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Growth and survival of river catfish *Mystus nemurus* (Cuvier & Valenciennes) larvae fed isocaloric diets with different protein levels during weaning

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Summary

The growth of river catfish *Mystus nemurus* (Cuvier & Valenciennes) larvae fed four isocaloric diets (4200 kcal kg^{-1}) with different protein levels during weaning was determined. Diets containing 45, 50, 55, and 60% protein were formulated by linear programming using amino acid profiles based on that of 2-day-old river catfish larvae. Artificial diets were fed to the larvae beginning at day 5 after being initially fed *Artemia* nauplii for 4 days. The larvae thrived solely on artificial diets from day 8 to day 16. On the other hand, the control larvae were fed *Artemia* nauplii from day 1 to day 16. Results of the feeding trial showed that growth and survival of *M. nemurus* larvae given the diet containing 60% protein were high and comparable to those of the larvae given only live food (control). Larvae fed the 55% protein diet had significantly lower growth and survival than the larvae on the control and 60% diets but significantly higher growth and survival rates than did larvae fed with 45 and 50% protein diets. Carcass moisture and total lipids after 16 days of feeding did not differ significantly ($P > 0.05$), but body protein increased with increasing dietary protein. Body protein of the control larvae was similar to that of larvae given the 60% protein diet.

Introduction

In Malaysia, hatchery operators use live food for the mass production of river catfish *Mystus nemurus* (Cuvier & Valenciennes) juveniles. To ensure a steady supply of live food, operators have high expenditures on labour costs and facilities (Biedenbach et al. 1989; Hayashi 1995). This often results in a poor return of investment thereby discouraging aquaculture entrepreneurs from engaging in the mass production of *M. nemurus*.

Commercially available artificial feeds are used to supplement the live food requirements in the mass production of juveniles. However, this use is based more on availability than suitability (Tidwell et al. 1992); the available artificial feeds are not designed specifically for *M. nemurus* larvae and fail to meet the actual protein and other essential nutrient requirements of the larvae. An economically viable commercial production of *M. nemurus* requires reliable diets that will support nutritional requirements for optimal growth and survival. In any diet formulation, protein is considered the most important nutrient as it represents a single group required in largest quantity for growth, and protein sources are normally the most expensive components of a feed (Murai 1992; van der Meer et al. 1995; Catacutan and Coloso 1995). An optimum amount of protein in the diet is important because extremely high protein levels may result in poor growth and increased susceptibility of fish to diseases and parasites due to poor water quality. When fed to fish, diets containing excessive amounts of protein cause toxicity since the fish tends to excrete high amounts of ammonia in the rearing water which may lead to growth depression (Zeitoun et al. 1976). On the other hand, reduction in fish growth could be due to lack of non-protein energy in diets containing high amounts of protein (Jauncey 1982), in which case some protein would be used for energy rather than growth. Moreover, excess dietary protein or amino acids which cannot be stored are catabolized preferentially over carbohydrates and fats and used for energy instead of growth by some fishes (Wilson 1989).

Studies on the protein requirements of *M. nemurus* have been focused on juveniles (Khan et al. 1993) and information regarding the optimum protein level in larval diet is scarce. Concerning amino acids, Wilson and Poe (1985) and Cowey (1994) observed a close relationship between amino acid requirements and the essential amino acid pattern of protein in the tissue or muscle of the fish. Although amino acid composition of the fish muscle may not vary much between species, the relative proportion of structural proteins and the metabolic or physiological needs for specific amino acids may differ between species (Cowey 1994). Furthermore, the success of any diet formulation will also depend on the qualitative and quantitative requirements of the target species. As a part of a larger project on nutrition of the river catfish larvae, the present study was therefore conducted to formulate artificial diets with varying protein levels specifically for *M. nemurus* larvae and to determine the effects of the diets on growth, survival and body composition of the larvae.

