Journal of Animal Feed Science and Technology 3 (2015) 11-17

Effects of dietary protein level on growth and body composition of asian catfish, *Clarias batrachus* fry

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Keywords:
Protein
Growth Rate
Carcass Composition
Clarias batrachus.

Abstract

This study aimed to evaluate the protein requirements of *Clarias batrachus* fry, were estimated by feeding isocaloric diets. The influence of dietary protein level on weight gain, survival, food utilization and body composition were estimated. The Asian catfish, *C. batrachus* fry were fed four diets containing 25% (diet 1), 30% (diet 2), 35% (diet 3) and 40% (diet 4) protein levels and 3580 kcal kg^{-1} of energy level for 30 days. Fry fed with diet 3 containing 35% protein showed the highest final body weight, survival, specific growth rate and protein efficiency ratio and were significantly ($P < 0.05$) affected by dietary treatments. *C. batrachus* fry raised at 25% protein (diet 1) had higher apparent feed conversion ratio (AFCR) (1.30±0.13) than 35% dietary protein level (0.63±0.07). Higher protein efficiency ratio (4.52±0.4) observed at higher protein level (35%) than 25% protein level (3.08±0.6). Body lipid and ash content observed to have been influenced ($P < 0.05$) by diets with increasing protein level. Further, crude protein was not affected ($P > 0.05$) with increase in dietary protein level. The study concluded that the diet 3 containing 35% protein was optimal for growth of *C. batrachus* fry.

Introduction

In aquaculture practices, more emphases are generally given to the dietary protein requirements to achieve optimal fish growth and production. Protein is one of the major dietary nutrients, affecting growth performance of fish and also feed cost (Lovell 1989). Regular intake of protein is required by fish to build new tissues and to repair old tissues. Protein deficiency in the diet result in reduction of growth and loss of weight due to utilization of body protein to maintain the functions like building of new tissues and repairing of new tissues (Rath 1993; Singh et al. 2009). Hence, fish require high levels of protein in their diets, which vary from fish species to species. In addition, extremely high protein levels may result in poor growth and increased susceptibility of fish to diseases and parasites due to poor water quality. When fed to fish, diets containing excessive amounts of protein cause toxicity since the fish tend to excrete high amounts of ammonia in the rearing water which may lead to growth depression (Zeitoun et al. 1976). On the other hand, reduction in fish growth could be due to lack of non-protein energy in diets containing high amounts of protein (Jauncey 1982), in which case some protein would be used for energy rather than growth. Moreover, excess dietary protein which cannot be stored are catabolized preferentially over carbohydrates and fats and used for energy instead of growth by some fishes (Wilson 1989). Requirements of protein in the diets for silver carp, were reported to be between 37% and 42% (Singh 1989), for common carp between 31% and 54% (Varghese et al. 1976; Sen et al. 1978; Pandian 1989) and for pearl gourami 45% (Singh et al. 2003).

The Asian catfish, *Clarias batrachus*, is widely distributed throughout Indian continents and is known as a species of aquaculture importance. The

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twin qualities; rapid growth and high marketability, make it a good candidate fish species for aquaculture production (Goda et al. 2007; Singh et al. 2009). Commercially available artificial feeds are used to supplement the protein requirements in the mass production of fry. However, this use is based more on availability than suitability (Tidwell et al. 1992; Gupta et al. 2007); the available artificial feeds are not designed specifically for catfish and fail to meet the actual protein requirements of the fry. An economically viable commercial production of *C. batrachus* requires reliable diets that will support nutritional requirements for optimal growth and survival. Studies on the protein requirements of *C. batrachus* have been focused on juveniles (Khan et al. 1993) and information regarding the optimum protein level in fry diet is scarce. For sustainable aquaculture of this species, knowledge of optimal dietary protein requirements, growth and feed utilization need to be investigated. Therefore, the objective of this study was to determine the optimum level of dietary protein, growth, feed utilization and body composition of Asian catfish, *C. batrachus* fry.

