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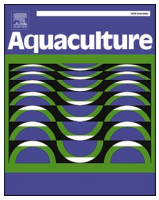

Genetic selection for high growth improves the efficiency of gilthead sea bream (*Sparus aurata*) in using novel diets with insect meal, single-cell protein and a DHA rich-microalgal oil

Marta Carvalho [a,*], Rafael Ginés [a], Ignacio Martín [a], María Jesús Zamorano [a], Félix Acosta [a], Ramon Fontanillas [c], Silvia Torrecillas [a,b,1], Daniel Montero [a,1]

[a] Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Telde, Las Palmas, Canary Islands, Spain
[b] Aquaculture Program, Institut de Recerca i Tecnologia Agroalimentàries (IRTA) Centre de Sant Carles de la Ràpita (IRTA-SCR), Sant Carles de la Ràpita, Spain
[c] Skretting Aquaculture Research Centre, Stavanger, Norway

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ABSTRACT

Genetic selection and novel raw materials for aquafeeds are current key tools in the ongoing effort to increase the productivity, efficiency, and sustainability of the aquaculture sector. Selective breeding could also improve the utilization of novel dietary formulations with emergent ingredients. Gilthead sea bream juveniles, either coming from a selective breeding program based on growth traits, or a non-selected population, were nutritionally challenged with two novel dietary formulations that were compared with a Control diet based on 15% FM and 6% FO dietary commercial levels for this species. The novel formulations included an insect meal diet (INS) at 5% of the diet to replace 33.3% of the dietary FM, or a single-cell protein diet (SCP) at 10% of the diet and to replace 66.7% of the dietary FM. Fish oil was also totally replaced in these diets by a blend of poultry oil and Veramaris algal oil. Better growth and feed utilization of the selected genotype compared to non-selected fish was observed, at any of the diets assayed. INS and SCP novel diets reduced general performance of fish by reducing feed intake. However, selected fish fed novel diets showed very similar growth and lower feed conversion ratio compared with non-selected fish fed a control diet. The novel formulations increased n-3 LC-PUFA in fish tissues, particularly DHA, irrespective of the genotype, as a result of the dietary inclusion of the DHA-rich microalgal oil. Neither genetic selection nor the use of novel raw materials affected fillet proximate composition and consequently, sea bream fillet quality in terms of texture and sensorial perception of consumers. Overall, the results reaffirm the positive effects of selective breeding programs in improving sea bream key productive indicators, as well as support the use of novel dietary formulations, using insect meal from *H. illucens,* single-cell protein from *M. capsulatus* as partial replacers of FM in diets for gilthead sea bream (33 and 66% of replacement, respectively), and a blend of DHA-rich microalgal and poultry oils as total replacer of FO.

1. Introduction

Fish meals (FM) and oils (FO) are not considered indispensable ingredients for aquafeeds anymore (Tacon and Metian, 2008). Alternatively, the use of vegetable ingredients in fish diets is a widely used practice, due to their greater abundance and lower cost in relation to FM and FO (Bandara, 2018). On one hand, among the most used sources of vegetable proteins to replace FM are soybean, wheat, or corn meals (Hardy, 2010), some of them with a high protein content (Bandara, 2018). On the other hand, FO can be partially replaced by vegetable oils like soya, palm, rapeseed, sunflower, or flax (Hardy, 2010). It has been generally established for many fish species, including marine piscivorous species like gilthead sea bream, that FM and FO can be successfully partially replaced by vegetable protein and lipid sources until 75% (Benedito-Palos et al., 2007; De Francesco et al., 2007). However, the total replacement of FM/FO by plant raw materials often leads to reduced fish performance by reducing feed acceptability and digestibility due to the presence of antinutrients, low protein content, or

* Corresponding author.
E-mail address: marta.carvalho@ulpgc.es (M. Carvalho).
[1] These authors contributed equally to the study.

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an unbalanced amino acid profile (Francis et al., 2001). In addition, the inclusion of vegetable oils in fish feeds changes the dietary fatty acid profile, significantly reducing the contents of important fatty acids for fish, including the arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), with pivotal functions in fish growth and health (Izquierdo, 2005). Indeed, for marine carnivorous fish like gilthead sea bream, these fatty acids are also considered essential nutrients (EFA) due to the inefficiency of these species for endogenously synthetize them (Izquierdo, 2005). Therefore, when fish oil is replaced by vegetable oils, it is challenging to meet fish EFA requirements without an additional dietary source of n-3 long-chain polyunsaturated fatty acids (LC-PUFA) (Montero et al., 2003). This is because vegetable oils do not contain ARA, EPA and DHA, and usually contain high contents of n-6 fatty acids like linoleic acid, which could lead to an imbalance in the n-3/n-6 ratio of the feeds, that could also affect fish performance and health, as well as impact the nutritional quality of fish fillets for consumers (Turchini et al., 2009; Montero and Izquierdo, 2010). For all these reasons, to meet the future demand for aquaculture products and to reduce completely the dependence on FM and FO as well as on some raw materials from vegetable origin, it is necessary to find new profitable and more sustainable sources of protein and lipids for the aquafeed industry.

In July 2017, the European Union (EU) approved the use of insect meal for aquaculture (Regulation EU 2017/893). Since then, insect meals have been one of the most studied novel alternatives for replacing FM. Insect meals are placed in a favored position as FM replacer, based on their low dependence on natural resources, high reproduction rates, efficient production and feed conversion, as well as their high protein content (60–70% in dry weight) (Ferrer Llagostera et al., 2019) and well-balanced amino acid profile (van Huis, 2013; Lock et al., 2018; Ferrer Llagostera et al., 2019). Black soldier fly (*Hermetia illucens*) has been the most studied insect for aquafeed production due to its higher feed conversion ratios and higher nutritional quality compared to other insects, such as crickets or tenebrios (Oonincx et al., 2015). Several studies with fish supported a partial replacement of FM by insect meals without negative consequences on fish growth (reviewed by Tran et al., 2022). In gilthead sea bream, *H. illucens* can be incorporated at 5%, replacing 33.3% of the dietary FM on a practical diet with low basal levels of FM, without affecting fish performance, the biochemical composition of fish tissues, or the expression gut health-related molecular markers, after 112 days of feeding (Carvalho et al., 2023).

Single-cell proteins (SCP) are also considered novel raw materials with potential to replace FM, and have been studied in the most recent years. SCPs are defined as the protein product from microbial sources such as yeasts, fungi, bacteria, or microalgae (Jones et al., 2020; Glencross et al., 2020; Sharif et al., 2021). The production of SCPs presents a broad methodology and microorganisms, and its high potential relies on its high efficiency for substrate conversion and its remarkable productivity at low cost and low footprint (Nasseri et al., 2011). Regarding their nutritional value, SCPs potentially have a high protein content (60–80% dry matter), and are rich in vitamins, minerals, and essential amino acids, such as lysine or methionine, that often are limited in plant raw materials (Sharif et al., 2021). . Among the SCPs, those obtained through bacterial fermentation have been recently deserved special attention. For instance, *Methylococcus capsulatus* is a gram-negative methotroph bacterium (Foster and Davis, 1966), that have been commercially produced by a few companies worldwide. The inclusion of this microbial source can constitute 52% and 38% of the dietary protein in diets for Atlantic Salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), respectively, without compromising growth performance (Øverland et al., 2010). Recently, *M. capsulatus* incorporated at 10% of the diet could replace 66% of FM in diets for gilthead sea bream juveniles without compromising growth performance, feed utilization, and fish health (Carvalho et al., 2023).

In addition, microalgae algae oils are possibly the most promising source for replacing the FO in aquafeeds in the future, due to their very rich content in n-3 LC-PUFA, particularly DHA. Microalgal products supported a reduction in the dependence of FM/FO in feeds for many farmed fish species, including gilthead sea bream (Kousoulaki et al., 2015; Carvalho et al., 2020, 2022; Sarker et al., 2016). Furthermore, since microalgae oils are still an expensive raw material, their combination with low-cost lipid sources like poultry oil (PO) was shown to be suitable and flexible alternatives to totally replace FO by balancing the nutritional fatty acid profile of the diets (Carvalho et al., 2020, 2022).

Added to the implementation of innovative feed formulations, selection programs may have as selection objectives the adaptation of individuals to meet current aquaculture needs, including high robustness of farmed fish and their plasticity to deal with nutritional innovations and challenging feeds with low FM/FO. Several studies demonstrated that different genotypes have diverse abilities to deal with dietary shifts, for instance from FM/FO to plant meals and oils (Dupont-Nivet et al., 2009; Le Boucher et al., 2010, 2012; Yamamoto et al., 2015; Callet et al., 2021). Furthermore, selection for high growth has been shown in some species to increase the ability of fish to utilize plant-based diets (Yamamoto et al., 2015). However, little is still known about the effect of genetic selection in farmed fish to use recent novel aquafeeds with low levels of the traditional marine ingredients and containing emergent raw materials like SCP, insect meals, or microalgal oils. Furthermore, some studies, for example in rainbow trout, reported that fish selective breeding programs for fast growth carried out using diets based on FM/FO, also resulted in higher growth on plant-based diets (Palti et al., 2006). However, other studies, for instance in amago salmon (*Oncorhynchus masou ishikawae*) or European sea bass (*Dicentrarchus labrax*), reported a significant genotype x diet interaction. This interaction resulted in a non-successful fish performance when fish are fed a challenging diet with low levels of FM (Geay et al., 2011; Yamamoto et al., 2016), thus pointing out the importance of evaluating the potential genotype x diet interactions in selected farmed fish when challenged with novel diet formulations. Therefore, the present study aimed to determine the effectiveness of selective breeding for high growth in gilthead sea bream, in response to a challenging low FM/FO-based diet, that aimed to partially replace FM with two emergent ingredients: an insect meal from black soldier fly or a single-cell protein from *M. capsulatus*. These diets were additionaly formulated to totally replace FO with a blend of poultry oil and a novel DHA rich-microalgae oil. The response of selected for high growth sea bream to the novel dietary interventions was assessed on fish productive parameters, proximate and fatty acid composition of fish tissues, as well as on fillet quality properties at commercial size, and compared with a reference population. The independent effects of genotype, diet as well as their potential interactions were addressed.

2. Material 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 12/2020).

2.2. Experimental fish

A total of 6122 gilthead sea bream adults from the Canary Islands and the 3rd generation of the National Breeding Program (PROGENSA®) were evaluated for growth (León Bernabeu, 2022). The estimated breeding values (EBV) of these stock range between −159.14 for reference population and + 223.18 for High Growth selected fish, with an average value of 8.59 and a standard deviation value of 52.84. A total of 192 breeders were selected on the basis of their EBV and relationship coefficient. Two groups of breeders were established: High Growth

selected group (HG) (2×, with 46 and 48 breeders per broodstock) and the reference population group (REF). These breeders showed opposite values in their EBV, which corresponds to a value of +39.68 in the group of High Growth values of HG and REF breeders contained almost 47% of the evaluated population.

Fertilized eggs from spontaneous spawning from the different broodstock groups were collected. The two resulting populations from HG or REF fish were incubated separately until hatching. Hatched larvae were kept in separate tanks. The larval production protocol used to grow larvae was the standardized methodology of the ULPGC facilities (Eryalçın et al., 2020). Briefly, hatched larvae were maintained at pre-weaning and weaning tanks and fed rotifers (*Brachionus plicatilis*) enriched with DHA Protein Selco ® (INVE) until 20 days post hatching (dph), and a commercial diet afterwards. Progeny from either selected for growth (HG) or reference fish (REF) was kept at similar conditions during the pre-weaning, weaning, and early juvenile growing phases.

2.3. Experimental diets

Three isoproteic and isoenergetic diets were formulated to meet the described nutritional requirements of gilthead sea bream juveniles. Control diet (C) contained 15% of FM and 5.9% of FO to mimic the composition of a current commercial diet, that was completed with some vegetable meals and rapeseed oil as protein and lipid sources, respectively. Insect meal (InnovaFeed, France) was included at 5% of the diet to replace 33.3% of the dietary FM and single-cell protein meal (Calysta, USA) was included at 10% of the diet and replaced 66.7% of the dietary FM, corresponding to INS and SCP diets, respectively. FO was also totally replaced in both diets by a blend of poultry oil and microalgae algal oil (Veramaris, Netherlands). The dietary inclusions of these novel ingredients were based on previous studies showing no detrimental effects for sea bream juveniles (Carvalho et al., 2020, 2023). Diets were manufactured by Skretting (Skretting ARC, Stavanger, Norway). Feeds formulation and proximate composition are shown in Table 1 and their amino acid and fatty acid profile are detailed in Tables S1 and S2, respectively.

