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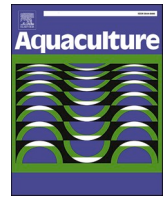
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## Nutritional innovations in superior European sea bass (*Dicentrarchus labrax*) genotypes: Implications on fish performance and feed utilization

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### ABSTRACT

The sustainable development of the aquaculture industry relies on the use of alternative conventional and emergent raw materials that contributes to a circular economy and to reduce the dependency on fish meals and fish oils coming from oceanic fish populations. Additionally, the genetic selection of farmed fish that can display higher growth and feed utilization when fed alternative feeds, is pointed out to be a complementary valuable tool to facilitate the implementation of circular economy approaches. The main purpose of the present study was to determine the effectiveness of genetic selection for growth in European sea bass, in response to a challenge with an alternative diet that aimed to partially replaced fishmeal (FM) by poultry meal (PM) and totally replace fish oil (FO) by a blend of poultry oil (PO) with a novel microalgae oil. The two families of fish juveniles were obtained by *in vitro* fertilization of selected for a multi-trait including high growth (genetically selected, GS) or non-selected (wild type, WT) broodstocks and then were nutritionally challenged with a control diet that mirrored a standard commercial diet with fishmeal (20%) and fish oil (7%), or a Future diet that partially replaced the FM by PM and totally replaced the FO by a blend of rapeseed oil, PO, and a novel DHA rich-algal oil. From the second month of feeding until the end of the trial, European sea bass that was selected since for 7 generations performed better in terms of growth than the wild-type genotype, possibly related with an apparent favored feed and nutrient utilization. Furthermore, selection decreased the perivisceral fat and increased the nutritional value of flesh by increasing DHA (in g/ 100 g flesh) and ARA contents. In contrast, the dietary treatment showed little effect on fish growth performance, denoting the successful partial replacement of FM by PM and the total replacement of FO by a blend of poultry oil and an emergent microalgal oil. However, Future diet tended to reduce the ADCs of some amino acids, as well as showed an additive effect to genotype in increasing the n-3 PUFA of flesh. Altogether, our data demonstrate that multi-trait genetic selection of European sea bass improve fish plasticity to cope with the variations of ingredients in alternative feeds with low FM/FO.

**Abbreviations:** ADC, apparent digestibility coefficient; ARA, arachidonic acid; C, Control diet; DGI, daily growth index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; F, Future diet; FCR, feed conversion ratio; FO, fish oil; FM, fishmeal; GS, genetically selected; HSI, hepatosomatic index; K, condition factor; LC-PUFA, long-chain polyunsaturated fatty acids; LER, lipid efficiency ratio; MUFA, monounsaturated fatty acids; PCA, principal component analysis; PFI, perivisceral fat index; PER, protein efficiency ratio; PM, poultry meal; PO, poultry oil; SGR, specific growth rate; SFA, saturated fatty acids; SL, standard length; TGC, thermal growth coefficient; WT, wild type.

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## 1. Introduction

The limited supply of finite marine raw materials, such as fishmeal (FM) and fish oil (FO) deriving from wild fish stocks and largely used in aquafeeds for many decades, has driven the industry towards alternative ingredients as a tool to reduce the dependency on FM and FO and maintain the sustainability of the aquafeed sector (FAO, 2020). Among the most used alternatives to FM and FO, plant raw materials have been intensively studied, given their wide availability and/or competitive price. Resulting of the intensive research done on these raw materials, moderate to high replacement levels of FM and FO with alternative sources of protein and lipids have been accomplished in many farmed species, including in European sea bass (*Dicentrarchus labrax*) (Kaushik et al., 2004; Torrecillas et al., 2017). However, despite the great progress in reducing and replacing FM in marine aquafeeds, the total replacement of FO remains a major challenge, considering that the aquafeed sector accounts for 75% of the world's total production of FO (Colombo and Turchini, 2021). Furthermore, as aquaculture continues to expand globally as well as the awareness and efforts in meeting sustainability criteria arise, the continuous reduction of the dietary levels of FM and FO to the minimum possible is considered urgent to level up aquaculture as a more responsible food production sector. Unfortunately, the complete replacement of FM or FO by plant meals or oils, often negatively affects fish productive performance, health, and/or the nutritional quality of the flesh, particularly in some carnivorous marine species (Turchini et al., 2013; Nasopoulou and Zabetakis, 2012). These consequences are often associated to an unbalanced dietary protein, amino acid (particularly methionine and lysine) and/or fatty acid profile of plants in comparison with marine raw materials. Therefore, a new approach in aquaculture nutrition is addressing the formulation of alternative sustainable diets that blend emergent and conventional raw materials and use at minimum of FM and FO coming from finite resources, that do not compromise fish performance and maintain the quality and cost of the feeds, to achieve the maximum production potential and maximize the contribution of the sector to a circular economy (Sprague et al., 2017). These emerging ingredients should compensate the dietary imbalances, for instance, those caused by plant sources in the dietary n-3 long-chain polyunsaturated fatty acids (LC-PUFA), which are essential nutrients for marine carnivorous fish and play important functions also in human health. One of those novel lipid sources are algae oils rich in n-3 LC-PUFA, that have been shown to support a reduction in the use of FM/FO of the feeds for a wide number of aquatic species, including European sea bass (Haas et al., 2016; Kousoulaki et al., 2015; Carvalho et al., 2020, 2022; Sarker et al., 2016). In addition to algae, terrestrial animal by-products, such as poultry meal (PM) and poultry oil (PO) are also considered conventional, already used, suitable alternatives to FM and FO, respectively, that can be easily blended with more novel but still expensive ingredients (for example algae oils) to balance the nutritional profile of the feeds (Carvalho et al., 2020, 2022), increasing the flexibility in the formulation. PM is very rich in protein, thus being a good FM replacer for maintaining fish growth performance and feed utilization (Galkanda-Arachchige et al., 2020), whereas PO constitutes a good energy source for fish as it is rich in saturated (SFA) and monounsaturated fatty acids (MUFA).

Genetic selection is another way, potentially complementary to the formulation of novel diets, for solving the FM/FO dependency, by developing fish strains with more plasticity to face future feeding-associated changes in aquafeed formulas and to revert the possible negative impacts of alternative new ingredients on growth and feed efficiency (Gjedrem et al., 2012). In several fish species, some genotypes can experimentally use more efficiently plant protein- or -lipid based diets than others (Dupont-Nivet et al., 2009; Le Boucher et al., 2010; Le Boucher et al., 2012; Yamamoto et al., 2015; Callet et al., 2017). By continuously selecting faster-growing fish fed on low FM/FO diets, the performance and the capacity of fish to utilize alternative diets are increased in the forthcoming generations (Yamamoto et al., 2015). In

this regard, the use of selective breeding in European sea bass, which is an economically important species in the Mediterranean region, is still relatively limited compared with other farmed species, such as Atlantic salmon (*Salmo salar*), or rainbow trout (*Oncorhynchus mykiss*). Indeed, the domestication of this species is relatively recent, with only eight generations away from the wild origin populations (Chavanne et al., 2016). Even though European sea bass shows a large genetic variation for growth, a considerable response to selection in terms of growth rate has been reported (in the range of 20–40% per generation) (Dupont-Nivet et al., 2008; Vandeputte et al., 2009, 2016).

Despite the current knowledge on the enhancing effect of genetically selected fish in utilizing plant-based diets, the ability of selected fish to grow and properly utilize alternative modern aquafeeds with other, more emergent, blends of raw materials, like PM, PO and microalgae oils, that aim to compensate for the low levels of FM/FO, remains to be investigated. Accordingly, the main purpose of the present study was to determine the effectiveness of genetic selection for growth in European sea bass, in response to a challenge with an alternative diet that aimed to partially replace FM by PM and totally replace FO by a blend of PO with a novel microalgae oil. The influence of genetic selection, alternative diet, and their interaction effect were addressed on fish productive parameters, fish proximate and fatty acid composition, the apparent digestibility coefficients of the dietary nutrients, as well as on fish morphological traits.

## 2. Materials and methods

### 2.1. Ethical statement

The animal experiments comply with the guidelines of the European Union Council (2010/63/EU) for the use of experimental animals. The Bioethical Committee of the University of Las Palmas de Gran Canaria approved all the protocols used in the present study (approval no OEBA-ULPGC 27/2019).

### 2.2. Populations design and fish production

On March 2019, sea bass families were produced in one day by mating one origin of dams of the MARBEC-IFREMER broodstock in experimental facility of Palavas-les-flot (France) with two origins of sires named wild-type (WT) or genetically selected (GS).

The 7 dams were derived from the experimental broodstocks mass selected for growth at commercial size for 3 generations (see for the selection protocol Vandeputte et al., 2009). Eggs were collected by stripping and pooled in equal representation between dams, and transferred into 65 tubes (one tube per sire). The *in vitro* fertilization was performed using thawed sperm from 33 sires (genetically selected, GS) derived from the breeding nucleus of the EMG Ecloserie Marine de Gravelines (Gravelines, France) breeding company or 32 wild sires captured in the Gulf of Lion (Wild-type genotype, WT).

GS males were from 7th generations (>35 years) of multitrait mass selection on growth between 350 g to 1.2 kg depending on sex and generations, external morphology on a “wild type” and a limitation in muscular lipid content measured indirectly by microwave with the Torry Fish Fat Meter according to Haffray et al. (2007). The selection pressure varied between 3% and 1% according to generations. Reproduction and fry production were done in the sanitary protected EMG hatchery. The growing and the selection were performed in concrete race-way in Aquanord (Gravelines, France), a land-based fish farm with non-sanitary treated water from the English Channel enriched in liquid oxygen and with a temperature varying yearly from 16 °C to 24 °C. Fish were fed with various type of commercial feed across the generations following a progressive shift in composition with increasing amount of plant protein and lipids ingredients.

This mating scheme by the sire pathway allowed to evaluate half of the selection response (additive genetic effect) in supposing a limited

non-additive effect according to Guinand et al. (2017). The use of dams previously selected during 3 generations may also limit the expression of the additive effects when compared to a pure wild x wild type as the real comparison was then done between 1.5 generations of selection (WT genotype; [3 generations by the dams +0 generation by the sires] divided by 2) and 5 generations (GS genotype; [3 generations by the dams +7 generations by the sires] divided by 2). The selection response is then reported in the publication for 3.5 generations of selection. This response has to be doubled to estimate the real selection response when compared to the wild x wild cross.

The two resulting genotypes from selected sires (GS) or wild sires (WT) were incubated separately at 14 °C until hatching. One-day-old hatched larvae were pooled by equi-representation of each dam and shipped to the University of Las Palmas de Gran Canaria (ULPGC; Las Palmas de Gran Canaria, Spain) by airplane into oxygen-saturated water within transport bags that were kept in insulated boxes. Larvae were grown in separated tanks following the standardized methodology of the Research Group in Aquaculture at the ULPGC facilities (Betancor et al., 2011; Atalah et al., 2011). Progenies from either selected for growth (selected sires x selected dams –GS) or reference fish (wild sires x selected females –WT) were kept at similar conditions during the pre-weaning, weaning, and early juvenile growing phases. At 294 days post hatching (dph), juveniles (34 g) were nutritionally challenged.

### 2.3. Diets of the nutritional challenge

Two isoenergetic and isonitrogenous diets were formulated to meet the nutritional requirements of European sea bass, irrespective of the different formulations. The Control diet (C) mirrored a standard commercial diet with FM (20%) and FO (7%) (Table 1). The Future diet (F) decreased the FM content to 10%, which was replaced by PM (equivalent to 50% of replacement), and the FO content was completely replaced by a blend of rapeseed oil, poultry oil, and a novel DHA rich-algal oil (Table 1). Yttrium premix was added at 0.1% to both diets to further determine the apparent digestibility coefficients (ADC) of

**Table 1**

Ingredients and proximal composition of the experimental control (C) or future (F) diets.

Ingredients (%)	Control diet C	Future diet F
Corn gluten	5	5
Hi Pro Soybean meal <sup>1</sup>	6	6
Wheat gluten	9.04	10.2
Faba bean dehulled <sup>2</sup>	8	8
Wheat	20.25	19.95
Soy protein concentrate <sup>3</sup>	15	15
Fish oil <sup>4</sup>	7.03	
Fish meal <sup>5</sup>	20	10
Rapeseed oil	6.68	8.98
Phosphate	0.6	0.35
Vitamin & mineral mix <sup>6</sup>	0.3	0.3
Poultry meal <sup>7</sup>		10
Poultry oil <sup>8</sup>		1.37
DHA oil <sup>9</sup>		2.75
Lecithin	2	2
Proximal composition (% dry matter)		
Moisture	7.3	7.3
Crude protein	52.3	52.3
Crude fat	17.7	17.8
Ash	5.2	5.2

<sup>1</sup> Soya bean meal: CJ Selecta S.A (Brasil).

