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Genetic selection and novel feeds containing single cell protein as a substitute for fishmeal in European sea bass: Effects on growth, fatty acid profile and E-sensing analysis of fillets

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ABSTRACT

The development of sustainable aquaculture relies on replacing marine raw materials like fish meal (FM) and fish oil (FO). Emerging alternatives, such as single-cell proteins and alternative lipids, offer promise. This study explored the effects of partially substituting FM with 10% bacterial protein (*Methylococcus capsulatus*) and completely replacing FO with a blend of poultry oil (PO) and DHA-rich microalgae oil in European sea bass (*Dicentrarchus labrax*) of unselected (WT) and selected (HG) genotypes. The results indicated that bacterial protein had no adverse impact on fish growth. The HG group demonstrated better growth and feed conversion due to genetic selection. This study also analysed the dietary and genotype effects on body lipid composition and fatty acid profiles. Notably, the HG fish had lower levels of major fatty acids (EPA, DHA, n-3 FAs, and n-3 LC-PUFAs) in their fillets, but not in their whole-body composition. These differences influenced sensory and qualitative aspects. Electronic sensory analyses (the first e-sensory profiling conducted for genetic purposes in fish) showed more significant differences due to diet in the WT group, with a less variable pattern in the e-tongue score in for the HG group. The volatile profiles showed no significant differences. In summary, combining selected fish genotypes with innovative feeds is a step forward in aquaculture. It maximizes nutrient utilization, enhances fish growth, and improves product quality. This approach becomes increasingly important in scenarios with limited FM/FO availability, promoting sustainability in aquaculture.

1. Introduction

Aquaculture is an indispensable food production system to meet global food needs and sustainability challenges. The rapid increase in global aquaculture production over the past 30 years is expected to continue due to the growing world population and rising average per capita income (FAO, 2018). With 90% of the world's wild fish stocks overfished or fished at capacity, the potential for seafood production from wild stocks has likely reached a ceiling or is declining. Future expansion of seafood production should come from aquaculture, which is one of the fastest growing sectors of food production in the world (FAO, 2022). However, such rapid development can also have negative environmental impacts, such as overfishing for feed raw materials. Finfish species have traditionally relied on protein and lipid sources from the sea, such as fish meal (FM) and fish oil (FO), to thrive – but aquafeeds can no longer rely on these raw materials. Therefore, research is rapidly evolving into new and sustainable aquafeed ingredients that could reduce the use of marine commodities (FM and FO) or eventually

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replace them without compromising farming efficiency (Tacon and Metian, 2015).

Commodities currently used as FM substitutes to make intensive aquaculture production more sustainable include terrestrial plants, fish by-products, insects, marine algae, and microbial biomass (Naylor et al., 2009; Bandara, 2018; Glencross et al., 2020a). The latter, generally defined as single-cell proteins (SCPs), refers to dried cells of microorganisms, such as yeast, bacteria, fungi, or microalgae that are grown in large-scale culture systems to be used as protein sources in animal feed. The production and use of SCPs in aquafeeds as an alternative source of marine-derived protein has attracted particular interest due to the microorganisms' high crude protein content, rapid growth, and ability to grow on a variety of substrates and convert non-food waste materials into high-quality feed (Glencross et al., 2020b; Sharif et al., 2021). Soybean meal and FM, traditionally used to produce high-protein fish feed, have significant environmental impacts and are also associated with a high carbon footprint. Microorganisms can instead be easily grown in a controlled environment without depending on seasonal and climatic conditions. They can also tolerate different growing conditions and be genetically modified to obtain specific products. In addition, these products do not compete with food crops for arable land and fresh water. Therefore, SCPs could be a real alternative to FM and/or soybean meal in aquafeeds (Mekonnen and Hoekstra, 2014; Vermeulen et al., 2012).

Most SCP sources (from bacteria and yeast) generally contain 50–80% protein on a dry weight basis and an amino acid profile comparable to FM with similar lysine, methionine, and cysteine content and higher levels of tryptophan and threonine (Skrede et al., 1998). They also contain a wide range of macro- and micronutrients with high nutritional value and biological relevance, such as phospholipids, vitamins, and omega-3 fatty acids, as well as other bioactive molecules such as astaxanthin and peptidoglycans (Jones et al., 2020; Zamani et al., 2020).

The two major obstacles to the use of SCPs are related to their high content of nucleic acids, which can cause metabolic disorders in many farm animals. However, studies have shown that fish species, unlike terrestrial animals, can tolerate and metabolize excess dietary nucleic acids without adverse effects (Rumsey et al., 1991; Oliva-Teles et al., 2006). In addition, the production costs of SCPs are higher than those of conventional feedstocks such as FM or vegetable meals, limiting their viability as a protein source for large-scale feed production. Reducing production costs is a matter of ongoing investigation, development and investment. However, to reduce costs while maximizing yields and cell growth, substrate selection is a very important factor as it represents a significant portion (45–75%) of the total production cost (Ritala et al., 2017; Jones et al., 2020). For this reason, the use of substrates from low-cost waste materials from the food and beverage industry or directly from forestry and agricultural sources represents a key strategy. At the same time, these substrates help to reduce the environmental footprint of the entire production chain without compromising the quality of the final microbial biomass (Øverland and Skrede, 2017; Sharif et al., 2021; Xu et al., 2021).

Methylococcus capsulatus is a gram-negative methanotrophic bacterium (Foster and Davis, 1966) that is commercially produced by a few companies worldwide. Inclusion of this microbial source can account for 52% and 38% of dietary protein in the diets of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), respectively, without negatively affecting growth performance (Øverland et al., 2010). In spotted sea bass (*Lateolabrax maculatus*), *M. capsulatus* showed no negative effects on fish growth when it constituted up to 14% of the total diet to replace 40% FM (Yu et al., 2022, 2023). In gilthead sea bream (*Sparus aurata*), a diet containing 10% *M. capsulatus* to replace 66.6% of FM resulted in similar growth performance and feed conversion as a FM-based control diet (Carvalho et al., 2023). To our knowledge, only one very recent study has attempted to include bacterial meal from *M. capsulatus* in the diet of European sea bass (*Dicentrarchus labrax*) (Vasilaki et al., 2023). The results of this 71-day feeding experiment showed that the diet containing 15% of a mixture of SCPs (bacteria: yeast: algae - 9.4:4.7:1), in which the bacterial protein consisted of *M. capsulatus* meal, had no negative effects on fish growth parameters.

However, the long-term effects of replacing FM with SCPs such as *M. capsulatus* and their impact on fillet quality and potential consumer perception remain to be evaluated. Sensory characteristics of fish fillets such as appearance, colour, odour, texture, and taste may be affected by changes in diet and should therefore be considered when novel raw materials are incorporated into fish diets. In addition, sensory characteristics of fish are of great importance to consumers as they are a major determinant of fish consumption and are also extremely important in evaluating the freshness of this product.

In addition to FM replacement with alternative protein sources, it is also of utmost urgency for aquafeeds to reduce dependence on FO as a source of long-chain n-3 polyunsaturated fatty acids (n-3 LC-PUFA). In this regard, algal oils from microalgae could be the most promising source to replace the entire FO of aquafeeds due to their very high content of n-3 LC-PUFA, especially DHA. Microalgae products supported a reduction in the reliance on FM/FO in feeds for many farmed fish species (Kousoulaki et al., 2015; Sarker et al., 2016; Carvalho et al., 2020). Moreover, since microalgal oils are still an expensive raw material, their combination with low-cost lipid sources, such as poultry oil (PO) has proven to be a suitable and flexible alternative to completely replace FO and balance the nutritional profile of feeds (Carvalho et al., 2020).

According to recent studies, the challenge of gradually replacing FM in aquafeeds should be addressed with a holistic research strategy tailored to industry needs rather than replacing single or multiple commodities (Turchini et al., 2019). In this view, another approach is breeding programs and genetic selection that can improve fish growth, health, and feed conversion. Genetic selection is an important innovation tool that has completely changed the way breeding is done in terrestrial animals. It has already been taken up by the fish farming sector and is playing an increasingly important role in the global production of several farmed species (Small et al., 2016; Chavanne al., 2016; Boudry et al., 2021). Growth improvements of 5–20% per generation have been observed in many genetically selected fish species (Vandeputte et al., 2009; Gjedrem et al., 2012).

At the same time, breeding programs have dramatically changed the physiology of farmed fish and the associated nutrient requirements. As a result, some of the current unbalanced diets do not fully meet the nutrient requirements of "modern" fish, and this is considered by breeders to be one of the main obstacles to the viability of the sector. Therefore, there is an urgent need to develop tailored feeds for genetically selected fish to ensure the production of healthier, more nutritious, and resource-efficient animals. In addition, selection breeding programs can be used as a complementary tool to improve the implementation of novel formulations with emerging commodities, as selected fish have a higher tolerance and plasticity to changes in feed formulations (Dupont-Nivet et al., 2009; Le Boucher et al., 2013; Yamamoto et al., 2015; Callet et al., 2017; Carvalho et al., 2023) and may be able to utilize novel alternative feeds with low FM/FO levels more efficiently, as recently reported in European sea bass for growth, carcass yield, FCR, protein and lipid retention, but also for gut microbiota, gut health or immune response and stress resistance (Montero et al., 2023; Torrecillas et al., 2023; Serradell et al., 2023; Rimoldi et al., 2023).

