




Is a taurine supplement necessary in fishmeal-based feeds for juvenile European sea bass (*Dicentrarchus labrax*)?

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Abstract

The effect of taurine supplementation of fishmeal-based feeds for juvenile European sea bass (*Dicentrarchus labrax*) was investigated. Fish (initial weight ca. 5 g) were reared for 90 days in 12 concrete tanks (1 m³), with 50 fish in each tank. Four feeds (44% crude protein (CP) and 17% lipids (L)) containing 0 (control (CTRL)), 0.4 (T4), 0.7 (T7) and 1.0 (T10) % taurine were prepared and were fed to apparent visual satiation to triplicate groups of fish. The effects on fish growth, feed utilization, proximate chemical composition, haematology, immune biomarkers and muscle morphometry were examined. An increase in the dietary taurine incorporation resulted in progressive increases in the growth metrics, with the highest values being observed for fish receiving the T10 feed (weight gain 22 vs 18 g/fish in CTRL; protein productive value 31 vs 28% in CTRL). Taurine addition at all levels significantly increased the fish protein percentage, but the percentage lipid was reduced significantly in T4 fish only (7.8% compared with 9.4% in CTRL fish). Taurine supplementation resulted in elevated blood lymphocyte and monocyte counts and increased serum phagocytic and lysozyme activities. Taurine addition at 1% (T10 feed), the suggested level to boost growth, altered the dorsal muscle cellularity and myofibril ultrastructure, suggesting enhanced muscle function and firmness in comparison with that of the fish given the CTRL feed.

Keywords Diet formulation · Growth metrics · Haematology · Immune biomarkers · Muscle ultrastructure

Abbreviations

CTRL	Control
T4	4 g taurine/kg diet (0.4%)
T7	7 g taurine/kg diet (0.7%)

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T10	10 g taurine/kg diet (1%)
TEM	Transmission electron microscopy

Introduction

Taurine is a simple free amino sulphonic acid present in protein sources of animal origin, and it must be added to feed formulations for farmed carnivorous fish when plant protein sources are used (*El-Sayed 2014*). Taurine concentrations in formulated feeds are lower than those in raw fish even when fishmeal (FM) is used as the only protein source (*Kato et al. 2014; Satriyo et al. 2017*). The ability of fish to biosynthesize taurine is species-specific and depends on both fish size and life stage, and taurine has been classified as an essential, non-essential or conditionally essential nutrient based upon these factors (*Salze and Davis 2015*). Many marine fish species have a low or negligible ability to synthesize taurine due to limited activity or deficiency of the enzyme cysteine sulphinate decarboxylase (CSD) which is involved in the metabolism of methionine to cystathionine (*El-Sayed 2014*). The addition of taurine to feeds has positive effects on growth, health, muscle quality and reproduction in a variety of farmed finfish (reviewed by *El-Sayed 2014; Salze and Davis 2015*) and reduces the percentage of body lipids (*Espe et al. 2012*). Taurine is essential for many body functions, including lipid metabolism (*Satriyo et al. 2017*), eyesight and normal behavior in fish (*El-Sayed 2014; Salze and Davis 2015*).

Numerous studies have shown that European sea bass (*Dicentrarchus labrax*) production can be increased by improving diet formulations and management practices or by adding key nutrients to support nutritionally balanced diets (*Kousoulaki et al. 2015; Villegas and Mulero 2015*). Inclusion of 0.2–1.0% dietary taurine has been reported to improve the growth performance of sea bass fry (*Martinez et al. 2004*), juveniles (*Kotzamanis et al. 2012*) and adults (*Rimoldi et al. 2016*) fed fish/soybean meal-based diets. *Feidantsis et al. (2014)* mentioned that taurine-enriched diets (0.2, 0.5 and 1% taurine) induce important protective biochemical mechanisms particularly in the sea bass liver. Despite the increasing interest in taurine supplementation in sea bass feeds, very limited information is available on the effects of this specific nutrient on fish health status and immunity. The main objective of the study was to determine whether, and at what concentration, addition of taurine to FM-based feeds for juvenile European sea bass was beneficial for growth, feed utilization, health and immunity. The work included an investigation of the effects of dietary taurine supplementation on aspects of muscle ultrastructure that may influence muscle firmness and fillet characteristics.

