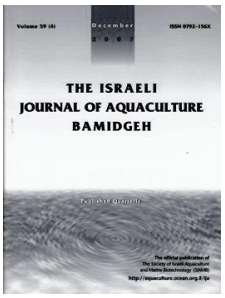

Dietary Protein Requirement of Juvenile Ide, *Leuciscus idus* in Relation to Growth Performance, Whole-body Composition and Plasma Parameters

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Keywords: Ide *Leuciscus idus*; Protein; Growth performance; Whole-body composition

Abstract

A 60-day feeding trial was conducted to determine the dietary protein requirements of juvenile ide, *Leuciscus idus*. Six iso-energetic experimental diets were formulated to contain graded protein levels ranging from 26.5% to 50.9% of dry diet. At the end of feeding trial, final weight (FW), weight gain (WG), and specific growth rate (SGR) increased significantly with increasing dietary protein levels up to 36.4%, and thereafter decreased with further increase of dietary protein levels ($P<0.05$). Feed conversion ratio (FCR) showed a converse trend ($P<0.05$). Hepatosomatic index and viscerosomatic index were negatively related to dietary protein levels ($P<0.05$). Increase of dietary protein levels significantly improved whole-body protein content and decreased lipid content ($P<0.05$). White blood cell counts, red blood cell, hemoglobin, and hematocrit, were independent of dietary protein levels ($P>0.05$). Plasma protein content was not significantly affected by dietary protein levels ($P>0.05$), while plasma triglyceride and glucose concentrations were decreased significantly with increasing dietary protein levels ($P<0.05$). The highest aspartic transaminase activity was observed in fish fed diet 26.5% protein, and the lowest value was observed in 36.4% protein group ($P<0.05$). Based on SGR and FCR, the dietary protein requirement of juvenile ide was determined to be 36.9%-37.7% of dry diet. Dietary methionine and lysine levels for optimal growth was calculated to be 0.83% and 2.25% of dry diet, respectively.

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Introduction

The ide (*Leuciscus idus*) is a freshwater fish species of the family *Cyprinidae* which is distributed across northern Europe and Asia (Rohtla et al., 2015). This fish species is an important natural component of the aquatic ecosystem, and is popular in sport fishing (Błachuta, 1998). Aquaculture of this species began with the success of artificial propagation. Ide is considered one of the most promising cultured fish species in China because of its rapid growth, and high market price. However, information about its nutritional requirements is limited. Formulated feed production of ide relies on formulae for grass carp *Ctenopharyngodon Idella,* or common carp *Cyprinus carpio*. Basic nutritional research is needed to find optimal feeds for this species.

Protein is an essential component in fish diets and plays an important role in supplying energy and amino acids for fish growth. It is the most expensive compound in fish diets, and excess dietary protein levels results in high nitrogen emission into ambient waters, which in turn affects feed intake and fish growth (Webb and Gatlin, 2003; Mohanta et al., 2007; Zehra and Khan, 2012). The protein requirement corresponding to essential amino acids (EAA) profile is generally the first nutritional parameter to be determined for a cultured fish species, and this information provides the basis for formulation of commercial diets when little knowledge is available on EAA, lipid, and other nutrient requirements for this fish species (NRC, 2011).

To the best of our knowledge, there are no available studies concerning dietary protein requirements for ide. Thus, the aim of the present study was to investigate the effects of dietary protein levels on growth, whole-body composition, and plasma parameters of juvenile ide, and to determine the optimum dietary protein level for this species with semi-purified diets.

Materials and methods

Diet preparation. Formulation of the experimental diets are presented in Table 1. Six iso-energetic diets were formulated to contain graded levels of protein (26.5%, 31.5%, 36.4%, 41.2%, 46.0% and 50.9% of dry weight, respectively) using fish meal, casein and gelatin as protein sources, and fish oil as the lipid source. These protein levels were chosen based on the results of the protein requirements of other *Cyprinidae* species (Deng et al, 2013; NRC, 2011). The ratio of casein and gelatin was 4:1 to provide a balanced dietary amino acid profile (Deng et al., 2011). Corn starch levels were adjusted accordingly to make the diets iso-energetic. All the ingredients were ground into powder and thoroughly mixed with fish oil and water, then forced though a pelletizer (4-2 style, Xinchang machinery LTD, China) and dried in a ventilated oven at 30°C. After drying, all diets were sealed in bags and stored at -15°C until further use.

