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Effect of Ration Size and Feeding Frequency on Growth, Feed Utilization, Body Composition and Some Haematological Characteristics of Juvenile Snapper, *Lutjanus johnii* (Baloch, 1792)

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Abstract.- The effect of ration size and feeding frequency on growth rate, feed utilization efficiency, body composition and haematological characteristics of juvenile snapper, *Lutjanus johnii* (body weight 27.1−140.0 g) were examined. Fish were maintained in recirculating system (rearing tanks of 0.28 m^3, water temperature 27ºC) and fed with a test diet (protein 40%, lipid 18.4% and energy 321.0 kcal 100 g^{-1} dry diet) at six ration levels of 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5% body weight per day (BW d^{-1}) for four feeding frequencies per day. Each ration level and feeding frequency was randomly assigned to three tanks of fish with 10 fish per tank for 75 days. Significantly higher weight gain, specific growth rate and feed conversion efficiency were observed at ration level from 2.5 to 4.5% BW d^{-1} and feeding frequency of three to four times daily ($P< 0.05$). Broken-line regression on weight gain yielded an estimated ration requirement of 2.45% BW ($Y = 0.52 - 0.007(R - X_{LR})$, $R = 2.45 \pm 0.33$) with 2.9 times daily ($Y = 0.41 - 0.005(R - X_{LR})$, $R = 2.99 \pm 0.21$). There were no significant ($P>0.05$) differences in protein efficiency and retention of protein and energy among treatments. Digestibility of protein, lipid and energy was significantly ($P<0.05$) higher at 2.0 to 2.5% BW d^{-1} and three to four meals daily. The moisture, protein and ash contents of fish whole body, muscle, liver and viscera were not significantly ($P>0.05$) affected by either ration level or feeding frequency. The highest lipid contents of whole body, liver and viscera were observed in fish at ration levels from 2.5 to 4.5% BW d^{-1} and feeding frequency of three to four times daily. The muscle lipids decreased significantly ($P<0.05$) with increasing feeding frequency, whereas that of liver increased. The cholesterol level, plasma triglycerides and haematocrit values remained similar ($P>0.05$) among the fish fed on different ration level and feeding frequency. Total plasma lipids of fish fed 2.5% BW d^{-1} showed the maximum level which statistically differed ($P<0.05$) only from that of fish fed 2.0% BW d^{-1} while total plasma lipids were similar in fish receiving one to four meals daily. The condition factor, viscerosomatic index, hepatosomatic index and mesenteric fat index were significantly ($P< 0.05$) higher in fish at ration levels from 2.5 to 4.5% BW d^{-1} and feeding frequency of three to four times daily. These results suggest that under similar culture conditions, the optimum ration level and feeding frequency of the juvenile snapper (from initial weight of 27.1 to 140.0 g) are 2.5% BW d^{-1} and three times daily, respectively.

Key words: Snapper *Lutjanus johnii*, feed efficiency, hematological characteristics.

INTRODUCTION

The snapper family Lutjanidae constitutes the major demersal fish resource of the northern Arabian Sea, and snappers are costly food fish in local markets. Although there is a considerable commercial fishery (Anonymous, 2012), the demand has increased to such extent that there is now interest in the development of culture methods for commercial production and stock enhancement of snappers (Abbas and Siddiqui, 2009). However, if sustainable aquaculture methods are to be developed, fish farmers and scientists must have a better understanding of the snapper nutrient requirements, which are influenced by ration level and feeding frequency (Houlihan *et al.*, 2008; Jobling, 2012). The optimum ration level and feeding frequency are important for maximum growth, greater food intake, higher feed utilization, higher nutrient retention efficiency, and for stable body conformation and composition in fish (Booth *et al.*, 2008). Feeding at levels higher than the

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optimum, increase in growth is negligible (Jobling, 2012) whereas a sub-optimal ration may result in reduced growth (Du *et al.,* 2006; Abbas and Siddiqui, 2009). Moreover, a ration in excess of the maximum may also lead to a decreased digestive efficiency which has a detrimental effect on water quality (Ng *et al.,* 2000). Therefore, an optimum ration may be supportive to minimize the feed loss, prevent water quality deterioration and reduce production cost in aquaculture of snappers.

Snappers (65 species belonging to genus *Lutjanus*) are mainly confined to the tropical and sub-tropical waters (Coniza *et al.,* 2012). Among these species, the snapper *Lutjanus johnii* (Baloch, 1792) is considered as an esteemed food fish having great potential for export and culturing in South-east Asia (Anonymous, 2012; Coniza *et al.,* 2012; FAO, 2012). It has a good market demand because of its delicious taste. Although some work has been done on the protein and lipid requirements of some snappers (Catacutan *et al.,* 2001; Catacutan and Pagador, 2004), data regarding ration level and feeding frequency of *L. johnii* is scarce. The objectives of this study were to determine the best feeding frequency and rate for juvenile snapper, *L. johnii* growing from 27.1 to 140.0 g at 27°C.

MATERIALS AND METHODS

Feed preparation

A feed was formulated to contain 40% protein using fishmeal and soybean meal as the major sources of protein (Table I). Tapioca was used as a source of carbohydrates. Ingredients were ground and mechanically mixed for 15 min to ensure homogeneity, and fish oil was added, and then mixed again for 15 min. Water (250 ml kg^{-1} dry ingredients mixture) was added and mixed for another 15 min to attain a consistency appropriate for pelleting. The wet mixture was then passed through a mincing machine; the resultant vermicellies were oven-dried at 30°C for 24 h. Thereafter, the crispy material was cut into 2-mm diameter pellets which were stored in polythene bags at –20°C until used. About 50 g of diet was ground in a mortal for chemical analysis. Chromic oxide (0.5%) was well mixed into the diet as an indicator for digestibility determination (De Silva *et al.,* 2000).

