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RESEARCH PAPER

Preliminary Effects of Dietary Protein Levels on Muscle Quality and Digestive Enzyme Activities in GIFT-*Oreochromis niloticus*

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Abstract

The effects of 10%, 15%, 20%, 25%, 30%, and 35% dietary protein levels on muscle quality, and digestive enzyme activities in GIFT-*O. niloticus* were investigated for 12 weeks. A total of 720 juvenile GIFT-*O. niloticus* with initial wet body weight 3.50±0.2 g and length 4.6±0.5 cm were randomly divided into six triplicate groups according to the above protein levels. The results show that muscle quality of GIFT-*O. niloticus* increased with increasing muscle protein and lipid, amino acid level, fiber area, and muscle water-holding capacity, but decreasing flesh fiber density. The optimal protein level was 25%. Protease activity increased first with the 10%–25% dietary protein level, followed by a decrease as the proportion increased. In conclusion, the 25% dietary protein level was optimal to improve muscle quality and protease activities, which could be regarded as a reference level to produce GIFT-*O. niloticus* feed.

Introduction

Genetically improved farmed tilapia-GIFT (*Oreochromis niloticus*) is a commercially important freshwater fish, being widely farmed in more than 135 countries (Gobi *et al*., 2016).

Pond culture has become an economically important activity in China and other countries because of the high nutritional value and increasing market demand for GIFT-*O. niloticus* (Hu, Chen, Pan, & Huang, 2006). The muscle quality of aquatic fish is affected by different dietary protein levels. Protein is one of the main components of a fish diet, and its quality and quantity are pivotal factors influencing fish quality (Conceição, Dersjant-Li, & Verreth, 1998; Lovell, 1989). Fish muscle quality are studied by analyzing muscle cellularity, which involves determining muscle fiber area and density (Johnston *et al*., 2006; Kiessling, Storebakken, & Asgard, 1991). Muscle growth is the result of two different processes: fiber hyperplasia and hypertrophy. Hyperplasia is the recruitment of new fibers and is more frequent during the earlier stages of fish development, whereas hypertrophy is associated with an increase in already existent fibers and occurs in an area until the maximum fiber size is reached (Aguiar, Barros, Padovani, Pezzato, & PaiSilva, 2005; Alami-Durante, Fauconneau, Rouel, Escaffre, & Bergot, 1997; Campos *et al*., 2013; Galloway, Kjørsvik, & Kryvi, 1999).

The digestive function is vital for gut health and maintaining the general health of fish. Impaired organ function causes weak digestion and increased susceptibility to bacterial infection (Sundh, 2009). Digestive function is affected by several factors, such as dietary composition, environmental stress, gut microbial population, and immune function of the fish (Kim *et al*., 2016). Digestive enzymes found in the fish alimentary system directly affect digestion and absorption of nutrients, animal growth, and

adaptability to the environment (Xia *et al*., 2017). The growth and digestibility responses to different dietary protein levels have been reported for several species, such as redclaw crayfish *Cherax quadricarinatus* (Figueiredo, Kricker, & Anderson, 2001), sea cucumber *Apostichopus japonicus* (Xia *et al*., 2017; Bai, *et al*., 2016), meagre *Argyrosomus regius* (Saavedra, *et al*., 2018), and pacific white shrimp *Penaeus vannamei* (Huang, Wang, & Lu, 2003). Although Kim *et al*. (2016) evaluated the individual and combined effects of oxygen concentration and diet composition on growth and nutrient utilization of *O. niloticus* (Ling, Hashim, Kolkovski, Chong, & Chien, 2006), the effects of dietary protein levels on muscle quality and digestive enzyme activities of GIFT-*O. niloticus* have not been analyzed. In general, related data on this species are lacking.

A low protein supply is often associated with decreases in muscle growth and digestive enzyme activity, but higher dietary protein levels also have disadvantages, such as increased feed cost and higher nitrogen excretion resulting in increased organic loading in the tanks and effluent volume. Adequate protein levels in the diet minimize the use of protein as an energy source (Xu *et al*., 2015). However, improper dietary protein levels can have adverse effects on flesh growth and digestive function (Huang *et al*., 2003; Kim *et al*., 2016). Although commercial feeds are available for tilapia, special use feeds are seldom used for GIFT-*O. niloticus*. Therefore, a study on muscle quality and digestive enzyme activities of GIFT-*O. niloticus* is required to quantify their optimal protein level.

In the present study, we evaluated the effects of dietary protein levels of 10%, 15%, 20%, 25%, 30%, and 35% on muscle quality and digestive enzyme activities in GIFT-*O. niloticus*. The aims of the present study are to provide a reference for the effects of nutrients on physiological and biochemical levels in this species and to provide a preliminary optimal protein level.

