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**Effect of reducing 3.2% dietary protein level on the growth performance and immunity of Nile tilapia (*Oreochromis niloticus*) with supplementation of multi amino acids**

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Received: September 2013

Accepted: July 2014

**Abstract**

Reducing dietary protein content in fish feeds will reduce cost of production if growth performance can be maintained. In this study, we assessed the effects of reduced dietary protein content from 33.5% to 27.4% with ideal amino acids profile on the growth, immune parameters, intestinal microvilli length and total ammonia nitrogen discharge of tilapia. After 8 weeks of feeding, growth performance and feed efficiency were not affected by reducing dietary protein content from 33.5% to 30.3%, while fish fed 27.4% CP had the lowest weight gain. Total ammonia nitrogen discharged into the water 9 hours after the feeding was decreased by about 35%. Serum lysozyme activity, blood respiratory burst activity and serum ACH50 were not significantly affected by dietary protein content. Fold height, enterocyte height and microvillus height of proximal and middle intestine were significantly increased with reducing of dietary protein. Results indicated that 3.2% dietary protein content can be reduced, which had no effects on growth performance and immunity of Nile tilapia in practical diet.

**Keywords:** Tilapia, Ideal amino acids profile, Dietary protein, Growth

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## Introduction

Protein is a major component in aquaculture feeds because it provides the essential and nonessential amino acids to synthesize body protein and in part provides energy for maintenance. Reducing dietary protein content is very important to formulate cost effective and low pollution diets in aquaculture (Gan *et al.*, 2012). Fish growth is determined by the limiting amino acids and reducing the dietary protein content is a strategy to increase the sustainability of aquaculture production via reducing the environmental pollution as well as decreasing feed costs if growth performance can be maintained with less nitrogen intake (Gaylord and Barrows, 2009). The ideal amino acids or protein concept should be used to balance amino acids profile of fish diets (Furuya *et al.*, 2004). The possibility of reducing the dietary protein had been demonstrated in diets for rainbow trout (Cheng *et al.*, 2003, Gaylord and Barrows, 2009) and carp (Viola *et al.*, 1991) through balancing amino acids profile with crystalline amino acids. Fish growth was not affected when dietary protein content was reduced about 4%~10%.

Tilapia has become the most important fish in aquaculture after carp and salmon, worldwide production exceeded 3 million tons in 2010 and increases annually (FAO, 2012). In China, tilapias are typically fed a commercial feed with 26%-34% crude protein content. Protein is the most expensive ingredient in fish feeds. Protein deficiency or amino acids

imbalance have been demonstrated to reduce growth performance, immune function and increase the susceptibility of fish to infectious diseases (Kiron *et al.*, 1995, Oliva-Teles, 2012). The present study was conducted to evaluate the growth performance, ammonia excretion and immune responses of tilapia when dietary protein content were reduced based on ideal amino acids profile with supplementation of crystalline lysine, methionine, threonine and tryptophan.

## Materials and methods

### *Diets preparation.*

Four diets were formulated to assess the effect of reducing dietary protein content with crystalline amino acids based on ideal amino acids profile for tilapia. The formulation is presented in Table 1. The amino acids profiles of the experimental diets are shown in Table 2, detection value of threonine were slightly lower than calculation value. Diet 1 was a typical practical diet formulated to contain 34% dietary crude protein (CP) with methionine supplementation. Diet 2, 3 and 4 were formulated through adjusting the inclusion rate of soybean meal, CP content were respectively 32%, 30% and 28%. The dietary lysine, methionine, threonine and tryptophan of diets 2, 3 and 4 were equal to those of diet 1 by adding commercial available crystalline Lysine-HCl, MHA-Ca, L-threonine and L-tryptophan. All diets were isoenergetic through adjusting the inclusion rate of cellulose. All feed ingredients and supplements were

thoroughly mixed with oil, and produced pellets of approximate 2.0 mm in diameter, respectively. Subsequently, the pellets were dried in the dark and then stored at -20°C until

used. Proximate compositions of diets were shown in Table 1, which were measured with standard methods (AOAC, 1984).

