Journal of Agricultural Technology 2013 Vol. 9(7):1775-1788
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Assessment of dietary protein requirement on growth performance of young Mekong giant catfish (*Pangasianodongigas*), cultured in floating net cages

SuthusPhuakgeen* **and PraneetNgamsnae**

Faculty of Agriculture, UbonRatchathani University UbonRatchathani, 34190 Thailand

SuthusPhuakgeen and PraneetNgamsnae (2013) Assessment of dietary protein requirement on growth performance of young Mekong giant catfish (*Pangasianodongigas*), cultured in floating net cages. Journal of Agricultural Technology 9(7):1775-1788.

Mekong Giant Catfish (*Pangasianodongigas*), is one of the largest freshwater fish in the world, native to Mekong River basin with the fact that the species is critically endangered. However, most of its nutritional requirements have not been determined. The study on protein requirement of young Mekong Giant Catfish (*Pangasianodongigas*) was conducted using five formulated diets containing 20, 25, 30, 35 and 40% protein with digestible energy of 433 kcal/100 g. The fishes average weight 41.00 g, were maintained in each floating net cages with 1.0 x 1.0 x 1.5 m^3 size, at the stocking rate of 50 fishes/cage. Fishes were fed to apparent satiation twice daily for five months. The experiment was conducted at Kalasin Inland Fisheries Station, Thailand during November 2009-March 2010. The results showed that the maximum growth was significantly ($p<0.05$) attained at 35% protein while the fishes fed with 20% protein was the lowest. The growths of fish fed 25, 30, and 35% dietary protein were not significantly different. Protein efficiency ratio (PER), feed conversion ratios (FCR) and apparent net protein retention (ANPR) all decreased with increasing dietary protein levels, while survival rate was not significantly affected. On the basis of percentage weight gain, daily weight gain, and specific growth rate the dietary protein requirement of young Mekong Giant Catfish was approximately 35%. The dietary protein level producing maximum growth, calculated by broken line regression was 35.01%. The relationship between protein level (X; %) and weight gain (Y; g) was expressed as $Y = 14.46X – 10.87$. Fish muscle moisture, NFE, fiber and ash were not clearly related to dietary protein level but low dietary protein levels resulted in significantly higher ($P<0.05$) lipid but lower protein content.

Keywords: Mekong Giant Catfish *(Pangasianodongigas*), Dietary protein requirement, Growth performances

Introduction

Mekong Giant Catfish (*Pangasianodongigas*), is one of the largest freshwater fish in the world, native to Mekong River basin with the fact that the

*Corresponding author: SuthusPhuakgeen; e-mail: suthus_53@hotmail.com

species is critically endangered.The viability culture of this species depends on the largest production cost lines in feed, with protein comprising the most expensive component.It is crucial, therefore, that the optimal protein levels are known to ensure the best growth and survival at the low cost.

Unlike mammals, proteins act both as a structural component and as an energy source in fish. Consequently, the dietary protein requirements of these organisms tend to be higher. Protein requirements of fish are about 2 to 3 times higher than that of mammals (Pandian, 1987). Requirements for protein in fish are quite variable, and an adequate supply of dietary protein is essential if fish are to thrive and grow well. Jobling (1994) summarized results from laboratory studies conducted on several carnivorous fish species such as salmonids, percids and marine flatfish, and concluded that optimum growth is achieved when around half of food energy is supplied from proteins. Thus, it is generally recognized that protein should provide 40-50% of the dietary energy of these species. Other species, such as cyprinids, tilapias and some ictalurids, are capable of maintaining excellent rates of growth when fed diets of lower protein content. For these species, diets formulated to contain 30-40% protein will be adequate to fulfill the requirements.

The increasing cost of traditional protein sources and the associated use of non-traditional protein sources together with the environmental cost of using excess protein in fish diets mean that more precise estimates of protein of cultured species will be required in the future. Protein often constitutes the most expensive item of fish diets. Thus, research on potentially cultural species invariably starts with the determination of their gross protein requirements. This information however, is of limited value without data on essential amino acid requirements since protein quality depends largely on its amino acid composition and digestibility (Benitez, 1989).

