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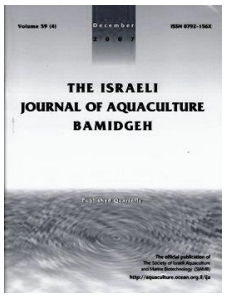
ISSN 0792 - 156X

PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il



Effects of Dietary Isoleucine Levels on the Growth Performance, Feed Utilization, and Serum Biochemical Indices of Juvenile Golden Pompano, *Trachinotus ovatus*

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(Received 15.6.2015, Accepted 30.8.2015)

Key words: *Trachinotus ovatus*, isoleucine requirement, growth performance, serum biochemical parameters

Abstract

A 56-day growth trial was conducted to determine the isoleucine requirement of juvenile *Trachinotus ovatus*. Six diets with different concentrations of L-isoleucine (13.2, 15.7, 18.2, 20.7, 23.2 and 25.7g/kg dry diet, defined as diet Ile-1, diet Ile-2, diet Ile-3, diet Ile-4, diet Ile-5 and diet Ile-6, respectively) were formulated to contain 430g/kg crude protein with fish meal, soybean meal, peanut meal, and pre-coated crystalline amino acids. Each diet was randomly assigned to triplicate treatments of 20 fish (initial body weight 6.36±0.03g) in seawater floating net cages. Results indicated that weight gain increased with increasing isoleucine concentrations up to 18.2g/kg, whereas diets containing higher isoleucine concentration reduced the growth performance significantly (P<0.05). The highest muscle protein content, protein efficiency ratio, body protein deposition, viscerasomatic index, hepatosomatic index, and lowest feed conversion ratio, serum AST activities were also found in 18.2g/kg dietary isoleucine treatment (P<0.05). The highest lipid content of whole fish was found in 15.7g/kg dietary isoleucine treatment (P<0.05). Survival rates in treatments Ile-5 and Ile-6 were significantly lower (P<0.05) than those in other treatments. Results of polynomial regression based on weight gain, feed conversion ratio, protein efficiency ratio and body protein deposition indicated that the optimal dietary isoleucine requirement for *Trachinotus ovatus* reared in seawater floating netcages was 17.39-17.50g/kg isoleucine of dry diet, correspondingly 40.44-40.70g/kg of dietary protein.

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Introduction

Golden pompano (*Trachinotus ovatus*) is a warm-water species (25-32°C), which belongs to the family Carangidae, genus *Trachinotus*. It is widely distributed in China, Japan, Australia, and other countries (Yang, 2006; Niu et al., 2013). The growth rates of most fish species may be highly variable and appear to be limited by food availability. Information on amino acid requirements of an animal provides a basis for evaluation of the nutritional value of protein sources and for formulating feeds. Proteins with a poorly balanced amino acid profile are of low nutritional value while a well-balanced, complete protein has high nutritional value (Abidi and Khan, 2004; Kou et al, 2015). Any single protein source cannot however, provide well-balanced essential amino acids (EAAs) for pompano.

Isoleucine is an essential amino acid with a hydrocarbon side chain. It is involved in many metabolic pathways. It is considered essential for protein synthesis and optimal fish growth. It is also necessary for repair and growth of tissue, maintaining nitrogen balance in the body, and assists in energy production (Abidi and Khan, 2004; Ahmed and Khan, 2006). A deficiency of isoleucine in diets can cause severe biochemical malfunction, including growth retardation (Millamena et al., 1999; Abidi and Khan, 2004; Liu et al., 2014a). Both deficiency and excess of isoleucine reduce utilization of dietary protein. This triggers the excretion of nitrogen which in turn impacts on the environment.

Although supplementation of isoleucine in formulated diets for pompano is necessary, to date the optimal isoleucine requirement has not been reported. In the present study, crystalline amino acids (CAA) were used as supplementation to adjust the concentration of EAAs to match those in 430g/kg whole body protein of golden pompano. This study investigated the effects of dietary isoleucine on the growth performance, feed utilization and serum biochemical indices of golden pompano, in order to determine the isoleucine requirement.

Materials and Methods

Experimental diets. Ingredients and proximate composition of the experimental diets are presented in Table 1. Amino acid composition of diets is showed in Table 2. Six graded concentrations of crystal isoleucine with 0, 2.5, 5, 7.5, 10, 12.5g/kg were added to the diets, defined as diet Ile-1, diet Ile-2, diet Ile-3, diet Ile-4, diet Ile-5, and diet Ile-6, respectively.

