Received: 14 June 2024 | Revised: 29 November 2024 | Accepted: 7 April 2025

DOI: 10.1111/jwas.70021

APPLIED RESEARCH

Journal of the World Aquaculture Society Open Access | WORLD AQUACULTURE Society | WILEY

Toward the use of innovative environmentally sustainable feed in organic aquaculture: Impact on growth performance, health, and welfare of gilthead seabream (*Sparus aurata*)

Lola Toomey[1] | Sébastien Alfonso[1,2] | Elena Mente[3] | Isabella Bitetto[1] | Eleonora Fiocchi[4] | Amedeo Manfrin[4] | Phelly Vasilaki[5] | Walter Zupa[1] | Marie-Laure Bégout[6] | Maria Teresa Spedicato[1] | Giuseppe Lembo[1] | Pierluigi Carbonara[1]

[1]Fondazione COISPA ETS, Bari, Italy

[2]ECOSEAS, Université Côte d'Azur, CNRS, Nice, France

[3]School of Veterinary Medicine, Laboratory of Aquaculture and Aquatic Animal Diseases, Aristotle University of Thessaloniki, Thessaloniki, Greece

[4]Reference Laboratory for Fish, Mollusc and Crustacean Disease, Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro, Italy

[5]IRIDA S.A, Nea Artaki, Greece

[6]MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, Palavas-Les-Flots, France

Correspondence
Lola Toomey, Fondazione COISPA ETS, via dei Trulli 18-20, 70126 Bari, Italy.
Email: toomey@fondazionecoispa.org

Funding information
H2020 FutureEUAqua, Grant/Award Number: 817737

Abstract

Organic aquaculture aims to provide sustainable aquatic products, and utilizing innovative aquafeeds with less fish meal is crucial. This study evaluated the impact of a cost-effective and environmentally friendly diet (51% replacement with plant/animal protein sources like fermented soy, pea, krill, squid, and yeast meals) on gilthead seabream (*Sparus aurata*) growth, health, and welfare using a multi-parametric approach (growth, swimming activity, blood health, and welfare indicators). The innovative diet showed no adverse effects on growth performance and survival.

Lola Toomey and Sébastien Alfonso these authors contribute equally to this work.

Hematological and biochemical blood indicators demonstrated minimal alterations, with only lactate levels increasing, suggesting no compromise in overall welfare. Immune features indicated a potentially stronger innate immune response in fish fed the innovative diet, as shown by higher levels of total proteins, alpha 1, and beta 1. Finally, a slight difference was observed in swimming activity between diets, but primarily occurred at the end of the day. A comprehensive multiparametric analysis and multi-criteria decision analysis indicated better global welfare and health status with the innovative diet compared to the control. While the innovative diet showed promising results for gilthead seabream organic aquaculture, further long-term investigations are necessary to explore the underlying causes of the observed immune parameter changes.

KEYWORDS
acoustic telemetry, fish meal, innovative diet, organic aquaculture, welfare

1 | INTRODUCTION

Organic aquaculture is gaining global prominence as a response to the escalating demand for sustainable aquatic products (Gould et al., 2019). Organic aquaculture practices rely on the establishment of ecologically integrated systems that prioritize animal welfare, environmental preservation, biodiversity conservation, and the generation of high-quality and healthy products (Gould et al., 2019). Despite the impressive overall production figures in world aquaculture, organic aquaculture remains confined to a niche market. For example, the total organic aquaculture production in the European Union reached an estimated 74,032 tons in 2020, constituting only 6.4% of the total European aquaculture production (EUMOFA, 2022). The hindrance in the development of organic aquaculture production is primarily attributed to a myriad of challenges encountered by the organic sector from its inception. These challenges encompass technical production difficulties, diminished profitability, compliance issues with regulations, limited market demand, and competition with other certification schemes (EUMOFA, 2022; Gambelli et al., 2019; Mente et al., 2011; Sicuro, 2019).

A critical facet of organic aquaculture revolves around fish nutrition, addressing both societal and environmental concerns (Mente et al., 2011; Sicuro, 2019). While significant research has been conducted in conventional aquaculture to develop sustainable fish feeds (e.g., Aragão et al., 2022; Naylor et al., 2021), there remains a need for research specific to organic aquaculture (but see e.g., Lund et al., 2011; Carbonara, Alfonso, et al., 2020; Estévez & Vasilaki, 2023; Tefal et al., 2023; Toomey et al., 2024). Formulating new organic feeds aims at minimizing the reliance on fish meal and fish oil, aligning with the fundamental principle of sustainability, mitigating the pressure on declining wild stocks, and limiting issues related to availability and price fluctuations (Gasco et al., 2018; Mente et al., 2019; Oliva-Teles et al., 2015). The specific regulations governing organic practices, including limitations on the use of plant-origin ingredients (not exceeding 60% of total ingredients), restrictions on certain raw materials, and the prohibition of synthetic free amino acid supplementation, add complexity to meeting the nutritional requirements of

essential nutrients throughout the entire organic production cycle. This challenge is particularly pronounced for carnivorous fish species (Busacca & Lembo, 2019; Mente et al., 2011, 2019). In addition to meeting the nutritional needs of the fish, the competitiveness of new fish meal substitutes in terms of price must also be considered. Alternative raw ingredients need to be available year-round, and the final cost of the feed must be sustainable for farmers. Overall, formulating novel organic feeds poses difficulties and costs (Mente et al., 2019), emphasizing the urgent need for the development of innovative and cost-effective organic feeds.

Various alternative protein sources have gained prominence in the formulation of new organic aquafeeds. Currently, soybean meal is widely advocated as a plant-based protein source, owing to its high-protein content, suitable amino acid profile, availability, and cost-effectiveness. However, its inclusion rate is constrained due to the presence of anti-nutritional factors (Gatlin et al., 2007; Mitra, 2021; Zhou et al., 2005), a limitation shared by many plant substitutes (Hossain et al., 2024) that can be partly overcome thanks to recent biotechnological developments (Ghosh & Ray, 2017) and the supplementation of exogenous digestive enzymes (Anwar et al., 2020; Magalhães et al., 2018). Recently, pea protein has also been promoted as a promising substitute (González-Rodríguez et al., 2016). Studies have demonstrated, for instance, that replacing up to 25% of fish meal with organic green pea protein does not adversely affect the growth, health, and final product quality of gilthead seabream (*Sparus aurata*; Estévez & Vasilaki, 2023). Microbial ingredients, derived from sources, such as yeasts, also appear promising (Agboola et al., 2021; Hua et al., 2019), particularly due to their potential contribution to the circular economy through production from various waste sources (Mente et al., 2019). Additionally, meals from other marine animals, such as krill or squid meals, are widely considered for their interesting characteristics, although they may present availability challenges (Choi et al., 2020; Kader & Koshio, 2012; Mitra, 2021; Torrecillas et al., 2021). To address limitations in inclusion rate, intensifying competition with other sectors, and fluctuations in the availability and prices of individual protein ingredients, there has been a gradual shift toward aquafeeds with ingredient blends from both plant and animal sources. These blends aim to effectively replace, at least partially, dietary fish meal (Kader & Koshio, 2012; Kissinger et al., 2016).

The development of new organic aquafeeds must ensure the fulfillment of the developmental, physiological, and behavioral needs of animals, aiming at sustainable production performance. Moreover, achieving a high level of immunity to cope with potential pathogens and diseases, along with ensuring the health and welfare of farmed species, is crucial, particularly in the context of organic aquaculture where the use of antibiotics is limited (Aragão et al., 2022; Glencross et al., 2020). Various indicators are currently employed at different biological levels, encompassing morphological indicators, behavioral metrics, and physiological and molecular indices (Carbonara, Zupa, et al., 2020; Dara et al., 2023; Huntingford & Kadri, 2014). Growth-related traits, being of economic significance, are particularly scrutinized among morphological indicators. Welfare assessments often involve a combination of behavioral indicators (e.g., swimming speed, group cohesion) and physiological variables (e.g., blood and immunological indicators), with advancements in technology allowing for more in-depth evaluations. In the realm of precision fish farming, the development of tags utilizing telemetry technology now enables the monitoring of fish energy expenditure. For example, accelerometer sensors have been employed to assess the impact of acute stressors or rearing conditions, including the use of new aquafeeds, on fish welfare (e.g., Alfonso et al., 2023; Brijs et al., 2018; Carbonara et al., 2022; Carbonara, Alfonso, et al., 2019; Gesto et al., 2020; Toomey et al., 2024). However, the potential negative impacts of these tags are still debated (see for instance, Alfonso, Zupa, Manfrin, Fiocchi, Dioguardi, et al., 2020a and Georgopoulou et al., 2022 for the same species) and should be thoroughly assessed when employing this emerging technology.

In this study, we conducted an investigation into the effects of an innovative and environmentally friendly diet, with a lower fish meal content (51% replacement by a blend of plant and animal protein sources, including notably fermented soy, pea, krill, squid, and yeast meals), on the growth, health, and welfare of *S. aurata*. *Sparus aurata* is a key species in Mediterranean aquaculture, and extensive research has been conducted on the use of alternative raw materials (Hodar et al., 2020). Although its organic production is relatively small, the production is growing rapidly. For example, European organic production of *S. aurata* and *Dicentrarchus labrax* increased by 38% between 2015

and 2020 (EUMOFA, 2022), and recent consumer studies indicate good market potential. The search for nutritionally adequate, economically viable, and environmentally sustainable diets is crucial and therefore ongoing (e.g., Estévez & Vasilaki, 2023; Tefal et al., 2023). In this study, fish meal substitutes were selected based on their cost-effectiveness and availability at the time of the study. The research involved monitoring the growth performance of fish fed two organic diets, either a commercial diet (i.e., control) or the innovative diet over a four-month period. Various physiological and molecular parameters, encompassing stress, immunity, health, and welfare indicators, were also monitored. Additionally, a subset of fish was implanted with physiological sensors, specifically accelerometer tags, enabling continuous monitoring of acceleration as a proxy for energy expenditure (Alfonso et al., 2021) throughout the experiment. The impact of employing this technological tool was assessed using mortality and growth indicators. Overall, this study offers a comprehensive evaluation of the seabream physiological state under an innovative diet regime, addressing the environmental sustainability challenges of the European organic aquaculture sector.

2 | MATERIALS AND METHODS

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and ARRIVE guidelines. All the experiments were performed in accordance with EU recommendation (Directive 2010/63/EU), with the authorization of the Health Ministry number code n° 488/2021-PR.

2.1 | Fish, feeding, and experimental protocol

Gilthead seabream individuals (about 1.5 years old) were purchased from the commercial farm REHOMARE (Gallipoli, Italy) and transported to Fondazione COISPA ETS facilities (Bari, Italy). Fish were maintained undisturbed for 2 weeks in a flow-through system with sea water replacement of 25 L/min. Sea water pH (7.30 ± 0.05), temperature (18 ± 1°C), oxygen saturation (~80%), and salinity (35 PSU) were checked weekly. Fish were kept at a stocking density of approximately 20 kg/m^3. Photoperiod was 12 L:12D (light: dark; from 6 am to 6 pm). The fish were fed with a commercial feed (Skretting Marine 3 P, Italy) amounting to about 1% of their body mass, six to seven times per week, using automatic feeders between 8 am and 12 pm. After this two-week acclimatization period, all fish were individually pit-tagged with ID100 radio frequency identification (RFID) tags (DORSET ID, Trovan, Netherlands) under anesthesia (hydroalcoholic clove oil solution at 30 ppm; Erbofarmosan, Bari, Italy). Fish were then randomly distributed into four fiberglass tanks of 1.2 m^3 (about 70 fish per tank; 12 kg/m^3) with the same water quality parameters and photoperiod specified earlier.

Two weeks after PIT-tagging (Figure 1), fish were fed with two organic diets: either a commercial diet, serving as a control (two tanks), or with an innovative diet (two tanks). Both commercial (control) and innovative feeds were

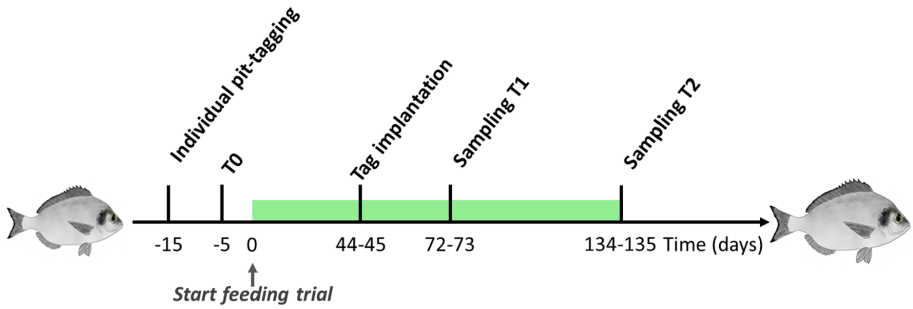

FIGURE 1 Experimental protocol for the feed trial in seabream (*Sparus aurata*).

produced by the Greek fish feed company IRIDA SA. The innovative diet used in this study was formulated to contain fishmeal replacers such as fermented soy, yeast (derived from *Saccharomyces cerevisiae*), squid, and krill meals (51% fish meal replacement) and used a Synergen™ (Alltech, USA) supplementation. The innovative feed was nutritionally balanced with other ingredients commonly used in aquafeeds, as well as with macro and micronutrients. The formulation and feeds' composition are shown in Table 1 and Table 2, respectively. The essential and nonessential amino acid ratios and fatty acid composition of the two diets are presented in Tables 3 and 4, respectively. The innovative feed utilized in this study adhered to all regulations governing organic feed formulation.