**Table 1: Formulation and composition of experimental diets.**

Diet	1	2	3	4
Ingredient				
Soybean meal <sup>a</sup>	360	300	240	180
Canola meal <sup>a</sup>	170	170	170	170
Cotton meal <sup>a</sup>	110	110	110	110
Rice bran meal <sup>a</sup>	90	90	90	90
Wheat flour <sup>a</sup>	191.6	230.8	269.8	308.7
Mineral mix <sup>b</sup>	5	5	5	5
Vitamin mix <sup>c</sup>	5	5	5	5
Soy oil <sup>a</sup>	30	30	30	30
Choline chlorine (50%) <sup>a</sup>	3	3	3	3
Monocalcium phosphate <sup>a</sup>	20	20	20	20
78%Lysine-HCl <sup>d</sup>	0	1.9	3.8	5.8
84%MHA-Ca <sup>e</sup>	4.4	4.6	5	5.3
98%L-Threonine <sup>f</sup>	0	0.9	2	2.9
98%L-Tryptophan <sup>f</sup>	0	0.3	0.6	0.8
Cellulose	0	17.5	34.8	52.5
Phospholipid <sup>a</sup>	10	10	10	10
VC Ascorbic acid	1	1	1	1
Total	1000	1000	1000	1000
Proximate analysis (g kg <sup>-1</sup> dry diets)				
Moisture	80.2	73.0	70.0	72.1
Crude protein	335	319	303	274
Crude fat	54.0	53.4	53.2	52.6
Gross Energy kj g <sup>-1</sup> <sup>g</sup>	14.4	14.4	14.4	14.4

<sup>a</sup> Zhuhai Shihai Feed Corporation Ltd, Zhuhai, China

<sup>b</sup> Mineral mix (mg kg<sup>-1</sup> of

diet):MgSO<sub>4</sub>.7H<sub>2</sub>O,4061.5;ZnSO<sub>4</sub>.7H<sub>2</sub>O,525.46;FeSO<sub>4</sub>.7H<sub>2</sub>O,238.83;MnSO<sub>4</sub>.H<sub>2</sub>O,49.22;CoCl<sub>2</sub>.6H<sub>2</sub>O,0.2;KI,5.23;CuSO<sub>4</sub>.5H<sub>2</sub>O,11.82;Na<sub>2</sub>SeO<sub>3</sub>,0.66; KCl,600;NaCl,400 (Guangzhou Chengyi Aquatic Technology Ltd, Guangzhou, China)

<sup>c</sup> Vitamin mix (mg kg<sup>-1</sup> of diet):thiamin,20;riboflavin,20;vitmin A,2;vitamin E,50;vitamin D<sub>3</sub>,0.05 ;menadione,10;pyridoxine,10;cyanocobalamin,0.02;biotin,1;calcium pantothenate,50;folic acid,5;niacin,100;inositol,500. , (Guangzhou Chengyi Aquatic Technology Ltd, Guangzhou, China)

<sup>d</sup> L-Lysine.HCl contained L-Lysine≥78%(CJ Co., Ltd., Liaocheng, China)

<sup>e</sup> MHA.Ca contained DL-HMTBA (2-hydroxy-4-methylthio butanoic acid) ≥ 84% (Novus International Inc. Zhibo, China)

<sup>f</sup> supplied as L-form,( Shanghai Cangda Amino acid Company Ltd,Shanghai,China)

<sup>g</sup> calculated from dietary crude protein, lipid and carbohydrate content

