Songklanakarin J. Sci. Technol.
34 (1), 37-43, Jan. - Feb. 2012

SJST SONGKLANAKARIN JOURNAL OF SCIENCE AND TECHNOLOGY
http://www.sjst.psu.ac.th

Original Article

Growth, feed utilization, survival and body composition of fingerlings of Slender walking catfish, *Clarias nieuhofii*, fed diets containing different protein levels

Suphada Kiriratnikom* and Anut Kiriratnikom

Aquaculture Biotechnology Research Unit, Faculty of Science, Thaksin University Phatthalung Campus, Pa Phayom, Phatthalung, 93110 Thailand.

Received 24 May 2011; Accepted 8 November 2011

Abstract

The effects of test diets, containing different dietary protein levels (32, 36, 40, and 44%), on growth, feed conversion rate and survival of slender walking catfish (*Clarias nieuhofii*) fingerlings were studied. Fish with an average weight of 8.35-8.46 g were fed with isocaloric test diets containing 32, 36, 40 and 44% protein, for 12 weeks. The average body weight of the fish fed 40% - 44% protein was significantly higher than that of the others within 4 weeks of feeding (P<0.05). At termination of the experiment, average body weight and weight gain of the fish fed the diet containing 40% and 44% protein were highest and were significantly different from those of the fish fed 32% to 36% protein (P<0.05). The lowest feed conversion rate (FCR) was observed in the fish fed 40% and 44% protein. Condition factors (CF) were lowest in the fish that received 32% and 36% protein test diets, and hepatosomatic indices (HSI) were increased by the dietary protein levels but were not significantly different in the fish fed protein in the range of 36% to 44%. Carcass composition analysis indicated a positive correlation between dietary protein level and fish body protein content, but moisture, lipid level and ash content in the fish body were not significantly different among treatments. In conclusion, the 40% protein diet gave the maximum growth performance, lowest feed conversion ratio and high body protein content in slender walking catfish fingerling during the 12 weeks of the feeding trial.

Keywords: slender walking catfish, *Clarias nieuhofii*, protein requirement, growth performance, condition factor, hepatosomatic index

1. Introduction

Slender walking catfish (*Clarias nieuhofii*) has been categorized in the group of endangered fish in the Songkla lake basin, South of Thailand. Two major attempts have been made towards conservation of this species, *i.e.* the restoration of the peat swamp forest, which is the main habitat of this catfish, and the hatchery production of larvae to increase their population before restocking into nature. Because of their desirable taste and high market price, slender walking catfish have a high potential for development as a new cultured fish species in Thailand. Although the production of larvae from wild-catch broodstock has been successful, very few data about effects of water quality, nutrient requirement, and diseases have been reported for this catfish. Because feed and feeding are the highest variable cost in commercial aquaculture, the use of suitable feed is essential. Determination of nutritional requirements is very important as a basis for increasing growth of cultured species, reducing the

* Corresponding author.
Email address: ksuphada@yahoo.com

culture period and increasing the efficiency of production (Halver and Hardy, 2002). Protein is the energy-yielding nutrient that is important for optimizing physiological performance, locomotion, optimum growth, and disease resistance. Therefore, it is the first nutritional parameter that should be determined for formulated feed production for a newly established cultured fish species. Several studies have been reported on the protein requirement of *Clarias* species, and the relationship between variation in fish size, dietary protein source, and environmental conditions. A summary of the protein requirements of some *Clarias* species has been reported by Van Weerd (1995). This stated that the *Clarias* are omnivorous fish, and their dietary protein requirement is about 40%, with an energy requirement ranging between 13 and 17 kJ/g. Singh *et al.* (2009) reported that the daily protein retention in *Clarias batrachus* fry depended on water temperature. At higher temperatures the catfish required higher dietary protein level to maximize the protein retention. Also, as advised by Robinson *et al.* (2001), small fish need higher dietary protein levels. Giri *et al.* (2003) showed that the protein requirement of hybrid *Clarias* catfish (*Clarias batrachus* X *Clarias gariepinus*) post-larvae is 36.5%. Optimum levels of dietary protein for slender walking catfish have not been reported, however. The objectives of the present study were therefore, to determine the effects of dietary protein levels on growth, survival, feed conversion ratio, protein efficiency ratio, apparent net protein utilization, condition factor, hepatosomatic index, and chemical compositions of slender walking catfish.

