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Effects of dietary lipid levels on growth performance of marbled spinefoot rabbitfish *Siganus rivulatus*

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ABSTRACT

The present study was carried out to evaluate the effect of dietary lipid levels on growth performance and body composition of juvenile marbled spinefoot (*Siganus rivulatus*). Six iso-nitrogenous (301.0 g kg^{-1} crude protein) and iso-energetic diets (16.0 MJ gross energy kg^{-1}) were formulated to contain 25, 45, 65, 85, 105, and 125 g lipid kg^{-1}. Twenty juvenile fish (2.9 g ± 0.01; mean ± SE) were randomly assigned to triplicate treatments and offered the test diets three times daily to apparent satiation for 7 weeks. Survival was greater than 95% in all treatments. Fish offered the diet with the lowest lipid content (25 g kg^{-1}) exhibited lowest growth performance. No significant differences in growth performance were detected among all other treatments. However, analysis of final body weight by second order polynomial regression suggested that the dietary lipid requirement for optimal growth in juvenile marbled spinefoot is 98 g kg^{-1}.There were no significant differences in condition indices (K), fillet yield and hepatosomatic index (HSI) values among treatments. Viscerosomatic index (VSI) (13.35% ± 0.42) of fish offered the diet with 125 g kg^{-1} lipid was significantly greater than the VSI (10.00% ± 0.35) of fish offered the diet with 25 g kg^{-1} lipid. A trend of increasing VSI with an increase in dietary lipid was obvious. Increasing dietary lipid levels beyond 85 g kg^{-1} resulted in increase in whole body lipid content. No significant differences in moisture content (69.3–71.6%), protein content (13.1–14.6%), and ash content (2.8–3.2%) were observed among treatments. Based on our results, marbled spinefoot rabbitfish have low dietary lipid requirements and increasing dietary lipids increased the lipid proportion of body weight significantly.

1. Introduction

Marbled spinefoot rabbitfish (*Siganus rivulatus*) is an economically valuable species in the Eastern Mediterranean and Indo-West Pacific regions (Lam, 1973; Woodland, 1983). It is a euryhaline marine fish of the herbivorous family Siganidae and can reach sizes of 31.9 cm and 318.2 g in length and body weight, respectively (Bariche, 2005). Marbled spinefoot is identified as a potential candidate for marine warmwater aquaculture because of its good taste, herbivorous feeding habits (Lam, 1973), tolerance to high stocking densities (Saoud et al., 2008a) and tolerance to wide ranges of environmental factors such as salinity (Saoud et al., 2007a) and temperature (Saoud et al., 2008b). Currently, only limited amounts of the fish are aquacultured in offshore cages in Cyprus and Saudi Arabia mostly because of a dearth in information about proper aquaculture protocols and suitable feeds. Diet development would be a significant step towards establishment of viable aquaculture production of marbled spinefoot as feed is the single greatest operational expense in aquaculture industry.

Dietary lipids are an important source of energy that also provide essential fatty acids, phospholipids, sterols and fat-soluble vitamins necessary for proper functioning of physiological processes and maintenance of biological structure and function of cell membranes (Watanabe, 1982; Sargent et al., 1989). Lipid used for energy can also spare dietary proteins and reduce nitrogenous waste production (Bromley, 1980; Vergara et al., 1999). Increasing dietary lipid levels improves feed efficiency and growth by sparing protein, particularly in coldwater carnivorous fish, as they generally have a lesser ability to utilize carbohydrates for energy than do warmwater species (Watanabe, 1982; Beamish and Medland, 1986; Hardy, 1999). In salmonid aquaculture, up to 30% lipid is used in diets whereas in warmwater species diets contain less than 10% lipid (Gaylord and Gatlin, 2000). Benefits of dietary lipids notwithstanding, excessive lipid can cause a decrease in feed consumption and reduce the utilization of other nutrients resulting in reduced growth (Page and Andrews, 1973; Watanabe, 1982; Daniels and Robinson, 1986; Ellis and Reigh, 1991) as well as increasing fat deposition (Bromley, 1980; Hillestad and Johnsen, 1994).

The natural diet of marbled spinefoot consists mainly of macrophytes (Bariche, 2006) with low lipid content; probably resulting in suboptimum growth and inconsistent production. However, under

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culture conditions, the fish exhibits omnivorous feeding habits and readily accepts commercial and experimental diets, making them suitable for commercial aquaculture (Saoud et al., 2008a,b). Studies on dietary lipid requirements of rabbitfish are scarce and the purpose of the present study was to determine the effect of increasing dietary lipid levels from 25 to 125 g kg^{-1} lipid on growth performance and lipid deposition in whole body, fillet, liver and viscera of juvenile marbled spinefoot in an effort to determine lipid inclusion levels in specialized siganid diets.