Materials and Methods

Fish

Juvenile GIFT-*O. niloticus* with initial wet body weight 3.50 ± 0.2 g and length 4.6 ± 0.5 cm, collected from the national tilapia breeding grounds, Nanning, Guangxi Province, China, were transported rapidly to our laboratory, and acclimatized for 2 weeks in 1500-L fiberglass tanks at 28.1 ± 1.8°C with dissolved oxygen of 5.2 ± 0.3 mg/L. During this experimental period, the fish were fed normal feed twice per day at 08:00 and 17:00. These holding tanks were kept static and 50% of the water was exchanged daily. The 12-week study was carried out at the Guangxi Key Laboratory of Aquatic Genetic Breeding and Healthy Aquaculture, Nanning, Guangxi Province, China.

Experimental Design

The formulation and different proportions of protein in the experimental diets are shown in Table 1.

Table 1. Ingredients and chemical composition of experimental diets for GIFT-*O. niloticus* (g/kg)

Ingredient	Groups					
	G10%	G15%	G20%	G25%	G30%	G35%
Fish meal	100	100	100	100	100	100
Soybean meal	5	30	55	80	105	130
Peanut meal	5	30	55	80	105	130
Wheat bran	507	464.2	421.4	478.6	335.8	293
Cassava powder	300	300	300	300	300	300
Fish oil	5	5	5	5	5	5
Soybean oil	22	22	22	22	22	22
Ca contained $(H_2PO_4)_2$	36	28.8	21.6	14.4	7.2	0
Vitamin premix[a]	12	12	12	12	12	12
mineral premix[b]	8	8	8	8	8	8
Total	1,000	1,000	1,000	1,000	1,000	1,000
	Chemical composition					
Crude protein	100.3	152.2	199.8	250.7	301.6	349.8
Crude lipid	8.9	10.2	10.8	9.3	9.6	10.4
Ash	207.6	209.8	208.5	208.2	209.8	208.4
Moisture	33.2	31.8	32.9	33.8	32.4	30.7

[a] Vitamin premix contained the following ingredients and amount which were diluted in cellulose (g/kg premix): L-ascorbic acid, 100; DL-a-tocopheryl acetate, 2; thiamin hydrochloride,8; riboflavin,10; pyridoxine hydrochloride,15; niacin, 45; Ca-D-pantothenate, 18; myo-inositol, 80; D-biotin, 0.3; folic acid, 1.5; menadione,4; retinyl acetate, 3.2; cholecalciferol, 1; cyanocobalamin,0.004; ethoxyquin 16.

[b] Mineral premix contained the following ingredients and amount which were diluted in zeolite (g/kg premix): $MgSO_4 \cdot 7H_2O$, 80.5; Ferric citrate, 16; $ZnSO_4 \cdot H_2O$, 9; $CuSO_4 \cdot 5H_2O$, 3; $AlCl_3 \cdot 6H_2O$, 6; KIO_3, 0.04; $MnSO_4 \cdot H_2O$, 2; $CoCl_2 \cdot 6H_2O$, 0.04.

Six experimental diets were prepared with 10% (G10%), 15% (G15%), 20% (G20%), 25% (G25%), 30% (G30%), and 35% (G35%) protein levels. Peanut meal, soybean meal, and fish meal were used as protein sources, and cassava powder was the carbohydrate source. Other than the fish oil and soybean oil, assigned quantities of the ingredients were evenly ground, stirred, and subsequently sieved through a 40-μm sifter. Then, the quantities of fish oil, soybean oil, and 30% deionized water, which accounted for the balance of the mixture, were added. The feed was formed into 2-mm-diameter pellets using a single-screw extruder (SLP-45; Chinese Fishery Machinery and Instrument Research Institute, Shanghai, China), which were air dried and stored at 4℃ until use. After 1 day of starvation, 720 of normal GIFT-*O. niloticus* with initial wet body weight 3.50 ± 0.2 g and length 4.6 ± 0.5 cm were chosen at random from the collected fish. The fish were allocated at random into six triplicate groups, and 40 individuals allocated and reared in each dietary replicate in a separate 150-L fiberglass tank. During the experiment, water was kept aerated to maintain dissolved oxygen content of more than 5.0 mg/L. The pH ranged from 7.2 to 8.0 and water temperature was controlled at 28.1 ± 1.8℃. GIFT-*O. niloticus* in each tank were fed manually at 08:00 and 17:00 with a daily feeding rate of 4% of body weight. At the end of the experiment, all fish in each tank were collected withn 24 h after the final feeding on week 12.

