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RESEARCH ARTICLE

Effect of different inclusion levels of defatted *Hermetia illucens* larvae meal on fillet quality of gilthead sea bream (*Sparus aurata*)

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Abstract

In recent years, insect meal has attracted increasing interest as an innovative protein source to replace fish meal in feed formulation due to its valuable nutritional profile. This research aimed to compare the effects of different dietary inclusion levels (5, 10, and 15%) of *Hermetia illucens* (HI) larvae meal on *Sparus aurata* (initial weight: 98.6 ± 0.6 g) sensorial, technological, and nutritional fillets quality. Fish were fed experimental diets over 113 days. Results showed that the inclusion of defatted HI larvae meal did not induce off-flavours in gilthead sea bream fillets. No significant differences were found in appearance, mouthfeels, and texture, while a difference emerged in the trait 'cooked chicken breast' for odour and flavour characteristics. Moreover, fillets' quality traits and proximate composition analyses performed did not show significant differences between the treatments. The fillets' fatty acid content showed that higher inclusion of HI meal leads to higher saturated fatty acids content, while no significant difference in polyunsaturated fatty acids was observed among treatments. Results have a positive implication as dietary HI did not negatively affect the fatty acids composition or quality of sea bream fillets.

Keywords

sensory profile – fillet technological quality – insect meal – fish meal substitution – fillet nutritional quality

1 Introduction

In recent decades, the demand for sustainably produced proteins for human consumption has grown so considerably that the current protein production would have to double by 2050. This poses a huge challenge considering that the European Union (EU) still has a deficit for high-quality protein materials (30-50%). Consequently, the great protein demand is now largely met by imported proteins with severe concerns regarding feed and food security and the general competitiveness of the EU (FEFAC, 2018).

Considering the increasing standard of living and the fast growth of the world population, there is a rising demand for seafood (Alfiko *et al.*, 2022). Today the aquaculture industry plays a key role as a world-wide supplier of high-protein quality products, and farmed fish products are expected to rise from 114.5 million tons in 2018

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to 201 million tons by 2030 (FAO, 2020). Fish nutritional requirements, particularly for carnivorous fish, are quite high in terms of quality and quantity of protein. For this reason, fish meal (FM) has been traditionally considered the best protein source in feed formulation (Kok *et al.*, 2020). However, FM is a limited available product (Hidalgo *et al.*, 2022), and finding alternative protein sources that are sustainable, circular, and environmentally friendly, needs to be urgently addressed (Colombo *et al.*, 2022). Promising alternative protein sources, such as insect meal, are already eyeing market adoption. Among the positive aspects of insect meal utilisation, its application as a sustainable aquafeed ingredient is a very interesting topic, especially for its high nutritional value. As such, insects present a high protein content that varies according to the species from 25 to 75% (Colombo *et al.*, 2022). They have valid amino acids (AAs) profiles in good correlation with fish dietary requirements (Henry *et al.*, 2015; NRC, 2011). Additionally, insects are rich in lipids, vitamins (e.g. pyridoxine, riboflavin, folic acid, and vitamin B12), and minerals (potassium, calcium, iron, magnesium, zinc, and selenium) (Henry *et al.*, 2015).

In particular, *Hermetia illucens* (HI) has attracted increasing interest both as an alternative protein source to replace FM and as an ingredient with an excellent nutritional profile. It contains about 35-46% (DM) protein with an essential AAs profile similar to that of FM with the exception of lysine which may be deficient in some insect species (Fisher *et al.*, 2020). HI lipid content ranges between 15-49%, depending on the larvae's diet. Specifically, some studies have shown that by altering insect larval feed intake, the fatty acids (FAs) profile of larvae can be manipulated (Barroso *et al.*, 2017; Ewald *et al.*, 2020; Liu *et al.*, 2017). Larval age may also have an important effect, with an increase in saturated fatty acids (SFAs) and a decrease in unsaturated fatty acids in older larvae (Liu *et al.*, 2017). Also, the HI larvae lipid content varies whether the defatting process was done or not (Huyben *et al.*, 2019).

HI meal has been recently utilised as a protein source in fish feed with the purpose of investigating its potential effects on growth performance, feed utilisation efficiency, fish welfare, and health (Abdel-Latif *et al.*, 2021; Abu Bakar *et al.*, 2021; Bruni *et al.*, 2020a, 2020b; Caimi *et al.*, 2020a,b, 2021; Xu *et al.*, 2020). Sensory analysis and more specifically Quantitative Descriptive Analysis (QDA) is a valid method for providing information on the sensory properties of food. Several studies utilised QDA in order to investigate relationships between fish flavour, fillet texture, and fillet nutritional profile (Izquierdo *et al.*, 2005). Studies conducted on Atlantic salmon (*Salmo salar*) and Rainbow trout fillets (*Oncorhynchus mykiss*) did not show sensory significant differences between diets despite a high percentage of HI inclusion in substitution of FM (Lock *et al.*, 2016; St-Hilaire *et al.*, 2007).

Furthermore, it should be considered that diet ingredients influence the fillet technological properties, with special reference to muscle pH which plays a crucial role in assuring a proper level of water holding capacity (WHC) to fish fillets (Iaconisi *et al.*, 2018).

In this study, sea bream (*Sparus aurata*) was chosen because is the species of major interest in Mediterranean aquaculture. Although several studies have been conducted on sea bream fed diets containing HI in substitution of FM (Bosi *et al.*, 2021; Carvalho *et al.*, 2023; Fabrikov *et al.*, 2021, 2020; Mastoraki *et al.*, 2022; Oteri *et al.*, 2022; Panteli *et al.*, 2021; Pulido *et al.*, 2022), to the best of our knowledge, only a few studies tested low and moderate HI inclusion (5-15%) (Carvalho *et al.*, 2023; Oteri *et al.*, 2022), and no studies investigated on nutritional, technological, and sensory fillet quality at the same time. The present study aimed to assess the effects of different inclusion levels of HI larvae meal on the nutritional, technological, and sensory quality of sea bream fillets.

