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Dietary taurine improves the growth performance, health status and liver histopathology of meagre (*Argyrosomus regius*) fed a reduced fish meal diet

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Running head: Effects of dietary taurine on meagre

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Abstract

Research has focused on alternative plant materials or additives that can be used instead of fish meal due to limited stocks and increased feed prices, although it is important for carnivorous fish species. Taurine is a functional amino acid supplemented to low fish meal diets to improve the growth performance of fish. In this study, eight experimental diets with 0%, 0.5%, 1% and 2% ratios of taurine supplemented (T0, T0.5, T1 and T2) high (HFM) and low fish meal (LFM) diets were prepared (defined as HFMT0, HFMT0.5, HFMT1, HFMT2, LFMT0, LFMT0.5, LFMT1 and LFMT2). The experiment was carried out in twenty-four 400 L rectangular fiberglass tanks conducted in a marine water system, and fish (initial mean weight of 23.5 g) were stocked in each tank. The nutrition trial was carried out for 90 days with three replicates. Meagre fed with the HFMT1 diet had a higher final mean weight

(FMW) and specific growth rate (SGR) than fish fed with HFMT0 and all LFM diets. Including taurine in all LFM diets also enhanced growth performance. The FMW and SGR of fish fed the LFMT0.5 diet were similar when compared to HFMT0 ($P>0.05$) and increased than that of other LFM diets with taurine ($P<0.05$). Meagre fed the LFM0.5 diet had a lower feed conversion rate (FCR) than those provided for all HFM diets, although there was no significant difference between treatments. Dietary taurine has decreased the hepatosomatic index (HSI), viscerosomatic index (VSI) and total ammonia-nitrogen excretion (TAN), irrespective of taurine and fish meal levels. Including dietary taurine at both fish meal levels enhances the number of red blood cells (RBC) and whole-body amino acids of meagre. The most remarkable conclusion of this study is the inclusion of taurine in low fish meal diets improved growth performance and profitability in meagre, and the level of 0.5% has been recommended.

Key words: mariculture, plant ingredients, amino acid, dietary replacement

It is expected that the feeds used in aquaculture will be cost-effective, provide maximum growth, and cause minimal environmental pollution. In enterprises with intensive aquaculture, fish feed costs constitute more than 60% of the operating expenses and the protein in the feed is defined as the most costly nutritional component (Lovell, 2002). Dietary protein level and quality are among the most critical factors affecting fish growth performance and health (Wilson, 2002). Researchers have focused on strategies such as reducing dietary protein levels, using high plant protein sources instead of fish meals and using low-cost raw materials in feed formulation to reduce feed costs. As a result of feeding the fish, which is among these strategies, with low fish meal and oil-containing diets, an unhealthy fish with low growth performance and high feed utilization rate and loss of quality-related nutrient composition is obtained (Matos et al., 2012; Sarker et al., 2012; Zhang et al., 2012; Ribeiro et al., 2015; Sitja-Bobadilla et al., 2015). In the case of feeding fish with diets containing high plant proteins, various additives are used to increase or maintain the growth performance of the fish. Among these additives; synthetic amino acids (lysine, methionine, arginine, glutamic acid, alanine, etc.), vitamin mixtures or attractive additives (betaine, nucleotide, organic acids, etc.) are included (Smith et al., 2007; Grey et al., 2009; Kader et al., 2010).

When the low fish meal is used in marine aquafeeds, two of the most crucial limiting amino acids that can be added externally are lysine and methionine. These amino acids are used to synthesize nitrogen-containing compounds (Li et al., 2009). Likewise, taurine (2-aminoethanesulfonic acid) is abundant in fish meals, but it is almost absent in low and non-fish meal containing aquafeeds (Li et al., 2022). Mainly if the carnivorous fish species' diet contains high amounts of plant protein sources and low levels of fish meal, the taurine content of these feeds will also be low. After the 2000s, studies focused on using taurine in fish feeds. The inclusion of dietary taurine has beneficial effects on growth performance, feed utilization, proximate composition, muscle texture, disease resistance, multiple metabolic functions (osmoregulation, intestinal immune function, cell membrane stabilization, protein, lipid and

carbohydrate metabolism), reproduction performance and immunity of fish (Salze and Davis, 2015; Sampath et al., 2020). Previous studies conducted on marine species revealed that dietary taurine enhanced the growth performance, serum parameters, gene expression and organoleptic characteristics at least one of the species of European seabass (*Dicentrarchus labrax*), black sea bream (*Acanthopagrus schlegelii*), Japanese flounder (*Paralichthys olivaceus*), Senegalese sole (*Solea senegalensis*) and turbot (*Scophthalmus maximus*) (Wei et al., 2019; Kotzamanis et al., 2020; Tong et al., 2020; Nakamura et al., 2021; Aragão et al., 2023). Because of these beneficial effects, taurine is considered a functional feed additive/amino acid. However, the use of dietary taurine does not have a significant undesirably impact on some fish's performance and health. Whereas taurine deficiency negatively affects the growth and health of the fish, an overdose of taurine in aquafeed will cause hepatic pathological changes in fish and deterioration of the structure and function of the distal intestine (Liu et al., 2018; Li et al., 2022). Therefore, including an appropriate level of taurine in fish feeds can improve fish's growth performance and health. In this context, dietary taurine levels or effect varies depending on the species, size, basal taurine level and level of plant ingredients in the feed.

Because of meagre, relatively easy broodstock management, lack of reproductive maturation during growth, rapid growth, reasonable feed conversion rate, excellent processing efficiency, low oil content, excellent flavor and firm tissue content for the marine fish market has become an important species (Duncan et al., 2013; Güroy et al., 2017). In recent years, studies have been carried out on the nutritional requirements, feed formulation, and feeding regime of the meagre, and the information obtained has contributed significantly to the development of the species' production strategy. In previous studies on the use of taurine in meagre diets (de Moura et al., 2018, 2019; Matias et al., 2020), the fish size, feed content and taurine use levels were used in our study are different. Therefore, this study aims to evaluate the effects of low or high fish meal diets containing different taurine levels on meagre (23 g) growth performance and health.

Material and methods

Ethics statement

This trial was approved by the Turkish Republic Ministry of Agriculture and Forestry, Mediterranean Fisheries Research Production and Training Institute Animal Experiments (Antalya, Türkiye), Local Ethics Committee with the protocol dated 23.03.2016.

Fish and culture conditions

Meagre (*Argyrosomus regius*) were produced in the Beymelek Unit of Mediterranean Fisheries Research Production and Training Institute, Antalya, Turkey. Following the spawning of meagre broodstocks, incubation and hatching of eggs were completed. Larvae and postlarvae were fed with algae, *Artemia* and the different larvae diets until the juvenile stage. Meagre weighing 18-25 grams from the juvenile population produced in the hatchery of the institute were subjected to the adaptation period to be used in the experiment. Before the start of the experiment, the fish were acclimatized to experimental conditions for two weeks. During the acclimation period, fish were fed with a commercial sea bass diet

nutritionally complete feed for marine species. After the adaptation period, three hundred and sixty unsatisfied fish with an initial stocking density of 15 fish per tank and an average starting weight of 23 g were randomly distributed into twenty-four tanks (450 L). All groups were performed in triplicate for this trial. The water quality for meagre throughout the experiment was as temperature (20.7±0.2) (Oxy Guard Handy Polaris), dissolved oxygen (10.2±0.1 mg/L) (Oxy Guard Handy Polaris), and pH (8.48±0.08) (HANNA HI9125) under a 12 h light photoperiod was implemented with fluorescent lights and timers. The meagre was hand-fed three times daily (09:00, 13:00 and 17:00 hours) to satiation for 12 weeks. Feed was meticulously applied by dropping a few pellets until the feeding activity ceased.

Diet formulation and experimental design

Experimental diets were formulated by Chatzifotis et al. (2010, 2012) and Güroy et al. (2017) on the nutritional requirements for meagre. Two sets of four experimental diets contained 48% crude protein and 18% lipid. The first set of diets included 38.5% fish meal as the primary protein source (defined as HFM, which is the high fish meal diet), while the second set had 11% fish meal (defined as LFM, which is the low fish meal diet). Within each fish meal level, dietary taurine has supplemented the levels of 0% (T0), 0.5% (T0.5), 1% (T1) and 2% (T2) in the experimental diets (HFMT0, HFMT0.5, HFMT1, HFMT2, LFMT0, LFMT0.5, LFMT1 and LFMT2). The proximate composition of the feed ingredients is presented in Table 1, and the formulation and proximate composition of all experimental diets are detailed in Table 2. Dry mixing ingredients with fish oil manufactured diets before utilizing a laboratory food mixer. The mixing was continued until a homogenous blend was obtained. The resulting paste was extruded through a cold press extruder (PTM P6 Extruder System) using a 3 mm aperture die. All diets were subsequently dried in a dehumidifying oven for 24 h at 40°C and then stored in plastic bags at –18°C until required.