Fish were fed six to 7 days per week, amounting to about 1% of their body mass during the whole experiment using automatic feeders between 8 a.m. and 12 p.m. The feeding started on day 0 (11th of July 2022) and lasted 135 days until the 23rd of November 2022 (Figure 1). Growth and physiological factors were evaluated across the experiment at three sampling points: T0 (5 days before the start of the experiment to serve as a control), T1 (72nd-73rd experimental days), and T2 (134th–135th experimental days) (Figure 1).

2.2 | Growth measurements, blood, and organs sampling

At each sampling time (i.e., T0, T1, and T2), all fish were gently caught from the rearing tanks and anesthetized with hydroalcoholic clove oil solution (15 ppm) to measure total weight (g). Mortalities were recorded daily in each tank, as well as the quantity of feed provided per tank. Specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated between T0 and T2. The SGR was calculated individually using the following equation: $SGR = 100 \times (\ln(W_0) - \ln(W_2))/T$, where W is the total weight of the fish (g) at t0 (W_0) and at the end of the experiment (W_2), and T is the number of feeding days. The FCR was calculated using the following equation: FCR = Feed intake (kg)/biomass gain (kg). Finally, the PER was calculated in the following way: PER = fish weight gain/estimated total amount of protein ingested.

At T0, 12 fish were randomly sampled, while at T1 and T2, eight fish were randomly sampled per tank (i.e., $n = 16$ per diet) for blood sampling. Fish were gently caught from the rearing tanks and bathed in an anesthetic solution (clove oil, 30 ppm) for 2–3 min before proceeding to blood sampling. Blood was retrieved from the caudal

TABLE 1 Feedstuff (%) used in the two organic diets: Control (i.e., commercial) and innovative diet.

Feedstuff	Control diet	Innovative diet
Fish meal	51	25
Soybean meal	23.5	–
Fermented soy	–	13
Wheat	15	12.45
Fish oil	10	10.8
Pea protein	–	10
Krill meal	–	6
Squid meal	–	6
Yeast	–	5
Corn gluten meal	–	5
Wheat gluten meal	–	5
Monocalcium-phosphate	–	1.2
Synergen	–	0.05
Premix	0.5	0.5

TABLE 2 Chemical composition (%) of the two organic feeds (control and innovative) used in the experiment according to AOAC 1995 protocols.

Proximate composition analysis	Control diet	Innovative diet
Proteins (%) ($n = 5$; mean ± se)	46.58 ± 0.12	47.07 ± 0.21
Lipids (%) ($n = 5$; mean ± se)	14.72 ± 0.08	14.93 ± 0.32
Ash (%) ($n = 5$; mean ± se)	12.21 ± 0.03	9.74 ± 0.03
Energy (Kj/g) ($n = 5$; mean ± se)	21.90 ± 0.04	22.29 ± 0.03
Additives (per 1 kg)		
Vitamin A (I.U)	5	10
Vitamin D3 (I.U)	1	2
Vitamin E (mg)	250	250
Choline Chloride (mg)	750	1250
Vitamin C (mg)	200	200
Zinc (mg)	60	60
Manganese (mg)	35	35
Copper (mg)	7	7
Iron (mg)	45	70
Iodine (mg)	4	6
Toccopherol rich extract (mg)	300	–
BHT (mg)	–	45.4
BHA (mg)	–	30.1
Propyl Gallate (mg)	–	15
Cictric Acid (mg)	–	30
Lecithins	Not present	Present

vein using a heparinized syringe. At T2, the fish used for blood sampling were then euthanized using an overdose of anesthetics (clove oil, 60 ppm), and 100 mg of spleen, kidney, gill, liver, and brain were collected for subsequent quantitative real-time PCR analyses. Each sample was stored in a tube containing 1 mL of RNAlater (QIAGEN), maintained for 48 h at 4°C, and then stored at −80°C until further processing.

2.3 | Analysis of hematological and biochemical indicators

Hematocrit was determined using a heparinized micro-hematocrit tube filled with blood directly from the syringe needle, which was then centrifuged at 15,000 × g for 3 min and immediately read. Hematocrit was expressed as the percentage of red blood cells relative to the entire blood volume. The red blood cell count (RBCC) was carried out in a Bürker counting chamber under a light microscope (Nikon 400E, Japan). Hemoglobin was measured using a commercial kit (H7379; Sigma, USA). The remaining blood was transferred to a tube with K3EDTA (VACUMED, Torreglia, Italy) and was centrifuged at 15,000 × g for 3 min to obtain plasma samples, which were stored at −20°C until further analyses. Plasma cortisol was measured using a commercial competitive electrochemiluminescent immunoassay kit (Elecsys Cortisol II Gen, Roche Diagnostics) in an automatic analyzer Cobas E601 (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer's instructions. Plasma glucose, lactate, and total protein concentrations were measured in the Biochemical analyzer Cobas C501 (Roche Diagnostics) using commercial kits (Glucose HK Gen.3, Lactate Gen.2, and Total Protein Gen.2, respectively; Roche Diagnostics), following manufacturer

TABLE 3 Essential and nonessential amino acid ratio (A/E, corresponding to the percentage of each amino to total amino acids) for the organic control and innovative feeds used in the experiment.

Essential amino acid ratio (A/E, %)		
	Control diet	**Innovative diet**
Arginine	15.79	12.47
Histidine	2.77	2.92
Isoleucine	8.52	9.83
Leucine	16.41	19.90
Lysine	18.20	13.43
Methionine	4.01	2.68
Phenylalanine	9.99	11.36
Threonine	7.63	9.40
Valine	10.62	11.17
Tyrosine	6.07	6.86
Nonessential amino acid ratio (A/E, %)		
	Control diet	**Innovative diet**
Alanine	15.16	11.42
Aspartic acid	16.14	20.34
Glycine	16.83	12.59
Serine	10.99	11.64
Glutamic acid	25.66	28.39
Proline	15.21	15.62

instructions. Total serum IgM levels were analyzed using an enzyme-linked immunosorbent assay kit (BT LAB™, China) following the manufacturer's instructions.

In order to measure the protein fractions content in plasma (albumin, alpha1, alpha2, beta1, beta2, and gamma), electrophoresis was carried out using the Minicap Flex Piercing system (Sebia, Bagno A Ripoli, Italy) following the manufacturer's instructions. The Minicap Flex Piercing is a multitasking automated capillary electrophoresis instrument equipped with two capillaries. Serum proteins were separated within silica capillaries based on their electrophoretic mobility and electrosmotic flow under high-voltage conditions and in the alkaline buffer. Proteins were detected directly during migration by UV absorbance at 200 nm, and they were quantified. The Minicap system allows all electrophoresis sequences to be performed automatically from the primary tube until the electrophoretic profile is obtained. The instrument is equipped with a Phoresis (Sebia) software program that allows the processing of results. Identification of the fractions was performed automatically, and the electrophoretic profiles were analyzed on the fly.

2.4 | Quantification of HSP70 using qPCR

The absolute quantification of heat shock protein 70 (*Hsp70*) was conducted with quantitative real-time PCR analysis according to the method developed by Fiocchi et al. (2020) using a specific plasmid previously developed for seabream *Hsp70* (Carbonara, Alfonso, et al., 2019). For each sample, the different organs were pooled together before being treated with TissueLyser II (QIAGEN, Hilden, Germany) for 1 min at 15 Hz. RNA was extracted from 30 mg of

TABLE 4 Fatty acid composition (% of total fatty acids) for the organic control and the innovative feeds used in the experiment.

Fatty acid	Control diet	Innovative diet
14:0	4.11	3.94
14:1	0.05	0.05
15:0	0.31	0.31
16:0	14.61	14.47
16:1 n-9	0.25	0.26
16:1 n-7	4.46	4.32
17:0	0.26	0.27
17:1 n-7	0.22	0.23
18:0	2.98	3.15
18:1 n-9	22.24	23.77
18:1 n-7	3.10	3.24
18:2 n-6	12.91	11.99
18:3 n-3	2.93	3.19
18:4 n-3	1.81	1.71
20:0	0.33	0.34
20:1 n-11	0.60	0.60
20:1 n-9	5.39	5.34
20:2 n-6	0.48	0.53
20:3 n-6	0.22	0.22
20:4 n-6 (arachidonic acid; ARA)	0.65	0.72
20:3 n-3	0.48	0.49
20:4 n-3	0.83	0.80
20:5 n-3 (eicosapentaenoic acid; EPA)	5.50	5.27
22:0	0.28	0.29
22:1 n-9	6.67	6.19
22:1 n-7	0.92	0.97
24:0	0.18	0.18
22:6 n-3 (docosahexaenoic acid; DHA)	6.46	6.38
24:1 n-9	0.76	0.79
Total saturated fatty acids	23.06	22.96
Total monounsaturated fatty acids	44.66	45.74
Total polyunsaturated fatty acids	32.27	31.29
Total n-9 long-chain polyunsaturated fatty acids	35.31	36.36
Total n-6 long-chain polyunsaturated fatty acids	14.26	13.46
Total n-3 long-chain polyunsaturated fatty acids	18.01	17.83
EPA + DHA	11.95	11.64
n-3/n-6	1.26	1.32

each sample using the RNeasy® Mini Kit (QIAGEN) following the manufacturer's instructions. All RNA extracts were checked using a NanoDrop Lite Spectrophotometer (ThermoFisher Scientific) for quantification and quality

assessment (A260/A280 ratio). Extracts were standardized in RNAase-free water to obtain a final concentration of 10 ng/μL.

To obtain first-strand cDNA, 10 ng of RNA was reverse transcribed into cDNA using Random Examers and SuperScript™ II Reverse Transcriptase (Invitrogen) following the protocol provided by the manufacturer. The primer set for *HSP70* (Forward primer: 5′-ATCAGCGACAACAAGAGAGC -3′ and Reverse primer: 5′-GGTGATGGAGGTGTAGAAGTC -3′) was designed in the context of a previous study using the sequences deposited in GenBank (DQ524995.1 EU805481.1). One μL of cDNA and one μL in triplicate of plasmid were used in 25 μL PCR reactions that also included SYBR Green Master Mix (QIAGEN) and 0.4 μM of forward and reverse primers. The fragment generated by primers for *HSP70* was 176 bp. All reactions having been conducted using the SYBR Green technology, a dissociation stage was added after the amplification cycle to estimate the specificity of the products. To evaluate the limit of detection of assays, 10-fold dilutions in triplicate of the plasmid were amplified.

2.5 | Implantation of accelerometer tags and recording of swimming activity

A subsample of fish ($n = 5$ fish per tank; $n = 10$ per diet; 312.5 ± 34.9 g and 298.9 ± 30.4 g for control and innovative diets, respectively) was implanted on the 44th–45th experimental days (corresponding to the 24th and 25th of August 2022; Figure 1) with VEMCO V9A-2x accelerometer tags (AMIRIX Systems Inc., Nova Scotia, Canada). Tags were implanted into the body cavity through an incision of about 1.5 cm that was carefully sutured as described elsewhere (Alfonso et al., 2021; Carbonara et al., 2022; Carbonara, Alfonso, et al., 2019). The whole procedure was performed under anesthesia (clove oil, 30 ppm). After the surgery, an antibiotic injection (sodic-ampicillin–cloxacillin; 1 mg/kg) was carried out and fish were released into their original tank. Feeding and swimming behaviors of the tagged fish were monitored daily in the days following the surgical implantation to ensure good recovery. All fish started feeding 2–3 days following the surgery and displayed normal swimming behavior, testifying that all tagged fish recovered well. Since the tag implantation represents an invasive procedure, blood analyses were not performed on tagged fish.

Data recording started 12 days after tag implantation, in order to ensure full recovery of tagged fish from the stress induced by the tagging procedure before data acquisition (Alfonso et al., 2021), and lasted until the end of the experiment (T2; Figure 1). Tags were programmed to measure acceleration over two axes (X and Z; tailbeat acceleration). Acceleration was used as a proxy for energy expenditure (Alfonso et al., 2021). The accelerometer tag implanted in fish transmitted the tag ID and coded values, corresponding to the swimming activity of tagged fish with a sampling rate of 12.5 Hertz and low-power frequency (40–80 s of transmitting delay), to an acoustic receiver Vemco VR2W (AMIRIX Systems Inc.) located on the bottom of each tank. At T2, receivers were retrieved from each tank and data were downloaded using the software VUE (AMIRIX Systems Inc.). The swimming activity values obtained from the accelerometer tags ranged from 0 to 255 and were displayed in arbitrary units (AU). The values obtained could be converted into acceleration using the following equation: acceleration (m/s^2) = 0.01955(x), where *x* is the acomponental value returned by tags.

2.6 | Statistical analyses

All statistical analyses were carried out using the R software version 4.3.1 (R Core Team, 2023) at a significance level of 95%. Data are presented as mean ± sd (standard deviation) and fish individual was used as the statistical unit, except for mortality for which the tank was the statistical unit. Initially, interactions between the different fixed effects and random effects (tank, fish) were included in all models, where applicable. When an interaction was not significant or when some random effects presented a null variance, they were excluded from the final models. For all linear models, graphical checks were conducted to verify the assumptions of normality of residuals, linearity, absence of outliers, and homoscedasticity. Analyses are summarized below, and details can be found in Annex A1.