Previous nutritional studies on Mekong Giant Catfishhave focused on supplementing of microalgae (Spirulina) in the fish diet for improve growth and maturation performance (Meng-umphan and Saengkrachang, 2008), replacing fishmeal with Spirulina in the fish diet (Tongsiri *et al.* 2010) and growth performance of the fish fed with commercial feed (Ungsethaphand and Hangsapreurke, 2008).

However, there is very little known about its protein requirement though its feed and feeding habits in nature are well documented (Thongsagaand Pholprasith. 1991; Pholprasith and Tavarutmaneegul, 1997; Leelapatra*et,al*, 2000)

The objectives of this study were to determine the quantitative protein requirement and the effects of dietary levels on muscle composition of Mekong Giant Catfish (*Pangasianodongigas*), raised in cages.

Materials and methods

Experimental design and feeding

Mekong Giant Catfish juveniles were taken from the induced breeding project of Kalasin Inland Fisheries Station and transported to the culture facility and acclimatized to pellet diet feeding 2 weeks. The fishes average weight 41.00 g, were maintained in each floating net cages with 1.0 x 1.0 x 1.5 m^3 size, at the stocking rate of 50 fishes/cage. Group of fish in triplicate were randomly assigned to one of the experimental diets. Fish were fed to satiation twice daily at 09.30 and 15.30 h. Fish in each replicate were counted and weighed in bulk, in water fortnightly. The experiments lasted 5 months. Fish were fed to apparent satiation twice a day (09.00 and 17.00 h local time). The amount of feed consumed by the fish was recorded daily in each treatment. Final weight (g), standard length, and survival were recorded.

Experimental Diets

Five practical experimental diets containing 20, 25, 30, 35 and 40% protein with average digestible energy of 433 Kcal/100 g were formulated. Diets were designed to contain increasing protein level (20, 25, 30, 35, and 40%), using fish meal as the main ingredient. Each diet was prepared by first mixing the macro ingredients; fish meal, rice starch and soybean protein meal, in a Kitchen blender Aid for 5 min. The dry ingredients were pulverized, sieved through 0.5 mm. The ingredients were thoroughly mixed in a food mixer prior to the addition of fish oil, vegetable oil and soybean.

The final product was extruded at room temperature with a meat grinder and a 2-mm die, and the resulting pellets were air-dried at room temperature. To verify the composition of the experimental diets the proximate composition was carried out in UbonRatchathani University laboratory. The percentage of dry matter, crude protein (N × 6.25), ether extract, crude fiber, ash, and nitrogen-free extract (NFE) was calculated by standard AOAC (1995) methods, for the experimental diet. The ingredient composition and proximate analysis of the experimental diets are given in Table 1.

Table 1. Formulation and proximate analysis of the experimental diets

Ingredients	Dietary Protein Levels				
	20%	**25%**	**30%**	**35%**	**40%**
Fishmeal	16.0	24.0	31.0	40.0	50.0
Soybean meal	15.0	15.0	15.0	15.0	15.0
Rice starch	35.0	30.0	30.0	20.0	8.0
Fish oil	2.5	2.5	2.5	2.5	2.5
Vegetable oil	2.5	2.5	2.5	2.5	2.5
Choline Chloride	0.2	0.2	0.2	0.2	0.2
Vitamin premix	1.0	1.0	1.0	1.0	1.0
Trace Mineral Premix	1.0	1.0	1.0	1.0	1.0
Ascorbic acid	0.1	0.1	0.1	0.1	0.1
Cellulose	26.7	23.7	16.7	17.7	19.7
Total	100	100	100	100	100
Proximate analysis					
Moisture %	7.50	7.10	6.70	6.80	6.90
Crude protein %	20.84	25.63	31.00	36.10	40.20
Ether extract %	8.10	8.35	8.20	8.10	8.20
Crude fiber %	4.90	3.91	2.95	3.00	2.95
Ash %	5.90	5.90	5.70	5.60	5.70
NFE	52.76	48.41	45.45	40.40	35.80
GE(Kcal/l00g)	430	431	435	434	435