2. Materials and Methods

2.1 Experimental diets and design

A feeding trial was conducted with a completely randomized design. Four experimental diets (gross energy 17.3-17.9 kJ/g) were formulated to contain levels of 32, 36, 40, or 44% crude protein. Fish meal was used as the individual protein source. All dry ingredients were ground; according to each formulation in Table 1, mixed in a Hobart mixer (Hobart, A200) for 15 min, then oil was added before further thorough mixing. After that 350 ml of freshwater were added per kilogram of feed and the whole mixed well for another 15 min before being passed through a meat grinder to give pellets of 3.0 mm in diameter and these were dried in an air-flow oven at 60°C for 10 to 12 hrs or until the moisture was less than 10%. Each test diet was analyzed for lipid, protein, and ash contents (AOAC, 1997). The nitrogen-free extract content of fish was calculated as 100 - (% protein + % lipid + % ash). The gross energy (GE) value of each diet was determined by using values of 16.7 kJ/g protein or carbohydrate and 37.6 kJ/g lipid (Garling and Wilson, 1976). The dry pellets were crushed into desirable particle sizes and stored at -20°C until used.

Table 1. Feed formula (%) and proximate chemical composition of the experimental diet

	T1 32% Protein	T2 36% Protein	T3 40% Protein	T4 44% Protein
Fish meal	56	64	72	80
Rice flour	20	14.5	8	1.5
Wheat flour	10	10	10	10
Rice bran	2.5	2.5	2.5	2.5
Mineral premix[1]	2.0	2.0	2.0	2.0
Vitamin premix[2]	2.0	2.0	2.0	2.0
Soybean oil	7.5	5	3.3	2
Moisture (%)	3.4	3.9	3.3	3.5
Crude protein (%)	32.8	36.9	40.5	44.8
Crude lipid (%)	18.3	18.3	18.2	18.9
Ash (%)	15.5	18.0	19.7	20.3
NFE[3] (%)	33.4	26.8	21.6	16.0
GE[4] (kJ/g)	17.9	17.5	17.2	17.3

[1] Mineral mixture (g/kg diet) KH_2PO_4 4.2; $CaHPO_4$ 3.3; NaH_2PO_4 6.25; KCl 2; KI 1.6; $MgSO_4.7H_2O$ 2; $ZnSO_4$ 0.1; $MnSO_4$ 0.1.
[2] Vitamin mixture (mg/kg diet): thiamine hydrochloride 30; riboflavin 30; nicotinic acid 140; calcium pantothenate 100; pyridoxine hydrochloride 25; vitamin B_{12} 0.005; inositol 2,000; biotin 6; folic acid 6; choline chloride 1,000; ascorbyl- polyphosphate 250; vitamin E 200; vitamin K_3 12; vitamin A 12,000 IU; vitamin D_3 2400 IU.
[3] NFE : Nitrogen-free extract = 100- (%protein + %lipid + %ash).
[4] GE : Gross energy using values of 16.7 kJ/g protein or carbohydrate and 37.6 kJ/g lipid.

2.2 Experimental fish and feeding trial

Hatchery-reared juvenile slender walking catfish (*Clarias nieuhofii*) from the hatchery of the Thaksin University Aquacultural Biotechnology Research Unit (ABRU) were used in this experiment. Before the feeding experiment began, the fish were acclimated to the experimental conditions for two weeks. During acclimation, fish were fed to satiation twice daily, with a diet containing 36% protein. Fish with the initial weight of 8.35-8.46 g were used and were randomly distributed to each of 12 experimental concrete tanks (LxWxH, 100x100x80 cm, 15 fish per tank) with aeration. Each of the experimental diets was fed to three replicate groups of fish at a feeding rate of 3-4% biomass per day (the actual amount consumed by the fish fed to satiation) for 12 weeks. The fish were fed twice a day at 08:30 and 18:00 hrs, seven days a week. Growth was measured every two weeks and the feeding rate was adjusted accordingly.

