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Abstract

In the issue of fishmeal replacement, besides maintaining optimal growth, a key area of investigation for continuing to improve modern aquafeeds includes the evaluation of the effects of plant ingredients on fish quality. It is generally accepted that farmed fish quality can be influenced by the formulation of composition of their feed. Hence, the aim of the present research was to evaluate plant protein inclusion up to 84% of the overall protein content in an integrated study on growth and quality traits of European sea bass. Three diets were formulated to contain increasing plant protein levels (50, 67 and 84%; 50PP, 67PP and 84PP, respectively), with fishmeal dietary levels at 30, 20 and 10%, respectively. No significant differences due to reducing fishmeal content were observed after 118 days in terms of growth (final body weight and specific growth rate) and feed intake, even though a trend towards lower growth performance at higher fishmeal replacement levels was observed. Fish fed diet 50PP showed lower feed conversion rate in comparison to those fed diet 84PP, while no differences were recorded between diet 50PP and 67PP. No significant differences among treatments were found in protein efficiency rate. On the contrary, fish fed diet 84PP showed lower gross protein efficiency in comparison to those fed diet 50PP and 67PP. No significant differences in biometric indices and fillet composition were observed. No significant differences were found in pH, liquid holding capacity and skin colour measurements between treatments, while regarding fillet colour, significant differences were found only for $H°_{ab}$. In conclusion, our findings demonstrated that dietary plant proteins up to 84% of the overall protein content had no effects on quality traits of European sea bass

in comparison with 50% and 67%. All experimental groups showed similar growth even though 84% plant protein inclusion negatively influenced feed and protein utilisation.

Keywords: European sea bass; plant proteins; fishmeal; growth; quality; fillet

1. Introduction

In the issue of fishmeal replacement, besides maintaining optimal growth, a key area of investigation for continuing to improve modern aquafeeds includes the evaluation of the effects of plant ingredients on fish quality. With the progressive reduction of fishmeal dietary content, feeds are composed of different ingredients mixed in various proportions to complement each other. Altering the composition of feed has an important impact on several parameters directly influencing the quality of the fish, such as colour and appearance, smell and taste, texture, and nutritional quality (Lie, 2001).

Texture is an important quality attribute of acquatic foods and its shear force determination requires careful attention due to the unique fish muscle structure being organised in myotomes held together by thin membranous myocommata (Cheng et al., 2014). Variable blade and Allo-Kramer shear have been proposed in literature as instrumental texture methods and comparable sensitivity in detecting variation in fillet texture were evidenced (Aussanasuwannakul et al. 2012). Among the many plant proteins available for aquafeed, soy products are one of the most interesting alternatives because of the advantages of supply, price, protein and amino acid composition (Parma et al., 2016). Glutens have high protein level, are low in fibre, rich in vitamins B and E and are known to contain no antinutritional factors (Bonaldo et al., 2011, 2015).

European sea bass (*Dicentrarchus labrax*) is an important aquaculture marine finfish species in Europe, particularly in the Mediterranean, cultured for food production. Several studies have compared wild *vs* cultured (Alasalvar et al., 2002; Fasolato et al., 2010; Fuentes et al., 2010) and organic *vs* conventionally-farmed (Trocino et al., 2012; Di Marco et al., 2017) European sea bass in terms of quality traits. To our knowledge, limited information are available on the effects of dietary plant proteins on fillet quality of European sea bass. Hence, the aim of the present research was to evaluate plant protein inclusion up to 84% of the overall protein content in an integrated study on growth and quality traits of European sea bass.

2. Materials and methods

2.1. Experimental diets

Three isonitrogenous and isolipidic experimental sinking diets were formulated to contain increasing plant protein levels (50, 67 and 84%; 50PP, 67PP and 84PP, respectively), with fishmeal dietary levels at 30, 20 and 10%, respectively. Fishmeal was replaced by adding a combination of wheat gluten and soy protein concentrate. The small variation of soya extract between diets was due to the balancing of nutrient composition of the diets. DL-methionine and L-lysin were added in order to obtain similar diets in terms of amino acid content when changing the inclusion of the protein raw materials. The diets were produced by extruded process by Skretting Aquaculture Research Centre, Stavanger, Norway. The diameter of the pellet was 4 mm. Ingredients and proximate composition of the experimental diets are presented in Table 1, while

proximate and amino acid composition of the plant protein ingredients used are shown in Table 2 as provided by NRC, 2011.