2 Materials and methods

Experimental diets

Four experimental diets were produced via extrusion technology by Sparos Lda (Olhão, Portugal) with a different inclusion level of HI larvae meal (0% CTRL, 5% HI5, 10% HI10, and 15% HI15) in substitution for FM on a protein basis. All powder ingredients were mixed accordingly to the target formulation in a double-helix mixer (model 500 L, TGC Extrusion, Roullet-Saint-Estèphe, France) and ground (below 400 μm) in a micro pulveriser hammer mill (model SH1, Hosokawa-Alpine, Augsburg, Germany). Diets (pellet size: 4.5 mm) were manufactured with a twin-screw extruder (model BC45, Clextral, Firminy, France). Extrusion conditions: feeder rate (78-83 kg/h), screw speed (256-267 rpm), water addition (340 ml/min), temperature barrel 1 (34-37 °C), temperature barrel 3 (109-114 °C). Extruded pellets were dried in a vibrating fluid bed dryer (model DR100, TGC Extrusion) for a target moisture level of approximately 8%. After cooling, oils were added by vacuum coating (model PG-10VCLAB, Dinnissen, Sevenum, the Netherlands). Coating conditions: pressure (700 mbar); spray-

TABLE 1 Ingredients and proximate composition of the four experimental diets provided to sea bream (*Sparus aurata*) over 113 days.[1]

Ingredients (%)	Experimental diets			
	CTRL	HI5	HI10	HI15
FM[2]	22.0	18.1	14.1	10.1
Hermetia illucens meal[3]	0.00	5.01	10.0	15.0
Wheat flour	9.82	8.47	7.12	5.79
Wheat gluten meal	3.07	3.08	3.08	3.09
Soybean meal	11.4	11.5	11.5	11.5
Maize gluten meal	26.4	26.4	26.5	26.5
Soy protein concentrate	13.2	13.2	13.2	13.3
Rapeseed oil	7.52	6.89	6.37	5.76
Fish oil	3.22	3.71	4.09	4.56
DL-methionine	0.26	0.30	0.33	0.36
HCl lysine	0.26	0.33	0.41	0.50
Taurine	0.22	0.24	0.26	0.27
Monammonium phosphate	0.79	1.01	1.24	1.43
Vitamin C	0.07	0.07	0.07	0.07
Premix vitamins and minerals[4]	0.69	0.69	0.69	0.69
Hydrolysed shrimp protein (liquid)	1.03	1.03	1.03	1.03
Proximate composition (%)				
Moisture	7.29	7.62	7.40	7.13
Crude protein	51.1	51.5	51.6	53.1
Crude fat	13.5	14.4	13.6	13.6
Ash	6.44	6.37	6.28	6.17
Crude fibre	1.95	4.31	2.56	4.80
NFE[5]	19.72	15.80	18.56	15.20
Crude energy in feed (cal/g)	5249.3	5227.9	5206.5	5186.5

1 Three diets contained different inclusion levels of *Hermetia illucens* (HI) larvae meal in substitution of fish meal (FM). CTRL = control diet; HI5 = 5% of HI meal; HI10 = 10% of HI; HI15 = 15% of HI meal.

2 Protein content in FM: 66%.

3 Origin: Mutatec (France). Proximate composition (g/100 g): proteins 55, fibres 10, lipids 10, saturated fatty acids 6 (lauric acid 40.1%, palmitic acid 15.1%, myristic acid 8.5%), monounsaturated fatty acids 2 (oleic acid 13.5%), polyunsaturated fatty acids 2 (linoleic acid 12.8%), ash 11, gross energy (KJ/100 g) 2041.

4 Vitamins and mineral premix (mg/kg diet, *in vivo* NSA: Portugal): vitamin D 0.05 mg, vitamin A 2.38 mg, vitamin E 324.68 mg, inositol 158.40 mg, niacin 182.17 mg, pantothenic acid 67.69 mg, vitamin B2 27.44 mg, vitamin B1 27.44 mg, vitamin B6 24.27 mg, folic acid 6.52 mg, vitamin K 5.39 mg, biotin 0.96 mg, vitamin B12 0.05 mg, choline 1314.58 mg, vitamin C 250.25 mg, calcium 0.87 mg, cobalt 0.38 mg, copper 48.62 mg, iron 494.70 mg, magnesium 21.27 mg, manganese 25.89 mg, molybdate 0.97 mg, nickel 0.80 mg, phosphorus 0.51 mg, potassium 0.83 mg, sodium 0.14 mg, selenium 0.83 mg, sulphur 0.35 mg, zinc 52.67 mg.

5 NFE (nitrogen free extracts) (%) = 100% – (moisture + protein + lipid + ash).

ing time under vacuum (approximately 90 seconds), return to atmospheric pressure (120 s). Immediately after coating, diets were packed in sealed.

Insect meal was produced from black soldier fly larvae reared on organic substrates by the company Mutatec (Châteaurenard-Provence, France). Diets' ingredients and their proximate composition are shown in Table 1. The composition by weight of the FAs contained in the experimental diets is shown in Table 2. Moisture content was gained by weight loss after drying samples in an oven at 105 °C overnight. Crude protein was detected as total nitrogen (N*6.25) using Kjeldahl's method in accordance with AOAC International (2010). Total lipids were obtained following the Bligh and Dyer's (1959) extraction method. Ash content was estimated by incineration in a muffle oven at 450 °C overnight.

TABLE 2 List of fatty acids composition of the control diet and the three diets containing different inclusion levels of *Hermetia illucens* (HI) larvae meal.[1]