Evaluation of growth performance

All fish were weighed individually at the trial's beginning and end. During the experiment, fish bulk weight in each tank was weighed every 15 days with a precision balance (Precisa XT 4200C). Before all samplings, all fish were lightly sedated with clove oil at 30 ppm (Cárdenas et al., 2016) and wiped dry with a paper towel. Fish were starved one day before the weighing day. At the end of the experiment, growth measurements and feed utilization parameters such as specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were determined as the following equations:

$$\text{FCR} = \text{Feed intake} / \text{Weight gain},$$

$$\text{SGR (\%/day)} = 100 \times [(\text{Ln Final fish weight}) - (\text{Ln Initial fish weight})] / \text{Experimental days},$$

$$\text{PER} = [(\text{Final fish weight}) - (\text{Initial fish weight})] / \text{Protein intake (PI)}$$

where PI was calculated as PI = (Feed intake x Protein ratio of feed) / 100].

Sampling protocols

Thirty fish before the trial and two fish for each tank at the end of the trial were randomly sampled for whole-body proximate analysis. The fish fasted one day before sampling. At the termination of the feeding trial, fifteen fish per treatment (five fish per tank)

were randomly selected for somatic indexes, liver histology analysis, tissue and blood collection. Meagre was sacrificed by lethal anesthesia using clove oil. Blood samples were taken from the caudal vein using a 32-gauge needle and a 2 mL heparinized syringe. The study collected 240 µL of blood (2 fish x 40 µL sample x 3 tanks) from fish in each experimental group. We washed the injector several times with Nevrapin (Heparin sodium) to prevent blood coagulation. Then gathered the blood from each fish. The amount of blood obtained for each fish was used to prepare blood smears and hematocrit values.

Evaluation of somatic indices

Six fish were randomly taken from the initial pool at the beginning of the experiment. Three fish from each tank (nine fish per treatment) were randomly sampled at the end of the trial to determine somatic indices. The somatic indices, including condition factor (CF), hepatosomatic index (HSI), viscerosomatic index (VSI) and mesenteric fat index (MFI) were determined according to the following formulas:

$$CF\ (\%) = (\text{Final body weight} / (\text{Fork length})^3) \times 100,$$

$$HSI\ (\%) = (\text{Liver weight} / \text{Final body weight}) \times 100,$$

$$VSI\ (\%) = (\text{Viscera weight} / \text{Final body weight}) \times 100,$$

$$MFI\ (\%) = (\text{Body fat weight} / \text{Final body weight}) \times 100.$$

Proximate analysis

Feed ingredients, experimental diets, and body composition analysis performed throughout this study were according to AOAC methods (AOAC, 2000). Dry matter was determined by drying at 105 °C until a constant weight was obtained. Ash content was determined by incineration in a muffle furnace at 525 °C for 12 h. Crude protein (N*6.25) was analyzed by the Kjeldahl method after acid digestion using the Gerhardt system. The crude fiber was determined by acid/alkali hydrolysis, recovering filtered residue and ignition of the dried sample for 3 hr. Ingredients, diets and whole-body lipids were extracted according to Folch et al. (1957) with chloroform/methanol (2:1 v/v). Nitrogen-free extract (NFE) was calculated by taking the sum values for moisture, crude protein, lipid, ash and crude fiber and then subtracting this value from 100.

Amino acid analysis

The amino acid (AA) composition of the experimental diets is given in Table 3. Sixty mg of sample (diet or fish) was hydrolyzed in 10 mL of 6 M HCl in screw-capped tubes to analyse amino acids. The tubes were flushed with N and then heated at 110 °C for 24 h. The hydrolysates were rotary evaporated to dryness under vacuum at 40 °C and then re-dissolved in a sodium citrate buffer at pH 2.2. The amino acids were separated by ion-exchange chromatography on a sodium column and detected following post-column derivatization with ninhydrin by measuring absorbance at 350-450 nm. Identification and quantification of the detected AA were performed using external standards after adjustments by linear regression. The standard AA was purchased from Sigma-Aldrich Co, USA, as a synthetic mixture of AAs.

Hematological analysis

Hematocrit was determined with a micro-hematocrit centrifuge (Nüve NF 048, Turkey) for 5 min at 12500 rpm and was calculated as packed cell volume (PCV%), and it was read using a Hawksley Hematocrit Reader, which represented the volume of the whole blood occupied by the red blood cells. Hemoglobin levels (Hb) were assessed by using Sahli's method. White and red blood cells (WBC and RBC) were counted using a Neubauer chamber (Blaxhall and Daisley, 1973; Güroy et al., 2017). Blood smear slides were prepared using five μL of whole blood to assess the total leukocyte counts. The smear was air-dried and then fixed in 95% methanol. Slides were stained with 6% Giemsa (BDH) for 20 min and mounted in DPX (BDH). Images were taken using a digital camera using the Olympus BH light microscope. Counts were assessed using digital pictures and levels were determined as the total number of leukocytes per 1000 blood cells.

The corpuscular calculations, including mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were calculated following equations:

$$MCV\ (\mu m^3) = \text{Packed cell volume} / \text{Red blood cell count} \times 10,$$

$$MCHC\ (\%) = \text{Hemoglobin concentration} / \text{Packed cell volume} \times 100,$$

$$MCH\ (pg) = \text{Hemoglobin concentration} / \text{Red blood cell count} \times 10.$$

Histological analysis

At the termination of the trial, three fish from each tank were sacrificed with an overdose of clove oil before dissection, followed by cerebral destruction. Sampled livers were immediately fixed in the neutral buffered formalin (10%). Livers were dissected into small size cubic tissue. Dehydration, clearing and paraffin wax process were performed manually. Paraffin blocks were sectioned (5 μm) by using a rotary microtome (Leica RM2125 RTS) and stained with hematoxylin and eosin (H&E). Light microscopy (Nikon Eclipse E200) and a digital camera (Nikon DS-Fi1) with NIS-Elements Microscope Imaging Software (Nikon Corporation, Tokyo, Japan) was used for the examination of hepatopancreas tissues (Roberts et al., 2012; Güroy et al., 2017).

Determination of total ammonia-nitrogen (TAN) excretion

Fish were starved for three days to ensure the gut was empty after the nutrition trial. On the morning of the fourth day, tanks were thoroughly cleaned, and fish in all groups were fed with their experimental diets to apparent satiation. Thirty minutes postprandial, water flow to each tank was discounted and uneaten food was removed. After 12 h postprandial, the water samples were taken with an automatic pipette from the tanks and transferred to the NH_4-N reagent cuvettes (Hach Lange LCK 304). Separate (and new) cuvettes were used taking for each tank's water samples. TAN concentrates were analyzed with the ammonia salicylate method and measured in a Hach Lange DR 2800 spectrophotometer. TAN excretion levels were determined by subtracting the baseline value of each tank according to the following formula:

$$TAN\ (\text{mg-N} / 100\ \text{g fish} / 6\ \text{h}) = [(\text{Final ammonia concentration} - \text{Initial ammonia concentration}) \times \text{Water volume}] / \text{Final mean fish weight} / \text{Sampling period}.$$

Economic analysis

The economic analysis in terms of economic conversion ratio (ECR) and economic profit index (EPI) was developed by Martínez-Llorens et al. (2007), and it was determined using the following formulae:

ECR (€/g) = Feed conversion ratio x Feed price

EPI (€/fish) = (Final mean weight x Fish sale price) – (Economic conversion ratio x Weight gain).

The cost of feed ingredients was listed by IndexMundi (http://www.indexmundi.com/commodities). Meagre sale price is determined as 4.5 €/kg according to global fish markets.

Statistical analysis

The Shapiro-Wilk W test and Levene test were subjected to verify normality and homogeneity of variance before further analysis was undertaken. A 2 x 4 factorial arrangement of treatments (2 fish meal levels x 4 taurine levels) was used. All data were analyzed via one and two-way analysis of variance (ANOVA) for multiple pairwise comparisons. Significance levels were determined using Tukey's post hoc test. For statistical assessment of this study data, the statistical package program (Statgraphics Centurion XVI, Statpoint Technologies Inc., The Plains, VA) was used (Zar, 1999). All means were presented with standard errors (±SE). Differences were considered significant at the 95% confidence interval.