**Table 2: Amino acid composition of experimental diets for tilapia (g kg<sup>-1</sup> dry diets).**

Diet	1	2	3	4	Requirement (34% diet protein)(NRC, 2011)
<b>Essential amino acids g kg<sup>-1</sup></b>					
Lysine	17.3(5.16)	17.4(5.45)	17.2 (5.68)	17.2(6.28)	17.3(5.09)
Methionine	9.55(2.85)	9.54(2.99)	9.56 (3.16)	9.55(3.49)	9.52(2.80)
Threonine	12.1(3.61)	12.3(3.86)	12.5 (4.13)	12.4(4.53)	12.8(3.76)
Tryptophan	3.83(1.14)	3.83(1.20)	3.83 (1.26)	3.83(1.40)	3.40(1.00)
Phenylalanine	14.3(4.27)	13.4(4.20)	11.5(3.80)	11.4(4.16)	12.9 (3.79)
Histidine	7.23(2.16)	6.69(2.10)	6.16(2.03)	5.63(2.05)	5.74(1.69)
Arginine	21.6 (6.45)	19.9(6.24)	18.2(6.01)	16. 5(6.02)	14.2(4.18)
Isoleucine	13.6 (4.06)	12.4(3.89)	11.3(3.73)	10.1 (3.69)	10.5(3.09)
Leucine	21.6(6.45)	19.9(6.24)	18.0(5.94)	16.2(5.91)	11.6(3.41)
Valine	15.4 (4.60)	14.2 (4.45)	13.2(4.36)	12.1(4.42)	9.52(2.80)
<b>Non-essential amino acids g kg<sup>-1</sup></b>					
Serine	13.0	11.8	10.6	9.5	
Proline	15.7	14.9	14.0	13.2	
Cystine	1.38	1.38	1.28	1.17	
Tyrosine	8.29	7.65	6.91	6.16	
Aspartic acid	29.0	26.1	23.2	20.2	
Glutamic acid	60.2	56.5	52.9	49.3	
Glycine	13.7	12.6	11.6	10.5	
Alanine	13.6	12.4	11.3	10.2	
∑AA	291	273	253	235	

Amino acids were analyzed following acid hydrolysis using high-pressure liquid chromatography (HPLC; Hewlett Packard 1090, Palo Alto, USA)

Tryptophan is calculated from NRC.

As an analog of methionine, MHA-Ca can not be detected by the amino acid analyzer, so methionine value was analyzed the sum of MHA-Ca and Methionine.

(Data):amino acid percent of dietary protein.

### *Fish maintenance and experimental design*

Juveniles tilapia from our facilities were used in this experiment and their initial wet weight was 15.1±0.11 g. All experimental fish were maintained as previously described by Xiong *et al.* (2014). Before the experiment, the fish were acclimated to the experimental conditions for 2 weeks and fed a commercial diet containing 32% protein and 4% lipid to satiation. Acclimated fish were then randomly divided into 4 groups, and each group had three tanks

containing 20 tilapia for each. The fish were fed with diets 1, 2, 3 and 4 by 7% of body weight thrice a day for 8 weeks. During the trial period, the diurnal cycle was 12L/12D. The water was oxygenated, passed through artificial sponge (3 cm thickness), coral-sand (25 cm thickness) and active-carbon filter (25 cm thickness) to remove chlorine. Water quality parameters monitored weekly as follows: temperature, 27.1±1.1°C; pH, 7.22±0.17, dissolved oxygen, 6.08±0.10 mg L<sup>-1</sup> and total ammonia-nitrogen, 0.05±0.01 mg L<sup>-1</sup>.

At the beginning of the feeding trial, 18 fish were randomly sampled from the initial fish and killed for analyses of whole body composition. At the end of the eight weeks experiment, three fish from each tank were randomly collected for analysis of whole-body composition and five fish were anaesthetized with tricaine methanesulphonate (MS-222) ( $50 \text{ mg L}^{-1}$ ) for blood collection from caudal vein and to obtain weights of whole body, viscera, liver and intraperitoneal fat. Anticoagulated blood (0.2 ml) and non-anticoagulated blood (1.5 ml) were collected from each fish. Plasma was stored at  $4^{\circ}\text{C}$ , and serum was stored at  $-70^{\circ}\text{C}$  until analyzed. White muscle from both sides of the fillets without skin and liver were dissected, frozen immediately in liquid nitrogen and then stored at  $-70^{\circ}\text{C}$  until used. Entire gastrointestinal tract of two fish per tanks were collected, fixed in Bouin's solution and then transferred into 70% ethanol until used.