Materials and methods

Four isocaloric diets with 45, 50, 55 and 60% protein were formulated (Table 1) using DHLLP linear programming software. The amino acid profile of the *Mystus nemurus* larvae (Table 2) was used as the basis for the amino acid profile of the diets. The main sources of proteins were locally produced brown fish meal, squid meal, shrimp meal and soybean meal whose amino acid profiles were also determined (Table 2). These protein sources were selected because they are relatively cheap and readily available. Casein and crystalline essential amino acids were also included in order to attain the desired protein levels and amino acid profile of the diets. Fish oil supplied additional gross energy that was fixed at about 4200 kcal kg^{-1} of feed. The gross energy of each feed ingredient was determined directly using Parr Adiabatic Bomb Calorimeter. The diets were pro-

Table 1
Composition of formulated diets for *Mystus nemurus* larvae (g/100 g diet)

Ingredients	Diet			
	1 45% protein	2 50% protein	3 55% protein	4 60% protein
Shrimp meal	14.60	32.06	44.72	42.20
Squid meal	4.83	26.63	41.55	24.76
Soybean meal	29.69	29.99	1.72	–
Fish meal	41.17	4.99	4.99	4.99
Binder	4.00	4.00	4.00	4.00
Min. premix [1]	1.00	1.00	1.00	1.00
Vitamin premix [2]	1.00	1.00	1.00	1.00
Oil (fish)	2.84	0.41	–	–
Casein	–	–	–	21.07
Methionine	0.50	0.56	0.61	0.38
Histidine	0.59	–	–	–
Tryptophan	0.26	0.28	1.02	0.97
Gross energy, GE (kcal 100 g^{-1})	420.0	420.0	420.0	420.0

[1] Mineral content per 100 g diet: 45 mg Mn, 157 mg Zn, 100 mg Fe, 15 mg Cu, 90 mg iodine, 126 mg Ca.
[2] Vitamin content per 100 g diet: 2750 IU vit A, 550 IU vit D, 25 IU vit E, 5 mg vit K, 250 mg choline, 50 mg niacin, 1 mg riboflavin, 10 mg pyrodixine, 10 mg thiamine, 25 mg calcium D-pantothenate, 0.05 mg biotin, 2.5 mg folacin, 0.01 mg vit B_{12}.

Table 2
Essential amino acid (EAA) ratio of the larvae and feed ingredients[1]

Amino acid	Fish meal	Shrimp meal	Squid meal	Soybean meal	2-day-old larvae
Histidine	2.85	5.63	7.15	6.21	4.77
Threonine	11.17	9.88	8.45	7.42	10.73
Arginine	16.87	15.74	14.45	11.75	9.94
Methionine	0.43	0.42	0.30	2.30	5.11
Valine	12.61	9.14	9.86	7.77	11.12
Tryptophan	1.71	3.02	1.69	10.66	12.14
Phenylalanine	19.20	19.34	19.57	20.85	5.85
Isoleucine	8.28	6.73	8.79	6.91	9.31
Leucine	16.31	15.00	14.69	11.05	17.41
Lysine	10.55	15.06	15.02	15.03	13.60

[1] Essential amino acid ratio = (EAA/total EAA) × 100 (Peñaflorida 1989).

cessed in micro-bound form using carrageenan as binder (Teshima et al. 1982). When administered, the feed particles gradually sank to the bottom of the aquaria. Particle size of feed given on the first week was about 450 μm and was gradually increased to 700 μm as the feeding trial progressed.

Experimental fish, treatments and set-up

Two-day-old *M. nemurus* larvae (mean total length = 5.98 $\pm$ 0.66 mm) produced at the University Putra Malaysia Fish Hatchery Complex were used in the experiment. Individual total length and weight of an initial sample of 50 larvae were measured. Total length (to the nearest 0.1 mm) of larvae in a moist Petri dish was determined using a Nikon profile projector at 10 × magnification. Fish were weighed to the nearest 0.1 mg using a platform balance.

The feeding treatments, each with three replicates, were as follows: Treatment I (control) – fish were given newly hatched *Artemia* nauplii twice a day for the entire 16-day rearing period at 5 nauplii ml^{-1} of water for the first week and then 10 nauplii ml^{-1} thereafter; treatment II – fish were given artificial diet containing 45% protein from the start of weaning at day 5 until the end of the experiment; treatment III – fish were fed diet with 50% protein from weaning; treatment IV – fish fed diets containing 55% protein from weaning; and treatment V – fish were given a diet with 60% protein from weaning onwards.