Materials and Methods

Test animals and experimental system

Fry of Asian catfish *C. batrachus* were procured from a local fish seed supplier (M/s Jawhar fish seed supplier, Fazilka, Punjab) and acclimatized to laboratory conditions for one week in two plastic trough containing 50 L water. Fry were fed to satiation, a mixture of groundnut oil cake and wheat flour (1:1). Daily removal of 50% water from plastic trough was done to remove uneaten food and excreta and replenishment of same volume of fresh water. Groups of 30 catfish fry of average 0.2 g body weight were stocked in 12 plastic troughs of 15 litre capacity. Triplicate troughs were allotted randomly for each feeding treatment. The water quality was maintained through daily 50% water exchange and siphoning out of the waste material. Continuous aeration was provided by air stones using an air compressor to ensure oxygen saturation. The experiment was lasted for 30 days.

Water quality parameters were determined daily and there were no significant differences between dietary treatments at any sampling period. Ranges of temperature, pH, dissolved oxygen, ammonia, nitrates and nitrites were from 34.2 to 34.6^{o}C, 8.0 to 8.37, 7.62 to 8.50 mg L^{-1}, 0.12 to 0.16 mg L^{-1}, 0.09 to 0.16 mg L^{-1} and 0.04 to 0.07 mg L^{-1}, respectively, during the experimental period. The water quality parameters were within the acceptable range as reported for *C. batrachus* (Rao et al. 1994) rearing and culture.

Diet preparation and feeding trial

Four isocaloric diets were formulated with different proportions of ingredients to contain 25% (diet 1), 30% (diet 2), 35% (diet 3) and 40% (diet 4) protein level. The ingredients and proximate composition of the experimental diets are shown in Table 1. The ingredients of each diet were mixed thoroughly and an aliquot of water were added to the mixture. The resulting dough was cooked for 20 min in a pressure cooker. After cooling, the dough was pelletized by using a hand pelletizer. Pellets of 1mm diameter size were dried at 50^{o}C. The dried diets were then stored in airtight plastic containers. Experimental diets were fed to the fry at 7% of body weight, twice a day (09:00 and 17:00 h) for a period of 30 days. After every 10 days, ten fishes from each trough were weight individually for recording weight and calculating daily food ration.

Diet and body composition analysis

At the end of the experiment, ten fishes from each replicate were sacrificed for the analysis of chemical composition of whole body. Diet and body tissue at the end of the experiment were analysed for dry matter (DM), crude protein (CP), lipid and total ash according to the method of AOAC (2000). Organic matter (OM) of diets was calculated by subtracting the total ash value from DM. Total carbohydrates was estimated by subtracting CP and lipid values from OM. Gross energy of diets was calculated on the basis of gross energy values of crude protein, total carbohydrate and lipid of the respective diets. The digestible energy values of the diets were calculated as 14.6 MJ kg^{-1} proteins, 33.9 MJ kg^{-1} lipids and 10.5 MJ kg^{-1} carbohydrate on the basis of values adapted for channel catfish (NRC 1993).

Growth parameters

At the end of the feeding trial, the water from each plastic trough was siphoned out and the total number of fry in each trough was counted. Randomly the individual body weights of fry were recorded and the mean final body weight was multiplied with total number of fry to arrive at final biomass production from each trough. Feed conversion ratio was considered to be apparent as no correction was made for uneaten food left (if any). The following formulae were used to calculate growth performance in *C. batrachus* fry:

1. Specific growth rate (SGR) = 100 ($\log_e$ final body weight - $\log_e$ initial body weight) / culture period (days).

2. Apparent feed conversion ratio (AFCR) = total dry feed fed (g) / total live weight gain (g).
3. Protein efficiency ratio (PER) = weight gain / protein intake.

Analysis of data

The experimental data were subjected to one-way ANOVA (Snedecor & Cochran 1968) and results were considered to be statistically significant at 0.05 probability level or smaller.

Results

Growth performance

The diets are formulated in such a way that gross energy level remains almost same in all the experimental diets. Estimated digestible energy for different experimental diets ranged from 11.13 to 11.46 kJ g^{-1} of diet (Table 1). The data of final body weight, biomass gain, percent survival, feed intake, specific growth rate (SGR), apparent feed conversion ratio (AFCR) and protein efficiency ratio (PER) are summarised in Table 2.

