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Effects of feeding low fishmeal diets with increasing soybean meal levels on growth, gut histology and plasma biochemistry of sea bass

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The aquaculture industry depends upon the development of sustainable protein sources to replace fishmeal (FM) in aquafeeds and the products derived from soybeans are some of the most studied plant feedstuffs. A key area of investigation for continuing to improve modern aquafeeds includes the evaluation of varying proportions and combinations of plant ingredients to identify mixtures that are more efficiently utilized by the fish. This study investigated the effects of increasing soybean meal (SBM) by replacing a mix of plant ingredients in low FM (20%) diets on growth, blood biochemistry profile and gut histology on European sea bass. Five isonitrogenous and isolipidic experimental diets were formulated: four diets containing increasing SBM levels (0, 10, 20 and 30%; 0SBM, 10SBM, 20SBM and 30SBM, respectively) with a low content of FM (20%) and one control diet (0% SBM; 35% FM). Diets containing SBM brought to comparable performance and protein utilization, while 0SBM had negative impact on feed conversion rate and protein utilization. Blood parameters suggested an optimal nutritional status under all feeding treatments, even though slightly decreased values were reported at increasing dietary SBM. Histology examination did not show any changes indicative of soy-induced enteritis. We can conclude that for European sea bass: (i) different blends of plant protein did not affect feed intake despite the 20% FM dietary level; (ii) the inclusion of SBM maintains optimal growth and feed utilization in low FM diets; (iii) blood biochemistry profile showed a good nutritional status under all feeding regimes; (iv) no evidence of soy-induced enteritis was reported in any group fed low FM diets. For formulation of practical diets in on-growing of European sea bass, SBM up to 30% can be successfully incorporated into feeds containing low FM inclusion.

Keywords: European sea bass, soy, low fishmeal, blood biochemistry, histology

Implications

Nowadays, aquaculture is the fastest-growing food production system. However, reducing the dependence of aquaculture on fishmeal (FM) and fish oil in feeds is essential for a sustainable growth of the sector. A key area of investigation to improve modern aquafeeds includes the evaluation of varying proportions and combinations of plant ingredients in low FM diets to identify mixtures that are more efficiently utilized by the fish. This study compared the effects of diets containing a low amount of FM and different plant protein blends containing increasing levels of soybean meal (SBM) on the performance of European sea bass.

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Introduction

For several years, there has been continuing interest in identifying and developing ingredients as alternatives to FM for use within aquafeeds, resulting in a substantial decrease of this ingredient in the feed formulation of many species. Nowadays aquaculture relies on a basket of common input plant protein ingredients such as SBM, corn gluten (CGM), wheat gluten (WGM) or sunflower meal (SFM), for which it competes in the marketplace with the animal husbandry sector as well as with use for direct human consumption. Based on evidence indicating that mixed dietary plant proteins outperform single sources, a key area of investigation for continuing to improve modern aquafeeds includes the evaluation of varying proportions and combinations of plant ingredients to identify mixtures that are more

efficiently utilized by the fish (De Santis *et al*., 2016). Corn gluten and WGM have high protein level, are low in fiber, rich in vitamins B and E and are known to contain no antinutritional factors (Bonaldo *et al*., 2011 and 2015). Sunflower meal is highly palatable and has low antinutritional factors (Merida *et al*., 2010). SBM is one of the most interesting alternatives to FM because of the advantages of supply, price, protein and amino acid composition (Parma *et al*., 2016). However, SBM has been found to induce a variety of histological and functional changes in the gastrointestinal tracts of several species, especially in salmonids, such as subacute enteritis of the distal epithelial mucosa including morphological alteration and inflammation (Krogdahl *et al*., 2010). To our knowledge, only a few studies have explored in European sea bass (*Dicentrarchus labrax*) the effects of different plant blends with increasing levels of SBM in low FM diets, and most of the data in the literature were restricted to replacing FM with SBM. Hence, this study investigated the effects of different plant protein blends, with increasing SBM by replacing a mix of plant ingredients (WGM, CGM and SFM) in low FM diets (20%), on growth, blood biochemistry profile and gut histology in European sea bass.

