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The tolerance and safety assessment of taurine as additive in a marine carnivorous fish, *Scophthalmus maximus* L.

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Abstract

The effects of dietary taurine on growth performance, liver and intestine morphology, serum physiological and antioxidant parameters, serum thyroid hormone level, muscle taurine content and fatty acid composition of turbot were first evaluated, for the safe utilization in marine fish feed and for human food safety. Four experimental diets were formulated to contain 0, 10, 50 and 100 g/kg taurine. Each diet was randomly assigned to six replicates of 30 juvenile turbot (initial mean weight of 7.46 g). The feeding trial lasted for 10 weeks. The growth performance of fish was significantly enhanced by 10 g/kg dietary taurine. The integrity of the distal intestine was impaired and the absorptive surface was found to be significantly reduced by 100 g/kg dietary taurine. The obvious pathological changes in liver were observed in fish fed 100 g/kg taurine. Dietary taurine with 10 and 50 g/kg significantly increased the activities of serum superoxide dismutase, lysozyme and thyroid hormone. The taurine content in muscle was found to be significantly increased by dietary taurine; however, no significant differences were observed among taurine-supplemented treatments. This study suggested that 10 g/kg taurine was safe in turbot feed, and fivefold of safety margin was obtained.

KEYWORDS
growth, health, morphology, safety assessment, taurine, turbot

1 | INTRODUCTION

Taurine, as a conditionally essential amino acid, has been reported attributing a wide diversity of roles in mammal physiology. In 1990s, taurine gained a growing attention for use in aquafeed because several studies reported the superior survival and growth of fish fed diets with taurine supplementation (Conceição et al., 1997; Luizi, Gara, Shields, & Bromage, 1999; Park, Takeuchi, Seikai, & Nakazoe, 1997). Marine fish have inferior taurine biosynthesis ability than mammals or even freshwater fish to meet the maximal growth, due to the low or negligible ability of a key enzyme cysteinesulfinate decarboxylase (CSD; El-Sayed, 2014; Goto et al., 2001; Kim, Takeuchi, Akimoto, et al., 2005; Park, Takeuchi, Yokoyama, & Seikai, 2002; Yokoyama, Takeuchi, Park, & Nakazoe, 2001). Furthermore, as the limited supply and high cost of fish meal is becoming a limited factor of rapidly growing aquaculture and aquafeed industry, the substitution with plant protein sources increased significantly. As is known, carnivorous fish is susceptible to plant protein and no taurine exists in plant protein source. Thus, it is quite necessary to supplement exogenous taurine in feeds of marine carnivorous fish. Recently, many studies have shown that the taurine supplementation in diets could alleviate the negative effects of high level of plant proteins (Bañuelos-Vargas, López, Pérez-Jiménez, & Peres, 2014; Boonyoung, Haga, & Satoh, 2013; Chatzifotis, Polemitou, Divanach, & Antonopoulou, 2008; Jirsa, Davis, Salze, Rhodes, & Drawbridge, 2014; Jirsa, Stuart, et al., 2014; Takagi et al., 2005, 2010, 2011; Takagi, Murata, Goto, Hayashi, et al., 2006; Takagi, Murata, Goto, Ichiki, et al., 2006; Yun et al., 2012). Takagi et al. (2005, 2010, 2011) and Takagi, Murata, Goto, Ichiki, et al. (2006) have reported that

the incidence of green liver of yellowtail and red sea bream fed soy protein concentrate (SPC)-based diets could be decreased by supplementing 2–20 g/kg taurine in diets. Taurine also exhibited antioxidant effects in the study on zebrafish in which 150 and 400 mg/L taurine administration could restore the activity of superoxide dismutase (SOD) decreased by ethyl alcohol (EtOH; Rosemberg et al., 2010). In some mammal species, supplementing 10 g/kg dietary taurine for 2 weeks enhanced the cholesterol degradation and significantly decreased the serum cholesterol level in rats fed high-cholesterol diet (Yokogoshi et al., 1999). However, body lipid content decreased by taurine inclusion in the soybean meal-based diet of turbot (Yun et al., 2012) might have a potential impact on the quality and flavour of this fish, which has low lipid contents. Espe, Ruohonen, and El-Mowafi (2012) have reported that 1 g/kg taurine supplement in diet could cause a slightly negative effect on the weight gain of juvenile Atlantic salmon. Moreover, excessive taurine supplementation could cause excessive loss of free amino acids and reduce the utilization efficiency of amino acids in fish (Matsunari, Furuita, et al., 2008; Zhou, Wang, & Ye, 2015). As for humans, it is still unclear whether taurine deposit in fish could exceed the observed safety level (OSL) and the no observed adverse effect level (NOAEL) of taurine intake (100–1000 mg taurine per kg body weight per day for people; Aguilar et al., 2009; EFSA, 2012) and cause hazards when humans ingest normal amount of fish.

Taurine was designated as a feed additive in all species of fish in China (Declaration No. 2045 of the Ministry of Agriculture of China). The concentration of taurine used wildly in flatfish diets in previous studies ranged from 10 to 15 g/kg (Enterria et al., 2011; Kim, Takeuchi, Yokoyama, & Murata, 2003; Kim, Takeuchi, Akimoto, et al., 2005; Matsunari, Furuita, et al., 2008; Qi et al., 2012; Yun et al., 2012). However, to date little information about its safety limit and the adverse effects of high levels on fish was available. Turbot (*Scophthalmus maximus* L.) is a typical marine carnivorous fish cultured in north China and Europe due to its high-quality flesh and rapid growth. Therefore, this study was conducted to evaluate the effects and safety limit of taurine level on juvenile turbot, according to "Technical Guidance: Tolerance and efficacy studies in target animals" (EFSA Panel on Additives and Products or Substances used in Animal Feed, 2011) and "The Guidelines for Tolerance Test of Feeds and Feed Additives in Target Aquatic Animals" (Ministry of Agriculture of China, 2012).

