Optimum protein requirement for the growth of Jelawat fish (*Leptobabrbus hoevenii*)

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Abstract

A study was conducted to evaluate the growth performance of juvenile Jelawat fish, *Leptobarbus hoevenii*, fed 4 levels of crude protein (CP): 25, 32, 38 and 44% with isocaloric diets of 15.20 MJ/kg. All experimental feeds were formulated using digestible protein values of local feedstuffs; namely poultry offal meal, palm kernel expeller and rice bran. The fish were reared in 1000-litre circular polytanks with 3 replicates with 50 fish per replicate per diet. The feeding trial was conducted for 168 d from fingerling stage of 7.62 cm to 25.40 cm in size. Fish fed 38% CP showed the best growth performance in terms of weight gain (39.86 g), feed conversion ratio (1.70) and specific growth rate (0.72) but were not significantly different (P>0.05) from fish fed 32% and 44%. Lower protein levels used in the experimental diets resulted in lower performance of the Jelawat fish. The lowest protein efficiency ratio (PER) was found in fish fed with 44% CP, and as the protein level in the diet increased, the PER value decreased. Feed formulated with 38% CP was found to be the optimum protein level needed for the growth of Jelawat fish.

Keywords*:* Leptobarbus hoevenii, poultry offal meal, palm kernel expeller, rice bran, growth performance

Introduction

In developing a formulated feed for aquaculture, the main concern is to understand and know the requirements needed of the particular fish species. The nutritional requirements of fish vary from species to species, habitat, feeding habit and others. *Leptobarbus hoevenii* or locally known as Jelawat is one of the most popular high valued freshwater river fish in Malaysia (Mohsin and Ambak, 1983). It is also famously known as White Sultan fish because it was considered as royal meat in the old community long time ago. This fish is an omnivorous fish and can grow up to 60 cm long (Kamarudin *et al.*, 2013). *L. hoevenii* belongs to the carp family (Cyprinidae) and is native to Malaysia. This fish inhabits big rivers of the eastern part of Peninsular Malaysia. Kamarudin *et al.* (2013) also reported that the population of wild *L. hoevenii* has depleted due to overfishing, water pollution from human activities and also disruption in the ecosystem. One of the succesful efforts in increasing the population of *L. hoevenii* in Malaysia was by producing the fish in captivity and applying induced breeding techniques (Ahmad Tajuddin *et al.*, 1983).

However, few studies have been conducted on this particular species, especially on the aspect of nutrition. Information on the digestibility of agricultural by-products and other feed ingredients in locally cultured fish is also lacking, thus research in finding suitable local feed ingredients for local fish becomes imperative (Law, 1984). To date, most of the studies conducted were on breeding techniques carried out in 1970's to 1990's with only few reports on nutrition (Chan *et al.,* 1981; Pathmasothy and Rafiah, 1982; Law, 1984). Therefore, specific nutrients requirement for the growth of this species is still unknown and more research is needed to fully understand its requirements.

River fishes require high protein in their diets due to their physiology and original habitat where protein is utilized for muscle build up. Some Malaysian farmers used pelleted marine feed to feed the fish, as marine feed contains higher crude protein (more than 40%), compared to freshwater fish feed. Source, strain, age, previous nutritional history and the condition of the fish were some of the factors that may influence nutritional studies (NRC, 2011). Protein requirement in fish also differs due to habitat, feeding behaviour and species of fish. Feed cost is one of the constraints in fish production, therefore it is vital to have a comprehensive table of nutritional requirements of this species in order to optimize the use of protein and other nutrients for the development of this species. Besides being the most expensive component in a fish diet, protein also plays a big role in the structure and metabolism of all living organisms (Gore, 2006). The use of local feed ingredients in fish feed is seen to be one way in reducing the operational cost through the cost of fish feed. The aim of this study was to determine the nutrient digestibility of selected local feed ingredients and evaluate the optimal protein requirement for Jelawat fish using their digestible nutrients values.

Materials and Methods

All studies were conducted at the Fish Research Unit, Animal Science Research Centre, Malaysian Agricultural Research & Development Institute (MARDI), Serdang, Selangor.

Fish preparation

A total of 900 Jelawat (*Leptobarbus hoevenii)* fingerlings (7.62 cm in average length) were purchased from a local fish farm in Selangor. The fish were then kept in a 3-tonnes rectangular fiber glass tank for acclimatization prior to digestibility and feeding trials. The fish were acclimatized for about 2 wk and fed to satiation with commercial pellet and aeration was supplied continuously. A day before the trial, fish were fasted in order to ensure that all feed materials in the gut were fully emptied. Fish were graded according to sizes to ensure uniformity prior for study.

