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Effect of Dietary Protein Level on Growth Performance, Protein Utilization and Body Composition of Nile Tilapia Cultured in Low Salinity Water

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Abstract

The juveniles of Nile tilapia, Oreochromis niloticus (body weight 1.0±0.03 g) were reared in seawater tanks (35 liters each) in order to examine their optimum dietary protein requirements. They were fed four isoenergetic (20.3 kJg^{-1}) diets containing 25%, 30%, 35% and 40% protein at a daily ration of 5% body weight for 42 days. Fish fed diets of 35% and 40% protein produced higher weight gain and growth rate than those of the other diets. Broken line regression analysis yielded an optimal protein level of 35%. Feed conversion and protein efficiency were significantly higher at 35% and 40% protein diets than remaining diets. Fish whole body composition showed that moisture, protein and ash content of the fish fed diets of 35% and 40% protein was significantly higher than that of fish fed diets containing protein levels of 25% and 30%, although the lipid contents were lower. Fish fed 35% and 40% protein diets showed higher nitrogen gain and nitrogen retention efficiency than those fed on other diets. Based on the biological data, it was estimated that the optimal level of protein for O. niloticus weighing between 1.0 g and 5.7 g was 35%.

Key words: Nile tilapia, growth, protein requirements, feed conversion, body composition.

Introduction

Tilapia (Oreochromis spp) are known as commercially important food fishes for aquaculture throughout several regions of the world such as China, South-east Asia, Africa, USA and Latin America/Caribbean (Lim and Webster, 2006; Chowdhury, 2011). According to FAO (2012), global tilapia production, which totaled less than 500,000 metric tons in the early 1990s, topped 3.5 million metric tons in 2011. In 2012 it increased up to 2.7 percent. Further increase was recorded as 3.4 percent in 2013 and this year it is expected to approach around 3.9 million tons.

In Pakistan, tilapia is highly prized for its good quality meat. Although there is a considerable commercial fishery (Anon, 2012), the demand has increased to such an extent that there is now interest in the culture of Nile tilapia. It is suitable for aquaculture because of its hardiness, rapid growth, resistance against stress and diseases, short generation interval and low supplementary feed requirement (El-Sayed, 1999). In addition, It is an omnivorous fish that can use high proportion of inexpensive plant sources in their feeds, stands well in wide range of environmental conditions like temperature, salinity, low dissolve oxygen (Asche et al., 2008; Chowdhury, 2011). Some work has been done on the culture of some species of tilapia but data are scarce (Jamil et al., 2004). Aquaculture of Nile tilapia would require the formulation of efficient food with optimum potency to meet the protein requirements during grow-out period (Kenawy, 1993).

Protein is considered as the main constituent of the fish body thus sufficient dietary supply is needed for optimum growth. Since protein is the most expensive component of the diet, therefore, the amount of protein in the diet should be just enough for fish growth where the excess protein in fish diets may be wasteful and cause diets to be unnecessary expensive (Ahmad, 2004). Thus, reducing feeding costs could be a key factor for successful development of aquaculture.

Information on the nutritional requirements of Nile tilapia is available to some extent. Dietary protein requirement has been stated to be between 32 to 50% for juvenile tilapia and for larger tilapia 25 to 30% (Nguyen et al., 2009., El-Saidy and Gaber., 2005; Ali et al., 2008; Abdel-Tawab et al., 2010). The optimum dietary lipid requirement for tilapia is 5 to 12% (Lim et al., 2011), and Han et al. (2010) found significantly better growth by increasing dietary lipid from 55 to 85 g per kg diet. According to Lim et al. (2011) tilapia requires linoleic (n-6) series fatty acids (18:2n-6 or 20:4n-6) and it can enhance the growth better than the n-3 series (18:3n-3, 20:5n-3 or 22:6n-3). However, these studies did not reveal the changes in liver lipid and hepatosomatic index of the fish when dietary protein level is increased as somatic growth strongly correlates with hepatosomatic index (Dos Santos et al., 1993; Jobling, 1988; Lie et al., 1988; Abbas and Siddiqui, 2009, 2013).

The present study describes the optimal level of dietary protein to achieve good growth of Oreochromis niloticus fed the diets containing protein of 25%, 30%, 35% and 40%, keeping in view that the optimum protein level for cultured fish would help in reducing the cost and maximize the feed conversion efficiency (Charles et al., 1984; Sampath, 1984; Chiu et al., 1987; Chen et al., 1994).

Materials and Methods

Experimental diet

Four isoenergetic (20.2 kJ g^{-1} digestible energy) diets were formulated on dry matter basis (g 100^{-1}) in one batch to supply calculated protein levels of 25%, 30%, 35% and 40% with fishmeal providing the majority of dietary protein (Table I). A mixture of minerals and vitamins were added to the ingredients (rice bran, wheat bran, mustered oil cake and wheat flour) of diets. All these ingredients were purchased from the local markets and were ground to 500µm and mechanically mixed for15 min to ensure homogeneity. Fish oil was added and then mixed again for 15 min. Water (250 mL kg^{-1} dry ingredients mixture) was added and mixed for another 15 minutes to attain a consistency appropriate for pelleting. The wet mash was pelleted with a California Laboratory Pellet Mill (model CL-type 3, California pellet Mill Company, San Francisco, CA, USA.) using a 2-mm die. No heating or steam was used in the pelleting process and the wet pellets were air-dried at room temperature for 20 hours. The experimental feeds were then stored at –20°C for feeding trials.