Materials and methods

Experimental design

Hatchery-bred sea bass (*D. labrax*) juveniles of almost uniform size (4.0–6.0 g) were obtained from the Marine Fish Hatchery, National Institute of Oceanography and Fisheries (NIOF), and transported to El-Max Experimental Fish Farm, where they were maintained within a 40 m³ outdoor concrete reservoir tank filled with filtered, UV-treated and continuously aerated well-seawater. Before the start of the experiment, fish were acclimatized to the experimental unit for 2 weeks and fed a commercial diet (45% crude protein (CP) and 16% lipids (L), Aller blue, Aller Aqua, Egypt). At the beginning of the

experiment, 15 fish were taken and frozen at $-80\text{ }^{\circ}\text{C}$ for initial fish biochemical analysis. Four dietary groups of fish were established in triplicate and randomly distributed into 12 blue-coated concrete tanks (1 m^3 each) with a stocking density of 50 fish per tank, and the initial body weight for each tank was obtained (IBW, ca. 5 g/fish). Tanks were supplied with filtered sea water (salinity 32 ppt, flow rate of 120 l/h), and the oxygen concentration was maintained at 6–7 mg /l. Water quality parameters were monitored throughout the experiment to ensure the welfare of the fish.

Diet formulation and feeding protocol

Four experimental FM-based diets of similar composition were produced in the Fish Nutrition Laboratory, NIOF, Alexandria, to contain approximately 44% CP and 17% L, which are adequate proportions for the growth of sea bass juveniles. Experimental diets with progressively increasing taurine supplementation levels of 0, 4, 7 and 10 g taurine/kg diet were prepared and designated as the control (CTRL), T4, T7 and T10 diets, respectively. All solid ingredients were milled well, screened and mixed together. To add taurine to the diets, the determined amount of taurine was dissolved in the oil and added slowly with mixing to the feed ingredients. The water was added slowly to form a dough, and pellets were then formulated in a proper size using a mincer (*Abdel-Mohsen et al. 2018*). The pelleted diets were dried in an oven at $60\text{ }^{\circ}\text{C}$ for 24 h, packed into plastic bags and stored at $-20\text{ }^{\circ}\text{C}$ until use. The diet composition, proximate analyses and amino acid (AA) profile are given in Tables 1 and 2, respectively.

Fish were hand-fed the diets to apparent visual satiety in three meals per day (7:00, 12:00 and 17:00) for four consecutive days and fasted on the fifth day according to the protocol suggested by *Türkmen et al. (2012)*. Then, the amount of feed consumed was quantified for each tank. Overall, there were 72 feeding days during the entire 90-day feeding trial.

Measurement indicators and methods

Growth and biometric indices

At the end of the experimental period, 24 h after the last feeding, all fish in each tank were collectively weighed, and the final body weight (FBW, g/fish) for each tank was assessed. For biometric measurements, 10 anaesthetized fish from each tank were individually measured (total length, cm, and weight, g) after blood sampling. Then, each fish was dissected, and the liver and viscera were excised carefully and separately weighed to calculate somatic indices.

Growth and feed utilization indices were calculated according to the following equations: total weight gain (WG, g) = FBW – IBW; specific growth rate (SGR, %/day) = $100 (\ln \text{FBW} - \ln \text{IBW})/t$; feed intake (%body weight/day) = $100 \times [\text{dry feed consumed}/(\text{IBW} + \text{FBW})/2/t]$; feed conversion ratio (FCR) = dry feed consumed (g)/WG (g); protein efficiency ratio (PER) = WG (g)/protein intake (g); and protein productive value (PPV) = $100 (\text{protein gain, g})/(\text{protein fed, g})$, where “ t ” is the trial duration (days). The survival rate (S) was determined as the percentage of live fish at the end of the trial relative to the initial number of fish. Hepatosomatic (HSI) and viscerosomatic (VSI) indices and the Fulton condition factor (K) were calculated for the biometric study. $\text{HSI} = 100 \times \text{liver}$

Table 1 Formulation and proximate composition (% dry matter (DM)) of the taurine (T)-supplemented diets

Ingredient	Diet (g/kg)			
	CTRL	T4	T7	T10
Fish meal (FM) ^a	460	460	460	460
Corn gluten (CG) ^b	130	130	130	130
Soybean meal ^c	75	75	75	75
Yellow maize (YM) ^d	125	125	125	125
Wheat flour (WF)	80	76	73	70
Fish oil (FO) ^e	100	100	100	100
Vitamin and mineral mix ^f	30	30	30	30
Added taurine ^g	0	4	7	10
Proximate composition (% DM)				
Crude protein (CP)	43.95	44.26	44.37	44.19
Total lipids (L)	16.80	16.90	16.90	16.90
Ash	16.00	17.20	17.30	17.80
Fibre	1.30	1.30	1.30	1.30
Moisture	7.90	6.50	8.30	7.40
Nitrogen-free extract (NFE) ^h	14.1	13.8	11.9	12.4
Measured taurine (% of diet)	0.125	0.522	0.850	1.130
Gross energy (GE, MJ/kg) ⁱ	19.43	19.50	19.19	19.24
CP/GE ratio	22.63	22.70	23.12	22.96