Table 1 *Ingredients and proximate composition of the experimental diets*

	CD	0SBM	10SBM	20SBM	30SBM
Ingredient (% of the diet)					
FM North Atlantic	35.00	20.00	20.00	20.00	20.00
Hi-pro SBM	0.00	0.00	10.00	20.00	30.00
Wheat	21.43	19.31	15.13	10.94	6.75
Corn gluten	12.00	18.00	16.00	14.00	12.00
Wheat gluten	12.05	18.07	15.98	13.89	11.80
Sunflower meal	4.00	8.00	6.00	4.00	2.00
Fish oil North Atlantic	15.02	16.12	16.40	16.67	16.95
Vitamin/mineral premix[1]	0.50	0.50	0.50	0.50	0.50
Proximate composition (% dry weight basis)					
Protein	45.41	46.10	47.00	46.63	46.57
Lipid	19.56	19.13	19.19	19.83	20.17
Ash	5.64	4.42	4.72	5.00	5.31
Moisture	6.26	6.61	6.66	6.30	7.22
NfE	23.13	23.74	22.43	22.24	20.73
Energy (cal/g)	5259	5355	5223	5268	5244

FM = fishmeal; SBM = soybean meal; CD = control diet; 0SBM = 0 g/kg SBM diet; 10SBM = 100 g/kg SBM diet; 20SBM = 200 g/kg SBM diet; 30SBM = 300 g/kg SBM diet; NfE = nitrogen-free extract, calculated.
[1]Vitamin and mineral premix; Skretting, Stavanger, Norway (fulfilling recommendations for marine fish species given by NRC, 2011).

Material and methods

Experimental diets

Five isonitrogenous and isolipidic experimental diets were formulated: four diets containing increasing SBM levels (0%, 10%, 20% and 30%; 0SBM, 10SBM, 20SBM and 30SBM, respectively) with a low content of FM (20%) and one control diet (CD) (0% SBM; 35% FM). SBM was replaced by adding a combination of WGM, CGM and SFM, as recently described by Parma *et al*. (2016) on gilthead seabream (*Sparus aurata* L.). The diets were produced by Skretting Aquaculture Research Centre, Stavanger, Norway. According to the feed manufacturer, protein and lipid levels were within the range of commercial diets for sea bass. The CD, containing 35% FM, was included to exclude any deleterious effect of low FM on the performance and health during the on-growing of European sea bass, adopting the same approach used by Torrecillas *et al*. (2017) and Parma *et al*. (2016).

The diameter of the pellet was 4 mm. Ingredients and proximate composition of the experimental diets are presented in Table 1.

Fish and feeding trial

The experiment was carried out at the Laboratory of Aquaculture, Department of Veterinary Medical Sciences of the University of Bologna, Cesenatico, Italy. European sea bass juveniles were obtained from Ecloserie Marine de Gravelines (Gravelines, France). One week before the beginning of the trial, fish were fed with a mixture of the five experimental diets. At the beginning of the trial, 60 fish (initial average weight 68.9 ± 1.7 g) per tank were randomly distributed into 15 1000 l square tanks with a conical bottom. Each diet was administered to triplicate groups, assigned in a completely random manner, over 91 days. Tanks were provided with natural seawater and connected to a closed recirculation system (overall water volume: 18 m^3). The rearing system consisted of a mechanical sand filter (Astralpool, Spain), ultraviolet lights (Philips, the Netherlands) and a biofilter (Astralpool, Spain). The water exchange rate within each tank was 100% every hour, while the overall water renewal amount in the system was 5% daily. During the trial, the temperature was kept constant at 22 ± 1.0°C and the photoperiod was maintained at 12 h light and 12 h dark through artificial light. The oxygen level was kept constant at 100% saturation by a liquid oxygen system regulated by a software program (B&G Sinergia snc, Chioggia, Italy). Ammonia (total ammonia nitrogen ⩽0.1 ppm), nitrite (⩽0.2 ppm) and salinity (25 g/l) were spectrophotometrically monitored daily (Spectroquant Nova 60, Merck, Lab business, Darmstadt, Germany). Sodium bicarbonate was added on a daily basis to keep pH constant at 7.8 to 8.0. Feed was provided to 10% overfeeding by automatic feeders, twice a day for 6 days a week, while one meal was supplied on Sundays, as reported by Mongile *et al*. (2014). Each meal lasted 1 h, after that the uneaten pellets in each tank were gathered, dried overnight at 105°C, and their weight was deducted for overall calculation.