Experimental procedure. Fish husbandry was conducted in an indoor re-circulating freshwater system consisting of 18 fiberglass tanks (300L) with equal supplemental aeration (Wuxi, China). Juvenile ide were obtained from a commercial farm (Tianjin, China). Prior to the feeding trial, the fish were fed a commercial diet (35% crude protein and 5% crude lipid) for 2 weeks to acclimate to the experimental conditions. After fasting for 24 h, juvenile fish (initial weight 33 g) were randomly sorted into eighteen tanks with 15 fish per tank. Each diet was randomly assigned to triplicate tanks. Fish were hand-fed three times daily at 8:00, 12:00, and 16:00, until apparent satiation, on the basis of visual observation, 7 days a week. During the 60 day feeding trail, water quality parameters were kept as follows: temperature was constant (24±1°C), pH was 7.0-7.5, ammonia nitrogen was lower than 0.05 mg/L, and dissolved oxygen was not less than 6.0 mg/L. Photoperiod was natural (light-dark cycle) throughout the experiment. No mortality was observed in all dietary treatments during the 60 day feeding trial.

Table 1. Formulation and proximate composition of the experimental diets (% dry matter)

Ingredients	*Diet Number*					
	1	*2*	*3*	*4*	*5*	*6*
Fish meal[1]	19.0	19.0	19.0	19.0	19.0	19.0
Casein[1]	12.0	16.5	21.0	25.5	30.0	34.5
Gelatin[1]	3.00	4.13	5.25	6.38	7.50	8.63
Fish oil	2.00	2.00	2.00	2.00	2.00	2.00
Soybean lecithin	2.50	2.50	2.50	2.50	2.50	2.50
Vitamin premix3	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix4	2.00	2.00	2.00	2.00	2.00	2.00
Corn starch	40.0	33.5	27.0	20.5	14.0	7.50
Cellulose	5.34	6.22	7.09	7.97	8.84	9.72
Carboxymethyl cellulose	8.00	8.00	8.00	8.00	8.00	8.00
Ethoxyquin	0.01	0.01	0.01	0.01	0.01	0.01
$Ca(H_2PO_4)_2$	2.00	2.00	2.00	2.00	2.00	2.00
Bentonite	2.00	2.00	2.00	2.00	2.00	2.00
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin C	0.05	0.05	0.05	0.05	0.05	0.05
Proximate analysis (% of dry diet)						
Crude protein	26.5	31.5	36.4	41.2	46.0	50.9
Crude lipid	6.30	6.20	6.24	6.24	6.25	6.31
Gross energy (KJ g-1)5	15.6	15.6	15.7	15.7	15.7	15.7
Essential amino acid						
Arginine	1.13	1.41	1.61	1.81	2.01	2.22
Histidine	0.62	0.80	0.91	1.01	1.11	1.21
Isoleucine	1.06	1.26	1.42	1.63	1.81	2.02
Leucine	1.81	2.31	2.64	3.08	3.41	3.70
Lysine	1.57	1.89	2.18	2.42	2.69	3.01
Methionine	0.58	0.66	0.78	0.89	1.02	1.12
Phenylalanine	1.06	1.23	1.45	1.65	1.85	2.03
Threonine	0.81	1.06	1.23	1.42	1.51	1.73
Valine	1.24	1.55	1.80	2.02	2.31	2.53

[1]Casein, obtained from Hua'an Biological Products Lit. (Gansu, China), crude protein 90.0%; gelatin, obtained from Zhanyun chemical Lit. (Shanghai, China), crude protein 90.3%; fish meal, obtained from Copeinca (Lima, Peru), crude protein 68.4%, crude lipid 10.3%.
[2]Vitamins premix (IU or mg/kg of diet): vitamin A, 25000 IU; vitamin D3, 20000 IU; vitamin E, 200 mg; vitamin K3, 20 mg; thiamin, 40 mg; riboflavin, 50 mg; calcium pantothenate, 100mg; pyridoxine HCl, 40mg; cyanocobalamin, 0.2 mg; biotin, 6mg; folic acid, 20 mg; niacin, 200mg; inositol, 1000mg; Vitamin C, 2000 mg; Choline, 2000 mg and cellulose was used as a carrier.
[3]Mineral premix (g/kg of diet): calcium biphosphate, 20g; sodium chloride, 2.6; potassium chloride, 5g; magnesium sulphate, 2g; ferrous sulphate, 0.9g; zinc sulphate, 0.06g; cupric sulphate, 0.02; manganese sulphate, 0.03g; sodium selenate, 0.02g; cobalt chloride, 0.05g; potassium iodide, 0.004; and zeolite was used as a carrier.
[4]Digestible energy calculated based on 23.6 kJ/g for protein, 39.5 kJ/g for lipid and 17.2 kJ/g for starch (Ye et al., 2017).

