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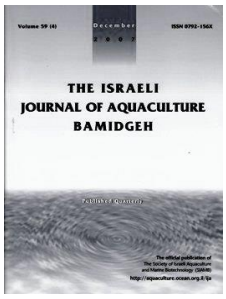

Effect of Dietary Tryptophan Levels on Growth and Hemato-Immunological Parameters in Juvenile Nile Tilapia *Oreochromis niloticus*

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Keywords: dietary tryptophan; growth performance; hematological parameters; non-specific immune responses; Nile tilapia juvenile; index of blood immune function

Abstract

An 8-week feeding trial was conducted to evaluate the effects of dietary tryptophan on growth performance, feed utilization, hematological parameters and non-specific immune responses of juvenile Nile tilapia. Six isonitrogenous and isoenergetic diets were formulated to contain graded levels of L-tryptophan (0.102%, 0.171%, 0.223%, 0.284%, 0.331% and 0.391% of dry weight respectively) from dietary ingredients and crystalline tryptophan. Each diet was randomly assigned to triplicate groups of 25 juvenile fish (1.41±0.01g) three times daily (8:30, 12:30, and 17:00 hours) to apparent satiation. Results showed that weight gain (WG) and special growth rate (SGR) increased with increased dietary tryptophan levels up to 0.284% and remained nearly the same thereafter. Dietary tryptophan requirement was best expressed by the quadratic regression equation $y=-6251x^2+3525x+538.1$ $R^2= 0.895$; based on weight gain against dietary tryptophan levels, with optimal dietary tryptophan requirement estimated to be 0.282% of the diet; corresponding to 6.24% of dietary protein on a dry weight basis. Tryptophan supplementation had no impact on the survival rate, body composition, antioxidant activities, hematological parameters, and non-specific immune parameters of tilapia. Our study indicated that tryptophan supplementation enhanced growth and promoted normal physiology. Our findings could be useful in the aquaculture industry.

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Introduction

Tilapia is widely cultured in many tropical and subtropical regions of the world. Tilapia represents the third largest group of farmed finfish species after the cyprinids and salmonids (FAO, 2007). Several species of tilapia are cultured commercially, but *Oreochromis niloticus* is the predominantly cultured species worldwide. Tryptophan is one of the essential amino acids for most aquatic animals (NRC, 2011).

Besides being involved in the synthesis of protein, tryptophan functions as a biochemical precursor for several compounds, including 5-HT (serotonin), a neurotransmitter, which is synthesized via tryptophan hydroxylase. Serotonin can be converted into melatonin (a neurohormone) via N-acetyltransferase and 5-hydroxyindole-O-methyltransferase activities.

After lysine and methionine, tryptophan is one of the most limiting amino acids in plant proteins used for fish feed such as corn meal and wheat grain meal (Kim and Lall, 2000; Coloso et al., 2004). In fish, the antioxidant defense mechanism involves both enzymatic and nonenzymatic antioxidants. Under normal physiological conditions, cells contain a complex network of antioxidant defense that scavenges the generation of ROS thus evading damage related to their high reactivity (Halliwell and Gutteridge, 1989).

To our knowledge, information on the effect of limiting amino acid tryptophan, on the antioxidant defense system is limited but worth investigation. Previous studies have shown that tryptophan deficiency can reduce growth performance and feed efficiency of fish. Furthermore, tryptophan deficiency can disturb routine mineral metabolism (Walton et al., 1984; Halver and Shanks, 1960; Akiyama et al., 1986). It is therefore imperative to determine the tryptophan requirements of fish, especially for those species, such as tilapias, which are fed diets formulated from plant protein sources. The dietary tryptophan requirement has been evaluated for a limited number of cultured fish species such as Mud crab *Scylla Serrata* (Peñaflorida, 2004), channel catfish *Ictalurus punctatus* (Wilson et al., 1978), common carp *Cyprinus carpio* (Nose, 1979), hybrid striped bass *Morone chrysops* × *M. saxatilis* (Gaylord et al., 2005), Mrigal carp *Crirrhinus mrigala* (Ahmed and Khan, 2005), and rainbow trout *Oncorhynchus mykiss* (Rodehutscord et al., 1997).