Sample Collection

At the end of the feeding trial, GIFT-*O. niloticus* were taken off feed and 60 individuals in each group were collected within 24 h. Of these, eight 3-g of flesh blocks sampled from fifteen fish were chosen to measure water-holding capacity (WHC) immediately. The dorsal fin muscle samples (2 × 2 × 2 cm) were cut from fifteen fish of the 60 collected above to store at −60℃ until measurements were taken of crude protein, crude lipid, ash, moisture, and amino acids. Another fifteen fish were killed, and cross-fin muscle samples (1 × 1 cm) were cut and fixed in 10% buffered formalin for 3 days, dehydrated, and paraffin-embedded for muscle cellularity analysis. The intestines of the remaining fifteen fish were extracted with a scalpel and rinsed with 4℃ distilled water, gently dried with bibulous paper, and stored at −60℃ until digestive enzyme measurements were taken.

Proximate Composition of Flesh and Diets

Crude protein, crude lipid, moisture, and crude ash content were determined according to the methods of William (2003) method. Crude protein content was determined using the Kjeldahl system method (2300 Auto analyzer; FOSS Tecator, AB, Hoganas, Sweden), and the conversion coefficient of 6.25 was adopted. After ether extraction, crude lipid content was measured using a Soxtherm (SOX 416 Macro, Gerhardt, Germany). Ash content was determined by combusting samples in a muffle furnace at 550℃ for 6 h (SXL-1008; Jing Hong Laboratory Instrument Co., Ltd., Shanghai, China). After the dry diet and muscle samples were hydrolyzed in a sealed glass tube with 6 M HCl at 120℃ for 12 h and amino acid analysis was performed using an automated amino acid analyzer (Hitachi 835-50, Hitachi Co., Ltd., Tokyo, Japan). The samples were hydrolyzed with 2.5 mL performic acid for 20 min at 55℃ and injected into a unit with a sodium exchange column for methionine (Met) analysis. Hyp (hydroxyproline) was extracted with a kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and measured at 560 nm using an ultraviolet spectrophotometer (UV759, Shanghai Precision Scientific Instruments, Shanghai, China). The amino acid composition of the experimental diets is shown in Table 2.

Water-Holding Capacity of Flesh

The water of cooking loss and steaming loss in the muscle were measured following a method described previously (Wongthahan & Thawornchinsombut, 2015) with some modifications. Two flesh blocks were steamed in gauze at 100℃ for 5 min to determine steaming loss. Then another two blocks were cooked in boiling water at 100℃ for 5

Table 2. Amino acid composition of the experimental diets (g/kg, dry matter)

Groups	EAA										NEAA							TAA
	Thr	Val	Met	Ile	Leu	Phe	His	Lys	Arg	Asp	Ser	Glu	Gly	Ala	Cys	Tyr	Pro	
G10%	9.5	8.1	3.6	6.2	20.2	15.5	16.2	14.0	25.0	31.7	15.6	55.2	17.0	14.0	3.7	8.0	13.0	276.4
G15%	9.5	7.8	3.8	6.3	20.3	15.6	16.1	14.3	24.9	31.6	15.6	55.1	16.9	14.0	3.6	8.2	12.9	276.5
G20%	9.4	8.1	3.9	5.9	20.1	15.3	16.3	14.1	24.7	31.9	16.4	55.0	16.8	14.1	3.8	7.8	13.0	276.6
G25%	9.3	7.9	3.7	6.0	20.0	15.4	16.0	14.3	24.8	32.9	16.0	55.2	17.0	13.8	3.5	7.9	13.1	276.8
G30%	9.1	7.9	3.9	5.9	20.3	15.6	16.0	14.3	24.6	31.7	16.3	55.3	17.1	14.1	3.5	7.9	13.2	276.7
G35%	9.2	8.0	3.6	5.8	20.1	15.7	15.9	14.5	25.0	31.8	16.1	55.4	17.1	13.9	3.6	8.1	13.0	276.8

EAA, essential amino acids; NEAA, non-essential amino acids; TAA, total amino acids.

min to determine cooking loss. Subsequently, the samples were cooled at room temperature, the surface water of the muscle was wiped off with bibulous paper, and weighed. Steam loss and cooking loss were measured using an equivalent calculation as the percentage (%) of weight lost from the initial sample weight. Two blocks were centrifuged at 5000 × g for 15 min for the centrifugal loss measurement and stored at −20℃ for 24 h. Subsequently two blocks were thawed at room temperature for the freezing loss measurement. Centrifugal loss and freezing loss were calculated and expressed as the percentage (%) of weight lost to initial sample weight.

Quantification of Muscle Cellularity

Muscle cellularity was quantified according to the method described by Sun, Xu, Li, Pan and Leng (2017). The above paraffin blocks were cuts into four sections (10 μm) and stained with hematoxylin and eosin. The epaxial region (muscle area above the vertebral cord) was photographed using an image analysis program (AxioVision Release 4.8.2. SP2) connected to a light microscope (Zeiss-Axioplan) and a video camera (AxioCamER5s). Four sections of the epaxial region were randomly chosen to quantify the fiber area and density of the white muscle using image processing and analysis software (Image J). Fiber density was expressed as the number of fibers/muscle area.