2.2. 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 Panittica Pugliese (Torre Canne di Fasano, Brindisi, Italy). At the beginning of the trial 60 fish (initial average weight: 66.2 ± 1.7 g) per tank were randomly distributed into nine 900 L square tanks with a conical bottom. Each diet was administered to triplicate groups, assigned in a completely random manner, over 118 days. Tanks were provided with natural seawater and connected to a closed recirculating system (overall water volume: 18 m^3). The rearing system consisted of a mechanical sand filter (PTK 1200, Astralpool, Barcelona, Spain), ultraviolet lights (SH-2500 38 W, Philips, Amsterdam, the Netherlands) and a biofilter (PTK 1200, Astralpool, Barcelona, 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 - TAN), nitrite and salinity (25 g L^{-1}) were daily monitored spectrophotometrically (Spectroquant Nova 60, Merck, Lab business, Darmstadt, Germany). Values recorded for TAN and nitrite during the trial have been always below 0.1 ppm and 0.2 ppm,

respectively. Sodium bicarbonate was added on a daily basis to keep pH constant at 7.8–8.0.

Feed was provided to apparent satiation by oversupplying the feed by approximately 10% of the daily ration, twice a day for six days a week, while one meal was supplied on Sundays, as reported by Mongile et al. (2014). Each meal lasted 1 hour. Feed delivered was recorded daily and the uneaten feed was collected by an uplift system 30 min post feeding. The uneaten pellets of each tank were gathered, dried overnight at 105°C and their weight was deducted for overall calculation.

2.3. Sampling

At the beginning and at the end of the experiment, all the fish in each tank were anaesthetised 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 ratio (FCR) were calculated. Survival rate was calculated as a percentage of the initial number of fish. The proximate composition of the carcasses was determined at the beginning of the trial on a pooled sample of 10 fish and on a pooled sample of 5 fish per tank at the end of the trial. Protein efficiency ratio (PER) and gross protein efficiency (GPE) were calculated. Furthermore, at the end of the trial, wet weight, viscera, liver, mesenteric fat and fillet weight were individually recorded for 10 fish per tank to determine viscerosomatic index (VSI), hepatosomatic index (HSI), mesenteric fat index (MFI) and fillet yield (FY). The proximate composition of the fillet was determined at the end of the trial on a pooled sample of 5 fish per tank. Moreover, at the end of the trial, quality assessment

was performed. 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.

2.4. Calculations

The formulae employed were as follows:

Specific growth rate (SGR) (% day^{-1}) = 100 * (ln FBW- ln IBW) / days (where FBW and IBW represent the final and the initial body weights). Voluntary Feed Intake (VFI) (g feed/fish) = g feed ingested / fish number. Feed conversion ratio (FCR) = feed intake / weight gain. Viscerosomatic index (VSI) (%) = 100 * (viscera weight / body weight). Hepatosomatic index (HSI) (%) = 100 * (liver weight / body weight). Mesenteric fat index (MFI) (%) = 100 * (mesenteric fat weight / body weight). Fillet yield (FY) (%) = 100 * (skinned fillet weight / body weight). Protein efficiency rate (PER) = (FBW – IBW) / protein intake. Gross protein efficiency (GPE) (%) = 100 * [(% final body protein * FBW) - (% initial body protein * IBW)] / total protein intake fish. Economic efficiency ratio (ECR) (€/kg) = (total feed intake * feed cost) / weight gain. Diet cost (50PP, 1.09 €/kg; 67PP, 0.99 €/kg; 84PP, 0.96 €/kg) was calculated at formulation level according to the average current prices of raw materials provided by Skretting Italy, Mozzecane VR, Italy.