2.4. Feeding trial and sampling conditions

The nutritional trial was carried out at the experimental facilities of the ULPGC. Gilthead sea bream from each experimental group (HG genotype *vs* REF genotype) with an initial body weight of 49.91 g (average body weight), were randomly distributed in 12 experimental tanks, at a density of 45 fish/tank (3 tanks/treatment). Fish were initially allocated in cylinder-conical tanks of 500 L. 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 6 and 8 ppm and 19.2 to 21 °C, respectively. Salinity was 37 g/ L. Fish were manually fed until apparent satiation with one of the three experimental diets for 12 weeks (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 every 4 weeks until the end of the feeding trial. Before samplings, fish were fasted for 24 h. For monitoring fish growth during the feeding trial, all fish were anesthetized with clove oil diluted in ethanol at 1:1 (0.04 mL/Ll) and individually weighed and measured. Specific growth rate (SGR), thermal growth coefficient (TGC), and feed conversion ratio (FCR) were calculated based on fish weight and/or feed intake to estimate growth performance, using the following equations: SGR, Specific Growth Rate (SGR) = [(Ln (final weight-Ln (initial weight)) / number of days x 100]; Thermal Growth Coefficient (TGC) = [(final weight$^{1/3}$ - Initial weight$^{1/3}$) / (temperature x number of days) x 1000)]; Feed Conversion Ratio (FCR) = feed intake/ weight gain.

At the end of the 12 week-trial, 12 fish per tank (3 tanks per

Table 1
Ingredients (%) and proximate composition (% dry matter) of the experimental diets.

Ingredients (%)	Control (C) – 1.8 mm	Insect meal (INS)-1.8 mm	Single-cell protein (SCP)-1.8 mm	Control (C) – 4 mm	Insect meal (INS)-4 mm	Single-cell protein (SCP)-4 mm
Corn gluten	5.4	10.0	5.0	6.0	6.0	6.0
Wheat gluten	18.8	20.0	20.0	20.0	20.0	17.0
Faba bean[1]	8.0	8.0	8.0	8.0	8.0	8.0
Soya protein concentrate[2]	25.0	22.0	25.0	12.6	14.7	18.0
Fish oil[3]	5.9	0	0	6.9	0	0
Fish meal[4]	15.0	10.0	5.0	15.0	10.0	5.0
Rapeseed oil	3.9	4.3	5.1	4.6	5.8	6.3
Ytrium premix	0.1	0.1	0.1	0.1	0.1	0.1
Phosphate	0.8	1.0	1.3	1.0	1.1	1.4
Wheat	14.9	13.2	13.7	23.8	21.9	20.5
Poultry oil[5]	0	2.0	2.2	0	2.6	2.7
Veramaris oil[6]	0	2.3	2.5	0	2.6	2.9
Lecithin	2.0	2.0	2.0	2.0	2.0	2.0
Insect meal[7]	0	5.0	0	0	5.0	0
Single-cell protein[8]	0	0	10	0	0	10
Premix vitamins and minerals[9]	0.1	0.1	0.1	0.1	0.1	0.1
Proximate composition (% dry matter)						
Protein	46.9	46.8	48.3	46.9	46.8	48.3
Ash	4.7	4.6	4.6	4.7	4.6	4.6
Lipids	16.6	16.9	17.2	16.6	16.9	17.2
Moisture	8.5	8.2	8.3	8.5	8.2	8.3

[1] Faba beans: Cefetra BV (The Netherlands).
[2] Soya protein concentrate: CJ Selecta S.A (Brasil).
[3] Fish oil: Copeinca, S. A. (Perú).
[4] Fish meal: Norsildmel AS (Norway).
[5] Poultry oil: Sonac (Belgium).
[6] DHA microalgal oil: Veramaris (Evonik).
[7] Produced from *Hermetia illucens*. InnovaFeed (France) (Protein: 57–62%; Lipids: 8–11; Ash: 8–10; Moisture: 2.5).
[8] FeedKind© produced from *Methylococcus capsulatus* fermentation. Calysta (USA) (Protein: 70.6%; Lipids: 9.8; Ash: 7.1; Moisture: 6).
[9] Mineral and Vitamin premix: Trouw Nutrition (The Netherlands).

experimental group) were euthanized with an excess of clove oil, and whole-body samples from 6 fish per tank, as well as livers and fillets from other 6 fish per tank were sampled and pooled by tank for proximate composition and fatty acid profile analyses. After 12 weeks of feeding and the respective sample collection, the remaining fish were transferred to 1000 L-cylinder tanks (2 tanks per treatment) to be continuously fed with their corresponding diet until reaching commercial size (~300 g; 330 days) to study fillet organoleptic characteristics (texture and sensorial attributes). When fish reached the commercial size, 12 fish per experimental group were euthanized and fillets were collected for analyzing texture attributes, and muscular fibres morphology, whereas other 3 fillets per treatment were collected for the sensorial analysis. All samples were conserved at −80 °C until analysis, except muscle samples for the histological study that were conserved in buffered formaldehyde at 4%.

2.5. Proximate and fatty acid composition analysis

Proximate composition analysis of feeds and fish samples was carried out accordingly with the standardized procedures described by AOAC (2019). Crude protein content (Nx6.25) was analyzed following the Kjeldahl method. The amino acid composition of feeds 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. The total lipid content of the samples followed the method described by Folch et al. (1957), where lipids were extracted with chloroform/methanol (2:1 *v*/v), except for fillet samples in which lipid content was analyzed by Near-Infrared Spectroscopy (NIR) method (FoodScan™, FOSS, Hillerød, Denmark). 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).

2.6. Fillet quality: texture and sensory properties

2.6.1. Texture properties

Texture analyses were performed on raw fillets on days 1 and 4 post-slaughter. For that, two rectangular portions of 2 × 2 cm were collected from the dorsal part of the skinless fillet, which were analyzed with a texturometer TA-XT2 (Stable Micro Systems Ltd., Surrey, UK). The strength of the texturometer was calibrated at 5 kg of mass. Fracture capacity, hardness, elasticity, cohesivity, gumminess, chewiness, adhesiveness, and resilience were calculated according to Ginés et al. (2004) and measured as texture properties with a compression plate of 100 mm Ø at a speed of 0.8 mm/s, until achieving a deformation in crude of 60% of the sample thickness (Ginés et al., 2004).

2.6.2. Sensory properties

For evaluating the sensory properties of fish fillets, 4 sensory panels composed of 8 evaluators were set. Evaluators were specifically trained according to ISO guidelines (ISO 8586-2:2008). To determine the intensity of tested attributes, fillet portions of 3 × 4 cm were cooked in lidded aluminum boxes in an air-heated oven (Compact; Eurofred, Barcelona, Spain) at 115 °C for 12 min. Fourteen sensory attributes related to odor, appearance, texture, flavor, and aftertaste were evaluated by each panelist using a continuous intensity scale from 0 (low intensity of the attribute) to 100 (the highest intensity of the attribute). The sensory evaluation was developed in 4 different sessions where each panelist scored the sensory attributes of 4 random pieces per session , which were stored in a temperate maintainer (Clatronic International GmbH, Kempen, Germany). The sensory evaluation took place in the test room of SABE (Service of Aquaculture and Biotechnology of High Specialization), that was designed according to ISO guidelines (ISO 8589:2007).

2.7. Histological study of white and red muscular fibres

To ensure the presence of white and red muscular fibres, fish muscle samples were collected, dehydrated in a graded ethanol series and embedded in paraffin. Sections were cut at 3 μm with an AUTOCUT JUNG 2055 microtome (LEICA BIOSYSTEMS, Deer Park, IL, United States) and stained with hematoxylin and eosin (H&E) (Martoja and Martoja-Pierson, 1970) for evaluation under a light microscope (CX41, Olympus, Tokyo, Japan) equipped with an Olympus XC50 camera (Olympus, Tokyo, Japan). Two micrographs were obtained from each sample, one from white muscle (x10), and the other from red muscle (x20). The number and diameter of white and red muscular fibres were quantified and measured using the image processing software Image Pro-Plus for Windows, Version 6.0 (Media Cybernetics, Inc. Rockville, MD, United States). White and red mucle muscle fibres area was delimited between 100 and 3,000,000 μm^2, and between 50 and 800,000 μm^2, respectively, and the number of muscular fibres was quantified according to their diameter ($\leq$20 μm, 20 < d $\leq$ 40 μm, 40 < d $\leq$ 60 μm, 60 < d $\leq$ 80 μm, 80 < d $\leq$ 100 μm, and > 100 μm, for white muscle fibre and $\leq$ 20 μm, 20 < d $\leq$ 30 μm, 30 < d $\leq$ 40 μm, 40 < d $\leq$ 50 μm, and > 50 μm, for red muscle fibres).

2.8. Statistical analyses

All data are presented as mean ± SD and were tested for normality and homogeneity of variances using Shapiro–Wilk and Levene's tests, respectively. To evaluate the effects of genotype and diet and their potential interactions, a two-way ANOVA was performed, using diet and genotype as fixed factors. For texture post-sacrifice data, a three-way ANOVA was performed including time as an additional fixed factor. Differences were considered significant at $P < 0.05$. When significant interactions were detected ($P < 0.05$), a one-way ANOVA was applied to the data to check the differences between groups, using Tukey's as a posthoc test (Tukey, 1949). These analyses were carried out using the SPSS Statistical Software System v24.0 (SPSS, Chicago, IL, USA). Additionally, principal components (PCA) were carried out over the fatty acid profile of sea bream tissues, using the R Project for Statistical Computing software.

3. Results

3.1. Growth performance and feed utilization

After 12 weeks of feeding, selected genotype increased fish growth in terms of body weight and productive parameters like SGR and TGC, as well as lowered FCR, irrespective of the diet ($P < 0.05$; Table 2). INS and SCP diets led to lower growth in terms of body weight, length, and/or SGR and TGC than the C diet from the 4th week of feeding until the end of the 12-week feeding trial ($P < 0.05$; Table 2). INS and SCP diets also significantly decreased fish FI compared to C diet, irrespective of the genotype ($P < 0.05$), but showed no effect on FCR (Table 2). However, it is noteworthy that HG fish fed INS or SCP diets showed similar growth as REF fish feeding a C diet (Table 2). No significant interactions g x d were observed in any productive parameter of sea bream after the 12-week feeding trial (Table 2).

3.2. Proximate composition and fatty acid profile of fish tissues

The proximate compositions of whole-body, muscle, and liver were not significantly affected by genotype, diet, nor by an interaction g x d (Table 3). Regarding the fatty acid profile of the tissues, genotype did not significantly affect FA profile of whole-body and fillet (Tables 4 and

Table 2
Growth performance of genetically selected for high growth and reference gilthead sea bream fed the experimental diets.

								Two-Way ANOVA (*p*- value)		
Weeks		HG-*C*	HG-*INS*	HG-*SCP*	REF-*C*	REF-*INS*	REF-*SCP*	Genotype	Diet	GxD
Initial	BW (g)[1]	49.85 ± 2.47	49.77 ± 1.31	50.40 ± 2.55	50.13 ± 0.42	49.90 ± 0.42	50.20 ± 0.26	*n.s*	*n.s*	*n.s*
	TL (cm)[2]	14.60 ± 0.14	14.43 ± 0.15	14.55 ± 0.21	14.53 ± 0.15	14.55 ± 0.07	14.53 ± 0.06	*n.s*	*n.s*	*n.s*
4	BW (g)[1]	82.78 ± 0.16	76.44 ± 2.53	75.36 ± 0.91	78.90 ± 3.75	75.48 ± 0.42	72.84 ± 0.05	*n.s*	$P = 0.001$ C > INS, SCP	*n.s*
	TL (cm)[2]	16.77 ± 0.06	16.46 ± 0.23	16.44 ± 0.02	16.59 ± 0.20	16.4 ± 0.01	16.29 ± 0.10	*n.s*	$P = 0.036$ C > SCP	*n.s*
8	BW (g)[1]	110.26 ± 0.82	99.28 ± 1.41	99.69 ± 0.94	101.70 ± 1.79	96.63 ± 2.34	94.71 ± 1.08	$P = 0.000$	P = 0.000 C > INS, SCP	*n.s*
	TL(cm)[2]	18.63 ± 0.03	18.25 ± 0.03	18.24 ± 0.01	17.82 ± 0.87	18.25 ± 0.30	17.94 ± 0.17	*n.s*	*n.s*	*n.s*
12	BW (g)[1]	133.32 ± 2.91	116.46 ± 2.18	118.48 ± 1.37	120.41 ± 1.52	114.61 ± 1.93	112.00 ± 0.60	P = 0.000	P = 0.000 C > INS, SCP	*n.s*
	TL (cm)[2]	19.80 ± 0.14	19.05 ± 0.15	20.36 ± 1.41	19.41 ± 0.09	19.20 ± 0.23	19.06 ± 0.23	*n.s*	*n.s*	*n.s*
	SGR [3]	1.09 ± 0.08	0.94 ± 0.02	0.95 ± 0.04	0.97 ± 0.02	0.92 ± 0.01	0.89 ± 0.01	$P = 0.002$	P = 0.001 C > INS, SCP	*n.s*
	TGC[4]	1.57 ± 0.13	1.33 ± 0.04	1.35 ± 0.05	1.38 ± 0.01	1.30 ± 0.01	1.24 ± 0.01	P = 0.002	P = 0.000 C > INS, SCP	*n.s*
	FI (kg/tank)[5]	5.31 ± 0.07	4.57 ± 0.15	4.84 ± 0.05	5.15 ± 0.11	4.98 ± 0.02	4.67 ± 0.08	*n.s*	P = 0.001 C > INS, SCP	*n.s*
	FCR[6]	1.45 ± 0.16	1.55 ± 0.05	1.60 ± 0.04	1.65 ± 0.04	1.73 ± 0.02	1.76 ± 0.05	P = 0.001	*n.s*	*n.s*

C: Control diet; INS: Insect meal diet; SCP: Single-cell protein diet; HG: high-growth genotype; REF: reference (non-selected) 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. Values are expressed in mean ± SD. ($n = 3$ tanks/diet/genotype; 12 weeks of feeding). Two-way ANOVA, $p < 0.05$, Genotype and Diet as fixed factors. n.s = not significant.

