Aquacult Int (2015) 23:1189–1201
DOI 10.1007/s10499-014-9876-1

Effects of dietary protein levels on growth performance and haemato-immunological parameters of juvenile genetically improved farmed tilapia (GIFT), *Oreochromis niloticus*

Mathew Didlyn Kpundeh · Jun Qiang · Jie He · Hong Yang · Pao Xu

Received: 20 February 2014 / Accepted: 29 December 2014 / Published online: 3 February 2015

Abstract This study evaluated the effect of dietary protein level on growth performance and haemato-immunological parameters of juvenile genetically improved farm tilapia, *Oreochromis niloticus*, fed five iso-caloric diets of dietary protein levels 25–45 %, formulated using white fish, casein, gelatine, soybean, cottonseed and rapeseed meals as protein sources. The diets influenced the significant increase in final weight, condition factor and whole body lipid composition ($P < 0.05$). Conversely, the 25 % protein diet resulted in a significantly ($P < 0.05$) lower weight gain and higher feed conversion ratio than the 30–45 % protein diets. Protein efficiency ratio and protein retention efficiency of the fish significantly reduced as dietary protein level increased ($P < 0.05$). Meanwhile, hepatosomatic index increased with dietary protein. Haemato-immunological parameters, white blood cell, red blood cell, immune globulin M, superoxide dismutase and lysozyme activity, were not significantly affected by the dietary protein levels ($P > 0.05$). Quadratic regression model, $y = -0.493x^2 + 41.03x + 267.3$, informed that 41.6 % dietary protein supported maximum growth of tilapia juvenile. Fish fed 35 % protein diet had a similar growth performance, but better protein utilization than the 40 % group. These results demonstrated possibility of reducing protein level in commercial feeds, thus lowering feed costs.

M. D. Kpundeh (✉) · P. Xu
Wuxi Fisheries College, Nanjing Agricultural University, Wuxi 214081, China
e-mail: mathdidlyndeh@yahoo.com

M. D. Kpundeh · J. Qiang · J. He · H. Yang · P. Xu (✉)
Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization, Ministry of Agriculture, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, Jiangsu, China
e-mail: xup@ffrc.cn

M. D. Kpundeh
Department of Aquaculture and Fisheries Management, Njala University, P.M.B, Freetown, Sierra Leone

Keywords GIFT tilapia · Protein level · Growth performance · Feed utilization · Immunological function

Introduction

Protein intake is essential for metabolism and tissue repair in fish (Bowden 2008). According to Jauncey and Ross (1982), dietary energy for fish can be provided by protein, lipid and carbohydrate with total energy content approximately 5.5, 9.1 and 50 4.1 kcal/g, respectively. Proteins constitute a larger proportion of fish feed and, at the same time, are one of the most important dietary nutrients (Singh et al. 2008). Protein generally is the most expensive component in fish diet, and diets used to feed small tilapia must contain higher protein levels (normally higher levels of fish meal) than grow-out diets (Trosvik et al. 2012). The protein required for body maintenance is called the minimum protein requirement or maintenance requirement; optimal protein on the other hand is the proportion of protein in the diet which produces the highest fish growth per unit of protein consumed. The nutritional value of a dietary ingredient is dependent on its ability to supply energy. Protein as a major dietary nutrient can influence the growth performance of fish and other animals (Lovell 1989; Takagi et al. 2001; Cho et al. 2007; Ye et al. 2011). Additionally, its supplies essential and non-essential amino acids necessary for muscle formation, enzymatic function as well as energy for maintenance (Yang et al. 2002). Determination of optimal dietary protein requirement is a critical step in developing feed for any new species in aquaculture, given that excess protein diets may be wasteful and makes diets to be gratuitously expensive (Bahnasawy 2009).

In formulating an optimum diet, the ratio of protein to energy must be determined separately for each fish species. Dietary protein requirement is affected by several factors including species, age of fish, dietary protein quality, energy level and the protein-to-energy balance (Wilson 2002). Dietary ingredients including protein (e.g. fish meal, soybean meal), lipid (e.g. fish oil, soybean oil, corn oil) and carbohydrates (e.g. corn starch, wheat starch) used in fish diets can be sourced from either plant or animals. These ingredients must be able to meet the needs of the cultured animal when included in feed formulation. Caution must be taken when selecting dietary ingredients that should boost optimal fish growth and well-being. Since apparent digestibility of protein depends upon its degree of purity, experimental diets, therefore, must contain some purified protein such as casein and gelatine, for their higher digestibility. Dietary protein and lipid contents are crucial factors affecting the growth performance of teleost fish and the feed cost (Glencross et al. 2007; Arredondo-Figueroa et al. 2012). Fish meal is essentially the first choice of raw material in aquafeed production due to its high quality of protein and well-balanced amino acid profile; meanwhile, global fish meal production for the past two decades have remained relatively stable (Ye et al. 2011). It is therefore prudent to maximized lipid or carbohydrates in fish diets so as to spare dietary protein from being used as energy. Protein sparing occurs when energy levels in the diet are sufficient to 'spare' protein from being used as an energy source. For instance, protein sparing effect by lipid supplementation has been well demonstrated for salmonid and sea bass (Watanabe 1982; Beamish and Medland 1986; Dias et al. 1998; Torstensen et al. 2001). Other studies have reported that differences in whole body protein contents are small in fish fed high-lipid diets due to lipid dilution (Wang et al. 2005; Song et al. 2009).