Fifteen 60-L capacity glass aquaria (80 cm × 40 cm × 40 cm) were used. Each aquarium was filled with 30 L water and stocked with 150 larvae. Water in the aquaria was static but 50% of it was removed and replaced twice daily after siphoning faecal material and uneaten food. Water temperature was determined daily and ranged from 26.5 to 29.0 °C with up to 3 °C change per day. The dissolved oxygen was monitored before each sampling and ranged from 6.2 to 8.9 mg L^{-1}; pH, 7.4–8.2; and total un-ionized ammonia (NH_3), 0.02–0.20 mg L^{-1}. The photoperiod was approximately 12 h light and 12 h dark by using fluorescent bulbs.

Feeding management and sampling

The larvae were initially given newly hatched *Artemia* at 5 nauplii ml^{-1} for 4 days. The volume of *Artemia* nauplii concentrate (V) given twice daily was estimated using the following formula: V (ml) = (FR/Asc) × V_t, where: FR = feeding rate, 5 nauplii ml^{-1} or lower during weaning; V_t = total volume of rearing water (30 000 ml including the added volume of water with *Artemia*); and Asc = *Artemia* nauplii count in stock, nauplii ml^{-1} which should be determined first.

Weaning started on the fifth day. After cleaning and changing the water in the aquaria in the morning, the artificial diet was immediately administered while the larvae were very hungry. The feed particles sank to the bottom of the aquaria where the larvae fed on them. After 1 h, the diet was supplemented with *Artemia* nauplii which were given at decreasing density (from 5 nauplii ml^{-1} or 100% density for the first 4 days to 75% density on day 5, 50% density on day 6, and 25% density on day 7). Larvae were fed 100% artificial diet to satiation four times a day (0800, 1100, 1400 and 1800 h) on day 8 onwards. With this scheme, the fish consumed on the average about 300 mg feed day^{-1} per aquarium on days 5 to 8 and about 800 mg feed day^{-1} per aquarium on days 9 to 16.

Twenty fish per aquarium were sampled for total length measurements at days 4, 8, 12 and 16 before feeding in the morning. Relative growth rate [(length at t_1 – initial length/initial length)/$t_1 - t_0$] × 100 (Hopkins 1992) was determined every 4 days. Final weight and survival (%) were also determined at day 16. Body composition (protein, lipid and moisture) after 16 days of rearing was analysed.

Biochemical analysis

Moisture content of the larvae after 16 days of feeding was determined by freeze-drying the preweighed individuals and then weighing the dried samples (AOAC 1984). Quantitative analysis of protein was carried out following the method of Bradford (1976) using the Bio-Rad assay kit after pretreatment with 0.5N NaOH at 100 °C for 20 min followed by neutralization with HCl (Meyer and Walther 1988). Bovine serum albumin was used as a standard. The method described by Holland and Hannant (1973) was adopted for total lipid determination using tripalmitin as a standard. Absorbance was read at 375 nm (Shimadzu UV 160; UV visible spectrophotometer).

Samples of fish larvae and feed ingredients were analysed for amino acids as follows: (1) protein precipitation and defattening of approximately 20 mg freeze-dried samples; (2) hydrolysis of sample protein with 4N methanosulphonic acid (MSA) containing 0.2% tryptamine (Simpson et al. 1976) with the following modified step – about 2 mg of purified protein was hydrolysed in 200 μl of the acid at 115 °C for 22 h; (3) partial neutralization with 3.5N NaOH to pH 2.2 ± 0.1 and filtration through Whatman cellulose nitrate membrane filter (0.45 μm pore size). Amino acids were derivatized using *o*-phthaldehyde/2 mercaptoethanol reagent (Lee and Drescher 1978). The amino acid composition of the samples was determined through a reverse phase high performance liquid chromatography equipped with a Bio-Rad prepared column (Bio-Sil ODS-5S; 150 mm × 4 mm) with tetra-hydrofuran (THF) solution prepared by mixing (2 : 2 : 96) 50 mM sodium acetate and 50 mM dibasic sodium phosphate (pH adjusted to 6.8), and 65% methanol as solvent. Amino acids were identified from the retention indices obtained from a Sigma amino acid standard. Percentage amino acid was quantified using Millennium software version 2.0.