Therefore, the production of novel and improved feed formulations specifically adapted to genetically superior genotypes from breeding programs can reduce dependence on traditional marine ingredients and improve the use of cost-effective and sustainable raw materials in aquafeeds, taking into account the principles of waste prevention and circular economy. This will enable the improvement of fish health and the production of nutritious products for consumers while applying the environmentally friendly principles of waste prevention and circular economy.

Accordingly, the present study aimed to evaluate the effects of the simultaneous replacement of FM and FO with newly emerging proteins (derived from *Methylococcus capsulatus*) and lipid sources (a mixture of DHA-rich microalgal oil, and PO) on the growth performance, filet quality, and sensory characteristics of unselected and selected genotypes of European sea bass (*Dicentrarchus labrax*).

2. Material and methods

2.1. Ethical statements

Animal experimentation is in accordance with the European Union Council Directives (2010/63/EU) for the use of experimental animals. The Bioethics Committee of the University of Las Palmas de Gran Canaria approved all protocols used in the present study (approval no OEBA-ULPGC 13/2020).

2.2. Fish rearing conditions and sampling

The fish feeding experiment was conducted in the flow-through seawater system of the Parque Científico-Tecnológico Marino, University of Las Palmas de Gran Canaria (Telde, Canary Islands, Spain) (ULPGC). The additive effect of genetic selection was evaluated by comparing the effects of 2.5 generations of selection on the same female stock (half of the effect) through the sire pathway (see details in Montero et al., 2023a). Frozen sperm from selected sires of EMG or Ecloserie Marine de Graveline (France) or from wild sires (Golf du Lion, France) were used to fertilize eggs from the experimental broodstock selected by Ifremer. The EMG line has been selected for 5 generations by multi-trait selection (growth, carcass yield, external morphology, robustness on farm) and the Ifremer line has been experimentally selected for growth for 3 generations. The mean breeding value of the HG genotype (male EMG x female Ifremer) is (5 + 3)/2 or 4 equivalent generations and the mean breeding value of the WT genotype is (0 + 3)/2 or 1.5 equivalent generations, so that the genetic difference between the HG and WT genotypes is 2.5 selection generations (= 4 – 1.5).

The hatched larvae of genotypes HG and WT were sent from the Ifremer Palavas Research Station (France) to the ULPGC for juvenile rearing. HG and WT sea bass were cultured at ULPGC under similar conditions during the preweaning, weaning, and early juvenile stages until they reached the initial experimental size of 31.95 $\pm$ 1.01 g. Then, fish were randomly distributed into 12 tanks of 500 L (6 tanks per genotype), at a density of 35 fish/tank and fed for 300 days with two diets: a commercially-like control diet (C) based on 20% FM and 5.09–7.14 FO, and an alternative diet (SCP) containing 15% FM, 0% FO, 10% SCP and 2–4% PO and 2–3% DHA oil from algae.

During the feeding experiment, water quality was monitored daily (salinity: 37 mg/L, dissolved oxygen 6.0 $\pm$ 0.5 ppm, and temperature 22 $\pm$ 1 °C). Fish were fed three times daily for 6 days per week until apparent satiety. Excess unconsumed fish feed was gathered on a daily basis by allowing water to drain after each meal. The collected feed was then subjected to a 24-hour drying process in an oven and subsequently weighed for a more precise determination of feed intake (FI) and feed conversion. The progress of fish growth was assessed at four-week intervals throughout the feeding experiment, with the fish undergoing a 24-hour fasting period before sampling. In order to monitor fish growth during the feeding trial, all fish were anesthetized using clove oil (0.04 mL/L; Guinama S.L; Spain, ref. Mg83168) and individually weighed and measured. Body weight (BW) was measured and growth performance indices, such as specific growth rate (SGR), thermal growth coefficient (TGC), protein efficiency ratio (PER), and feed conversion ratio (FCR) were calculated using the following equations:

SGR = 100 x [ln (final BW) - ln (initial BW)]/days;

TGC = [(final weight$^{\circ}$ - initial weight$^{1/3}$) / (temperature x number of days) x 1000];

PER= weight gain / protein intake; FCR = total feed fed/ weight gain;

Protein gain (%) = (final BW x final protein content, %) - (initial BW x initial protein content, %);

Lipid gain (%) = (final BW x final lipid content, %) - (initial BW x initial lipid content, %).

At the end of the experiment, 12 fish per tank were euthanized with an excess of clove oil (5 mL/L), and whole-body samples from 6 fish and fillets from other 6 fish per tank were collected and pooled for proximate composition and fatty acid profile analysis. In addition, 20 sea bass fillets (white dorsal muscle) were collected and analysed for two sensory characteristics using e-nose and e-tongue. All samples were stored at −80°C until analysis.

2.3. Experimental diets

Two isonitrogenous, isolipidic, and isoenergetic diets named C (control) and SCP (single cell protein), were formulated for the feeding trial by Skretting (Skretting ARC, Norway). Pellet size was adjusted to fish size during the experiment, following the recommendations of the feed manufacturer (Skretting ARC, Norway). The ingredients and proximate composition of the diets for both pellet sizes (C 1.8, SCP 1.8; C 4, SCP 4) are shown in Table 1. The control diet (C) was a commercial-like diet containing 20% FM and 5.09–7.14% FO (C 1.8 and C 4, respectively), while the SCP diet was based on novel ingredients to reduce the use of FM and FO. Thus, in the SCP diet, FM was partially replaced (25%) by 10% bacterially derived protein produced by a *M. capsulatus* fermentation (FeedKind©, Calysta, USA) and FO was completely replaced by a combination of poultry oil and a DHA-rich microalgae oil (Veramaris, Netherlands). The amino acid and fatty acid profiles of the feeds are shown in Tables 2 and 3, respectively.

Table 1
Ingredients and proximate composition of the experimental diets used in the European sea bass experiment.

	Experimental diets			
Name/pellet size	**C 1.8**	**SCP 1.8**	**C 4**	**SCP 4**
Ingredients (%)				
Fish meal[1]	20.0	15.0	20.00	15.00
Single-cell meal[2]		10.00		10.00
Soya protein concentrate[3]	19.57	15.38	13.93	10.00
Wheat[4]	20.36	21.76	24.57	22.37
Faba bean[5]	8.0	8.0	8.0	7.03
Corn gluten[6]	1.28	3.08	3.00	3.00
Wheat gluten[7]	18.00	15.00	13.50	13.50
Fish oil[8]	5.09		7.14	
Rapeseed oil[9]	4.60	4.77	6.64	8.44
Poultry oil[10]		1.65		4.02
DHA-algal oil[11]		2.00		3.04
Vitamin premix[12]	0.10	0.10	0.10	0.10
Mineral premix[13]	0.30	0.30	0.30	0.30
Phosphate	0.61	0.86	0.73	1.10
Lecitin	2.00	2.00	2.00	2.00
Proximate composition (%) (dry weight basis)				
Moisture	7.36	7.47	7.00	7.00
Crude protein	49.92	49.80	40.49	41.18
Crude fat	15.79	15.79	20.00	22.00
Ash	4.57	4.54	5.56	5.53

C: control diet; SCP: single-cell protein-based diet.[1] Norsildmel AS (Norway); [2] Calysta (USA); [3] CJ Selecta S.A (Brasil); [4] Lantmnnen Ek For (Sweeden); [5] Cefetra BV (The Netherlands); [6,7] CARGILL (The Netherlands); [8] Copeinca, S. A. (Perú); [9] AAK, AB (Sweeden); [10] Sonac B.V. (Belgium); [11] Veramaris (The Netherlands); [12,13] Trouw Nutrition (The Netherlands).

Table 2
Amino acid composition (g/100 g feed) of the experimental feeds.

Amino acid	Control (C)	Single-cell protein (SCP)
Arginine	2.78	2.26
Histidine	1.09	0.95
Isoleucine	1.91	1.65
Leucine	3.59	3.07
Lysine	2.29	2.29
Methionine	0.84	0.86
Cysteine	0.65	0.49
Valine	2.05	1.86
Phenylalanine	2.18	1.05
Threonine	1.62	1.49
Tyrosine	1.25	1.88
Alanine	2.17	2.04
Glutamic acid	10.11	8.43
Glycine	2.14	1.83
Aspartic acid	3.71	3.3
Proline	3.31	2.81
Serine	2.20	1.86

Amino acid composition refers to the 1.8-mm feed. However, the various pellet-sized feeds were formulated to have very similar AA compositions.

2.4. Proximate and fatty acid composition analyses

The proximate composition analyses of both the feed and fish samples were conducted in accordance with standardized procedures outlined in AOAC (2019) guidelines. Crude protein content (Nx6.25) was determined using the Kjeldahl method. The ash content was established by subjecting the samples to a 12-hour incineration at 600 °C in a muffle furnace, while moisture content was ascertained by drying the samples in an oven at 110 °C until a constant weight was achieved.