The initial body weight (0.2 g) of the fish fry was similar among the treatments. The final body weight attained by diet 1 treatment was the lowest (0.68±0.08 g), followed by diet 2 (0.82±0.05 g), however, both were lower than that of diet 3 (0.99±0.07 g) and diet 4 (0.91±0.05 g) fed fish treatments (Table 2). It was observed that net body weight gain in fish fed on 35% and 40% protein diet was significantly higher ($P < 0.05$) than that of fish fed on 30% protein diet, which was also significantly higher than that of 25% protein diet fed fish. Dietary protein levels had significant effect on AFCR and PER values in catfish fry. AFCR value of 25% protein diet was significantly higher ($P < 0.05$). Fish fed diet containing 35% protein showed significantly lower ($P < 0.05$) AFCR value and better than those obtained with the diets containing 40% and 30% level protein (Table 2). Fry fed the 35% (diet 3) protein diet showed the highest PER followed by diet 2, 4 and 1 treatment.

Body composition

Whole body composition of *C. batrachus* fry is shown in Table 3. There were significant differences ($P < 0.05$) for whole-body lipid and ash contents. Both body lipid and ash content decreases significantly with increase in protein content of the diet, whereas, protein values were increased. However, fry fed 25% protein (diet 1) recorded lower protein content ($P > 0.05$) compared with the other treatments.

Table 1: Ingredient and chemical composition of the experimental diets

Item	Diet 1	2	3	4
Ingredient (g kg^{-1} as-is basis)				
Fish meal	175	220	285	325
Meat meal	50	50	50	50
Soybean meal	100	170	220	290
Groundnut oilcake	50	50	50	50
Wheat flour	150	110	80	50
Rice bran	250	190	120	85
Corn starch	185	170	155	110
Sunflower oil	20	20	20	20
Vitamin & mineral mixture *	15	15	15	15
Iodized salt	5	5	5	5
Proximate composition (% dry weight) #				
Dry matter	89.33	89.49	89.62	89.84
Crude protein (calculated)	25.00	30.00	35.00	40.00
Crude protein (analyzed)	25.41	30.21	35.03	39.92
Lipid	7.70	6.96	6.16	5.81
Ash	7.24	8.13	10.47	12.35
Organic matter †	82.09	81.36	79.15	77.49
Total carbohydrate §	48.98	44.19	37.96	31.76
Gross energy (kJ g^{-1}) $	1746.5	1748.5	1723.9	1719.3
Digestible energy (kJ g^{-1}) ¶	11.46	11.41	11.18	11.13

* Vitamin & mineral per gram of premix contained: Vitamin A 8000 i.u., vitamin B2 2.8 mg, vitamin B12 5 mg, vitamin D3 1500 i.u., vitamin E 5 mg, vitamin K3 5 mg, vitamin PP 12.5 mg, D calcium pantothenate 5 mg, copper sulphate 0.7 mg, zinc sulphate 2.5 mg, ferrous sulphate 6.2 mg, potassium iodide 0.4 mg, manganese sulphate 3.8 mg, sorbitol 20 mg.
# Values are the mean of three determinations.
† Organic matter = dry matter – total ash.
§ Total carbohydrates = organic matter – (crude protein + total lipid).
$ Calculated using gross caloric values of 5.65, 9.45 and 4.1 kcal g^{-1} for protein, fat and carbohydrate, respectively, according to Brett (1973).
¶ Calculated as 14.6 MJ kg^{-1} protein, 33.9 MJ kg^{-1} lipid and 10.5 MJ kg^{-1} carbohydrate, according to NRC (1993).

Table 2: Growth performance of C*larias batrachus* fry fed with increasing levels of dietary protein for 30 days*

Nutritional indices	Diet 1	Diet 2	Diet 3	Diet 4
Initial weight (g)	0.2±0.02 ^{a}	0.2±0.01 ^{a}	0.2±0.01 ^{a}	0.2±0.02 ^{a}
Final weight (g)	0.68±0.08 ^{c}	0.82±0.05 ^{b}	0.99±0.07 ^{a}	0.91±0.05 ^{a}
Net weight gain (g)	0.48±0.03 ^{c}	0.62±0.05 ^{b}	0.79±0.06 ^{a}	0.71±0.02 ^{a}
Survival (%)	45.6±11.2 ^{c}	62.3±8.1 ^{b}	76.5±7.2 ^{a}	65.8±9.5 ^{b}
Biomass gain (g)	33.2±9.5 ^{d}	93.3±10.9 ^{c}	167.7±13.6 ^{a}	120.2±7.4 ^{b}
Total feed intake (g)	43.1	74.6	106.0	82.9
SGR $	2.45±0.4 ^{c}	2.82±0.3 ^{b}	3.2±0.2 ^{a}	3.03±0.3 ^{a}
AFCR †	1.30±0.13 ^{a}	0.80±0.11 ^{b}	0.63±0.07 ^{c}	0.69±0.09 ^{b}
PER #	3.08±0.6 ^{d}	4.16±0.7 ^{b}	4.52±0.4 ^{a}	3.62±0.6 ^{c}