2.3 Sample collection and analytical methods

At the beginning of feeding trial, 30 fish were randomly collected and killed with an overdose of tricaine methanesulfonate (MS-222) and analyzed for initial chemical composition. All the fishes in each concrete tank were weighed every two weeks in order to adjust the ration to mean body weight. On day 84 of the feeding trial, weight gain, feed conversion ratio, protein efficiency ratio, and specific growth rate were calculated. Six fish in each concrete tank were sampled and killed by overdose of tricaine methanesulfonate (MS-222) for analysis of the condition factor. K= 100 Wt/L^3, where Wt is body weight in g and L is total length in cm, by the method described by Toko *et al.* (2007) and for the hepatosomatic index, HSI=100 x [liver weight (g)]/[total body weight (g)], by the method reported by Anwar and Jafri (1995).

The remaining six fish from each tank were collected and killed with an overdose of anesthetic and sampled for biochemical analysis. Pooled samples of six fish per tank were freeze-dried, homogenized in a grinder and analyzed for lipid, protein and ash contents (AOAC, 1997). The nitrogen-free extract content of the fish was calculated as 100-(%protein + %lipid + %ash). Protein efficiency ratio (PER) was calculated by the method described by Zeitoun *et al.* (1973) and the apparent net protein utilization (ANPU) of the fish in each treatment was calculated by the method of Robinson and Wilson (1985). Data from each experimental unit were submitted to one-way analysis of variance (ANOVA) by means of SPSS software version 17.0 (Chicago, IL, USA). When significant differences ($P<0.05$) found, the means were compared by the Duncan's multiple range test (DMRT).

3. Results

3.1 Growth performance

The average results for various parameters of slender walking catfish fed the experimental diets during 12 weeks are shown in Table 2. The average body weight of catfish fed the test diet containing 40%-44% protein was significantly higher than the others within four weeks of the trial ($P<0.05$). At the end of experiment, the lowest average body weight was found in the catfish fed 32% and 36% protein and the highest found in the catfish fed the test diets containing 40% and 44% protein ($P<0.05$).

Weight gain, feed conversion ratio (FCR) and specific growth rate (SGR) of the slender walking catfish fed the test diet with different protein levels are given in Table 3. All those parameters were significantly different among treatments ($P<0.05$). The fish fed diets that contained 40% or 44% protein level exhibited the highest weight gain, FCR, and SGR, whereas the lowest parameters were found in the fish fed 32% and 36% dietary protein. However, the survival of catfish fed all the test diets was not significantly different ($P>0.05$).

3.2 Condition factor and hepatosomatic index

Condition factor and hepatosomatic index (HSI) of the slender walking catfish fed test diets with different protein levels for 12 weeks are shown in Table 4. The highest condition factor was found in the fish fed 44% protein and was significantly different ($P<0.05$) from the others, followed by the 40% protein level, and the lowest condition factor was found in the fish fed 32% or 36% protein. The HSI of catfish fed the test diets containing 36% to 44% protein were not

Table 2. Average body weight (g) of *C. nieuhofii* fed each experimental diet for 12 weeks