Statistical analysis

Data on total length and relative growth rate at 4-day intervals, and growth, survival and body composition at day 16 obtained from three replicates per treatment were analysed by analysis of variance. Duncan's multiple range test was used to compare differences between means. Statistical analyses were done with the use of STATS and MGLH modules for IBM-PCs by Systat Inc. (Wilkinson 1987).

Results

Rapid growth in length was observed in all treatments from day 0 to day 4. Total length of the larvae ranged from 11.2 to 11.4 mm at day 4 (Table 3) and did not differ significantly ($P > 0.05$). Subsequently, length increase was observed for all groups, but larvae in treatments I and V grew faster. At day 8, total length of larvae in treatments I (control) and V (60% protein) as well as in treatment IV (55% protein) was higher than that of larvae in treatments III (50% protein) and II (45% protein) (Table 3). This trend was sustained until day 16 of the experiment at which time total length of larvae in treatment I and treatment V did not differ significantly ($P > 0.05$). With regard to the relative growth rate (RGR) of the larvae, a decreasing trend was noted (Table 4). RGR at day 4 ranging from 21.8 to 22.7% per day dipped to 5.5 to 8.9% per day at day 8 and decreased further to 1.3 to 2.2% per day at day 12. However from day 12 to day 16, RGR increased to 1.4 to 2.3% per day except in treatment I (control) which increased to 3.8% per day. Mean final body weight of the larvae increased with increasing protein level in the diets (Table 5). Thus, among the larvae fed artificial diets, those fed the 60% protein diet (treatment V) had the highest final body weight followed by larvae fed the 55% protein diet (treatment IV). The mean final total length followed a trend similar to body weight, with larvae fed the 60% protein diet attaining the highest total length among the weaned fish (Table 5). The control larvae (no weaning) had the highest final weight (78.2 mg) and total length

Table 3
Total length (mm) of *M. nemurus* larvae at 4-day intervals during 16 days of feeding [1]

Treatment (dieatry protein)	Day 4	Day 8	Day 12	Day 16
I (control)	11.3 ± 0.7^{a}	15.1 ± 0.9^{ab}	16.3 ± 1.3^{b}	19.2 ± 1.5^{a}
II (45%)	11.3 ± 0.6^{a}	13.8 ± 1.1^{c}	14.5 ± 1.0^{d}	15.6 ± 1.5^{c}
III (50%)	11.2 ± 0.5^{a}	13.8 ± 0.9^{c}	14.8 ± 0.8^{d}	16.1 ± 1.5^{bc}
IV (55%)	11.4 ± 0.6^{a}	14.6 ± 0.9^{b}	15.4 ± 1.2^{c}	16.8 ± 1.8^{b}
V (60%)	11.4 ± 0.6^{a}	15.5 ± 1.0^{a}	17.0 ± 1.1^{a}	18.8 ± 1.1^{a}

[1] Mean initial total length (day 0), 5.98 ± 0.66 mm.
Means within a column followed by a common letter are not significantly different ($P > 0.05$).

Table 4
Relative growth rate (RGR,% per day) [1,2] of *M. nemurus* larvae determined at 4-day intervals

Treatment (dietary protein)	Day 4	Day 8	Day 12	Day 16
I (control)	21.9 ± 0.8^{ns}	8.4 ± 0.5^{a}	1.8 ± 0.4^{ns}	3.8 ± 0.1^{a}
II (45%)	21.8 ± 0.8	5.5 ± 0.7^{c}	1.3 ± 0.5	1.4 ± 0.5^{c}
III (50%)	22.3 ± 1.0	5.8 ± 0.5^{bc}	1.6 ± 0.5	2.1 ± 0.6^{bc}
IV (55%)	22.5 ± 0.9	6.9 ± 0.8^{b}	1.3 ± 0.3	2.0 ± 0.5^{bc}
V (60%)	22.7 ± 0.7	8.9 ± 0.5^{a}	2.2 ± 0.4	2.3 ± 0.1^{b}

[1] Adopted from Hopkins (1992): RGR = [(length at $time_2$ – length at $time_1$)/(length at $time_1$) × (4 days)] × 100.
[2] Mean initial total length (day 0), 5.98 ± 0.66 mm. Means within a column followed by a common letter are not significantly different ($P > 0.05$).
[ns] no significant difference.