^a 67% CP, 9% L; ^b local CG (60% CP) produced from maize (*Zea mays*); ^c 48% CP; ^d local products (YM, 7% CP; WF, 13% CP); ^e Iceland SR; ^f vitamin and mineral premix (mg/kg): p-amino benzoic acid (9.48), D-biotin (0.38), inositol (379.20), niacin (37.92), Ca pantothenate (56.88), pyridoxine HCl (11.38), riboflavin (7.58), thiamine HCl (3.79), L-ascorbyl-2-phosphate Mg (APM) (296.00), folic acid (0.76), cyanocobalamin (0.08), menadione (3.80), vitamin A palmitate (17.85), α -tocopherol (18.96), calciferol (1.14), K₂PO₄ (2.011), Ca₃(PO₄)₂ (2.736), Mg SO₄·7H₂O (3.058), NaH₂PO₄·2H₂O (0.795); ^g NOW Foods, Bloomingdale, IL 60108, USA; ^h calculated by difference; ⁱ gross energy was quantified based on 23.6, 39.5 and 17.2 kJ/g for proteins, lipids and carbohydrates, respectively (NRC 1993)

weight (g)/fish body weight (g), VSI = 100 × viscera weight/fish weight and $K = 100 \times \text{fish weight}/\text{total fish length}^3$.

Biochemical and amino acid composition

At the end of the experiment, five fish from each tank were randomly sampled and killed by sudden icing and stored at $-80\text{ }^\circ\text{C}$ for subsequent biochemical analyses. The biochemical compositions of the experimental diets and fish were determined according to the standard methodology of the AOAC (2005). Moisture and ash were determined by drying in an oven at $105\text{ }^\circ\text{C}$ and combustion in a muffle furnace at $550\text{ }^\circ\text{C}$ to a constant weight. CP was determined according to the Kjeldahl nitrogen method (VELP Scientifica, Italy), and crude lipid was measured gravimetrically using a mixture of chloroform and methanol as the solvent. For taurine determination, diet extraction was performed using 0.1 M HCl; derivatization was performed using dansyl chloride to form a fluorescent derivative determined by reversed-phase HPLC (Agilent 1090 system, Palo Alto, CA) (McCarthy et al. 2000). To quantitatively determine AAs in diets, samples were treated with 10% trichloroacetic acid (TCA) to precipitate proteins. Sample proteins obtained were hydrolysed with 4N methane sulphonic acid containing 0.2% tryptamine (Simpson et al. 1976). AA compositions were analysed with an automatic AA analyser (Hitachi, Ibaraki, Japan).

Table 2 Amino acid composition (g per 100 g protein) of taurine (T)-enriched diets ($n = 2$)

Amino acid (AA)	CTRL	T4	T7	T10
Essential amino acid (EAA)				
Arginine	7.11	6.02	5.81	5.99
Histidine	2.17	2.17	2.27	2.2
Isoleucine	4.23	4.03	4.35	4.46
Leucine	9.07	8.61	9.31	8.63
Lysine	6.65	6.8	6.75	7.39
Methionine	1.59	1.62	1.61	1.61
Phenylalanine	4.74	4.55	4.78	4.95
Threonine	3.93	3.72	3.67	4.25
Tryptophan	1.03	1.02	0.88	1.13
Valine	4.54	4.55	4.43	4.84
Non-essential amino acid (NEAA)				
Alanine	6.18	6.37	6.6	7.25
Aspartic acid	8.43	8.96	8.51	6.41
Cysteine	1.0	0.85	1.0	1.0
Glycine	6.36	6.71	6.61	6.3
Glutamic acid	17.14	17.86	17.67	17.35
Proline	4.13	6.5	6.1	6.62
Serine	6.05	4.0	4.15	4.4
Tyrosine	3.68	3.68	3.64	4.16

Haemato-immunological assays

Five fish were randomly removed from each tank and lightly anaesthetized with clove oil (20 mg/l) for 3 min for haematological analyses. Blood samples were immediately obtained from the caudal vasculature using heparinized syringes, placed into microtubes and pooled for each tank. The major blood constituents of the fish were measured immediately after blood collection. Moreover, another blood sample was collected with a non-heparinized syringe and left to clot at 4 °C and then centrifuged at 3500×g for 10 min. Serum samples were stored at –80 °C until subsequent immunological assays.

A full automatic blood counter (model PCE210 N, AGD Biomedicals (P) Ltd., India) was used to assess the red blood cell/erythrocyte count (RBCC, $10^6/\mu\text{l}$), haemoglobin concentration (Hb, g/dl), haematocrit value (Ht, %), mean corpuscular volume (MCV, fl), mean corpuscular haemoglobin (MCH, pg), mean corpuscular haemoglobin concentration (MCHC) and white blood cell/leukocyte count (WBCC, $10^3/\mu\text{l}$) and their differentiations.