<sup>2</sup> Faba beans: Cefetra BV (The Netherlands).

<sup>3</sup> Soya protein concentrate: CJ Selecta S.A (Brasil).

<sup>4</sup> Fish oil: Copeinca, S. A. (Perú).

<sup>5</sup> Fish meal: Norsildmel AS (Norway).

<sup>6</sup> Mineral and Vitamin premix: Trouw Nutrition (The Netherlands).

<sup>7</sup> Poultry meal: Sonac (Belgium).

<sup>8</sup> Poultry oil: Sonac (Belgium).

<sup>9</sup> DHA: Veramaris (Evonik).

protein, amino acids and fatty acids. Diets were manufactured by Skretting (Skretting ARC, Stavanger, Norway) at 1.8, 4.0, and 6.0 mm in diameter and were shipped and analyzed for proximate (Table 1), amino acid (Table 2) and fatty acid composition (Table 3) at ULPGC facilities.

Yttrium premix: 0.1%.

### 2.4. Experimental conditions of the nutritional challenge

The nutritional trial was carried out at the experimental facilities of the ULPGC. Sea bass at 294 dph from each experimental group (GS genotype vs WT genotype) and with an initial body weight of 34.6 ± 1.66 g (mean ± SD), were randomly distributed in 16 experimental tanks, at a density of 50 fish/tank (4 tanks/treatment). Fish were initially allocated in cylinder-conical tanks of 500 L and then transferred to 1000 L-tanks when fish were bigger to maintain the optimal density. All tanks were provided with filtered seawater in a flow-through system under natural photoperiod (12 h light: 12 h dark). Dissolved oxygen and water temperature ranged between 7.5 and 7.7 ppm and 17.3–23.3 °C, respectively. Salinity was 37 g/L. Fish were manually fed until apparent satiation with one of the two experimental diets for 300 days (4 times a day, 6 days a week). Wasted (uneaten) feed was daily recovered in a net by opening the water outlet after meals, dried in an oven for 24 h and weighed to estimate feed intake (FI) and feed conversion ratio more accurately. Fish growth performance was monitored monthly.

### 2.5. Sampling protocol

Prior to each monthly sampling, fish were fasted for 24 h. For growth samplings, all fish were anesthetized with clove oil (4 mL clove oil / 100 L water) and individually weighed and measured. At the end of the experimental period, 16 fish per tank were euthanized with an excess of clove oil, and whole-body samples from 8 fish as well as livers and fillets from other 8 fish were collected and pooled by tank for proximate composition and fatty acid profile analysis. Livers and perivisceral fat were weighed to calculate the hepatosomatic (HSI) and perivisceral fat indices (PFI).

### 2.6. Mathematical equations of fish productive parameters

Fish productive parameters related with growth performance and feed utilization were calculated according to the following equations:

Condition factor (K) = [(weight) x 100/(length)<sup>3</sup>]; Daily Growth Index (DGI) = [(final weight<sup>1/3</sup> - initial weight<sup>1/3</sup>)/number of days x 100]; SGR, Specific Growth Rate (SGR) = [(Ln (final weight)-Ln (initial weight)) / number of days x 100]; Thermal Growth Coefficient (TGC) = [(final weight<sup>1/3</sup> - Initial weight<sup>1/3</sup>) / (temperature x number of days) x

**Table 2**

Amino acid composition (% of feed) of the control (C) or future (F) experimental diets.

Amino acid (% feed)	Control diet C	Future diet F
Alanine	2.17	2.24
Arginine	2.78	2.83
Aspartic acid	3.71	3.81
Cysteine	0.65	0.66
Glutamic acid	10.11	10.33
Glycine	2.14	2.31
Histidine	1.09	1.11
Isoleucine	1.91	1.95
Leucine	3.59	3.70
Lysine	2.29	2.31
Methionine	0.84	0.84
Phenylalanine	2.18	2.28
Proline	3.31	3.44
Threonine	1.62	1.65
Tyrosine	1.25	1.33
Serine	2.20	2.24
Valine	2.05	2.08

**Table 3**

Fatty acid composition (% of total fatty acids) of the control (C) or future (F) experimental diets.

Fatty acid (% total fatty acids)	Control diet C	Future diet F
14:0	2.24	1.92
14:1n-5	0.04	0.03
14:1n-7	0.11	0.09
15:0	0.24	0.22
15:1n-5	0.02	0.02
16:0iso	0.05	0.04
16:0	11.66	10.72
16:1n-7	2.17	2.02
16:1n-5	0.08	0.08
16:2n-6	0.00	0.01
16:2n-4	0.13	0.13
17:0	0.07	0.07
16:3n-4	0.19	0.18
16:3n-3	0.11	0.10
16:3n-1	0.01	0.01
16:4n-3	0.11	0.11
16:4n-1	0.01	0.01
18:0	2.24	2.18
18:1n-9	32.78	32.06
18:1n-7	2.25	2.24
18:1n-5	0.18	0.18
18:2n-6	18.02	17.95
18:2n-4	0.05	0.05
18:3n-6	0.05	0.08
18:3n-4	0.04	0.04
18:3 n-3	4.46	4.60
18:3n-1	0.00	0.00
18:4n-3	0.98	1.06
18:4n-1	0.02	0.03
20:0	0.44	0.45
20:1n-9	0.39	0.42
20:1n-7	4.47	4.55
20:1n-5	0.14	0.14
20:2n-9	0.00	0.01
20:2n-6	0.19	0.20
20:3n-9	0.02	0.02
20:3n-6	0.04	0.03
20:4n-6	0.24	0.25
20:3n-3	0.07	0.07
20:4n-3	0.28	0.30
20:5n-3	2.96	3.38
22:6n-3	4.74	5.80
∑ SFA	16.90	15.55
∑ MUFA	49.84	49.36
∑ n-3	14.05	15.87
∑ n-6	18.68	18.69
∑ n-9	34.03	33.37
∑ n-3 LC-PUFA	8.40	9.99
n-3/n-6	0.75	0.85

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; LC-PUFA: long-chain polyunsaturated fatty acids.

1000)]; Feed Conversion Ratio (FCR) = total feed fed/ weight gain; Protein gain = [((final weight x final protein content of fish) – (initial weight x initial protein content of fish)) / number of days]; Protein efficiency ratio (PER) = weight gained/weight of protein consumed; Lipid gain = [((final weight x final lipid content of fish) – (initial weight x initial lipid content of fish)) / number of days]; LER (lipid efficiency ratio) = weight gained/weight of lipid consumed; Perivisceral fat index (PFI) = perivisceral fat /body weight x 100; Hepatosomatic index (HSI) = wet liver weight /wet body weight x 100.

## 2.7. Biochemical composition

Proximate composition analyses of feed and fish samples (whole-body, flesh and liver) were carried out accordingly with the standardized procedures described by AOAC (1975). Crude protein content (Nx6.25) was analyzed following the Kjeldahl method. Amino acid composition of feeds and feces was determined according to the

principles and methods provided in Commission Regulation (EC) No 152/2009, 2009. Ash content was determined by incineration at 600 °C for 12 h in a muffle furnace, whereas moisture content was determined after drying samples in an oven at 110 °C until constant weight. Total lipid content of the samples was extracted with chloroform/methanol (2:1 v/v) (Folch et al., 1957). Then, fatty acid methyl esters were obtained by transmethylation of total lipids (Christie, 1989) and separated by gas chromatography following the conditions described by Izquierdo et al. (1990). Fatty acid methyl esters were quantified (in % of total fatty acids) by a flame ionization detector and identified by comparison with external and well-characterized FO standards (EPA 28, Nippai, Ltd. Tokyo, Japan).

## 2.8. Apparent digestibility coefficients of the diets

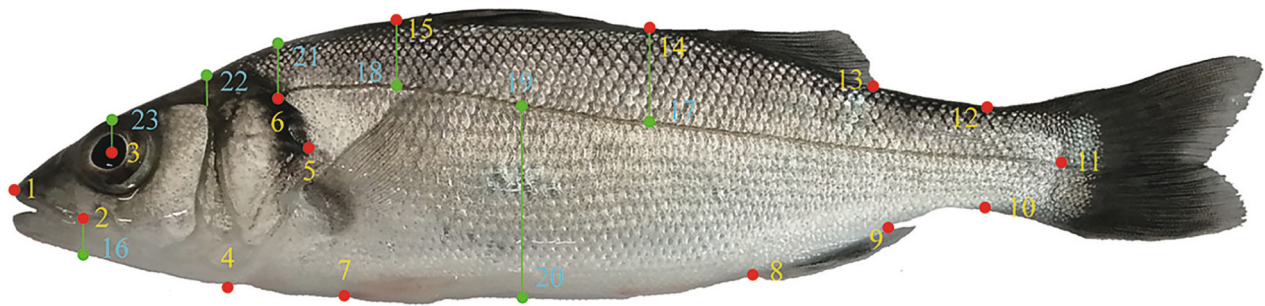
To determine the apparent nutrient digestibility of the experimental diets, feces of fish fed with the different experimental diets were obtained by dissection of fish guts and removal of the digested material. The apparent digestibility coefficients (ADC) of proteins and lipids were determined (Cho et al., 1982) using the following equation: ADC diet (% on a dry matter basis) =  $100 - 100 \times (\%Y_{\text{feed}} / \%Y_{\text{faeces}}) \times (\% \text{Nutrient}_{\text{faeces}} / \% \text{Nutrient}_{\text{feed}})$ , where  $Y_{\text{feed}}$  and  $Y_{\text{faeces}}$  are the dietary and fecal yttrium oxide content, respectively, whereas  $\text{Nutrient}_{\text{faeces}}$  and  $\text{Nutrient}_{\text{feed}}$  are the fecal and dietary nutrient content, respectively.

## 2.9. Morphological analyses

Geometric and morphometric analyses performed for evaluating fish body shape followed standardized protocols (Fernández-Montero et al., 2018). All experimental fish were photographed using digital cameras (Fuji Finepix S2000HD, resolution 10.0 MP; Canon 50D, resolution 10.0 MP, and macro lens F18/100). The body shape of each individual was analyzed using a landmark-based method (Rohlf and Marcus, 1993). Seventeen homologous landmarks (fixed homologous points) and six semi-landmarks (sliding or mobile non-homologous points) on the left side of the body were selected (Fig. 1). The coordinates of these landmarks for each individual were acquired using the tpsDig v. 2.30 software (Rohlf, 2017a). A Generalized Procrustes Analysis (GPA) was performed (Rohlf and Slice, 1990; Dryden and Mardia, 2016) on the raw landmarks data to superimpose all specimens to a common location and remove the effects of size and orientation from landmark coordinates. The tpsSmall v. 1.34 software package (Rohlf, 2017b) was used to evaluate the approximation of the distribution of the specimens in the Kendall's shape space relative to the linear tangent space for each analyzed view (Dryden and Mardia, 2016). The correlation coefficient between tangent distances and the Procrustes distances was high ( $r = 1$ ), indicating that the amount of shape variation was small enough to allow statistical analyses using only the Procrustes distances. Differences in geometric scale were removed during the GPA. The centroid size was computed as the square root of the sum of squared distances of a set of landmarks from their centroid and used as a proxy for body shape ( $r = 0.582$ ,  $p < 0.001$ ).