Table 1. Proximate composition of the experimental diets.

Diets	*Ile-1*	*Ile-2*	*Ile-3*	*Ile-4*	*Ile-5*	*Ile-6*
Ile content (g/kg)	*13.2*	*15.7*	*18.2*	*20.7*	*23.2*	*25.7*
Ingredient (g/kg)						
Fish meal	150	150	150	150	150	150
Soybean meal	100	100	100	100	100	100
Peanut meal	150	150	150	150	150	150
Wheat flour	226	226	226	226	226	226
Soy protein concentrate	100	100	100	100	100	100
Beer yeast powder	30	30	30	30	30	30
Amino acid mixture*	75.5	75.5	75.5	75.5	75.5	75.5
Fish oil	80	80	80	80	80	80
Soya lecithin	20	20	20	20	20	20
Vitamin premix†	20	20	20	20	20	20
Mineral premix**	20	20	20	20	20	20
Choline chloride (50%)	5	5	5	5	5	5
Phagostimulant	5	5	5	5	5	5
Ascorbic phosphate ester	6	6	6	6	6	6
Crystalline Isoleucine	0	2.5	5	7.5	10	12.5
Crystalline Glutamic acid	12.5	10	7.5	5	2.5	0
Proximate analysis (g/kg dry matter)						
Crude protein	438.6	440.0	439.8	438.3	437.2	444.2
Crude lipid	112.8	113.8	113.0	113.1	113.8	114.0
Ash	91.6	90.9	90.9	90.3	91.3	90.3
Isoleucine (g/kg dry diet)	12.9	15.1	17.5	19.5	21.8	24.3
Isoleucine (g/kg dry protein)	29.4	34.3	39.8	44.5	49.9	54.7

* Amino acid mix (g/kg diet): L-methionine, 8.17; L-lysine, 6.78; L-valine, 5.45; L-arginine, 3.15; L-leucine, 4.39; L-aspartic acid, 14.72; L-alanine, 17.43; L-cystine, 1.00; and L-glycine, 14.41.

† Vitamin premix provides the following per kg of diet: thiamine, 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; cyanocobalamin, 0.1 mg; menadione, 10 mg; inositol, 800 mg; D-calcium pantothenate, 60 mg; nicotinic acid, 200 mg; folic acid1.2 mg; biotin, 32 mg; cholecalciferol, 5 mg; all-rac-a-tocopheryl acetate, 120 mg; Ascorbic phosphate ester, 2.0 g; choline chloride 2.0 g; ethoxyquin, 150 mg; manna-croup, 14.52 g.

** Mineral premix provides the following per kg of diet: NaF, 4 mg; KI, 1.6 mg; $CoCl_2 \cdot 6H_2O$(1%), 100 mg; $CuSO_4 \cdot 5H_2O$, 20 mg; $FeSO_4 \cdot H_2O$, 160 mg; $ZnSO_4 \cdot H_2O$, 100 mg; $MnSO_4 \cdot H_2O$, 120 mg; $MgSO_4 \cdot 7H_2O$, 2.4 g; $ZnSO_4 \cdot H_2O$, 100 mg; $Ca(H_2PO_4)2 \cdot H_2O$, 6.0 g; NaCl, 200 mg; zeolite power, 30.90 g.

Table 2. Amino acid compositions of diets

Diets	*Ile-1*	*Ile-2*	*Ile-3*	*Ile-4*	*Ile-5*	*Ile-6*	*Whole-body protein (g/kg)*
Ile content (g/kg)	*13.2*	*15.7*	*18.2*	*20.7*	*23.2*	*25.7*	
EAA							
Threonine	11.9	11.9	11.9	11.7	11.7	11.8	11.7
Valine	19.3	19.4	19.8	19.5	19.7	19.6	20.3
Methionine	10.8	11.1	11.5	11.2	11.4	11.4	11.3
Leucine	25.0	25.1	25.3	25.1	25.2	25.7	28.0
Phenylalanine	15.4	15.5	15.6	15.4	15.5	15.8	16.1
Histidine	6.0	6.0	6.1	6.0	6.1	6.3	5.8
Lysine	20.5	20.7	20.0	20.0	19.4	20.2	25.3
Arginine	25.4	25.6	25.3	25.1	25.1	25.8	26.5
Isoleucine	12.9	15.1	17.5	19.5	21.8	24.3	19.4
NEAA							
Aspartic acid	46.7	46.8	47.3	46.5	46.2	47.2	38.0
Serine	15.3	15.3	15.3	15.2	15.1	15.4	8.0
Glycine	25.9	25.9	25.5	25.5	25.1	26.0	24.8
Alanine	33.7	33.9	34.2	33.5	33.2	33.9	20.2
Tyrosine	8.1	8.1	8.2	7.9	8.1	8.0	6.6
Proline	18.0	18.1	18.0	18.0	17.8	18.1	13.5
Glutamic acid	73.3	71.1	68.5	65.9	63.4	62.6	55.7