Table I. Formulation and chemical analysis of the experimental diet.

Ingredients[1]	g 100 g^{-1} diet (dry)
Soybean meal (defatted)	25.5
Fish meal	16.5
APC (poultry feathers meal)	13.9
Tapioca flour	10.0
Wheat flour	13.0
Lupin seed meal	6.0
Blood meal (ring dried)	4.5
Algal SCP	1.0
Fish oil	5.0
Vitamin/mineral premix[2]	3.5
L-methionine	0.1
L-lysine	0.1
Chromic oxide (Cr_2O_3)	0.5
Chemical analysis[3]	
Crude protein	39.5±0.5
Crude lipid	18.4±0.4
Crude fiber	1.5±0.6
Ash	7.3±0.7
NFE[5]	33.3±0.5
Energy (kcal 100 g^{-1})	321.0±0.5
P/E (mg protein $kcal^{-1}$)	135.2±0.7

[1]Defatted soybean meal, *Glycine max* (CP = 45.7%); fish meal (CP = 61.3%); poultry feathers meal (CP = 84.3%); tapioca flour, *Metroxylon sago* (CP = 3.1%); wheat flour, *Triticum aestivum* (CP = 16.4%); lupin seed meal, *Lupinus albus* (CP = 43.1%); blood meal (CP = 89.5%) and algal SCP, *Spirulina maxima* (CP = 61.5%) purchased from the local markets of Karachi. CP represents crude protein.

[2]Vitamin and mineral mixture contained the following ingredients (g 100 g^{-1} diet): Ascorbic acid (vit C), 15.3; thiamin HCl (vit B6), 1.0; inositol, 39.5; calcium, 1.25; zinc, 1.0; retinol (vit A), 1.0; phosphorus, 3.5; choline chloride, 3.5; magnesium, 2.5; copper, 1.0; pyridoxine (vit B6), 1.3; phospholipids, 3.5; α-tocopherol acetate (vit E), 5.5; folic acid, 0.4; cholecalciferol (vit D3), 7.5; cyanocobalamine (vit B12), 0.006; riboflavin (vit B2), 1.5; menadione sodium bisulphite (vit K3), 0.03; manganese, 2.0; iodine, 2.0; sodium, 1.0; iron, 1.0; nicotinic acid, 4.3; biotin, 0.35.

[3]Dry matter basis (%); mean ± SE, number of determination = 5.

[4]Nitrogen-free extract = 100 – (% protein + % fat + % ash + % fiber)].

Fish and culture facilities

Juvenile snapper (*L. johnii*) were collected from Sonari Channel, Hawks Bay, Karachi in April, 2014 and placed in a circular plastic tank (0.28 m^3) provided with aeration through an air compressor.

The juvenile fish were acclimated to experimental condition and diet for 15 days. During acclimating period, fish were fed experimental diet (40% crude protein) about 2% body weight per day (BW d^{-1}). Feeding was discontinued 48 h before the transfer of fish into the experimental tanks (0.28 m^3) that were connected as a closed recirculating system containing seawater. The system was provided with a biological filter, aeration through an air compressor and thermostat to keep water temperature at 27.0±0.3°C, a temperature within the preferred range of snapper (Emata *et al.*, 1994). Artificial lighting of 630 lx was also provided by a set of two 40-W fluorescent tubes from 08:00 to 21:00 on the water surface. Water quality was measured daily. All the experimental tanks were cleaned every day after each meal.

Experimental design

Two consecutive experiments were conducted in aquaculture laboratory of the Centre of Excellence in Marine Biology, University of Karachi to investigate the effects of ration level and feeding frequency on the growth performance, feed efficiency and body composition of juvenile snapper.

In experiment I, fish (mean initial body weight 27.1±0.7 g) were stocked in the rearing plastic circular tanks in triplicate groups (10 fish per tank). Prior to the stocking, each fish was taken randomly, weighed and distributed among the tanks. Each fish was weighed to the nearest 0.1 g in a tarred water-filled glass beaker (2000 ml) after blotting dry with paper towel. Fish were fed the test diet (Table I) at one of six levels (2.0, 2.5, 3.0, 3.5, 4.0 and 4.5% of their body weight per day, BW d^{-1}) and to apparent satiation, three times a day (09.30, 13.30 and 17.30 h) for 75 days.

In experiment II, juvenile snapper (mean initial body weight 27.1±0.5 g) reared in the same culture system and under the same conditions used in experiment I. There were 10 fish per tank with three replicates of four feeding frequencies a day (once daily at 0800 h, twice daily at 0800 and 1800 h, three times daily at 0800, 1300 and 1800 h, and four times daily 0800, 1120, 1440 and 1800 h) and a feeding level of 2.5% BW d^{-1} (the optimal feeding level attained from experiment I). The experiment was lasted for 75 days.

In both experiments, fish were weighed fortnightly and the daily rations were adjusted accordingly. Feed pellets were spread by hand evenly across the water surface of rearing tanks. The uneaten feed and feces were siphoned out from each tank after 2 h of feeding and in the morning before feeding, respectively. They were dried at 105°C for 12 h, and then kept at −20°C for digestibility estimations (Du *et al.*, 2006).

