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Supplementing taurine to plant-based diets improves lipid digestive capacity and amino acid retention of Senegalese sole (*Solea senegalensis*) juveniles

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Abstract

Recent studies reported positive effects of taurine supplementation to plant protein-based diets on fish growth performance and feed conversion ratio but the mechanisms involved remain unclear. The objective of this work was to provide a first insight on the importance of taurine on dietary protein and lipid absorption and metabolic utilisation, in Senegalese sole (*Solea senegalensis*) juveniles fed a plant-based diet. Seven practical diets were tested: a fishmeal-based diet (FM) and a plant protein-based formula (PP85) to which taurine was added in graded levels: 0.2% (TAU1), 0.3% (TAU2), 0.4% (TAU3), 0.7% (TAU4) and 1.5% (TAU5). Short-term metabolic trials were performed by tube-feeding the different experimental diets labelled with different tracers: ^{14}C-taurine, ^{14}C-triolein (as tracer of dietary triacylglycerol) and a mixture of ^{14}C-amino acids. Feeding sole juveniles with the PP85 diet affected taurine metabolism, since a higher proportion of dietary taurine was directed to gallbladder and probably used for bile salt synthesis. Consistent with this, fish receiving the plant-based low taurine-content diets (PP85 and TAU1) displayed a poor triacyglycerol digestion capacity. A higher proportion of ^{14}C-amino acids was retained in fish muscle when fed the TAU5 compared to the PP85 diet. These short-term trials suggested that taurine supplementation to a plant-based diet might be beneficial to sole performance by enhancing bile salt synthesis and triacylglycerol digestion as well as muscle amino acid retention. However, this dietary supplementation should be tested in long-term trials to verify the effect on fish growth and to assess the beneficial effects on protein and lipid metabolism suggested here.

1. Introduction

To face the decreasing availability of the main and traditional ingredients used in the formulation of aquafeeds (i.e. fish oil and fishmeal), further development of the aquaculture sector implies a search for new ingredients. During the last two decades, intense research efforts have specifically focused on the potential of plant-derived ingredients as an alternative to gradually substitute ingredients of marine origin in aquafeeds formulation (e.g. Benedito-Palos et al., 2007; Cabral et al., 2013; Kaushik et al., 1995; Richard et al., 2006; Torstensen et al., 2008). On account of this, incorporation of consequent levels of vegetable oils and plant proteins in standard commercial pellet feeds is nowadays common practice for the main European farmed species (Olsen, 2011). However, these plant raw materials have various inconveniences compared to the marine ones (Oliva-Teles et al., 2015). Vegetable oils and fish oils have different fatty acid compositions and above all, vegetable oils are devoid of essential long chain polyunsaturated fatty acids of the n-3 series (i.e. 20:5n-3 and 22:6n-3), which are essential for marine fish species and confers the nutritional quality of fish flesh for human consumption (Oliva-Teles et al., 2015). Regarding plant meals, these have a lower protein content compared to fishmeal and usually contain some deleterious compounds, commonly called anti-nutritional factors, not found in fishmeals. These compounds negatively affect feed intake, digestion and/or absorption of nutrients, may cause intestinal inflammation and affect fish health status (Hardy, 2010). Additionally, plant proteins frequently have an imbalanced amino acid profile compared to proteins from animal origins, being deficient in one or several amino acids that are essential for fish, such as lysine, methionine, threonine, tryptophan and/or arginine, depending on the plant source, and are almost devoid of taurine (Oliva-Teles et al., 2015).

Senegalese sole (*Solea senegalensis*) is a high value flatfish species, considered an essential species to sustain the diversification of Portuguese and Southern European marine finfish aquaculture in the nearest future (Morais et al., 2014). Even for a carnivorous species, the juvenile stage of Senegalese sole requires the incorporation of particularly high levels of proteins in the feed (60% DM crude protein) to reach maximum protein accretion (Rema et al., 2008). A few recent studies showed that despite its high dietary protein requirement, Senegalese sole seems to show a high tolerance for plant-protein based diets either during the juvenile stage (Aragão et al., 2003; Cabral et al., 2011; Silva et al., 2009; Silva et al., 2010) or in market-sized fish (Cabral et al., 2013; Dias et al., 2010; Valente et al., 2011). However, Valente et al. (2011) observed a few severe signs of hepatic degeneration with necrosis and increased vacuolisation in sole (market-sized, > 300g) fed a plant protein-based formulation compared to a fishmeal-based one (high fat diets containing 15%

DM crude lipid), together with higher hepatosomatic index, viscerosomatic index and body lipid content, though fish growth performances were not affected. The authors suggested that a taurine supplementation to the plant protein-based diet might avoid these hepatic damages (Valente et al., 2011).

Taurine is an amino acid found at high levels in feedstuffs from animal sources and in particular from marine animal sources (El-Sayed, 2014). Taurine can be endogenously synthesised from methionine/cysteine through the transsulfuration pathway, but the capacity of taurine synthesis in fish varies interspecies and along the development (reviewed in El-Sayed, 2014 and Salze and Davis, 2015). Taurine is neither used by the cells for protein synthesis, nor oxidised for energy purposes but it is the most abundant amino acid of the free amino acid pool in animal blood and tissues. Even if its role is not yet well understood, taurine is involved in many important physiological processes such as cellular osmoregulation, bile acid metabolism, control of plasma cholesterol level, immune response modulation, myocardial contractibility, retina development, regulation of endocrine tissues physiology, and it also presents some antioxidant and hypoglycaemic properties (reviewed in El-Sayed, 2014 and Salze and Davis, 2015). Taurine requirements appear to be species specific (besides also differing according to the developmental stage of the animal) and according to the species investigated so far, taurine requirements seem to be much higher in marine fish compared to freshwater fish due to an extremely low capacity to synthesise it (reviewed in El-Sayed, 2014 and Salze and Davis, 2015). Therefore, taurine is considered an essential nutrient for marine fish (NRC, 2011). Thus, when feeding marine fish with terrestrial plant protein-based diets taurine requirements may not be fulfilled, since taurine is virtually absent in plants (Kataoka and Ohnishi, 1986).

Recent studies revealed positive effects of taurine supplementation to plant protein-based diets on fish growth performance and feed conversion ratio (Al-Feky et al., 2016; Bañuelos-Vargas et al., 2014; Chatzifotis et al., 2008; Li et al., 2016; Takagi et al., 2011). However, it remains unclear if taurine improves growth through mechanisms that increase protein retention or decrease protein catabolism, which may result from a more effective use of lipids as energy substrates. Taurine is involved in lipid metabolism through its important role in bile salt synthesis. In teleost, taurine is virtually the only amino acid that conjugates with bile acids (Hofmann et al., 2010).