Sample collection. At the end of the feeding trial, total numbers and mean body weight of fish in each tank were determined after fasting 24h. Five fish per tank were euthanized using MS-222 (100mg/L), and then blood samples were collected immediately from the caudal vein using heparinized syringes. Following centrifugation (3500×g, 10 min, 4°C), the plasma was separated. All the samples were stored at -80°C until analysis. Per cage, ten fish at the beginning, and five fish at the end of the experiment were sampled and stored at -20°C for analysis of whole-body composition.

Laboratory analysis. Dry matter, crude protein, and lipid were determined according to the established methods of AOAC (2003): dry matter after drying in an oven at 105°C until constant weight; crude protein (N×6.25) by Kjeldahl method after acid digestion; lipid by ether extraction using Soxhlet. Amino acid concentrations were determined by a professional laboratory (Jiangnan University, Wuxi, China). Diets were freeze-dried overnight, and then hydrolyzed for 24 hours in 6 N HCl at 110°C. After pretreatment, all

samples were analyzed with an Agilent-1100 amino acid determination system (Agilent Technologies Co., Ltd., Santa Clara, USA). Tryptophan could not be detected after acid hydrolysis.

Red blood cell (RBC) count, white blood cell (WBC), hemoglobin (HGB), and hematocrit (HCT) were measured using an Auto Hematology Analyzer (BC-5300Vet, Mindray, PR China) with a test kit from Shenzhen Mindray Medical International Co. Ltd., PR China. Aspartate aminotransferase (AST) activity was determined by a colorimetric test kit (Mindray Bio Medical Co., Ltd., Shenzhen, China) according to Reitman and Frankel (1957). Analyses of glucose, total protein, and total triglyceride levels in plasma were carried out with an automatic biochemical analyzer (Mindray BS-400, Mindray Medical International Ltd., Shenzhen, China) as in the previous description (Ren et al., 2015).

Statistical analysis. Data were transformed if necessary after evaluating assumptions of normality, equality of variances, and outliers, and subjected to one-way analysis of variance (ANOVA) using the software of the SPSS 13.0 for Windows. Significant differences in the means between dietary treatments were evaluated by Tukey's multiple range test. Mean differences were considered significant at a P value equal or less than 0.05. The second-order polynomial regression model (Zeitoun et al., 1976) was used to estimate the optimum dietary protein requirement for juvenile ide on the basis of SGR and FCR after comparing the estimation coefficient (R2) between broken-line regression model and second- order polynomial regression model.

Results

Growth performance. Results of growth performance of the juvenile ide are presented in Table 2. The lowest final weight (FW, 71.8g), weight gain (WG, 115.5%), and specific growth rate (SGR, 1.28 %/day) were observed in fish fed a diet which contained 26.5% protein (P<0.05). FW, WG, and SGR values significantly increased with increasing dietary protein levels up to 36.4% of dry diet, and thereafter decreased (P<0.05). Feed conversion ratio (FCR) presented a reverse trend (P<0.05). Hepatosomatic index (HSI) and viscerosomatic index (VSI) decreased with the increasing dietary protein levels (P<0.05).

Table 2. Growth performance of juvenile ide fed with the experimental diets

Protein	*IW (g)*[1]	*FW (g)*[2]	*WG (%)*[3]	*SGR (%/day)*[4]	*FCR (%)*[5]	*HSI (%)*[6]	*VSI (%)*[7]
26.5	33.3±0.08	71.8±0.81^{a}	115.5±2.30^{a}	1.28±0.02^{a}	2.36±0.03^{d}	2.09±0.12^{b}	9.82±0.91^{b}
31.5	33.2±0.04	77.6±0.70^{bc}	133.8±2.14^{bc}	1.42±0.02^{bc}	2.12±0.04^{ab}	2.00±0.11^{b}	9.65±0.27^{b}
36.4	33.9±0.06	79.3±0.84^{c}	137.5±2.59^{c}	1.44±0.02^{c}	2.04±0.04^{a}	1.89±0.06^{b}	9.53±0.29^{b}
41.2	33.3±0.10	76.9±0.96^{bc}	130.7±2.69^{bc}	1.39±0.02^{bc}	2.17±0.05^{ab}	1.79±0.18^{b}	8.37±0.40^{a}
46.0	33.1±0.10	73.6±0.77^{ab}	121.6±2.36^{ab}	1.33±0.03^{ab}	2.25±0.06^{bc}	1.71±0.12^{b}	8.21±0.45^{a}
50.9	33.4±0.13	73.1±0.77^{ab}	118.7±2.52^{ab}	1.31±0.03^{ab}	2.30±0.05^{cd}	1.32±0.13^{a}	7.62±0.26^{a}