Growth, hematological, and biochemical indices were analyzed using linear models or a linear mixed model (LMM; *lme4* package, Bates et al., 2014), with the sampling time (T1 vs. T2) and the diet groups (control vs. innovative) as fixed effects also the tagging status (tagged vs. non tagged) for growth indicators while the fish ID was added as a random effect when required (see details in Annex A1). Linear models were followed by estimated marginal means post hoc tests with Benjamini–Hochberg correction for multiple comparisons to test for differences between groups. Because parametric test assumptions were not respected, the FCR was analyzed using the Scheirer Ray Hare test with the tagging status and the diet as fixed effects. A Wilcoxon test was used to compare mortality rates between diets. Finally, to compare the swimming activity between the two diet groups (control vs. innovative), we employed the same statistical approach as Toomey et al. (2024) using a generalized linear mixed model (individual fish as a random factor and diet and period of the day [day vs. night] as fixed factors) and an analysis of frequency distribution (see details in Annex A1).

In order to have a global overview of the diet effect on fish health and welfare (22 variables in total), a multivariate analysis was performed. Only values at T2 were considered to investigate the long-term effect of diets. After scaling data, a Principal Component Analysis (PCA) was performed using the library *FactoMineR* (Le et al., 2008). The relevant components of the PCA were selected using the *nFactors* package (Raiche & Magis, 2022). Visualization of the PCA results was conducted using the *factoextra* library (Kassambara & Mundt, 2020). The principal component scores of the relevant axes were then extracted and Mann–Whitney-Wilcoxon tests were performed to assess the difference between the two diets for each component. Variables contributing the most to each PCA component were identified. Among the 22 variables used for the PCA, nine were selected based on their contribution to the component for which a statistical differentiation between diets was highlighted: total proteins, beta 1, beta 2, alpha 1, alpha 2, prealbumin, glucose, gamma, and lactate. On the basis of these selected nine parameters, a multicriteria decision analysis (MCDA) was designed using a nonstructural fuzzy decision support system (Tam et al., 2002; see detailed methodology in Annex A1) using a freeware Excel sheet (Tong, 2008). The analysis was led under two hypotheses: differential importance of variables (based on their contribution to the first component of the PCA) or under the hypothesis of equivalence of variables for ranking diets ("equal importance of criteria" hypothesis) (see methodology details in Annex A1).

3 | RESULTS

3.1 | Growth performance

Overall, the mortality over the experiment was similar between the two diet groups (7.14 ± 2.02% for control vs. 7.02 ± 1.85% for innovative diet; $W = 2.5$, $p = 1$), and no mortality was recorded in tagged fish over the experiment. In addition, fish did grow over time ($F = 2248.83$, $p < 0.001$) but neither the tagging ($t = 1.92$, $p = 0.06$) nor the diet treatment ($t = -0.02$, $p = 0.99$) affected the fish weight (Table 5). In addition, the SGR and the PER were not found to be significantly different between the two diet groups (SGR: $t = 0.81$, $p = 0.42$; PER: $t = 1.02$, $p = 0.31$; Table 5) nor regarding the tagging status (SGR: $t = -1.44$, $p = 0.15$; PER: $t = 0.33$, $p = 0.74$, Table 5) over the experimental period. The FCR was not significantly different between diet groups ($H = 1.20$, $p = 0.27$) nor between tagged and untagged fish ($H = 0.02$, $p = 0.89$) (Table 5).

3.2 | Hematological and biochemical indicators

3.2.1 | Stress, health, and welfare parameters

Regarding blood physiological parameters related to stress and welfare, hemoglobin and RBCC were overall not affected either by diet (hemoglobin: $t = -1.88$, $p = 0.07$; RBCC: $t = -0.38$, $p = 0.70$) or the sampling time

TABLE 5 Growth parameters in the control and innovative diet groups and in the untagged and tagged groups: Total weight (mean ± SD; in grams) measured at the three sampling points (T0, T1, and T2), specific growth rate (SGR, %/day), feed conversion ratio (FCR) and protein efficiency ratio (PER).

Variable	Control diet	Innovative diet	Untagged fish	Tagged fish
Weight at T0 (g)	215.9 ± 47.5	212.3 ± 54.2	–	–
Weight at T1 (g)	306.4 ± 59.8	310.9 ± 69.4	307.2 ± 65.9	326.3 ± 46.4
Weight at T2 (g)	397.2 ± 80.5	395.9 ± 91.2	393.8 ± 87.2	429.5 ± 60.5
SGR (%/day)	0.56 ± 0.15	0.58 ± 0.16	0.57 ± 0.16	0.53 ± 0.13
FCR	2.10 ± 1.50	2.08 ± 1.79	2.11 ± 1.71	1.85 ± 0.61
PER	1.26 ± 0.39	1.31 ± 0.40	1.28 ± 0.39	1.31 ± 0.37

Note: Weight at T0 is not reported for the tagged/untagged groups because tag implantation occurred between T0 and T1.

(hemoglobin: $t = -1.05$, $p = 0.30$; RBCC: $=1.68$, $p = 0.10$) (Figure 2). The sampling time did affect the levels of plasma cortisol ($t = -2.43$, $p = 0.02$), plasma glucose ($t = -4.42$, $p < 0.001$), and hematocrit ($t = 5.37$, $p < 0.001$) over the trial but without any effect of the diet (Hematocrit: $t = -0.55$, $p = 0.64$; Glucose: $t = 0.28$, $p = 0.78$; Cortisol: $t = 1.60$, $p = 0.11$) (Figure 2). In more detail, the level of both cortisol and glucose was higher at T1 compared to T2, while the levels of hematocrit were lower at T1 than at T2, regardless of the diet group (Figure 2). For lactate levels in plasma, there was no significant effect of diet ($t = 0.72$, $p = 0.48$) or sampling time ($t = 1.07$, $p = 0.29$) individually, but a significant interaction effect was shown between the two fixed factors ($t = 2.47$, $p < 0.05$) (Figure 2). Post hoc tests indicated that the lactate levels in fish fed the innovative diet were higher at T2 compared to T1, higher than in control fish at T1 and T2, but there was no time effect in fish fed the control diet (Figure 2). In addition, there was no significant difference in the expression of *Hsp70* in the organs targeted between the two diet groups at the end of the organic feed trial in seabream ($t = -1.20$, $p = 0.24$; Figure A1).

3.2.2 | Immune parameters and protein content

No significant difference across time was found in beta 2 levels ($t = 1.94$, $p = 0.06$), while a significant effect of time was evident on total proteins ($t = 9.07$, $p < 0.001$), prealbumin ($t = 5.34$, $p < 0.001$), albumin ($t = 7.90$, $p < 0.001$), alpha 1 ($t = 3.28$, $p < 0.01$), alpha 2 ($t = 4.13$, $p < 0.001$), beta 1 ($t = 5.27$, $p < 0.001$), gamma ($t = 2.24$, $p = 0.03$), and immunoglobulin M ($t = 77.90$, $p < 0.001$), with globally higher levels at T2 compared to T1 (Figure 3). There was no diet effect on prealbumin ($t = 1.66$, $p = 0.10$), albumin ($t = -0.44$, $p = 0.66$), alpha 2 ($t = 1.29$, $p = 0.20$), beta 2 ($t = 0.65$, $p = 0.58$), and gamma ($t = 1.66$, $p = 0.10$) levels (Figure 3). Total proteins ($t = 2.35$, $p = 0.02$), alpha 1 ($t = 3.28$, $p < 0.01$), beta 1 ($t = 2.20$, $p = 0.03$), and Immunoglobulin M ($t = 45.04$, $p < 0.001$) values were higher in plasma of fish fed the innovative diet than in fish fed the control diet (control group) (Figure 3).

3.3 | Swimming activity data recorded by tags

Overall, the swimming activity recorded by tags was affected by the period of the day ($t = -199.32$, $p < 0.001$) and the diet treatment ($t = -2.15$, $p = 0.03$), even if the difference is mainly localized at the end of the day-time (Figure 4a). More precisely, swimming activity was lower at night than during the day, regardless of the diet. Post hoc tests failed to find significant differences between diet groups within the period of the day (day or night) (Figure 4b).

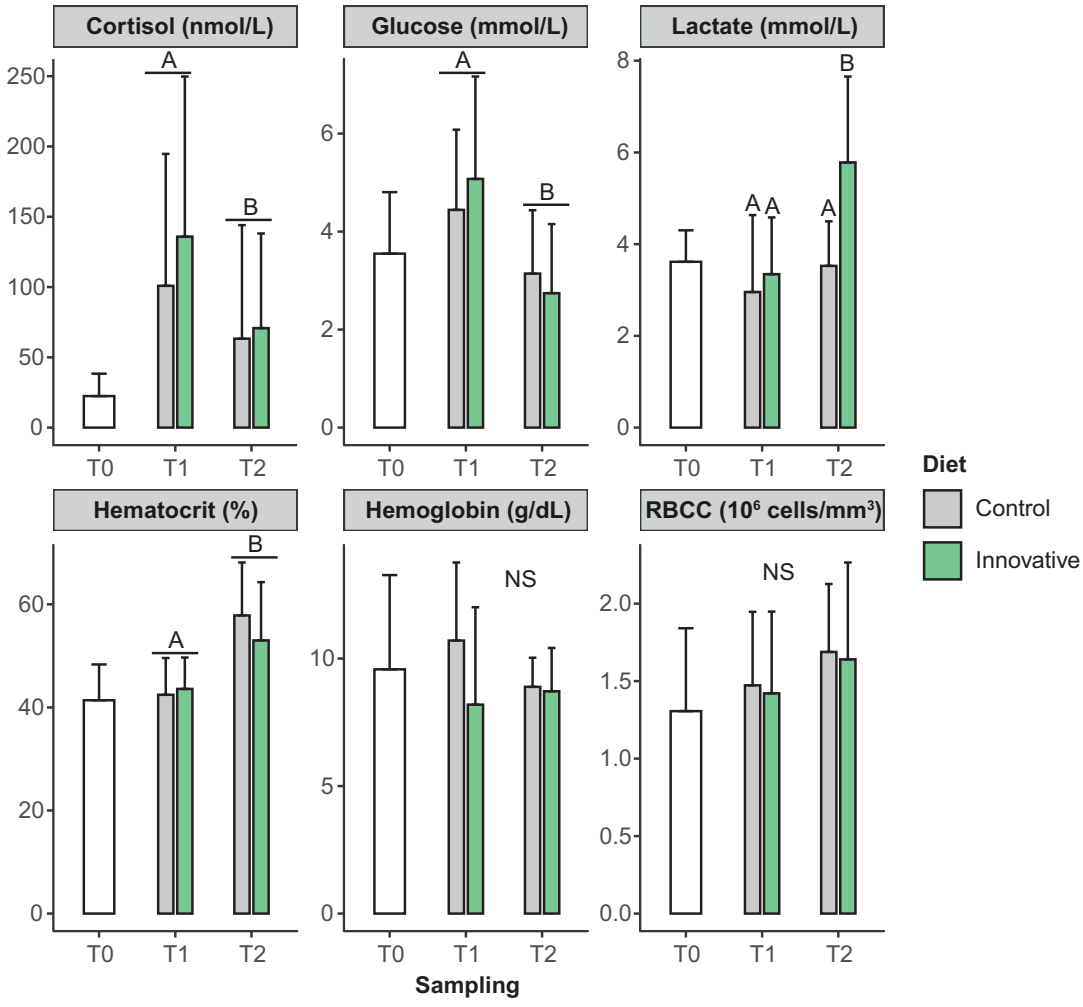

FIGURE 2 Stress, health, and welfare blood physiological parameters (mean ± sd) were measured at the two different sampling points (T1 and T2) in the control (in gray; $n = 13–16$) and innovative diet (in green; $n = 13–16$) groups in *Sparus aurata*. Parameters monitored were cortisol (nmol/L), glucose (mmol/L), lactate (mmol/L), hematocrit (%), hemoglobin (g/dL), and red blood cell count (RBCC; 10^6 cells/mm^3). Significant differences between the two sampling times and/or between diets are indicated in the figure by different letters for each parameter independently; NS (not significant) indicates the absence of time and diet effects. Values obtained at T0 (i.e., before the beginning of the feeding trial) were added in white as reference values.

When looking at the frequency distribution of data registered by tags, the distribution shapes look overall very similar between the two diets, but there is a significant difference in frequency distribution between diets ($\chi^2 = 800.10$, $p < 0.001$). The distribution of swimming activity values for fish fed the control diet is slightly skewed toward higher values than the fish fed the innovative diet (Figure A2).

