Songklanakarin J. Sci. Technol.
43 (2), 453-459, Mar. - Apr. 2021

http://www.sjst.psu.ac.th

Original Article

Dietary protein requirements for growth performance and effects on carcass composition of young Siamese spiny eel, *Macrognathus siamen*sis (Günther, 1861)

Theerachai Pongjanyakul[1*], Jongkon Boonngarm[2], Praneet Ngamsnae[3], Kanjana Payooha[3], and Jarungjit Grudpan[3]

[1] *Inland Fisheries Research and Development Center Regional 4 (Ubon Ratchathani), Department of Fisheries, Mueang, Ubon Ratchathani, 34000 Thailand*

[2] *Rayong Inland Fisheries Research and Development Center, Department of Fisheries, Pluak Daeng, Rayong, 21140 Thailand*

[3] *Department of Fisheries, Faculty of Agriculture, Ubon Ratchathani University, Warin Chamrap, Ubon Ratchathani, 34190 Thailand*

Received: 2 September 2019; Revised: 30 January 2020; Accepted: 9 March 2020

Abstract

The study on protein requirement of young Siamese spiny eel, *Macrognathus siamen*sis (GÜnther, 1861) was conducted using six different protein level (35, 40, 45, 50, 55 and 60 % protein) with average gross energy of 450 kcal·100/g. The results demonstrated the maximum specific growth rate (SGR), % weight gain and daily weight gain were achieved at 55% protein while the fishes fed with 35% protein was the lowest. It was estimated by broken line regression that dietary protein level producing maximum growth was 46.50% protein. There was no significant difference (P>0.05) on survival rate amongst treatments. Protein efficiency ratio (PER) and apparent net protein retention (ANPR) were not significantly (P>0.05) affected by diet protein levels. No significant (P>0.05) effect of dietary protein levels was found on carcass moisture, crude protein, crude lipid and ash. However, carcass moisture, protein and ash were apparently increased in all fish groups after feeding trial, comparing to fish before the experiment.

Keywords: Siamese spiny eel (*Macrognathus siamen*sis), dietary protein requirement, growth performances

1. Introduction

The Siamese spiny eel (*Macrognathus siamen*sis) naturally lives in canal, streams, swamp, ponds, paddy field and the mud, throughout Thailand, Lao, Cambodia and Vietnam (Khachonpisitsak, 2007; Rainboth, 1996). In Thailand, the species is an economically important native freshwater food fish and has very high demand of consumers who are willing to pay 150-200 baht/kg live fish and processed fish are usually traded in the market. During rainy and rice harvesting season, fish could be drawn out from trap ponds into the rice field areas to be caught. Although Siamese spiny eel is a small fish, this fish is a rich source of animal protein, essential fatty acids, vitamin and minerals. As well as many small fish species, Siamese spiny eel are eaten whole, including head, visceral and bones, they are particularly rich in bioavailable calcium and also rich in vitamin A, iron and zinc (Thilsted, 2010). However, the population of this species in natural water bodies becomes fast decreasing, due to degradation of environments. Not recently, this broodstock of this fish were successfully induced its ovulations by hormone LHRH-a (Luteinizing Hormone Releasing Hormone analog)

*Corresponding author
Email address: ptheerachai@gmail.com

injection followed by natural spawning or artificial fertilizations (Saowakoon & Saowakoon, 2007).

Appropriate feeding process for nursing 1-14 days old Siamese spiny eel larvae was feeding with *Moina* that obtained the best growth and survival. The larvae were then fed with live food such as blood worm and earthworm. Nevertheless, the substitution of a compound diet for live foods is necessary for reducing production costs and for sustaining production of high and constant quality juveniles (Cahu & Zambonino Infant, 2001).

It is well known that proteins play a part in both as a structural component and provides energy for maintenance in fish. Inevitably, the dietary protein requirements of these organisms tend to be higher than other animals (National Research Council [NRC], 1993). Protein requirements for fish are 2-3 times higher than that of mammals (Pandian, 1987). However, requirements for protein from plant protein and fishmeal in fish are quite variable, and an adequate supply of dietary protein is essential if fish are to thrive and grow well (Walker & Berlinsky, 2011). Protein is the most expensive and significant in the optimization of economic feed costs nutrient of fish diets (Yao-Ping *et al.*, 2009). Therefore, research on potentially cultural fish species starts with the determination of their dietary protein requirements. However, there is very little known about protein requirement of Siamese spiny eel, despite its feeding habits and breeding method are well documented (Saowakoon & Saowakoon, 2007). Furthermore, we now that the digestive tract of Siamese spiny eel is a straight tube, and the morphology of the fish similar to the carnivorous species, such as rice field eel (*Monopterus albus*) and the optimal protein content was 45 % protein (Ma, Wang, He, Feng, & Lu, 2014). Thus, objectives of this study were to determine the quantitative protein requirement and the effects of dietary levels on carcass composition of young Siamese spiny eel (*Macrognathus siamen*sis), maintained in aquarium.