Some aspects of tilapia nutrition have been studied in the past and recently reviewed (Yue and Yang, 2011). According to relevant literature and experiments, amino acid requirements have been determined for Nile tilapia fry with mean initial weight 0.06g (Santiago and Lovell, 1988); however, with the exception of arginine, threonine, methionine, and lysine (He et al., 2013; Yue et al., 2013; Yue et al., 2014), not enough research information is available on any of its indispensable amino acid requirements. Moreover, indispensable amino acid requirements differ at different growth stages (Yue et al., 2013, Yue et al., 2014). For this reason, efforts have curbed in developing amino acid-balanced practical feeds required for the intensive culture of this nutritionally commercial aquaculture species. Since hematological parameters have been proven to be essential tools for analyzing health status of fish in response to dietary manipulation (Hrubec et al., 2001; Ahmed, 2012; Qiang et al., 2013), the present study was designed to determine the optimum dietary tryptophan requirement for tilapia using growth and hemato-immunological parameters.

Materials and methods

Experimental diets.

Six isonitrogenous and isoenergetic diets, using casein and gelatin as protein sources, and soybean oil as the lipid source, were formulated to contain graded levels of tryptophan (0.102%, 0.171%, 0.223%, 0.284%, 0.331% and 0.391% of dry weight respectively). The diets were made isonitrogenous by adjusting the levels of L-aspartame/L-glutamate (1:1). Composition and proximate analysis of the experimental diets is presented in Table 1.

Table 1. Composition of experimental diets (%)

Ingredients	*Diets*					
	T1	*T2*	*T3*	*T4*	*T5*	*T6*
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00
Casein	5.00	5.00	5.00	5.00	5.00	5.00
Gelatin	1.25	1.25	1.25	1.25	1.25	1.25
Soybean oil	6.00	6.00	6.00	6.00	6.00	6.00
Amino acid	20.27	20.27	20.27	20.27	20.27	20.27
Choline chloride	0.50	0.50	0.50	0.50	0.50	0.50
Ascorbic acid	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin mixture§	2.00	2.00	2.00	2.00	2.00	2.00
Monocalcium	2.75	2.75	2.75	2.75	2.75	2.75
Mineral mixture	2.00	2.00	2.00	2.00	2.00	2.00
Corn starch	45.00	45.00	45.00	45.00	45.00	45.00
Cellulose	7.78	7.78	7.78	7.78	7.78	7.78
Carboxymethy	5.00	5.00	5.00	5.00	5.00	5.00
L-aspartame/L-	0.25	0.20	0.15	0.10	0.05	0.00
L-tryptophan	0.00	0.05	0.10	0.15	0.20	0.25
Proximate						
Dry matter	89.18	90.64	90.2	89.94	90.13	90.92
Crude protein	28.18	28.20	28.39	28.52	28.43	28.89
Crude lipid	6.26	6.191	6.45	6.16	6.17	6.17
Ash	6.86	6.81	6.78	6.76	6.75	6.84
Tryptophan	0.102	0.171	0.223	0.284	0.331	0.391

[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 B_{12}, 0.02 mg; Vitamin K_3, 10 mg; Vitamin D_3, 0.05 mg.
2) Per kg diet contain $ZnSO_4 \bullet 7H_2O$, 525.5 mg; $MnSO_4 \bullet H_2O$, 49.2 mg; KI, 5.23 mg; $FeSO_4 \bullet 7H_2O$, 238.8mg; $MgSO_4 \bullet 7H_2O$, 4.62 g; $CuSO_4 \bullet 5H_2O$, 11.8 mg; $CoCl \bullet 6H_2O$, 0.2 mg; Na_2SeO_4, 0.66 mg; KCl, 600 mg; NaCl, 107.1 mg.
Amino acid mixture† (g/100 g diet): Arginine, 1.50; Histidine, 0.51; Isoleucine,1.51; Leucine, 1.84; Lysine, 1.28; Methionine, 0.73; Phenylalanine, 1.21; Threonine,1.15; Valine, 1.38; Aspartic acid, 2.37; Serine, 1.87; Glycine, 0.44; Alanine, 1.24; Cystine, 0.10; Tyrosine, 0.93; Glutamic acid, 2.30; Proline 0.26. Amino acids obtained from Feeer Co., LTD (Shanghai, China).