Feed preparation

All raw materials used in formulating experimental feeds were purchased from local suppliers. The raw materials and processed feeds were analyzed for moisture, crude protein, lipid, fiber and ash content (AOAC, 2012). The raw materials were ground and pulverized to very fine particles (0.6 mm) and mixed thoroughly according to the formulations as presented in Table 1. The pre mixed ingredients were then extruded through a twin screw extruder (SIMA Brand) to form extruded pellets. The pellets were then dried in an oven at 60˚C for 1 d to achieve moisture content of 10%. All feeds were then subjected to proximate analysis.

Digestibility study

Digestibility of 3 local feedstuffs: poultry offal meal (POM), palm kernel expeller (PKE) and rice bran (RB), was measured using direct or total collection method. The reference diet consisted of fishmeal, which was used as a sole protein source. The experimental diet consisted of 70% of the reference diet and 30% of the test ingredient (POM, PKE, RB) by weight (Lim *et al.*, 2005). A total of 15 Jelawat juveniles (mean weight of 17±1 g) were stocked in a 100-L aquarium glass tank and each tank was assigned to 1 of the 4 dietary diets which were the reference diet (fishmeal as sole protein source) and test diets (poultry offal meal, palm kernel meal and rice bran) with 3 replicates. The fish were fed once a day till satiation at 0900.

All uneaten food was collected an h after feeding. After 4 to 5 h feeding, fresh faeces were collected. Faeces were also collected the next morning before feeding time. The collection of faeces was done by siphoning the faeces into a plankton-net to avoid any damage or break off of the faecal strand which could cause leaching of nutrients. Faecal samples of 15-d collection were pooled, kept in freezer and then dried in the oven at 60˚C for 1 d and ground for analysis. Analysis of dry matter, crude protein, amino acids and energy content of the feed and faeces were performed following the methods of AOAC (2012). The nutrient digestibility was calculated according to the following formula described by Belal (2005) and Lim *et al.* (2005) as presented below:

Apparent Digestibility Coefficient (ADC) of nutrient (%)
= 100 x [amount of nutrient ingested-amount of nutrient egested)/ amount of nutrient ingested]

ADC of test ingredient (%)
= ADC of test diet + [(0.7/0.3) x (% nutrient in reference diet / % nutrient in test ingredient) x (ADC of test diet-ADC of reference diet)]

Feeding trial

A total of 750 fingerlings were randomly transferred in equal number into 15 1000-liters polyethylene tanks and were assigned to the 5 dietary diets with 3 replicates per diet. The diets were A: commercial diet with 43% CP, B: formulated diet with 25% CP, C: formulated diet with 32% CP, D: formulated diet with 38% CP and E: formulated diet with 44% CP. Diet A was designated as Positive Control. Diets B, C, D and E were formulated to be isocaloric (15.2 MJ/kg) with different levels of crude protein (Table 1) using the digestible values of protein and digestible energy. The values of digestible nutrients for poultry offal meal, palm kernel expeller and rice bran were obtained from the present digestibility study (Table 2).

Table 1: Composition of the experimental diets as fed basis (g kg^{-1})

Ingredients	Diet				
	A[1] Control	B 25%CP	C 32%CP	D 38%CP	E 44%CP
Poultry offal meal	na[2]	240.00	300.00	400.00	500.00
Palm kernel expeller	na	140.00	100.00	50.00	40.00
Rice bran	na	100.00	70.00	50.00	50.00
Maize	na	80.00	50.00	50.00	0.00
Soybean meal	na	60.00	50.00	90.00	70.00
Fishmeal	na	80.00	130.00	130.00	130.00
Wheat pollards	na	0.00	40.00	0.00	0.00
Crude palm oil	na	120.00	80.00	50.00	30.00
Cassava	na	120.00	120.00	120.00	120.00
Limestone	na	10.00	10.00	10.00	10.00
NaCl	na	10.00	10.00	10.00	10.00
Premix	na	40.00	40.00	40.00	40.00
Total	na	1000.00	1000.00	1000.00	1000.00
Proximate chemical composition (based on calculation)					
Dry matter	88.00	94.43	93.97	91.02	91.15
Crude protein	43.00	25.00	32.00	38.00	44.00
Crude fat	6.00	19.85	15.65	15.13	14.46
Crude fibre	na	5.38	4.72	4.14	3.70
Gross energy	na	15.19	15.02	15.36	15.45

