Dietary protein requirement of bighead catfish (*Clarias macrocephalus* Gunther, 1864) fingerling

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Abstract-The study was conducted in order to find the protein requirement of bighead catfish (*Clarias macrocephalus*) fingerling. The initial weight of fish was 6.29 g/ind and raised them in 8 weeks. The experiment was set up with six dietary treatments including six protein levels such as 25%, 30%, 35%, 40%, 45% and 50% with an isoenergy of approximately 4.5 Kcal/g and an isolipidic diets of 8%. Results show that the protein level effected on the survival rate. The 25% protein diet had the lowest survival rate of 67.78% while those in the remaining treatments varied from 91.1% to 100%. The specific growth rates (SGR) of fish were maximal at 3.26% per day in the 45% protein diet and minimal at 1.92% per day in the 25% protein diet. The protein efficiency ratios (PER) decreased as the protein levels in diets increased. The protein content affected significantly by the dietary protein levels. Using the Broken-line method and based on the SGR, the dietary protein requirement for bighead catfish was 46.1%.

Keywords: bighead catfish, *Clarias macrocephalus*, protein requirement

I. INTRODUCTION

Freshwater fish species have been developed in the past such as red tilapia, Nile tilapia, snakehead and catfish. Among these species, catfishes have lower mouth, bottom eaters, omnivorous, easy to grow, fast growth, resistance with diseases, live in polluted water, high density, and high productivity. Vietnam is currently been exploited and cultivated various catfish species such as *C. macrocephalus*, *C. gariepenius*, *C. batrachus*, and hybrid catfish. In particular, bighead catfish is a popular species with a high economic value and easy to raise. Many studies investigated the nutritional needs of different catfish species such as walking catfish *C. batrachus* (Singh *et al.*, 2009), hybrid catfish (*C. batrachus* × *C. gariepinus*) (Giri *et al*., 2003). Mollah *et al.* (1990) studied the possibility of using carbohydrate of walking catfish. The other research of Machiels and Henken (1985) studied on the effect of protein and energy on growth using food and energy metabolism.

Protein is one of the most important factors influencing growth and feed cost in aquaculture (Lovell *et al*., 1989). Increasing protein in feed usually improves fish yields, especially for fish eaters, but also increases feed costs. Although there have been many studies on nutrition in different catfish species but studying in nutrition of bighead catfish are limited. Therefore, a research on nutrients for bighead catfish is necessary for the establishment of feed formula and contributes to the development of commercial catfish farming.

II. MATERIALS AND METHODS

2.1 Experimental diets

The experiment consisted of 6 treatments fed with isoenegy of 4.5kcal/g and 25, 30, 35, 40, 45 and 50% crude protein, respectively. Where the ratio of fish meal/soybean meal is 5:2 and the ratio of animal oil for plant oil is 1: 1. The main feed ingredients are fish meal, soybean meal, and wheat flour. The ingredients were mixed, pelletized in 1.5-2 mm, dried and stored at -20°C in freezer during the experiment.

2.2 Fish and experimental condition

Bighead catfish fingerlings used in the experiments were bought from a hatchery in An Giang province. Before starting experiment, fish were acclimated in a 2000-L circular tank and fed with commercial diet for 15 days. The average initial weight varied from 6-7 g/tail. The experiment was conducted in 18 composite tanks (volume of 200 L/tank), water exchanged every 3 days and continuous aeration. Bighead catfish was stocked with a density of 30 fish/tank. The experiment was completely randomized, with three replications.

Fish were fed twice a day (9:00 and 16:00) to satiation. The amount of consumed feed and uneaten feed in each tank was recorded daily. The remained feed was siphoned out 30 minutes after feeding then dried and weighed. Any fish mortality was daily recorded, removed and weighed immediately. The experiment prolonged for 8 weeks.

Water parameters were weekly recorded. Temperature ranged from 28.2-28.5°C, pH from 7.4-7.5 and dissolved oxygen from 4.7-5.1 mg/L, TAN 0.02-0.11 mg/L. The water quality parameters in all treatments were in a suitable range for the normal growth and development of this species. At the end of the experiments, the survival rate and growth were determined by counting and weighing all fish in each tank. Each specimen was kept in the freezer at -20°C for the chemical compositions analysis of the fish according to the method of AOAC (2000).