Experimental design

Juveniles of Nile tilapia, Oreochromis niloticus (mean weight 1±0.03g and mean length 3.8±0.02 cm) collected from Government Fish Hatchery, Chilya, Thatta, Sindh were held in seawater for fifteen days before starting the experiment. After the acclimatization phase, fish were randomly distributed in twelve plastic experimental indoor tanks (10 fish per tank). The water carying capacity of each tank was 35 liters. Oxygenation was provided by aerators throughout the entire experiment which lasted 42 days (27^{th} June 2011 to 8^{th} August 2011). Fish were subjected to a natural photoperiod and all tanks had similar light conditions. Physico-chemical parameters i-e., temperature, salinity, pH, ammonia and dissolved oxygen were monitored daily.

Feeding protocol

Experimental diets containing 25%, 30% and 35% protein concentration were tested to find out the optimum protein level of Nile Tilapia (O. niloticus). During this experiment, each diet was supplied to triplicate tanks in three equal meals per day at 9:00, 13:00 and 17:00 hours. Fish were hand-fed on daily ration of 5% wet body weight per day for 42 days. The

daily feed supplied was recorded and uneaten feed was collected two hours after the start of feeding. The amount of food to be provided being adjusted following weekly sampling for the determination of gain in weight and length per treatment which lasted 42 days. Each tank was completely drained and thoroughly scrubbed on the day of sampling.

Measurement and analysis

Five fish were randomly sampled from each tank, dissected and their livers weighed for estimations of the hepatosomatic index (HSI). The remaining five fish were removed from each tank, killed and pooled for whole body composition analysis. Fish whole-body samples were taken out of the –20 °C cold store and thawed at room temperature using a fan. Subsequently, all these samples were homogenized, dried and then ground into a powder before chemical composition analysis.

At the beginning of the experiment, three replicate samples with 10 fish per replicate were taken and kept frozen at –20 °C for subsequent analysis of the fish whole body composition. The moisture, protein, lipid and ash contents of experimental diets and samples were analyzed according to the standard methods (Association of Official Analytical Chemists 2000). Moisture was determined by drying in an oven (Labostar-LG 122, Tabai Espec, Osaka, Japan) at 105 °C for 24 h; ash by burning in a muffle furnace (Isuzu Seisakusho, Tokyo, Japan) at 550 °C for 18 h; crude protein by the Kjeldahl method (N × 6.25) using an automatic Kjeldahl System (Buchi 430/323, Flawil, Switzerland); crude fiber by acid detergent fiber analysis; and crude lipid by the chloroform/methanol (2:1, v/v) extraction procedure (Folch et al., 1957). The carbohydrate content was calculated by subtracting the content of lipids, total protein and ash from the dry weight, and gross energy estimation was made using an automatic bomb-calorimeter (Parr Instrument, model1265, Moline, IL, USA). All chemical analyses were performed in triplicate and averaged.

Calculation of growth parameters

At the end of the experiment, all fish from each tank were individually weighed and their total length was measured for calculation of the condition factor [CF = (100 × body weight in g)/(TL in cm)^3]. Growth and feed efficiency were monitored in terms of the final weight, weight gain (expressed as the percent of initial body weight at the end of the experiment), specific growth rate (SGR) (ln final body weight – ln initial body weight/time, expressed as % per day), feed conversion ratio (FCR) (feed fed / wet weight gain), protein efficiency ratio (PER) (wet weight gain/protein intake), protein productive value (PPV) [(protein gain / total protein intake)] and protein growth rate (PGR) [100 (ln final protein content of fish – ln initial protein content of fish) / number of days in the feeding period.

Statistical analysis

The data on fish growth, feed utilization efficiency and whole fish body constituents were subjected to one-way analyses of variance (ANOVAs) to determine whether there was a significant difference ($P<0.05$) among fish fed at different protein levels. Differences between means were assessed at the 5% probability level using Duncan's multiple range test, as described by Steel and Torrie (1980). The data are presented as mean±SE of the replicate groups. The optimal dietary protein requirements were estimated from percent weight gain of initial weight using the broken line regression analysis (Robbins et al., 1979; Cowey, 1992).

Results

Water quality:

The water temperature was maintained at 28±0.43°C (mean±SD). Salinity was 15±0.5 ‰ and pH ranged from 7.5 to 7.7 with a mean of 7.6±0.07 throughout the study period. Dissolve oxygen was 5.8±0.1 ml/l. Ammonia never exceeded 0.1±0.006 ml/l.

Growth performance:

Growth performance of Nile tilapia juveniles was significantly affected by dietary protein level (Table II). Body weight gain and SGR of the fish fed 35% and 40% protein diets were significantly ($P<0.05$) higher than of those fed the 25% and 30% protein diets. Weight gain and SGR tended to plateau at around 470.0 g and 4.14% day^{-1} respectively. Based on weight gain, the appropriate supplementation of dietary protein for the fish was estimated to be 35% of diet using broken line regression analysis (Fig. 1).

Feed conversion and condition indices :

Feed intake, expressed on a dry matter basis, increased slightly with an increase in dietary protein level. Fish fed the 35% and 40% protein diets showed significantly higher ($P<0.05$) feed intake than the other groups (Table II). The same trend was observed in feed conversion ratio (FCR). The hepatosomatic index (HSI) of fish fed diets containing 35% and 40% protein were significantly ($P<0.05$) higher than for those fed diets of 25% and 30% protein (Table II). There were no significant differences in condition factor (CF) between all the groups where survival remained 100%.