Quantification of Digestive Enzymes

The intestine samples were homogenized 1:10 (w/v) in 4℃ distilled water, centrifuged (4,500 × *g*) at 4℃ for 30 min, and the supernatant was used to measure enzyme activities. Protease was quantified according to the method described by Sun *et al*. (2017) with some modifications. The optimal pH and temperature conditions of protease (28.1℃ and pH 1.9), amylase (31.5℃ and pH 7.1), and cellulase (54℃ and pH 5.7) were set for the activity analysis. Protease activity was determined using casein as the substrate. Amylase activity was measured according to a method described previously (Robyt, & Whelan, 1968). Cellulase activity was quantified according to a previous description of Pan and Wang (1997). Lipase was extracted and activity was determined according to the method of Bier (1955). The activity was evaluated as degradation of monoacylglycerols, diacylglycerols, and triacylglycerols to free fatty acids.

Statistical Analysis

Data are presented as mean ± standard error and statistically analyzed using ORIGIN 7.0 software (OriginLab, Northampton, MA, USA). The square root transformation was performed prior to one-way analysis of variance. Significant differences were detected by Tukey's post-hoc test. A p-value < 0.05 was considered significant.

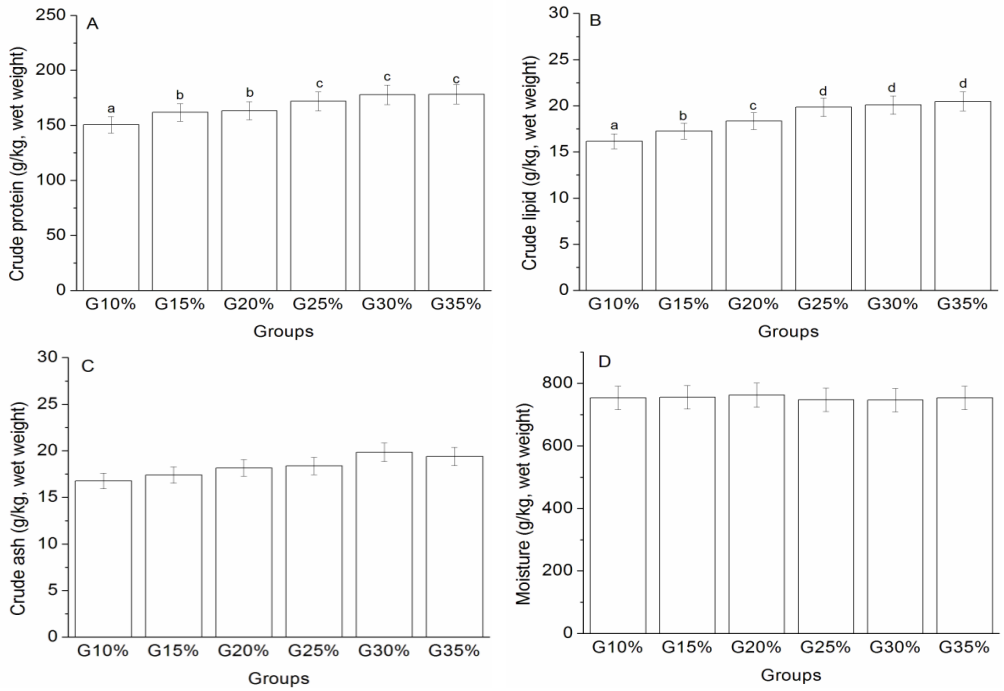

Figure 1. Proximate composition in muscle of GIFT-*O. niloticus* fed diets with different dietary protein levels for 12 weeks, (A) crude protein, (B) crude lipid, (C) crude ash, and (D) moisture. Different letters indicate significant differences (P< 0.05).

Results

Muscle Composition

As shown in Figure 1, no significant differences in muscle moisture, crude protein, or crude ash were observed among the groups (P>0.05), but crude lipid tended to increase with increasing dietary protein level, and crude lipid content of fish in the G25%, G30%, and G35% groups were 19.85 ± 0.82, 20.09 ± 0.54, and 20.48 ± 0.62 g/kg, respectively, which were significantly higher than those of the G10%, G15%, and G20% groups (P<0.05)

Muscle Amino Acid Composition

As shown in Table 3, total non-essential amino acids (NEAA), essential amino acids (EAA), delicious amino acids (DAA), and total amino acids (TAA) in muscle of GIFT-*O. niloticus* fed the 25% protein level were 492.1 ± 7.6, 404 ± 8.7, 388 ± 9.5, and 904.8 ± 9.6 g/kg, respectively. These were significantly higher than those of other groups (P<0.05).