## 2.10. Statistical analyses

The statistical treatment of the data followed the method outlined by Sokal and Rolf (1995). All data were tested for normality and homogeneity of variance. The individual effects of diet and genotype were analyzed by two-way ANOVA, with diet and genotype as fixed factors. Differences were considered significant at  $p < 0.05$ . At  $t = 0$ , a student's  $t$ -test was applied for testing differences between the two genotypes.  $P$ -values obtained for each parameter evaluated are detailed in the corresponding tables of the Results section. Additionally, a one-way ANOVA was carried out when applicable to generally compare all the dietary treatments when significant interaction GxD was detected with two-way ANOVA ( $P < 0.05$ ). Statistical analyses were performed using

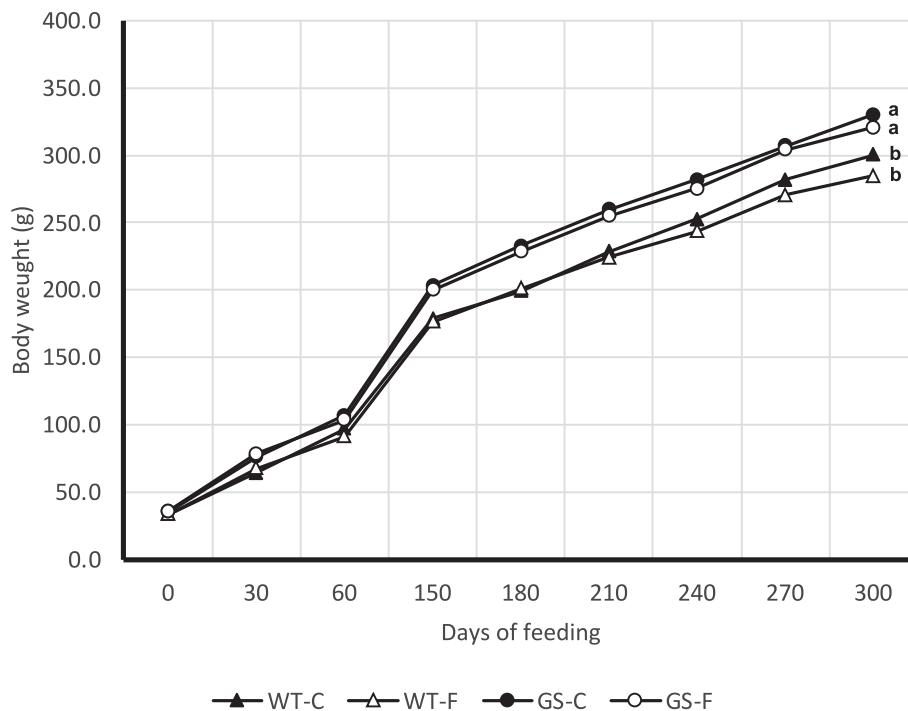


**Fig. 1.** Position and meaning of landmarks (red) and semi landmarks (green) in the body of European seabass: 1- anterior tip of the snout; 2- most posterior-superior point of the premaxilla; 3- midpoint of eye; 4- ventral point of the operculum; 5- limit posterior of the operculum; 6- limit dorsal of the operculum and beginning of the line lateral; 7- insertion of the ventral fin; 8 and 9- anterior and posterior insertion of the anal fin, respectively; 10 and 12- lower and upper of caudal peduncle, respectively; 11- insertion of lateral line with midpoint of the hypural notch; 13 and 14- posterior and anterior insertion of the second dorsal fin, respectively; 15- anterior insertion of the first dorsal fin; 16- ventral projection of premaxilla, 17 and 18- projection on lateral line of the anterior insertion of second and first dorsal, respectively; 19- midpoint between the 17 and 18 semi landmarks; 20- ventral projection of 19 semi landmarks; 21- dorsal projection of 6 initial lateral line, 22- projection dorsal of preopercle; 23- dorsal projection of eye. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the SPSS Statistical Software System v21.0 (SPSS, Chicago, IL, USA) and GraphPad Prism 8 software (GraphPad Software, Inc., La Jolla, CA, USA). Principal components (PCA) were carried out with the R Project for Statistical Computing software, using the statistical packages “FactoMiner 2.4” for data analysis and “Factoextra 1.0.7” for graphical representation. As a prerequisite for the analysis, data normality was verified through multivariate skewness and Kurtosis analysis (Wang and Du, 2000).

For analyzing the morphological traits of fish, a Procrustes MANOVA on shape was performed with centroid size, diet, and genotype as factors implemented in the `procD.lm` function of the `geomorph` package in R environment. After examining the allometric effect on shape, the coordinates of the Procrustes mean configuration were subtracted, defining the Procrustes residuals. For that, we analyzed how shape allometries varied by group (genotype) for the selection of a common or

unique slope using the pairwise function to test the variability of slopes between groups. Also, the morphological disparity (morphol.disparity function) was estimated as the Procrustes variance for comparing the degree of inter-groups variability. A principal component analysis (PCA) was also performed on the Procrustes residuals to determine how the shape varied by group. The scores of the first two PC components, which reached ca. to 50% of the total morphological variation, were used to plot the morphological position of each specimen in a two-dimensional graphic (morphospace). Additionally, these PC components were used to estimate the kernel density for the clustering of morphological patterns using a Gaussian function (Farré et al., 2016). Finally, thin-plate splines were built for the visualization of the induced changes in the shape (Bookstein, 1989). These data and analysis were processed in `geomorph` v. 4.0 package in R environment and the thin plane-splines were obtained in PAST v. 4.04 (Hammer et al., 2001).



**Fig. 2.** Fish weight (g) tendency along the feeding period (days) between genetically selected fish (GS) and wild-type genotype (WT) fed either Control or Future diet. Different letters within the same row denote significant differences among the dietary treatments ( $p < 0.05$ ; one-way ANOVA; Post-hoc test Tukey) for the significant interaction GxD ( $p < 0.05$ ; two-way ANOVA).

### 3. Results

#### 3.1. Fish growth and feed utilization

Two months from the beginning to the end of the feeding trial (300 days), fish from the WT genotype showed a significantly ( $p < 0.05$ ) lower growth (body weight, standard length and DGI) than fish from the GS genotype, irrespective of the diet (Fig. 2; Table 4). The two-way ANOVA analyses revealed the effect of genotype on final fish weight ( $F = 57.11; p = 0.001$ ), SGR ( $F = 25.81, p = 0.001$ ), DGI ( $F = 43.20, p = 0.022$ ) and TGC ( $F = 46.87, p = 0.001$ ). A significant interaction between the genotype and the diet was detected for body weight ( $F = 8.63; p = 0.01$ ), with GS sea bass showing a higher body weight than WT sea bass, irrespective of the diet, as well as for TGC ( $F = 5.84; p = 0.028$ ), with GS fish fed the Future diet showing a higher ( $p < 0.05$ ) TGC than WT fish fed the same diet (Table 4).

**Table 4**

Growth and feed utilization of wild (WT) and genetically selected for growth (GS) European sea bass fed the experimental control (C) or future (F) diets.

Days of feeding		GS-C	GS-F	WT-C	WT-F	Two-Way ANOVA (p-value)		
						Genotype	Diet	GxD
Initial	BW (g) <sup>1</sup>	35.6 ± 1.12	35.8 ± 0.93	33.5 ± 0.96	33.5 ± 1.64	NS	NS	NS
	SL (cm) <sup>2</sup>	12.2 ± 0.2	12.4 ± 0.3	12.3 ± 0.4	12.5 ± 0.3	NS	NS	NS
	K <sup>3</sup>	1.9 ± 0.2	1.9 ± 0.2	1.8 ± 0.1	1.7 ± 0.1	NS	NS	NS
30	BW (g) <sup>1</sup>	76.1 ± 1.2	78.6 ± 2.1	64.6 ± 4.0	67.7 ± 5.4	NS	NS	NS
	SL <sup>2</sup>	16.8 ± 0.6	16.9 ± 0.6	15.7 ± 0.4	15.5 ± 0.5	0.004	NS	NS
	K <sup>3</sup>	1.6 ± 0.2	1.7 ± 0.2	1.7 ± 0.2	1.8 ± 0.1	NS	NS	NS
	DGI <sup>4</sup> (%/day)	3.2 ± 0.2	2.6 ± 1.4	2.6 ± 0.4	2.8 ± 0.4	0.009	0.009	NS
60	BW (g) <sup>1</sup>	107.1 ± 2.3	103.8 ± 1.6	96.9 ± 6.8	91.2 ± 2.2	NS	NS	NS
	SL <sup>2</sup>	17.7 ± 0.3	17.7 ± 0.6	16.7 ± 0.5	16.3 ± 0.3	0.001	NS	NS
	K <sup>3</sup>	2.0 ± 0.1	1.9 ± 0.2	2.1 ± 0.3	2.1 ± 0.2	NS	NS	NS
	DGI <sup>4</sup> (%/day)	2.4 ± 0.1	2.3 ± 0.1	2.3 ± 0.2	2.1 ± 0.1	0.009	NS	NS
150	BW (g) <sup>1</sup>	203.3 ± 2.61	200.3 ± 3.38	178.6 ± 1.43	176.4 ± 2.79	0.000	0.001	NS
	SL <sup>2</sup>	18.2 ± 0.4	18.6 ± 1.23	18.7 ± 0.3	17.8 ± 0.3	NS	NS	NS
	K <sup>3</sup>	3.4 ± 0.3	3.1 ± 0.5	2.7 ± 0.2	3.1 ± 0.1	NS	NS	NS
	DGI <sup>4</sup> 0–150 (%/day)	2.0 ± 0.0	1.7 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	NS	NS	NS
180	BW (g) <sup>1</sup>	232.9 ± 3.91	228.5 ± 2.53	199.3 ± 5.15	201.0 ± 2.68	0.000	0.02	NS
	SL <sup>2</sup>	18.5 ± 0.4	19.0 ± 1.0	19.1 ± 0.3	18.2 ± 0.3	NS	NS	NS
	K <sup>3</sup>	3.7 ± 0.2	3.4 ± 0.5	2.9 ± 0.2	3.4 ± 0.2	NS	NS	NS
	DGI <sup>3</sup> (%/day)	1.6 ± 0.0	1.6 ± 0.0	1.5 ± 0.0	1.5 ± 0.0	NS	NS	NS
210	BW (g) <sup>1</sup>	259.7 ± 8.48	254.7 ± 4.52	228.4 ± 2.90	224.3 ± 4.60	0.000	NS	NS
	SL <sup>2</sup>	21.8 ± 0.3	21.3 ± 0.2	22.1 ± 0.3	21.2 ± 1.5	NS	NS	NS
	K <sup>3</sup>	2.5 ± 0.1	2.6 ± 0.1	2.1 ± 0.1	2.4 ± 0.5	NS	NS	NS
	DGI <sup>4</sup> (%/day)	1.5 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	0.020	NS	NS
240	BW (g) <sup>1</sup>	281.8 ± 6.41	275.4 ± 2.10	252.5 ± 3.04	243.7 ± 2.24	0.000	0.01	NS
	SL <sup>2</sup>	21.8 ± 0.3	21.3 ± 0.2	22.1 ± 0.3	21.2 ± 1.5	NS	0.017	NS
	K <sup>3</sup>	2.4 ± 0.1 <sup>ab</sup>	2.5 ± 0.2 <sup>a</sup>	2.1 ± 0.1 <sup>b</sup>	2.3 ± 0.2 <sup>ab</sup>	0.015	0.05	0.045
	DGI <sup>4</sup> (%/day)	1.4 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	0.022	NS	NS
270	BW (g) <sup>1</sup>	307.1 ± 9.17	303.9 ± 13.66	282.0 ± 9.98	270.6 ± 4.39	0.001	NS	NS
	SL <sup>2</sup>	26.4 ± 0.3	25.5 ± 0.6	25.3 ± 1.0	25.1 ± 0.6	NS	NS	NS
	K <sup>3</sup>	1.7 ± 0.0	1.8 ± 0.1	1.8 ± 0.2	1.7 ± 0.1	NS	NS	NS
	DGI <sup>4</sup> (%/day)	1.3 ± 0.0	1.3 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	NS	NS	NS
300	BW (g) <sup>1</sup>	330.2 ± 3.35 <sup>a</sup>	320.6 ± 5.96 <sup>a</sup>	300.5 ± 11.59 <sup>b</sup>	284.7 ± 10.97 <sup>b</sup>	0.00	NS	0.01
	SL <sup>2</sup>	26.4 ± 0.4	26.1 ± 0.2	25.7 ± 0.6	25.1 ± 0.8	0.049	NS	NS
	K <sup>3</sup>	1.8 ± 0.1	1.8 ± 0.0	1.7 ± 0.0	1.8 ± 0.1	NS	NS	NS
	DGI <sup>4</sup> (%/day)	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.1 ± 0.0	0.022	NS	NS
	SGR <sup>5</sup> (%/day)	0.8 ± 0.0	0.8 ± 0.0	0.7 ± 0.0	0.7 ± 0.1	0.001	NS	NS
	TGC <sup>6</sup>	0.58 ± 0.0 <sup>a</sup>	0.59 ± 0.0 <sup>a</sup>	0.56 ± 0.0 <sup>ab</sup>	0.53 ± 0.0 <sup>b</sup>	0.001	NS	0.028
	Feed intake (g feed/100 g BW/day)	0.8 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	NS	NS	NS
	FCR <sup>7</sup>	1.5 ± 0.1	1.6 ± 0.0	1.9 ± 0.1	1.8 ± 0.1	0.001	NS	NS
	Protein gain (g protein gained/fish/day)	2.98 ± 0.06	2.79 ± 0.12	2.55 ± 0.0	2.71 ± 0.1	0.001	NS	NS
	PER <sup>8</sup>	1.21 ± 0.03	1.24 ± 0.07	1.02 ± 0.05	1.07 ± 0.02	0.001	NS	NS
	Lipid gain (g lipid gained/fish/day)	2.21 ± 0.02	2.34 ± 0.14	2.71 ± 0.1	2.11 ± 0.1	0.001	NS	NS
LER <sup>9</sup>	3.59 ± 0.09	3.64 ± 0.21	3.03 ± 0.14	3.13 ± 0.1	0.000	NS	NS	
PFI <sup>10</sup> (%)	5.44 ± 0.3 <sup>b</sup>	5.04 ± 0.2 <sup>b</sup>	7.63 ± 1.2 <sup>a</sup>	6.28 ± 1.4 <sup>ab</sup>	0.001	NS	0.001	
HSI <sup>11</sup> (%)	1.3 ± 0.1	1.4 ± 0.2	1.9 ± 0.1	1.8 ± 0.1	0.001	NS	NS	

