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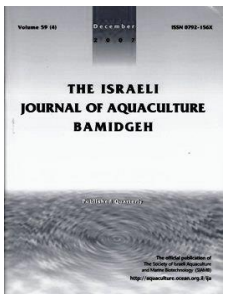

Effect of Dietary Methionine on Taurine Distribution and Non-specific Immune Responses in Juvenile Blunt Snout Bream, *Megalobrama amblycephala* at A Constant Dietary Cystine Level

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Keywords: *Megalobrama amblycephala;* methionine; cystine; taurine; non-specific immune responses

Abstract

A 9-week feeding trial was conducted to investigate taurine distribution by supplementation of methionine in juvenile *Megalobrama amblycephala* at a constant dietary cystine level. Six semi-purified diets were formulated to contain graded dietary methionine levels from 0.39-1.54% in about 0.25% increments. At the end of feeding trial, plasma methionine content significantly increased with increasing dietary methionine level from 0.39 to 1.0% ($P < 0.05$) and thereafter reached a plateau. The taurine content of muscle, intestine, liver, and eye in the fish fed dietary methionine level ranged from 1.24-1.54% and was significantly higher than that of the control group ($P < 0.05$). Taurine content of the brain in fish fed 1.54% methionine diet was higher than the fish fed 0.39% ($P < 0.05$). Cysteine sulfinic acid decarboxylase content significantly increased with increasing dietary methionine level from 0.39-0.85% ($P < 0.05$) and thereafter stayed at a similar level. Supplementation with methionine significantly increased ($P < 0.05$) plasma total protein (TP) content, complement 3 (C3) content and superoxide dismutase (SOD) activity (0.85% methionine diet groups), aspartate transaminase (AST) activity (1.24% methionine diet groups) and albumin content (1.0% methionine diet groups). Supplemented groups had significantly decreased plasma urea content (0.85% methionine diet groups) ($P < 0.05$). In conclusion, these results indicate that crystalline methionine can be utilized efficiently, juvenile blunt snout bream were able to convert methionine to taurine directly, and appropriate dietary methionine supplementation improves non-specific immune responses in blunt snout bream.

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Introduction

Blunt snout bream, *Megalobrama amblycephala*, also known as Wuchang bream, is a principal species in Chinese freshwater polyculture systems and is considered a delicacy. Aquaculture of this fish has rapidly expanded because of its fast growth, high feed efficiency ratio, tender flesh, and high disease resistance (Zhou et al., 2008). Blunt snout bream are currently the most widely cultivated freshwater fish in China and its production rapidly increased to approximately 0.70 million tons in 2012 (Ministry of Agriculture of the People's Republic of China 2013).

Protein quantity and dietary amino acid profile are two major factors that influence growth of fish. It is important to incorporate protein ingredients in the formulation of fish feed by taking care of an essential amino acid balance (Sardar et al., 2009). Methionine is an essential amino acid for optimal growth of fish, and involved in many physiological and biochemical processes, including growth, protein retention, immune function, and haemato-biochemical status (Chen et al., 2004; Mai et al., 2006; Yan et al., 2007; Sardar et al., 2009; Zhou et al., 2011; Mukhtar & Shabi 2013). Methionine plays an important role in protein synthesis and stimulating growth of aquatic animals (Alam et al., 2001; Luo et al., 2005; Espe et al., 2008; Zhou et al., 2011). Nutrient substance is the foundation of immune organ development and contribution; deficiencies of nutrient substance result in reduced immunity (Susanna et al., 2005). Normal growth of fish is often associated with immune response (Meeker et al., 1987). We hypothesize that dietary methionine plays a key role in innate immune function enhancement in blunt snout bream.