In the present study, we aimed to provide a first insight on the importance of taurine on dietary protein and lipid absorption and metabolic utilisation, in Senegalese sole juveniles fed diets with high levels of fishmeal replacement by plant proteins. For this, several short-term metabolic trials were designed, to enable the assessment of absorption and metabolic utilisation (tissue retention or catabolism) of radiolabelled tracers of interest. In a first metabolic trial, we assessed the

influence of dietary taurine content of a plant-based dietary formulation on taurine retention. In a second trial, we studied the impact of dietary taurine content on dietary triacylglycerol absorption and subsequent metabolic utilisation, using triolein as tracer. And in a third trial, we investigated the effect of dietary taurine content on dietary amino acid utilisation.

2. Materials and methods

2.1. Experimental diets

For this experiment seven practical diets were formulated (Table 1) using two basal formulations previously used by Aragão et al. (2014): a CONTROL fishmeal-rich diet (FM) and an EXPERIMENTAL plant protein-based diet (PP85). In the FM diet, which presented a formulation similar to a commercial diet, the total marine-derived ingredients, accounting as major protein sources, represented 60% of the formula and fish oil was the main lipid source. In PP85 diet, plant protein sources replaced 85% of proteins from marine origin. Based on the PP85 formulation, five additional experimental diets were further supplemented with taurine, at the expense of whole wheat, in order to reach five graded levels of taurine (% DM): 0.2 (TAU1), 0.3 (TAU2), 0.4 (TAU3 - corresponding to a taurine level similar to the one found in the FM diet), 0.7 (TAU4) and 1.5 (TAU5 - corresponding to the level of taurine found in polychaetes, the natural food of sole in the wild). All the diets were isonitrogeneous, isolipidic and isoenergetic and fulfilled the known nutritional requirements (essential amino acids and phosphorus) of juvenile Senegalese sole..

All diets were manufactured and extruded at SPAROS Lda. (Olhão, Portugal), as described in Aragão et al. (2014). Briefly, powdered ingredients were mixed in a double-helix mixer and diets (pellet size 2.0 mm) were extruded (twin-screw extruder, model BC45, Clextral, France) and dried in a convection oven (OP 750-UF, LTE Scientifics, United Kingdom). After cooling, the oils were added to the pellets by vacuum coating (model PG-10VCLAB, Dinnisen, The Netherlands). Samples of all diets were taken for proximate composition and taurine content analysis (Table 1).

Proximate composition analysis of the diets was done by the following procedures: dry matter by drying at 105 °C for 24 h; ash by combustion at 550 °C for 12 h; crude protein (N × 6.25) by a flash combustion technique followed by a gas chromatographic separation and thermal conductivity detection (LECO FP428, Leco Instruments,USA); crude fat after dichloromethane extraction by the Soxhlet method; and gross energy in an adiabatic bomb calorimeter (model C2000, IKA-Werke GmbH & Co. KG, Staufen, Germany). For taurine content analysis, diet samples were firstly homogenised (0.1 M HCl, on ice) and then the supernatant (obtained after centrifugation at

1500 x *g*, 15 min, 4ºC) was deproteinised by centrifugal ultrafiltration (10 kDa cut-off, 2500 x *g*, 20 min, 4 ºC). Samples were then pre-column derivatised with Waters AccQ Fluor Reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) using the AccQ Tag method (Waters, USA) and analysed by ultra-high-performance liquid chromatography (UPLC) as described in Aragão et al. (2014).

2.2. Fish husbandry and metabolic trials design

A group of Senegalese sole juveniles (23.7 ± 10.4 g body weight) was obtained from Estação Piloto de Piscicultura de Olhão (IPMA, Portugal) and transported to the experimental rearing facilities at CCMAR (Faro, Portugal). Fish were adapted to the new conditions (flat-bottomed tanks with a fish density <5 kg m^{-2}, recirculated water circuit supplied with aerated natural seawater at 33 ± 2‰ salinity, 18.8 ± 1.0 °C and 12 h L:12 h D photoperiod) and fed with the Control (FM) diet during this acclimation period (feeding at 1.5% body weight with automatic feeders during 24h day^{-1}) until the different metabolic trials.

All animal manipulations were carried out in compliance with the European (Directive 2010/63/EU) and Portuguese legislation for the use of laboratory animals. All animal protocols were performed under a Group-1 license from the Direção-Geral de Alimentação e Veterinária, Ministério da Agricultura e do Mar (Portugal).

After two weeks of acclimation to the new conditions, 35 fish were isolated in the tank and received the PP85 diet during two additional weeks. This period was chosen to diminish the taurine reserves in fish and be able to observe the taurine flux as a function of the dietary taurine content. Taurine content was shown to decrease almost four times in liver of Japanese flounder fed diets devoided of taurine during six weeks (Kim et al., 2007) and our own results (unpublished data, C. Aragão) showed a decrease of 2.5 times in liver taurine content of Senegalese sole fed during four weeks with the PP85 diet. After these two weeks of PP85 feeding, fish were fasted during 24 hours and transferred to the nutrient flux laboratory to perform the first metabolic trial. This first metabolic trial assessed the effect of dietary taurine content on taurine absorption and retention by Senegalese sole juveniles, as described in section 2.3.

After the first metabolic trial, the remaining fish in the tank (those not used for metabolic trial 1) were randomly distributed into seven tanks (maintaining the same density and environmental conditions), fed during two weeks with PP85 diet (as in metabolic trial 1), but then received one of the seven experimental diets during 5 days (conditioning period). This conditioning period was chosen to avoid possible disruptions in the metabolic flux of taurine, as suggested from

results of metabolic trial 1. Following this conditioning period on the experimental diets, fish of all treatments were fasted during 24 hours before the second and third metabolic trial. In the second metabolic trial, our goal was to assess the effect of dietary taurine content on dietary triacylglycerol absorption and subsequent metabolic utilisation. Radiolabelled triolein was used as triacylglycerol tracer. Finally, in the third metabolic trial, we aimed at studying the influence of dietary taurine content on dietary amino acid metabolic utilisation.

2.3. Metabolic trials with radiolabelled nutrients

The *in vivo* method of tube-feeding described by Costas (2011), which in turn was an up scaling of the method firstly described by Rust et al. (1993) and modified by Rønnestad et al. (2001) was used to perform the metabolic trials.