2. Materials and Methods

2.1 Experimental fish design and water quality

Siamese spiny eel juveniles were prepared by induced breeding at culture facility of Inland Fisheries Research and Development Center Regional 4 (Ubon Ratchathani), thereafter fishes were fed with moina for 30 days followed by weaning to acclimate to artificial diet 30 days. The experimental fishes average weight (0.22±0.04 g, n=20) were randomly selected and grouped in triplicate and assigned to one of the experimental diets, at the stocking rate of 25 fishes/aquarium. Culture facilities consist of 18 glass aquaria (45x90x45 cm) in a recirculating culture system with 35 cm water depth (140-liter). Each aquarium was provided with aeration and two shelters of diameter 1.5 inches, 14 cm long polyvinylchloride (PVC) pipes. Fishes were fed to apparent satiation three times daily at 08.00, 12.00 and 16.00 hr for the experimental period of 12 weeks. Fish of each replicate were counted and weighed fortnightly in bulk. The amount of feed consumed by the fish in each treatment was recorded daily. Final weight (g), total length (cm) and survivals were recorded.

Water temperature and dissolved oxygen was monitored daily using YSI 550 DO meter. Water quality analyses were conducted three times a week for concentrations of total ammonia–nitrogen using a DR/2500 spectrophotometer and pH measured with a YSI 60 electronic pH meter. Alkalinity, Free CO_2 and hardness were measured once a week by digital titration (HACH Company). All aquaria, some water were siphoned off and filled in prior to the first daily feeding to remove accumulated maters and kept maintaining a water depth 35 cm.

2.2 Experimental diets and feeding

Six experimental diets were formulated to contain increasing protein level 35, 40, 45, 50, 55, and 60% protein with average gross energy of 450 Kcal•100/g. Diets were prepared using fish meal and krill meal as the main protein sources. Each diet was prepared by first mixing the macro ingredients; fish meal, krill meal, rice bran and wheat flour, in Hammer mill for 5 min. The dry ingredients were pulverized, sieved through 0.5 mm. All ingredients were thoroughly mixed and followed by addition of squid oil, tuna oil, vegetable oil, α-starch, cellulose, vitamin and mineral premixes, choline chloride and ascorbic acid. The mixed product was extruded with a grinder-Hobart at room temperature with 1.6-mm die and the outcome pellets were air-dried at temperature of 28-40 °C for about three days. In order to match the fish mouth size, pellets were then grinded by Molinex grinder and passed through 800 and 1,000 µm mesh sieves. The diets were kept at -18 °C in a freezer until the time of feeding. Analysis of the proximate composition of the experimental diets was carried out in Chonburi Aquatic Animal Feed Technology Research and Development Center. Determination for percentage of dry matter of the experimental diet i.e crude protein (Nx6.25), ether extract, crude fiber, ash and nitrogen-free extract (NFE) was conducted by standard (AOAC, 1985) methods. The ingredient composition and proximate analysis of the experimental diets are given in Table 1.

2.3 Growth performance parameters and carcass composition analyses

Six fishes from each dietary treatment (2 per replicate) were sampled and pooled for their proximate carcass composition analysis, at the beginning and the end of the experiment. Dry matter analysis was performed by drying for 24 hr at 105 °C, crude protein by Kjeldahl method, and crude lipid using a Soxhlet extraction apparatus, ash by combustion in muffle furnace at 550 °C for 7 hr, and energy by burning in a Gallenkamp bomb calorimeter (Leicestershire, England). Determination of carbohydrate content (nitrogen-free extract; NFE = 100 - (% protein + % lipid + % fiber + % ash) was calculated by difference. Specific growth rate (SGR), feed conversion ratios (FCR), weight gain (DWG), feed efficiency ratio (FE), daily feeding rate (DFR), protein efficiency ratio (PER), apparent net protein retention (ANPR) and survival ratio (SUR), were calculated as follows:

SGR (%) = 100× (ln final wt - ln initial wt)/ t (days)

FCR = Food consumed in g (dry weight)/ Live weight gain in g

DWG (g/day) = (Mean final weight–Mean initial weight) / Culture period (days)

Table 1. Formulation and composition analysis of the experimental diets (% Dry wt. basis)