All dry ingredients were thoroughly blended in a Hobart-type mixer, and then water and lipids were added and thoroughly homogenized. Mineral and vitamin premixes were incorporated in the diets and then adjusted to pH 7.0 with 6 N NaOH. A 2.0 mm diameter die was used to produce wet-extruded, air-dried pellets with 10% moisture, which were then sealed in vacuum-packed bags and stored frozen at -20°C until feeding (Table 2).

Table 2. Amino acid composition of ingredients (% dry matter)

Ingredients	*Experimental diets*					
	T1	*T2*	*T3*	*T4*	*T5*	*T6*
EAA[a]						
Arginine	1.753	1.783	1.769	1.772	1.791	1.782
Histidine	0.661	0.641	0.651	0.646	0.631	0.671
Isoleucine	1.481	1.470	1.465	1.467	1.412	1.481
Leucine	2.317	2.377	2.326	2.312	2.377	2.277
Lysine	1.827	1.820	1.812	1.826	1.789	1.782
Methionine	0.854	0.905	0.891	0.885	0.815	0.895
Phenylalanine	1.632	1.622	1.567	1.612	1.624	1.609
Threonine	1.417	1.377	1.389	1.369	1.352	1.381
Valine	1.831	1.811	1.781	1.798	1.765	1.679
Tryptophan	0.102	0.171	0.223	0.284	0.331	0.391
NEAA[b]						
Aspartic acid	3.021	3.001	3.110	3.320	3.198	3.241
Serine	1.972	1.924	1.982	1.770	1.876	1.857
Glycine	1.828	1.801	1.768	1.802	1.742	1.815
Alanine	1.721	1.660	1.762	1.672	1.682	1.701
Cystine	0.102	0.103	0.102	0.101	0.101	0.102
Tyrsine	0.889	0.886	0.887	0.891	0.912	0.798
Gulmatic acid	3.868	3.792	3.882	4.012	3.982	3.892
Proline	1.010	0.962	1.020	0.989	0.986	0.965

[a]EAA, essential amino acid; [b]NEAA, non-essential amino acid.

Fish rearing.

Healthy tilapia juveniles obtained from Freshwater Fishery Research Center, Chinese Academy of Fishery Sciences, Wuxi, China, were acclimatized in 350 L fiberglass tanks for two weeks. During the acclimatization period, the fish were fed diet L1. At the beginning of the experiment, healthy fish with initial body weight of 1.41±0.01g were

distributed randomly in 18 fiber glass tanks with 350 L of water in each, and stocked equally with 25 fish. All the tanks were connected to a recirculation system. Fish were hand-fed to apparent satiation three times daily (08:30, 12:30 and 17:00 h) for 8 weeks. Water quality parameters were monitored daily. During the feeding trial, temperature ranged from 28-30°C, dissolved oxygen was never less than 6.0 mg/L, and ammonia nitrogen was lower than 0.05 mg/L. The fish were reared and fed the diets under a natural day light cycle (12h light: 12h dark).

Sample collection and analytical methods.

At the start of experiment, thirty 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. Five fish from each tank were used for whole body composition analysis.

Proximate analysis.

Crude protein, crude lipid, moisture, and ash in diets, and whole-body fish 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 method using a Soxtec System HT (Soxtec System HT6, Tecator, Sweden). Moisture 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. Amino acid concentrations in the diets were determined in a professional laboratory at the Institute of Feed Science, Jiangnan University, Wuxi China, using an automatic amino acid analyzer (Hitachi, Model 835-50, Hitachi, Tokyo, Japan) with a column Hitachi custom ion exchange; resin no. 2619.

Blood analysis.

Blood samples were drawn from the caudal vein of 9 fish from each tank with heparinized needles, and centrifuged at 4°C, 3000 g, for 15 min to obtain the serum. Blood serum was used for lysozyme activity (Lyz), immune globulin M (IgM) concentration, and complement C3, complement C4 level analysis. All blood serum samples were quickly frozen and kept at -80°C until analysis. After whole blood samples were collected, red blood cells (RBCs), white blood cell (WBCs), hemoglobin (Hb) and hematocrit (Ht) were then measured using Auto Hematology Analyzer (BC-5300Vet, Mindray, P.R. China), with a commercial kit from Shenzhen Mindray Medical International Co. Ltd. P.R. China.