Mean values and standard error (M±SE) are presented for each parameter. Significant differences within the diets are indicated by different superscript letters (P<0.05)

[1]IW (g): initial weight.

[2]FW (g): final weigh.

[3]WG (%):weight gain=100 × (final weight -initial weight) /initial weight.

[4]SGR (%/day): specific growth rate=100×((ln FBW−ln IBW)/ experimental days).

[5]FCR (%): feed conversion ratio=100×dry feed intake /wet weight gain.

[6]HSI (%): hepatosomatic index = 100 × (liver weight /body weight).

[7]VSI (%): viscerosomatic index = 100 × (visceral weight/body weight).

Using the second-order polynomial regression model, the optimal dietary protein level for juvenile ide was estimated to be 36.9% of dry diet ($y = -0.0009x^2 + 0.0665x + 0.1548$, $R^2 = 0.6555$, Fig.1) and 37.5% of dry diet ($y = 0.0016x^2 - 0.1205x + 4.3959$, $R^2 = 0.6141$, Fig. 2) on the basis of SGR and FCR, respectively. Based on SGR, dietary methionine and lysine level for optimal growth was 0.83% of dry diet ($y = -1.7242x^2 + 2.8632x + 0.2338$, $R^2 = 0.6094$) and 2.25% of dry diet ($y = -0.2444x^2 + 1.1022x + 0.1738$, $R^2 = 0.6444$), respectively.

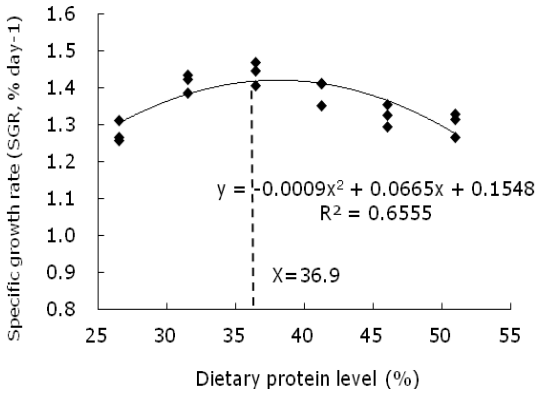

Figure 1. Protein requirement for juvenile ide *Leuciscus idus* calculated by second-order polynomial regression analysis of specific growth rate (SGR) against dietary protein levels.

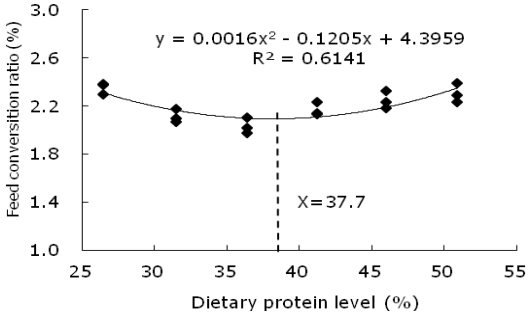

Figure 2. Protein requirement for juvenile ide *Leuciscus idus* calculated by second-order polynomial regression analysis of feed conversion ratio (FCR) against dietary protein levels.

Whole-body composition. Whole-body composition is presented in Table 3. No significant differences were observed in whole-body moisture and ash content between the different dietary treatments ($P>0.05$). Significantly lower whole-body protein content was observed in fish fed 26.5% protein diets compared to those fed diets with 36.4-50.9% protein ($P<0.05$). A decreasing trend of whole-body lipid was found in fish fed diets with increasing protein levels ($P<0.05$).