Table 5
Mean body weight, total length, and survival of *M. nemurus* larvae after 16 days of feeding [1,2]

Treatment (dietary protein)	Body weight (mg)	Total length (mm)	Survival (%)
I (control)	78.2 ± 9.0[a]	19.2 ± 1.5[a]	76.6 ± 5.3[a]
II (45%)	42.0 ± 2.0[c]	15.6 ± 1.5[c]	54.2 ± 3.7[c]
III (50%)	47.0 ± 5.2[c]	16.1 ± 1.5[bc]	55.2 ± 4.7[c]
IV (55%)	53.0 ± 1.2[b]	16.8 ± 1.8[b]	72.6 ± 8.3[b]
V (60%)	69.3 ± 3.4[a]	18.8 ± 1.1[a]	76.6 ± 1.7[a]

[1] Means within a column followed by a common letter are not significantly different ($P > 0.05$).
[2] Initial weight = 1.96 mg; initial mean total length = 5.98 ± 0.66 mm.

(19.2 mm) among all treatments; however, no significant difference ($P > 0.05$) was detected between the control larvae and larvae on 60% protein diet. Survival rates at the final sampling were significantly different among treatments ($P < 0.05$), but comparison of means revealed no significant difference between treatment I (control) and treatment V (60% protein) (Table 5).

Moisture content of the larvae ranged from 89.2 to 92.0% and did not differ significantly (Table 6). However, the control and treatment V larvae had significantly higher body protein (64.5 and 64.8%, respectively) than that of larvae in all other treatments. Lipid content of the larvae showed no significant difference (Table 6).

Discussion

The exponential growth pattern observed from day 1 up to day 8 coincided with the period of feeding with *Artemia* before and during weaning. Live food, particularly *Artemia*, when given in small amounts can be used as an essential component of food during weaning since it contains substances necessary for the development and growth of larvae (Fluchter 1982; Szlaminska and Pryzbyl 1986; Verreth and Den Bieman 1987). The slow growth from day 8 to day 12 indicates that the larvae were adjusting to the artificial diet (weaning). A similar trend was observed during weaning of *Clarias gariepinus* larvae (Verreth and van Tongeren 1989). According to Kestemont and Stalmans (1992), the rapid increase in body weight and decrease in specific growth rate is related to the rapidly changing dietary requirements of the fish larvae. Hogendoorn (1980) noted in weaning *Clarias lazera* that growth rate remained almost constant from day 1 to day 27 and subsequently decreased from 68% to only 9% after 28 days of rearing. *Mystus nemurus* larvae seem to require a shorter weaning period compared with other catfish species. In the present study, however, RGR started to increase again on day 16 when the fish were presumed to have adjusted to the artificial diet. Present results are similar to an earlier experiment on weaning *M. nemurus* larvae (Eguia and Kamarudin, unpubl.). The unexpected slow growth of the control larvae from day 8 to day 12 is difficult to explain because the density of *Artemia* was adjusted from 5 to 10 nauplii ml^{-1} on day 8.

Among the weaned larvae, those fed the 60% protein diet had the best overall performance comparable to that of the control (live food only). The high performance of the larvae fed the 60% protein diet may be partly due to the presence of casein and the absence of soybean meal in the formulation. Casein is known to have a high biological value in fish and was added in the 60% diet mainly to obtain the desired high protein level. Soybean meal was present in high quantities both in the 45 and 50% protein diets, but was added in small amounts in the 55% protein diet and absent in the 60% protein diet. The incorporation of soybean meal in the other diets probably had a negative effect on the nutrient utilization by the fish larvae. Larvae from treatment IV (55% protein) grew much better than larvae fed the 50 and 45% protein diets despite the fact that all the artificial diets were well accepted during the weaning period.

