# Effects of varying dietary protein level on the growth, feed efficiency and body composition of lemon fin barb hybrid fingerlings

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Received: April 2014 Accepted: November 2014

**Abstract**

This study was conducted to determine the optimal dietary protein requirement for lemon fin barb hybrid fingerlings. Triplicate groups of fish (1.00 ± 0.05 g) were fed twice a day until apparent satiation with five isocaloric (16 kJ/g) diets containing varying protein level ranging from 20 to 40% for 60 days. Survival was not affected by the dietary protein level. The weight gain and specific growth rate were improved with dietary protein level up to 35 %. The best feed conversion ratio (1.61) was achieved at 35% dietary protein level. Protein efficiency ratio was not influenced by the dietary protein level. Using a broken line regression model, the dietary protein requirement of lemon fin barb hybrid was estimated at 34.6 % based on weight gain response but the value was not significantly better than the 30% protein diet. Proximate composition of the fish showed that dietary protein level only affected the protein content of the lemon fin barb hybrid. The study showed the protein retention was increased until 30% and decreased above this level. Lipid retention decreased as the dietary protein increased. It can be concluded that lemon fin barb hybrid required 30-35% protein at gross energy 16 kJ/g for the best growth and feed efficiency.

**Keywords:** Lemon fin barb hybrid, Protein requirement, Growth, nutrition, Body composition

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## Introduction

The Department of Fisheries Malaysia (DOF) has successfully produced a new carp hybrid using male lemon fin barb, *Hypsibarbus wetmorei* (locally named Keraikunyit) and female silver barb, *Barbodes gonionotus* (locally named Lampam Jawa) since 2004. Lemon fin barb is a high value carp in Malaysia. This species is found in Kuala Tahan, Pahang River, Malaysia. It is an omnivore that occasionally consumes plant materials (Rainboth, 1996). Meanwhile, silver barb is an important carp species cultured in Indonesia, Malaysia, Thailand and Vietnam. It is a fast growing omnivorous carp that feeds filamentous algae, submerged plants and some invertebrates (Mohanta *et al*., 2008).

The lemon fin barb hybrid, named by DOF as Kerailampam, has a high market value (up to USD 6.26 $kg^{-1}$). It has a meat quality and the external features of lemon fin barb which has tiny tubercles at the tip of the snout with the anal fin and paired fin are brightly colored. In contrast to lemon barb, the hybrid has the fast growing characteristic of silver barb. Interestingly, this hybrid can breed in captivity and is seen as a potential aquaculture candidate for the commercial freshwater fish farming in Malaysia. The successful production of lemon fin barb hybrid in DOF hatcheries has proved that a continuous supply of fry for intensively culturing this species is possible and feasible.

Although the culture of this hybrid species is still at the infancy state, the interest among the small-scaled fish farmers has been overwhelming. Fish farmers have been using various commercial feeds to raise this fish. The hatchery operators used a $F_1$ broodstock with 200-250 g of body weight for breeding purposes and the fry can achieve a 500-600 g body weight within a 6-month culture period. Smaller fish of 150-200 g (after 3 months) also receive a good demand for the production of smoked and salted fish, respectively.

As protein represents the most expensive component in a fish feed, the dietary protein content should be maintained at the optimal requirement level for growth and survival to minimize feed cost (Shyong *et al*., 1998; Lee *et al*., 2000). Inadequate protein in diets will result in a reduction or cessation of growth (Ebrahimi and Ouraji, 2012). On the other hand, providing the optimum energy level in fish diet is also important because low dietary energy will result in the partial utilization of dietary protein for energy rather than fully for protein synthesis (Salhi *et al*., 2004).

Being a new commercial aquaculture fish, many aspects of the culture requirements of lemon fin barb hybrid including its feeding and nutritional requirements have not been determined. The readiness of this hybrid to accept artificial feed should facilitate studies into its nutritional requirements, feeding preference, feeding behavior and others. Therefore, the objective of the present study was to determine the optimal dietary protein requirement of lemon fin barb hybrid fingerlings.