2. Materials and methods

2.1. Experimental diets

The present work was performed at the American University of Beirut aquaculture research laboratory. Diets used (Table 1) were manufactured at the Auburn University Department of Fisheries and Aquaculture, Alabama. Six iso-nitrogenous and iso-energetic diets were formulated to contain 301 g kg^{-1} crude protein and 16 MJ gross energy kg^{-1}. The diets were kept iso-energetic by adjusting the corn starch and cellulose content in the diets. The gross energy content of the diets was calculated based on 23.7, 39.5 and 17.2 kJ g^{-1} for protein, lipid and nitrogen-free extract, respectively (Brett and Groves, 1979). Crude lipid contents of the diets were 25, 45, 65, 85, 105 and 125 g kg^{-1}. Alpha-cellulose was added as a non-nutritive binder that helps impart water stability to the formulated diets. Dry ingredients were mixed thoroughly in a Hobart mixer, then boiling water was added, mixed for another 5 min and the mash extruded in a meat grinder with a 1-mm die. The diets were dried in a forced air oven at 40 °C to a moisture content of 8% and stored at −20 °C until used.

Table 1
Ingredients and chemical composition of the experimental diets (g kg^{-1} in dry weight) offered to marbled spinefoot *Siganus rivulatus* juveniles.

Ingredients (g kg^{-1})	Dietary lipid level (g kg^{-1})					
	25	45	65	85	105	125
Fishmeal[a]	32	32	32	32	32	32
Poultry by product meal[b]	100.0	100.0	100.0	100.0	100.0	100.0
Soybean meal solvent extracted[c]	400.0	400.0	400.0	400.0	400.0	400.0
Menhaden Fish Oil[d]	0.0	20.0	40.0	60.0	80.0	100.0
Corn Starch[e]	270.0	220.0	180.0	140.0	100.0	70.0
Whole wheat[e]	115.0	115.0	115.0	115.0	115.0	115.0
ASA Trace Mineral premix[f]	5.0	5.0	5.0	5.0	5.0	5.0
ASA Vitamin premix w/o choline[g]	10.0	10.0	10.0	10.0	10.0	10.0
Choline chloride	2.0	2.0	2.0	2.0	2.0	2.0
Stay C 250 mg kg^{-1} using 25%[h]	1.0	1.0	1.0	1.0	1.0	1.0
Lecithin (soy refined, USB)	10.0	10.0	10.0	10.0	10.0	10.0
Cellufill[i]	55.0	85.0	105.0	125.0	145.0	155.0
Chemical composition (g kg^{-1} in dry matter)						
Crude protein	301.0	301.0	301.0	301.0	301.0	301.0
Crude lipid	25.0	45.0	65.0	85.0	105.0	125.0
Crude ash	50.0	46.0	52.0	55.0	60.0	68.0
Gross energy (MJ kg^{-1})	16.0	15.8	15.8	15.8	15.8	16.0

[a] Omega Protein Inc., Hammond, LA, USA.
[b] Griffin Industries, Inc. Cold Springs, KY, USA.
[c] De-hulled solvent extracted soybean meal, Southern Sates Cooperative Inc., Richmond VA, USA.
[d] Omega Protein Inc., Reedville, Virginia, USA.
[e] United States Biochemical Corporation, Cleveland, Ohio, USA.
[f] Content as g 100 g^{-1} premix: cobalt chloride 0.004, cupric sulfate pentahydrate 0.250, ferrous sulfate 4.0, magnesium sulfate heptahydrate 28.398, manganous sulfate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulfate heptahydrate 13.193, and filler 53.428.
[g] Content as g kg^{-1} premix: thiamin HCl 0.5, riboflavin 3.0, pyrodoxine HCl 1.0, DL Ca-Pantothenate 5.0, nicotinic acid 5.0, biotin 0.05, folic acid 0.18, vitamin B12 0.002, choline chloride 100.0, inositol 5.0, menadione 2.0, vitamin A acetate (20,000 IU g^{-1}) 5.0, vitamin D3 (400,000 IU g^{-1}) 0.002, dl-alpha-tocopheryl acetate (250 IU g^{-1}) 8.0, Alpha-cellulose 865.266.
[h] 250 mg kg^{-1} active C supplied by Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), Roche Vitamins Inc., Parsippany, New Jersey, USA.
[i] Alpha-cellulose, Unites States Biochemical Corporation, Cleveland, Ohio, USA.

