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# The Optimal Protein Requirements of Juvenile Mangrove Red Snapper, *Lutjanus argentimaculatus* Fed Isoenergetic Diets

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**Abstract.-** In order to investigate optimum dietary protein requirement, juvenile mangrove red snapper *Lutjanus argentimaculatus* (body weight 8.0±0.3 g) were reared in seawater tanks (125 liters each) and fed one of the experimental diets at a daily ration of 2% body weight for 90 days. Six isoenergetic (22.4 $kJg^{-1}$) diets were formulated to contain protein levels of 20%, 25%, 30%, 35%, 40% and 45%. Fish fed diets of 40% and 45% protein produced higher weight gain and growth rate than those of the other diets. Broken line regression analysis yielded an optimal protein level of 42.8%. Fish whole body, muscle, liver and visceral composition showed that moisture content of fish fed diets of 40% and 45% protein was significantly higher than that of fish fed diets containing protein levels of 20% to 35% in 5% increments, although the lipid contents were lower. No significant difference was observed in protein and ash contents of whole fish or body organs for the diets of 20% to 45% protein. Fish fed 40% and 45% protein diets showed higher nitrogen gain and nitrogen retention efficiency than those fed on other diets. The mesenteric fat, hepato- and viscerosomatic indices of fish fed diets of 40% and 45% protein were significantly higher than those of fish fed diets of 20%, 25%, 30% and 35% protein. Based on the biological data, it was estimated that the optimal level of protein for *L. argentimaculatus* weighing between 8.0 g and 110 g was 40% to 42.8%.

**Key words:** Mangrove red snapper, growth, protein requirements, nutrient retention, body composition.

## INTRODUCTION

The mangrove red snapper, *Lutjanus argentimaculatus* (Forsskal 1775) is a marine carnivorous fish that has been identified as a potential candidate for aquaculture in South-east Asia, Southern China and the Middle East (Emata *et al*., 1994; Leung *et al*., 1999; Estudillo *et al*., 2000; Catacutan *et al*., 2001; Emata, 2003; Catacutan and Pagador, 2004). In Pakistan, it is known for its good quality meat. Owing to its rapid growth and high commercial value (Anonymous, 2002), there is an interest in its culture (Abbas, 2002; Abbas and Siddiqui, 2009). The sustainable aquaculture of this fish depends on nutritionally balanced fish feed. Since protein is the most expensive component in fish feed, optimizing dietary concentration is essential to minimize feed cost and to formulate feed, which allows good growth and protein utilization (Cowey, 1992, 1995; Serrano *et al*., 1992; Chen and Tsai, 1994; Alvarez-Gonzalez *et al*., 2001; Hecht *et al*., 2003). Information on the nutritional requirements of mangrove red snapper is available to some extent. Dietary protein requirement has been stated to be between 40% and 44% (Catacutan *et al*., 2001; Abbas, 2002). However, these studies did not reveal the changes in liver lipid content and hepatosomatic index of the fish when dietary protein level is increased.

The present study describes the approximate level of dietary protein and energy necessary to achieve optimal growth of mangrove red snapper fed the diets containing protein of 20% to 45% in 5% increments. Further, it gives more insight concerning the association of dietary lipid intake, amount of lipid contents in liver, and hepatosomatic index with the increase in protein levels in diet of the fish during grow-out phase, keeping in view that somatic growth strongly correlates with hepatosomatic index (Dos Santos *et al*., 1993; Jobling, 1988; Lie *et al*., 1988).

## MATERIALS AND METHODS

### *Experimental diet*

Six isoenergetic (22.4 kJ $g^{-1}$ digestible energy) diets were formulated on dry matter basis (g

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$100g^{-1}$) in one batch to supply calculated protein levels from 20% to 45% in 5% increments with fishmeal providing the majority of dietary protein (Table I). Tapioca was used as a source of carbohydrates. A mixture of minerals and vitamins was added to the ingredients of diets. Ingredients were ground to 500µm and mechanically mixed for15 min to ensure homogeneity, 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 mash was pelleted with a California Laboratory Pellet Mill (model CL-type 3, California pellet Mill Company, San Francisco, CA, USA.) using a 2-mm die. No heating or steam was used in the pelleting process and the wet pellets were air-dried at room temperature for 24 hours. The experimental feeds were then stored at –20°C until used.

*Experimental design and feeding trial*

Mangrove red snapper juveniles were obtained from a private fish farm located at Sonmiani, Balochistan, Pakistan. Fish juveniles were acclimated to the experimental conditions for 2 weeks. During the acclimatization period, fish were fed a commercial feed (Marubeni Nisshin Feed, Tokyo, Japan). The analyzed composition of this feed was: 40% protein, 5% fat, 22% carbohydrates, 2.5% fiber, 7.5% ash and 21.5 MJ $kg^{-1}$ gross energy content. After the acclimatization phase, groups of 10 fish (8.0± 0.3 g $fish^{-1}$) were randomly distributed into the experimental circular opaque plastic tanks (volume 0.35 $m^3$) supplied with a continuous flow (4 L $min^{-1}$) of sand filtered seawater with continuous aeration. Fish were subjected to a photoperiod of 12L:12D (light: 08:00-20:00 h) and all tanks had similar light conditions. Each diet was randomly assigned to triplicate tanks. Fish were hand-fed on daily ration of 2% wet body weight per day ($BWday^{-1}$) for 90 days. They were given their daily rations divided into three equal meals per day at 09:30, 13:30 and17:30 h. The daily feed supplied was recorded and uneaten feed was collected 2 h after the start of feeding. The remaining pellets in the tanks were collected by siphoning or netting. Feed intake was calculated from the amount of feed supplied minus collected uneaten feed. The water temperature was maintained at 24±0.5°C (Mean±SD). Salinity was 35.3±0.2‰. Dissolved oxygen was kept constant at 7.5±0.5 mL $L^{-1}$, and pH was 7.5±0.4. Ammonia and nitrites never exceeded 0.1±0.007 mL $L^{-1}$. At the end of the feeding trial, the fish were fasted for 24 h and fish in each tank were weighed and counted.

