



## Effects of dietary taurine supplementation to a casein-based diet on growth performance and taurine distribution in two sizes of juvenile turbot (*Scophthalmus maximus* L.)

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### ABSTRACT

Feeding trials were conducted to investigate the effects of dietary taurine on feeding, growth, feed utilization and taurine distribution in tissues of juvenile turbot (*Scophthalmus maximus* L.) with initial average weight of  $6.3 \pm 0.01$  g (Trial 1) and  $165.9 \pm 5.01$  g (Trial 2). The basal diet was supplemented with 0.0% (control), 0.5%, 1.0% and 1.5% taurine to formulate four isonitrogenous and isolipidic diets (Diet 1 (0.0%), Diet 2 (0.5%), Diet 3 (1.0%) and Diet 4 (1.5%)). The analyzed taurine concentrations were 0.16%, 0.64%, 1.15%, 1.66%, respectively. Each diet was randomly assigned to triplicate groups of 30 fish (6.3 g) in 200 L tank and 15 fish (165.9 g) in 500 L tank. Fish were fed twice daily (06:30 and 18:30) to apparent satiation for 10 weeks. With the increase of dietary taurine, growth, feed efficiency ratio (FER) in fish with initial body weight of 6.3 g first increased (0.0% to 1.0%) ( $P=0.000$ ,  $P=0.000$ ) and thereafter reached a plateau, while growth in fish with initial body weight of 165.9 g first increased (0.0% to 0.5%) ( $P=0.006$ ) and then gradually decreased. Feed intake (FI) in fish with both body weights (6.3 g; 165.9 g) increased with increasing dietary taurine ( $P=0.000$ ;  $P=0.001$ ) and thereafter gradually declined. With increasing dietary taurine, crude protein contents in fish of both body weights increased ( $P=0.049$ ;  $P=0.000$ ) and then reached a plateau, while the crude lipid content in fish with 165.9 g weight increased with increasing dietary taurine ( $P=0.000$ ). The contents of taurine in body, muscle, eye, liver and brain of turbot were correlated with consumed taurine, and the correlated coefficients in fish with 6.3 g body weight were separately 0.955 ( $P=0.000$ ), 0.945 ( $P=0.000$ ), 0.881 ( $P=0.001$ ), 0.947 ( $P=0.000$ ) and 0.924 ( $P=0.002$ ) while those fish with 165.9 g body weight were 0.967 ( $P=0.000$ ), 0.964 ( $P=0.000$ ), 0.977 ( $P=0.000$ ), 0.946 ( $P=0.000$ ) and 0.997 ( $P=0.000$ ). This result suggested that 1.0% taurine in diet of turbot with  $6.3 \pm 0.01$  g weight and 0.5% taurine in diet of turbot with  $165.9 \pm 5.01$  g weight are probably optimal.

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### 1. Introduction

Taurine (2-aminoethanesulfonic acid) is an important amino acid derivative for vertebrates (Hayes, 1976; Hayes and Trautwein, 1989; Knopf et al., 1978), especially for juveniles (Sturman, 1988), which plays important roles in fat digestion (Garbutt et al., 1971), antioxidative defense (Huxtable, 1992; Tadolini et al., 1995), cellular osmoregulation (Pasantes et al., 1998) as well as development of visual (Militante and Lombardini, 2002), neural (Lima, 2004) and muscular systems (Omura and Inagaki, 2000). Many marine larvae and juvenile are unable to synthesize enough taurine (Jacobsen and Smith, 1968; Yokoyama et al., 2001). Lack of taurine in low fishmeal diets results in anemia (Takagi et al., 2008), green liver syndrome (Goto et al., 2001; Takagi, 2006a; Takagi et al., 2011) and inferior growth (Takagi et al., 2010). Therefore, an exogenous supplementation of taurine is probably required in the certain life stages of some fish fed diets with low fishmeal inclusion.

The promotion effects of taurine on feeding and growth have been reported in numerous studies for some marine fish species including yellowtail (Matsunari et al., 2005), Japanese flounder (Kim et al., 2007) and red sea bream (Matsunari et al., 2008). Compared with most marine fish (more than 1%), the optimum dietary taurine level for growth of freshwater fish was relatively low (less than 0.5%) (Chatzifotis et al., 2008; Gaylord et al., 2006; Qiu et al., 2006). Even, growth was not significantly improved by supplementation of taurine in juvenile carp (Kim et al., 2008a) and hybrid striped bass (Savolainen, 2008). However, to some degree, the optimum supplementation of taurine varies with not only fish species but fish size, diet and experimental conditions.

