Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol.25 (5): 645 – 660 (2021)
www.ejabf.journals.ekb.eg

Effects of different dietary protein and lipids levels on growth performance and digestive enzymes of the rabbitfish (*Siganus rivulatus*), reared in well water

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ARTICLE INFO

Article History:
Received: July 10, 2021
Accepted: Sept. 30, 2021
Online: Oct. 30, 2021

Keywords:
Rabbitfish;
Siganus rivulatus;
Growth;
Digestive enzymes;
Lipid;
Protein.

ABSTRACT

A trial has been evaluated the effects of different levels of dietary protein and lipid on the growth performance, feed utilization, and digestive enzymes of the rabbitfish (*Siganus rivulatus*). Four diets were formulated as follows: diet 1 (P30L6) (protein 30% and lipid 6%), diet 2 (P30L12) (protein 30% and lipid 12%), diet 3 (P40L6) (protein 40% and lipid 6%) and diet 4 (P40L12) (protein 40% and lipid 12%). Diets were fed to the Rabbitfish (*S. rivulatus*) juveniles (0.32 ± 0.16 g /fish) for 56 days. Fish fed on P40L12 and P40L6 diets showed the best growth, followed by P30L6 and P30L12. Better feed utilization was obtained in fish-fed diets with 40% protein compared to 30% protein regardless of the dietary lipid levels. The highest value of protease has been recorded in P40 fish groups with both lipid levels followed by P30L12. Fish groups of P30L6, P30L12, P40L6 to P40L12, respectively, showed an increase in lipase upward. The highest result of amylase was detected in P30L6 followed by P30L6 and P40L6. The highest value of glutamate dehydrogenase was given in P30L6 followed by P30L12. Thus, the P40L6 diet is suitable for the optimal growth performance and digestion functions of the Rabbitfish (*S. rivulatus*) juveniles.

INTRODUCTION

Marbled spinefoot (*Siganus rivulatus*) belongs to the Family Siganidae, commonly known as rabbitfish **(Woodland, 1983)**. Wild *S. rivulatus* is a coastal dweller feeding on various macrophytes **(Anastasiades, 2011)**. Rabbitfish has been found as a potential warm water candidate for marine aquaculture production due to its good taste and being omnivore feeding on artificial diets **(Lam, 1974; Abou-Daoud *et al.*, 2014)**. Thus, it can provide a cheap protein source on a large production scale. Until now, the rabbitfish

aquaculture has not been introduced on a commercial large scale. The main reason for that shortage in mass production of the rabbitfish aquaculture may be attributed to the lack of its nutritional requirements knowledge regarding the growth performance. Therefore, an effective grow-out dietary formulation is highly required to not only support the optimal growth, but also to promote the disease resistance in juvenile fish.

From a nutritional perspective, dietary protein is the most important macronutrient in formulated diets affecting growth, health, reproduction, and physiological performance of farmed fish as well as the production costs **(Kaushik & Seiliez, 2010** and **Lovell, 1998)**. In turn, dietary protein requirements are affected by different factors such as species, fish size, age, water temperature, rearing environment and protein quality, as well as genetic characters and feeding regime of fish **(Halver & Hardy, 2002)**. In specific, the optimal protein level in fish diets is also affected by other dietary factors such as the digestibility efficiency of the protein, its amino acid composition, and the amount of non-protein energy sources **(Halver & Hardy 2002)**.

In parallel, dietary lipid is a main source of energy and essential fatty acids that cannot be *de novo* synthesized by fish **(López *et al*., 2009)**. Lipids are the main source of metabolic energy for growth from an egg to the adulthood **(Mohanta *et al*., 2008)**. Fish, as all animals, have specific energy requirements. If the diet does not contain enough non-protein energy sources, then fish will use the protein for energy to satisfy its maintenance at the expense of its growth **(NRC, 1993)**. In similar, excess dietary protein will be used for energy and not for the production of new tissues resulting in growth retardation and an increase in ammonia excretion **(Mohanta *et al*., 2008)**. Moreover, use of protein as a source of energy leads to an increase in the production cost **(Lee *et al*., 2002)**. Thus, a sufficient dietary lipid level was found to improve the feed utilization and growth rate by sparing the protein **(Watanabe, 1982; Beamish & Medland, 1986** and **Hardy, 1999)**. In other words, adequate levels of dietary lipid can minimize the protein utilization. However, exceeding the optimal level of energy can lead to fat deposition, reduced feed consumption and decreased growth performance in fish **(Martino *et al*., 2002; Lin & Shiau, 2003** and **Chaitanawisuti *et al*., 2011)**. Thus, a protein-to-energy balance in the feed is essential.