2 | MATERIALS AND METHODS

2.1 | Experimental diets

Taurine (>994 g/kg purity) was obtained from Yongan Pharmaceutical Co. Ltd. (Qianjiang, China), and the batch number was STP14042149. By detecting related hygienical indicators, harmful substance contents in taurine met the criterion of National Feed Hygiene. A basal diet (control) was formulated to contain approximately 560 g/kg crude protein and 120 g/kg crude lipid (Table 1), which have been demonstrated to be sufficient for the optimal growth of turbot (Kaushik, 1998). The dose design of taurine was determined according to "Technical Guidance: Tolerance and efficacy studies in target animals" (EFSA Panel on Additives and Products or Substances used in Animal Feed, 2011) and "The Guidelines for Tolerance Test of Feeds and Feed Additives in Target Aquatic Animals" (Ministry of Agriculture of China, 2012). Supplementing 10 g/kg taurine was the maximum recommended

TABLE 1 The formulation of experimental diets and component analysis (dry matter, g/kg)

	Taurine supplementation (g/kg)			
Ingredients	**0**	**10**	**50**	**100**
Fish meal[a]	420	420	420	420
Soybean meal	100	100	100	100
Corn gluten meal	150	150	150	150
Beer yeast	50	50	50	50
Fish oil	46	46	46	46
Soybean oil	23	23	23	23
Vitamin mixture[b]	10	10	10	10
Mineral mixture[c]	5	5	5	5
Soy lecithin	10	10	10	10
Choline chloride	3	3	3	3
Monocalcium phosphate	3	3	3	3
Betaine	5	5	5	5
Calcium propionate	1	1	1	1
Ethoxyquin	0.5	0.5	0.5	0.5
Sodium alginate	5	5	5	5
Yttria	1	1	1	1
Amino acid mixture[d]	20.9	20.9	20.9	20.9
Taurine[e]	0	10	50	100
α-Starch	146.6	136.6	96.6	46.6
Sum	1,000	1,000	1,000	1,000
Component analysis				
Dry matter content	979.8	981.0	985.2	985.0
Crude protein (g/kg)	516.2	525.1	552.8	593.4
Crude lipid (g/kg)	119.6	124.4	128.7	128.7
Crude ash (g/kg)	89.8	91.5	90.0	90.8
Taurine content (g/kg)	2.5	12.6	47.7	95.2

[a]Purchased from *Qingdao Seven Great Bio-tech Company Limited (Qingdao, China)*, crude protein: 720.7 g/kg; crude lipid: 89 g/kg.

[b]Vitamin mixture: providing for per kg diet: VA 16,000 IU, VD 2,500 IU, VB_1 25 mg, VB_2 45 mg, VB_6 20 mg, VB_{12} 10 mg, niacinamide 200 mg, creatine 800 mg, calcium pantothenate 60 mg, VH 60 mg, folate 20 mg, VE 240 mg, VK 10 mg, VC phosphate 2,000 mg, antioxidants 3 mg, microcrystalline cellulose 6,470 mg.

[c]Mineral mixture: providing for per kg diet: Mg 313.0 mg, Fe 79.1 mg, Zn 62.6 mg, Mn 46.6 mg, I 2.0 mg, Se 0.9 mg, Cu 6.4 mg, zeolite powder 3,485 mg.

[d]Amino acid mixture: providing for per kg diet: Arg 7.92 mg, His 1.80 mg, Lys 5.37 mg, Met 4.16 mg, Thr 1.61 mg.

[e]Taurine: purchased from *Yongan Pharmaceutical Company Limited (Qianjiang, China)*, and the batch number is STP14042149. The marked taurine content is 985–1,015 g/kg, and the measured taurine content is 994 g/kg.

dose according to the previous study in turbot (Qi et al., 2012). Two tolerance treatments were designed to supplement 50 g/kg (5×) and 100 g/kg (10×) dietary taurine. The corresponding levels of dietary taurine were 2.5, 12.6, 47.7 and 95.2 g/kg, respectively.

Ingredients were ground into fine powder through a 180-μm mesh. All the ingredients including taurine were thoroughly mixed with fish oil and soybean oil, and water was added to produce stiff dough. Then, the dough was pelleted with an experimental feed mill (F[II]-26, South China University of Technology, Guangzhou, China), dried for about 12 hr in a ventilated oven at 45°C and stored at −20°C prior to usage.

2.2 | Experimental procedure

Juvenile turbot (*S. maximus* L.) in this experiment were subyearlings obtained from Three Mountain Islands Seafood Co. Ltd. (Laizhou, Shandong, China). After arriving, all the fish were fed the control diet and reared in experimental system for 2 weeks to acclimate the experimental conditions. At the initiation of the experiment, all the fish were fasted for 24 hr and weighed. Fish of similar size (initial mean weight 7.64 ± 0.01 g) were randomly distributed into 24 tanks. Four experimental diets were randomly assigned to six replicate tanks and 30 fish in each tank were fed by hand to apparent satiation twice daily (8:00 a.m., 18:00 p.m.) for 10 weeks. The uneaten feed was collected 30 min after each meal, dried to a constant weight and weighed to allow the calculation of feed intake. During the feeding period, water conditions and fish behaviour were observed and noted every day. The water temperature was 14~20°C, dissolved oxygen (DO) > 7.0 mg/L, salinity 24–26 g/L, NH_4^+-N < 0.3 mg/L and pH 7~8.

2.3 | Sample collection

After fasted for 24 hr, all the fish were weighed after anaesthetized with eugenol (1:10,000; Shanghai Reagent) to ameliorate suffering. Five fish were randomly selected in each tank, sampled for whole-body composition analysis and stored at −20°C prior to analysis. Livers and intestines of three fish from each tank were sampled for morphological observation and measurements. Three fish in each tank were randomly selected, and the individual body weight, body length, liver weight and visceral weight were recorded to calculate the condition factor (CF), hepatosomatic index (HSI) and viscerosomatic index (VSI). Blood samples from 10 fish in each tank were collected from the caudal veins and centrifuged (836 *g*, 10 min) at 4°C to obtain serum samples. Then, the serum samples were immediately stored at −80°C prior to analysis. Muscle samples from five fish in each tank were scraped from the fishbone on both sides and stored at −20°C.

2.4 | Chemical analysis

2.4.1 | Body composition assays

Moisture, crude protein, crude lipid and ash were analysed for fish samples and diets. Moisture content was determined by drying the samples to constant weight at 105°C. Crude protein was measured via the Kjeldahl method (Association of Official Analytical Chemist, AOAC, 1995) and estimated by multiplying nitrogen by 6.25 (FOSS Kjeltec TM 8400, Tecator, Hoganas, Sweden). Crude lipid was determined by ether extraction via Soxhlet method (FOSS Soxhlet TM 8000, Denmark). Ash was examined by combustion in a muffle furnace at 550°C for 16 hr. Duplicate analyses were conducted for each sample.