All dry ingredients, except CAA and binders, were finely ground into powder through a 60-mesh sieve and thoroughly mixed with oil and water until homogenous using a Hobart mixer (A-200T Mixer Bench Model unit, Resell Food Equipment, Ottawa, ON, Canada). Soy lecithin was added to a pre-weighed premix of fish oil, and blended until homogenous, and then added to the mixture. The semi-moist mixture was placed in a pelletizer (F-26, South China University of Technology, Guangzhou, China) and extruded through a 2.5-mm-diameter mesh. The diets were air-dried to contain approximately 10% moisture, sealed in bags, and stored in a deep freezer at -20°C until fed.

Experimental procedure. Experimental fish, *Trachinotus ovatus*, were obtained from Shenzhen Trial Base of South China Sea Fisheries Research Institute (Shenzhen, China). Prior to the feeding trial, juvenile fish were acclimated to the experimental conditions for 2 weeks and fed the commercial diet during this period. A total of 360 healthy fish with an initial body weight of 6.36±0.03g were chosen from 1000 fish and then randomly distributed into 18 seawater floating net cages (1m ×1m × 1m, 3 cages per diet, 20 fish per cage). Fish were fed by hand to apparent satiation twice daily (08:00 and 16:00) for 8 weeks. During the 8 week feeding trial, the number and weight of dead fish and feed consumption were recorded every day. During the experimental period, water temperature, salinity, pH, total ammonia-nitrogen, and dissolved oxygen ranged from 21.0 to 29.0 °C,13-17g/L, 7.6 to 7.8,<0.1mg/L and>5mg/L, respectively.

Sampling and analytical methods. At the end of the 8-week experiment, fish were fasted for 24 h before sampling, and then they were counted and weighed. Three fish from each cage were randomly collected for measurement of whole body composition, and another four fish per cage were used for serum and muscle collection after being euthanized with 10mg/L eugenol (Shanghai Medical Instruments Co., Ltd, Shanghai, China). Approximately 1.0mL of blood was withdrawn from the caudal vein into a 2mL sterile syringe. The serum was placed in a 1.5mL Eppendor tube, and then centrifuged at 3000 rpm, for 10min, at 4 °C. The supernatant was stored at -80°C until analysis of total protein (TP), glucose (GLU), triacylglycerol (TG), cholesterol (CHO), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Another three fish per cage were used to determine hepatosomatic index (HSI) and viscerosomatic index (VSI). Viscera and liver were collected and weighed and the ratios were expressed as a percentage of body weight. Then the muscle samples (dorsal muscle on both sides of the fish) were removed and stored frozen (-20°C) until analyzing the composition of the muscle.

Amino acids in diets were determined after acid hydrolysis. For total amino acid content analysis, the diet was freeze-dried overnight, and then hydrolyzed for 24h in 6N HCl at 110 °C. After pretreatment, all the samples were analyzed with an L-8900 amino

acid analyzer (Hitachi, Tokyo, Japan) for free amino acid content analysis. Using fish samples, moisture, crude ash, crude protein, and crude lipid of the experimental diets were determined using standard methods (AOAC, 2005). Moisture was determined by oven-drying at 105°C until reaching constant weight; ash was determined using a muffle furnace (FO610C, Yamato Scientific Co., Ltd, Tokyo, Japan) at 550°C for 8h. Crude protein (N×6.25) was analyzed by Kjeldahl method using Kjeltec (FOSS 2300, Hoganas, Sweden) after acid digestion; crude lipid was determined by petroleum ether extraction using Soxtec TM 205 (FOSS, Hoganas, Sweden). Serum CHO, TG, and GLU contents were measured using the enzymatic (cholesterol oxidase) and colorimetric method, the enzymatic (glycerol phosphate oxidase) and colorimetric (PAP) method, and the glucose oxidase method, respectively, using test kits purchased from Junshi Biotechnology Co., Ltd. (Shanghai, China). Serum ALT, AST, and ALP activities were tested by ROCHE-P800 automatic biochemical analyzer (Roche, Basel, Switzerland). Serum TP content was tested by ROCHE-P800 automatic biochemical analyzer (Roche, Basel, Switzerland).