Changes in blood parameters are governed in part by the nutritional state of the fish (Kumar et al. 2005; Eslamloo et al. 2012; Zhou et al. 2012). For instance, Sakthivel (1988) reported that haemoglobin concentration and red blood cell count of common carp (*Cyprinus carpio*) fed 38 % dietary protein level were higher than those fed 14 and 58 % dietary protein levels. Abdel-Tawwab et al. (2010) further suggested that increased dietary protein level can raise haemoglobin concentration and red blood cell count in Nile tilapia, *Oreochromis niloticus*. Serum immunoglobulins are major components of the humoral immune system; in particular, immunoglobulin M (IgM) is the main immunoglobulin found in fish (Sun et al. 2010). Moreover, lysozyme also acts as vital bio-defence effectors of innate immunity (Simser et al. 2004) which can be affected by feeding regime and dietary protein level. In China currently, the increased culture of GIFT (genetically improved farmed tilapia) strain tilapia is mainly due to its many advantages such as rapid growth rate, high fillet yield and good disease-resistance ability (Qiang et al. 2012). Although several nutritional studies on Nile tilapia have been conducted (Jauncey 1982; Shiau and Huang 1989; El-Sayed and Teshima 1992), there are, however, limited studies on the improved fast-growing strain (GIFT). Since nutritional state of an animal can play a major role in its growth and well-being, the current study was conducted to evaluate the effects of dietary protein levels on the growth performance, haematological parameters and non-immune response parameters and to determine the optimal dietary protein requirement of GIFT strain Nile tilapia juvenile, *Oreochromis niloticus.*

Materials and methods

Experimental diets

Composition and proximate analysis of the experimental diets (Table 1) consisted of five iso-caloric diets using white fish meal, casein, gelatine, soybean meal, cottonseed meal and rapeseed meal as protein sources and fish oil and soybean oil as lipid sources. The diets were formulated to contain graded levels of protein of dry weight (25.03, 31.08, 35.94, 41.25 and 45.82 %) corresponding to D1, D2, D3, D4 and D5, respectively. Diets were made isoenergetic by adjusting levels of carbohydrate (corn starch). All the ingredients were weighed according to the composition of the diet; dry ingredients for the respective diets were homogenously blended using Hobart-type mixer; lipid and mineral and vitamin premixes were then incorporated into the diets. Water, approximately 50 % diet weight, was added to the respective diet ingredient mixture and thoroughly mixed. A 2.0-mm-diameter die was then used to produce the wet-extruded, air-dried pellets, with moisture content of 10 % and then sealed in vacuum-packed bags and stored at −20 °C until feeding.

Animal rearing

Healthy GIFT tilapia juveniles, of the sixteenth generation, bred at the Yixing farm of Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences in China, were used as test object in this trial. Prior to the official experiment, fish were acclimatized for 2 weeks in a concrete tank. During the acclimatization period, the fish were fed diet D1. At the commencement of the official experiment, 20 individual agile fish (initial body weight 3.77 ± 0.01 g) were distributed randomly in 15 fibre glass tanks connected to a recirculation system with 350 l of water each. All the tanks were equally aerated to supply

Table 1 Composition and nutrient levels of experimental diets (expressed as %)

Ingredients	Experimental diets				
	*D*1	*D*2	*D*3	*D*4	*D*5
White Fish meal[1]	5.00	5.00	5.00	5.00	5.00
Casein[2]	4.0	8.70	13.40	18.20	22.90
Gelatine[3]	1.0	2.20	3.40	4.50	5.70
Corn starch	35.8	29.90	24.00	18.10	12.20
Fish oil	4.50	4.50	4.50	4.50	4.50
Soybean oil	4.50	4.50	4.50	4.50	4.50
Soybean meal	11.0	11.0	11.0	11.0	11.0
Cottonseed meal	15.0	15.0	15.0	15.0	15.0
Rapeseed meal	15.0	15.0	15.0	15.0	15.0
[a]Vitamin premix[1]	1.0	1.0	1.0	1.0	1.0
[a]Mineral premix[2]	1.0	1.0	1.0	1.0	1.0
Choline chloride	0.50	0.50	0.50	0.50	0.50
Vitamin C sodium phosphate	0.20	0.20	0.20	0.20	0.20
$Ca(H_2PO_4)_2$	1.50	1.50	1.50	1.50	1.50
Total	100	100	100	100	100
Nutrient levels					
Dry matter	87.30	86.50	87.30	87.00	82.50
Crude protein	25.03	31.08	35.94	41.25	45.82
Crude lipid	9.72	9.38	9.26	9.48	9.47
Ash	6.39	6.24	6.56	7.58	7.89

[1] White fish meal, obtained from Copeinca (Lima, Peru), crude protein 67.4 %, crude lipid 9.3 %

[2] Casein, obtained from Hualing Casein Company Ltd. (Gansu, China), crude protein 91.2 %

[3] Gelatine, obtained from Rousselot Gelatin Company Ltd (Guangdong, China), crude protein 91.6 %

Soybean meal, obtained from Tongwei Shihai Feed Corporation Ltd, Wuxi, China, crude protein 41.4 %, crude lipid 1.15 %

Cottonseed meal, obtained from Tongwei Shihai Feed Corporation Ltd, Wuxi, China, crude protein 38.4 %, crude lipid 0.3 %

Rapeseed meal, obtained from Tongwei Shihai Feed Corporation Ltd, Wuxi, China, crude protein 36.2 %, crude lipid 1.2 %