2.5. Quality assessment

2.5.1. Determination of fillet pH, Liquid holding capacity (LHC) and Allo-Kramer shear

We adopted the approach used in the studies by Álvarez et al. (2008) for pH and Veiseth-Kent et al. (2010) for Liquid holding capacity (LHC). For pH, a sample from 3 fish per tank was collected from the dorsal-left skinned fillet, homogenised and then pooled into one sample (one pool per tank). Ten grams of each sample were blended with 100 ml distilled water and the pH value of the fish homogenate was measured using a pH-meter (Crison Instruments S.A., Barcelona, Spain), standardised at pH 4.01 and 7.00.

For LHC, a sample from 5 fish per tank was collected from the dorsal-left skinned fillet and individually analysed. Muscle samples were weighed and placed in a tube with a weighted absorbing paper (Schleicher & Schuell GmbH, Dassel, Germany) (V1). The tubes were centrifuged at 45 *g* for 10 min at 10°C (TJ-25 Centrifuge, Beckman Coulter, Brea, CA, U.S.A.) and the wet paper was weighed (V2) before drying at 50 °C until constant weight (V3). The formulae employed were as follows: Liquid loss (LL) = 100* (V2 - V1) * S^{-1} (where S = weight of muscle sample). Water loss (WL) = 100 * (V2 - V3) * S^{-1}. Fat loss (FL) = 100 * (V3 - V1) * S^{-1}. All losses were expressed as a percentage of muscle wet weight.

For Allo-Kramer share test, a sample from 3 fish per tank was collected from the dorsal skinned fillet near the central backbone and then the fillets were diced (5 × 5 mm), pooled into one sample (one pool per tank) and formed into bricks (33 × 24 × 15 mm) using a metallic mould. Six bricks were prepared for each tank. The Allo-Kramer test was performed either on a raw brick at a time or after thoroughly moist-heating it at 80°C core temperature in a Lainox Minimix steam oven (Lainox S.r.l., Vittorio Veneto,

Italy), as checked with a digital thermometer upon removal from the oven. Texture was analysed using an Instron Model 3365 operated by Bluehill 2 software, version 2.19 (Instron Engineering Corp., Canton, MA, U.S.A.). The five-blade Allo-Kramer shear compression cell (adapted to a 2 kN load cell) was operated at 100 mm/min over a fixed distance to determine peak shear force. Allo-Kramer shear values were reported as kilograms shear per gram of sample.

2.5.2. Skin and fillet colour

Colour measurements were evaluated with a Minolta Chroma Meter CR400 (Minolta, Osaka, Japan), according to Álvarez et al. (2008). Colours were expressed as CIELab coordinates. In this system, L^* denotes lightness on a 0–100 scale of black to white; a^*, (+) red or (−) green; b^*, (+) yellow or (−) blue. Colour intensity is expressed by chroma (C^*_{ab}) value and hue ($H°_{ab}$) is the name of a colour as it is found in its pure state on the spectrum. Values were calculated using the formulae: $C^*_{ab} = (a^{*2} + b^{*2})^{1/2}$ and $H°_{ab} = \arctan (b^*/ a^*)$.

Skin colour measurements were estimated on 5 fish per tank. Four colour measurements were performed on each individual on the left side, as described by Pavlidis et al. (2006): two on the dorsal skin area, (i) at the positions where the vertical line to the longitudinal body axis passes through the anterior margin of the dorsal fin (D1) and (ii) through the anus (D2); two at the ventral skin area, (i) below the pelvic fin (V1) and (ii) at the position where the vertical line to the longitudinal body axis passes through the anus, crossing the parallel line to the longitudinal body axis as it passes through the ventral margin of the caudal peduncle (V2) (Fig. 1).

Fillet colour measurements were estimated on 5 fish per tank. Two colour measurements were performed on each individual on the left side, both on the dorsal side: (i) at the positions where the vertical line to the longitudinal body axis passes through the anterior margin of the dorsal fin ($D1_f$); (ii) through the anus ($D2_f$). European sea bass is characterised by a white colour of the fillet, thus two Whiteness index (Wtn1 and Wtn2) were computed on the fillet of each fish, according to Schubring (2010). Values were calculated using the formulae: $Wtn1 = L^* - 3b^*$ and $Wtn2 = 100 – ((100-L^*)^2+a^{*2}+b^{*2})^{1/2}$.

2.6. Analytical methods

Diets, whole body and fillets were analysed 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, U.S.A.).