Protein utilization:

Protein utilization was evaluated through protein dependent parameters such as protein efficiency ratio (PER), protein productive value (PPV) and protein growth rate (PGR). Fish fed the 35% and 40% protein diets showed significantly higher ($P<0.05$) PER than the other groups (Table II). PPV and PGR decreased as dietary protein level increased ($P<0.05$).

Body composition:

The chemical composition of whole body showed that the protein and moisture content of fish fed diets of 35% and 40% protein was significantly ($P<0.05$) higher than that of the fish fed diets containing protein levels of 25% and 30%, although the lipid contents were lower (Table III). No significant differences were observed in the protein and ash contents of fish fed the diets in all treatments ($P>0.05$).

Nutrient deposition:

Nutrient deposition in whole body of Nile tilapia juveniles was significantly affected by dietary protein level ($P<0.05$). Nitrogen intake increased with an increase in dietary protein (Table IV). The amount of protein taken in by the fish fed 35% and 40% protein diets was significantly different ($P<0.05$) from that of fish fed diets containing 25% and 30% protein diet being intermediate. A similar trend was observed in nitrogen gain of the fish whole body. Fish fed 35% and 40% protein diets showed higher nitrogen gain than those fed on all other diets ($P<0.05$). However, there seemed to be a different trend in the values of nitrogen retention efficiency (NRE) which decreased consistently as dietary protein level increased. Fish fed diets containing 40% and 45% protein had a significant better NRE than those of fish given 25% and 30% protein (Table IV). Gross energy intake (GEI) of fish showed a linear decrease as protein level increased over the whole range of dietary protein levels. Although GEI in the fish fed 40% protein was lower (817.13kJ) than that of 35% protein diet (829.29kJ), the differences were not statistically significant ($P>0.05$); GEI ranging from 901.3 kJ to 906.2 kJ at remaining four diets (25% to 35% protein) did not appear to differ significantly ($P>0.05$, Table IV). The highest energy gain of 829.29 kJ was obtained with fish fed 35% protein, resulting in the highest energy retention efficiency (ERE) of 69.4%.

Discussion

Dietary protein is generally considered to be of crucial importance in fish nutrition and feeding, therefore sufficient supply of dietary protein is required for rapid growth (Jauncey and Ross, 1982; Lovell, 1989). In the present study, the dietary protein levels of 35% and 40% with 20.2 kJ g^{-1} digestible energy were adequate to optimize both the weight gain

and the feeding efficiency in juvenile Nile tilapia, O. niloticus growing from 1.0 g to 5.8 g. On the basis of maximum weight gain, the estimated protein requirement of the fish was 35.0%. These findings are in agreement with those of Wang et al. (1985), Siddiqui et al. (1988), Omar (1994), Abdul-Hakim et al. (2001), Wilkinson (2003), Coyle et al. (2004) and Bahnasawy (2009), which have shown that growth and FCRs improve with high protein diets. Some studies on tilapia nutrition and feeding show conflicting results. For intance, Jauncy and Ross (1982) found that the dietary protein requirement for fry is high and ranges from 35% to 56%. According to Wilson (1989), Pillay (1990) and, El-Sayed and Teshima (1991, 1992), dietary protein requirements decreased with increasing fish size, and age. Furthermore, Balarin and Halfer (1982) investigated that fry of tilapia less than 1 g requires diet with 35-50% protein, 1-5 g fish requires diet with 30-40% protein and 5-25g fish requires diet with 25-35% protein. These results may be due to the fact that each fish size has a certain protein limit after which excess protein level could not be utilized efficiently. Wee and Tuan (1988) found that the minimum dietary protein requirements for non-spawning and spawning Nile tilapia was 27.5% and 35% crude protein, respectively. De Silva et al. (1989) demonstrated that the most economical dietary protein requirement for young tilapia (1 to 5 g) was 28%. However maximum growth was achieved at about 34%. In addition, Santiago et al. (1982) reported that the optimum dietary protein level for O. niloticus fry was between 35 and 40%. These findings reveal that the optimal dietary protein level of Nile tilapia juveniles ranged from 20% to 55%. The considerable variations in the results mentioned above for optimum dietary protein requirements for maximum growth might have been due to the variations in fish size, stocking density, dietary protein quality, feeding protocol and environmental conditions (Bahnasawy, 2009). In the present study, when dietary protein concentration was above 35%, mean percent weight gain did not increase significantly ($P<0.05$). This indicates that weight gain maxima may be identified in a range of dietary protein concentration from 35% to 40% as suggested by Cowey (1992). According to him, broken line model or an asymptotic model is preferable in attempting weight gain maxima similar in the present study.

Feed conversion ratio (FCR) significantly decreased as the dietary protein level increased and ranged from 3.27 to 3.39. The best FCR was obtained from 35% and 40% protein diets, although there were no statistically significant differences among them ($P>0.05$). These results are similar to the findings of some studies on tilapia species (Siddiqui et al., 1988, Omar, 1994, Kheir,1997, Abdel-Hakim et al., 2001 and Bahnasawy, 2009). According to Wee and Tuan (1988) better FCR values were obtained with increasing dietary protein level up to 42.55 and deteriorated slightly by diet containing 50%.