For each of the three metabolic trials, tube-feeding was performed on anaesthetised fish (2-phenoxyethanol, 300 ppm, Sigma, Spain) by inserting a flexible silicone tube with the labelled feed pellets in the fish mouth. Feed pellets were then gently pushed into the oesophagus. The quantity of feed pellets administered to each fish corresponded to a 0.4% body weight ration. Five fish were tube-fed with each experimental feed (FM, PP85, TAU1, TAU2, TAU3, TAU4 and TAU5) tested (n=5), being the seven experimental diets labelled with [1,2-^{14}C-]-taurine in the first metabolic trial (American Radiolabeled Chemicals Inc., The Netherlands), with [U-^{14}C]-triolein (Perkin Elmer, USA) in the second metabolic trial and with [U-^{14}C]-L-amino acid mixture (15 amino acids; American Radiolabeled Chemicals Inc.) in the third metabolic trial. For labelling the feed, each tracer was diluted in an appropriate volume of ethanol (70%) and a known volume of diluted tracer was deposed with a micropipette on individual pellets. The pellets were then dried at 50ºC for 30 minutes and reserved for subsequent tube-feeding. Pellets presented a mean value of disintegrations per minute (DPM) of 207 455 DPM for ^{14}C-taurine, 220 587 DPM for ^{14}C-triolein, and 300 093 DPM for ^{14}C-amino acid mixture. In the second and third metabolic trials, for each group of five fish, the labelled feed used for the tube-feeding experiment was the same as the one received in the previous conditioning period. It should be noted that all the metabolic trials were aimed at testing the effect of the dietary taurine content (provided by the feed) on the metabolic fate of the several nutrients, used in the radiolabelled form as a tracer.

After being tube-fed, each fish was allowed to recover from anaesthesia in clean seawater with aeration and was then transferred into individual incubation chambers containing 2 L of clean seawater. Each chamber was hermetically closed, supplied with a gentle oxygen flow and connected to CO_2 traps (three in series, each containing 10 ml of 0.5 M KOH), in order to collect $^{14}CO_2$ produced

by the fish from catabolism of ^{14}C-labelled nutrient. After 24 hours of incubation, oxygen flow was stopped and fish were sacrificed inside the chambers by a lethal dose of anaesthetic (ethyl 3-aminobenzoate - MS-222, Sigma). Fish were then taken for sampling.

After fish removal, incubation chambers were immediately resealed and hydrochloric acid (0.1 M HCl) was added in a series of gradual steps, resulting in a progressive decrease of pH that caused the rapid diffusion of any remaining $^{14}CO_2$ from the water into the CO_2 traps (based on the method of Rønnestad et al., 2001 and further adapted by Costas, 2011). Samples of the CO_2 traps as well as of the water from incubation chambers (considered to contain ^{14}C resulting from fish evacuation) were collected for radioactive counting. Concerning fish, they were individually weighed and from each fish, dorsal and ventral muscles (without removing the skin part) and liver were collected and weighed. Gallbladder was only sampled at the metabolic trial 1, since taurine was the only radiolabelled nutrient that could be used for bile production. Samples of the dissected tissues were taken, weighed and completely dissolved in an appropriate volume of Solvable (Perkin Elmer) at 50°C for 24 hours. For each ^{14}C-tracer and dietary treatment, samples from incubation seawater, CO_2 traps, liver, muscle, and gallbladder fractions were added Ultima Gold XR scintillation cocktail (Perkin Elmer) and DPM were counted in a Tri-Carb 2910TR low activity liquid scintillation analyser (Perkin Elmer).

2.4. Data analysis

All samples were corrected for quench and lumex. Nutrient utilization as a function of dietary taurine content was expressed as nutrient percentage evacuated, catabolised or retained in the different tissues, estimated as follows (based on Canada et al., 2016):

$$\text{Evacuation (\%)} = (R_{SW}) / (R_{SW} + R_{Trap} + \Sigma R_{Tissue}) \times 100;$$

$$\text{Catabolism (\%)} = (R_{Trap}) / (R_{Trap} + \Sigma R_{Tissue}) \times 100;$$

$$\text{Retention}_{Tissue}\ (\%) = (R_{Tissue}) / (R_{Trap} + \Sigma R_{Tissue}) \times 100;$$

where R_{SW}, R_{Trap}, R_{Tissue} are the total radioactivity contents (dpm) found in incubation seawater, CO_2 trap, and each tissue (liver, muscle or gallbladder; ΣR_{Tissue} is the sum of the different tissues).

Results are presented as mean and standard deviation. Results expressed as percentages were transformed (arcsin square root) before statistical analysis, according to Ennos (2007). Data were checked for normal distribution and homogeneity of variances. Significant differences among groups were assessed by one-way ANOVA. When significant differences were detected, the Tukey's multiple-comparison test was used to assess differences among groups. Differences were considered

to be significant when $p < 0.05$. Statistical analyses were performed using the STATISTICA version 8.0 software (StatSoft Inc., 2007).

3. Results

3.1. Assessment of taurine retention depending on dietary taurine content in sole juveniles fed on a plant protein-based diet

The proportions of tube-fed ^{14}C-taurine that were absorbed (found in the liver, muscle and gallbladder) or evacuated (undigested tracer evacuated to the water) by the fish depending on the experimental labelled feed used (FM, PP85, TAU1, TAU2, TAU3, TAU4 or TAU5), are presented in Fig. 1. Absorption of ^{14}C-taurine ranged between 58.6 and 71.2% (in average) of label and did not differ significantly between the different dietary treatments.

Repartition of the absorbed ^{14}C-taurine between the liver, muscle and gallbladder of the fish after 24 hours of incubation in the chambers and depending on the different tube-fed diets, is shown in Fig. 2. As already mentioned, taurine is not catabolised by Vertebrates, therefore CO_2 traps were not taken into consideration in this metabolic trial. Among the three body compartments analysed, ^{14}C-taurine was mainly found in the liver for the fish tube-fed with all the diets tested except PP85 (56.1-67.4% of absorbed ^{14}C-taurine in average) and gallbladder contained about 11.4-23.6% of absorbed ^{14}C-taurine (in average). For the fish tube-fed with PP85 diet, the distribution profile of ^{14}C-taurine was different, with 35.8% of ^{14}C-taurine found in the liver and 42.8% of ^{14}C-taurine found in the gallbladder. Proportion of ^{14}C-taurine found in gallbladder was significantly higher in fish tube-fed with PP85 compared to fish tube-fed with FM, TAU2, TAU3, TAU4 and TAU5 diets and the gallbladder of fish tube-fed with TAU1 contained an intermediary level of ^{14}C-taurine. In liver, on the contrary, a significantly lower proportion of ^{14}C-taurine was found in fish tube-fed with PP85 diet compared to fish tube-fed with TAU2, TAU4 and TAU5 diets, and liver of fish tube-fed with FM, TAU1 and TAU3 diets contained an intermediary proportion of ^{14}C-taurine. Muscle of the fish contained between 14.9 and 22.8% of ^{14}C-taurine in average and this proportion was not affected by the feed tube-fed.

3.2. Effect of taurine content of a plant protein-based diet on dietary triolein absorption and subsequent metabolic utilisation

Differences were observed in the absorption of ^{14}C-triolein depending on the diet the fish received, as displayed by Fig. 3. ^{14}C-triolein absorption was lower in fish fed PP85 and TAU1 diets

(38.2% and 42.5% of tube-fed tracer, respectively) compared to fish fed the other diets (averages ranging from 59.3% to 74.6% of tube-fed tracer) but the differences were statistically significant only when compared to fish fed FM diet.