2.7. Statistical analysis

All data are presented as mean ± standard deviation (SD). Data were analysed by a one-way ANOVA and in case of significance ($P \leq 0.05$) Tukey’s post hoc test was

performed. The normality and/or homogeneity of variance assumptions were validated for all data preceding ANOVA. Linear regression models have been applied in order to measure the effect of the increasing percentages of dietary plant protein on FBW, SGR, VFI, FCR, PER and GPE. Allo-Kramer shear values were analysed by two-way ANOVA using diet and state (raw and cooked) as independent factors and in case of significant interaction ($P \leq 0.05$) Tukey's post hoc test was performed. All statistical analyses were performed using GraphPad Prism 6.0 for Windows (Graph Pad Software, San Diego, CA, USA).

3. Results

3.1. Growth

Growth performances, survival, nutritional indices and ECR are summarised in Table 3. No significant differences due to reducing fishmeal content were observed after the 118 days in terms of growth performance (final body weight and SGR), survival and feed intake (VFI) among treatments. Fish fed diet 50PP showed lower FCR in comparison to those fed diet 84PP, while no differences were recorded between diet 50PP and 67PP. No significant differences ($P \leq 0.05$) among treatments were found in PER, even if a marginally significance ($P < 0.1$) was evident. Fish fed diet 84PP showed lower GPE in comparison to those fed diet 50PP and 67PP. No significant differences among treatments were found in the ECR. The effects of the increasing percentages of plant protein on FBW, SGR, VFI, FCR, PER and GPE are shown in Fig.

2. A significant correlation ($P \leq 0.05$) was observed for FCR, PER and GPE while a marginally significant correlation ($P < 0.1$) was observed for SGR.

3.2. Quality assessment

Data on biometric indices and fillet composition are shown in Table 4. No significant differences in VSI, HSI, MFI and FY were observed. Regarding fillet proximate composition, no significant differences were found between fish fed the different experimental diets.

pH and LHC of the fillets are shown in Table 5. No significant differences were found in pH and in the three parameters analysed for the LHC (Liquid loss; Water loss; Fat loss). Allo-Kramer shear values are shown in table 6. No significant effect of diet was found while a significant effect of the state and a significant interaction between diet and state was displayed. Cooked fillet showed higher shear values in comparison to raw fillet. Skin and fillet colour measurements are shown in Tables 7 and 8 respectively. No significant differences among treatments were found in skin colour measurements. Regarding fillet colour, significant differences were found only for $H°_{ab}$; fish fed diet 84PP showed lower values in comparison to those fed diet 67PP and 50PP.

4. Discussion

Several studies have investigated the utilization of plant dietary inclusion in European sea bass, as a fishmeal replacement; but to our knowledge, few studies investigated the effects on both growth and quality traits.

In the present study, increasing the dietary levels of plant protein up to 84% of the overall protein content did not lead to a depletion in terms of body weight and specific growth rate, even though a trend towards lower growth performance at higher fishmeal replacement was observed. The growth performances registered in this trial are similar to those found in other studies on European sea bass (Guerreiro et al., 2015). As reported by Médale and Kaushik (2009), a blend of plant protein sources can replace 75 to 95% of fishmeal in almost all species, thus reducing the pressure of aquaculture on marine resources. Kaushik et al. (2004) observed that an almost total replacement of fishmeal by a mixture of several plant protein sources had no influence on final weight at commercial size in European sea bass.

Concerning feed conversion rate, in fish fed diet 67PP and 50PP values registered are similar to those found in other studies on European sea bass. As previously reported, European sea bass fed blends of plant protein at 20% fishmeal inclusion showed no negative effects on growth, feed intake and feed efficiency (Bonvini et al., 2017). On the other hand, although we did not find significant differences between groups in growth and feed intake, the 84% plant protein dietary inclusion determined a negative effect on feed conversion rate and protein utilisation. Generally, one of the reasons for reduced performances in fish fed diets containing plant ingredients is a decrease of feed intake, due to reduced feed palatability. Interestingly, according to our findings, the highest inclusion of plant protein had an effect only on feed utilisation, while no influence on palatability was recorded. Robaina et al. (1999) reported high digestibility coefficient of protein from wheat gluten in European sea bass. Moreover, Messina et al. (2013), showed that replacing up to 70% fishmeal protein with wheat gluten in diets supplemented with the most limiting amino acids did not adversely affect feed intake,