To analyze the fatty acid composition, we extract the total lipids from all samples, including fish fillets. This extraction was done according to the method described by Folch et al. (1957). Lipids were extracted using a chloroform/methanol mixture (2:1 v/v). Fillet lipid content was analysed through near-infrared spectroscopy (NIR) with a FoodScan™ instrument from FOSS in Hillerød, Denmark. The preparation of fatty acid methyl esters involved transmethylation of the total lipids following the protocol outlined by Christie (1989). These esters were then separated through gas chromatography, provided with a flame ionization detector (FID) utilizing the conditions specified by Izquierdo et al. (1990). The fatty acids of the fish samples were identified by comparing the relative retention times of the fatty acid methyl esters with those of a standard mixing solution, such as EPA 28 from Nippai, Ltd. in Tokyo, Japan, under the same analytical conditions. FA concentrations were expressed as a percentage of the total fatty acid methyl esters identified.

2.5. Fillet quality: texture characteristics

Texture analysis was conducted on the uncooked fillets from each experimental group. Two 2×2 cm rectangular sections were extracted from the skinless fillet's dorsal part and examined with a texturometer TA-XT2, manufactured by Stable Micro Systems Ltd. in Surrey, UK. The texturometer's strength was calibrated to a 5 kg mass.

Fracture strength, hardness, elasticity, cohesiveness, gumminess, chewiness, stickiness, and resilience were computed according to the methodology outlined by Ginés et al. (2004). These textural properties were measured using a 100 mm Ø pressure plate at a speed of 0.8 mm/s, continuing until a raw deformation equivalent to 60% of the specimen's thickness was achieved, as described in the works of Ginés et al. (2004) and Castro et al. (2021).

2.6. Instrumental sensory evaluation of the organoleptic characteristics of sea bass fillets

Fillets (white dorsal muscle) of 20 sea bass were collected from fish of 4 experimental groups: genetically selected (HG) and wild-type (WT) sea bass fed either control (C) or single cell protein (SCP) diets.

Table 3
Fatty acid composition (% total fatty acids) of the experimental feeds.

Fatty acids	Control (C)	Single-cell protein (SCP)
14:0	1.49	0.70
14:1n7	0.01	0.02
14:1n5	0.08	0.03
15:0	0.18	0.14
15:1n5	0.02	0.01
16:0 iso	0.04	0.03
16:0	9.21	13.37
16:1n7	1.81	1.91
16:1n5	0.08	0.03
16:2n6	0.00	0.00
16:2n4	0.12	0.03
17:0	0.08	0.03
16:3n4	0.18	0.09
16:3n3	0.10	0.02
16:3n1	0.03	0.02
16:4n3	0.12	0.04
16:4n1	0.01	0.01
18:00	2.18	2.65
18:1n9	33.96	38.09
18:1n7	2.45	2.17
18:1n5	0.15	0.06
18:2n9	0.01	0.01
18:2n6	16.54	19.85
18:2n4	0.05	0.03
18:3n6	0.06	0.05
18:3n4	0.06	0.04
18:3n3	4.91	4.88
18:3n1	0.01	0.01
18:4n3	1.17	0.28
18:4n1	0.03	0.01
20:0	0.50	0.55
20:1n9	0.51	0.12
20:1n7	4.97	1.90
20:1n5	0.13	0.06
20:2n9	0.01	0.01
20:2n6	0.21	0.10
20:3n9	0.03	0.01
20:3n6	0.04	0.04
20:4n6	0.25	0.47
20:3n3	0.11	0.09
20:4n3	0.29	0.17
20:5n3	3.39	2.87
22:1n11	7.26	1.25
22:1n9	0.77	0.46
22:4n6	0.08	0.06
22:5n6	0.23	0.38
22:5n3	0.39	0.26
22:6n3	5.72	6.59
SFA	13.68	17.47
MUFA	52.20	46.11
n9-UFA	35.28	38.70
n6-PUFA	17.41	20.96
n3-PUFA	16.18	15.20
EPA/ARA	13.30	6.10
EPA/DHA	0.59	0.44
EPA+DHA	9.11	9.46
n3 LC-PUFA	9.89	9.98

Fatty acid composition refers to the 1.8-mm feed. However, the various pellet-sized feeds were formulated to have very similar FA compositions.

2.6.1. E-sensing analysis: e-nose

An e-nose (FOX 4000, Alpha MOS, Toulouse, France) equipped with an array of 18 MOS (metal oxide semiconductors) whose resistance is modulated in the presence of a carrier gas (air), was used to evaluate the volatile profile of the fish fillets. The instrument was also equipped with a stirring oven and an HS100 headspace autosampler. To perform the analysis, the samples were minced with a knife and then 2 g of each fillet was weighed and placed in a 10 mL glass vial with a magnetic cap. Then the vials were placed in the autosampler. For each sample, 4 vials were prepared and analysed, resulting in a total of 80 analyses (4 replicates x

20 samples). The biological samples were incubated at 60 °C for 5 minutes and then shaken at 500 rpm for 5 seconds. Injection was performed at a temperature of 70 °C using a syringe. The injection volume was 3500 µl and the injection rate was 500 µl/s.

2.6.2. E-sensing analysis: e-tongue

Artificial taste analysis was performed using a commercially available electronic tongue (Astree, Alpha MOS, Toulouse, France) equipped with a set of seven potentiometric sensors (ANS, PKS, CTS, NMS, CPS, ANS, and SCS). This equipment included an Ag/AgCl reference electrode (Metrohm, Pte Ltd., Singapore), a mechanical stirrer, a 48-position autosampler, and an electronic unit for final amplification and analogue-to-digital conversion. To perform the analysis, the samples were crushed with a knife. Then, 5 g of each sample was immersed in 50 mL of double distilled water and homogenised for 2 minutes using an Ultra Turrax (IKA T25 Basic). The homogenised samples were centrifuged at 3000 rpm for 15 minutes at 4°C. The solution was then filtered and poured into 25-mL beakers for analysis (Trabelsi et al., 2021). The analysis of a single sample was repeated 30 times to obtain the most stable sensor response, and the last 15 measurements were used for data processing. The signal was acquired every second for 120 seconds, and the average intensity of the last 20 seconds was measured. Before measurement, the sensors were conditioned with one of the samples as standard. After each measurement, the sensors were rinsed with double-distilled water.

2.7. Statistical analysis

All data, except for the results of the e-sensing analyses, are expressed as mean ± SD and were tested for normality and homogeneity of variances using the Shapiro–Wilk test and Levene's test, respectively. To assess the effects of genotype and diet and their possible interactions, a two-way ANOVA was performed using diet and genotype as fixed factors. When significant interactions were found ($p < 0.05$), a one-way ANOVA was applied to the data to test for differences between groups, using Tukey's as a post-hoc test (Tukey, 1949). All statistical analyses were performed using SPSS Statistical Software System v24.0 (SPSS, Chicago, IL, USA). For the e-sensing analyses, exploratory data analysis was first performed for each set of measurements using principal component analysis (PCA). Then, the sensors with higher discriminatory power were selected and the reduced data set was subjected to another PCA to improve the ability to analyse the results and avoid the redundancy of sensor response. Data evaluation was expressed in terms of the discrimination index (DI), which provides an evaluation of the discrimination quality on the selected plan from the surface between groups and the size of each group. In addition, based on the organoleptic distance, the pattern discrimination index (PDI%) between the 4 groups was calculated. All data analyses were performed using the native instrument AlphaSoft statistical software v12.44 (Alpha- MOS, Toulouse, France).

3. Results

3.1. Fish growth performance

After 300 days of feeding with the experimental diets, the HG-selected fish exhibited significantly higher body weight and length as well as higher growth parameters such as FCR, PER, protein, and lipid gain than the WT genotype, regardless of diet ($p<0.05$; Table 4). However, the biometric parameters were not affected by diet and no significant interactions were observed between the genotype and the diet fed during the feeding experiment (Table 4).

3.2. Proximate and fatty acid composition of fish tissues

Whole-body fat content was significantly higher in fish of genotype HG than in WT, regardless of diet ($p<0.05$; Table 5). The SCP diet decreased whole-body fat and moisture content compared to the C diet, regardless of fish genotype. No significant GxD interactions were observed for whole-body proximate composition. In addition, fat content in fillets decreased significantly in the HG genotype ($p<0.05$), while no effect was observed for protein content. A significant interaction ($p<0.05$) GxD was observed for moisture content in the fillet, with fish of the HG genotype fed SCP having the highest moisture content (Table 5).

As for the whole-body fatty acid profile, a significant decrease in total MUFA was observed in the HG genotype, while total n-6 PUFA and n-3 PUFA increased ($p<0.05$) regardless of diet (Table 6). Instead, the SCP diet increased 16:3n-1, 18:2n-6, 18:3n-3, 20:0, 20:2n-6, 20:3n-6, 22:5n-6, and total n-6 PUFA, while 14:0, 15:0, 16:0, total SFA, 16:1n-7, 16:1n-5, total MUFA, 16:2n-4, 16:3n-4, 18:2n-9, 18:4n-3, 18:4n-1, and 22:1n-11 decreased ($p<0.05$), regardless of the genotype of the fish. Significant GxD interactions were observed for several fatty acids, including 18:1n-9, 18:1n-7, 20:4n-6, 20:5n-3, 22:6n-3, total n-3 PUFA, EPA+DHA, and n-3 LC-PUFA. In addition, the fish of genotype HG, fed SCP, had the highest levels of 20:4n-6, 20:5n-3, 22:6n-3, total n-3 PUFA, EPA+DHA, and n-3 LC-PUFA compared to the fish of the other experimental groups (Table 6).