## Materials and Methods

### *Experimental set up*

The feeding trial was conducted at the Wet Laboratory of Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia for a period of 8 weeks. Fifteen 100-L glass aquaria (76 cm × 35 cm × 35 cm) each equipped rectangular top filters were used for the fish rearing. Dechlorinated water was used to fill each aquarium (60-L). An adequate level of oxygen was also provided through individual air stones. The walls of each aquarium were partially covered with dark plastics and the top was covered with a net to minimize distraction and to prevent the fish from jumping out.

*Test animals*

Lemon fin barb hybrid fingerlings were supplied by the Perlok Aquaculture Extension Centre, Jerantut, Pahang, Malaysia. The fingerlings (1.00±0.05 g) were induced bred from the F1 hybrid broodstock of *H. wetmorei*× *B. gonionotus*. Upon arrival at the laboratory, the fish were acclimatized in a 1000 L fiberglass tank for two weeks and fed a commercial tilapia diet (Dinding, 32% crude protein). After the acclimation period, twenty fish were randomly stocked into each glass aquarium.

*Test diets*

Five isocaloric (16 kJ/g) test diets with varying protein levels (20, 25, 30, 35 and 40 %) were tested (Table 1). This gross energy level was based on the optimal energy requirement of *B. gonionotus* (Mohanta *et al*., 2008). The diets were processed using a single screw extruder (Brabender KE-19) at 2 mm Ø. The pellets were oven dried at 50°C for about 12h (Lee *et al*., 2000). The test diets were broken into 1 mm crumbles and packed in air tight containers with silica gel.

**Table 1: Feed and proximate composition (as fed basis) of the experimental diets.**

| | Dietary Protein (%) | | | | |
|---|---|---|---|---|---|
| **Ingredient** | 20 | 25 | 30 | 35 | 40 |
| **Fishmeal** | 10 | 10 | 10 | 18.03 | 49.07 |
| **Soybean meal** | 18.27 | 31.23 | 46.38 | 49.92 | 19.15 |
| **Corn meal** | 19.09 | 32.2 | 10 | 10 | 10 |
| **Rice bran** | 20.89 | 22.74 | 10.65 | 0 | 0 |
| **Tapioca starch** | 20 | 20 | 20 | 20 | 20 |
| **Vitamin premix[a]** | 1 | 1 | 1 | 1 | 1 |
| **Mineral premix[b]** | 1 | 1 | 1 | 1 | 1 |
| **Vegetable oil** | 0 | 0 | 1.73 | 2.79 | 1.13 |
| **α-cellulose** | 9.74 | 10.81 | 8.24 | 6.26 | 7.65 |
| **Proximate composition (%)** | | | | | |
| **Moisture** | 8.2 ± 0.30 | 8.4 ± 0.36 | 8.1 ± 0.40 | 8.2 ± 0.26 | 8.5 ± 0.20 |
| **Protein** | 19.10 ± 0.58 | 24.20 ± 0.77 | 29.50 ± 0.78 | 34.60 ± 0.25 | 38.98 ± 0.46 |
| **Lipid** | 6.39 ± 0.10 | 6.49 ± 0.11 | 6.82 ± 0.08 | 6.91 ± 0.11 | 7.02 ± 0.11 |
| **Ash** | 8.12 ± 0.26 | 8.31 ± 0.47 | 8.41 ± 0.09 | 8.75 ± 0.16 | 8.93 ± 0.13 |
| **Crude fiber** | 1.15 ± 0.05 | 1.51± 0.14 | 1.36 ± 0.11 | 1.03 ± 0.03 | 1.33± 0.07 |
| **NFE[c]** | 57.04 | 51.09 | 45.81 | 40.51 | 35.24 |
| **Gross energy (kJ/g)** | 16.17 ± 0.22 | 16.41 ± 0.12 | 16.55 ± 0.14 | 16.32 ± 0.12 | 16.18 ± 0.16 |
| **P:E [d]** | 11.79 ± 0.27 | 14.71 ± 0.43 | 17.79 ± 0.59 | 21.17 ± 0.05 | 24.09 ± 0.36 |