The leucocyte phagocytosis activity (PA) was determined by the method of Kawahara et al. (1991). In brief, 1 ml of heparinized total blood was incubated with 50 μl of formalin-killed cells (FKC) at 25 °C, and aliquots of the blood were taken at 1, 3 and 6 h during the incubation period. Smears of the total blood were stained with Wright-Giemsa solution. The percentage of cells showing phagocytosis was calculated by counting 100 cells.

The serum total proteins were estimated by the Biuret reaction (Dumas et al. 1981) using commercial kits (Pasteur Commercial Ltd., France) and bovine albumin as the standard following the manufacturer's instructions. The absorbance of light is proportional to the concentration of total protein in the sample. The turbidimetric method (Ellis 1990) with slight modifications was used for the determination of lysozyme activity based on the ability of lysozyme to lyse the bacterium *Micrococcus lysodeikticus*. A suspension of *M. lysodeikticus* (0.2 mg/ml 0.05 M sodium phosphate buffer, pH=6.2) was mixed with varying sample

amounts to give 2 ml as a final volume in a microtray. The microtray was then incubated at 25 °C, and the absorbance was measured spectrophotometrically at 530 nm (Stat Lab, Germany). The lysozyme activity unit was defined as the amount of sample that caused an absorbance depletion of 0.001 min⁻¹.

Dorsal muscle ultrastructure

For muscle extraction, four fish from the CTRL and T10 fish groups (uniform in size and representing the three tanks of each treatment) were euthanized with a clove oil overdose and placed immediately on an ice block. A small piece of muscle tissue ($\leq 1 \text{ cm}^3$) was removed from the left side of the fish in the region between the dorsal fin insertion and the lateral line and then placed immediately into fixative solution (Bouin's formalin/glutaraldehyde, 4F1G; buffered with 0.1 M phosphate buffer, pH = 7.4, for 1 h at 4 °C). The tissue was then dehydrated through a graded series of ethanol, cleared with propylene oxide and embedded in pre-dried gelatine capsules. Ultrathin sections were stained with uranyl acetate for 20 min and lead citrate for 5 min, attached to grids and imaged using a JEOL 100CX transmission electron microscope (TEM). Measurements of different muscle bands/areas were taken from digital TEM images (one for each fish) at magnifications of $\times 4000$ and $\times 10,000$. The sarcomere length (z-z), number and diameter of myofibrils (M-line), anisotropic (A) band and isotropic (I) band were all measured, and the average was then calculated for each parameter.

Statistical analysis

All data are presented as the mean \pm standard deviation (SD). One-way ANOVA was applied to determine differences among treatment mean values ($n = 3$, unless otherwise stated) at the 5% significance level. The data were first assessed for normality (Shapiro-Wilk test) and homogeneity of variance (univariate procedure) (SAS 2004), and the results indicated that the data were distributed normally (Shapiro-Wilk test). When appropriate, means were compared by Duncan's multiple range tests. For muscle histology, at least four measurements were averaged for each individual fish (mean \pm SD, $n = 4$). Group differences in mean muscle zone/line measurements between the two dietary treatments (T10 and CTRL) were further compared by *t* tests.

Results

Growth, feed utilization evaluation and somatic indices

Under the experimental conditions, sea bass (*D. labrax*) grew well throughout the study duration, and no mortality occurred in the T7 and T10 fish groups (Table 3). Taurine incorporation caused a progressive increase in all measured growth criteria and the highest weight gain was 22 g/fish in T4 vs 18 g/fish in CTRL, with no significant difference between the T10 and T7 groups.

Unexpectedly, dietary taurine addition had no significant ($P > 0.05$) effect on voluntary feed intake, and all diets were equally consumed by fish with no appreciable differences among treatments. However, the PPV was 31% in T10 vs 28% in CTRL (Table 3). Regarding the

Table 3 Growth, feed utilization and biometric indices (mean \pm SD) of sea bass fed taurine (T)-supplemented diets