	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
T1 (32% protein)	8.46±0.11^{ns}	9.89±0.51^{ns}	10.66±1.21^{a}	12.28±1.49^{a}	13.78±1.50^{a}	14.74±1.53^{a}	15.70±1.26^{a}
T2 (36% protein)	8.35±0.28^{ns}	9.59±0.60^{ns}	11.30±0.81^{ab}	13.01±0.87^{a}	14.7±1.08^{a}	16.02±1.00^{a}	14.88±1.44^{a}
T3 (40% protein)	8.42±0.21^{ns}	10.53±0.22^{ns}	12.66±0.39^{bc}	15.71±0.63^{b}	18.38±0.63^{b}	20.38±0.54^{b}	21.89±0.51^{b}
T4 (44% protein)	8.37±0.29^{ns}	10.46±0.56^{ns}	13.31±0.98^{c}	16.20±1.73^{b}	19.26±2.31^{b}	21.35±2.61^{b}	22.89±3.00^{b}

Means within columns not sharing the same superscript are significantly different ($P<0.05$).
ns = non-significant.

Table 3. Weight gain (%), feed conversion ratio (FCR), specific growth rate (SGR) and survival (%) of *C. nieuhofii* fed each experimental diet for 12 weeks

	Weight gain (%)	FCR	SGR (%)	Survival (%)
T1 (32% protein)	85.46±12.39^{a}	3.55±0.93^{a}	0.73±0.08^{a}	97.78±3.85^{ns}
T2 (36% protein)	78.46±20.56^{a}	3.88±1.04^{a}	0.68±0.14^{a}	100.00±0.00^{ns}
T3 (40% protein)	160.03±10.12^{b}	1.85±0.07^{b}	1.14±0.05^{b}	100.00±0.00^{ns}
T4 (44% protein)	173.25±30.43^{b}	1.86±0.35^{b}	1.19±0.14^{b}	100.00±0.00^{ns}

Means within columns not sharing the same superscript are significantly different (P<0.05).
ns = non-significant.

significantly different (P<0.05) and the lowest HSI was found in the catfish fed 32% dietary protein.

3.3 Protein efficiency ratio and apparent net protein utilization

Juvenile slender walking catfish fed the test diet containing 40% to 44% protein had the highest Protein efficiency ratio (PER) and this was significantly different (P<0.05) from that of the catfish fed diets containing 32% to 36% protein (Table 4). The apparent net protein utilization (ANPU) of the catfish fed 40% dietary protein was higher than the fish fed the test diet containing 36% protein (Table 4), but not significantly different from that of the catfish fed 32% or 44% dietary protein (P>0.05).

3.4 Proximate body composition

Carcass chemical compositions of the catfish fed diet containing different protein levels are presented in Table 5. The analysis data showed a positive correlation of fish body's crude protein and dietary protein levels. Crude proteins in the catfish fed test diets containing 40% to 44% protein were higher than the catfish fed diets with 32% to 36% protein, but there was no significant difference in crude protein among the catfish fed the diets containing 36% and 44% protein. Crude lipid, moisture, and ash content in the catfish fed test diets contain 32% to 44% protein were not significantly different (P>0.05).

4. Discussion

This study showed that body weight, weight gain, and specific growth rate of the juvenile of slender walking catfish increased as dietary protein level was increased from 32% to 40% but no improvement in growth performance was observed at 44% crude dietary protein level. The results were similar to those reported for other *Clarias* catfish, especially *Clarias gareipinus*, *Clarias anguillaris* and *Heterobranchus bidorsalis*, for which 40% dietary protein was the optimum protein level for maximum growth performance (Van Weerd, 1995; Wilson and Moreau, 1996) and similar to the protein requirements (42%–44%) of fingerlings of the bagrid catfish (*Pseudobagrus fulvidraco*) (Kim and Lee, 2005) and Malaysian freshwater catfish *Mystus nemurus* (Khan *et al.*, 1993; Ng *et al.*, 2001). Adebayo (2005) reported the maximum growth performance of hybrid catfish (*Clarias gariepinus* × *Heterobranchus bidorsalis*) fingerlings in the fish fed 40% protein diet with 5% feeding rate daily. However, the protein requirement of slender walking catfish from our study is higher than

Table 4. Condition factor, hepatosomatic index (HSI), protein efficiency ratio (PER), and apparent net protein utilization (ANPU) of *C. nieuhofii* fed each experimental diet for 12 weeks