2.2. Experimental design

Juvenile rabbitfish, *S. rivulatus*, were caught in traps off the Beirut beach and transported to the aquaculture research laboratory at the American University of Beirut. The fish were quarantined and trained to accept artificial feed (Zeigler Silver, Slow sinking, Zeigler Bros., USA) for 2 weeks in a recirculating system composed of 180-L fiberglass tanks. Fish were then sorted by hand to a uniform size and stocked in a recirculating system comprised of 18, 52-L (58 × 30 × 30 cm; L × W × H) glass aquaria connected to a biological filter, a mechanical filter, a water pump and submersible heating unit. Aeration was provided via a regenerative blower and submerged air diffusers. The flow rate of filtered seawater into each aquarium was 55 L h^{-1}. Photoperiod was maintained at 14:10 h (light:dark). Dissolved oxygen (DO) concentration, salinity, pH and water temperature were measured daily and maintained at 6.11 mg L^{-1} ± 0.06 (mean ± SE), 33.9 ppt ± 0.63, 8.1 ± 0.14 and 26.3 °C ± 0.20, respectively. Ammonia-N and nitrite-N were measured weekly using the Solorzano (1969) method and Parsons et al. (1985) method, respectively and averaged 0.06 mg L^{-1} ± 0.01 and 0.21 mg L^{-1} ± 0.06, respectively.

Twenty fish (2.9 g ± 0.01; mean ± SE) were stocked in each of the 18 aquaria. Each diet was randomly offered to three tanks, giving six treatments with three replicate tanks per treatment. Fish were offered experimental diets to apparent satiation three times daily (7:30 h, 13:00 h and 18:00 h), six days a week. Fish were considered satiated when two to three pellets were not consumed within 3 min of being offered. Fish were group weighed biweekly after a day of fasting and the experiment was terminated after 7 weeks.

2.3. Sampling and chemical analysis

At the start of the experiment, 18 fish were euthanized using an overdose of Tricaine-S (Tricaine Methanesulfonate, MS 222, Western Chemical Inc., Ferndale, WA, USA). Eight of these fish were individually weighed and liver, viscera and fillet removed, weighed and stored at −20 °C for subsequent analysis. Another ten fish were euthanized and stored whole at −20 °C. At the end of the growout period all fish were harvested, anesthetized using Tricaine-S, and group-weighed. Every fish was individually weighed and its length determined. Seven fish from each tank were euthanized and livers, viscera and fillets removed and weighed for estimation of hepatosomatic and viscerosomatic indices and then stored at −20 °C for analysis. Five fish from each tank were macerated in a blender and also stored at −20 °C. All stored samples were freeze dried (Freezone Plus 6 Liter Freeze Dry System, Labconco Corporation, Kansas City, MO, USA) and moisture content estimated. Total lipids were extracted from whole fish, viscera, livers and fillets 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. Lipid content of the experimental diets was confirmed using the same procedure.

Samples from whole fish were analyzed for protein content using a nitrogen analyser (Thermo Finnigan/EA1112 elemental analyser, Thermo Electron Corporation, Madison, WI, USA) with aspartic acid as a calibration standard. Samples were weighed in tin containers and inserted into the machine. Every tenth sample was duplicated for quality control and the machine was recalibrated after every thirtieth sample analysis. Nitrogen values were multiplied by 6.25 to estimate protein content of samples (see Alavanese and Orto, 1963). A known weight sample from whole fish was combusted in a furnace at 500 °C for 8 h to estimate ash content. All proximate analysis results were then reported on a wet weight basis.

2.4. Data analysis and statistics

Hepatosomatic index (HSI) and viscerosomatic index (VSI) were calculated using the following formulas: [HSI (%) = (weight of liver (g) / total body weight (g)) × 100] and [VSI (%) = (weight of viscera and associated fat tissue (g) / total body weight (g)) × 100], respectively. Feed conversion ratio (FCR) was estimated using the formula: $FCR = F/(Wf - Wi)$, where F is the weight of feed offered to fish, Wf is the final weight of the fish and Wi is the initial weight of the fish at stocking (Hopkins, 1992). Protein efficiency ratio (PER) was calculated using the following formula: wet weight gain (g)/protein intake (g). Apparent protein utilisation (APU) = fish protein gain (g)/protein offered (g). Length and weight measurements were used to calculate Fulton's condition index: $K = (W/L^3) \times X$, where W = fish weight (g) and L = total length (mm) and X is a constant equal to 100,000 (Anderson and Gutreuter, 1983). Proportion of fat in fillet, liver, and viscera out of total body fat was also calculated.