[a] Vitamin mix and mineral mix were provided by Guangzhou Chengyi Aquatic Technology Ltd (Guangzhou, China)

[1] Per kg diet contain thiamine, 20 mg; riboflavin, 20 mg; pyridoxine, 10 mg; nicotinic acid, 100 mg; calcium pantothenate, 50 mg; biotin, 1 mg; folacin, 5 mg; inositol, 500 mg; vitamin E, 50 mg; vitamin A, 2 mg; vitamin B12, 0.02 mg; vitamin K_3, 10 mg; vitamin D_3,0.05 mg

[2] Per kg diet contain $ZnSO_4 \cdot 7H_2O$, 525.5 mg; $MnSO_4 \cdot H_2O$, 49.2 mg; KI, 5.23 mg; $FeSO_4 \cdot 7H_2O$, 238.8 mg; $MgSO_4 \cdot 7H_2O$, 4.62 g; $CuSO_4 \cdot 5H_2O$, 11.8 mg; $CoCl \cdot 6H_2O$, 0.2 mg; Na_2SeO_4, 0.66 mg; KCl, 600 mg; NaCl, 107.1 mg

fish with dissolved oxygen. At a start, the diets were fed to the GIFT strain tilapia juveniles at a rate of 10 % their body weight, three times a day (08:30, 12:30 and 17:00 h). Each diet was assigned to triplicate groups. Feeding level was reduced to 8 % body weight at the start of the second month. Fish were group weighed at an interval of 15 days, and daily amount of feed per tank re-adjusted on the basis of the new average weight. Fish readily accepted the diets, but when the diets were not completely eaten 15 min after they have

been served (which in most cases were a few pellets), they are removed using a fine-meshed scoop net. Faeces when seen floating in the cultured water were siphoned out of the tanks. During the feeding trial, water quality parameters were monitored daily, temperature ranged from 28 to 29 °C, dissolved oxygen was greater than 6.0 mg/l, and ammonia nitrogen was lower than 0.05 mg/l. The fish were reared and fed the respective diets under natural photoperiod (12-h light: 12-h dark) for 8 weeks.

Sample collection and analytical methods

At the start of experiment, 25 fish were sampled and stored frozen at −20 °C for analysis of whole body composition. At the end of the 8-week feeding trial, fish in each tank were individually weighed and sampled for tissue analysis 24 h after the last feeding.

Proximate analysis

At the end of the experiment, five fish from each tank were used for whole body proximate composition. Crude protein, crude lipid, moisture, ash content in diets and fish whole body samples were determined following standard methods (AOAC 1995). Crude protein (N × 6.25) was determined by the Kjeldahl method after acid digestion using an Auto Kjeldahl System (1030-Auto-analyzer, Tecator, Hoganos, Sweden). Crude lipid was determined by ether extraction using Soxtec System HT (Soxtec System HT6, Tecator, Sweden). Moisture content was determined by oven drying at 105 °C until a constant weight was achieved. Ash content was measured after placing the samples in a muffle furnace at 550 °C for 24 h.

Blood analysis

Blood samples were drawn from the caudal vein of five fish per tank using heparinized needles and centrifuged at 3,000*g*, at 4 °C for 15 min to obtain the serum. Whole blood samples were used for white blood cell count, red blood cell count, haematocrit and haemoglobin content measurement. White blood cell count, haematocrit and red blood cell count were determined using an automatic blood analyser (Hitachi 7170A). The Haemoglobin (Hb) level was measured using the Diagnostic Kit (Sekisui medical, Tokyo, Japan), with complete conversion to cyanmethemoglobin and read at 540 nm (nm).

Blood serum was used for lysozyme activity, superoxide dismutase activity and immune globulin M (IgM) concentration. The blood serum samples were quickly frozen and kept at −80 °C until analysis. Serum lysozyme activity was determined based on lysis of the lysozyme-sensitive Gram-positive bacterium, Micrococcus lysodeikticus (Sigma). The dilutions of hen egg white lysozyme (Sigma) ranging from 0 to 20 μg/ml (in 0.1 M phosphate citrate buffer, pH 5.8) were taken as the standard and evaluated against the test serum (25 μL) in 96 wells of flat-bottomed microtitre plates with 175 μL of M. lysodeikticus. After rapid mixing and change in turbidity, it was measured after every 30 s for 5 min at an approximate temperature of 20 °C using a microplate reader at 450 nm.

Superoxide dismutase (SOD) activity was measured based on its ability to inhibit superoxide anion generated by xanthine and xanthine oxidase reaction system according to Wang and Chen (2005), using SOD detection kit (Nanjing Jiancheng Bioengineering Institute, China). Serum immune globulin M (IgM) level was measured by an enzyme-linked immunosorbent assay (ELISA) using commercial kit (Cusabio, Wuhan, Hubei,

China), as described by Sun et al. (2010). Succinctly, flat-bottomed 96-well plates were coated with serum samples for 2 h at 37 °C and liquid removed. Serum samples were then diluted each with sample diluent (1:5,000) according to the manufacturer's recommended protocols. The samples were then blocked with 100 mL of biotin antibody for an hour at 37 °C. Each well was aspirated and washed three times using wash buffer (350 mL). Samples were incubated with 100 mL of horseradish peroxidase–avidin (HRP-avidin) working solution for an hour at 37 °C and developed with tetramethylbenzidine (TMB) for 30 min at 37 °C. Each well was aspirated and washed three times with wash buffer (350 mL). The reaction was stopped by adding 50 mL of stop solution per well. The plates were read at 450 nm in a plate reader. Negative controls included samples without biotin antibody. The mean absorbance of the negative control for each plate was then subtracted from the optical density at 450 nm. All assay kits are specially designed for fish.