#### *Ammonia excretion experiment*

For ammonia excretion analysis, acclimated fish were randomly distributed to four groups with 40 fish each, and each group had two tanks (98 L×48 W×42 H cm, water volume of 200 L). After feeding of fish at 0800 h with diets 1, 2, 3 and 4 by 2.5% of body weight, all tanks were cleaned, the water supply to the tanks was shut-off, and oxygen was supplied to the tanks. At 0800 h and 1700 h, water samples were collected for total ammonia nitrogen (TAN) analyses for 2 days (Gan *et al.*, 2012). Water samples were

analyzed for TAN concentration by HACH water quality analyzer (DR850, American).

#### *Histology*

Segments (0.5-1cm length) of proximal and middle intestine were sliced transversely into 4-mm sections, fixed in Bouin's fixative for 24 h (at room temperature), dehydrated in ethanol, cleared in xylene, embedded in paraffin, and stained with hematoxylin and eosin (H&E). The slides were examined under a light microscope (Motic) equipped with a camera and Motic Images Advanced 3.0 software for image acquisition and assessing dimensions of intestinal folds, enterocytes and microvilli in different enteric sections (10 measurements per fish) (Cheng *et al.*, 2011).

#### *Biochemical compositions of serum*

The concentrations of total protein (TP), albumin (ALB), cholesterol (CHO), triacylglycerol (TG), glucose (GLU), high density lipoprotein (HDL) and low density lipoprotein (LDL) in serum were determined using an automatic blood analyzer (Hitachi 7170A, Japan) in a clinical laboratory.

#### *Respiratory burst activity*

Blood leukocyte respiratory burst activity was detected according to the method described by Anderson *et al.* (1995). In brief, 0.1 ml of anticoagulated blood was added to the equal volume of 0.2% Nitro blue tetrazolium buffer, and incubated at  $28^{\circ}\text{C}$  for 30 min. 0.05 ml of this

mixture was then added to a 1.5 ml EP tube containing 1 ml dimethylformamide and centrifuged at 8000 rpm for 5 min. Subsequently, the absorbance of the supernatant at 540 nm was measured.

#### *Lysozyme activity*

Serum lysozyme activity was measured using an LZM test kit (Nanjing Jiancheng Bioengineering Institute, China). In brief, 0.2 ml of serum, distilled water or standard liquid containing 2.5 µl/ml lysozyme was added to 2 ml bacteria solution, mixed gently, incubated at 37 °C for 15 min, and then immediately transferred to ice water for 3 min. The solution was then transferred into a 1 cm optically clear colorimetric dish and the transmittance (T15) at 530 nm was measured. The transmittance of the water at 530 nm was adjusted to 100% before the detection. Lysozyme content of the samples was calculated by using the following formula:

Lysozyme content (µg/ml) =  $(UT15 - OT15) / (ST15 - OT15) \times \text{standard concentration (100 µg/ml is namely 2000 U/ml)} \times \text{sample dilution factor}$ ,

where:

UT15: transmittance of test tube;

OT15: transmittance of blank tube;

ST15: transmittance of standard tube

#### *Alternative complement activity*

Serum alternative complement activity was detected using a Fish ACH50 ELISA Kit (R&D). In brief, 10 µl of serum was added to the microtiter plates, diluted 5 times with sample

dilution, mixed gently, and incubated at 37 °C for 30 min. After discard liquid, the plates were washed 5 times with wash buffer, and 50 µl HRP-conjugated anti-complement antibody was added to each well and incubated at 37 °C for 30 min. After washing five times, 50 µl of chromogen solution A and chromogen solution B were added to each well, and incubated at 37 °C for 15 min. The reaction was stopped with stop solution, and the absorbance was determined at 450 nm. The sample complement activities were determined by comparing absorbance with a standard curve.

#### *Growth performance*

Growth performance was evaluated by the following formulas:

WG (weight gain, %)=  $100 \times (\text{final body weight} - \text{initial body weight}) / \text{initial body weight}$ .

SGR (specific growth rate, % day<sup>-1</sup>) =  $100 \times (\ln \text{ final body weight} - \ln \text{ initial body weight.}) / 56 \text{ days}$ .

Survival (%) =  $100 \times (\text{final fish number}) / (\text{initial fish number})$ .

