Taurine supplementation in high-soy diets affects fillet quality of European sea bass (*Dicentrarchus labrax*)

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ABSTRACT

This study evaluated the effects of taurine supplementation to diets containing a high dietary inclusion of soybean meal and soy protein concentrate on growth performance and fillet quality of juvenile European sea bass (*Dicentrarchus labrax*). A control diet (C+) was produced containing high levels of fishmeal (30% FM) and soybean meal (20% SBM). Three other experimental diets were prepared to contain a lower FM inclusion (25%), and a higher amount of soy products (20% of SBM plus 12% soy protein concentrate, SPC) supplemented with three graded levels of crystalline taurine, 0.2%, 0.5% and 1.0% (T0.2, T0.5 and T1.0), respectively. A fifth diet was also prepared having a similar composition as the latter three diets but without the addition of crystalline taurine (negative control diet, C-). All diets were iso-nitrogenous (44%), iso-lipidic (20%) and iso-energetic (22 MJ kg^{-1}) and were fed to five triplicate groups of sea bass (initial weight 86 g) over the course of a 12-week trial. Dietary taurine supplementation did not affect the growth performance, and feed efficiency ($P > 0.05$). Proximate composition of whole body and muscle were similar among groups ($P > 0.05$). Taurine dietary supplementation had no effect on the level of intraperitoneal fat deposition ($P > 0.05$). However, muscle taurine concentration was found to increase gradually in sea bass fed the elevated levels of taurine ($P < 0.05$). Interestingly, the hardness and chewiness of the fillet, recorded by texture analysis, increased significantly at higher dietary taurine levels ($P < 0.05$). The highest adhesiveness values were obtained in sea bass fed the C- diet, whereas the lowest ones were found in fish fed the T1.0 diet ($P < 0.05$). No significant ($P > 0.05$) impact of diets on texture fillet springiness and cohesiveness was found ($P > 0.05$). In general, no significant differences were observed by the test panel, however, fish fed the diet supplemented with 1.0% taurine exhibited lower fillet elasticity,

thus indicating a potential textural difference in accordance with those obtained from the texture analysis of fish muscles. The histological analysis did not indicate any differences in the gut and liver of the fish fed the experimental diets. Overall, the findings of the present study showed that 1.0% taurine supplementation in diets incorporating high levels of soy products might have a pronounced effect on flesh quality of European sea bass.

Keywords: European sea bass, fishmeal substitution, soy, taurine, fillet quality, test panel.

1. Introduction

Fishmeal (FM) has been the major protein source in aquafeeds during the last decades. However, because FM is a finite resource and an expensive ingredient, the development of feeds with significantly lower dietary FM levels has been acknowledged as the only way for the sustainable expansion of the aquaculture industry (Tacon and Metian, 2008; Shepherd and Jackson, 2013). The level of FM inclusion within compound diets for marine finfish has steadily declined during the last years due to the incorporation of plant proteins (PP) (Tacon and Metian, 2008) or microalgae and heterotrophic bacteria (Kousoulaki et al., 2015; García-Ortega et al., 2016). In search for alternative economically viable protein sources for aquafeeds, many plant raw materials have been tested, for example soybean meal, rapeseed meal, sunflower meal, lupin seed meal and pea seed meal, among others (Francis et al., 2001; Kousoulaki et al., 2015) with varying degree of success. In most cases, high inclusion of plant proteins showed reduced fish performances, which were attributed partly due to the presence of anti-nutritional substances in plant feedstuffs, and to the impaired nutrient digestibility and bioavailability (Francis et al., 2001; Krogdahl et al., 2010).

Among different plant proteins, soya derived plant proteins have been received most interest by the researchers due to their consistent nutritional composition, comparatively balanced amino acid profile, availability, and reasonable price (El-Sayed, 1999; Storebakken et al., 2000). However, the use of soy protein in feeds developed for carnivorous fish represents several challenges which include low methionine and cysteine content, lower protein digestibility, indigestible oligosaccharides, low phosphorus availability, anti-nutritional factors, poor palatability and undetectable levels of taurine (Francis et al., 2001). In these circumstances, the supplementation strategy, *i.e.* supplementation of different functional feed additives and limiting amino acids of soybean (e.g. methionine, lysine, taurine etc.), is one of the most effective approaches. This would help to overcome the challenges of using soybean meal in aquafeeds through improving palatability, digestibility and overall nutritional quality of soya-based diets (Floreto et al., 2000; Hossian and Koshio, 2017).

Taurine is a non-proteinaceous beta-amino acid and considered as essential for felines, rhesus monkeys and human infants and a conditionally indispensable amino acid for humans and nonhuman primates (Schuller-Levis & Park 2006). In certain fish species taurine is also considered an essential nutrient and it is synthesized from methionine via cysteine by a series of enzymatic reactions, a process that is considered as species- and developmental stage dependent (Yokoyama et al., 2001; Kim et al., 2003, 2005). Taurine is typically found in relatively high concentrations (0.5 – 1.0%) in FM and animal by-products (Zhao et al., 1998), but is almost non-existent in PP (Espe et al., 2008). Even when all essential amino acid requirements are met in plant-based diets for carnivorous fish, growth performance still is often reduced when compared to diets containing high FM levels (Gaylord et al., 2006).