In the present study, PER was significantly affected by protein levels and noticeable that protein utilization was obtained at low protein level. High protein utilization of low protein diets has been observed in many fish species including tilapia (Jauncey, 1982; Wee and Tuan, 1988; Shiau and Huang, 1989; Kheir, 1997; El-Dahhar and Lovell, 1995; Webster et al., 1995; Ahmad et al., 2004 and Abbas and Siddiqui, 2013). This might have been due to the fact that more dietary protein is used as energy when high protein diets are fed to fish (Kim et al., 1991 and Bahnasawy, 2009). Evidence to support this is available in another study of Shimeno et al. (1981). He found that increasing dietary carbohydrate and fat caused a reduction in the activities of amino acid degrading enzymes in the hepatopancreas and resulted in a low nitrogen excretion rate and a high protein efficiency ratio. Moreover, Dabrowski (1977) reported different patterns of changes in PER in relation to dietary protein level and found that the relationship between dietary protein and PER differ from species to species.

In the present study, although the diets of protein levels 25% and 30% had significantly high PER, the SGR values were low. This indicates that Nile tilapia could have efficiently utilized the low protein diet for protein synthesis, thus increasing PER value and suggesting a compensatory mechanism (Berger and Halver, 1987; Catacutan et al., 2001;

Bahnasawy, 2009). Although an increase in dietary protein causes a decrease in PER, PPV, PGR and NRE (Lee and Putnam, 1973; Bromly, 1980; Pongmaneerat and Watanabe, 1991; Bahnasawy, 2009), a linear increase in nitrogen gain is generally observed until the requirement level is met. This indicates that excess protein is catabolized to provide energy for growth (Lied and Braaten, 1984; Cowey, 1992, 1995). Similar trend was observed in Arctic char (Gurure et al., 1995), haddock (Kim and Lall, 2001) and Nile tilapia in the present study.

It is well known that protein and fat contents are the principle components to evaluate quality of fish flesh (Caulton and Bursell, 1977). In the present study, whole body lipid was significantly higher for fish fed with diets 35% and 40% than for fish fed with diets 25% and 30%. When dietary protein level increased, lipid content decreased as in sea bass (Metailler et al., 1981; Ballestrazzi et al., 1994), tilapia (Jauncey and Ross, 1982; Wee and Tuan, 1988; Shiau and Huang, 1989; Kheir, 1997; Al-hafedh, 1999), grass carp (Dabrowski, 1977), guppy (Fah and Leng, 1986) . The increase in whole body protein and decrease in lipid content with increasing dietary protein levels may be endorsed to the high carbohydrate and low protein content in the diet having low protein concentration. The surplus carbohydrate in the diet may be converted into body fat for storage (Fah and Leng, 1986). These results corroborate with the findings of Zeitler et al. (1984), Reis et al. (1989), Al-Asgah (1992), Mahboob et al. (1996) and Maithya (1998). In the present study, a clear inverse relationship between fat and water content was found and there appeared to be a mechanism for some homeostasis of tissue volume. Additional energy stored as fat replaced body water and did not adversely affect the deposition of protein. Tveranger (1985) reported that the dry matter and fat in muscle of rainbow trout were positively correlated. A variation in dry content was caused mainly by a variation in fat content. Fat and water to a certain degree substitute each other. With increasing fat content the protein content (% of dry matter) is reduced with a simultaneous increase in dry matter. These findings are in line with the results of the present study and with those of Shimma (1986), who reported significantly negative correlation between moisture content and fat content in two races Yamato and Mirror of carp, Cyprinus carpio. In the present study, body fat contents reflected the same of the diets. The apparent protein retention (APR) varied inversely with dietary protein. The APR was significantly different in fish fed with diets of 35% and 40% protein than fish fed with diets of 25%–30%.

In conclusion, the diet containing 35% dietary protein with P/E ratio of 17.1 mg protein kJ^{-1} could be considered as optimum for the growth of Nile tilapia juveniles under the experimental conditions of the present study.

References

Abbas, G and P. J. A. Siddiqui, 2009. Effects of different feeding level on the growth, feed efficiency and body composition of juvenile mangrove red snapper, Lutjanus argentimaculatus (Forsskal 1775). Aquacult. Res., 40: 781–789.

Abbas, G. and P. J. A. Siddiqui, 2013. The effect of varying dietary protein level on growth, feed conversion, body composition and apparent digestibility coefficient of juvenile mangrove red snapper, Lutjanus argentimaculatus (Forsskal 1775). Aquacult. Res., 44: 807–818.

Abdel-Hakim, N. F., M. S. Hussein, M. N. Bakeer and M. A. Soltan, 2001. Effect of protein level and stocking density on growth performance of Nile tilapia (Oreochromis niloticus) cultured in tanks. Egyptian J. Nutr. Feed, 4: 763–780.

Abdel-Tawwab, M., M. H. Ahmad, Y. A. E Khattab and A. M. E. Shalaby, 2010. Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, Oreochromis niloticus (L.). Aquaculture, 298: 267–274.

Ahmad, M. H., M. Abdel-Tawwab and Y. A. E. Khattab, 2004. Effect of dietary protein levels on growth performance and protein utilization in Nile tilapia (Oreochromis niloticus) with different initial body weights. In: The sixth international symposium on tilapia in aquaculture, Manila, Philippine. Pp. 249–263.

Al-Asgah, N. A., 1992. Variation in the carcass composition of Orechromis niloticus in relation to body weight and length. Pakistan J. Zool., 24**:** 47–51.

Al-Hafedh, Y. S., 1999. Effects of dietary protein on growth and body composition of Nile tilapia, Oreochromis niloticus L. Aquacult. Res., 30: 385**–**393.

Ali A., S. M. Al-Ogaily, N. A. Al-Asgah, J. S. Goddard and S. I. Ahmed, 2008. Effect of feeding different protein to energy (P/E) ratios on the growth performance and body composition of Oreochromis niloticus fingerlings. J. Appl. Ichthy, 24: 31–37.