[1]Positive control 43% Crude Protein (CP)
[2] Not available

The feeding trial was conducted for 168 d from 7.62 cm to 25.40 cm in length using fingerlings. Aeration was supplied continuously throughout the experiment. The fish were fed twice daily (0830 and 1600) with the feeding rate of 3-4% of their body weight. The fish were weighed fortnightly and feeding portion was adjusted depending on the fish weight. Water parameters including dissolved oxygen, temperature, pH and ammonia level were monitored every alternate wk using a multiprobe meter (YSI Proplus model, Yellow Springs) throughout the feeding trial. This was to ensure that these parameters were stable and within the range of the environmental requirement of cultured freshwater fish. In order to maintain water quality, the water in each tank was changed at 50% of the total water volume at least twice a wk depending on the quality of water.

Data on fish performance included body weight gain, percentage of weight gain, feed intake, feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER) which were calculated using the following equations:

Weight gain = Mean final weight – Mean initial weight

Feed intake: Amount of feed consumed throughout the duration of the experiment

Feed conversion ratio (FCR):

$$FCR = \frac{\text{Total feed consumed (g)}}{\text{Live weight gained (g)}}$$

Specific Growth rate (SGR- % per day):

$$SGR = \frac{\ln W_2 - \ln W_1}{T} \times 100$$

where
W_1 = Initial live body weight (g)
W_2 = Final live body weight (g)
T = Experimental period (day)

Protein Efficiency Ratio (PER):

$$PER = \frac{\text{Live weight gain (g)}}{\text{Protein fed (g)}}$$

Statistical analysis

All data were analysed using General Linear Model (GLM) analysis of variance and significant differences between means were separated by Tukey test using the SAS Version 9.3 (ref).

Results and Discussion

Digestibility trial

The digestible coefficients of dry matter, protein and energy for the 3 locally available feed ingredients: poultry offal meal (POM), palm kernel expeller (PKE) and rice bran (RB) are shown as in Table 2. POM showed a very efficient digestibility performance where it was 100% digested by the fish, whereas for rice bran, the digestibility of protein and energy was 78% and 84%, respectively. PKE had the poorest digestibility for protein (74%) and energy (60%). Poultry by-product meal and any other terrestrial animal by-products were found to contain high protein with good essential amino acids profiles (Tacon, 1993). In tilapia, poultry offal meal can be utilised up to 25% in the diet with no adverse effects (Farahiyah *et al.*, 2014) indicating that the digestibility of this ingredient could be high in the fish. Emre *et al.* (2003) also reported that up to 20% of poultry by-product meal could be included in carp diet. Nile tilapia exhibited a 90% digestibility of crude protein from poultry by-product meal (Koprucu and Ozdemir, 2005; Guimaraes *et al.*, 2008). Poultry by-product meal seemed to be one of the potential substitutes for the depleting source of fishmeal (El-Sayed, 1999). In another study by Chan *et al.* (1981), growth performance of Jelawat fish fed poultry processing waste without any diet was found to be promising, indicating that the fish could feed readily on the processing wastes.

Table 2: Apparent digestibility coefficients of dry matter, protein and energy of poultry offal meal, palm kernel expeller and rice bran in Jelawat fish

Ingredients	Dry matter	Protein	Energy
Poultry offal meal	1.00±0.00	1.00±0.00	1.00±0.00
Palm kernel expeller	0.57±0.05	0.74±0.09	0.60±0.06
Rice bran	0.83±0.03	0.78±0.06	0.84±0.04

Digestible coefficient values are presented as Means±SD

Feeding trial

The initial weight gain of individual fish is tabulated in Table 3. The initial weight ranged from 16.67 g to 17.33 g. The final weight of the fish ranged from 47.38 g to 56.53 g. It could be seen that the weight gain of the fish increased as the dietary protein increased, and slightly reduced when the dietary protein exceeded 38%. The net increase in terms of weight gain showed that diet 38% CP had the highest weight gain (39.86 g) over the 24 wk of culture.

Table 3: Mean initial and final body weights (means) of Jelawat fish

Diet[1]	Initial weight (g)	Final weight (g)	Weight gain (g)
A	16.67±0.58	47.87±2.60	31.20±2.69
B	17.33±1.15	47.38±0.20	30.05±1.14
C	16.67±1.15	52.75±1.03	36.09±1.34
D	16.67±1.15	56.53±2.40	39.86±2.56
E	17.33±1.15	56.00±1.74	38.67±2.60

[1]Diets A: commercial diet with 43% CP, B: formulated diet with 25% CP, C: formulated diet with 32% CP, D: formulated diet with 38% CP and E: formulated diet with 44% CP.
No significant difference (P>0.05) between all diets within the same column

At the end of the trial, the weight gain, feed intake and specific growth rate (SGR) increased as the dietary protein levels in the feed increased (Table 4). The feed conversion ratio (FCR) decreased with increasing protein level. The lowest FCR (1.70±0.08) was found in fish fed 38% of crude protein but was not significantly different (P>0.05) compared to the other diets. The highest SGR (0.72±0.04) was also recorded in diet D but was not significantly different from each other except for diet B (0.59±0.03).