Results

Meagre had readily consumed the experimental diets, and no health problems were observed during the nutrition trial. Growth performance parameters of fish fed with the experimental diets for 12 weeks are given in Table 4. From the 12-week feeding period, fish fed diets with HFMT1 grew significantly more ($P<0.05$) with improved final mean weight (FMW) and specific growth rate (SGR) than those fed the HFMT0 and all LFM groups. Including taurine in the LFM diets also improved growth performance and feed utilization. For instance, the FMW and SGR of fish fed the diets containing LFM supplemented with 0.5% taurine were found to be similar when compared to HFM without the inclusion of taurine diet ($P>0.05$) and higher than that of LFM supplemented without taurine ($P<0.05$). Although there was no significant difference among the treatments, fish fed the diets containing LFM supplemented with 0.5% taurine had a lower feed conversion ratio (FCR) than those provided for all HFM diets. The protein efficiency ratio (PER) of the LFMT0.5 group was higher than all other LFM groups ($P<0.05$) and similar to all HFM groups ($P>0.05$). No significance was recorded in the survival rates (SR) of meagre fed with experimental diets ($P>0.05$). In two-way ANOVA, both fish meal levels and taurine levels were affected by the FMW ($P=0.0000$ for fish meal level and $P=0.0065$ for taurine level) and SGR ($P=0.0001$ for fish meal level and $P=0.0044$ for taurine level). However, the FCR

(P=0.0023) and PER (P=0.0049) also affected fish meal levels. No interaction was recorded between independent variables (P>0.05).

The effects of fish meal levels, taurine and their interaction on the biological parameters of treated groups are presented in Table 5. The condition factor (CF) of the HFMT0 and HFMT0.5 groups was significantly higher when compared to the LFMT1 group (P<0.05). The hepatosomatic index (HSI) was affected considerably by fish meal levels, taurine and their interaction. The HSI and viscerosomatic index (VSI) of the fish fed the diets containing taurine at both fish meal levels were significantly reduced compared to the test diets without taurine inclusion (P<0.05). No significant difference in the mesenteric fat index (MFI) was found between dietary treatment groups (P>0.05). In two-way ANOVA, fish meal and taurine levels affected the CF (P=0.0199) and VSI (P=0.0001), respectively. The HSI affected both independent variables (P=0.0042 for fish meal level and P=0.0001 for taurine level). There was an interaction in the independent variables affecting the HSI (P=0.0415).

The whole-body analyzes of experimental fish have resulted in Table 6. The lowest moisture content in meagre was recorded in the HFMT0 group (P<0.05). Fish fed LFMT1 recorded significantly lower protein content than fish fed all HFM diets and recorded considerably higher values (P<0.05). The body lipid content was significantly higher in fish fed LFMT0.5 than in LFMT0 and HFMT0. The level of crude ash was found to be substantially lower in meagre fed LFMT0.5 compared with all other groups, except LFMT1 (P<0.05). In two-way ANOVA, fish meal levels were affected by the protein content (P=0.0171) and taurine levels were affected by the lipid (P=0.0139) and ash (P=0.0235) contents. Both independent variables have affected the moisture (P=0.0084 for fish meal level and P=0.0301 for taurine level). Interactions were noted in the independent variables affecting the moisture (P=0.0001) and the crude ash (P=0.0138).

The whole-body amino acid composition of meagre was presented in Table 7. The highest and lowest arginine contents were found in the HFMT1 and LFMT0 groups, respectively (P<0.05). Histidine level of the HFMT2 was higher than HFMT0.5, HFMT1 and HFMT2 (P<0.05). Leucine contents of all LFM groups were the highest (P<0.05). The lysine level of the LFMT0.5 was lower than the HFMT1 and HFMT2 (P<0.05). The methionine ratio of the HFMT1 was higher than the HFMT0, LFMT0, LFMT0.5 and LFMT1 groups (P<0.05). The highest and the lowest phenylalanine levels were noted in all HFM and LFM groups, respectively (P<0.05). Significance in the taurine contents of the experimental fish was increased with the further supplementation level (P<0.05). Threonine ratios of the HFMT2 and LFMT0.5 were higher than the HFMT0, LFMT1 and LFMT2 (P<0.05). No significant differences were recorded in cysteine, isoleucine, tryptophan, tyrosine and valine contents (P>0.05). In two-way ANOVA, both fish meal and taurine levels were affected by arginine (P=0.0000 for fish meal level and P=0.0000 for taurine level), leucine (P=0.0000 for fish meal level and P=0.0180 for taurine level), methionine (P=0.0012 for fish meal level and P=0.0240 for taurine level), taurine (P=0.0000 for fish meal level and P=0.0000 for taurine level) and threonine (P=0.0212 for fish meal level and P=0.0376 for taurine level) contents. Histidine (P=0.0002), lysine (P=0.0004), phenylalanine (P=0.0000) and valine (P=0.0128) were influenced by fish meal level. Interactions were recorded in the independent variables

affecting the arginine (P=0.0005), isoleucine (P=0.0245), taurine (P=0.0064) and threonine (P=0.0013).

The hematological parameters in fish fed with the experimental diets are shown in Table 8. Fish fed the LFMT0.5 diet recorded significantly higher packed cell volume (PCV) values than fish fed the remaining dietary treatments, except HFMT1 and HFMT2 (P<0.05). The LFMT0.5 resulted in considerably higher hemoglobin (Hb) values than the HFMT0 and LFMT0 groups (P<0.05). White blood cell counts (WBC) were significantly higher in meagre fed with HFMT0.5, HFMT1 and HFMT2 diets compared to all LFM groups (P<0.05). The red blood cell counts (RBC) were higher in meagre fed diets with a high fish meal when compared with fish fed diets with a low fish meal, regardless of taurine levels (P<0.05). Moreover, the supplementation of taurine into the diets at both fish meal levels increased the number of red blood cells. Mean corpuscular volume (MCV) of the LFMT0 and LFMT1 were statistically higher than all HFM groups (P<0.05). The mean corpuscular hemoglobin concentration (MCHC) of the LFMT0.5 was significantly lower than the HFMT0.5 and LFMT2 groups (P<0.05). The mean corpuscular hemoglobin (MCH) of the LFMT0 was higher than all experimental groups, except the LFMT0.5 (P<0.05). In two-way ANOVA, taurine levels affected the Hb (P=0.0118), and fish meal levels affected the WBC (P=0.0001). Both independent variables were affected by the RBC (P=0.0001 for fish meal level and P=0.0002 for taurine level), MCV (P=0.0000 for fish meal level and P=0.0023 for taurine level) and MCH (P=0.0000 for fish meal level and P=0.0000 for taurine level). Interactions were recorded in the independent variables affecting the MCV (P=0.0083), MCHC (P=0.0093) and MCH (P=0.0176).

Typical tissue structure was determined for fish in all treatments. Therefore, according to histopathological evaluation, no pathological findings were found in the treatments. It can be observed from the results that the LFMT1 yielded significantly elevated hepatic lipid accumulation among the experimental diets, as shown in Figure 1 (P<0.05). The dietary treatment groups could be ranked to the following effects based on the hepatic lipid deposition: LFMT1 < HFMT2 < HFMT0.5.

The ammonia excretion rates of fish fed HFMT1, HFMT0.5, and LFMT2 diets were significantly lower than those fed LFMT0 diet (P<0.05) (Figure 2). The supplementation of taurine into all diets also reduced the ammonia excretion rates of fish.

The economic conversion ratio (ECR) of the LFMT0.5 group was significantly lower than the other groups, except for the HFMT0.5 and HFMT1 groups (Table 9). It was determined that all diets containing a high fish meal and LFMT0.5 diet were more elevated in terms of the economic profit index (EPI) than all other treatments. Although there was no statistical difference between HFMT0 and HFMT0.5 groups, the EPI value of the LFMT0.5 group was slightly higher. In two-way ANOVA, fish meal levels and taurine levels have affected the EPI (P=0.0006 for fish meal level and P=0.0467 for taurine level). No interaction was recorded between independent variables (P>0.05).