2.3. Data collection and calculations

Growth rate was calculated and expressed as weight gain

(WG), daily weight gain (DWG), specific growth rate (SGR) according to the following equations: WG(g) = Wf – Wi, DWG (g/day) = WG/t, SGR (%/day) = ((ln (Wf) - ln (Wi))/t) x 100. The survival rate of the fish in each tank was counted using the following formula: survival rate (%): SR = (the number of the fish after 8 weeks per the number fish at commencement) x 100. Feed conversion ratio (FCR) and protein efficiency rate (PER) were calculated by the following equations: FCR = consumed feed (dry weight (g))/weight gain of fish (g), PER = (Wf - Wi)/protein intake. Where Wi is the initial weight of fish (g), Wf is the final weight of fish (g), t is the experimental period (day).

2.4 Statistical analysis

Mean differences in growth parameters and feed efficiency among treatments were tested using ANOVA followed by DUNCAN tests at the significant level of 0.05. Statistical analyses were performed using SPSS 16.0. Protein requirements were calculated using to a model of broken line of Zeitoun *et al.* (1976).

III. RESULTS AND DISCUSSION

3.1 Survival rate of bighead catfish of different dietary protein levels

The survival rates of bighead catfish range from 67.78 to 100%. The survival rate of fish increases with an increasing rate of protein in food. The lowest rate was 67.78% in the 25% protein treatment and significantly different ($p < 0.05$) from the remaining treatments. The highest survival rates were in treatments of 45 and 50% protein. The results showed that different dietary protein levels had a significant effect on the survival rate of bighead catfish.

This finding is consistent with the study by Loum *et al.* (2013) on tilapia (*Oreochromis niloticus*) (1.25 g/fish) in which the survival rate tends to increase with high protein levels from 21-45%. In addition, other studies have shown that dietary protein levels have an effect on the survival rate of aquaculture animals. According to Tran Thi Thanh Hien *et al.* (2005), *Channa micropeltes* fed with dietary protein content of 14 and 24% were significantly lower than those with high levels of protein ($p < 0.05$). Similarly, experiments on *Channa striatus* showed that the survival rate increased as the protein content in the feed increased and there were significant differences between treatments (Trieu *et al*., 2001). In diets, when the protein content is sufficiently supplied, fish grow fast with a high survival rate. When the protein content is lower than demand but still enough for the maintenance and growth activities, fish grow slowly. However, when the protein content in the food is too low compared to the fishes' needs, the fishes use the body's own protein to maintain the minimum functions to survive (Tran Thi Thanh Hien and Nguyen Anh Tuan, 2009). This might be the reason for the survival rate of the treatments with too low protein levels.

3.2 Growth's performances of bighead catfish with different dietary protein levels

Experiment results show that WG and DWG growth of bighead catfish increased when dietary protein content increased from 25% to 45% and tended to decrease when dietary protein content in food is up to 50%. Weight gain and daily growth rate of the fishes were the lowest in treatments with a protein content of 25% (11.01 g and 0.21 g / day), which are not significantly different ($p > 0.05$) from diets of 30 and 35% protein content but from the other treatments. In the 45% protein treatments, gain and daily growth rate were at the highest (27.93 g and 0.54 g / day), which were not significantly different ($p > 0.05$) from the 50% protein treatments but from the other treatments.

When analyzing the correlation between relative growth rate (SGR) and protein content in feeds, we obtained the equation $y = 0.055x + 0.543$ with $R^2 = 0.906$. This shows a strong correlation between the protein content in the feed with the relative growth rate (SGR) of catfish. Figure 1 shows the optimal dietary protein content of the bighead fin catfish during breedings with the best growth rate of 46.1%.

This result shows that when the protein content in the food is low, the fish grows slowly because the fish use the body's own protein to maintain its function to survive. However, when the feed was supplied with too much protein (exceeding the optimal requirement of 46.1%), the growth rate and growth of bighead carp tended to decrease. According to Tran Thi Thanh Hien and Nguyen Anh Tuan (2009), if the food supply is too high protein, the excess protein is not absorbed by the body to synthesize new protein that is used to convert energy into or out. In addition, the body also consumes more energy for the process of digesting excess protein, so the growth of the body decreases. This result is consistent with the results of research on Basa catfish, Tilapia and Pangasius fingerlings at the breeding stage of Tran Thi Thanh Hien *et al*. (2003), Mystus catfish (Khan *et al.*, 1993).