Sampling

At the beginning and at the end of the experiment, all the fish in each tank were anesthetized and individually weighed. In case of any mortality, fish were immediately

removed and the weight was recorded for overall calculation. Specific growth rate (SGR), voluntary feed intake (VFI) and feed conversion rate (FCR) were calculated. The proximate composition of the carcasses was determined at the beginning of the trial on a pooled sample of 10 fish and on pooled samples of five fish per tank at the end of the trial. Protein efficiency rate (PER), gross protein efficiency (GPE) and gross lipid efficiency (GLE) were calculated. Furthermore, at the end of the trial, wet weight, viscera, liver and visceral fat weight were individually recorded for five fish per tank to determine viscerosomatic index (VSI), hepatosomatic index (HSI) and fat index (FaI). At the end of the trial five fish per tank (15 fish per dietary treatment) were sampled for intestine histology examination. After the end of the trial, the fish left were kept in the same rearing and feeding conditions for 3 more days and then were sampled to perform blood analyses of hematocrit, serum total protein, triglycerides and cholesterol. Blood from five fish per tank was collected 5 h postprandial from the caudal vein. Samples were then centrifuged ($3000 \times \mathbf{g}$ for 10 min at 4°C) and serum aliquots were stored at 4°C and analyzed during the same day.

All experimental procedures were evaluated and approved by the Ethical-Scientific Committee for Animal Experimentation of the University of Bologna, in accordance with European directive 2010/63/UE on the protection of animals used for scientific purposes.

Analytical methods

Diets and whole body were analyzed for proximate composition. Moisture content was obtained by weight loss after drying samples in a stove at 105°C until a constant weight was achieved. Crude protein was determined as total nitrogen (N) by using the Kjeldahl method and multiplying N by 6.25. Total lipids were determined according to Bligh and Dyer's (1959) extraction method. Ash content was estimated by incineration to a constant weight in a muffle oven at 450°C. Gross energy was determined by a calorimetric bomb (Adiabatic Calorimetric Bomb Parr 1261; PARR Instrument, IL, USA).

The hematocrit value was obtained as packed cell volume % using microhematocrit tubes on a dedicated centrifuge (Hettich Haematokrit 210, Tuttingen, Germany). Serum total protein, cholesterol and triglycerides were measured using colorimetric methods (Total protein OSR6232, Cholesterol OSR6216, Triglyceride OSR61118; Beckman Coulter, Brea, CA, USA) on an automated analyzer (AU 400; Beckman Coulter).

Calculations

The formulae employed were as follows:

Specific growth rate (SGR) (per day) $= 100 \times$ (ln FBW − ln IBW)/days (where FBW and IBW represent the final and the initial BWs). Voluntary feed intake (VFI) (g feed/fish) = g feed ingested/fish number. Feed conversion ratio (FCR) = feed intake/weight gain. Viscerosomatic index (VSI) (%) $= 100 \times$ (viscera weight/BW). Hepatosomatic index (HSI) (%) $= 100 \times$ (liver weight/BW). Fat index (FaI) (%) $= 100 \times$ (visceral fat weight/FBW). Protein efficiency ratio (PER) = (FBW–IBW)/protein intake. Gross protein efficiency (GPE) (%) $= 100 \times$ [(% final body protein $\times$ FBW)−(% initial body protein $\times$ IBW)]/total protein intake fish. Gross lipid efficiency (GLE) (%) $= 100 \times$ [(% final body lipid $\times$ FBW) − (% initial body lipid $\times$ IBW)]/total lipid intake fish. The survival rate was calculated as a percentage of the initial number of fish.

