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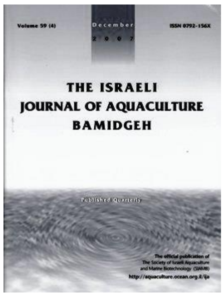

Effects of Supplemental Nucleotides, Taurine, and Squid Liver Paste on Feed Intake, Growth Performance, Serum Biochemical Parameters, and Digestive Enzyme Activities of Juvenile GIFT Tilapia *(Oreochromis sp.)* Fed Low Fishmeal Diets

Qing Zou [1,2], Yanhua Huang [2], Junming Cao [2*], Hongxia Zhao [2], Guoxia Wang [2], Yongjuan Li [2], Qing Pan [1*]

[1] College of Animal Science, South China Agricultural University, Guangzhou 510642, China
[2] Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangdong Provincial Key Laboratory of Animal Breeding and Nutrition, Guangzhou 510640, China

Key words: feeding stimulants; nucleotides; taurine; squid liver paste; low fishmeal diets.

Abstract

This 8 week feeding trial evaluated the effects of three feeding stimulants (FS) on feed intake, growth performance, body composition, serum biochemical parameters, and digestive enzyme activities for juvenile GIFT tilapia fed low fishmeal diets. Four test diets were supplemented with 0, 16 g/kg taurine (Tau), 0.4 g/kg mixed nucleotides (Mix-NT), and 30 g/kg squid liver paste (SQLP) respectively, and fed to juvenile GIFT strain of *Oreochromis niloticus* (3.34±0.01 g). The results showed that feed intake (FI) significantly increased with SQLP. Specific growth rate (SGR) in fish fed SQLP or Mix-NT diet was significantly higher than that of the Tau and control group. The feed conversion ratio (FCR) ranged from 1.34 in the group fed Mix-NT to 1.50 in the group fed Tau, with no significant differences compared to the control group. Three feeding stimulants had no significant influence on whole body composition of tilapia. Fish fed Mix-NT diet showed significantly higher intestinal protease activity and intestinal amylase activity than that of other groups. High-density lipoprotein content in serum was significantly higher in fish fed SQLP diet than that of other groups. In conclusion, supplementation of 0.4 g/kg mixed nucleotides or 30 g/kg squid liver paste in low fishmeal diets could provide better growth performance for juvenile tilapia, and SQLP could act as an effective FS under these conditions. Supplementation of Mix-NT could reduce the FCR in these fish.

* Corresponding authors. Junming Cao, E-mail: junmcao@163.com; Qing Pan, E-mail: qpan@scau.edu.cn

Introduction

Plant proteins are the most important alternatives to fishmeal in fish diets. However, partial or total replacement of fishmeal increases anti-nutritional factors and lowers palatability in the feed, leading to decreased feed intake (FI) and poor performance (Kissil et al., 2000, Arndt et al., 1999, Freitas et al., 2011). Tilapia usually reject feed like Pakistan rapeseed meal in which there is excessive use of plant proteins. For effective utilization of higher levels of plant proteins in fish feed must be palatable. Addition of feeding stimulants (FS) or feed additives increases FI while maintaining feed palatability and attractiveness (Yun et al., 2014; Chatzifotis et al., 2009, Martínez-Alvarez et al., 2015).

Studies have demonstrated that compounds like nucleotides, amino acids, and organic acids, which are abundant in marine extracts, are potent FS in aquatic feeds (Lian et al., 2005). Certain nucleotides act as taste enhancers for fish, mixed nucleotides being superior to single ones (Harada, 1986, Kasumyan and DÖving, 2003, Li and Gatlin, 2006). Taurine (Tau), a β-sulfonic-amino acid, is only found in animal tissues. It is a potential FS for some fish (Aragão et al., 2014, Chatzifotis et al., 2009). Pellets coated in a Tau solution (2 g/kg) were more readily consumed than control pellets by Sea Bream (*Sparus aurata*) (Chatzifotis et al., 2009). Squid liver paste (SQLP) is a type of hydrolysate from squid processing byproducts. Aquatic animal extracts or hydrolysates like squid or krill extract/hydrolysates are FS for fish (Yun et al., 2014; Chatzifotis et al., 2009, Martínez-Alvarez et al., 2015).