growth, feed and nutrient conversion efficiency in European sea bass. However, in these studies, the fishmeal level ranged between 19 and 45 % and was higher in comparison to a 10% of fishmeal in diet 84PP tested in the present trial. In addition, it is worth to mention that despite the negative trend in feed utilization at increasing fishmeal replacement no significant differences in the economic efficiency ratio was found among treatments. Regarding fish quality, it is encouraging to note that inclusion of high levels of plant proteins up to 84% did not affect morphological traits and fillet proximate composition. In particular, fillet yield ranged between 47% and 49%. Those values are comparable to those reported by Poli et al. (2001) and Tibaldi et al. (2015) (calculated on fillet with skin). Moreover, fillet protein composition values found in our trial are comparable to those reported for organic, conventionally-farmed and fed experimental diets, ranging between 18.6% and 19.2% (Trocino et al., 2012; Tibaldi et al., 2015). The quality of the edible portion, destined to the consumer, seemed not to be affected by high plant protein inclusion. Also, increasing dietary plant proteins did not affect Allo-Kramer shear values of the fillet. Differences were only recorded between the raw and cooked fillet, with increasing values for the cooked state in all the treatments. Comparing data on flesh texture among different studies can be difficult due to the different analysis methods utilised. Moreover, the few references available on the effects of diet composition on these traits are generally related to dietary lipids: different fat contents and lipid sources could have an effect and modify the texture. Different authors found that an increase in fat content led to a decrease in firmness (Johnston et al., 2006; Fuentes et al., 2010).

The colour of fish products deserves particular attention from the point of view of consumer acceptance because together with price and freshness, this is one of the

choosing traits that may influence purchasing decision criteria. The skin and fillet colour of fish fed the different diets were similar and were not substantially affected by the difference in amount of dietary plant protein. To our knowledge, few references are available on the effects of a blend of plant protein on skin and fillet colour, and where they exist they are generally focused on the effects relative to a specific ingredient. In salmonids and gilthead sea bream, dietary corn gluten meal has a notable effect on fillet colour (Hardy 1996; Robaina et al., 1997; de Francesco et al., 2004). In European sea bass, increased greenish skin pigmentation was associated with a slightly enhanced yellowish flesh in fish fed the diets containing dried microalgae (Tibaldi et al., 2015). On the other hand, fish colour could also be influenced by other factors such as rearing environment and temperature, swimming possibilities, feeding regime, storage period (Fuentes et al., 2010). In addition, differences in moisture content may also explain the changes in colour between fish: higher moisture content contributes to the creation of refractive indices within the food matrix leading to a lighter colour (Fuentes et al., 2010: Trocino et al., 2012). In our trial, only a slight decrease in $H°_{ab}$ in fish fed diet 84PP was found. Despite the significant difference, such a slight change in colour cannot be detected by the human eye.

In conclusion, our findings demonstrated that dietary plant proteins up to 84% of the overall protein content had no effects on quality traits of European sea bass in comparison with 50% and 67%. All experimental groups showed similar growth even though 84% plant protein inclusion negatively influenced feed and protein utilisation.

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

	Experimental diets		
	50PP	67PP	84PP
Ingredients, % of the diet			
Wheat gluten	8.00	10.00	16.19
SPC 60%	8.00	13.00	17.00
Fishmeal LT	30.00	20.00	10.00
Corn gluten	9.62	8.91	9.00
Soya extract	18.18	17.00	15.94
Wheat	11.62	8.60	7.00
Fish oil	9.62	10.11	10.59
Rapeseed oil	9.62	10.11	10.59
DL-Methionine	0.06	0.12	0.20
Phosphate	0.23	1.11	2.02
L-Lysine	0.20	0.59	1.00
Vit/Min premix[1]	0.46	0.46	0.46
Proximate composition[2], %			
Protein	44.4	43.0	43.5
Lipid	25.0	24.9	24.9
Ash	6.2	5.5	4.7
Moisture	5.7	6.1	6.5
Gross Energy (MJ)	23.3	23.5	23.2

[1]Vitamin and mineral premix; Skretting, Stavanger, Norway (fulfilling recommendations for marine fish species given by NRC, 2011).
[2]Values are reported as mean of duplicate analyses.