Histology

After euthanasia, the gut was removed and the intestine was divided into two segments (proximal and distal). From each segment, a 5-mm-long piece was sectioned and fixed in 10% buffered formalin. Samples were then processed for routine histology to obtain a transversal section, which was stained with hematoxylin and eosin. Sections were evaluated in blind fashion under a light microscope regarding treatments for degenerative and inflammatory changes. Photographs of the sections were made with a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan) equipped with a Nikon Digital Sight SD-MS camera and the Nikon software NIS-Elements. Adobe Photoshop CS3 Extended was used for the final photographic preparation without altering the original integrity of the pictures. Regarding diet CD and 30SBM, also morphometry and scoring were conducted for proximal and distal tracts. Morphometry consisted in measurements at 100× magnification of five randomly selected fields per intestinal tract. Width of *lamina propria*, width of mucosal layer in the middle of the folds, height of mucosal layer at the base of the folds and height of *submucosa* were measured and expressed in micron (μ). Goblet cells were counted in three fields per intestinal tract at 40× magnification. A semiquantitative scoring system, with the range of scores set at 0 to 3, was used for evaluation of inflammatory cells infiltration, number of mast cells and number of intraepithelial lymphocytes.

Statistical analysis

All data are presented as mean. Tank was used as the experimental unit for analyzing growth and performance, a pool of five sampled fish was considered the experimental unit for analyzing carcass composition, whereas individual fish was used for analyzing VSI, HSI, FaI, and all blood parameters. Diets with low FM (0SBM, 10SBM, 20SBM and 30SBM) were analyzed by a one-way ANOVA followed by a Tukey's multiple comparison test. *T*-test was used to compare diets CD and 0SBM. Normal distribution of morphometric and histologic data sets was tested by Shapiro–Wilk. If normality criteria were not met, Mann–Whitney *U* testing was run. The differences among treatments were considered significant at $P \leqslant 0.05$. All statistical analyses were performed using software Statistica 8 (StatSoft Inc., Tulsa, OK, USA).

Results

Growth

Growth performances are summarized in Table 2. No significant differences were found in final BW, SGR and VFI. Fish fed diet 0SBM showed lower FCR in comparison to those fed diet CD and 20SBM. No significant differences were recorded in

Table 2 *Growth performance, intake and survival of European sea bass fed experimental diets over 91 days*

	CD	0SBM	10SBM	20SBM	30SBM	*t*-test	RMSE	*P* value
IBW (g)	68.2	68.7	70.0	69.4	68.3	0.5401	3.897	0.7156
FBW (g)	231.6	219.3	230.7	228.3	228.4	0.0647	36.12	0.1775
SGR (per day)	1.34	1.28	1.31	1.31	1.33	0.1369	0.001	0.2800
VFI	209.6	211.3	219.1	209.5	215.5	0.6158	29.22	0.2104
FCR	1.28^{x}	1.38^{by}	1.35^{ab}	1.31^{a}	1.35^{ab}	0.0496	0.001	0.0268
Survival (%)	100.0	100.0	100.0	100.0	99.4	–	0.2315	0.4411

CD = control diet; 0SBM = 0 g/kg SBM diet; 10SBM = 100 g/kg SBM diet; 20SBM = 200 g/kg SBM diet; 30SBM = 300 g/kg SBM diet; RMSE = root mean square error; IBW = initial BW; FBW = final BW; SGR = specific growth rate (% per day) = 100 × (ln FBW − ln IBW)/days (where FBW and IBW represent the final and the initial BWs); VFI = voluntary feed intake (g feed/fish) = g feed ingested/fish; FCR = feed conversion rate = feed intake/weight gain.
Data are presented as mean of three measurements.
[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$ for one-way ANOVA analysis.
[x,y]Values within a row with different superscripts differ significantly at $P < 0.05$ for *t*-test analysis.