Bighead catfish is the carnivorous species feeding mainly on small insects and larvae of aquatic insects, worms, small crustaceans, so the high protein requirement of 46.1% is suitable for the breeding stage. Compared to some catfish species, bighead fishes require a high level of protein comparing with *C. batrachus* (0.1 g) 30% (Chuapoehu, 1987), *C. gariepinus* (40 g) 30-40% (Henken *et al*., 1986), *Mystus nemurus* 42% (Khan *et al.*, 1993). According to Phuong (1998), the best protein level for basa catfish's growth with fish sizes of 16-17g and 75-81g are 36.7% and 34.9%, respectively. Hung et al., (2000) reported that basa and tra catfish of 5-6 g had protein requirements of 27.8 and 32.2%. Liem and Tu (2000) reported that the asphalted fish had a maximum growth rate at a 37.9% level of protein in the feed. However, the protein requirements of catfish were lower than those of tilapia (48.5%) (Tran Thi Thanh Hien and Duong Thuy Yen, 2006), 47% (Trieu *et al*., 2014) and 50.8% of snakehead fish (Tran Thi Thanh Hien *et al*., 2005).

3.3 Feed utilization efficiency of bighead catfish with different dietary protein levels

The amount of food intake (FI) increased with increasing levels of protein in the food. The FI at 363 ± 45 mg / day / day (25% protein) was not significantly different ($p > 0.05$) from the 30% and 35% protein treatments but to the other treatments. The FCR in the experiment decreased as the protein content in the feed increased. FCR ranges from 1.06 to 1.84. The lowest FCR of 1.06 was found in the treatments of 45% protein. The difference was statistically significant ($p < 0.05$) compared to the 25% and 30% protein treatments but not statistically significant (p>0.05) compared to the other treatments. The highest FCR was found in the 25% protein treatment which was significantly different (p<0.05) compared to the 45% protein treatments but not to the remaining treatments. However, the FCR increased when the dietary protein content was too high (>45% protein), suggesting that the ability of catfish feed conversion decreased when fish fed diets with protein content Low 45% or higher 50%.

These results is similar to that of Tran Thi Thanh Hien *et al*. (2003) studying the protein requirements of Basa fish, Tilapia, and Tra catfish species. The results show that the feed conversion ratio (FCR) decreases as the protein content in the feed increases. However, when the demand exceeds the fish, the FCR begins to increase. This was reported similarly in many fish species, such as Asiatic fish, non-catfish from Morennike and Akinola (2010*)* *Channa punctatus* (Zehra and Mukhtar, 2011).

The efficiency of protein utilization (PER) ranged from 1.63 to 2.54 and tended to decrease as dietary protein content increased. PER was the highest in the 25% protein treatment (2.54±0.92). The difference was statistically significant (p<0.05) compared to the 50% protein treatment but not statistically significant (p>0.05) compared to the other treatments. Similarly, the lowest PER in the 50% protein treatment (1.63±0.09) was statistically significantly different (p<0.05) compared to the 25% protein treatment but not to other treatments. According to Tran Thi Thanh Hien and Nguyen Anh Tuan (2009), with the same source of protein for feed, protein efficiency will be high in low protein foods as fish will maximize the usage of protein source to build the body. The results of this study are similar to those of previous studies e.g. the protein utilization efficiency of tilapia (*Oreochromis niloticus*) decreased from 2.35 to 1.58 (fry stage) and from 1.92 to 1.19 (seed stage) and from 1.53 to 0.99 (grow-out stage) (Mohsen *et al*., (2010). The study of Khan *et al*. (1993) on *Mystus nemurus*, the highest PER was in the 27% protein treatment and the lowest in the 50% protein treatment.

NPU is the protein's ability to accumulate protein from the fish body, which is used to evaluate the effectiveness of different protein sources. The cumulative protein efficiency (NPU) varied between 25.76-34.16% and the same as for feed efficiency (PER), the NPU also tended to decrease as the protein content in the diet increased. The highest NPU was 34.16% (35% protein), followed by 32.37% (25% protein) and lowest was 25.76% (50% protein) but not statistically significant (p>0.05) between treatments. This result is consistent with the study by Meyer and Fracalossi (2004) on jundia fingerlings *Rhamdia quelen*.