* Values are the mean of triplicate groups of ten random fishes. Mean values in the rows with different superscripts are significantly different ($P < 0.05$).
$ Specific growth rate (SGR) = 100 ($\log_e$ final body weight - $\log_e$ initial body weight)/culture period (days).
† Apparent feed conversion ratio (AFCR) = total dry feed fed (g)/total live weight gain (g).
# Protein efficiency ratio (PER) = weight gain/protein intake.

Table 3: Carcass composition of C*larias batrachus* fry (% dry weight) fed increasing levels of dietary protein for 30 days*

Constituents	Diet 1	Diet 2	Diet 3	Diet 4
Dry matter	23.8±2.6 ^{a}	22.5±1.4 ^{a}	21.0±3.0 ^{a}	20.2±1.2 ^{a}
Crude protein	13.53±1.3 ^{a}	13.90±2.6 ^{a}	14.25±0.98 ^{a}	14.31±1.9 ^{a}
Total lipids	4.77±0.82 ^{a}	3.56±0.56 ^{b}	2.55±0.27 ^{c}	2.07±0.31 ^{d}
Total ash	2.54±0.06 ^{a}	2.26±0.11 ^{b}	2.02±0.05 ^{c}	1.87±0.08 ^{d}

* Values are the mean of triplicate groups of ten random fishes. Mean values in the rows with different superscripts are significantly different ($P < 0.05$).

Discussion

The protein level was increased steadily from diet 1 to diet 4, which was required to study the protein requirements of fish. Because of the protein level increased, some other constituent must vary among the dietary treatments, and in the present study total carbohydrate concentration decreased steadily from diet 1 (48.98%) to diet 4 (31.76%). The decrease in carbohydrate concentration was due to decreased levels of rice bran and wheat flour in the diets. The increased ash content in diet 4 was due to the presence of higher level of fish meal and soybean meal as a major feed ingredient. Fish meal and soybean meal are generally considered as good quality protein sources with essential amino acid index (EAAI) of around 0.90. The initial body weight of the *Clarias* fry was similar among the treatments. The final body weight attained by diet 1 was the lowest, followed by diet 2; however, both were lower than that of diet 3 and 4 fed fish ($P < 0.05$). Since the diets were offered at 7% of the wet body weight and the fish consumed the diets within 25 min, voluntary intake of the diets was not a factor that affected growth, which corroborates the observations of Moon and Gatlin (1994); Giri et al. (2000) and Giri et al. (2003). The inferior growth performance of *Clarias* fry fed diet 1 and 2 might be due to the presence of higher level of carbohydrates in the diets, which agrees with the observation of Venkatesh et al. (1986) in *Clarias batrachus* fed a diet containing more than 50% carbohydrate. It is seen in the present study that fry were capable possibly of tolerating carbohydrate up to 37.96% diet without exhibiting growth reduction, which was much higher than the values reported for channel catfish (28%) (Garling & Wilson 1977), but less than the values reported for Nile tilapia (40%) (Anderson et al. 1984), hybrid catfish of *C. macrocephalus* × *C. gariepinus* (50%) (Jantrarotai et al. 1994), and *C. batrachus* (51%) (Giri et al. 2000). The poor performance of fry on diets with lower protein levels may have been influenced by the relationship between the protein and energy in the diets. Information on the gross energy requirements of the test fish is lacking, but the energy level used in the present study was based on the available information from other studies on catfish. Earlier, Khan et al. (1993) concluded that the optimum protein requirement of juvenile *M. nemurus* is 42% with protein to digestible energy ratio of 113.82 mg kcal^{-1}. The 40% protein diet used in this study has protein to gross energy ratio of 111.7 mg kcal^{-1}. The protein and energy level in fish diets is important because fish, like any other animal, eat primarily to satisfy their energy requirements and they tend to adjust their feed intake in accordance with their energy requirements (Cho & Kaushik 1985; Smith 1989; Kim et al. 1991). Excessive energy levels also have deleterious effects such as deposition of fat (Hajra et al. 1988) which is undesirable.