2.4.2 | Morphological assays

Liver and distal intestine samples were routinely dehydrated in ethanol, equilibrated in xylene and embedded in paraffin wax according to the standard histological procedure (Hu et al., 2015; Li et al., 2014; Ross & Pawlina, 2006). Then, the samples were cut in 7-μm longitudinal sections with a rotary microtome (Lecia Jung RM 2016, Germany) and stained with haematoxylin–eosin method (HE). Observation of sections was performed by Nikon eclipse Ti-S microscope (Japan).

2.4.3 | Haematological assays

Total protein (TP), albumin (ALB), globulin (GLO), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and glucose (GLU) in serum were determined with a fully automatic biochemical analyser (HITACHI 7600, Tokyo, Japan). The activities of lysozyme, superoxide dismutase (SOD) and catalase (CAT) in serum were determined with commercial kits (Nanjing Jiancheng Biotechnology Co. Ltd., Nanjing, China). Triiodothyronine (T_3) and thyroxine (T_4) contents in serum were determined with efficient and dedicated commercial kits (Tianjin Jiuding Medical Biological Engineering Co. Ltd., Tianjin, China).

2.4.4 | Muscle assays

Muscle samples of fish were lyophilized for 24 hr in a lyophilizer (CHRIST Alpha 1-4, Germany) and ground to powdery. After the pretreatment, the samples were determined with LC2000 high-performance liquid chromatograph (Guangdong Food Quality Supervision Institute Station, Guangzhou, China) for determining taurine level, and with 2010 plus gas chromatograph (Shimadzu, Japan) to determine the fatty acid compositions. The standards of fatty acids were purchased from Sigma Aldrich Co. LLC. (USA).

2.5 | Calculations and Statistical analysis

Survival rate, SR (kg^{-1}) = 100 × (final amount/initial amount)

Weight gain rate, WGR (kg^{-1}) = 100 × [(final body weight − initial body weight)/initial body weight]

Specific gain rate, SGR (kg^{-1} day^{-1}) = (ln final body weight – ln initial body weight)/days × 100

Feed intake, FI (kg^{-1} day^{-1}) = 100 × total amount of feed consumption (g)/[(initial body weight + final body weight)/2]/days

TABLE 2 Effects of dietary taurine supplementation on growth performance of turbot

Growth parameters	Dietary taurine supplementation (g/kg)			
	0	10	50	100
SR (kg^{-1})	99.17 ± 0.83	99.17 ± 0.83	99.17 ± 0.83	100.00 ± 0.00
IMW (g)	7.66 ± 0.01	7.62 ± 0.02	7.65 ± 0.01	7.64 ± 0.01
FMW (g)	55.54 ± 0.65^{a}	58.13 ± 0.78^{b}	57.57 ± 0.31^{ab}	56.18 ± 0.43^{ab}
WGR (kg^{-1})	626.36 ± 3.67^{a}	647.63 ± 5.60^{b}	643.54 ± 2.95^{ab}	634.20 ± 2.80^{ab}
SGR (kg^{-1} day^{-1})	2.67 ± 0.02	2.71 ± 0.02	2.70 ± 0.02	2.70 ± 0.02
FER	1.25 ± 0.01^{a}	1.33 ± 0.03^{b}	1.26 ± 0.01^{ab}	1.23 ± 0.01^{a}
FI (kg^{-1})	1.63 ± 0.01	1.61 ± 0.03	1.65 ± 0.01	1.69 ± 0.01
CF	3.68 ± 0.05^{ab}	3.74 ± 0.04^{b}	3.71 ± 0.03^{b}	3.54 ± 0.03^{a}
HSI (kg^{-1})	1.14 ± 0.02^{b}	1.07 ± 0.02^{b}	1.12 ± 0.01^{b}	1.01 ± 0.01^{a}
VSI (kg^{-1})	5.46 ± 0.12	5.45 ± 0.04	5.62 ± 0.08	5.64 ± 0.09

SR, survival rate; IMW, initial mean weight; FMW, final mean weight; WGR, weight gain rate; FER, feed efficiency ratio; FI, feed intake; SGR, specific growth rate; CF, condition factor; HSI, hepatosomatic index; VSI, viscerosomatic index.
Different superscript letters in the same row indicate significant difference ($p < .05$).

Feed efficiency ratio, FER = weight gain (g)/total amount of feed consumption (g)
Condition factor, CF = 100 × average weight/average length^{3}
Hepatosomatic index, HSI (kg^{-1}) = 100 × liver weight/body weight
Viscerosomatic index, VSI (kg^{-1}) = 100 × visceral weight/body weight

Absorptive surface (perimeter ratio, PR) = IP/EP, arbitrary units. IP is the internal perimeter of the gut lumen (villi and mucosal folding length) and EP is the external perimeter of the gut lumen (perimeter of internal muscularis mucosa; Dimitroglou et al., 2009).

SPSS 17.0 microcomputer software was used for all statistical analyses. Data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's multiple-range test. Homogeneity of variance test was conducted to ensure that variance is homogeneous. The results were presented as means ± standard error. In all statistical testing, differences between means at $p < .05$ were considered as significant.

3 | RESULTS

3.1 | Growth performance

No significant difference ($p > .05$) in survival rate was observed among all experimental treatments (Table 2). The final mean weight, weight gain rate and feed efficiency ratio of turbot were significantly increased by 10 g/kg dietary taurine ($p < .05$); however, the feed intake showed no remarkable difference among all treatments ($p > .05$). Fish fed 100 g/kg dietary taurine showed the lowest condition factor and hepatosomatic index ($p < .05$, Table 2).

With increasing dietary taurine supplementation, the moisture content of fish increased remarkably ($p < .05$). Higher levels of taurine (50 and 100 g/kg) significantly increased the crude body protein content ($p < .05$). Compared to the control treatment, fish fed 100 g/kg dietary taurine significantly decreased the crude body lipid and ash contents of turbot ($p < .05$, Figure 1).