Weight gain was lowest in diet 25% CP with 30.05±1.14 g, followed by diet 43% CP, diet 32% CP, diet 44% CP and diet 38% CP. Since the specific protein requirement is unknown for this species, 25% CP may not be sufficient enough to support the growth of the fish, thus resulting in lower weight gain. Fish fed with 38% CP showed the best growth in terms of weight gain (39.86±2.56 g) and was not significantly different when compared with diet E (38.67±2.60 g). As the dietary protein exceeded 38%, the FCR and SGR were slightly reduced (P>0.05) compared the other diets. This showed that at 38% CP, protein dietary intake was already sufficient for the growth of the Jelawat fish. This however, is in contrast with Pathmasothy and Rafiah (1982), who reported that the optimum crude protein requirement for *L.hoevenii* juveniles was between 29 to 34%. The feed intake was lowest in diet 25% CP, but the FCR was slightly better than diet 43% CP with no significant difference (P>0.05). The feed intake in diet A was high compared to diets B, C and D, however, the weight gain was still poor and the FCR seemed to be the poorest.

Table 4: Mean weight gain, feed intake, feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER) of Jelawat fish

Parameters	Diets				
	A	B	C	D	E
Weight gain (g)	31.20±2.69[bc]	30.05±1.14[c]	36.09±1.34[ab]	39.86±2.56[a]	38.67±2.60[a]
Feed intake (g)	67.66±2.95[a]	61.55±2.56[b]	65.81±2.43[ab]	67.54±1.12[ab]	68.91±1.39[a]
FCR	2.18±0.24[a]	2.06±0.15[ab]	1.83±0.14[ab]	1.70±0.08[b]	1.79±0.12[ab]
SGR	0.63±0.04[ab]	0.59±0.03[b]	0.68±0.05[ab]	0.72±0.04[a]	0.70±0.05[ab]
PER	1.07±0.11[d]	1.95±0.15[a]	1.71±0.13[ab]	1.55±0.07[bc]	1.28±.09[cd]
Cost of feed/kg (RM)	4.50	2.88	3.42	3.43	3.44
Cost of fish/kg (RM)	9.81	5.93	6.26	5.83	6.16

[1]A-Commercial (43%CP), B-25%CP, C-32%CP, D-38%CP, E-44%CP
[ab]Means with different superscripts within the same row differ significantly (P<0.05).

The highest PER (1.95) was found in fish fed with diet B, containing the lowest protein percentage (25% CP). The protein efficiency ratio (PER) decreased as the protein content of the feed increased. This effect was also observed by Pathmasothy and Rafiah (1982), where the PER was highest in fish fed with lowest protein level. PER was poorest in diet A (1.07±0.11) when compared with the others (P<0.05) even though the protein content of diet A was high at 43% CP. The low PER in the diet A (commercial diet) could be due to the diet was formulated using total value of the nutrients in the ingredients, whereas for the experimental diets were formulated using digestible values of the ingredients. The low weight gain in diet A supported this assumption. Dietary protein level in feed was found to be directly proportional to weight gain, feed intake, FCR and SGR, but inversely proportional to PER.

The cost of fish feed production was lowest in diet 25% CP and as the inclusion of CP increased the cost of fish feed production also increased. The cost of fish production however was highest in diet A as the FCR was highest, and the cost of the feed was high with RM 9.81 per kg. Lowest cost of fish production was in diet with 38% CP with only RM 5.83 per kg of meat. The cost of production for a kg of fish also needs to be considered in determining the optimum inclusion of CP for the cost effective product.

Conclusion

From this study, the best growth performance of the Jelawat fish *Leptobarbus hoevenii* was attained when fed with 38% CP diet indicating that it might be the optimum dietary protein required for growth. Although there was no significant difference in terms of growth parameters for fish fed diets with 32% CP, 38% CP and 44% CP, but in terms of economic view, fish fed with 38%CP had the lowest cost of production with highest weight gain attained and lowest FCR compared to the others. Utilization of diets containing more than 38% CP did not show any significant difference to further increase the growth rate of the fish.

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