FCR (feed conversion ratio) =  $\text{feed consumed} / (\text{FBW} - \text{IBW})$ .

NR (nitrogen retention) =  $100 \times \text{retained nitrogen (g)} / \text{nitrogen fed (g)}$ .

LR, lipid retention =  $100 \times \text{retained lipid (g)} / \text{lipid fed (g)}$ .

VSI,viscerasomatic index =  $100 \times \text{viscerasomatic weight (g)} / \text{body weight (g)}$ .

HSI, hepatopancreasomatic index =  $100 \times \text{liver weight (g)} / \text{body weight (g)}$ .

IPF, intraperitoneal fat ratio =  $100 \times \text{intraperitoneal fat weight (g)} /$

body weight (g).

CF, condition factor= $100 \times \text{body weight (g)} / \text{body length (cm)}^3$ .

SSI, spleensomatic index= $100 \times \text{spleen weight (g)} / \text{body weight (g)}$ .

#### *Statistical analysis*

All data are presented as means $\pm$ S.E.M and subjected to one-way ANOVA and Duncan's multiple range tests using the SPSS software version 13.0 for Windows (SPSS Inc., Chicago, IL, USA Ver. 13.0, USA). Differences were considered significant at  $p < 0.05$  (McKillup *et al.*, 2006).

#### **Results**

Fish readily accepted the experimental diets and survival rate was very high during the 8 weeks feeding trial. Growth performance and nutrient retention of fish were presented in Table 3. There were no significant differences in survival among fish fed all the diets. After the feeding trial, FBW, WG, SGR and FCR were not affected with reducing dietary protein content from 33.5% to 30.3%. NR and protein efficiency ratio (PER) of fish were significantly improved with reducing dietary protein content ( $p < 0.05$ ). Total ammonia nitrogen discharged into the water after 9 hours of feeding (Fig.1) showed an gradually decreasing trend with reducing dietary protein content

( $p < 0.05$ ).

The proximate body composition (moisture, protein, and lipid) and morphometric index of tilapia are shown in Table 4. Lipid content of liver and muscle were significantly increased when fish was fed lower dietary protein ( $p < 0.05$ ), which showed a reduced trend with increasing in dietary protein level. VSI, HSI and IPF were significantly increased with reducing of dietary protein content among diet treatments ( $p < 0.05$ ). There was no difference of SSI among the treatments. Fish fed diets containing lower dietary protein had lower condition factor.

Serum biochemical parameters were provided in Table 5. CHO and TG content were significantly increased with reducing dietary protein content ( $p < 0.05$ ). There were no differences in TP, ALB, HDL and LDL contents. Serum lysozyme activity, alternative complement activity and blood respiratory burst activity were also not affected by dietary protein content.

Micromorphology of the intestine of tilapia is shown in Table 6. Fold height, enterocyte height and microvillus height of intestine were lower when fish fed diet 1. Results indicated that fold height, enterocyte height and microvillus height of intestine were significantly increased with reducing of dietary protein.

**Table 3: Effect on growth and nutrients retention of tilapia fed experimental diets.**

Diet	1	2	3	4
Initial mean body weight, g	15.2±0.06	15.0±0.15	15.1±0.14	15.0±0.01
Final mean body weight, g	105±0.78 <sup>b</sup>	101±1.95 <sup>ab</sup>	100±1.55 <sup>ab</sup>	95.4±2.13 <sup>a</sup>
Weight gain, %	596±3.72 <sup>b</sup>	572±6.21 <sup>ab</sup>	560±12.7 <sup>ab</sup>	536±13.9 <sup>a</sup>
Specific growth rate, % day <sup>-1</sup>	3.46±0.01 <sup>b</sup>	3.40±0.02 <sup>ab</sup>	3.37±0.03 <sup>ab</sup>	3.30±0.04 <sup>a</sup>
Survival, %	100±0.00	98.3±1.67	98.3±1.67	98.3±1.67
Feed conversion ratio	1.01±0.01 <sup>a</sup>	1.06±0.02 <sup>ab</sup>	1.10±0.02 <sup>ab</sup>	1.15±0.03 <sup>b</sup>
Nitrogen retention, %	35.9±0.21 <sup>a</sup>	36.1±0.62 <sup>a</sup>	36.8±0.75 <sup>ab</sup>	38.7±1.00 <sup>b</sup>
Lipid retention, %	128±3.53	128±0.31	129±3.66	130±1.25

Means±S.E.M. of three replicates, Different letters indicate significant differences among groups in the same row. ( $p < 0.05$ ).