Many studies have focused on the importance of determining the optimal ratio of dietary protein to lipid for fishes, such as juvenile Asian seabass, *Lates calcarifer* **(Catacutan & Coloso,1995);** the rabbitfish, *Sigunus guttatus* **(Parazo, 1990)**; the brown trout, *Salmo trutta fario* **(Wang *et al*., 2018)** and the loach, *Misgurnus anguillicaudatus* **(Yan *et al*., 2017**). In particular, two previous studies have been carried out to determine the optimum dietary protein ratio for *Siganus rivulatus* **(El-Dakar *et al*., 2011** and **Abou-Daoud *et al*., 2014)**. They estimated the optimum protein level for the juvenile *S. rivulatus* as 40%. In parallel, only one study **(Ghanawi *et al*., 2011)** has addressed the lipid requirement for *S. rivilatus*, which suggested that the dietary lipid requirement for the optimal growth in juvenile marbled spinefoot is 9.8%. Therefore, it is clearly noticeable

that the optimum protein to lipid ratio rather than the separate protein and lipid levels was not studied yet for the rabbitfish (*S. rivulatus*).

On the other side, digestion and nutrients absorption are the key processes in the efficient use of diets. Nutrient absorption capacity is highly affected by the diet composition **(Buddington *et al*., 1997)**. Consequently, the feed utilization efficiency and growth performance will be also affected **(García-Meilán *et al.*, 2013)**. In some species, digestive enzymes such as protease, amylase and lipase increase with any increase in dietary protein, lipids or carbohydrates **(Buddington & Krogdahl, 2004** and **García-Meilán *et al*., 2013).** For instance, the nutrient absorption capacity is modified in response to the inclusion of high levels of plant protein sources **(Santigosa *et al*., 2011)**. Thus, digestive enzymatic activities often reflect the feeding habits of teleosts **(German *et al*., 2010)**. However, less is known about how the digestive and absorptive processes interact with the dietary modifications in *S. rivulatus*. In our knowledge, no studies have been carried out to assess the effect of different protein to lipid ratios on digestive and digestive absorptive processes of the rabbitfish (*S. rivulatus*).

Accordingly, the purpose of this study was to determine the optimal protein to lipid ratio and its effects on growth performance, feed utilization, digestive enzymes and the body composition of the rabbitfish (*S. rivulatus*) juveniles.

MATERIALS AND METHODS

1. Experimental diets:

Four semi-purified diets were formulated to contain two crude protein levels, 30% and 40%; and each with two crude lipid levels, 6% and 12% **(Table 1)**. All dry ingredients were finely ground (<250 μm) using a laboratory mill and combined with the other ingredients and micronutrients (vitamins and minerals) prior to mixing. Then, the ingredients were thoroughly mixed together, and then, the lipid was added and thoroughly mixed. The diet mixtures were then extruded into pellets with an appropriate size by a meat grinder (Tornado, MG-2000). All diets were dried in an electric oven at 60°C for 24 hours, and then stored in plastic bags at −20°C until use. The fish were fed the diets until apparent satiation 5 times a day.

2. Experimental fish:

Rabbitfish (*S. rivulatus*) juveniles were obtained from the coast of Mediterranean Sea, Alexandria, Egypt. The Rabbitfish juveniles were maintained at the Fish Nutrition Laboratory at the National Institute of Oceanography and Fisheries (NIOF). The experimental system consisted of 12 hapas (50 × 50 × 70 cm; L × W × H). Each three hapas placed in one concrete tank (5.0 × 2.0 × 1.5 m) at least 1 m apart. Filtered well water (salinity 40‰) was provided to each tank with a continuous aeration. The water quality parameters were monitored every 2 days throughout the experimental period.

Water temperature, salinity, dissolved oxygen, ammonia-N, pH were maintained at 26 ± 2.3°C, 40‰, 6 mgL^{-1}, 0.5 mgL^{-1} and 7.8, respectively. The fish were acclimatized to the hapas for 2 weeks, and they were fed the control diet as an adaptation period prior to the feeding on the experimental diets.