Therefore, taurine supplementation may be required for plant-based diets and indeed, dietary taurine addition improves weight gain and feed efficiency in several fish species like olive flounder (*Paralichthys olivaceus*) (Park et al., 2002; Kim et al., 2005), rainbow trout (*Oncorhynchus mykiss*) (Gaylord et al., 2006), cobia (*Rachycentron canadum*) (Lunger et al., 2007), yellowtail (*Seriola quinqueradiata*) (Khaoian et al., 2014), totoaba (*Totoaba macdonaldi*) (Bañuelos-Vargas et al., 2014) and red snapper (*Lutjanus colorado*) (Hernandez et al., 2018). In addition, due to its amino acid structure, taurine possesses the ability to stimulate feeding in fish (Carr, 1982).

European sea bass (*Dicentrarchus labrax*, L.) is one of the main species produced in Mediterranean aquaculture. Recently, Martins et al. (2018) estimated the taurine requirement of sea bass juveniles fed on high PP based diets. Coutinho et al. (2017) found that taurine supplementation (1.0%) modulated both hepatic and intestinal antioxidant response but did not affect sea bass growth. The benefits of taurine inclusion in a high-FM diet have also been reported for European sea bass fry (Brotons-Martínez et al., 2004). However, there is still limited available data on the effects of taurine supplementation in moderate and low FM diets on growth performance during the on-growing phase, and especially on fillet quality in European sea bass. Thus, the present study aimed to evaluate the impact of dietary taurine supplementation in diets incorporating high levels of soy products on growth, feed intake, body and muscle composition and histology of intestine and liver of juvenile sea bass during the on-growing phase, as well as on the evaluation of fillet quality by means of texture and sensory analyses.

2. Materials and methods

2.1. Experimental diets, fish, rearing conditions and sampling

Five isoenergetic and isonitrogenous experimental diets were manufactured at the pilot production plant of BioMar AS in Brande (Denmark) by cooking extrusion. A control diet (C+) was formulated to contain 30% FM and 20% soybean meal (SBM), whereas other three experimental diets (T0.2, T0.5 and T1.0) contained 25% FM, and a higher amount of soy products (20% of SBM and 12% soybean protein concentrate, SPC) and three different levels of crystalline taurine (0.2, 0.5 and 1.0%). In addition, a fifth diet was formulated to have similar composition of the former three diets, but without any addition of crystalline taurine (negative control, C-). The ingredients and chemical composition of the diets are provided in Table 1. The proximate composition of the diets (Table 1) revealed no differences between the different experimental diets. The taurine values obtained by the amino acid analysis of the experimental feeds (Table 2) were in agreement with the theoretical / calculated values.

Juvenile European sea bass of an initial body weight of 86.0 ± 1.6 g (mean ± SD) were obtained from a commercial fish farm (Hellenic Fisheries, Marathon, Attica Greece), and transferred to the HCMR's facilities in Agios Kosmas, Athens (Greece). Fish were individually weighted to the nearest 0.1 g and distributed into 15 cylindro-conical tanks of 1000-L volume equipped with feed waste collectors, with 40 fish per tank, 3 tanks per diet, receiving flow-through sea water (salinity 35 ppt). In each tank, water was renewed at a rate of 400 L h^{-1} and aerated to over 75% oxygen saturation. Water temperature throughout the experimental period was 27.9 ± 1.5 ºC, while natural photoperiod was applied. After stocking, fish were fed a commercial diet and acclimated to experimental tanks for 2 weeks. At the end of this acclimatization period, five fish from the initial population were sampled at random, sacrificed using an overdose of anaesthetic (50 mg/kg, MS-222), pooled, minced,

140 freeze-dried and grounded to be analyzed for initial whole-body composition. Each
141 experimental diet was randomly assigned in triplicate groups. Fish were hand-fed to visual
142 apparent satiation, twice daily, at 09:00 and 15:00 h, six days a week. Uneaten feed was
143 collected, dried and weighted after each meal and the feed consumption was monitored daily.
144 The trial lasted for a period of 88 days.

145 At the end of the feeding trial, all fish were anaesthetized and weighed individually as
146 previously indicated. Ten fish were randomly sampled from each tank (30 fish per diet); the
147 fillets from five specimens were pooled and stored immediately at -80º C for taurine
148 analysis, while their livers and intestines were used for histological examination. In addition,
149 the perivisceral fat and liver weight were measured to the nearest 0.1 g. The remaining five
150 fish from each tank were pooled and analyzed for carcass composition. Three other fish from
151 each tank (nine fish per diet) were sampled for colour and texture analyses. Finally, six
152 equally sized fish from the diets C+, C- and T1.0 (the most likely diets to exhibit differences
153 among groups) were sampled and ice-killed for sensory analysis. All animal handling and
154 sampling procedures were conducted in accordance with the directive 2010/63/EU on the
155 protection of animals used for scientific purposes.

156

157 ***2.2 Calculations***

158 The following formulae were used to evaluate growth performance and feed efficiency
159 parameters:

160 Specific growth rate, (SGR) (%/d) = 100 × [(ln BWf – ln BWi)/days], where BWf and
161 BWi were the final and initial body weight of fish, respectively.

Total feed intake, (TFI) per fish = g DM feed/fish, where DM is the dry matter of the mean feed consumption per fish.

Relative feed intake, (FI) (%/d) = 100 x (TFI / BWi).

Daily growth index, DGI (%) = $(BWf^{1/3} - BWi^{1/3}) / days \times 100$.