[a] Vitamin premix (g $kg^{-1}$ premix): ascorbic acid, 45; myo-inositol, 5; choline chloride, 75; niacin, 4.5; riboflavin, 1; pyridoxine, 1; thiamin mononitrate, 0.9; Ca-pantothenate, 3; retinyl acetate, 0.6; cholecalciferol, 0.08; vitamin K menadione, 1.7; a-tocopheryl acetate (500 IU $g^{-1}$), 8; biotin, 0.02; folic acid, 0.1; vitamin B12, 0.001;cellulose, 845.1.

[b] Mineral premix (g $kg^{-1}$ premix): KCl, 90; KI, 0.04; $Ca(H_2PO_4).H_2O$,500; NaCl, 40; $CuSO_4.5H_2O$, 3; $ZnSO_4.7H_2O$, 4; $CoSO_4$, 0.02; $FeSO_47H_2O$, 20; $MnSO_4.H_2O$, 3; $CaCO_3$, 215; MgOH, 124; $Na_2SeO_3$, 0.03; NaF, 1.

[c]Nitrogen-free extract (NFE ) = 100 – (moisture + protein + lipid + ash + fiber)

[d] P:E =protein to energy ratio in mg protein / kJ

*Feeding and sampling of fish*

Each test diet was randomly assigned to three aquaria (as replicates). The fish were hand fed to apparent satiation two times daily (0900 and 1600 h). Fish were held under natural photoperiod conditions throughout the feeding trial. Samplings were conducted at every two weeks to minimize stress to the fish (Ingram *et al*., 2005). The fish were bulk weighed using a digital electronic balance (Hossain *et al*., 2012). Specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated at the end of the experiment.

For whole body proximate analysis, 30 fish from the stock at the beginning of the experiment and 15 fish from each aquarium at the end of the experiment were randomly sacrificed, kept in -20°C freezer until the analysis. Five fish from each replicate were also sampled for determination of hepatosomatic index (HSI) and viserosomatic index (VSI).

*Water quality parameters*

Water quality parameters such as water temperature, dissolved oxygen, pH and total ammonia were measured on non-sampling days and the water was 50% changed during samplings (Kamarudin *et al*., 2011). During the experiment, water temperature ranged 28.1-29.7 °C, dissolved oxygen 7.6-7.9 mg/L, pH 7.2-7.7, and ammonia nitrogen ($NH_3$-N) 0.01-1.15 mg/L.

*Chemical Analysis*

The proximate composition of fish samples and experimental diets were analyzed in triplicates (AOAC, 1990). Dry matter was estimated by oven drying the samples at 105°C till a constant weight and crude protein percentage was obtained by a protein analyzer (Foss 2400 Kjeltec Analyzer Unit). Lipid was determined by solvent extraction with petroleum ether (Foss Tecator Lipid Analyzer). Total ash content was determined by incinerating samples at 600°C for 6 h and crude fiber was estimated by acid digestion followed by alkaline digestion (Fibertec 2010 Hot Extractor Foss Tecator). Gross energy content was determined using a bomb calorimeter (Leco AC-350, USA). All percentage of protein, lipid and carbohydrate retentions in the fish body was calculated at the end of experiment.

*Statistical analysis*

Data on survival, growth performance and feed utilization were analyzed by one-way analysis of variance (ANOVA) using Statistical Analysis System 9.3 for Windows (SAS Inc., USA). All percentage data were arcsine transformed prior to analysis. Mean differences between dietary treatments were tested by Tukey's test.