3.4 Body composition of bighead catfish with different dietary protein levels

The chemical composition of the fish varies depending on the species, the stage of development and strongly on the quality of the food. For bighead catfish when fed with protein feed from 25 to 50%, protein content in the fish body was 60.13-63.94%, lipid 12.62-17.57% (Table 5). The results show that protein and lipid levels in fish tend to increase as the dietary protein levels increase. Especially the protein content in fish is proportional to protein content in food; the lowest of 60.13% in the 25% protein treatment was statistically significantly different (p<0.05) compared to the other treatments. The highest of 65.01% in the 50% protein treatment was not statistically significant (p>0.05) with 40-45% treatment but statistically significant difference (p<0.05) with the remaining treatments. Lipid tended to increase as dietary protein content increased but the difference was not statistically significant (p>0.05) between treatments (Table 5).

This result is similar to the results of the experiments of Morenike and Akinola (2010) when feeding *C. gariepinus* with protein content of 25, 30, 35, the protein content in the body Fish increased from 56.01% to 60.57%. In addition, the amount of lipid in the body increased when the protein content in food increased from 2.81% to 4.8%. Some other experiments showed similar results to the experiment on giant snakehead (*Chana marulius*), when feeding fish with feed with protein levels of 30, 36, 42, 48, 54 and 60%, the protein content in the fish body increased from 56% in the 30% level to 66.63% in the 60% levek. Similarly, lipid levels were also increased from 30% to 6.68% in the 60% level (Raizada *et al.*, 2012).

IV. CONCLUSION

Protein content in feed has an influence on survival rate, growth rate, feed efficiency and chemical composition of bighead catfish. Appropriate protein requirements for fish development are in the range of 45-50% in which the optimal protein content for bighead catfish is 46.1%.

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Table 1. Formulation and proximate composition of experimental diets (dry matter basis)

Ingredients ($g.kg^{-1}$)	Experimental diets (% Crude Protein)					
	25	**30**	**35**	**40**	**45**	**50**
Fish meal	29.0	35.1	41.6	47.4	53.6	60.1
Defatted soybean meal	12.7	15.3	18.0	20.7	23.4	26.2
Cassava meal	53.1	44.4	35.7	27.0	18.3	9.62
Oil	3.30	2,98	2.66	2.34	2.02	1.15
Mineral and vitamin premix	1.76	1.76	1.76	1.76	1.76	1.76
Carboxylmethyl Cellulose (CMC)	0.12	0.34	0.56	0.78	1.00	1.22
Chemical composition (%)						
- Crude Protein	24.8	29.9	34.0	39. 6	45.6	49.9
- Crude fat	8.04	8.59	8.21	7.68	7.87	7.80
- Ash	9.1	10.7	12.7	13.6	14.9	18.4
Gross energy ($Kcal. g^{-1}$)	4.69	4.39	4.53	4.41	4.51	4.31

Premix mineral and vitamin ($unit.kg^{-1}$): Vitamin A 2,000,000 IU; Vitamin D 400,000 IU; Vitamin E 6g; Vitamin B_1 800mg; Vitamin B_2 800mg; Vitamin B_{12} 2mg; Calcium D Pantothenate 2g; Folic acid 160mg; Choline Chloride 100g; Iron (Fe^{2+}) 1g; Zinc (Zn^{2+}) 3g; Manganese (Mn^{2+}) 2g; Copper (Cu^{2+}) 100mg; Iodine (I^-) 20mg; Cobalt (Co^{2+}) 10mg.

Table 2 : The survival rates of bighead catfish of different dietary protein levels

Treatment	Survival Rate (%)
Treatment 1 (25% CP)	67.8±8.39^{a}
Treatment 2 (30% CP)	91.1±7.70^{bc}
Treatment 3 (35% CP)	92.2±3.85^{bc}
Treatment 4 (40% CP)	85.6±8.39^{b}
Treatment 5 (45% CP)	100±0,00^{c}
Treatment 6 (50% CP)	100±0,00^{c}

Values (mean ± SD) in a column followed by the same superscript letter are not significantly different (P>0.05).