Thermal growth coefficient, (TGC) = $(BWf^{1/3} - BWi^{1/3}) \times (\Sigma D^0)^{-1}$, where ΣD^0 is the thermal sum (days × average temperature, ºC).

Feed conversion ratio (FCR) = dry feed consumed (g) / body weight gain (g).

Protein efficiency ratio (PER) = body weight gain (g) / protein intake (g).

Lipidosomatic Index (LSI, %) = 100 x (visceral fat (g) / body weight (g)).

Hepatosomatic Index (HSI) = 100 x (liver weight (g) / body weight (g)).

2.3 Chemical analyses

Samples of diets, fish whole bodies and fillets from each dietary group were analysed for dry matter and ash according to AOAC (1995), for crude protein content by the Kjeldahl method (N × 6.25) and for crude fat using the Soxtec™ extraction (FOSS, 2050 automated analyser, Denmark) with petroleum ether. The Folch's procedure was used for determining the lipid content in the fillet. The energy content in diets was calculated as Energy (MJ/kg) = 23.6 × P + 39.5 × F + 17.3 × CH, where P, CH and F are the crude levels of proteins, fat and carbohydrates, respectively.

The amino acid composition of the diets and fillets was analyzed after acid hydrolysis (6N, 110 °C, 24 h) and derivatization by AccQ-Tag™ Ultra according to the amino acid analysis application solution (Waters Corporation, Milford, MA, USA). DL- Norvaline (Sigma) 2.5 mM was used as an internal standard. UPLC was performed on an Acquity

system (Waters Corporation) equipped with PDA detector and the detection wavelength was set at λ = 260 nm. The column used was a BEH C18 column (100mm × 2.1 mm i.d., 1.7μm) from Waters. The flow rate was 0.7 ml/min and the column temperature were kept at 55 ºC. Peak identification and integration were performed by the software Empower v.2.0 (Waters) using an Amino Acid Standard H (Pierce) as an external standard. All analyses were performed in duplicate. In case that the values between replicates did not meet the standardized acceptance criteria based on the mean and standard deviation (<5%), new duplicate analyses were performed according to established procedures.

2.4 Histological examination

At the end of the trial period, three fish per tank were collected and killed with an overdose of MS 222 as previously described. The liver and intestines were removed from fish and immediately fixed in neutralized formalin solution (10%) and processed according to the standard procedures described by Bancroft & Gamble (2007). The samples were embedded in paraffin, cut in thin sections (5 μm) and stained with haematoxylin and eosin. Examination for any pathological condition was performed using a light microscope at different magnifications.

2.5 Fillet quality evaluation

Fillet colour change was evaluated by means of the measurement of CIELAB values (L*: lightness, a*: redness and greenness, b*: yellowness and blueness) using a CR-Minolta Chromameter® (Minolta Co., Chuo-Ku, Osaka, Japan). The instrument was calibrated according to the CIE (Commission International de l' Eclairage) using a standard white

reference tile (calibration plate CR-200, L = 97.50, a = -0.31, b = -3.83). Each fish was filleted by hand and measurements were taken at two different points. All measurements were carried out on four different single specimens (fillets) (Tsironi et al., 2009).

Texture parameters were defined using a texture analyzer with a load cell of 5 kg (MODEL TA-XT2i, Stable Micro Systems, Godalming, Surrey, United Kingdom). A flat-ended cylinder of 20 mm diameter was selected to simulate the human finger. Constant penetration depth was applied on the fish flesh and penetration depth of 2.0 mm was selected as the maximum distance that could be applied without affecting the muscle structure by erupting and leaving a mark on the fish flesh. Double compression was applied to construct the Texture Profile Analysis (TPA) parameters of the fillets of two different specimens. The cylinder approached the sample at the speed of 0.5 mm/s and penetrated 2 mm into the fish flesh. Then, the force was reduced and the sample was allowed to rebound for 5 s. The cylinder was pressed on the sample a second time, force-distance curves were recorded and analyzed using the Texture Expert Exceed Application (Version 2.64, Stable Micro Systems Ltd) and texture analysis parameters (hardness, springiness, cohesiveness, adhesiveness and chewiness) were calculated (Sigurgisladottir et al., 1999).

A descriptive sensory analysis was conducted by 12 trained panellists (*BS ISO 13300-1:2006*). For this purpose, fish of equal sizes (200 g) from three different dietary treatments (C+, C- and T1.0) were manually filleted. Whole fillets from these fish were wrapped separately in aluminium foil and steam-cooked for 20 minutes. Each panellist received whole fillets (three-digit coded samples) from each group. Although emphasis was mainly given to textural characteristics of fish, colour, taste and flavour intensity were also considered. The textural characteristics that were evaluated were those described by Szczesniak (1998) for