Statistical analysis. The present experiment was a regression design and all data were expressed by mean ± SD. The significance level (P<0.05) of each variable was first detected by F-test for regression design, and then Duncan's multiple range test was used to rank the treatments. The analysis was performed using SPSS 21.0 (SPSS, Chicago, IL, USA). The optimal isoleucine requirement of juvenile fish was determined using weight gain, feed conversion ratio, protein efficiency ratio and body protein deposition by a polynomial regression method (Zeitoun et al., 1976; Shiau and Lo, 2000).

Results

Growth performance and morphometry index. Effects of dietary isoleucine concentration on growth, feed utilization, and survival of juvenile golden pompano are presented in Figs. 1, 2, 3, and Table 3. Final body weight (FBW) and weight gain (WG) increased with increasing isoleucine concentration up to 18.2g/kg dietary isoleucine treatment (diet Ile-3), whereas the diets containing higher isoleucine concentration reduced the growth performance significantly (P<0.05). The changes of protein efficiency ratio (PER), viscerasomatic index (VSI) and hepatosomatic index (HSI) were similar with WG. Compared to treatment Ile-1, Ile-5 and Ile-6, the PER, VSI and HSI of treatment Ile-3 showed significantly higher values (P<0.05). In contrast, feed conversion ratio (FCR) decreased to 18.2g/kg dietary isoleucine (diet Ile-3), when the isoleucine concentration was above 18.2g/kg, a significant increase was observed (P<0.05). The survival rates in treatment Ile-5 and Ile-6 were significantly lower than those in other four treatments (P<0.05).

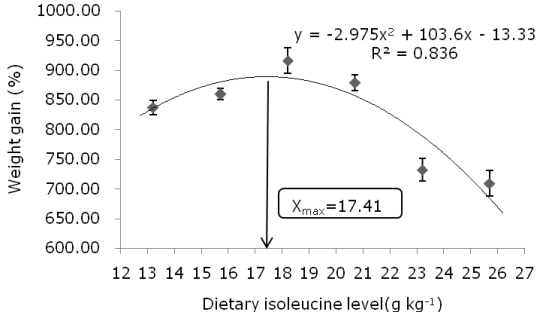

Fig. 1. Relationship between weight gain and dietary isoleucine level for juvenile golden pompano based on polynomial regression method (P<0.05; Weight gain (%)= (final weight – initial weight)×100/(initial weight); the points in this Figure are mean points).

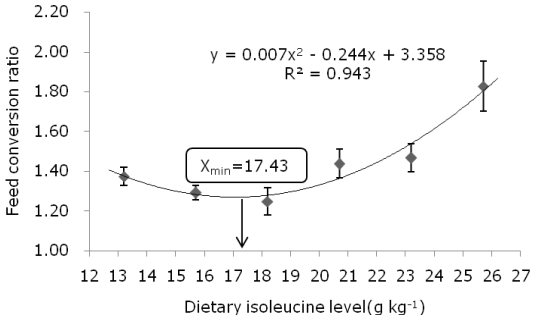

Fig. 2. Relationship between feed conversion ratio and dietary isoleucine level for juvenile golden pompano based on polynomial regression method (P<0.05; Feed conversion ratio= (shrimp weight gain)/(feed intake); the points in this Figure are mean points).

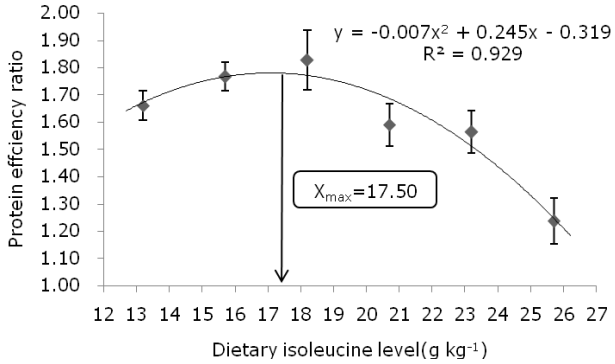

Fig. 3. Relationship between protein efficiency ratio and dietary isoleucine level for juvenile golden pompano based on polynomial regression method (P<0.05; Protein efficiency ratio= (g weight gain)/(g protein fed); the points in this Figure are mean points).

