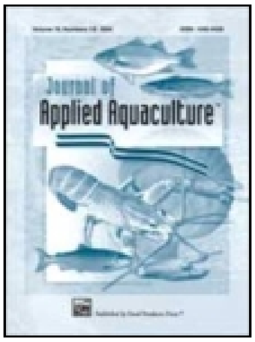

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Effect of Dietary Protein Level on Growth Performance and Blood Parameters of Marbled Spinefoot *Siganus rivulatus*

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*The present study evaluated the effect of dietary protein levels on growth performance and blood parameters of juvenile marbled spinefoot (*Siganus rivulatus*). Five semipurified, isoenergetic (14.2 MJ digestible energy/kg; 84 g lipids/kg) diets were formulated to contain 30, 35, 40, 45, and 50 g crude protein (CP)/100 g diet. Groups of 15 juvenile fish (7.4 g individual weight) were randomly assigned in triplicate to each of the five treatments and offered the test diets three times daily ad libitum for 9 weeks. Total erythrocyte and differential leukocyte counts, hematocrit, hemoglobin, blood glucose, protein, and serum aspartate aminotransferase and alanine aminotransferase were evaluated. Survival was greater than 95% in all treatments. Maximum growth was observed in fish offered the diet containing 40 g CP/100 g diet crude protein. Differences in dietary protein level had no effect on the hepatosomatic and viscerosomatic indices of the fish, but whole body composition was influenced by diet. Blood parameters showed no physiological or metabolic dysfunctions among fish offered any of the five diets. Findings of the present work strongly suggest that diets containing 40 g CP/100 g and 14.2 MJ digestible energy/kg are suitable for good growth of juvenile spinefoot rabbitfish* Siganus rivulatus *and do not affect hematological parameters.*

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KEYWORDS Rabbitfish, protein requirement, hematology

INTRODUCTION

Marbled spinefoot rabbitfish *Siganus rivulatus* is a potential candidate for warm water marine aquaculture diversification. It is an herbivorous, euryhaline, eurythermal, schooling species with commercial importance in the Eastern Mediterranean and East Indian Ocean regions (Lam 1974; Saoud et al. 2007, 2008; Woodland 1983). Currently, the aquaculture of marbled spinefoot is in its infancy, with limited production in offshore cages in Cyprus and Saudi Arabia. Development of the marbled spinefoot industry is partly restricted by a lack of knowledge of the nutritional requirements of the species. The commercial farm growing it in Cyprus offers sea bass feed, and the result is very fatty rabbitfish that is not readily accepted by consumers.

Dietary protein is the most important macronutrient in formulated feeds, as it is the most significant factor affecting growth, health, reproduction, and physiological well-being of farmed fish as well as production costs (Catacutan and Coloso 1995; Kaushik and Seiliez 2010; Lazo et al. 1998; Lovell 1998). Dietary protein requirements are influenced by species, age, fish size, rearing environment, water temperature and quality, as well as the genetic predisposition and feeding regimen of the fish (Halver and Hardy 2002; NRC 1993). The optimal protein level in a fish feed is also affected by factors in the formulated feed such as digestibility of the protein, its amino acid composition, and the amount of nonprotein energy sources (Halver and Hardy 2002). Protein requirements as a proportion of the diet are generally thought to be less for noncarnivorous fish than they are for carnivorous fish (Cowey 1979; Wee and Tacon 1982). The natural diet of marbled spinefoot rabbitfish consists of selective grazing of available macrophytes where the choice of the macrophyte species is influenced by its seasonal availability and fish size (Lundberg and Lipkin 1993). Although both juveniles and adults are primarily herbivorous, they exhibit omnivorous feeding habits in captivity, and they consume a variety of food items (El-Dakar et al. 2011).