Turbot (*Scophthalmus maximus* L.), an important commercial carnivorous fish, has been widely farmed in Europe and East Asia because of its delicious meat and rapid growth. A previous study found that growth rate of turbot larvae was positively correlated with dietary taurine concentration (Conceição et al., 1997), suggesting a dietary dependency for taurine. The up-to-date study illustrated dietary taurine (1%) increased growth of juvenile turbot fed high

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plant protein diets (Yun et al., 2012). However, to our knowledge, no information is available on the effects of taurine supplemented in casein-based diets for juvenile turbot with different body weight. Therefore, the purpose of this study was to investigate the effects of taurine on growth performance and taurine distribution in turbot with two body weight.

## 2. Materials and methods

### 2.1. Experimental diets

The basal diet was formulated to contain approximately 53% crude protein (35% casein, 11% gelatin and 10% white fish meal) and 12.5% crude lipid (Table 1), which have been shown to be sufficient to support the optimal growth of turbot (Kaushik, 1998). Taurine (purity, 99%; Shanghai Chemical Reagent co., Ltd, China) was supplemented separately to the basal diet at the expense of -cellulose to obtain 0.0% (control), 0.5%, 1.0% and 1.5% (of dry weight) taurine, respectively.

Ingredients were ground into fine powder through 246-um mesh. All ingredients including taurine were thoroughly mixed with fish oil and soybean oil, and water was added to produce a stiff dough. The dough was then pelleted with an experimental feed mill and dried for about 12 h in a ventilated oven at 60 °C. After drying, the diets were broken up and sieved into proper pellet size and were stored at -20 °C until used.

### 2.2. Experimental procedure

Turbot juveniles were obtained from Yellow Sea Fisheries Co., Ltd (Haiyang, Shandong, China). Prior to the start of the experiment, the fish were selected for two sizes, reared in 200 L and 500 L tank,

respectively, and fed the control diet for 2 weeks to acclimate to the experimental diet and conditions.

At the initiation of the experiment, the fish were fasted for 24 h and weighed. Fish of similar sizes ( $6.3 \pm 0.01$  g) were randomly distributed into 12 tanks (200 L) and each tank was stocked with 30 fish. Fish of similar sizes ( $165.9 \pm 5.01$  g) were randomly distributed into 12 tanks (500 L) and each tank was stocked with 15 fish. Each diet was randomly assigned to triplicate tanks. Fish were hand-fed to apparent satiation twice daily (06:30 and 18:30). Any uneaten feed was collected 1 h after each meal, dried to constant weight at 70 °C and reweighed. Leaching loss in the uneaten diet was estimated by leaving five samples of each diet in tanks without fish for 1 h, recovering, drying and reweighing. Both feeding trials lasted for 10 weeks.

During the experimental period, water temperature was controlled at 15–20 °C, pH 7.5–8.0, the salinity 30–33‰. Ammonia nitrogen was lower than 0.25 mg/L; nitrite was lower than 0.1 mg/L, and dissolved oxygen was higher than 6.0 mg/L. Aeration was supplied to each tank 24 h daily. At the termination of the experiment, the fish were fasted for 24 h before harvest. Total number and body weight of fish in each tank were measured.

### 2.3. Sample collection

Before the start of experiment, eight fish were randomly selected for determination of initial whole-body proximate composition. At the end of the experiment, all experimental fish were anesthetized with eugenol (1:10,000) (Shanghai Reagent Corporation, Shanghai, China), five fish in each tank were sampled and stored at -20 °C for whole body composition analysis, and other 5 fish of each tank were sampled for morphometric parameters. Individual body weight, body length, liver weight and visceral weight were recorded to calculate condition factor, hepatosomatic index and viserosomatic index. The samples (the whole liver, partial muscle from the back of existed eye side (at the same position of fish), the whole brain, and both eyes) were frozen in liquid nitrogen and stored at -80 °C for subsequent determination of taurine concentration.

### 2.4. Chemical analysis

#### 2.4.1. Chemical composition assays

Moisture, crude protein, crude lipid and ash were analyzed for fish samples and diets. Moisture was analyzed by drying the samples to constant weight at 105 °C. Crude protein was determined using the Kjeldahl method (AOAC, 1995) and estimated by multiplying nitrogen by 6.25. Crude lipid was measured by ether extraction using Soxhlet method. Ash was examined by combustion in a muffle furnace at 550 °C for 16 h. Duplicate analyses were conducted for each sample.

#### 2.4.2. Taurine assay

The assay of taurine was carried out with High Performance Liquid Chromatography (HP1100, America) according to the method of Sakai and Nagasawa (1992).

### 2.5. Calculations and statistical analysis

Parameters were calculated as follows:

Specific growth rate (SGR)(%/d)

$$= 100 \times [(\ln(\text{final body weight}) - \ln(\text{initial body weight}))/\text{days}].$$

Feed intake(FI)(%/d) = 100 × total amount of the feed consumed

$$/ [(\text{initial body weight} + \text{final body weight})/2]/\text{days}.$$

**Table 1**

Formulation (%), proximate composition (%) and energy content (MJ/kg) of the trial diets for juvenile turbot with two body weight.