Table 3
Proximate composition of tissues (% dry matter) from genetically selected for high growth and reference gilthead sea bream fed the experimental diets.

								Two-Way ANOVA (*p*- value)		
		HG-*C*	HG-*INS*	HG-*SCP*	REF-*C*	REF-*INS*	REF-*SCP*	Genotype	Diet	GxD
Whole-body	Protein	48.78 ± 1.77	48.89 ± 0.88	48.53 ± 1.27	47.99 ± 1.24	49.31 ± 1.09	50.39 ± 2.55	*n.s*	*n.s*	*n.s*
	Ash	8.70 ± 0.21	8.69 ± 0.37	9.06 ± 0.30	8.40 ± 0.56	7.96 ± 0.94	8.65 ± 0.26	*n.s*	*n.s*	*n.s*
	Lipids	41.97 ± 1.87	43.34 ± 1.77	40.08 ± 0.08	41.93 ± 1.17	40.78 ± 1.06	40.96 ± 0.71	*n.s*	*n.s*	*n.s*
	Moisture	67.59 ± 1.32	69.63 ± 0.90	69.40 ± 1.24	68.40 ± 0.82	68.22 ± 0.63	69.18 ± 0.75	*n.s*	*n.s*	*n.s*
Muscle	Protein	67.66 ± 1.19	68.84 ± 4.13	69.18 ± 3.60	66.52 ± 3.65	67.62 ± 1.62	67.96 ± 2.22	*n.s*	*n.s*	*n.s*
	Ash	4.80 ± 0.28	5.35 ± 0.04	5.29 ± 0.40	4.84 ± 0.33	5.05 ± 0.41	5.00 ± 0.04	*n.s*	*n.s*	*n.s*
	Lipids	27.05 ± 1.65	26.43 ± 2.20	26.33 ± 2.32	27.40 ± 2.61	26.46 ± 0.70	27.91 ± 1.10	*n.s*	*n.s*	*n.s*
	Moisture	69.69 ± 0.31	70.47 ± 0.69	70.49 ± 1.08	70.11 ± 0.27	70.01 ± 0.74	70.40 ± 0.09	*n.s*	*n.s*	*n.s*
Liver	Protein	28.54 ± 0.86	30.41 ± 2.89	31.04 ± 2.89	28.66 ± 0.63	31.24 ± 1.13	29.31 ± 0.76	*n.s*	*n.s*	*n.s*
	Ash	2.28 ± 0.40	2.53 ± 0.47	2.42 ± 0.50	2.67 ± 0.26	2.13 ± 0.26	2.27 ± 0.37	*n.s*	*n.s*	*n.s*
	Lipids	36.91 ± 1.32	32.79 ± 6.10	30.52 ± 6.10	33.07 ± 2.64	29.96 ± 4.30	35.24 ± 4.22	*n.s*	*n.s*	*n.s*
	Moisture	62.24 ± 0.47	64.14 ± 2.08	64.51 ± 2.69	62.96 ± 0.70	64.61 ± 1.30	62.55 ± 1.70	*n.s*	*n.s*	*n.s*

C: Control diet; INS: Insect meal diet; SCP: Single-cell protein; HG: high-growth genotype; REF: reference (non-selected) genotype. Values are expressed in mean ± SD. (n = 3 tanks/diet/genotype; 12 weeks of feeding). Two-way ANOVA, p < 0.05, Genotype and Diet as fixed factors. n.s = not significant.

5), and, in the liver, it increased the contents of some important fatty acids, such as 20:5n-3 ($P < 0.05$; Table 6). This lack of effect was also clearly noted by the overlapping of the different genotypic groups fed the same diet in the PCA analysis (Figs. 1, 2, and 3). In contrast, diet was the main factor affecting the fatty acid profile of the three tissues. Indeed, PCA analysis showed that fish fed INS or SCP diets formed a well-defined group towards the left of the plot in the three analysed tissues, which was separated from fish fed C diet (Figs. 1, 2, and 3). PC1 explained the 55.1, 65.3 and 44.2% of the total variability in fatty acid profiles between the experimental groups, whereas PC2 explained 28.2, 19.6 and 32.7% in whole-body, fillet, and liver, respectively (Figs. 1, 2, and 3). Therefore, fish tissues, particularly fillets, of sea bream fed INS or SCP diets were characterized by a higher content of 18:0, 18:1n-9, 18:2n-6, 18:3n-3 and 22:6n-3, whereas those of fish fed C diet were characterized by a higher content of 14:0, 20:1n-7, 20:5n-3 and 22:1n-11 ($P < 0.05$; Tables 4, 5 and 6). These were also the FA that, according to the three PCAs most drove the variability between the fatty acid profile of the different experimental groups (Figs. 1, 2, and 3).

3.3. Fillet quality: texture and sensory properties

At commercial size, fillet proximate composition was only affected by genotype in their ash content, with fillets from REF sea bream showing lower ash than those from HG genotype ($P < 0.05$; Table 7). As expected, time post-slaughter significantly affected many texture properties of fish fillets, irrespective of the diet or genotype, with the values for fracturability, hardness, elasticity, gumminess, and resilience decreasing with time (P < 0.05; Table 7). However, no significant effect of genotype or diet was noted in the texture properties of fish fillets, neither at 1 nor 4 days post-slaughter. A significant interaction g x d x t interaction for fillet cohesivity values was found, with HG fish fed INS diets presenting significant lower muscle cohesivity compared with their HG and REF counterparts at 1 days post-sacrifice (P < 0.05; Table 7). Similarly, fish from REF groups and fed diets C and SCP showed lower fillet cohesivity at 4 days post-slaughter compared with those fed HG and fed INS at only 1 day post-sacrifice (P < 0.05; Table 7).

Concerning the sensory properties of sea bream fillets, no significant effects of diet, genotype, nor interaction between g x d were observed in any of the evaluated sensorial attributes of fish fillets (Table 8).

3.4. Histological study of white and red muscular fibres

In white-muscle, fish from REF genotype showed higher density of muscular fibres from higher diameter (d > 100 μm), compared to those

Table 4
Fatty acid composition (% total fatty acids) of whole-body from genetically selected for high growth and reference gilthead sea bream fed the experimental diets.

							Two-Way ANOVA (p-value)		
Fatty acids (% total FA)	HG-*C*	HG-*INS*	HG-*SCP*	REF-*C*	REF-*INS*	REF-*SCP*	Genotype	Diet	GxD
14:0	1.48 ± 0.10[a]	1.14 ± 0.03[b]	0.90 ± 0.04[c]	1.65 ± 0.10[a]	1.11 ± 0.06[b]	0.90 ± 0.03[c]	*n.s*	P = 0.000	$P = 0.047$
14:1n-7	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	*n.s*	P = 0.001 INS > C, SCP	*n.s*
14:1n-5	0.06 ± 0.00 [a]	0.02 ± 0.00 [b]	0.02 ± 0.00[b]	0.07 ± 0.00 [a]	0.02 ± 0.00 [b]	0.02 ± 0.00[b]	*n.s*	P = 0.000	$P = 0.028$
15:0	0.18 ± 0.01 [a]	0.14 ± 0.00 [b]	0.15 ± 0.00[b]	0.20 ± 0.01[a]	0.13 ± 0.00 [b]	0.15 ± 0.00[b]	*n.s*	P = 0.000	$P = 0.009$
15:1n-5	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	*n.s*	*n.s*	*n.s*
16:0ISO	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	*n.s*	P = 0.000 C > SCP > INS	*n.s*
16:0	13.05 ± 0.60	13.12 ± 0.21	13.07 ± 0.63	13.56 ± 0.30	13.07 ± 0.27	13.48 ± 0.70	*n.s*	*n.s*	*n.s*
16:1n-7	3.02 ± 0.10	2.59 ± 0.08	2.84 ± 0.02	3.16 ± 0.13	2.66 ± 0.02	2.03 ± 1.53	*n.s*	*n.s*	*n.s*
16:1n-5	0.09 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.09 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	*n.s*	P = 0.000 C > SCP,INS	*n.s*
16:2n-6	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	*n.s*	*n.s*	*n.s*
16:2n-4	0.11 ± 0.01	0.06 ± 0.00	0.05 ± 0.00	0.11 ± 0.01	0.06 ± 0.01	0.05 ± 0.00	*n.s*	P = 0.000 C > INS > SCP	*n.s*
17:0	0.07 ± 0.01	0.05 ± 0.00	0.05 ± 0.00	0.07 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	*n.s*	P = 0.000 C > SCP,INS	*n.s*
16:3n-4	0.23 ± 0.01	0.15 ± 0.01	0.15 ± 0.00	0.23 ± 0.00	0.15 ± 0.00	0.15 ± 0.01	*n.s*	P = 0.000 C > SCP,INS	*n.s*
16:3n-3	0.07 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.07 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	*n.s*	P = 0.000 C > SCP,INS	*n.s*
16:3n-1	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.01	*n.s*	*n.s*	*n.s*
16:4n-3	0.05 ± 0.00	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.04 ± 0.00	0.04 ± 0.01	*n.s*	P = 0.002 C > SCP,INS	*n.s*
16:4n-1	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	*n.s*	*n.s*	*n.s*
18:0	3.25 ± 0.06	3.26 ± 0.08	3.46 ± 0.22	3.26 ± 0.21	3.33 ± 0.19	3.64 ± 0.23	*n.s*	$P = 0.029$ SCP > C	*n.s*
18:1n-9	36.02 ± 0.50	38.20 ± 0.66	37.94 ± 1.23	36.16 ± 0.85	37.42 ± 0.90	38.83 ± 0.56	*n.s*	P = 0.001 SCP,INS > C	*n.s*
18:1n-7	2.51 ± 0.12	2.24 ± 0.18	2.26 ± 0.16	2.45 ± 0.02	2.26 ± 0.12	2.28 ± 0.13	*n.s*	$P = 0.017$ C > SCP,INS	*n.s*
18:1n-5	0.16 ± 0.00	0.08 ± 0.00	0.10 ± 0.00	0.16 ± 0.00	0.07 ± 0.00	0.10 ± 0.00	*n.s*	P = 0.000 C > SCP > INS	*n.s*
18:2n-9	0.42 ± 0.07	0.39 ± 0.10	0.38 ± 0.02	0.33 ± 0.05	0.36 ± 0.05	0.35 ± 0.01	*n.s*	*n.s*	*n.s*
18:2n-6	15.28 ± 0.15	18.73 ± 0.27	18.61 ± 0.53	15.36 ± 0.25	18.51 ± 0.41	18.40 ± 0.64	*n.s*	P = 0.000 SCP,INS > C	*n.s*
18:2n-4	0.06 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.06 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	*n.s*	P = 0.000 C > SCP,INS	*n.s*
18:3n-6	0.44 ± 0.07	0.50 ± 0.09	0.51 ± 0.03	0.38 ± 0.05	0.45 ± 0.06	0.46 ± 0.05	*n.s*	*n.s*	*n.s*
18:3n-4	0.08 ± 0.00	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	*n.s*	$P = 0.003$ C > SCP,INS	*n.s*
18:3n-3	3.38 ± 0.07	3.72 ± 0.11	3.91 ± 0.30	3.37 ± 0.14	3.78 ± 0.24	3.77 ± 0.23	*n.s*	P = 0.003 SCP,INS > C	*n.s*
18:3n-1	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	*n.s*	P = 0.003 SCP,INS > C	*n.s*
18:4n-3	0.76 ± 0.07	0.33 ± 0.03	0.31 ± 0.03	0.72 ± 0.07	0.33 ± 0.03	0.28 ± 0.05	*n.s*	P = 0.000 C > SCP,INS	*n.s*
18:4n-1	0.04 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	*n.s*	P = 0.000 C > SCP,INS	*n.s*
20:0	0.33 ± 0.01	0.34 ± 0.02	0.33 ± 0.03	0.32 ± 0.01	0.32 ± 0.02	0.36 ± 0.01	*n.s*	*n.s*	*n.s*
20:1n-9	0.57 ± 0.04	0.19 ± 0.01	0.14 ± 0.01	0.56 ± 0.01	0.18 ± 0.01	0.15 ± 0.02	*n.s*	P = 0.000 C > INS > SCP	*n.s*
20:1n-7	3.31 ± 0.07	1.59 ± 0.09	1.41 ± 0.11	3.31 ± 0.08	1.60 ± 0.13	1.47 ± 0.02	*n.s*	P = 0.000 C > INS > SCP	*n.s*
20:1n-5	0.14 ± 0.00	0.09 ± 0.01	0.08 ± 0.01	0.13 ± 0.00	0.09 ± 0.01	0.08 ± 0.01	*n.s*	P = 0.000 C > SCP,INS	*n.s*
20:2n-9	0.36 ± 0.01	0.33 ± 0.04	0.32 ± 0.05	0.35 ± 0.03	0.35 ± 0.03	0.34 ± 0.04	*n.s*	*n.s*	*n.s*
20:2n-6	0.45 ± 0.01	0.45 ± 0.03	0.45 ± 0.02	0.46 ± 0.03	0.49 ± 0.04	0.46 ± 0.00	*n.s*	*n.s*	*n.s*
20:3n-9	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	*n.s*	$P = 0.005$ C > SCP	*n.s*
20:3n-6	0.23 ± 0.02	0.27 ± 0.01	0.29 ± 0.01	0.23 ± 0.01	0.27 ± 0.01	0.28 ± 0.01	*n.s*	P = 0.000 SCP,INS > C	*n.s*
20:4n-6	0.27± 0.01	0.42 ± 0.02	0.47 ± 0.06	0.26 ± 0.01	0.44 ± 0.02	0.44 ± 0.04	*n.s*	P = 0.000 SCP,INS > C	*n.s*
20:3n-3	0.18 ± 0.00	0.17 ± 0.01	0.18 ± 0.00	0.18 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	*n.s*	*n.s*	*n.s*
20:4n-3	0.53 ± 0.03	0.32 ± 0.02	0.32 ± 0.03	0.52 ± 0.04	0.34 ± 0.02	0.31 ± 0.03	*n.s*	P = 0.000 C > SCP,INS	*n.s*
20:5n-3	2.07 ± 0.20	1.87 ± 0.16	1.95 ± 0.34	1.95 ± 0.18	2.01 ± 0.20	1.86 ± 0.28	*n.s*	*n.s*	*n.s*
22:1n-11	3.39 ± 0.06	0.69 ± 0.06	0.43 ± 0.08	3.26 ± 0.08	0.65 ± 0.07	0.45 ± 0.04	*n.s*	P = 0.000 C > INS > SCP	*n.s*
22:1n-9	0.86 ± 0.00	0.50 ± 0.04	0.43 ± 0.06	0.81 ± 0.04	0.48 ± 0.06	0.46 ± 0.02	*n.s*	P = 0.000 C > SCP,INS	*n.s*