Sample collection and chemical analysis

In order to evaluate the product (harvested fish) quality, biochemical constituents like protein, carbohydrate, lipid and ash were determined. In addition, fish health was assessed through haematological parameters i-e haematocrit, total lipids, triglycerides and cholesterol. Thus, the biochemical and haematological samples were collected in the following pattern.

A single pool of 50 fish at the beginning and a pool of 10 fish per tank at the end of the experiment were captured and killed by an excess of ethylene glycol-monophenyl ether. The body weight, body length, liver weight and mesenteric fat were measured. Back muscle was dissected without skin. Fish, muscle and liver were then freeze-dried at −20°C until analysis. The moisture, protein, lipid and ash contents of diets and samples were analyzed according to the standard methods (AOAC 2000). Moisture was determined by drying into an oven (Labostar-LG 122, Japan) at 105°C for 24 h; ash by burning in a muffle furnace (Isuzu, Japan) at 550°C for 18 h; crude protein by the Kjeldahl method ($N \times 6.25$) using an automatic Kjeldahl System (Buchi 430/323, Switzerland); crude fiber by acid detergent fiber analysis, crude lipid by chloroform/methanol (2:1, v/v) extraction procedure (Folch *et al.*, 1957). The carbohydrate content was calculated by subtracting the content of lipids, total protein and ash from the dry weight, and gross energy estimation was made using an automatic bomb-calorimeter (Parr Instrument, model 1265, USA). Chromic oxide in the diet and fecal samples from the fish fed the test diet was determined by a wet-acid digestion method (Furukawa and Tsukahara, 1966). All chemical analyses were performed in triplicate and averaged.

Blood samples were taken by cardiac puncture of a single pool of 50 fish at the beginning and a pool of 10 fish per tank at the end of the experiment for analyses of haematocrit, blood plasma total lipids, cholesterol and triglyceride. The haematocrit values were obtained by centrifuging the blood in a readacrit centrifuge and the determinations of plasma constituents were made by using the calorimetric methods of the Boehringer Corp as described by Papoutsoglou and Voutsinos (1988).

Calculations

The data obtained from experiments I and II were analyzed for the weight gain (WG), specific growth rate (SGR), feed conversion efficiency (FCE), protein efficiency ratio (PER), protein retention efficiency (PRE), energy retention efficiency (ERE), apparent digestibility coefficient (ADC) of protein, lipid and energy, condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI) and mesenteric fat index (MFI) using the following formula:

WG (%) = 100 × (final body weight – initial body weight) / initial body weight.

SGR (%) = 100 × (ln final body weight – ln initial body weight) / rearing period in days.

FCE (%) = 100 × wet weight gain (g) / dry food intake (g).

PER = wet weight gain (g) / protein intake (g).

PRE (%) = 100 × [(final whole body protein − initial whole body protein) / total protein intake].

ERE (%) = 100 × [(final whole body energy – initial whole body energy) / total energy intake].

ADC of nutrients or energy (%) = 100 × [1 − {(dietary Cr_2O_3 / fecal Cr_2O_3) × (fecal nutrient or energy / dietary nutrient or energy)}].

CF = 100 × [(wet body weight, g) / (body length, cm)^3].

VSI (%) = 100 × [wet weight of visceral organs and associated fat tissue (g) / wet body weight (g)].

HSI (%) = 100 × [wet weight of liver (g) / wet body weight (g)].

Statistical analysis

The data regarding fish growth rates, feed utilization efficiency and body composition were subjected to one-way analyses of variance (ANOVA) to determine if significant difference (p <0.05) occurred among fish fed different feeding levels and feeding frequencies. Differences between means were assessed at the 5% probability level using Duncan's new multiple range test, as described by Steel and Torrie (1980). The data are presented as mean ± SE of the replicate groups. The optimum feeding level and feeding frequency were determined according to the broken-line regression method (Robbins *et al.,* 2006).

RESULTS

Water quality

Over the duration of the study, salinity was maintained at 36.2±0.7‰. Dissolved oxygen concentration was kept constant at 6.8 ml l^{-1}, and pH was close to 7.7. The concentration of total ammonia (NH_4-N) and nitrites (NO_2-N) did not exceed 0.1±0.008 ml l^{-1} and nitrates remained below 0.02 ml l^{-1}. The water flow rate to the tanks was controlled at 8.0±0.05 l min^{-1}.

Effects of ration size

The survival remained 100% and all fish appeared healthy at the end of this experiment. Final weight, percent weight gain (WG) and specific growth rate (SGR) of fish fed 2.0% BW d^{-1} increased significantly (P<0.05) with increasing feeding level up to 2.5% BW d^{-1} (Table II) but no further increases were observed when feeding level was increased away from 2.5% BW d^{-1}. Broken-line regression of WG against feeding level yielded ($Y = 0.52 - 0.007(R - X_{LR})$, $R = 2.45 \pm 0.33$) an estimated ration requirement of 2.45% BW d^{-1}. Feed conversion efficiency (FCE) for fish fed 2.0% BW d^{-1} was 140.0% and this increased to 200% as fish were fed 2.5% BW d^{-1} and decreased significantly (P<0.05) when ration level was increased to 3% BW d^{-1} and beyond (Table II). Protein efficiency ratio (PER) were similar among treatments. The apparent digestibility coefficient (ADC) of protein did not differ for fish fed 2.0 and 2.5% BW d^{-1}, while it decreased significantly for fish fed 3.0–4.5% BW d^{-1} (P<0.05; Table II). Similar pattern was found for the ADC of lipid and energy.