Ingredients	Diet no. (taurine supplementation level)			
	Diet 1 (0.0%)	Diet 2 (0.5%)	Diet 3 (1.0%)	Diet 4 (1.5%)
White fish meal	10.00	10.00	10.00	10.00
Casein	35.00	35.00	35.00	35.00
Gelatin	11.00	11.00	11.00	11.00
Dextrin	19.95	19.95	19.95	19.95
-Starch	8.00	8.00	8.00	8.00
Soybean lecithin	1.00	1.00	1.00	1.00
Feed oil	11.00	11.00	11.00	11.00
Choline chloride (50%)	0.20	0.20	0.20	0.20
Vitamin mix <sup>a</sup>	0.60	0.60	0.60	0.60
Mineral mix <sup>b</sup>	0.20	0.20	0.20	0.20
Ethoxy quinoline	0.05	0.05	0.05	0.05
Calcium propionate	0.10	0.10	0.10	0.10
Dicalcium phosphate	0.50	0.50	0.50	0.50
L-Methionine	0.20	0.20	0.20	0.20
L-Arginine	0.20	0.20	0.20	0.20
L-Phenylalanine	0.50	0.50	0.50	0.50
-cellulose	1.50	1.00	0.50	0.00
Taurine	0.00	0.50	1.00	1.50
<i>Analyzed nutrients compositions (dry matter basis)</i>				
Taurine	0.16	0.64	1.15	1.66
Crude protein	52.90	53.40	53.90	54.40
Crude lipid	12.50	12.50	12.50	12.50
Gross energy (MJ/kg)	19.10	19.10	19.10	19.10

<sup>a</sup> Vitamin premix (mg or IU/kg diet): thiamin 25, riboflavin (80%) 45, pyridoxine hydrochloride 20, vitamin B<sub>12</sub> (1%) 10, niacin 200, Ca-pantotenatate 60, inositol 800, biotin (2%) 60, folic acid 20, vitamin K<sub>3</sub> 10, retinyl acetate 16,000, vitamin D<sub>3</sub> 2500, DL-α-tocopherol acetate (50%) 240, L-ascorbyl-2-monophosphate-Na (35%) 2000, microcrystalline cellulose 2473.

<sup>b</sup> Mineral premix (mg/kg diet): MgSO<sub>4</sub>·7H<sub>2</sub>O 1200, FeSO<sub>4</sub>·H<sub>2</sub>O 80, CuSO<sub>4</sub>·5H<sub>2</sub>O 10, ZnSO<sub>4</sub>·H<sub>2</sub>O 50, MnSO<sub>4</sub>·H<sub>2</sub>O 45, CoCl<sub>2</sub> (1%) 50, Na<sub>2</sub>SeO<sub>3</sub> (1%) 20, CaI<sub>2</sub> (1%) 60, Zeolite 485.

Feed efficiency ratio (FER)

$$= \text{wet weight gain (g)} / \text{total amount of the feed consumed (g)}.$$

Protein efficiency rate (PER) = wet weight gain (g)

$$/ \text{total amount of the feed consumed (g)} / \text{protein percent in diets}.$$

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

Condition factor (CF) =  $100 \times \text{fish weight (g)} / [\text{body length (cm)}]^3$ .

Hepatosomatic index (HSI)(%) =  $100 \times (\text{liver weight} / \text{body weight})$ .

Viscerosomatic index (VSI)(%)

$$= 100 \times (\text{visceral weight} / \text{body weight}).$$

The Software SPSS, 11.0 microcomputer software package (SPSS, Chicago, IL, USA) was used for all statistical evaluations. A homogeneity test for the variance was conducted. All data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test. Differences were regarded as significant when  $P < 0.05$ . Data are expressed as means with S.E.M.,  $F$ -value and  $P$ -value. The relationship between consumed taurine and various taurine concentrations in tissues is expressed as regressive equations, correlation coefficients ( $r$ ) and  $P$ -value.

### 3. Results

#### 3.1. Growth performance and survival

The FI in fish with initial body weight of 6.3 g significantly increased with increasing dietary taurine from 0.0% to 0.5% ( $P < 0.05$ ), and thereafter significantly declined ( $P < 0.05$ ). As dietary taurine increased from 0.0% to 1.0%, The SGR significantly increased ( $1.5$ – $2.0 \text{ d}^{-1}$ ) ( $P < 0.05$ ) and thereafter reached a plateau ( $P > 0.05$ ). FER, PER followed the same pattern as SGR. The survival rate (SR) ranged

from 97.8% to 100.0%, but no significant difference was found among dietary treatments ( $P > 0.05$ ) (Table 2).

The FI in fish with initial body weight of 165.9 g significantly increased with increasing dietary taurine from 0.0% to 0.5% ( $P < 0.05$ ), reached a plateau from 0.5% to 1.0% ( $P > 0.05$ ) and then significantly decreased ( $P < 0.05$ ). The SGR followed the same pattern as FI. However, no significant differences in FER, PER were detected among dietary treatments ( $P > 0.05$ ). The SR was found to be no significant difference among dietary treatments ( $P > 0.05$ ), which ranged from 93.6% to 100.0% (Table 2).