(*continued on next page*)

Table 4 (*continued*)

Fatty acids (% total FA)	HG-*C*	HG-*INS*	HG-*SCP*	REF-*C*	REF-*INS*	REF-*SCP*	Two-Way ANOVA (*p*-value)		
							Genotype	Diet	GxD
22:4n-6	0.10 ± 0.00	0.11 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	*n.s*	*P* = 0.012 SCP,INS > C	*n.s*
22:5n-6	0.15 ± 0.01	0.34 ± 0.03	0.37 ± 0.04	0.14 ± 0.01	0.36 ± 0.03	0.36 ± 0.05	*n.s*	P = 0.000 SCP,INS > C	*n.s*
22:5n-3	0.92 ± 0.09	0.86 ± 0.09	0.89 ± 0.07	0.89 ± 0.09	0.97 ± 0.08	0.88 ± 0.14	*n.s*	*n.s*	*n.s*
22:6n-3	5.20 ± 0.72	6.38 ± 0.75	6.73 ± 0.96	4.74 ± 0.46	6.97 ± 0.69	6.45 ± 1.23	*n.s*	*P* = 0.006 SCP,INS > C	*n.s*
SFA	18.40 ± 0.74	18.06 ± 0.30	17.99 ± 0.92	19.11 ± 0.39	18.04 ± 0.42	18.60 ± 0.94	*n.s*	*n.s*	*n.s*
MUFA	50.16 ± 0.63	46.29 ± 0.86	45.75 ± 1.39	50.22 ± 0.99	45.53 ± 1.08	45.96 ± 1.87	*n.s*	P = 0.000 C > SCP,INS	*n.s*
N-9	38.24 ± 0.44	39.63 ± 0.71	39.21 ± 1.33	38.23 ± 0.89	38.80 ± 0.93	40.13 ± 0.56	*n.s*	*P* = 0.038 SCP > C	*n.s*
N-6	16.93 ± 0.22	20.83 ± 0.19	20.81 ± 0.59	16.93 ± 0.31	20.65 ± 0.45	20.54 ± 0.79	*n.s*	P = 0.000 SCP,INS > C	*n.s*
N-3	13.16 ± 1.17	13.71 ± 1.12	14.37 ± 1.73	12.49 ± 0.97	14.67 ± 1.13	13.81 ± 1.97	*n.s*	*n.s*	*n.s*
EPA/ARA	7.57 ± 0.41	4.46 ± 0.18	4.17 ± 0.20	7.39 ± 0.54	4.57 ± 0.24	4.18 ± 0.24	*n.s*	P = 0.000	*n.s*
EPA/DHA	0.40 ± 0.02	0.29 ± 0.01	0.29 ± 0.01	0.41 ± 0.01	0.29 ± 0.02	0.29 ± 0.02	*n.s*	P = 0.000	*n.s*
EPA + DHA	7.27 ± 0.91	8.25 ± 0.91	8.68 ± 1.30	6.68 ± 0.64	8.98 ± 0.86	8.31 ± 1.51	*n.s*	P = 0.036	*n.s*
N-3 LC-PUFA	8.89 ± 1.04	9.60 ± 1.02	10.08 ± 1.40	8.28 ± 0.76	10.49 ± 0.93	9.69 ± 1.69	*n.s*	*n.s*	*n.s*

C: Control diet; INS: Insect meal diet; SCP: Single-cell protein; HG: high-growth genotype; REF: reference (non-selected) genotype. Values are expressed in mean ± SD. (n = 3 tanks/diet/genotype; 12 weeks of feeding). 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.

from HG genotype ($P < 0.05$; Table 9). Furthermore, a significant g x d interaction was observed for those fibres with diameter between 60 and 80 μm, with REF fish fed INS diet showing higher density of these fibres compared to those from HG genotype fed the same diet ($P < 0.05$; Table 9).

In red muscle, diet affected muscular fibres with diameter between 20 and 30 μm, with INS increasing the density of those fibres compared to SCP diet ($P < 0.05$; Table 9). A significant g x interaction was observed for muscular fibres with <20 μm as well those with diameter between 40 and 50 μm. Therefore, fish from HG genotype and fed the INS diet also showed higher density of muscular fibres from small diameter ($d \leq 20$ μm) in red muscle compared with REF sea bream fed SCP diet. REF fish fed INS diet also showed the highest density of red fibres from high diameter ($40 < d \leq 50$ μm).

4. Discussion

Combining genetic selection and novel nutritional strategies and formulae in aquaculture can help to improve the growth, health, and sustainability of the sector. Previous studies have reported that genetically selected fish have higher plasticity to deal with dietary challenges, and, consequently, a higher tolerance to alternative diet formulations with low FM/FO contents (Gjedrem et al., 2012; Montero et al., 2023a, 2023b). However, most of the available studies were conducted with the replacement of FM/FO by plant-origin alternatives. Indeed, other novel raw materials, like insect meals, single-cell proteins or microalgae oils have been emerging in the market more recently as promising alternatives not only to FM and FO but also to other traditional ingredients from vegetal origin in fish feeds for the near future. Therefore, based on the previous literature on the effectiveness of genetic selection of farmed fish for supporting the replacement of FM/FO in aquafeeds by alternative ingredients, it was hypothesized that selective breeding could also improve the utilization of novel dietary formulations with emergent ingredients. In the present study, gilthead sea bream, which was genetically selected for 3 generations (PROGENSA®) based on growth performance trait, showed improved growth and feed utilization compared to non-selected sea bream. The improvement capacity of genetic selection in growth and feed conversion obviously depends on feed origins but in the present study an improvement of approximately 10% of magnitude was observed in growth (when fed control diet) and particularly on feed conversion ratio in all diets, similarly to what was observed in a similar study with European sea bass (11.2%; Montero et al., 2023a, 2023b). Therefore, these results confirm the success of the selective breeding program PROGENSA® applied to gilthead sea bream for 3 generations in improving the productive performance of fish (León-Bernabeu et al., 2021, León Bernabeu, 2022), denoted by the better growth and feed utilization of the selected genotype compared to non-selected fish, at any of the diets assayed. However, despite the generally improved growth and feed utilization caused by the applied selective breeding program, a decreasing effect on fish growth was associated to the novel diets, probably related with a reduced feed intake, which might suggest a lower acceptability of these alternative diets by sea bream. Interestingly, despite this lowering effect of the novel diets in fish feed intake, feed conversion ratio was not affected by the diets, which might suggest that the reduced feed intake observed in sea bream fed these diets might probably be related to a lower palatability of the novel ingredients used, rather than an effect in the digestibility of the novel proteins. Indeed, a previous study using the same novel protein sources at the same dietary levels (5% for insect meal and 10% for SCP) in non-selected sea bream, reported no effect on growth or feed intake with these replacement levels, contrary to what was observed in the present study (Carvalho et al., 2022). Besides the slightly different dietary formulations between the two studies, it should also be noted that the diets assayed in the present study were more challenging compared to those used in Carvalho et al. (2022) study, since it aimed concomitantly replace the dietary FM and the total FO, which might also partially explain the slight differences observed between the two studies. However, it is noteworthy that selected fish fed novel diets (INS but particularly SCP) showed very similar growth and lower feed conversion ratio compared with non-selected fish fed a control diet based on FM and FO. These results suggest that genetic selection for high growth improved gilthead sea bream utilization of novel dietary formulations with high replacement levels of FM and FO by emergent ingredients, similarly to what was observed with diets rich in plant ingredients previously (Perera et al., 2019). In agreement, previous studies with other fish species also reported improved tolerance to alternative diets in selected fish (Gjedrem et al., 2012; Montero et al., 2023a, 2023b). Therefore, it is possible to replace 33% and 66% of the total fishmeal in the diet by insect meal and single cell protein, respectively, as well as the total FO by a combination of poultry oil and a DHA-rich microalgal oil, in diets for genetically selected sea bream and achieve a similar performance when a FM/FO diet is used and no selective breeding program is applied. Other studies in gilthead sea bream or European sea bass reported similar FM replacement levels (25–30%) by black soldier fly meal,

Table 5
Fatty acid composition (% total fatty acids) of muscle from genetically selected for high growth and reference gilthead sea bream fed the experimental diets.