In our previous study, three independent trials were conducted to determine the optimal inclusion levels of mixed nucleotides (Mix-NT), Tau, and SQLP for relative FI quantification in 2 weeks (Zou et al., 2015). Additional research is needed into the effect of these supplements and their optimal long-term inclusion levels. The present study was conducted to investigate the effects of supplementation of Mix-NT, Tau, and SQLP in diets with low levels of fishmeal, on FI, growth performance, body composition, serum biochemical parameters, and digestive enzyme activities of juvenile GIFT tilapia.

Materials and Methods

Test diets. Four isonitrogenous (350 g/kg crude protein), isolipidic (65 g/kg total lipid) and isoenergetic (18 MJ/kg) experimental diets were formulated. The basal (control) diet was a commercial formulation for juvenile tilapia in China. It contained 4% fish meal, 18% soybean meal, 6% canola meal, 20% Pakistan rapeseed meal, and 19% cottonseed meal as protein with soybean oil and lecithinase as lipid sources. The three supplemented diets were prepared by adding 16 g/kg taurine (Tau), 0.4 g/kg mixed nucleotides (Mix-NT), 30 g/kg squid liver paste (SQLP) to the basal diet. To ensure that all the diets were isonitrogenous, isolipidic and isoenergetic, wheat flour, microcrystalline cellulose, canola meal, cottonseed meal, and soybean oil were adjusted in SQLP diet, while wheat flour and microcrystalline cellulose were adjusted in the other diets. The ingredient composition, chemical analysis, and amino acid composition of the four diets are shown in Table 1 and Table 2, respectively.

Table 1 Ingredients and nutrients levels of the test diets (air-dry basis g/kg)

Ingredients	*Control*	*Mix-NT*	*Tau*	*SQLP*
Fish meal[1)]	40.0	40.0	40.0	40.0
Soybean meal[1)]	180.0	180.0	180.0	180.0
Canola meal[1)]	60.0	60.0	60.0	70.0
Pakistan rapeseed meal[1)]	200.0	200.0	200.0	200.0
Cottonseed meal[1)]	190.0	190.0	190.0	185.0
Wheat flour[1)]	260.7	260.7	244.7	230.7
Lecithin[1)]	20.0	20.0	20.0	20.0
Soybean oil[1)]	30.0	30.0	30.0	25.0
Calcium dihydrogen phosphate[1)]	2.0	2.0	2.0	2.0
Vitamin premix[2)]	5.0	5.0	5.0	5.0
Mineral premix[3)]	5.0	5.0	5.0	5.0
Vitamin C ester[1)]	0.3	0.3	0.3	0.3
Choline chloride[1)]	2.0	2.0	2.0	2.0
Microcrystalline Cellulose[1)]	5.0	4.6	5.0	5.0
Mix-NT(98 %) [4)]	0.0	0.4	0.0	0.0
Tau (99.9 %) [5)]	0.0	0.0	16.0	0.0
SQLP [6)]	0.0	0.0	0.0	30.0
Nutrient levels(g/kg)				
Moisture	72.0	69.3	65.8	77.1
Crude protein	354.2	362.9	364.6	359.1
Crude lipid	53.7	59.7	61.2	66.8
Crude ash	80.2	81.1	81.4	78.7
Gross energy（MJ/kg）	17.95	17.81	17.92	17.95