All statistical analyses were performed using SPSS statistical software (V.12 for Windows, SPSS Inc., Chicago, IL, USA). Data were expressed as the mean ± SE of three replicates. Survival, growth, K, FCR, APU, PER, HSI, VSI and lipid contents were analyzed using one-way ANOVA and Student Newman–Keuls multiple-range test to determine significant differences ($P < 0.05$) among treatment means. Dunnett's test was used to determine significant differences ($P < 0.05$) between initial means and final means of various parameters. A second order polynomial regression model between final body weight and dietary lipid level was used for estimation of dietary lipid levels that promote maximum somatic weight gain of juvenile marbled spinefoot (Zeitoun et al., 1976).

3. Results

Survival was greater than 95% in all treatments and no signs of stress or other health problems were observed. Growth was least in fish offered the diet with 25 g crude lipid kg^{-1} but there were no differences in growth among fish in all other treatments (Table 2). Second order polynomial regression analysis between final body weight and dietary lipid levels suggest that dietary lipid level required for maximum weight gain in marbled spinefoot juveniles is 98 g kg^{-1} (Fig. 1). There were no significant differences in K (1.16–1.23) or fillet yield (26.7%–28.1%) among treatments. Fish offered diet 25 g kg^{-1} consumed significantly less feed (176.9 g) than fish in all other treatments. Feed conversion ratio of fish offered the diet with lipid content of 105 g kg^{-1} (1.78 ± 0.02; mean ± SE) was significantly less than FCR of fish offered the diet with lipid content of 25 g kg^{-1} (2.05 ± 0.03) but not significantly different from FCR in all other treatments (Table 2). No significant differences in APU (20.0–23.1%) were observed among treatments (Table 2). Protein efficiency generally increased with increase in dietary lipid levels (Table 2).

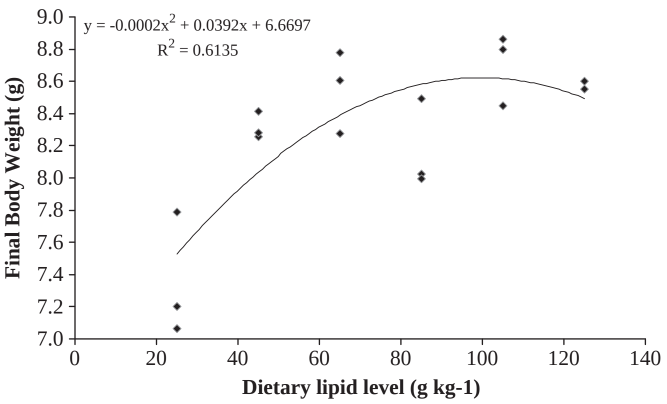

Fig. 1. Second order polynomial regression analysis between final body weight (g) and dietary lipid level (g kg^{-1}) for juvenile marbled spinefoot.

There were no significant differences in HSI and in total lipid liver content among treatments (Table 3). Viscerosomatic index of fish offered the diet containing 125 g lipid kg^{-1} (13.35% ± 0.42) was significantly greater than VSI of fish offered the diet containing 25 g lipid kg^{-1} (10.00% ± 0.35). A trend of increasing VSI with an increase in dietary lipid was obvious although not statistically significant. Viscera of rabbitfish contained the majority of whole body lipid store accounting for 52.2–68.9% of total body fat. No statistical differences in visceral lipid contents were detected among treatments at the end of the experiment (Table 3). Fillet lipid content of fish offered the diet containing 125 g kg^{-1} lipid (3.0% ± 0.30) was significantly greater than fillet lipid content of fish offered the diet containing 25 g lipid kg^{-1} (0.9% ± 0.30). Although no significant differences were observed in fillet lipid content among fish in the other treatments, there was an increasing trend of fillet lipid content with increasing dietary lipid levels (Table 3).