C: Control diet; F: Future diet; WT: wild type genotype; GS: genetically selected for growth genotype. Values are expressed in mean ± SD. ( $n = 4$  tanks/diet/genotype). <sup>1</sup>Body weight (BW) (g); <sup>2</sup>Standard length (SL) (cm); <sup>3</sup>Condition factor ( $K = [(weight) \times 100 / (length)^3]$ ); <sup>4</sup> Daily Growth Index ( $DGI = [(final\ weight^{1/3} - initial\ weight^{1/3}) / (number\ of\ days \times 100)]$ ); <sup>5</sup>Specific Growth Rate ( $SGR = [(\ln\ (final\ weight) - \ln\ (initial\ weight)) / (number\ of\ days \times 100)]$ ); <sup>6</sup>Thermal Growth Coefficient ( $TGC = [(final\ weight^{1/3} - Initial\ weight^{1/3}) / (temperature \times number\ of\ days \times 1000)]$ ); <sup>7</sup>Feed Conversion Ratio ( $FCR = total\ feed\ fed / weight\ gain$ ); <sup>8</sup>Protein efficiency ratio ( $PER = weight\ gained / weight\ of\ protein\ consumed$ ); <sup>9</sup>LER (lipid efficiency ratio) = weight gained/weight of lipid consumed; <sup>10</sup>Perivisceral fat index (PFI) (perivisceral fat /body weight)  $\times 100$ ; <sup>11</sup>Hepatosomatic index (HSI) = (liver weight /body weight)  $\times 100$ . Different letters within the same row denote significant differences among the dietary treatments ( $p < 0.05$ ; one-way ANOVA; Post-hoc test Tukey). Two-way ANOVA,  $p < 0.05$ , Genotype and Diet as fixed factors. NS = not significant.

**Table 5**

Proximate composition, in wet weight (ww) of whole-body, flesh and liver of wild (WT) and genetically selected for growth (GS) European sea bass fed the experimental diets.

Proximate composition (% ww)	GS-C	GS-F	WT-C	WT-F	Two-way ANOVA ( <i>p</i> -value)		
					Genotype	Diet	G x D
<b>Whole-body</b>							
Crude lipid	11.0 ± 0.4 <sup>a</sup>	12.1 ± 1.3 <sup>a</sup>	15.6 ± 0.6 <sup>b</sup>	12.92 ± 1.1 <sup>a</sup>	0.003	0.013	0.001
Crude protein	15.4 ± 0.2	16.5 ± 0.4	15.1 ± 0.2	16.4 ± 1.0	NS	NS	NS
Moisture	69.08 ± 0.9	68.2 ± 0.3	67.9 ± 0.4	68.0 ± 0.8	NS	NS	0.02
Ash	4.70 ± 0.11 <sup>a</sup>	4.62 ± 2.16 <sup>a</sup>	4.36 ± 0.12 <sup>b</sup>	4.88 ± 0.12 <sup>a</sup>	0.001	NS	0.001
<b>Muscle</b>							
Crude lipid	5.33 ± 0.1 <sup>a</sup>	4.77 ± 0.2 <sup>a</sup>	4.48 ± 0.3 <sup>b</sup>	4.4 ± 0.1 <sup>b</sup>	0.000	0.002	NS
Crude protein	20.77 ± 0.5	20.15 ± 0.1	20.53 ± 2.1	20.45 ± 2.2	NS	NS	NS
Moisture	74.39 ± 0.5	74.20 ± 0.1	73.35 ± 0.2	74.31 ± 0.2	NS	NS	0.02
Ash	6.40 ± 0.1 <sup>a</sup>	5.56 ± 0.1 <sup>a</sup>	6.53 ± 0.1 <sup>b</sup>	7.11 ± 0.2 <sup>b</sup>	NS	NS	NS
<b>Liver</b>							
Crude lipid	26.9 ± 1.3	30.7 ± 1.6	25.7 ± 2.6	27.9 ± 1.0	0.013	0.005	NS
Crude protein	11.4 ± 0.2	10.3 ± 1.8	11.1 ± 0.6	9.7 ± 1.0	0.032	NS	NS
Moisture	58.9 ± 1.6	52.89 ± 4.07	55.95 ± 2.11	56.39 ± 0.78	NS	NS	NS
Ash	0.9 ± 0.2	0.7 ± 0.1	0.9 ± 0.2	0.8 ± 0.1	0.019	NS	NS

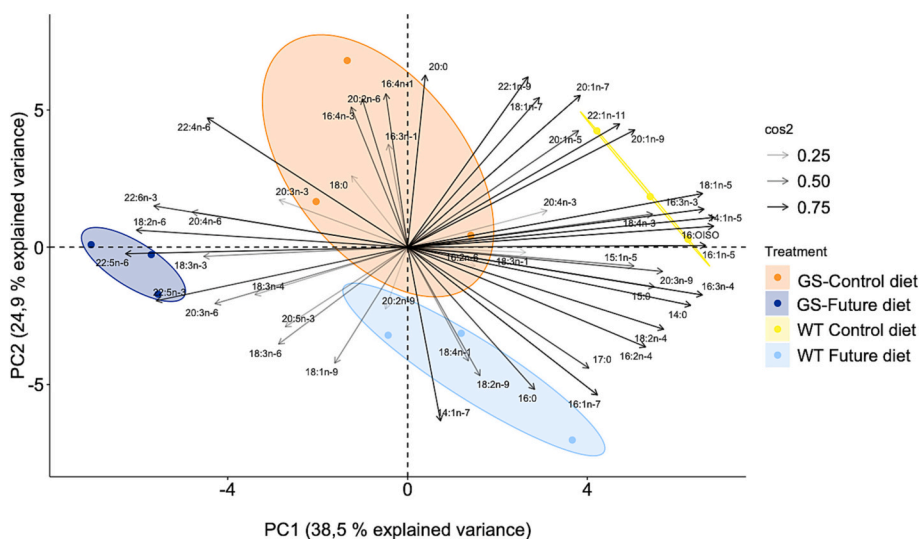
C: Control diet; F: Future diet; WT: wild type genotype; GS: genetically selected for growth genotype. Values are expressed in mean ± SD. (*n* = 4 tanks/diet/genotype). Different letters denote significant differences analyzed with one-way ANOVA, *p* < 0.05. Two-way ANOVA, *p* < 0.05, Genotype and Diet as fixed factors. NS = not significant.

three tissues, but Future diet led to a higher lipid content in the liver (*p* = 0.005) and lower in the flesh (*p* = 0.002) compared to Control diet (Table 5). Furthermore, a significant interaction between diet and genotype was observed for whole-body lipid and ash contents, with WT sea bass fed the Control diet showing the highest lipids and the lowest ash (*p* = 0.001) (Table 5).

Regarding the fatty acid profile (in % total fatty acids) of sea bass flesh, the PCA showed that the fatty acid profiles of GS fish fed the Future diet formed a well-defined group towards the left of the plot and were well described by PC1, which explained 38.5% of the total variability between the experimental groups (Fig. 3). In contrast, the flesh' fatty acid profiles of both genotypes of fish fed the Control diet, were more described by PC2, which explained 24.9% of the total variability among the experimental groups (Fig. 3). There was no overlap between the different experimental groups. Interestingly, almost all LC-PUFA, both from n-6 and n-3 series, particularly 20:4n-6, 22:5n-6, 22:5n-3, and 22:6n-3, but also their precursors 18:2n-6 and 18:3n-3, respectively, were highly negatively correlated with PC1 and thus characterizing flesh fatty acid profile of GS sea bass fed the Future diet (Fig. 3). Indeed, all those fatty acids (20:4n-6, 22:5n-6, 22:5n-3, 22:6n-3 and 18:2n-6) were significantly affected by genotype (*p* < 0.05), showing the highest levels in GS fish compared with WT (Table 6). Accordingly, some

of them were also increasingly affected by Future diet (18:2n-6, 22:5n-3 and 22:5n-6), whereas 18:3n-3 showed an interaction between genotype and diet (Table 6). The highest DHA and ARA contents of GS fish was also reflected in their highest (*p* < 0.05) DHA content in g/100 g, but EPA was similar irrespective of the diet or genotype (Fig. 4). Contrary to genotype, that affected a wide number of fatty acids of the flesh, diet had a fewer independent effect, increasing (*p* < 0.05) the contents of 18:2n-6, 22:5n-6 or n-6 as well as 22:5n-3 in Future diet compared to Control diet, whereas decreasing (*p* < 0.05) MUFA content (Table 6). Furthermore, significant interactions between genotype and diet were observed for some fatty acids (Table 6). For instance, GS fed the Future diet showed the highest (*p* < 0.05) 18:3n-3 in flesh, as well as higher total n-3 compared with WT fed the same diet (Table 6). Furthermore, 16:0 and the total SFA content in GS fish flesh were lower (*p* < 0.05) compared with WT fish fed the Future diet (Table 6).

The PCA of liver fatty acid profiles (in % total fatty acids) showed similar tendencies as those observed for fish flesh, with the hepatic fatty acid profile of sea bass fed Future diet being well defined by PC1, accounting for 40.2% of the total variance, and PC2 explaining 36.6% of the total variance (Fig. 5). PCA clearly separated the hepatic fatty acid profile of WT sea bass fed Control diet from the other experimental groups (Fig. 5). In contrast to the pattern observed in flesh, where the



**Fig. 3.** PCA represents the variability in the flesh fatty acid profile of wild (WT) and genetically selected for growth (GS) European sea bass fed the experimental diets. Percent of total fatty acids are represented as cos2 function by an intensity scale, and confidence ellipses are generated around mean group points. The points correspond to the replicates and are colored according to genotype and diet fed. The fatty acids are plotted in the PCA as arrows indicating the level of each fatty acid contribution to the formation of PC1 and PC2. The stronger the correlation of a fatty acid to PC1 or PC2, the closer its arrowhead to the circle plotted.



**Table 6**

Flesh fatty acid composition (% total fatty acids) of wild (WT) and genetically selected for growth (GS) European sea bass fed the experimental diets.