Regarding the fatty acid composition of the fillets, the content of 16:0, 16:4n-1, 18:1n-9, and total n-9 increased in the muscles of the genotype HG, while the content of 16:4n-3, 18:0, 22:6n-3, total n-3 fatty acids, EPA+DHA, and n-3 LC-PUFA decreased regardless of the diet

Table 4
Growth performance, feed conversion ratio, and nutrient gain of WT and HG European sea bass fed the experimental diets.

					Two-way ANOVA (p=value)		
	WT-*C*	**WT-*SCP***	**HG-*C***	**HG-*SCP***	Genotype	Diet	GxD
Growth performance							
BW (g)[1]	186.70±13.43	214.11±33.36	275.65±23.75	248.99±18.76	*p*=0.002	*n.s*	*n.s*
TL (cm)[2]	24.56±0.16	25.45±1.90	27.44±0.47	26.61±0.55	*p*=0.009	*n.s*	*n.s*
SGR[3]	0.59±0.02	0.64±0.06	0.71±0.04	0.69±0.02	*p*=0.006	*n.s*	*n.s*
TGC[4]	0.38±0.02	0.42±0.05	0.49±0.03	0.47±0.02	*p*=0.003	*n.s*	*n.s*
FI (g/fish)[5]	291.92±21.66	332.29±44.33	320.45±22.35	299.21±11.36	*n.s*	*n.s*	*n.s*
FCR[6]	1.89±0.10	1.82±0.10	1.32±0.04	1.38±0.08	*p* < 0.001	*n.s*	*n.s*
PER[7]	1.07±0.06	1.10±0.06	1.52±0.05	1.46±0.08	*p* < 0.001	*n.s*	*n.s*
Nutrient gain							
Protein gain (%)	72.32±6.24	87.03±14.50	118.06±13.50	99.32±9.40	*p*=0.002	*n.s*	*n.s*
Lipid gain (%)	83.01±19.89	75.57±21.13	154.47±22.51	113.66±21.21	*p*=0.002	*n.s*	*n.s*

C: Control diet; SCP: Single-cell protein diet; WT: wild-type (non-selected) genotype; HG: high-growth genotype. [1]BW: body weight; [2]TL: total length; [3]SGR: specific growth rate; [4]TGC: thermal growth coefficient; [5]FI: feed intake; [6]FCR: feed conversion ratio; [7]PER: protein efficiency ratio; Values are expressed in mean ± SD. (n = 3 tanks/diet/genotype). Two-way ANOVA, $p < 0.05$, Genotype and Diet as fixed factors. Different letters denote significant differences analysed with one-way ANOVA, $p < 0.05$ for significant g x d interactions. n.s= not significant.

Table 5
Proximate composition of tissues (% wet weight) of WT and HG European sea bass fed the experimental diets.

						Two-Way ANOVA (*p=value*)		
		WT-*C*	**WT-*SCP***	**HG-*C***	**HG-*SCP***	Genotype	Diet	GxD
Whole-body	Protein	14.98±0.65	15.49±0.35	15.39±0.07	15.12±0.09	*n.s*	*n.s*	*n.s*
	Ash	1.09±0.03	1.22±0.18	1.30±0.32	1.10±0.02	*n.s*	*n.s*	*n.s*
	Lipids	16.61±2.41	13.52±1.17	18.99±0.71	16.41±1.88	*p*=0.022	*p*=0.010	*n.s*
	Moisture	68.28±0.60	67.65±0.067	68.77±0.50	67.73±0.78	*n.s*	*p*=0.031	*n.s*
Muscle	Protein	20.52±0.46	20.72±0.81	20.57±0.74	20.37±0.95	n.s	n.s	n.s
	Lipids	6.13±1.22	5.65±1.31	5.15±1.66	4.52±1.27	*p*=0.004	n.s	n.s
	Moisture	74.12±2.21[b]	73.14±1.93[b]	73.06±1.71[b]	76.71±1.74[a]	*p*=0.012	*p*=0.008	*p*<0.001

C: Control diet; SCP: Single-cell protein diet; WT: wild-type (non-selected) genotype; HG: high-growth genotype. Values are expressed in mean ± SD. (n = 3 tanks/diet/genotype). Two-way ANOVA, p<0.05, Genotype and Diet as fixed factors. Different letters denote significant differences analyzed with one-way ANOVA, p<0.05 for significant g x d interactions. n.s= not significant.

(p<0.05; Table 7). Regardless of the genotype of the fish, the SCP diet increased only the 20:3n-6 content in the fillet. However, numerous significant GxD interactions (p<0.05) were observed for many fatty acids, including 18:2n-6, 20:4n-6, 20:5n-3, total n-6 PUFA, and total MUFA (Table 7).

3.3. Fillet quality: texture properties

The results of the texture analysis are shown in Table 8. Fillet chewiness was significantly (p<0.05) decreased in the HG genotype compared to the WT genotype, regardless of diet. Instead, the SCP diet significantly (p<0.05) decreased fillet hardness and adhesiveness compared to the C diet and regardless of fish genotype. Regarding GxD interactions, significant results were found for fillet elasticity, with HG genotypes fed diet C having a significantly lower value than fillets from fish belonging to the WT-C and HG-SCP groups, which had similar values. A significant GxD interaction was also observed for fillet cohesion within the same genotype groups. Specifically, fillets of genotype WT, fed the C diet, had significantly (p<0.05) higher values than those of genotype WT, fed the SCP diet, but similar values compared to genotype HG, fed both diets.

3.4. Instrumental sensory evaluation of organoleptic properties of sea bass fillets

Fig. 1a shows the PCA diagram of the volatile profiles of the fillets from the four dietary groups and Fig. 1b shows the responses of the e-tongue. In both cases, the discrimination index (DI) used by the same software of both instruments (Alpha Soft) is negative, highlighting the lack of discrimination between the samples of the 4 groups. However, as shown by DI, the e-tongue data had better spatial separation than the e-nose data. This result was also confirmed by the PCA plots obtained considering genotype and diet separately, as shown in Supplementary Figures S1 and S2.

Indeed, a more detailed investigation revealed a combined effect of diet and genotype on the organoleptic characteristics of the fillets, and more so for the taste qualities than for the volatile profile. As shown in Table 9, pairwise comparisons between experimental groups showed no significant differences in e-nose analysis, with the exception of fillets from WT sea bass fed different diets (p=0.01). In contrast, significant differences (p<0.05) are reported in e-tongue scores, even in groups fed the same diet (Table 9). The different diets showed a greater effect on the taste of the WT sea bass fillets, which had a higher organoleptic distance (285.53) and PDI (32.89%) than the fillets of the HG group (178.67 and 17.01%, respectively). These results were also confirmed by the spatial differentiation of the data (positive DI) in Fig. 2.

4. Discussion

Replacing FM and FO in aquafeeds is an ongoing challenge. The use of plant-based ingredients is already a widespread practice, as these raw materials are more readily available and less expensive compared to FM, and FO (Bandara, 2018). However, although their use as partial substitutes for marine ingredients is widely tolerated, the complete replacement of FM and FO with vegetable raw materials often results in reduced growth performance of fish and lower nutritional quality of fillets for human consumption (Francis et al., 2001). In this context, the use of alternative raw materials, such as raw or refined proteins and oils from microorganisms (yeast, fungi, microalgae, and bacteria), in combination with low-cost sources, such as poultry and vegetable meal and oil, has the potential to completely replace FO and FM and balance the nutrient profile of feeds (Carvalho et al., 2020, 2021; Jones et al., 2020; Glencross et al., 2020a; Sharif et al., 2021).

The results of the present study confirm that a diet with a partial replacement (25%) of FM with 10% *M. capsulatus* meal and a complete replacement of FO with a mixture of PO and a DHA-algal oil had no negative effects on the growth performance of the fish. These results are consistent with those of previous studies in which *M. capsulatus* was used as a partial replacement for FM in formulated diets for juvenile largemouth bass (*Micropterus salmoides*) (Zhang et al., 2022), genetically selected tilapia (*Oreochromis niloticus*) (Chama et al., 2021), and Jian carp (*Cyprinus carpio* var. Jian) (Yu et al., 2022, 2023). The authors proposed that the beneficial effects of the methanotrophic bacteria could be due to the nutrient composition of the microbial biomass, which is characterized by a balanced amino acid profile and a higher proportion of unsaturated than saturated fatty acids (Kuźniar et al., 2019; Glencross et al., 2020b).

In addition to novel nutritional strategies, the implementation of genetic selection programs can be considered as a complementary tool to improve the robustness of farmed fish and their adaptability to nutritional innovations. Indeed, the number of studies showing that different fish genotypes have different abilities to cope with nutritional changes, is constantly increasing (Dupont-Nivet et al., 2009; Le Boucher et al., 2013; Yamamoto et al., 2015; Callet et al., 2017). From this point of view, the present study will increase knowledge about which feed formulations can be administered and be well accepted by genetically selected animals. This information is important because little is known about the specific nutritional needs of genetically selected fish and how they can take advantage of novel aquafeeds with low FM/FO and with novel raw materials (Vandeputte et al., 2009).