							Two-Way ANOVA (*p*-value)		
Fatty acids (% total FA)	HG-*C*	HG-*INS*	HG-*SCP*	REF-*C*	REF-*INS*	REF-*SCP*	Genotype	Diet	GxD
14:0	1.35 ± 0.22	0.95 ± 0.02	0.71 ± 0.06	1.40 ± 0.10	0.97 ± 0.09	0.64 ± 0.08	*n.s*	P = 0.000 C > INS > SCP	*n.s*
14:1n-7	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	*n.s*	P = 0.009 INS > SCP	*n.s*
14:1n-5	0.06 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.06 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	*n.s*	P = 0.000 C > INS,SCP	*n.s*
15:0	0.17 ± 0.02	0.12 ± 0.00	0.12 ± 0.01	0.17 ± 0.01	0.12 ± 0.00	0.11 ± 0.01	*n.s*	P = 0.000 C > INS,SCP	*n.s*
15:1n-5	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	*n.s*	*P* = 0.022 C > SCP	*n.s*
16:0ISO	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	*n.s*	P = 0.000 C > INS,SCP	*n.s*
16:0	12.34 ± 1.22	12.16 ± 0.42	12.08 ± 0.27	12.54 ± 0.31	12.30 ± 0.48	11.95 ± 0.73	*n.s*	*n.s*	*n.s*
16:1n-7	2.85 ± 0.27	2.29 ± 0.05	2.49 ± 0.19	2.89 ± 0.11	2.30 ± 0.12	2.36 ± 0.11	*n.s*	P = 0.000 C > INS,SCP	*n.s*
16:1n-5	0.08 ± 0.02	0.04 ± 0.01	0.04 ± 0.00	0.08 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	*n.s*	P = 0.000 C > INS,SCP	*n.s*
16:2n-6	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	*n.s*	*n.s*	*n.s*
16:2n-4	0.11 ± 0.00	0.05 ± 0.01	0.04 ± 0.00	0.11 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	*n.s*	P = 0.000 C > INS > SCP	*n.s*
17:0	0.08 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.07 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	*n.s*	P = 0.000 C > INS > SCP	*n.s*
16:3n-4	0.21 ± 0.01	0.13 ± 0.00	0.13 ± 0.01	0.21 ± 0.00	0.13 ± 0.00	0.12 ± 0.00	*P* = 0.046	P = 0.000 C > INS,SCP	*n.s*
16:3n-3	0.07 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.07 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	*n.s*	P = 0.000 C > INS,SCP	*n.s*
16:3n-1	0.04 ± 0.00	0.06 ± 0.02	0.06 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	*n.s*	*n.s*	*n.s*
16:4n-3	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.01	*n.s*	*n.s*	*n.s*
16:4n-1	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	*n.s*	*n.s*	*n.s*
18:0	3.07 ± 0.09	3.33 ± 0.15	3.54 ± 0.15	3.15 ± 0.18	3.46 ± 0.12	3.54 ± 0.09	*n.s*	P = 0.000 INS,SCP > C	*n.s*
18:1n-9	34.34 ± 0.72	36.05 ± 0.31	36.31 ± 1.00	34.25 ± 0.25	36.35 ± 0.14	36.65 ± 0.32	*n.s*	P = 0.000 INS,SCP > C	*n.s*
18:1n-7	2.38 ± 0.19	2.15 ± 0.20	2.26 ± 0.08	2.44 ± 0.05	2.15 ± 0.16	2.40 ± 0.01	*n.s*	P = 0.017 C > INS	*n.s*
18:1n-5	0.15 ± 0.01	0.07 ± 0.00	0.10 ± 0.00	0.16 ± 0.00	0.07 ± 0.00	0.09 ± 0.00	*n.s*	P = 0.000 C > SCP > INS	*n.s*
18:2n-9	0.31 ± 0.00	0.37 ± 0.05	0.30 ± 0.07	0.36 ± 0.03	0.31 ± 0.01	0.32 ± 0.02	*n.s*	*n.s*	*n.s*
18:2n-6	15.52 ± 0.11	18.58 ± 0.34	18.27 ± 0.22	15.34 ± 0.27	18.51 ± 0.41	18.34 ± 0.09	*n.s*	P = 0.000 INS,SCP > C	*n.s*
18:2n-4	0.06 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	*n.s*	P = 0.000 C > INS,SCP	*n.s*
18:3n-6	0.37 ± 0.01	0.47 ± 0.04	0.43 ± 0.09	0.40 ± 0.05	0.40 ± 0.02	0.41 ± 0.02	*n.s*	*n.s*	*n.s*
18:3n-4	0.09 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.09 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	*n.s*	P = 0.000 C > INS,SCP	*n.s*
18:3n-3	3.64 ± 0.05	3.76 ± 0.15	3.89 ± 0.14	3.55 ± 0.06	3.78 ± 0.04	3.97 ± 0.03	*n.s*	P = 0.000 SCP > INS > C	*n.s*
18:3n-1	0.01 ± 0.00^{ab}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{a}	*n.s*	*n.s*	*P* = 0.018
18:4n-3	0.84 ± 0.01	0.33 ± 0.01	0.28 ± 0.02	0.82 ± 0.06	0.31 ± 0.02	0.26 ± 0.01	*n.s*	P = 0.000 C > INS > SCP	*n.s*
18:4n-1	0.05 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	*n.s*	P = 0.000 C > SCP > INS	*n.s*
20:0	0.31 ± 0.03	0.33 ± 0.01	0.34 ± 0.01	0.32 ± 0.01	0.33 ± 0.02	0.36 ± 0.02	*n.s*	P = 0.009 SCP > C	*n.s*
20:1n-9	0.55 ± 0.03	0.18 ± 0.00	0.13 ± 0.01	0.53 ± 0.01	0.18 ± 0.02	0.14 ± 0.00	*n.s*	P = 0.000 C > INS > SCP	*n.s*
20:1n-7	3.24 ± 0.21	1.52 ± 0.08	1.43 ± 0.08	3.20 ± 0.02	1.60 ± 0.07	1.45 ± 0.06	*n.s*	P = 0.000 C > INS,SCP	*n.s*
20:1n-5	0.12 ± 0.01	0.08 ± 0.01	0.08 ± 0.00	0.12 ± 0.01	0.08 ± 0.01	0.08 ± 0.00	*n.s*	P = 0.000 C > INS,SCP	*n.s*
20:2n-9	0.33 ± 0.02	0.31 ± 0.04	0.31 ± 0.01	0.36 ± 0.03	0.33 ± 0.04	0.35 ± 0.02	*n.s*	*n.s*	*n.s*
20:2n-6	0.46 ± 0.03	0.45 ± 0.05	0.48 ± 0.02	0.46 ± 0.03	0.49 ± 0.01	0.47 ± 0.02	*n.s*	*n.s*	*n.s*
20:3n-9	0.02 ± 0.00^{a}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{b}	0.02 ± 0.00^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{ab}	*n.s*	P = 0.000	*P* = 0.004
20:3n-6	0.24 ± 0.02	0.30 ± 0.03	0.31 ± 0.02	0.25 ± 0.01	0.28 ± 0.01	0.32 ± 0.01	*n.s*	P = 0.000 INS,SCP > C	*n.s*
20:4n-6	0.32 ± 0.02	0.53 ± 0.05	0.55 ± 0.08	0.32 ± 0.01	0.50 ± 0.01	0.52 ± 0.02	*n.s*	P = 0.000 INS,SCP > C	*n.s*
20:3n-3	0.19 ± 0.01	0.18 ± 0.02	0.20 ± 0.01	0.19 ± 0.01	0.19 ± 0.00	0.19 ± 0.01	*n.s*	*n.s*	*n.s*
20:4n-3	0.61 ± 0.04	0.36 ± 0.03	0.34 ± 0.01	0.62 ± 0.02	0.35 ± 0.00	0.33 ± 0.01	*n.s*	P = 0.000 C > INS,SCP	*n.s*

(*continued on next page*)

Table 5 (*continued*)

Fatty acids (% total FA)	HG-*C*	HG-*INS*	HG-*SCP*	REF-*C*	REF-*INS*	REF-*SCP*	Two-Way ANOVA (*p*-value)		
							Genotype	Diet	GxD
20:5n-3	2.68 ± 0.20	2.38 ± 0.11	2.30 ± 0.12	2.63 ± 0.03	2.27 ± 0.02	2.26 ± 0.07	*n.s*	P = 0.000 C > INS,SCP	*n.s*
22:1n-11	3.30 ± 0.47	0.68 ± 0.05	0.44 ± 0.05	3.26 ± 0.03	0.68 ± 0.06	0.42 ± 0.02	*n.s*	P = 0.000 C > INS,SCP	*n.s*
22:1n-9	0.81 ± 0.13	0.50 ± 0.03	0.46 ± 0.00	0.81 ± 0.03	0.51 ± 0.03	0.50 ± 0.03	*n.s*	P = 0.000 C > INS,SCP	*n.s*
22:4n-6	0.11 ± 0.01	0.13 ± 0.02	0.14 ± 0.01	0.10 ± 0.01	0.13 ± 0.01	0.17 ± 0.02	*n.s*	P = 0.000 INS,SCP > C	*n.s*
22:5n-6	0.18 ± 0.03	0.45 ± 0.02	0.50 ± 0.07	0.18 ± 0.01	0.44 ± 0.01	0.50 ± 0.01	*n.s*	P = 0.000 SCP > INS > C	*n.s*
22:5n-3	1.16 ± 0.17	1.07 ± 0.12	1.14 ± 0.03	1.18 ± 0.06	1.13 ± 0.01	1.11 ± 0.09	*n.s*	*n.s*	*n.s*
22:6n-3	7.04 ± 1.07	9.29 ± 0.33	9.49 ± 1.27	7.01 ± 0.08	8.90 ± 0.16	9.23 ± 0.56	*n.s*	P = 0.000 INS,SCP > C	*n.s*
SFA	17.34 ± 1.51	16.94 ± 0.57	16.85 ± 0.17	17.67 ± 0.48	17.24 ± 0.63	16.66 ± 0.72	*n.s*	*n.s*	*n.s*
MUFA	47.94 ± 0.18	43.63 ± 0.36	43.79 ± 1.19	47.85 ± 0.15	44.02 ± 0.24	44.17 ± 0.38	*n.s*	P = 0.000 C > INS,SCP	*n.s*
N-9	36.37 ± 0.55	37.41 ± 0.36	37.53 ± 0.93	36.33 ± 0.29	37.68 ± 0.15	37.97 ± 0.33	*n.s*	P = 0.001 INS,SCP > C	*n.s*
N-6	17.20 ± 0.13	20.93 ± 0.34	20.68 ± 0.07	17.05 ± 0.32	20.75 ± 0.44	20.74 ± 0.12	*n.s*	P = 0.000 INS,SCP > C	*n.s*
N-3	16.28 ± 1.44	17.43 ± 0.27	17.70 ± 1.29	16.11 ± 0.14	16.99 ± 0.18	17.40 ± 0.73	*n.s*	*P* = 0.043 SCP > C	*n.s*
EPA/ARA	8.41 ± 0.04	4.45 ± 0.26	4.22 ± 0.35	8.25 ± 0.16	4.54 ± 0.07	4.37 ± 0.06	*n.s*	P = 0.000 C > INS,SCP	*n.s*
EPA/DHA	0.38 ± 0.03	0.26 ± 0.01	0.24 ± 0.02	0.38 ± 0.00	0.26 ± 0.00	0.24 ± 0.01	*n.s*	P = 0.003 C > INS,SCP	*n.s*
EPA + DHA	9.72 ± 1.26	11.67 ± 0.43	11.80 ± 1.39	9.64 ± 0.09	11.17 ± 0.18	11.49 ± 0.62	*n.s*	P = 0.003 INS,SCP > C	*n.s*
N-3 LC-PUFA	11.68 ± 1.47	13.27 ± 0.28	13.47 ± 1.41	11.64 ± 0.07	12.84 ± 0.17	13.12 ± 0.72	*n.s*	P = 0.017 INS,SCP > C	*n.s*

C: Control diet; INS: Insect meal diet; SCP: Single-cell protein; HG: high-growth genotype; REF: reference (non-selected) genotype. Values are expressed in mean ± SD. (n = 3 tanks/diet/genotype; 12 weeks of feeding). 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.

without consequences on fish performance or gut morphology (Karapanagiotidis et al., 2014; Magalhães et al., 2017; Di Rosa et al., 2023), while for other fish species like Atlantic salmon (*Salmo salar*), FM could be totally replaced by insect meal (Lock et al., 2018; Belghit et al., 2019). Replacement levels between 30 and 100% of the dietary FM in fish diets by *M. capsulatus* meal were also reported in different fish species, including in sea bream (Rhodes et al., 2015; Biswas et al., 2020; Xu et al., 2021). However, all of those studies were carried out with non-selected fish, and, to our knowledge, studies using these novel ingredients for fish selected lines are scarce. Indeed, a parallel study using selected gilthead sea bream of the present trial showed that selected fish have also higher activity of proteases, which consequently resulted in a higher apparent digestibility coefficient for all dietary amino acids compared to non-selected fish, which can partially explain the improved utilization of alternative diets with these novel ingredients by selected sea bream (Montero et al., 2023a, 2023b). Furthermore, in the mentioned study, the use of novel diets unaffected the digestibility coefficients of dietary protein and amino acids, supporting that the dietary inclusion and FM replacement levels used in the present trial do not compromise digestibility of the feeds, at least in which concerns the protein component of the diet. Therefore, if the observed lowering effect of novel diets in fish growth is caused by a lower palatability of these alternative raw materials, the addition of feed attractants are speculated to overcome the palatability issues and probably mitigate the negative consequences in fish feed intake and, ultimately, in growth. Further studies in this regard would thus be interesting to carry out to corroborate the potential of the novel raw materials used in the present study for genetically selected fish feeds. Furthermore, the lack of significant g x d interactions in fish productive key parameters also suggests that high-growth genotype gilthead sea bream will perform better than the non-selected genotype, irrespective of the nutritional background, in agreement with previous studies (Palti et al., 2006), and which further motivates for the adoption of more novel nutritional strategies.

In addition to the proven enhancing effects of selective breeding programs on fish productive performance and plasticity to deal with alternative feeds, they have been also used to select genotypes with desirable fillet traits, for improving fillet quality (Barrows et al., 2008; Gjedrem et al., 2009). Indeed, selection for high growth was reported to have both positive and negative impacts on fish fillet quality. For instance, selected for high growth fish genotypes were reported to have lower perivisceral fat, fillet fat, and/or improved texture properties, resulting in an improved quality of the final product for the consumers (Katsika et al., 2021; Montero et al., 2023a, 2023b). This is because selected genotypes may have optimized lipid utilization, absorption, and transport (Jin et al., 2020). However, the high growth rates of genetically selected fish can lead to increased water content and consequently affect fillet texture properties like hardness as well as flavor. In the present study, genetic selection did not have a positive nor a negative effect on fillet quality. This was denoted by the similar flesh proximate composition of both genetically selected sea bream and non-selected fish, as well as the similar texture attributes and sensorial perceptions scored by the evaluation panel. Accordingly, genetic selection did not affect whole-body and fillet fatty acid profiles, with both fish genotypes providing similar EPA + DHA levels to consumers. It is well recognized the role of n-3 LC-PUFA, particularly EPA, and DHA, in improving human health, by reducing the risk of cardiovascular diseases, chronic inflammation diseases, as well as neurodegenerative problems (Simopoulos, 2002; Kidd, 2007; Calder, 2010). Thus, the present results suggest the similar nutritional antiatherogenic and anti-thrombogenic benefits of consuming flesh from farmed fish, irrespective of its genetic background (Ulbricht and Southgate, 1991). On the contrary, dietary composition often strongly influences the composition of fish cells and

Table 6
Fatty acid composition (% total fatty acids) of liver from genetically selected for high growth and reference gilthead sea bream fed the experimental diets.