3.4 | PCA & MCDA results

Based on the parallel analysis, the optimal coordinates, and the acceleration factor methods, the first two components of the PCA were retained, all together explaining 47.34% of the total data variability (Figure 5a). The

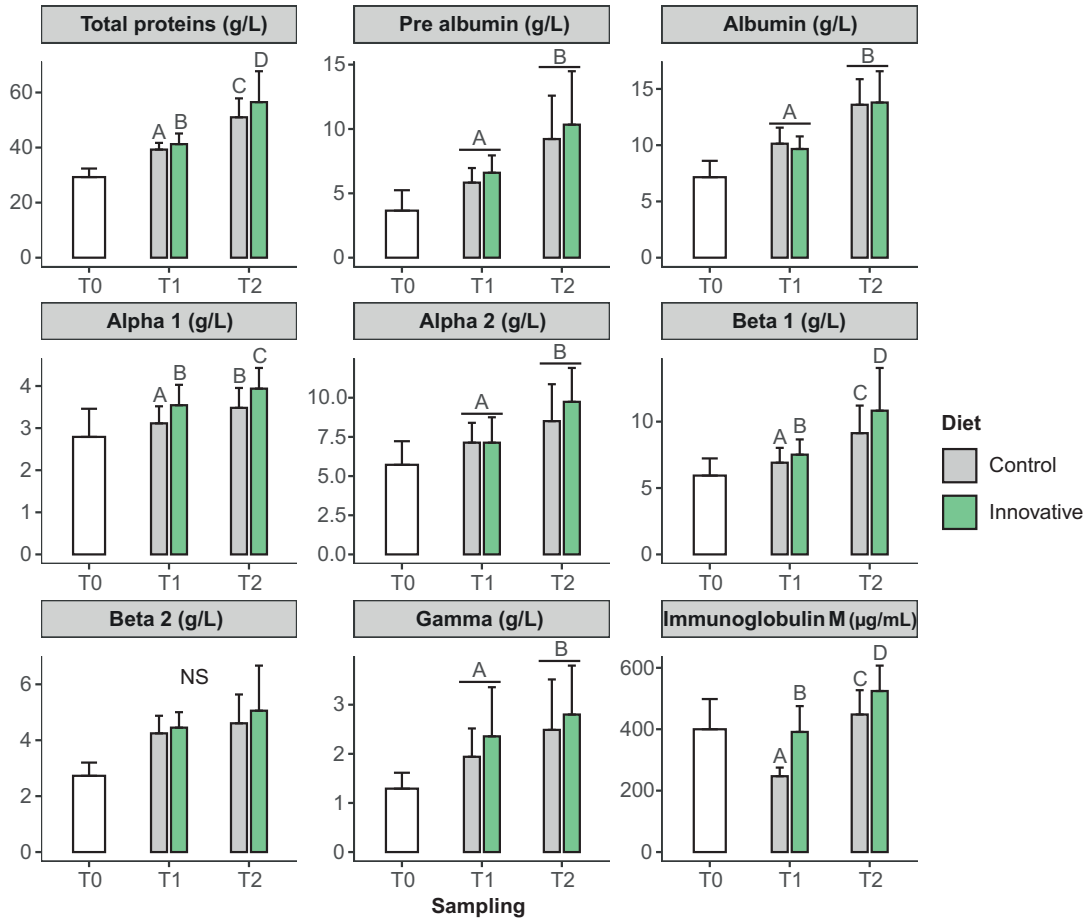

FIGURE 3 Immune parameters and protein content (mean ± sd) were measured at the two different sampling points (T1 and T2) in the control (gray; $n = 15–16$) and innovative diet (green; $n = 10–16$) groups in *Sparus aurata*. Parameters monitored were total proteins (g/L), prealbumin (g/L), albumin (g/L), alpha 1 (g/L), alpha 2 (g/L), beta 1 (g/L), beta 2 (g/L), gamma (g/L), and immunoglobulin M (µg/mL). Statistically significant differences between the two sampling times and/or between diets are indicated in the figure with different letters for each individual parameter; NS (not significant) indicates the absence of time and diet effects. Values obtained at T0 (i.e., before the beginning of the feeding trial) were added in white as reference values.

contribution of the different variables varied greatly within each component (Figure 6). Looking at variables contributing to the first PCA component, total proteins were the most influencing factor (14.03%), followed by beta 2 (10.41%), alpha 1 (10.15%), beta 1 (8.77%), gamma (7.50%), prealbumin (7.33%), glucose (7.16%), lactate (6.05%), and alpha2 (5.20%) (Figure 6). Other variables contributed to the first component less than the expected average contribution if the contribution of the variables were uniform. Globally, individuals displaying low total protein values presented lower values in the different protein fractions, lower lactate values, and higher glucose values (Figure 6). This suggests that individuals with high values on the first PCA component (Figure 5a) generally exhibit lower welfare, despite showing lower lactate levels. Along the second component (Figure 6), individuals with higher weight values also demonstrate higher SGR and PER, lower FCR, and reduced hemoglobin levels. Overall, fish with higher values on the second PCA component (Figure 5a) display better growth performance but lower hemoglobin levels. Looking at the individual fish positioning along the different PCA components, the two organic diets seemed slightly different in the first component (i.e., distinction of two ellipses along component 1 despite a slight overlap in

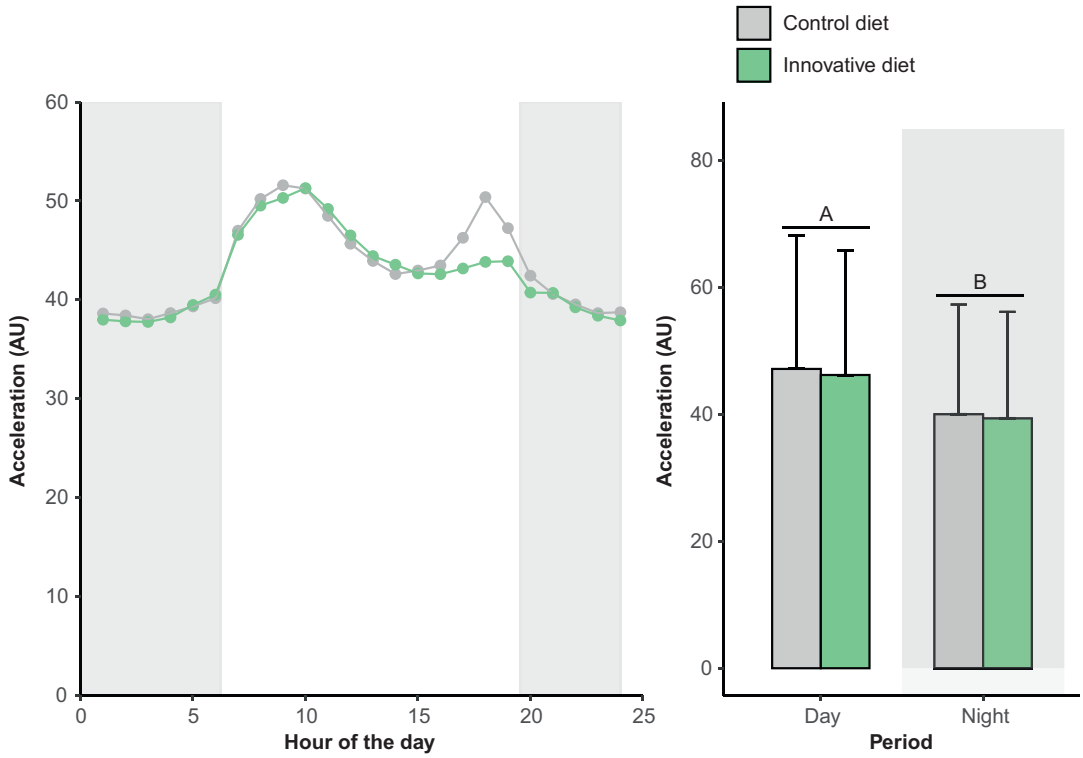

FIGURE 4 Swimming activity (mean ± sd; AU) as a function of (a) hours of the day and (b) period of the day (i.e., day and night) for control diet (gray; $n = 10$) and innovative diet (green; $n = 10$) in *Sparus aurata*. Gray rectangles indicate the dark period of the photoperiod. Different letters indicate significant differences between groups according to post hoc tests.

Figure 5a) but there was a larger overlap in the second component (Figure 5a). Analyses of the principal component scores showed that there was a significant difference between diets for the first component ($W = 109$, $p = 0.03$; Figure 5b) while no significant difference could be seen for the second ($W = 49$, $p = 0.20$; Figure 5c) component.

Regarding the nonstructural fuzzy decision support system analysis, scores of the two diets obtained for each of the nine decision factors considered are reported in Annex A2. For glucose, prealbumin, albumin, beta 2, and gamma, the control diet was set equivalent to the innovative diet. On the other hand, for total proteins, alpha 1, and beta 1, the innovative diet presented the highest score, while the control diet presented a higher score for lactate. Under the hypothesis using weighting scores, the MCDA showed that the parameter characterized by the highest score was total proteins, followed by beta2, while albumin and lactate had the lowest scores (Annex A2). The best score relative to the welfare status of fish was achieved by the innovative diet, regardless of the weighting hypothesis considered (Annex A2).

4 | DISCUSSION

In this study, we conducted a comprehensive evaluation of the effects of an innovative, environmentally friendly, and cost-efficient diet. We evaluated the effects of this diet, characterized by lower fish meal content (51% replacement by a blend of plant and animal protein sources, including notably fermented soy, pea, krill, squid, and yeast meals) on the health and welfare of *S. aurata*.

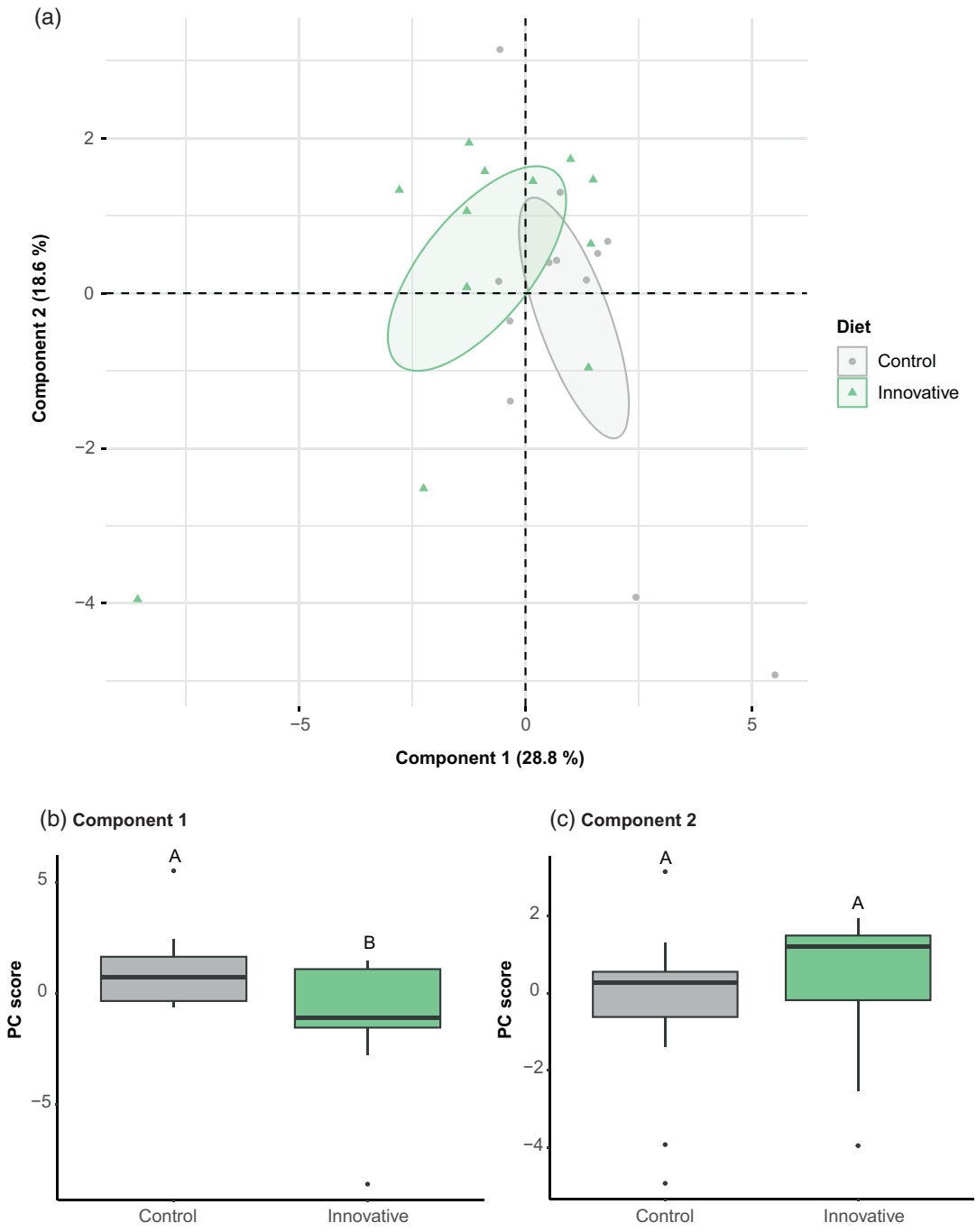

FIGURE 5 Principal component analysis. (a) Visualization of the individual fish positioning on the PCA components 1 and 2 as a function of diet (control diet in gray, innovative diet in green); confidence ellipses were drawn around diet groups with a confidence level of 0.95. (b) PC scores for the first component as a function of diet. (c) PC scores for the second component as a function of diet. For B and C, different letters on boxplots indicate a significant statistical differentiation between diets ($p < 0.05$).