Table II.- The growth rate, feed utilization efficiency and apparent digestibility of protein, lipid and energy of juvenile snapper fed at different levels for 75 days (Experiment I).

Parameters	Ration level (% BW d^{-1})					
	2.0	2.5	3.0	3.5	4.0	4.5
Final weight (g)	70.2±10.1[a]	139.5±13.2[b]	139.6±12.8[b]	139.8±12.5[b]	139.9±14.9[b]	139.9±17.8[b]
WG[1]	159.0±1.2[a]	414.7±1.8[b]	415.2±2.1[b]	415.8±2.4[b]	416.1±2.8[b]	416.1±2.8[b]
SGR[2]	1.3±0.01[a]	2.2±0.03[b]	2.2±0.19[b]	2.2±0.08[b]	2.2±0.08[b]	2.2±0.10[b]
FCE[3]	140.0±0.4[c]	200.1±0.3[d]	110.2±0.2[b]	70.1±0.3[a]	70.4±0.4[a]	68.7±0.2[a]
PER[4]	1.3±0.4[a]	1.3±0.2[a]	1.4±0.3[a]	1.4±0.4[a]	1.4±0.2[a]	1.4±0.3[a]
ADC[5] (protein)	81.0±1.3[e]	81.3±1.1[e]	78.6±1.4[d]	77.1±1.2[c]	75.4±0.9[b]	73.2±1.1[a]
ADC[5] (lipid)	83.0±0.8[b]	83.4±1.1[b]	79.2±0.6[a]	79.1±1.2[a]	79.4±0.6[a]	79.1±1.2[a]
ADC[5] (energy)	87.0±1.3[b]	87.2±1.0[b]	84.2±1.2[a]	84.0±0.7[a]	84.3±0.9[a]	84.4±1.1[a]

Values (mean ± SE, $n = 3$ and each n consists of 10 fish per replicate) in the same row with similar superscripts are not significantly different ($p > 0.05$). Initial body weight of the fish was 27.1±0.7 g.
[1]Weight gain = 100 × [(final body weight – initial body weight) / initial body weight].
[2]Specific growth rate (%) = 100 × [(ln final body weight – ln initial body weight) / time in days].
[3]Feed conversion efficiency (%) = 100 × [wet weight gain (g) / dry food intake (g)].
[4]Protein efficiency ratio = wet weight gain (g) / protein intake (g).
[5]Apparent digestibility coefficient of nutrients or energy (%) = 100 × [1 − {(dietary Cr_2O_3 / fecal Cr_2O_3) × (fecal nutrient or energy / dietary nutrient or energy)}].

The proportions of moisture, protein and ash contents of fish whole body, muscle, liver and viscera were not significantly ($P>0.05$) affected by feeding levels (Table III). The lipid content of fish fed 2.0% BW d^{-1} was significantly ($P<0.05$) lower than that of fish fed higher rations (Table III). The liver lipid content of fish fed 2.0 to 3.5% BW d^{-1} increased significantly ($P<0.05$) but no further increases were observed when feeding level was increased away from 3.5% BW d^{-1}. The lipid contents of muscle and viscera, and viscerosomatic index (VSI), hepatosomatic index (HSI) and condition factor (CF) of fish fed 2.5, 3.0, 4.0 and 4.5% BW d^{-1} were significantly ($P<0.05$) greater than for the fish fed 2.0% BW d^{-1} (Table III). The mesenteric fat index (MFI) of fish fed 2.0% BW d^{-1} increased significantly ($P<0.05$) with increasing feeding level up to 3.0% BW d^{-1} (Table III) but no further increases were observed when feeding level was increased away from 3.0% BW d^{-1}. The protein retention efficiency (PRE) and energy retention efficiency (ERE) of fish were similar among treatments (Table III). No statistically important differences were observed between groups regarding the cholesterol level, plasma triglycerides and haematocrit values ($P>0.05$; Table IV). Total plasma lipids of fish fed 2.5% BW d^{-1} showed the maximum level which significantly differed ($P<0.05$) only from that of fish fed 2.0% BW d^{-1}.

Effects of feeding frequency

There was no mortality among treatments. Final weight, percent WG, SGR and FCE of the fish fed three and four times daily were significantly ($P<0.05$) greater than for the fish fed once and twice daily (Table V). PER and ADC of protein, lipid and energy for the fish fed one, two, three and four times daily did not appear to differ significantly ($P>0.05$; Table V). Broken-line regression of WG against feeding frequency yielded an estimated number of meals per day of 2.99 ($Y = 0.41 - 0.005(R - X_{LR})$, $R = 2.99 \pm 0.21$).

The relationship between feed ration and body composition alongwith some haematological parameters such as haematocrit, plasma total lipids and cholesterol values was examined, keeping in view that the biological and haematological characteristics could be used as satisfactory criteria for the observation of the general hygienic situation and rearing progress of this fish from fingerling stage to their marketable size as suggested by Abbas and Siddiqui (2003). The moisture, protein, lipid and ash contents of fish whole body, muscle, liver and viscera were not significantly ($P>0.05$) affected by feeding frequency (Table VI). The lipid content of fish fed three and four times daily was

Table III.- Proximate composition (% wet weight basis) of whole body, muscle, liver and viscera, retention efficiency and biological variables of juvenile snapper fed at different levels for 75 days (Experiment I).