A significant decrease ($P < 0.05$) in net biomass gain in diet 2 and 1 treatment with decreasing dietary protein was observed. Low dietary protein was also associated with decreased survival of the fry. The works of Kandasami et al. (1987) and Fiogbe et al. (1996) also indicated similar decrease in growth as well as survival of fish, fed low levels of protein (less than 30% diet). The low fish survival in diet 1 (45.6±11.2 %) and diet 2 (62.3±8.1 %) as compared to diet 3 (76.5±7.2 %) can be attributed to the dietary treatments. Fish in diet 1 and 2 treatments were observed to feed well on artificial diets but increased mortalities were observed in these treatments during the latter part of the experiment. Cannibalism was also noted in the aforementioned treatments. Cannibalism is known to be triggered by internal factors such as variable sizes and weak state of the fry in the culture system, which is influenced by the fry diet (Ehrlich et al. 1989; Qin & Fast 1996; Watanabe et al. 1996).

There was a significant ($P < 0.05$) increase in SGR and PER, together with an improved AFCR with increased dietary protein (Table 2). Earlier work in *C. batrachus* fry (Chuapoehuk 1987) also indicated that dietary low level of protein (less than 35% diet) decreased the PER values in catûsh, which is also supported by others (Shyong et al. 1998; Giri et al. 2003).

Using the final body weights as the indicator it was estimated that the dietary protein requirement was 35% for *Clarias batrachus* fry with a corresponding energy value of about 1719.3-1748.5 kJ g^{-1} diet. This dietary protein requirement value for catfish fry is much higher than that reported for *Clarias batrachus* fry (30%) and *Pangasius* sp. (30%) by Chuapoehuk (1987) and Aizam et al. (1980), respectively, but lower than those found for other tropical catfishes, particularly *C. gariepinus*, 40% (Degani et al. 1989), hybrid catûsh of *C. macrocephalus* × *C. gariepinus*, 40% (Jantrarotai et al. 1998) and *Mystus nemurus*, 42% diet (Khan et al. 1996).

Result of present study, indicated that dietary protein level influenced the whole body DM, CP, lipid and ash content (Table 3). Protein concentration in the carcass of catfish increased with increase in dietary protein levels from 25% to 40% but these changes were not significant ($P > 0.05$). A similar increasing trend of tissue protein has also been observed for other fish species, Zeitter et al. (1984) in *Cyprinus carpio*; Mohanty & Samantaray (1996) in *Channa striatus*; Shyong et al. (1998) in *Zacco barbata* and Giri et al. (2003) in hybrid post-larvae of *Clarias batrachus* x *Clarias gariepinus*. Shearer (1994) pointed out that the proximate composition of fish is influenced by both endogenous factors such as fish size and sex as well as exogenous factors such as diet composition. This may partly explain the lack of agreement concerning the influence of various levels of dietary protein on the protein content of the fish body.

The carcass ash content decreased significantly ($P < 0.05$) with increase of dietary CP content. There was a direct relation between whole body DM and lipid with dietary protein levels, which was reported earlier also by Juancey (1982) for juveniles of tilapia *Sarotherodon mosambicus*; Shiau & Huang (1988) for hybrid tilapia *Oreochromis niloticus* x *O. aurea*; Khan et al. (1996) for catfish *Mystus nemurus* and Giri et al. (2003) for hybrid post-larvae of *Clarias batrachus* x *C. gariepinus*. The increased level of dietary carbohydrate in fish fed low protein diets might have stimulated several tissue lipogenic enzyme activities and converted dietary carbohydrate into fat. The resultant fat thus synthesised might have been deposited in the adipose tissues. Similar studies of Likimani & Wilson (1982) and Giri et al. (2003) also observed that feeding higher level of carbohydrate increased tissue DM as well as lipid levels in channel catfish and hybrid catfish, respectively.

In conclusion, based on the growth performance, best feed utilisation and highest survival observed in this study, a diet with dietary protein level of 35% is required for *C. batrachus* fry, when fish meal and soybean meal were used as the primary sources of protein and the diets were fed at 7% of the wet body weight.