Calculations and statistical analysis

The indices for growth performance assessment were calculated as follows:

$$\text{Weight gain (WG \%)} = 100 \times (W_2 - W_1)/W_1$$

$$\text{Specific growth rate; SGR } (\%/d) = [(\ln W_2 - \ln W_1)/(t_2 - t_1)] \times 100$$

where W_1 and W_2 are body weights (g) at starting (t_1) and ending time (t_2)

$$\text{Percentage survival } (\%) = 100 \times \text{final number/initial number}$$

$$\text{Feed conversion ratio (FCR)} = \text{dry feed fed}(g)/\text{wet weight gain}(g)$$

$$\text{Protein efficiency ratio (PER)} = \text{wet weight gain(g)/protein in take(g)}$$

$$\text{Protein retention efficiency (PRE)} = 100 \times \text{protein gain(g)/protein in take(g)}$$

$$\text{Hepatosomatic index (HSI\%)} = 100 \times \text{liver weight(g)/body weight(g)}$$

$$\text{Condition factor (CF)} = \left[\left(W/L^3\right) \times 100\right],$$

where W is the wet weight of the fish and L is the standard length.

Results were expressed as mean ± SD. Furthermore, all data were subjected to one-way analysis of variance. When significant differences occurred, group means were further compared with Duncan's multiple-range tests. All statistical analyses were performed using the SPSS 19 (SPSS, IL, USA).

Results

During the 8-week feeding trial, the experimental diets were well accepted by GIFT tilapia juveniles. Mean survival rates were generally high in all treatments, ranging from 95.0.0 to 100.0 % and were unaffected by the dietary protein levels ($P > 0.05$) (Table 2). Increasing dietary protein from 25 to 30 % resulted in an increase in final weight gain ($P < 0.05$); however, further increases of 35, 40 and 45 % resulted to reduced final weights, respectively. Weight gain and final weight by the fish showed a similar trend. The relationship between weight gain and dietary protein was best described using a quadratic equation

Table 2 Growth performance and feed utilization of juvenile tilapia fed the experimental diets

Item	Experimental diets				
	*D*1	*D*2	*D*3	*D*4	*D*5
Initial weight (g)	3.77 ± 0.01	3.76 ± 0.01	3.77 ± 0.01	3.76 ± 0.01	3.78 ± 0.01
Final weight (g)	41.1 ± 1.30a	43.3 ± 1.46ab	45.9 ± 0.82b	45.7 ± 2.18b	45.8 ± 0.77b
Weight gain (%g)	988.9 ± 35.6a	1,051.8 ± 37.4ab	1,120.8 ± 25.7b	1,113.3 ± 58.5b	1,112.0 ± 17.6b
FCR	1.50 ± 0.09b	1.43 ± 0.12ab	1.29 ± 0.06a	1.29 ± 0.07a	1.38 ± 0.02a
SGR (%/day)	3.41 ± 0.05a	3.49 ± 0.05ab	3.57 ± 0.03b	3.56 ± 0.12b	3.56 ± 0.02b
Survival rate	96.7 ± 2.89	95.0 ± 5.00	98.3 ± 2.89	100.0 ± 0.00	96.7 ± 2.89
PRE	42.28 ± 2.34d	37.55 ± 3.32c	35.74 ± 1.62c	30.76 ± 1.59b	26.39 ± 0.43a
PER	2.69 ± 0.15d	2.35 ± 0.21c	2.21 ± 0.10c	1.95 ± 0.10b	1.62 ± 0.03a
CF	3.02 ± 0.22a	3.12 ± 0.21ab	3.26 ± 0.15b	3.28 ± 0.19b	3.25 ± 0.18b
HSI	3.01 ± 0.33	3.08 ± 0.30	3.19 ± 0.33	3.10 ± 0.35	3.23 ± 0.42

Values are presented as mean ± SD ($n = 3$), in the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while values with different small letter superscripts mean significant difference ($P < 0.05$). SGR = specific growth rate. Diets include D1, 25 %; D2, 30 %; D3, 35 %; D4, 40 %; and D5, 45 % protein level. Total number of fish per group (N) = 20

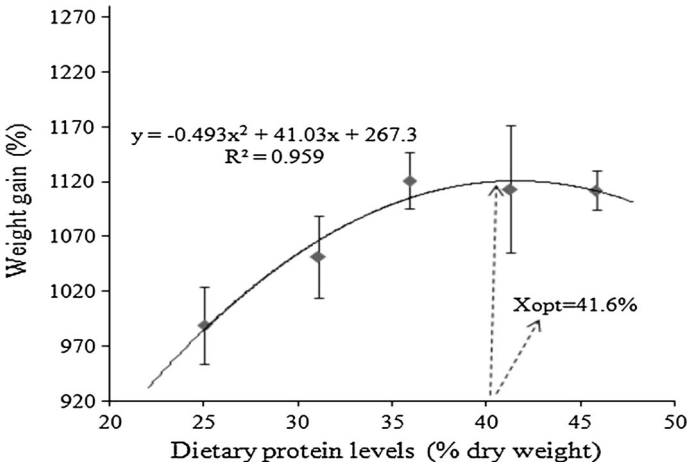

Fig. 1 Relationship between weight gain (WG) and dietary protein levels based on quadratic regression analysis, where Xopt represents the optimal dietary protein level for the maximum WG of GIFT tilapia juvenile

(Fig. 1), informing that maximum weight gain occurred at 41.6 % dietary protein level. There was also a significant difference in fish condition factor ($P < 0.05$), which increased with increasing dietary protein levels. There was a somewhat gradual increase in HSI as dietary protein level increased; meanwhile, no significant difference in HSI existed among the respective dietary groups ($P > 0.05$).