Taurine is an important amino acid derivative for fish and has been credited with playing an important role in numerous physiological functions including antioxidation, improved visual, neural and muscular systems (Huxtable 1992; Tadolini et al., 1995; Militante & Lombardini 2002; Lima 2004; Omura & Inagaki 2000). Previous studies have demonstrated that taurine is synthesized from cysteine in rainbow trout, *Oncorhynchus mykiss* (Yokoyama et al., 1997) and methionine in Atlantic salmon, *Salmo salar* L. (Espe et al., 2008), but the rate of synthesis may be inadequate to fulfill the taurine needs of fish. Two enzymes, cysteine dioxygenase (CDO) and cysteine sulfinic acid decarboxylase (CSAD) are involved in the conversion of cysteine to hypotaurine. Cats are inherently deficient of CSAD, the limiting enzyme for taurine biosynthesis (Hayes, Carey & Schmidt, 1975). In addition, CSAD has low activity in rainbow trout, O. mykiss, and other fish relative to mammalian omnivores (Goto et al., 2003; Goto et al., 2001a; Goto et al., 2001b). However, recently several nutritional studies have reported that marine larvae and juvenile are unable to synthesize enough taurine (Jacobsen & Smith 1968; Yokoyama et al., 2001). In a previous study in our lab, we found that dietary optimum concentration of methionine (8.4 and 8.5 g/kg) can promote growth and maintain high survival rates of juvenile blunt snout bream (Liao et al., 2014). Up to now, we do not know if the methionine can affect the taurine distribution and non-specific immune responses in juvenile blunt snout bream. Therefore, it is important to incorporate taurine in the formulation of fish feed by taking care of taurine synthesis. Based on this information, the current study was undertaken to examine taurine synthesis and blood biochemical parameters by supplementation of methionine in juvenile blunt snout bream.

Materials and Methods

Experimental diets.

Ingredients and proximate composition of the experimental diets are presented in Table 1 and the amino acid compositions (% dry diet, L-form, 99%, Shanghai Feeer Technology Development Co. Ltd., P.R. China) of dietary ingredients are shown in Table 2. Experimental diets contained 34.0% crude protein and 7.0% crude lipid which were identified to be optimal for growth of juvenile blunt snout bream (Shi, Shan, Liu, Yan, Huang, Zhou & Shen 1988; Li, Jiang, Liu & Ge 2012). Six isonitrogenous and isoenergetic semi-purified experimental diets were formulated to contain graded levels of methionine (0.4% to 1.65% dry diet) at about 0.25% increments, replaced by the same proportions of glycine. Fish meal, casein, and gelatin were used as intact protein sources supplemented with a crystalline amino acid mixture and soybean oil as lipid source. The final levels of methionine were confirmed by amino acid analysis, and the values were 0.39%, 0.56%, 0.85%, 1.0%, 1.24% and 1.54%, respectively.

Table 1. Ingredients and proximate composition analysis of experimental diets (% dry diet).

Ingredients	*Diet no.*					
	Diet1 (0.39)	*Diet2(0.56)*	*Diet3(0.85)*	*Diet4 (1.0)*	*Diet5(1.24)*	*Diet6(1.54)*
Fish meal[1]	5.0	5.0	5.0	5.0	5.0	5.0
Casein[2]	12.0	12.0	12.0	12.0	12.0	12.0
Gelatin[3]	3.0	3.0	3.0	3.0	3.0	3.0
Soybean oil[4]	6.0	6.0	6.0	6.0	6.0	6.0
Soybean lecithin[4]	1.0	1.0	1.0	1.0	1.0	1.0
Amino acid mix[5]	15.61	15.61	15.61	15.61	15.61	15.61
Choline chloride[4]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin C[6]	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin mix[7]	2.0	2.0	2.0	2.0	2.0	2.0
Monocalcium phosphate8	2.75	2.75	2.75	2.75	2.75	2.75
Mineral mix[9]	2.0	2.0	2.0	2.0	2.0	2.0
Dextrin[8]	35.0	35.0	35.0	35.0	35.0	35.0
Microcrystalline cellulose8	8.49	8.49	8.49	8.49	8.49	8.49
Carboxymethylcellulose8	5.0	5.0	5.0	5.0	5.0	5.0
Ethoxy quinoline8	0.5	0.5	0.5	0.5	0.5	0.5
Glycine	1.25	1.0	0.75	0.5	0.25	0
L-methionine	0	0.25	0.5	0.75	1.0	1.25
Total	100	100	100	100	100	100
Proximate analysis[10]						
L-methionine	0.39	0.56	0.85	1.0	1.24	1.54
Cystine	0.21	0.2	0.21	0.21	0.2	0.19
Crude protein	33.01	33.18	33.15	33.51	33.17	33.73
Crude lipid	8.32	8.40	8.54	8.26	8.35	8.42
Ash	4.94	5.25	5.52	5.58	5.44	5.38