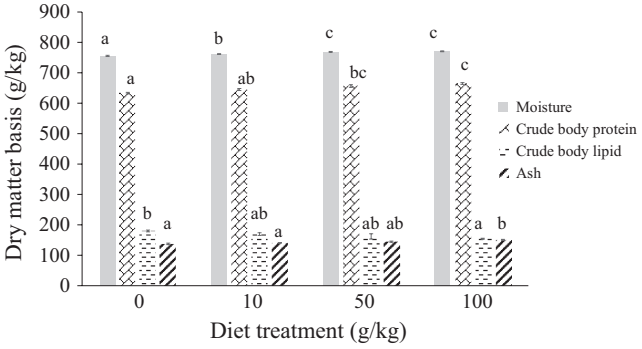

FIGURE 1 Effects of dietary taurine supplementation on body composition of turbot (dry matter basis). Different superscript letters in the same row indicate significant difference ($p < .05$)

3.2 | Morphological examination and measurements

The overall morphological conditions are summarized in Table 3 and Figure 2. No apparent morphological changes in liver and distal intestine of turbot juveniles were observed in treatments with 10 or 50 g/kg taurine inclusion compared to the control treatment. Fish fed the diet with 100 g/kg dietary taurine showed significantly decreased absorptive surface compared to other treatments ($p < .05$). Livers from 10 or 50 g/kg taurine treatments presented regularly shaped hepatocytes, clearly stained nuclei and well-ordered cell cords; however, in 100 g/kg dietary taurine treatment, 10 of 12 sampled fish showed irregularly shaped and darkly stained hepatocytes, obscure nucleoli, atrophied cytoplasm, disordered cell cords, as well as expanded hepatic sinusoids (Figure 3). Similar to the control treatment, fish fed 10 or 50 g/kg dietary taurine in this study showed normal morphology of distal intestine, characterized by an intact epithelial barrier and a significantly higher absorptive surface including tall finger-like mucosal folds, more complex villi structures, thin lamina propria, well-arranged enterocyte nucleus position and well-developed microvilli. However, in the treatment with 100 g/

TABLE 3 Occurrence of morphological changes in the liver and distal intestine of turbot fed experimental diets (n = 12)

	Dietary taurine supplementation (g/kg)			
Parameters	**0**	**10**	**50**	**100**
Liver				
Normal[a]	9	7	8	2
Abnormal-1[a]	1	2	2	10
Abnormal-2[a]	2	3	2	0
Distal intestine				
Normal[b]	10	10	7	4
Abnormal[b]	2	2	5	8

[a]Normal, the hepatic cell cords are well ordered. Hepatocytes have regularly shaped, clearly stained nuclei and basal eosinophilic cytoplasm. Abnormal-1, cell cords of liver are disordered. Hepatocytes are shaped irregularly and stained darkly. The nucleoli are obscure and the cytoplasm is also atrophied. Parts of the sinusoids are expanded. Abnormal-2, hepatocytes are swollen and stained lightly.
[b]Normal, the section of distal intestine has an intact epithelial barrier and a significantly higher absorptive surface that includes tall finger-like mucosal folds, more complex villi structures, thin lamina propria, well-arranged enterocyte nucleus position and well-developed microvilli. Abnormal, the intestinal epithelium is characterized by irregularly shaped enterocytes, reduced height of mucosal folds, wider lamina propria, increased goblet cells and disarranged microvilli. In addition, the nucleoli of enterocytes are obscure.

kg taurine inclusion, 8 of 12 sampled fish presented irregularly shaped enterocytes, reduced height of mucosal folds, wider lamina propria, increased goblet cells and disarranged microvilli (Figure 4).

3.3 | Haematological parameters

The total protein (TP), albumin (ALB), globulin (GLO) and triglyceride (TG) contents in serum of turbot were found to be significantly decreased by 100 g/kg dietary taurine (p < .05). The total cholesterol (TC) content in serum of fish was found to be remarkably reduced by taurine supplementation (p < .05). The serum glucose level of turbot fed the diet with 10 g/kg taurine supplementation was the lowest compared with other treatments (p < .05, Table 4).

The activities of lysozyme and superoxide dismutase (SOD) in serum were found to be significantly increased by 50 and 10 g/kg dietary taurine, respectively (p < .05); however, no significant difference in the activity of catalase (CAT) was observed among all treatments (p > .05; Table 5).

Dietary taurine supplementation at the level of 50 and 10 g/kg remarkably improved the triiodothyronine (T_3) and thyroxine (T_4) contents in the serum, respectively (p < .05, Figure 5).

3.4 | Muscular parameters

The taurine content in fish muscle was found to be increased by dietary taurine supplementation (p < .05); however, no significant differences were observed among the taurine treatments (p > .05; Figure 6).

Supplementing 100 g/kg dietary taurine to fish significantly decreased arachidic acid (20:0) content in muscle (p < .05). Supplementing 100 g/kg taurine remarkably increased the palmitoleic acid (16:1n-7)

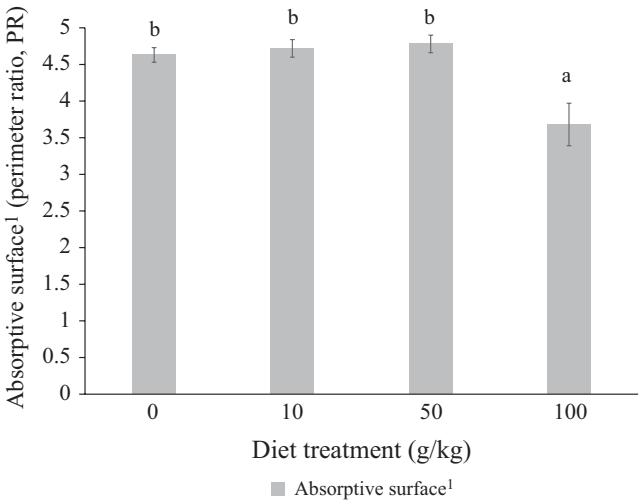

FIGURE 2 Morphology of distal intestine of turbot fed experimental diets (n = 12/treatment). Different superscript letters in the same row indicate significant difference (p < .05). [1]Arbitrary units

content (p < .05) but decreased the oleic acid (18:1n-9) content (p < .05). In polyunsaturated fatty acids (PUFAs), dietary 50 and 100 g/kg taurine significantly increased the contents of α-linolenic acid (α-ALA; 18:3n-3) and docosahexaenoic acid (DHA; 22:6n-3), respectively, in turbot muscle (p < .05, Table 6).