Table 2. Proximate and amino acid composition of the plant protein ingredients (as-fed basis) used for the experimental diets (data from NRC, 2011).

	Wheat gluten	SPC 60 %	Corn gluten	Soya extract
Proximate composition, %				
Dry matter	89	92	91	90
Protein	80.7	63.6	63.7	48.5
Lipid	1.5	0.5	2.2	0.9
Ash	0.7	—	1.6	5.8
Amino acid composition, %				
Arginine	3.80	4.64	1.90	3.60
Histidine	2.00	1.58	1.20	1.30
Isoleucine	3.70	2.94	2.30	2.60
Leucine	6.30	4.92	9.40	3.80
Lysine	4.90	3.93	1.07	2.24
Methionine	1.60	0.81	1.90	0.70
Cystine	—	0.89	1.10	0.71
Phenylalanine	4.50	3.28	3.80	2.70
Tyrosine	—	2.30	0.87	1.25
Threonine	1.60	2.47	2.00	2.00
Tryptophan	1.05	0.84	0.30	0.70
Valine	4.00	3.06	2.70	2.70

Table 3. Growth performance, feed intake and nutritional indices of European sea bass fed experimental diets over 118 days.

	Experimental diets			
	50PP	67PP	84PP	*P* value
IBW (g)	65.6 ± 1.0	66.2 ± 1.1	66.8 ± 2.7	0.7153
FBW (g)	214.2 ± 15.1	213.3 ± 8.8	199.7 ± 6.2	0.2597
SGR (%day^{-1})	1.00 ± 0.05	0.99 ± 0.04	0.93 ± 0.03	0.1511
VFI (g feed/fish)	190 ± 7.3	201 ± 5.9	193 ± 5.3	0.1721
FCR	1.28 ± 0.08^{a}	1.37 ± 0.04^{ab}	1.45 ± 0.05^{b}	0.0372
Survival (%)	99.4 ± 1.0	96.7 ± 4.4	99.4 ± 1.0	0.3955
Nutritional indices				
PER	1.76 ± 0.11	1.70 ± 0.05	1.58 ± 0.06	0.0722
GPE	29.6 ± 1.10^{b}	28.8 ± 0.79^{b}	25.2 ± 1.17^{a}	0.0045
ECR (€/kg)	1.40 ± 0.09	1.36 ± 0.04	1.40 ± 0.05	0.6556

Data are given as the mean (n=3) ± SD. In each line, different superscript letters indicate significant differences among treatments ($P \leq 0.05$).
IBW = Initial body weight.
FBW = Final body weight.
SGR = Specific growth rate (% day^{-1}) = 100 * (ln FBW- ln IBW) / days.
VFI = Voluntary Feed Intake (g feed/fish) = g feed ingested / number of fish.
FCR = Feed conversion rate = feed intake / weight gain.
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.
ECR = Economic efficiency ratio (€/kg) = (total feed intake * feed cost) / weight gain.

Table 4. Biometric indices and fillet composition of European sea bass fed the experimental diets.

	Experimental diets			
	50PP	67PP	84PP	*P* value
Biometric indices				
VSI	11.1 ± 1.7	11.5 ± 1.4	11.3 ± 1.5	0.5691
HSI	2.9 ± 1.5	2.7 ± 0.5	2.3 ± 0.6	0.0677
MFI	5.7 ± 1.7	5.8 ± 1.6	5.8 ± 1.5	0.9292
FY	48 ± 4.3	49 ± 3.5	47 ± 3.9	0.2214
Fillet proximate composition				
Protein	19.2 ± 0.4	19.0 ± 0.4	18.6 ± 0.4	0.3158
Lipid	11.7 ± 0.7	12.3 ± 1.1	12.6 ± 1.8	0.7057
Ash	1.3 ± 0.0	1.2 ± 0.0	1.2 ± 0.1	0.1250
Moisture	68.8 ± 0.4	68.7 ± 0.9	67.7 ± 1.6	0.4456

Data are given as the mean (n=3; n=30 for VSI, HSI, MFI, FY) ± SD.
VSI = Viscerosomatic index (%) = 100*(viscera weight/FBW).
HSI = Hepatosomatic index (%) = 100*(liver weight/FBW).
MFI = Mesenteric fat index (%) = 100*(mesenteric fat weight/FBW).
FY = Fillet yield (%) = 100 * (skinned fillet weight/FBW).