[1]Fish meal, crude protein 67.4%, crude lipid 9.3%, provided by Coprinca, Brazil.
[2]Casein, crude protein 90.2%, purchased from Hua'an biological products, Ltd. (Gansu, China).
[3]Gelatin, crude protein 91.3%, purchased from Shanghai zhan yun chemical Co., Ltd. (Shanghai, China).
[4]Supplied by Cargill, Shanghai, China.
[5]Supplied as L-form (99%, Shanghai Feeer Technology Development Co. Ltd., Shanghai, China).
[6]Provided by Tongwei Feed Group Co. (Jiangsu, China).
[7]Vitamin mix(IU or mg/kg of diet) : Vitamin A, 900,000 IU; Vitamin D, 250,000 IU; Vitamin E, 4500 mg; Vitamin K3, 220 mg; Vitamin B1, 320 mg; Vitamin B2, 1090 mg; Vitamin B5,2000 mg; Vitamin B6, 5000 mg; Vitamin B12, 116 mg; Pantothenate, 1000 mg; Folic acid, 165 mg; Choline, 60,000 mg; Biotin, 50 mg; Niacin acid, 2500 mg; provided by Tongwei Feed Group Co. (Jiangsu, China).
[8] Supplied by Guangzhou Hinter Biotechnology Co., Ltd. (Guangdong, China).
[9]Mineral mix (g/kg of diet): $CuSO_4 \cdot 5H_2O$, 2.5 g; $FeSO_4 \cdot 7H_2O$, 28 g; $ZnSO_4 \cdot 7H_2O$, 22 g; $MnSO_4 \cdot 4H_2O$, 9 g; Na_2SeO_3, 0.045 g; KI, 0.026 g; $CoCl_2 \cdot 6H_2O$, 0.1 g; provided by Tongwei Feed Group Co. (Jiangsu, China).
[10]Values for the proximate analysis of the test diets are means of triplicate analyses.

Table 2. Amino acid composition of ingredients used to prepare experimental diets (%dry matter).

Amino acids[1]	*Amount in*					
	12.0 g Casein	*3.0 g Gelatin*	*5.0 g Fish meal*	*Crystalline amino acid premix*	*Total*	*34.0% Whole body protein*
EAAs						
Methionine	0.29	0.02	0.09	Variable	Variable	0.9
Histidine	0.26	0.01	0.11	0.38	0.76	0.76
Isoleucine	0.5	0.04	0.14	0.81	1.49	1.49
Leucine	0.97	0.07	0.23	1.13	2.4	2.4
Lysine	0.8	0.09	0.23	1.32	2.44	2.44
Arginine	0.34	0.19	0.18	1.3	2.01	2.01
Phenylalanine	0.5	0.05	0.14	0.78	1.47	1.47
Threonine	0.44	0.04	0.11	0.83	1.41	1.41
Valine	0.62	0.06	0.16	0.73	1.57	1.57
NEAAs						
Aspartic acid	0.77	0.12	0.27	1.68	2.84	2.84
Serine	0.56	0.07	0.12	0.7	1.45	1.45
Glycine	0.19	0.55	0.19	Variable	Variable	2.5
Alanine	0.34	0.22	0.2	1.39	2.15	2.15
Cystine	0.03	0.0	0.02	0.17	0.22	0.22
Tyrosine	0.55	0.02	0.1	0.4	1.07	1.07
Gulmatic acid	2.23	0.26	0.42	1.73	4.64	4.64
Proline	0.95	0.31	0.1	0.56	1.92	1.92

EAAs = essential amino acids; NEAAs = non-essential amino acids. [1]Supplied as L-form (99%, Shanghai Feer
[1]Supplied as L-form (99%, Shanghai Feeer Technology Development Co. Ltd., Shanghai, China).

Ingredients were ground and sieved through a 60 mesh sieve. All the ingredients of each diet were homogenized and blended with distilled water. Then the dough was extruded through a 1 mm die with a grinder (Type Y90L-2, Xinchang Chenshi MACHINERP Co., Ltd., Zhejiang, P.R. China), then dried at 25^{0}C for 72 h. After drying, the diets were packed into airtight plastic bags and stored at -20^{0}C for further use.

Experimental fish and feeding trial.

Juvenile blunt snout bream were obtained from the breeding farm of Freshwater Fisheries Research Centre (FFRC) of Chinese Academy of Fishery Sciences. Prior to the start of the experiment, healthy fish of similar size were selected, held in cages in a pond (1.0 m × 1.0 m × 1.0 m), and fed the control diet (0.39% methionine diet) for two weeks to acclimate to the experimental diet and conditions. At the initiation of the experiment, fish (3.34 ± 0.03 g) were randomly chosen and sorted into eighteen cages with 30 fish per cage. Each experimental diet was randomly assigned to triplicate cages in a completely randomized design. The fish were hand-fed three times daily (08:00, 12:00 and 16:00) until apparent satiation based on visual observation of the fish feeding behavior. They were fed slowly to prevent waste of diets.