Table 3 : Growth performance of bighead catfish with different dietary protein levels

Treatment	Wi (g)	Wf (g)	WG (g)	DWG (g/day)	SGR (%/day)
Treatment 1 (25% CP)	6.25±0.10	17.26±3.79^{a}	11.01±3.69^{a}	0.21±0.071^{a}	1.92±0.43^{a}
Treatment 2 (30% CP)	6.26±0.06	17.98±1.88^{a}	11.72±1.88^{a}	0.23±0.036^{a}	2.02±0.20^{a}
Treatment 3 (35% CP)	6.35±0.09	21.03±2.07^{a}	14.67±1.98^{a}	0.28±0.038^{a}	2.30±0.17^{a}
Treatment 4 (40% CP)	6.36±0.10	28.41±2.83^{b}	22.05±2.88^{b}	0.42±0.055^{b}	2.87±0.21^{b}
Treatment 5 (45% CP)	6.27±0.08	34.20±1.24^{c}	27.93±1.17^{c}	0.54±0.023^{c}	3.26±0.05^{b}
Treatment 6 (50% CP)	6.33±0.08	31.62±1.62^{bc}	25.30±1.60^{bc}	0.49±0.031^{bc}	3.09±0.10^{b}

Values (mean ± SD) in a column followed by the same superscript letter are not significantly different (P>0.05).

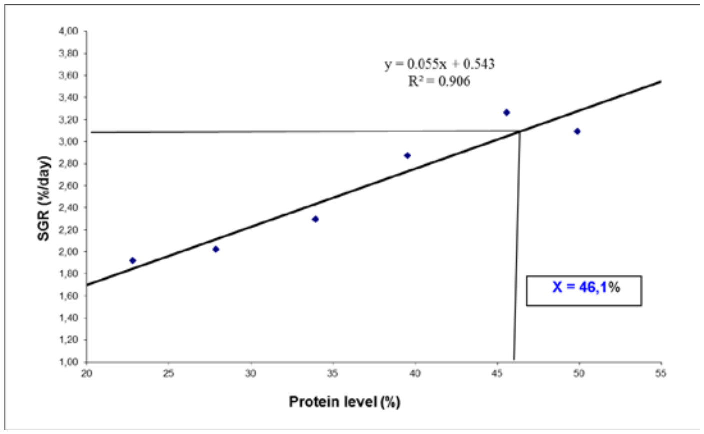

Figure 1. The relationship between specific growth rate (SGR) with different dietary protein levels of bighead catfish.

Table 4: Feed intake (FI), feed conversion ratio (FCR), protein efficiency (PER) and net protein utilization (NPU) of bighead catfish with different dietary protein levels

Treatment	FI (mg/fish/day)	FCR	PER	NPU (%)
Treatment 1 (25% CP)	363±45[a]	1.84±0.72[a]	2.54±0.92[a]	32.37±2.37[a]
Treatment 2 (30% CP)	406±12[a]	1.79±0.24[a]	1.96±0.27[ab]	28.63±3.54[a]
Treatment 3 (35% CP)	395±32[a]	1.40±0.30[ab]	2.10±0.40[ab]	34.16±7.93[a]
Treatment 4 (40% CP)	518±42[b]	1.21±0.13[ab]	2.04±0.22[ab]	30.01±3.94[a]
Treatment 5 (45% CP)	581±37[c]	1.06±0.02[b]	2.07±0.03[ab]	30.54±2.85[a]
Treatment 6 (50% CP)	588±04[c]	1.19±0.07[ab]	1.63±0.09[b]	25.76±2.02[b]

Values (mean ± SD) in a column followed by the same superscript letter are not significantly different (P>0.05).

Table 5: Effect of dietary protein levels on body composition of bighead catfish

Treatment	Moisture (%)	Body composition (% dry weight)		
		Crude protein (%)	Crude fat (%)	Ash (%)
Treatment 1 (25% CP)	76.82± 0.32[a]	60.13± 0.92[a]	15.55±2.26[a]	15.84±1.38[a]
Treatment 2 (30% CP)	76.79± 0.24[a]	62.74± 0.61[b]	15.90±0.47[a]	13.41±1.61[b]
Treatment 3 (35% CP)	74.84± 0.40[b]	62.80± 0.04[b]	17.57±0.17[a]	11.18±1.29[b]
Treatment 4 (40% CP)	77.04± 0.13[a]	63.94± 0.65[bc]	15.66±2.00[a]	11.98±0.50[b]
Treatment 5 (45% CP)	76.69± 0.81[a]	63.37± 1.08[bc]	17.25±1.26[a]	12.28±1.33[b]
Treatment 6 (50% CP)	76.20± 0.44[a]	65.01± 2.21[c]	16.91±0.76[a]	11.00±1.42[b]

Values (mean ± SD) in a column followed by the same superscript letter are not significantly different (P>0.05).