Water Quality

Temperature and dissolved oxygen were monitored daily using a YSI 550 DO meter (YSICompany,Yellow Springs, CO, USA). Water quality analyses were conducted three times a week for concentrations of total ammonia–nitrogen and nitrite–nitrogen using a DR/2500 spectrophotometer (HACH Company, Lovel and CO, USA) and pH measured with a YSI 60 electronic pH meter (YSI Company). Alkalinity and hardness were determined once a month by digital titration (HACH Company) and tanks were siphoned three times a week to remove accumulated solids. Unionized ammonia was calculated based on total ammonia–nitrogen, water temperature, and pH according to Boyd (1979).

Growth performance parameters and muscle composition analyses

The experiments lasted 150 days, at the beginning and the end of the trial 3 fish from each dietary treatment (1 per replicate) were sampled and pooled for muscle composition analysis. Proximate composition of fish muscle and experimental diets was performed on most of the dietary treatments. Analysis of dry matter was performed by drying for 24h at 105°C, crude protein by Kjeldahl method, and crude fat using a Soxhlet extraction apparatus, ash by combustion in muffle furnace at 550 °C for 7h, and energy by burning in a Gallenkamp bomb calorimeter (Leicestershire, England). Carbohydrate content (nitrogen-free extract; NFE = 100 - (% protein + % lipid + % fiber + % ash) was determined by difference. Specific growth rate (SGR), feed conversion ratios (FCR), weight gain (DWG), feed efficiency ratio (FE),daily feed intake (DFI), protein efficiency ratio (PER), and apparent net protein retention (ANPR), were calculated using the following formulae:

$$\text{SGR (\%)} = \frac{\ln \text{final wt} - \ln \text{initial wt}}{t \text{ (days)}} \times 100$$

$$\text{FCR} = \frac{\text{Food consumed in g (dry weight)}}{\text{Live weight gain in g}}$$

$$\text{DWG (g/day)} = \frac{\text{(Mean final weight–Mean initial weight)}}{\text{Culture period (day)}}$$

$$\text{FE} = \frac{\text{Mean weight gain}}{\text{Mean feedintake}}$$

$$\text{DFI (\%/day)} = \frac{\text{Daily feed intake}}{\text{)Initial weight +Final weight/(2}} \times 100$$

$$\text{PER} = \frac{\text{Body weight gain (wet)}}{\text{Apparent protein intake (dry)}}$$

$$\text{ANPR} = \frac{\text{(Final body protein-initial body protein)}}{\text{Apparent protein intake}} \times 100$$

Statistical analysis

A completely randomized design with three replicates per treatment was used in the experiment. All data on growth, feed conversion and survival rates were subjected to one-way analysis of variance and Tukey's test to determine the significant differences amongst treatments.The broken line regression model (Robins *et al.* 1979) was used to estimate the breakpoint in the growth curve which represented the optimum dietary protein level for fish.

Results

During the study period, overall water quality values averaged (±SD): dissolved oxygen, 6.0 ± 0.4 mg/L; temperature, 22.2 ± 0.4 C; pH, 7.2 ±0.3; unionized ammonia–nitrogen, 0.014 ± 0.007 mg/L; total ammonia–nitrogen, 0.94 ± 0.13 mg/L; nitrite–nitrogen, 0.74 ± 0.28 mg/L; alkalinity, 93.6 ±14 mg/L; and total hardness, 156 ± 14.4 mg/L. Water quality parameters were within ranges suitable for health and growth of Mekong giant catfish (Pholprasith and Tavarutmaneegul, 1997).