Anonymous, 2012. Hand Book of Fisheries Statistics of Pakistan. A publication of Marine Fisheries Department, Government of Pakistan. Ministry of Ports & Shipping, 20: 215.

Asche, F., K. H. Roll and S. Tveterås, 2008. Future Trends in Aquaculture: Productivity Growth and Increased Production, In: M. Holmer, Black, K., Duarte, C. M., Marbà, N. and Karakassis, I. (eds.), Aquaculture in the Ecosystem, Springer Netherlands. pp. 271-292.

Bahnasawy, M. H., 2009. Effect of dietary protein levels on growth performance and body composition of mono-sex Nile tilapia, Oreochromis niloticus L. reared in fertilized tanks. Pak. J. Nutr., 8: 674-678.

Balarin, J. D. and R. D. Halfer, 1982. The intensive culture of tilapia in tanks, raceways and cages. In: J.F. Muir and R.J. Roberts (eds.). Recent Advances in Aquaculture, Croom Helm, London. 265-356 pp.

Ballestrazzi, R., D. Lanari, E. D. Agaro and A. Mion, 1994. The effect of dietary protein level and source on growth, body composition, total ammonia and reactive phosphate excretion of growing sea bass (Dicentrarchus labrax). Aquacult., 127: 197–206.

Berger, A. and J. E. Halver, 1987. Effect of dietary protein, lipid and carbohydrate content on the growth, feed efficiency and carcass composition of striped bass, Morone saxatilis (Walbaum) fingerlings. Aquacult. Fish. Manag., 18: 345–356.

Bromly, P. J., 1980. Effect of dietary protein, lipid and energy content on the growth of turbot (Scophthalmus maximus L.). Aquacult., 19: 359–369.

Catacutan, M. R., G. E. Pagador, and S. Teshima, 2001. Effect of dietary protein and lipid levels and protein to energy ratios on growth, survival and body composition of the mangrove red snapper, Lutjanus argentimaculatus (Forsskal 1775). Aquacult. Res., 32: 811–818.

Caulton, M. S. and E. Bursell, 1977. The relationship between changes in condition and body composition in young Tilapia rendolli Boulenger. J. Fish Biol., 11: 143–150.

Charles, P. M., S.M. Sebastian, M. C. Raj, and M.P. Marian, 1984. Effect of feeding frequency on growth and food conversion of Cyprinus carpio fry. Aquaculture, 40: 293-300.

Chen, H. Y. and J. C Tsai, 1994. Optimal dietary protein level for the growth of juvenile grouper, Epinephelus malabaricus, fed semi purified diets. Aquacult., 119: 265–271.

Chiu, Y. N., N. S. Sumagaysay, and M. S. Sastrillo, 1987. Effect of feeding frequency and feeding rate on the growth and feed efficiency of milkfish (Chanos chanos). Asian Fish. Sci., 1: 27-31.

Chowdhury, D. K., 2011. Optimal feeding rate for Nile tilapia (Oreochromis niloticus). MSc thesis. Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Submitted 2011-05-16. Pp 76.

Cowey, C. B., 1992. Nutrition: estimating requirements of rainbow trout. Aquacult., **100:** 177–189.

Cowey, C.B., 1995. Protein and amino acid requirements: A critique of methods. J. Appl. Ichthy., 11: 199–204.

Coyle, S. D., G. J. Mengel, J. H. Tidwell and C. D. Webster, 2004. Evaluation of growth, feed utilization and economics of hybrid tilapia, Oreochromis aureus, fed diets containing with distillers dried grains with solubles. Aquacult. Res., 35: 365–370.

Dabrowski, K., 1977. Protein requirments of grass carp fry (Ctenopharyngodon idella Val.). Aquacult.,12: 663–73.

De Silva, S.S., R. M. Gunasekera and D. Atapattu, 1989. The dietary protein requirements of young tilapia and an evaluation of the least cost dietary protein levels. Aquaculture, 80: 271–284.

Dos Santos, J. D., I. C. Burkow, and Jobling, M. 1993. Patterns of growth and lipid deposition in cod (Gadus morua) fed natural prey and fish based feeds. Aquaculture, 110: 173–189.

El-Dahhar, A. A. and R. T. Lovell, 1995. Effect of protein to energy ratio in purified diets on growth performance, feed utilization and body composition of Mozambique tilapia, Oreochromis mossambicus (Peters). Aquaculture, 97: 383–394.

El-Saidy, D. M. S. D. and M . M. A. Gaber, 2005. Effect of dietary protein levels and feeding rates on growth performance, production traits and body composition of Nile tilapia, Oreochromis niloticus (L.) cultured in concrete tanks. Aquacult. Res., 36:163–171.

El-Sayed A. F. M. and S. I. Teshima, 1991. Tilapia nutrition in aquaculture. Rev. Aqua. Sci., 5: 247−265.

El-Sayed, A-F.M. and S. I. Teshima, 1992. Protein and energy requirements of Nile tilapia, Oreochromis niloticus fry. Aquaculture, 103: 55–63.

El-Syed A.-F.M., 1999. Alternative dietary protein source for farmed tilapia, Oreochromis spp. Aquaculture, 179: 149–168.

Fah, S. K. and C. Y. Leng, 1986. Some studies on the protein requirement of the guppy, Poecilia retculata (peters). J. Aquacult. Aquatic Sci., 44, 1–12.

FAO, 2012. The State of World Fisheries and Aquaculture 2012. Published by Food and Agriculture Organization, Rome. 209 pp.

Folch, A. C., Leed, M. and G. M. Sloane-Stanley, 1957. A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497–509.