Protein requirements of marbled spinefoot are not well studied, but a few studies on other rabbitfish species have been published. Parazo (1990) evaluated the protein requirement for *Siganus guttatus* and found that a diet containing 35 g CP/100 g diet was optimal. Tacon et al. (1990), Bwathondi (1982), and Ismail et al. (1986) reported that a diet containing 31 g CP/100 g diet was optimal for the growth of *Siganus canaliculatus*. El-Dakar et al. (2011) reported that the minimum dietary protein requirement for *S. rivulatus* was 40 g CP/100 g diet. However, protein content in the experimental feeds of El-Dakar et al. (2011) varied by 10 g CP/100 g diet from one feed to the next, which makes it difficult to precisely pinpoint dietary protein requirements. Additionally, El-Dakar et al. (2011) fed their fish to apparent satiation, so theoretically fish offered low-protein diets were offered a bit less protein than those offered a high-protein diet. Protein requirements for marbled spinefoot need to be better refined, and requirements under various culture protocols need to be evaluated if we are to develop a specialized diet for the species.

In addition to affecting growth, nutritional factors influence health and affect hematology of fish (Sampath and Pandian 1984). An ideal dietary protein level should ensure maximum growth of fish and also maintain good health. Dietary protein is necessary for maintenance of physiological functions such as oxygen transport, acid-base balance, metabolic adjustments, and immunochemical defense mechanisms (Oprea and Georgescu 2000), all of which affect hematological parameters. Thus, general hematological analyses could act as a diagnostic tool to assess physiological or metabolic dysfunctions caused by malnutrition or nutritional deficiency. Well-balanced nutrition for a specific fish should result in "normal" values of the main hematological indices (Misăilă 1998). There are no published studies on the effects of nutrition and dietary protein content on hematological indices of rabbitfish, yet such information would be very important in assessing the nutritional suitability of diets for the fish. The present study was performed to refine values of the optimal dietary protein requirement for juvenile marbled spinefoot and to evaluate effects of dietary protein on blood measures of fish health.

MATERIALS AND METHODS

The present work was performed at the aquaculture research laboratory of the American University of Beirut (AUB). Five semipurified isoenergetic experimental diets containing 30, 35, 40, 45, and 50 g/100 g crude protein were prepared (Table 1). Ingredients were mixed with hot water using a dough mixer and extruded in a meat grinder using a 3 mm die. Pellets were dried in a forced-air oven at 40°C to a moisture content of 8%. The dried pellets were then ground to a suitable size, packed in airtight bags, sealed and stored at –20°C.

Experimental Design

Juvenile rabbitfish, *S. rivulatus*, were caught in traps off the Beirut beach and transported to AUB. The fish were quarantined and trained to accept artificial feed (Rangen EXTR 350, Rangen Inc., Buhl, ID, USA) for 10 days in a recirculating system composed of six 1-m cube circular tanks. Fish were then sorted by hand to a uniform size and stocked in a recirculating system comprised of fifteen 180 L (60 × 60 × 50 cm; L × W × H) fiberglass tanks connected to a biological filter, a water pump, and a sump tank, all filled

TABLE 1 Ingredients and chemical composition of the experimental diets offered to juvenile marbled spinefoot (*Siganus rivulatus*) for 9 weeks.

	Dietary Crude Protein Level (%)				
Ingredients	30	35	40	45	50
Fish meal[1] (g/100 g diet)	41.7	48.7	55.8	62.8	69.8
Cod liver oil[2] (g/100 g diet)	3.4	2.6	1.7	0.9	0.0
Vit& Min[3] (g/100 g diet)	2.5	2.5	2.5	2.5	2.5
Corn Starch (g/100 g diet)	30.9	24.8	18.9	12.9	7.0
Vit C[4] (g/100 g diet)	0.1	0.1	0.1	0.1	0.1
Cellufill[5] (g/100 g diet)	19.4	19.3	19.0	18.8	18.6
D-L Methionine (g/100 g diet)	0.1	0.1	0.1	0.1	0.1
Gelatin[6] (g/100 g diet)	2.0	2.0	2.0	2.0	2.0
Chemical Composition (g/100 g in dry matter)					
Crude Protein	30.4	34.7	39.8	45.6	49.2
Digestible energy (MJ/kg)[†]	14.2	14.2	14.2	14.2	14.2
Crude Lipids (g/100 g)[†]	8.4	8.4	8.4	8.4	8.4
Fibers (g/100 g)	19.8	19.5	19.4	19.3	19.8