A total of 600 larvae (average weight: 0.32 ± 0.16 g /fish) were randomly allocated into 12 hapas at a density of 50 fish per hapa. Each hapa was then randomly assigned to one of three replicates of the four dietary treatments. Faeces were removed after feeding by siphoning. The amount of feed consumed in each treatment was calculated weekly by the difference in the weight of the food containers before and after feeding. The experiment was subjected to natural light conditions.

3. Productive performance parameters:

All fish were bulk weighed and measured individually at the beginning, every two weeks, and the end of the experimental period. Survival was measured by the percentage of the final number of fish divided by the initial number of fish. Growth performance and feed utilization were calculated as the following equations:

- $\textbf{Weight gain rate (WGR \%)} = \frac{\textbf{(final weight − initial weight)}}{\textbf{initial weight}} \times \textbf{100}$
- $\textbf{Specific growth rate (SGR) (\%/ day)} = \frac{\textbf{ln (final weight) − ln (initial weight)}}{\textbf{56 days}} \times \textbf{100}$
- $\textbf{Daily growth index (DGI)} = \textbf{100} \times \frac{\textbf{final weight}^{\frac{1}{3}} - \textbf{initial weight}^{\frac{1}{3}}}{\textbf{56 days}}$
- $\textbf{Feed efficiency (FE) \%} = \textbf{100} \times \frac{\textbf{body weight gain (g)}}{\textbf{feed intake (g)}}$
- $\textbf{Feed conversion ratio (FCR)} = \frac{\textbf{total dry feed intake (g)}}{\textbf{total fish weight gain (g)}}$
- $\textbf{Protein efficiency ratio (PER)} = \frac{\textbf{fish weight gain (g)}}{\textbf{total dry protein intake (g)}}$
- $\textbf{Protein retention (PR) (\% of intake)} = \frac{\textbf{protein gain (g /kg weight gain)}}{\textbf{protein intake (g /kg weight gain)}} \times \textbf{100}$

4. Sample collection:

At the beginning of the trial, 50 fish were randomly collected and frozen at -20°C for further initial whole-body composition analysis. At the end of the feeding trial, the fish were starved for 24 h, and then survival and mass weight per replicate were recorded in all hapas. Twenty fish per hapa were sampled for further analyses. All samples were frozen immediately and stored at -20 °C until being analyzed.

Table (1): Composition and proximate analysis (%) of the experimental diets fed to *S. rivulatus*

Feed ingredients (%)	Diets			
	P30L6	**P30L12**	**P40L6**	**P40L12**
Fishmeal[1]	8.5	8.5	28.7	28.7
Maize gluten[2]	9.2	9.2	11.0	11.0
Soybean meal[3]	31.5	31.5	26.7	26.7
Wheat meal[4]	25.0	25.0	8.0	8.0
Maize starch	10.0	3.6	10.0	4.0
Ulva sp. powder	6.0	6.0	6.0	6.0
Brewer's yeast[5]	2.0	2.0	2.0	2.0
Sunflower oil	4.40	10.8	4.2	10.2
Vitamin and Mineral mix[6]	1.0	1.0	1.0	1.0
Gelatin as a binder	1.0	1.0	1.0	1.0
$Ca(H_2PO_4)_2$	0.8	0.8	0.8	0.8
Essential amino acid mixture[7]	0.36	0.36	0.36	0.36
Choline chloride	0.12	0.12	0.12	0.12
Betaine	0.1	0.1	0.1	0.1
Vitamin C	0.02	0.02	0.02	0.02
Proximate composition (%)				
Crude protein	30.10	30.56	41.03	40.65
Ether extract	6.34	12.48	6.84	13.04
Protein lipid ratio	4.75	2.45	6.00	3.12
Ash	5.23	5.08	6.73	6.67
Moisture	11.38	12.81	10.32	12.78
Carbohydrate	58.33	51.88	45.40	39.64
Gross energy (MJ kg^{-1})[8]	19.34	20.68	19.83	21.11
P/E ratio (g MJ^{-1})[9]	15.56	14.78	20.69	19.25

[1] Fish meal: crude protein 671.0 g kg^{-1}, crude lipid 50.6 g kg^{-1};