Parameter	CTRL	T4	T7	T10
Initial body weight (IBW, g)	4.79 \pm 0.12	4.70 \pm 0.04	4.70 \pm 0.04	4.71 \pm 0.04
Weight gain (WG, g/fish)	17.92 \pm 0.34 ^c	18.28 \pm 1.30 ^c	20.43 \pm 0.31 ^{ab}	21.97 \pm 0.17 ^a
Specific growth rate (SGR, %/day)	1.73 \pm 0.03 ^b	1.76 \pm 0.07 ^b	1.86 \pm 0.01 ^{ab}	1.93 \pm 0.02 ^a
Feed intake (% BW/day)	2.65 \pm 0.13	2.90 \pm 0.12	2.88 \pm 0.25	2.93 \pm 0.45
Feed conversion ratio (FCR)	1.59 \pm 0.02 ^{ab}	1.69 \pm 0.05 ^a	1.52 \pm 0.07 ^b	1.59 \pm 0.08 ^{ab}
Protein efficiency ratio (PER)	1.49 \pm 0.51	1.39 \pm 0.32	1.57 \pm 0.58	1.60 \pm 0.51
Protein productive value (PPV)	28.27 \pm 0.61 ^b	28.39 \pm 0.79 ^b	30.19 \pm 0.70 ^a	30.99 \pm 0.64 ^a
Survival rate (S, %)	95.0 \pm 3.0 ^b	98.3 \pm 4.2 ^a	100 \pm 0.00 ^a	100 \pm 0.00 ^a
Condition factor (K)	0.89 \pm 0.13 ^b	1.00 \pm 0.17 ^{ab}	1.06 \pm 0.11 ^a	1.02 \pm 0.08 ^a
Hepatosomatic index (HSI)	2.35 \pm 0.66	2.96 \pm 0.50	3.04 \pm 0.50	2.62 \pm 0.95
Viscerosomatic index (VSI)	8.18 \pm 2.30 ^{ab}	6.70 \pm 0.71 ^b	8.71 \pm 1.66 ^a	8.31 \pm 2.08 ^{ab}

Means in the same row with different letters are significantly different ($P < 0.05$)

somatic indices, the results (Table 3) illustrate that taurine-enriched diets led to a significant ($P < 0.05$) improvement in the K of sea bass, particularly for T7 and T10 fish (1.06 and 1.02, respectively) relative to the CTRL group (0.89). No significant differences in the HSI were observed among the dietary groups, whereas the VSI was the lowest in fish fed the T4 diet (6.70).

Fish biochemical composition

The whole-fish proximate composition data (Table 4) showed that the protein percentage was significantly ($P < 0.05$) elevated in taurine-fed fish but that the relative lipid content was significantly reduced in T4-fed fish (7.90%) compared with CTRL-fed fish (9.38%). No significant differences due to addition of dietary taurine were observed in percentages of either ash or moisture contents ($P > 0.05$) (Table 4).

Haemato-immunological parameters

Supplying juvenile sea bass with taurine as a feed additive significantly affected their major blood constituents, with Hb and RBCC being increased by all dietary taurine levels (Table 5). WBCC was not affected by taurine addition, but the lymphocyte and monocyte percentages were significantly higher in taurine-fed fish, particularly at higher taurine levels (T7 and T10), than in CTRL-fed fish. Additionally, serum immunity indicators were found to be influenced by dietary taurine (Fig. 1). Total protein, lysozyme and phagocytic activities were substantially

Table 4 Fish biochemical composition (% wet weight) of sea bass fed taurine (T)-supplemented diets

Parameter	Initial	CTRL	T4	T7	T10
Crude protein	17.32 \pm 0.25	18.06 \pm 0.35 ^b	19.60 \pm 0.61 ^a	19.45 \pm 0.40 ^a	19.11 \pm 0.31 ^a
Lipids	7.54 \pm 0.38	9.38 \pm 0.15 ^a	7.90 \pm 0.79 ^b	8.19 \pm 0.15 ^{ab}	8.48 \pm 0.23 ^{ab}
Ash	5.28 \pm 0.16	4.99 \pm 0.31	4.97 \pm 0.72	4.77 \pm 0.21	4.70 \pm 0.40
Moisture	68.23 \pm 0.52	67.10 \pm 0.46	68.03 \pm 0.57	67.20 \pm 0.10	67.99 \pm 0.21

Means in the same row with different letters are significantly different ($P < 0.05$)

Table 5 Haematological profiles of sea bass fed taurine (T)-supplemented diets

Parameter	CTRL	T4	T7	T10
Haemoglobin (Hb, g/dl)	12.77 ± 0.25 ^b	12.83 ± 0.62 ^{ab}	13.55 ± 1.11 ^a	13.50 ± 0.53 ^a
Haematocrit (Ht, %)	31.67 ± 3.79	34.00 ± 3.46	33.67 ± 3.06	33.00 ± 1.00
RBCC (× 10 ⁶ /μl)	3.69 ± 0.18 ^b	4.17 ± 0.35 ^a	4.23 ± 0.15 ^a	4.13 ± 0.06 ^a
MCV (fl)	85.83 ± 5.20 ^a	81.46 ± 3.30 ^a	70.60 ± 6.31 ^b	79.80 ± 1.85 ^a
MCH (pg)	34.62 ± 0.50 ^a	30.77 ± 3.42 ^b	32.03 ± 1.96 ^{ab}	32.80 ± 1.60 ^{ab}
MCHC (mg/dl)	40.33 ± 2.17	37.73 ± 3.73	40.27 ± 2.21	40.93 ± 1.14
WBCC (× 10 ³ /μl)	31.33 ± 6.28	32.00 ± 7.00	31.67 ± 6.03	30.33 ± 9.87
Neutrophils (%)	60.31 ± 4.54 ^a	56.51 ± 5.07 ^{ab}	52.67 ± 4.94 ^{ab}	50.33 ± 3.98 ^b
Lymphocytes (%)	35.33 ± 3.06 ^c	37.60 ± 3.06 ^{bc}	39.00 ± 4.04 ^{ab}	42.67 ± 4.58 ^a
Monocytes (%)	3.67 ± 1.53 ^b	4.89 ± 1.51 ^b	7.00 ± 1.00 ^a	6.00 ± 1.00 ^a
Eosinophils (%)	0.69 ± 0.58	1.00 ± 1.00	1.33 ± 0.58	1.00 ± 1.00