Fig. 4 presents the proportion of absorbed ^{14}C-triolein found in liver, muscle and CO_2 trap. Catabolism of absorbed ^{14}C-triolein tended to increase with increasing amount of taurine supplemented to the plant protein-based diet (average of catabolised ^{14}C-triolein in TAU1 group was 13.1%, 16.8% in TAU2 group, 17.7% in TAU3 group, 27.9% in TAU4 group and 31.4% in TAU5 group) but no statistically significant differences were observed among the dietary treatments, due to high inter-individual variabilities. Proportions of ^{14}C-triolein retained in the liver and the muscle of the fish were highly variable between the groups, ranging from 21.3 ± 9.5% (TAU4 group) to 39.9 ± 10.4% (TAU3 group) for the liver and from 39.2 ± 15.5% (TAU5 group) to 61.6 ± 15.6% (TAU1 group) for the muscle, and they did not differ significantly between the groups due to the high variability between individuals for both of the tissues.

3.3. Effect of taurine content of a plant protein-based diet on dietary amino acid absorption and subsequent metabolic utilisation

After five days of metabolic conditioning on the different experimental diets, the absorption of the tube-fed ^{14}C-amino acid mixture was similar between the various treatments, ranging from 66.2 to 74.6% of tube-fed ^{14}C-tracer in average, as shown in Fig. 5.

Concerning the repartition of the absorbed ^{14}C-amino acid mixture between the CO_2 trap (catabolised portion) and the different body tissues analysed (liver and muscle) depending on the dietary treatment received, results are reported in Fig. 6. Amino acid tracer was mostly retrieved in the liver of the fish (from 39.3 to 63.7% of absorbed tracer in average) and the proportion of tracer measured in this tissue was significantly lower in fish from TAU5 group compared to those from FM group while the values were intermediary in fish for the five other groups. The proportion of tube-fed ^{14}C-amino acid mixture measured in the muscle of the fish ranged from 15.0 to 28.8% of absorbed tracer in average and it was significantly higher in fish fed TAU5 diet compared with fish fed PP85 and TAU3 diets. The proportions were intermediary for fish fed the four other diets.

4. Discussion

4.1. Assessment of taurine retention depending on dietary taurine content in sole juveniles fed on a plant protein-based diet

The metabolic trial performed with radiolabelled taurine indicated that intestinal taurine absorption is not affected by the taurine content of a meal in sole juveniles. However, depending on the taurine content of the tube-fed diet, the absorbed radiolabelled taurine was distributed in different proportions between the liver and the gallbladder of the fish, indicating a different utilisation. In sole receiving a meal containing more than 2.3g kg^{-1} of taurine (feed TAU1, TAU2, FM, TAU3, TAU4, TAU5) radiolabelled taurine was mainly directed to the liver suggesting that liver, besides its important involvement in taurine metabolism, might also play an immediate buffering role when a higher taurine load is supplied. Liver was reported to be one of the richest taurine content tissues, together with the brain (the other measured tissues usually being eyes and muscle) in several flatfish species like turbot juveniles and post-larvae as well as in Japanese flounder juveniles fed with a low-taurine diet (Kim et al., 2008a; Qi et al., 2012). It was shown that when increasing dietary taurine content of numerous species like turbot (Qi et al., 2012), Japanese flounder (Kim et al., 2008a,b; Park et al., 2002), rainbow trout (Yokoyama and Nakazoe, 1992) or cobia (Watson et al., 2014), tissue taurine content increases in liver, brain, muscle and eye but accumulates in bigger proportions in muscle compared to the other tissues. However, in other species like carp, taurine proportionally accumulates more in liver than in muscle, brain or eyes when increasing taurine content of their diet (Kim et al., 2008b). No information is yet available regarding taurine distribution among Senegalese sole tissues. However, in the present study, radiolabelled taurine of a single meal was retrieved in much smaller proportion in muscle tissue (mean values ranging from 14.9 to 22.8% of absorbed tracer) compared to liver (mean values ranging from 35.6 to 67.4% of absorbed tracer), 24 hours after the meal. Altogether, these results suggest a different tissue distribution and use of taurine according to fish species and even with fish age. In the case of Senegalese sole, more investigations are needed to bring information regarding taurine distribution among body tissues as well as their response depending on dietary taurine supply, since this study was a short-term trial and not a growth trial as the other studies cited.

Compared to all the other groups, in sole fed with the PP85 diet, which was the poorest in taurine (0.9 g kg^{-1} taurine), radiolabelled taurine was preferentially retained in the gallbladder and less allocated to the liver, highlighting the importance of taurine in bile salt metabolism. In vertebrates, bile acids are synthesised from cholesterol in the liver and are conjugated with taurine or glycine before their storage in the gallbladder or secretion in the gut (Hofmann et al., 2010). However, in Senegalese sole, like in other teleost species, bile acids are conjugated to taurine (Velez et al., 2009), implying that taurine has a paramount role in bile salt synthesis in these animals. Our results suggest that when sole juveniles are fed on a plant-based diet poor in taurine (like PP85 diet),

dietary taurine is preferentially used for bile salt synthesis. It is known that plant ingredients, like soy protein and wheat gluten used in PP85 diet, contain proteins and other components that have the ability to bind bile salts in the intestinal lumen (Kahlon and Woodruff, 2002; Omoni and Aluko, 2005). Bile acid enterohepatic circulation is therefore affected since the fraction of bile salts bound to those plant proteins are then excreted instead of being absorbed and recycled. This might be an explanation for the higher allocation of dietary taurine to the gallbladder in sole fed the PP85 diet whose poor content in taurine would be used in priority for conjugation with neosynthesised bile acids. Proportion of radiolabelled taurine allocated to the gallbladder of sole then decreased with increasing taurine amount supplied by the meal and remained similar for diets supplying above 3.3g kg^{-1} of taurine (diets TAU2, TAU3, TAU4, TAU5). This realocation of taurine to the gallbladder suggest a possible disruption in bile salt synthesis due to insuficient taurine intake.

4.2. Effect of taurine content of a plant protein-based diet on dietary triolein absorption and subsequent metabolic utilisation

Since the results of the taurine metabolic trial suggest a possible disruption in bile salt synthesis when sole was fed with a plant-based and low taurine content diet, we next decided to investigate if there was an impact on dietary lipid absorption and metabolism. Indeed, the main role of bile salts is to promote dietary lipids emulsification in the intestine lumen, by facilitating micelles formation through their amphipathic characteristics. The participation of bile salts in the intestinal digestion process thus greatly enhances the digestion of dietary lipids and also their absorption by the intestinal brush border membrane.