Table 3 *Biometric indices, body composition and nutritional indices of European sea bass fed the experimental diets*

	CD	0SBM	10SBM	20SBM	30SBM	*t*-test	RMSE	*P* value
Biometric indices								
VSI	12.7	12.4	11.8	11.0	12.1	0.6939	4.275	0.3004
HSI	2.25	2.12^{c}	1.91^{bc}	1.58^{a}	1.68^{ab}	0.2917	0.106	0.0001
FaI	9.18	9.16	8.38	7.78	8.42	0.9878	3..999	0.3189
Whole body composition								
Protein	16.1	16.0	16.2	16.3	16.0	0.6895	0.166	0.7376
Lipid	18.5	19.0	18.6	18.8	17.8	0.6461	0.988	0.5021
Ash	3.36	3.02	3.13	3.30	3.17	0.1848	0.025	0.2661
Moisture	59.2	59.2	58.7	59.9	57.9	0.9910	4.346	0.6798
Nutritional indices								
PER	1.72^{x}	1.55^{y}	1.56	1.63	1.60	0.0395	0.002	0.0666
GPE	26.6^{x}	23.6^{y}	24.3	25.7	24.6	0.0077	0.870	0.1298
GLE	79.1	77.0	76.3	77.9	69.5	0.6791	27.12	0.2549

CD = control diet; 0SBM = 0 g/kg SBM diet; 10SBM = 100 g/kg SBM diet; 20SBM = 200 g/kg SBM diet; 30SBM = 300 g/kg SBM diet; RMSE = root mean square error; VSI = viscerosomatic index (%) = 100 × (viscera weight/BW); HSI = hepatosomatic index (%) = 100 × (liver weight/BW); FaI = fat index (%) = 100 × (visceral fat weight/FBW); PER = protein efficiency ratio = ((FBW − IBW)/protein intake); GPE = gross protein efficiency = 100 × [(%final body protein × FBW) − (% initial body protein × IBW)]/total protein intake fish; GLE = gross lipid efficiency = 100 × [(% final body lipid × FBW) − (% initial body lipid × IBW)]/total lipid intake fish.
Data are presented as mean of 3 measurements, 15 measurements for VSI, HSI, FaI.
[a,b,c]Values within a row with different superscripts differ significantly at $P < 0.05$ for one-way ANOVA analysis.
[x,y]Values within a row with different superscripts differ significantly at $P < 0.05$ for *t*-test analysis.

survival rate among groups. Data on biometric indices, body composition and nutritional indices are shown in Table 3. No significant differences were found in VSI and FaI. Within the groups fed low FM, HSI significantly decreased from 0SBM to 20SBM. No significant differences were found in the whole body proximate composition. Fish fed diet CD showed higher PER and GPE in comparison to those fed diet 0SBM, while no differences were recorded between low FM treatments. No significant differences among treatments were found in GLE.

Blood biochemistry

Hematocrit, serum total protein, triglycerides and cholesterol levels are shown in Table 4. Data on hematocrit analyzed by ANOVA were significantly different ($P = 0.0355$), even though the Tukey's multiple comparison test did not underline specific differences between groups. Fish fed diet 0SBM showed higher serum total protein in comparison to those fed diet 30SBM and higher serum triglycerides in comparison to CD. Within low FM diets, cholesterol values decreased with the increasing of SBM inclusion.

Histology

Inflammatory and/or degenerative changes indicative of soy-induced enteritis were not present in any histological section from all subjects examined (Figure 1). Regarding proximal tract, no differences were recorded for morphometry and scoring while a depletion of goblet cells was found in animals fed 30SBM in comparison with CD. Considering distal tract, no differences were recorded for all the parameters evaluated. All data are shown in Table 5.

Discussion

A few studies have investigated the utilization of plant dietary inclusion in European sea bass, but mainly as a FM

Table 4 *Hematocrit, serum total protein, triglycerides and cholesterol concentrations of European sea bass fed the experimental diets*

	CD	0SBM	10SBM	20SBM	30SBM	*t*-test	RMSE	*P* value
Hematocrit (%)	44.3	46.2	45	41.7	41.4	0.3139	27.84	0.0355
Total protein (g/dl)	5.69	5.61^{b}	5.28^{ab}	5.47^{ab}	5.08^{a}	0.7453	0.245	0.0296
Triglycerides (mg/dl)	923.2^{x}	1885.1^{y}	1629.7	1648.7	1339.4	0.0003	575 050	0.2827
Cholesterol (mg/dl)	334.7	369.0^{b}	354.5^{ab}	350.2^{ab}	287.9^{a}	0.3016	6851	0.0468

CD = control diet; 0SBM = 0 g/kg SBM diet; 10SBM = 100 g/kg SBM diet; 20SBM = 200 g/kg SBM diet; 30SBM = 300 g/kg SBM diet; RMSE = root mean square error. Data are presented as mean of 15 measurements.
[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$ for one-way ANOVA analysis.
[x,y]Values within a row with different superscripts differ significantly at $P < 0.05$ for t-test analysis.

replacement. More recently, the combination of maximum replacement of FM and fish oil by alternative meals and oils was evaluated (Torrecillas *et al*., 2017). The present study compared the effects of diets containing different plant protein blends, maintaining constant the FM level (20%), on the performance of European sea bass.