	Condition factor	HSI	PER	ANPU (%)
T1 (32% protein)	0.56±0.02^{a}	0.89±0.17^{a}	0.93±0.23^{a}	23.92±3.99^{ab}
T2 (36% protein)	0.55±0.03^{a}	1.01±0.20^{ab}	0.85±0.20^{a}	20.10±1.82^{a}
T3 (40% protein)	0.58±0.02^{b}	1.14±0.15^{b}	1.70±0.07^{b}	27.85±0.93^{b}
T4 (44% protein)	0.61±0.03^{c}	1.17±0.22^{b}	1.74±0.31^{b}	24.44±3.99^{ab}

Means within columns not sharing the same superscript are significantly different (P<0.05).
ns = non-significant.

Table 5. Proximate body composition of *C. nieuhofii* fed each experimental diet for 12 weeks

	Crude protein (% dry basis)	Crude lipid (% dry basis)	Moisture (%)	Ash (% dry basis)
Initial fish	55.89±1.53	10.12±0.51	74.85±0.04	10.64±0.23
T1 (32% protein)	48.34±1.04[a]	12.79±2.68[ns]	73.44±0.71[ns]	12.00±0.45[ns]
T2 (36% protein)	49.53±2.14[ab]	11.24±0.67[ns]	73.39±1.19[ns]	11.59±0.82[ns]
T3 (40% protein)	52.84±1.81[c]	12.52±1.13[ns]	74.75±0.47[ns]	10.94±1.27[ns]
T4 (44% protein)	52.14±1.02[bc]	11.34±1.21[ns]	74.80±1.00[ns]	11.17±0.61[ns]

Means within columns not sharing the same superscript are significantly different (P<0.05).
ns = non-significant.

that (36.5% dietary protein) reported in hybrid *Clarias* catfish (*Clarias bratachus* × *Clarias gareipinus*) post-larvae (Giri *et al.*, 2003).

From previous reports, insufficient dietary protein levels resulted in poor growth performance in many fish species (Yang *et al.*, 2002; Giri *et al.*, 2003; Kim and Lee, 2005) due to insufficiency of amino acids supplied to maintain the body composition (Halver and Hardy, 2002). Excess dietary protein level, however, did not improve the growth of the fish because the excess amino acid is metabolized by oxidative deamination and used to generate energy (Cho *et al.*, 1985; Shiau and Huang, 1989; Vergara *et al.*, 1996; Kim and Lee, 2009). Using excess protein levels in the diet therefore cannot enhance fish growth, and therefore show negative effects on the fish production. Excess protein levels in the feed increased the amino acid catabolism in the fish body, and this resulted in higher ammonia excretion and accumulation of nitrogen waste in the culture system (Yang *et al.*, 2002; Webb Jr. and Gatlin III, 2003). Moreover, increasing excess protein in a practical diet results in higher feed costs, which is the major variable cost in the aquaculture production system (Goddard, 1996).

Feed conversion rates of slender walking catfish in our study were similar to the growth performance data. FCR of the fish fed 32% to 36% dietary protein were extremely high and rapidly became lower when the fish received 40% or higher protein diet. These results implied that a high proportion of the dietary protein in the low-protein test diet was metabolized to maintenance energy (Guillaume *et al.*, 1999). The condition factor, which is described as an indicator of fatness, gross nutritional state (Love, 1970), and the level of reserve nutrients (Gershanovich *et al.*, 1984), increased for the slender walking catfish in the present study as the dietary protein level increased, possibly indicating that higher dietary protein level increases the nutrient content in the fish body (Ali *et al.*, 2005). Especially, the proximate chemical composition analysis indicated an increase of carcass crude protein in the fish fed 40% to 44% protein.