231 solid foods. The scale for each attribute was 0-10 (increasing with intensity of organoleptic
232 attribute respectively).
233
234 ***2.6 Statistical analysis***
235 Tanks were considered as experimental units and fish represented the sample units. All data
236 from the individual observations were tested for normality and homogeneity of variance
237 prior to be subjected to one–way ANOVA using Kolmogorov-Smirnov and Levene's tests,
238 respectively. Tank means were used for comparisons. Significant differences between means
239 were determined by Tukey's test. All statistical tests were performed using the General
240 Linear Model (STATISTICA version 7.0). The results from organoleptic test panel were
241 statistically evaluated by non-parametric Kruskal –Wallis test. In all cases, the level of
242 significance was set at P = 0.05. Correlation between different variables (*i.e.* dietary *vs.* fillet
243 taurine levels, and dietary taurine levels vs. fillet texture parameters) were evaluated by
244 means of the Pearson product moment correlation test.
245
246 **3 Results**
247 ***3.1 Growth performance and feed efficiency parameters***
248 Survival rate was similar among groups with values ranging between 94.0 and 99.0% (Table
249 3, P > 0.05). Data on growth performance of sea bass fed the different experimental diets are
250 shown in Table 3. A *ca.* 2.5-fold increase in BW was found over the course of the 12-week
251 trial period. Fish fed the T1.0 diet showed a numerically higher average BWf compared to
252 the other groups, although the differences were not statistically significant (P > 0.05).
253 Similar trend was observed in WG, TGC and DGI for this group in comparison to the fish

fed the other diets ($P > 0.05$; Table 3). Similar values were found regarding the HSI and LSI between diets differing in their FM and taurine levels ($P > 0.05$; Table 3). Feed utilization parameters (FER, FCR and PER) were not significantly different among the dietary groups ($P > 0.05$; Table 3).

3.2 Whole body composition and fillet taurine content

There were no statistically significant differences in whole body proximate composition between the fish fed the diets differing in their level of FM and taurine inclusion ($P > 0.05$; Table 4). In contrast, fillet taurine levels were significantly affected by dietary taurine supplementation. The levels of taurine in the fillet of fish fed graded levels of taurine was positively correlated to dietary taurine levels ($r^2 = 0.963$, $n = 12$, $P = 0.037$; Table 5).

3.3 Histological analysis

No significant differences in the histological structure of the intestine were found among groups. The overall morphology of the intestine in all fish examined was in general terms normal, while in some individuals of all groups, small areas of mild hyperplastic enteritis were observed in all segments of the intestine. This mild enteritis was characterized by slight thickening of the intestinal folds, due to infiltration of various white blood cells, mainly lymphocytes, in the *lamina propria*. Small areas of oedema could also be seen, both in the *lamina propria,* as well as in the submucosa. All liver samples collected from fish from the different groups exhibited mild to moderate fatty infiltration of the hepatocytes. The infiltration was characterized by mild or more pronounced swelling of the hepatocytes and displacement of their nuclei to the periphery of the hepatocytes.

3.4 Fillet quality: colour and texture analysis, and organoleptic characteristics

Sea bass fed the C- diet showed the lower a* values (redness), whereas the highest values were recorded in fish fed the C+ diet ($P < 0.05$). Fish fed diets with SBM and SPC supplemented with taurine at different levels (T0.2, 0.5 and T1.0) showed intermediate values. In contrast, no statistically significant differences were found with regard to the other colour variables measured (L* and b*) (Table 6, $P > 0.05$).

Fillet hardness and chewiness, as recorded by texture analysis, increased significantly at higher dietary taurine levels (Table 6, $P < 0.05$). The highest hardness and chewiness values were found in fish fed the T1.0 diet, whereas the lower ones in the C+ groups ($P < 0.05$), while the rest of dietary treatments showed intermediate values. Fillet hardness and chewiness characteristics were positively correlated as indicated by the Pearson product moment correlation test ($r^2 = 0.996$, $P < 0.001$). The highest adhesiveness values were obtained in sea bass fed the C- diet, whereas the lowest ones were found in fish fed the T1.0 diet ($P < 0.05$) and the other groups showed intermediate values. These differences in adhesiveness between groups were not correlated to dietary taurine levels ($P > 0.05$). Other texture parameters such as springiness and cohesiveness showed small changes among groups due to sample variability, but average values were similar among different experimental groups ($P > 0.05$). The results from the taste panel are presented in Figure 1. In particular, no significant differences were found between the C+ and C- groups ($P > 0.05$), whereas fish fed the T1.0 showed lower fillet elasticity, even though was not statistically significant ($P < 0.10$), indicating potential textural difference compared to the other groups.

299 Similar tendencies ($P < 0.10$) were observed for taste intensity and fillet darkness, where the
300 T1.0 group exhibited slightly lower values.
301
302 **4 Discussion**
303 Earlier studies indicated that European sea bass fry (BWi = 0.79 g) fed with 0.2-0.3%
304 taurine-supplemented diets, in which FM was the primary protein source, exhibited enhanced
305 somatic growth when compared to those that were fed on diets supplemented with 0 and
306 0.1% taurine (Brotons Martinez et al., 2004). Somatic growth enhancement by taurine
307 supplementation has been observed in many other fish species during larval and juvenile
308 stages (Park et al., 2002; Kim et al., 2003, 2005; Lunger et al., 2007; Chatzifotis et al., 2008;
309 Matsunari et al., 2005; Pinto et al., 2010; Qi et al., 2012, Salze et al., 2012, Martins et al.,
310 2018). In contrast, sea bass juveniles fed low-FM diets and supplemented with taurine (1%)
311 did not improve significantly growth and feed utilization (Feidantsis et al., 2014; Coutinho et
312 al., 2017). Under current experimental conditions, where sea bass juveniles were fed with
313 diets containing moderate levels of FM (25%) and high content of soy products (20% SBM
314 and 12% SPC) and graded taurine levels (0.2, 0.5 and 1.0 %), no significant differences were
315 found in final average BW values between experimental groups with regard to the control
316 group (C+). Our results are in agreement with those previously reported in this species
317 (Feidantsis et al., 2014; Coutinho et al., 2017). These results might be attributed to the
318 endogenous capacity of taurine biosynthesis in sea bass, as it was postulated by Coutinho et
319 al. (2017), even though some other authors have reported the lack of taurine biosynthesis
320 capacity in other fish species (Park et al., 2002). Regardless of no statistically significant
321 differences found in BWf values among groups in the present study, sea bass fed 1.0 %