As reported by Médale and Kaushik (2009), a blend of plant protein sources can replace 75 to 95% of FM in almost all species, thus reducing the pressure of aquaculture on marine resources. The authors also reported that to limit the negative effects of each raw material, the strategy adopted is to replace FM with a mixture of vegetable protein sources. Kaushik *et al*. (2004) observed that an almost total replacement of FM by a mixture of several plant protein sources had no influence on final weight at commercial size in European sea bass. Geay *et al*. (2011) found that European sea bass fed an exclusively vegetable-based diet exhibited significantly lower final weight and daily growth index than those fed a fish-based diet. Overall, one of the reasons of reduced growth in fish fed diets containing plant protein is a decrease of feed intake, due to a reduced feed palatability. Interestingly, in the present study no differences were recorded in voluntary feed intake among all feeding regimes and despite the low FM level, the inclusion of different blends of different plant feedstuff did not cause palatability problems in European sea bass.

Considering the low FM treatments, diets containing a plant blend with SBM (10SBM, 20SBM and 30SBM) brought to similar performance and protein utilization. Indeed, fish fed 0SBM diet showed lower feed and protein efficiency in comparison with 20SBM and CD. The highest inclusion of CGM in diet 0SBM may have led to a negative impact on feed conversion rate and protein utilization, since this ingredient is characterized by a low protein digestibility coefficient, as reported by Dias *et al*. (2005).

No significant differences were found in VSI and this result is in agreement with those previously reported for this species (Kaushik *et al*., 2004). The authors reported no differences in VSI in European sea bass fed diets containing different levels of FM incorporation. Instead, HSI values decreased on increasing the inclusion of SBM. Geay *et al*. (2011) found lower HSI values in European sea bass fed vegetable-based diets in comparison with fish-based diets; on the contrary, Kaushik *et al*. (2004) reported no differences in European sea bass fed diets with graded levels of FM replacement. A possible explanation of the trend in HSI, found in our study, can be related to the decrease in level of wheat and nitrogen-free extract in SBM diets. As has been observed in sea bass (Dias *et al*., 1998; Peres *et al*., 1999), hepatosomatic index increased with the increase of dietary digestible carbohydrate.

Information on the nutritional status and health in fish species can be achieved through the study of blood metabolites (Peres *et al*., 2014; Bonvini *et al*., 2015). To our knowledge, few studies have assessed blood parameters in response to SBM inclusion in low FM diets in marine species. In aquaculture, this practice is not widely spread and it is to be expected that, as occurs in relation to land animals, blood analysis will become a useful tool (Peres *et al*., 2014). Data obtained in our study are within the ranges reported for European sea bass under good nutritional status (Peres *et al*., 2014). However, we found some differences between feeding treatments. Hematocrit averaged between 41.4 and 46.2, with lower values recorded in fish fed diet 20SBM and 30SBM. As reported for juvenile beluga (*Huso huso*) (Hosseini and Khajepour, 2013), hematocrit was significantly lowered by increasing SBM of diets. On the contrary, Moradi *et al*. (2013) reported that hematocrit concentration decreased parallel to plant protein inclusion in diet in *Cyprinus carpio*. Plasma proteins averaged between 5.7 and 5.1 g/dl, with the lowest value recorded in diet 30SBM (5.1 ± 0.4 g/dl). Plasma protein level is usually very stable in well-nourished animals but decreases under fasting conditions (Peres *et al*., 2014). Triglycerides and cholesterol levels are in agreement with previously reported values for this species (Couto *et al*., 2015), but higher than those reported by Peres *et al*. (2014). Regarding cholesterol, values decreased with the increasing of SBM inclusion within low FM diets. Our data confirm the hypocholesterolemic effect of soybean components in European sea bass, as previously reported in several researches (Gómez-Requeni *et al*., 2004; Couto *et al*., 2015).