[1)] Obtained from Fishtech Fisheries Science & Technology Company, LTD, Institute of Animal Science, Guangdong Academy of Agricultural Sciences (Guangzhou, China).
[2)] The vitamin premix was provided by Fishtech Fisheries Science & Technology Company, LTD, Institute of Animal Science, Guangdong Academy of Agricultural Sciences (Guangzhou, China). One kg of vitamin premix contained: vitamin A, 2000 IU; vitamin D_3, 700 IU; vitamin E, 10 mg; vitamin K_3, 2.5 mg; thiamin, 2.5 mg, riboflavin, 5 mg; pyridoxine, 3 mg; vitamin B_{l2}, 0.01 mg; niacin, 17.5 mg; D-calcium pantothenate, 10 mg; folic acid, 0.8 mg; biotin, 0.045 mg; inositol, 25 mg.
[3)] The mineral premix was provided by Fishtech Fisheries Science & Technology Company, LTD, institute of Animal Science, Guangdong Academy of Agricultural Sciences (Guangzhou, China). One kg of mineral premix contained: Ca 230 g, K 36 g, Mg 9 g, Fe 10 g, Zn 8 g, Mn 1.9 g, Cu 1.5 g, Co 250 mg, I 32 mg, Se 50 mg, moisture≤10%.
[4)] The nucleotides were purchased from Nanjing Biotogether Co., LTD, Nanjing, China. The Mixed nucleotides (Mix-NT) consisted of AMP (adenosine-5′-monophosphate sodium salt, 99.9%), CMP (cytidine-5′- monophosphate disodium salt, 99.9%), UMP (uridine-5′-mono-phosphate disodium salt, 99.9%), IMP (inosine-5′-monophosphate disodium salt, 99.9%), GMP (guanosine-5′-monophosphate disodium salt, 99.9%) (1:1:1:1:1 W/W).
[5)] The taurine (99.9%) was purchased from Aladdin Industrial Co., LTD, Shanghai, China.
[6)] The squid liver paste was purchased from Zhejiang Industrial Group Co., LTD. Oil feed plant, Zhoushan, China. One kg of Squid liver paste contained dry matter 312.3 g, Cude lipid 253.1 g; Crude protein. 321.2 g. Amino acid composition: Arginine (Arg) 15.6 g, Glycine (Gly) 19.2 g, Histidine (His) 6.1 g, Isoleucine (ILe) 14.6 g, Leucine (Leu) 21.2 g, Lysine (Lys) 17.8 g, Methionine (Met) 7.6 g, Phenylalanine (Phe) 13.7 g, Threonine (Thr) 12.5 g, Tyrosine (Tyr) 11.1g, Valine (Val) 16.0 g, Alanine (Ala) 23.3 g, Aspartic acid (Asp) 29.8 g, lutamic acid (Glu) 42.9 g, Serine (Ser) 12.9 g, total 264.0 g.

Table 2 Amino acid composition of the test diets (AA g/kg air-dry diet)

Amino acides	*Control*	*Mix-NT*	*Tau*	*SQLP*
Essential amino acid				
Arginine	25.0	24.7	25.1	25.3
Histidine	8.0	8.4	8.5	8.8
Isoleucine	13.6	13.4	13.4	13.9
Leucine	23.3	23.0	23.1	23.7
Lysine	16.2	16.3	16.3	16.9
Methionine	3.8	3.8	3.8	4.0
Phenylalanine	17.0	17.1	17.3	17.4
Threonine	12.2	12.3	12.4	12.8
Valine	16.7	16.5	16.4	17.0
Tryptophan	3.8	3.6	3.6	3.8
Non-essential amino acid				
Aspartic acid	28.7	28.3	28.4	29.3
Glutamic acid	76.1	75.5	75.3	76.0
Serine	15.6	15.4	15.7	15.8
Glycine	16.6	16.6	16.7	17.2
Alanine	15.4	14.6	26.2	15.3
Tyrosine	8.9	8.6	8.5	9.0
Taurine	0.01	0.01	1.29	0.03

Experimental fish. The feeding trial was conducted in an indoor re-circulating aquaculture system in Guangdong Academy of Agricultural Sciences, Guangzhou, China. The fish were transported from a farm to the laboratory at the larval stage and grown in a 5000 L outdoor tank, using commercial tilapia feeds. After being acclimated, the fish were fasted for 24 h and then weighed. Fish of similar sizes (weight 3.34 ± 0.01 g) were randomly distributed into 12 circular tanks (Blue PVC cone cylinder, 300 L) with freshwater, and each tank was stocked with 25 fish.