The biometric data showed a significantly higher value for final body weight and length in the selected fish (HG groups) compared to the WT animals. Even without a clear effect of diet within the two genotypes (HG; WT), these results demonstrate the effectiveness of genetic selection for high growth in improving overall performance, particularly SGR and FCR, of European sea bass. Similar results were

obtained in previous studies with selected fish strains fed alternative diets with low FM and/or FO both in sea bass and in other fish species, such as rainbow trout (*O. mykiss*), amago salmon (*Oncorhynchus masou ishikawae*) (Overturf et al., 2013; Yamamoto et al., 2015), European sea bass and gilthead sea bream (Montero et al., 2023a, 2023b; Carvalho et al., 2023).

Table 6
Fatty acid composition (% total fatty acids) of the whole-body of HG and WT European sea bass fed the experimental diets.

					Two-way ANOVA (p=value)		
Fatty acids	**WT-*C***	**WT-*SCP***	**HG-*C***	**HG-*SCP***	Genotype	Diet	GxD
14:0	0.75±0.36	0.32±0.44	1.30±0.15	0.21±0.19	*n.s*	*p*=0.003	*n.s*
14:1n7	0.02±0.01	0.03±0.02	0.03±0.02	0.03±0.01	*n.s*	*n.s*	*n.s*
14:1n5	0.04±0.02	0.03±0.02	0.06±0.03	0.03±0.02	*n.s*	*n.s*	*n.s*
15:0	0.10±0.06	0.06±0.04	0.15±0.07	0.04±0.02	*n.s*	*p*=0.026	*n.s*
15:1n5	0.04±0.03	0.02±0.00	0.02±0.01	0.03±0.02	*n.s*	*n.s*	*n.s*
16:0 iso	0.02±0.01	0.02±0.00	0.03±0.01	0.02±0.01	*n.s*	*n.s*	*n.s*
16:0	6.08±0.48	4.93±3.17	6.38±0.76	2.88±0.09	*n.s*	*p*=0.040	*n.s*
16:1n7	1.97±0.23	1.15±0.56	1.65±0.40	0.59±0.21	*n.s*	*p*=0.003	*n.s*
16:1n5	0.09±0.06	0.03±0.02	0.10±0.04	0.03±0.01	*n.s*	*p*=0.017	*n.s*
16:2n4	0.04±0.03	0.03±0.01	0.06±0.02	0.02±0.01	*n.s*	*p*=0.033	*n.s*
17:0	0.03±0.01	0.03±0.01	0.04±0.01	0.03±0.01	*n.s*	*n.s*	*n.s*
16:3n4	0.13±0.06	0.09±0.02	0.14±0.06	0.06±0.00	*n.s*	*p*=0.038	*n.s*
16:3n3	0.05±0.01	0.03±0.00	0.05±0.01	0.04±0.02	*n.s*	*n.s*	*n.s*
16:3n1	0.04±0.01	0.07±0.02	0.03±0.01	0.06±0.01	*n.s*	*p*=0.019	*n.s*
16:4n3	0.04±0.01	0.05±0.02	0.04±0.01	0.05±0.01	*n.s*	*n.s*	*n.s*
16:4n1	0.03±0.01	0.02±0.01	0.02±0.01	0.05±0.06	*n.s*	*n.s*	*n.s*
18:0	2.46±0.51	3.30±1.04	2.66±0.98	3.31±1.13	*n.s*	*n.s*	*n.s*
18:1n9	37.06±0.79^{ab}	34.00±0.40^{b}	37.23±0.53^{ab}	38.05±2.48^{a}	*p*=0.027	*n.s*	*p*=0.037
18:1n7	5.21±1.72^{b}	13.40±0.02^{a}	2.90±0.35^{b}	3.39±1.09^{b}	*p*<0.001	*p*<0.001	*p*<0.001
18:1n5	2.86±2.46	1.20±1.86	1.07±0.75	0.35±0.20	*n.s*	*n.s*	*n.s*
18:2n9	0.43±0.18	0.18±0.10	0.86±0.60	0.19±0.02	*n.s*	*p*=0.036	*n.s*
18:2n6	12.15±0.81	16.93±0.53	14.73±0.75	17.09±1.87	*n.s*	*p*=0.001	*n.s*
18:2n4	0.15±0.13	0.10±0.10	0.91±0.76	0.11±0.05	*n.s*	*n.s*	*n.s*
18:3n6	0.12±0.02	0.12±0.00	0.65±0.79	0.12±0.06	*n.s*	*n.s*	*n.s*
18:3n4	0.08±0.03	0.07±0.04	0.04±0.01	0.21±0.21	*n.s*	*n.s*	*n.s*
18:3n3	2.94±0.74	4.07±0.27	3.74±0.84	4.68±0.25	*n.s*	*p*=0.016	*n.s*
18:3n1	0.08±0.08	0.06±0.07	0.15±0.12	0.26±0.35	*n.s*	*n.s*	*n.s*
18:4n3	0.48±0.15	0.19±0.05	0.47±0.20	0.22±0.06	*n.s*	*p*=0.009	*n.s*
18:4n1	0.04±0.01	0.02±0.01	0.04±0.02	0.03±0.01	*n.s*	*p*=0.037	*n.s*
20:0	0.46±0.11	0.67±0.17	0.44±0.25	0.93±0.31	*n.s*	*p*=0.026	*n.s*
20:1n9	0.67±0.16	0.24±0.07	1.72±1.42	1.18±1.49	*n.s*	*n.s*	*n.s*
20:1n7	6.11±1.02	4.56±0.89	5.23±2.66	4.82±0.59	*n.s*	*n.s*	*n.s*
20:1n5	0.44±0.13	0.26±0.23	0.68±0.43	0.35±0.22	*n.s*	*n.s*	*n.s*
20:2n9	0.06±0.02	0.09±0.08	0.14±0.10	0.16±0.11	*n.s*	*n.s*	*n.s*
20:2n6	0.65±0.13	0.80±0.14	0.61±0.31	0.98±0.12	*n.s*	*p*=0.033	*n.s*
20:3n9	0.03±0.01	0.04±0.03	0.09±0.07	0.13±0.13	*n.s*	*n.s*	*n.s*
20:3n6	0.07±0.02	0.09±0.02	0.11±0.07	0.36±0.17	*n.s*	*p*=0.031	*n.s*
20:4n6	**0.26±0.06^{c}**	**0.51±0.10^{b}**	**0.22±0.14^{c}**	**0.78±0.02^{a}**	*n.s*	*p*<0.001	*p*=0.018
20:3n3	0.12±0.03	0.08±0.02	0.13±0.04	0.17±0.06	*n.s*	*n.s*	*n.s*
20:4n3	0.29±0.10	0.19±0.04	0.24±0.13	0.38±0.07	*n.s*	*n.s*	*n.s*
20:5n3	**2.41±0.54^{b}**	**2.30±0.14^{b}**	**2.66±0.32^{b}**	**3.93±0.35^{a}**	*p*=0.002	*p*=0.024	*p*=0.011
22:1n11	5.77±0.87	2.16±0.17	5.50±1.60	2.00±0.21	*n.s*	*p*<0.001	*n.s*
22:1n9	1.16±0.37	1.23±0.08	1.76±0.37	1.25±0.13	*n.s*	*n.s*	*n.s*
22:4n6	0.05±0.02	0.08±0.05	0.11±0.05	0.22±0.18	*n.s*	*n.s*	*n.s*
22:5n6	0.15±0.02	0.34±0.08	0.13±0.04	0.92±0.42	*n.s*	*p*=0.004	*n.s*
22:5n3	0.48±0.12	0.26±0.08	0.38±0.15	0.84±0.50	*n.s*	*n.s*	*n.s*
22:6n3	**7.30±0.30^{a}**	**5.50±0.26^{b}**	**4.26±0.43^{b}**	**8.44±0.97^{a}**	*n.s*	*p*=0.007	*p*<0.001
SFA	9.91±0.57	9.32±2.23	11.01±1.11	7.42±0.59	*n.s*	*p*=0.025	*n.s*
MUFA	61.44±1.41	58.31±2.13	57.97±1.79	52.09±1.43	*p*=0.001	*p*=0.002	*n.s*
n9-UFA	39.41±0.89	35.79±0.33	41.80±2.44	40.95±1.20	*p*=0.002	*p*=0.027	*n.s*
n6-PUFA	13.44±1.00	18.92±0.68	16.56±0.94	20.46±1.22	*p*=0.003	*p*<0.001	*n.s*
n3-PUFA	14.10±1.28^{b}	12.68±0.58^{b}	11.96±1.38^{b}	18.74±0.44^{a}	*p*=0.010	*p*=0.002	*p*<0.001
EPA/ARA	9.20±0.73	4.63±0.81	17.65±14.15	5.05±0.53	*n.s*	*n.s*	*n.s*
EPA/DHA	0.33±0.09^{b}	0.42±0.04^{ab}	0.63±0.10^{a}	0.47±0.08^{ab}	*p*=0.006	*n.s*	*p*=0.028
EPA+DHA	**9.71±0.38^{b}**	**7.80±0.21^{c}**	**6.92±0.54^{c}**	**12.37±0.87^{a}**	*p*=0.024	*p*=0.001	*p*<0.001
n3 LC-PUFA	10.60±0.61^{b}	8.34±0.26^{c}	7.67±0.75^{c}	13.75±0.29^{a}	*p*=0.003	*p*<0.001	*p*<0.001

C: Control diet; SCP: Single-cell protein diet; WT: wild-type (non-selected) genotype; HG: high-growth genotype. Values are expressed in mean ± SD. (n = 3 tanks/diet/genotype). Two-way ANOVA, p<0.05, Genotype and Diet as fixed factors. Different letters denote significant differences analyzed with one-way ANOVA, p<0.05 for significant g x d interactions. n.s= not significant.