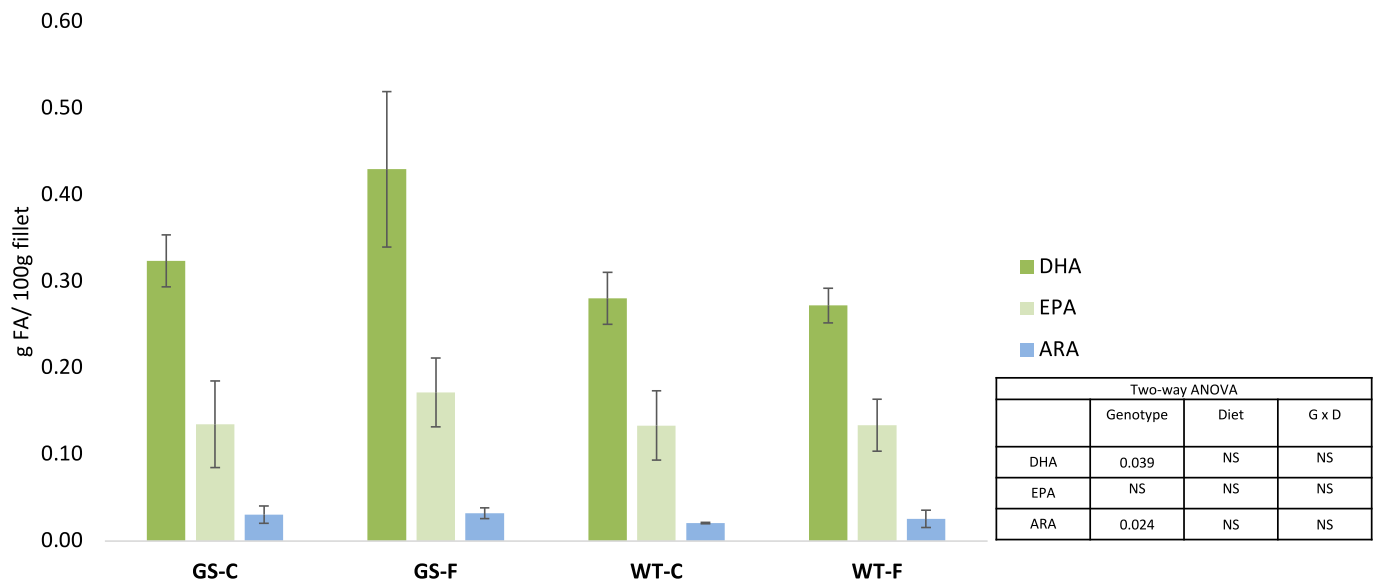
Fatty acids (% total fatty acids)	GS-C	GS-F	WT-C	WT-F	Two-way ANOVA ( <i>p</i> -value)		
					Genotype	Diet	G x D
14:0	1.55 ± 0.09	1.28 ± 0.05	1.78 ± 0.09	1.78 ± 0.30	0.005	NS	NS
14:1n-5	0.06 ± 0.01 <sup>b</sup>	0.04 ± 0.00 <sup>c</sup>	0.07 ± 0.00 <sup>a</sup>	0.05 ± 0.01 <sup>b</sup>	0.000	0.000	0.000
14:1n-7	0.03 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	0.05 ± 0.01	NS	0.014	NS
15:0	0.23 ± 0.01	0.19 ± 0.01	0.24 ± 0.00	0.25 ± 0.04	0.021	NS	NS
15:1n-5	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.022	NS	NS
16:0iso	0.03 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	0.03 ± 0.01	0.000	0.001	NS
16:0	16.22 ± 0.53 <sup>b</sup>	15.68 ± 0.26 <sup>b</sup>	16.25 ± 0.38 <sup>b</sup>	18.24 ± 1.16 <sup>a</sup>	0.011	NS	0.012
16:1n-7	2.82 ± 0.28	2.74 ± 0.15	3.04 ± 0.22	3.38 ± 0.31	0.017	NS	NS
16:1n-5	0.08 ± 0.01	0.06 ± 0.00	0.10 ± 0.01	0.09 ± 0.01	0.000	0.004	NS
16:2n-6	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.022	NS	NS
16:2n-4	0.11 ± 0.01 <sup>ab</sup>	0.09 ± 0.01 <sup>b</sup>	0.12 ± 0.01 <sup>ab</sup>	0.13 ± 0.01 <sup>a</sup>	0.002	NS	0.035
17:0	0.07 ± 0.01 <sup>ab</sup>	0.06 ± 0.01 <sup>b</sup>	0.08 ± 0.01 <sup>ab</sup>	0.09 ± 0.01 <sup>a</sup>	0.006	NS	0.021
16:3n-4	0.19 ± 0.02	0.17 ± 0.01	0.21 ± 0.01	0.21 ± 0.02	0.007	NS	NS
16:3n-3	0.07 ± 0.01 <sup>b</sup>	0.05 ± 0.01 <sup>c</sup>	0.08 ± 0.00 <sup>a</sup>	0.07 ± 0.01 <sup>b</sup>	0.002	0.002	0.002
16:3n-1	0.14 ± 0.06	0.08 ± 0.01	0.09 ± 0.03	0.10 ± 0.01	NS	NS	NS
16:4n-3	0.17 ± 0.07	0.11 ± 0.02	0.12 ± 0.04	0.11 ± 0.01	NS	NS	NS
16:4n-1	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	NS	NS	NS
18:0	4.29 ± 0.27	3.86 ± 0.23	3.84 ± 0.09	4.08 ± 0.35	NS	NS	NS
18:1n-9	33.27 ± 0.54	33.70 ± 0.44	33.07 ± 0.65	33.90 ± 0.69	NS	NS	NS
18:1n-7	2.47 ± 0.05	2.24 ± 0.03	2.62 ± 0.13	2.20 ± 0.20	NS	0.002	NS
18:1n-5	0.12 ± 0.01 <sup>b</sup>	0.09 ± 0.00 <sup>c</sup>	0.15 ± 0.01 <sup>a</sup>	0.12 ± 0.01 <sup>b</sup>	0.000	0.000	0.000
18:2n-9	0.19 ± 0.05	0.26 ± 0.02	0.27 ± 0.05	0.29 ± 0.04	0.044	NS	NS
18:2n-6	15.31 ± 0.54	15.98 ± 0.24	13.77 ± 0.20	14.47 ± 0.34	0.000	0.011	NS
18:2n-4	0.07 ± 0.01	0.06 ± 0.00	0.07 ± 0.01	0.08 ± 0.01	0.028	NS	NS
18:3 n-9	0.18 ± 0.03	0.21 ± 0.02	0.18 ± 0.02	0.19 ± 0.00	NS	NS	NS
18:3n-4	0.06 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	NS	NS	NS
18:3n-3	3.12 ± 0.17 <sup>b</sup>	3.52 ± 0.05 <sup>a</sup>	3.14 ± 0.09 <sup>b</sup>	3.01 ± 0.06 <sup>b</sup>	0.003	NS	0.002
18:3n-1	0.00 ± 0.00	0.00 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	NS	NS	NS
18:4n-3	0.35 ± 0.05 <sup>b</sup>	0.34 ± 0.02 <sup>b</sup>	0.54 ± 0.04 <sup>a</sup>	0.36 ± 0.02 <sup>b</sup>	0.000	0.001	0.002
18:4 n-1	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	NS	NS	NS
20:0	0.35 ± 0.03	0.30 ± 0.01	0.32 ± 0.01	0.28 ± 0.02	NS	0.003	NS
20:1n-9	0.28 ± 0.04 <sup>b</sup>	0.20 ± 0.01 <sup>c</sup>	0.39 ± 0.02 <sup>a</sup>	0.20 ± 0.02 <sup>c</sup>	0.002	0.000	0.001
20:1n-7	3.20 ± 0.36 <sup>a</sup>	2.54 ± 0.02 <sup>b</sup>	3.65 ± 0.05 <sup>a</sup>	2.38 ± 0.23 <sup>b</sup>	NS	0.000	0.037
20:1n-5	0.14 ± 0.02	0.10 ± 0.00	0.14 ± 0.01	0.11 ± 0.01	NS	0.001	NS
20:2n-9	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	NS	NS	NS
20:2n-6	0.71 ± 0.03	0.66 ± 0.01	0.66 ± 0.02	0.60 ± 0.07	0.039	0.030	NS
20:3n-9	0.01 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.022	NS	NS
20:3n-6	0.11 ± 0.01	0.12 ± 0.01	0.09 ± 0.00	0.11 ± 0.01	0.004	0.014	NS
20:4n-6	0.64 ± 0.09	0.60 ± 0.04	0.46 ± 0.09	0.58 ± 0.01	0.024	NS	NS
20:3n-3	0.16 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.14 ± 0.02	NS	NS	NS
20:4n-3	0.26 ± 0.04	0.26 ± 0.01	0.30 ± 0.01	0.25 ± 0.03	NS	NS	NS
20:5n-3	2.82 ± 0.38	3.22 ± 0.18	2.98 ± 0.15	3.06 ± 0.13	NS	NS	NS
22:1n-11	1.62 ± 0.27 <sup>b</sup>	1.03 ± 0.03 <sup>c</sup>	2.70 ± 0.11 <sup>a</sup>	0.94 ± 0.11 <sup>c</sup>	0.001	0.000	0.000
22:1n-9	0.54 ± 0.06 <sup>a</sup>	0.43 ± 0.01 <sup>b</sup>	0.59 ± 0.02 <sup>a</sup>	0.37 ± 0.03 <sup>b</sup>	NS	0.000	0.028
22:4n-6	0.10 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.002	NS	NS
22:5n-6	0.24 ± 0.03	0.26 ± 0.02	0.18 ± 0.03	0.22 ± 0.02	0.007	0.042	NS
22:5n-3	0.68 ± 0.13	0.86 ± 0.05	0.62 ± 0.03	0.70 ± 0.07	0.038	0.022	NS
22:6n-3	6.78 ± 0.43	8.06 ± 0.59	6.56 ± 0.89	6.52 ± 0.47	0.039	NS	NS
∑SFA	22.72 ± 0.43 <sup>b</sup>	21.38 ± 0.42 <sup>b</sup>	22.51 ± 0.40 <sup>b</sup>	24.71 ± 1.26 <sup>a</sup>	0.006	NS	0.003
∑MUFA	44.65 ± 0.83	43.22 ± 0.53	46.58 ± 0.80	43.79 ± 0.72	0.018	0.001	NS
∑ n-3	14.41 ± 0.67 <sup>ab</sup>	16.59 ± 0.87 <sup>a</sup>	14.48 ± 0.98 <sup>ab</sup>	14.22 ± 0.77 <sup>b</sup>	0.044	NS	0.035
∑ n-6	17.29 ± 0.57	17.92 ± 0.27	15.44 ± 0.26	16.24 ± 0.36	0.000	0.012	NS
∑ n-9	34.35 ± 0.55	34.64 ± 0.47	34.37 ± 0.71	34.82 ± 0.74	NS	NS	NS
∑n-3 LC-PUFA	10.70 ± 0.67	12.56 ± 0.81	10.60 ± 1.05	10.67 ± 0.71	NS	NS	NS
ARA/EPA	0.23 ± 0.05	0.19 ± 0.02	0.16 ± 0.02	0.19 ± 0.01	NS	NS	NS
DHA/EPA	2.43 ± 0.37	2.51 ± 0.10	2.20 ± 0.19	2.13 ± 0.07	0.039	NS	NS
DHA/ARA	10.68 ± 0.81 <sup>b</sup>	13.40 ± 0.83 <sup>a</sup>	14.38 ± 0.68 <sup>a</sup>	11.33 ± 0.63 <sup>b</sup>	NS	0.022	0.001
n-3/n-6	0.83 ± 0.06	0.93 ± 0.06	0.94 ± 0.05	0.88 ± 0.05	NS	NS	0.040

C: Control diet; F: Future diet; WT: wild type genotype; GS: genetically selected for growth genotype. Values are expressed in mean ± SD. (n = 4 tanks/diet/genotype). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; LC-PUFA: long-chain polyunsaturated fatty acids. Different letters denote significant differences analyzed with one-way ANOVA, *p* < 0.05. Two-way ANOVA, *p* < 0.05, Genotype and Diet as fixed factors. NS = not significant.