Table 5. pH and LHC (liquid holding capacity) of fillet of European sea bass fed experimental diets.

	Experimental diets			
	50PP	67PP	84PP	*P* value
pH	6.54 ± 0.06	6.48 ± 0.02	6.54 ± 0.04	0.2255
Liquid holding capacity				
LL (%)	10.33 ± 1.82	10.37 ± 0.87	8.68 ± 0.43	0.2203
WL (%)	0.92 ± 0.56	0.91 ± 0.07	0.60 ± 0.24	0.4951
FL (%)	9.41 ± 1.27	9.46 ± 0.84	8.08 ± 0.38	0.1878

Data are given as the mean (n=3) ± SD.
LL = Liquid loss.
WL = Water loss.
FL = Fat loss.

Table 6. Allo-Kramer shear values of the fillets at the raw and cooked state, expressed as kg/g.

	Experimental diets				*P-values*		
	50 PP	67 PP	84 PP	Diet effect	*Diet*	*State*	*Interaction*
Raw	0.54 ± 0.06^{a}	0.52 ± 0.07^{a}	0.52 ± 0.07^{a}				
Cooked	1.00 ± 0.12^{b}	1.13 ± 0.07^{b}	1.10 ± 0.18^{b}	0.79 ± 0.29	0.2551	0.0001	0.0384
State effect	0.76 ± 0.25	0.81 ± 0.31	0.80 ± 0.32				

Data are given as the mean (n=3) ± SD. Values with different superscript letters between lines and columns are significantly different ($P \leq 0.05$).

Table 7. Skin colour measurements of European sea bass fed experimental diets.

	Experimental diets			
	50PP	67PP	84PP	*P* value
Dorsal				
L*	49.0 ± 9.1	50.4 ± 9.6	47.8 ± 8.1	0.6198
a*	-0.77 ± 0.60	-0.70 ± 0.59	-0.91 ± 0.56	0.4957
b*	6.09 ± 1.76	6.48 ± 1.10	6.57 ± 1.03	0.4509
C^*_{ab}	6.19 ± 1.64	6.56 ± 1.03	6.68 ± 0.94	0.4077
$H°_{ab}$	-1.03 ± 0.39	-1.02 ± 0.49	-1.09 ± 0.36	0.8490
Ventral				
L*	84.6 ± 2.3	84.6 ± 2.9	84.6 ± 1.7	0.6146
a*	0.04 ± 0.97	0.05 ± 0.88	-0.16 ± 1.03	0.8057
b*	7.05 ± 2.65	7.12 ± 2.71	7.24 ± 2.38	0.9683
C^*_{ab0}	7.14 ± 2.60	7.20 ± 2.66	7.36 ± 2.33	0.9571
$H°_{ab}$	-0.21 ± 1.01	-0.29 ± 1.03	-0.38 ± 1.00	0.8497

Data are given as the mean (n=3) ± SD.
C^*_{ab} = chroma = $(a^{*2}+b^{*2})^{1/2}$.
$H°_{ab}$ = hue = arctan (b^*/a^*).

Table 8. Fillet colour measurements of European sea bass fed experimental diets.

	Experimental diets			
	50PP	67PP	84PP	*P* value
L*	42.9 ± 1.1	43.1 ± 2.2	43.8 ± 1.6	0.6471
a*	0.87 ± 0.93	0.88 ± 0.94	0.68 ± 0.66	0.9009
b*	-1.36 ± 0.80	-1.56 ± 1.09	-1.07 ± 1.02	0.6896
C^*_{ab}	1.95 ± 0.11	2.14 ± 0.37	1.87 ± 0.30	0.2824
$H°_{ab}$	-0.41 ± 0.15^b	-0.44 ± 0.08^b	-0.10 ± 0.28^a	0.0139
Wtn1	47.2 ± 0.9	47.8 ± 1.1	47.0 ± 1.4	0.5107
Wtn2	42.8 ± 1.2	43.0 ± 2.2	43.7 ± 1.6	0.6471