Parameters	Feeding level (% BW d^{-1})					
	2.0	2.5	3.0	3.5	4.0	4.5
Whole body						
Moisture	71.6±2.4[a]	71.5±2.1[a]	71.6±2.9[a]	71.5±1.9[a]	71.8±3.7[a]	71.7±3.5[a]
Protein	18.5±0.4[a]	19.2±0.3[a]	19.4±0.6[a]	18.6±0.2[a]	19.0±0.2[a]	19.4±0.8[a]
Lipid	9.1±0.9[a]	11.1±0.6[b]	10.5±1.6[b]	10.7±1.4[b]	10.9±0.3[b]	11.3±0.7[b]
Ash	4.4±0.3[a]	4.3±0.5[a]	4.3±0.2[a]	3.9±0.1[a]	3.7±0.3[a]	4.3±0.1[a]
Muscle						
Moisture	71.8±1.8[a]	72.3±1.6[a]	71.9±1.5[a]	71.7±1.8[a]	72.2±2.7[a]	71.5±2.5[a]
Protein	12.6±1.1[a]	13.4±1.5[a]	13.1±1.2[a]	12.9±0.8[a]	13.1±1.3[a]	13.4±1.2[a]
Lipid	1.4±0.6[a]	1.5±1.3[b]	1.5±1.2[b]	1.6±0.9[b]	1.5±0.6[b]	1.5±1.0[b]
Ash	0.9±0.1[a]	0.7±0.3[a]	0.8±0.2[a]	0.9±0.4[a]	1.1±0.2[a]	0.9±0.2[a]
Liver						
Moisture	62.4±2.0[a]	62.3±1.5[a]	63.3±1.7[a]	63.1±1.5[a]	62.8±2.2[a]	62.9±2.4[a]
Protein	10.5±1.5[a]	11.3±1.2[a]	10.7±1.3[a]	11.4±0.9[a]	10.8±1.3[a]	11.2±1.0[a]
Lipid	9.2±0.06[a]	11.4±0.05[b]	15.9±0.03[c]	19.5±0.07[d]	25.2±0.15[d]	28.5±0.1[d]
Ash	1.1±0.3[a]	1.0±0.4[a]	0.9±0.5[a]	1.2±0.1[a]	0.8±0.6[a]	1.2±0.3[a]
Viscera						
Moisture	57.5±1.5[a]	57.8±2.0[a]	58.2±1.9[a]	58.4±1.3[a]	57.8±2.5[a]	58.3±1.6[a]
Protein	13.8±0.5[a]	14.2±1.3[a]	13.6±1.0[a]	14.4±0.9[a]	14.2±1.2[a]	14.4±1.3[a]
Lipid	10.5±1.3[a]	13.3±1.6[b]	16.5±2.2[c]	17.5±1.8[d]	19.8±1.4[e]	22.5±1.5[f]
Ash	0.6±0.1[a]	0.8±0.1[a]	1.2±0.3[a]	1.4±0.4[a]	1.3±0.3[a]	0.9±0.2[a]
Retention efficiency						
PRE[1]	36.4±1.9[a]	36.5±2.5[a]	36.1±2.6[a]	35.5±0.4[a]	35.6±0.4[a]	36.6±0.5[a]
ERE[2]	37.8±0.5[a]	38.3±1.2[a]	37.9±0.6[a]	38.4±0.5[a]	38.3±0.8[a]	36.6±0.5[a]
Biological analysis						
CF[3]	10.1±1.2[a]	12.2±0.9[b]	12.4 ± 0.8[b]	12.0±1.0[b]	12.1±1.2[b]	12.3±0.6[b]
VSI[4]	6.3±0.1[a]	7.5±0.1[b]	7.6±0.4[b]	7.6±0.2[b]	7.8±0.1[b]	7.7±0.2[b]
HSI[5]	0.8±0.2[a]	1.5±0.1[b]	1.6±0.1[b]	1.3±0.3[b]	1.4±0.1[b]	1.5±0.2[b]
MFI[6]	1.3±0.5[a]	2.4±0.3[b]	2.9±0.5[c]	3.3±0.4[c]	3.8±0.6[cd]	4.2±0.3[cd]

Values (mean ± SE, $n = 3$ and each n consists of 10 fish per replicate) in the same row with similar superscripts are not significantly different ($p > 0.05$). Initial body proximate composition was: moisture 78.1%, protein 17.3%, lipid 4.2% and ash 4.3%, and total lipid contents of liver, viscera and muscle were 8.5%, 13.4% and 0.7%, respectively.

[1]Protein retention efficiency (%) = 100 × [(final whole body protein – initial whole body protein) / total protein intake].

[2]Energy retention efficiency (%) = 100 × [(final whole body energy – initial whole body energy) / total energy intake].

[3]Condition factor = 100 × [(wet body weight, g) / (body length, cm)^3].

[4]Viscerosomatic index = 100 × [wet weight of visceral organs and associated fat tissue (g) / wet body weight (g)]; that of the initial fish was 4.28%.

[5]Hepatosomatic index = 100 × [wet weight of liver (g) / wet body weight (g)]; that of the initial fish was 0.43%.