FAs (g/100 g)[2]	Experimental diets			
	CTRL	HI5	HI10	HI15
10:0	0.0010 ± 0.00	0.006 ± 0.001	0.010 ± 0.002	0.014 ± 0.003
12:0	0.032 ± 0.006	0.083 ± 0.018	0.154 ± 0.033	0.011 ± 0.002
14:0	0.345 ± 0.053	0.412 ± 0.059	0.458 ± 0.064	0.523 ± 0.070
15:0	0.023 ± 0.004	0.024 ± 0.005	0.026 ± 0.005	0.035 ± 0.007
16:0	1.53 ± 0.18	1.67 ± 0.20	1.70 ± 0.20	1.82 ± 0.22
17:0	0.027 ± 0.005	0.030 ± 0.006	0.023 ± 0.006	0.031 ± 0.006
18:0	0.332 ± 0.052	0.363 ± 0.055	0.353 ± 0.054	0.373 ± 0.056
20:0	0.053 ± 0.011	0.065 ± 0.014	0.060 ± 0.013	0.058 ± 0.012
22:0	0.018 ± 0.004	0.026 ± 0.005	0.018 ± 0.004	0.021 ± 0.005
24:0	0.007 ± 0.001	0.014 ± 0.003	0.010 ± 0.002	0.009 ± 0.002
SFAs total	2.41 ± 0.20	2.75 ± 0.22	2.88 ± 0.22	2.96 ± 0.24
16:1 total	0.42 ± 0.84	0.49 ± 0.42	0.51 ± 0.42	0.52 ± 0.42
16:1t n-7	0.01 ± 0.42	0.01 ± 0.42	0.01 ± 0.42	0.01 ± 0.42
16:1 n-7	0.413 ± 0.059	0.479 ± 0.066	0.500 ± 0.068	0.508 ± 0.068
17: 1 total	0.008 ± 0.001	0.008 ± 0.001	0.016 ± 0.002	0.016 ± 0.003
17:1 n-7	0.008 ± 0.002	0.008 ± 0.002	0.009 ± 0.002	0.010 ± 0.002
18:1 total	5.22 ± 0.45	5.38 ± 0.46	4.90 ± 0.43	4.56 ± 0.40
18:1t n-9	0.017 ± 0.004	0.0010 ± 0.00	0.019 ± 0.004	0.025 ± 0.005
18:1t n-8	0.0010 ± 0.00	0.019 ± 0.004	0.0010 ± 0.00	0.0010 ± 0.00
18:1t n-7	0.0010 ± 0.00	0.057 ± 0.012	0.060 ± 0.013	0.068 ± 0.014
18:1 n-9	4.76 ± 0.44	4.90 ± 0.45	4.46 ± 0.42	4.12 ± 0.39
18:1 n-7	0.373 ± 0.056	0.394 ± 0.058	0.361 ± 0.055	0.348 ± 0.054
18:1 n-6	0.012 ± 0.002	0.013 ± 0.003	0.004 ± 0.001	0.0010 ± 0.00
20:1 total	0.136 ± 0.029	0.149 ± 0.032	0.132 ± 0.028	0.133 ± 0.028
20:1 n-9	0.136 ± 0.029	0.149 ± 0.032	0.132 ± 0.028	0.133 ± 0.028
22:1 total	0.047 ± 0.007	0.059 ± 0.009	0.050 ± 0.007	0.040 ± 0.006
22:1 n-11	0.022 ± 0.005	0.027 ± 0.006	0.026 ± 0.005	0.016 ± 0.003
22:1 n-9	0.026 ± 0.005	0.032 ± 0.006	0.024 ± 0.005	0.025 ± 0.005
24:1 n-9	0.019 ± 0.004	0.030 ± 0.006	0.016 ± 0.003	0.024 ± 0.005
MUFAs total	5.85 ± 0.61	6.12 ± 0.62	5.63 ± 0.60	5.30 ± 0.58
18:2 total	2.28 ± 0.25	2.39 ± 0.26	2.24 ± 0.25	2.13 ± 0.24
18:2c,t n-6	0.013 ± 0003	0.016 ± 0.003	0.015 ± 0.003	0.016 ± 0.003
18:2 n-6	2.26 ± 0.25	2.37 ± 0.26	2.22 ± 0.25	2.11 ± 0.24
20:2 n-6	0.015 ± 0.003	0.016 ± 0.003	0.017 ± 0.003	0.016 ± 0.003
18:3 total	0.714 ± 0.088	0.735 ± 0.090	0.655 ± 0.083	0.625 ± 0.077
18:3t,c,c n-3	0.012 ± 0.003	0.015 ± 0.003	0.014 ± 0.003	0.008 ± 0.002
18:3 n-6	0.007 ± 0.001	0.0010 ± 0.00	0.0010 ± 0.00	0.010 ± 0.002
18:3c,c,t n-3	0.0010 ± 0.00	0.0010 ± 0.00	0.0010 ± 0.00	0.019 ± 0.004
18:3 n-	0.695 ± 0.088	0.711 ± 0.090	0.641 ± 0.083	0.587 ± 0.077
20:3 total	0.011 ± 0.002	0.013 ± 0.002	0.010 ± 0.002	0.011 ± 0.002
18:4 n-3	0.097 ± 0.021	0.109 ± 0.023	0.108 ± 0.023	0.107 ± 0.023
20:4 total	0.054 ± 0.012	0.060 ± 0.013	0.057 ± 0.012	0.053 ± 0.011
20:4 n-6 (ARA)	0.054 ± 0.012	0.060 ± 0.013	0.057 ± 0.012	0.053 ± 0.011
20:5 n-3(EPA)	0.696 ± 0.088	0.83 ± 0.11	0.783 ± 0.098	0.807 ± 0.100
22:5 total	0.080 ± 0.015	0.100 ± 0.019	0.082 ± 0.016	0.083 ± 0.017

TABLE 2 (Continued.)

FAs (g/100 g)[2]	Experimental diets			
	CTRL	HI5	HI10	HI15
22:5 n6	0.015 ± 0.003	0.017 ± 0.004	0.012 ± 0.003	0.008 ± 0.002
22:5 n3	0.065 ± 0.014	0.083 ± 0.018	0.069 ± 0.015	0.074 ± 0.016
22:6 n3(DHA)	0.389 ± 0.057	0.456 ± 0.064	0.354 ± 0.054	0.355 ± 0.054
PUFAs > C20	0.475 ± 0.059	0.566 ± 0.067	0.440 ± 0.057	0.448 ± 0.057
PUFAs total	4.34 ± 0.29	4.72 ± 0.31	4.31 ± 0.29	4.20 ± 0.28
Total n-3	1.96 ± 0.14	2.22 ± 0.16	1.98 ± 0.14	1.97 ± 0.14
Total n-6	2.39 ± 0.25	2.52 ± 0.26	2.34 ± 0.25	2.23 ± 0.24
n-3/n-6	0.82 ± 0.10	0.88 ± 0.11	0.85 ± 0.11	0.88 ± 0.12
PUFAs/MUFAs	0.742 ± 0.092	0.771 ± 0.093	0.766 ± 0.097	0.79 ± 0.10
PUFAs/SFAs	1.80 ± 0.19	1.72 ± 0.18	1.50 ± 0.16	1.42 ± 0.15

1 Three diets contained different inclusion levels of *Hermetia illucens* (HI) larvae meal in substitution of fish meal. CTRL = control diet; HI5 = 5% of HI meal; HI10 = 10% of HI; HI15 = 15% of HI meal.