*Measurement and analysis*

Five fish were randomly sampled from each tank, dissected and their livers and viscera weighed for estimations of the hepatosomatic index (HSI) and the viscerosomatic index (VSI). These indices were calculated as a percentage of organ or tissue to the whole body weight of the fish. Viscera comprised the liver, gastrointestinal tract and intraperitoneal fat. After weighing, the liver and viscera samples of the five fish from each tank were pooled and stored frozen at –20°C for subsequent proximate composition analysis. The remaining five fish were removed from each tank, killed and pooled for whole body composition analysis. Back muscle was dissected without skin. Fish muscle, viscera and whole-body samples were taken out of the –20°C cold store and thawed at room temperature using a fan. Subsequently, all these samples were homogenized, dried and then ground into a powder before chemical composition analysis. At the beginning of the experiment, three replicate samples with 10 fish per replicate were taken and kept frozen at –20°C for subsequent analysis of the viscera, liver, muscle and whole body composition.

The moisture, protein, lipid and ash contents of experimental diets and samples were analyzed according to the standard methods (Association of Official Analytical Chemists, 2000). Moisture was determined by drying in an oven (Labostar-LG 122, Tabai Espec, Osaka, Japan) at 105°C for 24 h; ash by burning in a muffle furnace (Isuzu Seisakusho, Tokyo, Japan) at 550°C for 18 h; crude protein by the Kjeldahl method (N × 6.25) using an automatic Kjeldahl System (Buchi 430/323, Flawil, Switzerland); crude fiber by acid detergent fiber analysis; and crude lipid by the 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

**Table I.- Formulation and proximate composition of the experimental diets.**

| Ingredients[1] (%) | Dietary protein (% dry matter DM) | | | | | |
|---|---|---|---|---|---|---|
| | **20** | **25** | **30** | **35** | **40** | **45** |
| Fish meal | 19.5 | 24.5 | 29.5 | 34.5 | 39.5 | 44.5 |
| Soybean meal | 9.0 | 10.0 | 11.0 | 12.0 | 13.0 | 14.0 |
| Shrimp meal | 2.8 | 2.9 | 3.0 | 3.1 | 3.2 | 3.4 |
| Rice bran | 15.6 | 13.7 | 11.8 | 9.9 | 8.0 | 6.1 |
| Wheat meal | 6.5 | 7.3 | 8.1 | 8.9 | 9.7 | 10.5 |
| Tapioca | 15.0 | 13.0 | 11.0 | 9.0 | 7.0 | 5.0 |
| Dextrin | 19.6 | 17.2 | 14.8 | 12.4 | 10.0 | 7.6 |
| Cod liver oil | 6.5 | 5.9 | 5.3 | 4.7 | 4.1 | 3.5 |
| Vitamin/mineral premix[2] | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Soy lecithin | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Fish protein hydrolysate | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| **Proximate composition**[3] | | | | | | |
| Moisture | 7.9 | 9.4 | 9.5 | 10.5 | 11.6 | 12.1 |
| Crude protein[4] | 19.6 | 24.5 | 29.8 | 34.5 | 39.3 | 44.5 |
| Crude lipid | 7.5 | 7.5 | 7.3 | 7.4 | 7.5 | 7.5 |
| Crude fiber | 2.4 | 4.2 | 6.6 | 9.5 | 10.6 | 14.2 |
| Ash | 5.5 | 6.5 | 7.3 | 8.8 | 10.8 | 11.8 |
| NFE[5] | 65.0 | 57.3 | 49.0 | 39.8 | 31.8 | 22.8 |
| Energy ($kJg^{-1}$) | 22.6 | 22.3 | 22.5 | 22.6 | 22.4 | 22.3 |
| P/E (mg crude protein $kJ^{-1}$) | 8.7 | 11.0 | 13.2 | 15.4 | 17.6 | 20.0 |

[1]Fish meal (CP=61.3%); soybean meal, *Glycine max* (CP=45.7%); shrimp meal, *Acetes* sp. (CP=56.0%); rice bran, *Oryza sativa* (CP=6.1%); wheat flour, *Triticum aestivum* (CP=16.4%); tapioca flour, *Metroxylon sago* (CP=3.1%); soluble fish protein hydrolysate (CP=75.3%) purchased from the local market 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 $B_6$), 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 $B_6$), 1.3; phospholipids, 3.5; α-tocopherol acetate (vit E), 5.5; folic acid, 0.4; cholecalciferol (vit $D_3$), 7.5; cyanocobalamine (vit $B_{12}$), 0.006; riboflavin (vit $B_2$), 1.5; menadione sodium bisulphite (vit $K_3$), 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]Measured as nitrogen × 6.25.