Whole body composition at the end of the trial is presented in Table 4. Moisture content of fish at the start of the experiment (75.2% ± 0.28) was significantly greater than moisture content of fish offered the experimental diets (69.3–71.6%), with no significant differences observed among treatments. Similarly, no significant differences were observed in whole body protein content. However, a decreasing trend was observed where fish offered diets containing lipid of 8.5 g kg^{-1} and greater had lower body protein content (Table 4). Proportion of protein in initial fish (16.2% ± 0.20) was significantly greater than in fish in all treatments at the end of the trial. Increasing dietary lipid levels beyond 85 g kg^{-1} resulted in increased whole body lipid content (Table 4). Proportion of lipids in fillet (6.4–8.9%; $P > 0.05$), liver (2.1–3.9%; $P > 0.05$),

Table 2
Survival, initial body weight (BW), final body weight (BW), Fulton-type condition index (K), feed conversion ratio (FCR), feed offered, fillet yield, apparent protein utilisation (APU), and protein efficiency ratio (PER) of juvenile rabbitfish *Siganus rivulatus* offered experimental diets for seven weeks. Values (mean ± SE) in the same row with different superscripts are significantly different from each other ($P < 0.05$).

	Dietary lipid level (g kg^{-1})					
	25	45	65	85	105	125
Survival (%)	98.3 ± 1.67^a	96.7 ± 1.67^a	96.7 ± 1.67^a	96.7 ± 1.67^a	100.0 ± 0.00^a	97.5 ± 7.50^a
Initial BW (g)	2.9 ± 0.00^a	2.9 ± 0.00^a	3.0 ± 0.03^a	2.9 ± 0.00^a	3.0 ± 0.03^a	3.0 ± 0.05^a
Final BW (g)	7.4 ± 0.22^b	8.3 ± 0.05^a	8.6 ± 0.15^a	8.2 ± 0.16^a	8.7 ± 0.13^a	8.5 ± 0.00^a
K	1.16 ± 0.01^a	1.18 ± 0.03^a	1.18 ± 0.03^a	1.23 ± 0.01^a	1.18 ± 0.01^a	1.16 ± 0.01^a
FCR	2.05 ± 0.03^a	1.88 ± 0.07^{ab}	1.86 ± 0.07^{ab}	1.95 ± 0.05^{ab}	1.78 ± 0.02^b	1.81 ± 0.04^{ab}
Feed Offered (g)	176.9 ± 1.43^b	198.0 ± 2.00^a	202.6 ± 4.00^a	206.0 ± 1.91^a	205.4 ± 3.92^a	195.5 ± 4.77^a
Fillet Yield (%)	26.7 ± 0.84^a	27.4 ± 1.36^a	27.3 ± 0.60^a	26.8 ± 0.75^a	26.7 ± 1.86^a	28.1 ± 1.63^a
APU	21.7 ± 1.56^a	21.8 ± 1.17^a	23.1 ± 0.91^a	20.0 ± 0.44^a	23.1 ± 1.35^a	22.5 ± 0.05^a
PER	1.62 ± 0.03^a	1.77 ± 0.07^{ab}	1.79 ± 0.70^{ab}	1.70 ± 0.04^{ab}	1.86 ± 0.02^b	1.84 ± 0.05^{ab}

Table 3
Hepatosomatic index (HSI), Viscerosomatic index (VSI) and lipid content (% wet weight) of fillet, liver and viscera of juvenile rabbitfish, *Siganus rivulatus*, offered experimental diets with increasing lipid levels.

	Dietary lipid level (g kg^{-1})						
	Initial	25	45	65	85	105	125
HSI (%)	0.71 ± 0.11	$1.76 \pm 0.12^{a*}$	$1.89 \pm 0.10^{a*}$	$1.60 \pm 0.11^{a*}$	$1.68 \pm 0.09^{a*}$	$1.88 \pm 0.14^{a*}$	$1.70 \pm 0.15^{a*}$
VSI (%)	8.08 ± 0.43	10.00 ± 0.35^{c}	$11.06 \pm 0.34^{bc*}$	$11.20 \pm 0.31^{bc*}$	$12.20 \pm 0.48^{ab*}$	$12.26 \pm 0.22^{ab*}$	$13.35 \pm 0.42^{a*}$
Lipid content of							
Fillet	0.6 ± 0.06	0.9 ± 0.30^{a}	1.4 ± 0.07^{ab}	1.6 ± 0.58^{ab}	$2.2 \pm 0.26^{ab*}$	$2.6 \pm 0.38^{ab*}$	$3.0 \pm 0.30^{b*}$
Liver	5.4 ± 0.19	9.8 ± 2.75^{a}	10.3 ± 0.10^{a}	9.4 ± 0.60^{a}	$12.6 \pm 1.20^{a*}$	$13.4 \pm 1.10^{a*}$	$13.0 \pm 1.45^{a*}$
Viscera	10.1 ± 1.47	$34.8 \pm 2.72^{a*}$	$37.9 \pm 1.59^{a*}$	$39.5 \pm 2.50^{a*}$	$41.1 \pm 0.66^{a*}$	$42.9 \pm 2.43^{a*}$	$42.4 \pm 1.95^{a*}$

*Values (mean ± SE) in the same row are significantly different when compared to initial means using Dunnette's test ($P<0.05$).
Values (mean ± SE) in the same row with different superscripts are significantly different from each other using SNK mean separation test ($P<0.05$).

and viscera (52.2–68.9%; $P>0.05$) out of the total body fat (0.5–1.1 g) did not change significantly among treatments. No significant differences in ash content (2.8–3.2%) were observed among treatments.