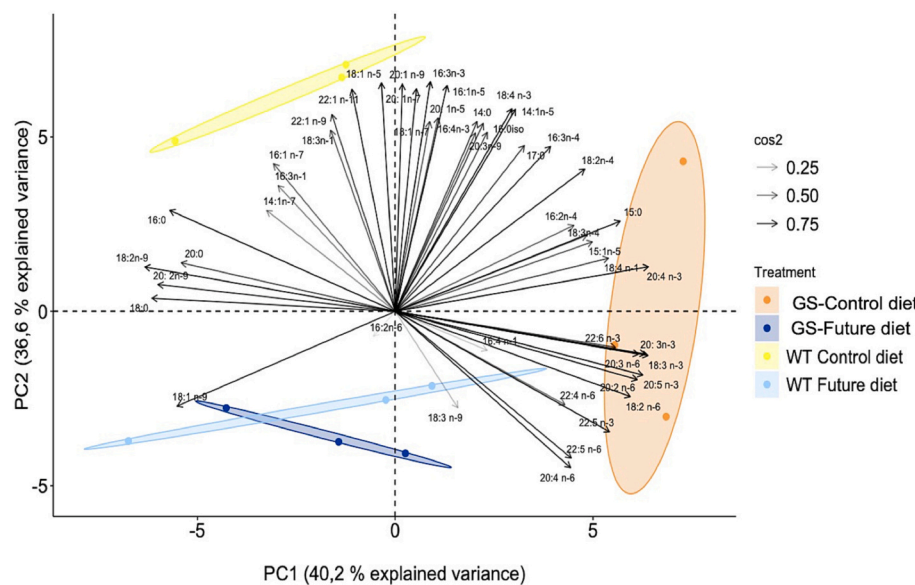
most biological active FA, like 20:5n-3, 22:6n-3 or 20:4n-6, as well as their 18:C precursors (18:3n-3 and 18:2n-6, respectively), were mainly affected by genotype, in liver these FA were positively correlated to PC1, but showed a significant interaction between genotype and diet (*p* < 0.05). Therefore, the highest contents on those FA characterized the hepatic fatty acid profile of GS fish fed the Control diet (Table 7). SFA also showed a significant interaction GxD (*p* < 0.05), presenting in GS fed the Control diet a lower content than in WT fed the same diet (Table 7).

### 3.3. Apparent digestibility coefficients of the diets

Although the protein ADC was not significantly affected by genotype or diet, GS sea bass showed highest ADC of cysteine (*p* = 0.04) and histidine (*p* = 0.03), as well as higher ADCs for SFA (*p* = 0.04) and n-3 FA (*p* = 0.04) (Table 8). Future diet reduced the ADCs of alanine (*p* = 0.04), aspartic acid (*p* = 0.01), proline (*p* = 0.04), tyrosine (*p* = 0.02), as well as MUFA (*p* = 0.02) and total FA (*p* = 0.03) (Table 8). A significant interaction between genotype and diet was observed for glycine (*p* =



**Fig. 4.** Amount of n-3 and n-6 PUFAs per 100 g of flesh wild (WT) and genetically selected for growth (GS) European sea bass fed the experimental diets. C: Control diet; F: Future diet; WT: wild type genotype; GS: genetically selected for growth genotype. DHA: Docosahexaenoic acid, 22:6n-3; EPA: Eicosapentaenoic acid, 20:5n-3; ARA: Arachidonic acid, 20:4n-6.



**Fig. 5.** Principal component analysis (PCA) representing the variability in the liver fatty acid profile of wild (WT) and genetically selected for growth (GS) European sea bass fed the experimental diets. Percent of total fatty acids are represented as cos2 function by an intensity scale, and confidence ellipses are generated around mean group points. The points correspond to the replicates and are colored according to genotype and diet fed. The fatty acids are plotted in the PCA as arrows indicating the level of each fatty acid contribution to the formation of PC1 and PC2. The stronger the correlation of a fatty acid to PC1 or PC2, the closer its arrowhead to the circle plotted.

0.03), isoleucine ( $p = 0.04$ ), threonine ( $p = 0.01$ ) and serine ( $p = 0.04$ ), with GS sea bass fed the Control showing the highest value ADCs of these amino acids, particularly when compared with WT fed the Future diet (Table 8). Concerning the lipids, the ADC of total fatty acids showed an increasing effect of GS genotype, particularly SFA ( $p = 0.04$ ), and a decreasing effect of the Future diet, particularly MUFA ( $p = 0.02$ ) (Table 8). No interactions between genotype and diet were observed for fatty acids ADC.

### 3.4. Effects on fish body shape

The Procrustes ANOVA only showed significant differences in the centroid size ( $F = 6.648$ ,  $p = 0.001$ ), caused by the genotype ( $F = 21.510$ ,  $p = 0.001$ ), and the interaction of genotype and the diet ( $F = 3.285$ ,  $p = 0.0017$ ) (Table 9). Pairwise comparisons of shape model growth of centroid with several genes indicated significant differences in

the slopes (distance = 0.113,  $Z = 2.475$ ,  $p = 0.005$ ).

The morphological disparity (variance genotypes,  $H = 0.0024$  and  $U = 0.0019$ ) of specimens from the GS genotype was higher than the WT genotype ( $p = 0.005$ ). This was noted in the morphospace and kernel density graphics (Fig. 6 a, b) shown by the PCA analysis. With some exceptions, most specimens from the WT genotype were close to the center of the morphospace (red colour in Fig. 6b), whereas GS individuals presented different morphological patterns along PC1 and PC2. In the PCA analysis, the first 14 PC components explained 90.6% of the total variance. The PC1 encompassed 33.9% of the total variation between specimens with a higher height of body shape (negative scores, Fig. 6 c, d) and anomalous specimens with vertebral deformations between the second dorsal and anal fins producing a haemal lordosis (positive values, Fig. 6 c, d). The PC2 (15.8%) values exhibited morphological alterations affecting anterior or posterior fish body shape. The positive scores showed vertebral deformations at the first

**Table 7**  
Liver fatty acid composition (% total fatty acids) of wild (WT) and genetically selected for growth (GS) European sea bass fed the experimental diets.

Fatty acids (% total fatty acids)	GS-C	GS-F	WT-C	WT-F	Two-way ANOVA (p-value)		
14:0	1.47 ± 0.26	1.22 ± 0.07	1.64 ± 0.19	1.11 ± 0.15	NS	0.006	NS
14:1n-5	0.04 ± 0.02	0.02 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	NS	0.003	NS
14:1n-7	0.03 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	NS	NS	0.017
15:0	0.20 ± 0.04	0.12 ± 0.02	0.15 ± 0.03	0.11 ± 0.04	NS	0.011	NS
15:1n-5	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	NS	NS	NS
16:0iso	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.02 ± 0.01	NS	0.008	NS
16:0	16.23 ± 0.03	18.97 ± 1.61 <sup>ab</sup>	21.53 ± 1.09	18.54 ± 1.67 <sup>ab</sup>	0.011	NS	0.005
16:1 n-7	3.56 ± 0.32	3.87 ± 0.50	4.27 ± 0.24	3.61 ± 0.24	NS	NS	0.040
16:1n-5	0.10 ± 0.03	0.07 ± 0.00	0.12 ± 0.01	0.07 ± 0.01	NS	0.003	NS
16:2n-6	n.d.	n.d.	0.01 ± 0.01	0.02 ± 0.03	NS	NS	NS
16:2n-4	0.07 ± 0.02	0.04 ± 0.01	0.06 ± 0.02	0.05 ± 0.04	NS	NS	NS
17:0	0.05 ± 0.00	0.03 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	NS	0.016	NS
16:3n-4	0.25 ± 0.05	0.20 ± 0.01	0.24 ± 0.03	0.19 ± 0.03	NS	0.024	NS
16:3n-3	0.05 ± 0.02	0.03 ± 0.00	0.07 ± 0.01	0.03 ± 0.01	0.048	0.000	NS
16:3n-1	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	0.02 ± 0.00	NS	NS	0.022
16:4n-3	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	NS	0.028	NS
18:0	2.72 ± 0.17	3.63 ± 0.13	4.27 ± 0.63	4.38 ± 0.88	0.007	NS	NS
18:1 n-9	37.86 ± 1.46	42.91 ± 0.94	40.69 ± 1.2	43.89 ± 2.56	0.081	0.003	NS
18:1 n-7	2.40 ± 0.18	2.26 ± 0.06	2.55 ± 0.13	2.39 ± 0.12	NS	NS	NS
18:1 n-5	0.13 ± 0.03	0.11 ± 0.00	0.17 ± 0.01	0.11 ± 0.00	NS	0.002	NS
18:2n-9	0.56 ± 0.08	0.85 ± 0.08	1.01 ± 0.09	0.90 ± 0.29	0.027	NS	NS
18:2n-6	16.58 ± 2.42 <sup>a</sup>	12.80 ± 1.88 <sup>ab</sup>	9.26 ± 1.42 <sup>b</sup>	11.73 ± 3.15 <sup>ab</sup>	0.014	NS	0.047
18:2n-4	0.06 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	NS	0.016	NS
18:3 n-9	0.47 ± 0.00	0.49 ± 0.02	0.42 ± 0.04	0.43 ± 0.01	0.001	NS	NS
18:3n-4	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	NS	NS	NS
18:3n-3	3.52 ± 0.37 <sup>a</sup>	2.52 ± 0.40 <sup>ab</sup>	1.92 ± 0.30 <sup>b</sup>	2.17 ± 0.64 <sup>b</sup>	0.005	NS	0.041
18:4n-3	0.40 ± 0.11	0.25 ± 0.02	0.42 ± 0.06	0.24 ± 0.04	NS	0.002	NS
18:4n-1	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	NS	NS	NS
20:0	0.13 ± 0.02	0.15 ± 0.00	0.19 ± 0.02	0.19 ± 0.03	0.002	NS	NS
20:1 n-9	0.28 ± 0.13	0.17 ± 0.01	0.43 ± 0.02	0.19 ± 0.03	0.046	0.002	NS
20:1n-7	2.10 ± 0.28	1.74 ± 0.10	2.60 ± 0.22	1.87 ± 0.17	0.026	0.002	NS
20:1n-5	0.07 ± 0.02	0.05 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	NS	0.028	NS
20:2n-9	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	NS	NS	NS

**Table 7 (continued)**

Fatty acids (% total fatty acids)	GS-C	GS-F	WT-C	WT-F	Two-way ANOVA (p-value)		
20:2n-6	0.61 ± 0.03	0.49 ± 0.07	0.41 ± 0.06	0.49 ± 0.11	0.046	NS	NS
20:3n-9	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	NS	NS	NS
20:3n-6	0.08 ± 0.01 <sup>a</sup>	0.07 ± 0.01 <sup>ab</sup>	0.06 ± 0.01 <sup>b</sup>	0.07 ± 0.01 <sup>ab</sup>	NS	NS	0.021
20:4n-6	0.39 ± 0.05 <sup>a</sup>	0.33 ± 0.04 <sup>ab</sup>	0.24 ± 0.02 <sup>b</sup>	0.37 ± 0.04 <sup>a</sup>	0.033	NS	0.002
20:3n-3	0.25 ± 0.02 <sup>a</sup>	0.14 ± 0.02 <sup>b</sup>	0.15 ± 0.02 <sup>b</sup>	0.13 ± 0.02 <sup>b</sup>	0.003	NS	0.009
20:4n-3	0.12 ± 0.03 <sup>a</sup>	0.12 ± 0.02 <sup>b</sup>	0.15 ± 0.02 <sup>b</sup>	0.13 ± 0.05 <sup>b</sup>	0.012	0.009	0.029
20:5n-3	2.27 ± 0.01 <sup>a</sup>	1.63 ± 0.16	1.33 ± 0.09	1.63 ± 0.26	0.001	NS	0.001
22:1n-11	0.66 ± 0.27 <sup>b</sup>	0.36 ± 0.03 <sup>b</sup>	1.49 ± 0.12 <sup>a</sup>	0.49 ± 0.04 <sup>b</sup>	0.001	0.000	0.004
22:1n-9	0.29 ± 0.04	0.24 ± 0.02	0.40 ± 0.04	0.29 ± 0.01	0.002	0.003	NS
22:4n-6	0.06 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	NS	NS	0.029
22:5n-6	0.12 ± 0.02 <sup>a</sup>	0.10 ± 0.01 <sup>ab</sup>	0.07 ± 0.01 <sup>b</sup>	0.10 ± 0.02 <sup>ab</sup>	NS	NS	0.019
22:5n-3	0.65 ± 0.09 <sup>a</sup>	0.46 ± 0.08 <sup>ab</sup>	0.23 ± 0.02 <sup>c</sup>	0.40 ± 0.11 <sup>bc</sup>	0.001	NS	0.005
22:6 n-3	4.86 ± 0.69 <sup>a</sup>	3.22 ± 0.46 <sup>b</sup>	2.93 ± 0.31 <sup>b</sup>	3.21 ± 0.43 <sup>b</sup>	0.009	0.043	0.009
∑SFA	20.80 ± 0.12 <sup>b</sup>	24.12 ± 1.77 <sup>ab</sup>	27.83 ± 1.53 <sup>a</sup>	24.37 ± 2.37 <sup>ab</sup>	0.005	NS	0.008
∑MUFA	47.54 ± 2.24	51.87 ± 1.25	52.92 ± 0.68	53.06 ± 2.25	0.011	NS	NS
∑Total n-3	12.18 ± 0.35 <sup>a</sup>	8.36 ± 1.05 <sup>b</sup>	7.14 ± 0.53 <sup>b</sup>	7.94 ± 1.50 <sup>b</sup>	0.001	0.027	0.003
∑Total n-6	17.84 ± 2.48 <sup>a</sup>	13.83 ± 2.00 <sup>ab</sup>	10.09 ± 1.51 <sup>b</sup>	12.83 ± 3.34 <sup>ab</sup>	0.014	NS	0.043
∑Total n-9	39.50 ± 1.62	44.73 ± 0.99	43.02 ± 1.15	45.77 ± 2.84	NS	0.005	NS
∑n-3 LC-PUFA	8.18 ± 0.74 <sup>a</sup>	5.55 ± 0.73 <sup>b</sup>	4.71 ± 0.30 <sup>b</sup>	5.47 ± 0.82 <sup>b</sup>	0.002	0.045	0.003
ARA/EPA	0.17 ± 0.02	0.20 ± 0.02	0.18 ± 0.01	0.23 ± 0.02	NS	0.003	NS
DHA/EPA	2.14 ± 0.31	1.97 ± 0.10	2.22 ± 0.32	1.99 ± 0.20	NS	NS	NS
DHA/ARA	12.35 ± 1.49	9.85 ± 1.09	12.32 ± 1.58	8.67 ± 0.18	NS	0.002	NS
n-3/n-6	0.69 ± 0.11	0.61 ± 0.06	0.72 ± 0.06	0.63 ± 0.05	NS	NS	NS