**Table 4: Body compositions and morphometric index of fish fed experimental diets for 56 days.**

Diet	1	2	3	4
<b>Whole body Composition (g kg<sup>-1</sup>)<sup>1</sup></b>				
Moisture	742±0.91 <sup>b</sup>	740±0.46 <sup>b</sup>	738±0.95 <sup>b</sup>	735±1.56 <sup>a</sup>
Protein	135±0.22	134±0.55	135±0.33	134±0.49
Lipid	75.0±1.95	76.8±1.15	79.5±0.81	82.2±1.21
<b>Muscle</b>				
Moisture	749±31.6	737±41.1	735±15.3	741±30.2
Protein	180±0.37	181±0.90	179±0.27	179±0.38
Lipid	8.73±0.32 <sup>a</sup>	9.37±0.22 <sup>a</sup>	9.30±0.10 <sup>a</sup>	10.9±0.76 <sup>b</sup>
<b>Liver</b>				
Moisture	706±14.2	687±4.66	683±5.84	681±28.0
Protein	113±0.31	114±0.95	114±0.42	115±0.90
Lipid	42.4±0.75 <sup>a</sup>	43.3±0.58 <sup>a</sup>	43.0±0.27 <sup>a</sup>	48.2±0.26 <sup>b</sup>
<b>Morphometry<sup>2</sup></b>				
Viscerasomatic index	9.76±0.89 <sup>a</sup>	9.97±1.11 <sup>ab</sup>	10.8±0.67 <sup>b</sup>	10.7±1.47 <sup>b</sup>
Hepatopancreasomatic index	2.05±0.33 <sup>a</sup>	2.53±0.51 <sup>b</sup>	2.86±0.45 <sup>bc</sup>	3.10±0.95 <sup>c</sup>
Intraperitoneal fat ratio	0.92±0.36 <sup>a</sup>	1.22±0.40 <sup>ab</sup>	1.24±0.32 <sup>ab</sup>	1.23±0.47 <sup>b</sup>
Condition Factor	4.19±0.38	4.13±0.28	3.98±0.17	4.17±0.29
Spleensomatic index	0.26±0.07	0.22±0.09	0.28±0.10	0.24±0.11

<sup>1</sup> Means±S.E.M. of three replicates, Different letters indicate significant differences among groups in the same row. ( $p < 0.05$ ).

<sup>2</sup> Means±S.E.M. of 18 replicates.

**Table 5: Biochemical compositions of serum and non-specific immune of tilapia fed experimental diets.**

Diet	1	2	3	4
TP g L <sup>-1</sup>	30.6±2.06	33.7±3.66	32.7±3.99	32.5±1.95
ALB g L <sup>-1</sup>	10.4±1.16	11.4±1.27	11.0±1.53	11.0±0.51
CHO mmol l <sup>-1</sup>	2.44±0.34 <sup>a</sup>	3.17±0.26 <sup>ab</sup>	3.25±0.44 <sup>ab</sup>	3.44±0.50 <sup>b</sup>
TG mmol l <sup>-1</sup>	2.00±0.15 <sup>a</sup>	2.49±0.42 <sup>a</sup>	3.97±1.76 <sup>ab</sup>	3.74±0.16 <sup>b</sup>
HDL mmol l <sup>-1</sup>	0.56±0.07	0.57±0.08	0.46±0.19	0.47±0.04