The metabolic trial performed with radiolabelled triolein evidenced that absorption capacity of dietary triacylglycerols fell down when substituting dietary proteins from marine origin by plants proteins (about 75% of ^{14}C-triolein was absorbed by fish fed FM diet compared to 38% of ^{14}C-triolein absorbed in fish fed PP85 diet) in sole juveniles. This was probably related with a lower bile salt availability for lipid digestion and absorption, due to the low dietary taurine content, and maybe to some extent to the bile salt binding ability of plant proteins as already referred upper (Kahlon and Woodruff, 2002; Kortner et al., 2013). Triacylglycerol absorption was not improved in the plant-based diet by a taurine supplementation of 2.3 g kg^{-1} feed, but when increasing dietary taurine content of the plant-based dietary formulation above 3.3 g kg^{-1} feed (diet TAU2) (and until 15.2 g kg^{-1} feed, diet TAU5 – Fig.3), triacylglycerol absorption capacity of sole was enhanced and reached a level that was not significantly different from the one measured in fish fed the FM diet. Besides enhancing lipid emulsification in the intestinal lumen as previously mentioned, bile salts also promote dietary

lipid digestion and absorption through their ability to stimulate the activity of some lipases (Wang et al., 1997). In common dentex, Chatzifotis et al. (2008) reported a considerable enhancement of bile salt activated lipase activity in pyloric caeca and liver of juveniles when supplementing soybean meal-containing dietary formulations with taurine. Likewise, lipase activity measured in the intestinal digesta of yellowtail fed several soybean meal-based formulations was increased when supplementing the diets with taurine (Nguyen et al., 2013). The improved triolein absorption capacity observed in sole fed TAU2, TAU3, TAU4, TAU5 diets compared to the fish fed PP85 and TAU1 diets might be at least ascribed to higher availability of bile salts for dietary lipid emulsification and activation of lipid hydrolysis in the lumen of the gut.

Concerning the utilisation of the absorbed fraction of labelled triolein, we did not observe any significant differences in the proportions of triolein that were catabolised, retained in the liver or retained in the muscle of the fish, depending on the dietary treatments, maybe because of the high inter-individual variability. Based on the results, there is a possibility that if the number of fish used were increased, increasing the statistical power, we might find some clear tendency. However, we should keep in mind that these metabolic trials were short-term trials with fish being conditioned to the diets during five days. Thus, although the beneficial effect of taurine supplementation was almost immediate on sole lipid digestion and absorption, five days might not be sufficient to observe any effects on their subsequent metabolic utilisation. Longer-term studies are needed to conclude on the impact of taurine supplementation on lipid metabolism.

4.3. Effect of taurine content of a plant protein-based diet on dietary amino acid absorption and subsequent metabolic utilisation

Numerous studies evidenced that supplementing plant protein-based diets with taurine enable a compensation of the negative impact induced by the substitution of dietary marine ingredients on growth performances of marine fish species (e.g. Al-Feki et al., 2016; Bañuelos-Vargas et al., 2014; Chatzifotis et al., 2008; Li et al., 2016; Takagi et al., 2011). These studies generally pointed out an enhancement of protein efficiency. Some authors hypothesised that the increased protein efficiency obtained when supplementing a soy-based diet with taurine could be due to a potential alleviation of intestinal mucosal damage (that can be induced by soy ingredients) resulting in an improvement of intestinal nutrient absorption (Bañuelos-Vargas et al., 2014). In the present study, results of the amino acid mixture metabolic trial showed that taurine content of the feed supplied to Senegalese sole juveniles did not have any direct and significant impact on intestinal

amino acid absorption (at least up to 15.2g kg^{-1} feed – Fig.5). However, has already stated for lipids, these were short-term trials and long-term studies are needed to confirm the absence of effects.

In our study, catabolism of dietary amino acids in sole juveniles did not differ significantly depending on the taurine content of the plant-based diet and was not impaired by the incorporation of plant proteins at the expense of dietary marine proteins. These results are not in accordance to what was observed in the study of Bañuelos-Vargas et al. (2014) carried out in totoaba juveniles. In this study, supplementing a plant-based dietary formulation with taurine enabled the restoration of several hepatic enzyme activities involved in amino acid catabolism (and also in gluconeogenesis) to levels similar of those measured in totoaba fed a fishmeal-based control diet (Bañuelos-Vargas et al., 2014). The fact that taurine did not affect amino acid catabolism in our experiment can be due to metabolic differences between species or can be related with the duration of the experiments since ours was a short-term metabolic trial whose primary goal was to assess immediate effects of taurine supplementation.

Concerning the distribution of labelled amino acids between sole tissues, even after only five days of feeding on the different diets, amino acids were retained in bigger proportion in the muscle fraction of sole fed the diet with the highest taurine content, instead of being directed towards the liver. These results suggest that taurine supplementation might favour muscle protein accretion in sole juveniles. A metabolic trial conducted in Senegalese sole larvae reported an increased amino acid retention in the body of larvae fed with taurine supplemented microcapsules when a concomitant increase in taurine body content was observed (Pinto et al., 2010). Taurine is an amino acid that cannot be used for protein synthesis nor oxidised for energy production. However, taurine is known to be involved in so many physiological processes that a higher dietary supply in taurine might enable the sparing of amino acids sharing similar role(s) and render them available in bigger quantities for protein accretion or energy production. For example, Salze and Davis (2015) suggested that a dietary supply in taurine could induce a spare of amino acids involved in cellular osmoregulation, such as glycine and arginine. Indeed the increase in muscle amino acid retention observed in our study, may suggest that some amino acids would probably be spared due to the higher dietary taurine supply and were used for protein synthesis. Together, these data suggest that when supplied in sufficient amount, dietary taurine might enable an enhancement of muscular protein deposition and muscular tissue accretion in sole. In other species, like turbot and cobia, higher carcass protein content and higher muscle ratio were respectively reported in fish fed diets with increasing taurine content (Lunger et al., 2007; Qi et al., 2012) also indicating an enhancement of muscular protein accretion. A longer-term trial with sole juveniles is needed to see if the trend we observed in the short-term of the present study is confirmed.

5. Conclusion

In conclusion, results of the present study pointed out the importance of taurine supplementation when sole juveniles are fed a plant-based dietary formulation. We showed that feeding sole juveniles with a plant-based dietary formulation affects taurine metabolism such that a higher proportion of dietary taurine is allocated to the gallblader and probably used for bile salts synthesis. Consistent with this, those fish also displayed a poorer triacylglycerol digestion capacity, which was improved with increasing dietary taurine supplementation. These short-term experiments did not reveal any significant effect of taurine supplementation on the metabolic utilisation of dietary triacylglycerol but a higher retention of dietary amino acids was observed in muscle for fish fed the plant-based diet containing the highest level of taurine. Globally, the results obtained with both the taurine and the triolein metabolic trials seem to indicate that when fed on a plant-based dietary formulation, sole juveniles probably benefit from a supply of at least 3.3 g of taurine per kilogram of feed, in order to avoid disruption of bile salt synthesis and maintain lipid digestion and absorption capacity similar to those obtained with a marine-based dietary formulation.