C: Control diet; F: Future diet; WT: wild type genotype; GS: genetically selected for growth genotype. Values are expressed in mean ± SD. (n = 4 tanks/diet/genotype). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; LC-PUFA: long-chain polyunsaturated fatty acids Different letters denote significant differences analyzed with one-way ANOVA, p < 0.05. Two-way ANOVA, p < 0.05, Genotype and Diet as fixed factors. NS = not significant.

dorsal fin generating pre-haemal lordosis (Fig. 6 c, d), and the negative values in the skull gave rise to skull lordosis (Fig. 6 c, d). These deformations also affected the position of the caudal peduncle in relation to the second dorsal and anal fins, being closer to positive values, and, therefore, to the swimming capacity of fish.

**Table 8**

Total protein, amino acids and fatty acids apparent digestibility coefficients (ADC, %) of wild (WT) and genetically selected for growth (GS) European sea bass fed the experimental diets.

	GS-C	GS-F	WT-C	WT-F	Two-Way ANOVA (p-value)		
					Genotype	Diet	GxD
Protein	84.02 ± 1.56	83.24 ± 1.00	82.09 ± 1.39	77.96 ± 2.57	NS	NS	NS
Alanine	85.18 ± 2.77	82.74 ± 3.56	83.55 ± 2.46	77.19 ± 3.48	NS	0.04	NS
Arginine	88.46 ± 3.21	86.84 ± 3.04	86.82 ± 5.54	82.33 ± 2.97	NS	NS	NS
Aspartic acid	77.32 ± 2.75	71.47 ± 2.72	74.17 ± 2.98	67.28 ± 2.44	NS	0.01	NS
Cysteine	73.40 ± 3.22	69.95 ± 2.09	67.60 ± 2.38	65.07 ± 2.03	0.04	NS	NS
Glutamic acid	91.81 ± 2.43	91.20 ± 1.43	90.92 ± 1.97	88.78 ± 2.16	NS	NS	NS
Glycine	81.07 ± 2.22 <sup>a</sup>	76.85 ± 2.00 <sup>b</sup>	77.59 ± 2.47 <sup>b</sup>	71.59 ± 1.37 <sup>c</sup>	NS	NS	0.03
Histidine	84.12 ± 1.68	81.86 ± 2.26	79.53 ± 1.17	77.41 ± 1.35	0.03	NS	NS
Isoleucine	85.18 ± 4.01 <sup>a</sup>	84.42 ± 2.94 <sup>a</sup>	83.83 ± 3.75 <sup>ab</sup>	78.97 ± 3.18 <sup>b</sup>	NS	NS	0.04
Leucine	87.61 ± 3.98	82.95 ± 3.22	82.04 ± 2.21	81.26 ± 2.54	NS	NS	NS
Lysine	84.00 ± 2.38	82.95 ± 2.61	82.04 ± 2.12	76.71 ± 3.87	NS	NS	NS
Methionine	83.35 ± 1.65	83.12 ± 2.92	81.40 ± 2.02	77.03 ± 1.97	NS	NS	NS
Phenylalanine	87.09 ± 3.23	85.98 ± 3.29	87.24 ± 3.98	80.38 ± 4.31	NS	0.03	NS
Proline	90.12 ± 3.41	88.96 ± 1.88	90.15 ± 1.98	86.08 ± 2.91	NS	0.04	NS
Threonine	80.00 ± 2.51 <sup>a</sup>	76.57 ± 1.92 <sup>b</sup>	76.39 ± 1.23 <sup>b</sup>	70.61 ± 1.44 <sup>c</sup>	NS	NS	0.01
Tyrosine	83.45 ± 1.95	81.84 ± 1.64	84.14 ± 2.51	74.02 ± 2.41	NS	0.02	NS
Serine	83.61 ± 3.95 <sup>a</sup>	81.38 ± 3.91 <sup>a</sup>	81.31 ± 3.43 <sup>a</sup>	76.36 ± 3.18 <sup>b</sup>	NS	NS	0.04
Valine	83.42 ± 3.41	81.78 ± 2.96	80.27 ± 3.28	76.40 ± 3.57	NS	NS	NS
∑Amino acids	85.86 ± 2.48	84.09 ± 2.59	84.15 ± 5.21	79.63 ± 3.87	NS	NS	NS
∑SFA	85.10 ± 4.45	78.69 ± 2.62	77.75 ± 3.01	75.54 ± 3.43	0.04	NS	NS
∑MUFA	87.09 ± 5.18	79.42 ± 2.77	73.60 ± 4.46	72.07 ± 2.25	NS	0.02	NS
∑n-3	83.41 ± 4.01	83.49 ± 2.34	81.88 ± 2.47	79.86 ± 2.02	0.04	NS	NS
∑n-6	87.41 ± 4.59	83.69 ± 2.26	83.15 ± 2.66	77.16 ± 4.03	NS	NS	NS
∑Fatty acids	86.22 ± 4.63	81.43 ± 2.01	80.16 ± 3.03	78.25 ± 3.04	0.05	0.03	NS

C: Control diet; F: Future diet; WT: wild type genotype; GS: genetically selected for growth genotype. Values are expressed in mean ± SD. (n = 4 tanks/diet/genotype). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids. Different letters denote significant differences among experimental groups (one-way ANOVA, p < 0.05). Two-way ANOVA, p < 0.05, Genotype and Diet as fixed factors. NS = not significant.

**Table 9**

Results of Factorial MANOVA of wild (WT) and genetically selected (GS) European sea bass shape fed the experimental diets, using centroid (CS), genotype, and diet as factors. Df: Degree of freedom; SS: Sum of squares; MS: mean squares.

Variable	Df	SS	MS	F	p-value
log(CS)	1	0.014	0.014	6.648	0.001
Genotype (gen)	1	0.046	0.046	21.510	0.001
Diet (diet)	3	0.009	0.003	1.417	0.123
log(CS) × gen	1	0.007	0.007	3.285	0.007
log(CS) × diet	3	0.010	0.003	0.567	0.056
gen × diet	3	0.009	0.003	1.431	0.096
log(CS) × gen × diet	3	0.007	0.002	1.069	0.358
Residuals	322	0.693	0.002		
Total	337	0.796			

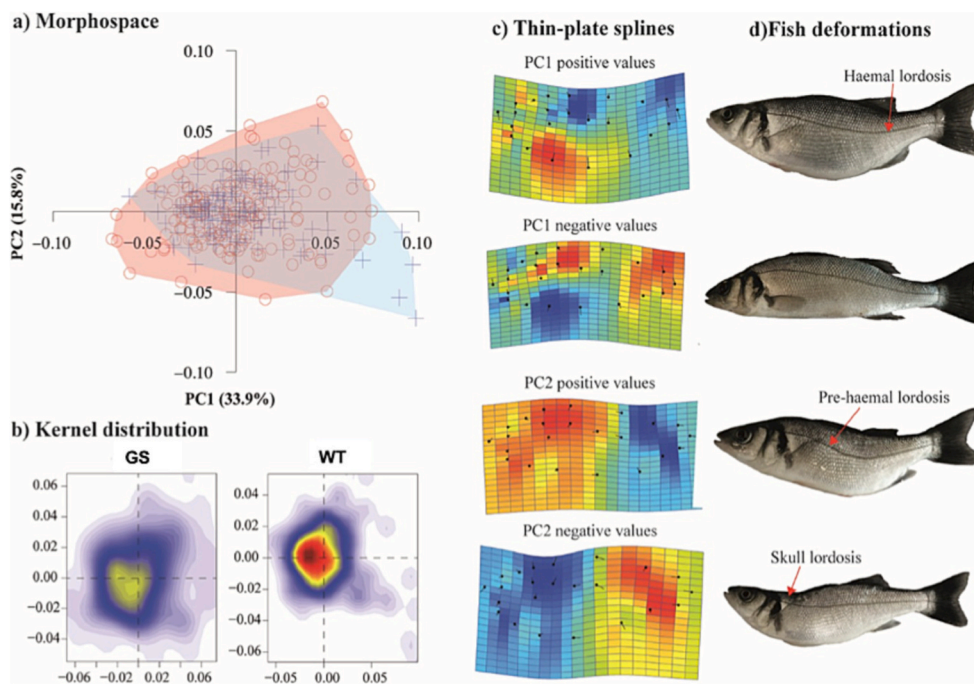
#### 4. Discussion

The sustainable development of the aquaculture industry relies on the use of novel and alternative raw materials that contribute to reduce the dependency on FM and FO coming from oceanic fish populations. Additionally, the multitrait genetic selection of farmed fish which can display higher growth and feed utilization rates when fed alternative feeds is pointed out to be a complementary valuable tool to facilitate the implementation of circular economical approaches. Indeed, although the domestication of European sea bass is relatively recent, this species has been proposed as a good candidate for selective breeding in aquaculture, whose response to selection has been demonstrated in its growth performance (23–42% per generation) (Vandeputte et al., 2009).