Feeding. The test diets were randomly assigned to identical tanks. The fish were hand fed to apparent satiation twice daily (9:00 and 16:00). During feeding, the water was not circulated and compressed air was not supplied. After one hour, the uneaten feed was removed and the water circulation and compressed air supply renewed. The amount of feed consumed in each tank was recorded every day, and the daily amount of feed was adjusted according to the amount consumed on the previous day. During the feeding trial, water temperature ranged from 27 to 30 ℃, pH from 7.4 to 7.8, ammonia nitrogen from 0.20 to 0.26 mg/L, DO>7.0 mg/L, and a natural photoperiod of 12 h light and 12 h dark. The feeding trial lasted for 8 weeks.

Sample collection. At the end of the feeding trial, fish in each tank were fasted for 24 h, then weighed and counted to determine weight gain rate (WGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate (SR). All fish were anesthetized with 100 mg MS-222/L before they were dissected. Three fish from each group were killed and stored at -20℃ for analysis of whole-body composition. Seven fish from each tank were individually weighed and body length, viscera weight, and liver weight were measured for calculation of condition factor (CF), viscerosomatic index (VSI) and hepatosomatic index (HSI) respectively. The intestines and livers were dissected on ice, homogenized in 9 volumes (w/v) of ice-cold saline solution and then centrifuged (5000 g/min) at 4℃ for 5 min. The supernatants were stored at -80℃ for enzyme activity analysis. Blood samples were collected from caudal vein of seven fish from each tank, and serum was separated from blood samples by centrifugation at 5000 g/min for 5 min at 4℃ and stored at -80℃ for analysis.

Chemical analysis. Proximate compositions including dry matter, crude protein, crude lipid and ash of the test diets and the fish whole body samples were determined by standard AOAC methods (AOAC, 1995). Dry matter content was determined by oven drying to constant weight at 105℃, and crude ash by combustion at 550℃. Crude protein (N×6.25) was determined by the Kjeldahl method using a semi-automatic Kjeldahl System after acid digestion. Crude lipid was determined by using the Soxhlet extraction method, and energy by using an adiabatic bomb calorimeter (C2000, IKA-WERKE®, Germany). The amino acid composition of the test diets was analyzed by high-performance liquid chromatography (HPLC) system (LC1260, Agilent Technologies Inc., Germany) equipped with Agilent ZORBAX Eclipse Plus C_{18} columns (150 × 5 μm, Australia) after acid hydrolysis. Tryptophan amino acid was determined by the colorimetric method of Spies (1967) using standard curve of pure tryptophan (Aladdin, China) and detected at 590 nm, with spectrophotometer (UV 1800-Mapada, China). Serum total protein (TP), cholesterol (CHO), triacylglycerol (TG), low density lipoprotein (LDL), high-density lipoprotein (HDL), glutamate pyruvate transaminase (ALT), glutamic-oxaloacetic transaminase (AST), urea nitrogen (UN) and glucose (GLU) were analyzed by automatic blood analyzer (Hitachi 7170A, Japan) in Kingmed Diagnostics, Guangzhou, China. Proteinase activity was measured according to the method of McDonald and Chen (1965). Lipase, amylase activities, and total protein in supernatant were assayed using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute following the manufacturer's instructions.

Statistical analysis. Data from each treatment were subjected to one-way ANOVA. Duncan’s test was used to compare mean values between individual treatments when overall differences were significant at a level that was less than 0.05. Statistical analysis was performed using SPSS (IBM® SPSS® Statistics version 20).