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

ash from the dry weight, and gross energy estimation was made using an automatic bomb-calorimeter (Parr Instrument, model 1265, Moline, IL, USA). All chemical analyses were performed in triplicate and averaged.

*Calculation and statistical analysis*

At the end of the experiment, all fish from each tank were individually weighed and their total length was measured for calculation of the condition factor [CF = (100 × body weight in g)/(TL in cm)$^3$]. Growth and feed efficiency were monitored in terms of the final weight, weight gain (expressed as the percent of initial body weight at the end of the experiment), specific growth rate (SGR) [In (final body weight) – In (initial body weight)/time, where In = natural log, expressed as % per day), feed conversion ratio (FCR) (feed fed /wet weight gain), protein efficiency ratio (PER) (wet weight gain/protein intake), protein retention efficiency [(final whole body protein – final body weight) – (initial whole body protein – initial body weight)/total protein intake] and energy retention efficiency [(final whole body energy – final body weight) – (initial whole body energy – initial body weight)/total energy intake].

The data regarding fish growth rate, feed utilization efficiency and body constituents were subjected to one-way analyses of variance (ANOVAs) to determine whether there was a significant difference ($P$<0.05) among fish fed at different protein levels. Differences between means were assessed at the 5% probability level using Duncan's multiple range test, as described by Steel

and Torrie (1980). The data are presented as mean±SE of the replicate groups. The optimal dietary protein requirements were estimated from percent weight gain of initial weight using the broken line regression analysis (Robbins *et al*., 1979; Cowey, 1992).

## RESULTS

*Growth, feed conversion and condition indices*

Body weight gain and SGR of juvenile mangrove red snapper fed the 40% and 45% protein diets were significantly ($P$<0.05) higher than of those fed the 20%, 25%, 30% and 35% protein diets (Table II). Weight gain and SGR tended to plateau at around 1277.5 g and 2.91% day$^{-1}$ respectively. Based on weight gain, the appropriate supplementation of dietary protein for the fish was estimated to be 42.8% of diet using broken line regression analysis (Fig. 1). Feed intake, expressed on a dry matter basis, decreased slightly with an increase in dietary protein level. Fish fed the 40% and 45% protein diets showed significantly lower ($P$<0.05) feed intake than the other groups. The same trend was observed in FCR and PER values. The HSI, VSI and mesenteric fat index (MFI) of fish fed diets containing 40% and 45% protein were significantly (P<0.05) higher than for those fed diets of 20% to 35% protein (Table II). The survival remained 100% among all groups.

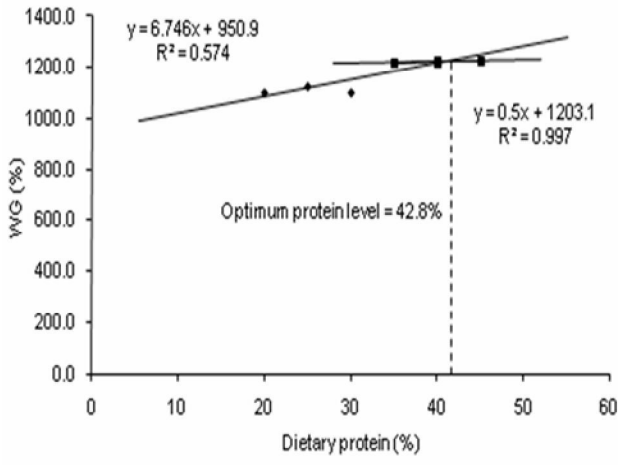


Fig. 1. Optimum protein level based on percent weight gain as determined by the broken line model.

*Body composition*

The chemical composition of whole body, muscle, liver and viscera showed that the moisture content of fish fed diets of 40% and 45% protein was significantly ($P$<0.05) higher than that of fish fed diets containing protein levels of 20% to 35% in 5% increments, although the lipid contents were lower (Table III). No significant differences were observed in the protein and ash contents of fish fed the diets in all treatments.

*Nutrient and energy balance*

Nitrogen intake increased with an increase in dietary protein (Table IV). The amount of protein taken in by the fish fed 40% and 45% protein diets was significantly different ($P$<0.05) from that of fish fed diets containing 20% to 30% protein and 35% protein diet being intermediate. A similar trend was observed in nitrogen gain of the fish whole body. Fish fed 40% and 45% protein diets showed higher nitrogen gain than those fed on all other diets ($P$<0.05). However, there seemed to be a different trend in the values of nitrogen retention efficiency (NRE) which decreased consistently as dietary protein level increased. Fish fed diets containing 40% and 45% protein had a significant better NRE than those of fish given 20%, 25%, 30% and 35% protein (Table IV).

Gross energy intake (GEI) of fish showed a linear decrease as protein level increased over the whole range of dietary protein levels. Although GEI in the fish fed 45% protein was lower (600.67 kJ) than that of 40% protein diet (607.94 kJ), the differences were not statistically significant ($P$>0.05); GEI ranging from 677.31 kJ to 663.20 kJ at remaining four diets (20% to 35% protein) did not appear to differ significantly ($P$>0.05, Table IV). The highest energy gain of 518.33 kJ was obtained with fish fed 40% protein, resulting in the highest energy retention efficiency (ERE) of 85.26%.