4 | DISCUSSION

4.1 | Growth performance

This study showed that the growth weight gain rate (WGR) of turbot was found to be significantly increased by 10 g/kg dietary taurine; however, no significant difference in the feed intake (FI) was observed among treatments. This indicated that lower level (10 g/kg) of dietary taurine improved the growth performance of turbot by increasing the feed utilization, which was supported by the significantly increased feed efficiency ratio (FER). Takagi, Murata, Goto, Hayashi, et al. (2006), Takagi et al. (2010), and Yun et al. (2012) also reported that dietary taurine (10 g/kg) improved the WGR but not FI in red sea bream and turbot. However, as a kind of amino acid derivative, taurine plays an important role of appetite promotion in red sea bream (Matsunari, Yamamoto, Kim, Goto, & Takeuchi, 2008; Matsunari, Furuita, et al., 2008; Takagi et al., 2011), tilapia (Wang, 2013; Zhou et al., 2015) and white seabass (Jirsa, Davis, et al., 2014). The discrepancy between the present study and previous studies might be due to the fact that different fish species had different appetitive reactions to taurine. Diet formulations may also contribute to the discrepancy. Qi et al. (2012) reported that the WGR, FER and FI of turbot were found to be significantly increased by 10 g/kg dietary taurine in a casein-basal diet. The formulation with fish meal (420 kg^{-1}) as the main protein source in the present study might increase taurine content as well as the acidity of diets and thus impacted the palatability of diets (Takaoka, Takii, Nakamura, Kumai, & Takeda, 1990). However, in common carp, it was also pointed out that exogenous taurine (10 and 30 g/kg) had

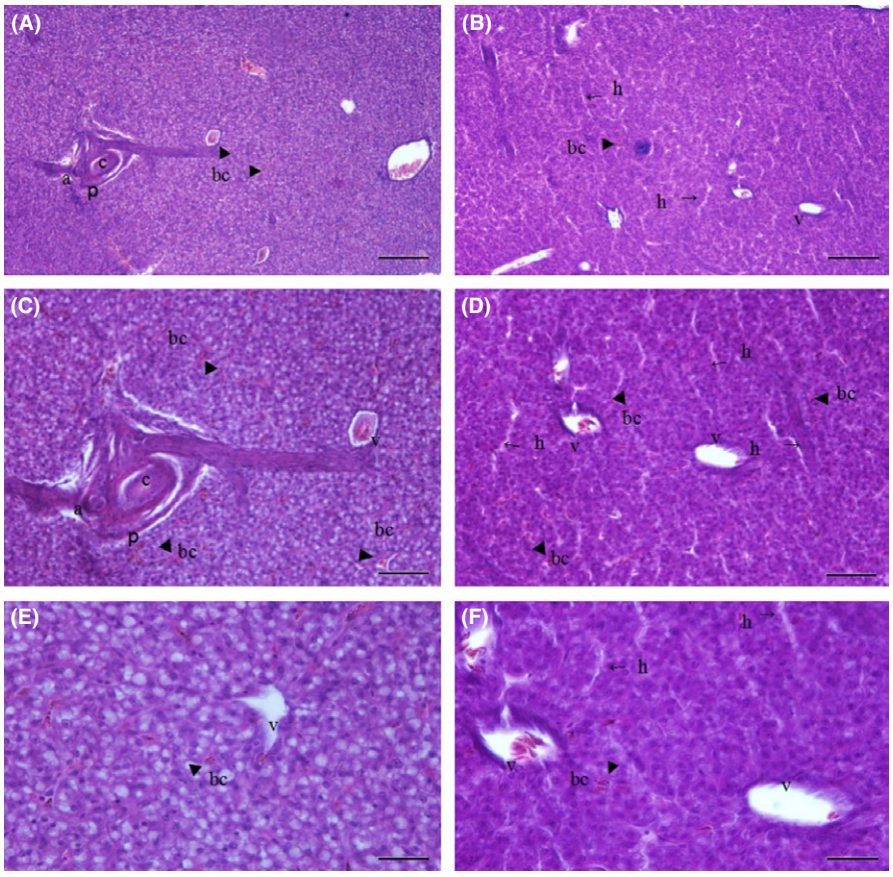

FIGURE 3 Photomicrograph of a 7-μm-thick section of juvenile turbot liver stained with haematoxylin–eosin. Fish were fed 0 taurine (A, C and E) and 100 g/kg taurine (B, D and F). p, portal area; v, central vein; a, interlobular artery; c, interlobular bile duct; h, hepatic sinusoid (arrows); bc, blood cell (arrowheads). Scale bar of A and B, 100 μm; scale bar of C and D, 50 μm; scale bar of E and F, 20 μm