[6]Mesenteric fat index = 100 × [mesenteric fat weight (g) / wet body weight (g)]; that of the initial fish was 1.2%.

significantly ($P<0.05$) higher than that of fish fed one and two times daily. The lipid contents of liver and viscera increased significantly ($P<0.05$) with increasing feeding frequency, whereas muscle lipid decreased (Table VI). PRE and ERE were similar among treatments. CF, VSI, HSI and MFI of fish fed three and four times daily were significantly ($P>0.05$) higher than those of the fish fed one and two times daily (Table VI). The plasma triglycerides, plasma total lipids and cholesterol levels did not vary among treatments while haematocrit values increased significantly ($P<0.05$; Table VII).

Table IV.- Haematological parameters of juvenile snapper fed at different levels for 75 days (Experiment I).

Parameters	Feeding level (% BW d^{-1})					
	2.0	**2.5**	**3.0**	**3.5**	**4.0**	**4.5**
Haematocrit[1]	43.5±6.2[a]	43.4±8.3[a]	44.0±6.6[a]	44.5±8.4[a]	45.1±9.2[a]	45.5±9.3[a]
Total lipids[2]	1496.7±163.1[a]	1843.9±154.8[b]	1536.6±166.6[ab]	1552.8±170.6[ab]	1583.1±152.3[ab]	1576.5±149.3[ab]
Triglycerides[2]	173.6±53.5[a]	196.7±66.1[a]	211.4±56.6[a]	167.4±57.3[a]	201.2±52.8[a]	187.5±54.6[a]
Cholesterol[2]	154.3±50.3[a]	175.2±51.5[a]	186.7±56.7[a]	164.9±60.7[a]	173.2±55.7[a]	163.2±54.5[a]

Values (mean ± SE, n = 3 and each n consists of 10 fish per replicate) in the same row with similar superscripts are not significantly different ($P > 0.05$). Initial fish blood analysis was: haematocrit 40.5%, total plasma lipids 1385.1 mg 100 ml^{-1}, triglycerides 169.8 mg 100 ml^{-1} and cholesterol 150.7 mg 100 ml^{-1}.
[1]Measured as %.
[2]Measured as mg 100 ml^{-1}.

Table V.- The growth rate, feed utilization efficiency and apparent digestibility coefficient of protein, lipid and energy of juvenile snapper fed at different frequencies for 75 days (Experiment II).

Parameters	Feeding frequency (number of meals d^{-1})			
	1.0	**2.0**	**3.0**	**4.0**
Final weight (g)	72.9±7.9[a]	74.4±7.5[a]	141.3±9.4[b]	139.8±8.6[b]
WG[1]	170.0±1.5[a]	175.1±1.5[a]	423.3±2.5[b]	417.7±1.3[b]
SGR[2]	1.3±0.1[a]	1.4±0.1[a]	2.1±0.2[b]	2.2±0.1[b]
FCE[3]	182.2±0.4[a]	186.0±0.2[a]	199.8±0.4[b]	203.0±0.3[b]
PER[4]	1.3±0.2[a]	1.3±0.3[a]	1.4±0.1[a]	1.4±0.1[a]
ADC[5] (protein)	79.6±1.0[a]	79.5±1.3[a]	80.1±1.1[a]	79.8±1.2[a]
ADC[5] (lipid)	83.6±1.2[b]	83.5±1.3[b]	81.3±1.3[a]	81.4±1.2[a]
ADC[5] (energy)	86.8±1.3[b]	87.3±1.1[b]	85.1±1.2[a]	85.3±1.0[a]

Values (mean ± SE, n = 3 and each n consists of 10 fish per replicate) in the same row with similar superscripts are not significantly different ($P > 0.05$). Initial body weight of the fish was 27.1±0.7g.
[1-5]See footnote of Table II.

DISCUSSION

In the present study, juvenile snapper (*L. johnii*) were shown to require feeding level from 2.5 to 4.5% BW d^{-1} and feeding frequency 3-4 times daily for the highest growth performance, associated with significantly higher weight gain and better feed conversion. Since growth and feed conversion are influenced by feeding rate and higher feeding rates are counterproductive (Ng *et al.*, 2000; Houlihan *et al.*, 2008; Jobling, 2012). Therefore, feeding to fish should be reduced to a minimal level. This minimal level corresponds to 2.5% BW d^{-1} with three times a day in the present study. Feeding above 2.5% BW d^{-1} with four times a day was over the satiety level of the fish and some food waste might occur. Moreover, when feeding was at 2.0% BW d^{-1} with one and two times a day, fish showed the lowest growth which might have been due to the nutrient requirement for maintenance. It appears that a large proportion of nutrient in the diet was used to maintain life, and only a small proportion (2.0% BW d^{-1}) was available for growth. Higher growth of snapper accompanied with higher food conversion parallels the findings in other fishes of the same family, such as mangrove red snapper *Lutjanus argentimaculatus* (Abbas and Siddiqui, 2009; Abbas *et al.*, 2011).

As the total daily amount of feed supply was constant with various feeding frequencies at different ration levels, improvement in weight gain, growth rate and feed conversion was found in snapper juveniles with higher feeding frequency at lower feeding level. Evidence to support this is available in other studies showing that two or three feedings a day was sufficient for maximum growth

Table VI.- Proximate composition (% wet weight basis) of whole body, muscle, liver and viscera, retention efficiency and biological variables of juvenile snapper fed at different frequencies for 75 days (Experiment II).