*Growth parameter and body constituent relationship*

Significant positive correlation was observed between the CF and the length and the weight of the fish ($P$<0.05, Table V). Regression equations 2, 6, 10, 14, 18 and 22 in Table VI, give the most precise estimates ($R^2$ = 89.73%, 85.59%, 81.56%, 90.15%,

**Table II.- The growth rate and feed utilization of juvenile mangrove red snapper fed at different levels of protein for 90 days.**

| Parameters | Dietary protein (%DM) | | | | | |
|---|---|---|---|---|---|---|
| | 20 | 25 | 30 | 35 | 40 | 45 |
| Final weight (g) | 78.1±0.9[a] | 89.4±0.8[b] | 97.4±0.5[c] | 109.5±0.8[cd] | 110.2±0.4[d] | 110.3±0.6[d] |
| WG, % of initial weight[1] | 876.3±0.3[a] | 1017.5±0.4[b] | 1117.5±0.2[c] | 1268.8±0.6[cd] | 1277.5±0.4[d] | 1278.8±1.3[d] |
| SGR[2] | 2.53±0.06[a] | 2.68±0.06[a] | 2.77±0.05[a] | 2.90±0.03[a] | 2.91±0.01[b] | 2.92±0.01[b] |
| FI[3] (g fish$^{-1}$) | 34.5.±1.5[a] | 33.8±1.3[a] | 33.5±1.2[a] | 33.4±0.5[a] | 30.0±1.7[b] | 29.8±1.1[b] |
| FCR[4] | 0.43±0.02[a] | 0.37±0.01[a] | 0.33±0.01[a] | 0.29±0.02[a] | 0.27±0.01[b] | 0.26±0.01[b] |
| PER[5] | 1.50±0.01[a] | 1.37±0.04[a] | 1.29±0.02[a] | 1.24±0.01[a] | 1.19±0.03[b] | 1.06±0.01[b] |
| CF[6] | 3.6±0.01 | 3.7±0.03 | 3.7±0.01 | 3.6±0.01 | 3.7±0.10 | 3.6±0.11 |
| VSI[7] | 5.8±0.4[a] | 5.9±0.4[a] | 6.7±0.9[a] | 6.9±0.1[a] | 8.7±0.3[b] | 8.8±0.8[b] |
| HSI[8] | 2.6±0.1[a] | 2.8±0.2[a] | 2.8±0.1[a] | 2.7±0.3[a] | 3.7±0.5[b] | 3.9±0.03[a] |
| MFI[9] | 4.5±0.1[a] | 4.6±0.1[a] | 5.0±0.7[a] | 5.1±0.6[a] | 5.8±0.9[b] | 5.9±0.4[b] |
| Survival (%) | 100 | 100 | 100 | 100 | 100 | 100 |

Values (means±SE, n = 3 and each n consists of 10 fish per replicate) in the same row with different superscripts are significantly different ($P$<0.05). Initial body weight of the fish was 8.0 ± 0.3 g.
[1]Weight gain, % of initial weight = 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 intake = total feed fed as % body weight – total uneaten feed.
[4]Feed conversion ratio = total feed fed (g) / total wet weight gain (g).
[5]Protein efficiency ratio = wet weight gain / protein ($N$ × 6.25) intake.
[6]Condition factor (CF) = 100 × (weight / length$^3$).
[7]Viscerosomatic index (VSI) = 100 × [wet weight of visceral organs and associated fat tissue (g) / wet body weight (g)]; that of the initial fish was 5.73%.
[8]Hepatosomatic index (HSI) = wet liver weight (g) / empty fish weight (g) × 100; that of the initial fish was 1.24%.
[9]Mesenteric fat index (MFI) = 100 × [mesenteric fat weight (g) / wet body weight (g)]; that of the initial fish was 1.33%.

**Table III.- Chemical composition (% wet weight basis) of whole body, muscle, liver and viscera of juvenile mangrove red snapper fed at different levels of protein for 90 days.**