Acknowledgements

The study was undertaken by the corresponding author for the partial fulfilment of M.Phil. degree (Enrolment no: 6MZ013430002) from Periyar University, Salem. Authors are grateful to the Director, Periyar Institute of Distance Education, Periyar University, Salem, India for according approval and Principal, D.A.V. College, Abohar, Punjab, India for providing infrastructure and laboratory facilities to conduct this study.

References

1. Aizam, Z.A., Che Roos, S. & Sharr, H.A. The growth of Ikan Patin: *Pangasius sutchi* (Hamiltan) fingerlings fed varying dietary protein level. Partanika, 1980; 6: 49-54.
2. Anderson, J., Jackson, A.J., Matty, A.J. & Capper, B.S. The effects of dietary carbohydrate and fibre on the tilapia *Oreochromis niloticus* (Linn). Aquaculture, 1984; 37: 303-314.
3. AOAC (2000) Official Methods of Analysis. 16th edn, Association of Official Anal. Chem., Washington, DC, USA, 2000; pp. 1094.

4. Brett, J.R. Energy expenditure of sockeye salmon *Oncorhynchus nerka*, during sustained performance. J. Fish. Res. Board of Canada, 1973; 30: 1799-1809.

5. Cho, C.Y. & Kaushik, S.J. Effect of protein intake and metabolized and net energy values in fish diets. In: Nutrition and feeding of fish. (Cowey, C.B., Mackie, A.M. & Bell, J.G. eds.), London, Academic Press, 1985; pp. 95-117.

6. Chuapoehuk, W. Protein requirements of walking catfish, *Clarias batrachus* (Linnaeus) fry. Aquaculture, 1987; 63: 215-219.

7. Dabrowska, H. & Wojno, T. Studies on the utilization by rainbow trout (*Salmo gairdneri* Richardson) on feed mixtures containing soybean meal in addition to amino acid. Aquaculture, 1977; 10: 297-310.

8. Degani, G., Ben-zvi, Y. & Levanon, D. The effect of dietary protein levels and temperature on feed utilization, growth and body composition of *Clarias geriepinus* (Burchell 1822). Aquaculture, 1989; 76: 293-301.

9. Ehrlich, K.F., Claude Cantin, M. & Rust, M. Growth and survival of larval and post larval smallmouth bass fed a commercially prepared dry feed and / or *Artemia nauplii.* J. World Aquacult. Soc., 1989; 20: 3-6.

10. Fiogbe, E.D., Kestemont, P., Melard, C. & Micha, J.C. The effects of dietary crude protein on growth of the European perch *Perca fluviatilis*. Aquaculture, 1996; 144: 239–249.

11. Garling, D.L. Jr. & Wilson, R.P. Optimum dietary protein to energy ratio for channel catfish fingerlings, *Ictalurus punctatus*. J. Nutr., 1977; 106: 1368-1375.

12. Giri, S.S, Sahoo, S.K., Sahu, A.K. & Meher, P.K. Effect of dietary protein level on growth, survival, feed utilisation and body composition of hybrid *Clarias* catfish (*Clarias batrachus* × *Clarias gariepinus*). Anim. Feed Sci. Technol., 2003; 104: 169-178.

13. Giri, S.S., Sahoo, S.K., Sahu, A.K. & Mukhopadhyay, P.K. Growth feed utilization and carcass composition of catfish *Clarias batrachus* (Linn.) fingerlings fed on dried fish and chicken viscera incorporated diets. Aqua. Res., 2000; 31: 767-771.

14. Goda, A.M., El-Haroun, E.R. & Chowdhury, M.A.K. Effect of total or partially replacing fish meal by alternative protein sources on growth of African catfish *Clarias gariepinus* (Burchell, 1822) reared in concrete tanks. Aqua. Res., 2007; 38: 279-287.

15. Gupta, A., Sehgal, H.S. & Sehgal, G.K. Growth and carcass composition of giant freshwater prawn, *Macrobrachium rosenbergii* (De Man), fed different isonitrogenous and isocaloric diets. Aqua. Res., 2007; 38: 1355-1363.

16. Hajra, A.S., Ghosh, A. & Mandal, S.K. Biochemical studies on the determination of optimum dietary protein to energy ratio for tiger prawn (*Penaeus monodon* Fabricius) juveniles. Aquaculture, 1988; 71: 71-79.