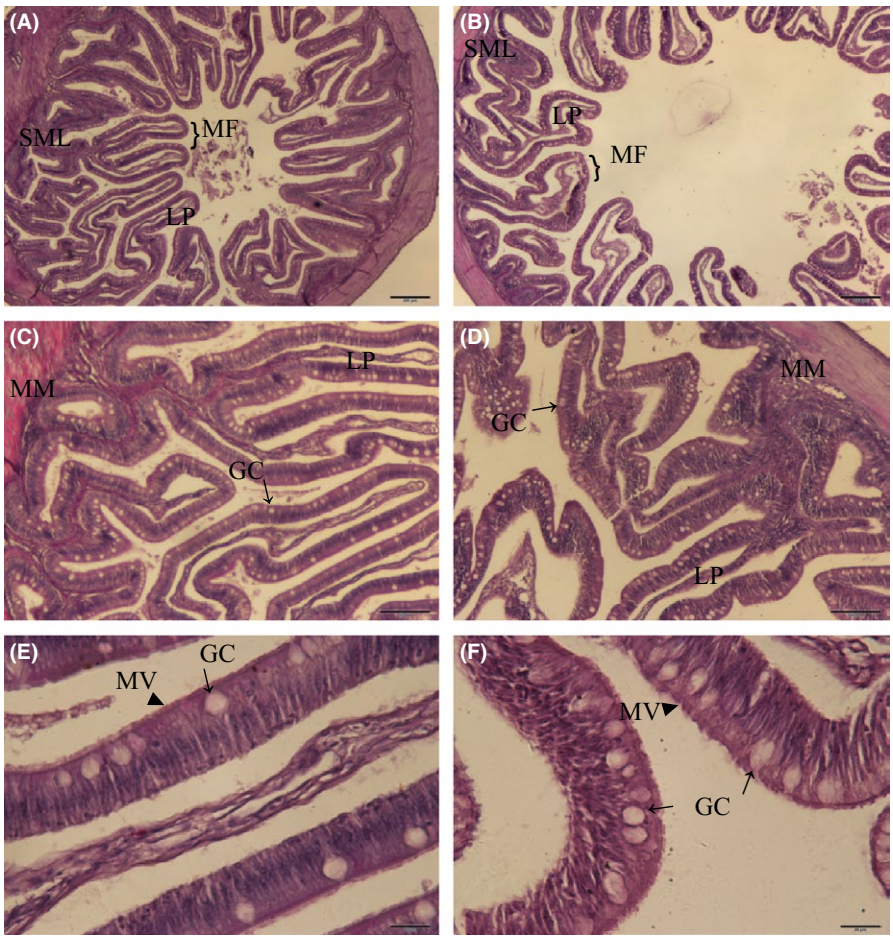

FIGURE 4 Details of distal intestine section from fish fed 0 taurine (A, C and E) and 100 g/kg taurine (B, D and F). MF, mucosal fold; MM, muscularis mucosa; SML, submucous layer; LP, lamina propria; GC, goblet cell (arrows); MV, microvilli (arrowheads). Scale bar of A and B, 200 μm; scale bar of C and D, 100 μm; scale bar of E and F, 20 μm

TABLE 4 Effects of dietary taurine supplementation on physiological and biochemical analyses in serum of turbot

Parameters	Dietary taurine supplementation (g/kg)			
	0	10	50	100
TP (g/L)	32.00 ± 0.77^{b}	31.39 ± 0.43^{b}	31.14 ± 0.27^{ab}	29.13 ± 0.41^{a}
ALB (g/L)	6.82 ± 0.18^{b}	6.67 ± 0.17^{ab}	6.51 ± 0.07^{ab}	6.15 ± 0.07^{a}
GLO (g/L)	25.18 ± 0.59^{b}	24.72 ± 0.31^{ab}	24.63 ± 0.42^{ab}	23.08 ± 0.72^{a}
ALB/GLO (kg^{-1})	27.20 ± 0.20^{ab}	27.40 ± 0.40^{b}	26.20 ± 0.20^{a}	26.20 ± 0.20^{a}
TG (mmol/L)	2.56 ± 0.07^{b}	2.13 ± 0.08^{ab}	2.08 ± 0.18^{ab}	2.01 ± 0.15^{a}
TC (mmol/L)	3.49 ± 0.15^{b}	2.58 ± 0.15^{a}	2.48 ± 0.09^{a}	2.16 ± 0.10^{a}
HDL-C (mmol/L)	2.62 ± 0.21	2.67 ± 0.09	2.59 ± 0.11	2.52 ± 0.15
LDL-C (mmol/L)	0.34 ± 0.00	0.32 ± 0.03	0.36 ± 0.01	0.31 ± 0.02
GLU (mmol/L)	2.37 ± 0.01^{b}	1.64 ± 0.11^{a}	2.26 ± 0.03^{b}	2.51 ± 0.18^{b}

TP, total protein; ALB, albumin; GLO, globulin; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; GLU, glucose.
Different superscript letters in the same row indicate significant difference ($p < .05$).

TABLE 5 Effects of dietary taurine supplementation on immune and antioxidant capacity in serum of turbot

Parameters	Dietary taurine supplementation (g/kg)			
	0	10	50	100
Lysozyme (μg/ml)	3.65 ± 0.04^{a}	3.71 ± 0.01^{ab}	3.77 ± 0.03^{b}	3.66 ± 0.02^{ab}
SOD (U/ml)	243.19 ± 4.75^{a}	257.24 ± 2.72^{b}	255.64 ± 1.27^{ab}	251.95 ± 3.13^{ab}
CAT (U/ml)	4.41 ± 0.54	4.90 ± 0.24	4.30 ± 0.34	4.39 ± 0.35

SOD, superoxide dismutase; CAT, catalase.
Different superscript letters in the same row indicate significant difference ($p < .05$).

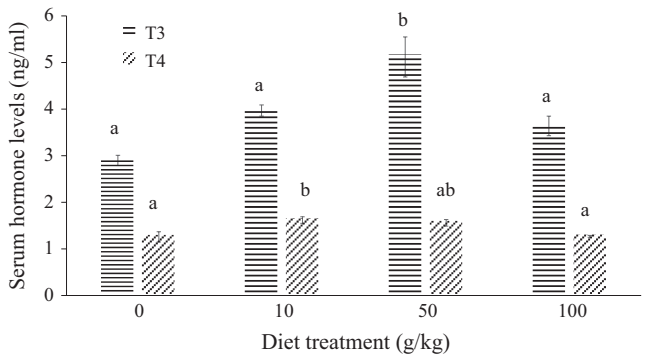

FIGURE 5 Effects of dietary taurine supplementation on hormone levels in serum of turbot. Different superscript letters in the same row indicate significant difference ($p < .05$). T_3, triiodothyronine; T_4, thyroxine

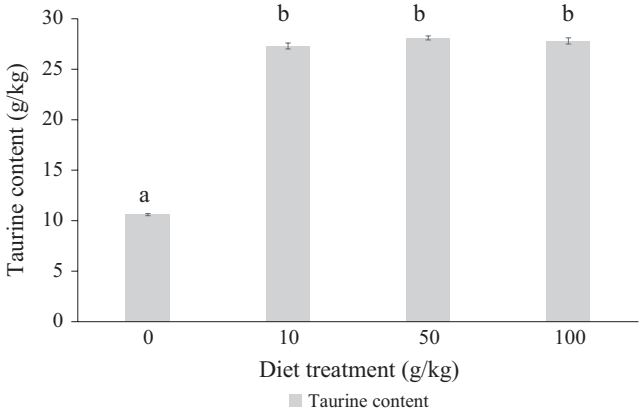

FIGURE 6 Effects of dietary taurine supplementation on taurine content in muscle of turbot. Different superscript letters in the same row indicate significant difference ($p < .05$)

no effect on fish growth (Kim et al., 2008). Supplementation of 1 g/kg taurine in the diet even caused a slightly negative impact on weight gain of juvenile Atlantic salmon (initial mean weight 2 g; Espe et al., 2012). This is probably due to the fact that freshwater species had the ability to biosynthesize taurine. Additionally, developmental stages of fish might also contribute to the differences; for example, dietary taurine enhanced the growth of juvenile Japanese flounder but not the fingerling period (Kim et al., 2003) and the gained weight of juvenile yellowtail fed taurine increased rapidly in later stage, but not in the former stage (Takagi et al., 2005).