| Parameters | Dietary protein (%DM) | | | | | |
|---|---|---|---|---|---|---|
| | 20 | 25 | 30 | 35 | 40 | 45 |
| **Whole body** | | | | | | |
| Moisture | 72.4±1.7[a] | 71.8±1.6[a] | 73.1±1.4[ab] | 73.3±1.5[ab] | 73.5±1.5[c] | 73.7±2.0[c] |
| Protein | 18.5±0.5 | 18.8±0.5 | 18.4±0.6 | 18.5±0.4 | 18.6±0.8 | 18.7±0.7 |
| Lipid | 8.8±0.5[a] | 8.1±0.5[a] | 7.3±0.8[a] | 7.1±1.8[a] | 6.4±1.1[b] | 6.1±0.7[b] |
| Ash | 1.4±0.6 | 1.3±0.7 | 1.4±1.1 | 0.7±0.8 | 2.0±0.6 | 1.6±1.8 |
| **Muscle** | | | | | | |
| Moisture | 71.8±0.9[a] | 71.6±1.0[a] | 72.8±0.6[ab] | 73.4±0.5[ab] | 73.7±1.3[c] | 73.9±1.3[c] |
| Protein | 18.3±0.6 | 18.5±0.2 | 18.4±0.8 | 18.3±0.4 | 18.5±0.8 | 18.4±0.4 |
| Lipid | 1.8±0.9[a] | 1.7±0.3[a] | 1.5±0.7[a] | 1.5±0.5[a] | 0.7±0.6[b] | 0.8±0.4[b] |
| Ash | 0.9±0.5 | 0.9±0.2 | 1.4±0.3 | 1.5±0.5 | 1.6±0.8 | 1.5±0.6 |
| **Liver** | | | | | | |
| Moisture | 58.5±1.0[a] | 58.6±1.3[a] | 59.2±0.5[ab] | 60.4±0.3[ab] | 62.8±0.7[c] | 62.5±1.3[c] |
| Protein | 16.1±0.3 | 16.3±0.4 | 16.3±0.5 | 16.1±0.6 | 16.4±0.9 | 16.2±0.7 |
| Lipid | 9.3±0.6[a] | 9.1±0.5[a] | 9.3±1.7[a] | 9.4±0.6[a] | 7.2±0.4[b] | 7.3±0.8[b] |
| Ash | 0.8±0.4 | 0.9±0.4 | 0.8±0.5 | 1.3±0.7 | 0.8±1.2 | 1.3±0.5 |
| **Viscera** | | | | | | |
| Moisture | 47.2±0.6[a] | 47.4±1.1[a] | 48.5±0.6[ab] | 49.2±0.6[ab] | 51.1±0.5[c] | 51.3±0.4[c] |
| Protein | 18.3±0.5 | 18.7±0.5 | 18.2±0.6 | 18.4±0.4 | 18.7±0.8 | 18.5±0.7 |
| Lipid | 12.6±0.2[a] | 12.5±0.8[a] | 13.3±0.5[a] | 13.4±1.0[a] | 11.4±1.3[b] | 11.4±0.9[b] |
| Ash | 1.3±0.5 | 1.3±0.7 | 1.4±0.4 | 0.9±0.5 | 1.6±0.2 | 1.8±0.9 |

Values (mean±SE, n =3 and each n consists of 10 fish per replicate) in the same row with different superscripts are significantly different ($P$<0.05). Chemical composition of initial body was: moisture 72.8%, protein 18.7%, lipid 6.3% and ash 1.5%, and total lipid contents of muscle, liver and viscera were 0.6%, 7.4% and 12.9%, respectively.

**Table IV.- Nitrogen and energy utilization of juvenile mangrove red snapper fed at different levels of protein for 90 days.**

| Parameters | Dietary protein (%DM) | | | | | |
|---|---|---|---|---|---|---|
| | 20 | 25 | 30 | 35 | 40 | 45 |
| N intake[1] | 1.32±0.02[a] | 1.38±0.03[a] | 1.43±0.01[a] | 1.50±0.02[ab] | 1.61±0.04[b] | 1.93±0.02[b] |
| N gain[2] | 0.23±0.01[a] | 0.24±0.03[a] | 0.24±0.02[a] | 0.25±0.01[a] | 0.27±0.01[b] | 0.26±0.02[b] |
| N retention[3] | 17.42±0.04[a] | 17.39±0.06[a] | 16.78±0.08[a] | 16.67±0.11[a] | 16.77±0.12[ab] | 13.47±1.05[b] |
| E intake | 677.31±1.62[a] | 658.79±2.19[a] | 658.53±3.66[a] | 663.20±2.11[a] | 607.94±1.99[b] | 600.67±0.58[b] |
| E gain[4] | 436.65±0.82[a] | 441.72±0.99[a] | 447.55±2.18[a] | 450.18±4.49[a] | 518.33±2.56[b] | 494.77±3.12[b] |
| E retention[5] | 64.46±0.11[a] | 67.05±0.73[a] | 67.96±0.42[a] | 67.88±0.08[a] | 85.26±2.36[b] | 82.37±1.62[b] |

Values (means±SE, n = 3 and each n consists of 10 fish per replicate) in the same row with different superscripts are significantly different ($P$<0.05). Initial body weight of the fish was 8.0±0.3 g.
[1]Nitrogen intake (g fish$^{-1}$) = feed intake per fish × nitrogen content of feed.
[2]Nitrogen gain (g fish$^{-1}$) = nitrogen in whole body of final fish – nitrogen in whole body of initial fish.
[3]Nitrogen retention (%) = nitrogen gain / nitrogen intake × 100.
[4]Energy gain (kJ fish$^{-1}$) = energy in whole body of final fish – energy in whole body of initial fish.
[5]Energy retention (%) = energy gain / energy intake × 100.

96.25%, 95.66% and SD = 0.034, 0.052, 0.063, 0.079, 0.051, 0.071, respectively) regarding the effect of crude protein on body weight of the fish fed diets containing 20% to 45% protein. Fat content showed a highly significant ($P$<0.01) positive correlation with the length, weight and condition factor of the fish among all groups (Table VI). Regression between ash contents and body weight of the fish in tanks yielded significant ($P$<0.05) relationship with precision ($R^2$ = 39.53% to 55.59% and SD = 0.026 to 0.099).