Table 3. Effect of dietary isoleucine levels on growth performance and morphometry index of juvenile golden pompano fed experimental diets for 8 weeks

Diets	*Ile-1*	*Ile-2*	*Ile-3*	*Ile-4*	*Ile-5*	*Ile-6*
Ile content (g/kg)	*13.2*	*15.7*	*18.2*	*20.7*	*23.2*	*25.7*
Survival(%)*	93.33±2.89^{c}	91.67±2.89^{c}	91.67±7.64^{c}	93.33±7.64^{c}	80.00±5.00^{b}	65.00±8.66^{a}
FBW (g) *	59.57±1.07^{b}	61.26±0.77^{bc}	64.85±1.24^{a}	62.15±0.65^{c}	52.82±1.34^{a}	51.35±1.32^{a}
WG(%)†	837.10±12.08^{b}	859.75±9.30^{bc}	915.95±21.76^{d}	879.19±13.21^{c}	731.81±18.77^{a}	709.06±21.79^{a}
FCR‡	1.37±0.05^{ab}	1.29±0.04^{a}	1.25±0.07^{a}	1.44±0.07^{b}	1.47±0.07^{b}	1.83±0.13^{c}
PER**	1.66±0.05^{bc}	1.77±0.05^{cd}	1.83±0.11^{d}	1.59±0.08^{b}	1.56±0.08^{b}	1.24±0.09^{a}
VSI (%)††	5.53±0.13^{b}	5.66±0.07^{cd}	5.79±0.12^{e}	5.74±0.14^{de}	5.58±0.08^{bc}	5.31±0.09^{a}
HSI (%)‡‡	0.95±0.08^{b}	1.00±0.06^{bc}	1.04±0.04^{c}	0.97±0.09^{bc}	0.92±0.08^{b}	0.73±0.05^{a}

^{a,b,c} Values are means ± SD of three replications. Means in the same row with different superscripts are significantly different (P<0.05).

* Survival (%)=100 × (final fish number)/(initial fish number).

* FBW (g fish-1), final mean body weight.

† WG (Weight gain, %)= (final weight – initial weight)×100/(initial weight).

‡ FCR (Feed conversion ratio)= (fish weight gain)/(feed intake).

** PER (Protein effciency ratio)=g weight gain/g protein fed

†† VSI (Viscerasomatic index, %)= viscerosomatic weight×100/ fish weight.

‡‡ HSI (Hepatosomatic index, %)= liver weight×100/ fish weight.

Whole-body and muscle composition. Some parameters of the whole-body and muscle composition of fish were significantly (P<0.05) affected by the isoleucine concentration (Fig. 4 & Table 4).

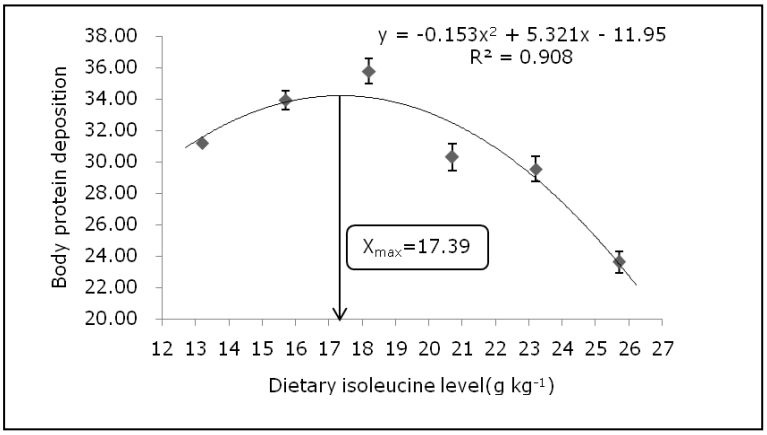

Fig. 4. Relationship between body protein deposition and dietary isoleucine level for juvenile golden pompano based on polynomial regression method (P<0.05; Body protein deposition= [(Final bodyweight × final body crude protein) – (Initial body weight × initial body crude protein)] × 100/ protein fed; the points in this figure are mean points).