**Results**

Fish were observed to readily accept and actively feed all the test diets. The survival, growth and feed efficiency of lemon fin barb hybrid fed varying dietary protein level are shown in Table 2. No mortality was observed in all treatments during the feeding trial. Weight gain (WG) and SGR increased when the dietary protein level was increased from 20 to 35 % protein and then decreased at 40% protein. The diet containing 35% protein gave the maximum growth (Fig. 1), but the growth was not significantly better ($p$>0.05) than the 30% protein. The lowest growth was observed when the barb hybrid fed a 20% protein diet. The best FCR (1.61) was achieved at 35% dietary protein level which produced the highest efficiency in feed utilization. However, no significant differences ($p$>0.05) in FCR were observed among the treatments. There was a decreasing trend in PER with increasing of dietary protein level. PER ranged between 1.21-2.48 and no significant differences were observed among the high PER group (20-30% dietary protein). HSI and VSI were unaffected by the dietary protein level (Table 2).

**Table 2: Growth performance and feed utilization of lemon fin barb hybrid fed with test diets for 8 weeks**

| | **Dietary Protein (%)** | | | | |
|---|---|---|---|---|---|
| **Parameter** | **20** | **25** | **30** | **35** | **40** |
| Initial body weight (g) | 1.05± 0.11[a] | 0.93± 0.16[a] | 1.00± 0.08[a] | 0.96± 0.03[a] | 1.07± 0.05[a] |
| Final body weight (g) | 1.39± 0.06[c] | 1.48± 0.17[c] | 2.21± 0.10[ab] | 2.36± 0.10[a] | 2.06± 0.04[b] |
| Body Weight gain (g) | 0.34± 0.05[c] | 0.55± 0.05[c] | 1.21± 0.11[ab] | 1.40± 0.13[a] | 0.98± 0.09[b] |
| Body Weight gain (%) | 33.11± 8.49[d] | 66.52± 14.78[cd] | 121.30± 19.28[ab] | 145.92± 20.05[a] | 92.40± 13.49[bc] |
| Initial total length (cm) | 3.92±0.02[a] | 3.92±0.05[a] | 3.90±0.01[a] | 3.91±0.02[a] | 3.90±0.04[a] |
| Final total length (cm) | 5.17± 0.51[a] | 5.21± 0.19[a] | 5.86± 0.23[a] | 5.96± 0.43[a] | 5.44 ± 0.21[a] |
| SGR (% day$^{-1}$) | 0.47±0.10[d] | 0.76± 0.11[cd] | 1.31± 0.14[ab] | 1.52± 0.11[a] | 1.08 ± 0.11[bc] |
| Daily FI[3] (% BW day$^{-1}$) | 1.23± 0.09[b] | 1.65±0.47[b] | 3.57±0.74[a] | 3.65±0.42[a] | 3.62±0.16[a] |
| FCR | 2.07± 0.08[a] | 1.91 ± 0.24[a] | 1.74±0.22[a] | 1.61±0.15[a] | 2.06 ±0.19[a] |
| PER | 2.48± 0.22[a] | 2.11± 0.28[ab] | 1.93±0.26[ab] | 1.77± 0.18[bc] | 1.21± 0.11[c] |
| HSI (%) | 2.55± 0.11[a] | 2.23± 0.19[a] | 2.27 ± 0.12[a] | 2.38± 0.13[a] | 2.50± 0.13[a] |
| VSI (%) | 7.80± 1.70[a] | 7.71± 1.70[a] | 7.72± 1.07[a] | 7.94± 1.14[a] | 7.82± 1.46[a] |
| Survival (%) | 100 | 100 | 100 | 100 | 100 |

Means (± SD) within a row with the same superscripts are not significant ($p$>0.05).