[†]Based on a calculated value.
[1]FF Skagen Denmark. Havnevagtvej 12.9990 Skagen.
[2]Seven Seas LTD., Great Britain. Mar Fleet, Hull, England HU9 5NJ.
[3]The vitamin and mineral premix provided the following per kg of experimental diet: vitamin A retinyl acetate 1 million IU, vitamin D3 cholecalciferol 0.1 million IU, vitamin E alpha-tocoph acet 7 g, vitamin K 0.5 g, folic acid niacin 0. 1 g, niacin 4 g, calcium pantothenate 2.5 g, riboflavin (B2) 0.6 g, vitamin B12 0.001 g, thiamine (B1 nitrate) 0.5 g, pyridoxine (B6 HCl) 0.5 g, biotin 0.0125 g, vitamin C (ascorbic acid) 0.25 g, inositol 5 g, selenium (as sodium selenite) 0.0045 g, iodine (as calcium iodate) 0.25 g, iron (as sulphate monohydr) 2 g, zinc (as oxide) 5 g, copper (as sulphate pentahydrate) 0.25 g, manganese (as sulphate monohydrate) 3.5 g, chlorine chloride 75, phosphorus (as monodicalcium phosphate) 2.5, sodium chloride (salt) 225 g, and cellulose 75 g. Calcium carbonate carrier to balance.
[4]DSM Nutritional Products, Inc., USA, 45 Waterview Boulevard, Parsippany, NJ, USA.
[5]ICN, Aurora, OH, USA.
[6]Himedia laboratories Pvt. Ltd., 23, Vadhani Ind. Est., LBS Marg, Mumbai, India.

with filtered seawater. Photoperiod was maintained at 14:10 h (light:dark). Dissolved oxygen (DO) concentration, salinity, pH, and water temperature were measured daily and maintained at 6.0 ± 0.37 mg/L (mean ±SE), 36.1 ± 0.69 mg/L, 8.0 ± 0.24, and 26.0 ± 0.87°C, respectively. Ammonia-N and nitrite-N were measured weekly using the Solorzano (1969) method and Parsons et al. (1985) methods, and averaged 0.1 ± 0.07 mg/L and 0.1 ± 0.06 mg/L, respectively.

Fifteen fish (7.4 ± 0.09 g; mean ±SE) were stocked in each of the 15 tanks. Each diet was offered to three tanks in a complete randomized design, resulting in five dietary treatments with three replicate tanks per treatment. Fish were offered experimental diets ad libitum three times daily (7:00, 13:00, and 19:00). When active feeding behavior stopped, a few feed pellets were added to each tank so that fish could consume them later if needed. Fish were group weighed biweekly after a day of fasting and the experiment was terminated after 9 weeks.

Sampling and Chemical Analysis

At the start of the trial, 25 fish were euthanized using a 250 ppm Tricaine-S (Tricaine Methanesulfonate, MS 222, Western Chemical Inc., Ferndale, WA, USA) solution, and individually weighed and total length measured to estimate condition index (K). Hepatosomatic (HSI) and viscerosomatic (VSI) indices of 15 of these fish was then determined. The remaining ten fish were stored whole at −20°C for proximate analysis. At the end of the grow-out period all fish were harvested, anesthetized using Tricaine-S, and group weighed. Every fish was individually weighed and its length determined. Three fish from each tank were euthanized and livers and viscera removed and weighed for estimation of HSI and VSI. Another three fish from each tank were randomly taken, dried at 95°C to a constant weight, moisture content estimated, and then ground and stored at −20°C for further analysis. Total lipids were extracted from whole fish and experimental diets using a reflux extractor (Ankom^{xt10} Extractor, Ankom Technology Corporation, Macedon, NY, USA) with a mixture of 20% diethyl ether, 80% petroleum ether, as solvent. Also, samples from whole fish and experimental diets were analyzed for protein content using a nitrogen analyzer (Thermo Finnigan/EA1112 elemental analyzer, Thermo Electron Corporation, Madison, WI, USA) with aspartic acid as a calibration standard. Every tenth sample was duplicated for quality control, and the machine was recalibrated after every 20 samples analyzed. Nitrogen values were multiplied by 6.25 to estimate protein content of samples (Alavanese and Orto 1963). Samples of dried fish were weighed and combusted in a furnace at 550°C for 12 h to estimate ash content. All proximate analysis results were then reported on a wet weight basis.