Ingredients	Dietary Protein levels					
	35%	40%	45%	50%	55%	60%
Fish meal	34.00	39.00	43.00	43.80	47.10	46.57
Krill meal	12.00	16.00	20.00	21.00	25.80	33.10
Gluten	3.00	3.00	3.00	3.00	3.00	3.00
Wheat flour	14.00	9.50	5.00	3.75	0.50	1.00
Rice bran	13.50	9.50	5.00	3.75	0.50	0.50
Squid oil	2.45	1.88	1.80	1.14	0.58	0.00
Vegetable oil	6.65	6.20	5.80	4.65	3.95	1.50
Tuna oil	2.45	1.88	1.80	1.14	0.58	0.00
Ascorbic acid	0.10	0.10	0.10	0.10	0.10	0.10
Choline Chloride	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix[1]	1.00	1.00	1.00	1.00	1.00	1.00
Mineral Premix[2]	1.00	1.00	1.00	1.00	1.00	1.00
α-starch	7.00	7.00	7.00	7.00	7.00	7.00
Cellulose	2.65	3.74	5.30	8.47	8.69	5.03
Total	100	100	100	100	100	100
Proximate analysis (% Dry wt. basis)						
Moisture (%)	8.41	7.56	8.01	8.69	7.45	9.14
Crude protein	36.20	41.84	46.02	49.90	54.04	60.06
Ether extract	15.74	13.71	13.68	11.31	10.03	6.98
Ash	7.31	7.86	8.21	8.25	8.68	9.07
Crude fiber	5.88	5.82	7.15	10.26	10.43	7.15
NFE	26.46	23.21	16.93	11.59	9.37	7.60
GE (kcal·100/g)	463.08	462.27	459.54	436.91	438.99	436.84
GE:P	12.79	11.05	9.99	8.76	8.12	7.27

[1]Vitamin mix (g/kg premix): vitamin A retinol acetate, 0.80; vitamin D3 cholecalciferol, 0.06; vitamin E a-tocopherol acetate, 4.00; vitamin K3 menadione, 8.00; thiamin, 2.00; riboflavin, 2.00; pantothenic acid, 6.00; pyridoxine, 2.00; folic acid, 0.50; niacin, 15; vitamin B12 cyanocobalamin, 0.02; inositol, 40.00; corn starch, 920.62. [2]Mineral premix (g/kg mix): $FeSO_4.7H_2O$, 15; $CuSO_4 .H_2O$, 0.3; $ZnSO_4 .7H_2O$, 10; $MnSO_4.H_2O$, 0.5; NaCl, 30;$MgSO_4$, 40; $Ca(H_2PO_4)2$, 400; KI, 0.05; Na_2SeO_3, 0.005; $CoCl_3 .6H_2O$, 0.5; zeolite, 503.645

FER = Mean weight gain/ Mean feed intake

DFR (%/day) = 100 x (Daily feed intake)/(Initial weight+ Final weight)/2

PER = Body weight gain (wet)/Apparent protein intake (dry)

ANPR (%) = 100× (Final body protein-Initial body protein)/Apparent protein intake

SUR (%) = 100 × (Final number of fish)/ Initial number of fish.

2.4 Statistical analysis

To determine the significant differences amongst treatments at the 0.05 significance level, all data on growth, feed conversion and survival rates were subjected to one-way analysis of variance and Tukey's test. The equation broken-line regression model is Y = L+U(R-X), which Y is the growth parameter (% weight gain) chosen to predict the protein requirement, L is the ordinate and R is the x-axis of the breakpoint R is taken as the predicted requirement and U is the slope of the line for X, which represented the optimum dietary protein level for fish. (Robbins, Norton, & Baker, 1979: Robbins, Saxton, & Southern,2006).

3. Results

Overall water quality values ranged during the study period, water temperature, 25.7-28.7 ºC; air temperature, 27.50-30.50 ºC; dissolved oxygen, 4.30-5.80 mg/l; free CO_2, 1.35-2.80 mg/l; pH, 5.0-7.9; hardness, 35.60-52.20 mg/l as $CaCO_3$; alkalinity, 15.20-18.75 mg/l as $CaCO_3$; and total ammonia–nitrogen, 0.029-0.403 mg/l (Table 2). These major water quality parameters ranges were within suitable levels for living and growth of tropical freshwater fishes (Boyd, 1979), and similar reported water quality results of juvenile rice field eel (Ma *et al.*, 2014).

3.1 Growth performance

Weekly increments in mean body weight of young Siamese spiny eels are shown in Figure 1 overall growth performances and other related parameters are given in Table 3. The weights of fish in all treatments were increased and varied with the dietary protein content (Figure 1). However, mean survival rates did not significantly different (P>0.05) among treatments and mortalities were not affected by treatments (Table 3). The highest final weight 0.85 g occurred at 55% dietary protein level while the lowest final weight gain of 0.50 g observed at the lowest dietary protein level of 35%. Similarly, the highest final length 6.42 cm was obtained from fish fed 55% protein diet, which was significantly (P<0.05) different from 35% protein. Percentage weight gain was significantly different (P<0.05) between 35% protein and the rest dietary protein levels. Although fish fed the 55% protein diets showed the highest specific growth rate, but did not significantly (P> 0.05) differ from those fed 45, 55, and 60% protein diets.