taurine (T1.0 diet) showed higher somatic growth and FI values. Taurine possesses the ability to stimulate feeding in fish due to its amino acid structure (Carr, 1982). In this sense, it is generally accepted that taurine may act as an attractant by stimulating the olfactory and/or gustation organs of vertebrates, as it has been demonstrated in mammals and fish; thus, the inclusion of taurine in the diet may improve its palatability (Kuzmina et al., 2010). This is of special relevance under the actual scenario of high FM replacement by PP sources in aquafeeds that may lead to reduced feed palatability and consequently, reduced growth performance (Kissil et al., 2000; Sánchez-Lozano et al., 2009). In the present study, a gradual increase in TFI, although not statistically significant, was revealed in fish fed the diets with taurine supplementation.

Recently, Martins et al. (2018) reported that taurine requirements of European sea bass juveniles fed diets based on high plant feedstuffs were 0.47-0.51% DM in terms of guaranteeing a maximum growth performance and N retention, respectively. However, these authors included very low levels of FM (12%) in their dietary formulations compared to our study, in which a 25% FM was employed in order to match the amount of FM currently used in the commercial aqua feeds for this species (Bonvini et al., 2018; Martins et al., 2018). High FM replacement with PP is known to cause a significant reduction in growth performance of fish because the plant derived ingredients contain anti-nutritional factors (Francis et al., 2001; Krogdahl et al., 2010), whereas on the other hand, FM has been hypothesized to contain unidentified growth factors that are necessary for maximizing fish growth and efficiency (Hardy, 2010).

Fillet proximate composition was not influenced by diet composition, which is in agreement with most of the studies dealing with FM replacement by soybean-derived

ingredients (Tibaldi et al., 2006; Bonaldo et al., 2008; Kousoulaki et al., 2015). The results of the present study showed that dietary taurine supplementation did not affect the proximate composition of the fillet in sea bass, which is in agreement with those previously reported in cobia (*Rachycentron canadum*) (Lunger et al., 2007), totoaba (*Totoaba macdonaldi*) (López et al., 2015) and sea bass Coutinho et al. (2017). Moreover, Poppi et al. (2018) reported that the taurine supplementation to a plant-based diet did not alter significantly the carcass proximate composition of juvenile barramundi (*Lates calcarifer*). However, taurine accumulation in the muscle was correlated to its dietary content, which was in agreement with data reported on taurine deposition in the fillet (Park et al., 2002; Kim et al., 2005; Matsunari et al., 2005) and whole body (Martinez-Brotons et al., 2004; Boonyoung et al., 2012).

The knowledge of the effect of taurine supplementation on the microscopic structure of liver and intestine in fish is currently limited. Although direct comparison with previous studies is difficult due to different experimental designs and the taurine requirements of different species (Salze and Davis, 2015), most studies reveal a positive and even mitigating effect against the effect of soybean meal, particularly in the intestine (López et al. 2015; Rimoldi et al. 2016). The mechanism of action of taurine is still not quite clear, but based on the previous studies, it might be due to its role in the osmoregulation, or the inhibition of the production of the inflammatory mediators. In the liver, taurine deficiency is also linked to the 'green liver syndrome' (Tagagi et al. 2005). Hoseini et al. (2017); however, using a semi-qualitative method reported some alterations (increased fatty infiltration, areas of necrosis, hyperemia and melanomacrophage aggregates) in the liver of Persian sturgeon (*Acipenser persicus*), even when the taurine supplementation was as low as 0.25%. A negative effect of

368 the supplementation on the growth was also observed in the above-mentioned study (El-
369 Sayed, 2014). The former authors speculated that this may have been due to the production
370 of toxic substances, following the oxidation of this compound in the liver. It should be noted
371 that many freshwater fish species have the ability to synthesize taurine and thus taurine
372 supplementation may not be necessary or may even have negative effects. In our study, the
373 histological examination of liver and intestine samples of sea bass from the different
374 experimental groups, revealed no significant differences among diets, regardless of the level
375 of FM replacement and taurine supplementation. The fatty infiltration observed in all the
376 liver samples is a common finding in sea bass fed diets containing 20% crude fat diets
377 (Caballero et al., 2004; Baeza-Ariño et al., 2016). Furthermore, the mild enteritis observed in
378 all experimental groups is also considered a common finding, due to the presence of some
379 antinutritional factors that are present in soybean meal (Tibaldi et al., 2006). In Atlantic
380 salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), such alterations and
381 inflammatory response are particularly evident in the distal intestine (Krogdahl et al., 2003;
382 Venold et al., 2012).