**Table 5 continued:**

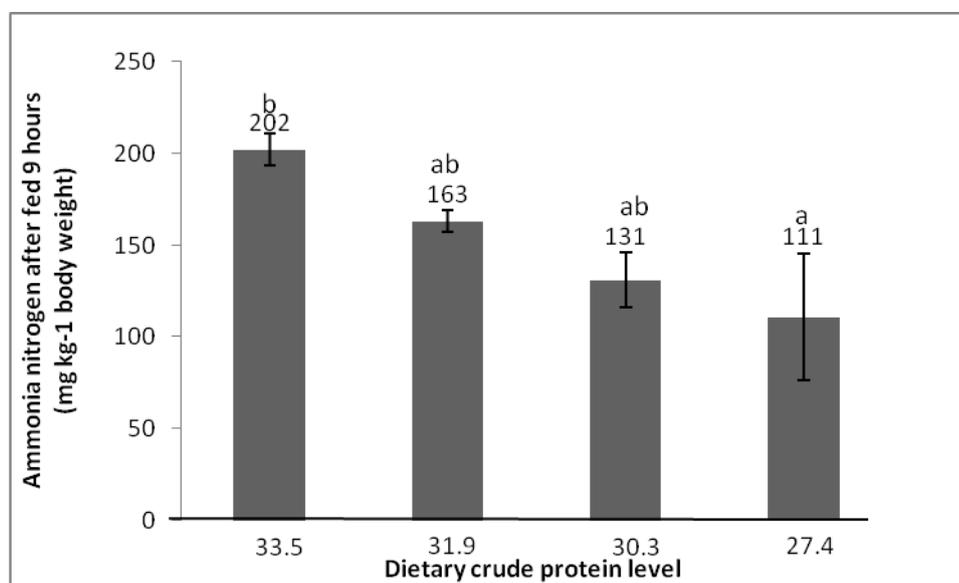
LDL mmol l <sup>-1</sup>	0.13±0.04	0.22±0.04	0.21±0.06	0.26±0.06
Respiratory burst (OD 540 nm)	0.25±0.07	0.25±0.09	0.28±0.08	0.29±0.04
Lysozyme U ml <sup>-1</sup>	256±30.1	239±26.7	219±35.3	250±56.5
ACH50 U ml <sup>-1</sup>	82.7±6.33	100±6.15	86.6±2.70	83.4±3.98

Means±S.E.M. of three replicates, Different letters indicate significant differences among groups in the same row ( $p < 0.05$ ).

**Table 6: Micromorphology of the intestine of tilapia fed with different experimental diets.**

Diet	1	2	3	4
Proximal intestine				
hF(μm):Fold height	738±32.0 <sup>a</sup>	1155±117 <sup>b</sup>	1482±152 <sup>c</sup>	1735±63.8 <sup>c</sup>
hE(μm):Enterocyte height	134±6.07 <sup>a</sup>	192±13.6 <sup>b</sup>	197±8.9 <sup>b</sup>	258±20.6 <sup>c</sup>
hMV(μm):Microvillus height	7.45±0.47 <sup>a</sup>	14.4±0.92 <sup>b</sup>	15.7±1.09 <sup>b</sup>	14.7±0.79 <sup>b</sup>
Mid-intestine				
hF(μm):Fold height	907±59.7 <sup>a</sup>	726±57.6 <sup>a</sup>	1162±93.3 <sup>b</sup>	1231±88.2 <sup>b</sup>
hE(μm):Enterocyte height	182±8.77 <sup>a</sup>	216±29.0 <sup>ab</sup>	236±13.8 <sup>ab</sup>	244±15.0 <sup>b</sup>
hMV(μm):Microvillus height	8.63±0.49 <sup>a</sup>	11.6±0.66 <sup>b</sup>	13.9±1.06 <sup>c</sup>	10.7±0.64 <sup>ab</sup>

Values are means of two fish from each of three replicate groups (10 measurements for each fish). Different letters indicate significant differences among groups in the same row ( $p < 0.05$ ).