Along with the partial replacement of FM with 10% commercial protein from bacteria (*M. capsulatus*), the present study investigated the effects of the complete replacement of FO with a mixture of poultry and algal oil. Substitution of lipid fractions from the sea is currently one of the major challenges for the aquaculture sector. However, dietary intake of the minimum daily requirement of essential fatty acids (EFAs) is paramount for both fish and human nutrition. It is well known that marine fish species are particularly affected by low dietary EFAs due to their limited ability to synthesize endogenous long chain poly-unsaturated fatty acids (LC-PUFAs) (Montero et al., 2010; Turchini et al., 2009), whereas freshwater fish are less so. As numerous studies have shown, expression of desaturases, particularly Δ9 and Δ6, increases in sea bream and sea bass in concert with limited EFA intake. However, synthesis of 20:5n3 and 22:6n3 (EPA and DHA, respectively), the major highly unsaturated FAs for fish and human nutrition, from 18-PUFA precursors is insufficient and hardly effective in these species (Tocher, 2015; Carvalho et al., 2021; Terova et al., 2021; Oteri et al., 2022).

The growth performance of the fish in this study confirmed that the combination of different lipid sources as an alternative to FO met the nutritional requirements of the animals, as no significant changes and no

Table 7
Fatty acid composition (% total fatty acids) of muscle from WT and HG European sea bass fed the experimental diets.

					Two-way ANOVA (p=value)		
Fatty acids	**WT-*C***	**WT-*SCP***	**HG-*C***	**HG-*SCP***	Genotype	Diet	GxD
14:0	1.40±0.09^{ab}	0.86±0.09^{b}	0.90±0.06^{b}	1.67±0.40^{a}	*n.s*	*n.s*	*p=0.001*
14:1n7	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.01	*n.s*	*n.s*	*n.s*
14:1n5	0.06±0.00^{a}	0.03±0.01^{b}	0.03±0.00^{b}	0.07±0.01^{a}	*n.s*	*n.s*	*p<0.001*
15:0	0.20±0.02^{ab}	0.15±0.02^{b}	0.17±0.01^{ab}	0.21±0.02^{a}	*n.s*	*n.s*	*p=0.007*
15:1n5	0.03±0.01^{a}	0.01±0.00^{c}	0.01±0.00^{bc}	0.02±0.01^{ab}	*n.s*	*n.s*	*p=0.002*
16:0 iso	0.05±0.01	0.03±0.00	0.03±0.00	0.05±0.01	*n.s*	*n.s*	*p=0.005*
16:0	13.83±0.16	14.96±0.22	15.33±0.83	15.22±0.86	*p=0.038*	*n.s*	*n.s*
16:1n7	2.31±0.14	2.37±0.05	2.42±0.19	2.72±0.38	*n.s*	*n.s*	*n.s*
16:1n5	0.08±0.00	0.04±0.00	0.11±0.13	0.07±0.01	*n.s*	*n.s*	*n.s*
16:2n6	0.10±0.00^{a}	0.00±0.00^{b}	0.04±0.01^{b}	0.10±0.00^{a}	*p<0.001*	*p<0.001*	*p<0.001*
16:2n4	0.24±0.01^{a}	0.04±0.01^{c}	0.06±0.01^{c}	0.21±0.01^{b}	*n.s*	*p=0.004*	*p<0.001*
17:0	0.07±0.01^{a}	0.04±0.01^{b}	0.03±0.00^{b}	0.06±0.01^{a}	*n.s*	*n.s*	*p<0.001*
16:3n4	0.19±0.01^{a}	0.11±0.01^{b}	0.10±0.01^{b}	0.20±0.02^{a}	*n.s*	*n.s*	*p<0.001*
16:3n3	0.10±0.02^{a}	0.05±0.02^{b}	0.03±0.01^{b}	0.06±0.00^{b}	*p=0.007*	*n.s*	*p<0.001*
16:3n1	0.16±0.04	0.13±0.04	0.09±0.02	0.11±0.05	*n.s*	*n.s*	*n.s*
16:4n3	0.24±0.03	0.26±0.03	0.14±0.08	0.17±0.07	*p=0.016*	*n.s*	*n.s*
16:4n1	0.04±0.01	0.03±0.01	0.19±0.15	0.35±0.14	*p=0.004*	*n.s*	*n.s*
18:0	3.33±0.09	4.01±0.43	2.89±0.40	2.86±0.26	*p=0.003*	*n.s*	*n.s*
18:1n9	33.75±1.20	34.78±0.39	37.41±1.16	36.57±0.59	*p=0.001*	*n.s*	*n.s*
18:1n7	2.39±0.34	1.70±0.15	2.14±0.31	2.41±0.29	*n.s*	*n.s*	*p=0.019*
18:1n5	0.16±0.03^{ab}	0.08±0.00^{b}	0.13±0.07^{ab}	0.18±0.02^{a}	*n.s*	*n.s*	*p=0.021*
18:2n9	0.21±0.05	0.21±0.12	0.13±0.04	0.28±0.09	*n.s*	*n.s*	*n.s*
18:2n6	13.82±0.13^{b}	16.54±0.21^{a}	17.41±0.57^{a}	13.33±0.63^{b}	*n.s*	*p=0.030*	*p<0.001*
18:2n4	0.10±0.04	0.04±0.00	0.06±0.04	0.08±0.06	*n.s*	*n.s*	*n.s*
18:3n6	0.14±0.01	0.12±0.04	0.13±0.03	0.17±0.04	*n.s*	*n.s*	*n.s*
18:3n4	0.07±0.03	0.03±0.00	0.05±0.03	0.06±0.03	*n.s*	*n.s*	*n.s*
18:3n3	4.02±0.06	4.04±0.07	4.16±0.21	3.90±0.12	*n.s*	*n.s*	*n.s*
18:3n1	0.04±0.03	0.02±0.00	0.06±0.06	0.03±0.02	*n.s*	*n.s*	*n.s*
18:4n3	0.77±0.02^{a}	0.28±0.00^{b}	0.24±0.02^{b}	0.70±0.07^{a}	*n.s*	*n.s*	*p<0.001*
18:4n1	0.04±0.01^{a}	0.02±0.00^{b}	0.03±0.01^{ab}	0.04±0.01^{a}	*n.s*	*n.s*	*p=0.009*
20:0	0.24±0.01^{c}	0.29±0.01^{ab}	0.31±0.02^{a}	0.27±0.01^{bc}	*p=0.016*	*n.s*	*p=0.001*
20:1n9	0.32±0.02^{a}	0.15±0.04^{b}	0.13±0.02^{b}	0.32±0.04^{a}	*n.s*	*n.s*	*p<0.001*
20:1n7	3.27±0.08^{a}	2.01±0.19^{b}	2.02±0.11^{b}	3.61±0.26^{a}	*n.s*	*n.s*	*p<0.001*
20:1n5	0.16±0.04	0.08±0.00	0.11±0.04	0.18±0.09	*n.s*	*n.s*	n.s
20:2n9	0.03±0.01	0.03±0.00	0.03±0.01	0.03±0.00	*n.s*	*n.s*	*n.s*
20:2n6	0.47±0.04	0.50±0.05	0.46±0.02	0.43±0.06	*n.s*	*n.s*	*n.s*
20:3n9	0.00±0.00	0.00±0.00	0.01±0.00	0.03±0.00	*p<0.001*	*p=0.000*	*n.s*
20:3n6	0.05±0.01	0.07±0.01	0.05±0.00	0.06±0.01	*n.s*	*p=0.016*	*n.s*
20:4n6	**0.50±0.03^{b}**	**0.85±0.04^{a}**	**0.62±0.11^{b}**	**0.32±0.05^{c}**	*p=0.001*	*n.s*	*p<0.001*
20:3n3	0.10±0.01^{a}	0.06±0.00^{b}	0.05±0.01^{b}	0.09±0.01^{a}	*n.s*	*n.s*	*p<0.001*
20:4n3	0.25±0.02^{a}	0.16±0.00^{b}	0.15±0.01^{b}	0.22±0.01^{a}	*p=0.010*	*n.s*	*p<0.001*
20:5n3	**3.57±0.06^{a}**	**2.96±0.07^{b}**	**2.22±0.18^{c}**	**2.67±0.27^{b}**	*p<0.001*	*n.s*	*p=0.001*
22:1n11	2.36±0.10^{b}	0.75±0.08^{c}	0.69±0.04^{c}	2.75±0.09^{a}	*p=0.007*	*p=0.001*	*p<0.001*
22:1n9	0.49±0.05^{a}	0.31±0.01^{b}	0.32±0.02^{b}	0.51±0.05^{a}	*n.s*	*n.s*	*p<0.001*
22:4n6	0.06±0.03	0.05±0.00	0.05±0.01	0.05±0.01	*n.s*	*n.s*	*n.s*
22:5n6	0.21±0.02	0.48±0.01	0.39±0.06	0.14±0.01	*p=0.003*	*n.s*	*p<0.001*
22:5n3	0.49±0.04^{a}	0.33±0.03^{b}	0.26±0.02^{c}	0.37±0.02^{b}	*p<0.001*	*n.s*	*p<0.001*
22:6n3	**9.50±0.44**	**9.90±0.30**	**7.60±1.24**	**6.01±0.95**	*p<0.001*	*n.s*	*n.s*
SFA	19.10±0.33	20.33±0.18	19.65±1.30	20.34±1.07	*n.s*	*n.s*	*n.s*
MUFA	45.42±0.92^{b}	42.34±0.35^{c}	45.54±1.69^{b}	49.46±0.48^{a}	*p<0.001*	*n.s*	*p<0.001*
n9-UFA	34.81±1.23	35.47±0.53	38.02±1.19	37.75±0.68	*p=0.001*	*n.s*	*n.s*
n6-PUFA	15.34±0.14^{b}	18.61±0.32^{a}	19.16±0.41^{a}	14.61±0.75^{b}	*n.s*	*n.s*	*p<0.001*
n3-PUFA	19.02±0.59	18.05±0.40	14.84±1.39	14.19±1.31	*p<0.001*	*n.s*	*n.s*
EPA/ARA	7.47±0.62^{a}	3.56±0.27^{b}	3.75±0.29^{b}	8.59±1.50^{a}	*n.s*	*n.s*	*p<0.001*
EPA/DHA	0.38±0.02^{b}	0.30±0.01^{b}	0.30±0.03^{b}	0.45±0.04^{a}	*n.s*	*p=0.044*	*p<0.001*
EPA+DHA	**13.07±0.50**	**12.87±0.36**	**9.82±1.43**	**8.68±1.18**	*p<0.001*	*n.s*	*n.s*
n3 LC-PUFA	13.90±0.54	13.42±0.40	10.28±1.43	9.36±1.18	*p<0.001*	*n.s*	*n.s*