Gurure, R.M., R. D. Moccia, and J. L. Atkinson, 1995. Optimal protein requirements of young Arctic char (Salvelinus alpinus) fed practical diets. Aquacult. Nutr., 1: 227–234.

Han C.Y., X. B. Wen Q. M. Zheng and H. B. Li., 2010. Effects of dietary lipid levels on lipid deposition and activities of lipid metabolic enzymes in hybrid tilapia (Oreochromis niloticus × O. aureus). Journal of Animal Physiology and Animal Nutrition. Article first published online: 26 Nov 2010.

Jamil, K., M. Shoaib, F. Ameer and L. Hong, 2004. Salinity tolerance and growth response of juvenile Oreochromis niloticus at different sailinity levels. J. of Ocean Univ. China, 3: 53–54.

Jauncy, K. and B. Ross, 1982. A guide to tilapia feeds and feeding. Institute of Aquaculture. University of Stirling, Scotland, UK.

Jobling, M., 1988. A review of the physiological and nutritional energetics of cod, Gadus morhua L., with particular reference to growth under farmed conditions. Aquaculture, 70: 1–19.

Kenawy, S. M., 1993. Performance of food organisms and tested diets in fish nursing. J. Int. Fish. Soc. India, 25: 39–41.

Kheir, M.T., 1997. Growth of Oreochromis niloticus (Linnaeus, 1758) raised on feeds with different protein levels. Egypt. J. Zool., 28: 65–76.

Kim, J. D. and Lall, S.P., 2001. Effects of dietary protein level on growth and utilization of protein and energy by juvenile haddock (Melanogrammus aeglefinus). Aquacult., 195: 311–319.

Kim, K., T. B. Kayes and C. H. Amundson, 1991. Purified diet development and re-evaluation of the dietary protein requirement of fingerling rainbow trout (Oncorhyncus mykiss). Aquaculture, 96: 57-67.

Lee, D.J. and G. B. Putnam, 1973. The response of rainbow trout to varying protein/energy ratios in a test diet. J. Nutr., 103: 916–922.

Lie, O., E. Lied and G. Lambertsen, 1988. Feed optimization in Atlantic cod (Gadus morhua): fat versus protein content in the feed. Aquacult., 68: 333–341.

Lied, E. and B. Braaten, 1984. The effect of feeding and starving, and different ratios of protein energy to total energy in the feed on the excretion of ammonia in Atlantic cod (Gadus morhua). Comp. Biochem. Physiol., 78A: 49–52.

Lim, C. E. and C. D. Webster, 2006. Tilapia: biology, culture, and nutrition. The Haworth Press Inc., 10 Alice Street, Bringhamton, NY 13904-1580, USA.

Lim, C., M. Oksoy, and P. Klesius, 2011. Lipid and fatty acid requirements of tilapia. North Amer. J. Aquacult., 73: 188-193.

Lovell, R. T., 1989. Nutrition and Feeding of Fish. Van Nostrand reinhold. New York. 260 pp.

Mahboob, S., A. N. Sheri and S. H. Raza, 1996. Proximate composition of major, common and some Chinese carps as influenced by pond fertilization and feed supplementation in composite culture system. J. Aquacult. Trop., 11: 227–284.

Maithya, J., 1998. The evolution of body muscle composition of the African catfish (Clarias gariepinus) (Burchell 1822), Naga, The ICLARM quarterly, 21: 24–26.

Metailler, R., J. F. Aldrin, J. L Messager, G. Mavel, and G. Stephan, 1981. Feeding of European sea bass (Dicentrarchus labrax): role of protein level and energy source. J. World Maricult. Soc., 12: 117–118.

Nguyen, T. N., D. A. Davis and I. P. Saoud, 2009. Evaluation of alternative protein sources to replace fish meal in practical diets for juvenile tilapia, Oreochromis spp. J. World Aquacult. Soc., 40:113–121.

Omar, E.A., 1994. Optimum protein to energy ratio for tilapia (Oreochromis niloticus) fingerlings. Alex. J. Agri. Res., 39: 73–93.

Pillay, T. V. R., 1990. Aquaculture: Principles and Practices. Fishing News Books. Blackwell Scientific Publication, Ltd., Oxford UK. Pp. 575.

Pongmaneerat, J. and T. Watanabe, 1991. Nutritive value of protein of feed ingredients for carp. Nippon Suisan Gakkaishi, 57: 503–510.

Reis, L. M., E. M. Reutebuch and R. T. Loveil, 1989. Protein to energy ratios in production, diets and growth, feed conversion and body composition of channel catfish, Ictalurus punctatus. Aquacult., 77: 21–27.

Robbins, K. R., H. R. Norton, and D. H. Baker, 1979. Estimation of nutrient requirements from growth data. J. Nutr., 109: 1710–1714.

Sampath, K., 1984. Preliminary report on the effect of feeding frequency in Channa striatus. Aquaculture, 40: 301–306.

Santiago, C.B., M. Banes-Aldaba and M. A. Laron, 1982. Dietary crude protein requirement of Tilapia Nilotic fry. Kalikasan Philipp. J. Biol., 11: 255–265.

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

Shimma, H., 1986. Growth and proximate composition of two races, Yamato and Mirror of carp Cyprinus carpio. Bull. Nat. Res. Inst. Aquaculture, 9: 15–20.

Shimmeno, S., M. Takeda, S. Takayama and H. Sasaki, 1981. Response of nitrogen excretion to change of dietary composition in carp. Bull. Japan. Soc. Sci. Fish., 47: 191-195.