Growth performance

Monthly changes in mean body weight of Mekong Giant Catfish juveniles are shown in Fig.1 and details on the overall growth performances and other related parameters are given in Table.2. In all treatments, the fish increased in weight, the increase varied with the dietary protein content (Fig.1). Mean survival rates did not differ significantly ($P<0.05$) among treatments and mortalities were not treatment-related (Table2). The greatest final weight 568.8 gram occurred at 35% dietary protein level while the lowest final weight gain of 343.3 gram observed at the lowest dietary protein level of 2%. Weight gain was significantly different ($P< 0.05$) between 20 and 35%, 20 and 40%, 35 and 40% dietary protein levels. Fish fed the 35% protein diets had the best specific growth rate (Table.2). At the higher protein levels than this level there were decline in % SGR, feed efficiency ratio (FE) and daily feed intake (DFI). Protein efficiency ratio (PER) tended to decrease as the dietary protein level increased. The best feed conversion ratio was at the protein level of 35% and was significantly ($P< 0.05$) different from that of other dietary treatments.

Table 2. Growth performance, feed utilization efficiency and protein utilization efficiency of fish fed experimental diets for 150 days

Parameters	Dietary protein levels				
	20%	25%	30%	35%	40%
Initial weight (g)	15.18±0.09[ns]	15.08±0.08[ns]	15.07±0.18[ns]	15.19±0.01[ns]	15.25±0.09[ns]
Final weight (g)	41.4±1.67[ns]	40.77±0.40[ns]	41.2±1.22[ns]	40.73±0.12[ns]	41.13±0.49[ns]
Initial length (cm)	33.60±0.20[a]	33.93±2.68[a]	35.46±1.70[ab]	37.98±0.77[b]	36.82±0.25[b]
Final length (cm)	343.33±47.97[a]	375.47±94.37[ab]	423.07±38.25[ab]	568.83±41.02[c]	456.97±21.66[b]
Daily weight gain (DWG)	2.01±0.32[a]	2.23±0.63[ab]	2.55±0.26[ab]	3.52±0.27[c]	2.77±0.14[b]
Specific growth rate (SGR)	1.41±0.09[a]	1.47±0.16[ab]	1.55±0.06[ab]	1.76±0.05[c]	1.61±0.03[bc]
Survival rate	100.0±0.0[ns]	100.0±0.0[ns]	100.0±0.0[ns]	100.0±0.0[ns]	100.0±0.0[ns]
Feed conversion ratios (FCR)	1.53±0.07[b]	1.46±0.09[b]	1.32±0.02[a]	1.23±0.03[a]	1.32±0.07[a]
Feed efficiency ratio (FE)	0.66±0.03[a]	0.68±0.04[a]	0.76±0.01[b]	0.81±0.02[b]	0.76±0.04[b]
Daily feed intake (DFI)	1.60±0.05[b]	1.55±0.04[b]	1.45±0.03[a]	1.43±0.02[a]	1.46±0.06[a]
Protein efficiency ratio (PER)	3.27±0.15[e]	2.75±0.16[d]	2.52±0.04[c]	2.31±0.05[b]	1.91±0.10[a]
App.net protein retention (ANPR)	42.96±5.44[c]	39.98±6.18[bc]	33.59±2.60[ab]	34.54±1.17[ab]	30.90±2.36[a]

Row means with common superscripts are not significantly different ($P>0.05$)

On the basis of percentage weight gain, daily weight gain, and specific growth rate, the dietary protein requirement of young Mekong Giant Catfish was experimentally observed at 35%.Protein efficiency ratio (PER), feed conversion ratios (FCR) and apparent net protein retention (ANPR) all decreased with increasing dietary protein levels, while survival rate was not significantly affected.

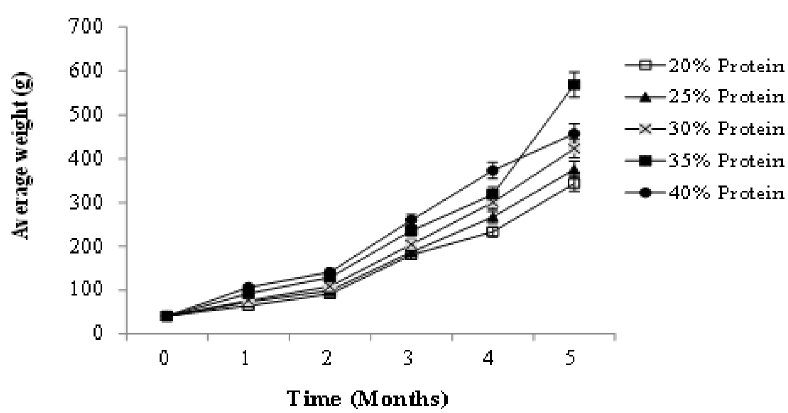

Fig.1. Changes in average weight of Mekong Giant Catfish juveniles fed with different protein content diets