Flesh Water-Holding Capacity

As shown in Figure 2, the steaming and cooking losses of the fish in the G25%, G30%, and G35% groups were 14.42 ± 0.44%, 14.76 ± 0.36%, and 14.55 ± 0.81%, respectively, which decreased significantly compared with those in the G10%, G15%, and G20% groups (P< 0.05). No significant differences were found among the G25%, G30%, and G35% groups (P>0.05). There were no significant differences in

Table 3. Muscle amino acid composition of GIFT-*O. niloticus* fed diets with different dietary protein levels for 12 weeks (g/kg, dry matter)

Amino acids		G10%	G15%	G20%	G25%	G30%	G35%
EAA	Thr	30.7 ± 0.3	31.6 ± 0.4	31.1 ± 1.5	32.4 ± 1.2	32.6 ± 1.1	31.8 ± 0.8
	Val	23.5 ± 0.9	24.0 ± 1.1	23.9 ± 1.3	24.1 ± 0.9	24.5 ± 1.5	23.8 ± 1.2
	Met	21.5 ± 1.2a	21.3 ± 0.7a	22.1 ± 1.7ab	23.6 ± 1.2bc	22.8 ± 1.4ab	22.7 ± 1.1ab
	Ile	21.5 ± 1.1a	20.8 ± 1.0a	21.6 ± 1.4a	22.4 ± 1.3b	21.7 ± 1.2a	21.6 ± 1.5a
	Leu	63.3 ± 2.7b	61.2 ± 4.8b	63.9 ± 3.3	65.2 ± 3.5	64.2 ± 2.8	63.8 ± 3.7
	Phe	33.9 ± 2.3a	32.8 ± 2.2ab	34.4 ± 2.6bc	34.8 ± 2.4bc	33.9 ± 2.0a	32.1 ± 2.1a
	His	48.3 ± 2.7a	50.2 ± 4.8b	49.9 ± 3.4a	52.2 ± 3.5b	48.0 ± 2.8a	64.8 ± 4.1c
	Lys	75.4 ± 3.1a	76.3 ± 2.4a	77.8 ± 3.2a	80.1 ± 2.5b	79.2 ± 4.1b	77.5 ± 2.3a
	Arg	48.9 ± 2.3a	50.8 ± 2.2ab	49.4 ± 2.6a	51.3 ± 2.4bc	49.1 ± 2.0a	50.7 ± 2.1ab
	Total	383.6 ± 10.1a	391.6 ± 9.6b	395.17 ± 11.2b	404 ± 8.7c	395.9 ± 10.8b	394.7 ± 9.8b
NEAA	Asp	98.4 ± 5.1a	98.7 ± 4.1a	100.5 ± 7.3ab	103.1 ± 5.2c	101.3 ± 8.5b	100.8 ± 8.2ab
	Ser	38.4 ± 2.1	39.3 ± 2.7	41.6 ± 3.0	44.4 ± 2.8	42.6 ± 2.7	41.9 ± 2.0
	Glu	131.5 ± 8.8a	138.7 ± 10.0b	141.1 ± 9.2b	144.3 ± 7.5bc	140.7 ± 8.6b	141.0 ± 9.7b
	Gly	39.3 ± 2.7a	42.2 ± 4.8a	49.9 ± 3.4b	53.2 ± 3.5c	50.8 ± 2.8b	50.0 ± 4.1b
	Ala	51.1 ± 2.7a	57.1 ± 2.4b	59.1 ± 3.2b	68.2 ± 3.0c	63.0 ± 2.8bc	62.8 ± 2.0bc
	Cys	4.4 ± 1.1	4.8 ± 1.4	5.0 ± 1.2	5.1 ± 1.5	5.0.9 ± 1.0	4.8 ± 1.3
	Tyr	21.4 ± 2.1a	21.8 ± 2.4a	24.6 ± 1.1b	28.1 ± 1.5c	26.9 ± 2.1bc	24.5 ± 1.3bc
	Pro	15.9 ± 2.3a	16.8 ± 2.2a	17.4 ± 2.6ab	18.3 ± 2.4ab	18.9 ± 2.0ab	17.1 ± 2.1ab
	Total	427.33 ± 8.9a	449.42 ± 9.3ab	470.2 ± 8.8b	492.1 ± 7.6c	479.7 ± 10.0b	473.6 ± 9.8b
	DAA	339.6 ± 6.8a	358 ± 7.0b	373.7 ± 8.2c	388 ± 9.5d	378.5 ± 7.9c	378.6 ± 6.7c
	TAA	821.3 ± 10.1a	850.2 ± 9.1b	876.7 ± 8.7c	904.8 ± 9.6d	886.4 ± 10.4c	878.1 ± 11.1c

EAA, essential amino acids; NEAA, non-essential amino acids; DAA (Asp, Gly, Glu, Ala), delicious amino acids; TAA: total amino acid. Values in the same row with different letters indicate significant differences (P<0.05).