Blood Parameters

At the end of the experiment, fish were fasted for 24 h, three fish from each tank were anaesthetized in a buffered solution of 100 mg/L Tricaine-S, and blood samples were collected by cardiac puncture using 27.5 gauge needles coated with heparin and attached to 1 mL syringes. Approximately 0.6 mL of blood were collected and transferred to heparin-coated microcentrifuge tubes. A second blood sample was collected using the same procedure but without an anticoagulant. Blood samples were held on ice until all samples were collected. Hematology and plasma biochemistry analyses were performed within 2 h of blood collection.

The heparinized samples were used for hematological examination. Haemoglobin (Hb) was determined using the cyanmethaemoglobin technique (Drabkin and Austin 1935). Hematocrit measurements were done in duplicates by drawing well-mixed blood into heparin-coated microhaematocrit tubes (75 mm length, inside diameter 1 mm, ABCO, Dealers, Inc., Elmhurst, IL, USA) and centrifuged at 10,000 g for 5 min in a microhaematocrit centrifuge at room temperature (Morris and Davey 1996). Whole blood was diluted 1:200 in modified Natt-Herrick's solution (Natt and Herrick 1952; Noga 1995), and total cell counts, red blood cells (RBCs), and white blood cells (WBCs) were counted manually using a Neubauer haemocytometer. Differential blood cell counts were done on blood films fixed with absolute methanol and stained with modified Wright-Giemsa stain. A total of 600 white blood cells (WBCs) per slide were identified, recorded, and described according to Ellis (1976) and Ainsworth (1992). Each type of blood cell was expressed as a percentage of the total number of blood cells examined.

For biochemical analysis, nonheparinized blood samples were placed on ice for 1 h and centrifuged at 950 g for 10 min at 4°C to isolate the serum, which in turn was collected as a supernatant and stored at –78°C until analyzed. Activities of aspartate aminotransferase (AST, U/L) and alanine aminotransferase (ALT, U/L) in the serum were determined using commercial kits (DIALAB GmbH, Wiener Neudorf, Austria). Total plasma protein (TPP, g/dL) was determined by placing a drop of serum onto a veterinary refractometer (RHC-200ATC, Westover Scientific, Inc., Mill Creek, WA, USA). Glucose (GLU, mg/dL) was measured using an Accutrend glucose meter (Accutrend® Plus System, Roche Diagnostics GmbH, Mannheim, Germany).

Data Analysis and Statistics

Hepatosomatic index and viscerosomatic index were calculated using the following formulas: HSI % = (weight of liver/total body weight) × 100 and VSI % = (weight of viscera and associated fat tissue/total body weight) × 100, respectively. Length and weight measurements were used to calculate Fulton's condition index: $K = (W \times L^{-3}) \times X$, where W = fish weight (g) and L = total length (mm) and X is a constant equal to 100,000 (Anderson and Neumann 1996). Protein gain was calculated as: Protein gain = protein weight at the end of the experiment – protein weight at beginning of the experiment. Protein conversion efficiency was not calculated because the fish were offered more feed than they consumed.

Results were compared using one-way ANOVA. Significant ANOVA was followed by Student-Newman-Keuls multiple comparison test. Differences were considered significant at $P < 0.05$. Regression of final weight versus dietary protein was performed to estimate best dietary crude protein inclusion. All statistical analyses were performed using SPSS (V.8 for Windows, SPSS Inc., Chicago, IL, USA).

RESULTS

Survival was greater than 95.5% in all dietary treatments with no significant differences among treatments (Table 2). Final weight was greater than 200% of initial weight even for fish offered the 30 g/100 g protein diet. The

TABLE 2 Survival, initial weight (W_i), final weight (W_f), weight gain protein gain, final condition index (K_f), hepatosomatic index (HSI) and viscerosomatic index (VSI) for juvenile *Siganus rivulatus* offered diets with various protein levels for 9 weeks.