Dietary protein requirement

The broken line regression model was used to estimate the breakpoint in the growth curve which represented the optimum dietary protein level for fish in this study was applied with two assumptions; the first procedure assumed a linear or straight line relation between weight gain and dietary protein level at or below the requirement and when the requirement was met, the weight gain abruptly plateaued as the horizontal line (Fig.2), the second procedure assumed the weight gain after the requirement point was decreased corresponding to the existing results recorded. Thus, the dietary protein level producing maximum growth, calculated by broken line regression was 35.01%.

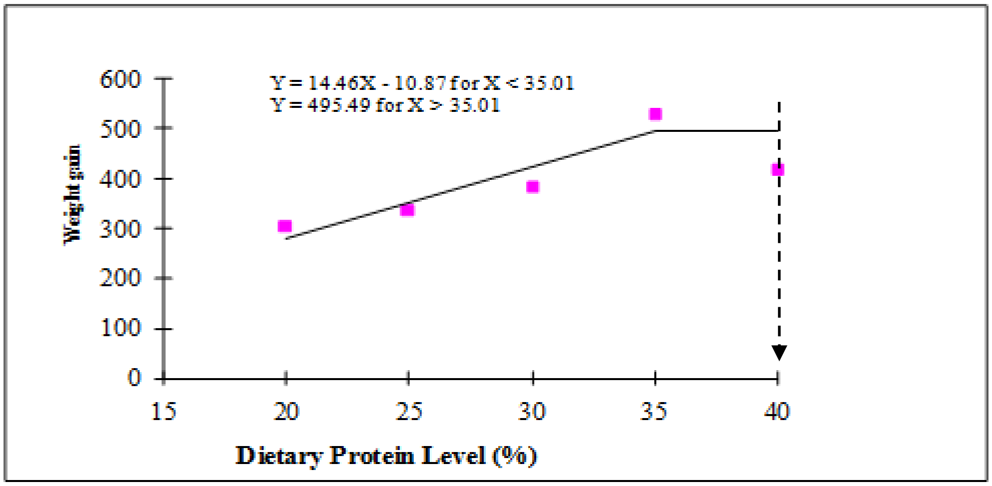

Fig.2. Optimum dietary Methionine level for Mekong giant catfish juveniledetermined by Broken-line regression analysis.

Muscle Composition

The influence of dietary protein levels on muscle composition of Mekong Giant Catfish juveniles is presented in Table 3. Fish muscle moisture content, crude fibre, NFE and ash did not differ significantly among treatments. There were significant differences (P< 0.05) in protein compositions between treatments. The protein content was significantly lower, and higher (P< 0.05) in fish fed the 20% and 40% protein diets, respectively but did not differ significantly among fish fed the 25-35% protein diets.

Table 3. Muscle proximate composition of Mekong giant catfish fed diets containing graded levels of protein over 5 months