During the experimental period, fish were held under natural photoperiod. Water quality measurement such as water temperature fluctuated from 26-31^{o}C, pH from 7.2-7.8, ammonia nitrogen was lower than 0.01 mg/L, and dissolved oxygen concentration was higher than 5 mg/L throughout the experimental period. Aeration was supplied to each cage daily for 24 h.

Sample collection techniques and chemical analysis.

At the end of the experiment, approximately 24 h after the last feeding, all fish from each cage were counted and individually weighed. Three fish were chosen and anesthetized with MS-222 (100 mg/L, Sigma Chemical Company, St Louis, MO, USA), and blood samples were collected immediately from the caudal vein with disposable medical syringes. The pooled blood sample per cage was centrifuged at 3,500 g for 10 min at 4^{o}C to extract the blood plasma, which was separated and stored at -80^{o}C for further analysis. The whole liver, dorsal muscle, whole brain, and intestine were frozen in liquid nitrogen and stored 80^{0}C for subsequent determination of taurine concentration. All parameters in this study were measured in triplicate.

Proximate composition of moisture, crude protein, crude lipid, and ash content in ingredients and diets were analyzed in triplicate using standardized methods (AOAC 2003). Moisture was analyzed by drying the samples to constant weight at 105^{o}C. Crude protein content was determined by the Kjeldahl method using semi-automatic Kjeldahl system (1030 Auto-analyzer, Tecator, Hoganos, Sweden) after acid digestion and estimated by multiplying nitrogen by 6.25. Crude lipid content was measured by ether extraction method using Soxhlet system HT6 (Soxtec System, Tecator, Sweden). Ash content was examined by combustion in a muffle furnace at 560^{o}C for 16 h.

Amino acid assays.

The amino acid composition of all samples including ingredients, diets and plasma were analyzed with Agilent-1100 Automatic amino acid analyzer (Agilent Technologies Co., Ltd., Santa Clara, USA) provided by an amino acid analysis laboratory (Institute of Food Science and Technology, Jiangnan University, Wuxi, China) after the acid hydrolysis. Briefly, performic acid oxidation was performed prior to hydrolysis to oxidize methionine at -10^{o}C for 3 h to get methionine sulfone. Then sodium metabisulfite was added to decompose surplus performic acid after which amino acid was liberated from protein by hydrolysis with 6 N HCl for about 22 h. Plasma samples were deproteinized by additing $HCLO_4$ followed by neutralization with K_2CO_3. Hydrolyzed samples were diluted with sodium citrate buffer, and pH was adjusted to 2. Hydrolyzed and neutralized samples were filtered through 0.44 μm polycarbonate syringe filters followed by precolumn derivatization with o-phthaldialdehyde. Cystine content in diets was determined from the same acid hydrolysate after treatment with dithiothreitol and sodium tetrathionate (Inglis & Liu 1970). In this study, tryptophan could not be detected after acid hydrolysis.

Taurine assay.

After the removal of excess water by lyophilization, taurine was extracted from fish tissue in 2 mL cold 70% ethanol for 20 min sonication at 4°C cold-room. The assay of taurine was analyzed using High Performance Liquid Chromatography (HP 1100, USA) in the laboratory of Institute of Food Science and Technology, Jiangnan University, according to the method of Watson, Barrows & Place (2015).

Liver cysteine sulfinic acid decarboxylase (CSAD) and cysteine dioxgenase (CDO) content assay.

The CSAD and CDO content in the samples were measured using the kits according to the manufacturer's protocol; purified fish CSAD and CDO antibody was used to coat the microtiter plate wells and made solid-phase antibodies. Then CSAD and CDO were added to the wells, CSAD and CDO antibodies were combined with HRP to become antibody-antigen-enzyme-antibody complex, after washing completely. Then TMB (tetramethyl benzidine) substrate solution was added; it turned blue at HRP (horse radish peroxidase) enzyme-catalyzed. Finally, the reaction was terminated by the addition of sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm using multiskan (Labsustems Multiskan MS-352, Thermo Electron Corporation, Finland). The concentrations of CSAD and CDO in the samples were determined by comparing the O.D. of the samples to a standard curve.

Plasma biochemical parameters, antioxidization enzyme activity, and immune parameter measurements.

