of the protein source, experimental design, and culture environmental conditions.

The highest feed utilization from FCR, PER, and NPU were obtained in diet (45/12%) protein to lipid levels, these results were in agreement with the values recorded in the same species (*Solea aegyptiaca*) by (Yones and Abdel-Hakim, 2011; Yones and Metwally, 2014), and Senegalese sole (Guerreiro *et al.*, 2012).

Fish, like other animals, control in their feed intake to meet their energy requirements (Kaushik and Medale, 1994), but excessive energy in the diets is undesirable because it may reduce feed intake, these results in a depression in growth and feed efficiency because a lack of necessary nutrients such as protein for normal growth (Mohanta *et al.*, 2007). In the current study, both dietary protein and lipid levels affected the feed intake, whereas it decreased with treated diet (55/12%, protein/lipid) than that of other dietary diets. These results are similar to other studies (McGoogan and Gatlin 1999; Kim and Lee 2005, 2017; Mohanta *et al.*, 2007).

Apparent digestibility of the tested diets was not affected by different dietary protein and lipid levels. Similar results were reported in juveniles of Senegalese sole fed 540-560 g protein /kg diet (Cabral *et al.*, 2011; Borges *et al.*, 2013). Moreover, other species such as rainbow trout for dry matter, crude lipid, carbohydrate, and crude protein digestibility was significantly different (Gai *et al.*, 2016). However, Sales–Leiton *et al.* (2018) displayed low digestibility values in Senegalese sole. The controversial digestibility values might influenced by the different origin, processing conditions of raw material and the genetic origin of the fish.

Sole, *Senegalese sole* is considered a lean fish with lipid contents, 6% (Dais *et al.*, 2004; Rema *et al.*, 2008), these findings agree with the values reported in the present study. Also, whole-body lipid significantly ($P < 0.05$) increased with increasing dietary lipid level from 8 to 12%, which in accordance with the results in this species (Dias *et al.*, 2004; Borges *et al.*, 2009, 2013; Cabral *et al.*, 2013), and in other flatfish species (Van Ham *et al.*, 2003; Fang *et al.*, 2010; Lv *et al.*, 2015).

In the present study, HSI values ranged from 1.0 to 1.2%, within the ranges were found in the same species (Yones and Abdel-Hakim, 2011; Yones and Metwally, 2014) and in other flatfish species (Hemre *et al.*, 2003; Peres and Oliva-Teles 2005; Martins *et al.*, 2007). HSI was not affected by dietary lipid levels, indicating that the liver was not the main site for body fat storage. However, Dais *et al.* (2004) and Borges *et al.* (2009) observed that the HSI was higher in Senegalese sole fed with high-fat diet.

Hepatic glycogen concentrations, lipids, AST, and ALT in fish liver were also significantly affected by dietary lipid levels. AST and ALT in fish liver were significantly ($P < 0.05$) increased with increasing dietary lipid level. The activities of AST and ALT are useful to evaluate the feeding status in some fish (Moyano *et al.*, 1991; Melo *et al.*, 2006).

High protein level or protein/energy ratio usually causes an increase of liver AST and ALT (Meton *et al.*, 1999; Liu *et al.*, 2013). In this study, the increase of hepatic ALT and AST suggests that 12% lipid level in diets of Sole may affect protein metabolism. Rueda-Jasso *et al.* (2004) also reported that the high dietary lipid affected the oxidative status of Senegalese sole and inhibited the growth rate of fish.

Therefore, more studies are needed to elucidate the optimum dietary lipid level and the metabolism of the Sole in the future.

CONCLUSION

In conclusion, the results of this investigation confirmed a maximal dietary inclusion level of 45% protein and 12% lipid was recommended for both optimal growth and nutrient utilization for Sole juveniles, *Solea aegyptiaca*. In addition, a protein-sparing effect by dietary lipid was recorded.

Future studies are required to better understanding on both nutrient digestibility and metabolic changes induced by dietary protein/lipid ratio should allow adjustment the diet composition to the metabolic efficiency of Egyptian sole (*Solea aegyptiaca*) juveniles.

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ARABIC SUMMARY

تأثير التغذية بمستويات عدة من البروتين والدهن على القياسات الغذائية ووظائف الكبد لأصبعيات أسماك موسى

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معمل تغذية الأسماك - شعبة تربية الأحياء المائية - المعهد القومي لعلوم البحار والمصايد - مصر.

أجريت هذه التجربة لتقييم تأثير استخدام مستويات مختلفة من البروتين والدهن لتحديد المستوى الأمثل منهما في تغذية أسماك موسى ، حيث تم تكوين ٨ علائق تحتوي على أربع مستويات بروتين مختلفة (٤٠ ، ٤٥ ، ٥٠ ، ٥٥ %) مع مستويين من الدهون (٨ ، ١٢ %). أظهرت القياسات الغذائية لأداء الأسماك مثل عائد النمو ومعدل النمو النوعي ومعامل الحالة ومعدل التحويل الغذائي وكفاءة استخدام البروتين نتائج أعلى لمجموعة الأسماك المغذاة على العليقة المحتوية (٤٥% بروتين و ٨% دهن) عن مجموعات الأسماك المغذاة على العلائق الأخرى . أشارت معدلات إستهلاك الغذاء للأسماك انخفاضاً مع إرتفاع مستوى الدهون بالعليقة في حين لم تظهر معاملات الهضم للعلائق المختبرة أى إختلافات معنوية بين مجاميع الأسماك المرياة ولم تظهر أسماك موسى أيضاً أى إختلافات في تركيب جسم الأسماك من (المادة الجافة والبروتين والرماد) ومع ذلك أظهر محتوى الأسماك من الدهون ونشاط إنزيمات الكبد تأثيرات إيجابية مع زيادة مستوى الدهون بالعليقة . أشارت الدراسة الحالية إلى أن العليقة المحتوية على مستوى ٤٥% بروتين و ١٢% دهن مع طاقة كلية ٢٢.٥ ميغا جول/كجم من العليقة كانت المثلى وكان لها فعل توفيري للبروتين عند تغذيتها لأصبعيات أسماك موسى .