The results from these short-term trials can provide a fast-screening of requirements within a short-time and using a limited number of fish. Although some indications on the dietary needs for taurine in Senegalese sole species can be obtained from this work, future work is needed to establish the taurine requirement for this species. The conduction of longer-term growth trials are now required is this endeavour and to verify the effect of dietary taurine supplementation on fish growth, as well as to assess more precisely the potentially beneficial effects on protein and lipid metabolism suggested here.

Acknowledgements

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The authors declare no conflict of interest.

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Fig. 1

Proportions of the tube-fed ^{14}C-taurine that were absorbed (opened square) or evacuated (undigested fraction found in water compartment - shaded square) by Senegalese sole juveniles tube-fed experimental diets with different taurine levels. Values are means ± standard deviations (n = 5). Mean values were not significantly different among treatments (ANOVA, $p > 0.05$).

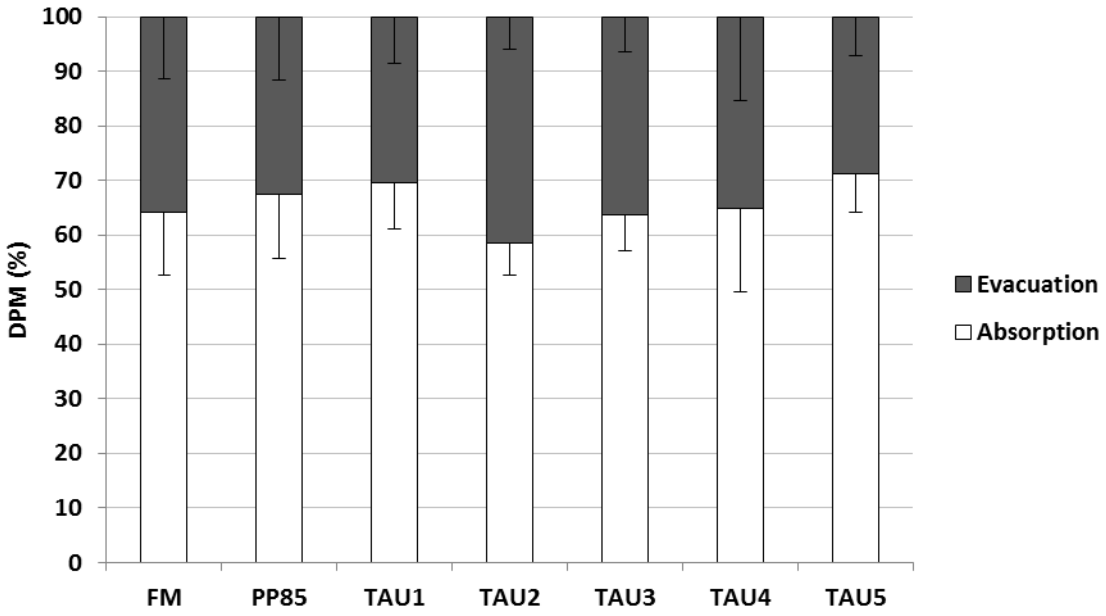

Fig. 2

Proportions of the absorbed (sum of fractions found in fish tissues) tube-fed ^{14}C-taurine that were retained in the liver, muscle or gallbladder of Senegalese sole juveniles tube-fed experimental diets with different taurine levels. Values are means ± standard deviations (n = 5). Statistically significant differences among treatments, for a selected tissue, are indicated by different letters (Tukey test, $p < 0.05$).

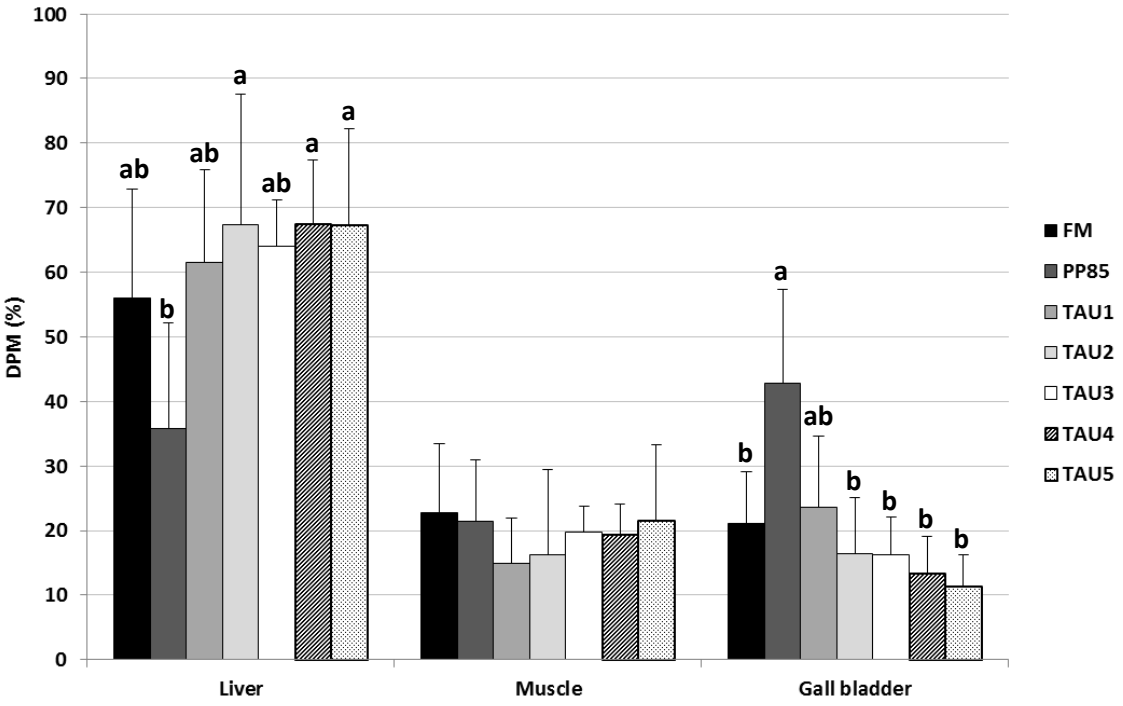

Fig. 3

Proportions of the tube-fed ^{14}C-triolein that were absorbed (opened square) or evacuated (undigested fraction found in water compartment - shaded square) by Senegalese sole juveniles fed experimental diets with different taurine levels. Values are means ± standard deviations (n = 5). Statistically significant differences among treatments, for a selected compartment, are indicated by different letters (Tukey test, $p < 0.05$).