Siddiqui, A.Q., M. S. Howlader and A. A. Adam, 1988. Effects of dietary protein levels on growth, feed conversion and protein utilization in fry and young Nile tilapia, Oreochromis niloticus. Aquaculture, 70: 63–73.

Steel, R.G.D. and J. H. Torrie, 1980. Principles and Procedures of Statistics. 2nd edn. McGraw-Hill. New York, NY. 631 pp.

Tveranger, B., 1985. Variation in growth rate, liver weight and body composition at first sexual maturity in rainbow trout. Aquaculture, 49: 89–99.

Wang, K., T. Takeuchi and T. Watanabe, 1985. Optimum protein and digestible energy levels in diets for Tilapia niloticus. Bull. Japan Soc. Sci. Fish., 51: 141–146.

Webster, C.D., L. Tiu, J. H. Tidewell, P. Van Wyk and R. Howerton, 1995. Effects of dietary protein and lipid levels on growth and body composition of sunshine bass (Morone chrysops × M. saxatillis) reared in cages. Aquacult., 131: 291–301.

Wee, K.L. and N. A. Tuan, 1988. Effects of dietary protein levels on growth and reproduction in Nile tilapia (Oreochromis niloticus). In: R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.). The Second International Symposium on Tilapia in Aquaculture, ICLARM, Manila, Philippines. pp. 401-410.

Wilkinson, S., 2003. Aquaculture fundamentals getting the most out of your feed. Part II: The role of macronutrients. Aquacult. Asia, 2: 6–9.

Wilson, R.P., 1989. Protein and amino acid requirements of fishes. Pp. 51–76. In: S. Shian (ed.): Progress in Fish Nutrition. National Taiwan Ocean University, Keeling, Taiwan.

Zeitler, M. H., M. Kirchgessner and F. J. Schwarz, 1984. Effects of different protein and energy supplies on carcass composition of carp (Cyprinus carpio L.). Aquacult., 36: 37–48.

Table I.- Ingredients and proximate composition of the experimental diets.

	Dietary protein (% dry matter DM)			
Ingredients (%)	**25**	**30**	**35**	**40**
Fish meal	24.5	29.5	34.5	39.5
Wheat bran	20.0	18.0	16.0	14.0
Rice bran	15.0	13.0	11.0	9.0
Mustered oil cake	15.0	14.0	13.0	12.0
Wheat flour	20.0	20.0	20.0	20.0
Vitamin-mineral premix[1]	2.5	2.5	2.5	2.5
Fish oil	3.0	3.0	3.0	3.0
Proximate composition[2]				
Moisture	7.5±0.5	7.2±0.3	7.0±0.5	7.0±0.5
Crude protein[3]	24.7±0.7	29.8±0.9	34.6±0.5	34.6±0.5
Crude lipid	5.5±0.4	5.6±0.5	5.8±0.6	5.8±0.6
Crude fiber	4.4±0.6	5.2±0.6	5.9±0.4	5.9±0.4
Ash	5.3±0.8	6.1±0.5	7.0±0.7	7.0±0.7
NFE[4]	60.1±0.3	53.3±0.4	46.7±0.3	46.7±0.3
Energy (kJg^{-1})	20.3±0.5	20.1±0.4	20.2±0.6	20.3±0.5
P/E (mg crude protein kJ^{-1})	12.2±0.4	14.8±0.3	17.1±0.5	17.1±0.5

[1]Vitamin and mineral mixture contained the following ingredients (g 100 g^{-1} diet): Ascorbic acid (vit C), 15.2; thiamin HCl (vit B_6), 1.1; inositol, 39.5; calcium, 1.25; zinc, 1.0; retinol (vit A), 1.5; phosphorus, 3.5; choline chloride, 3.5; magnesium, 2.0; copper, 1.0; pyridoxine (vit B_6), 1.3; phospholipids, 3.5; α-tocopherol acetate (vit E), 5.5; folic acid, 0.4; cholecalciferol (vit D_3), 7.5; cyanocobalamine (vit B_{12}), 0.006; riboflavin (vit B_2), 1.5; menadione sodium bisulphite (vit K_3), 0.03; manganese, 2.0; iodine, 2.0; sodium, 1.0; iron, 1.0; nicotinic acid, 4.3; biotin, 0.35.

[3]Dry matter basis (%): mean ± SE, number of determination = 3.

[4]Measured as nitrogen × 6.25.

[5]Nitrogen-free extract = 100 – (% protein + % fat + % ash + % fiber).

Table II.- The growth rate and feed utilization of juvenile Nile tilapia fed at different levels of protein for 42 days.

	Dietary protein (%DM)			
Parameters	**25**	**30**	**35**	**40**
Final weight (g)	3.8±0.2[a]	4.8±0.1[b]	5.7±0.2[c]	5.8±0.2[c]
Weight gain, % of initial weight[1]	280.0±2.5[a]	380.0±2.7[b]	470.0±3.2[c]	480.0±3.0[c]
Specific growth rate[2]	3.17±0.02[a]	3.73±0.06[b]	4.14±0.05[c]	4.18±0.05[c]
Feed intake[3] (g fish^{-1})	9.5.±1.0[a]	12.8±1.3[b]	15.6±1.3[c]	15.7±1.1[c]
Feed conversion ratio[4]	3.39±0.1[a]	3.37±0.2[a]	3.32±0.4[b]	3.27±0.3[b]
Protein efficiency ratio[5]	2.45±0.02[a]	1.87±0.03[b]	1.18±0.02[c]	1.19±0.01[c]
Condition factor[6]	3.5±0.03 [a]	3.5±0.01 [a]	3.5±0.01 [a]	3.6±0.01 [a]
Hepatosomatic index[7]	1.7±0.1[a]	1.8±0.2[a]	2.0±0.1[b]	2.2±0.3[b]
Protein productive value[8]	34.87±0.2[a]	27.8±0.1[b]	25.8±0.3[c]	25.6±0.1[c]
Protein growth rate[9]	3.8±0.4[a]	3.7±0.2[b]	3.2±0.1[c]	3.3±0.1[c]
Survival (%)	100	100	100	100

Values (means±SE, n = 3 and each n consists of 10 fish per replicate) in the same row with different superscripts are significantly different (P>0.05). Initial body weight and length of the fish was 1±0.03 g and 3.9 cm ± 0.02 respectively.