Data are given as the mean (n=3) ± SD. In each line, different superscript letters indicate significant differences among treatments ($P \leq 0.05$).
C^*_{ab} = chroma = $(a^{*2}+b^{*2})^{1/2}$.
$H°_{ab}$ = hue = arctan (b^*/ a^*).
Wtn1 = whiteness1 = $L^* - 3b^*$.
Wtn2 = whiteness2 = $100 – ((100- L^*)^2+a^{*2}+b^{*2})^{1/2}$.

Figure caption

Fig. 1. Representation of the points (D1-2, dorsal; V1-2, ventral) on the European sea bass where colour was measured.

Fig 2. Linear regression models showing the effect of the increasing percentages of dietary plant protein (PP) on final body weight (FBW, g), specific growth rate (SGR, % day^{-1}), voluntary feed intake (VFI, g), feed conversion rate (FCR), protein efficiency rate (PER) and gross protein efficiency (GPE, %). Significant relations at $P \leq 0.05$

Figure 1

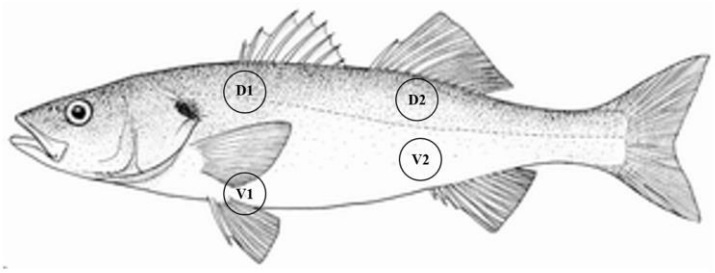
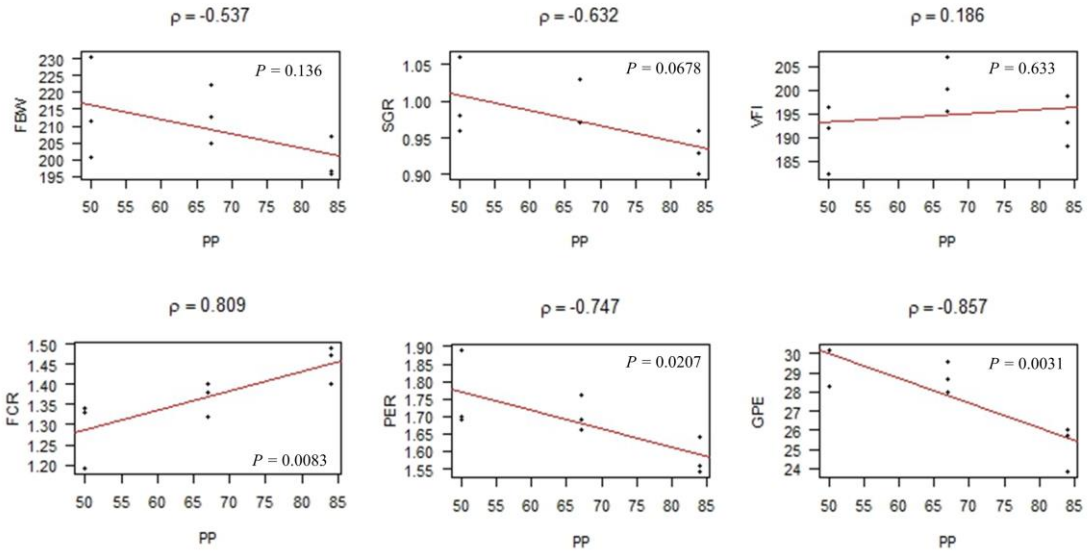


Figure 2


ρ = -0.537
P = 0.136
FBW
PP
ρ = -0.632
P = 0.0678
SGR
PP
ρ = 0.186
P = 0.633
VFI
PP
ρ = 0.809
P = 0.0083
FCR
PP
ρ = -0.747
P = 0.0207
PER
PP
ρ = -0.857
P = 0.0031
GPE
PP