							Two-Way ANOVA (*p*-value)		
Fatty acids (% total FA)	HG-*C*	HG-*INS*	HG-*SCP*	REF-*C*	REF-*INS*	REF-*SCP*	Genotype	Diet	GxD
14:0	1.12 ± 0.12	0.91 ± 0.04	0.62 ± 0.08	1.19 ± 0.14	0.98 ± 0.08	0.68 ± 0.07	*n.s*	P = 0.000 C > INS > SCP	*n.s*
14:1n-7	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	*n.s*	P = 0.003 INS > C, SCP	*n.s*
14:1n-5	0.05 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.04 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	*n.s*	P = 0.000 C > INS, SCP	*n.s*
15:0	0.14 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.13 ± 0.02	0.10 ± 0.02	0.09 ± 0.01	*n.s*	P = 0.001 C > INS, SCP	*n.s*
15:1n-5	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	*n.s*	*n.s*	*n.s*
16:0ISO	0.03 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	*n.s*	P = 0.000 C > SCP > INS	*n.s*
16:0	12.95 ± 0.74	13.60 ± 0.12	12.48 ± 1.02	14.50 ± 1.99	13.60 ± 0.44	13.30 ± 0.53	*n.s*	*n.s*	*n.s*
16:1n-7	2.67 ± 0.22	2.26 ± 0.15	2.32 ± 0.12	2.97 ± 0.46	2.30 ± 0.16	2.36 ± 0.14	*n.s*	P = 0.004 C > INS,SCP	*n.s*
16:1n-5	0.10 ± 0.02	0.04 ± 0.00	0.05 ± 0.00	0.12 ± 0.03	0.04 ± 0.01	0.04 ± 0.00	*n.s*	P = 0.000 C > INS,SCP	*n.s*
16:2n-6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	*n.s*	*n.s*	*n.s*
16:2n-4	0.08 ± 0.01	0.03 ± 0.00	0.02 ± 0.00	0.07 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	*n.s*	P = 0.000 C > INS > SCP	*n.s*
17:0	0.06 ± 0.01	0.04 ± 0.01	0.03 ± 0.00	0.06 ± 0.00	0.04 ± 0.00	0.03 ± 0.01	*n.s*	P = 0.000 C > INS > SCP	*n.s*
16:3n-4	0.25 ± 0.02	0.15 ± 0.01	0.14 ± 0.01	0.24 ± 0.02	0.15 ± 0.01	0.13 ± 0.00	*n.s*	P = 0.000 C > INS, SCP	*n.s*
16:3n-3	0.07 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.07 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	*n.s*	P = 0.000 C > INS, SCP	*n.s*
16:3n-1	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	*P* = 0.023	*n.s*	*n.s*
16:4n-3	0.03 ± 0.00	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	P = 0.000	*n.s*	*n.s*
16:4n-1	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	*n.s*	*n.s*	*n.s*
18:0	4.20 ± 0.18	4.75 ± 0.16	4.70 ± 0.44	4.81 ± 0.44	4.55 ± 0.61	5.37 ± 0.11	*n.s*	*n.s*	*n.s*
18:1n-9	37.05 ± 1.14	39.34 ± 1.34	39.40 ± 1.67	38.01 ± 1.44	40.09 ± 1.53	40.97 ± 0.92	*n.s*	P = 0.012 INS,SCP > C	*n.s*
18:1n-7	2.49 ± 0.11	2.03 ± 0.11	2.20 ± 0.07	2.44 ± 0.20	2.08 ± 0.13	2.16 ± 0.09	*n.s*	P = 0.000 C > INS, SCP	*n.s*
18:1n-5	0.17 ± 0.01	0.07 ± 0.00	0.13 ± 0.00	0.16 ± 0.00	0.07 ± 0.00	0.13 ± 0.01	*n.s*	P = 0.000 C > SCP > INS	*n.s*
18:2n-9	0.60 ± 0.03	0.64 ± 0.06	0.59 ± 0.04	0.76 ± 0.24	0.57 ± 0.26	0.63 ± 0.03	*n.s*	*n.s*	*n.s*
18:2n-6	13.77 ± 0.16	16.57 ± 0.22	16.71 ± 0.93	12.52 ± 1.48	16.83 ± 1.81	15.21 ± 1.00	*n.s*	P = 0.000 INS,SCP > C	*n.s*
18:2n-4	0.06 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	0.02 ± 0.01	*n.s*	P = 0.000 C > INS, SCP	*n.s*
18:3n-6	0.80 ± 0.06	0.90 ± 0.08	0.91 ± 0.15	0.89 ± 0.25	0.79 ± 0.33	0.83 ± 0.07	*n.s*	*n.s*	*n.s*
18:3n-4	0.10 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	0.08 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	P = 0.036	P = 0.000 C > INS, SCP	*n.s*
18:3n-3	2.84 ± 0.14	2.90 ± 0.03	3.16 ± 0.11	2.49 ± 0.37	3.05 ± 0.56	2.92 ± 0.19	*n.s*	*n.s*	*n.s*
18:3n-1	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	*n.s*	*n.s*	*n.s*
18:4n-3	0.63 ± 0.05	0.29 ± 0.00	0.29 ± 0.03	0.58 ± 0.04	0.28 ± 0.02	0.25 ± 0.02	*P* = 0.042	P = 0.000 C > INS, SCP	*n.s*
18:4n-1	0.04 ± 0.01	0.02 ± 0.01	0.03 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	*n.s*	P = 0.000 C > INS, SCP	*n.s*
20:0	0.22 ± 0.03	0.21 ± 0.01	0.20 ± 0.02	0.20 ± 0.04	0.25 ± 0.09	0.23 ± 0.01	*n.s*	*n.s*	*n.s*
20:1n-9	0.72 ± 0.02[a]	0.16 ± 0.02[c]	0.12 ± 0.01[cd]	0.65 ± 0.04[b]	0.18 ± 0.02[c]	0.09 ± 0.01[d]	P = 0.017	P = 0.000 C > INS > SCP	*P* = 0.016
20:1n-7	2.53 ± 0.17	1.14 ± 0.02	1.13 ± 0.19	2.32 ± 0.37	1.37 ± 0.28	1.28 ± 0.20	*n.s*	P = 0.000 C > INS, SCP	*n.s*
20:1n-5	0.13 ± 0.00	0.08 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	*n.s*	P = 0.000 C > INS, SCP	*n.s*
20:2n-9	0.83 ± 0.05	0.59 ± 0.04	0.73 ± 0.13	0.94 ± 0.24	0.68 ± 0.30	0.94 ± 0.19	*n.s*	*n.s*	*n.s*
20:2n-6	0.56 ± 0.01	0.50 ± 0.02	0.55 ± 0.05	0.51 ± 0.10	0.55 ± 0.10	0.57 ± 0.05	*n.s*	*n.s*	*n.s*
20:3n-9	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.02	0.01 ± 0.00	*n.s*	*n.s*	*n.s*
20:3n-6	0.50 ± 0.03	0.44 ± 0.02	0.54 ± 0.04	0.47 ± 0.06	0.46 ± 0.19	0.56 ± 0.04	*n.s*	*n.s*	*n.s*
20:4n-6	0.42 ± 0.01	0.82 ± 0.14	0.84 ± 0.16	0.42 ± 0.05	0.62 ± 0.15	0.69 ± 0.11	*n.s*	P = 0.001 INS,SCP > C	*n.s*
20:3n-3	0.22 ± 0.00	0.20 ± 0.00	0.23 ± 0.01	0.20 ± 0.04	0.20 ± 0.04	0.22 ± 0.01	*n.s*	*n.s*	*n.s*
20:4n-3	0.68 ± 0.02	0.30 ± 0.01	0.32 ± 0.01	0.58 ± 0.10	0.32 ± 0.04	0.31 ± 0.02	*n.s*	P = 0.000 C > INS, SCP	*n.s*
20:5n-3	2.17 ± 0.26	1.85 ± 0.17	1.75 ± 0.19	1.99 ± 0.23	1.60 ± 0.09	1.59 ± 0.10	*P* = 0.044	P = 0.004 C > INS, SCP	*n.s*
22:1n-11	2.27 ± 0.28	0.35 ± 0.04	0.24 ± 0.05	1.91 ± 0.41	0.43 ± 0.19	0.19 ± 0.03	*n.s*	P = 0.000 C > INS, SCP	*n.s*
22:1n-9	0.94 ± 0.08	0.45 ± 0.02	0.44 ± 0.08	0.83 ± 0.14	0.51 ± 0.06	0.51 ± 0.10	*n.s*	P = 0.000 C > INS, SCP	*n.s*
22:4n-6	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.00	0.06 ± 0.02	0.08 ± 0.01	0.09 ± 0.01	*n.s*	*n.s*	*n.s*

(*continued on next page*)

Table 6 (*continued*)

Fatty acids (% total FA)	HG-*C*	HG-*INS*	HG-*SCP*	REF-*C*	REF-*INS*	REF-*SCP*	Two-Way ANOVA (*p*-value) Genotype	Diet	GxD
22:5n-6	0.14 ± 0.02	0.34 ± 0.05	0.36 ± 0.05	0.12 ± 0.04	0.29 ± 0.03	0.33 ± 0.02	*n.s*	P = 0.000 INS,SCP > C	*n.s*
22:5n-3	1.15 ± 0.11	0.77 ± 0.07	0.86 ± 0.04	0.91 ± 0.27	0.83 ± 0.12	0.80 ± 0.08	*n.s*	*P* = 0.025 C > INS	*n.s*
22:6n-3	6.06 ± 0.78	6.81 ± 1.13	7.45 ± 1.76	5.41 ± 1.33	5.75 ± 0.80	6.22 ± 0.60	*n.s*	*n.s*	*n.s*
SFA	18.73 ± 0.88	19.63 ± 0.25	18.15 ± 1.54	20.92 ± 2.37	19.53 ± 0.89	19.72 ± 0.70	*n.s*	*n.s*	*n.s*
MUFA	49.14 ± 0.83	45.98 ± 1.19	46.14 ± 2.15	49.61 ± 1.05	47.20 ± 1.34	47.85 ± 1.32	*n.s*	P = 0.009 C > INS, SCP	*n.s*
N-9	40.16 ± 1.19	41.20 ± 1.47	41.29 ± 1.84	41.21 ± 1.78	42.05 ± 1.91	43.14 ± 1.16	*n.s*	*n.s*	*n.s*
N-6	16.28 ± 0.19	19.65 ± 0.25	20.00 ± 1.19	15.00 ± 1.39	19.63 ± 1.27	18.28 ± 1.17	*n.s*	P = 0.000 INS,SCP > C	*n.s*
N-3	13.85 ± 1.34	13.18 ± 1.29	14.09 ± 2.11	12.25 ± 2.31	12.06 ± 1.30	12.32 ± 0.92	*n.s*	*n.s*	*n.s*
EPA/ARA	5.17 ± 0.51	2.28 ± 0.33	2.12 ± 0.22	4.75 ± 0.39	2.69 ± 0.67	2.32 ± 0.27	*n.s*	P = 0.000 C > INS, SCP	*n.s*
EPA/DHA	0.36 ± 0.01	0.27 ± 0.02	0.24 ± 0.03	0.38 ± 0.05	0.28 ± 0.02	0.26 ± 0.01	*n.s*	P = 0.000 C > INS, SCP	*n.s*
EPA + DHA	8.23 ± 1.04	8.66 ± 1.29	9.19 ± 1.95	7.40 ± 1.57	7.34 ± 0.89	7.80 ± 0.70	*n.s*	*n.s*	*n.s*
N-3 LC-PUFA	10.28 ± 1.16	9.93 ± 1.28	10.60 ± 1.98	9.09 ± 1.92	8.70 ± 1.08	9.12 ± 0.74	*n.s*	*n.s*	*n.s*

C: Control diet; INS: Insect meal diet; SCP: Single-cell protein; HG: high-growth genotype; REF: reference (non-selected) genotype. Values are expressed in mean ± SD. (n = 3 tanks/diet/genotype; 12 weeks of feeding). 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.