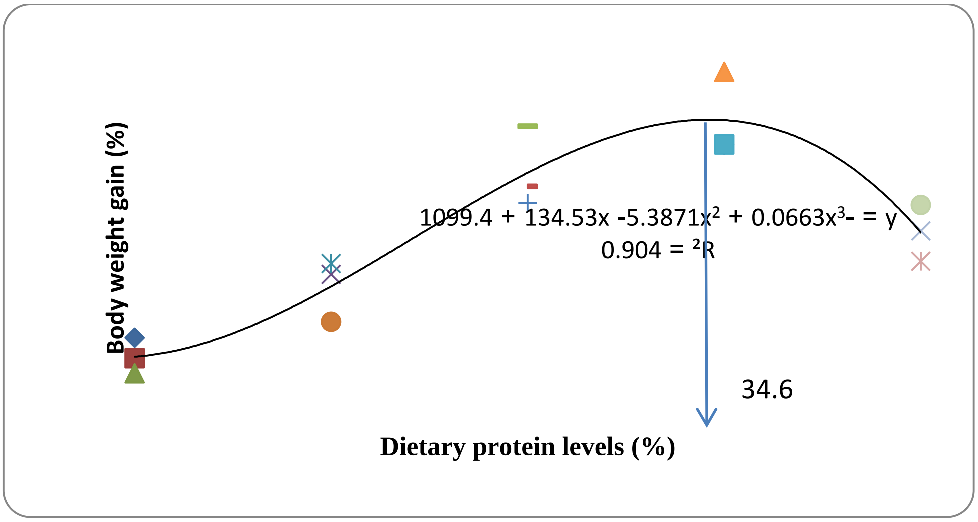


**Figure 1: Polynomial relationship between fish body weight gain (%) and dietary protein levels (%) for lemon fin barb hybrid fingerlings.**

*Body composition*

The whole body proximate compositions of lemon fin barb hybrid at the beginning and end of the experiment are shown in Table 3. The results showed that the dietary protein level only affected the protein content of the barb hybrid. Fish fed 35% protein had the highest body protein but the moisture and body lipid content were similar for all treatments. Fish fed 20-25% protein diets had significantly lower ($p$<0.05) body protein than those fed higher protein diets.

**Table 3: Whole body proximate composition (% wet weight basis) of lemon fin barb hybrid fingerlings after 8 weeks culture period.**

| | | Dietary protein (%) | | | | |
|---|---|---|---|---|---|---|
| | Initial | 20 | 25 | 30 | 35 | 40 |
| Moisture | 73.95 ± 0.96 | 73.20±0.13[a] | 73.08±0.90[a] | 72.50±0.52[a] | 72.35±0.85[a] | 72.72±0.89[a] |
| Crude protein | 15.35± 0.58 | 14.06± 0.20[b] | 14.59± 0.80[b] | 16.55± 0.70[a] | 17.04± 0.33[a] | 16.26± 0.79[a] |
| Crude lipid | 6.42± 0.14 | 9.21 ± 0.80[a] | 8.71± 1.03[a] | 8.34± 0.30[a] | 8.24± 0.75[a] | 8.45± 1.56[a] |
| Ash | 2.22± 0.20 | 1.56± 0.11[a] | 1.59± 0.06[a] | 1.49 ± 0.17[a] | 1.48 ± 0.26[a] | 1.50 ± 0.15[a] |
| Fiber | 0.19 ± 0.01 | 0.16 ± 0.01[a] | 0.15±0.01[a] | 0.18±0.01[a] | 0.11±0.02[a] | 0.14±0.01[a] |
| NFE | 1.87 | 1.81 | 1.88 | 0.94 | 0.78 | 0.93 |
| Gross energy (kJ/g) | 21.68± 0.28 | 24.49 ± 0.38[a] | 23.82 ± 2.23[a] | 22.88± 0.88[a] | 22.77± 0.48[a] | 22.97± 0.97[a] |

Mean values with the same superscripts in each row are not significant ($p>0.05$). Values are means of three replicates of each experimental diet ± SD.

Protein, lipid and carbohydrate retentions of hybrid fingerlings were significantly affected by the dietary protein level (Table 4). Lipid retention was high (>74%) while protein retention was low (<35%) and carbohydrate retention was the lowest (<6%). The study showed the protein retention increased until the dietary protein level reached 30% and then decreased at higher dietary protein levels. Lipid retention decreased as the dietary protein increased. Juveniles fed a 20% dietary protein diet had a significantly higher ($p<0.05$) lipid retention (exceeding 100%) than those fed diets containing higher than 30% protein (<86 %).