Table 3. Whole-body composition (wet basis) of juvenile ide fed with the experimental diets

Protein level (%)	*Moisture (%)*	*Protein (%)*	*Lipid (%)*	*Ash (%)*
26.5	67.9±0.58	17.1±0.09^{a}	12.5±0.31^{bc}	3.44±0.23
31.5	65.9±0.73	17.8±0.19^{ab}	12.7±0.16^{c}	3.39±0.24
36.4	66.3±0.27	18.3±0.23^{b}	11.6±0.16^{b}	3.53±0.26
41.2	66.8±0.13	18.2±0.18^{b}	9.82±0.18^{a}	3.54±0.28
46.0	67.9±0.59	18.2±0.18^{b}	9.60±0.33^{a}	3.48±0.25
50.9	67.6±0.44	18.1±0.25^{b}	10.2±0.13^{a}	3.49±0.22

Mean values and standard error (M±SE) are presented for each parameter. Significant differences within the diets are indicated by different superscript letters ($P<0.05$).

Hematological parameters. There were no significant differences ($P>0.05$) in the hematological parameters including white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), and hematocrit (HCT) among the groups (Table 4).

Table 4. Blood hematological parameters of juvenile ide fed with the experimental diets

Protein level (%)	*WBC (10^9/L)*	*RBC (10^{12}/L)*	*HGB (g/L)*	*HCT (%)*
26.5	195.7±3.63	1.65±0.05	95.5±2.56	25.7±1.19
31.5	202.5±5.53	1.64±0.03	98.6±3.45	27.4±2.24
36.4	201.3±2.85	1.66±0.03	98.4±1.97	26.5±1.65
41.2	207.0±8.10	1.53±0.10	92.5±3.48	24.9±2.46
46.0	211.0±4.74	1.68±0.06	101.3±2.45	26.9±2.51
50.9	200.9±5.02	1.58±0.09	93.7±3.52	28.1±1.56

White blood cell (WBC), red blood cell (RBC), hemoglobin (HGB) and hematocrit (HCT).
Mean values and standard error (M±SE) are presented for each parameter. Significant differences within the diets are indicated by different letters ($P<0.05$).

Plasma parameters. Results of plasma parameters are presented in Table 5. Plasma total protein content was not significantly affected by dietary protein levels ($P>0.05$). The highest AST activity was observed in fish fed diet containing 26.5% protein and the lowest value was observed in fish fed diets with 36.4% protein ($P<0.05$). A decreased

trend was found in plasma glucose and total triglyceride content with the increasing dietary protein levels ($P<0.05$).

Table 5 Plasma parameters of juvenile ide fed with the experimental diets

Protein level (%)	*AST (U/L)*	*TP (g/L)*	*TG (mmol/L)*	*Glucose (mmol/L)*
26.5	591.8±49.06^{b}	37.6±1.75	8.99±0.83^{b}	16.5±1.09^{b}
31.5	567.0±41.62^{ab}	40.6±1.52	8.53±0.52^{ab}	16.2±1.27^{ab}
36.4	422.4±31.63^{a}	41.7±0.83	7.57±0.66^{ab}	13.5±0.83^{ab}
41.2	480.8±39.79^{ab}	39.6±1.67	7.46±0.51^{ab}	12.7±1.22^{ab}
46.0	440.3±27.11^{ab}	40.0±1.66	7.61±0.35^{ab}	11.9±0.70^{a}
50.9	497.6±34.49^{ab}	38.8±2.25	6.91±0.61^{a}	12.4±0.99^{ab}

Aspartate aminotransferase (AST), total protein (TP) and total triglyceride (TG).
Mean values and standard error (M±SE) are presented for each parameter. Significant differences within the diets are indicated by different superscript letters ($P<0.05$).

Discussion

In the present study, FW, WG, and SGR of juvenile ide were significantly affected by dietary protein levels. This has been well documented in scientific literature for other fish species (Parazo, 1990; Tibbetts et al., 2000; Yang et al., 2003; Kim and Lee, 2005; Kaushik and Seiliez, 2010). Feed conversion ratio of juvenile ide in the present study decreased in relation to increasing dietary protein levels; the lowest FCR was found in fish fed a diet with 36.4% protein, and thereafter showed an increasing trend. Similar results have been noted in other fish species, such as in olive flounder *Paralichthys olivaceus* (Kim et al., 2002), tiger puffer, *Takifugu rubripes* (Kim and Lee, 2009), and silver barb, *Puntius gonionotus* (Mohanta et al., 2008). The present study indicated that it is necessary to provide adequate dietary protein for the growth of juvenile ide. However, it is also important to avoid excessive dietary protein which inhibits growth and feed utilization, and increases the cost of diets for juvenile ide.