Our results are comparable to the data of Catacutan and Coloso (1995) who reported a significantly better condition factor in Asian sea bass (*Lates calcarifer*) when the fish were fed a diet containing optimum levels of protein and lipid. The hepatosomatic index of slender walking catfish was positively correlated to the dietary protein levels, and increased to the maximum value when the fish received test diet containing at least 36% protein, with no further improvement beyond this point. Our results differed from those reported by Yang *et al.* (2002) who demonstrated that the hepatosomatic index of the silver perch (*Bidyanus bidyanus*) was inversely related to dietary protein levels but positively related to the carbohydrate levels, which implied that the increase of hepatosomatic index was due to the deposition of glycogen in the liver. However, the hepatosomatic index was positively correlated to the intraperitoneal ratio and reflects the high proportional accumulation of energy in the abdominal cavity and the liver (Brown *et al.*, 1992). Protein efficiency ratio, which indicates the utilization of dietary protein by means of the gain of biomass and apparent net protein utilization that describes the gain in body protein of slender walking catfish, showed in this study the maximum values for the fish fed 40% to 44% protein diets. The data were in agreement with those of Giri *et al.* (2003) who reported that low levels of dietary protein decrease the PER value in hybrid catfish (*Clarias bratachus* × *Clarias gariepinus*), but no improvement of PER was observed in the hybrid catfish when the catfish received test diet containing high levels of dietary protein (>35% protein).

A number of factors have been reported to affects the protein requirements of many fish species. Protein to energy ratio is very important to establish a formulated feed for particular fish species and usually affects the protein requirement (Ramseyer and Garling, 1998; Somsueb and Boonyaratpalin, 2001; Kim *et al.*, 2005). The gross energy of test diets used in our study (17.2-17.9 kJ/g) and dietary lipid content of all test diets (18.2%-18.9%) were comparable to the optimum energy level for *Clarias* catfish as described by Van Weerd (1995). Even though, increasing dietary energy provision tends to reduce the optimum protein requirement value due to the protein-sparing effect, as reported in jundia fingerlings (*Rhamdia quelen*) (Meyer and Fracalossi, 2004). Using high energy feed (excess lipid content) showed adverse effects on the carcass fat content (Samantary and Mohanty, 1997) and resulted in off-flavor in fish products (Ng *et al.*, 2003). However, the protein requirement of the slender walking catfish in this study is only based on the fish-meal test diet. Fish meal is one of the most expensive ingredients in prepared fish diets. Our further studies aim to minimize the amount of fish-meal in the practical diet by replacement with less expensive plant protein sources (Webster *et al.*, 1995). Applications of alternative protein sources or protein replacements also affect the protein requirement data, probably because of the digestible efficiency of feed ingredients (Singh *et al.*, 2005; Yildirim and Turan, 2010). In the present study, in which dietary lipid ranged between 18.2% to 18.9% and energy level between 17.3 to 17.9 kJ/g gross energy, the test diet containing 40% crude protein gave the maximum growth performance and feed utilizations. This value is recommended for use in the practical feed for slender walking catfish fingerlings.

Acknowledgements

The authors are grateful to G. Britton (University of Liverpool, UK) for his help in the preparation of this manuscript and they would like to thank K. Supamattaya (deceased) and W. Phromkunthong (Prince of Songkla University, Thailand) for their suggestions to study endangered fish. Thanks also to P. Choksawatdikorn and K. Reungklay for their assistance in preparation of the test animal and experimental system.

References

Adebayo, O.T. 2005. Dietary protein level and feeding rate for hybrid Clariid catfish, *Clarias gariepinus* × *Heterobranchus bidorsalis*, in homestead tanks. Journal of Applied Aquaculture. 9(1), 97-106.

Ali, M., Iqbal, F., Salam, A., Iram, S. and Athar, M. 2005. Comparative study of body composition of different fish species from brackish water pond. International Journal of Environmental Science and Technology. 2(3), 229 – 32.

Anwar, M.F. and Jafri, A.K. 1995. Effect of varying dietary lipid levels on growth, feed conversion, nutrient retention and carcass composition of fingerling catfish, *Heteropneustes fossilis.* Asian Fisheries Science. 8, 55-62.