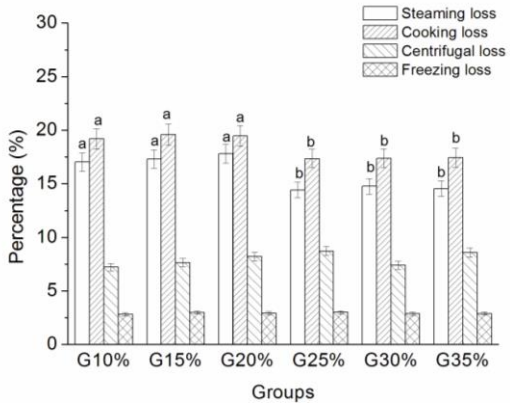

Figure 2. The flesh water-holding capacity of GIFT-*O. niloticus* fed diets with different dietary protein levels for 12 weeks (%). Different letters indicate significant differences (P<0.05).

centrifugal loss or freezing loss among the groups (P>0.05).

Muscle Cellularity

As shown in Figure 3, the fiber area values of the fish fed the G25%, G30%, and G35% protein levels were 841.4 ± 80.1, 949.5 ± 92.4, and 1,006.7 ± 103.7 μm^2, respectively. These were significantly higher than those of fish fed the G10%, G15%, G20% protein levels (P< 0.05). No significant differences were found among G25%, G30%, or G35% groups (P>0.05). The

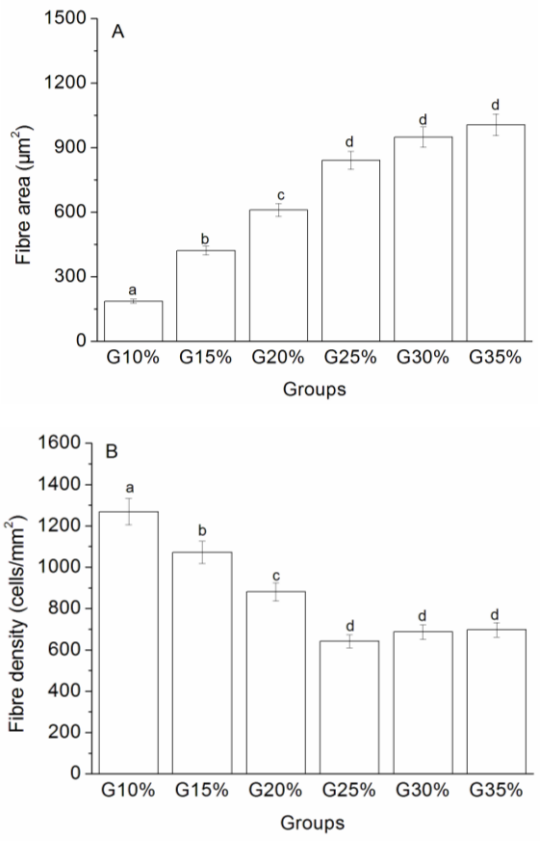

Figure 3. The muscle fibre characteristics of GIFT-*O. niloticus* fed diets with different dietary protein levels for 12 weeks, (A) fibre area and (B) fibre density. Different letters indicate significant differences (P<0.05).

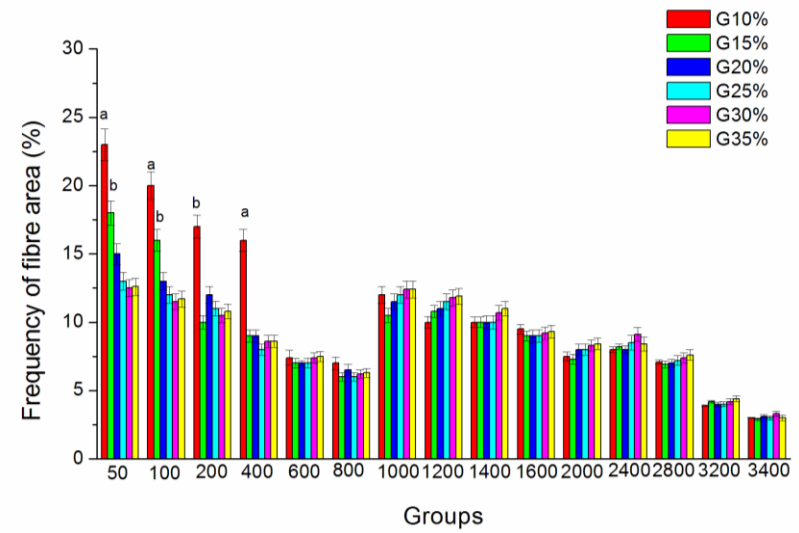

Figure 4. The fibre area frequency of GIFT-*O. niloticus* fed diets with different dietary protein levels for 12 weeks. Different letters indicate significant differences (P<0.05).