Fish whole body proximate analysis is presented in Table 3. The result shows that there was no significant difference in whole body moisture, protein and ash content of fish fed the respective diets ($P > 0.05$); meanwhile, a significant difference in whole body lipid content existed among the dietary groups ($P < 0.05$).

White blood cell, red blood cell, haematocrit and haemoglobin contents of GIFT tilapia juvenile fed the experimental diets are presented in Table 4. Result shows that haematological parameters were not significantly affected by the dietary protein levels ($P > 0.05$). Serum lysozyme activity, superoxide dismutase activity and immune globulin M (IgM) concentration of GIFT strain tilapia fed the diets are presented in Table 4. Serum lysozyme activity (LYS), superoxide dismutase activity (SOD) and immune globulin M (IgM) concentration were also not significantly affected by the dietary protein levels ($P > 0.05$).

Table 3 Whole body composition of juvenile tilapia fed the experiments diets

Item	Experimental diets				
	*D*1	*D*2	*D*3	*D*4	*D*5
Moisture (%)	70.8 ± 2.05	71.3 ± 0.35	71.4 ± 1.25	71.5 ± 0.37	70.5 ± 0.65
Protein (%)	15.5 ± 0.53	16.3 ± 0.71	16.7 ± 0.51	16.9 ± 0.52	16.10 ± 0.54
Lipid (%)	9.89 ± 1.26b	7.27 ± 0.13a	8.12 ± 1.63a	7.43 ± 0.52a	7.41 ± 0.41a
Ash (%)	3.46 ± 0.27	3.44 ± 0.40	3.59 ± 0.33	3.58 ± 0.18	3.68 ± 0.10

Values are presented as mean ± SD ($n = 3$), in the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while values with different small letter superscripts mean significant difference ($P < 0.05$). Diets include D1, 25 %; D2, 30 %; D3, 35 %; D4, 40 %; and D5, 45 % protein level. Total number of fish per group (N) = 20

Table 4 Haematological indices of GIFT juvenile tilapia fed the experimental diets

Item	Experimental diets				
	*D*1	*D*2	*D*3	*D*4	*D*5
WBC (10^{-9}/L)	310.1 ± 22.1	319.4 ± 29.1	307.9 ± 27.7	301.4 ± 24.4	308.1 ± 19.6
RBC (10^{-12}/L)	2.47 ± 0.26	2.43 ± 0.24	2.61 ± 0.30	2.60 ± 0.30	2.61 ± 0.26
Haemoglobin (g/L)	79.7 ± 5.51	83.0 ± 7.13	82.3 ± 8.02	79.7 ± 3.84	84.3 ± 4.91
Haematocrit (%)	32.9 ± 3.40	34.8 ± 3.49	34.4 ± 3.19	35.8 ± 2.01	33.2 ± 3.67

WBC White blood cell count, *RBC* red blood cell count; values are presented as mean ± SD ($n = 3$), in the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$). Diets include D1, 25 %; D2, 30 %; D3, 35 %; D4, 40 %; and D5, 45 % protein level. Total number of fish per group (N) = 20

Discussion

Numerous studies have shown that growth and feed utilization are significantly affected by dietary protein level (Brecka et al. 1995; muskellunge (*Esox masquinongy*); Al-Hafedh 1999 (Nile tilapia; *O. niloticus*); Chou et al. 2001 (Cobia, *Rachycentron canadum*)). The results of this study show that weight gain improved as dietary protein level increased from 25 to 30 %, suggesting that the fish efficiently utilized the moderately high protein diets by converting them into body tissue protein. Viola and Zohar (1984) also reported similar trends and utilization of protein in hybrid tilapia (*O. niloticus* × *O. aureus*). However, when protein levels increased further, up to 35 and 45 %, respectively, specific growth rate and weight gain of the GIFT strain tilapia did not increase significantly. The relationship between weight gain and dietary protein level was best expressed using the quadratic regression analysis, $y = -0.493x^2 + 41.03x + 267.3$ with $R^2 = 0.959$, indicating that maximum weight gain occurred at 41.6 % protein level (Fig. 1). This estimation is within the reported range of protein requirement (35–45 %) for other tilapia species and strain including, *O. aureus* (Davis and Stickney, 1978; Winfree and Stickney, 1981) and *O. niloticus* (El-Sayed and Garling, 1988; Siddiqui, 1988, Al-Hafedh, 1999; Abdel-Tawwab et al. 2010). In this present study, fish fed diet containing 40 % crude protein exhibited similar growth performance but lower PER and PRE compared to those fed diets with 30 and 35 % protein levels (Table 2). It is suggested that some 5 or 10 % of the protein may have been utilized for energy or stored as fat in the body of the fish. This phenomenon has also been reported by Jauncey (1982) and Shiau and Huang (1989), for other tilapia species, which give credence to (Dabrowski 1977), who reported different patterns of changes in PER in relation to dietary protein level, and further observed that the relationship between dietary protein level and PER differ between and among species. GIFT strain tilapia juveniles fed the 35 % dietary protein feed have similar growth performance but better protein utilization (PER and PRE) than those fed the 40 % dietary protein feed, which means for commercial production of GIFT strain tilapia, optimal protein levels may be reduced to 35 % based on their effect on growth performance and protein utilization. Dietary nutrients are essential for the development of tissue in fish and are a source of stored energy for fish digestion, absorption, growth, reproduction and other metabolic processes.