Results

Growth performance, feed utilization, and biometric indices of GIFT tilapia fed the four diets are given in Table 3. FBW and FI in fish fed SQLP diet were significantly higher than that of Tau group and control ($P<0.05$). SGR in fish fed SQLP diet or Mix-NT diet was significantly higher than that of Tau group and control ($P<0.05$). The highest FCR and lowest PER was found in fish fed Tau diet and was significantly different compared to the Mix-NT or SQLP group ($P<0.05$). No significant difference in SR was observed among all dietary treatments. Three FS had no significant influence on CF and HSI of tilapia, while VSI increased significantly with SQLP ($P<0.05$).

Table 3 Growth parameters, feed utilization and biometric indices in juvenile tilapia fed test diets for 8 weeks

Items	*Control*	*Mix-NT*	*Tau*	*SQLP*
IBW (g)	3.34±0.01	3.30±0.04	3.34±0.00	3.38±0.05
FBW (g)	26.14±0.32 ^{ab}	28.95±1.82^{bc}	24.64±0.08^{a}	30.39±1.06^{c}
SGR (%/d)	3.64±0.04^{a}	3.85±0.11^{b}	3.50±0.03^{a}	3.88±0.04^{b}
FI	858.38±14.50^{a}	925.18±45.59^{ab}	840.88±21.22^{a}	975.01±27.19^{b}
PER	1.98±0.02^{ab}	2.06±0.04^{b}	1.83±0.06^{a}	2.01±0.02^{b}
FCR	1.43±0.01^{ab}	1.34±0.02^{a}	1.50±0.05^{b}	1.38±0.02^{a}
SR (%)	97.33±2.67	98.72±1.28	96.00±2.31	97.22±2.78
CF (100 g/cm^3)	3.62±0.06	3.65±0.12	3.61±0.10	3.62±0.14
VSI (%)	9.44±0.14^{a}	9.98±0.31^{ab}	10.36±0.42^{ab}	10.47±0.30^{b}
HSI (%)	1.63±0.06	1.59±0.03	1.78±0.09	1.78±0.07

Values are means ± standard error (n = 3). Values within the same row with different letters are significantly different ($P<0.05$). IBW(g), initial body weight; SGR (specific growth rate, % / d) = 100 ×[ln (total final body weight + dead fish body weigh) − ln total IBW)] /56; FI (feed intake, g feed/100 g IBW /d) = Σ_1^{56} (total dry feed consumed on day i / fish number on day i) / IBW × 1/56 × 100; PER (protein efficiency rate) = weight gain / total dry protein intake × 100; FCR (feed conversion ratio) = dry feed intake / wet weight gain; SR (survival rate, %) = 100 × (final number of fish/ initial number of fish). CF (condition factor, 100 g/cm^3) = weight of fish / (length of fish)^3 × 100; VSI (viscerosomatic index, %) = weight of viscera / weight of fish × 100; HSI (hepatosomatic index, %) = weight of liver / weight of fish × 100.

Whole body composition of juvenile tilapia fed four diets for 8 weeks are given in Table 4. Three FS had no significant influence on the whole body dry matter, crude protein, crude lipid content and crude ash content in fish.

Table 4 Whole body proximate analysis (%) of juvenile tilapia fed test diets for 8 weeks

Items	*Control*	*Mix-NT*	*Tau*	*SQLP*
Dry matter	25.74±0.43	26.12±0.52	26.86±0.47	27.35±0.94
Crude protein	15.31±0.13	15.13±0.30	15.12±0.13	16.03±0.47
Crude lipid	7.63±0.31	7.58±0.20	8.41±0.43	7.90±0.33
Crude ash	3.01±0.03	3.00±0.12	2.89±0.05	2.96±0.12

Values are means ± standard error (n = 3). Values within the same row with different letters are significantly different ($P<0.05$).