fiber density values of the fish fed 25%, 30%, and 35% protein levels were 641.4 ± 53.4, 686.4 ± 56.4, and 697.4 ± 63.4 cells/mm^2, respectively, which decreased significantly compared with G10%, G15%, and G20% ($P<0.05$). No significant differences were found among G25%, G30%, or G35% ($P>0.05$). As shown in Figure 4, several differences were found in the frequency of fibre areas, mainly between the beginning and end of the trial, independently of treatment. When dietary protein level was 10 %, there was a significantly higher percentage of fibres with an area smaller than 400 µm^2 compared to the other protein levels. No significant differences were observed in the frequency of muscle fibre with areas between 800 and 3,400 µm^2.

Digestive Enzyme Activities

As shown in Figure 5, the dietary protein levels had no significant effects on amylase or lipase activities of GIFT-*O. niloticus* ($P>0.05$), but had significant effects on protease activity ($P<0.05$). Protease activity increased first, and then decreased, as the proportion of dietary protein increased. Protease activity reached a maximum of 8.42 U/g protein at the 25% dietary protein level, and the lowest was 4.22 U/g protein at G10%. In addition, dietary protein level had no significant effect on cellulase activity of GIFT-*O. niloticus* ($P<0.05$), and activity was low.

Discussions

The increased protein content produces high nutritional value, flavor, texture, and tenderness of muscle (Haard, 1992). This study showed that protein content increased with increasing dietary protein level, up to 25% protein, where it plateaued, which indicated that the 25% dietary protein level is the optimal percentage for GIFT-*O. niloticus* protein formation. Muscle lipid content reserves can be utilized as energy and as a source of highly unsaturated fatty acids (Ling *et al*., 2006). The conversion of excess dietary protein *in vivo* is quite an expensive process, and it increases ammonia, so there is a need to enhance lipid content in muscle as an energy source (Xu *et al*., 2015). The lipid increased significantly in our study, similar results also have been reported in giant croaker *Nibea japonica* (Lee, Cho, Lee, & Yang, 2001), black sea bass *Centropristis striata* (Shah-Alam, Watanabe, & Carroll, 2008), and juvenile Manchurian trout *Brachymystax lenok* (Xu *et al*., 2015). However, this was in contrast to silver perch *Bidyanus didyanus* (Yang, Liou, & Liu, 2002) and spotted barbell *Hemibarbus maculates* (Chen, Ye, Pan, Shen, & Wang, 2010). The current results that lipid content increased as dietary protein level was increasing might be the reason.

Amino acids are important for synthesis of protein and other nitrogenous compounds involved in the regulation of metabolic pathways (Jobgen, Fried, Fu, Meininger, & Wu, 2006). Total EAA and TAA in grass carp muscle relate to dietary substance and level (Sun *et al*., 2017). Dietary protein promotes the generation of serine, glutamic acid, and lysine in muscle (Lovell, 1989). The present study showed that total NEAA, DAA, EAA, and TAA in muscle increased up to the 25% protein level and then decreased, suggesting that dietary protein promotes the biosynthesis of NEAA as well as accumulation of EAA in muscle. However, further study on the mechanism for the increase in amino acids in response to the different protein levels is necessary.

The WHC is commonly used as a key index of

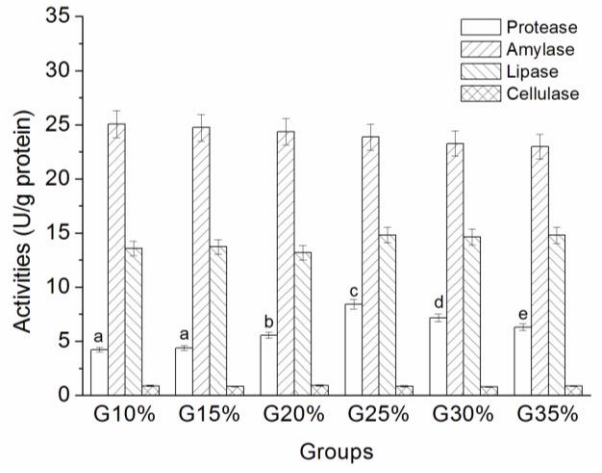

Figure 5. The protease, amylase, lipase and cellulase activities of GIFT-*O. niloticus* fed diets with different dietary protein levels for 12 weeks. Different letters indicate significant differences ($P<0.05$).

muscle quality, and correlates with collagen synthesis in muscle (Kauffman, Eikelenboom, Wal, Engel, & Zaar, 1986; Chang 1986). Cooking loss reflects the combined loss of liquid and soluble matter from the muscle during cooking, and the main loss is water (Aaslyng, Bejerholm, Ertbjerg, Bertram, & Andersen, 2003). In this study, both steaming loss and cooking loss decreased significantly after adding 10%–20% dietary protein, and then plateaued at the 25% protein level, when centrifugal loss and freezing loss were similar. These results indicate that the WHC decreased probably due to the promotion of collagen synthesis in response to the increased protein level, which led to increased adhesion and hardness of the muscle. Collagen content in muscle reflects taste of food (Haard, 1992). In our study, the WHC did not change significantly in fish fed the 25%–35% dietary protein level, indicating that protein used for collagen synthesis was limited to 25% and that flesh quality was optimal at that level. However, the correlation between collagen synthesis and dietary protein needs further study.