2 FAs = fatty acids; SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; ARA = arachidonic acid.

Crude energy was measured by a calorimetric bomb (Adiabatic Calorimetric Bomb Parr 1261; PARR Instrument, Moline, IL, USA). The crude fibre was determined according to EU Regulation EC 152/09 (EC, 2009). Fatty analysis composition of diets was performed according to ISO16958:2015 (ISO, 2015).

Fish, feeding trial, and sampling

The experiment was carried out at the Laboratory of Aquaculture, Department of Veterinary Medical Sciences of the University of Bologna, Cesenatico, Italy. Sea bream specimens were obtained from Panittica Italia (Torre Canne di Fasano, Brindisi, Italy). At the beginning of the trial, 50 fish (initial weight: 98.6 ± 0.6 g) per tank were randomly distributed into 12,450 l square tanks. Experimental diets were assigned randomly and administered by hand to triplicate groups to visual satiation twice a day (8:30 and 16:00) for 6 days a week over 113 days. Tanks were provided with natural seawater and connected to a closed recirculation system (RAS) (overall water volume: 6 m^3. Oxygen level 8.0 ± 1.0 mg/l; temperature 24 ± 1.0 °C, salinity 25 g/l, artificial photoperiod of 12 h light and 12 h dark.). The oxygen level was kept constant through a liquid oxygen system regulated by a software program (B&G Sinergia snc, Chioggia, Italy). Ammonia (total ammonia nitrogen ≤0.1 mg/l) and nitrite (≤0.2 mg/l) were daily monitored spectrophotometrically (Spectroquant Nova 60, Merck, Darmstadt, Germany). Salinity was measured by a salt refractometer (106 ATC, Giorgio Bormac S.r.l., Carpi, Italy), and sodium bicarbonate was added daily to keep pH at 7.8-8.2. (Pelusio *et al.*, 2021). At the end of the trial a total of 48 sea bream (4 fish/tank) for technological analysis, and a total of 84 fish (7 fish/tank) for sensory analysis, were ice-killed.

Technological analysis

The fish were eviscerated, stored in ice until the complete resolution of *rigor mortis*, and filleted at 48 h post mortem. For each sea bream, one fillet was marinated (8% NaCl, 1% CH_3COOH for 48 h at 4 °C) and used for the determination of purge loss, which represents the ability of meat to retain the marinade solution during refrigerated storage. The other fillet was used for the measurement of ultimate pH as described by Jeacocke (2007), total protein solubility as proposed by Sotelo *et al.* (1994), and oxidative status of both lipid and protein fractions through the determination of thiobarbituric acid reactive substances (i.e. TBARS) and carbonyls content, respectively, following the procedures proposed by Bao and Ertbjerg (2015) and Soglia *et al.* (2016).

Fillets cooking for sensory profile

The specimens of sea bream were filleted manually, keeping the skin as a protection of the fillet both for storage and for subsequent cooking. Each examined diet (CTRL, HI5, HI10, HI15) was retained and quickly transported to the sensory analysis laboratory where the samples were washed and dried and each sea bream fillet was individually wrapped with aluminium foil to protect them from light and therefore from oxidation. Finally, the processed fillets were placed under vacuum

and then frozen within 12 h of tank capture, at –80 °C until analysis.

The fillets to be analysed on the day were previously thawed by placing them at 3 °C for about 12 h and keeping them in their packaging. The next morning, tap water was boiled in a stainless-steel pot (Ø 32 cm) by placing it on a Schott Ceran glass-ceramic induction plate. A large stainless-steel sieve was placed on top of the pot and all the fillets were allocated, with the skin facing down, taking care to completely cover them. The fillets' cooking time was standardised based on their weight (100 g each) and set to 4 min. After this time, each fillet was delicately removed and placed inside a plastic food box equipped with a cover (750 cc capacity), aligned with the others on the panellist tray.

The sensory panel

The sensory analysis group (Panel) was made up of 10 individuals of both sexes working at the aquaculture centre in Cesenatico, University of Bologna, and operating under the guidance of an experienced panel leader. The sex ratio, Panel leader excluded, was M:F = 5:4 and the age range was 23:58 years. Each panellist attended a 20-h course aimed at verifying their normosensitivity (ISO 8586:2012; ISO, 2012) as well as their proficiency in discriminant tests (mainly triangular tests) (ISO 4120:2021; ISO, 2021a) and descriptive tests (quantitative descriptive analysis, QDA) according to Stone and Sidel (1993).

Because of the Italian government restrictions due to the COVID 19 pandemic, the entire process of creating and training, as well as the triplicate final evaluation, the ballot, and the final evaluation was conducted remotely (Teams platform), with the help of a rather large number of physical references provided each panellist, aimed at anchoring and therefore stabilising the sensory response as suggested by Rainey (1986).

Set-up and performing of sensory analysis

4 h were spent familiarising with the main sensory traits of sea bream from different origins. Each sample was named with a random three-digit code. For each sample, the panellists identified 40 traits according to Civille and Lyon (1996) and Hyldig (2012), and then reduced them to 31 according to ISO 11035:1994 (ISO, 1994). Supplementary Table S1 shows traits along with the definitions and references, as derived from the panel's work during 6 dedicated sessions. In these sessions, the initial work of naming and defining the descriptors was carried out remotely, each panellist being placed in an odourless room connected to all others via the Teams platform. This led the panel to use a single set of widely agreed traits. Once the final ballot was assembled, the actual samples were prepared as described above, and analysis was performed within the following week to allow panellists to have stabilise and remember all references. Also, natural mineral water at room temperature and the soft inside of Tuscan unsalted bread were utilised as neutralisers. The panellists were instructed to give priority to the evaluation of the olfactory notes (direct ways) as they are rather labile, to then move on to the examination of the appearance, then to the aroma (retronasal ways), to the basic taste, flavour, and to the mouth feels, to finish with the texture aspects of a mechanical, geometric, and chemical type. Therefore, each panellist tray contained as many permitted plastic boxes as there were experimental theses (including control) plus a dozen references duly kept warm. The panel test was blind according to ISO 11132-2021 (ISO, 2021b).