**Table 4: Protein, lipid and carbohydrate retention of lemon fin barb hybrid fingerlings during 8 weeks rearing period.**

| Dietary protein (%) | Protein retention (%) | Lipid retention (%) | Carbohydrate retention (%) |
|---|---|---|---|
| 20 | 25.02± 4.28 [bc] | 133.15±16.46[a] | 4.98± 0.66[a] |
| 25 | 28.89± 2.60 [abc] | 100.68 ±8.31[ab] | 5.92 ± 1.16[a] |
| 30 | 34.81 ± 2.37[a] | 84.48 ±11.21[b] | 3.15± 0.22[b] |
| 35 | 32.63± 1.51[ab] | 85.41 ±11.24[b] | 2.71± 0.15[b] |
| 40 | 21.58± 3.27[c] | 73.89 ±22.28[b] | 4.32±0.11[ab] |

Mean values with the same superscripts in each row are not significant ($p>0.05$). Values are means of three replicates of each experimental diet ± SD

## Discussion

The present study indicated that 34.6 % was the optimal dietary protein requirement of lemon fin barb hybrid fingerlings. This value was higher than the optimal requirements of a very closely related species, silver barb (30%) (Mohanta *et al*., 2008) and bighead carp (30%) (Santiago and Reyes, 1991), but lower than those of *Labeo rohita* (Narejo *et al*., 2011). Other carp fingerlings likes *Leptobarbus hoeveni* (Suhenda and Tahapari, 1997), *Tor tambroides* (Misieng *et al*., 2011), *Aristichthys nobilis* (Santiago and Reyes, 1991) and *Catla catla* (Dars *et al*., 2010) have optimal dietary protein requirement of 30-40%. The optimal dietary protein requirement may change with fish species, size, diet formulation, culture conditions, water temperature, dietary energy level and protein quality used in the studies (Elangovan and Shim, 1997; Webster and Lim, 2002). Moreover, different protein sources can affect the protein requirement of a fish (Siddiqui *et al*., 1988). Ogunji *et al*. (2008) showed some of the factors that may contribute to the variation in the results obtained, such as protein composition, amino acid profile, phosphorus content and palatability of feeds.

Providing sufficient dietary energy level is critical because a portion of protein is utilized as an energy source when the fish feed is deficient in energy (Lovell, 1991; Salhi *et al*., 2004; Ali *et al*., 2008). However when fed a high protein diet, protein is deaminated (Winfree and Stickney, 1981) and partially catabolized to provide energy for maintenance, thus reducing the protein conversion efficiency for growth of fish (Lee and Putnam, 1973; Mohanta *et al*., 2008).

Utilization of dietary protein by an organism also depends on the types of diet, digestibility of dietary protein, amino acid profile, the ratio of energy to protein in diet and the amount of protein supplied (Mohanta *et al*., 2008). According to Lim *et al*. (1979), other factors that affect protein utilization are animal size, sex, genotype and environmental conditions.

The feed intake for a 35% protein diet was higher (3.65 % BW $d^{-1}$) than those of other groups and the weight gain was also proportionately more (142.95%) in that group when compared to other groups. It was clear that when fish fed more feed, the results of body weight gain could be more. This implied that the amount of feed consumed in 35% protein was efficiently utilized and contributed to the maximum growth, resulting in lowest FCR compared to other treatments. In this study, high quality fish meal and soybean meal were used as a protein source.

In the present study, the FCR value of the 35% protein diet was 1.61. This result was very close to the FCR obtained for *B. gonionotus* (Mohanta *et al*., 2008). The lowest dietary protein level (20%) produced the highest FCR, it should be probably due to insufficient intake of protein levels to promote growth. When fed a low protein diet, the pressure on body protein seems to be high because dietary nutrient needs to meet the demand of tissue building, repair and metabolism (Winfree and Stickney, 1981).