4. Discussion

In the present study, marbled spinefoot offered 25 g dietary lipid kg^{-1} exhibited reduced growth and feed utilization whereas no significant differences in growth performance and feed utilization were found for fish when dietary lipid levels increased from 45 to 125 g kg^{-1}. This suggests that dietary lipid levels of 45 g kg^{-1} probably meet the minimum lipid requirements of marbled spinefoot. However, regression analysis of final body weight vs. dietary lipid content suggests that dietary lipid requirement for optimal growth is 98 g kg^{-1}. Similar low dietary lipid requirement was observed for other herbivorous fishes such as grass carp *(Ctenopharyngodon idella)* (Du et al., 2005) suggesting a low dietary energy requirement for herbivorous fishes.

When fish are offered diets with insufficient lipids, reduced growth performance might be because of insufficient digestible energy or deficiencies in essential fatty acids. In the present study, diets containing 25 g lipid kg^{-1} had the same gross energy content as all other diets. Thus the reduced growth of marbled spinefoot offered 25 g kg^{-1} dietary lipid is probably a result of essential fatty acid deficiencies in the diet or because rabbitfish cannot efficiently utilize carbohydrates. Most fish do not utilize carbohydrates as well as terrestrial animals but utilization differs among species, and is also influenced by carbohydrate source, dietary level, processing, as well as the complexity of the carbohydrate (Wilson, 1994; Hemre and Hansen, 1998; Rawles and Gatlin, 1998. Generally, warmwater species utilize carbohydrates more efficiently than coldwater or marine fish (Wilson, 1994). Moreover, herbivorous and omnivorous fish species utilize carbohydrate better than carnivorous fishes (Shimeno et al., 1977; El-Sayed and Garling, 1988). For example, Sabapathy and Teo (1993) reported that carbohydrases (amylase, laminarinase, maltase, sucrase and trehalase) are present in greater amounts and are more active in the herbivorous rabbitfish *Siganus canaliculatus* than in the carnivorous sea bass *Lates calcarifer*. The authors suggested that the higher amylase activity in rabbitfish indicates easy starch hydrolysis and thus an important role for carbohydrates in energy metabolism. If this quality holds true for all siganids, then fish in the present study offered diets with low lipid but high starch content exhibited low growth performance and FCR values because of a lack of lipids for structural uses and not because of a lack of digestible energy. Another reason might be because excessive dietary carbohydrates can depress growth in some fishes (see Hemre et al., 2002).

Table 4
Effect of dietary lipid levels on whole body composition of marbled spinefoot rabbitfish *Siganus rivulatus* juveniles. Values for protein, lipid and ash are reported as a proportion of wet tissue weight.

Dietary lipid level (g kg^{-1})	Moisture (%)	Crude protein (%)	Crude lipid (%)	Crude ash (%)
Initial	$75.2 \pm 0.28^{*}$	$16.2 \pm 0.20^{*}$	$2.6 \pm 0.21^{*}$	$3.8 \pm 0.18^{*}$
25	71.6 ± 0.88^{a1}	14.6 ± 0.33^{a}	6.5 ± 0.55^{b}	3.2 ± 0.09^{a}
45	72.0 ± 0.38^{a}	14.0 ± 0.19^{a}	6.1 ± 0.15^{b}	3.0 ± 0.16^{a}
65	71.3 ± 0.81^{a}	14.1 ± 0.45^{a}	6.4 ± 0.55^{b}	3.1 ± 0.10^{a}
85	70.3 ± 0.56^{a}	13.1 ± 0.49^{a}	9.0 ± 0.61^{a}	2.8 ± 0.19^{a}
105	70.1 ± 0.55^{a}	13.5 ± 0.38^{a}	8.1 ± 1.15^{a}	3.1 ± 0.10^{a}
125	69.3 ± 0.26^{a}	13.7 ± 0.20^{a}	9.5 ± 0.20^{a}	3.0 ± 0.09^{a}

*Values (mean ± SE) were significantly different from final values for all treatments using Dunnett's test ($P<0.05$).
[1] Values (mean ± SE) in the same column with different superscripts are significantly different from each other using SNK mean separation test ($P<0.05$).