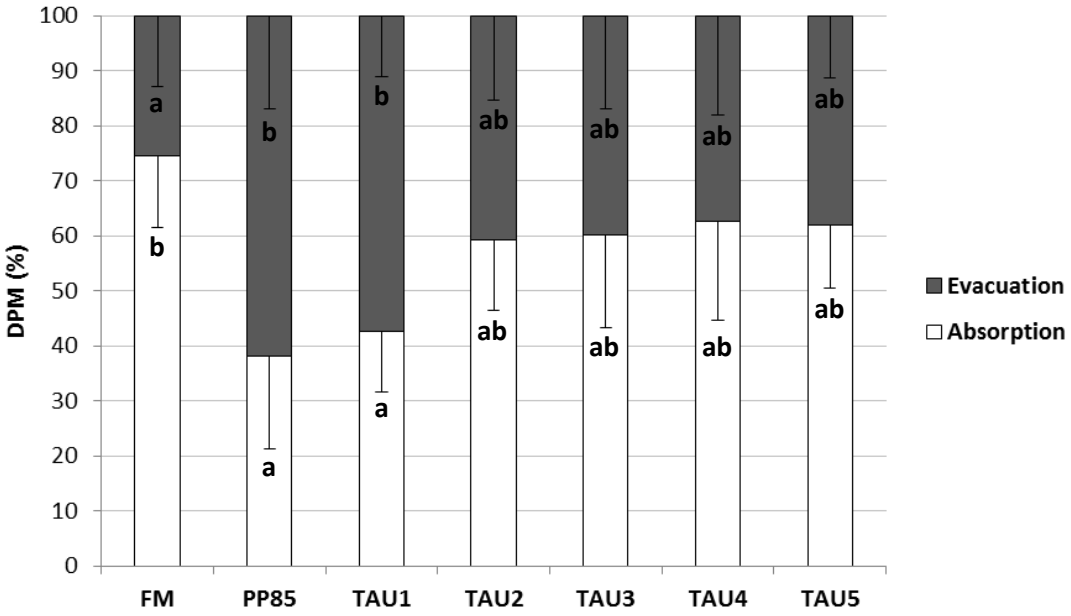

Fig. 4

Proportions of the absorbed (sum of fractions found in CO_2 trap and fish tissues) tube-fed ^{14}C-triolein that were catabolised, retained in the liver or in the muscle of Senegalese sole juveniles fed experimental diets with different taurine levels. Values are means ± standard deviations (n = 5). Mean values were not significantly different among treatments (ANOVA, $p > 0.05$).

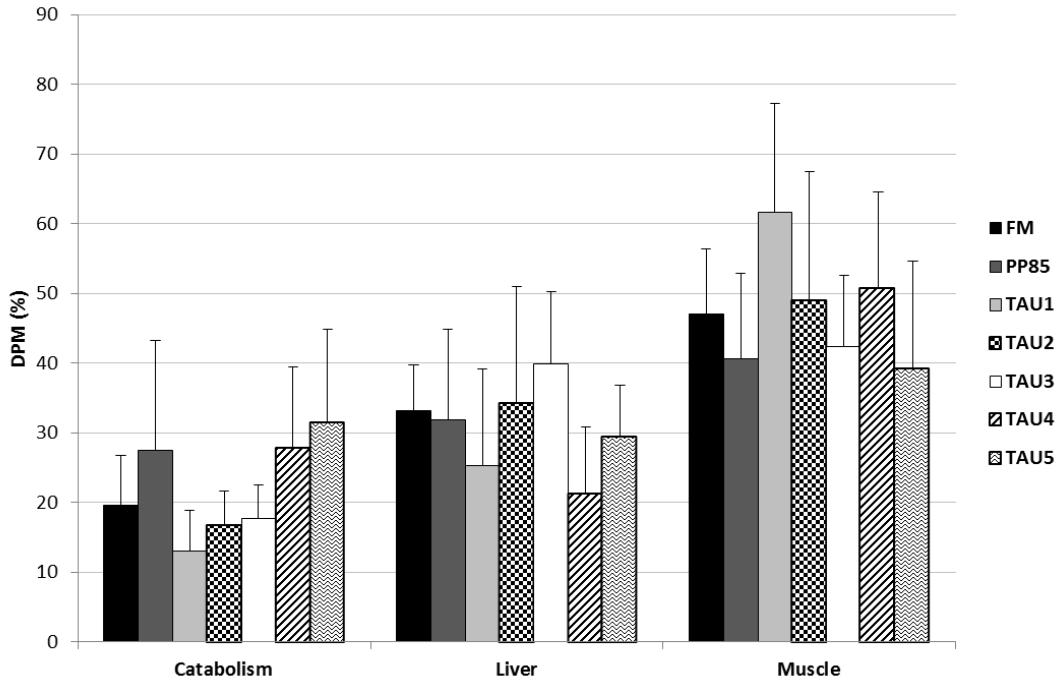

Fig. 5

Proportions of the tube-fed ^{14}C-amino acid mixture that were absorbed (opened square) or evacuated (undigested fraction found in water compartment - shaded square) by Senegalese sole juveniles fed experimental diets with different taurine levels. Values are means ± standard deviations (n = 5). Mean values were not significantly different among treatments (ANOVA, $p > 0.05$).

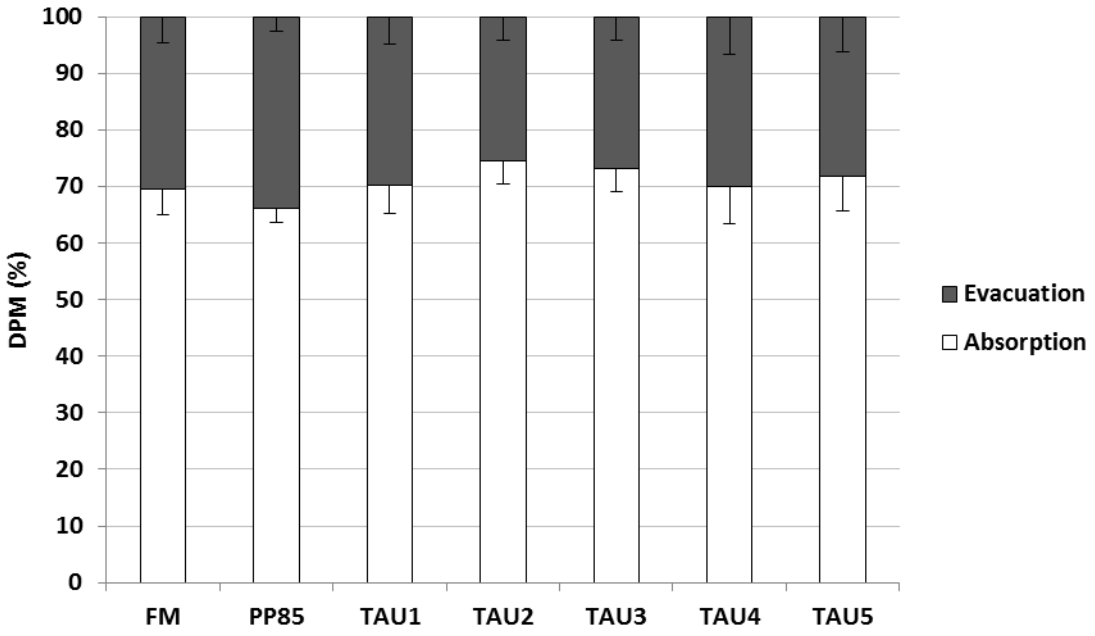

Fig. 6

Proportions of the absorbed (sum of fractions found in CO_2 trap and fish tissues) tube-fed ^{14}C-amino acid mixture that were catabolised, retained in the liver or in the muscle of Senegalese sole juveniles fed experimental diets with different taurine levels. Values are means ± standard deviations (n = 5). Statistically significant differences among treatments, for a selected tissue, are indicated by different letters (Tukey test, $p < 0.05$).