[2] corn gluten: crude protein 607.1 g kg^{-1}, crude lipid 22.0 g kg^{-1};

[3] soybean meal: crude protein 483.1 g kg^{-1}, crude lipid 9.4 g kg^{-1};

[4] wheat meal: crude protein 151.2 g kg^{-1}, crude lipid 31.2 g kg^{-1};

[5] Brewer's yeast: crude protein 438.6 g kg^{-1}, crude lipid 50.1 g kg^{-1};

[6] Vitamin and Mineral mix : VA, 15000 IU; VC, 1000 mg; VD3, 2500 IU; VK3, 50 mg; VB1, 50 mg; VB2, 20 mg; VB6, 30 mg; VB12, 0.5 mg; VE, 300 mg; Niacin, 260 mg; Calcium pantothenate, 150 mg; Folacin 20 mg; Biotin, 2.5 mg; Inositol, 100 mg. Cu, 8; Zn, 250; Mn, 45; Fe, 100; I, 2.4; Co, 2; Mg, 4;

[7] Contained: L-methonine, 0.1%; L-lysine, 0.2%; L-valine, 0.06%;

[8] Calculated using gross and digestible energy values of 23.01, 38.05 and 17.15 kJg^{-1}; 16.84, 33.47 and 10.46 kJg^{-1} for protein, fat and carbohydrate, respectively;

[9] P/E: protein/energy (mg crude protein/kJ gross energy) = CP/ GE × 1,000.

5. Biochemical analysis:

Moisture, ash, crude protein, and lipid contents of all samples were analyzed according to **AOAC (2009)** methods. Crude protein was determined by the Kjeldahl method using an auto Kjeldahl System (K358/355, BUCHI, Flawil, Switzerland). Crude

lipid was determined by the ether extraction method using a Soxlet System (VELP Scientific a, SER 248, Italy). Moisture was determined in an oven at 105°C for 24 hr. Ash was determined using a muffle furnace at 600°C for 6 hr.

6. Analysis of the digestive enzymes activities:

Tissue samples from foregut, midgut and hindgut were homogenized in 4 volumes of ice-cold physiological saline (0.85% w/v NaCl). Homogenates were centrifuged at 3,500 rpm for 15 min at 4℃, and the resulting supernatants were aliquoted and stored at -80 ℃ until subsequent analysis. Amylase activity was assayed by SIGMA-ALDRICH kit (Catalog number MAK009). Amylase activity was determined using a coupled enzymatic assay, which resulted in a colorimetric (405 nm) product, proportional to the amount of the substrate, ethylidenepNP- G7, cleaved by the amylase. One unit was the amount of amylase that cleaved ethylidene-pNP-G7 to generate 1.0 mmole of p-nitrophenol per minute at 25 °C. Glutamate Dehydrogenase (GDH) activity was assayed by SIGMA-ALDRICH kit (Catalog number MAK099). GDH activity was determined by a coupled enzyme assay in which glutamate was consumed by GDH generating NADH, which reacted with a probe generating a colorimetric (450 nm) product proportional to the GDH activity. One unit of GDH was the amount of enzyme that generated 1.0 mmole of NADH per minute at pH 7.6 and 37°C. Lipase activity was assayed by SIGMA-ALDRICH kit (Catalog number MAK046). Lipase activity was determined using a coupled enzyme reaction, which resulted in a colorimetric (570 nm) product proportional to the enzymatic activity. One unit of lipase is the amount of enzyme that generated 1.0 µmole of glycerol from triglycerides per minute at 37 ℃. Protease was assayed by SIGMA-ALDRICH kit (Catalog number PF0100). This kit used a modification of the published procedure **(Twining, 1984)**. Protease activity was detected using casein labeled with fluorescein isothiocyanate (FITC) as the substrate. Protease activity resulted in the cleavage of the FITC-labeled casein substrate into smaller fragments, which did not precipitate under acidic conditions. After incubation of the protease sample and substrate, the reaction was acidified with the addition of trichloroacetic acid (TCA). The mixture was then centrifuged with the undigested substrate, forming a pellet and the smaller acid-soluble fragments remaining in solution. The supernatant was neutralized, and the fluorescence of the FITC-labeled fragments was measured.