Serum lysozyme activity assay.

Serum lysozyme activity was determined based on lysis of the lysozyme-sensitive Gram-positive bacterium, *Micrococcus lysodeikticus* (Sigma). Dilutions of hen egg white lysozyme (Sigma) ranging from 0-20 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, the change in turbidity was measured every 30 s for 5 min at 450 nm at approximately 20°C using a microplate reader.

Preparation of liver tissue extracts.

Liver tissue samples were extracted from each specimen (n=9 per treatment) were dissected out and immediately homogenized in ten volumes (w/v) of 100 mM Tris–HCl buffer pH 7.5. Each homogenate was sonicated briefly (2–3 s) using ultrasonic processor and centrifuged at 9000g at 4°C for 15 min (Manduzio et al., 2004). After centrifugation, supernatants were collected and immediately used for the determination of enzymatic activities and the malondialdehyde concentration (MDA). All assays were performed in triplicate and results were evaluated with reference to the total protein content of the samples (Biorad Protein Assay) using bovine serum albumin as standard.

Determination of liver tissue homogenate biochemical parameters.

Levels of glutathione (GSH), total antioxidant capacity (TAC) and malondialdehyde (MDA), as well as activities of, glutathione peroxidase (Gpx), and catalase (CAT) in the liver tissue homogenate were measured using commercial kits (Jiancheng Institute of Biotechnology, Nanjing, China). The liver GSH content was estimated by a colorimetric method as described by Lora et al. (2004) and expressed as units per milligram protein. TAC was measured following Benzie and Strain (1996). TBARS assay was used for the estimation of MDA, final concentration of MDA was expressed as nanomoles per milligram protein (Ohkawa et al., 1979).

Immune globulin M (IgM) assay.

Serum immune globulin (IgM) level was measured by an ELISA assay using a commercial kit (Cusabio, Wuhan, Hubei, China), as described by Sun et al. (2010); flat-bottomed 96-well plates were coated with serum samples for 2 h at 37°C and the liquid removed. The samples were blocked with 100ml of biotin-antibody for an hour at 37°C. Each well was aspirated and washed three times using wash buffer (350ml). Samples were incubated with 100ml of horseradish peroxidase avidin (HRPeavidin) working solution for an hour at 37°C and developed with TMB for 30 min at 37°C. Each well was aspirated and washed three times with wash buffer (350ml). The reaction was stopped by adding 50ml 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 controls for each plate was then subtracted from the optical density at 450 nm. All assay kits were specially designed for fish detection.

Complement C3 and C4 assay.

The serum complements C3 and C4 levels were assayed using a C3 and C4 kit respectively (Elikan, Wenzhou, Zhejiang, China) according to (Sun et al., 2010). Analysis of C3 level included measurement of the increase in turbidity after immunity response of C3 and its increased antibody; results are presented as C3 mg/ml. Analysis of C4 level included measurement of the increase in turbidity after immunity response of C4 and its increased antibody; results are presented as C4 mg/ml.

Calculations and statistical analysis.

The parameters of growth performance were calculated as follows:

Weight gain (WG; %) =100× (Wt−W0) /W0

Specific growth rate (SGR) = (LnWt−LnW0) × 100/time

Where, W0 is the initial weight and Wt is the final weight

Survival (%) =100 × Final number/Initial number

Feed conversion ratio (FCR) = dry feed fed (g)/ Wet weight gain (g)

Protein efficiency ratio (PER) = Wet weight gain (g)/protein intake (g)

Protein retention efficiency (PRE) = 100 × protein gain (g) / protein intake (g)

Results were expressed as mean ± SD. All data were subjected to one-way analysis of variance. When significant differences occurred, the group means were further compared with Duncan's multiple-range tests. All statistical analyses were performed using the SPSS 19.0 (SPSS, IL, USA).

Results

Growth performance.