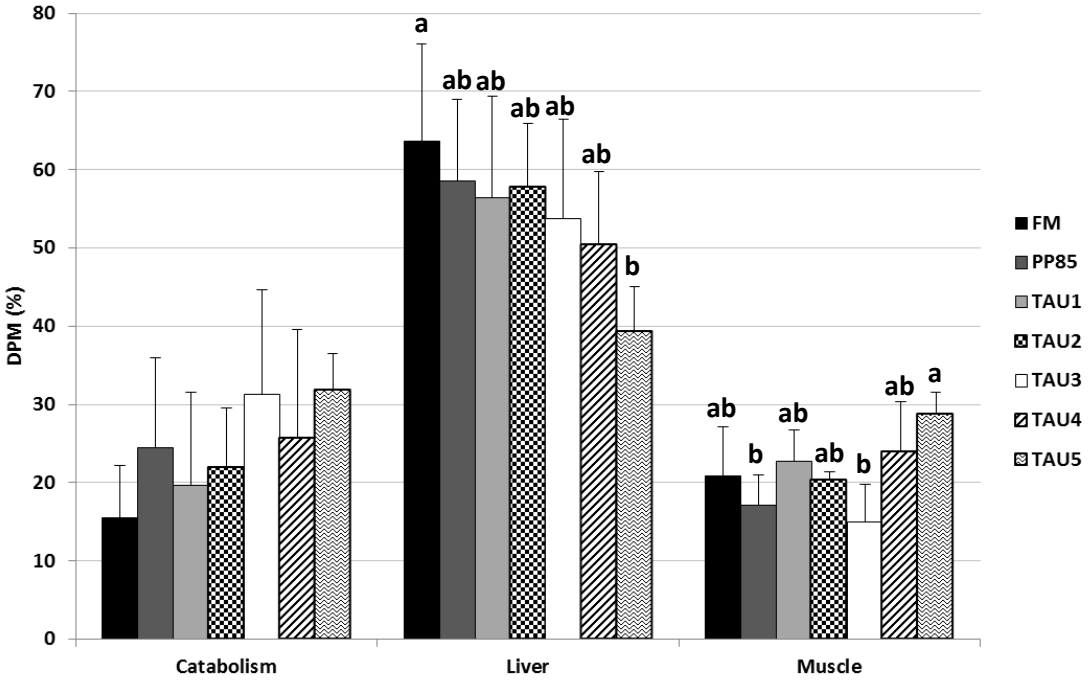

Table 1. Formulation and proximate composition of the experimental diets.

Ingredients (%)	**FM**	**PP85**	**TAU1**	**TAU2**	**TAU3**	**TAU4**	**TAU5**
Fishmeal 70 LT[a]	37	3	3	3	3	3	3
Fishmeal 60[b]	12.5	0	0	0	0	0	0
Fish solubles protein concentrate[c]	7.5	3	3	3	3	3	3
Squid meal[d]	7.5	3	3	3	3	3	3
Pea protein concentrate[e]	0	17	17	17	17	17	17
Soy protein concentrate[f]	0	5	5	5	5	5	5
Soybean meal[g]	10	10	10	10	10	10	10
Potato protein concentrate[h]	0	5	5	5	5	5	5
Wheat gluten[i]	0	17	17	17	17	17	17
Corn gluten meal[j]	0	8	8	8	8	8	8
Dehulled pea grits[k]	10	6.5	6.5	6.5	6.5	6.5	6.5
Whole wheat[l]	10.5	8.2	8.0	7.9	7.8	7.5	6.7
Fish oil[m]	2	6.6	6.6	6.6	6.6	6.6	6.6
Vitamin & Mineral Premix[n]	1	1	1	1	1	1	1
Di-calcium phosphate[o]	0	4	4	4	4	4	4
L-Lysine[p]	0	0.5	0.5	0.5	0.5	0.5	0.5
DL-Methionine[q]	0	0.2	0.2	0.2	0.2	0.2	0.2
Guar gum[r]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Diatomaceous earth[s]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Taurine[t]	0	0	0.2	0.3	0.4	0.7	1.5
Proximate composition							
Dry matter, DM (%)	94.11	93.84	94.17	94.00	93.80	93.80	93.80
Crude protein (% DM)	58.21	58.16	58.25	58.21	58.22	58.17	58.17
Crude fat (% DM)	8.73	8.71	8.72	8.73	8.74	8.74	8.74
Ash (% DM)	8.60	7.16	7.17	7.18	7.14	7.14	7.14
Gross energy (kJ·g^{-1} DM)	20.79	20.77	20.77	20.78	20.78	20.78	20.79
Taurine (% DM)	0.44	0.09	0.23	0.33	0.42	0.72	1.52

[a] Peruvian fishmeal LT, EXALMAR, Peru.

[b] Fair average quality (FAQ) fishmeal, COFACO, Portugal.

[c] CPSP 90, Sopropêche, France.

[d] Super prime without guts, Sopropêche, Spain.

[e] NUTRALYS F85F, ROQUETTE, France.

[f] Soycomil P, ADM, The Netherlands.

[g] Micronized soybean meal, SORGAL SA, Portugal.

[h] Potato protein concentrate, AgroKorn, Denmark.

[i] VITAL, ROQUETTE, France.

[j] GLUTALYS, ROQUETTE, France.

[k] Aquatex G2000, SOTEXPRO, France.

[l] Whole wheat, Casa Lanchinha, Portugal.

[m] Marine oil omega 3, Henry Lamotte Oils GmbH, Germany.

[n] PV040.01 Premix for marine fish, PREMIX Lda., Portugal.

[o] Di-calcium phosphate, Fosfitalia, Italy.

[p] L-Lysine HCl 99%, Ajinomoto Eurolysine SAS, France.

[q] DL-Methionine 99%, Evonik Degussa GmbH, Germany.

[r] Guar gum HV109, SEAH International, France.

[s] Kielseguhr, LIGRANA GmbH, Germany.

[t] L-Taurine 98.5%, Ajinomoto Eurolysine SAS, France.

Statement of relevance in the general field of aquaculture (limited to 60 characters)

Adding taurine to a plant-based diet is beneficial for sole

Highlights

- Taurine supplementation to a plant-based diet is beneficial to sole performance.
- Feeding plant-based diets affect taurine flux in sole.
- Adding taurine to a plant-based diet improved triacylglycerol digestion in sole.
- Amino acid retention was enhanced in sole muscle by dietary taurine supplementation.