Regarding histology, no inflammatory and/or degenerative changes were recorded in any of the histological sections. European sea bass seems to be less sensitive to certain soy-anti-nutritional factors, which induce intestinal disturbances in salmonids (Tibaldi *et al*., 2006). Bonaldo *et al*. (2008) also reported no evidence of soy-induced enteritis in European sea bass juveniles fed diets with 35% of FM and 30% of SBM. Our results support the thesis that SBM, even at the

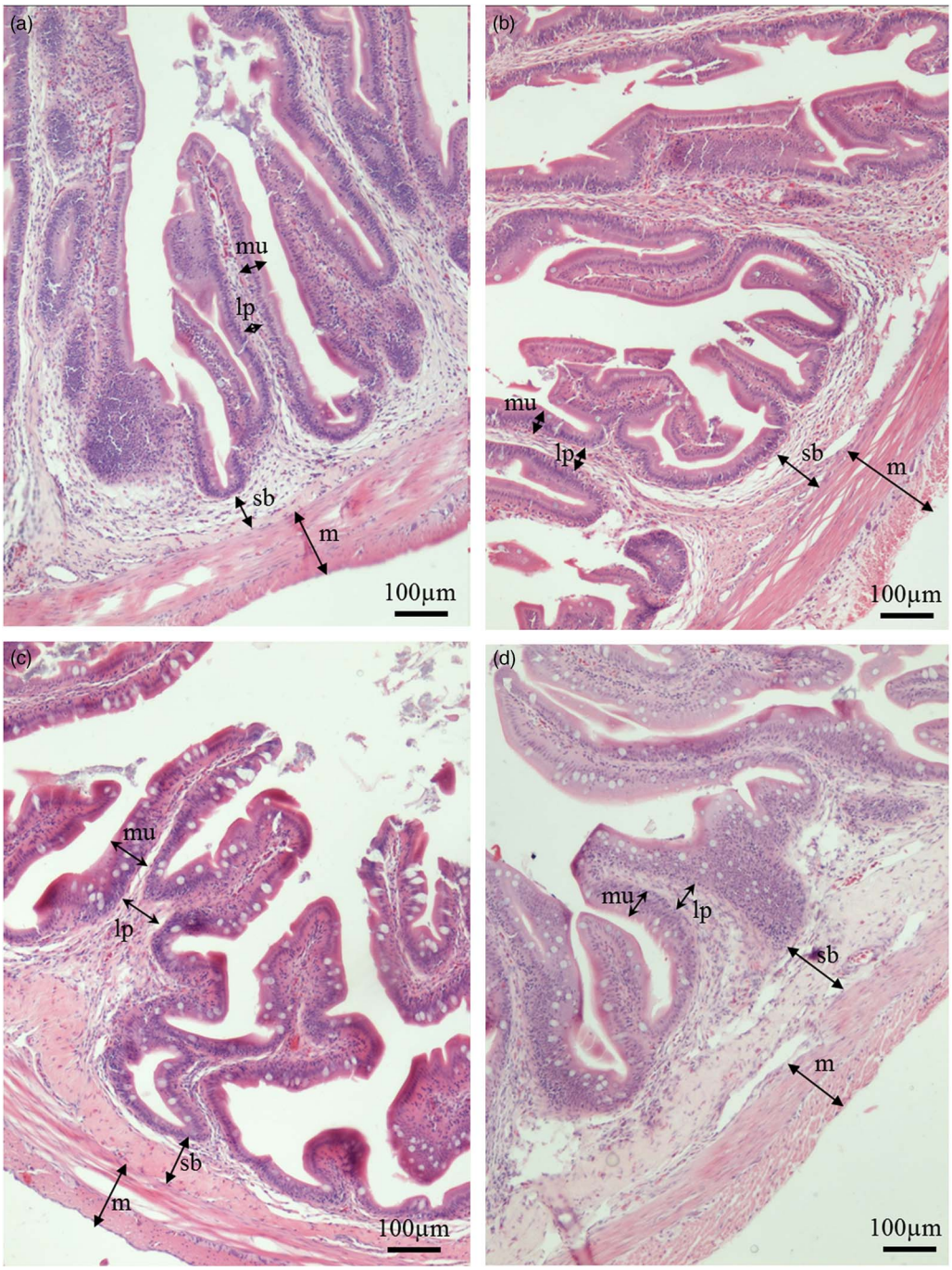

Figure 1 Histology of the proximal and distal intestine of sea bass fed the control diet (CD) (a, c) and 30SBM diet (b, d). Sections show a normal architecture of the mucosal layer (mu) that displays a regular columnar epithelium with polarized and basally located nuclei, of the *lamina propia* (lp) and submucosal layer (sb) that show a loose connective tissue rich in capillary network, and of the muscular layer (m) for both treatments (hematoxylin and eosin, bar = 100 µm).

high inclusion rate of 30% in a diet with only 20% of FM, did not lead to soy-induced enteritis in European sea bass. Further, no differences were recorded in measurement and scoring in proximal and distal tracts in CD and 30SBM diets, and a slight depletion of goblet cells in proximal tracts in 30SBM diet was the only difference reported. A few references are available on this subject. Olsen *et al.* (2007) observed in cod (*Gadus morhua*) goblet cell hypertrophy and hyperplasia in the distal parts of the gastrointestinal tract in fish fed high plant protein levels. Notwithstanding the above, a few studies reported a depletion in number of goblet cells in fish fed a diet with other vegetable raw materials like Jojoba meal (Saleh and Toutou, 2015) and vegetable oil (Perez-Sanchez *et al.*, 2013), but to our knowledge the reasons for this decrement are still unknown. Further investigations will be necessary to determine and understand the effects of the diets on the depletion of these cells.