Plasma alanine transaminase (ALT) and aspartate transaminase (AST) activities were measured by the IFCC method. The total protein (TP) content was determined by direct assay, the Glucose (Glu) content was determined by hexokinase method, the urea (UREA) content was determined by UV-glutamate dehydrogenase method, and the albumin (ALB) level was measured using the Bromcresol green method. All these kits were bought from Shenzhen Mindray Bio-Medical Electronics Co., Ltd. The measurements were conducted in a Mindray Auto Bio-chemical Analyzer (BS-400, Mindray, P.R. China). Superoxide dismutase (SOD) activity was measured by its ability to inhibit superoxide anion generated by a xanthine and xanthine oxidase reaction system using a SOD detection kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) as described by Zhou et al. (2012a). Plasma complement 3 (C3), and complement 4 (C4) concentrations were determined by the method of Welker et al. (2007) and test kits were bought from Nanjing Jiancheng Biological Engineering Research Institute (Jiangsu, China).

Statistical analysis. All data were subjected to one-way analysis of variance (ANOVA).

Significant differences between means were evaluated by Duncan New Multiple Range Test (DMRT) in SPSS version 16.0 (SPSS, Chicago, IL, USA). Difference was regarded as significant when $P < 0.05$. Data were expressed as means with standard error of means (S.E.M.).

Results

Effect of dietary methionine level on the plasma methionine content. Dietary methionine levels significantly affected plasma methionine content in juvenile blunt snout bream ($P < 0.05$). Plasma methionine showed an increasing trend with increasing dietary methionine levels from 0.39-1.0% and thereafter kept a constant level ($P < 0.05$) (Figure 1).

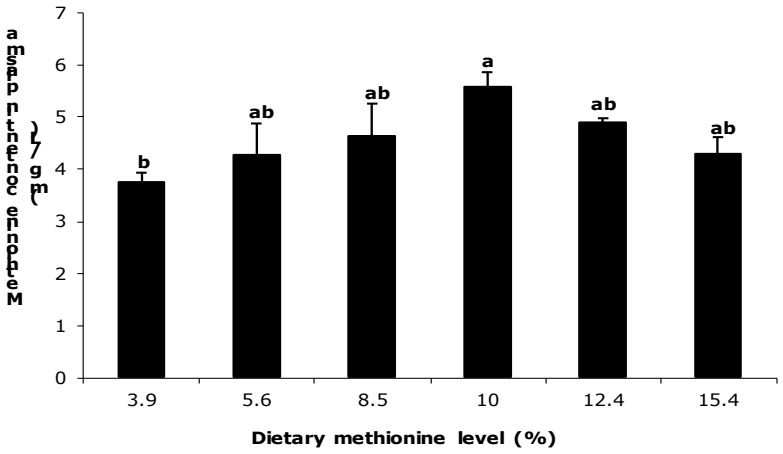

Fig. 1. Effect of dietary methionine level on methionine content of plasma in juvenile blunt snout bream. Each point represents the means of three replicate groups; different superscript letters indicate significant differences ($P<0.05$).

Effect of dietary methionine level on the taurine content of muscle, liver, intestine, brain and eye.

With increasing dietary methionine level, taurine content of muscle, liver, intestine, brain and eye significantly increased (Table 3). Besides, the muscle taurine content in fish fed 1.0, 1.24 and 1.54% dietary methionine levels was significantly higher than in the fish fed 0.39% ($P < 0.05$). The intestine taurine content in fish fed 1.24 and 1.54% methionine diet was significantly higher than in the fish fed 0.39% ($P < 0.05$). The brain taurine content in fish fed 1.54% methionine diet was significantly higher than in the fish fed 0.39% ($P < 0.05$). The liver taurine content in fish fed 0.85, 1.0, 1.24 and 1.54% methionine diet was significantly higher than in the fish fed 0.39% ($P < 0.05$). The eye taurine content in fish fed 0.85, 1.24 and 1.54% methionine diet was significantly higher than in the fish fed 0.39% ($P < 0.05$).

Table 3. Effect of dietary methionine level on the taurine content of muscle, liver, intestine, brain and eye of juvenile blunt snout bream.