#### 3.2. Morphological index

The CF, HSI and VSI in turbot with initial weight of 6.3 g first increased, then decreased with increasing dietary taurine ( $P > 0.05$ ) (Table 3).

The VSI in fish with initial weight of 165.9 g first decreased, then increased with increasing dietary taurine, while CF, HSI first increased, then kept stable ( $P > 0.05$ ) (Table 3).

Neither CF, HSI or VSI were affected by treatment in either of the two fish trials.

#### 3.3. Body composition

The moisture, crude protein content in fish with initial weight of 6.3 g showed an increasing trend with increasing dietary taurine, and fish fed the diet with 1.0% taurine (Diet 3) showed the highest crude protein content (15.7%), which was significantly higher compared with the control group ( $P < 0.05$ ). However, crude lipid, ash content showed decreasing trend, but fish fed diets with taurine supplementation showed significantly lower ash content than fish fed the control diet ( $P < 0.05$ ). No significant differences were observed in moisture and lipid content among dietary treatments ( $P > 0.05$ ) (Table 4).

The crude protein content in fish with initial weight of 165.9 g significantly increased with increasing dietary taurine from 0.0% to 0.5%

**Table 2**  
Growth performance of juvenile turbot with two body weight (means  $\pm$  S.E.M.).\*

	Diet no. (taurine supplementation level)				F-value	P-value
	Diet 1 (0.0%)	Diet 2 (0.5%)	Diet 3 (1.0%)	Diet 4 (1.5%)		
<i>Trial 1 (initial body weight of 6.3 g)</i>						
IBW <sup>1</sup>	6.3 $\pm$ 0.01	6.3 $\pm$ 0.01	6.3 $\pm$ 0.01	6.3 $\pm$ 0.01		
FBW <sup>2</sup>	17.8 $\pm$ 0.23 <sup>a</sup>	20.8 $\pm$ 0.76 <sup>ab</sup>	25.4 $\pm$ 1.22 <sup>b</sup>	24.6 $\pm$ 0.60 <sup>b</sup>	20.177	0.000
WGR <sup>3</sup>	182.5 $\pm$ 0.45 <sup>a</sup>	230.2 $\pm$ 1.09 <sup>a</sup>	303.2 $\pm$ 1.34 <sup>b</sup>	290.5 $\pm$ 0.77 <sup>b</sup>	20.593	0.000
SGR <sup>4</sup>	1.5 $\pm$ 0.02 <sup>a</sup>	1.7 $\pm$ 0.04 <sup>b</sup>	2.0 $\pm$ 0.10 <sup>c</sup>	2.0 $\pm$ 0.04 <sup>c</sup>	24.720	0.000
FI <sup>5</sup>	2.3 $\pm$ 0.03 <sup>a</sup>	2.6 $\pm$ 0.04 <sup>b</sup>	2.5 $\pm$ 0.04 <sup>b</sup>	2.4 $\pm$ 0.04 <sup>a</sup>	20.244	0.000
FER <sup>6</sup>	0.5 $\pm$ 0.02 <sup>a</sup>	0.6 $\pm$ 0.02 <sup>a</sup>	0.8 $\pm$ 0.03 <sup>b</sup>	0.8 $\pm$ 0.03 <sup>b</sup>	41.157	0.000
PER <sup>7</sup>	0.9 $\pm$ 0.08 <sup>a</sup>	0.9 $\pm$ 0.08 <sup>a</sup>	1.3 $\pm$ 0.07 <sup>b</sup>	1.2 $\pm$ 0.06 <sup>b</sup>	36.644	0.000
SR <sup>8</sup>	100.0 $\pm$ 0.00	98.9 $\pm$ 1.11	97.8 $\pm$ 1.11	98.9 $\pm$ 1.11	0.889	0.487
<i>Trial 2 (initial body weight of 165.9 g)</i>						
IBW <sup>1</sup>	165.5 $\pm$ 0.64	170.5 $\pm$ 4.04	162.0 $\pm$ 3.30	165.8 $\pm$ 4.45		
FBW <sup>2</sup>	347.7 $\pm$ 5.38 <sup>a</sup>	395.2 $\pm$ 7.65 <sup>b</sup>	381.1 $\pm$ 5.52 <sup>b</sup>	359.3 $\pm$ 3.31 <sup>a</sup>	14.150	0.001
WGR <sup>3</sup>	110.1 $\pm$ 3.71 <sup>a</sup>	131.8 $\pm$ 4.01 <sup>b</sup>	135.2 $\pm$ 6.52 <sup>b</sup>	116.7 $\pm$ 3.03 <sup>a</sup>	15.492	0.001
SGR <sup>4</sup>	1.0 $\pm$ 0.02 <sup>a</sup>	1.2 $\pm$ 0.03 <sup>b</sup>	1.2 $\pm$ 0.04 <sup>b</sup>	1.0 $\pm$ 0.02 <sup>a</sup>	9.141	0.006
FI <sup>5</sup>	1.3 $\pm$ 0.02 <sup>a</sup>	1.8 $\pm$ 0.03 <sup>c</sup>	1.5 $\pm$ 0.04 <sup>b</sup>	1.3 $\pm$ 0.03 <sup>a</sup>	17.608	0.001
FER <sup>6</sup>	0.4 $\pm$ 0.02	0.4 $\pm$ 0.00	0.5 $\pm$ 0.03	0.4 $\pm$ 0.01	0.099	0.959
PER <sup>7</sup>	0.8 $\pm$ 0.02	0.8 $\pm$ 0.01	0.8 $\pm$ 0.02	0.8 $\pm$ 0.01	0.152	0.925
SR <sup>8</sup>	95.6 $\pm$ 2.22	97.6 $\pm$ 2.38	93.6 $\pm$ 3.61	100 $\pm$ 0.00	1.283	0.345