Table 2. Water quality parameters of all treatments during the study period

Water quality Parameters	Dietary protein levels (%)					
	35	40	45	50	55	60
Water temperature (ºC)	25.80-29.50	26.00-30.43	25.70-29.70	26-28.70	25.70-28.70	25.70-28.70
Air temperature (ºC)	27.50-30.50	27.50-30.50	27.50-30.50	27.50-30.50	27.50-30.50	27.50-30.50
Dissolved oxygen (mg/l)	4.30-5.73	4.30-5.70	4.30-5.80	4.30-5.20	4.30-5.30	4.40-5.80
Free CO_2 (mg/l)	1.50-2.60	1.45-2.60	1.35-2.50	1.50-2.80	1.50-2.60	1.60-2.50
pH	6.5-7.5	6.5-7.0	6.9-7.5	6.8-7.9	6.6-7.5	6.5-7.0
Hardness (mg/l as $CaCO_3$)	35.80-45.70	35.60-47	35.80-50	35.80-45.70	36.20-52.20	36.67-51.67
Alkalinity (mg/l as $CaCO_3$)	16.20-17.58	16.70-18.75	17.40-18.05	15.20-18.25	16.45-17.15	17.30-18.67
Total ammonia-N (mg/l)	0.037-0.110	0.029-0.207	0.045-0.095	0.051-0.403	0.039-0.082	0.053-0.236

Table 3. Growth performance, feed utilization efficiency and protein utilization efficiency of fish fed experimental diets for 12 weeks

Growth Parameters	Dietary protein levels (%)					
	35	40	45	50	55	60
Initial weight (g)	0.22±0.04	0.22±0.04	0.22±0.04	0.22±0.04	0.22±0.04	0.22±0.04
Final weight (g)	0.50±0.03^{a}	0.69±0.04^{b}	0.78±0.03^{c}	0.84±0.03^{c}	0.85±0.06^{c}	0.84±0.07^{c}
Initial length (cm)	4.69±3.34	4.69±3.34	4.69±3.34	4.69±3.34	4.69±3.34	4.69±3.34
Final length (cm)	5.32±0.69^{a}	5.78±1.09^{ab}	5.38±0.40^{ab}	5.81±0.40^{ab}	6.42±0.24^{b}	6.34±0.37^{ab}
Specific Growth Rate (SGR)	1.11±0.09^{a}	1.56±0.78^{b}	1.73±0.06^{c}	1.83±0.06^{c}	1.84±0.10^{c}	1.82±0.13^{c}
Daily Weight Gain (DWG)	3.77±0.57^{a}	6.33±0.57^{b}	7.68±0.58^{c}	8.67±0.58^{c}	8.67±0.58^{c}	8.33±0.12 ^{c}
% Weight gain	123.37±14.85^{a}	208.42±18.56^{b}	249.12±14.03^{c}	274.18±15.31^{c}	276.11±27.17^{c}	273.15±34.22^{c}
% Daily Feeding Rate (DFR)	2.39±0.26^{a}	2.69±0.37^{ab}	2.82±0.22^{ab}	2.96±0.37^{b}	2.56±0.19^{ab}	2.31±0.15^{a}
Average Daily Feed intake (g)	0.63±0.09^{a}	0.89±0.09^{b}	1.03±0.81^{bc}	1.13±0.11^{c}	0.99±0.11^{bc}	0.89±0.12^{b}
Protein Efficiency Ratio (PER)	0.98±0.21^{a}	1.04±0.27^{a}	1.04±0.16^{a}	1.02±0.15^{a}	0.98±0.07^{a}	1.05±0.08^{a}
Feed Efficiency Ratio (FER)	0.36±0.08^{a}	0.43±0.11^{ab}	0.48±0.08^{ab}	0.51±0.07^{bc}	0.53±0.04^{bc}	0.63±0.05^{c}
App. Net Protein Retention (ANPR)	15.60±0.49^{a}	17.81±3.55^{a}	16.53±1.31^{a}	15.12±2.39^{a}	15.46±1.96^{a}	15.57±0.50^{a}
Average Protein Intake (g)	0.23±0.04^{a}	0.37±0.04^{b}	0.48±0.04^{c}	0.56±0.05^{c}	0.53±0.06^{c}	0.53±0.07^{c}
Feed Conversion Ratio (FCR)	2.27±0.36^{b}	1.91±0.34^{ab}	1.84±0.16^{ab}	1.85±0.28^{ab}	1.69±0.29^{a}	1.62±0.29^{a}
Survival Rate	82.67±14.05^{a}	72±4.00^{a}	76±8.00^{a}	84±4.00^{a}	72±10.58^{a}	80±10.58^{a}

Row means with the same letters are not significantly different (P>0.05).

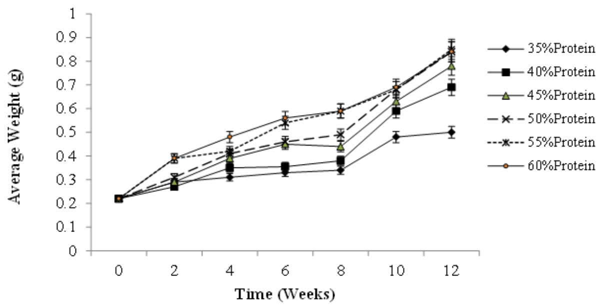

Figure 1. Changes in average weight of young Siamese spiny eel (*Macrognathus siamensis*), fed with different protein content diets

The fish fed with the higher protein levels were increased in %SGR (1.11-1.84) and feed efficiency ratio (FER) (0.36-0.63). Daily feeding rate (DFR), Protein efficiency ratio (PER) tended to increase as the dietary protein level increasing from 35 to 50% but were decreased at the protein levels of 55 to 60%. The highest feed conversion ratio (FCR) was observed at the protein level of 35%, while the lowest was at 60% but was not significantly (P>0.05) different when fish were fed diets with protein levels from 40, 45, 55, and 60%. Apparent net protein retentions (ANPR) were not significantly different (P >0.05) in all dietary protein levels. The overall survival rate was 72-84%, with no significant differences (P>0.05) amongst groups.