Nutritional analysis and total lipids and fatty acids composition

Five fish per tank were sacrificed and the dorsal-left skinned fillet was collected for proximate composition analysis. Moisture content was obtained by weight loss after drying samples on a stove at 105 °C overnight. Crude protein was determined as total nitrogen (N) by using the Kjeldahl method and multiplying N by 6.25 Behr (for nitrogen determination: BehrS5 equipment). Total lipids were determined according to Bligh and Dyer's (1959) extraction method (equipment: VELP ser 148 e VELP HU6). Ash content was estimated by incineration to a constant weight in a muffle oven at 550 °C for 3 h.

Energy values, expressed in Kcal/100 g, were derived multiplying the grams of protein and fat by factors 4 and 9, respectively (USDA, 2016). Fatty acids composition analysis was performed according to ISO16958:2015 (ISO, 2015) and shown in Table 3.

Statistical analysis

All data are presented as mean ± standard deviation (SD). The tank was used as the experimental unit for analysing growth performance, and a pool of five sampled fish was considered the experimental unit for analysing carcass composition. Data from sensorial, technological, and nutritional analyses were analysed by a one-way ANOVA. The differences among treatments were considered significant at $P \leq 0.05$, and in this case, Tukey's post hoc test was performed. The data were checked for normality of variance by the Shapiro-

TABLE 3 List of total fatty acids composition of sea bream fillets fed the control diet and the three diets containing different inclusion levels of *Hermetia illucens* (HI) larvae meal.[1,2]

FAs (g/100 g)	Experimental diets				*P*-value[3]
	CTRL	HI5	HI10	HI15	
12:0	$0.005^{a} \pm 0.001$	$0.005^{a} \pm 0.009$	$0.066^{b} \pm 0.014$	$0.117^{c} \pm 0.025$	**<0.0001**
14:0	$0.207^{a} \pm 0.043$	$0.297^{b} \pm 0.049$	$0.292^{b} \pm 0.049$	$0.344^{b} \pm 0.053$	**0.005**
15:0	$0.016^{a} \pm 0.003$	$0.020^{b} \pm 0.004$	$0.020^{ab} \pm 0.004$	$0.020^{b} \pm 0.004$	**0.013**
16:0	1.273 ± 0.153	1.68 ± 0.200	1.487 ± 0.180	1.607 ± 0.193	0.117
17:0	$0.016^{a} \pm 0.003$	$0.020^{b} \pm 0.004$	$0.019^{ab} \pm 0.004$	$0.020^{b} \pm 0.004$	**0.021**
18:0	0.302 ± 0.050	0.386 ± 0.057	0.345 ± 0.053	0.346 ± 0.053	0.182
20:0	$0.021^{a} \pm 0.004$	$0.027^{b} \pm 0.006$	$0.025^{ab} \pm 0.005$	$0.025^{ab} \pm 0.005$	**0.025**
22:0	$0.011^{a} \pm 0.002$	$0.015^{b} \pm 0.003$	$0.013^{ab} \pm 0.003$	$0.013^{ab} \pm 0.003$	**0.048**
Total SFAs	1.943 ± 0.167	2.597 ± 0.217	2.363 ± 0.200	2.6 ± 0.213	0.459
16:1 total	0.373 ± 0.079	0.497 ± 0.420	0.457 ± 0.308	0.503 ± 0.307	0.090
17:1 total	0.022 ± 0.004	0.026 ± 0.004	0.024 ± 0.004	0.025 ± 0.004	0.375
18:1 total	3.207 ± 0.300	4.057 ± 0.360	3.593 ± 0.333	3.627 ± 0.333	0.175
20:1 total	0.147 ± 0.031	0.187 ± 0.040	0.174 ± 0.037	0.173 ± 0.036	0.112
22:1 total	0.098 ± 0.015	0.12 ± 0.019	0.108 ± 0.016	0.109 ± 0.016	0.205
Total MUFAs	3.893 ± 0.517	4.947 ± 0.553	4.417 ± 0.537	4.493 ± 0.533	0.165
18:3 total	0.325 ± 0.048	0.394 ± 0.054	0.351 ± 0.050	0.348 ± 0.050	0.102
18:4 n3	$0.048^{a} \pm 0.010$	$0.062^{b} \pm 0.013$	$0.056^{ab} \pm 0.012$	$0.060^{ab} \pm 0.013$	**0.032**
20:4 n6 (ARA)	$0.036^{ab} \pm 0.001$	$0.042^{b} \pm 0.001$	$0.041^{b} \pm 0.003$	$0.034^{a} \pm 0.010$	**0.024**
20:5 n3 (EPA)	$0.283^{a} \pm 0.012$	$0.372^{b} \pm 0.013$	$0.355^{ab} \pm 0.032$	$0.373^{ab} \pm 0.035$	**0.021**
22:6 n3 (DHA)	0.441 ± 0.012	0.502 ± 0.020	0.476 ± 0.019	0.422 ± 0.020	0.096
EPA + DHA	0.724 ± 0.024	0.874 ± 0.032	0.832 ± 0.050	0.823 ± 0.067	0.061
PUFAs (>C20)	0.657 ± 0.073	0.774 ± 0.082	0.744 ± 0.079	0.714 ± 0.077	0.067
Total PUFAs	2.527 ± 0.167	3.1 ± 0.197	2.893 ± 0.187	2.89 ± 0.187	0.066
PUFAs / MUFAs	0.649 ± 0.096	0.627 ± 0.081	0.658 ± 0.091	0.649 ± 0.090	0.592
PUFAs / SFAs	$1.300^{cb} \pm 0.143$	$1.193^{ab} \pm 0.127$	$1.230^{ab} \pm 0.133$	$1.120^{a} \pm 0.123$	**0.020**

1 FAs = fatty acids; SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; ARA = arachidonic acid; CTRL = control diet; HI5 = 5% of HI meal; HI10 = 10% of HI; HI15 = 15% of HI meal.

2 Values with different superscript letters do something. ######

3 Significant values are indicated in bold.

Wilk tests. GraphPad Prism (La Jolla, CA, USA) was used to conduct the statistical analyses.