Whole body lipid content, determined in this study, was significantly affected by the dietary protein levels; lipid content of fish fed 25 % protein diet was significantly higher

than those fed other diets. Excess energy relative to the protein levels in the diet could have resulted to the high-lipid deposition, especially when all the diets had equal levels of lipid content. The higher the protein levels in the diets, the lower the starch level in present (Table 1). Previous studies have shown that high starch diet fed to fish resulted in higher glycogen accumulation in the liver (Daniels and Robinson 1986; Hidalgo and Alliot 1988; Brown et al. 1992), which resulted in higher HSI values. In this study, however, there was no significant difference in HSI among the respective dietary groups. GIFT strain tilapia fed low-protein diet (D1) could have effectively utilized the excess starch in the diet, thus converting them into body lipid, and supported the assertion that tilapia have higher ability of utilizing carbohydrate than most cultured fish (Anderson et al. 1984).

Blood parameters can be useful to help determine the health status of fish in response to dietary supplements (Congleton and Wagner 2006; Buentello et al. 2007). In the current study, contents of white blood cell (WBC), red blood cell (RBC), haematocrit and haemoglobin of GIFT strain tilapia juvenile were not significantly affected by the dietary protein levels, and the levels obtained for the haematological parameters were in accordance with those of healthy tilapia as reported by Welker et al. (2007), which informs that 25 % dietary protein (D1) can ordinarily meet the requirement for the formation and development of blood cells in this strain tilapia. IgM is the main immunoglobulin present in fish (Sun et al. 2010). In this study, IgM levels were not found to be significantly affected by the dietary protein levels (Table 4). Immunoglobulin M plays an important role in defending the host from infectious diseases (Li et al. 2007). Antioxidant system in fish involves enzymes such as superoxide dismutase (SOD) (Box et al. 2007). In this present study, SOD activity of the respective dietary groups was not significantly affected by the diets. Under normal physiological conditions, cells contain a complex network of antioxidant defence that scavenges the generation of reactive oxygen species (ROS) thus evading damages related to their high reactivity (Halliwell and Gutteridge 1989). Lysozyme acts as a defence though its ability to lysis bacteria (Jollès and Jollès 1984), thus acting as a vital effector of the fish's innate immune response (Simser et al. 2004). The serum lysozyme activity of fish fed the various diets was not significantly affected by the dietary protein levels (Table 5). On the contrary, Kiron et al. (1995) reported that serum lysozyme activity in rainbow trout (*Oncorhynchus mykiss*) fed a protein deficient diet (10 % dietary protein level) was significantly lower than those fed high protein diets (35 and 50 %). This can be explained in the sense that if dietary protein level becomes too low, lower than the maintenance protein requirement, normal immunological function of the animal could be hindered. The tilapia juveniles fed the lower-protein diet (D1) in this study had a similar rate of survival to those fed diets containing higher levels of protein.

Table 5 Non-specific immune parameters of GIFT tilapia juvenile fed the experimental diets

Item	Experimental diets				
	*D*1	*D*2	*D*3	*D*4	*D*5
IgM (mg/L)[a]	27.8 ± 2.33	28.8 ± 3.16	29.1 ± 3.53	29.2 ± 2.63	28.9 ± 3.91
SOD (U/mL)[b]	121.9 ± 5.24	124.4 ± 5.42	126.7 ± 8.65	125.9 ± 6.35	126.9 ± 8.99
LYS (U/mL)[c]	13.02 ± 1.51	14.25 ± 1.64	14.36 ± 1.58	14.12 ± 1.63	14.37 ± 1.72

[a] Immune globulin M; [b] superoxide dismutase; [c] lysozyme activity

Values are presented as mean ± SD ($n = 3$), in the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$). Diets include D1, 25 %; D2, 30 %; D3, 35 %; D4, 40 %; and D5, 45 % protein level. Total number of fish per group (N) = 20

Generally, it can be suggested that the dietary protein requirement for optimal growth performance of tilapia juvenile may be higher than that ordinarily needed to maintain normal physiology and immunity. Our findings can be useful for the development of cost-effective diets that support optimal growth and well-being of tilapia.

Conclusion

Based on second-degree polynomial regression analysis of growth performance, the optimal protein level for GIFT tilapia juvenile is 41.6 % based on maximum growth rate and 35 % when growth rate, feed conversion ratio and protein utilization are considered together, and is similar with other tilapia species and strains. The dietary protein requirement for optimal growth performance of GIFT tilapia juveniles is higher than that needed to maintain normal physiology and immunity and may have a high ability to utilize carbohydrate as dietary energy source.

Acknowledgments The study was supported by Special Fund for Agro-scientific Research in the Public Interest (200903046-02) and Postgraduate Scientific Research Innovation Program for Jiangsu Ordinary Higher Colleges and Universities (CXLX11-0708). The authors are grateful to Mr. Zhu Zhixiang of Yixing Experimental Base of Chinese Academy of Fishery Sciences for providing biological material, site and other technical assistance. The authors are also indebted to the Ministry of Fisheries and Marine Resources, Sierra Leone, for the moral support. Anonymous reviewers are greatly appreciated for their brilliant suggestions.