	Dietary Protein Level (g CP/100 g diet)					
	30	35	40	45	50	PSE*
Survival (%)	100	100	100	97.8	95.6	2.22
W_i (g)	7.5	7.4	7.4	7.4	7.3	0.14
W_f (g)	25.1[b]	26.4[b]	28.8[a]	26.2[b]	27.2[b]	0.62
Weight gain (%)	236.6[b]	255.6[b]	291.7[a]	254.1[b]	271.3[b]	7.02
Protein gain (g)	10.5	10.3	11.6	11.1	10.5	0.47
K_f	1.2	1.2	1.3	1.2	1.2	0.03
HSI	2.0	2.3	2.1	1.9	1.9	0.21
VSI	10.8	11.4	11.9	10.9	11.2	0.67

*PSE: pooled standard error.
Values with different superscripts within a row are significantly different from each other ($P < 0.05$).

highest growth was observed in fish offered diets containing 40 g CP/100 g diet, while there was no significant difference among the other experimental groups of fish. The relationship between growth and crude protein inclusion was described by the equation: $y = -0.2639x^2 + 22.189x - 190.18$ ($r^2 = 0.587$, $n = 15$ tanks), where y is percent growth and x is crude protein level in the diet. The shape of the relationship is parabolic with maximum growth at 42.04% protein. There were no significant differences in the protein gain, condition index, HSI, and VSI among fish offered any of the various dietary protein levels (Table 2).

At the end of the experiment, moisture content of whole fish was not significantly different among samples obtained from fish offered 30, 35, 40, and 45 g/100 g protein in their diets. However, fish in these treatments contained less body moisture than did fish at stocking and more body moisture than fish offered the 50 g/100 g protein diet (Table 3). The protein proportion in the body of the fish was not significantly different ($P < 0.05$) among samples obtained from fish offered 30, 35, 40, and 45 g/100 g protein

TABLE 3 Proximate composition of juvenile marbled spinefoot (*Siganus rivulatus*) offered experimental diets containing various protein levels for 9 weeks.

	Dietary Protein Level (g CP/100 g diet)						
	Initial	30	35	40	45	50	PSE*
Moisture (%)	74.6[a]	67.9[b]	68.3[b]	68.9[b]	69.1[b]	65.4[c]	0.65
Protein (%)	15.8[b]	18.5[a]	18.5[a]	19.2[a]	18.9[a]	13.2[c]	0.55
Lipids (%)	5.2[c]	8.3[b]	9.6[b]	8.8[b]	7.8[b]	12.4[a]	0.49
Ash (%)	3.3	3.3	3.2	3.5	3.1	3.0	0.20

*PSE: pooled standard error.
Values with different superscripts within a row are significantly different from each other ($P < 0.05$).

in their diets, but was significantly greater than protein proportion in initial fish samples. In turn, protein proportion of initial fish was significantly greater than protein proportion of fish offered the 50 g/100 g diet (Table 3). However, we know that high lipids in the sample affect nitrogen measurements in the nitrogen analyzer and suspect protein content to be slightly more than reported. Lipid proportions were not significantly different from each other ($P < 0.05$) among samples obtained from fish offered 30, 35, 40, and 45 g/100 g protein in their diets, but were significantly greater than lipid proportion in initial fish samples and significantly less than lipid in fish offered 50 g/100 g protein in their diets (Table 3). Ash content of the fish body was not significantly different among treatments or between fish in all treatments and initial fish (Table 3).

Hematocrit, hemoglobin, and blood glucose values were not significantly different from each other ($P < 0.05$) among fish in all dietary treatments (Table 4). Plasma protein values were not significantly different from each other ($P < 0.05$) in fish offered 35, 40, 45, and 50 g/100 g protein in their diets, but were significantly less than plasma protein of fish offered 30 g/100 g protein in their diets (Table 4). Total RBC counts were not significantly different from each other ($P < 0.05$) in fish offered 30, 35, 45, and 50 g/100 g protein in their diets, all of which were significantly greater than counts in fish offered 40 g/100 g protein in their diets. ALT activities were not significantly different from each other ($P < 0.05$) in the serum of fish from all treatments. However, activities of AST were significantly different from each other ($P < 0.05$) in serum of fish offered the various dietary protein levels, showing maximum activity in fish offered the 35 g/100 g protein diet and decreasing in all other treatments (Table 4).