Discussion

In this study, taurine addition to low fish meal diets appears significant for balancing growth performance comparable to high fish meal diets. But the higher taurine levels did not

enhance growth parameters within the high fish meal diets and as in the stated in the previous studies, addition of proper level of taurine to the plant-based diets could enhance the performance and health of fish (Zhang et al., 2018; Kalhoro et al., 2020; Tong et al., 2020). The final mean weight (FMW) of the HFMT1 was higher than the HFMT0.5 and HFMT2, while there was no statistical difference among these groups ($P>0.05$). A comparable result was noted in a study conducted with 26.8 g rainbow trout (*Oncorhynchus mykiss*). The authors revealed a decrease in the weight gains with the high taurine levels in fish meal diets (Gaylord et al., 2006). Also, we found that the FMWs of the HFMT0 and LFMT0.5 were recorded at the same level, statistically. Chatzifotis et al. (2008) fed the 39.1 g common dentex (*Dentex dentex*) with fish meal and soy protein concentrate (25% and 40% SPC) diets with or without taurine, and they declared the FMWs of fish meal + taurine and 25% SPC + taurine groups were significantly similar. The most critical point here is that incorporating taurine into low fish meal diets improves the growth of fish species at a level comparable to that of high fish meal diets. Reminiscent results were also reported in previous studies carried out with 5.94 g black carp (*Mylopharyngodon piceus*) and 14.4 g giant grouper (*Epinephelus lanceolatus*) (Zhang et al., 2018; Lin and Lu, 2020). However, high taurine levels added to low fish meal diets may not constantly improve growth. Dehghani et al. (2020) added 2, 4, 8, 12 and 16 g/kg taurine to yellowfin seabream (*Acanthopagrus latus*) diets, and they declared the FMWs of the fish fed 8 g/kg and above taurine supplemented diet groups did not improve. The same result was noted in the 10.3 g African catfish (*Clarias gariepinus*) study (Adeshina and Abdel-Tawwab, 2020). Our study found an increment in the FMW of meagre fed the LFMT0.5 diet, while a decrease was observed in the LFMT1 and LFMT2 groups. These differences in research on the inclusion of taurine to plant protein-based diets may be due to the fish's size, species-specific response, and the dietary fish meal level and quality. Likewise, this study recorded similar results as in the FMWs in the specific growth rates (SGR) of the meagre fed diets with and without taurine addition at both fish meal levels. Previous studies proved SGR improvement with taurine supplementation in low fish meal diets. Lunger et al. (2007) replaced fish meal in the diets of 9.8 g cobia (*Rachycentron canadum*) with the ratios of 50:50 and 25:75 with a yeast protein plus taurine addition, and they concluded the SGRs of the fish fed with fish meal and replaced diet groups were significantly similar. Taurine supplemented (4.5%) non- or low-fish meal diets also enhanced the SGRs of common dentex (*D. dentex*), yellowtail (*Seriola quinqueradiata*) and red seabream (*Pagrus major*) (Chatzifotis et al., 2008; Takagi et al., 2008, 2010; Khaoian et al., 2014). Wu et al. (2015) conducted a fish meal replacement study with 40% and 80% SPC on 14.3 g golden pompano (*Trachinotus ovatus*) and added 5 g/kg taurine to all experimental diets. The authors revealed the feed conversion ratio (FCR) of the SPC + taurine groups were statistically similar to the fish meal group. A comparable result was recorded in the feed efficiency of 7.5 g totoaba (*Totoaba macdonaldi*) juveniles (López et al., 2015). As in these previous studies, in our trial, the FCR of the LFMT0.5 group reached the HFM + taurine supplemented groups and was significantly similar to HFMT0.5, HFMT1 and HFMT2 groups. The 0.5%, 1% and 2% addition of taurine to non-fish meal diets (SPC and fermented soybean meal based) improved the protein efficiency ratio (PER) of 11.25 g black seabream (*Acanthopagrus schlegelii*) juveniles (Tong et al., 2020). Results of Tong et al. (2020) and other fish meal replacement

studies by non- or low-fish meal diets conducted on rainbow trout (*O. mykiss*) and totoaba (*T. macdonaldi*) supported our PER results in the present study (Gaylord et al., 2006; López et al., 2015).

In previous studies, the condition factors (CF) of 12.5 g largemouth bass (*Micropterus salmoides*) decreased with the further ratios of plant protein in the diets (Wu et al., 2021; Ren et al., 2022). These studies supported the CF results recorded in our trial. However, no significant differences were noted in CF in previous studies conducted on several fishes fed with taurine supplemented plant-based, non- or low-fish meal-containing diets, including yellowtail, *S. quinqueradiata* (Khaoian et al., 2014), golden pompano, *T. ovatus* (Wu et al., 2015), black carp, *M. piceus* (Zhang et al., 2018), yellowfin seabream, *A. latus* (Denghani et al., 2020) and black seabream, *A. schlegelii* (Tong et al., 2020). In this study, the viscerosomatic indexes (VSI) of meagre fed with high and low fish meal diets without taurine supplementation (HFMT0 and LFMT0) were markedly the highest of all other experimental feeding groups and the taurine level affected this parameter (P=0.0001 in two-way ANOVA). Similar results were presented in the VSIs of cobia (*R. canadum*) fed with fish meal replacement diets by yeast protein plus taurine addition and also yellowfin seabream (*A. latus*) fed with low fish meal diets supplemented with six different levels of taurine (Lunger et al., 2007; Denghani et al., 2020). Likewise, similar findings to the VSI were recorded in the hepatosomatic indexes (HSI) of meagre in the current study and cobia (*R. canadum*), black carp (*M. piceus*), yellowfin seabream (*A. latus*) and black seabream (*A. schlegelii*) in the previous studies (Lunger et al., 2007; Zhang et al., 2018; Denghani et al., 2020; Tong et al., 2020). Song et al. (2021) clearly stated that the taurine regulates the liver metabolism and lipid profile. Therefore, these differences can be expected in experimental groups fed with taurine. In contrast, Aragão et al. (2023) recorded a significant decrease in the HSI and VSI of Senegalese sole (*Solea senegalensis*) fed with fish meal-based diets compared to plant-based diets and 0.5% and 1.5% taurine-included diets. However, no significant differences in HSIs were reported in previous studies carried out with rainbow trout (*O. mykiss*), common dentex (*D. dentex*) and totoaba (*T. macdonaldi*) juveniles (Gaylord et al., 2006; Chatzifotis et al., 2008; López et al., 2015). In our study, no significant difference was found in the mesenteric fat indexes (MFI) of the meagre fed with taurine supplemented high and low fish meal diets as in the previous study conducted on common dentex (*D. dentex*) by Chatzifotis et al. (2008). However, Gaylord et al. (2006) found higher intraperitoneal fat in rainbow trout (*O. mykiss*) fed with non-fish meal diet groups than in fish meal groups, while Tong et al. (2020) stated reverse findings in black seabream (*A. schlegelii*). All these findings show that the somatic indices of finfish species will be modified when plant proteins replace the fish meal at high rates, but this can be regulated by dietary taurine addition.

This study showed significant differences between the whole-body compositions of meagre fed with diets containing different levels of fish meal and taurine. The effects of taurine on the body composition of fish were evaluated in various studies. Whole body protein levels were decreased depending on fish meal levels, and the LFMT1 group was lower than all HFM groups. In contrast, Biasato et al. (2022) declared that the crude protein level of 7.7 g rainbow trout (O. mykiss) juveniles fed with low fish meal diets were higher than that of the fish fed with high fish meal diets. In our study, dietary taurine levels affected the lipid

levels of meagre as recorded in the previous studies conducted with yellow catfish (*Pelteobagrus fulvidraco*) and black carp (*M. piceus*) juveniles (Li et al., 2016; Zhang et al., 2018), while Qi et al. (2012) declared no significant difference in 6.3 g turbot (*Scophthalmus maximus*). The authors also stated the body ash content of turbot fed with a non-taurine supplemented diet was higher than 0.5%, 1% and 1.5% taurine groups as in the LFM groups in our study, while Tong et al. (2020) noted controversy results in black seabream (*A. schlegelii*). In largemouth bass (*M. salmoides*), the inclusion of taurine in the diet did not influence crude protein and lipid but increased moisture values and decreased ash content (Wu et al., 2021). Contrary to the results of our research, no significant differences were reported in whole body compositions, including moisture, crude protein, crude lipid and crude ash contents of common dentex (*D. dentex*), sablefish (*Anoplopoma fimbria*), totoaba (*T. macdonaldi*), golden pompano (*T. ovatus*) and yellowfin seabream (*A. latus*) species (Chatzifotis et al., 2008; Johnson et al., 2015; López et al., 2015; Wu et al., 2015; Dehghani et al., 2020). All the differences in whole body composition results of all these studies are due to factors such as feed content, protein/lipid ratio of the diets, fish species, taurine levels and fish size.