7. Statistical analysis:

The experiment was consisted of four treatments arranged in a 2×2 factorial design (2 protein and 2 lipid levels) with three replicates. Data were subjected to two-way analysis of variance (ANOVA), followed by a comparison of means (Tukey's HSD test) to test the effects of the dietary protein and lipid levels on fish performance. $P < 0.05$ was regarded as statistically significant. If significant ($p<0.05$) differences were found in factors, Duncan's multiple range test (Duncan, 1955) was used to rank the means. All statistics were processed using the SPSS package (version 23.0).

RESULTS

1. Growth performance:

Dietary protein and lipid levels either individually or in a combination had significant effects on final weight (FW), weight gain ratio (WGR), daily gain index (DGI) and specific growth rate (SGR) of fish **(Table 2)**. Fish fed on P40L12 and P40L6 diets gave the best FW, WGR, DGI and SGR, followed by P30L6 and P30L12. With respect to dietary protein and lipid levels as single factors, WG, FW, WGR, DGI and SGR were higher in fish fed diets with protein level 40% compared to fish fed diets with 30%, and they were not significantly different in fish fed diets with 6% or 12% lipid levels.

2. Nutrients utilization

The effects of the experimental diets on feed utilization of *S. rivulatus* are shown in **Table (3)**. The best feed conversion ratio (FCR), Feed efficiency (FE), protein retention (PR) and protein efficiency ratio (PER) were found in fish fed the P40L6 and P40L12 diets, while P30L6 and P30L12 gave the worst results of those parameters. With respect to dietary protein and lipid levels as single factors, the best results of FCR, FE, PR, PER and EG were obtained in fish fed the diets with 40% protein compared to fish fed the diets with 30% protein. However, FCR, FE, PER, PR and EG were not affected by dietary lipid levels.

Table (2): Effects of dietary protein and lipid levels on growth performance and survival of *S. rivulatus*

Diet		Growth performances					
Protein	Lipid	Survival (%)	IW (g)	FW (g)	WGR (%)	DGI (%)	SGR (% day^{-1})
Double factor							
P30	L6	80.0±0.12^{b}	0.33±0.02^{a}	1.55±0.07^{b}	375.7±0.16^{b}	0.73±0.04^{b}	2.79±0.01^{b}
	L12	83.3±3.34^{b}	0.33±0.01^{a}	1.85±0.06^{b}	461.8±34.60^{b}	0.91±0.05^{b}	3.08±0.11^{b}
P40	L6	96.7±3.33^{a}	0.32±0.01^{a}	2.57±0.01^{a}	713.5±14.42^{a}	1.34±0.01^{a}	3.74±0.03^{a}
	L12	96.7±2.34^{a}	0.30±0.00^{a}	2.31±0.01^{a}	666.5±6.97^{a}	1.20±0.01^{a}	3.64±0.01^{a}
Single factor							
P30		81.7±1.67^{b}	0.33±0.01^{a}	1.70±0.09^{b}	418.7±28.59^{b}	0.82±0.06^{b}	2.93±0.10^{a}
P40		96.7±1.93^{a}	0.31±0.01^{a}	2.45±0.08^{a}	690.1±15.05^{a}	1.27±0.04^{a}	3.69±0.03^{a}
	L6	88.3±5.00^{a}	0.32±0.01^{a}	2.06±0.29^{a}	544.6±97.72^{a}	1.03±0.18^{a}	3.26±0.28^{a}
	L12	90.0±4.30^{a}	0.32±0.01^{a}	2.08±0.13^{a}	564.2±60.86^{a}	1.05±0.09^{a}	3.36±0.17^{a}

Note: Means in the same column bearing different superscripts differ significantly at 0.05 levels. Values are means ± SD.