3.2 Optimum dietary protein level

Based on percentage weight gain, daily weight gain, and specific growth rate, the dietary protein requirement of young Siamese spiny eel was apparently observed at 55% protein. However, in accordance with the growth response to the feeding experimental, body weight gain as an index (y) and dietary protein level (x) were fitted using the broken-line model and a regression analysis map constructed (Figure 2). The appropriate level of dietary protein was calculated to be 46.50% protein of the dry feed that produced maximum growth for young Siamese spiny eel in this study.

3.3 Fish carcass composition

The effects of dietary protein levels on carcass composition of young Siamese spiny eel are presented in Table 3. Fish body moisture content (15.76-18.17), crude protein (68.86-70.68), crude lipid (6.54-8.30) and ash (16.40-18.44) did not differ significantly (P>0.05) among all fish groups after the experiment.

Fish body moisture, protein, lipid and ash were not clearly related to dietary protein level. Fishes in this study were young and sexually immature; however, carcass

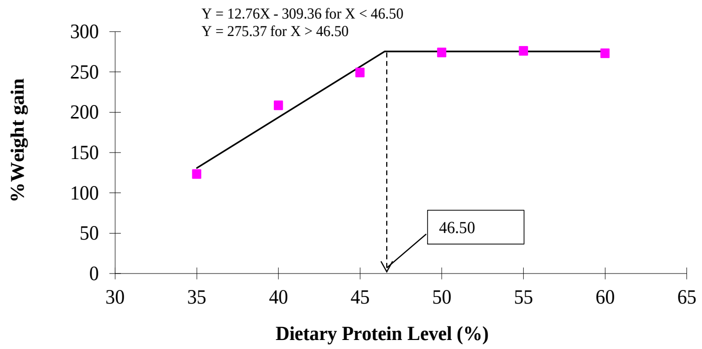

Figure 2. Optimum dietary Protein level for young Siamese spiny eel (*Macrognathus siamensis*), determined by Breakpoint regression analysis.

Table 4. Proximate carcass composition of young Siamese spiny eel (*Macrognathus siamensis*), fed diets containing graded levels of protein over 12 weeks

Dietary protein levels (%)	As percentage of dry matter (after 12 weeks)			
	Moisture	Protein	Lipid	Ash
Before the Expt.	14.39	62.43	6.94	11.75
35	15.76	70.68	6.54	17.84
40	17.54	70.21	7.25	17.58
45	18.17	68.86	8.12	16.96
50	17.85	69.82	8.30	16.89
55	17.15	70.50	7.82	16.40
60	17.58	69.84	6.68	18.44

moisture, protein and ash increased in all fish groups after feeding trial comparing to fish before the experiment, and there was no significant (P>0.05) effect of dietary protein on carcass lipid.

4. Discussion

4.1 Growth

From this study results, the dietary protein level producing maximum growth of this experimental fish, calculated by broken line regression was 46.50% protein. This optimum protein level for young *Macrognathus siamen*sis (GÜnther, 1861) is close to the demand for protein of other eel-like appearance species such as swamp eel: *Monopterus albus* 45% protein (Ma *et al.*, 2014) and Japanese eel; *Anguilla japonica* 45% protein (Okorie *et al.*, 2007). When compare to the protein demand (35-45% protein) of *Anguilla australis* (Engin & Carter, 2006), this study result is slightly higher, and probably due to the relatively small size of the fish that were used in this study. However, the present result is quite close to the dietary protein requirement of juvenile American eel; *Anguilla rostrata* 47% protein (Tibbetts, Lall, & Anderson, 2000). It was found that at an excessive dietary protein levels, the growth performances responding to dietary protein levels in Siamese spiny eel exhibited similar trend in decreasing growth rate to all previous studies.

Generally, the optimum dietary protein requirement is known to differ between different fish species. These may be due to differences in strains (NRC, 1993), and environmental factors and size or stages of their growth development. In the present study, with 35 and 40% protein diets, fish feed lower protein diets showed significantly lower growth than the higher protein groups. On the other hand, with diets containing 45-50% protein, fish had sufficient protein for their maximum growth. Further, increases in dietary proteins did not result in significantly increase in growths. Excessive dietary protein levels (55-60%) in this study trended to decrease in growth rates. This similar apparent growth-depressing effect of fish fed high protein diets has also been reported for other fish species, in carnivorous fish such as bagrid catfish; *Mystus nenurus* (Ng, Soon, & Hashim, 2001), snakehead; *Channa striata* (Mohanty & Samantaray, 1996; Wee & Tacon, 1982) and striped catfish; *Pangasianodon hypopthalmus* (Jayant *et al.*, 2018)**,** herbivorous fish such as tawes, *Puntius gonionotus* (Wee & Ngamsnae, 1987) and omnivorous freshwater fish such as rohu; *Labeo rohita* (Satpathy, Mukherjee, & Ray, 2003) and Nile tilapia; *Oreochromis niloticus* (Siddiqui, Howlader, & Adam, 1988).