383 Regarding fillet quality, the colour of the fillet was affected by the inclusion of PP
384 sources as data from C+ and C- revealed (a*, redness values); whereas the supplementation
385 of taurine did not substantially affected the colour of the fillet, since a* were within the
386 former groups, but closer to the C- diet. The relevance of these findings needs to be further
387 evaluated in terms of fillet quality consumers' preferences, since changes in a* were mild,
388 whereas L* and b* values remained constant among dietary groups. The textural
389 characteristics of the fillet are important for evaluating its quality in terms of consumer's
390 product acceptance. For instance, a salmon product with soft flesh has an unpleasant mushy

391 mouthfeel and will reduce acceptability by the consumers, leading to a quality downgrading
392 for this product in the fish processing industry (Ashton et al., 2010). Besides, being relevant
393 to mouthfeel, texture is a general quality property related to fillet freshness (Isaksson et al.,
394 2002). In this context, the present study provides novel findings regarding the significant
395 effect that taurine supplementation has on fillet quality and especially in its texture as
396 indicated by TPA. In this sense, sea bass fed the T1.0 diet showed significantly higher
397 hardness and chewiness values, which indicated the positive effect of dietary taurine and its
398 accumulation in the muscular tissue on the firmness of the fillet. However, in contrast to
399 TPA results, the organoleptic analysis failed to find significant differences in hardness or
400 chewiness even at the highest taurine inclusion level. This disagreement between both
401 methodologies might be explained by the fact that the organoleptic results referred to cooked
402 fillets, contrary to the TPA that was conducted on raw fillets. In addition, organoleptic
403 analysis indicated a tendency for reduced elasticity in the fish fed with the T1.0 diet, a result
404 that may be linked to the higher hardness and chewiness found by the TPA. Considering that
405 total muscular fat and protein contents have a major effect on sea bass fillet texture
406 (Grigorakis, 2007), but in the present study there were no differences in fillet proximate
407 composition among different experimental groups, the above-mentioned differences found in
408 texture may be attributed to taurine supplementation. Although the exact mechanism by
409 which taurine affects fillet texture is not yet known and no previous study has mentioned an
410 impact of dietary taurine on textural attributes of fish fillet, it has been reported that in
411 Atlantic salmon the addition of specific amino-acids, as for example glutamate and / or
412 arginine, changed the muscle cell density, resulting in firmer muscular texture
413 (WO/2010/082832A1). Mørkøre et al. (2009) correlated firmer texture in salmon fillets with

low fibre cross-sectional area, while fillets with larger muscle fibres exhibited a softer texture, as well as a more stable and less disordered collagen fibres (Moreno et al., 2012). In addition, Goodman et al. (2009) found that dietary taurine supplementation in rats increased skeletal muscle force production and protected muscle function during and after high-frequency *in vitro* stimulation. The results from the former authors were due to the key role of taurine in regulating muscular calcium levels and modulating its homeostasis from the muscular proteins (Schaffer et al., 2010). In our study, no such assessment was carried out and thus, we cannot confirm nor reject this hypothesis, so further research is needed to investigate the role of taurine in fillet texture properties.

Conclusions

The findings of the present study showed that juvenile European sea bass can be fed a diet with high levels of soy products that contains a mixture of soybean meal and soy protein concentrates up to 32%, without impeding fish performance either inducing alterations, morphological changes or inflammatory symptoms in the distal intestine. Taurine supplementation did not impact significantly somatic growth performance. Moreover, taurine supplementation did not amend the proximate composition of the fillet but led to an increased muscle taurine concentration. A key finding of the present study is that the increase in taurine muscular content as a result of the taurine addition affected significantly the hardness and chewiness as the TPA revealed, whereas the organoleptic analysis indicated a tendency for reduced fillet elasticity with increasing taurine levels, which represents a significant consumer added advantage. Further research is needed to clarify the beneficial role of taurine on organoleptic properties of sea bass fillet.

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650

651 **Table 1.** Diet formulation and calculated chemical composition of the experimental diets.

652

Ingredients **(g kg^{-1} diet)**	**C+**	**C-**	**T0.2**	**T0.5**	**T1.0**
Fish meal (71%)[a]	300.0	250.0	250.0	250.0	250.0
Soya cake 48[b]	200.0	200.0	200.0	200.0	200.0
Soya protein concentrate (60%) [c]	0.0	120.0	120.0	120.0	120.0
Corn gluten 60	183.0	120.0	117.0	112.0	104.0
Wheat	139.8	156.9	157.9	159.7	162.4
Fish oil [a]	66.0	72.0	72.0	72.0	72.0
Rapeseed oil	83.0	83.0	83.0	83.0	83.0
Methionine (98%)[d]	0.0	1.1	1.1	1.2	1.3
Vit and Min premix[d]	3.0	3.0	3.0	3.0	3.0
Monocalcium phosphate[e]	2.9	4.7	4.7	4.8	5.0
Taurine[f]	0.0	0.0	2.0	5.0	10.0
Analyzed chemical composition of diets (g kg^{-1} or specified)					
Protein	439	440	438	437	437
Fat	203	201	202	200	201
Ash	59	63	60	61	61
Moisture	96	50	70	71	76
Carbohydrate*	203	246	230	231	225
Gross energy (MJ kg^{-1})**	21.9	22.5	22.2	22.1	22.1

653 [a] Supplied by Norsildmel Innovation AS.