<sup>1</sup> IBW: initial body weight.

<sup>2</sup> FBW: final body weight.

<sup>3</sup> Weight gain rate (WGR) (%) =  $100 \times [(\text{FBW} - \text{IBW}) / \text{IBW}]$ .

<sup>4</sup> Specific growth rate (SGR) (%/d) =  $100 \times [(\text{LnFBW} - \text{LnIBW}) / \text{days}]$ .

<sup>5</sup> Feed intake (FI) (%/d) =  $100 \times \text{total amount of the feed consumed} / [(\text{IBW} + \text{FBW}) / 2] / \text{days}$ .

<sup>6</sup> Feed efficiency ratio (FER) = wet weight gain (g) / total amount of the feed consumed (g).

<sup>7</sup> Protein efficiency rate (PER) = wet weight gain (g) / total amount of the feed consumed (g) / protein percent in diets.

<sup>8</sup> Survival rate (%) =  $100 \times (\text{final fish number} / \text{initial fish number})$ .

\* Data are means of triplicate, values in the same row with different superscripts are significantly different ( $P < 0.05$ ).

**Table 3**  
Morphological indexes of juvenile turbot with two body weight (% Means  $\pm$  S.E.M.).<sup>a</sup>

	Trial 1 (initial body weight of 6.3 g)			Trial 2 (initial body weight of 165.9 g)		
	CF	HSI	VSI	CF	HSI	VSI
Diet 1 (0.0%) <sup>b</sup>	2.9 $\pm$ 0.06	1.1 $\pm$ 0.10	5.2 $\pm$ 0.26	2.8 $\pm$ 0.14	0.7 $\pm$ 0.07	3.6 $\pm$ 0.04
Diet 2 (0.5%)	2.9 $\pm$ 0.05	1.1 $\pm$ 0.14	5.0 $\pm$ 0.44	2.9 $\pm$ 0.10	0.8 $\pm$ 0.02	3.2 $\pm$ 0.08
Diet 3 (1.0%)	3.2 $\pm$ 0.16	1.2 $\pm$ 0.22	5.4 $\pm$ 0.84	3.3 $\pm$ 0.27	0.8 $\pm$ 0.03	3.2 $\pm$ 0.15
Diet 4 (1.5%)	3.0 $\pm$ 0.16	1.1 $\pm$ 0.12	4.8 $\pm$ 0.51	3.3 $\pm$ 0.24	0.8 $\pm$ 0.09	3.3 $\pm$ 0.10
F-value	1.193	0.098	0.430	1.795	0.487	3.793
P-value	0.372	0.959	0.737	0.226	0.701	0.058

CF: condition factor = 100  $\times$  body weight (g) / [body length (cm)]<sup>3</sup>.HSI: hepatosomatic index = 100  $\times$  (liver weight/body weight).VSI: viscerosomatic index = 100  $\times$  (visceral weight/body weight).<sup>a</sup> Data are means of triplicate, values in the same row with the same superscripts are not significantly different ( $P > 0.05$ ).<sup>b</sup> Diet no. (taurine supplementation concentration).

( $P < 0.05$ ), and thereafter kept stable ( $P > 0.05$ ), while crude lipid content significantly increased ( $P < 0.05$ ) with increasing dietary taurine. The moisture, ash content decreased with increasing dietary taurine, and fish fed the diet with 1.5% taurine (Diet 4) showed significantly lower moisture content than the control diet (Diet 1) ( $P < 0.05$ ). However, no significant difference was observed in ash content among dietary treatments ( $P > 0.05$ ) (Table 4).