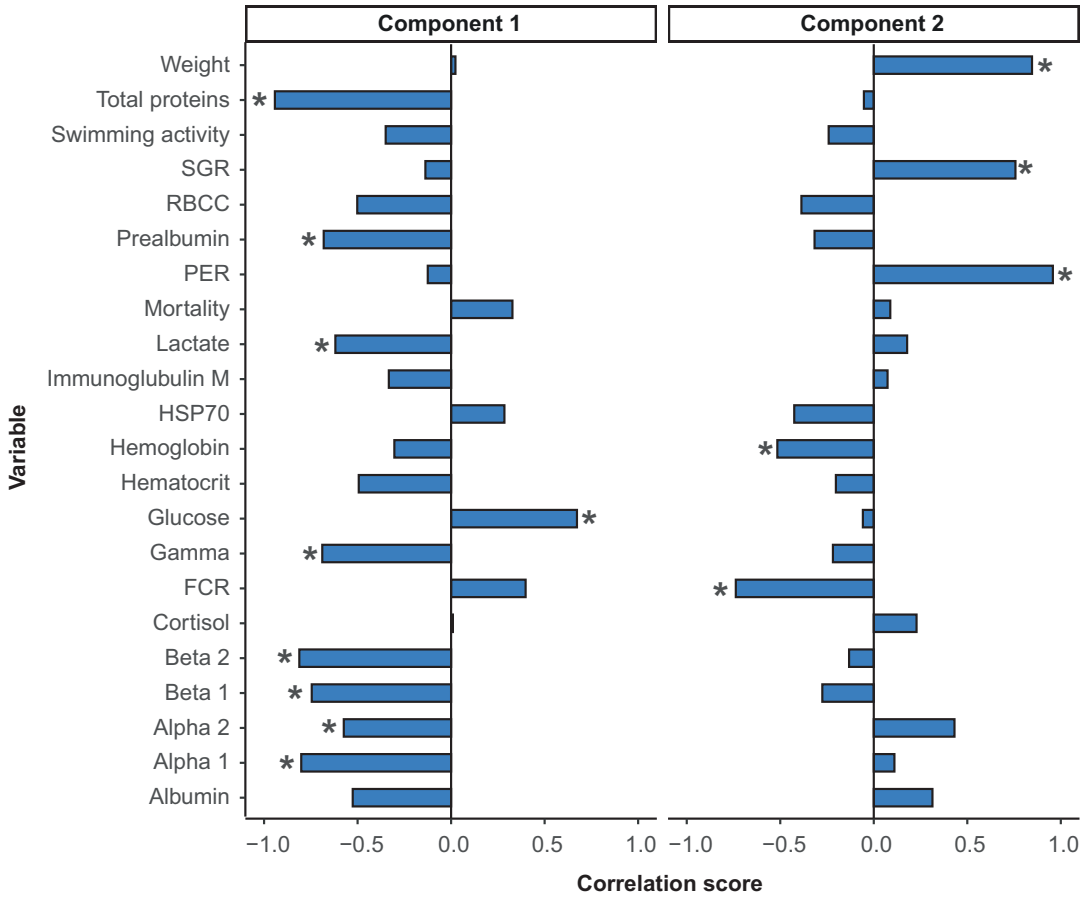

FIGURE 6 Contribution of the 22 variables to the first two components of the principal component analysis. Asterisks indicate variables contributing more than the expected average contribution if the contribution of the variables were uniform for each component.

4.1 | Growth and mortality performance

The introduction of the innovative diet had no discernible impact on the survival and growth performance of gilthead seabream. Irrespective of the diet, individuals exhibited comparable weights at the conclusion of the experiment and key metrics, including mortality rate, SGR, FCR, and PER, were not influenced by the dietary treatment. Previous studies have highlighted the specific effects of different ingredients on growth in various species. For example, fermented soybean meal has been shown to impair growth at high concentrations (e.g., in *Acanthopagrus schlegeli*, Zhou et al., 2011). However, no deleterious impact on growth was observed when used at appropriate concentrations (e.g., Alfonso et al., 2024; Azarm & Lee, 2014; Zhou et al., 2011). The use of exogenous enzymes and krill meal has also been reported to improve growth (Anwar et al., 2020; Saleh et al., 2018; Tharaka et al., 2020; Torrecillas et al., 2021), although this effect was not observed in the present study. As a last example, a previous study on *S. aurata* found that replacing fish meal with a mix of plant-based ingredients, krill meal, and squid meal in varying proportions did not negatively impact growth (Zaragozá et al., 2020), in agreement with what was observed in the present study. However, direct comparisons with studies evaluating the individual effects of ingredients remain limited, as the present study employed a mix of novel ingredients, making it challenging to isolate their individual effects. Overall, the growth parameters measured in this experiment align with existing literature on *S. aurata*

(e.g., Alfonso et al., 2024; Alfonso, Zupa, Manfrin, Fiocchi, Dioguardi, et al., 2020; Carbonara, Alfonso, et al., 2019), providing initial support for the potential future application of such an innovative diet in organic seabream aquaculture.

4.2 | Hematological and biochemical blood indicators

Hematological and biochemical parameters analyzed in this study serve as relevant indicators of fish health and welfare status and are sensitive to a broad spectrum of rearing conditions and associated potential stressors (Carbonara et al., 2015; Carbonara, Dioguardi, et al., 2019; Carbonara, Zupa, et al., 2020; Caruso et al., 2005; Ellis et al., 2012). The values obtained for these parameters fall within the range reported in previous studies on *S. aurata* (Alfonso et al., 2024; Alfonso, Zupa, Manfrin, Fiocchi, Dioguardi, et al., 2020a; Alfonso, Zupa, Manfrin, Fiocchi, Spedicato, et al., 2020b; Carbonara et al., 2022; Carbonara, Alfonso, et al., 2019; Fazio et al., 2020; Gallardo et al., 2003; Sala-Rabanal et al., 2003; Samaras et al., 2023). Our results also provide some valuable basal values, particularly for protein fractions for which there is not much information available in the literature for *Sparus aurata*.

While there was a temporal variation observed in most of the hematological and blood biochemical indicators related to stress and welfare, their levels remained unaffected by the use of the innovative diet. The only parameter directly impacted by the innovative diet was the concentration of lactate, which was higher at T2 in fish fed the innovative diet compared to control individuals. Lactate is implicated in the secondary stress response, and its elevation is associated with anaerobic metabolism resulting from hypoxia or locomotory performance. The rise in seabream fed the innovative diet at T2 could suggest higher stress levels (Schreck & Tort, 2016), but this interpretation was not corroborated by patterns observed in other monitored stress indicators (e.g., cortisol, glucose, *Hsp70*).

Immunological parameters measured in the plasma of seabream exhibited variations over time and between the two organic diets. The plasma of fish fed the innovative diet exhibited higher total protein content. Total proteins in blood play a crucial role in various physiological functions, including maintaining pH and osmotic pressure, transporting metabolites, and interacting with the immune system (Esmaeili, 2021), and are used as an indicator of health status (Coeurdacier et al., 2011; Mansour et al., 2021). In the presence of pathogens or stress factors, total protein concentration is often seen to decrease due to reduced synthesis capacity, decreased absorption, or protein catabolism resulting from increased energy costs of homeostasis (Das et al., 2006; Fazio et al., 2012; Xu et al., 2012; Yang & Chen, 2003). Different peptides, including anti-protease, lysozymes, antibodies, complement factors, and other lytic factors, are present in serum as a first line of defense. These peptides prevent bacteria from adhering to and colonizing the body, hence preventing infection and disease (Kaleeswaran et al., 2012). In this context, the higher level of total proteins in the innovative group could be associated with a stronger innate immune response (Carbonara et al., 2022; Coeurdacier et al., 2011; Mansour et al., 2021). This is also congruent with the swimming activity requiring more energy for the control group than the innovative group (see section 4.3). The higher level of total proteins is congruent with a previous study led on European seabass (*Dicentrarchus labrax*), which evaluated an innovative diet containing plant-based fish meal replacers (soy protein concentrate, fermented soya) and yeast (Alfonso et al., 2023), but total protein variations are different depending on fish meal substitutes and studied species (e.g., Alfonso et al., 2024; Choi et al., 2020; Tharaka et al., 2020; Wang et al., 2020). In more detail for globulin results, higher levels of IgM were observed in fish fed the innovative diet compared to those fed the control diet. IgM, known as a major component of the vertebrate humoral immune system in teleosts (Cuesta et al., 2004; Ellis, 1998; Wilson et al., 1995), typically shows increased levels in response to immunostimulant administration in seabream (Cuesta et al., 2004). This suggests a potential enhancement in the immune defenses of fish fed the innovative diet. Furthermore, specific changes were noted in the concentration of alpha 1 and beta 1 globulin in the plasma of fish fed the innovative diet compared to those fed the control diet. Both alpha and beta globulins constitute acute-phase inflammatory proteins in fish (Christiansen et al., 2015; Kumar et al., 2018; Manera & Britti, 2008) and their levels are regularly used as an indicator of immune response (Hassaan et al., 2019; Mansour et al., 2021).

These findings suggest a potential immunostimulant effect of the innovative diet since environmental conditions were the same among diets. Plant-based proteins have been reported in other studies to induce alterations in immune status (see Aragão et al., 2022 and references therein), while the inclusion of yeast and krill meal has been shown to enhance fish immunity (Mahdy et al., 2022; Sultana et al., 2024; Tharaka et al., 2020), likely due to the provision of β-glucans (Khanjani et al., 2022; Sultana et al., 2024) and chitin (Ringø et al., 2012). In a similar study, a potential immunostimulant effect of a diet containing plant-based (i.e., soy protein concentrate, fermented soy, pea protein, wheat gluten meal), yeast, and krill meals was shown in *S. aurata* (Alfonso et al., 2024). However, the specific effects of individual ingredients cannot be determined, as the innovative diet consisted of a mixture of alternative fishmeal substitutes. Further studies, both in vivo and in vitro, are needed to unravel the underlying reasons for the serum changes observed in this study.

4.3 | Swimming activity recorded by the tag

Swimming activity serves as a widely employed welfare marker in fish, providing a reliable indicator of oxygen consumption and serving as a proxy for energy expenditure in various fish species, including *S. aurata* (Alfonso et al., 2021, 2022, 2024; Carbonara et al., 2015, 2021; Carbonara, Alfonso, et al., 2019; Carbonara, Zupa, et al., 2020; Martins et al., 2012; Zupa et al., 2015, 2021). Higher swimming activity generally implies a greater reliance on anaerobic metabolism, suggesting lower availability of energetic reserves for other biological functions or potential future stressful events. Internally implanted acoustic transmitters are now widely utilized across various taxa (Lennox et al., 2023), offering various advantages compared to external or gastric tags (Thorstad et al., 2013). However, it is important to note that the implantation procedure is invasive and may potentially induce behavioral and physiological stress responses (Macaulay et al., 2021). Consequently, the potential impacts of tag implementation require investigation, not only at a species level but also for each case study (Alfonso, Zupa, Manfrin, Fiocchi, Dioguardi, et al., 2020). In adherence to the recommended "2 % rule," the study ensured that the tag mass/body mass ratio was within an acceptable range (1.65 ± 0.18%). Tagging was demonstrated to have no lethal impact, as no tagged fish experienced mortality, falling within the expected mortality range in tanks according to Macaulay et al. (2021) (mean: 2.5%, range = 0%–17%). Moreover, no significant differences were observed between tagged and untagged fish in terms of final weight, SGR, FCR, or PER, indicating a lack of detrimental impact on growth. However, it is crucial to acknowledge that the assessment of tagging effects in this study was limited to a specific set of traits related to mortality and growth. Conclusions should not be extrapolated to other biological functions, and further investigations are warranted on additional traits, considering that deleterious impacts of tag implantation have been reported in other species (e.g., Georgopoulou et al., 2022; Macaulay et al., 2021; Neves et al., 2018). Nevertheless, the current findings align with existing literature on *S. aurata*, which has reported either no impact (Alfonso, Zupa, Manfrin, Fiocchi, Dioguardi, et al., 2020a; Arechavala-Lopez et al., 2021) or minimal impact (e.g., no change in behavioral or physiological parameters except for higher cortisol values 9 days after surgery; Montoya et al., 2012) from transmitter implantation. The lack of negative impact from tag implantation has also been documented for other species (e.g., Ammann et al., 2013; Childs et al., 2011; Klinard et al., 2018; Koed & Thorstad, 2001). Overall, this supports the hypothesis that tagged individuals can be considered representative of the studied population, and hence the use of tags could be promising for monitoring the health and welfare of farmed fish in the context of organic aquaculture.

The acceleration values recorded throughout the experiment fall within the reported range for *S. aurata* (Alfonso et al., 2024; Carbonara et al., 2022; Carbonara, Alfonso, et al., 2019). The diet treatment affected the acceleration recorded by tags, but the difference was mainly localized at the end of the daytime (2 h before the dark phase of the photoperiod). Electromyogram analyses of red and white muscle activity during swimming in *S. aurata* indicate that swimming activity values below 80 AU are fueled by aerobic metabolism, while values above 80 AU involve progressive energy consumption from anaerobic metabolism until 255 AU (the limit of tag measurement; Alfonso

et al., 2021). In this study, fish fed the innovative diet, therefore, exhibited swimming activities principally fueled by aerobic metabolism. These fish are thus better equipped to handle stress due to their physiological state, which reduces the consumption of anaerobic reserves. The control group, on the other hand, used a higher percentage of anaerobic metabolism, which indicates a reduced metabolic energy storage that would be helpful in handling stressful conditions (Alfonso et al., 2021; Lembo et al., 2007). However, these differences, although statistically significant, are subtle and not reflected in either plasma lactate levels or fish growth. This suggests that the metabolic costs associated with higher swimming activity are either low or compensated by the control diet. The statistical significance of these differences should be interpreted cautiously, given the large amount of data collected by the tags (932,775 swimming speed values collected over the monitoring period).