In this study, regardless of the level of taurine added to the diet, dietary taurine in all fish meal levels improved the body amino acid composition of the fish. Similar results were achieved by Dehghani et al. (2020) in yellowfin seabream (*A. latus*). Leucine, lysine, phenylalanine, and taurine levels of fish were affected positively with the inclusion of graded taurine. In a study conducted by Al-Feky et al. (2016) on tilapia (*Oreochromis niloticus*) fed with diets containing different taurine levels to soy-based diets, it was declared that the body's taurine level increased with the dietary taurine, as in our study. Contrary to our results on body amino acid composition, Hongmanee et al. (2022) found that dietary taurine inclusion in non-fish meal diets had no noticeable effects on amino acid composition in juvenile snakehead fish (*Channa striata*). Since the body amino acid composition of fish may vary according to the dietary protein levels, fish levels and the diet formulation, differences may be observed among studies.

In the present study, regardless of the fish meal level, adding taurine to the feed increased the meagre's red blood cell count (RBC). Also, the inclusion of taurine in low fish meal diets generally improved the meagre's hematocrit (PCV) and hemoglobin (Hb) parameters. Including taurine in plant protein-based diets has improved hematological parameters in various fish species. For instance, the hematological parameters of yellowtail (*S. quinqueradiata*) fish fed with diets containing soy protein concentrate and non-fish meal supplemented with 4.5% taurine were similar to those of fish fed with diets containing fish meal and no taurine (Takagi et al., 2008). The PCV and Hb values of parrot fish (*Oplegnathus fasciatus*) fed taurine-added diets (optimally 0.8%) are higher than the fish meal without a taurine group (Lim et al., 2013). Khaoian et al. (2014) reported that adding taurine to low fish meal-containing diets increased the PCV of yellowtail (*S. quinqueradiata*) compared to groups that did not contain taurine. Yellow catfish (*P. fulvidraco*) fed with a diet with a high taurine level in all plant protein-based increased the PCV, Hb and RBC contents compared to the control diet (Li et al., 2016). All dietary taurine levels elevated the Hb and RBC of the European seabass (*D. labrax*) juveniles (Saleh et al., 2020). Unlike our data, Han et al. (2014)

observed that dietary taurine inclusion did not affect the PCV and Hb of Japanese flounder (*Paralichthys olivaceus*). The PCV, Hb and RBC of juvenile totoaba (*T. macdonaldi*) were not statistically different from those fed the control diet when taurine-fortified soy protein concentrates were substituted by dietary fish meal (López et al., 2015). Dietary taurine inclusion did not significantly affect hematological parameters in red seabream (*P. major*) fed with different soybean meals instead of fish meals (Gunathilaka et al., 2019). However, Takagi et al. (2010) recorded significant differences in the mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) of the same species (*P. major*) as in our study conducted with meagre (*A. regius*). There are differences between the hematological results obtained from various studies on the inclusion of taurine in finfish diets. Since this situation may vary depending on the type, size, dietary formulation, physiological state, and environmental conditions of the fish, more in-depth studies should be conducted to clarify the effects of taurine on fish health.

In this study, the liver was subjected to histological examination to determine the effects of fish meal and taurine levels on liver health. This study is the first to characterize the effect of dietary taurine on liver histomorphology in meagre. It has been observed that dietary taurine significantly impacts the histopathological changes in the liver. In our experimental unit, liver histological sections of similar-sized meagre fish fed with commercial seabass diet (Figure 3) were also compared with the livers of fish fed with the experimental diets. When the experimental groups were compared, the highest liver lipid accumulation was found to be LFMT1, HFMT2 and HFMT0.5, respectively. The other groups also were found to be similar to each other in terms of lipid accumulation. It was determined that the liver lipid accumulation of fish in all experimental groups was less than that of the group fed with commercial feed. Histological sections have also demonstrated the effects of taurine on the liver in fish in previous research. Similar results regarding histological outcomes have previously been observed in taurine-fed black carp, *M. piceus* (Tian et al., 2016), European seabass, *D. labrax* (Rimoldi et al., 2016, Martins et al., 2018) and totoaba, *T. macdonaldi* (López et al., 2015), less abnormal liver histopathology (López et al., 2015). For instance, totoaba were fed diets containing fish meal-based and either 30% or 60% of SPC supplemented or not with 1% of taurine diets and fat accumulation in the fish livers was studied (López et al., 2015). It was stated that the cytoplasm vacuoles in fish fed with a control diet showed smooth and consistent normal histology, while lipoid accumulation was noted in other groups. Also, it has been reported that the cytoplasmic nuclei become oval and intracellular syndromes occur in groups fed with feeds that do not contain taurine. The current study's findings were consistent with previous results in extensive macro-vesicular lipid deposition in the liver tissue of fish. Contrary to these studies, Kotzamanis et al. (2020) reported that dietary taurine was not a histological change in the liver of European seabass (*D. labrax*). Also, the authors determined lipid accumulation within the hepatocytes in European seabass fed all experimental diets. Therefore, more in-depth research is required to investigate taurine-fed fish's hepatic functionality.

The study revealed that the total ammonia nitrogen excretion (TAN) of the meagre fed with a diet low fish meal containing taurine-free (LFMT0) was higher than the other groups. The TAN of the other experimental groups fed with low fish meal supplementation with

taurine (LFMT0.5, LFMT1 and LFMT2) were higher than the feeding groups with the high fish meal (HFMT0.5, HFMT1 and HFMT2). Park et al. (2002) and Kim et al. (2005a, b) reported no significant difference among the NH_3 excretion of the experimental groups of Japanese flounder (*P. olivaceus*) fed with 0%, 0.5% and 1% taurine supplemented fish meal-based diets. Therefore, in the present study, there is a significance between HFMT1 (lower) and HFMT0 and HFMT2 (higher) groups. These results suggest that the absence or a high-level presence of taurine in fish meal diets increases ammonia-nitrogen excretion in meagre aquaculture. However, no comparative research was conducted on the TAN of fish fed with a low fish meal-containing diet (with taurine supplemented). In this study, it can be said that the TAN level decreased statistically with the increase of taurine level in low fish meal-containing diets. More detailed research should be done on improving the water quality parameters with dietary taurine addition to the aquafeeds.

Economic analysis revealed that the LFMT0.5 group was as profitable as the groups fed with the high fish meal diets (HFMs). Hien et al. (2015) fed 4.74 g snakehead (*C. striata*) fingerlings with the diets replaced fish meal with 20%, 30%, 40% and 50% soybean meal and also with taurine. Although they reported no statistical difference between the experimental groups' economic profit index (EPI), the best profitability was recorded in the groups fed with 50% soybean meal plus taurine. Accordingly, our results are supported by the findings of this previous research. The relative feed cost/kg of fish fed diets containing high fish meal and 2% level of taurine (HFMT2) was higher than all low-fish meal diets (100% vs 69-80%). The EPIs of the HFMT2 group with a feed cost of 1.16 €/kg and the LFMT0.5 groups with 0.83 €/kg were statistically similar. Therefore, reducing the level of fish meal provides profit in terms of production cost. Since the fish meal is the most costly component in the aquafeed, the inclusion of amino acids in the different fish meal levels, which positively affects growth parameters, also improves the profitability in meagre. Güroy et al. (2017), in our previous study on meagre, added amino acids to different protein levels diets and found that we increased profitability while reducing the cost of these diets. It has been reported that lower-cost diets with higher profitability can be prepared by adding amino acids by reducing the amount of fish meal in the aquafeed.

Conclusion

In conclusion, this research provided good evidence for the benefits of adding dietary taurine (0.5%) in conjunction with its high plant protein diets. This is becoming a vital strategy to deliver on the promise of significantly improving the growth performance and health parameters of fish fed low fish meal contained in the practical fish diets. Certainly, optimum taurine can be successfully used in commercial aquafeed as a functional amino acid to enhance meagre's growth, health, quality and profitability. It can be considered a commercially viable approach to producing low-cost and sustainable diets in mariculture. It is clear that more research and development in these areas are needed to fully evaluate taurine as a viable nutritional supplement for fish and especially meagre.