C: Control diet; SCP: Single-cell protein diet; WT: wild-type (non-selected) genotype; HG: high-growth genotype. Values are expressed in mean ± SD. (n = 3 tanks/diet/genotype). Two-way ANOVA, p<0.05, Genotype and Diet as fixed factors. Different letters denote significant differences analyzed with one-way ANOVA, p<0.05 for significant g x d interactions. n.s= not significant.

growth retardation were observed in either dietary group during the feeding trial. Regarding the lipid profile, it is known that diet is the most important factor influencing the FA composition of fish tissues. However, in this study, both variables considered (genotype and diet) affected body composition and fillet deposition of lipids. While the profile of the unselected animals reflects the FA composition of the diet, the data from the HG fish show a more complex combination of influences. Selected fish had significantly higher whole-body lipid accumulation than the WT animals on the same diet.

On the contrary, significantly lower lipid contents were found in the muscles of the selected animals than in the non-selected sea bass. These differences were also maintained in the FA profiles of the body parts. Major LC-PUFAs, including n-6 and n-3 PUFAs, such as 18:2 n-6, 18:3 n-3 20:4 n-6, EPA, and DHA were found in the whole-body profile according to the amount of feed, but not in the muscles of the HG animals, which, on the contrary, showed a significantly lower enrichment. These results are in contrast to those of previous studies, which described that the selected fish were better able to utilise and retain the dietary lipids in the fillet (Montero et al., 2023a, Cleveland et al., 2020, Jin, 2020). However, as has been well documented, lipid retention is not only

Table 8
Textural characteristics of fillets from WT and HG European sea bass fed the experimental diets.

	WT-*C*	WT-*SCP*	HG-*C*	HG-*SCP*	Genotype	Diet	GxD
Hardness	82.04±18.55	55.14±19.90	78.36±19.25	42.94±21.67	*n.s*	$p<0.001$	*n.s*
Elasticity	0.47±0.08^{a}	0.43±0.05^{ab}	0.39±0.04^{b}	0.46±0.04^{a}	*n.s*	*n.s*	$p<0.001$
Cohesivity	0.37±0.03^{a}	0.32±0.04^{b}	0.35±0.04^{ab}	0.34±0.03^{ab}	*n.s*	*n.s*	$p=0.024$
Gumminess	29.71±6.93	18.30±9.62	27.57±7.74	14.71±7.43	*n.s*	*n.s*	*n.s*
Chewiness	13.83±4.50	8.24±4.99	10.67±3.03	6.57±2.83	$p=0.014$	*n.s*	*n.s*
Adhesiveness	-0.27±0.25	-0.15±0.06	-0.18±0.14	-0.12±0.12	*n.s*	$p=0.036$	*n.s*
Resilience	0.11±0.02	0.11±0.03	0.11±0.02	0.11±0.01	*n.s*	*n.s*	*n.s*

C: Control diet; SCP: Single-cell protein diet; WT: wild-type (non-selected) genotype; HG: high-growth genotype. Values are expressed in mean ± SD. (n = 3 tanks/diet/genotype). Two-way ANOVA, $p<0.05$, Genotype and Diet as fixed factors. Different letters denote significant differences analysed with one-way ANOVA, $p<0.05$ for significant g x d interactions. N. s= not significant.

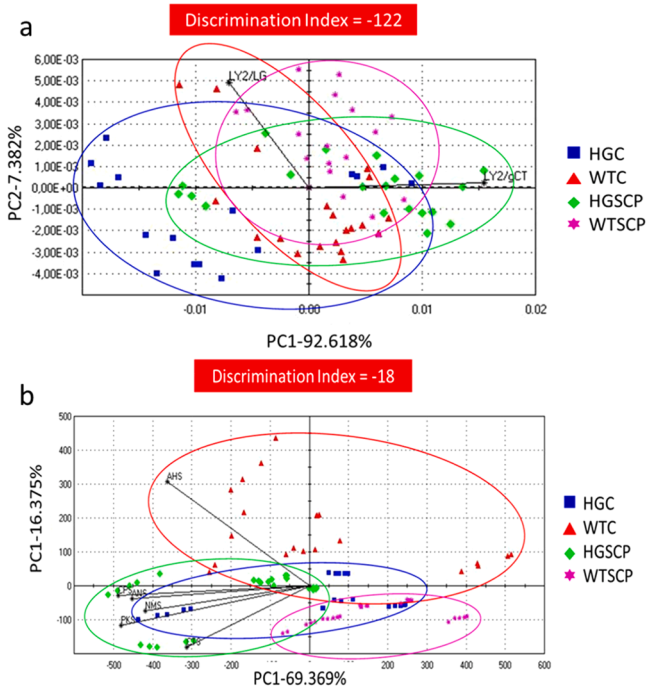

Fig. 1. PCA diagram of **A)** e-nose (smell map) and **B)** e-tongue (taste map) data in relation to European sea bass fillets from the four experimental groups (WT-C; WT-SCP; HG-C; HG-SCP).

species-specific but also highly variable and depends on numerous variables such as fish size, physiological conditions and diet composition (Tocher, 2003, De Verdal et al., 2018). In Atlantic salmon, for example, the mechanism of lipid accumulation is related to the content of n3 LC-PUFA, which is able to counteract triacylglycerol (TAG) in adipocytes.

However, the administration of feed with low FO and the resulting decrease in n3 PUFAs increases the TAG content, leading to a higher value of total lipid deposition (Todorčević et al., 2008). Similar results were also obtained in sea bream fed a diet containing wild-type camelina oil as a FO substitute (Betancor et al., 2016). One possible explanation for this phenomenon is related to the size of the animals. In particular, the better performance of the HG group indicates that they were in a phase of rapid growth during the experiment in which the energy sources were optimally utilised. Similar to gilthead sea bream, sea bass therefore preferentially mobilise fat stored in the liver and muscles rather than in visceral adipose tissue.

This could partly explain the different EFA levels found in the dorsal muscle of the selected fish compared to the wild-type specimens (Grigorakis and Alexis, 2005; Turchini et al., 2009; Katsika et al., 2021). Positive correlations between lower fat content in fillets and improved growth performance and FCR were also found in other fish species, such as rainbow trout and salmon, in which the selected leaner individuals had lower amounts of body and muscle fat but higher FCR (De Verdal et al., 2018). Overall, these results suggest that the relationship between lipid accumulation in different body regions and growth performance of European sea bass merits further investigation.

Table 9
Organoleptic distances and Pattern Discrimination Index (PDI%) between groups for smell (e-nose) and taste (e-tongue).

Compared groups	Organoleptic Distances	*p-Value*	Pattern Discrimination Index (%)
e-nose			
HG-C *vs* WT-C	0.01	n.s	23.46
HG-C *vs* HG-SCP	0.01	n.s	30.26
HG-C *vs* WT-SCP	0.01	n.s	35.76
WT-C *vs* HG-SCP	0.0001	n.s	5.6
WT-C *vs* WT-SCP	0.0001	0.01	9.58
HG-SCP *vs* WT-SCP	0.0001	n.s	3.55
e-tongue			
HG-C *vs* WT-C	251.26	<0.001	22.25
HG-C *vs* HG-SCP	178.67	n.s	17.01
HG-C *vs* WT-SCP	214.57	<0.001	27.33
WT-C *vs* HG-SCP	298.10	<0.001	30.98
WT-C *vs* WT-SCP	285.53	<0.001	32.89
HG-SCP *vs* WT-SCP	349.40	<0.001	52.78

Based on the observed differences in proximate and fatty acid composition of the fish between the four experimental groups, the present study also evaluated the textural and organoleptic properties of the raw fillets. Several studies have shown how FO and FM substitutions in the diet can affect sensory quality through chemical changes in the flavour compounds present in the fillets (Fountoulaki et al., 2009; Porcino and Genovese, 2022). The present study showed that both experimental variables (genotype and diet) play a role in the textural properties of sea bass flesh. Specifically, the SCP diet significantly decreased hardness and adhesiveness regardless of genotype. Similar results were obtained by Izquierdo et al. (2005) with gilthead sea bream, in which fillet hardness was reduced following a diet in which FM was partially replaced by soybean meal and up to 80% of FO was replaced by vegetable oils.