Table 4 Effect of dietary isoleucine levels on whole body and muscle composition of juvenile golden pompano fed experimental diets for 8weeks

Diets	*Initial*	*Ile-1*	*Ile-2*	*Ile-3*	*Ile-4*	*Ile-5*	*Ile-6*
Ile content (g/kg)		*13.2*	*15.7*	*18.2*	*20.7*	*23.2*	*25.7*
Whole body (%)							
Moisture	75.82±0.27	65.60±0.27	65.36±0.10	65.40±0.58	65.73±0.12	65.49±0.91	65.68±0.84
Protein	16.08±0.11	18.49±0.47	18.91±0.74	19.23±0.70	18.78±0.41	18.57±0.43	18.74±0.71
Lipid	3.18±0.10	12.82±0.03^{cd}	13.18±0.18^{e}	13.03±0.23^{de}	12.71±0.05^{c}	12.34±0.15^{b}	11.54±0.06^{a}
Ash	4.77±0.07	3.73±0.16	3.70±0.08	3.81±0.09	3.84±0.05	3.73±0.02	3.89±0.15
*Body protein deposition**		31.19±0.15^{c}	33.92±0.60^{d}	35.76±0.79^{e}	30.29±0.85^{bc}	29.54±0.80^{b}	23.61±0.71^{a}
Muscle(%)							
Moisture	--	72.37±0.25	71.76±0.67	72.54±0.67	72.02±0.19	71.69±0.70	72.08±0.24
Protein	--	19.78±0.18^{b}	20.37±0.13^{c}	20.74±0.26^{c}	20.31±0.29^{c}	20.41±0.32^{c}	19.30±0.23^{a}
Lipid	--	6.83±0.34^{b}	6.70±0.54^{b}	6.43±0.39^{b}	6.14±0.57^{b}	6.74±0.61^{b}	5.22±0.46^{a}
Ash	--	1.81±0.07^{b}	1.66±0.13^{ab}	1.51±0.06^{a}	1.53±0.12^{a}	1.57±0.09^{a}	1.56±0.13^{a}

^{a,b,c} Values are means ± SD of three replications. Means in the same row with different superscripts are significantly different (P<0.05).

* Body protein deposition=[(Final bodyweight × final body crude protein) – (Initial body weight × initial body crude protein)] × 100/ protein fed

Although there were no significant differences in the protein content of whole body among treatments, muscle protein content increased with increasing isoleucine concentration up to 18.2g/kg dietary isoleucine treatment (diet Ile-3), whereas the diets containing higher isoleucine concentration reduced the muscle protein content. Muscle protein content of treatments Ile-1 and Ile-6 was significantly lower than that of other treatments (P<0.05). The body protein deposition (BPD) significantly increased with increasing isoleucine concentration up to 18.2g/kg (diet Ile-3), whereas it decreased significantly (P<0.05) in treatments exceeding 18.2g/kg isoleucine. Whole-body lipid significantly increased with increasing isoleucine concentration up to 15.7g/kg (diet Ile-2), and then it decreased significantly (P<0.05). The muscle lipid content of treatment Ile-6 was significantly lower (P<0.05) than that of other treatments. The muscle ash content of fish fed with diet Ile-1 was higher than that of fish fed diets Ile-3~Ile-6 (P<0.05), but there was no difference among other treatments (P>0.05). There was no significant difference in whole-body moisture, whole-body ash and muscle moisture (P>0.05).

Serum biochemical parameters. Significant differences were found in the serum biochemical parameters of fish fed diets containing different concentrations of isoleucine (Table 5). The activity rates of AST decreased significantly with increasing isoleucine concentration up to 18.2g/kg, beyond which an increase was observed (P<0.05). The serum ALP activity rates of fish fed with diet Ile-4 was particularly lower than that of fish fed with diets Ile-2, Ile-5 and Ile-6 (P<0.05). The serum GLU, TP and TG content of fish fed diet Ile-6 was significantly lower than those of fish in other treatments (P<0.05). At the same time, the serum CHO content of treatment Ile-6 was lowest among six treatments, but it was only significantly lower than treatment Ile-2 (P<0.05). There was no significant difference in serum ALT activity rates (P>0.05).