Total WBC counts were greatest (121.8×10^3 cells/mm^3) in fish offered the diet containing 45 g CP/100 g diet but not significantly different from

TABLE 4 Hematological parameters for *S. rivulatus* offered experimental diets with various protein levels for 9 weeks. Total red blood cells (RBC) is reported as $\times 10^6$ cells/µL and total white blood cells (WBC) as $\times 10^3$ cells/mm^3.

	Dietary Protein Level (g CP/100 g diet)					
	30	35	40	45	50	PSE*
Hematocrit (%)	40.0	40.1	34.6	44.1	37.4	2.60
Hemoglobin (mg/dL)	14.2	14.0	14.3	14.5	13.8	0.62
Glucose (mg/dL)	36.8	31.7	33.8	37.2	33.8	3.55
Plasma protein (g/100 mL)	4.2[a]	3.5[b]	3.5[b]	3.9[b]	3.8[b]	0.14
Total RBC	2.6[a]	2.5[a]	1.7[b]	2.6[a]	2.2[a]	0.18
Total WBC	99.6[b]	97.3[b]	77.3[b]	121.8[a]	68.5[b]	9.51
Aspartate aminotransferase (U/L)	140.9[b]	195.5[a]	124.2[b]	86.5[b]	40.0[c]	20.11
Alanine aminotransferase (U/L)	85.6	86.3	89.0	107.1	110.3	19.52

*PSE: pooled standard error.

Values with different superscripts within a row are significantly different from each other ($P < 0.05$).

TABLE 5 Differential leukocyte counts of juvenile *Siganus rivulatus* offered experimental diets with various protein levels for 9 weeks.

	Dietary Protein Level (g CP/100 g diet)					
	30	35	40	45	50	PSE*
Thrombocytes (%)	81.7	81.7	82.0	82.3	84.1	10.5
Lymphocytes (%)	15.6	15.9	14.7	14.7	14.5	8.41
Eosinophils (%)	0.20	0.18	0.23	0.10	0.13	0.63
Basophils (%)	0.05	0.05	ND	0.06	0.06	0.33
Monocytes (%)	0.88	0.73	0.68	1.50	0.77	2.12
Neutrophils (%)	1.60^{a}	0.97^{b}	1.73^{a}	1.50^{a}	0.77^{b}	2.24

*PSE: pooled standard error.
Values with different superscripts within a row are significantly different from each other ($P < 0.05$).
ND: Not detected.

each other ($P < 0.05$) in fish offered feed with 30, 35, 40, and 50 g CP/100 g diet (Table 4). Values of thrombocytes, lymphocytes, eosinophils, basophils, and monocytes were not significantly different from each other ($P < 0.05$) among fish offered the five protein levels in their diets (Table 5). Values of neutrophils in fish blood were significantly less in fish offered feeds with 35 and 50 g CP/100 g diet than in all other treatments ($P < 0.05$) (Table 5).

DISCUSSION

As the need for fish production increases with increase in population, farmers need to shift their emphasis from carnivorous fishes to marine species that eat low on the food chain. Rabbitfish is a potential candidate for such an industry, and to be able to formulate a herbivorous diet for the species, its protein requirement should first be known.

In the present experiment, survival was excellent and growth typical of marbled spinefoot reared in our laboratory for more than 7 years. Maximum growth of juvenile *S. rivulatus* was observed in fish offered experimental diets containing 40 g CP/100 g feed and 14.2 MJ/kg gross energy. A polynomial regression of the growth data observed suggests that optimal crude protein content of the feed would be 42.04%. It is likely that fish receiving feeds with higher level of crude protein catabolized the additional amino acids for energy (Cowey 1980, 1995; Jauncey 1982; Parazo 1990).