## DISCUSSION

In the present study, the dietary protein levels of 40% and 45% with 22.4 kJ g$^{-1}$ digestible energy were adequate to optimize both the weight gain and the feeding efficiency in juvenile mangrove red snapper growing from 8.0 g to 110 g. On the basis of maximum weight gain, the estimated protein requirement of the fish was 42.8%. Similar results have been reported in other fish such as golden snapper, *Lutjanus johni* (Hussain and Abbas, 1995), red snapper, *Lutjanus campechanus* (Miller *et al*., 2005), blackspot seabream, *Pagellus bogaraveo* (Silva *et al*., 2006), gilthead seabream, *Sparus aurata* (Santinha *et al*., 1996), spotted sand bass, *Paralabrax maculatofasciatus* (Alvarez-Gonzalez *et al*., 2001); haddock *Melanogrammus aeglefinus* (Kim and Lall, 2001) and singhi, *Heteropneustes fossilis* (Siddiqui and Khan, 2009) which have shown that growth and FCRs improve with high protein diets. In this study, the dietary protein requirements for the growth of mangrove red snapper seem to be in the same range as other marine carnivorous fish species (NRC, 1993). Some studies in gilthead seabream (Santinha *et al.*, 1996), European seabass, *Dicentrarchus labrax* (Peres and Oliva-Teles, 1999), spotted sand bass (Alvarez-Gonzalez *et al.* 2001) and Japanese seabass, *Lateolabrax japonicus*, Cuvier **(**Ai *et al*.**,** 2004) have estimated 40% to 55% as the optimal dietary protein level in terms of growth performance. In the present study, when dietary protein concentration was above 42.8%, mean percent weight gain decreased significantly ($P$<0.05). This indicates that weight gain maxima may be identified in a range of dietary protein concentration from 40% to 45% as suggested by Cowey (1992). According to him, broken line model or an asymptotic model is preferable in attempting weight gain maxima similar in the present study.

Although an increase in dietary protein causes a decrease in PER and NRE (Lee and Putnam, 1973; Bromly, 1980; Pongmaneerat and Watanabe, 1991), a linear increase in nitrogen gain is generally observed until the requirement level is met. This indicates that excess protein is catabolized to provide energy for growth (Lied and Braaten, 1984; Cowey, 1992, 1995). Similar trend was observed in Arctic char (Gurure *et al*., 1995), haddock (Kim and Lall, 2001) and mangrove red snapper in the present study. As the dietary protein level increased, feed intake decreased resulting in a

**Table V.- Simple correlation between growth parameters and body constituents of juvenile mangrove red snapper fed at different levels of protein for 90 days (N = 75).**

| | Length | Weight | Condition factor | Moisture | Crude protein | Crude fat | Ash |
|---|---|---|---|---|---|---|---|
| **Diet: 20%DM** | | | | | | | |
| Weight | 0.993** | | | | | | |
| Condition factor | - 0.656** | -0.239$^{ns}$ | | | | | |
| Moisture | -0.210$^{ns}$ | -0.177$^{ns}$ | -0.161$^{ns}$ | | | | |
| Crude protein | -0.944** | -0.369$^{ns}$ | 0.311$^{ns}$ | -0.337$^{ns}$ | | | |
| Crude fat | 0.664** | 0.933** | 0.538** | 0.117$^{ns}$ | 0.124$^{ns}$ | | |
| Ash | 0.718** | 0.852** | -0.249$^{ns}$ | 0.300$^{ns}$ | -0.344$^{ns}$ | 0.222$^{ns}$ | |
| Gross energy | 0.311$^{ns}$ | 0.283$^{ns}$ | -0.270$^{ns}$ | 0.223$^{ns}$ | 0.642* | 0.551* | 0.182$^{ns}$ |
| **Diet: 25%DM** | | | | | | | |
| Weight | 0.990** | | | | | | |
| Condition factor | -0.823** | -0.173$^{ns}$ | | | | | |
| Moisture | -0.379$^{ns}$ | -0.057$^{ns}$ | -0.267$^{ns}$ | | | | |
| Crude protein | -0.651** | -0.419$^{ns}$ | 0.111$^{ns}$ | -0.227$^{ns}$ | | | |
| Crude fat | 0.835** | 0.813** | 0.648** | 0.402$^{ns}$ | 0.222$^{ns}$ | | |
| Ash | 0.851** | 0.532** | -0.227$^{ns}$ | 0.330$^{ns}$ | -0.216$^{ns}$ | 0.116$^{ns}$ | |
| Gross energy | 0.352$^{ns}$ | 0.333$^{ns}$ | -0.271$^{ns}$ | 0.323$^{ns}$ | 0.553* | 0.463* | 0.051$^{ns}$ |
| **Diet: 30%DM** | | | | | | | |
| Weight | 0.995** | | | | | | |
| Condition factor | -0.624** | -0.443$^{ns}$ | | | | | |
| Moisture | -0.301$^{ns}$ | -0.321$^{ns}$ | -0.462$^{ns}$ | | | | |
| Crude protein | -0.837** | -0.428$^{ns}$ | 0.442$^{ns}$ | -0.257$^{ns}$ | | | |
| Crude fat | 0.768** | 0.733** | 0.738** | 0.442$^{ns}$ | 0.404$^{ns}$ | | |
| Ash | 0.551** | 0.812** | -0.207$^{ns}$ | 0.313$^{ns}$ | -0.346$^{ns}$ | 0.332$^{ns}$ | |
| Gross energy | 0.192$^{ns}$ | 0.254$^{ns}$ | -0.141$^{ns}$ | 0.353$^{ns}$ | 0.553* | 0.541* | 0.194$^{ns}$ |
| **Diet: 35%DM** | | | | | | | |
| Weight | 0.890** | | | | | | |
| Condition factor | -0.539** | -0.415$^{ns}$ | | | | | |
| Moisture | -0.403$^{ns}$ | -0.163$^{ns}$ | -0.226$^{ns}$ | | | | |
| Crude protein | -0.882** | -0.599$^{ns}$ | 0.310$^{ns}$ | -0.115$^{ns}$ | | | |
| Crude fat | 0.547** | 0.753** | 0.558** | 0.412$^{ns}$ | 0.204$^{ns}$ | | |
| Ash | 0.877** | 0.842** | -0.217$^{ns}$ | 0.330$^{ns}$ | -0.316$^{ns}$ | 0.452$^{ns}$ | |
| Gross energy | 0.253$^{ns}$ | 0.284$^{ns}$ | -0.241$^{ns}$ | 0.203$^{ns}$ | 0.543* | 0.541* | 0.078$^{ns}$ |
| **Diet: 40%DM** | | | | | | | |
| Weight | 0.993** | | | | | | |
| Condition factor | -0.774** | -0.116$^{ns}$ | | | | | |
| Moisture | -0.331$^{ns}$ | -0.167$^{ns}$ | -0.301$^{ns}$ | | | | |
| Crude protein | -0.707** | -0.209$^{ns}$ | 0.316$^{ns}$ | -0.237$^{ns}$ | | | |
| Crude fat | 0.658** | 0.733** | 0.518** | 0.332$^{ns}$ | 0.424$^{ns}$ | | |
| Ash | 0.801** | 0.832** | -0.317$^{ns}$ | 0.350$^{ns}$ | -0.336$^{ns}$ | 0.105$^{ns}$ | |
| Gross energy | 0.441$^{ns}$ | 0.241$^{ns}$ | -0.437$^{ns}$ | 0.203$^{ns}$ | 0.665* | 0.566* | 0.113$^{ns}$ |
| **Diet: 45%DM** | | | | | | | |
| Weight | 0.997** | | | | | | |
| Condition factor | -0.624** | -0.269$^{ns}$ | | | | | |
| Moisture | -0.137$^{ns}$ | -0.132$^{ns}$ | -0.352$^{ns}$ | | | | |
| Crude protein | -0.767** | -0.249$^{ns}$ | 0.311$^{ns}$ | -0.357$^{ns}$ | | | |
| Crude fat | 0.663** | 0.763** | 0.588** | 0.422$^{ns}$ | 0.433$^{ns}$ | | |
| Ash | 0.845** | 0.842** | -0.115$^{ns}$ | 0.320$^{ns}$ | -0.300$^{ns}$ | 0.221$^{ns}$ | |
| Gross energy | 0.165$^{ns}$ | 0.264$^{ns}$ | -0.231$^{ns}$ | 0.229$^{ns}$ | 0.402* | 0.562* | 0.158$^{ns}$ |