Diets (methionine level, %)	*Muscle (mg/g)*	*Intestine (mg/g)*	*Brain (mg/g)*	*Liver (mg/g)*	*Eye (mg/g)*
Diet 1 (0.39)	31.01±4.62^{c}	39.52±3.13^{b}	38.91±0.45^{b}	34.72±1.28^{b}	37.31±0.05^{b}
Diet 2 (0.56)	32.44±3.20^{bc}	42.40±0.95^{ab}	39.87±1.45^{ab}	39.89±0.22^{ab}	36.83±0.26^{b}
Diet 3 (0.85)	33.36±2.74^{bc}	46.85±3.90^{ab}	43.02±0.01^{ab}	43.54±2.97^{a}	44.37±3.10^{ab}
Diet 4 (1.0)	43.05±3.09^{ab}	46.00±1.72^{ab}	43.83±1.50^{ab}	47.39±2.15^{a}	40.42±0.68^{bc}
Diet 5 (1.24)	44.33±3.17^{a}	47.74±1.84^{a}	43.83±1.98^{ab}	43.02±0.16^{a}	49.01±2.24^{a}
Diet 6 (1.54)	48.75±1.35^{a}	47.81±1.61^{a}	45.89±1.52^{a}	42.57±1.40^{a}	47.34±0.81^{a}

Values are presented as means ± S.E.M. (n = 3); values with different superscripts in the same row differ significantly ($P < 0.05$).

Effect of dietary methionine level on the liver cysteine dioxygenase (CDO) and cysteine sulfinic acid decarboxylase (CSAD).

Data on the liver CSAD and CDO content in juvenile blunt snout bream which were fed the different experimental diets are presented in Table 4. The CSAD content of liver increased significantly ($P < 0.05$) with increasing dietary methionine level up to 0.85% and then kept a similar level ($P > 0.05$). No significant differences were observed in the liver CDO content among the dietary treatments ($P > 0.05$).

Table 4 Effect of dietary methionine level on liver cysteine dioxgenase (CDO) and cysteine sulfinic acid decarboxylase (CSAD) in juvenile blunt snout bream.

	Diets (methionine level, %)					
	Diet 1 (0.39)	*Diet 2 (0.56)*	*Diet 3 (0.85)*	*Diet 4 (1.0)*	*Diet 5 (1.24)*	*Diet 6 (1.54)*
CSAD (ng/L)	33.57±0.77^{c}	36.58±0.99^{ab}	37.01±0.61^{a}	35.83±0.42^{abc}	34.27±0.71^{bc}	34.80±1.41^{abc}
CDO (ng/L)	59.93±1.64	61.17±1.00	61.73±1.59	61.75±0.90	60.24±1.62	61.36±1.48

Values are presented as means ± S.E.M. (n = 3); values with different superscripts in the same row differ significantly ($P < 0.05$).

Effect of dietary methionine level on the plasma biochemical parameters.

The effects of graded levels of dietary methionine on plasma biochemical parameters in juvenile blunt snout bream are described in Table 5. Aspartate Alanine aminotransferase (AST) activity in the fish fed 1.24 and 1.54% dietary methionine levels was significantly ($P < 0.05$) higher than in those fish fed 0.39, 0.56 and 0.85% methionine diet, while no significant differences ($P > 0.05$) were observed in alanine aminotransferase (ALT). Plasma total protein (TP) content significantly ($P < 0.05$) increased with increasing dietary methionine level from 0.39 to 1.0%, and thereafter kept stable ($P > 0.05$). Plasma urea content in the fish fed 0.39% dietary methionine level was significantly lower than in those fish fed 0.85% methionine diet ($P < 0.05$). No significant differences were observed on glucose content among the treatment groups ($P > 0.05$).

Table 5. Effect of dietary methionine level on plasma biochemical parameters in juvenile blunt snout bream.

	Diets (methionine level, %)					
	Diet 1 (0.39)	*Diet 2 (0.56)*	*Diet 3 (0.85)*	*Diet 4 (1.0)*	*Diet 5 (1.24)*	*Diet 6 (1.54)*
ALT (U/L)	5.51±0.84	4.42±0.62	5.32±1.05	5.05±0.79	6.10±0.91	4.47±0.44
AST (U/L)	23.00±1.2^{b}	24.72±2.741^{b}	24.13±1.56^{b}	30.05±3.92^{ab}	35.68±4.33^{a}	37.07±1.44^{a}
TP (mmol/L)	21.4±1.06^{b}	23.33±1.20^{ab}	25.34±1.03^{a}	25.91±1.45^{a}	24.16±1.06^{ab}	22.89±0.89^{ab}
Albumin (mmol/L)	2.80±0.46^{b}	2.63±0.43^{b}	4.36±0.68^{ab}	5.06±0.20^{a}	2.88±0.41^{b}	2.78±0.35^{b}
Urea (mmol/L)	0.73±0.03^{a}	0.64±0.04^{ab}	0.61±0.04^{b}	0.67±0.04^{ab}	0.65±0.02^{ab}	0.65±0.01^{ab}
Glucose (mmol/L)	2.66±0.16	2.97±0.25	2.98±0.29	3.16±0.36	2.76±0.36	2.33±0.91

ALT = alanine aminotransferase; AST = aspartate aminotransferase; TP = total protein.