Table 5 *Morphometry and histopathological scoring of European sea bass fed diets CD and 30SBM*

	Proximal					Distal				
	CD		30SBM			CD		30SBM		
	Median	Range	Median	Range	*P* value	Median	Range	Median	Range	*P* value
Morphometry (μ)										
Lamina propria	56.2	26.1 to 71.1	50.5	35.2 to 88.1	0.4428	46.2	22.8 to 62.2	45.9	38.4 to 72.1	0.5755
Width mucosa	39.0	27.2 to 53.4	33.2	25.0 to 46.9	0.1408	30.5	25.6 to 45.5	31.5	24.9 to 51.9	0.5755
Height mucosa	37.3	30.5 to 47.3	35.4	28.5 to 51.2	0.8519	37.1	28.1 to 49.8	36.8	28.4 to 56.3	0.7874
Submucosa	145.1	37.9 to 266.6	152.2	75.3 to 238.2	0.9174	124.7	83.5 to 404.8	162.6	82.0 to 220.1	0.3296
Goblet cells (number)	56.3[b]	24.0 to 116.3	24[a]	6 to 96.7	0.0016	147.3	43.3 to 265.7	103.0	6.3 to 298.0	0.2058
Scoring (0 to 3)										
Inflammatory cells	1	0 to 2	1	0 to 2	0.4553	1	1 to 3	1	0 to 3	0.4306
Mast cells	0	0 to 2	1	0 to 2	0.4553	1	0 to 3	1	0 to 3	0.8034
Intraepithelial lymphocytes	1	0 to 2	1	0 to 3	0.5069	1	0 to 3	1	0 to 2	0.4428

CD = control diet; 30SBM = 300 g/kg SBM diet.
Each value is median from 15 samples.
[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$.

In conclusion, from our findings we can state that for European sea bass: (i) different blends of plant protein did not affect feed intake despite the 20% FM dietary level; (ii) the inclusion of SBM maintains optimal growth and feed utilization in low FM diets; (iii) blood biochemistry profile showed a good nutritional status under all feeding regimes; (iv) no evidence of soy-induced enteritis was reported in any group fed low FM diets. For formulation of practical diets in on-growing of European sea bass, SBM up to 30% can be successfully incorporated in low FM.

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