Parameters	Feeding frequency (number of meals d^{-1})			
	1.0	2.0	3.0	4.0
Whole body				
Moisture	71.8±0.2[a]	71.5±0.1[a]	71.2±0.4[a]	71.4±0.2[a]
Protein	18.9±0.7[a]	19.1±0.6[a]	19.2±0.5[a]	18.5±0.3[a]
Lipid	9.5±0.7[a]	10.1±0.2[a]	11.8±0.4[b]	12.0±0.9[b]
Ash	4.5±0.4[a]	4.4±0.3[a]	4.4±0.4[a]	4.3±0.5[a]
Muscle				
Moisture	72.4±1.5[a]	72.0±1.3[a]	71.8±2.1[a]	72.3±1.6[a]
Protein	13.1±2.2[a]	13.2±1.0[a]	13.4±1.3[a]	13.1±1.5[a]
Lipid	1.6±0.5[c]	1.4±1.0[b]	1.0±1.2[a]	1.1±0.4[a]
Ash	1.1±0.2[a]	0.9±0.1[a]	1.3±0.4[a]	1.4±0.3[a]
Liver				
Moisture	63.1±1.6[a]	63.3±2.1[a]	63.0±1.5[a]	63.4±2.0[a]
Protein	11.4±1.3[a]	10.6±1.4[a]	11.2±1.3[a]	10.9±1.3[a]
Lipid	7.4±0.9[a]	8.3±0.7[b]	8.7±0.5[c]	9.5±0.5[d]
Ash	1.2±0.3[a]	1.3±1.0[a]	1.2±0.2[a]	1.4±0.1[a]
Viscera				
Moisture	58.1±2.3[a]	57.9±2.1[a]	58.0±1.4[a]	58.3±1.1[a]
Protein	14.4±2.3[a]	13.8±1.6[a]	14.3±1.9[a]	13.7±1.5[a]
Lipid	15.4±1.9[a]	18.2±2.5[b]	20.6±2.6[c]	23.5±0.4[d]
Ash	1.2±0.1[a]	1.4±0.3[a]	1.1±0.3[a]	1.3±0.1[a]
Retention efficiency				
PRE[1]	36.0±1.3[a]	35.9±0.7[a]	36.1±1.1[a]	36.3±0.8[a]
ERE[2]	38.2±0.6[a]	38.4±0.8[a]	38.1±0.7[a]	38.3±0.9[a]
Biological analysis				
CF[3]	11.2±0.5[a]	11.4±0.7[a]	12.6 ± 0.9[b]	13.3±0.7[b]
VSI[4]	6.2±0.2[a]	6.4±0.1[a]	7.6±0.3[b]	7.8±0.3[b]
HSI[5]	1.5±0.1[a]	1.6±0.1[a]	2.8±0.2[b]	2.7±0.3[b]
MFI[6]	2.3±0.65[a]	2.1±0.05[a]	2.5±0.03[b]	2.6±0.07[b]

Values (mean ± SE, n = 3 and each n consists of 10 fish per replicate) in the same row with similar superscripts are not significantly different ($P > 0.05$). Initial body proximate composition was: moisture 78.3%, protein 17.6%, lipid 4.01% and ash 4.2%, and total lipid contents of liver, viscera and muscle were 8.5%, 14.3% and 1.1%, respectively.
[1-6]See footnote of Table III.

Table VII.- Haematological parameters of juvenile snapper fed at different frequencies for 75 days (Experiment II).

Parameters	Feeding frequency (number of meals d^{-1})			
	1.0	2.0	3.0	4.0
Haematocrit[1]	32.1±4.5[a]	33.5±6.2[a]	34.0±5.1[a]	35.1±7.2[a]
Total lipids[2]	1688.6±149.2[a]	1795.4±137.6[a]	1823.7±150.4[a]	1840.3±164.5[a]
Triglycerides[2]	169.8±62.9[a]	186.6±67.6[a]	209.2±75.3[a]	232.3±55.1[a]
Cholesterol[2]	147.9±48.9[a]	156.7±49.3[a]	169.3±55.1[a]	174.1±62.2[a]

Values (mean ± SE, n = 3 and each n consists of 10 fish per replicate) in the same row with similar superscripts are not significantly different ($P > 0.05$). Initial fish blood analysis is given in Table IV
[1]Measured as %.
[2]Measured as mg 100 $ml^{-1.}$