Siganids are algaevorous fish, with a possibility that their protein requirements would be potentially less than those of carnivorous fish (Cowey 1979; Wee and Tacon 1982). However, fish in the present experiment had protein requirements of 43 g/100 g diet, similar to piscivorous fishes. This could be because of the small size and age of the fish used in the present experiment. In general, as fish increase in size and age, their protein requirements tend to decrease (Halver and Hardy 2002). Tacon et al. (1990) reported that large *Siganus canaliculatus* required dietary protein of about 31 g/100 g, less than the 43 g/100 g observed for juvenile *S. rivulatus* in the present work. Parazo (1990) found that 35 g/100 g dietary protein was optimal for the growth of juvenile *Siganus guttatus*. It appears that *S. rivulatus* has a protein requirement slightly greater than that of other siganids but all siganids investigated seem to have a protein requirement close to that of carnivorous fishes.

Fish, as all animals, have specific energy requirements. If the diet does not contain enough nonprotein energy, then protein will be used for energy to satisfy maintenance at the expense of growth (NRC 1993). Modifying the energy content of a fish's diet also modifies the optimal protein requirement of the fish (Cowey 1979). In the present study, the level of energy in the diets (14.2 MJ/kg) was less than that used in the study performed by El-Dakar et al. (2011). However, El-Dakar et al. fed their fish to apparent satiation, which may have limited the amount of energy intake, while in the present work we fed the fish ad libitum, which supposedly would have allowed the fish to regulate their energy intake. In both cases the requirement was about 40 g CP/100 g diet. When we compare these results to studies performed on other species of siganids, we find the majority suggest that diets with protein content between 30 g CP/100 g diet and 40 g CP/100 g diet and energy content between 14 and 18 MJ/kg are suitable for good growth of the fish. Parazo (1990) found that 35 g CP/100 g diet was optimal for the growth of *S. guttatus* when the level of gross energy used in the diets was 16 MJ/kg. Tacon et al. (1990), Bwathondi (1982), and Ismail et al. (1986) reported that 31 g CP/100 g diet was optimal for the growth of *S. canaliculatus*. The five experimental diets used in the present study were isoenergetic and contained similar amounts of lipids (8.4 g/100 g). This means that protein level in the diet was the only factor that affected growth, and thus we are quite confident that 43 g CP/100 g of a diet containing 14.2 MJ/kg is suitable for good growth of juvenile *S. rivulatus*. Even though we are confident of required dietary protein level, we are aware that we assumed complete starch digestibility of the tested diets. Such an assumption is partly justified because we are working with an algaevorous fish, but before specialized diets are formulated, starch digestibility should be determined.

When our fish were offered diets containing more than 40 g CP/100 g diet, protein gain tended to decrease. Because diets with high protein content tend to have high protein to energy ratio, part of the dietary protein is metabolized for energy, which results in a decrease in protein efficiency (Santiago and Reyes 1991) and thus a decrease in net protein gain when using high-protein diets. The low-protein diets probably did not contain enough protein to satisfy growth demands. Because the fish were fed ad libitum instead of being offered feed to apparent satiation or at a fixed rate, protein efficiency or protein production values (PPV) could not be calculated. Efficiency or PPV might have been better indicators of protein use, but artificially limiting protein offered to the fish would also have affected the results. The fact remains that previous work by El-Dakkar et al. (2011) that offered diets with various protein-inclusion levels to apparent satiation corroborates findings of the present work where the diets were offered *ad libitum*.

No differences in HSI and VSI among fish offered the five dietary protein levels were observed in the present work. Results were similar to those obtained by González et al. (2005), where no differences were obtained in HSI and VSI of southern flounder (*Paralichthys lethostigma*) when they were offered diets with various protein levels. This suggests that excess proteins used for lipogenesis and gluconeogenesis are stored in muscle and body compartments other than the liver and the viscera.

Body moisture and lipid contents of the fish offered the various diets appeared to be inversely correlated. Fish offered the 50 g/100 g protein diet had more lipids and less moisture as a proportion of their body than fish in other treatments. These results suggest that at high dietary protein levels exceeding fish requirements, excess amino acids absorbed are catabolized and used for lipogenesis. Similar results were described by El-Dakar et al. (2011), who found that *S. rivulatus* body lipids were least in fish offered the 10 g/100 g protein diet and most in fish offered the 60 g/100 g protein diet. However, increasing dietary protein level had no effect on fish body protein proportion. Similar findings were reported for various fish species, which also show no effect of dietary protein level on body protein levels (Jana et al. 2006; Salhi et al. 2004). Apparently, as long as the fish are getting enough energy for maintenance from nonprotein sources, and enough protein for production, their body protein content will not change. Moreover, body ash content did not vary with dietary protein level. Similar results were observed by Dabrowska and Wojno (1977) in *Salmo gairdneri* and by Jauncey (1982) in *Cyprinus carpio*.