The results of the effects of dietary tryptophan level on growth performance of juvenile tilapia are presented in Table 3. The tilapia readily accepted the diets. There were differences (P>0.05) in all the growth response parameters but compared to the control, significant differences were found only in T3, T4, T5, and T6. Survival rate ranged from 94.7%-100%; no mortality was recorded in the T1 dietary group. Fish fed tryptophan deficient diets (T1 and T2) showed reduced FW, SGR, and PER, compared to the other dietary groups; growth response and diet utilization improved with supplementation of tryptophan (P>0.05). Tryptophan requirement was best expressed by the quadratic equation: $y=-6251x^2+3525x+538.1$, with $R^2= 0.895$. Optimum tryptophan requirement for Nile tilapia was 0.282%, (Fig. 1).

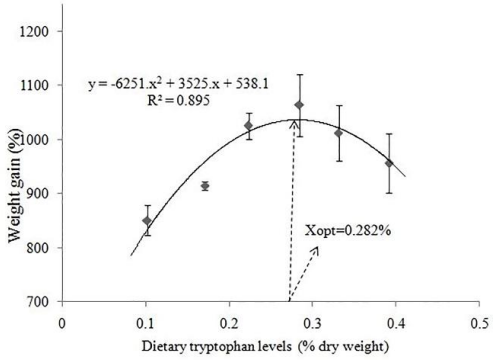

Figure 1. Relationship between weight gain (WG) and dietary tryptophan levels based on quadratic regression analysis, where Xopt represents the optimal dietary tryptophan level for the maximum WG of tilapia.

Table 3. Effects of dietary tryptophan level on growth performance of juvenile tilapia fed experimental diets for 8 weeks

Item	*Experimental diets*					
	T1	*T2*	*T3*	*T4*	*T5*	*T6*
Initial weight (g)	1.41±0.01	1.41±0.01	1.41±0.01	1.41±0.01	1.41±0.01	1.41±0.01
Final weigh (g)	20.0±0.57a	21.1±0.34ab	22.0±0.46bc	22.8±0.64c	22.3±0.78bc	22.2±1.16bc
FCR	1.77±0.05c	1.72±0.02bc	1.62±0.07ab	1.56±0.04a	1.62±0.08ab	1.68±0.03bc
Weight gain (%)	1325.9±41.0a	1407.7±24.22ab	1470.2±33.2bc	1531.0±45.5c	1492.0±55.9bc	1483.3±82.79bc
Survival (%)	100.0±0.0	97.3±2.31	98.7±2.31	98.7±2.31	97.3±4.62	94.7±4.62
SGR (%/day)	4.74±0.05a	4.84±0.03ab	4.92±0.04bc	4.98±0.05c	4.94±0.06bc	4.93±0.09bc
PRE (%)	35.57±1.08a	36.88±0.46ab	39.65±1.68c	40.45±1.04c	38.97±2.00bc	38.10±0.58bc
PER	2.02±0.06a	2.06±0.03ab	2.21±0.10c	2.25±0.06c	2.18±0.11bc	2.16±0.03bc

Values are presented as mean±SD (n=3); values with different superscripts in the same row differ significantly (P<0.05).

Whole body proximate composition.

Results of the effect of dietary tryptophan levels on whole body composition of juvenile tilapia fed experimental diets are presented in Table 4. The result showed that whole body moisture, crude protein, crude lipid and ash contents were not significantly affected by the dietary tryptophan supplementation in all treatment groups (P<0.05).

Table 4. Effect of dietary tryptophan on body composition in juvenile tilapia fed experimental diets for 8 weeks

Item	*Experimental diets*					
	T1	*T2*	*T3*	*T4*	*T5*	*T6*
Moisture	72.5±0.92	71.8±0.64	71.6±0.78	71.6±0.71	72.1±1.03	71.9±0.93
Crude	16.35±0.43	17.2±0.27	17.56±0.34	17.8±0.43	17.66±0.26	17.34±0.28
Crude	7.51±0.39	7.53±0.49	7.65±0.67	7.59±0.87	7.62±0.49	7.53±0.68
Ash	4.37±0.32	4.44±0.25	4.26±0.33	4.24±0.46	4.32±0.45	4.32±0.31

Values are presented as mean ± SD (n=3); values with different superscripts in the same row differ significantly (P<0.05).

Antioxidant enzymes activity.