654 [b] Purchased from Cargill

655 [c] Supplied by Imcopa

656 [d] DL-methionine and Vitamin/Mineral premix was supplied by Vilomix

657 [e] Supplied by Yara International

658 [f]Crystalline taurine was supplied by Omya Peralta GmbH

659 * Calculated by difference: 100 - (%protein + %fat + %ash + %moisture) (i.e. N-free

660 extractives + crude fiber)

661 **Gross energy was calculated using combustion values for protein, lipid and carbohydrate

662 of 23.6, 39.5 and 17.2 MJ kg^{-1}, respectively.

663

664

665 **Table 2.** Amino acid composition of the experimental diets as analyzed (g / 100 g feed).

666

	Diets				
	C+	C-	T 0.2	T 0.5	T 1.0
Tau	***0.23***	***0.15***	***0.31***	***0.55***	***0.91***
HyPro	0.03	0.10	0.05	0.09	0.07
His	0.69	0.77	0.72	0.80	0.63
Ser	2.73	2.21	2.03	2.06	2.00
Arg	2.34	2.78	2.50	2.64	2.66
Gly	2.34	2.12	2.03	2.00	2.01
Asp+Asn	4.07	4.41	4.22	4.08	4.18
Glu+Gln	7.48	8.05	7.61	7.44	7.44
Thr	1.85	1.84	1.75	1.73	1.77
Ala	2.96	2.70	2.56	2.52	2.52
Pro	2.53	2.43	2.33	2.27	2.36
Cys	0.25	0.26	0.25	0.24	0.25
Lys	2.37	2.63	2.53	2.49	2.52
Tyr	1.38	1.38	1.28	1.28	1.36
Met	0.91	0.96	0.78	0.83	0.93
Val	2.19	2.24	2.09	2.10	2.11
Ile	1.82	1.92	1.88	1.82	1.86
Leu	4.23	4.16	3.99	3.85	3.89
Phe	2.15	2.22	2.15	2.09	2.15

667

Table 3. Growth performance, survival and somatometric indices for sea bass fed the experimental diets containing graded levels of taurine. Values are means ± SD

	C+	***C-***	***T0.2***	***T0.5***	***T1.0***
Initial body weight (g)	86.0 ± 0.7	86.1 ± 0.6	86.7 ± 0.6	86.3 ± 0.3	86.5 ± 0.6
Final Body weight (g)	202.4 ± 6.4	201.3 ± 2.9	201.8 ± 15.7	201.4 ± 11.2	208.5 ± 5.1
Survival (%)	94	99	98	98	98
WG (g/fish)	116 ± 6.14	115 ± 2.4	115± 16.0	115 ± 11.3	122 ± 5.3
DGI (%)	1.94 ± 0.10	1.93 ± 0.02	1.91 ± 0.21	1.92 ± 0.23	2.0 ± 0.14
TFI (g DM $fish^{-1}$)	184.4 ± 6.9	175.1 ± 4.3	176.2 ± 25.1	180.9 ± 9.7	190.6 ± 2.8
FI (%)	214.3 ± 6.7	203.3 ± 6.3	203.2 ± 29.6	209.7 ± 11.3	220.2 ± 2.8
FCR	1.59 ± 0.05	1.52 ± 0.05	1.53 ±0.02	1.58 ±0.03	1.57 ± 0.09
PER	1.43 ± 0.04	1.50 ± 0.05	1.48 ±0.02	1.44 ±0.07	1.45 ± 0.08
SGR	1.14 ± 0.04	1.13±0.01	1.12 ±0.11	1.13 ±0.08	1.17 ± 0.04
TGC	0.69 ± 0.03	0.69 ±0.03	0.68 ±0.08	0.69 ±0.05	0.72 ± 0.02
LSI	7.9 ± 0.8	8.2 ± 0.3	8.0 ± 0.9	7.4 ±1.3	8.4 ± 1.2
HSI	2.3 ± 0.4	2.2 ± 0.4	2.3 ± 0.3	1.8 ±0.3	2.2 ± 0.5

WG= weight gain (g/fish); DGI= Daily growth index; TFI= Total feed intake g DM $fish^{-1}$; FI %= Feed intake, as % of initial body weight; FCR= Feed conversion ratio; PER= Protein efficiency ratio; SGR= Specific growth rate; TGC= Thermal growth coefficient; LSI= Lipidosomatix index; HSI= hepatosomatic index.

674 **Table 4**. Fish muscle composition (% wet weight) of European sea bass fed the experimental
675 diets at the end of the trial.

	Diets				
	C+	C-	T0.2	T0.5	T1.0
Water (%)	74.50 ± 0.70	75.30 ± 0.30	75.60 ± 0.40	75.70 ± 0.20	75.80 ± 0.60
Protein (%)	22.23 ± 0.74	22.17 ± 0.69	21.69 ± 0.47	21.98 ± 0.49	22.71 ± 1.19
Lipid (%)	2.07 ± 0.14	1.69 ± 0.33	1.96 ± 0.16	1.85 ± 0.24	1.83 ± 0.07
Ash (%)	1.50 ± 0.10	1.30 ± 0.10	1.40 ± 0.10	1.30 ± 0.00	1.30 ± 0.10

676 Data are mean ± SD

677

Table 5. Amino acid composition of sea bass muscles per dietary treatment (g per 100 g muscle).