Values are presented as means ± S.E.M. (n = 3); values with different superscripts in the same row differ significantly ($P < 0.05$).

Effects of dietary methionine levels on plasma superoxide dismutase (SOD), complement 3 (C3) and complement 4 (C4).

Plasma SOD activity, C3 and C4 concentrations, and survival rate in juvenile blunt snout bream fed graded levels of dietary methionine for 9 weeks are shown in Table 6. Maximum C3 concentration and SOD activity were observed in fish fed 0.85% dietary methionine level. No significant differences were observed in the liver C4 concentration among the dietary treatments ($P > 0.05$). No pathological signs were observed during the trial, and no anomalies occurred at the end of feeding experiment.

Table 6. Effects of dietary methionine levels on plasma superoxide dismutase (SOD), complement 3 (C3), complement 4 (C4) and survival rate of juvenile blunt snout bream.

Diets (methionine level, %)	*C3 (mg/L)*	*C4 (mg/L)*	*SOD (U/L)*
Diet 1 (0.39)	350.5±23.7^{d}	56.4±1.6	45.2±1.2^{b}
Diet 2 (0.56)	452.7±44.0^{bc}	54.4±6.9	50.8±2.6^{ab}
Diet 3 (0.85)	550.3±30.1^{a}	66.9±10.0	54.8±3.2^{a}
Diet 4 (1.0)	488.6±24.2^{ab}	55.9±8.2	54.2±1.8^{a}
Diet 5 (1.24)	344.3±18.4^{d}	49.3±9.8	48.5±2.3^{ab}
Diet 6 (1.54)	364.7±42.5^{cd}	68.5±3.7	51.0±0.8^{ab}

Values are presented as means ± S.E.M. (n = 3); values with different superscripts in the same row differ significantly ($P < 0.05$).

Discussion

Plasma amino acid levels are the result of a balance between influx from digested protein, endogenous catabolism, amino acid oxidation, and protein synthesis. Dietary amino acid profiles are known to influence the postfeeding levels of free amino acids in fish tissues, such as plasma, liver, muscle, and whole body (Twibell et al., 2000; Luo et al., 2005; Mai et al., 2006; Zhou et al., 2006; Espe et al., 2007). Free amino acid content in plasma initially reflects the content of amino acids in the dietary protein following absorption (Blasco et al., 1991). In the present study, juvenile blunt snout bream fed the methionine deficient diet showed lower methionine concentration in plasma. Similar results have been reported in rainbow trout, O. mykiss (Bae et al., 2011). The result of this study suggested that the crystalline methionine can be utilized by juvenile blunt snout bream. Plasma methionine concentration in juvenile cobia, *Rachycentron canadum* increased with increasing dietary methionine level and had no further increase beyond the optimum requirement (Zhou et al. 2006). Similarly, in this study, when the dietary methionine level ranged 1.0%, the plasma methionine kept a similar level with no further increase, however, it showed an increasing trend with increasing dietary methionine level from 0.39%-1.0%. The differences apparent in free methionine in the plasma might be due to the lack of methionine, and the demand for metabolic function would be closely related to the dietary quantity available. Therefore, it was clear that there was a low concentration of methionine in plasma when fish were fed a methionine deficient diet. Once the dietary methionine requirement was met, methionine could accumulate in the plasma. The results of these studies suggest that adequate methionine in a diet can enhance amino acid participation in protein synthesis and increase the amino acid pool in tissues (Mai et al., 2006). However, the free methionine content in the plasma was stable as the dietary methionine level exceeded the optimum requirement, which might result from the fact that the protein absorption with bound-amino acids (Plakas et al., 1981) and the capacity in fish of amino acid pool is limited.