AOAC. 1997. Official method of analysis Vol. 1. In K. Heltich, editor. Official method of analysis, Association of Official Analytical Chemists (AOAC), 15th Edition , Virginia, U.S.A., pp 1832.

Brown, M.L., Nematipour, G.R. and Gatlin III, D.M. 1992. Dietary protein requirement of juvenile sunshine bass at different salinities. Progressive Fish-Culturist. 54, 148-156.

Cho, C.Y., Cowey, C.B. and Watanabe, T. 1985. Finfish nutrition in Asia : Methodological Approaches to Research and Development. International Development Research Centre, Ottawa, Canada. pp. 10-80.

Catacutan, M.R. and Coloso, T.M.T. 1995. Effect of dietary protein to energy ratios on growth, survival, and body composition of juvenile Asian seabass *Lates calcarifer*. Aquaculture. 131, 125-133.

Garling, D.L.Jr. and Wilson, R.P. 1976. Optimum dietary protein to energy ratio for channel catfish fingerling, *Ictalurus punctatus.* Journal of Nutrition. 106, 1368-1375.

Gershanovich, A.D., Markevich, N.M. and Dergaleva, Z.T. 1984. Using the condition factor in ichthyological research. Journal of Ichthyology. 24, 78 – 90.

Giri, S.S., Sahoo, S.K., Sahu, A.K. and Meher, P.K. 2003. Effect of dietary protein level on growth, survival, feed utilisation and body composition of hybrid *Clarias* catfish (*Clarias batrachus X Clarias gariepinus*). Animal Feed Science and Technology. 104, 169–178.

Goddard, S. 1996. Feed Management in Intensive Aquaculture. Chapman & Hall, New York, U.S.A., pp. 17-20.

Guillaume, J., Kaushik, S., Bergot, P. and Metailler, R. 1999. Nutrition and Feeding of Fish and Crustaceans. Praxis Publishing, Chichester, United Kingdom, pp. 81-109.

Halver, J.E. and Hardy, R.W. 2002. Fish Nutrition, 3rd edition. Academic Press, New York, U.S.A., pp. 143-175.

Khan, M.S., Ang, K.J., Ambak, M.A. and Saad, C.R. 1993. Optimum dietary protein requirement of Malaysian freshwater catfish, *Mystus nemurus*. Aquaculture. 112, 227-235.

Kim, L.E. and Lee, S.M. 2005. Effects of the dietary protein and lipid levels on growth and body composition of bagrid catfish, *Pseudobagrus fulvidraco*. Aquaculture. 243, 323-329.

Kim, S.S. and Lee, K.J. 2009. Dietary protein requirement of juvenile tiger puffer (*Takifugu rubripes*). Aquaculture. 287, 219-222.

Love, R.M. 1970. The Chemical Biology of Fishes. Vol. 1, Academic Press, New York, U.S.A., pp. 1-78.

Mayer, G. and Fracalossi, D.M. 2004. Protein requirement of jundia fingerlings, *Rhamdia quelen,* at two dietary energy concentrations. Aquaculture. 240, 331-343.

Murthy, H.S. and Naik, A.T. 1999. Growth response of African catfish, *Clarias gariepinus* (Burchell) to varied protein and lipid levels. Indian Journal of Experimental Biology. 37(10), 986-989.

Ng, W.K., Soon, S.C. and Hashim, R. 2001. The dietary requirement of bagrid catfish, *Mystus nemurus* (Cuvier and Valenciennes), determined using semipurified diets of varying protein level. Aquaculture Nutrition. 7, 45-51.

Ng, W.K., Lim, P.K. and Boey, P.L. 2003. Dietary lipid and palm oil source affects growth, fatty acid composition and muscle α-tocopherol concentration of African catfish *Clarias gariepinus*. Aquaculture. 215, 229-243.

Robinson, E.H., Li, M.H. and Manning, B.B. 2001. A practical guide to nutrition, feeds, and feeding of catfish (second revision). Mississippi Agricultural and Forestry Experiment Station Bulletin No.1113. Mississippi State University, Mississippi State, Mississippi, U.S.A., pp. 7.