	Diets				
	C+	C-	T 0.2	T 0.5	T 1.0
Tau	***0.36***	***0.31***	***0.31***	***0.37***	***0.41***
HyPro	0.01	0.03	0.01	0.01	0.01
His	0.41	0.44	0.37	0.38	0.41
Ser	0.89	0.81	0.81	0.82	0.83
Arg	1.41	1.33	1.29	1.26	1.32
Gly	1.13	1.22	1.10	1.06	1.07
Asp+Asn	2.41	2.18	2.22	2.21	2.25
Glu+Gln	3.44	3.21	3.25	3.25	3.22
Thr	1.02	1.42	0.95	0.93	0.95
Ala	1.37	1.30	1.32	1.30	1.28
Pro	0.66	0.78	0.68	0.62	0.62
Cys	0.11	0.12	0.10	0.11	0.11
Lys	2.13	1.76	2.05	2.04	1.99
Tyr	0.79	1.02	0.75	0.72	0.73
Met	0.69	0.69	0.66	0.61	0.62
Val	1.08	1.01	1.03	1.00	0.99
Ile	0.99	0.84	0.93	0.95	0.91
Leu	1.77	1.62	1.74	1.75	1.65

Phe	0.95	1.10	0.90	0.91	0.89
Tau (%)*	1.63	1.40	1.42	1.68	1.81

*expressed as percentage of muscle protein

Table 6. Texture analyzed parameters and colour of fish fillets (n=4).

	Experimental diets				
Texture parameters	**C+**	**C-**	**T0.2**	**T0.5**	**T1.0**
Hardness (N)	1.83 ± 0.52^{a}	3.20 ± 0.47^{b}	2.36 ± 0.43^{bc}	3.34 ± 0.057^{bc}	4.27 ± 0.35^{c}
Chewiness (N)	0.88 ± 0.19^{a}	1.75 ± 0.17^{bc}	1.26 ± 0.06^{ab}	1.81 ± 0.38^{bc}	2.26 ± 0.04^{c}
Adhesiveness (N·s)	-0.029 ± 0.021^{ab}	-0.017 ± 0.018^{b}	-0.036 ± 0.013^{ab}	-0.032 ± 0.006^{ab}	-0.051 ± 0.010^{a}
Springiness	0.782 ± 0.072	0.782 ± 0.029	0.796 ± 0.110	0.778 ± 0.048	0.758 ± 0.044
Cohesiveness	0.684 ± 0.055	0.703 ± 0.017	0.681 ± 0.049	0.686 ± 0.055	0.701 ± 0.039
Colour parameters					
L*	41.5±1.9	42.1±1,6	43.8±2.0	45.4±5.6	42.8±1.1
a*	3.09 ± 0.42^{a}	2.19 ± 0.04^{b}	1.58 ± 0.09^{bc}	1.47 ± 0.20^{c}	1.91 ± 0.25^{bc}
b*	-2.62±0.29	-3.39±0.30	-3.05±0.39	-2.82±0.36	-3.31±0.17

Rows means not sharing the same superscript letters are significantly different ($P<0.05$)

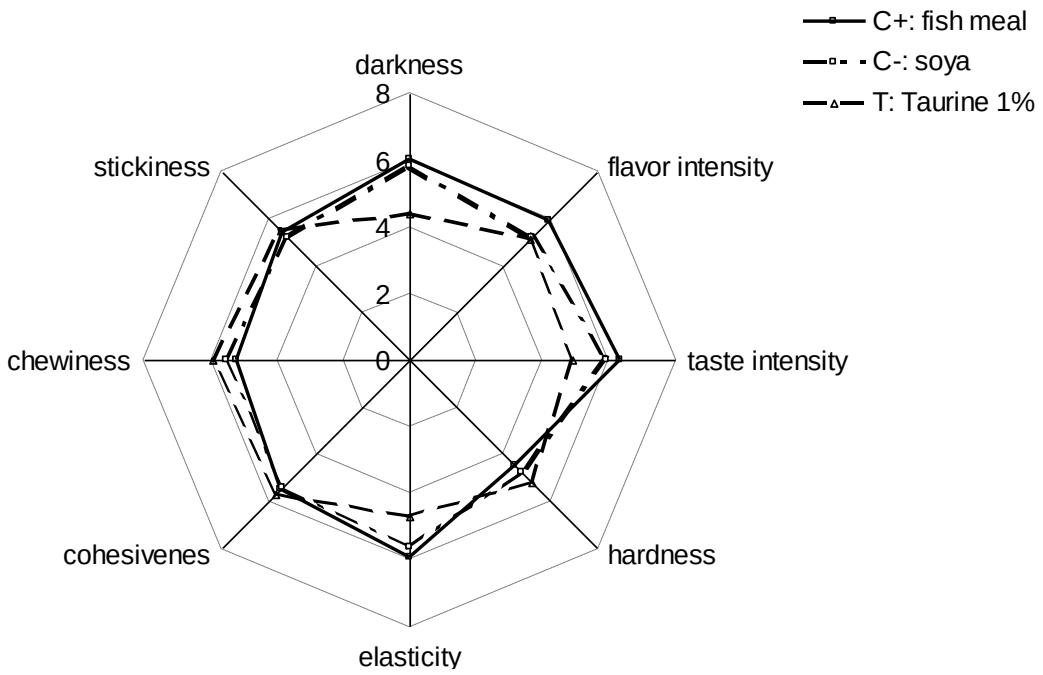

Fig. 1. Descriptive taste panel results for the three dietary groups studied.