**Figure 1: Total ammonia nitrogen discharged into water (mg kg<sup>-1</sup> body weight, mean±SD) after fed 9 hours. Different letters indicate significant differences among groups.**

## Discussion

Dietary protein satisfy fish requirement for essential amino acids, nitrogen need for the synthesis of non-essential amino acids as well as nitrogen-containing molecules (Young, 2000). Insufficient intake of amino acids or proteins

ultimately affects the cells' protein content and eventually incapacitates them, and fish growth performance would be impaired. The optimum growth of fry tilapia was obtained at 45% CP, while fingerling and advanced juvenile indicated optimum growth

performance with the 35%-CP (Abdel-Tawwab *et al.*, 2010). Results of the current trial suggest that protein level in tilapia diets could be reduced from 33.5 to 30.3% (analyzed values) by supplementing with methionine, lysine, tryptophan and threonine on an ideal protein basis. This is a net reduction of 3.2 g of crude protein per 100 g diet based on analyzed nitrogen. Supplementation of essential amino acids to reduce dietary protein content have been widely studied and used in the production of animal industry (Viola, 1991). Dietary crude protein of *Litopenaeus vannamei* could be reduced from 41.26 to 35.52% in the diets as long as synthetic amino acids were supplemented (Huai *et al.*, 2010). Cheng *et al.* (2003) also demonstrated that rainbow trout fed fish meal-based diet containing 37% CP grew as fast as those fed 42% CP diets supplemented with lysine, methionine, threonine, and tryptophan. In fact, there is a general agreement that reducing dietary crude protein content by 2–3% with crystalline amino acid supplementation does not reduce growth performance of growing and finishing pigs (Kerr *et al.*, 2003).

The excretion of TAN was affected by dietary protein content. TAN discharged into the water was decreased by 35% by reducing the dietary protein level from 33.5% to 30.3%, which was directly related to dietary nitrogen and protein intake in teleosts (Engin and Carter, 2001; Yang *et al.*, 2002). In the present study, high dietary protein resulted in higher excretion of ammonia,

which is in agreement with other reports (Cheng *et al.*, 2003; Gan *et al.*, 2012). Viola *et al.* (1992) reported that common carp could reduce nitrogen excretion about 20% by reducing dietary CP from 30% to 25% along with supplementing lysine (0.5%) and methionine (0.3%). In the present experiment, all diets were isoenergetic, the diets with less dietary protein contents had higher dietary starch content. Some researchers demonstrated increasing the dietary content of non-protein digestible energy could increase nitrogen retention by decreasing nitrogen losses (Médale *et al.*, 1995). In this study, protein retention of tilapia was increased with reducing dietary protein content, and ammonia is the major end product of protein catabolism (Elliott, 1976), so TAN excretion of fish was decreased.

In this study, reduction of dietary protein content caused an inverse increase in HSI, IPF and VSI of tilapia. Similar results have also been reported in several other studies (Gan *et al.*, 2012), which indicated that more fat was deposited in both the abdominal cavity and the liver.

The nutritional status could regulate host non-specific immune responses in fish (Oliva-Teles, 2012). Kiron *et al.* (1995) observed serum lysozyme activity and C-reactive proteins of rainbow trout were reduced when fed protein deficient diet, thus negatively affecting non-specific defense mechanisms. It was concluded that adequate protein level is necessary to maintain non-specific defense

mechanisms (Kiron *et al.*, 1993). Qiang *et al.* (2012) found that suitable dietary protein level can increase the non-specific immunity. In the present study, serum lysozyme activity, blood respiratory burst activity and serum ACH50 were not significantly affected by dietary protein content, which indicated that no effects of reducing dietary protein level based ideal amino acids profile on immunity of tilapia.

In this study, fold height, enterocyte height and microvillus height of proximal and middle intestine were significantly increased with reducing of dietary protein. Soybean meal inclusion was reduced from 36% to 18% with reducing of dietary protein content. High soybean meal content of diet can induce an inflammatory response in the distal intestine of fish, characterized by changes in absorptive cells, increased presence of inflammatory cells, endocytic blocking, shortening of villi and disruption of microvilli (Merrifield *et al.*, 2011).

In conclusion, the results of the present study showed dietary protein content of tilapia can be reduced from 33.5% to 30.3% based ideal amino acids profile.

### Acknowledgments

The work was financially supported by the fund of National Natural Science Foundation of China (31202007).

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