#### 3.4. Body taurine distribution

For turbot with 6.3 g initial weight, the taurine concentrations in tissues of the control group (without taurine supplementation) were 71 (in eye), 107 (in muscle), 150 (in body), 214 (in brain) and 226 (in liver) mg/100 g (Fig. 1A). Taurine contents in whole body, muscle, liver significantly increased with increasing dietary taurine from 0.0% to 1.0% ( $P < 0.05$ ), and kept stable from 1.0% to 1.5% ( $P > 0.05$ ), while taurine contents in eye, brain significantly increased with increasing dietary taurine from 0.0% to 0.5% ( $P < 0.05$ ), and thereafter kept stable ( $P > 0.05$ ). A significant positive relationship ( $r > 0.881$ ) was noted between the concentrations of taurine accumulated in whole body, muscle, eye, liver and brain and the consumed taurine ( $P < 0.05$ ) (Table 5).

For turbot with 165.9 g initial weight, the taurine concentrations in tissues of the control group (without taurine supplementation) were 100 (in eye), 184 (in muscle), 221 (in body) and 275 (in brain), 340 (in liver) mg/100 g, respectively (Fig. 1B). The taurine content in muscle significantly increased with increasing dietary taurine from 0.0% to 0.5% ( $P < 0.05$ ), and thereafter kept stable ( $P > 0.05$ ). The taurine content in liver significantly increased with increasing dietary taurine from 0.0% to 1.0% ( $P < 0.05$ ), and thereafter kept stable ( $P > 0.05$ ). Nevertheless, the taurine content in eye significantly increased with increasing dietary taurine from 0.0% to 0.5% ( $P < 0.05$ ), and then kept stable from 0.5% to 1.0% ( $P > 0.05$ ), and thereafter significantly increased ( $P < 0.05$ ). Taurine contents in whole body, brain significantly increased with increasing dietary taurine ( $P < 0.05$ ). A

significant positive relationship ( $r > 0.946$ ) was found between consumed taurine and taurine contents in tissues ( $P < 0.05$ ) (Table 5).

#### 4. Discussion

Taurine is generally considered as an acid derivative, which possesses the major characteristics of a feeding stimulant for fish in molecular weight, nitrogen content, water solubility and acid property (Carr, 1982). Actually, taurine has been used as an important attractant in sea bass (Martinez et al., 2004), red sea bream (Matsunari et al., 2008). In the present study, feeding intake in turbot with both body weight fed diets supplemented with 0.5–1.0% taurine was significantly higher than that in fish fed the control diet (without taurine) ( $P < 0.05$ ), which indicates that taurine is also an effective feeding stimulant for juvenile turbot.

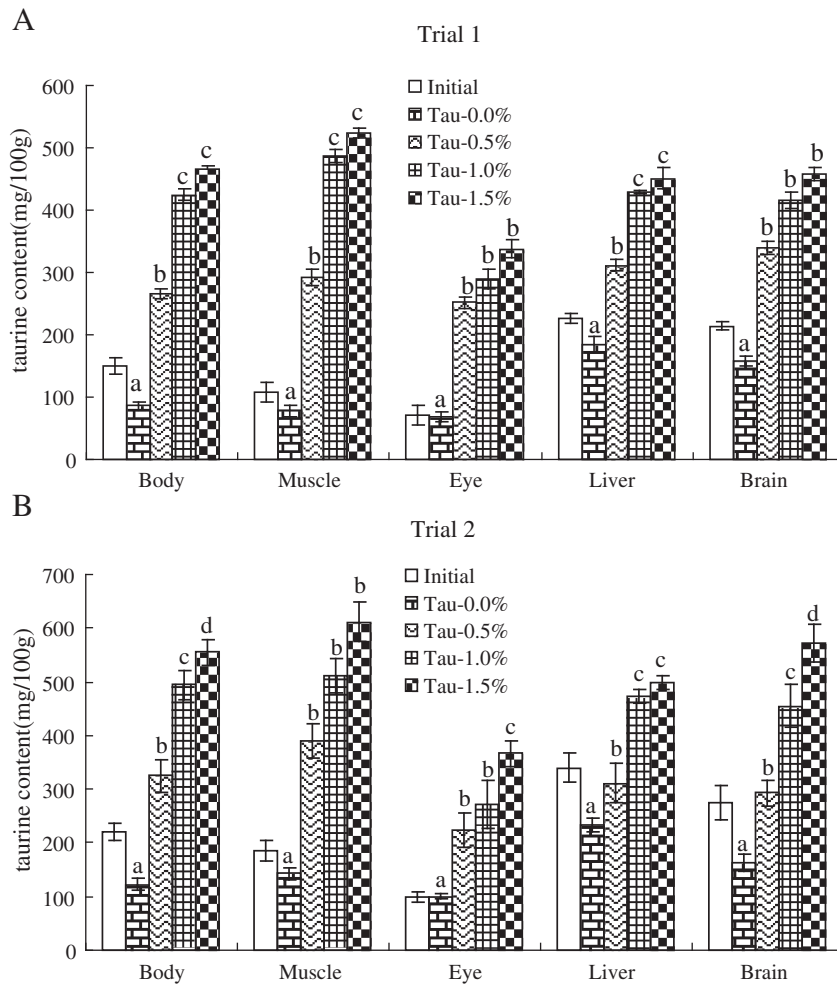
The promotion effect of taurine on growth has been reported in yellowtail (Matsunari et al., 2005) and Japanese flounder (Kim et al., 2007, 2008a,b). Similarly, growth of turbot with both initial body weight was significantly increased by supplementation of taurine in this study. For smaller turbot (6.3 g), both feed intake and feed utilization increased by supplementation of taurine, which paralleled with the result of growth. This indicated that increased growth was probably due to the increase of both feeding intake and feed utilization. For larger turbot (165.9 g), feed utilization was not significantly enhanced by supplementation of taurine although feed intake was significantly increased. Therefore, the increased growth was probably attributed to the increase of feed intake, but not feed utilization. This suggested that fish with different body weights responded to dietary taurine in different manner, which was confirmed by the studies on red sea bream (Matsunari et al., 2008; Takagi, 2006a; Takagi et al., 2011). Kuzmina et al. (2008) attributed this phenomenon to the taurine polyfunctionality appearing at different stages of the biota.