17. Jantrarotai, W., Sitasit, P. & Rajchapadee, S. The optimum carbohydrate to lipid ratio in hybrid *Clarias* catfish (*Clarias macrocephalus* × *Clarias gariepinus*) diets containing raw broken rice. Aquaculture, 1994; 127: 61-68.

18. Jantrarotai, W., Sitasit, P., Jantrarotai, P., Vipulhanumas, T. & Srabua, P. Protein and energy levels for maximum growth, diet utilization, yield of edible flesh and protein sparing of hybrid *Clarias* catfish (*Clarias macrocephalus* x *Clarias gariepinus*). J. World Aqua. Soc., 1998; 29: 281-289.

19. Jauncey, K. The effects of varying dietary protein level on growth, food conversion, protein utilization and body composition of juvenile tilapias (*Sarotherodon mossambicus*). Aquaculture, 1982; 27: 43-54.

20. Juancey, K. The effect of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapia *Sarotherodon mosambicus*. Aquaculture, 1982; 27: 34-54.

21. Kamarudin, M.S., Shah, Z.M. & Teshima, S. Amino acid composition of several feed stuff and local fish species. In: Advances in Fisheries and Marine Sciences. (Mohamed, M.I., Patimah, I., Law, A.T. & Cheah. Fac, S.H. eds.), Fisheries and Marine Science, University Putra Malaysia, Salangor, Malaysia. 1990; pp. 61-67.

22. Kandasami, D., Paul Raj, R. & Esterson, D.V.C. Effect of selected levels of dietary protein on the growth and feed efficiency of mullet *Liza macrolepis* fry. Indian J. Fish., 1987; 34: 306-311.

23. Kaushik, S.J., Craveldi, J.P., Lalles, J.P., Sumpter, J., Fauconneu, B. & Laroche, M. Partial or total replacement of fishmeal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 1995; 133: 257-274.

24. Ketola, H.G. Mineral supplementation of diets containing soybean meal as a source of protein for rainbow trout. Prog. Fish Cult., 1975; 37: 73-75.

25. Khan, M.S., Ang, K.J. & Ambak, M.A. The effect of varying dietary protein level on the growth, food conversion, protein utilization and body composition of tropical catfish *Mystus nemurus* (C. & V.) cultured in static pond water system. Aqua. Research, 1996; 27: 823-829.

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

27. Kim, K.I., Kayes, T.B. & Amundson, C.H. Purified diet development and protein requirements of fingerlings of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 1991; 96: 57-67.

28. Likimani, T.A. & Wilson, R.P. Effects of diet on lipogenic enzyme activities in channel catfish hepatic and adipose tissue. J. Nutr., 1982; 112: 112-117.

29. Lovell, R.T. Nutrition and Feeding of Fish. Van Nostrand-Reinhold, New York, 1989; pp. 260.

30. Mohanty, S.S. & Samantaray, K. Effect of varying level of dietary protein on the growth performance and feed conversion efficiency of snakehead, *Channa striatus*. Aqua. Nutr., 1996; 2: 89-94.

31. Moon, H.Y. & Gatlin, D.M. III Effects of dietary animal proteins on growth and body composition of the red drum (*Sciaenops ocellatus*). Aquaculature, 1994; 120: 337-340.

32. Ng, W.K., Soon, S.C. & Hashim, R. The dietary protein requirement of a bigrid catfish, *Mystus nemurus* (Cuvier & Valenciennes), determined using semipurified diets of varying protein level. Aqua. Nutr., 2001; 7: 45-51.

33. NRC (National Research Council) Nutrient Requirement of Fish. National Academy Press, Washington, DC, 1993; pp. 102.

34. Pandian, T.J. Protein requirements of fish and prawns cultured in Asia. In: Proceedings of the Third Asian Fish Nutrition Network Meeting, Topic in Fish Nutrition

Research in Asia (Desilva, S.S. ed.), Manila, Philippines, 1989; pp. 11-22.

35. Qin, J. & Fast, W.F. Size and feed dependent cannibalism with juvenile snakehead *Channa striatus.* Aquaculture, 1996; 144: 313-320.