The organoleptic characteristics of the flesh have instead been analysed using e-sensing instruments, specifically the e-nose, and e-tongue. These types of devices are increasingly used for the quality assessment of different food products, as they can faithfully substitute human olfactory and gustatory mechanisms (Di Rosa et al., 2017; Di Rosa and Leone, 2018). Consumer preference assessment in the seafood industry remains an important step in responding to market demand and ensuring a

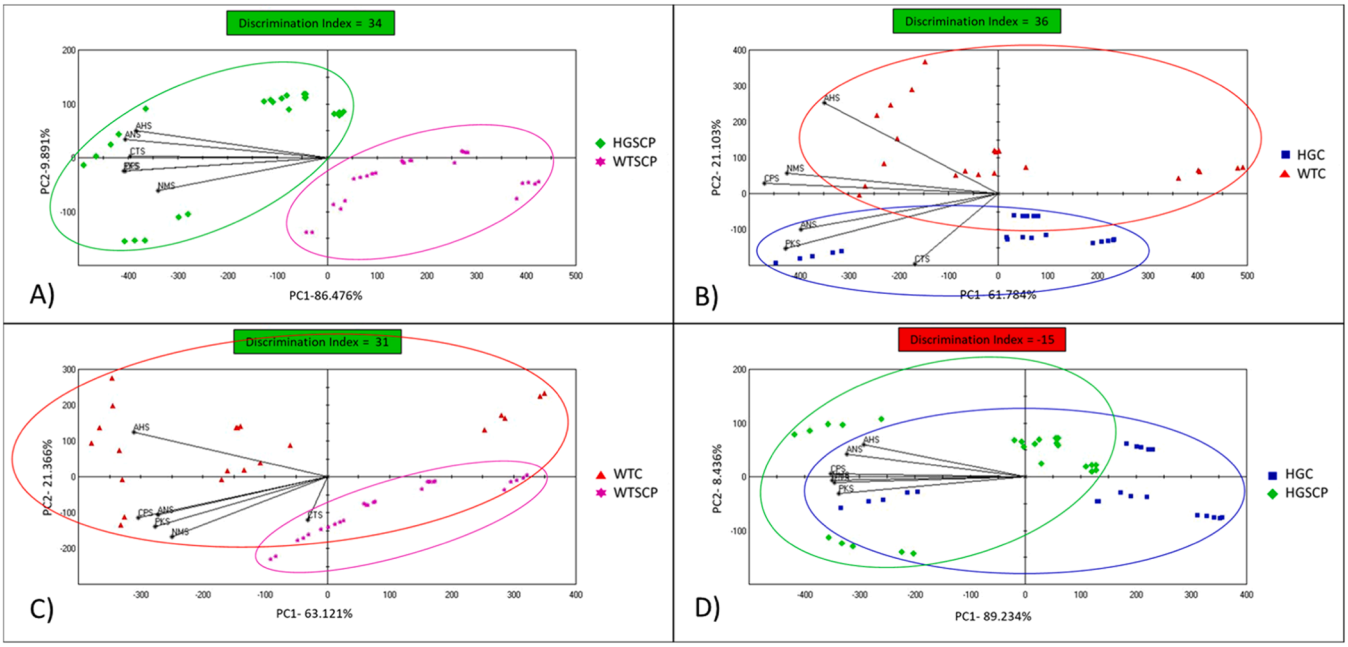

Fig. 2. A) PCA diagram of e-tongue (taste map) comparing groups HG-SCP and WT-SCP; **B)** PCA diagram of e-tongue (taste map) comparing groups HG-C and WT-C; **C)** PCA diagram of e-tongue (taste map) comparing groups WT-C and WT-SCP; **D)** PCA diagram of e-tongue (taste map) comparing the groups HG-C and HG-SCP.

high-quality product (Calanche et al., 2020; Basto et al., 2023). However, using trained panellists for sensory evaluation can lead to a lengthy process (Yu, Low and Zhou, 2018). In addition, the collected data are often inconsistent, as consumers 'general perceptions may be inaccurate or depend on personal preferences (Cheng et al., 2014, Turchini et al., 2009). Therefore, the use of novel approaches such as electronic tools, as described by Green-Petersen and Hyldig (2010), can improve the evaluation of aquaculture products and provide accurate and rapid results (Wojnowski et al., 2017; Di Rosa et al., 2017; Zaukuu et al., 2021; Oteri et al., 2021).

The combination of the sensory e-nose and e-tongue proved to be a powerful tool for discriminating different organoleptic profiles based on smell and taste. e-nose detected the volatile profiles of fish fillets from HG and WT, which were fed the two different diets, thanks to the sensors' response to different categories of volatiles. Pattarapon et al. (2018) was the first to demonstrate the ability of the tool to discriminate different flavours of grass carp by comparing the response of the different sensors for aromatic compounds (sulphides, mercaptan, thioethers, fatty hydrocarbon derivatives, helium, hydrocarbon, and sulphide) with the response of the panellists.

The results showed that despite some differences in fatty acid profiles, which are important precursors of volatile flavours, both genotype and diet had little effect on the volatile profile of the fillets. In contrast, the taste analyses of the fish fillets performed with the e-tongue showed a completely different pattern of responses. Apart from lipids, which are among the most important taste-related molecules, other relevant flavour compounds, such as inorganic salts, peptides, amino acids, and sugars, can determine different gustatory response (Zhang et al., 2012).

In agreement with the results of Montero et al. (2023b) and Carvalho et al. (2023), who used different alternative diet formulations for selected and unselected genotypes of *Sparus aurata*, the present analyses confirmed that diet was the main factor affecting the taste of fillets, especially in the WT fish group, which also showed the highest variability in e-sensing response. The HG fish, on the other hand, showed more homogeneous e-tongue data with a lower dispersion, indicating better utilization of the alternative ingredients, which was already evident in growth performance.

These results underscored the close relationships between variations in lipid content and fillet flavour and aroma (Grigorakis, 2007; Garduño-Lugo et al., 2007). These compounds, in combination with the amino acids (e.g., glutamic acid, aspartic acid, and monosodium glutamate), could have led to different responses on the e-tongue responses. As reported by Montero et al. (2023b), the different amino acid profile could be due to an enhanced protein digestion ability in genetically selected fish (HG), which correlates in particular with two key digestive proteases in particular: gastric pepsin and intestinal chymotrypsin.

5. Conclusions

The present study showed that the addition of 10% of a commercial bacterial meal consisting of *Methylococcus capsulatus* and a mixture of PO and a DHA-algae oil, used as partial and complete substitutes for FM and FO, provided an effective alternative to marine ingredients for sea bass. The effect of the diet in combination with genetic selection ensured the achievement of optimal growth performance of the animals, which is particularly evident in the higher final biomass of the genetically selected fish. The texture analysis revealed significant differences in fillet characteristics depending on genotype and diet. The HG genotype consistently showed a lower fillet chewiness compared to the WT genotype in all diets. In addition, the SCP diet significantly reduced the hardness and stickiness of the fillet compared to the C diet, regardless of genotype.

Further studies are needed to elucidate the mechanisms of lipid accumulation of key LC-PUFAs in sea bass fillets in relation to body growth. However, even with differences in lipid composition, the results of e-sensing analysis associated with growth performance underscored the ability of the selected fish to better utilize the alternative ingredients. To our knowledge, this is the first sensory profiling study conducted using artificial senses on genetically selected fish. Given these results, further insight into the lipid and amino acid profile of the fillets would be interesting, as e-nose and e-tongue have proven to be very powerful tools to study the sensory imprinting of fish fed innovative and more sustainable diets.

CRediT authorship contribution statement

Genciana Terova, Daniel Montero, Silvia Torrecillas: Conceptualization. **Marta Ribeiro Carvalho, Ambra Rita Di Rosa, Silvia**

Torrecillas, Pierrick Haffray, Francois Allal, Ramon Fontanillas, Aline Bajek: Methodology. **Federico Moroni, Marta Ribeiro Carvalho, Ambra Rita Di Rosa, Biagina Chiofalo, Silvia Torrecillas, Francois Allal:** Data collection, curation, and analysis. **Federico Moroni, Genciana Terova, Silvia Torrecillas, Marta Ribeiro Carvalho:** writing – original draft preparation. **Genciana Terova, Silvia Torrecillas, Ambra Rita Di Rosa, Biagina Chiofalo:** writing – review and editing. **Genciana Terova, Daniel Montero:** Funding acquisition. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare no competing interests.

Data Availability

All the data are reported in the manuscript

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2024.102021.

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