Table 5. Effect of dietary isoleucine levels on several serum biochemical indices of juvenile golden pompano fed experimental diets for 8weeks

Diets	*Ile-1*	*Ile-2*	*Ile-3*	*Ile-4*	*Ile-5*	*Ile-6*
Ile content (g/kg)	*13.2*	*15.7*	*18.2*	*20.7*	*23.2*	*25.7*
AST(U/L)	67.33±3.06^{c}	70.33±6.11^{c}	40.33±1.53^{a}	47.67±2.52^{a}	56.00±6.56^{b}	72.33±5.13^{c}
ALT(U/L	4.33±1.53	3.67±1.53	2.33±1.53	2.67±1.15	4.33±0.58	4.67±1.15
ALP(U/L)	57.00±6.00^{ab}	59.67±5.51^{b}	56.33±7.02^{ab}	48.00±7.00^{a}	62.33±2.52^{b}	61.67±3.79^{b}
TP(g/L)	34.33±1.43^{bc}	35.03±0.49^{c}	32.30±1.48^{b}	32.10±1.87^{b}	33.83±1.50^{bc}	24.20±0.17^{a}
GLU(mmol/L)	14.43±1.02^{bc}	13.82±0.65^{b}	14.56±0.45^{c}	13.40±0.35^{b}	15.31±0.64^{c}	6.11±0.78^{a}
CHO(mmol/L)	4.86±0.37^{ab}	5.32±0.41^{b}	4.86±0.50^{ab}	4.65±0.36^{ab}	4.54±0.23^{a}	4.42±0.45^{a}
TG(mmol/L)	1.62±0.19^{c}	1.47±0.20^{bc}	1.47±0.13^{bc}	1.33±0.06^{bc}	1.22±0.15^{b}	0.68±0.15^{a}

^{a,b,c} Values are means ± SD of three replications. Means in the same row with different superscripts are significantly different (P<0.05).

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, Alkaline phosphatase; TP, total protein; GLU, glucose; CHO, total cholesterol; TG, triglycerides.

On subjecting the WG, FCR, PER and BPD data to regression analysis (Zeitoun et al., 1976; Shiau and Lo, 2000), the break points were evident at 17.41, 17.43, 17.50, 17.39g/kg isoleucine of dry diet, corresponding to 40.49, 40.53, 40.70 and 40.44g/kg of dietary protein, respectively (Fig.1, 2, 3 and 4). The WG (Y_1), FCR (Y_2), PER (Y_3) and BPD (Y_4) to dietary concentration of isoleucine (x) relationship was estimated by the following second-degree polynomial regression equations:

$Y_1 = -2.975x^2 + 103.6x - 13.33$ (with $P<0.05$, $R^2 = 0.836$)

$Y_2 = 0.007x^2 - 0.244x + 3.358$ (with $P<0.05$, $R^2 = 0.943$)

$Y_3 = -0.007x^2 + 0.245x - 0.319$ (with $P<0.05$, $R^2 = 0.929$)

$Y_4 = -0.153x^2 + 5.321x - 11.95$ (with $P<0.05$, $R^2 = 0.908$)

On the basis of above polynomial equations, the optimal dietary isoleucine requirement for *Trachinotus ovatus* was 17.39-17.50g/kg isoleucine of dry diet, corresponding to 40.44-40.70g/kg of dietary protein.

Discussion

The present study used CAA as the supplement to adjust the EAA contents of each diet to the same levels as those in 430g/kg whole body protein of golden pompano, in order to investigate the effects of dietary isoleucine on the growth and feed utilization of golden pompano in seawater floating net cages. The growth performance of golden pompano indicated a positive response to the dietary isoleucine supplementation, with dietary isoleucine imbalance leading to reduced growth rate and feed conversion ratio as previously reported (Millamena et al., 1999; Ahmed and Khan, 2006; Abidi and Khan, 2007; Shang et al., 2009). Diets containing less than the optimum amount of isoleucine resulted in a reduced growth rate, as protein efficiency ratio was poor. Fish fed diets with excess nitrogen had less energy for growth and nutrient deposition, and the removal of surplus amino acids was an energy-consuming process (Macleod, 1997). The highest and lowest amounts of isoleucine in diets could affect the utilization of leucine and valine as a disproportionate amino acid (Coloso et al., 1999), and reduced whole protein deposition in muscle. Thus, the muscle protein content of treatments Ile-1 and Ile-6 was significantly lower than that of other treatments.