3 Results and discussion

Fillet's nutritional characteristics

At the end of the trial, no significant differences in growth and feed utilisation were detected between diets (final body weight, g: 271.6-282.3, $P = 0.400$; specific growth rate, % body weight/day: 0.89-0.93, $P = 0.184$; feed conversion rate: 1.29-1.32, $P = 0.492$). Analyses of fatty acid composition (Table 3) showed that some SFAs were significantly higher in fillets of fish-fed diets with HI meal inclusion.

Specifically, C14:0 presented higher values ($P = 0.005$) for fillets of fish-fed diets containing HI with respect to the CRTL; C15:0 and C17:0 presented higher values (respectively, $P = 0.00128$; $P = 0.0209$) for HI5 and HI15 compared to the control; C20:0 and C22:0 showed a significance (respectively, P 0.0253; $P = 0.0488$) in HI5 which had higher values in regard to the CTRL; C12:0 showed no differences among CTRL and HI5, while HI10 was significantly different concerning CTRL and HI5, and HI15 was higher compared to all the other diets ($P <$ 0.0001).

TABLE 4 Proximate composition of sea bream's (*Sparus aurata*) fillets fed the control diet and the three diets containing different inclusion levels of *Hermetia illucens* (HI) larvae meal.[1]

Proximate composition (%)	Experimental diets				*P*-value
	CTRL	HI5	HI10	HI15	
Moisture	68.0 ± 0.68	66.7 ± 0.89	67.6 ± 0.55	66.5 ± 1.33	0.224
Crude protein	21.5 ± 0.61	20.9 ± 0.06	21.0 ± 0.18	20.8 ± 0.10	0.113
Ash	1.55 ± 0.04	1.51 ± 0.04	1.52 ± 0.13	1.49 ± 0.07	0.851
Crude fat	7.38 ± 1.35	8.68 ± 1.92	8.64 ± 1.02	9.92 ± 3.15	0.539
Energy (Kcal/100 g)	100.0 ± 2.83	97.1 ± 0.23	97.6 ± 1.78	96.7 ± 0.83	0.173

1 Data are given as the mean (n = 3). CTRL = control diet; HI5 = 5% of HI meal; HI10 = 10% of HI; HI15 = 15% of HI meal.

It has been observed that SFAs C12:0 and C14:0 increase in the fillet with the increase of HI inclusion, in fish as well as in other species such as broiler chicks (Ross-308) (Altmann *et al.*, 2020; Borgogno *et al.*, 2017; Bruni *et al.*, 2020a,b; Caimi *et al.*, 2020b; Dalle Zotte, 2021; Mancini *et al.*, 2018; Renna *et al.*, 2017; Stejskal *et al.*, 2020). In this study, only C12:0 increased in fillets with the increase of HI meal inclusion, and the other statistically significant SFAs showed an increase in all diets containing HI meal compared to CTRL.

In the present study, although not significantly different, the total monounsaturated fatty acids (MUFAs) content was higher in the HI diets than in the control diet. In general, MUFAs showed no significant differences among treatments (P = 0.1648). Similar data were presented by Hoc *et al.* (2021), and Moutinho *et al.* (2021). On the contrary, Stejskal *et al.* (2020) stated that MUFAs followed a pattern similar to SFAs, increasing with the increase of HI meal inclusion.

In this study, no significant differences were found among treatments also in polyunsaturated fatty acids (PUFAs) (P = 0.0657). In particular, the level of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachinonic acid (ARA), DHA values did not show significant differences between diets (P = 0.0956). EPA was higher in the HI5 diet than the CTRL diet (P = 0.0211), while ARA displayed lower values in HI15 with respect to HI5 and HI10 diets (P = 0.0236). It is known that the fillet content in FAs reflects that of the diets in teleost species (Parma *et al.*, 2019; Pulido *et al.*, 2022). In this regard, it should be mentioned that insect meal, being made up of terrestrial insects, shows a deficiency in FAs that could result in a lowering of PUFAs content, especially n-3, in the fillet. This aspect is one of the main inconveniences of using insect meal, and indeed several studies reported that the PUFAs content decreased with the increase of HI inclusion (Altmann *et al.*, 2020; Borgogno *et al.*, 2017; Carvalho *et al.*, 2023; Lock *et al.*, 2016; Mancini *et al.*, 2018; Oteri *et al.*, 2022; Pulido *et al.*, 2022; Renna *et al.*, 2017; Secci *et al.*, 2019; Stejskal *et al.*, 2020; St-Hilaire *et al.*, 2007; Zarantoniello *et al.*, 2022). However, in the present study the quantities of PUFAs did not decrease and quite reflect those of the diets. This is due to the practical low fish meal level employed in the CTRL diet. Thus, the partial substitution of FM with HI did not give an overall effect on diet FAs composition. Data from this study seem to meet the results of previous assessments that tested HI in substitution of FM with a control diet rich in plant ingredients. Indeed, no significant difference in PUFAs n-3 was observed in trout (Bruni *et al.*, 2020b), a slight increase in PUFAs n-3 was observed in salmon (Bruni *et al.*, 2020a), and in pikeperch, DHA decreased with the increasing HI meal inclusion, while n-6 FAs showed the opposite trend, and no significant differences were found in total PUFAs among treatments (Stejskal *et al.*, 2023).

In addition, the fish fillets tested in this study show an excellent EPA + DHA content. According to EFSA Scientific Committee (2015), every adult should take 250 mg of EPA + DHA per day, about 1750 mg per week, consuming two portions of fish of 150 g each. Two portions of fillet from all four diets tested in this study exceed the weekly EPA + DHA requirement, in particular, HI diets which contain on average 119 mg/100 g more EPA + DHA compared to the control diet.

Data regarding the fillet's proximate composition are shown in Table 4. According to the results, no significant differences were found in moisture, crude protein, crude fat, ash, and energy values among treatments (P > 0.05).