The results of antioxidant enzyme activity in tilapia fed supplemented tryptophan levels are presented in Table 5. The results showed that there was no significant difference (P<0.05) in the antioxidant enzyme activity in tilapia fed dietary tryptophan supplementation.

Table 5. Serum antioxidant enzymes of tilapia fed the experimental diets for 8 weeks

Item	*Experimental diets*					
	T1	*T2*	*T3*	*T4*	*T5*	*T6*
GSH-Px（U/ml)	60.26±5.40	63.56±5.55	64.82±7.91	63.73±8.80	62.81±5.68	61.98±6.25
CAT（U/ml)	114.5±8.29	119.1±7.08	117.8±7.58	116.5±9.13	120.0±8.44	116.2±6.15
TAC（U/ml)	9.37±0.86	9.50±0.80	9.42±0.99	9.91±0.56	9.34±0.70	9.89±0.84
MDA（nmol/ml)	2.27±0.35	2.14±0.07	2.04±0.34	1.95±0.37	1.93±0.41	2.04±0.36

Values are presented as mean ± SD (n=3); values with different superscripts in the same row differ significantly (P<0.05).

Hematological parameters.

The results of hematological parameters including white blood cell (Wbc), red blood cells (Rbc), hemoglobin (Hb) and hematocrit (Ht) content of juvenile tilapia fed the experimental diets are presented in Table 6. The results showed that dietary tryptophan supplementation did not result in any significant difference in Wbc, Rbc, Hb and Ht (P<0.05); there was somewhat of a decrease in Wbc as dietary tryptophan levels increased.

Table 6. Concentration of hematological parameters of juvenile tilapia fed the experimental diets

Item	*Experimental diets*					
	T1	*T2*	*T3*	*T4*	*T5*	*T6*
[a]Wbc（$1\times10^{9}l^{1}$)	310.3±24.96	313.0±20.6	308.1±14.6	304.9±12.1	303.3±23.2	303.3±23.2
[b]Rbc ($1\times10^{12}l^{-1}$)	2.43±0.16	2.38±0.36	2.57±0.13	2.56±0.20	2.57±0.23	2.56±0.23
[c]Hb (gl^{-1})	80.5±6.03	82.5±10.34	80.2±11.04	83.3±9.02	80.6±7.20	80.5±7.30
[d]Ht (%)	33.4±2.80	35.3±2.53	34.9±1.20	36.3±1.76	33.7±2.68	33.6±2.66

[a]Values are presented as mean ± SD (n=3); values with different superscripts in the same row differ significantly(P<0.05).

[a]Wbc; white blood cell, [b]Rbc; Red blood cell, [c]Hb ; Hemoglobin, [d]Ht ; Hematocrit

Selected non- specific immune parameters.

The results of immune globulin M (IgM), lysozyme activity (Lsy), complement component, C3 and C4 are presented in Table 7. The results showed that dietary tryptophan supplementation resulted in no significant difference in IgM, Lsy, C3 and C4 levels of tilapia fed the experimental diets (P<0.05); there were some increases in these parameters as dietary tryptophan levels increased.

Table 7. Effects of different dietary tryptophan levels on several serum immune parameters in tilapia[a]

Item	*Experimental diets*					
	T1	*T2*	*T3*	*T4*	*T5*	*T6*
IgM(mg/l)	314.69±38.39	317.15±64.45	328.19±24.04	329.01±53.84	324.10±87.26	315.10±39.20
Lys(U/ml)	240.19±29.45	244.99±15.34	261.18±40.36	264.47±21.16	257.20±32.99	264.75±41.11
C3(mg/l)	55.40±8.97	59.45±10.53	61.48±11.32	63.79±7.91	58.60±11.13	58.56±12.70
C4(mg/l)	11.94±2.66	13.16±3.05	14.89±3.03	15.86±2.61	13.86±4.17	13.49±2.44

[a]Values are presented as mean ± SD (n=3); values with different superscripts in the same row differ significantly（P<0.05）

Lys, lysozyme activity; IgM, immune globulin M; Component C3; Component C4.