*($P < 0.05$); **($P < 0.01$); ns = non significant; CF represents condition factor.

**Table VI.- Regression coefficients of body constituents on mean body weight of juvenile mangrove red snapper fed at different levels of protein for 90 days (N = 75).**

| | Equation number | Regression coefficients | | SD[1] | *t*-ratio[2] | $R^2$ |
|---|---|---|---|---|---|---|
| | | Intercept | Slope | | | |
| **Diet: 20%DM** | | | | | | |
| Moisture | 1 | 23.73 | 0.47 | 0.085 | -0.33 | 3.18 |
| Crude protein | 2 | 13.56 | 3.09 | 0.044 | 5.82** | 88.72 |
| Crude fat | 3 | 19.72 | 0.36 | 0.003 | -0.10 | 1.39 |
| Ash | 4 | 23.11 | 0.04 | 0.029 | 1.77* | 38.52 |
| **Diet: 25%DM** | | | | | | |
| Moisture | 5 | 26.81 | 0.07 | 0.074 | -0.91 | 3.46 |
| Crude protein | 6 | 16.43 | 2.99 | 0.055 | 3.55** | 84.39 |
| Crude fat | 7 | 13.22 | 0.56 | 0.006 | -0.44 | 2.00 |
| Ash | 8 | 19.49 | 0.04 | 0.082 | 1.36* | 49.18 |
| **Diet: 30%DM** | | | | | | |
| Moisture | 9 | 21.74 | 0.31 | 0.080 | -0.18 | 2.59 |
| Crude protein | 10 | 9.99 | 2.82 | 0.052 | 3.87** | 81.55 |
| Crude fat | 11 | 23.54 | 0.73 | 0.009 | -0.20 | 2.47 |
| Ash | 12 | 18.87 | 0.11 | 0.043 | 1.96* | 44.37 |
| **Diet: 35%DM** | | | | | | |
| Moisture | 13 | 25.78 | 0.50 | 0.083 | -0.91 | 4.80 |
| Crude protein | 14 | 14.22 | 2.69 | 0.077 | 3.45** | 90.13 |
| Crude fat | 15 | 10.41 | 0.66 | 0.004 | -0.80 | 2.61 |
| Ash | 16 | 23.97 | 0.18 | 0.030 | 1.55* | 46.62 |
| **Diet: 40%DM** | | | | | | |
| Moisture | 17 | 17.9 | 0.12 | 0.069 | -0.53 | 4.31 |
| Crude protein | 18 | 15.42 | 2.79 | 0.050 | 3.61** | 95.13 |
| Crude fat | 19 | 11.22 | 0.49 | 0.003 | -0.49 | 1.97 |
| Ash | 20 | 18.57 | 0.08 | 0.099 | 1.29* | 54.47 |
| **Diet: 45%DM** | | | | | | |
| Moisture | 21 | 32.77 | 0.31 | 0.041 | -0.62 | 4.28 |
| Crude protein | 22 | 21.63 | 2.49 | 0.070 | 5.53** | 94.56 |
| Crude fat | 23 | 5.89 | 0.8 | 0.012 | -0.11 | 2.45 |
| Ash | 24 | 15.49 | 0.15 | 0.030 | 1.47* | 51.99 |