In the present study, results in smaller turbot (6.3 g) indicated that excessive taurine supplementation (1.5%) did not cause growth depression compared with optimal taurine inclusion (1.0%) as the

**Table 4**  
Whole body composition of juvenile turbot with two body weight (% wet weight; Means  $\pm$  S.E.M.).<sup>1</sup>

	Trial 1 (initial body weight of 6.3 g)				Trial 2 (initial body weight of 165.9 g)			
	Moisture	Crude protein	Crude lipid	Ash	Moisture	Crude protein	Crude lipid	Ash
Diet 1 (0.0%) <sup>2</sup>	77.7 $\pm$ 0.24	14.5 $\pm$ 0.15 <sup>a</sup>	2.7 $\pm$ 0.28	4.3 $\pm$ 0.07 <sup>b</sup>	79.5 $\pm$ 0.25 <sup>b</sup>	14.6 $\pm$ 0.22 <sup>a</sup>	1.2 $\pm$ 0.10 <sup>a</sup>	5.1 $\pm$ 0.14
Diet 2 (0.5%)	78.1 $\pm$ 0.18	15.2 $\pm$ 0.13 <sup>ab</sup>	2.5 $\pm$ 0.03	4.2 $\pm$ 0.10 <sup>a</sup>	78.7 $\pm$ 0.80 <sup>ab</sup>	15.9 $\pm$ 0.18 <sup>b</sup>	1.4 $\pm$ 0.09 <sup>ab</sup>	5.0 $\pm$ 0.17
Diet 3 (1.0%)	78.0 $\pm$ 0.40	15.7 $\pm$ 0.09 <sup>b</sup>	2.6 $\pm$ 0.10	4.0 $\pm$ 0.08 <sup>a</sup>	78.0 $\pm$ 0.30 <sup>ab</sup>	16.5 $\pm$ 0.06 <sup>b</sup>	1.7 $\pm$ 0.03 <sup>b</sup>	4.9 $\pm$ 0.20
Diet 4 (1.5%)	78.4 $\pm$ 0.22	14.7 $\pm$ 0.46 <sup>ab</sup>	2.5 $\pm$ 0.05	4.0 $\pm$ 0.05 <sup>a</sup>	76.8 $\pm$ 0.27 <sup>a</sup>	16.6 $\pm$ 0.18 <sup>b</sup>	2.3 $\pm$ 0.09 <sup>c</sup>	4.6 $\pm$ 0.17
F-value	0.942	4.092	0.551	6.227	6.068	26.775	37.819	1.819
P-value	0.464	0.049	0.662	0.017	0.019	0.000	0.000	0.222

<sup>1</sup> Data are means of triplicate, values in the same row with the same superscripts are not significantly different ( $P > 0.05$ ).<sup>2</sup> Diet no. (taurine supplementation concentration).



**Fig. 1.** Taurine distribution of turbot with  $6.3 \pm 0.01$  g (Trial 1, A),  $165.9 \pm 5.01$  g (Trial 2, B) initial body weight fed diets (% wet weight) with dietary supplemented and unsupplemented taurine.

reports in most previous studies (Kim et al., 2005a; Matsunari et al., 2005, 2008; Takagi et al., 2011). The possible reason is that excessive dietary taurine is excreted to keep body taurine at optimum concentration, which was supported by Pinto et al. (2012). However, both feed intake and growth in larger turbot ( $165.9$  g) significantly decreased with increasing dietary taurine from 1.0% to 1.5% ( $P < 0.05$ ),

**Table 5**

Correlation coefficients ( $r$ ) and of consumed taurine ( $X$ ) vs. various taurine concentrations in body, muscle, eye, liver and brain ( $Y$ ) at juvenile turbot with two body weight.<sup>a</sup>