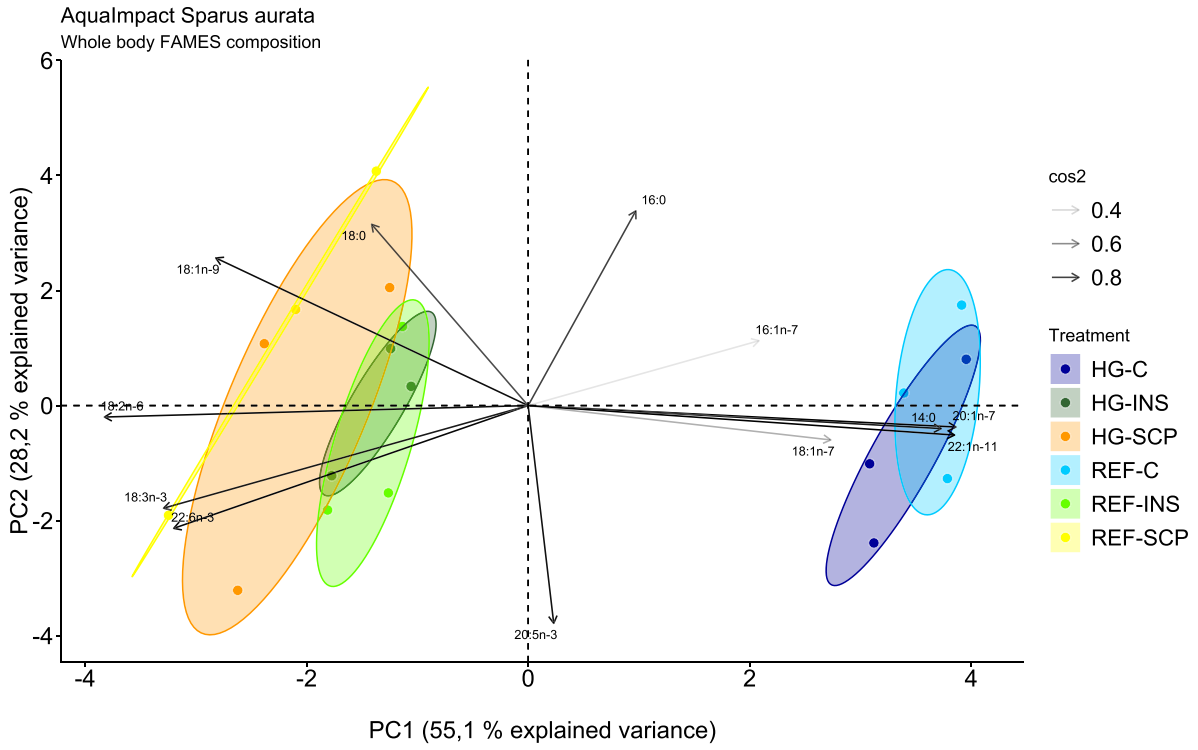

Fig. 1. PCA representing the variability in the whole-body fatty acid profile of high-growth genotype (HG) and reference genotype (REF) of gilthead sea bream fed the experimental diets. The percentage of total fatty acids is represented as cos2 function by an intensity scale, and confidence ellipses 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.

consequently, fish tissues, particularly in which concerns the lipid and fatty acid composition (Tocher, 2010). In agreement, in the present study, the fatty acid profiles of fish tissues were most influenced by the diet, clearly denoted by the very well separation in PCAs of fatty acid profiles of sea bream fed Control diet by one side, and those fed the novel diets with insect meal or single-cell protein and microalgae oil on the other. Besides FO, FM also contributes to the dietary n-3 LC-PUFA content and, for that reason, high replacement levels of FM by alternative protein sources that do not contain these fatty acids, often decrease the dietary levels (Tocher, 2015). This usually constrains the concomitant replacement of FM and FO in the diets because FO needs to supply the necessary contents of EPA and DHA for meeting fish requirements. In this sense, in all tissues, but particularly important in fillets for their value for consumers, fish fed the novel diets showed increased contents of n-3 LC-PUFA, mostly by their highest content in DHA. These results reflect the dietary inclusion of a DHA-rich microalgal oil in replacement of FO in these diets, supporting the concomitant replacement of FM and FO. Furthermore, the highest DHA contents of fish fillets caused by those

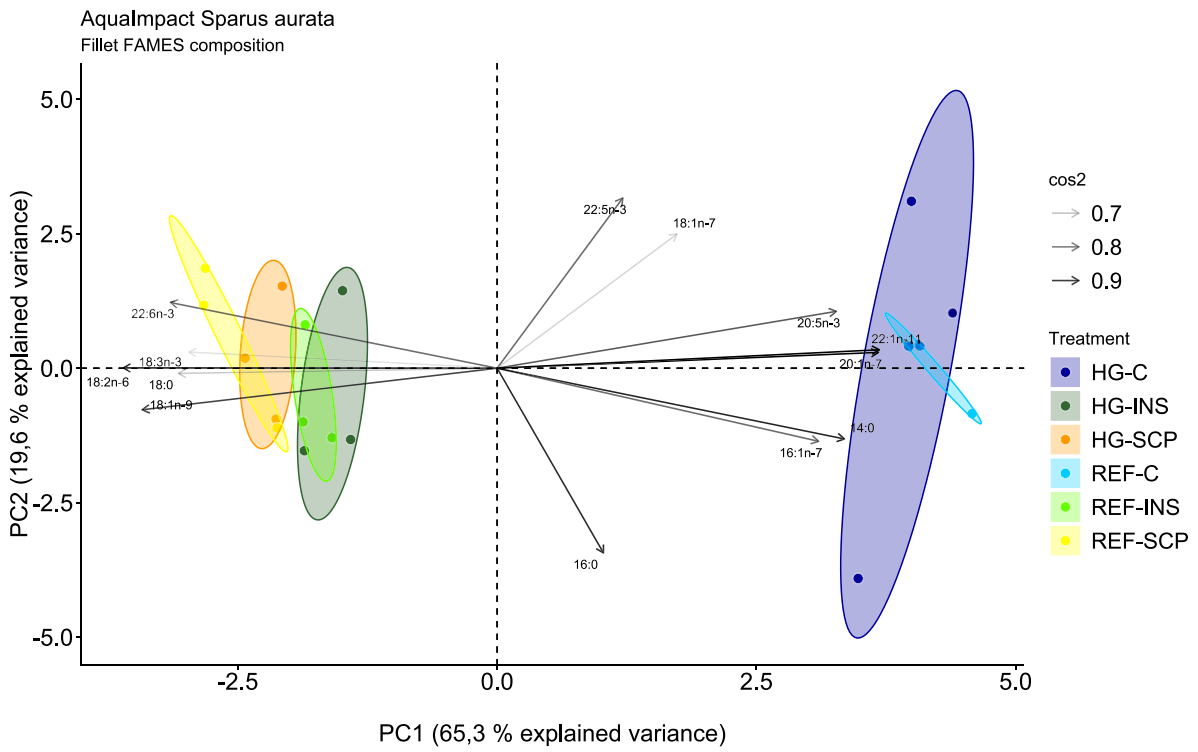

Fig. 2. PCA representing the variability in the flesh fatty acid profile of high-growth genotype (HG) and reference genotype (REF) of gilthead sea bream fed the experimental diets. The percentage of total fatty acids is represented as cos2 function by an intensity scale, and confidence ellipses are generated 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.

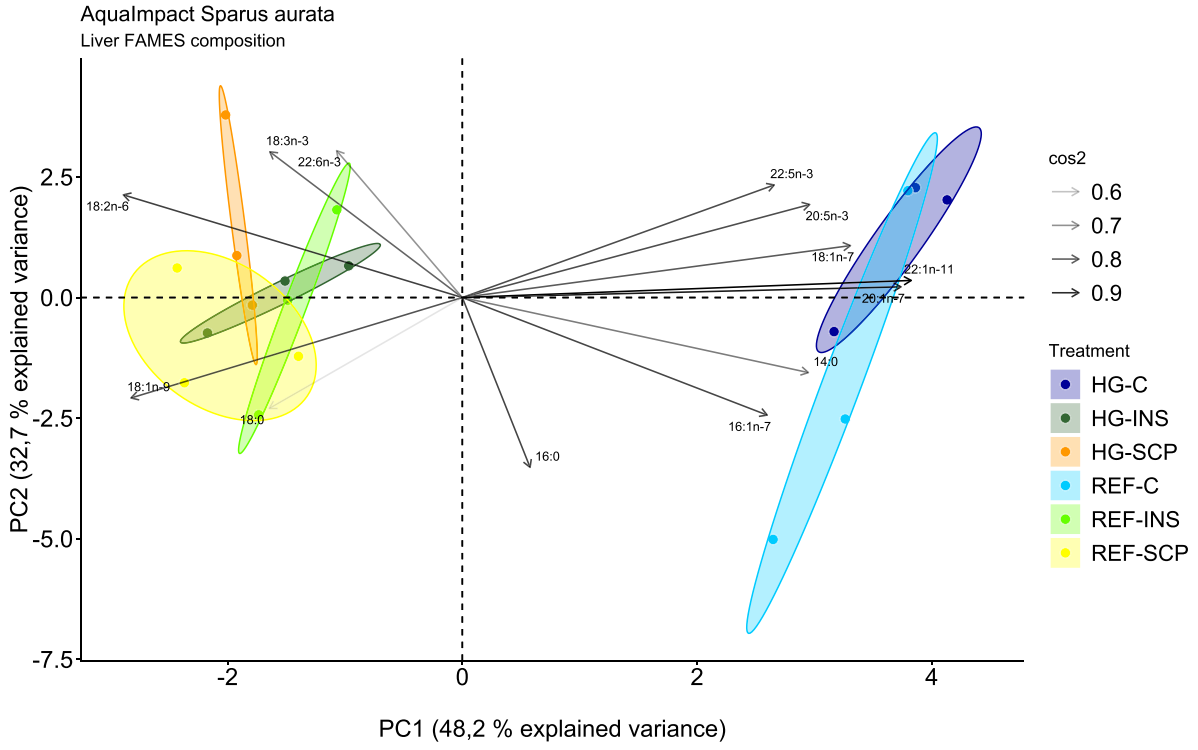

Fig. 3. PCA representing the variability in the liver fatty acid profile of high-growth genotype (HG) and reference genotype (REF) of gilthead sea bream fed the experimental diets. The percentage of total fatty acids is represented as cos2 function by an intensity scale, and confidence ellipses are generated 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 7
Texture characteristics of fillets from genetically selected for high growth and reference gilthead sea bream fed the experimental diets.

							Two-way ANOVA (*p*-value)				
	HG-*C*	HG-*INS*	HG-*SCP*	REF-*C*	REF-*INS*	REF-*SCP*	Genotype	Diet	Time	GxD	GxT; DxT; GxDxT
1 day post sacrifice											
Fracturability	90.55 ± 0.29	70.66 ± 2.49	93.54 ± 17.84	81.02 ± 9.50	85.56 ± 19.16	76.39 ± 0.57	*n.s*	*n.s*	*P* = 0.007	*n.s*	*n.s*
Hardness	75.34 ± 0.66	58.77 ± 2.75	77.03 ± 15.32	67.73 ± 7.22	71.13 ± 16.42	63.35 ± 0.15	*n.s*	*n.s*	P = 0.004	*n.s*	*n.s*
Elasticity	0.38 ± 0.04	0.39 ± 0.00	0.32 ± 0.04	0.39 ± 0.04	0.39 ± 0.06	0.29 ± 0.18	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*
Cohesivity	0.25 ± 0.02^{abc}	0.28 ± 0.01^{a}	0.23 ± 0.01^{abc}	0.25 ± 0.03^{abc}	0.26 ± 0.02^{bc}	0.26 ± 0.02^{bc}	*n.s*	*n.s*	P = 0.000	*n.s*	P = 0.028 (GxDxT)
Gumminess	18.66 ± 1.93	16.22 ± 0.46	17.23 ± 0.01	16.34 ± 0.43	18.18 ± 3.07	16.40 ± 0.80	*n.s*	*n.s*	P = 0.000	*n.s*	*n.s*
Chewiness	6.81 ± 1.05	6.35 ± 0.22	5.33 ± 0.05	6.19 ± 0.86	6.92 ± 0.12	5.11 ± 2.69	*n.s*	*n.s*	P = 0.000	*n.s*	*n.s*
Adhesiveness	−0.29 ± 0.09	−0.31 ± 0.02	−0.49 ± 0.10	−0.36 ± 0.05	−0.37 ± 0.14	−0.23 ± 0.07	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*
Resilience	0.12 ± 0.01	0.13 ± 0.00	0.11 ± 0.02	0.11 ± 0.02	0.13 ± 0.01	0.11 ± 0.01	*n.s*	*n.s*	P = 0.000	*n.s*	*n.s*
4 days post sacrifice											
Fracturability	86.76 ± 9.06	69.19 ± 5.27	58.99 ± 5.02	71.76 ± 7.84	70.17 ± 3.59	66.06 ± 7.48	–	–	–	–	–
Hardness	71.48 ± 8.62	55.76 ± 4.20	47.62 ± 4.91	58.31 ± 5.64	57.60 ± 1.60	53.64 ± 6.21	–	–	–	–	–
Elasticity	0.32 ± 0.03	0.32 ± 0.00	0.35 ± 0.02	0.34 ± 0.00	0.34 ± 0.01	0.33 ± 0.02	–	–	–	–	–
Cohesivity	0.23 ± 0.02^{abc}	0.20 ± 0.00^{c}	0.22 ± 0.00abc	0.21 ± 0.00^{bc}	0.23 ± 0.01^{abc}	0.21 ± 0.00^{bc}	–	–	–	–	–
Gumminess	16.06 ± 2.99	11.22 ± 0.65	10.43 ± 1.14	12.31 ± 0.73	13.25 ± 0.57	11.18 ± 1.11	–	–	–	–	–
Chewiness	5.01 ± 1.40	3.55 ± 0.10	3.64 ± 0.17	4.07 ± 0.15	4.55 ± 0.52	3.69 ± 0.67	–	–	–	–	–
Adhesiveness	−0.37 ± 0.03	−0.39 ± 0.07	−0.40 ± 0.07	−0.42 ± 0.13	−0.45 ± 0.13	−0.36 ± 0.10	–	–	–	–	–
Resilience	0.09 ± 0.01	0.08 ± 0.00	0.09 ± 0.00	0.08 ± 0.00	0.09 ± 0.01	0.08 ± 0.00	–	–	–	–	–

C: Control diet; INS: Insect meal diet; SCP: Single-cell protein; HG: high-growth genotype; REF: reference (non-selected) genotype. Values are expressed in mean ± SD. (n = 2 tanks/diet/genotype; 330 days of feeding). Two-way ANOVA, p < 0.05, Genotype and Diet as fixed factors. n.s = not significant.