4.4 | Multiparametric approach

The individual investigation of growth, physiological, and immunological traits in this study provides valuable and essential information on the effects of the innovative diet. However, due to the multifaceted nature of welfare, drawing conclusions can be challenging, given potentially contradictory indications from different traits (i.e., here, no diet difference for some traits, lactate highlighted the control diet as more suitable while for instance swimming activity indicated the innovative diet as more interesting from a welfare perspective). The multiparametric approach employed here offers a more comprehensive and global understanding of the impact of new aquafeeds on fish health and welfare by considering all pieces of evidence in a single analysis. Despite limitations, such as the loss of information for parameters providing more than one value per fish (e.g., swimming activity averaged per fish) or the consideration of a unique final sampling point to homogenize data among parameters (i.e., exclusion of T1 values for physiological parameters), the multiparametric approach facilitates assessment and allows for a global overview. In this study, akin to its application to sea bass in Carbonara, Alfonso, et al. (2020), the multiparametric approach highlighted a difference in welfare between the two diets, particularly in the first component of the PCA. Subsequent components did not reveal significant differences. The differentiation between dietary groups in the PCA was driven by four traits showing a diet effect in individual analyses (i.e., total proteins, alpha1, beta1, and lactate), and five traits that did not stand out in individual analyses (i.e., beta2, prealbumin, glucose, gamma, and albumin). Other traits did not significantly contribute to the first component, including two traits for which a diet effect was highlighted in single-trait analyses (i.e., IgM and swimming activity). Discrepancies with single-trait analyses can be attributed to various factors such as the consideration of solely T2 (e.g., stronger IgM difference between diets at T1 than at T2; Figure 3), value averaging per fish (e.g., slight diet difference in swimming activity visible only at the end of the day phase [Figure 4a]; averaging values per fish may erase differences), the fact that PCA considers correlations between traits, and/or the possibility that some traits partly explain inter-fish variability within the same diet group along the PCA first component (Figure 6a) and contribute less to inter-diet differentiation. Interpreting PCA results directly to determine which diet yields the optimal welfare and health status can be challenging, as certain parameters may favor different diets (e.g., lactate vs. total proteins). The MCDA offers a comprehensive approach and reveals that the innovative diet promotes the best health and welfare for fish, aligning with expectations from individual statistical analyses. Assessing fish welfare remains complex due to its multifaceted nature. Combining functional-based and feeling-based traits provides a comprehensive perspective, and employing multiparametric approaches facilitates reaching a consensus on fish welfare status under varying rearing conditions.

5 | CONCLUSION AND PERSPECTIVES

In conclusion, the introduction of the innovative diet did not have a negative impact on the growth performance and survival of seabream individuals. Furthermore, hematological and biochemical blood indicators remained mostly

unaffected by the innovative diet, collectively suggesting no adverse effects on welfare. The increased levels of total proteins and particular globulins in the plasma of fish fed the innovative diet may indicate an enhancement of innate immunity. The multiparametric analysis provided a comprehensive overview of the diet effects on fish welfare, indicating a differential impact of the two diets on fish welfare, with the innovative diet resulting in the best fish health and welfare. Overall, the cost-effective, innovative, and eco-friendly diet tested in this study appears promising for organic aquaculture. Nonetheless, further research is essential to investigate the underlying causes of the changes observed in immune parameters and long-term effects. Additionally, other aspects related to the use of this new diet should be assessed, such as its environmental impact, particularly in open systems like sea cages, where factors like waste discharge may potentially trigger eutrophication and impact benthic assemblages (see for instance, Ballester-Moltó et al., 2017 and Di Marco et al., 2017).

ACKNOWLEDGMENTS

The authors are grateful to the COISPA team (Pasquale Gallo, Stefano Fazi, and Cosmidano Neglia) for running the experiments and providing valuable help during sampling. The authors also thank Emilie Levavasseur (emilie.levavasseur@laposte.net) for the European sea bass drawing in Figure 1. The Synergen™ product was provided by Alltech (US).

FUNDING INFORMATION

The study was funded by the H2020 FutureEUAqua project under the grant agreement number 817737.

CONFLICT OF INTEREST STATEMENT

P.V. is an employee of IRIDA S.A. and the company is a partner in the FutureEUAqua project. Other co-authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Lola Toomey https://orcid.org/0000-0001-7060-6698

REFERENCES

Agboola, J. O., Øverland, M., Skrede, A., & Hansen, J. Ø. (2021). Yeast as major protein-rich ingredient in aquafeeds: A review of the implications for aquaculture production. *Reviews in Aquaculture*, *13*, 949–970. https://doi.org/10.1111/raq.12507

Alfonso, S., Mente, E., Fiocchi, E., Manfrin, A., Dimitroglou, A., Papaharisis, L., Barkas, D., Toomey, L., Boscarato, M., Losasso, C., Peruzzo, A., Stefani, A., Zupa, W., Spedicato, M. T., Nengas, I., Lembo, G., & Carbonara, P. (2023). Growth performance, gut microbiota composition, health and welfare of European Sea bass (Dicentrarchus Labrax) fed an environmentally and economically sustainable low marine protein diet in sea cages. *Scientific Reports*, *13*, 21269. https://doi.org/10.1038/s41598-023-48533-3

Alfonso, S., Toomey, L., Fiocchi, E., Manfrin, A., Boscarato, M., Vasilaki, P., Zupa, W., Bertazzo, V., Bégout, M.-L., Spedicato, M. T., Mente, E., Nengas, I., Lembo, G., & Carbonara, P. (2024). Growth performance, immune characteristics, and health and welfare of gilthead seabream (Sparus aurata) fed a tailor-made environmentally sustainable diet formulated using novel ingredients. *Aquaculture Research*, *2024*(1), 8234882. https://doi.org/10.1155/2024/8234882

Alfonso, S., Zupa, W., Manfrin, A., Fiocchi, E., Dioguardi, M., Dara, M., Lembo, G., Carbonara, P., & Cammarata, M. (2020a). Surgical implantation of electronic tags does not induce medium-term effect: Insights from growth and stress physiological profile in two marine fish species. *Animal Biotelemetry*, *8*, 21. https://doi.org/10.1186/s40317-020-00208-w

Alfonso, S., Zupa, W., Manfrin, A., Fiocchi, E., Spedicato, M. T., Lembo, G., & Carbonara, P. (2020b). Stress coping styles: Is the basal level of stress physiological indicators linked to behaviour of sea bream? *Applied Animal Behaviour Science*, *231*, 105085. https://doi.org/10.1016/j.applanim.2020.105085

Alfonso, S., Zupa, W., Spedicato, M. T., Lembo, G., & Carbonara, P. (2021). Mapping the energetic costs of free-swimming Gilthead Sea marine aquaculture. *Biology*, *10*, 1357. https://doi.org/10.3390/biology10121357

Alfonso, S., Zupa, W., Spedicato, M. T., Lembo, G., & Carbonara, P. (2022). Using telemetry sensors mapping the energetic costs in European Sea bass (Dicentrarchus Labrax), as a tool for welfare remote monitoring in aquaculture. *Frontiers in Animal Science*, *3*, 885850. https://doi.org/10.3389/fanim.2022.885850

Ammann, A. J., Michel, C. J., & MacFarlane, R. B. (2013). The effects of surgically implanted acoustic transmitters on laboratory growth, survival and tag retention in hatchery yearling Chinook Salmon. *Environmental Biology of Fishes*, *96*, 135–143. https://doi.org/10.1007/s10641-011-9941-9

Anwar, A., Wan, A. H., Omar, S., El-Haroun, E., & Davies, S. J. (2020). The potential of a solid-state fermentation supplement to augment white lupin (Lupinus albus) meal incorporation in diets for farmed common carp (Cyprinus carpio). *Aquaculture Reports*, *17*, 100348. https://doi.org/10.1016/j.aqrep.2020.100348

Aragão, C., Gonçalves, A. T., Costas, B., Azeredo, R., Xavier, M. J., & Engrola, S. (2022). Alternative proteins for fish diets: Implications beyond growth. *Animals*, *12*, 1211. https://doi.org/10.3390/ani12091211

Arechavala-Lopez, P., Lankheet, M. J., Díaz-Gil, C., Abbink, W., & Palstra, A. P. (2021). Swimming activity of gilthead seabream (Sparus aurata) in swim-tunnels: Accelerations, oxygen consumption and body motion. *Frontiers in Animal Science*, *2*, 679848. https://doi.org/10.3389/fanim.2021.679848

Azarm, H. M., & Lee, S. M. (2014). Effects of partial substitution of dietary fish meal by fermented soybean meal on growth performance, amino acid and biochemical parameters of juvenile black sea bream Acanthopagrus schlegeli. *Aquaculture Research*, *45*(6), 994–1003. https://doi.org/10.1111/are.12040

Ballester-Moltó, M., Follana-Berná, G., Sanchez-Jerez, P., & Aguado-Giménez, F. (2017). Total nitrogen, carbon and phosphorus digestibility in gilthead seabream (Sparus aurata) and European seabass (Dicentrarchus labrax) fed with conventional and organic commercial feeds: Implications for particulate waste production. *Aquaculture Research*, *48*, 3450–3463. https://doi.org/10.1111/are.13171

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2014). Fitting linear mixed-effects models using Lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01

Brijs, J., Sandblom, E., Axelsson, M., Sundell, K., Sundh, H., Huyben, D., Broström, R., Kiessling, A., Berg, C., & Gräns, A. (2018). The final countdown: Continuous physiological welfare evaluation of farmed fish during common aquaculture practices before and during harvest. *Aquaculture*, *495*, 903–911. https://doi.org/10.1016/j.aquaculture.2018.06.081

Busacca, E., & Lembo, G. (2019). EU regulation on organic aquaculture. In G. Lembo & E. Mente (Eds.), *Organic aquaculture: Impacts and future developments* (pp. 23–39). Springer. https://doi.org/10.1007/978-3-030-05603-2_2

Carbonara, P., Alfonso, S., Dioguardi, M., Zupa, W., Vazzana, M., Dara, M., Spedicato, M. T., Lembo, G., & Cammarata, M. (2021). Calibrating accelerometer data, as a promising tool for health and welfare monitoring in aquaculture: Case study in European Sea bass (Dicentrarchus labrax) in conventional or organic aquaculture. *Aquaculture Reports*, *21*, 100817. https://doi.org/10.1016/j.aqrep.2021.100817

Carbonara, P., Alfonso, S., Gai, F., Gasco, L., Palmegiano, G., Spedicato, M. T., Zupa, W., & Lembo, G. (2020). Moderate stocking density does not influence the Behavioural and physiological responses of rainbow trout (Oncorhynchus mykiss) in organic aquaculture. *Aquaculture Research*, *51*, 3007–3016. https://doi.org/10.1111/are.14640

Carbonara, P., Alfonso, S., Zupa, W., Manfrin, A., Fiocchi, E., Buratin, A., Bertazzo, V., Cammarata, M., Spedicato, M. T., & Lembo, G. (2022). Investigating the physiological response and antibody concentration of Gilthead Sea bream (Sparus aurata) following vibrio anguillarum vaccination depending on the stress coping style. *Frontiers in Animal Science*, *3*, 951179. https://doi.org/10.3389/fanim.2022.951179

Carbonara, P., Alfonso, S., Zupa, W., Manfrin, A., Fiocchi, E., Pretto, T., Spedicato, M. T., & Lembo, G. (2019). Behavioral and physiological responses to stocking density in sea bream (Sparus aurata): Do coping styles matter? *Physiology and Behavior*, *212*, 112698. https://doi.org/10.1016/j.physbeh.2019.112698

Carbonara, P., Dioguardi, M., Cammarata, M., Zupa, W., Vazzana, M., Spedicato, M. T., & Lembo, G. (2019). Basic knowledge of social hierarchies and physiological profile of Reared Sea bass Dicentrarchus labrax (L.). *PLoS One*, *14*, e0208688. https://doi.org/10.1371/journal.pone.0208688

Carbonara, P., Scolamacchia, M., Spedicato, M. T., Zupa, W., Mckinley, R. S., & Lembo, G. (2015). Muscle activity as a key indicator of welfare in farmed European Sea bass (Dicentrarchus labrax L. 1758). *Aquaculture Research*, *46*, 2133–2146. https://doi.org/10.1111/are.12369

Carbonara, P., Zupa, W., Bitetto, I., Alfonso, S., Dara, M., & Cammarata, M. (2020). Evaluation of the effects of the enriched-organic diets composition on European Sea bass welfare through a multi-parametric approach. *Journal of Marine Science and Engineering*, *8*, 934. https://doi.org/10.3390/jmse8110934

Caruso, G., Genovese, L., Maricchiolo, G., & Modica, A. (2005). Haematological, biochemical and immunological parameters as stress indicators in Dicentrarchus labrax and Sparus aurata farmed in off-shore cages. *Aquaculture International*, *13*, 67–73. https://doi.org/10.1007/s10499-004-9031-5