Muscle cellularity in many fish affects growth and muscle quality and is associated with the area and density of muscle fiber that continues to increase throughout life and can be modified by nutrition (Johnston *et al*., 2006; Rehfeldt *et al*., 2011). Therefore, a study on muscle dynamics would provide a better understanding of fish flesh quality. In the present study, fiber area increased significantly and density decreased significantly in fish fed the 10%–25% dietary protein level, whereas both plateaued above 25% protein. These results suggest that fiber hypertrophy prevailed, as many of the fibers enlarged with less production of recruited fibers, and excess protein nutrition had no significant effect. Hyperplasia is often reduced as fish size increases (Alami-Durante *et al*., 1997; Silva *et al*., 2009). Carp *Cyprinus carpio* (Alami-Durante *et al*., 1997) and Atlantic cod *Gadus morhua* (Galloway *et al*., 1999) show an increased muscle mass initially through hyperplasia and then partially replace it with fiber hypertrophy. In our study, the small fiber area (≦400 μm^2) only accounted for approximately 30% of the frequency distribution in all groups. These results are in accordance with other studies on juvenile stages (Alami-Durante *et al*., 1997; Silva *et al*., 2009), which further demonstrate that muscle growth is mainly due to fiber hypertrophy. Hyperplasia occurs via a stratified mechanism (within proliferation areas) or by a mosaic mechanism (in zones where larger fibers occur) in seabass *Dicentrarchus labrax* and meagre *Argyrosomus regius* (Alami-Durante, Olive, & Rouel, 2007; Saavedra *et al*., 2018). Mosaic hyperplasia is the main muscle growth process in juvenile and adult stages (Johnston *et al*., 2006). Salze, Alami-Durante, Barbut, Marcone and Bureau (2014) observed an adaptive mechanism consisting of a decrease in white muscle hyperplasia in favor of muscle fiber hypertrophy in rainbow trout when nutrition was modified. The results of the present study suggest that protein supplementation could regulate the GIFT-*O. niloticus* muscle growth mechanism.

The activities of digestive enzymes in aquatic animals change with different active dietary substances; thus, they are often used to evaluate animal protein utilization efficiency (Xia *et al*., 2017). In this study, protease activity increased first in fish fed the 10%–25% dietary protein level, following by a decrease as the protein proportion increased. These results are similar to those for pacific white shrimp *Penaeus vannamei* when fed 28%–48% dietary protein, and for catfish *Silurus soldatovi* as the proportion of dietary protein was increased (Huang *et al*., 2003). The results are similar to those of to Xia *et al*. (2017) who suggested that aquatic animals improve digestibility and absorption rate of dietary proteins by increasing protease activity and adapting to different levels of dietary protein. However, the absorption and utilization of protein plateaued, whereas protease activity decreased significantly in GIFT-*O. niloticus* fed the 25%–35% dietary protein level.

Amylase catalyzes the transformation of starch into maltose and facilitates absorption of nutrients (Bai *et al*., 2016). Amylase activity decreased slightly as carbohydrate level decreased with the increase in dietary protein level in the present study. Similar results were reported in grass carp *Ctenopharyngodon idella* (Das, & Tripathi, 1991), hybrid *Clarias* catfish *Clarias batrachus* × *Clarias gariepinus* (Giri, Sahoo, Sahu, & Meher, 2003), Jian carp *Cyprinus carpio* var. Jian (Liu, Feng, Jiang, Liu, & Zhou, 2009), and silver barb *Puntius gonionotus* (Mohanta, Mohanty, Jena, & Sahu, 2008), but not in *Labeo rohita* (Debnath *et al*., 2007). These results might be attributed to the fact that food habit, type of carbohydrate, water temperature and season can affect amylase activity in fish (Debnath *et al*., 2007). Further comparative study is needed to clarify the effect of dietary protein level on amylase activity and sugar transport in GIFT-*O. niloticus* and other fish with different food habits.

Lipase was not different among treatments, which might be ascribed to the constant lipid level in the diets. Similar results have been reported by Debnath *et al*. (2007) and Liu *et al*. (2009). Cellulase activities were very low, and no significant differences were observed among the treatments, probably because no cellulose was in the diets.

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