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Author contribution

D.G. is the project leader and responsible for every step of the experiment, calculations and statistical analysis, and wrote the manuscript draft, O.K. made the experimental feed, analyzed biological indices, hematological parameters, calculations and statistical analysis, and wrote the manuscript draft, B.G. designed experiment, made experimental feed, analyzed biological indices, hematological parameters, calculations and statistical analysis, and wrote the manuscript draft, Y.E. designed experiment, analyzed histological parameters and consulted during the study, N.E. analyzed biological indices, hematological and histological parameters, D.E. reared fish rearing and maintained system (RAS), F.B.Y. made biological indices, hematological and histological parameters, S.M. analyzed biological indices, hematological and whole-body parameters, amino acid analysis, and wrote the manuscript draft, A.D. reared fish rearing and maintained system (RAS).

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethics approval

The present study was carried out by animal welfare and the ethics of the experiment. This study complied with the Guidelines of the EU Directive 2010/63/EU for animal experiments.

Availability of data and material

All data generated and analyzed during this study are presented in this article.

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Table 1. Proximate compositions of feed ingredients (% on dry matter basis)

	Dry matter	Crude protein	Crude lipid	Crude ash	Crude fiber
Fish meal	91.45	65.33	12.90	11.50	1.00
Corn gluten	92.34	70.42	0.38	2.50	1.00
Dehulled soybean meal	90.15	44.42	0.73	6.00	4.00
Poultry by-product	91.45	60.48	11.98	14.00	2.50
Feather meal	92.10	85.31	5.73	3.00	1.46
Wheat gluten	90.82	73.38	0.88	1.00	1.59
Wheat flour	91.47	8.00	1.80	1.50	1.50
Sunflower seed meal	90.75	33.00	2.80	5.70	23.00

Table 2. Formulation and proximate composition (% on dry matter basis) of the experimental diets

Ingredients (% dry matter, DM)	HFMT0	HFMT0.5	HFMT1	HFMT2	LFMT0	LFMT0.5	LFMT1	LFMT2
Fish meal[1]	38.50	38.50	38.50	38.50	11.00	11.00	11.00	11.00
Corn gluten[2]	13.70	13.70	13.70	13.70	34.50	34.50	34.50	34.50
Dehulled soybean meal[3]	11.50	11.50	11.50	11.50	19.15	19.15	19.15	19.15
Poultry by-product[4]	6.20	6.20	6.20	6.20	6.00	6.00	6.00	6.00
Feather meal[4]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Wheat gluten[2]	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Wheat meal[5]	11.00	10.50	10.00	9.00	7.00	6.50	6.00	5.00
Sunflower seed meal[6]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Fish oil[7]	12.15	12.15	12.15	12.15	15.40	15.40	15.40	15.40
Vitamin premix[8]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral premix[8]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Choline chloride[8]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Taurine[9]	-	0.50	1.00	2.00	-	0.50	1.00	2.00
Chemical composition (%)								
Dry matter	92.11	92.25	92.08	92.34	92.19	92.07	92.24	92.15
Crude protein	47.74	47.70	47.81	47.60	47.68	47.64	47.40	47.52
Crude lipid	18.68	18.54	18.48	18.75	18.45	18.40	18.48	18.29
Crude ash	6.69	6.68	6.68	6.66	4.42	4.41	4.41	4.39
Crude fiber	1.83	1.82	1.82	1.80	2.00	2.00	1.99	1.97
Nitrogen free extract	25.06	25.26	25.21	25.19	27.45	27.55	27.72	27.83

[1]Anchovy fish meal. Sürsan Feed Mill Company, Samsun, Turkey.
[2]Cargill, İstanbul, Turkey.
[3]Kırcı Soya Company, Balıkesir, Turkey.
[4]Skretting Feed Mill Company, Muğla, Turkey.
[5]İpek Wheat Company, Nevşehir, Turkey.
[6]Matlı Feed Mill Company, Bursa, Turkey.
[7]Anchovy fish oil. Sürsan Feed Mill Company, Sinop, Turkey.
[8] DSM Nutritional Products, Turkey.
[9]L-taurine 99%>, Sigmaalgrichs.

Table 3. Amino acid compositions of the experimental diets (%)

	HFMT0	HFMT0.5	HFMT1	HFMT2	LFMT0	LFMT0.5	LFMT1	LFMT2
Arginine	2.05	2.10	2.14	2.18	1.82	1.85	1.84	1.90
Cysteine	0.54	0.56	0.58	0.55	0.56	0.57	0.58	0.55
Histidine	1.14	1.18	1.12	1.24	1.20	1.21	1.17	1.08
Isoleucine	1.74	1.74	1.75	1.80	1.95	1.85	1.76	1.81
Leucine	3.84	3.76	3.37	3.48	5.50	5.80	5.55	5.60
Lysine	2.65	2.69	2.71	2.47	1.66	1.70	1.61	1.74
Methionine	1.05	1.07	1.04	1.08	0.97	0.99	0.95	0.96
Phenylalanine	3.18	3.19	3.27	3.25	2.89	2.82	2.70	2.81
Taurine	0.25	0.73	1.29	2.21	0.08	0.61	1.11	2.04
Threonine	1.39	1.40	1.44	1.41	1.46	1.51	1.48	1.47
Tryptophan	0.45	0.54	0.49	0.41	0.40	0.35	0.37	0.34
Tyrosine	0.58	0.89	0.70	0.70	0.80	0.67	0.87	0.81
Valine	1.96	2.17	2.04	2.09	2.12	2.18	2.22	2.27

Table 4. Growth performance of meagre fed with diets containing different levels of fish meal and taurine during the 12 weeks

	High fish meal diets				Low fish meal diets				Two-way ANOVA		
	HFMT0	HFMT0.5	HFMT1	HFMT2	LFMT0	LFMT0.5	LFMT1	LFMT2	Fish Meal Level	Taurine Level	Interaction
Initial mean weight (g)	23.52±0.04	23.56±0.06	23.58±0.02	23.53±0.08	23.52±0.03	23.46±0.01	23.57±0.03	23.55±0.06	0.7957	0.5809	0.7359
Final mean weight (g)	81.51±2.71 c	87.51±2.05 cd	90.63±3.89 d	84.53±2.11 cd	57.2±0.89 a	81.85±1.15 c	69.26±1.47 b	63.5±0.93 ab	0.0000	0.0065	0.2737
Specific growth rate (%/day)	1.48±0.04 d	1.56±0.03 de	1.60±0.05 e	1.52±0.03 de	1.06±0.02 a	1.49±0.02 d	1.28±0.02 c	1.18±0.02 b	0.0001	0.0044	0.1246
Feed intake (g)	104.6±2.02 ab	100.77±11.29 ab	93.75±6.35 ab	99.63±11.45 ab	108.26±1.62 ab	83.99±11.06 a	110.8±7.71 b	111.51±5.09 b	0.5138	0.4938	0.8611
Feed conversion ratio	1.29±0.07 ab	1.16±0.16 a	1.04±0.09 a	1.19±0.16 a	1.89±0.06 c	1.02±0.12 a	1.61±0.15 bc	1.76±0.10 c	0.0023	0.0758	0.4481
Protein efficiency ratio	1.16±0.08 bc	1.37±0.18 c	1.51±0.13 c	1.33±0.2 c	0.65±0.03 a	1.48±0.17 c	0.88±0.08 ab	0.76±0.05 ab	0.0049	0.0639	0.7553
Survival rate (%)	93.33±0.00	97.78±3.85	100.00±0.00	91.11±15.40	100.00±0.00	95.55±3.85	100.00±0.00	100.00±0.00	0.1764	0.5846	0.3216

Different letters in the same line indicate statistically significant differences ($P<0.05$) among the groups.

Table 5. Somatic indexes of meagre fed with diets containing different levels of fish meal and taurine during the 12 weeks

	High fish meal diets				Low fish meal diets				Two-way ANOVA		
	HFMT0	HFMT0.5	HFMT1	HFMT2	LFMT0	LFMT0.5	LFMT1	LFMT2	Fish Meal Level	Taurine Level	Interaction
Condition factor (%)	1.00±0.01 b	1.03±0.01 b	0.99±0.01 ab	0.96±0.01 ab	0.98±0.01 ab	0.94±0.05 ab	0.90±0.02 a	0.93±0.01 ab	0.0199	0.0692	0.2464
Viscerosomatic index (%)	8.06±0.20 c	5.80±0.30 a	6.32±0.20 ab	6.12±0.24 ab	7.79±0.51 c	6.44±0.37 ab	6.83±0.22 b	6.39±0.22 ab	0.6652	0.0001	0.1353
Hepatosomatic index (%)	2.26±0.15 c	1.36±0.13 a	1.53±0.16 ab	1.42±0.08 a	1.79±0.1 b	1.38±0.09 a	1.40±0.08 a	1.28±0.09 a	0.0042	0.0001	0.0415
Mesenteric fat index (%)	0.06±0.02	0.07±0.04	0.08±0.09	0.06±0.01	0.07±0.02	0.06±0.01	0.09±0.01	0.06±0.01	0.6418	0.5129	0.3581

Different letters in the same line indicate statistically significant differences ($P<0.05$) among the groups.