Discussion

In the present study, fish fed tryptophan deficient diets (T1 and T2) showed reduced FW, SGR, and PER. Growth response and diet utilization improved with supplementation of tryptophan. The results indicate that tryptophan is essential for growth of juvenile tilapia and tilapia are able to utilize tryptophan. Deficiency of most amino acids causes certain pathological symptoms (Walton, 1985) and failure in weight gain and loss of appetite. Tryptophan deficiency has been reported to cause morphological abnormalities, for instance, lordosis and scoliosis in sockeye salmon (Halver, 1957; Halver and Shanks, 1960) and rainbow trout (Shanks et al., 1962; Poston and Rumsey, 1983). Scoliosis was also reported in chum salmon fed tryptophan deficient diet (Akiyama et al., 1986). Our results are similar to those of Tackman et al. (1990) and Mullen and Martin (1992). Tryptophan produces serotonin which is produced in the brain. Studies have reported that excess levels of amino acids may become toxic and may have an adverse effect on growth as the imbalanced intake of one amino acid affects the absorption and utilization of other amino acids (Harper et al., 1970; Borlongan and Coloso, 1993; Ahmed and Khan, 2005). Similar growth depressing effects of feeding amounts of tryptophan greater than the optimum amount were also evident in rainbow trout (Poston and Rumsey, 1983); Indian major carp, catla (Ravi and Devaraj, 1991), rohu (Murthy and Varghese, 1997) and mrigal (Ahmed and Khan, 2005). However, higher levels of dietary tryptophan, 0.34 g /100 g corresponding to 0.85 g/ 100 g of the protein were reported by (Ahmed, 2012) as optimum levels for maximum growth of Indian catfish, *Heteropneustes fossilis*. This discrepancy could be attributed to the differences in size and species of the test subjects. This further indicates that tryptophan requirements differ at different growth stages in fish. It was observed in this study that fish fed a tryptophan deficient diet had poor growth, but showed no obvious pathological symptoms. Protein synthesis can only be stimulated in the presence of a high supply of balanced amounts of essential amino acids. Therefore, excess dietary doses of tryptophan would not efficiently promote protein synthesis and would impair normal protein metabolism. This is in conformity with the lower levels of PER and PRE in tilapia fed diets containing high tryptophan concentrations (T6) suggesting that the imbalance of dietary amino acid levels resulted in poor growth performance in tilapia. In our study, tryptophan supplementation did not show any significant difference in whole body composition among the respective diets. The antioxidant system involves enzymes such as catalase (CAT) and glutathione peroxidase (GSH-PX) that act by detoxifying the generated ROS. CAT catalyses the production of oxygen and water from H_2O_2 and it has been demonstrated that this enzyme is active at high H_2O_2 concentrations (Chance et al., 1979). There was no significant difference between all treatment groups for antioxidant enzymes activities (GSH-Px, CAT, MAD and TAC) of tilapia fed the experimental diets; however, an inverse

trend was observed between MDA and CAT, GSH-PX, TAC. The same inverse trend was also reported by Pampanin et al. (2005). CAT and GSH-PX play a role in the catabolism of H_2O_2 at low production; meanwhile the oxidative metabolism of cells is a continuous source of reactive oxygen species (ROS), resulting from univalent reduction of O_2, which sequentially can damage most cellular components (Livingstone, 2001; Regoli et al., 2002). MDA showed a gradual decrease contrary to GSH-Px, CAT and TAC levels, as tryptophan concentration increased in the diets. There were no significant differences in hematological parameters of tilapia fed the graded levels of dietary tryptophan. Our results conform with those reported for healthy tilapia (Kiron et al., 2012; Yue et al., 2013). IgM plays an important role in defending the host from infectious diseases (Li et al., 2007); lysozyme has also been documented as an important defense element which causes lysis of bacteria (Jollès and Jollès, 1984) and acts as a vital bio-defense effector of innate immunity (Simser et al., 2004). The non-specific immune parameters assayed including IgM, Lys C3 and C4 did not reflect any significant difference in all the dietary groups. Tilapia fed graded levels of dietary tryptophan did not show any obvious pathological symptoms during the entire trial. It is suggested that dietary tryptophan supplementation can not only promote growth but can also initiate normal physiology and development of hemato-immunological parameters in tilapia especially at the optimal level. Immune defense mechanism in fish is vital since a direct relationship between the functioning immune system and the ability to counteract disease outbreaks has been established (Blaxhall, 1972).

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