Means in the same row with different letters are significantly different ($P < 0.05$)

RBCC, total erythrocyte count; *MCV*, mean cell volume; *MCH*, mean cellular haemoglobin; *MCHC*, mean cellular haemoglobin concentration; *WBCC*, total leukocyte count

increased ($P < 0.05$) in the taurine-supplemented groups, particularly in the T10 group, compared with the other dietary groups.

Muscle myofibril ultrastructure

The T10 diet resulted in changes to the ultrastructure of the dorsal musculature (Figs. 2 and 3) and significantly altered muscle cellularity compared with the CTRL fish (Table 6). The A-band (dark; myosin protein) and the I-band (light; actin protein) measurements were significantly different ($P < 0.05$) in fish fed the T10 diet (657 and 125 nm, respectively) and the CTRL diet (607 and 78 nm, respectively). There was a greater myofibril thickness in T10-fed fish than in CTRL-fed fish. Likewise, the Z-disc value was significantly higher in T10-fed fish than in CTRL-fed fish (772 and 670 nm, respectively). However, the number of myofibrils in the sarcomeres did not differ between the fish fed the T10 and CTRL diets. There was a shorter M-line (holds together the thick myosin filaments) in T10-fed fish (21 nm) than in CTRL-fed fish (32 nm) (Table 6).

Discussion

Juvenile sea bass had better survival, enhanced growth and a higher PPV and *K* when fed T7 and T10 diets than fish given the CTRL diet (Table 3). Accordingly, our results indicate that taurine is a functional nutrient, as previously described (Bouckennooghe et al. 2006). It appears to enhance fish performance despite having little role as a feeding attractant or stimulant. The positive effects of dietary taurine supplementation on the growth of sea bass juveniles may be attributed to a higher feed utilization efficiency rather than to a higher palatability of taurine-containing diets since feed intake was consistent among treatments. However, there was significant improvement in the PPV. Additionally, this improvement may be attributed to the fact that taurine has been shown to participate in a number of essential biological functions, although it is not incorporated into proteins (Huxtable 1992). Our findings regarding the beneficial effects of dietary taurine supplementation on growth and some feed utilization