Shrimp meal and squid meal are considered good protein sources with essential amino acid index (EAAI) of 0.98 and 0.96, respectively, in *Penaeus monodon* (Peñaflorida 1989). Fish meal and soybean meal are generally considered good quality protein sources with EAAI of around 0.90. However, soybean meal has been shown to cause problems in diets due to antinutritional factors (Kaushik et al. 1995), amino acid inadequacies (Kamarudin et al. 1990), inhibition of intestinal lipase (Robinson et al. 1981), inhibition of trypsin (Dabrowska and Wojno 1977) and bioavailability of minerals (Ketola 1975).

The poor performance of larvae on diets with lower protein levels may have been influenced by the relationship between the protein and energy in the diets. Although the formulations were isocaloric (4200 kcal kg^{-1}), the estimated protein and gross energy ratios varied with increasing dietary protein (107.1, 119.0, 130.5 and 142.8 mg $kcal^{-1}$ for the 45, 50, 55 and 60% protein diets, respectively). Information on the gross energy requirements of the test fish is lacking, but the energy level used in the present study was based on the available information from other studies on catfish. Earlier, Khan et al. (1993) concluded that the optimum protein requirement of juvenile *M. nemurus* is 42% with protein to digestible energy ratio of 113.82 mg $kcal^{-1}$. The 60% protein diet used in this study has protein to gross energy ratio of 142.8 mg $kcal^{-1}$. The protein and energy ratio in fish diets is important because fish, like any other animal, eat primarily to satisfy their energy requirements and they tend to adjust their feed intake in accordance with their energy requirements (Cho and Kaushik 1985; Smith 1989; Kim et al. 1991). Excessive energy levels also have deleterious effects such as deposition of fat (Hajra et al. 1988) which is undesirable. However, the total body lipids of *M. nemurus* larvae were not affected by the dietary treatments in the present study.

The low larval survival in treatments II (45% protein) and III (50% protein) as compared to treatment V (60% protein) and the control can be attributed to the dietary treatments. Fish in treatments II and III were observed to feed well on artificial diets but increased mortalities were observed in these treatments

Table 6
Body composition of *M. nemurus* larvae after 16 days of feeding [1,2]

Treatment (dietary protein)	Moisture (%)	Protein[2] (%)	Total lipids[2] (%)
I (control)	89.2 ± 0.9[a]	64.5 ± 2.1[a]	6.0 ± 0.6[a]
II (45%)	92.0 ± 0.7[a]	58.9 ± 2.3[b]	6.1 ± 0.6[a]
III (50%)	89.9 ± 1.0[a]	55.8 ± 1.4[b]	6.0 ± 0.5[a]
IV (55%)	90.5 ± 1.3[a]	59.0 ± 1.3[b]	6.2 ± 0.7[a]
V (60%)	89.7 ± 0.7[a]	64.8 ± 2.4[a]	6.4 ± 0.8[a]

[1] Means within a column followed by a common letter are not significantly different ($P > 0.05$).
[2] Dry matter basis.

during the latter part of the experiment (from day 12). Cannibalism was also noted in the aforementioned treatments. Cannibalism is known to be triggered by internal factors such as variable sizes and weak state of the larvae in the culture system, which are influenced by the larval diet (Ehrlich et al. 1989; Qin and Fast 1996; Watanabe et al. 1996).

With regard to body composition, only the body protein of the larvae was affected by the different protein levels in the diet; it increased as the dietary protein increased. The same was observed in the protein content of juvenile *M. nemurus* (Khan et al. 1993) and of bighead carp (Santiago and Reyes 1991).

The diet with a protein level of 60% supported high growth and survival in *M. nemurus* larvae, indicating a high dietary protein requirement by the larvae. The importance of the quality of protein sources in the efficiency of a formulated diet was also shown in the study. A follow-up study using different protein and energy levels is being undertaken to determine the optimum protein to energy ratio for *M. nemurus* larvae.

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