In another study, taurine distribution in fish varied throughout ontogenesis (Yokoyama et al. 2001). However, in our experiment, the average taurine concentrations in different tissues were all different. Higher taurine concentration was detected in the muscle compared to other tissues. Similarly, higher taurine concentration occurred in the liver and brain of juvenile Japanese flounder, *Paralichthys olivaceus* (9.7 g) and in the body and liver of fry (0.3 g) compared to other body tissues (Kim et al. (2007) and Kim et al. 2008). This implied that different fish size may result in a different taurine distribution in different tissues. Some studies observed that Mahi-mahi, *Coryphaena hippurus*, red hybrid tilapia, *Oreochromis sp*. and rainbow trout, *O. mykiss* presented some capacity for synthesis taurine (Divakaran et al., 1992; Yokoyama et al., 1997). Our data showed that supplementation of methionine, the taurine concentration significantly increased in the eye, brain, muscle, intestine, and liver of blunt snout bream, and that the trend of taurine content with increased levels of methionine in these tissues in our study were consistent with the variation of growth performance reported by Liao et al. (2014). This result suggested that juvenile blunt snout bream was able to convert methionine to taurine directly and thus meet the requirements of this fish. Furthermore, Atlantic salmon, *S. salar L.* were observed to have the capacity of taurine synthesis by supplementation of methionine and cystine (Nordrum et al. 2000). In the current study, different levels of cystine were not tested, and no attempt was been made to accurately measure the relationship between cystine intake and synthesis of taurine. Some studies indicated that taurine distribution was determined by the distribution of CASD (Yokoyama et al., 2001), which is a key enzyme for taurine synthesis. However, due to different CASD activity levels some fish species may be unable or poor at synthesizing taurine from methionine (Goto et al., 2001a; Goto et al., 2001b; Goto et al., 2003). In the present experiment, cysteine sulfinic acid decarboxylase (CSAD) concentration in the liver of juvenile blunt snout bream was upregulated by supplementation of methionine, but it failed to upregulate cysteine dioxygenase (CDO) concentration.

A similar result was observed in rainbow trout, O. mykiss (Yokoyama & Nakazoe 1996). In addition, the CDO and CSAD mRNA expressions were found to be influenced by dietary amino acid supplementation, for example CDO mRNA expression decreased as methionine supplementation increased, whereas CSAD mRNA expression increased with methionine supplementation except 0.5% (Gaylord et al. 2007). In summary, juvenile blunt snout bream is able to synthesis taurine, and thus taurine is not an essential amino acid.

Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are two important enzymes in the metabolism of amino acids (Luo et al., 2005). Those two enzymes can be seen in finfish response to toxins, stress, disease, and malnutrition (Groff & Zinkl 1999; Davis 2004). In the present study, the highest plasma AST was observed in the fish fed 1.24% and 1.56% dietary methionine level, which was inconsistent with the study reported by Luo et al. (2005). Earlier studies reported that excessive level of methionine may lead to accumulation and oxidation of methionine to ketones and other toxic metabolites in Indian major carp, *Labeo rohita* (Murthy & Varghese 1998) and red sea bream, *Pagrus major* (Takagi et al., 2001). A reasonable explanation for those findings could be that different fish species may have different tolerance to excessive dietary methionine level doses.

Plasma total protein, urea nitrogen, albumin, and glucose were related to animal health (Zhou et al., 2006). In the present study, plasma total protein was significantly affected by dietary methionine levels, which is similar to the results reported by Luo et al. (2005). Total protein is an important parameter for nitrogen metabolism. The increase of total protein levels is considered a diagnostic tool and a valuable test for evaluating the general physiological state of fish health (De-Pedro et al., 2005). In this study, the increased catabolism of dietary amino acids in juvenile blunt snout bream is supported by increased plasma nitrogen levels. Urea is a major product of protein catabolism in fish. Several studies reported that the protein retention efficiency was reduced, and urea concentration increased, in fish fed diets either deficient in, or with imbalanced amino acids levels, compared with fish were fed "whole protein" diets (Řehulka & Mina-řík 2003; Xie et al., 2012). These results indicated that dietary methionine levels could influence animal health. Where glucose was concerned, the parameters were stable and not related to dietary methionine. Currently, little information is available on the effect of dietary methionine on these blood characteristics and further investigations are needed to be needed in order to confirm these aspects.

Superoxide dismutase (SOD) plays an important role in the defense system and also has an important function in the immune system (Lin et al., 2011). In this study, SOD activity was significantly enhanced with increasing methionine levels. Although there is no report regarding the effect of dietary methionine on SOD activity, similar observations were reported in juvenile yellow grouper, *Epinephelus awoara* fed dietary arginine (Zhou et al., 2012b) and juvenile cobia, *R. canadum* fed vitamin C (Zhou et al., 2012a). This indicated that dietary methionine supplementation in this experiment had a positive effect on the oxidation resistance of juvenile blunt snout bream.

In conclusion, dietary methionine exhibited an effect on taurine concentration in the tissues and body of blunt snout bream in a dose-dependent manner. Furthermore, dietary methionine affected antioxidant enzyme activities and complement concentration in plasma, which could provide some evidence for improvement of methionine supplement in blunt snout bream.

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