Table (3): Effects of dietary protein and lipid levels on nutrient utilization of *S. rivulatus*

Diet		Nutrient utilization				
Protein	**Lipid**	**FCR**	**FE (%)**	**PER (g)**	**PR (%)**	**EG (Kcal)**
Double factor						
P30	**L6**	2.16±0.04^{a}	46.34±0.89^{b}	1.54±0.03^{b}	51.72±1.36^{c}	1.52±0.07^{b}
	L12	1.98±0.05^{b}	50.45±1.27^{b}	1.65±0.04^{a}	60.86±3.64^{b}	1.56±0.11^{b}
P40	**L6**	1.73±0.02^{c}	57.84±0.47^{a}	1.41±0.01^{c}	79.33±2.65^{a}	3.19±0.11^{a}
	L12	1.78±0.03^{c}	56.26±0.63^{a}	1.39±0.02^{c}	74.58±3.32^{a}	2.99±0.16^{a}
Single factor						
P30		2.07±0.06^{a}	48.39±1.34^{b}	1.60±0.04^{a}	56.29±3.08^{b}	1.54±0.05 ^{b}
P40		1.75±0.02^{b}	57.05±0.56^{a}	1.40±0.01^{b}	76.96±2.21^{a}	3.09±0.09^{a}
	L6	1.94±0.13^{a}	52.09±3.34^{a}	1.48±0.04^{a}	65.52±8.07^{a}	2.35±0.49^{a}
	L12	1.88±0.06^{a}	53.36±1.78^{a}	1.52±0.08^{a}	67.72±4.44^{a}	2.28±0.42^{a}

Note: Means in the same column bearing different superscripts differ significantly at 0.05 levels. Values are means ± SD.

3. Carcass chemical composition

There was a significant interaction between the dietary protein and lipid levels on crude protein, lipid contents and gross energy **(Table 4)**. Results showed that crude protein, crude lipid contents and gross energy of fish were influenced by dietary protein levels but not by lipid levels (P>0.05). The highest values of CP, CL and GE were recorded at P40 regardless the lipid level. On the other hand, there was no significant interaction between the dietary protein and lipid levels on moisture and ash contents.

4. Fish digestive enzymes

The dietary levels of protein and lipids have been well reflected on the digestive enzymes of fish **(Table 5)**. The highest value of protease has been recorded at protein level P40 regardless the lipid level followed by P30L12 treatment, while the lowest value has been obtained at P30L6. Fish fed on P30L6, P30L12, P40L6 to P40L12 showed an increase in lipase upward, respectively. The highest result of amylase has been detected in P30L6 followed by P30L12 and P40L6 at the same level, while the lowest value has been given at P40L12. The highest value of glutamate dehydrogenase has been given at P30L6 followed by P30L12 and then P40, regardless the lipid level.

TABLE (4): Effects of dietary protein and lipid levels on whole-body composition of *S. rivulatus* (% wet weight)

Diet		Body composition (%)				
Protein	Lipid	Moisture	Crude protein	Ether extract	Gross energy (KJ/g)	Ash
Double factor						
P30	**L6**	79.34±0.07 [a]	12.59±0.09[b]	4.06±0.15[c]	4.57±0.08[b]	4.13±0.01 [a]
	L12	75.85±1.54 [a]	13.09±0.77[b]	5.74±0.46[b]	5.35±0.36[ab]	5.17±0.26 [a]
P40	**L6**	74.63±0.37 [a]	14.46±0.35[a]	6.39±0.18[a]	5.93±0.15[a]	4.09±0.07 [a]
	L12	74.72±0.34 [a]	15.00±0.53[a]	6.73±0.16[a]	6.19±0.19[a]	3.55±0.12 [a]
Single factor						
P30		77.59±1.19 [a]	12.84±0.35[b]	4.90±0.52[b]	4.96±0.27[b]	4.65±0.32 [a]
P40		74.68±0.21 [a]	14.73±0.30[a]	6.56±0.14[a]	6.06±0.12 [a]	3.82±0.16 [a]
	L6	77.03±1.34 [a]	13.79±0.73[a]	5.39±0.78[b]	5.38±0.48 [a]	3.84±0.17 [a]
	L12	75.24±0.74 [a]	13.77±0.52[a]	6.06±0.27[a]	5.64±0.23 [a]	4.63±0.33 [a]

Note: Means in the same column bearing different superscripts differ significantly at 0.05 levels. Values are means ± SD.