Ramseyer, L.J. and Garling Jr., D.L. 1998. Effects of dietary protein to metabolizable energy ratios and total protein concentrations on the performance of yellow perch *Perca flavescens*. Aquaculture Nutrition. 4, 217-223.

Robinson, E.H. and Wilson, R.P. 1985. Nutrition and Feeding. In C.S. Tucker, editor. Channel Catfish Culture. Developments in Aquaculture and Fisheries Science, 15. Elsevier, Amsterdam, Netherlands, pp 323-404.

Samantaray, K. and Mohanty, S.S. 1997. Interactions of dietary levels of protein and energy on fingerling snakehead, *Channa striata*. Aquaculture. 156, 241-249.

Shiau, S.Y. and Huang, S.L. 1989. Optimal dietary protein level for hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) reared in seawater. Aquaculture. 81, 119-127.

Singh, R.K., Desai, A.S., Chavan, S.L. and Khandagale, P.A. 2009. Effect of water temperature on dietary protein requirement, growth and body composition of Asian catfish, *Clarias batrachus* fry. Journal of Thermal Biology. 34(1), 8-13.

Singh, P.K., Gaur, S.R., Barik, P., Sulochana, S.S. and Singh, S. 2005. Effect of protein levels on growth and digestibility in the Indian major carp, *Labeo rohita* (Hamilton) using slaughter house waste as the protein source. International Journal of Agriculture and Biology. 7(6), 939-941.

Somsueb, P. and Boonyaratpalin, M. 2001. Optimum protein and energy levels for the Thai native frog, *Rana rugulosa* Weigmann. Aquaculture Research. 32, 33-38.

Toko, I., Fiogbe, E.D., Koukpode, B. and Kestemont, P. 2007. Rearing of African catfish (*Clarias gariepinus*) and vundu catfish (*Heterobranchus longifilis*) in traditional fish ponds (whedos): Effect of stocking density on growth, production and body composition. Aquaculture. 262(1), 65-72.

Van Weerd, J.H.V. 1995. Nutrition and growth in *Clarias* species - a review. Aquatic Living Resources. 8, 395-401.

Vergara, J.M., Fernandez-Palacios, H., Robaina, L., Jauncey, K., De La Higuera, M. and Izquierdo, M. 1996. The effects of varying dietary protein level on the growth, feed efficiency, protein utilization and body composition of gilthead sea bream fry. Fisheries Science. 62, 620-623.

Webb, K.A. and Gatlin III, D.M. 2003. Effects of dietary protein level and form on production characteristics and ammonia excretion of red drum *Sciaenops ocellatus*. Aquaculture. 225, 17-26.

Webster, C.D., Tidwell, J.H., Tiu, L.S. and Yancey, D.H. 1995. Use of soybean meal as partial or total substitute of fish meal in diets for blue catfish, *Ictalurus furcatus*. Aquatic Living Resources. 8, 379-384.

Wilson, R.P. and Moreau, Y. 1996. Nutrient requirements of catfishes (Siluroidei). Aquatic Living Resources. 9, 103-111.

Yang, S.D., Liou, C.H. and Liu, F.G. 2002. Effects of dietary protein level on growth performance, carcass composition and ammonia excretion in juvenile silver perch (*Bidyanus bidyanus*). Aquaculture. 213, 363-372.

Yildrim, Y.B. and Turan, F. 2010. Effects of exogenous enzyme supplementation in diets on growth and feed utilization in African catfish, *Clarias gariepinus*. Journal of Animal and Veterinary Advances. 9(2), 327-331.

Zeitoun, I.H., Halver, J.E., Ullrey, D.E. and Tack, P.J. 1973. Influence of salinity on protein requirement of rainbow trout (*Salmo gairdneri*) fingerlings. Journal of the Fisheries Research Board of Canada. 30(12), 1867-1873.