Table 6. Whole body compositions of meagre fed with diets containing different levels of fish meal and taurine during the 12 weeks

	High fish meal diets				Low fish meal diets				Two-way ANOVA		
	HFMT0	HFMT0.5	HFMT1	HFMT2	LFMT0	LFMT0.5	LFMT1	LFMT2	Fish Meal Level	Taurine Level	Interaction
Moisture (%)	71.21±0.12 a	73.29±0.39 d	72.72±0.17 cd	72.01±0.01 b	72.79±0.09 cd	72.19±0.35 bc	72.06±0.16 b	72.60±0.25 bc	0.0084	0.0301	0.0001
Crude protein (%)	17.01±0.51 b	17.34±0.36 b	16.54±0.37 b	16.92±0.58 b	16.06±0.23 ab	16.01±0.92 ab	14.94±0.77 a	16.05±0.28 ab	0.0171	0.6005	0.7258
Crude lipid (%)	5.23±0.22 cd	3.84±0.34 a	4.31±0.25 ab	4.37±0.31 ab	4.83±0.13 bcd	5.37±0.40 d	3.95±0.19 a	4.45±0.28 abc	0.9739	0.0139	0.1144
Crude ash (%)	3.76±0.21 c	3.89±0.21 c	3.67±0.35 bc	3.88±0.28 c	4.29±0.22 c	2.36±0.16 a	2.96±0.29 ab	3.94±0.29 c	0.6853	0.0235	0.0138

Different letters in the same line indicate statistically significant differences ($P<0.05$) among the groups.

Table 7. Amino acid compositions of meagre fed with diets containing different levels of fish meal and taurine during the 12 weeks (%)

	High fish meal diets				Low fish meal diets				Two-way ANOVA		
	HFMT0	HFMT0.5	HFMT1	HFMT2	LFMT0	LFMT0.5	LFMT1	LFMT2	Fish Meal Level	Taurine Level	Interaction
Arginine	6.15±0.04 c	6.61±0.02 e	6.85±0.04 f	6.35±0.05 d	5.82±0.03 a	6.11±0.05 c	6.02±0.08 bc	5.91±0.05 ab	0.0000	0.0000	0.0005
Cysteine	0.68±0.05	0.71±0.08	0.65±0.08	0.68±0.08	0.67±0.03	0.68±0.07	0.71±0.07	0.69±0.03	0.9157	0.9881	0.9329
Histidine	4.86±0.08 ab	4.81±0.03 a	4.75±0.05 a	4.82±0.03 a	5.02±0.08 ab	4.91±0.05 ab	5.11±0.13 ab	5.18±0.08 b	0.0002	0.3293	0.2115
Isoleucine	3.18±0.08	3.25±0.08	3.57±0.10	3.50±0.10	3.27±0.08	3.54±0.10	3.31±0.04	3.35±0.08	0.8917	0.0855	0.0245
Leucine	4.67±0.07 a	4.98±0.14 ab	5.20±0.11 b	4.82±0.03 ab	5.94±0.06 c	6.22±0.05 c	5.98±0.12 c	6.01±0.14 c	0.0000	0.0180	0.0712
Lysine	3.42±0.05 ab	3.48±0.08 ab	3.61±0.10 b	3.58±0.08 b	3.24±0.03 ab	3.15±0.05 a	3.28±0.10 ab	3.45±0.11 ab	0.0004	0.0536	0.4796
Methionine	3.58±0.04 bc	3.65±0.08 bcd	3.82±0.08 d	3.75±0.10 cd	3.35±0.08 a	3.43±0.10 ab	3.52±0.04 ab	3.65±0.07 bcd	0.0012	0.0240	0.5962
Phenylalanine	4.18±0.09 b	4.11±0.04 b	4.24±0.08 b	4.19±0.11 b	2.89±0.10 a	2.82±0.03 a	2.70±0.04 a	2.81±0.08 a	0.0000	0.8201	0.3623
Taurine	0.18±0.02 a	0.76±0.03 b	1.41±0.08 d	2.02±0.04 e	0.04±0.01 a	0.57±0.03 b	1.01±0.05 c	1.98±0.06 e	0.0000	0.0000	0.0064
Threonine	3.52±0.10 bc	3.68±0.09 cd	3.70±0.04 cd	3.82±0.08 d	3.68±0.06 cd	3.85±0.15 d	3.22±0.09 a	3.27±0.11 ab	0.0212	0.0376	0.0013
Tryptophan	0.48±0.08	0.41±0.04	0.49±0.07	0.46±0.05	0.44±0.04	0.42±0.06	0.38±0.11	0.39±0.11	0.3078	0.9446	0.8836
Tyrosine	0.89±0.08	1.01±0.05	0.84±0.04	1.04±0.10	0.95±0.14	0.91±0.05	0.84±0.05	0.91±0.06	0.4354	0.3155	0.5606
Valine	2.62±0.08	2.68±0.07	2.71±0.08	2.61±0.10	2.82±0.08	2.91±0.05	2.78±0.11	2.79±0.10	0.0128	0.6824	0.7918

Table 8. Hematological parameters of meagre fed with diets containing different levels of fish meal and taurine during the 12 weeks

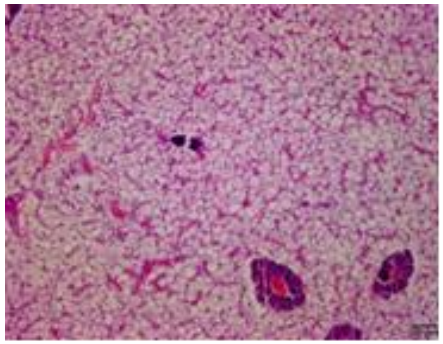
	High fish meal diets				Low fish meal diets				Two-way ANOVA		
	HFMT0	HFMT0.5	HFMT1	HFMT2	LFMT0	LFMT0.5	LFMT1	LFMT2	Fish Meal Level	Taurine Level	Interaction
Packed cell volume (%)	34.33±1.99 a	30.83±1.19 a	35.67±2.89 ab	36.33±1.63 ab	31.83±2.18 a	42.5±2.88 b	34.60±1.91 a	32.17±2.88 a	0.9527	0.2708	0.2597
Hemoglobin concentration (g/dL)	6.84±0.10 a	7.33±0.15 ab	7.2±0.20 ab	7.03±0.12 ab	6.83±0.12 a	7.40±0.10 b	7.04±0.27 ab	7.23±0.29 ab	0.8727	0.0118	0.8718
White blood cell count ($x10^3/\mu L$)	237.83±6.42 cd	246.33±9.51 d	251.33±6.43 d	250.33±10 d	201.83±6.34 ab	197.5±9.85 a	211.2±9.79 ab	222±6.33 bc	0.0001	0.1484	0.8160
Red blood cell count ($x10^6/\mu L$)	1.67±0.05 c	1.68±0.06 c	1.95±0.05 d	1.96±0.06 d	1.11±0.07 a	1.34±0.05 b	1.39±0.06 b	1.72±0.05 c	0.0001	0.0002	0.1472
Mean corpuscular volume (μm^3)	207.30±40.99 a	184.83±17.96 a	183.69±37.61 a	185.56±21.22 a	292.52±65.09 b	320.75±74.84 b	249.58±15.21 ab	188.78±46.40 a	0.0000	0.0023	0.0083
Mean corpuscular hemoglobin concentration (%)	20.24±2.98 ab	23.97±2.69 b	21.08±5.48 ab	19.49±1.52 ab	22.13±4.92 ab	17.80±2.85 a	20.51±2.57 ab	23.09±3.48 b	0.7605	0.9832	0.0093
Mean corpuscular hemoglobin (pg)	41.05±3.48 ab	44.01±3.55 ab	37.21±4.29 a	35.95±2.57 a	62.42±8.82d	55.59±6.72c d	51.15±6.76bc	42.30±5.37 ab	0.0000	0.0000	0.0176

Different letters in the same line indicate statistically significant differences ($P<0.05$) among the groups.