Taurine cannot be part of translated peptide chains (Bittner, Win, & Gupta, 2005; Lähdesmäki, 1987), but regulated the size of free amino acid pool (Boonyoung et al., 2013; Matsunari, Furuita, et al., 2008; Matsunari, Yamamoto, et al., 2008; Wang, 2013; Zhou et al., 2015). Some amino acids, such as glycine and arginine, involved in osmoregulation (Li, Mai, Trushenski, & Wu, 2009) of aquatic animals, could be spared by taurine to participate in protein synthesis (Ballatori & Boyer, 1992; Huxtable, 1992; King, Beyenbach, & Goldstein, 1982; Saha, Dutta, & Bhattacharjee, 2002; Saha, Dutta, & Haussinger, 2000; Takagi, Murata, Goto, Hayashi, et al., 2006). However, excessive taurine supplementation caused excessive loss of free amino acids and reduced the utilization efficiency of amino acids in fish (Matsunari, Furuita, et al., 2008; Zhou et al., 2015). In addition, thyroid hormones

TABLE 6 Effects of dietary taurine supplementation on composition of fatty acids in muscle of turbot (% of total fatty acids)

	Dietary taurine supplementation (g/kg)			
Fatty acids	**0**	**10**	**50**	**100**
14:0	2.28 ± 0.08	2.37 ± 0.09	2.41 ± 0.04	2.44 ± 0.13
16:0	17.08 ± 0.39	17.58 ± 0.30	17.10 ± 0.13	17.56 ± 0.28
18:0	5.54 ± 0.10	5.32 ± 0.12	5.32 ± 0.14	5.16 ± 0.18
20:0	0.31 ± 0.00^{b}	0.31 ± 0.01^{b}	0.29 ± 0.01^{ab}	0.27 ± 0.02^{a}
∑SFA^{A}	25.21	25.58	25.12	25.43
16:1n-7	2.74 ± 0.15^{a}	2.97 ± 0.16^{ab}	3.17 ± 0.02^{ab}	3.35 ± 0.07^{b}
18:1n-9	14.84 ± 0.04^{b}	14.57 ± 0.05^{b}	14.61 ± 0.14^{b}	13.97 ± 0.09^{a}
∑MUFA^{B}	17.58	17.54	17.78	17.32
18:2n-6	15.55 ± 0.07	15.72 ± 0.25	15.99 ± 0.12	15.82 ± 0.08
20:4n-6	1.09 ± 0.01	1.10 ± 0.05	1.05 ± 0.03	1.14 ± 0.06
∑n-6PUFA^{C}	16.64	16.82	17.04	16.96
18:3n-3	1.55 ± 0.04^{a}	1.69 ± 0.03^{ab}	1.75 ± 0.03^{b}	1.69 ± 0.05^{ab}
20:5n-3	5.46 ± 0.19^{a}	5.64 ± 0.04^{ab}	5.83 ± 0.10^{ab}	6.22 ± 0.17^{b}
22:6n-3	17.59 ± 0.70^{a}	17.65 ± 0.12^{a}	19.78 ± 0.34^{ab}	20.61 ± 0.68^{b}
∑n-3PUFA^{D}	24.60	24.98	26.50	28.22
∑SFA/∑PUFA	0.61	0.61	0.58	0.56
∑n-6PUFA/ ∑n-3PUFA	0.68	0.67	0.64	0.60

A: saturated fatty acid (SFA); B: monounsaturated fatty acid (MUFA); C: N-6 polyunsaturated fatty acid (N-6 PUFA); D: N-3 polyunsaturated fatty acid (N-3 PUFA).
Different superscript letters in the same row indicate significant difference ($p < .05$).

such as triiodothyronine (T_3) and thyroxine (T_4) are involved in metabolic regulation, growth and development and especially modulating protein synthesis (Chopra, 1977; Wang, 2013). In the present study, the inclusion of 50 and 10 g/kg taurine significantly increased the T_3 and T_4 contents, respectively, in turbot serum. Similar results were also reported in carp, which showed that supplementing 4 g/kg taurine in diet significantly increased serum T_3 and T_4 contents (Qiu & Zhao, 2006). Totally, low or medium levels of dietary taurine (10 and 50 g/kg) probably enhanced the metabolism and growth of juvenile turbot by sparing amino acids or increasing thyroid hormones. However, fish fed 100 g/kg dietary taurine showed no more improvement in T_3 and T_4 contents, which was in accordance with the result of growth.

4.2 | Hypolipidaemic and hypoglycaemic effect

In this study, fish fed high dietary taurine (100 g/kg) showed significant decreases in body lipid and serum total triglyceride (TG), and 10 g/kg taurine in the diet could cause decreased serum total cholesterol (TC) content. Comparative studies were carried out in rat, hamster and Japanese quail (Murakami et al., 2002, 2010; Yokogoshi et al., 1999) in which serum TC contents decreased with the inclusion of taurine in the high-cholesterol diets. That is probably due to the fact that taurine could mediate the lipid metabolism by combining bile acids to form taurocholic acids, which enhanced the capacity of related enzymes lipoprotein lipase (LPL) and hepatic lipase (HL; Zeng et al., 2012). An increasing trend of serum n-3 polyunsaturated fatty acids (PUFAs) of fish fed high level of taurine (100 g/kg) was also observed in this study. The increasing n-3 polyunsaturated acids (PUFA) contents could inhibit the synthesis of TG and the secretion of low-density lipoprotein by suppressing the transcription of lipogenic enzymes in liver (Jump & Clarke, 1999). Halvorsen et al. (2001) demonstrated that peroxisomal β-oxidation stimulated by n-3 PUFAs could direct fatty acids away from TG synthesis. However, supplementing 4–16 g/kg taurine in diets increased body lipid content in a freshwater species of fish, tilapia (Wang, 2013; Zhou et al., 2015). Additionally, juvenile turbot initially weighed 166 g but not 6.3 g fed 10 and 15 g/kg taurine in a casein-basal diet also increased the content of whole-body lipid (Qi et al., 2012). Fish species, diet formulation and growth stage should be paid attention to the usage of taurine in diet. In general, further studies need to be conducted to elucidate the manipulation of lipid metabolism by taurine in fish.