##### 4.1. The effect of genetic selection for growth in European sea bass nutritionally-challenged with low FM/FO diets

The results of the present trial demonstrate a 11.2% improvement in growth (in terms of body weight along the whole feeding period) in the progeny of genetically selected European sea bass, being this effect only half of the effect of the equivalence to 3.5 generations of selection (see

Material and Methods). This result agrees with other studies that reported a 12% higher growth in European sea bass offspring from experimentally selected sires when compared with those from wild dams after one generation of selection (Vandeputte et al., 2009). Indeed, in the present study, the multitrait selection was a much stronger factor in improving fish growth parameters, than the dietary treatment, which showed a lower influence on the productive performance of fish. The GS fish of the present study were also indirectly co-selected for other traits than those targeted by the breeding program during the rearing process along the 7 generations, such as their domestication and better adaptation to the rearing process implying farming manipulations, their resistance to different diseases, their higher social interactions at rearing density and their ability to adapt to different feed formulations and evolutions. At the end of the feeding period, only a significant interaction between genotype and diet was found for TGC, with wild sea bass fed the Future diet showing the lowest TGC. We cannot exclude that higher TGC for the selected genotype may not be associated with the higher rearing temperature during its selection *per se* regarding the facility using cooling water from a recirculated power plant. Whatever, this indicates that WT fish were less efficient when fed the Future diet, suggesting a possible interaction of genotype and diet with temperature. Indeed, a genotype × environment interaction was also previously reported for fish TGC when comparing different wild populations reared in different locations, and markedly occurring in a temperature-dependent manner (Vandeputte et al., 2014). In agreement with our study, other feeding trials have shown that selective breeding based on growth combined with a low FM diet was effective in increasing the growth performance of European sea bass (Geay et al., 2011) as well as other fish species, such as Atlantic salmon (Yamamoto et al., 2015), or gibel carp (*Carassius gibelio*) (Xu et al., 2019). Indeed, in our study, the GS sea bass also showed an important improvement of feed conversion ratio as well of protein and lipid utilization when compared to the WT strain, which supports this hypothesis and might partially explain the higher growth in GS fish, as often reported in studies with fish (Kolstad et al., 2006; Overturf et al., 2013; Verdal et al., 2017; Besson et al., 2018). The genetic effect created by the multitrait selection on growth and to limit



**Fig. 6.** a) Scatterplot of the PCA components 1 and 2 (PC1 and PC2) of the body shape of wild (WT, blue plus) and genetically selected for growth (GS, red circle) European sea bass shape fed the experimental diets; b) representation of Kernel density from morphospace for each genotype. Colors represent the clustering of specimens, from red (common shape) to grey (isolated shapes); c) Deformation grids (thin-plate splines) indicating the shape variation along the axes. Colors pointed out the points with higher variation; d) Possible morphological alterations observed in the body shape relying on vertebral deformations according to literature. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fat deposition in the viscera is important quantitatively with a gain in feed efficiency estimated between 11.2% to 21.1% depending on feed origins. Such gain was expected but not so high if only due to selection on growth. It is in agreement with a gain of 20% in FCR reported after 10 generations of selection in rainbow trout (Vandeputte et al., 2022). Indeed, these results are also in line with the higher ADCs of most amino acids observed in GS sea bass compared to WT fish, that were affected mainly by genotype. Selected rainbow trout fed on different plant ingredients also showed improved growth related to a higher digestibility of the dietary amino acids, by synchronization of amino acid absorption, which led to an improved amino acid bioavailability and utilization (Brezas and Hardy, 2020). In other studies, selected fish bass fed a plant-based showed a better growth performance associated with an upregulation of several genes involved in protein biosynthesis (Geay et al., 2011), digestive processes (Murashita et al., 2006), or amino acid intestinal transportation (Brezas et al., 2021; Callet et al., 2021). This higher feed and nutrient utilization is a major result of the study since it can suggest a better digestibility, resulting in lower production of feces, or/and an improved nutrient utilization of protein or amino acids for instance leading to lower ammonia excretion and thus improving the ecological impact of the system.

In addition, the optimization of protein retention is also related to the amount of other non-protein digestible energy sources, including fat (Dias et al., 2005). Several physiological and transcriptomic responses related to lipid metabolism have been described for different strains of several species genetically selected for faster growth, including European sea bass (Geay et al., 2011), Atlantic salmon (Morais et al., 2011; Jin et al., 2020), rainbow trout (Panserat et al., 2009; Callet et al., 2017; Cleveland et al., 2020), or Gibel carp (Xu et al., 2019). Selected fish have shown more effective lipid absorption as well as lipid transport, and/or a better ability for compensatory endogenous conversion and synthesis of lipids than unselected fish (Jin et al., 2020), leading to better utilization of the different dietary lipid sources, and thus giving more flexibility for feed formulations. These studies agree well with the results of the present study, where selected European sea bass showed a higher lipid efficiency ratio as well as a higher digestibility of SFA or n-3 FA. These results, added to the lower perivisceral fat observed in GS sea bass compared to WT genotype, particularly when fed Control diet, supports the hypothesis of a more efficient lipid utilization in selected European

sea bass. The accumulation of perivisceral fat is a result of the excess of dietary lipids that are not deposited in the flesh or used as energy sources for growth and can lead to fatty fish, decreasing the quality of the final product (Katsika et al., 2021). The liver and perivisceral adipose tissue are the primary sites of lipid storage in European seabass (Dias et al., 2005) but GS fish showed also a higher lipid deposition in muscle compared with WT fish, which might suggest a better utilization and mobilization of the lipids to the peripheral tissues. Therefore, the lower perivisceral fat and the higher lipid content in flesh suggest that multi-trait selection of European sea bass increases the quality of the final product compared with non-selected fish. In agreement with the present results, in European sea bass and gilthead sea bream, a reduction of the viscera portion (*i.e.* increase in gutted yield) and consequently, a reduction of perivisceral fat was previously estimated as high heritable traits (Saillant et al., 2009; Navarro et al., 2009), indicating that genetic variation concerning viscera weight is to some extent independent of the fish weight and could be explained by the variation of lipid deposition. This might be probably related to the genetic variability in the expression of genes related to lipid metabolism (Leaver et al., 2011; Morais et al., 2011; Overturf et al., 2013), such as *2-acylglycerol O-acyltransferase 2 (mogat2)*, *long-chain fatty acid-CoA ligase (acsb3)*, *monoacylglyceride lipase (magl)* (Ali et al., 2018; Cleveland et al., 2020), or the cholesterol-related *srebp2* (Leaver et al., 2011; Morais et al., 2011; Overturf et al., 2013), conferring to selected fish a better ability to efficiently grow when fed with alternative diets. Lean-selected fish strains also showed lower lipid biosynthesis by downregulating genes related to lipogenesis, mitochondrial oxidation, and glycerophospholipid metabolism, including *agpat*, *lpp2*, *ppara*, *pparβ* or *srebp-1* (Morais et al., 2011). Furthermore, added to the lipid content of fish flesh, its content in PUFA, particularly DHA, EPA and ARA, are of pivotal importance to satisfy consumers' demand and expectations. It is well known that European sea bass, as most of marine species, is unable to effectively convert 18:C PUFAs to LC-PUFAs (Izquierdo et al., 2003). Interestingly, in the present study, DHA and ARA, but not EPA, were increased in sea bass flesh by selection, suggesting a higher nutritional value of fish flesh for the customers. In agreement with the present results, a higher LC-PUFA content in the flesh of common Carp selected for growth was previously reported and associated with an upregulation of several genes encoding enzymes involved in fatty acid metabolism

(Zhang et al., 2018). In Atlantic salmon, selection for high flesh n – 3 LC-PUFA also increased the expression of lipid transport-related genes in the liver, and down-regulated the expression of genes encoding for acyl-CoA oxidase (ACOX1) and a long chain acyl-CoA dehydrogenase (ACADL) enzymes that metabolize n – 3 LC-PUFA, denoting an increased availability to transport those fatty acids to peripheral tissues (Leaver et al., 2011). Further determining the genetic variation in protein and lipid metabolism-related genes of WT and GS European sea bass would be an important step to better understand how genetic selection for growth can modulate the utilization of dietary nutrients in the current context of novel feeds.

In addition, the incidence of skeleton deformities was another quality trait that was modulated by genotype. Genetic trade-offs between growth and deformation traits have been well described (Vehviläinen et al., 2012). In a recent meta-analysis (Nguyen, 2021), the genetic correlations between growth and deformities varied from negative (Gjerde et al., 2005) to positive (Karahan et al., 2013), or non-significant (Kolstad et al., 2006). For marine fish, Lee-Montero et al. (2015) found a genetic correlation between sea bream growth and deformities, and the phenotypic variation of the presence of deformities (mainly vertebral deformities) was determined by an apparent relevant additive genetic component. In sea bass, intermediates heritability for lordosis, scoliosis and spinal malformations were also reported, with positive genetic correlations with body weight and body weight gain depending on age and sites (0.18 to 0.50) (Bardon et al., 2009; Karahan et al., 2013). These two last studies estimated differences between parents during a single generation and they were not able to differentiate these results from indirect effects of environmental factors (as flow velocity at some age) within the year of testing. In the present study, although PCA analysis did not show a very high significance for the deformities, the effect of selection on fish morphology was found to be limited, with GS fish showing a slightly higher incidence of deformities when compared to WT fish, suggesting that the real genetic correlation between growth and rate of spinal malformations is probably much lower in the present study than in the two previous reported studies (Bardon et al., 2009; Karahan et al., 2013).

#### 4.2. The effect of low FM/FO diets with alternative raw materials on European sea bass productive performance

The similar performance of European sea bass fed the Future diet with fish fed the Control diet, even after a long-term period of 300 days, highlights the success of the replacement of 50% of the dietary FM and total FO by PM and a blend of PO and microalgal oil, respectively. The use of animal fats, such as PO in diets for European sea bass juveniles, has been recently investigated showing that up to 50% of dietary FO could be replaced by PO with no negative effects on European sea bass growth (Monteiro et al., 2018; Campos et al., 2019). However, in those studies, higher replacement levels (above 50%) reduced dietary n-3 LC-PUFA in both the diets and the fish tissues, leading to a reduced fish growth performance. In the present study, the use of a blend of PO and a DHA-rich microalgal oil, allowed to maintain the n-3 LC-PUFA levels in the Future diet at an optimal level to meet the EFA requirements of sea bass, thus maintaining sea bass growth at a good rate during the whole feeding period. Based on the results of the present study, the supplementation of a microalgal oil rich in DHA can thus prevent the deleterious effects associated with the total replacement of FO in diets for European sea bass, as recently reported in other marine warm-water species, such as gilthead sea bream (*Sparus aurata*) (Carvalho et al., 2020), or meagre (*Argyrosomus regius*) (Carvalho et al., 2022). Furthermore, in previous studies, the growth of European sea bass was also highly dependent on the dietary FM content, especially when fish should have reached the commercial size (> 500 g) (Castro et al., 2021). For instance, the reduction of FM from 20 to 5% of the diet and its replacement by vegetable proteins caused a significant reduction of 33% and 9% in sea bass weight and SGR, respectively, after a grow-out period

of 556 days (Castro et al., 2021). In contrast, in the present study, the replacement of FM by PM did not affect sea bass growth, suggesting that PM had an appropriate profile of essential amino acids for proper somatic growth and nitrogen utilization (Kaushik et al., 2004), including lysine and methionine, which are usually the most limiting amino acids for fish growth when FM is reduced in the diet (Torrecillas et al., 2017). The good growth observed in the European sea bass fed the Future diet was also in agreement with the high protein ADC observed, but Future diet apparently reduced the ADC of some amino acids, in agreement with a previous study reporting the lower amino acid availability of PM compared to fish meals (Allan et al., 2000). The lower ADC of MUFA observed in Future diet compared to Control diet was also in line with the lower MUFA content observed in fish flesh fed this diet, corroborating a possible lower availability of these FA in Future diet and in line with the lower lipid content of the flesh from fish fed this diet. Future diet also increase the content of n-3 and n-6 docosapentaenoic acid (DPA; 22:5n-3 and 22:5n-6), probably due to the high content of n-6 DPA of the microalgae oil, as well as had an additive effect to selected genotype in increasing the total n-3 FA content of sea bass flesh, suggesting that combining this alternative diet with genetic selection can increase the omega-3 content in flesh and ultimately increase the nutritional value of the final product for human consumption.

## 5. Conclusions

In summary, from the second month of feeding until the end of the trial, European sea bass that was selected since for 7 generations performed better in terms of growth than the wild-type genotype, possibly related with an apparent favored feed and nutrient utilization. Furthermore, selection increased the nutritional value of flesh by decreasing the perivisceral fat and by increasing DHA and ARA contents. In contrast, the dietary treatment showed little effect on fish growth performance, denoting the successful partial replacement of FM by PM and the total replacement of FO by a blend of poultry oil and an emergent microalgal oil. However, Future diet tended to reduce the ADCs of some amino acids, as well as showed an additive effect to genotype in increasing the n-3 PUFA of flesh. Altogether, our data demonstrate that multitrait genetic selection of European sea bass improve fish plasticity to cope with the variations of ingredients in alternative feeds with low FM/FO.

## CRedit authorship contribution statement

**D. Montero:** Conceptualization, Funding acquisition, Data curation, Formal analysis, Investigation, Project administration, Writing – original draft, Writing – review & editing. **M. Carvalho:** Investigation, Writing – original draft, Writing – review & editing. **G. Terova:** Conceptualization, Writing – review & editing. **R. Fontanillas:** Writing – review & editing. **A. Serradell:** Data curation, Formal analysis. **R. Ginés:** Investigation. **F. Acosta:** Investigation. **S. Rimoldi:** Investigation. **A. Bajek:** Resources. **P. Haffray:** Writing – review & editing. **F. Allal:** Resources. **S. Torrecillas:** Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing.

## Declaration of Competing Interest

Daniel montero reports financial support was provided by European Union.

## Data availability

Data will be made available on request.

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