36. Rao, G.R.M., Tripathi, S.D. & Sahu, A.K. Breeding and seed production of the Asian catfish *Clarias batrachus* (Linnaues). Manual series (3), Central Institute of Freshwater Aquaculture, Orissa, India, 1994; pp. 29.

37. Rath, R.K. Freshwater Aquaculture. Scientific Publishers, India, 1993; pp. 256.

38. Robinson, E.H., Wilson, R.P., Poe, W.E. & Grizzle, S. Effect of residual antinutritional factors in processed soybean meal on fingerling of channel catfish. FASEB, 1981; 40: 882. (Abstract).

39. Sen, P.R., Rao, N.G.S., Ghosh, S.R. & Rout, M. Observations on the protein and carbohydrate requirements of carps. Aquaculture, 1978; 13: 121-129.

40. Shearer, K.D. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. Aquaculture, 1994; 119: 63-88.

41. Shiau, S.Y. & Huang, S.L. Optimal dietary protein level for hybrid tilapia (*Oreochromis niloticus* × *O. aurea*) reared in seawater. Aquaculture, 1988; 81: 119-127.

42. Shyong, W.L., Huan, C.H. & Chen, H.C. Effect of dietary protein concentration on growth and muscle composition of juvenile *Zacco barbata*. Aquaculture, 1998; 81: 119-127.

43. Singh, B.N. Protein requirements of young Silver carp, *Hypophthalmichthys molitrix*. In: Abstracts of International Symposium on Modern Trends in Fish Biology Research, June 1989; 17-20, Bhagalpur University, Bhagalpur, India.

44. Singh, R.K., Balange, A.K. & Vartak, V.R. Dietary protein, lipid and dry matter requirement of pearl gourami, *Trichogaster leeri* (Bleeker) fry. Indian J. Anim. Nutr., 2003; 20: 300-308.

45. Singh, R.K., Desai, A.S., Chavan, S.L. & Khandagale, P.A. Effect of water temperature on dietary protein requirement, growth and body composition of Asian catfish, *Clarias batrachus* fry. J. Thermal Biol., 2009; 34: 8-13.

46. Smith, R. Nutritional energetics. In: Fish Nutrition (Halver. San Diego, J. ed.), 2nd edn, CA National Academic Press Inc, 1989; pp. 2-28.

47. Snedecor, G.W. & Cochran, W.G. Statistical Methods. Oxford and IBH Publishing Co., Kolkata, 1968; pp. 594.

48. Tidwell, J.H., Webster, C.D. & Clark, J.A. Growth, feed conversion, and protein utilization of female green sunfish X male bluegill hybrids fed isocaloric diets with different protein levels. The Prog. Fish. Cult., 1992; 53: 234-239.

49. Varghese, T.J., Devaraj, K.V., Shantaram, B. & Shetty, H.P.C. Growth response of common carp, *Cyprinus carpio* var. *communis* to protein rich pelleted feed. Paper presented in Symposium on Development and Utilization of Inland Fishery Resources, Colombo, Sri Lanka. FAO Regional Office for Asia and the Far East, Bangkok, Thailand, 1976; pp. 408–416.

50. Venkatesh, B., Mukherjee, A.P., Mukhopadhyay, P.K. & Dehadrai, P.V. Growth and metabolism of the catfish *Clarias batrachus* (Linn.) fed with different experimental diets. Proceedings of Indian Academy of Science, 1986; 95: 457-462.

51. Watanabe, W.O., Ellis, S.E., Ellis, E.P. & Lopez, V.G. Evaluation of first feeding regiments for larval nassau grouper *Epinephelus striatus* and preliminary, pilot scale culture through metamorphosis. J. World Aquacult. Soc., 1996; 27: 323-331.

52. Wilson, R. P. Amino acid and proteins. In: Fish Nutrition (Halver, J.E. ed.), 2nd edn, New York Academic Press, 1989; pp. 111-115.

53. Zeitoun, I.H., Ullrey, D.E., Magee, W.T., Gill, J.L. & Bergen, W.G. Quantifying nutrient requirements of fish. Fish. Res. Board Can., 1976; 33: 167-172.

54. Zeitter, M., Kirchgessner, M. & Schwarz, F.J. (1984) Effect of dietary protein and energy supplies on carcass composition of carp, *Cyprinus carpio* (L). Aquaculture, 1984; 36: 37-48.