Table (5): Effects of dietary protein and lipid levels on digestive enzymes of S. *rivulatus*

Diet		Digestive enzymes			
Protein	Lipid	Protease (U mg^{-1}prot)	Lipase (U mg^{-1}prot)	Amylase (U mg^{-1}prot)	Glutamate dehydrogenase (U mg^{-1}prot)
Double factor					
P30	**L6**	28.00±3.00[c]	9.21±0.67[d]	0.62±0.03[a]	11.98±0.07[a]
	L12	45.00±3.00[b]	13.31±1.05[c]	0.46±0.02[b]	10.50±0.10[b]
P40	**L6**	90.00±2.00[a]	12.22±0.64[b]	0.45±0.01[b]	9.16±0.10[c]
	L12	92.50±2.50[a]	16.93±0.19[a]	0.34±0.05[c]	8.90±0.08[c]
Single factor					
P30		36.50±5.20[b]	11.26±1.03[b]	0.54±0.02[a]	11.24±0.83[a]
P40		91.25±1.49[a]	14.57±0.83[a]	0.40±0.04[b]	9.03±0.28[b]
	L6	59.00±7.96[b]	10.71±1.82[b]	0.53±0.02[a]	10.57±0.61[a]
	L12	68.75±3.80[a]	15.12±1.23[a]	0.39±0.03[b]	9.70±0.08[a]

Note: Means in the same column bearing different superscripts differ significantly at 0.05 levels. Values are means ± SD.

DISCUSSION

In the present study, the results showed that growth performance of fish significantly increased with an increase of the dietary protein level, regardless of the lipid level. The protein requirement for the best growth performance of the rabbitfish has been found at 40%. This protein requirement is in line with the previously reported protein level for *Siganus rivulatus* **(El-Dakar *et al*., 2011** and **Abou-Daoud *et al*., 2014).** However, the dietary protein requirement determined in this study for *S. rivulatus* is higher than those values reported for another rabbitfish species *S. guttutus* fry, which were 25-35%, **(Soletchnik, 1984** and **Parazo, 1990).** It is evident therefore that difference in protein requirements among *Siganus* species is logical and acceptable, which emphasizes a fact that says 'a protein requirement is species specific'. From another perspective, this high dietary protein requirement in the current study, comparing to other omnivore species, might be attributable to their feeding habits in that early life stage. It has been indicated that as fish increases in size and age, their protein requirement tends to decrease **(Halver & Hardy, 2002).** In the current study, fish fed on diets containing 30% protein gave lower growth performance and feed utilization efficiency values. This indicated that the dietary protein did not meet *Siganus* requirements at this early stage, which might be due to the insufficient amount of essential amino acids available to the fish **(Coutinho *et al*., 2016).** Indeed, fish at early stage have high requirements for protein to be broken down into amino acids, which are then used mainly for the rapid growth and as an energy source **(Conceicao *et al*., 1997).** In support, it was found that the optimal protein:energy ratio for the *Siganus canaliculatus* fingerlings was lower than that of the fry group. This may confirm that higher protein requirements for early development of fry decrease with increasing fish size **(Yousif *et al*., 1996).**

FCR and PER are known to decrease with an increase of dietary protein content **(Jauncey, 1982).** In the present study, FCR was significantly decreased by increasing dietary protein levels, regardless the dietary lipid level. However, at protein level 30%, it showed a tendency to decrease by increasing the lipid level from 6 to 12%, indicating that the low level of dietary lipid (6%) could meet the best feed conversion ratio needs of *Siganus rivulatus* at a protein level 40%. This is supported by **El-Dakar *et al*. (2011).** In the present study, the PER decreased with the increase in the dietary protein level, which is consistent with other reports **(Shapawi *et al*., 2013).** The decreased FCR and PER indicated that diets contained higher protein level in the present study were better utilized in *Siganus*.

Dietary protein impacts fish survival **(Arredondo-Figueroa *et al*., 2012).** In fact, after the eight-weeks experiment, fish fed on diets containing higher protein contents (P40L6 and P40L12) also showed a higher survival rate. This was parallel to the growth rate results, indicating that a 40% protein level was sufficient for the performance of *S. rivulatus*. This is in agreement with **Aliyu-Paiko *et al*. (2010)** who indicated that a lipid:

protein ratio of 6:45 gave the best survival rate in Snakehead (*Channa striatus*), but other diets with lower protein content produced a lower survival rate.

Furthermore, in the present study, the level of dietary lipid did not exhibit a significant effect on the growth performance or feed utilization of fish. This indicates that the low level of dietary lipid (6%) could meet the growth needs of *Siganus rivulatus* with protein level 40%. The lipid requirement recorded in the current study may be lower than that recorded for *S. rivulatus* in another study, which was 9.8% **(Ghanawi *et al*., 2011).** This may be attributed to the lower dietary protein level (30%) used in **Ghanawi *et al*. (2011)** which requires more lipid content to maintain the energy required for fish.