Table 8
Sensory scores for quality attributes evaluated at the sensorial panel of fillets from genetically selected for high growth and reference gilthead sea bream fed the experimental diets.

								Two-Way ANOVA (*p*- value)		
		HG-*C*	HG-*INS*	HG-*SCP*	REF-*C*	REF-*INS*	REF-*SCP*	Genotype	Diet	GxD
Odor	Intensity	69.42 ± 16.25	71.67 ± 16.18	71.92 ± 16.16	71.33 ± 17.13	73.13 ± 17.47	69.38 ± 18.76	*n.s*	*n.s*	*n.s*
	Seafood	65.75 ± 17.57	62.83 ± 22.49	63.50 ± 18.73	63.42 ± 23.88	64.88 ± 18.60	64.88 ± 19.99	*n.s*	*n.s*	*n.s*
	Oily	31.00 ± 24.61	31.67 ± 25.86	31.25 ± 23.57	32.00 ± 26.95	33.00 ± 23.84	32.75 ± 25.21	*n.s*	*n.s*	*n.s*
Appearance	Whiteness	75.75 ± 12.78	76.33 ± 11.79	75.00 ± 12.03	78.75 ± 10.95	74.75 ± 13.14	77.75 ± 13.36	*n.s*	*n.s*	*n.s*
	Shininess	74.67 ± 14.80	74.08 ± 12.72	75.42 ± 12.98	75.50 ± 13.37	76.50 ± 11.40	78.00 ± 12.71	*n.s*	*n.s*	*n.s*
Texture	Firmness	63.58 ± 12.41	65.08 ± 15.57	66.42 ± 14.24	65.00 ± 13.31	69.25 ± 14.49	63.88 ± 22.81	*n.s*	*n.s*	*n.s*
	Juiciness	63.75 ± 13.66	63.08 ± 14.58	59.17 ± 13.27	62.00 ± 12.72	60.75 ± 17.45	63.63 ± 16.37	*n.s*	*n.s*	*n.s*
	Chewiness	55.67 ± 12.11	59.83 ± 14.55	61.00 ± 14.15	59.25 ± 15.39	61.88 ± 14.15	56.00 ± 22.98	*n.s*	*n.s*	*n.s*
	Adhesiveness	57.50 ± 14.11	61.33 ± 11.00	61.17 ± 12–14	60.83 ± 15.31	61.75 ± 12.02	61.75 ± 16.49	*n.s*	*n.s*	*n.s*
	Fatness	30.50 ± 18.46	33.92 ± 19.56	28.83 ± 16.65	32.50 ± 17.49	32.63 ± 15.74	34.25 ± 17.36	*n.s*	*n.s*	*n.s*
Flavor	Intensity	66.92 ± 12.34	66.42 ± 11.99	66.75 ± 20.37	67.17 ± 12.41	71.75 ± 14.06	69.13 ± 14.61	*n.s*	*n.s*	*n.s*
	Seafood	41.17 ± 28.57	43.17 ± 27.92	42.17 ± 30.24	42.25 ± 30.18	45.50 ± 29.15	44.25 ± 29.07	*n.s*	*n.s*	*n.s*
	Oily	20.58 ± 19.48	20.92 ± 20.54	21.42 ± 20.92	22.58 ± 20.76	22.25 ± 21.10	23.13 ± 20.01	*n.s*	*n.s*	*n.s*
Aftertaste	Persistence	46.42 ± 30.55	47.92 ± 27.30	42.92 ± 30.77	48.08 ± 30.03	48.38 ± 32.97	50.25 ± 28.18	*n.s*	*n.s*	*n.s*

C: Control diet; INS: Insect meal; SCP: Single-cell protein diet; HG: high-growth genotype; REF: reference (non-selected) genotype. Values are expressed in mean ± SD. (n = 2 tanks/diet/genotype; 330 days of feeding). Two-way ANOVA, p < 0.05, Genotype and Diet as fixed factors. n.s = not significant.

novel diets suppose a positive effect in terms of improving the nutritional value of fish final products to meet consumer needs and expectations, and supporting the concomitant replacement of FM and FO in fish feeds without affecting the dietary and fillet fatty acid profiles. However, despite this microalgal oil was also rich in EPA, its present dietary inclusions were not enough to increase EPA levels of fish fillets, as previously demonstrated in gilthead sea bream and Atlantic Salmon fed microalgae diets (Carvalho et al., 2020; Santigosa et al., 2023).

Texture and sensorial attributes of fish fillets depend on both endogenous factors including the composition of fillets and contents on lipid, collagen, or fatty acid profile (Grigorakis et al., 2003; Olafsdottir et al., 2004), as well as exogenous factors like storage condition or time of storage (Zhu et al., 2013; Sampels, 2015). For instance, the replacement of FO in the diets by vegetable oils can have a direct effect on the texture of the fillet since saturated fatty acids, like 16:0 or 18:0, are reported to increase texture attributes of fish fillets (Xu et al., 2016; Álvarez et al., 2020). Higher content of fat in the flesh has been also associated with a softer and juicier flavor, whereas low levels of fat in the fillet increase dryness or fibrousness (Grigorakis et al., 2003). Therefore, high replacement levels of FM by plant proteins, for instance,

Table 9
White and red muscle fibres (accumulated %/ fish weight) from genetically selected for high growth and reference gilthead sea bream fed the experimental diets.

							Two-way ANOVA (*p*-value)		
	HG-*C*	HG-*INS*	HG-SCP	REF-*C*	REF-*INS*	REF-*SCP*	Genotype	Diet	GxD
White muscle							*n.s*	*n.s*	*n.s*
$d \leq 20$ μm	0.02 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	*n.s*	*n.s*	*n.s*
$20 < d \leq 40$ μm	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.02	0.06 ± 0.02	0.05 ± 0.01	*n.s*	*n.s*	*n.s*
$40 < d \leq 60$ μm	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.01	0.04 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	*n.s*	*n.s*	*n.s*
$60 < d \leq 80$ μm	0.04 ± 0.01^{ab}	0.03 ± 0.02^{b}	0.03 ± 0.01^{ab}	0.04 ± 0.01^{ab}	0.05 ± 0.02^{a}	0.03 ± 0.00^{ab}	*n.s*	*n.s*	P = 0.02
$80 < d \leq 100$ μm	0.04 ± 0.02	0.04 ± 0.01^{b}	0.04 ± 0.01^{ab}	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.01	*n.s*	*n.s*	*n.s*
$d > 100$ μm	0.06 ± 0.03	0.06 ± 0.03	0.05 ± 0.02	0.07 ± 0.03	0.07 ± 0.03	0.06 ± 0.02	P = 0.04	*n.s*	*n.s*
Red muscle									
$d \leq 20$ μm	0.03 ± 0.01^{ab}	0.05 ± 0.03^{a}	0.04 ± 0.02^{ab}	0.04 ± 0.02^{ab}	0.03 ± 0.02^{ab}	0.02 ± 0.01^{b}	*n.s*	*n.s*	P = 0.03
$20 < d \leq 30$ μm	0.07 ± 0.03	0.07 ± 0.03	0.05 ± 0.02	0.06 ± 0.03	0.07 ± 0.03	0.05 ± 0.02	*n.s*	P = 0.03 INS > SCP	*n.s*
$30 < d \leq 40$ μm	0.05 ± 0.02	0.06 ± 0.02	0.06 ± 0.01	0.06 ± 0.03	0.08 ± 0.03	0.05 ± 0.02	*n.s*	*n.s*	*n.s*
$40 < d \leq 50$ μm	0.06 ± 0.02^{b}	0.04 ± 0.01^{b}	0.05 ± 0.02^{b}	0.05 ± 0.02^{b}	0.09 ± 0.03^{a}	0.06 ± 0.02^{b}	n.s	*n.s*	P = 0.00
$50 < d \leq 60$ μm	0.02 ± 0.02	0.01 ± 0.02	0.02 ± 0.01	0.02 ± 0.03	0.02 ± 0.02	0.02 ± 0.01	*n.s*	*n.s*	n.s

C: Control diet; INS: Insect meal diet; SCP: Single-cell protein; HG: high-growth genotype; REF: reference (non-selected) genotype. Values are expressed in mean ± SD. (n = 2 tanks/diet/genotype; 330 days of feeding). 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.

are known to derive in lower acceptance of fish products by the consumers, probably related with a lower content of fat or fatty acids, and collagen (Fountoulaki et al., 2009). Despite the effects of the novel formulations tested in this study on fillet fatty acid composition, including a higher content of 18:0 in fillets , no effect of the diet on texture properties nor in the sensorial attributes of fish fillets was observed. This was probably because the total fat content of fish muscle was not affected, and therefore, fillets presented similar quality attributes. In addition, the distribution and composition of muscle fibres can influence parameters related to fillet quality, such as texture (Johnston et al., 2000; Rincón et al., 2016). For instance, there is a correlation between the transverse diameter of muscle fibres and texture, where a higher density of fibres from small diameter increases muscle firmness (Hurling et al., 1996; Periago et al., 2005). In the present study, non-selected fish showed higher density of muscular fibres from higher diameter ($d > 100$ μm) in white muscle, compared to the selected genotype. Fish from HG genotype and fed the INS diet also showed higher density of muscular fibres from small diameter ($d \leq 20$ μm) in red muscle compared with non-selected sea bream fed SCP diet, whereas non-selected fish fed INS diet showed the highest density of red fibres from high diameter ($40 < d \leq 50$ μm). Despite these results, that could have potentially resulted in differences in fillet texture, there was no clear correlations between the recruitment of muscle fibres and the textural or sensorial parameters. As expected, time postmortem was the only factor that affected fillet texture, decreasing almost all attributes of fillet texture properties after 4 days postmortem, in agreement with the proteolytic degradation of muscle fibres caused by cathepsins, calpains, and metalloproteins (Kubota et al., 2001; Chéret et al., 2007; Caballero et al., 2009). Therefore, the present results suggest that the partial replacement of the dietary FM by this insect meal or bacterial single-cell protein products, as well as the simultaneous replacement of FO by a combination of poultry oil and a DHA-rich microalgal oil, is not likely to affect the quality of fish fillets for consumers, supporting the use of novel dietary formulations in aquafeeds to meet consumers demand and expectations of aquatic products.

5. Conclusions

The results of the present study confirm the success of the selective breeding program PROGENSA® applied to gilthead sea bream for 3 generations in improving the productive performance of fish, denoted by the better growth and feed utilization of the selected genotype compared to non-selected fish, at any of the diets assayed. INS and SCP novel diets slightly reduced general performance of fish by reducing feed intake. However, selected fish fed novel diets (INS but particularly SCP) showed very similar growth and lower feed conversion ratio compared with non-selected fish fed a control diet based on FM and FO, suggesting that genetic selection for high growth in gilthead sea bream improved fish utilization of novel dietary formulations with high replacement levels of FM and FO by emergent ingredients (insect meal, single-cell protein meal and microalgal oil). Furthermore, these novel formulations increased n-3 LC-PUFA in fish tissues, particularly DHA, irrespective of the genotype, as a result of the dietary inclusion of the DHA-rich microalgal oil in replacement of FO, which might suppose a positive effect in terms of meeting consumer needs and expectations. However, neither genetic selection nor the use of the tested novel ingredientst affected fillet proximate composition and, consequently, sea bream fillet quality in terms of texture and sensorial perception of consumers.

Overall, the results reaffirm the positive effects of genetic selection in improving sea bream productive key indicators, as well as support the effective utilization of novel dietary formulations by selected sea bream, using insect meal from *H. illucens,* single-cell protein from *M. capsulatus* as partial replacers of FM , at 33 and 66% of dietary replacement levels, respectively.

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CRediT authorship contribution statement

Marta Carvalho: Formal analysis, Investigation, Writing – original draft. **Rafael Ginés:** Writing – review & editing, Investigation, Formal analysis, Resources. **Ignacio Martín:** Investigation, Formal analysis. **María Jesús Zamorano:** Resources, Investigation, Writing – review & editing. **Félix Acosta:** Resources, Investigation, Writing – review & editing. **Ramon Fontanillas:** Resources, Writing – review & editing. **Silvia Torrecillas:** Conceptualization, Investigation, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Daniel Montero:** Conceptualization, Formal analysis, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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Appendix A. Supplementary data

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