Fish eat satisfy to meet their energy requirements (Webster *et al*., 1995). According to Santiago and Reyes (1991), a significant decrease in weight gain of bighead carp occurs when it is fed excessive dietary protein (40-50%) because some part of the dietary protein is metabolized and used for energy. The present study also demonstrated a relatively poor weight gain of fish fed the highest protein diet (40%) and low protein diets (20-25%).

Fish fed higher crude protein tend to have lower PER (Siddiqui *et al*., 1988; Lee *et al*., 2000). A similar observation was made in this study. This reconfirmed that excess protein will be utilized to produce energy which leads to lower PER (Hidalgo and Alliot, 1988; Shyong *et al*., 1998). In contrast, the increase in dietary carbohydrate and fat levels cause a reduction in the activity of amino acid-degrading enzymes in the hepatopancreatic, lower nitrogen excretion rate and promote a higher PER (Shimeno *et al*., 1981). This phenomenon (higher PER) was apparent among lemon fin barb hybrid fed lower protein and higher carbohydrate diets.

The decreasing of protein retention with higher dietary protein levels were in agreement with the observations reported by Steffens (1981) in *Salmo gairdneri* and *Cyprinus carpio*. He found that raising the dietary protein level improves growth rate and food conversion but reduces PER and protein productive value (PPV). On the other hand, a reduced protein level causes deterioration of growth rate and food conversion whereas PER and PPV rise.

HSI and VSI of lemon fin barb hybrid were not affected by the varying dietary protein level. Similar findings have been reported for silver barb (Mohanta *et al*., 2008), gilthead seabream (Santinha *et al*., 1995) and juvenile *Dentex dentex* Labrax (Tibaldi *et al*., 1996). Jobling (1988) proposed that HSI in excess of 8–9% should probably be classified as "abnormal". However, HSI values of the present study was considered low and within the safe limit as no gross pathological signs of growth retardation were found in lemon fin barb hybrid.

The dietary protein level only affected the whole body protein content of lemon fin barb hybrid. The highest whole body protein content was achieved when the fingerlings were fed a 35% dietary protein. Hossain *et al*. (2002) noted that the body protein content of Mahseer is lower for fish fed with lower protein than those fed with high protein diets.

In general, lipid retention in lemon fin barb hybrid is high (73-133%) especially among lemon fin barb hybrid that fed lower protein and high carbohydrate. The value of protein and lipid retentions in fish fed 30-35% protein diet were found to be higher than Siberian sturgeon (29.5 and 71.8%) (Mazurkiewicz *et al*., 2009), sultan fish (28.63 and 67.06%) (Suhenda and Tahapari, 1997) and Indian major carps (< 33%) (Erfanullah and Jafri, 1998) fed with a 40% dietary protein. According to Suhenda and Tahapari (1997), lipid retention in the body does not totally come from the dietary lipid in a given diet but from the bioconversion of dietary carbohydrate and protein, respectively. The lipid retention values of more than 100% indicated that lemon fin barb hybrid was able to convert dietary carbohydrate into the body lipid. In addition, the extremely low carbohydrate retention in this hybrid indicated that the fish had efficiently converted most of the dietary carbohydrate to energy and body lipid.

Although the optimal dietary protein requirement was found at 34.6%, a diet containing 30% protein and a gross energy 16 kJ/g should be recommended for the practical culture of lemon fin barb hybrid fingerlings as it gave a similar performance and best protein retention. Further studies on amino acid and protein-energy ratio requirements should be conducted towards the improvement of its low cost practical diet.

**Acknowledgements**

The authors would like to thank the staffs of Perlok Aquaculture Extension Centre, Jerantut Pahang, Malaysia for their cooperation and fry supply. This study was funded by the Ministry of Education Malaysia through FRGS Grant no. 07-01-12-1122FR and RUGS 9303600.

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