[1]Weight gain, % of initial weight = 100 × [final body weight – initial body weight / initial body weight].

[2]Specific growth rate = 100 × [ln final body weight – ln initial body weight / time in days].

[3]Feed intake = total feed fed as % body weight – total uneaten feed.

[4]Feed conversion ratio = total feed fed (g) / total wet weight gain (g).

[5]Protein efficiency ratio = wet weight gain / protein (N × 6.25) intake.

[6]Condition factor (CF) = 100 × (weight / length^3).

[7]Hepatosomatic index (HSI) = wet liver weight (g) / empty fish weight (g) × 100; that of the initial fish was 1.24%.

[8]Protein productive value (PPV) =100 × protein gain / protein intake.

[9]Protein growth rate (PGR) = [100 (ln final protein content of fish – ln initial protein content of fish) / number of days in the feeding period.

Table III.- Whole body composition (% dry weight basis) of juvenile Nile tilapia fed at different levels of protein for 42 days.

	Dietary protein (%DM)			
Parameters	**25**	**30**	**35**	**40**
Moisture	71.5±1.3^a	72.1±1.2^b	72.6±1.4^c	72.5±1.3^c
Protein	53.1±1.5^a	54.8±1.7^b	56.4±1.8^c	56.3±1.6^c
Lipid	34.3±1.1^a	31.5±2.5^b	29.1±1.8^c	29.3±1.0^c
Ash	11.4±0.6^a	12.8±0.4^b	13.6±1.6^c	13.4±1.5^c

Values (mean±SE, n =3 and each n consists of 10 fish per replicate) in the same row with different superscripts are significantly different (P>0.05). Chemical composition of initial body was: moisture 72.5%, protein 53.7%, lipid 35.0% and ash 12.5%.

Table IV.- Nitrogen and energy utilization of juvenile Nile tilapia fed at different levels of protein for 42 days.

	Dietary protein (%DM)			
Parameters	**25**	**30**	**35**	**40**
Nitrogen intake[1]	1.22±0.03^a	1.27±0.07^a	1.51±0.02^b	1.52±0.06^b
Nitrogen gain[2]	0.44±0.01^a	0.45±0.04^a	0.6±0.01^b	0.6±0.02^b
Nitrogen retention[3]	36.1±1.3^a	34.4±1.6^a	39.7±1.8^b	39.5±1.9^b
Energy intake	906.2±4.6^b	901.3±3.8^b	829.29±4.9^a	817.13±3.5^a
Energy gain[4]	537.67±3.8^a	544.5±4.9^a	575.5±5.2^b	555.4±4.7^b
Energy retention[5]	59.3±1.0^a	60.4±1.3^a	69.4±1.2^b	67.9±2.0^b

Values (means±SE, n = 3 and each n consists of 10 fish per replicate) in the same row with different superscripts are significantly different (P>0.05). Initial body weight of the fish was 1.0±0.3 g.

[1]Nitrogen intake (g fish^{-1}) = feed intake per fish × nitrogen content of feed.

[2]Nitrogen gain (g fish^{-1}) = nitrogen in whole body of final fish – nitrogen in whole body of initial fish.

[3]Nitrogen retention (%) = nitrogen gain / nitrogen intake × 100.

[4]Energy gain (kJ fish^{-1}) = energy in whole body of final fish – energy in whole body of initial fish.

[5]Energy retention (%) = energy gain / energy intake × 100.

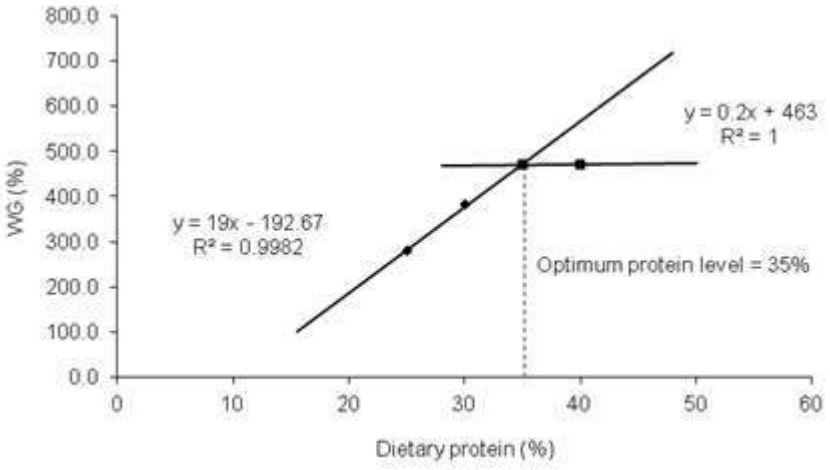

Fig:1 :Optimum protein level of Nile tilapia as determined by the broken line model.