	Linear regression	$r$	$P$
<i>Trial 1 (initial body weight of 6.3 g)</i>			
Body taurine content	$Y = 10,842X + 70,014$	0.955	0.000
Muscle taurine content	$Y = 12,788X + 60,748$	0.945	0.000
Eye taurine content	$Y = 7083.7X + 79,798$	0.881	0.001
Liver taurine content	$Y = 7680.1X + 173.04$	0.947	0.000
Brain taurine content	$Y = 8155.1X + 161.73$	0.924	0.000
<i>Trial 2 (initial body weight of 165.9 g)</i>			
Body taurine content	$Y = 12,225X + 102.51$	0.967	0.000
Muscle taurine content	$Y = 12,732X + 131.01$	0.964	0.000
Eye taurine content	$Y = 7015.9X + 84,303$	0.977	0.000
Liver taurine content	$Y = 8011.4X + 201.18$	0.946	0.000
Brain taurine content	$Y = 11,539X + 114.01$	0.997	0.000

<sup>a</sup> Consumed taurine (%/d) = feed intake (%/d)  $\times$  analyzed taurine content in diet (%); analyzed taurine content in diet Tau-0.0%, Tau-0.5%, Tau-1.0%, Tau-1.5%: 0.16%, 0.64%, 1.15%, 1.66%. Body, Muscle, Eye, Liver and Brain taurine content: mg/100 g wet matter weight.

suggesting that excessive taurine supplementation (1.5%) may retard growth by means of reducing feed intake as the reports in Japanese flounder (Park et al., 2002) and rainbow trout (Gaylord et al., 2006). It was presumed that inferior feed intake was attributed to dietary low palatability caused by high acid property of excessive taurine accumulated in diets (Carr, 1982; Takaoka et al., 1990).

The whole-body protein content in smaller turbot ( $6.3$  g) fed the diet with 1.0% taurine supplementation was significantly higher than that in fish fed the control diet. As to larger turbot ( $165.9$  g), higher carcass protein content was found in fish fed the diet with 0.5% taurine supplementation than that in fish fed the control diet. The results indicate that dietary taurine supplementation can increase whole-body crude protein level in juvenile turbot, which agrees well with the findings in common dentex (Chatzifotis et al., 2008). Another fact was that the crude protein of whole-body paralleled with growth of smaller or larger turbot in the present study. Thus, it is inferred that taurine probably promotes growth by increasing protein synthesis and deposit (Li et al., 2009).

A previous study indicated that taurine distribution in fish varied throughout ontogenesis (Yokoyama et al., 2001). Kim et al. (2007, 2008a) reported that higher taurine concentration was present in liver and brain of juvenile Japanese flounder ( $9.7$  g), and in body and liver of fry ( $0.3$  g) compared to other tissues. Thus, it is implied that different fish size may result in change of taurine distribution in tissues. Further studies indicated that taurine distribution was determined by the distribution of L-cysteinesulfinate decarboxylase (L-CSD) (Yokoyama et al., 2001), a key enzyme for taurine conversion

**Q8, Q9** (de la Rosa and Stipanuk, 1985; Oja and Kontro, 1983), which was expressed under the control of gene and distributed in distinctive tissues of fish throughout ontogenesis (Kim et al., 2003, 2005b). However, in this study, higher taurine level in turbot with two initial body weight was both shown in liver and brain than the other measured tissues, which indicates that turbot with different weight possibly exist slight change in tissue expression of L-CSD as reported in Japanese flounder (Kim et al., 2008a). Besides, taurine distribution in fish with both weight fed the diet with 1.5% taurine supplementation inclined to form an order of taurine level from muscle (high) to eye (low) compared to fish fed the control diet with an order from liver (high) to eye (low). The alternation indicates that taurine distribution also varies with dietary taurine supplementation (Matsunari et al., 2005; Takagi, 2006b; Takagi et al., 2010), which was further clarified by results in Japanese flounder (Kim et al., 2005b, 2007). It is explained that the capacity of accumulating excessive taurine differs with tissue distinction (Park et al., 2002; Pinto et al., 2010) and some important tissues (muscle, brain) may accumulate more taurine to play vital roles in physiological events (Pinto et al., 2012). However, excessive taurine in tissues was probably excreted ultimately to maintain a cycle process that keeps body taurine balance. Pinto et al. (2012) described it as a recycling pathway for taurine based on a high-affinity, low-capacity sodium/chloride-dependent taurine transporter (TauT; SLC6A6) (O'Flaherty et al., 1997).

In conclusion, results of the present study demonstrated that optimal taurine supplementation in diets improved feeding and growth of turbot with two body weight. Excessive taurine in diets had inhibitory effects on turbot feed intake and growth. Dietary taurine exhibited its effect on taurine concentration of body and tissues in a dose-dependent manner.

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