Dietary protein (%)	Moisture	As percentage of dry matter				
		Protein	Lipid	Fibre	NFE	Ash
20	8.67 ± 2.02[a]	67.98 ± 2.04[c]	14.90 ± 2.02[a]	0.08 ± 0.01[a]	7.03 ± 3.56[a]	5.36± 1.05[a]
25	6.94 ± 0.50[a]	73.22 ± 3.29[bc]	7.62 ± 1.33[b]	0.08 ± 0.02[a]	7.13 ± 3.22[a]	5.02± 0.79[a]
30	10.18 ± 3.37[a]	72.30 ± 5.41[bc]	6.90 ± 2.57[bc]	0.07 ± 0.01[a]	7.64± 5.14[a]	4.69± 0.06[a]
35	8.06 ± 0.47[a]	76.75 ± 0.85[b]	5.30 ± 1.68[bc]	0.08 ± 0.01[a]	4.73 ± 1.14[a]	5.10± 0.04[a]
40	7.54 ±1.17[a]	80.25 ± 1.05[a]	3.70 ± 0.70[c]	0.09 ± 0.02[a]	3.90 ± 0.26[a]	5.37± 0.36[a]

Column means with a commnon superscript are not significantly different (P>0.05)

Fish muscle moisture, NFE, Fiber and ash were not clearly related to dietary protein level

Low dietary protein levels resulted in significantly higher ($P<0.05$) lipid but lower protein content. Fishes in this study were sexually immature, and the differences in muscle protein content were attributed to dietary protein intake. The muscle protein and ash contents were not significantly affected by the dietary protein level.

Discussions

Growth

The dietary protein level producing maximum growth of Mekong giant catfish, calculated y broken line regression was 35.01%. This indicates that optimum protein level for young *P. igas* is likely to be closer to those required by other omnivorous species such as channel catfish, (32-36%, NRC, 1993) than carnivorous species such as red sea bream, *Chrysohrys major* (55%, Yone, 1976). This result is in agreement with the optimum protein requirement of Black ear catfish (*Pangasiuslarnaudii*Bocourt) was 41-43% (Chutjariyaves *et al.* 1996), while the optimum for Indian strain of common carp was 45% (Sen *et al.* 1978). The growth performances responding to dietary protein levels in Mekong giant catfish exhibited similar trends to all previous studies in decreasing growth rate at an excessive dietary protein levels.

The optimum dietary protein requirement is known to differ between different fish species. These may be due to differences in strains (Austreng and Refsite, 1979), and environmental factors and size of fish. The protein requirement reported for fish such as common carp,*Cyprinuscarpio* fingerling (Ogino and Saito, 1970), *Tilapia zilli*, (Teshima *et al*. 1978) and

Chrysophrysaurata (Sabaut and Luquet, 1973) fall within the range of 35-50% by dry weight. In contrast, a lower dietary protein requirement of less than 35% has been reported for herbivorous fish such as tawes,*Puntiusgonionotus* (Wee and Ngamsnae, 1987), *O . niloticus* (De Silva and Perera, 1985), *O. auratus* fingerling (Winfree and Stickney, 1981) and *Mugilcapito* (Papaperaskera-Papoutsoglou and Alexis, 1985).

Excessive dietary protein levels (40%) caused a significant decreased in growth rates. The apparent growth-depressing effect to high protein diets observed in this study has also been reported for other fish species, both in carnivorous fish such as snakehead (Wee and Tacon, 1982) and grouper (Teng *et al.* 1978), and herbivorous fish such as tilapia (Juancey, 1982), grass carp *Ctenopharyngynodonidella* (Val.) (Dabrowski, 1977), bighead carp, *Aristichthysnobilis* (Santiago and Reyes, 1991) and tawes, *Puntiusgonionotus* (Wee and Ngamsnae, 1987). It was postulated that the decrease in growth response at protein level above the optimum may be due to the reduction in dietary energy available for growth as extra energy is required to deaminate and excrete the excess amino acids absorbed (Jauncey, 1982). Lim, Sukhawongs and Pascual (1979) also reported that the slightly lower weight gain of milkfish (*Chanoschanos* (Forsskal), 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 form 1.23 to 1.53, with significant differences among the two groups (20%, 25% protein) and (30%, 35%, 40% protein) of experimental diets. However, there was a distinct trend for FCR to decrease with increasing dietary protein levels, similar to the trends observed in tilapia (Juancey, 1982), Rohu*Labeorohita* (Nandeesha *et al*. 1994) and tawes (Wee and Ngamsnae, 1987).