Childs, A. R., Næsje, T. F., & Cowley, P. D. (2011). Long-term effects of different-sized surgically implanted acoustic transmitters on the sciaenid Arygyrosomus japonicus: Breaking the 2% tag-to-body mass rule. *Marine and Freshwater Research*, *62*, 432–438. https://doi.org/10.1071/MF10219

Choi, J., Lee, K. W., Han, G. S., Byun, S.-G., Lim, H. J., & Kim, H. S. (2020). Dietary inclusion effect of krill meal and various fish meal sources on growth performance, feed utilization, and plasma chemistry of grower walleye Pollock (Gadus Chalcogrammus, Pallas 1811). *Aquaculture Reports*, *17*, 100331. https://doi.org/10.1016/j.aqrep.2020.100331

Christiansen, E. F., Cray, C., Lewbart, G. A., & Harms, C. A. (2015). Plasma protein electrophoresis and acute phase proteins in koi carp (Cyprinus carpio) following exploratory Coeliotomy. *Journal of Exotic Pet Medicine*, *24*, 76–83. https://doi.org/10.1053/j.jepm.2014.11.008

Coeurdacier, J.-L., Dutto, G., Gasset, E., & Blancheton, J.-P. (2011). Is total serum protein a good indicator for welfare in reared sea bass (Dicentrarchus labrax)? *Aquatic Living Resources*, *24*, 121–127. https://doi.org/10.1051/alr/2011130

Cuesta, A., Meseguer, J., & Esteban, M. A. (2004). Total serum immunoglobulin M levels are affected by immunomodulators in seabream (Sparus aurata L.). *Veterinary Immunology and Immunopathology*, *101*, 203–210. https://doi.org/10.1016/j.vetimm.2004.04.021

Dara, M., Carbonara, P., la Corte, C., Parrinello, D., Cammarata, M., & Parisi, M. G. (2023). Fish welfare in aquaculture: Physiological and immunological activities for diets, social and spatial stress on Mediterranean aqua cultured species. *Fishes*, *8*, 414. https://doi.org/10.3390/fishes8080414

Das, P. C., Ayyappan, S., & Jena, J. K. (2006). Haematological changes in the three Indian major carps, Catla catla (Hamilton), Labeo rohita (Hamilton) and Cirrhinus mrigala (Hamilton) exposed to acidic and alkaline water pH. *Aquaculture*, *256*, 80–87. https://doi.org/10.1016/j.aquaculture.2006.02.019

Di Marco, P., Petochi, T., Marino, G., Priori, A., Finoia, M. G., Tomassetti, P., Porrello, S., Giorgi, G., Lupi, P., Bonelli, A., & Parisi, G. (2017). Insights into organic farming of European sea bass Dicentrarchus labrax and gilthead sea bream Sparus aurata through the assessment of environmental impact, growth performance, fish welfare and product quality. *Aquaculture*, *471*, 92–105. https://doi.org/10.1016/j.aquaculture.2017.01.012

Ellis, A. E. (1998). Fish immune system. In P. J. Delves & I. M. Roitt (Eds.), *Encyclopedia of immunology* (pp. 920–926). Academic Press.

Ellis, T., Yildiz, H. Y., López-Olmeda, J., Spedicato, M. T., Tort, L., Øverli, Ø., & Martins, C. I. M. (2012). Cortisol and finfish welfare. *Fish Physiology and Biochemistry*, *38*, 163–188. https://doi.org/10.1007/s10695-011-9568-y

Esmaeili, M. (2021). Blood performance: A new formula for fish growth and health. *Biology*, *10*(12), 1236. https://doi.org/10.3390/biology10121236

Estévez, A., & Vasilaki, P. (2023). Organic production of Gilthead Sea bream (Sparus aurata) using organic certified green pea protein and seaweed. Effects on growth, feed conversion and final product quality. *Aquaculture*, *571*, 739490. https://doi.org/10.1016/j.aquaculture.2023.739490

EUMOFA. (2022). Organic Aquaculture in the EU. Current Situation, Drivers, Barriers, Potential for Growth. Report.

Fazio, F., Marafioti, S., Torre, A., Sanfilippo, M., Panzera, M., & Faggio, C. (2012). Haematological and serum protein profiles of Mugil cephalus: Effect of two different habitats. *Ichtyological Research*, *60*, 36–42. https://doi.org/10.1007/s10228-012-0303-1

Fazio, F., Saoca, C., Fortino, G., Piccione, G., & Zumbo, A. (2020). Seasonal biochemical changes in two aquaculture species Sparus aurata Linnaeus, 1758 and Dicentrarchus labrax Linnaeus, 1758. *Iranian Journal of Fisheries Sciences*, *19*, 1757–1769. https://doi.org/10.22092/ijfs.2019.119854

Fiocchi, E., Civettini, M., Carbonara, P., Zupa, W., Lembo, G., & Manfrin, A. (2020). Development of molecular and histological methods to evaluate stress oxidative biomarkers in sea bass (Dicentrarchus labrax). *Fish Physiology and Biochemistry*, *46*, 1577–1588. https://doi.org/10.1007/s10695-020-00811-x

Gallardo, M. Á., Sala-Rabanal, M., Ibarz, A., Padrós, F., Blasco, J., Fernández-Borràs, J., & Sánchez, J. (2003). Functional alterations associated with "winter syndrome" in Gilthead Sea bream (Sparus aurata). *Aquaculture*, *223*, 15–27. https://doi.org/10.1016/S0044-8486(03)00164-9

Gambelli, D., Vairo, D., Solfanelli, F., & Zanoli, R. (2019). Economic performance of organic aquaculture: A systematic review. *Marine Policy*, *108*, 1035542. https://doi.org/10.1016/j.marpol.2019.103542

Gasco, L., Gai, F., Maricchiolo, G., Genovese, L., Ragonese, S., Bottari, T., & Caruso, G. (2018). Fishmeal alternative protein sources for aquaculture feeds. In *Feeds for the aquaculture Secto* (pp. 1–28). Springer. https://doi.org/10.1007/978-3-319-77941-6_1

Gatlin, D. M., Barrows, F. T., Brown, P., Dabrowski, K., Gaylord, T. G., Hardy, R. W., Herman, E., Hu, G., Krogdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., J Souza, E., Stone, D., Wilson, R., & Wurtele, E. (2007). Expanding the utilization of sustainable plant products in aquafeeds: A review. *Aquaculture Research*, *38*, 551–579. https://doi.org/10.1111/j.1365-2109.2007.01704.x

Georgopoulou, D. G., Fanouraki, E., Voskakis, D., Mitrizakis, N., & Papandroulakis, N. (2022). European seabass show variable responses in their group swimming features after tag implantation. *Frontiers in Animal Science*, *3*, 997948. https://doi.org/10.3389/fanim.2022.997948

Gesto, M., Zupa, W., Alfonso, S., Spedicato, M. T., Lembo, G., & Carbonara, P. (2020). Using acoustic telemetry to assess behavioral responses to acute hypoxia and ammonia exposure in farmed rainbow trout of different competitive ability. *Applied Animal Behaviour Science*, *230*, 105084. https://doi.org/10.1016/j.applanim.2020.105084

Ghosh, K., & Ray, A. K. (2017). Aquafeed formulation using plant feedstuffs: Prospective application of fish-gut microorganisms and microbial biotechnology. In *Soft chemistry and food fermentation, handbook of food bioengineering* (pp. 109–144). Academic Press, Elsevier. https://doi.org/10.1016/B978-0-12-811412-4.00005-9

Glencross, B. D., Baily, J., Berntssen, M. H. G., Hardy, R., MacKenzie, S., & Tocher, D. R. (2020). Risk assessment of the use of alternative animal and plant raw material resources in aquaculture feeds. *Reviews in Aquaculture*, *12*, 703–758. https://doi.org/10.1111/raq.12347

González-Rodríguez, Á., Celada, J. D., Carral, J. M., Sáez-Royuela, M., & Fuertes, J. B. (2016). Evaluation of pea protein concentrate as partial replacement of fish meal in practical diets for juvenile Tench (Tinca tinca L.). *Aquaculture Research*, *47*, 2825–2834. https://doi.org/10.1111/are.12732

Gould, D., Compagnoni, A., & Lembo, G. (2019). Organic aquaculture: Principles, standards and certification. In G. Lembo & E. Mente (Eds.), *Organic aquaculture: Impacts and future developments* (pp. 11–22). Springer. https://doi.org/10.1007/978-3-030-05603-2_1

Hassaan, M. S., El-Sayed, A. I. M., Soltan, M. A., Iraq, M. M., Goda, A. M., Davies, S. J., El-Haroun, E. R., & Ramadan, H. A. (2019). Partial dietary fish meal replacement with cotton seed meal and supplementation with exogenous protease alters growth, feed performance, hematological indices and associated gene expression markers (GH, IGF-I) for Nile tilapia, Oreochromis niloticus. *Aquaculture*, *503*, 282–292. https://doi.org/10.1016/j.aquaculture.2019.01.009

Hodar, A. R., Vasava, R. J., Mahavadiya, D. R., & Joshi, N. H. (2020). Fish meal and fish oil replacement for aqua feed formulation by using alternative sources: A review. *Journal of Experimental Zoology India*, *23*, 13–21.

Hossain, M. S., Small, B. C., Kumar, V., & Hardy, R. (2024). Utilization of functional feed additives to produce cost-effective, ecofriendly aquafeeds high in plant-based ingredients. *Reviews in Aquaculture*, *16*, 121–153. https://doi.org/10.1111/raq.12824

Hua, K., Cobcroft, J. M., Cole, A., Condon, K., Jerry, D. R., Mangott, A., Praeger, C., Vucko, M. J., Zeng, C., Zenger, K., & Strugnell, J. M. (2019). The future of aquatic protein: Implications for protein sources in aquaculture diets. *One Earth*, *1*, 316–329. https://doi.org/10.1016/j.oneear.2019.10.018

Huntingford, F. A., & Kadri, S. (2014). Defining, assessing and promoting the welfare of farmed fish. *Revue Scientifique et Technique*, *33*, 233–244. https://doi.org/10.20506/rst.33.1.2286

Kader, M. A., & Koshio, S. (2012). Effect of composite mixture of seafood by-products and soybean proteins in replacement of fishmeal on the performance of Red Sea bream, Pagrus major. *Aquaculture*, *368*, 95–102. https://doi.org/10.1016/j.aquaculture.2012.09.014

Kaleeswaran, B., Ilavenil, S., & Ravikumar, S. (2012). Changes in biochemical, histological and specific immune parameters in Catla catla (ham.) by Cynodon dactylon (L.). *Journal of King Saud University, Science*, *24*, 139–152. https://doi.org/10.1016/j.jksus.2010.10.001

Kassambara, A., & Mundt, F. (2020). Package 'Factoextra': Extract and Visualize the Results of Multivariate Data Analyses. CRAN- R Package, 84.

Khanjani, M. H., Sharifinia, M., & Ghaedi, G. (2022). Β-glucan as a promising food additive and immunostimulant in aquaculture industry. *Annals of Animal Science*, *22*, 817–827. https://doi.org/10.2478/aoas-2021-0083

Kissinger, K. R., García-Ortega, A., & Trushenski, J. T. (2016). Partial fish meal replacement by soy protein concentrate, squid and algal meals in low fish-oil diets containing Schizochytrium limacinum for longfin yellowtail Seriola rivoliana. *Aquaculture*, *452*, 37–44. https://doi.org/10.1016/j.aquaculture.2015.10.022

Klinard, N. V., Halfyard, E. A., Fisk, A. T., Stewart, T. J., & Johnson, T. B. (2018). Effects of surgically implanted acoustic tags on body condition, growth, and survival in a Small, laterally compressed forage fish. *Transactions of the American Fisheries Society*, *147*, 749–757. https://doi.org/10.1002/tafs.10064

Koed, A., & Thorstad, E. B. (2001). Long-term effect of radio-tagging on the swimming performance of pikeperch. *Journal of Fish Biology*, *58*, 1753–1756. https://doi.org/10.1111/j.1095-8649.2001.tb02329.x

Kumar, V., Kumar, K., Raman, R. P., Prasad, K. P., Kumar, N., Kumar, S., Kumar, V., & Roy, S. (2018). Evaluation of cellular induction, soluble components of proteins and expression of pro-inflammatory genes in Labeo rohita fingerlings. *Journal of Environmental Biology*, *39*, 486–493. https://doi.org/10.22438/jeb/39/4/MRN-378

Le, S., Josse, J., & Husson, F. (2008). FactoMineR: An R package for multivariate analysis. *Journal of Statistical Software*, *5*, 1–18. https://doi.org/10.18637/jss.v025.i01

Lembo, G., Carbonara, P., Scolamacchia, M., Spedicato, M. T., & McKinley, R. S. (2007). Use of muscle activity indices as a relative measure of well-being in Cultured Sea bass Dicentrarchus Labrax (Linnaeus, 1758). *Hydrobiologia*, *582*, 271–280. https://doi.org/10.1007/s10750-006-0538-9

Lennox, R. J., Eldøy, S. H., Dahlmo, L. S., Matley, J. K., & Vollset, K. W. (2023). Acoustic accelerometer transmitters and their growing relevance to aquatic science. *Movement Ecology*, *11*, 45. https://doi.org/10.1186/s40462-023-00403-3