Technological analysis

Data concerning fillets' main technological properties and oxidative status are shown in Table 5. Overall data showed that the replacement of conventional protein sources with HI larvae meal up to a level of 15% did not affect the main technological properties and the

TABLE 5 Technological properties and oxidative status of sea bream's (*Sparus aurata*) fillets fed the control diet and the three diets containing different inclusion levels of *Hermetia illucens* (HI) larvae meal.[1]

Technological properties	Experimental diets				*P*-value
	CTRL	HI5	HI10	HI15	
pH	6.26 ± 0.06	6.27 ± 0.08	6.26 ± 0.08	6.24 ± 0.05	0.796
Purge loss (%)	1.75 ± 0.51	1.47 ± 0.56	1.58 ± 0.43	1.54 ± 0.38	0.706
Protein solubility (mg/g)	139.2 ± 21.0	125.1 ± 30.9	142.7 ± 22.4	137.6 ± 26.8	0.302
Oxidative status					
TBARS[2] (mg MDA/kg)	0.64 ± 0.04	0.66 ± 0.02	0.64 ± 0.06	0.66 ± 0.04	0.554
Carbonyls (nmol/mg)	1.77 ± 0.70	2.39 ± 0.65	1.98 ± 0.43	1.85 ± 0.33	0.078

1 Data are given as the mean (n = 12). CTRL = control diet; HI5 = 5% of HI meal; HI10 = 10% of HI; HI15 = 15% of HI meal.

2 TBARS = thiobarbituric acid reactive substances.

oxidative status of sea bream fillets. This outcome is corroborated by several recent studies aimed at evaluating the effects of the inclusion of HI larvae meal on technological properties not only of gilthead sea bream (Pulido-Rodriguez *et al.*, 2021) but also for other fish species, such as rainbow trout (Renna *et al.*, 2017).

It is worth mentioning that the inclusion of HI larvae meal did not affect the ultimate pH of fish fillets, thus suggesting that the pattern of post mortem acidification was not influenced by the dietary treatment. This result confirms what was previously found in research carried out by Renna *et al.* (2017), in which the muscular pH of rainbow trout fed with HI larvae meal up to 50% was not significantly modified. That is particularly relevant when considering the relationship between muscular pH and the technological properties of fish muscles, such as their ability to retain water (Liu *et al.*, 2010). This outcome is further corroborated by the results concerning purge loss and protein solubility obtained within this study (respectively, $P = 0.706$; $P = 0.302$). As for the oxidative status of fish muscles, the absence of significant differences in TBARS content ($P = 0.554$) suggests that the inclusion of HI larvae meal, regardless of its level, did not result in higher oxidation of the lipid fraction. As for protein oxidation, carbonyl levels tended to be higher in the HI5 group ($P = 0.078$). However, it should be noted that this difference is of a minor extent, and thus not relevant for the final quality of sea bream fillets.

Sensory analysis

At the end of the trial, no significant differences ($P > 0.05$) were detected in final body weights among treatments (CTRL 273.9 ± 7.86; HI5 282.3 ± 5.12; HI10 271.6 ± 8.69; HI15 273.3 ± 9.14). Results of different inclusion levels of HI larvae meal (0% CTRL, 5% HI5, 10% HI10, and 15% HI15) in place of FM, based on the list of sensory attributes developed for sea bream are shown in Figure 1. Most of the attributes considered were not significantly affected ($P \leq 0.05$) by the inclusion of HI. The same result has been encountered also in previous studies on several fish species where HI was included in the substitution of FM (Belghit *et al.*, 2019; Borgogno *et al.*, 2017; Lock *et al.*, 2016; Sealey *et al.*, 2011).

In the present study, a significant result was found regarding the odour ($P = 0.0124$) and flavour ($P = 0.0329$) trait 'cooked chicken breast' between HI5 and HI10 (Figures 2 and 3, respectively). Even if in different traits, also in other studies some significant differences were found in the odour and flavour modalities, highlighting that insect meal dietary inclusion can modulate the odour and flavour general intensity (Borgogno *et al.*, 2017, Belghit *et al.*, 2019). In particular, Borgogno *et al.* (2017) speculated on the possibility that the modalities' intensity may change with the increase of HI meal dietary inclusion. However, in this study the higher HI meal inclusion level did not show the highest significant values for the trait 'cooked chicken breast', an interesting result for which we have no plausible theories but that it would be desirable to investigate in future studies.

4 Conclusions

In conclusion, the HI larvae meal dietary inclusion up to 15% does not provoke a difference in the sensory evaluation of the fillet for most of the examined traits. Also, HI inclusion shows similar technological fish quality compared to the control diet, not compromising the fillet's technological characteristics and oxidative state. Anal-