References

Abdel-Tawwab M, Ahmad MH, Khattab YAE, Shalaby AME (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

Al-Hafedh YS (1999) Effects of dietary protein on growth and body composition of Nile tilapia, *Oreochromis niloticus* L. Aquac Res 5:385–393

Anderson J, Jackson AJ, Matty AJ, Capper BS (1984) Effects of dietary carbohydrate and fibre on the tilapia *Oreochromis niloticus* (Linn.). Aquaculture 37:303–314

Arredondo-Figueroa JL, Matsumoto-Soulé JJ, Ponce-Palafox JT, Shirai-Matsumoto K, Gómez-Márquez JL (2012) Effects of protein and lipids on growth performance, feed efficiency and survival rate in fingerlings of Bay Snook (*Petenia splendida*). International Journal of Animal and Veterinary Advances 4:204–213

Association of Official Analytical Chemists (AOAC) (1995) Official methods of analysis of official analytical chemists international, 16th edn. Association of Analytical Chemists, Arlington

Bahnasawy MH (2009) Effect of dietary protein levels on growth performance and body composition of mono sex nile tilapia, *Oreochromis niloticus* L reared in fertilized tanks. Pakistan J Nutr 8:674–678

Beamish FWH, Medland TE (1986) Protein sparing effects in large rainbow trout, *Salmo gairdneri*. Aquaculture 55:35–42

Bowden TJ (2008) Modulation of the immune system of fish by their environment. Fish Shellfish Immunol 25:373–383

Box A, Sureda A, Galgani F, Pons A, Deudero S (2007) Assessment of environmental pollution at Balearic Islands applying oxidative stress biomarkers in the mussel *Mytilus galloprovincialis*. Comp Biochem Physiol C 146:531–539

Brecka BJ, Kohler CC, Wahl DH (1995) Effects of dietary protein concentration on growth, survival, and body composition of muskellunge, *Esox masquinongy*, and tiger muskellinge, *Esox masquinongy* × *E. luscius*, fingerlings. J World Aquacult, Soc 26:416–425

Brown ML, Nematipour GR, Gatlin DM (1992) Dietary protein requirement of juvenile sunshine bass at different salinities. Progress Fish Cult 54:148–156

Buentello JA, Reyes-Becerril M, de Jesús Romero-Geraldo M, de Jesús Ascencio-Valle F (2007) Effects of dietary arginine on hematological parameters and innate immune function of channel catfish. J Aquat Anim Health 19:195–203

Cho JH, Min BJ, Chen YJ, Yoo JS, Wang Q, Kim JD, Kim IH (2007) Evaluation of FSP (fermented soy protein) to replace soybean meal in weaned pigs: growth performance, blood urea nitrogen and total protein concentrations in serum and nutrient digestibility. Asian Aust J Anim 20:1874–1879

Chou RL, Su MS, Chen HY (2001) Optimal dietary protein and lipid levels for juvenile cobia (*Rachycentron canadum*). Aquaculture 193:81–89

Congleton JL, Wagner T (2006) Blood-chemistry indicators of nutritional status in juvenile salmonids. J Fish Biol 69:473–490

Dabrowski K (1977) Protein requirements of grass carp fry (*Ctenopharyngodon idella* Val.). Aquaculture 12:63–73

Daniels WH, Robinson EH (1986) Protein and energy requirements of juvenile red drum (*Sciaenops ocellatus*). Aquaculture 53:243–252

Davis AT, Stickney RR (1978) Growth responses of *Tilapia aurea* to dietary protein quality and quantity. Trans Am Fish Soc 107:479–483

Dias J, Alvarez MJ, Diez A, Arzel J, Corraze G, Bautista JM, Kaushik SJ (1998) Regulation of hepatic lipogenesis by dietary protein/energy in juvenile European sea bass (*Dicentrarchus labrax*). Aquaculture 161:169–186

El-Sayed AFM, Garling DL Jr (1988) Carbohydrate-to-lipid ratios in diets for tilapia fingerlings. Aquaculture 73:157–163

El-Sayed AM, Teshima S (1992) Protein and energy requirements of Nile tilapia, *Oreochromis niloticus*, fry. Aquaculture 103:55–63

Eslamloo K, Falahatkar B, Yokoyama S (2012) Effects of dietary bovine lactoferrin on growth, physiological performance, iron metabolism and non-specific immune responses of Siberian sturgeon *Acipenser baeri*. Fish Shellfish Immunol 32:976–985

Glencross BD, Booth M, Allan GL (2007) A feed is only as good as its ingredients: a review of ingredient evaluation strategies for aquaculture feeds. Aquac Nutr 13:17–34

Halliwell B, Gutteridge JMC (1989) Free radicals in biology and medicine. Oxford University Press, Oxford

Hidalgo F, Alliot E (1988) Influence of water temperature on protein requirement and protein utilization in juvenile sea bass, *Dicentrachus labrax*. Aquaculture 72:115–129

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

Jauncey K, Ross K (1982) A guide of Tilapia feeds and feeding. University of Sterling, Institute of Aquaculture, Scotland, p 111

Jollès P, Jollès J (1984) What's new in lysozyme research? Mol Cell Biochem 63:165–189