Dietary protein requirements for juvenile ide ranging between 33g-80g is estimated to be 36.9-37.7% of dry diet based on SGR and FCR in this study; this is in the range of dietary requirements for omnivorous fish species. The present result is slightly lower than that reported for common carp (38%; NRC, 2011), gibel carp, *Carassius Auratus Gibelio* (40%; Yun et al., 2015), barbless carp, *Cyprinus pellegrini*, (37.3-43.6%), (Deng et al., 2013), and higher than the reports for silver barb (31.8%; Mohanta et al., 2008). However, dietary protein requirements were influenced by dietary EAA profile. The protein requirement corresponds to the well-documented requirements for specific EAA, especially limiting EAA such as methionine and lysine (NRC, 2011). In this study, dietary EAA was well balanced by modifying the ratio of protein sources, and the dietary methionine and lysine level for optimal growth of juvenile ide was calculated to be 0.83% and 2.25% of dry diet, respectively, which is similar to common carp (methionine 0.8% of diet and lysine 2.2% of diet; Nose, 1979). The present result is the first quantitative nutrient requirement data reported for the ide. Establishment of dietary protein levels for optimal growth of juvenile ide contributes to the development of specific feeds for the developing fish.

In this study, the increase of dietary protein levels significantly reduced HSI and VSI levels of juvenile ide. Similar results have been found in haddock, *Melanogrammus aeglfinus* (Kim and Lall, 2001), and obscure puffer, *Takifugu obscurus* (Ye et al., 2017). In our study, our results were not unexpected as the dietary starch levels were reduced with increasing dietary protein levels in order to keep the experimental diets iso-energetic. It is accepted that increasing dietary carbohydrate improves glycogen levels in fish liver, and the increase of water associated with glycogen in the liver causes higher HSI (Bergot, 19790, while carbohydrates can be converted to lipids (Brauge et al., 1995) and deposited in viscerosomatic tissues of fish which might then lead to higher VSI value.

In the present study, dietary protein levels significantly affected whole-body composition of the ide. The lowest whole-body protein content was found in fish fed a diet with the lowest dietary protein level. Similar results have been reported for other fish species (Ahmad, 2008; Kim and Lee, 2009; Zhang et al., 2010). However, there are no further increases of whole-body protein when the dietary protein level exceeds 36.4%, suggesting that excess that dietary protein is not used for body protein synthesis in juvenile ide.

In this study, whole-body lipid content of juvenile ide decreased in relation to increasing dietary protein levels. This result agrees with reports of bagrid catfish *Mystus nemurus* (Ng et al., 2001), yellow catfish *Horabagrus brachysoma* (Giri et al., 2006), Nile tilapia *Oreochromis niloticus* (Gaye-Siessegger et al., 2007), and obscure puffer (Ye et al., 2017). A possible explanation for this result in our study is that the decrease of dietary carbohydrate levels reduces glucose absorption and further affects lipid synthesis in juvenile ide.

In the present study, both plasma glucose and triglyceride levels decreased with increasing dietary protein (decreasing dietary starch) levels, which was in agreement with results in obscure puffer (Ye et al., 2017), and yellow croaker, *Pseudosciaena Crocea* (Yu et al., 2012).

Hematological parameters in the blood indicate the health status of fish (Tewary and Patra, 2011). In the present study, WBC, RBC, HGB, and HCT contents were independent of dietary protein levels. Similarly, another study found that dietary protein levels did not affect the RBC contents in obscure puffer (Ye et al. 2017). However, in blunt snout bream *Megalobrama amblycephala*, from our previous study, WBC content was not significantly affected by dietary protein levels, whereas RBC, HBG and HCT content was enhanced by increasing dietary protein levels (Habte-Tsion et al., 2013). AST activity is used as an indicator of liver function; high AST activity generally indicates weakened or damaged normal liver function in fish species (Kim and Lee, 2009). In the present study, the highest serum AST activity was observed in fish fed a diet with 26.5% protein; it was possibly affected by malnutrition as suggested by poor growth performance.

In conclusion, the present results indicate that dietary protein affects growth, feed utilization, whole-body composition, and plasma parameters in juvenile ide. The dietary protein requirement for this fish species was determined to be 36.9-37.7% of dry diet based on SGR and FCR, and the optimal dietary methionine and lysine was 0.83% and 2.25% of dry diet, respectively. However, dietary EAA, lipid, and other nutrient levels for optimal growth of ide needs to be investigated in the future.

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