It was advised that the content of dietary protein should be maintained at a suitable level to minimize feed costs. Otherwise, when dietary protein level the requirement, the fish excretes more ammonia nitrogen into the surrounding environment, thus reducing the quality of the culture water. Growth of the fish also might be affected since extra energy is required to delaminate the excess amino acids absorbed (Jauncey, 1982). This conclusion have been confirmed by Lim, Sukhawongs, & Pascaual (1979) who concluded that the lower weight gain of milkfish *Chanos chanos*, fed diets with protein levels above the optimum could be due to insufficient non-protein energy in the high protein diets which caused part of the dietary protein to be metabolized and use for energy.

Feed conversion (FCR) values ranged from 1.62 to 2.27, with significant differences between the lowest protein diet (35% protein) and the two high groups (55 and 60% protein) of experimental diets. However, similar distinct trend for FCR to decrease with increasing dietary protein levels was observed in Rohu *Labeo rohita* (Nandeesha *et al.*, 1994) and *Zacco barbata* (Shyong, Huang, & Chen, 1998). Protein efficiency ratio (PER) ranged from 0.98 to 1.05, with could not be easily categorized into groups. However, apparently PER decreased when dietary protein level increased. This trend was also reported in bighead carp (Santiago & Reyes, 1991) and *Zacco barbata* (Shyong *et al.*, 1998). This occurrence could explained that fish often show the greatest protein conversion efficiency when fed a dietary protein less than that yielding maximum growth and feed efficiency (Jobling, 1994).

Apparent net protein retention (ANPR) in this study for Siamese spiny eel ranged from 15.12% to 17.81%, with not significantly different amongst all treatments but apparently higher in the lower diets. Previous studies reported that the protein retention for different dietary protein levels depends on the type of energy provided by the ingredients (Ogino & Saito, 1970) and different between species (Boyd & McNevin, 2015). For example, while for striped catfish, *Pangasianodon hypopthalmus* and Nile tilapia, *Oeochromis niloticus* whole-body composition in retrieval is between 20.9-22.4% (Abdel-Tawwab, 2012; Jayant *et al.*, 2018), and for atlantic salmon, *Salmo salar* is up to 52.9% (Grisdale-Helland & Helland, 1997). Cowey, Blair, Adron, & Pope (1971) reported that ANPR and PER values for plaice were higher with diets containing carbohydrate than with diets without carbohydrate, even though those diets were isocaloric. In this study, the highest ANPR and PER of Siamese spiny eel were found with the 40% protein diet, nearly the lowest dietary protein concentration. This result empirically confirms the conclusion that the fish fed diets containing higher carbohydrate exhibit higher PER and ANPR values.

4.2 Fish carcass composition

The protein level in diets was not significantly affected on carcass composition of Siamese spiny at this stage of development. Although body moisture content tended to increase with the dietary protein level, changes in protein, fat and ash contents were not clearly related to the dietary treatment. Gunasekera, Silva, Collins, Gooley, & Ingram (2000) and Yao-Ping *et al.* (2009) found that gross body composition in Murray cod *Maccullochella peelii peelii* juvenile and *Barbodes caldwelli* juvenile were not affected by increasing dietary protein levels but the fish tended to have lower body protein and higher body lipid content when fed low protein diet. The carcass lipid levels generally increased with increasing dietary protein from 35% to 50% protein, then decreased after reached the optimum protein level for growth. A similar trend has been reported in other experiments with *Nibea diacanthus* (Li, Wen, Zhao, Li, & Zhu, 2016) and Japanese eel; *Anguilla japonica* (Nose & Arai, 1972).

5. Conclusions

The calculated dietary protein requirement for young Siamese spiny eel was 46.50% when determined by the broken-line regression model using % final weight gain as the indicator. However, the effectiveness of this recommended level practical diets, will depend on the essential amino acid composition provided by suitable plant and animal feedstuffs. Future study on practical diets for Siamese spiny eel emphasized on essential amino acid requirements and their optimum levels needed be conducted.

Acknowledgements

This research was sponsored by the Agriculture Research Development Agency (Public Organization), ARDA this support was appreciated. The authors would like to thank L. Krongpong, Director of Chonburi Aquatic Animal Feed Technology Research and Development Center, for providing research facilities for analysis of the proximate composition of the experimental diets and fish carcass, and S. Sripat, Director of Inland Fisheries Research and Development Regional 4 (Ubon Ratchathani) for providing facilities in feeding trial of the experimental fish.