Tacon et al. (1990) evaluated effect of various diets offered to rabbitfish *S. canaliculatus* and found that the best growth and feed efficiency was observed for rabbitfish offered a diet containing 31% crude protein 8% lipid and 38% carbohydrate. These results are very different from those of the present study but comparisons among species from various studies is difficult, as the effect of dietary lipid levels on fish growth performance vary considerably with species, size, age, diet formulation and composition, range of lipid levels tested, and rearing conditions. In the present study, marbled spinefoot with initial size of 2.9 to 3.0 g were reared in environmentally controlled tanks under optimal temperature and salinity conditions for the species (see Saoud et al., 2007a, 2008b) whilst Tacon et al. (1990) performed their study with fish (initial weight: 48–68 g) in cages under natural conditions. The differences in species, initial size and experimental conditions probably caused the large differences in results between these two studies with different rabbitfish species.

Increasing digestible energy content in fish diets by increasing mainly dietary lipid and less often carbohydrate levels is a common nutritional strategy to spare proteins without compromising growth (Cho and Bureau, 2001). A protein sparing effect by lipid supplementation has been well demonstrated for salmonids and sea bass (Watanabe, 1982; Beamish and Medland, 1986; Dias et al., 1998; Torstensen et al., 2001), and jade perch *Scortum barcoo* (Song et al., 2009). However, other researchers have reported no protein sparing effect of dietary lipid (McGoogan and Gatlin, 1999; Vergara et al., 1999; Regost et al., 2001; Wang et al., 2005; Ozorio et al., 2006; Sá et al., 2008). Parazo (1990) noted that rabbitfish *S. guttatus* offered high protein/high energy diets can utilize non-protein energy sources to spare proteins for growth. In the present study protein sparing effect of lipids on growth performance of marbled spinefoot was not evident. An increase in dietary lipid levels from 45 to 125 g kg^{-1} did not improve protein utilization and retention and showed no clear protein sparing effect. Although fish offered 105 g kg^{-1} dietary lipid exhibited significantly greater PER than fish offered 25 g lipid kg^{-1}, the fish offered 105 g lipid kg^{-1} had greater fat gain possibly causing the difference in PER. Additionally, APU values were not affected by increase in dietary lipid levels. A similar observation was reported by Regost et al. (2001) working with turbot (*Psetta maxima*) and they concluded that no protein sparing was evident in their data. Our results are also consistent with other studies on marine species such as mangrove snapper, (*Lutjanus argentimaculatus*) (Catacutan et al., 2001) and white sea bream (*Diplodus sargus*) (Ozorio et al., 2006; Sá et al., 2006, 2008) where increasing dietary lipid levels did not promote growth nor spare protein for growth purposes.

Dietary carbohydrates are also an important and inexpensive source of energy that can spare proteins in fish. In some cases carbohydrates can be digested as efficiently as lipids if a specific carbohydrate to lipid ratio is maintained in the diet. Furthermore, a balance between dietary lipids and carbohydrates affects protein sparing (see Wilson, 1994; Hemre et al., 2002). For some species optimal growth rates have been noted when carbohydrates to lipids occur in equal caloric quantities (Buhler and Halver, 1961). At present there are no published values of optimal caloric content division between lipid and carbohydrate in *S. rivulatus* diets and thus we maintain that at a dietary protein content of 301 g kg^{-1} and an energy density of 16 MJ kg^{-1}, minimum crude dietary lipid necessary for good growth of spinefoot rabbitfish juveniles is at or close to 45 g kg^{-1}.

Generally, an increase in dietary lipid levels is correlated with increases in whole body lipid content and excessive dietary lipids results in excessive fat deposition in visceral cavity, liver, and muscle tissues of fishes (see Peres and Oliva-Teles, 1999; Regost et al., 2001; Martino et al., 2002; Pei et al., 2004; Wang et al., 2005; Martins et al., 2007; Song et al., 2009). Excessive fat deposition in fish tissues may affect processing yield, product quality and storage stability of the final product and consequently its commercial value (Cowey, 1993). In the present study, whole body lipid content was observed to increase with increase in dietary lipid levels (>65 g kg^{-1}). Whole body lipid content in treatments of 25–65 g lipid kg^{-1} doubled as compared to wild marbled spinefoot and tripled in fish offered diets 85–125 g lipid kg^{-1}. These results suggest that the marbled spinefoot tend to increase lipid deposition with increasing dietary lipid levels. Additionally, increase in dietary lipid levels (>65 g kg^{-1}) resulted in decreasing trend of whole body protein proportion with no apparent effect on growth performance. Page and Andrews (1973) suggested that the differences in whole-body protein are lower in fish fed high-lipid diets as a result of dilution with lipid. Similar results where increase in dietary lipid levels results in decrease in whole body protein levels was reported for other fish species (see Parazo, 1990; Bjerkeng et al., 1997; Wang et al., 2005; Song et al., 2009). Although growth performance of marbled spinefoot was not affected by increasing dietary lipid levels (45–125 g kg^{-1}) the fish were fattier by the end of the experimental trial which affected muscle yield and could potentially affect marketability and shelf life of the fish.