Interestingly, the blood glucose (GLU) level in turbot serum was found to be significantly decreased by the lower taurine level (10 g/kg) in the present study. The treatment with taurine (10 g/kg) for 3 weeks decreased the plasma glucose and increased the plasma insulin levels in diabetic rats (Das, Vasan, & Sil, 2012). Tenner, Zhang, and Lombardini (2003) also pointed out that administration of feed with 5 g/kg taurine for 6 weeks resulted in a significant reduction in plasma glucose of rabbit. This might be due to the fact that taurine had a synergistic effect with insulin-accelerating glycolysis to decrease the blood glucose concentration in serum (Nakaya et al., 2000), or taurine could involve in the inhibition of glycogen breakdown to decrease the blood glucose concentration (Kulakowski & Maturo, 1989). However, the reason for the rise-first-fall-then

trend of blood glucose concentration with increasing taurine level in juvenile turbot is not yet clear, and further studies on hypoglycaemic effects of taurine will have great significance for poor utilization efficiency of glucose in fish.

4.3 | Antioxidation

This study suggested that 10 and 50 g/kg dietary taurine significantly improved the activity of superoxide dismutase (SOD) and lysozyme, respectively. Taurine could enhance non-specific immunity by increasing the activities of free radical scavenging enzyme SOD and lysozyme for preventing microbial invasion (Saurabh & Sahoo, 2008). In addition, Qiu, Zhao, Wang, and Bai (2008) have pointed out that carp fed diets with 0.5–2 g/kg taurine showed significantly increased activities of lysozyme and SOD in serum. Similar result has been reported in the study on zebrafish, showing that 150 and 400 mg/L taurine administration could restore the activity of SOD decreased by ethyl alcohol (EtOH; Rosemberg et al., 2010). Taurine was directly combined with the strong oxidant hypochlorous acid into taurine chloramine to protect body from the damage of strong oxidants (Salze & Davis, 2015). Taurine also improved the activity of antioxidase to eliminate superoxide radical in vivo and lower the level of lipid peroxidation (Divakaran, 2006).

4.4 | High level of taurine caused hepatosis

Green liver symptom was caused by a reduction in the excretion of bile pigments from the liver into the bile, and an overproduction of haemolytic biliverdin due to the deficiency of taurine in plant protein-based diets. Several studies have pointed out that 2–20 g/kg taurine supplementation remarkably decreased the incidence of green liver of yellowtail and red sea bream fed soy protein concentrate (SPC)-based diets (Takagi et al., 2005; Takagi, Murata, Goto, Ichiki, et al., 2006; Takagi et al., 2010; Takagi et al., 2011). In the present study, high inclusion of taurine (100 g/kg) significantly reduced the hepatosomatic index (HSI) and caused apparent expanded hepatic sinusoids from histological observation. The injured liver caused decreased serum protein contents such as total protein (TP), albumin (ALB) and globulin (GLO), which were mainly synthesized in liver and involved in the transport of other substances, immune defence and inflammation defence (Chen, Ning, & Yang, 2012). This might be due to the fact that expanded hepatic sinusoids caused by high taurine level could accelerate the interchange of materials between hepatocytes and blood, break the balance of liver metabolism and even induce hepatosis.

4.5 | Effects on taurine content and human health

In the present study, the taurine content in turbot muscle was found to be increased by 10 g/kg taurine in diets; however, there was no further deposition with more than 10 g/kg taurine supplementation. There might be a taurine recycling pathway to excrete excessive taurine in muscle for maintaining body balance, which was based on a high-affinity, low-capacity sodium/chloride-dependent taurine transporter (TauT; SLC6A6). The transporter worked as an active transport system that carried taurine against a concentration gradient, driven by transmembrane sodium, chloride gradients and membrane potentials (O'flaherty, Stapleton, Redmond, & Bouchier-Hayes, 1997; Pinto et al., 2012). In Japanese flounder and red sea bream, 1–15 g/kg dietary taurine made a linear growth of taurine contents in muscle with increasing taurine levels (Kim, Takeuchi, Akimoto, et al., 2005; Kim, Takeuchi, Yokoyama, et al., 2005; Kim et al., 2007, 2008; Matsunari, Yamamoto, et al., 2008). That might be due to the fact that taurine showed the depositional effect in low supplementation level but the maintenance effect in medium to high supplementation level.

The European Union stated that the observed safety level (OSL) and the no observed adverse effect level (NOAEL) for taurine consumption by humans were identified to be 100–1,000 mg taurine per kg body weight per day (Aguilar et al., 2009; EFSA, 2012). Hence, it is safe for humans to ingest normal amounts of fillet from juvenile turbot cultured with taurine-supplemented feeds. Besides that, n-3 PUFA contents in muscle of fish increased by dietary taurine. Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and α-linolenic acid (α-ALA) played important roles in the modulation and prevention of diseases for humans (Anderson, Connor, & Corliss, 1990; Connor, 2000; Lavie, Milani, Mehra, & Ventura, 2009; Politi, Rotstein, & Carri, 2001). Thus, taurine supplementation in fish diets could improve the convenience of n-3 PUFA intake for humans from the farmed fish.

In conclusion, a lower level of taurine supplementation in diet (10 g/kg) was necessary for juvenile turbot to support maximal growth rate, enhance serum thyroid hormone level, antioxidative ability and other physiological parameters. However, an excessive level of dietary taurine (100 g/kg) caused impairment in liver and distal intestine of turbot. This study suggested that 10 g/kg taurine supplementation in the diet was safe for turbot juveniles, and fivefold safety margin was obtained.

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