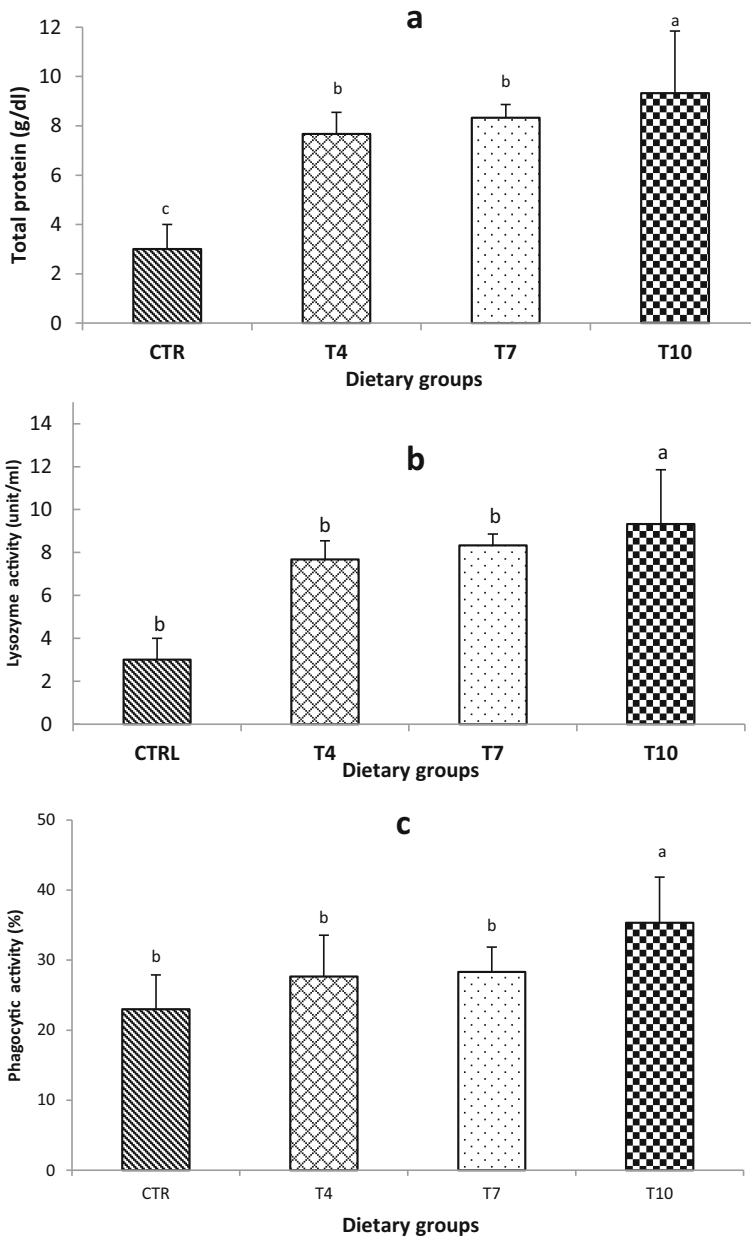


Fig. 1 Serum total protein (a), lysozyme (b) and phagocytic (c) activities in sea bass fed control (CTRL) and taurine (T)-supplemented diets

indices are mainly in agreement with earlier reports on the same species (Martinez et al. 2004; Kotzamanis et al. 2012; Rimoldi et al. 2016).

The increased percentage protein content of all taurine-fed fish compared with CTRL-fed fish could be related to the better utilization efficiency of the taurine-enriched diets than the CTRL diet. Our results are in agreement with Qi et al. (2012), who mentioned that dietary taurine

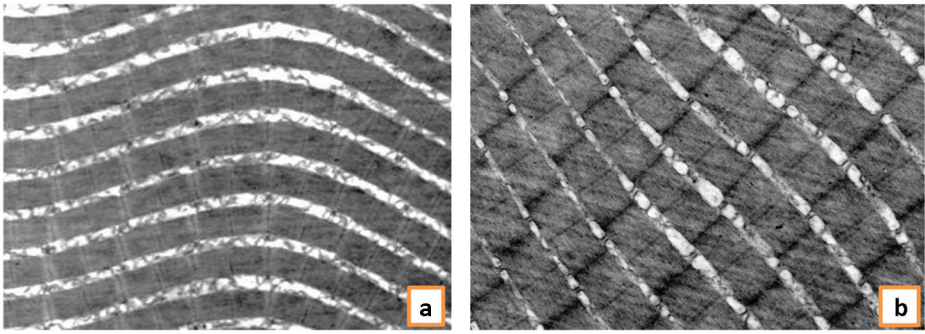


Fig. 2 **a, b** TEM photomicrographs of dorsal muscle from sea bass fed the CTRL (**a**) and 1% taurine (**b**) diets ($\times 4000$)

supplementation can increase the whole-body CP content in juvenile turbot (*Scophthalmus maximus*). The reduction in percentage carcass lipid of the sea bass, particularly in the T4-fed fish group, could be attributed to the more efficient use of protein and enhanced lipid metabolism. Our results are parallel to those of *Hoseini et al. (2017)*, who mentioned that dietary taurine led to a decrease in carcass lipid contents in Persian sturgeon (*Acipenser persicus*). According to *Huxtable (1992)*, taurine plays a role in lipid metabolism, enhancing intestinal fat absorption by stimulating bile acid synthesis and cholesterol degradation.

Information about blood parameters of sea bass is limited (*Roncarati et al. 2006; Wassef et al. 2017; Abdel-Mohsen et al. 2018*). The data obtained in the present experiment indicated that all dietary taurine levels had an impact on major blood constituent parameters such as Hb and the RBCC, suggesting a positive influence of taurine on general sea bass health. High RBCC values are associated with fish that swim quickly, have predatory natures and have high activity levels with streamlined bodies (cited from *Fazio et al. 2013*). Leukocytes (WBCs) are the defensive cells of the body, and their levels have implications for immune responses and the ability of an animal to fight infection (*Douglass and Jane 2010*). Some reports suggest that fish lymphocytes have phagocytic ability and participate in defence mechanisms and immune competence (*Buscaino*