Part of the whole-body and muscle composition of golden pompano was significantly affected by the isoleucine concentration. Similar effects had been reported in other fish species (Ahmed and Khan, 2006; Abidi and Khan, 2007; Shang et al., 2009; Liu et al., 2014a). Results from in vivo studies have suggested that isoleucine along with leucine and valine can regulate skeletal muscle protein metabolism (Jose et al., 2006) and increase intramuscular fat (Yu et al., 2007). But it has also been reported that protein synthesis can only be stimulated in the presence of a high supply of balanced amounts of essential amino acids (Norren Van et al., 2009). Muscle protein deposition during the present study significantly increased with increasing isoleucine concentration up to 18.2g/kg, whereas it significantly decreased in the treatments exceeding 23.2g/kg isoleucine. Whole-body lipids significantly decreased with increasing isoleucine concentrations up to 25.7g/kg (diet Ile-6) therefore excess dietary isoleucine would inhibit protein synthesis, impair normal protein metabolism, and reduce the body nutrient deposition. This is supported by significant changes of TP in serum between different isoleucine treatments in the present study, suggesting that an imbalance of dietary amino acid levels would alter protein metabolism (Coloso et al., 1999). When dietary isoleucine concentration is excessive, protein synthesis decreases while catabolism is increased, thereby inhibiting protein deposition and fish growth (Shang et al., 2009).

Dietary isoleucine requirements in the present study was estimated using the dose-response curve, considered a principal method for determining requirements (Cowey, 1995). Amino acid requirements derived by dose-response curve depend not only on the amount of dietary crude protein (CP) available for growth, but also on the balance of CP in the test diets. In the present study, the optimal isoleucine requirement of juvenile golden pompano was 17.39-17.50g of isoleucine per kg. of dry diet (corresponding to 40.44-40.70g/kg of dietary protein). This was determined using WG, FCR, PER, and BPD data analyzed by a polynomial regression method. This value was close to the requirements of *Chanos chanos* Forsskal (Borlongan and Coloso, 1993), *Labeo rohita*

(Abidi and Khan, 2007) and *Ctenopharyngodon idella* (Shang et al., 2009), which were determined as 40, 38.0-39.8, and 40.0-42.3g/kg isoleucine of dietary protein, respectively. The isoleucine requirement varies from 22.0 to 32.0g/kg protein among some other species, such as Chinook salmon, Rohu, and Nile tilapia (Ahmed and Khan, 2006), and the recommended requirement in the present study was much higher than this range. The large variation observed in requirements of isoleucine among different fish species may be due to differences in the fish species and the methodologies used to determine the requirement, such as salinity, protein quality, dietary energy, feeding rate, and availability of amino acid and amino acid sources (Millamena et al., 1999; Saoud et al., 2003; Abidi and Khan, 2004; Ahmed and Khan, 2006).

AST, ALT and ALP, the general indicators of fish liver function (Deng et al., 2010), were analyzed in the present study. The activity rates of AST and ALP decreased significantly with increasing isoleucine concentration up to 18.2g/kg, beyond which an increase was observed, suggesting that the liver function of fish was significantly influenced by the isoleucine concentration and optimal isoleucine concentration could help maintain normal function in liver. The changes of AST and ALP were consistent with not only the growth of fish, but also mortality. Diet-related mortality was observed in dietary isoleucine of 23.2-25.7g/kg, which was not found in *Cirrhinus mrigala* (Hamilton) (Ahmed and Khan, 2006) and *Labeo rohita* (Abidi and Khan, 2007). However, Extensive necrosis was found in the hepatopancreas of shrimp fed diets containing histidine at levels beyond that found in the tissue protein (Recodo 1991). Excess amount of dietary leucine was found to decrease survival rate of Pacific white shrimp (Liu et al. 2014b).

Conclusions

In conclusion, results of the present study indicate that both deficiencies and excessive amounts of dietary isoleucine can decrease growth performance of golden pompano (*Trachinotus ovatus*), and excess of dietary isoleucine will reduce survival, protein efficiency ratio and body nutrient deposition of fish. On subjecting the WG, FCR, PER and BPD data to regression analysis, the optimal dietary isoleucine requirement for *Trachinotus ovatus* reared in seawater floating net cages was determined to be 17.39-17.50g/kg isoleucine of dry diet, corresponding to 40.44-40.70g/kg of dietary protein.

Acknowledgement

The work was financially supported by the Special Scientific Research Funds for Central Non-profit Institutes, Chinese Academy of Fishery Sciences Special Scientific Research Funds for Central Non-profit Institutes, Chinese Academy of Fishery Sciences (2014ZD02), Special Scientific Research Funds for Central Non-profit Institutes, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (2014YD01), the Technology Extension Project of Fisheries Service and Fisheries Technology Extension Station of Shenzhen Municipality ([2014] No-34), and the Open Fund of Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization of the Ministry of Agriculture (KF201311).

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