Lund, I., Dalsgaard, J., Rasmussen, H. T., Holm, J., & Jokumsen, A. (2011). Replacement of fish meal with a matrix of organic plant proteins in organic trout (Oncorhynchus mykiss) feed, and the effects on nutrient utilization and fish performance. *Aquaculture*, *321*, 259–266. https://doi.org/10.1016/j.aquaculture.2011.09.028

Macaulay, G., Warren-Myers, F., Barrett, L., Oppedal, F., Føre, M., & Dempster, T. (2021). Tag use to monitor fish behaviour in aquaculture: A review of benefits, problems and solutions. *Reviews in Aquaculture*, *15*, 1565–1582. https://doi.org/10.1111/raq.12534

Magalhães, R., Díaz-Rosales, P., Diógenes, A. F., Enes, P., Oliva-Teles, A., & Peres, H. (2018). Improved digestibility of plant ingredient-based diets for European seabass (Dicentrarchus labrax) with exogenous enzyme supplementation. *Aquaculture Nutrition*, *24*, 1287–1295. https://doi.org/10.1111/anu.12666

Mahdy, M. A., Jamal, M. T., Al-Harb, M., Al-Mur, B. A., & Haque, M. F. (2022). Use of yeasts in aquaculture nutrition and immunostimulation: A review. *Journal Of Applied Biology & Biotechnology*, *10*, 59–65. https://doi.org/10.7324/JABB.2022.100507

Manera, M., & Britti, D. (2008). Assessment of serum protein fractions in rainbow trout using automated electrophoresis and densitometry. *Veterinary Clinical Pathology*, *37*, 452–456. https://doi.org/10.1111/j.1939-165X.2008.00070.x

Mansour, A. T., Fayed, W. M., Elkhayat, B. K., Omar, E. A., Zaki, M. A., Nour, A.-A. M., & Morshedy, S. A. (2021). Yucca schidigera extract dietary supplementation affects growth performance, hematological and physiological status of European seabass. *Annals of Animal Science*, *21*, 1043–1060. https://doi.org/10.2478/aoas-2021-0007

Martins, C. I. M., Galhardo, L., Noble, C., Damsgård, B., Spedicato, M. T., Zupa, W., Beauchaud, M., Kulczykowska, E., Massabuau, J. C., Carter, T., Planellas, S. R., & Kristiansen, T. (2012). Behavioural indicators of welfare in farmed fish. *Fish Physiology and Biochemistry*, *38*, 17–41. https://doi.org/10.1007/s10695-011-9518-8

Mente, E., Jokumsen, A., Carter, C. G., Antonopoulou, E., & Tacon, A. G. J. (2019). Nutrition in relation to organic aquaculture: Sources and strategies. In G. Lembo & E. Mente (Eds.), *Organic aquaculture: Impacts and future developments* (pp. 141–188). Springer. https://doi.org/10.1007/978-3-030-05603-2_8

Mente, E., Karalazos, V., Karapanagiotidis, I. T., & Pita, C. (2011). Nutrition in organic aquaculture: An inquiry and a discourse. *Aquaculture Nutrition*, *17*, e798–e817. https://doi.org/10.1111/j.1365-2095.2010.00846.x

Mitra, A. (2021). Thought of alternate aquafeed: Conundrum in aquaculture sustainability? *Proceedings of the Zoological Society*, *74*, 1–18. https://doi.org/10.1007/s12595-020-00352-4

Montoya, A., López-Olmeda, J. F., Lopez-Capel, A., Sánchez-Vázquez, F. J., & Pérez-Ruzafa, A. (2012). Impact of a telemetry-transmitter implant on daily behavioral rhythms and physiological stress indicators in gilthead seabream (Sparus aurata). *Marine Environmental Research*, *79*, 48–54. https://doi.org/10.1016/j.marenvres.2012.05.002

Naylor, R. L., Hardy, R. W., Buschmann, A. H., Bush, S. R., Cao, L., Klinger, D. H., Little, D. C., Lubchenco, J., Shumway, S. E., & Troell, M. (2021). A 20-year retrospective review of global aquaculture. *Nature*, *591*, 551–563. https://doi.org/10.1038/s41586-021-03308-6

Neves, V., Silva, D., Martinho, F., Antunes, C., Ramos, S., & Freitas, V. (2018). Assessing the effects of internal and external acoustic tagging methods on European flounder Platichthys flesus. *Fisheries Research*, *206*, 202–208. https://doi.org/10.1016/j.fishres.2018.05.015

Oliva-Teles, A., Enes, P., & Peres, H. (2015). Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. In D. A. Davis (Ed.), *Feed and feeding practices in aquaculture* (pp. 203–233). Elsevier Ltd. https://doi.org/10.1016/B978-0-08-100506-4.00008-8

R Core Team. (2023). *_R: A language and environment for statistical Computing_*. R Foundation for Statistical Computing. https://www.R-project.org/.

Raiche, G., & Magis, D. (2022). _nFactors: Parallel Analysis and Other Non Graphical Solutions to the Cattell Scree Test_. R package version 2.4.1.1. https://CRAN.R-project.org/package=nFactors

Ringø, E., Zhou, Z., Olsen, R. E., & Song, S. K. (2012). Use of chitin and krill in aquaculture – The effect on gut microbiota and the immune system: A review. *Aquaculture Nutrition*, *18*, 117–131. https://doi.org/10.1111/j.1365-2095.2011.00919.x

Sala-Rabanal, M., Sánchez, J., Ibarz, A., Fernández-Borràs, J., Blasco, J., & Gallardo, M. A. (2003). Effects of low temperatures and fasting on hematology and plasma composition of Gilthead Sea bream (Sparus aurata). *Fish Physiology and Biochemistry*, *29*, 105–115. https://doi.org/10.1023/B:FISH.0000035904.16686.b6

Saleh, R., Burri, L., Benitez-Santana, T., Turkmen, S., Castro, P., & Izquierdo, M. (2018). Dietary krill meal inclusion contributes to better growth performance of gilthead seabream juveniles. *Aquaculture Research*, *49*, 3289–3295. https://doi.org/10.1111/are.13792

Samaras, A., Tsoukali, P., Katsika, L., Pavlidis, M., & Papadakis, I. E. (2023). Chronic impact of exposure to low dissolved oxygen on the physiology of Dicentrarchus labrax and Sparus aurata and its effects on the acute stress response. *Aquaculture*, *562*, 738830. https://doi.org/10.1016/j.aquaculture.2022.738830

Schreck, C. B., & Tort, L. (2016). The concept of stress in fish. *Fish Physiology*, *35*, 1–34. https://doi.org/10.1016/B978-0-12-802728-8.00001-1

Sicuro, B. (2019). An overview of organic aquaculture in Italy. *Aquaculture*, *509*, 134–139. https://doi.org/10.1016/j.aquaculture.2019.05.024

Sultana, S., Biró, J., Kucska, B., & Hancz, C. (2024). Factors affecting yeast digestibility and Immunostimulation in aquatic animals. *Animals*, *14*, 2851. https://doi.org/10.3390/ani14192851

Tam, C. M., Tong, T. K. L., Leung, A. W. T., & Chiu, G. W. C. (2002). Site layout planning using nonstructural fuzzy decision support system. *Journal of Construction Engineering and Management*, *128*, 220–231. https://doi.org/10.1061/(ASCE)0733-9364(2002)128:3(220)

Tefal, E., Jauralde, I., Martínez-Llorens, S., Tomás-Vidal, A., Milián-Sorribes, M. C., Moyano, F. J., Peñaranda, D. S., & Jover-Cerdá, M. (2023). Organic ingredients as alternative protein sources in the diet of juvenile organic seabass (Dicentrarchus labrax). *Animals*, *13*, 3816. https://doi.org/10.3390/ani13243816

Tharaka, K., Benitez-Santana, T., Gunathilaka, B. E., Kim, M. G., Lee, C., Shin, J., & Lee, K. J. (2020). Evaluation of Antarctic krill (Euphausia superba) meal supplementation in diets for olive flounder (Paralichthys olivaceus). *Aquaculture Research*, *51*, 2291–2302. https://doi.org/10.1111/are.14573

Thorstad, E. B., Rikardsen, A. H., Alp, A., & Okland, F. (2013). The use of electronic tags in fish research – An overview of fish telemetry methods. *Turkish Journal of Fisheries and Aquatic Sciences*, *13*, 881–896. https://doi.org/10.4194/1303-2712-v13_5_13

Tong, T. K. L. (2008). Free Multi-Criteria Decision Aiding (MCDA) Tools for Research Students. https://sites.google.com/site/mcdafreeware/

Toomey, L., Gesto, M., Alfonso, S., Lund, I., Jokumsen, A., Lembo, G., & Carbonara, P. (2024). Monitoring welfare indicators of rainbow trout (Oncorhynchus mykiss) in a commercial organic farm: Effects of an innovative diet and accelerometer tag implantation. *Aquaculture*, *582*, 740549. https://doi.org/10.1016/j.aquaculture.2024.740549

Torrecillas, S., Montero, D., Carvalho, M., Benitez-Santana, T., & Izquierdo, M. (2021). Replacement of fish meal by Antarctic krill meal in diets for European Sea bass Dicentrarchus labrax: Growth performance, feed utilization and liver lipid metabolism. *Aquaculture*, *545*, 737166. https://doi.org/10.1016/j.aquaculture.2021.737166

Wang, J., Liang, D., Yang, Q., Tan, B., Dong, X., Chi, S., Liu, H., & Zhang, S. (2020). The effect of partial replacement of fish meal by soy protein concentrate on growth performance, immune responses, gut morphology and intestinal inflammation for juvenile hybrid grouper (Epinephelus fuscoguttatus♀× Epinephelus lanceolatus♂). *Fish & Shellfish Immunology*, *98*, 619–631. https://doi.org/10.1016/j.fsi.2019.10.025

Wilson, M. R., van Ravenstein, E., Miller, N. W., Clem, L. W., Middleton, D. L., & Warr, G. W. (1995). CDNA sequences and organization of IgM heavy chain genes in two Holostean fish. *Developmental and Comparative Immunology*, *19*, 153–164. https://doi.org/10.1016/0145-305X(94)00063-L

Xu, Q. Y., Wang, C. A., Zhao, Z. G., & Luo, L. (2012). Effects of replacement of fish meal by soy protein isolate on the growth, digestive enzyme activity and serum biochemical parameters for juvenile Amur sturgeon (Acipenser Schrenckii). *Asian-Australasian Journal of Animal Sciences*, *25*, 1588–1594. https://doi.org/10.5713/ajas.2012.12192

Yang, J. L., & Chen, H. C. (2003). Effects of gallium on common carp (Cyprinus carpio): Acute test, serum biochemistry, and erythrocyte morphology. *Chemosphere*, *53*, 877–882. https://doi.org/10.1016/S0045-6535(03)00657-X

Zaragozá, P., Martínez-Llorens, S., Fernández-Segovia, I., Vivancos, J.-L., Tomas-Vidal, A., Fuentes, A., Ros-Lis, J. V., Martínez-Máñez, R., & Barat, J. M. (2020). Study of fishmeal substitution on growth performance and shelf-life of Giltheadsea bream (Sparus aurata). *Fishes*, *5*(2), 15. https://doi.org/10.3390/fishes5020015

Zhou, F., Song, W., Shao, Q., Peng, X., Xiao, J., Hua, Y., Owari, B. N., Zhang, T., & Ng, W.-K. (2011). Partial replacement of fish meal by fermented soybean meal in diets for Black Sea bream, Acanthopagrus schlegelii, juveniles. *Journal of the World Aquaculture Society*, *42*, 184–197. https://doi.org/10.1111/j.1749-7345.2011.00455.x

Zhou, Q. C., Mai, K. S., Tan, B. P., & Liu, Y. J. (2005). Partial replacement of fishmeal by soybean meal in diets for juvenile cobia (Rachycentron canadum). *Aquaculture Nutrition*, *11*, 175–182. https://doi.org/10.1111/j.1365-2095.2005.00335.x

Zupa, W., Alfonso, S., Gai, F., Gasco, L., Spedicato, M. T., Lembo, G., & Carbonara, P. (2021). Calibrating accelerometer tags with oxygen consumption rate of rainbow trout (Oncorhynchus mykiss) and their use in aquaculture facility: A case study. *Animals*, *11*, 1496. https://doi.org/10.3390/ani11061496

Zupa, W., Carbonara, P., Spedicato, M. T., & Lembo, G. (2015). Modelling swimming activities and energetic costs in European Sea bass (Dicentrarchus labrax L., 1758) during critical swimming tests. *Marine and Freshwater Behaviour and Physiology*, *48*, 341–357. https://doi.org/10.1080/10236244.2015.1073456

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Toomey, L., Alfonso, S., Mente, E., Bitetto, I., Fiocchi, E., Manfrin, A., Vasilaki, P., Zupa, W., Bégout, M.-L., Spedicato, M. T., Lembo, G., & Carbonara, P. (2025). Toward the use of innovative environmentally sustainable feed in organic aquaculture: Impact on growth performance, health, and welfare of gilthead seabream (*Sparus aurata*). *Journal of the World Aquaculture Society*, *56*(2), e70021. https://doi.org/10.1111/jwas.70021