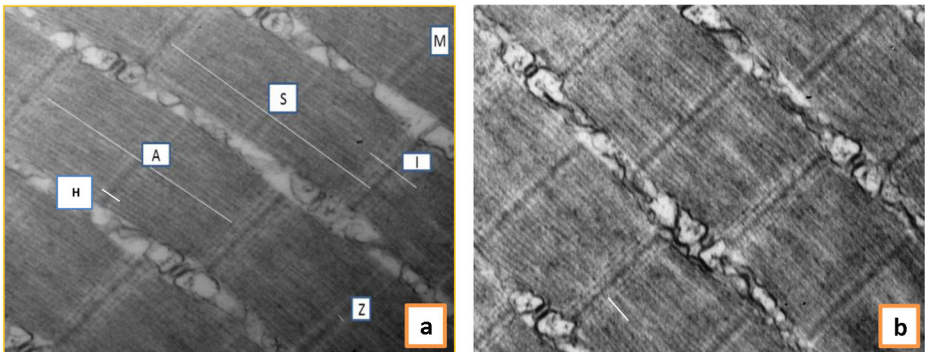


Fig. 3 **a, b** TEM photomicrographs of dorsal muscle from sea bass juveniles fed the CTRL (**a**) and 1% taurine (**b**) diets ($\times 10,000$) showing no lysis in myofilaments, normal distribution of the sarcoplasmic reticulum and organized striation of sarcomeres (s), which are parallel with almost regular size and are separated from each other for both treatments. The A-band (A), I-band (I), H-zone (H), Z-disc intersecting the I-band and M-line bisecting the A-band can be easily distinguished (scale bar = 100 nm)

Table 6 Electron microscopy morphometric measurements of sea bass dorsal muscle fibres

Muscle zone	CTRL	T10	<i>P</i> value
A-band (nm)	607.00 ± 18.52 ^b	657.00 ± 13.23 ^a	0.019
I-band (nm)	77.97 ± 15.26 ^b	124.67 ± 5.13 ^a	0.007
M-line (nm)	31.60 ± 3.04 ^a	20.60 ± 1.40 ^b	0.005
Z-disc (nm)	670.33 ± 17.67 ^b	772.33 ± 18.88 ^a	0.002
Myofibril number	27.75 ± 2.87 ^a	26.00 ± 1.41 ^a	0.058
Myofibril thickness (nm)	289.25 ± 22.90 ^b	331.25 ± 47.52 ^a	0.162

Means in the same row with different letters are significantly different ($P < 0.05$)

A-band, anisotropic band, mainly thick (myosin) filaments with an outer edge of thick and thin (actin) filament overlap; *I-band*, isotropic band, thin filaments only; *M-line*, thick filaments linked by accessory proteins; *Z-disc*, thin filaments

et al. 2010; Fazio et al. 2013). Accordingly, our results suggest that dietary taurine, particularly at the 1% level, enhanced the immune competence of sea bass juveniles, as indicated by the elevations in immune indicators such as lymphocytes, total serum protein and lysozyme and phagocytic activity. López et al. (2015) reported that taurine may enhance totoaba (*Totoaba macdonaldi*) growth performance and play an important role in fish haematological and blood biochemical homeostasis. Thus, taurine may play an important modulatory role in the haematological and blood biochemical status of sea bass fed both FM- and soy protein-based diets, contributing to the enhancement of overall fish health status and performance.

Muscle is the main edible portion of fish and is responsible for its nutritional value. White muscle cellularity is considered an important determinant of flesh texture (Hurling et al. 1996), taste and digestibility (Johnston 1999). In farmed fish, nutrition is a factor that has an influence on the proliferation and growth of muscle fibres. There is some information about muscle development and structure in both wild and farmed sea bass (Ayala et al. 2003; Abdel et al. 2005; Periago et al. 2005). In the present study, an increase in percentage carcass protein was observed in fish given diets supplemented with taurine (Table 4), and possible advantages of dietary T10 enrichment were indicated by measurements made on the myofibrils (Table 6). Johnston (1999) emphasized that the width of the Z-disc is probably related to muscle function, while a short M-line diameter indicates muscle firmness (Grigorakis 2007). Additionally, the width of the M-line reflects the numbers of myofibrils in the muscle fibres, and this influences muscle protein content (Barral and Epstein 1999). The significantly higher values observed for muscle bands in the current study may be associated with the greater proliferation of muscle fibrils in sea bass fed the T10 diet than in those fed the CTRL diet. In addition, the elevations in the quantities of myosin and actin myofilaments in T10-fed fish muscles relative to CTRL-fed fish muscles indicate that taurine enhanced the dorsal myofibril structure. In general, all measurements indicate an enhancement in muscle function and firmness in the T10 fish group compared with the CTRL group.

Conclusion

The overall results of this research suggest that the appropriate dietary supplementation level of taurine to promote growth, feed utilization efficiency, health status, immunity and dorsal muscle firmness in sea bass juveniles fed FM-based diets is 1.0%.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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