of a variety of fish species, for instance sea bass *Dicentrarchus labrax* (Tsevis *et al.*, 1992), grouper *Epinephelus akaara* (Kayano *et al.*, 1993), ayu *Plecoglossus altivelis* (Cho *et al.*, 2003), common carp *Cyprinus carpio* (Charles *et al.*, 1984), channel catfish *Ictalurus punctatus* (Andrews and Page, 1975) and rainbow trout *Oncorhynchus mykiss* (Grayton and Beamish, 1977). Davies (1963) reported that higher growth may be accompanied with an optimum feeding level and feeding frequency for apparent digestibility in fish, and by this ration, the ratio of utilizable energy is highest. In the present study, the apparent digestibility coefficient (ADC) of protein was significantly affected by the feeding level and not by the feeding frequency. The ADC of protein did not change significantly ($P<0.05$) with the increase in feeding level from 2.0 to 2.5% BW d^{-1}, whereas it decreased rapidly when the ration level exceeded 2.5% BW d^{-1}. The constancy of protein digestibility with increased amounts of food from feeding levels 2.0 to 2.5% BW d^{-1} may be because of increased enzyme secretion with increased food intake into the stomach at maintenance level (Windell, 1978; Lei, 2006) and longer retention of food in the stomach and intestine (Elliott, 1972; Garber, 1983). However, excessive food intake may prevent the effects of increased secretion and retention time (Garber, 1983; Lei, 2006), resulting in lower digestibility as meals offered to the fish fed over the satiety level. Over-feeding of the fish might be caused the overloading of stomach and intestine, and decreased the efficiency of ingestion, digestion and assimilation (Jobling, 1986), and thus reduced feed efficiency (Hung and Lutes, 1987). Moreover, the significantly higher digestibility of lipid and energy, and inferior whole body lipid content in fish receiving one and two meals daily at feeding level of 2% BW d^{-1} than those receiving three and four meals daily at feeding levels from 2.5 to 4.5% BW d^{-1} indicated that fish fed with the former level and frequency of feeding may have used lipids to mobilize energy to compensate for a greater energy demand for growth and an elevated metabolic rate. These results are in agreement with those reported in gilthead sea bream *Sparus aurata* (Ginés *et al.*, 2004), transgenic Atlantic salmon *Salmo salar* (Cook *et al.*, 2000), bagrid catfish *Mystus nemurus* (Ng *et al.*, 2000) and ayu *Plecoglossus altivelis* (Cho *et al.*, 2003).

Considering the effects of various feeding frequencies and feeding levels on whole body composition, protein content in fish remained at a comparatively stable level. It comes into view that the lowest feeding level and feeding frequency could provide dietary protein at or slightly above the maintenance level of the fish as suggested by Hung and Lutes (1987), Al-Asgah (1992) and Cho *et al.* (2003). This is also supported by the slight increase of body protein in the fish fed 2% BW day^{-1} as compared to their body protein before they were put on the experiment (18.5% versus 17.3%). This further suggests that body lipid is the preferred energy reserve for deposition or mobilization over protein in juvenile snapper which is inveterate by the lower lipid contents of fish whole body, liver, viscera and muscle were found at 2% BW d^{-1}. Similar conclusions were drawn by Love (1980), Hung and Lutes (1987) and Hung *et al.* (1993). Storebakken and Austreng (1987) believed the variation in the content of fat in salmonids was mainly a direct result of ration level. Likewise, Shimeno *et al.* (1997) while studying the metabolic response to ration level in common carp (*Cyprinus carpio*), mentioned that the activities of pentose phosphate cycle dehydrogenases, glucose-6-phosphate dehydrogenase (G6PDH) and phosphogluconate dehydrogenase (PGDH) were most susceptible to feeding level. According to him, these rapid actions together with the body fat content obviously increased as feeding level increased.

In the present study, an increase in the haematocrit value according to feeding level and feeding frequency might have been due to different metabolic rate of the fish caused by the different amount of food given. These results are similar to the findings of Papotsoglou and Voutsinos (1988). Since all fishes were found healthy over the duration of the experiment, the values of the fish fed 2.0 to 4.5% BW d^{-1} defined the normal range of haematocrit, taking the experimental conditions into consideration. Also, the plasma cholesterol levels, total lipids and triglycerides showed a high variability among individuals. Similar individual tendency has been reported in rainbow trout *Salmo*

gairdneri (McCarthy *et al.,* 1975; Papoutsoglou and Papaparaskeva-Papoutsoglou, 1979; Hile 1982; Papoutsoglou and Voutsinos, 1988) and red-spotted grouper *Epinephelus akaara* (Kayano *et al.,* 1993). In most of the above studies great differences in plasma lipids and cholesterol levels were observed among groups for chemically different foods. In the present study, feeding level and feeding frequency did not cause significant differentiation in the amount of these haematological parameters. Indices of condition, such as condition factor (CF), hepatosomatic index (HSI), viscerosomatic index (VSI) and mesenteric fat index (MFI) are well known as the indicators for the assessment of nutritional status of fish (Reintz, 1983; Cui and Wootton, 1988; Ng *et al*., 2000; Ali, 2001; Salam and Ali, 2001; Iqbal *et al.,* 2015). In the present study, there were no significant differences in CF, HSI, VSI and MFI among the fish fed at different levels and frequency of feeding except 2.0% BW day^{-1} with one and two meals a day where values of these indices were low, suggesting that this ration was suboptimal. Similar differences in the CF, HSI, VSI and MFI for fingerling and subadult of other fish species have been reported as tropical bagrid catfish *Mystus nemurus* (Ng *et al.,* 2000), striped bass *Morone saxatilis* (Hung *et al.,* 1993) and rainbow trout *Oncorhynchus mykiss* (Storebakken and Austreng, 1987; Storebakken *et al.,* 1991) and *Salmo gairdneri* (Reinitz, 1983) and white sturgeon *Acipenser transmontanus* (Hung and Lutes, 1987).

The findings of this study suggest that the optimum ration size and feeding frequency of juvenile snapper, *L. johnii* growing from 27.1 to 140.0 g were 2.5% BW d^{-1} and three meals a day, respectively. At these optimum feedings, improved weight gain, greater food intake, higher feed conversion efficiency, higher digestibility and superior retention efficiency are factors apparently responsible for the faster growth rate and lipid reserves in juvenile snapper under the present experimental conditions.

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