The serum biochemical parameters of the juvenile tilapia fed four diets are shown in Table 5. Three FS had no significant influence on serum ALT and AST activities. No significant difference was found in TP, LDL, TG, GLU and UN contents among fish fed the different test diets. HDL was significantly higher in fish fed SQLP diet than that of other groups ($P<0.05$).

Table 5 Serum biochemical parameters in juvenile tilapia fed test diets for 8 weeks

Items	*Control*	*Mix-NT*	*Tau*	*SQLP*
TP (g/L)	25.13±0.58	25.67±1.04	26.70±0.57	26.03±0.41
CHO (mmol/L)	2.09±0.11	2.20±0.07	2.24±0.23	2.48±0.04
LDL (mmol/L)	0.30 ±0.03	0.30 ±0.02	0.33 ±0.05	0.37 ±0.03
TG (mmol/L)	0.58 ±0.05	0.66 ±0.09	0.79 ±0.14	0.67 ±0.07
HDL (mmol/L)	1.84±0.10^{a}	1.94±0.05^{a}	1.81±0.13^{a}	2.23±0.03^{b}
ALT (U/L)	42.67±5.81	29.67±8.17	51.67±18.00	31.67±6.77
AST (U/L)	232.33±50.11	172.33±55.05	258.67±61.25	117.00±18.56
UN (mmol/L)	0.33 ±0.07	0.50 ±0.12	0.47 ±0.07	0.37 ±0.09
GLU (mmol/L)	4.75±0.57	4.44±0.35	4.54±0.51	5.15±0.29

Values are means ± standard error (n = 3). Values within the same row with different letters are significantly different ($P<0.05$). TP, total protein; CHO, cholesterol; TG, triglyceride; LDL, low density lipoprotein; HDL, high-density lipoprotein; ALT, glutamate pyruvate transaminase; AST, glutamic-oxaloacetic transaminase; UN, urea nitrogen; GLU, glucose.

Digestive enzyme activity in intestine and liver of juvenile tilapia in all treatments is shown in Table 6. The fish fed Mix-NT diet had significantly higher intestinal protease and amylase activities than those of other groups ($P<0.05$). Liver lipase activity in Mix-NT group was significantly higher than that of SQLP group ($P<0.05$). Liver protease, amylase and intestinal lipase activity was not significantly different among all treatments.

Table 6 Digestive enzyme activity in juvenile tilapia fed test diets for 8 weeks (U/mg prot)

Items		*Control*	*Mix-NT*	*Tau*	*SQLP*
Protease	Intestine	37.51±4.86^{a}	93.40±11.08^{b}	45.40±3.30^{a}	59.51±8.61^{a}
	Liver	12.77±3.59	32.71±16.96	14.12±7.18	13.35±2.81
Lipase	Intestine	12.88±1.75	20.92±5.69	26.86±10.37	22.42±6.89
	Liver	20.14±5.33^{ab}	47.72±15.70^{b}	34.63±10.70^{ab}	11.78±2.51^{a}
Amylase	Intestine	50.69±6.11^{a}	150.57±8.69^{c}	42.41±9.52^{a}	93.74±5.98^{b}
	Liver	114.39±25.08	135.49±10.91	101.09±8.67	90.28±23.57

Values are means ± standard error (n = 3). Values within the same row with different letters are significantly different ($P<0.05$).