Kiron V, Watanabe T, Fukuda H, Okamoto N, Takeuchi T (1995) Protein nutrition and defence mechanisms in rainbow trout *Oncorhynchus mykiss*. Comp Biochem Physiol Mol Integr Physiol 111:351–359

Kumar S, Sahu NP, Pal AK, Choudhury D, Yengkokpam S, Mukherjee SC (2005) Effect of dietary carbohydrate on haematology, respiratory burst activity and histological changes in *L. rohita* juveniles. Fish Shellfish Immunol 19:331–344

Li P, Yin YL, Li D, Woo Kim S, Wu G (2007) Amino acids and immune function. Br J Nutr 98:237–252

Lovell RT (1989) Nutrition and feeding of fish. New York, Van Nostrand-Reinhold, p 260

Qiang J, Yang H, Wang H, Kpundeh MD, Xu P (2012) Interactive effects of temperature-dietary protein level on somatotropic gene expression and its interrelationship with growth in juvenile GIFT tilapia *Oreochromis niloticus*. Aquaculture 364:263–271

Sakthivel M (1988) Effects of varying dietary protein level on the blood parameters of *Cyprinus carpio*. Proc Indian Natl 97:363–366

Shiau SY, Huang SL (1989) Optimal dietary protein level for hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) reared in seawater. Aquaculture 81:119–127

Siddiqui AQ, Howlander MS, Adam AA (1988) Effects of dietary protein levels on growth, diet conversion and protein utilization in fry and young Nile tilapia, *Oreochromis niloticus*. Aquaculture 70:63–73

Simser JA, Macaluso KR, Mulenga A, Azad AF (2004) Immune-responsive lysozymes from hemocytes of the American dog tick, *Dermacentor variabilis* and an embryonic cell line of the Rocky Mountain wood tick, *D. andersoni*. Insect Biochem Molec 34:1235–1246

Singh RK, Chavan SL, Desai AS, Khandagale PA (2008) Influence of dietary protein levels and water temperature on growth, body composition and nutrient utilization of *Cirrhinus mrigala* (Hamilton, 1822) fry. J Therm Biol 33:20–26

Song LP, An L, Zhu YA, Li X, Wang Y (2009) Effects of dietary lipids on growth and feed utilization of jade perch, *Scortum barcoo*. J World Aquacult Soc 40:266–273

Sun Y, Yang H, Ma R, Lin W (2010) Probiotic applications of two dominant gut Bacillus strains with antagonistic activity improved the growth performance and immune responses of grouper *Epinephelus coioides*. Fish Shellfish Immun 29:803–809

Takagi S, Shimeno S, Hosokawa H, Ukawa M (2001) Effect of lysine and methionine supplementation to a soy protein concentrate diet for red sea bream *Pagrus major*. Fish Sci 67:1088–1096

Torstensen BE, Lie O, Hamre K (2001) A factorial experimental design for investigation of effects of dietary lipid content and pro- and antioxidants on lipid composition in Atlantic salmon (*Salmo salar*) tissues and lipoproteins. Aquac Nutr 7:265–276

Trosvik KA, Rawles SD, Thompson KR, Metts LA, Gannam A, Twibell R, Webster CD (2012) Growth and body composition of Nile tilapia, *Oreochromis niloticus*, fry fed organic diets containing yeast extract and soybean meal as replacements for fish meal, with and without supplemental lysine and methionine. J World Aquacult Soc 43:635–647

Viola S, Zohar G (1984) Nutrition studies with market size hybrids of tilapia *Oreochromis* sp. in intensive culture. Isr J Aquacult Bamid 36:3–15

Wang SH, Chen JC (2005) The protective effect of chitin and chitosan against *Vibrio alginolyticus* in white shrimp, *Litopenaeus vannamei*. Fish Shellfish Immun 19:191–204

Wang JT, Liu YJ, Tian LX, Mai KS, Du ZY, Wang Y, Yang HJ (2005) Effect of dietary lipid level on growth performance, lipid deposition, hepatic lipogenesis in juvenile cobia (*Rachycentron canadum*). Aquaculture 249:439–447

Watanabe T (1982) Lipid nutrition in fish. Comp Biochem Physiol B 73:3–15

Welker TL, Lim C, Yildirim-Aksoy M, Klesius PH (2007) Growth, immune function, and disease and stress resistance of juvenile Nile tilapia (*Oreochromis niloticus*) fed graded levels of bovine lactoferrin. Aquaculture 262:156–162

Wilson RP (2002) Amino acids and proteins. In: Halver JE, Hardy RW (eds) Fish nutrition, 3rd edn. Academic Press, San Diego, pp 144–175

Winfree RA, Stickney RR (1981) Effects of dietary protein and energy on growth, feed conversion efficiency and body composition of *Tilapia aureus*. J Nutr 111:1001–1012

Yang SD, Liou CH, Liu FG (2002) Effects of dietary protein level on growth performance, carcass composition and ammonia excretion in juvenile silver perch (*Bidyanus bidyanus*). Aquaculture 213:363–372

Ye J, Liu X, Wang Z, Wang K (2011) Effect of partial fish meal replacement by soybean meal on the growth performance and biochemical indices of juvenile Japanese flounder *Paralichthys olivaceus*. Aquacult Int 19:143–153

Zhou QC, Wang LG, Wang HL, Xie FJ, Wang T (2012) Effect of dietary vitamin C on the growth performance and innate immunity of juvenile cobia (Rachycentron canadum). Fish Shellfish Immunol 32:969–975