References

Abdel-Tawwab, M. (2012). Effects of dietary protein levels and rearing density on growth performance and stress response of Nile tilapia, *Oreochromis niloticus* (L.). *International Aquatic Research*, *4*(1), 3. doi:10.1186/2008-6970-4-3

Association of Official Analytical Chemists. (1985). *Official Methods of Analysis* (16th ed.). Arlington, VA: Author.

Boyd, C. E. (1979). *Water quality in warmwater fish ponds*. Auburn, AL: Auburn University, Agricultural Experiment Station.

Boyd, C., & McNevin, A. (2015). *Aquaculture, Resource Use, and the Environment*. New Jersey, NJ: Willey Blackwell.

Cahu, C., & Zambonino Infante, J. (2001). Substitution of live food by formulated diets in marine fish larvae. *Advanced Biotechnology in Hatchery Production*, *2001*(1–2), 161–180. doi:10.1016/S0044-8486(01) 00699-8

Cowey, C. B., Pope, J. A., Adron, J. W., & Blair, A. (1971). Studies on the nutrition of marine flatfish. Growth of the plaice Pleuronectes platessa on diets containing proteins derived from plants and other sources. *Marine Biology*, *10*(2), 145–153. doi:10. 1007/BF00354830

Engin, K., & Carter, C. G. (2006). Growth and food utilization of the Australian short-finned eel, *Anguilla australis australis* (Richardson) given paired iso-energetic diets with increasing crude protein content. *Animal Science*, *82*(2), 169–174. doi:10.1079/ASC200528

Grisdale-Helland, B., & Helland, S. J. (1997). Replacement of protein by fat and carbohydrate in diets for atlantic salmon (*Salmo salar*) at the end of the freshwater stage. *Aquaculture*, *152*(1), 167–180. doi:10.1016/S 0044-8486(97)00003-3

Gunasekera, R. M., Silva, S. S. D., Collins, R. A., Gooley, G., & Ingram, B. A. (2000). Effect of dietary protein level on growth and food utilization in juvenile Murray cod Maccullochella peelii peelii (Mitchell). *Aquaculture Research*, *31*(2), 181–187. doi:10. 1046/j.1365-2109.2000.00417.x

Jauncey, K. (1982). The effects of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapias (*Sarotherodon mossambicus*). *Aquaculture*, *27*(1), 43–54. doi:10.1016/0044-8486(82)90108-9

Jayant, M., Muralidhar, A. P., Sahu, N. P., Jain, K. K., Pal, A. K., & Srivastava, P. P. (2018). Protein requirement of juvenile striped catfish, *Pangasianodon hypophthalmus*. *Aquaculture International*, *26*(1), 375–389. doi:10.1007/s10499-017-0216-0

Jobling, M. (1994). *Fish Bioenergetics*. London, England: Chapman and Hall.

Khachonpisitsak, S. (2007). *Taxonomy of Spiny Eels (Symbranchiformes: Mastacembelidae) Thailand*

(Master's thesis, Chulalongkorn University, Bangkok, Thailand).

Li, W., Wen, X., Zhao, J., Li, S., & Zhu, D. (2016). Effects of dietary protein levels on growth, feed utilization, body composition and ammonia–nitrogen excretion in juvenile *Nibea diacanthus*. *Fisheries Science, 82*(1), 137–146. doi:10.1007/s12562-015-0945-9

Lim, C., Sukhawongs, S., & Pascual, F. P. (1979). A preliminary study on the protein requirements of *Chanos chanos* (Forskal) fry in a controlled environment. *Aquaculture*, *17*(3), 195–201. doi:10. 1016/0044-8486(79)90123-6

Ma, X., Hu, Y., Wang, X.-Q., Ai, Q.-H., He, Z.-G., Feng, F.-X., & Lu, X.-Y. (2014). Effects of practical dietary protein to lipid levels on growth, digestive enzyme activities and body composition of juvenile rice field eel (*Monopterus albus*). *Aquaculture International*, *22*(2), 749–760. doi:10.1007/s10499-013-9703-0

Mohanty, S. S., & Samantaray, K. (1996). Effect of varying levels of dietary protein on the growth performance and feed conversion efficiency of snakehead *Channa striata* fry. *Aquaculture Nutrition*, *2*(2), 89–94. doi:10.1111/j.1365-2095.1996.tb00013.x

Nandeesha, M. C., Dathathri, K., Krishnamurthy, D., Vargese, T. J., Gangadhar, B. and Umesh, N. R. 1994. Effect of varied levels of protein on the growth and tissue biochemistry of stunted yearlings of rohu, Labeo rohita, in the absence and presence of natural food. In De silva, S. S. (Ed.), *Fish nutrition research in Asia, Proceedings of the 5th Asian Nutrition workshop, Asian Fisheries Society Publication 9*, p. 93-99, Manila, Philippines: Asian Fisheries Society.

National Research Council. (1993). *Nutrient Requirements of Fish*. Washington, DC: National Academy Press.