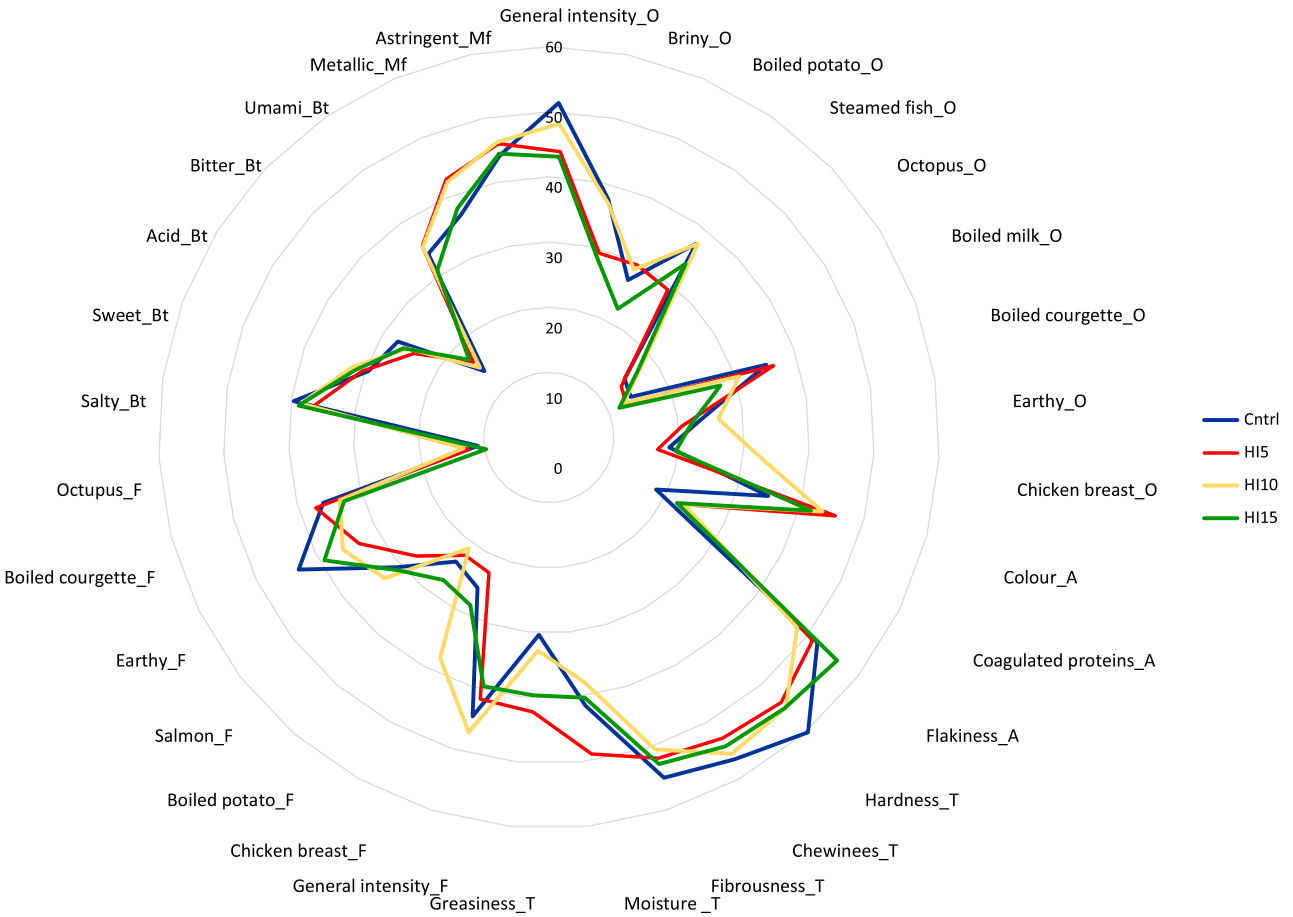

FIGURE 1 Complete sensory profile of sea bream from a feeding which control diet was compared with different percentages of insect diets. From right to left: O = odour, A = aspect, T = texture, F = flavour, Bt = based tastes, Mf = mouth feeling.

FIGURE 2 (A) Spider web for odour scope. From left to right: General intensity, briny, boiled potato, steamed fish, octopus, boiled milk, boiled courgette, earthy, chicken breast; (B) Statistical differences ($P = 0.0124$) in chicken breast odour descriptor among treatments.

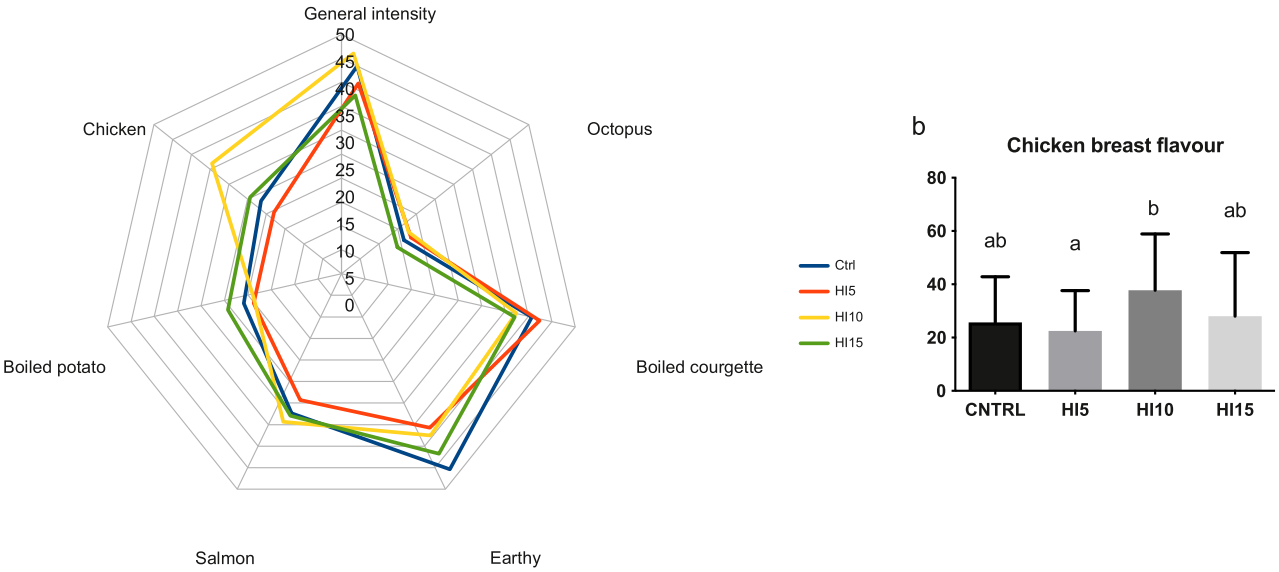

FIGURE 3 (A) Spider web for flavour scope. From left to right: General intensity, chicken breast, boiled potato, salmon, earthy, boiled courgette, octopus; (B) Statistical differences (P = 0.0329) in chicken breast flavour descriptor among treatments.

yses conducted on fillets' fatty acids content showed that SFAs were, in most cases, significantly higher in fillets fed the HI15 diet, confirming that a greater HI meal inclusion leads to higher SFAs content. Moreover, in this study, the inclusion of HI meal did not impair EPA and DHA content. The absence of a significant difference ($P > 0.05$) in most of the attributes examined provides further evidence to consumers that feeding sea bream with diets containing HI meal does not negatively impact fillets' taste and quality.

Supplementary material

Supplementary material is available online at: https://doi.org/10.6084/m9.figshare.23599179

Table S1. List of descriptors for quantitative descriptive analysis.

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Authors' contributions

Conceptualisation S.B, A.B., L.P., F.S., P.P.G., L.G., A.B., M.P.; Diets formulation F.B, S.B., A.B., L.P.; Methodology S.B., A.B., L.P., A.B., F.S., L.G., M.P.; Investigation S.B., M.M., M.S., A.B., F.S., G.B., M.P.; Writing-original draft S.B., M.M., G.B., F.S.; Writing-review and Editing S.B., A.B., L.P., M.M, A.B, F.S., G.B., M.P. All authors have read and agreed to the published version of the manuscript.

Conflict of 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

Data will be made available on request.

Ethical statement

The experiment was designed according to the guidelines of the current European Directive (2010/63/EU) on the protection of animals used for scientific purposes. The experimental protocol was approved by the Ethical Committee of the University of Bologna (Italy) (protocol No. 237050).

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