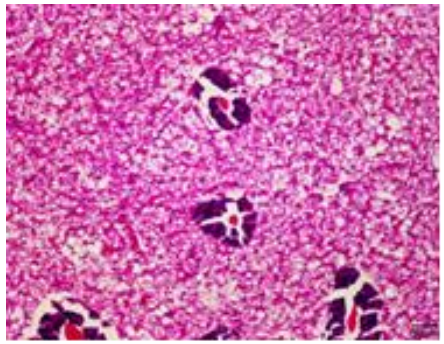
Table 9. Economic analysis of meagre fed with diets containing different levels of fish meal and taurine during the 12 weeks

	High fish meal diets				Low fish meal diets				Two-way ANOVA		
	HFMT0	HFMT0.5	HFMT1	HFMT2	LFMT0	LFMT0.5	LFMT1	LFMT2	Fish Meal Level	Taurine Level	Interaction
Feed cost (€)	1.03	1.06	1.09	1.16	0.8	0.83	0.86	0.93			
Economic conversion ratio (€/kg)	1.32±0.2 bc	1.23±0.17 abc	1.13±0.1 ab	1.38±0.19 bc	1.52±0.04 bc	0.85±0.07 a	1.38±0.13 bc	1.64±0.1 c	0.3398	0.4084	0.7572
Economic profit index (€/fish)	2.91±0.37 b	3.16±0.17 b	3.32±0.17 b	2.97±0.18 b	2.06±0.04 a	3.23±0.16 b	2.49±0.1 a	2.21±0.07 a	0.0006	0.0467	0.4931

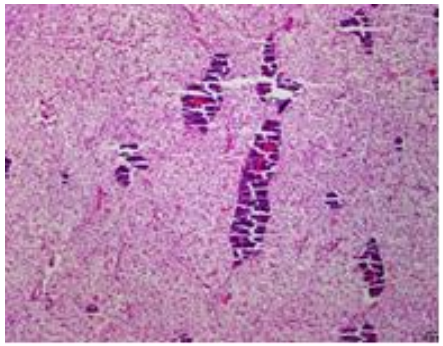
Different letters in the same line indicate statistically significant differences ($P<0.05$) among the groups.



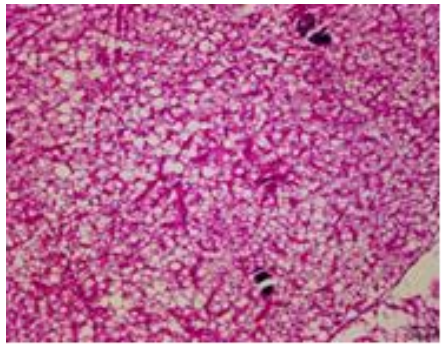
HFMT0



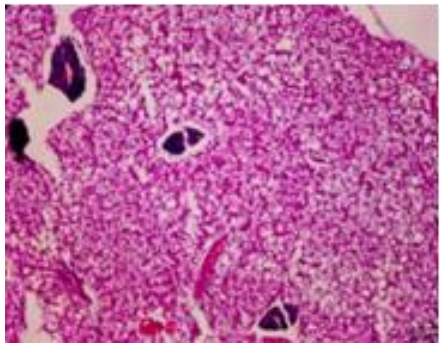
HFMT0.5



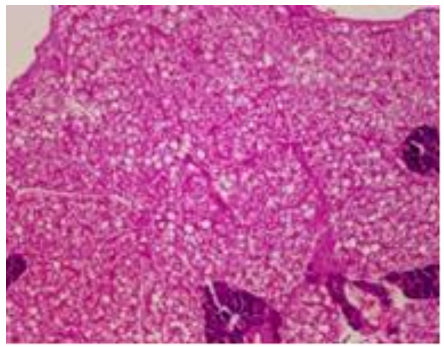
HFMT1



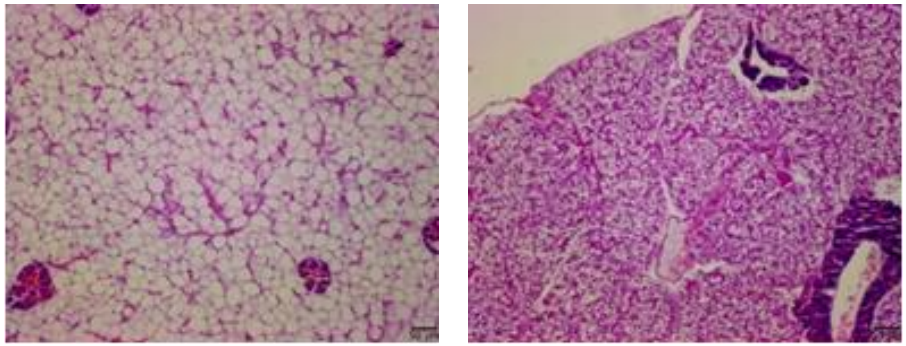
HFMT2



LFMT0



LFMT0.5



LFMT1 LFMT2

Figure 1. The liver histology sections of meagre fed with high and low fish meal containing experimental diets (HFM and LFM) with different levels of taurine as 0% (HFMT0 and LFMT0), 0.5% (HFMT0.5 and LFMT0.5), 1% (HFMT1 and LFMT1) and 2% (HFMT2 and LFMT2)

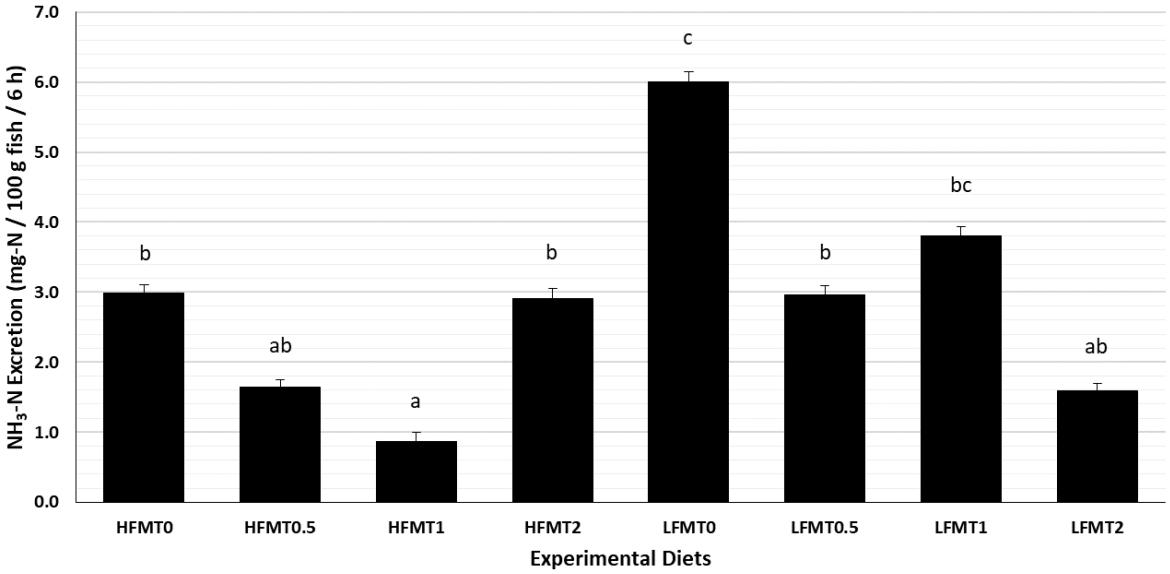


Figure 2. Total ammonia–nitrogen excretion (TAN) of meagre fed with high and low fish meal containing experimental diets (HFM and LFM) with different levels of taurine as 0% (HFMT0 and LFMT0), 0.5% (HFMT0.5 and LFMT0.5), 1% (HFMT1 and LFMT1) and 2% (HFMT2 and LFMT2) after 6 h. Different letters above the error bars indicate significant differences ($P<0.05$) among the groups

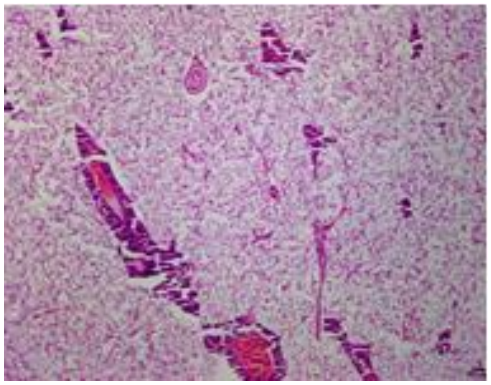


Figure 3. The liver histology section of meagre fed with a commercial European seabass diet