In salmonids, starvation and malnutrition can cause anemia accompanied with decrease in RBC counts, decreased hematocrit, hemoglobin, and total plasma proteins (Stoskopf 1993). In the present work, hematocrit and hemoglobin values were not affected by dietary protein levels. Hematocrit and hemoglobin values ranged from 34.6 to 44.1% and 13.8 to 14.5 mg/dL respectively and were similar to findings by Nasser (2012) working with *S. rivulatus*. Erythrocyte counts were similar to each other in all treatments except for fish offered the diet containing 40 g/100 g crude protein where they were less than in other treatments. However, the low RBC counts in fish offered diets containing 40 g/100 g protein were similar to those reported as normal for marbled spinefoot (Nasser 2012). Because of a lack of sufficient historical data on siganids hematology, it is impossible for us to decide if the reduction in RBC counts resulted from the dietary treatment or is an experimental error. Our results also suggest that even though fish do not grow well when offered a 30 g/100 g protein diet, they still are not so malnutritioned as to become anemic.

Plasma protein levels in the blood of *S. rivulatus* offered the various diets were similar to results observed in aquacultured *S. rivulatus* by Nasser (2012). Fish that were offered the low-protein diet had a significantly greater plasma protein value than fish in other treatments. This increase in plasma protein level may suggest catabolism of protein from the tissues due to its low availability in the diets. When the amount of dietary protein is less than the fish's requirement, it can cause retardation of growth due to withdrawal of protein from the less-vital tissues in order to maintain the functions of more-vital tissues (Halver and Hardy 2002). Considering the fact that rabbitfish are algaevorous and algae are not protein-rich feeds, the results are surprising and merit further investigation.

Blood glucose is used as a sensitive indicator of environmental stress in fish (Iwama 1998; Silbergeld 1974). In the present study, blood glucose values, ranging from 31.67 to 37.20 mg/dL, were not significantly different among dietary treatments. Similar results were reported for summer flounder (*Paralichthys dentatus*) offered diets containing increasing protein levels (Daniels and Gallagher 2000). It is possible that handling stress of the fish caused a surge in blood glucose that masked any effects the diets might have had. However, the lack of effect of dietary protein on most other haematological parameters could also indicate that the fish were not starved for energy, and thus blood glucose might not have been affected in the present work.

The aminotransferases AST and ALT are non-plasma-specific enzymes involved in the transfer of amino groups from one amino acid to another (Coz-Rakovac et al. 2008; Shahsavani et al. 2010). Although in fish ALT and AST are present in various organs, an alteration in their activity is usually, but not definitively, an indicator of liver damage or dietary stress (Gul et al. 2011; Wilson 1973). In the present study the levels of ALT activity were not significantly different from each other in serum of fish from the various treatments, although activity appears to increase with increasing dietary protein. Similar results were reported for juvenile tiger puffer (*Takifugu rubripes*) (Kim and Lee 2009). Conversely, AST activity increases significantly as dietary protein decreases.

Although neutrophil counts were significantly less in blood of fish offered the 50 g CP/100 g diet than in all other treatments, no apparent reason for the difference was observed. Neutrophils in teleosts proliferate in circulation because of infection, inflammation, and/or stress (Davis et al. 2008). No infection or inflammation was observed in our fish, and the neutrophil numbers do not suggest dietary stress.

Findings of the present work suggest that feeds containing 43 g CP/100 g diet and 14.2 MJ/kg digestible energy offer good growth of juvenile spinefoot rabbitfish *S. rivulatus* maintained at optimal salinity (35 mg/L; Saoud et al.

2007) and temperature (27°C; Saoud et al. 2008). Further work will determine if dietary energy will significantly affect dietary protein requirements. Results from blood parameter measurements showed that there were no physiological or metabolic dysfunctions in fish offered any of the five protein levels.

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