$R^2$ = Proportion of variation accounted for by the regression; *($P < 0.05$); **($P < 0.01$).
[1]Standard deviation of the estimate.
[2]Student's t distribution.

decrease in FCR. This shows that an increase in dietary protein energy could be more beneficial to feed utilization than an increase in lipid energy in the diet (Page and Andrews, 1973; Lovell, 1989). High protein utilization of low protein diets has been observed in many species (El-Dahhar and Lovell, 1995; Webster *et al.*, 1995). In this study, although the diets of protein levels 20%, 25%, 30% and 35% had significantly high PER, the SGR values were low. This indicates that snapper could have efficiently utilized the low protein diet for protein synthesis, thus increasing PER value and suggesting a compensatory mechanism (Berger and Halver, 1987; Catacutan *et al.*, 2001).

Protein and fat contents are generally known as the criterion constituents for determining the quality of fish flesh (Caulton and Bursell, 1977). In the present study, whole body fat was significantly higher for fish fed with diets 20%, 25%, 30% and 35% than for fish fed with diets 40% and 45%. As

dietary protein concentration increased, fat content decreased as in another fish such as sea bass (Metailler *et al*., 1981; Ballestrazzi *et al*., 1994). These results are substantiated by the findings of Zeitler *et al*. (1984), Reis *et al*. (1989), Al-Asgah (1992), Mahboob *et al*. (1996) and Maithya (1998). They observed that the fat contents of fish appeared to be influenced by feeding rhythm with age; correlation among them was positively significant. Similar strong correlation was also observed in the results of the present study. This relationship suggests that as the fish grows its weight increases and proportionately most of this increase is present in the form of fat in fish (Al-Asgah, 1992). The fish first consumes this fat from the liver and starts mobilizing muscle protein only when fat-derived energy has been nearly used up (Love, 1980). After that as protein is utilized, water moves in to take its place. Such a shift results an increase in moisture content of the body. Reinitz (1987) found that moisture content was not significantly correlated with either dietary protein or metabolizable energy for rainbow trout. In the present study, no statistically significant differences in moisture and crude protein contents were found among fish fed with diets 20% to 45%, though moisture content showed a clear inverse relationship with crude fat contents (Love, 1980; Al-Asgah, 1992; Shimma, 1986, Mahboob *et al*., 1996). Evidence to support this is available in other studies of the relationship between protein and water contents in different fish species. Eliassen and Vahl (1982), for example, found that in non-fatty fish, as protein is removed from the muscle, the moisture content rises steadily. Weatherly and Gill (1983) and Al-Asgah (1992) concluded that with increasing fat content, the water content fell (*i.e.*, the dry matter content increased and vice versa). A clear inverse relationship between fat and water content was found and there appeared to be a mechanism for some homeostasis of tissue volume. Additional energy stored as fat replaced body water and did not adversely affect the deposition of protein. The protein content was approximately constant since fat has a protein sparing action in fish. Tveranger (1985) reported that the dry matter and fat in muscle of rainbow trout were positively correlated. A variation in dry content was caused mainly by a variation in fat content. Fat and water to a certain degree substitute each other. With increasing fat content the protein content (% of dry matter) is reduced with a simultaneous increase in dry matter. These findings are in line with the results of the present study and with those of Shimma (1986), who reported significantly negative correlation between moisture content and fat content in two races Yamato and Mirror of carp, *Cyprinus carpio*. In the present study, body fat contents reflected the same of the diets. From this fact, it could be said that 5%–10% dietary lipid should be included in practical diets if appropriate protein and energy levels are provided (Sheen *et al*., 1994). The apparent protein retention (APR) varied inversely with dietary protein. The APR was significantly different in fish fed with diets of 40% and 45% protein than fish fed with diets of 20%–35%.

In the present study, except for lipid content, which was low in fish fed with diets containing 40% and 45% protein, whole body composition was not affected by the dietary treatments. Since protein constitutes the expensive component of the diet and its high concentrations in the diet are counterproductive (Cowey, 1995). Therefore, protein concentration when developing nutritionally balanced diet should be reduced to a minimum level as suggested by Cowey (1992). The dietary protein to energy ratio (P/E) that provided the maximal SGR (2.91% and 2.92%) was found to be 17.6–20.0 mg protein $kJ^{-1}$ digestible energy for juvenile mangrove red snapper fed diets of 40% and 45% protein. This ratio was very close to the reported optimum value (22.0 mg protein $kJ^{-1}$) for the same fish fed 42.6% dietary protein (Hidalgo and Alliot, 1988; De Silva and Anderson, 1995; Catacutan *et al*., 2001). In conclusion, the diet containing 40% to 42.8% dietary protein with P/E ratio of 17.6 mg protein $kJ^{-1}$ could be considered as optimum for the growth of mangrove red snapper juveniles under the experimental conditions of the present study.

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