Increase in dietary lipid level caused an increase in visceral lipid deposition, similar to reports by Martino et al. (2002), Wang et al. (2005), Hu et al. (2007), and Gao et al. (2010). As the dietary lipid increased, a significant proportion of the lipid was deposited in the viscera. Additionally, various authors report that increased dietary carbohydrate levels cause an undesirable body fat accumulation (Tung and Shiau, 1991; Lin and Shiau, 1995; Shiau and Chuang, 1995; Shiau and Lei, 1999). Marbled spinefoot appears to have the capacity to store lipids even when offered low dietary lipid levels if energy density of the diet is maintained by carbohydrates. Visceral lipid content of fish offered the diet with 25 g kg^{-1} with high carbohydrate content increased by about 30% compared to wild rabbitfish. These results suggest that rabbitfish diets could be made to contain less energy than the 16 MJ kg^{-1} found in the present study without adverse effects. Alternatively, protein content of the diet could be increased. El-Dakar et al. (in press) suggested that the minimum dietary requirement for suitable growth of *S. rivulatus* juveniles is 40% protein when digestible energy of the diet is 16–18 MJ kg^{-1}. An increase in dietary protein might improve muscle growth thus decreasing the proportion of stored fat to body protein.

Some authors maintain that the major site for fat and glycogen deposition in some fishes is the liver (see Hemre et al., 2002). Based on results of the present study, liver lipid content was not affected by dietary lipid levels; no statistical differences were noted in HSI and liver lipid contents among the six treatments. This indicates that liver does not contribute significantly to lipid deposition in the marbled spinefoot. These results were observed in various other fishes (Atlantic halibut *Hippoglossus hippoglossus* (Berge and Storebakken, 1991; Martins et al., 2007); sea bream *Diplodus sargus* (Sá et al., 2006); meagre *Argyrosomus regius* (Chatzifotis et al., 2010)) where no differences in hepatosomatic index due to increases in dietary lipid content were observed. However, muscle lipid content in marbled spinefoot in the present work increased with increase in dietary lipid levels, thus reflecting a tendency for some lipid deposition in muscle. These results are similar to those reported by Peres and Oliva-Teles (1999), Wang et al. (2005), and Song et al. (2009). However, marbled spinefoot is classified as a low fat fish (1.95% lipid in wet muscle tissue) (Saoud et al., 2007b) and this is also confirmed in the present study. Generally, low lipid content of muscle and high visceral lipid content is a characteristic of demersal species that are more sedentary than pelagic species (Sheridan, 1988), and this property is obvious in *S. rivulatus*. Aquacultured marbled spinefoot preferentially stores lipids in the viscera (52.2–68.9%), to a lesser extent in the muscles (6.4–8.9%), and least storage of lipids is in the liver (2.1–3.9%). Such lipid compartmentalization is similar to that of wild marbled spinefoot and other rabbitfish such as *S. javis* (Peiris and Grero, 1972). However, wild juvenile marbled spinefoot are two to three times leaner than farmed congenerics (see Abdel Aziz et al., 1992; Saoud et al., 2008a,b). This is probably because under culture conditions fish have an increased access to feed and greater protein and energy content of feed.

In conclusion, marbled spinefoot rabbitfish have less dietary lipid requirements than typically cultured carnivorous fishes and excessive lipids are stored in the viscera, not in the muscle. Diets could probably be formulated to contain a greater protein to energy (lipid) ratio to yield more rapid growth rates of the fish without excessive fat deposition. *S. rivulatus* were reared commercially in cages in Cyprus and offered a 45% protein, 18% lipid diet manufactured for sea bream but marketing was not very successful because fish were very fatty (personal communication, Giorgos Anastasiades). A low fat diet formulated specially for rabbitfish would help make the aquaculture and marketing of *S. rivulatus* a successful industry.

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