On the other side, this result is consistent with recorded lipid levels for other omnivorous fish species, such as 5.5–8.5% for hybrid tilapia (*Oreochromis niloticus x O. aureus*) **(Han *et al*., 2011**), 6 % For Nile tilapia (*Oreochromis niloticus*) **(Abdel-Ghany, 2017),** and 5% for Ussuri catfish (*Pseudobagrus ussuriensis*) fingerlings **(Wang *et al*., 2011)**. Many studies have pointed out that increasing dietary lipid or energy levels has a protein-saving effect on fish **(Luo *et al*., 2005** and **Chatzifotis *et al*., 2010).** However, this effect is not observed in the present study. Similarly, the protein-sparing effect of dietary lipid was not observed in previous studies **(Wang *et al*., 2005** and **Wang *et al*., 2017).** This discrepancy in results may be due to the differences in fish life stages, fish species, feed composition and feeding strategies **(Yan *et al*., 2015).**

Although the body composition of *S. rivulatus* was found to be dependent upon the dietary treatments in the current study; however, whole-body moisture was not correlated to the dietary protein level. This is in parallel with the result obtained by **Kim & Lee (2009).** In the current study, the protein content increased with the increase of dietary protein-to-energy ratio. Previous study reported similar trend **(Yousif *et al*., 1996)**. In the present study, the muscle lipid content in marbled spinefoot increased with increasing the dietary lipid levels, reflecting the tendency for some lipid deposition in the muscle. These results are similar to those reported by other authors **(Lee *et al*., 2002; Wang *et al*., 2005** and **Sandre *et al*., 2017)**. These results suggest that the marbled spinefoot used the surplus lipid to enhance the fat deposition rather than metabolizing energy to spare the protein. Although some authors indicated that the major site for fat and glycogen deposition in some fishes is the liver **(Hemre *et al*., 2002).** In the present study, liver did not contribute significantly to the lipid deposition in the marbled spinefoot, which is similar to results obtained by **Ghanawi *et al*. (2011).**

Digestive enzymes such as protease, amylase and lipase correlate positively with any increase in dietary protein, lipid and carbohydrates **(Buddington & Krogdahl, 2004; Zambonino *et al*., 2007** and **García-Meilán *et al*. 2013).** Following the same pattern, a difference in the protease activity among the different treatments was detected in the current study. The total protease activity progressively increased as the diet protein content increased from 30 to 40 %. This is in agreement with **García-Meilán *et al*. (2013)**

who found a progressive increase in the total protease activity in the sea bream (*Sparus aurata*) alimentary canal as the content of diet protein increased from 35 to 41%. As well, the lipase activity responded to the dietary lipid levels in the present study. Apparently, diets containing less crude lipid level, induced the lowest lipase activity throughout the alimentary tract **(Mohanta *et al.*, 2008** and **Dizhi *et al.*, 2018)**. In this study, the carbohydrate contents were varied among the different diets. This variation in carbohydrate content may be attributed to the different levels of starch included in each diet. Overall, a positive correlation between amylase activity and diets carbohydrate content was reported in the current study, which is supported by **Li *et al.* (2016).**

Glutamate dehydrogenase (GDH) is considered as a significant marker/regulator for nutrients utilization, especially protein utilization **(Rønnestad *et al.*, 1999).** Therefore, GDH could be a useful indicator of the metabolic utilization of dietary components by fish **(Rønnestad *et al.*, 1999).** Furthermore, the increased GDH activity is probably associated with a high-protein feeding **(Sanchez-Muros *et al.*, 1998)**. On the contrary, in the present study, the high level of protein level was accompanied with the lowest level of GDH and vice versa. These results were confirmed by **Cowey & Walton (1989)** who revealed that usually after long-term feeding with a low protein diet, the GDH expressions increased to induce protein metabolism and meet the compensatory growth.

The overall results in this study indicated that dietary protein and lipid levels at 40% and at 6%, respectively, gave the best growth rate and feed utilization efficiency. Increasing the level of lipid in the rabbitfish (*S. rivulatus*) juveniles diet to 12% does not differ significantly from the lower level.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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