Protein efficiency ratio (PER) ranged from 1.91 to 3.27, with the highest value in fish fed the diet with 20% protein and decreased as the level of protein increased. This trend was also observed in snakehead (Wee and Tacon, 1982), grass carp (Dabrowski, 1977), bighead carp (Santiago and Reyes, 1990). Fish often show the greatest protein conversion efficiency when fed a dietary protein less than that yielding maximum growth and feed efficiency (Davis and Stickney, 1978).

Apparent net protein retention (ANPR) in this study for Mekong Giant Catfish decreased from 42.96% to 30.90% with increasing dietary protein content. A similar decrease was also observed in snakehead (Wee and Tacon, 1982), common carp (Dabrowski, 1977), tilapia (Juancey, 1982) and brown trout *Salmotrutta* (Arzel et al., 1994). It has been reported that the protein retention for different dietary protein levels depends on the type of energy provided by the ingredients (Ogino and Saito, 1970). Cowey *et al*. (1972) found that ANPR and PER values for plaice were higher with diets containing carbohydrate than with diets without carbohydrate, even though both diets were isocaloric. The highest ANPR and PER obtained in this study on Mekong giant catfish were found with the 20% protein diet, the lowest dietary protein concentration. This result supports the postulation that the fish fed diets containing higher carbohydrate exhibit higher PER and ANPR values.

Muscle composition

Muscle composition of Mekong giant catfish was not evidently influenced by the dietary protein except for crude protein and lipid, which were significantly ($P<0.05$) affected by dietary protein concentration. The pattern of changes of muscle protein as influenced by dietary protein in this study reflected that of weight gain, and was mainly due to an increase in body protein. Although carcass moisture content increased with the dietary protein level, changes in protein, fat and ash contents were not clearly related to the dietary treatment.

This is in agreement with Juancey (1982), reported that gross body composition in hybrid tilapia and *O.mossambicus* was 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.

Muscle ash values were not affected by dietary protein levels. A similar trend has been noted in other experiments; with tilapia (Juancey, 1982); snakehead (Wee and Tacon, 1982); eel, *Anguilla japonica* (Nose and Arai, 1972 and sunshine bass,*Moronechrysops* (Brom *et al*. 1992).

The lipid levels generally decreased with increasing dietary protein. A similar trend has been noted in other experiments; with tilapia (Juancey, 1982); snakehead (Wee and Tacon, 1982); eel, *Anguilla japonica* (Nose and Arai, 1972) and sunshine bass,*Moronechrysops* (Brom *et al.* 1992). The higher lipid deposition in groups fed low protein diets can be explained by an overconsumption of non-protein nutrients per unit weight gain when protein limited growth, fish ingested more energy in the group fed low protein diet.(Arzel *et al.* 1994).

Conclusion

The present study indicate that, based on weight gain, relative growth, and feed efficiency, the optimum dietary protein levels for Mekong giant catfish juveniles were estimated to be approximately 35%. Mekong giant catfish is omnivorous and fed on wide variety of food, juvenile and adults readily take supplementary feed such as pellet, bread, insects, fish and crustacean flesh. Artificial foods are eaten even when other natural foods are available in ponds (Pholprasith *et al.* 1992). The optimum protein level for young Mekong giant catfish is likely to be closer to those required by other omnivorous species such as channel catfish, (32-36%, NRC, 1983) than carnivorous species such as red sea bream, *Chrysohrys major* (55%, Yone, 1976).

The effectiveness of practical diets, containing this recommended level, will depend on the amino acid composition produced by blending suitable plant and animal materials. Future refinement of practical diets for Mekong giant catfish requires the information on essential amino acid requirements and their optimum levels to be effectively incorporated.

In conclusion, This study indicate that the use of practical diets of 35% protein level for rearing juveniles of Mekong giant catfish is appropriated for obtaining an acceptable growth and feed utilization efficiency. However, a great deal of consideration is generally given to reducing feed costs, replacing fish meal by alternative protein sources that are of high quality, but less expensive for aqua feeds.

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(Received 29 November 2013; accepted 22 December 2013)