Ng, W.-K., Soon, S.-C., & Hashim, R. (2001). The dietary protein requirement of a bagrid catfish, *Mystus nemurus* (Cuvier & Valenciennes), determined using semipurified diets of varying protein level. *Aquaculture Nutrition*, *7*(1), 45–51. doi:10.1046/j. 1365-2095.2001.00160.x

Nose, T., & Arai, S. (1972). Optimum level protein in a purified diet for eel, *Anguilla japonica. Bulletin of Freshwater Fisheries Research Laboratory, 22*, 145-155.

Ogino, C., & Saito, K. (1970). Protein nutrition in fish. 1. The utilization of dietary protein by young carp. *Bulletin of the Japanese Society of Scientific Fisheries*, *36*(3), 250–254.

Okorie, E. O., Kim, Y.-C., Lee, S., Bae, jun-young, H. Yoo, J., Han, K., Bai, S., Park, G.-J., & Choi, S.-M. (2007). Reevaluation of the Dietary Protein Requirements and Optimum Dietary Protein to Energy Ratios in Japanese Eel, Anguilla japonica. *Journal of The World Aquaculture Society*, *38*, 418–426. doi:10.1111/j.1749-7345.2007.00113.x

Pandian, T. J. (1987). *Fish energetics. In Animal energetics. Volume 2*. New York, NY: Academic Press.

Rainboth, W. J. (1996). *Fishes of the Cambodia Mekong*. Rome, Italy: Food and Agriculture Organization.

Robbins, K. R., Norton, H. W., & Baker, D. H. (1979). Estimation of nutrient requirements from growth data. *Journal of Nutrition*, *109*, 1710-1714.

Robbins, K. R., Saxton, A. M., & Southern, L. L. (2006). Estimation of nutrient requirements using broken-line regression analysis. *Journal of Animal Science, 84*(Supplement E): E155-E165.

Santiago, C. B., & Reyes, O. S. (1991). Optimum dietary protein level for growth of bighead carp (*Aristichthys nobilis*) fry in a static water system. *Aquaculture*, *93*(2), 155–165. doi:10.1016/0044-8486(91)90214-R

Saowakoon, H., & Saowakoon, S. (2007). Breeding and Nursing of Spotted Spiny Eel (*Macrognathus siamensis*; Günther, 1861). *Proceeding of 45th Kasetsart University Annual Conference: Fisheries*, 712-721.

Satpathy, B. B., Mukherjee, D., & Ray, A. K. (2003). Effects of dietary protein and lipid levels on growth, feed conversion and body composition in rohu, *Labeo rohita* (Hamilton), fingerlings. *Aquaculture Nutrition*, *9*(1), 17–24. doi:10.1046/j.1365-2095. 2003.00223.x

Shyong, W.-J., Huang, C.-H., & Chen, H.-C. (1998). Effects of dietary protein concentration on growth and muscle composition of juvenile *Zacco barbata*. *Aquaculture*, *167*(1), 35–42. doi:org/10.1016/S00 44-8486(98)00313-5

Siddiqui, A. Q., Howlader, M. S., & Adam, A. A. (1988). Effects of dietary protein levels on growth, feed conversion and protein utilization in fry and young Nile tilapia, *Oreochromis niloticus*. *Aquaculture, 70*(1), 63–73. doi:10.1016/0044-8486(88)90007-5

Thilsted, S. H. (2010). The potential of nutrition-rich small fish species in aquaculture to improve human nutrition and health**.** *Proceeding of the Global Conference on Aquaculture*, 57–73.

Tibbetts, S. M., Lall, S. P., & Anderson, D. M. (2000). Dietary protein requirement of juvenile American eel (*Anguilla rostrata*) fed practical diets. *Aquaculture*, *186*(1–2), 145–155. doi:10.1016/S00 44-8486(99)00363-4

Walker, A. B., & Berlinsky, D. L. (2011). Effects of Partial Replacement of Fish Meal Protein by Microalgae on Growth, Feed Intake, and Body Composition of Atlantic Cod. *North American Journal of Aquaculture*, *73*(1), 76–83. doi:10.1080/15222055. 2010.549030

Wee, K. L., & A. G. J. Tacon. (1982). A preliminary study on the dietary protein requirement of juvenile snake head. *Bulletin of the Japanese Society for the Science of Fish, 48,* 1463-1468.

Wee, K. L., & Ngamsnae, P. (1987). Dietary protein requirement of fingerlings of the herbivorous carp tawes, *Puntius gonionotus* (Bleeker)*. Aquaculture and Fisheries Management*, *18*, 121-129.

Yao-Ping, L., Jian-Ming, C., Jin-Yun, Y., Xu-Xiong, H., Shen-Ying, L., Bin-Qian, S., Zi-Liang, Y., Jian-Lin, G., & Li-Ping, Y. (2009). The effects of diet protein levels on the growth, body composition and digestive enzyme activities of the *Barbodes caldwelli* juvenile. *Chinese Journal of Agricultural Biotechnology*, *6*(3), 199–205. doi:10.1017/S14792 36209990222