Discussion

FS have a direct effect on improving palatability of fish diets and as a consequence FI improves. In the present study, FI in fish fed with SQLP was the highest among all the treatments. SQLP is a squid liver hydrolysate containing many kinds of FS such as amino acids (Table 1), nucleotides, and quaternary ammonium base (Lian et al., 2005). Compared to Mix-NT and Tau, SQLP successfully increases FI in fish. Synthetic mixture was found to be inferior to natural FS as some effective components were absent from the synthetic mixture (Kohbara et al., 2000). Nucleotides act as taste enhancers for fish (Li and Gatlin, 2006), but in this study FI in fish fed with Mix-NT diet did not improve. This may be attributed to the different adaptabilities of fish to different FS. As in previous studies, some FS lost their potency when given for prolonged periods (de Oliveira and Cyrino, 2004). FI in fish fed with Tau diet was lower than that of the control in this study, but the difference was not significant. Positive results with Tau have occurred in different fish species e.g. European glass eel *Anguilla anguilla*, European sea bass *Dicentrarchus labrax* fry and gilthead sea bream *Sparus aurata* fry (El-Sayed 2014). Tau had a positive stimulating effect in red sea bream (Chatzifotis et al., 2009) but acted as a deterrent in marbled rockfish *Sebasticus marmoratus* (Salze and Davis, 2015). This suggests that stimulatory effects of Tau on FI are species-specific and might not be suitable for tilapia. FS have multiple functions. They not only alter feed palatability, but also play an important part in nutrient metabolism and animal growth (Gaber, 2005). In this study, SQLP and Mix-NT significantly improved tilapia growth. The growth promoting effect of SQLP might be largely attributed to its flavor, which promotes a higher feed intake. Many seafood protein hydrolysates like SQLP or Tuna viscera have been found to promote fish growth (Martínez-Alvarez et al. 2015). It is important to note that these diets were highly digestible and facilitated fast absorption of peptides and amino acids through the intestinal membrane (Martínez-Alvarez et al., 2015). The positive effect of Mix-NT on growth in the present study might be due to increased digestive enzyme activity in the intestine (Table 7). Dietary supplementation with nucleotides was found to be beneficial and increased digestive enzyme activity in rainbow trout, *Onchorchyncus mykiss* (Arzu, Özlüer, Hunt et al., 2014). Though nucleotides are not non-essential nutrients, dietary nucleotides have had multiple beneficial effects on the gastrointestinal tract in animal models (Li and Gatlin, 2006). In this context, a healthier gastrointestinal tract may lead to higher digestive enzyme activity. In the present study we observed that in fish fed Tau, FI decreased inhibiting growth performance.

PER and FCR are considerable economic indicators in aquaculture feeding practices. In terms of PER and FCR, the best performance in all treatments was recorded when feed diets were supplemented with Mix-NT containing high levels of plant proteins. Though SQLP significantly improved FI, we recommend using Mix-NT (0.4 g/kg) for tilapia rather than SQLP (30 g/kg) due to the lower PER and higher FCR in SQLP group. This also takes into account the environmental impact (Primavera, 2005).

Serum biochemical parameters serve as indicators for the physiological condition and welfare of fish. ALT and AST are often used for evaluation of liver function as they are released into the blood in damaged liver cells (Lemaire et al., 1991). ALT and AST activities were not affected by the three FS supplementation in the low fishmeal diets for tilapia. FS did not have significant benefits on liver function of the GIFT tilapia. In this study, higher serum content of HDL was found in fish supplemented with SQLP. These results were similar to those of Khosravi et al. (2015) who found that HDL increased in red sea bream (*Pagrus major*) fed with supplementation of marine protein hydrolysates. HDL assists in reversing cholesterol transport into the liver (Lewis and Rader, 2005). The lipid metabolism of fish may improve with supplementation of SQLP in low fishmeal diets.

In conclusion, inclusion of Mix-NT or SQLP in low fishmeal diets significantly improved growth performance for juvenile tilapia, and SQLP supplementation significantly improves FI. The growth promoting effects associated with Mix-NT in low fish meal diets are partly due to the higher intestinal protease and intestinal amylase activities.

Supplementation of SQLP is an effective FS. This might be due to the relatively comprehensive active ingredients in SQLP for increasing FI. When taking into consideration environmental and economic impacts, 0.4 g/kg Mix-NT is recommended as the appropriate feed additive in GIFT tilapia aquaculture.

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