Wiley
Aquaculture Research
Volume 2024, Article ID 8234882, 14 pages
https://doi.org/10.1155/2024/8234882

WILEY

Research Article

Growth Performance, Immune Characteristics, and Health and Welfare of Gilthead Seabream (*Sparus aurata*) Fed a Tailor-Made Environmentally Sustainable Diet Formulated Using Novel Ingredients

Sébastien Alfonso,[1,2] **Lola Toomey**,[1] **Eleonora Fiocchi**,[3] **Amedeo Manfrin**,[3] **Marilena Boscarato**,[3] **Phelly Vasilaki**,[4] **Walter Zupa**,[1] **Valentina Bertazzo**,[5] **Marie-Laure Bégout**,[6] **Maria Teresa Spedicato**,[1] **Elena Mente**,[7] **Ioannis Nengas**,[8] **Giuseppe Lembo**,[1] **and Pierluigi Carbonara**[1]

[1]*Fondazione COISPA ETS, Bari, Italy*
[2]*ECOSEAS, CNRS, Université Côte d'Azur, Nice, France*
[3]*Reference Laboratory for Fish, Mollusc and Crustacean Disease, Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro, Italy*
[4]*IRIDA S.A., Nea Artaki, Evia, Greece*
[5]*SCS3—Special Diagnostics, Histopathology and Parasitology, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy*
[6]*MARBEC, CNRS, Ifremer, IRD, Univ Montpellier, Palavas-Les-Flots, France*
[7]*School of Veterinary Medicine, Laboratory of Ichthyology—Culture and Pathology of Aquatic Animals, Aristotle University of Thessaloniki, Thessaloniki, Greece*
[8]*Hellenic Centre for Marine Research (HCMR), Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Anavyssos, Greece*

Correspondence should be addressed to Lola Toomey; toomey@fondazionecoispa.org

Received 9 May 2024; Accepted 4 October 2024

Academic Editor: Christyn Bailey

The use of fish meal/oil in carnivorous fish feeds remains a concern for the environmental sustainability of aquaculture. In this study, we investigated the impact of an innovative diet designed to be cost-effective and environmentally sustainable (i.e., 60% replacement of fish meal by a blend of plant, yeast [*Saccharomyces cerevisiae*], and krill meal feed ingredients) on the growth, health, and welfare of gilthead seabream (*Sparus aurata*). Over a 135-day experiment, fish were fed either the innovative or a commercial diet (control), and various parameters were evaluated, namely growth performance, levels of physiological blood parameters related to stress, immunity, health, and welfare, as well as swimming activity, serving as a proxy for energy expenditure. Results revealed that the innovative diet enhanced growth compared to fish fed the control diet. Hematological and biochemical indicators did not highlight any impaired welfare condition in fish fed innovative diet while higher levels of Immunoglobulin M were measured in plasma of fish fed innovative diet, potentially suggesting enhancement of humoral immunity. However, accelerometer tags data revealed that fish fed the innovative diet exhibited higher overall swimming activity, suggesting higher energy expenditure, which was consistent with greater prealbumin levels measured in the plasma. In conclusion, the higher energy metabolism in fish fed the innovative diet might be compensated by the diet's content, which may boost humoral immunity and hence help the fish develop a better adaptation to rearing environment, including its viral and bacterial load, ensuring overall better growth. Longer term investigations, including measurements of additional parameters, are required to validate these promising preliminary outcomes.

Keywords: acoustic telemetry; fish meal; health; innovative diet; krill; sustainability; welfare; yeast

1. Introduction

Due to the rising demand for fish products combined with the world's depleting wild resources, fish production through aquaculture has substantially increased in recent decades [1]. This rapid growth has also raised concerns regarding the environmental sustainability of the fish farming sector and the welfare of farmed species, dating back more than 20 years but still persisting [2–5]. The use of fish meal and oil as necessary dietary components to meet the nutritional needs of carnivorous marine fish in terms of proteins and omega-3 fatty acids puts strong pressure on marine ecosystems where fish stocks are facing overfishing [1, 2, 6]. Moreover, there can be strong fluctuations in the availability (e.g., finite resources, climate change impact), price, and quality of fish meal. This further emphasizes the need for the search and use of novel and sustainable raw ingredients with high nutritional value in aquafeed formulations, to reduce aquafeeds dependency on fish meal, particularly for omnivorous and carnivorous fish species [7–9].

Research on novel feed ingredients, such as plant, single cell, or animal proteins as potential substitutes for fishmeal in feeds while maintaining fish nutritional requirements, has been very active in recent years [6, 8, 10]. Substantial research has been made on the use of plant protein sources showing overall successful outcomes in diets, particularly for herbivorous species as they possess the requisite enzymes to process plant-based proteins, but also in carnivorous species (see, e.g., [11, 12]), such as gilthead seabream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*). Various plant substitutes presenting interesting characteristics (e.g., protein content, digestibility) have been used in aquafeeds such as soybean meal, processed soy protein concentrates, or pea protein (e.g., [7, 13–16]). In addition, the inclusion of single-cell ingredients, such as yeast, in aquafeed formulations is rising and is expected to play a significant role in future aquaculture sustainability due to their fast growth, high protein content, renewability, and cost-efficiency [7]. Finally, alternate ingredients originating from terrestrial or marine animal sources have been proposed as potential fish meal replacers [11, 12, 17, 18]. Among those, Antarctic krill (*Euphausia superba*) meal appears as an interesting appetite stimulant due to its high protein content, favorable amino acid and fatty acid profiles, palatability enhancement properties, and high levels of astaxanthin, a powerful antioxidant [19, 20].

To overcome limitations encountered by the use of a single source of alternate protein (e.g., antinutritional factors in plant ingredients), offer cost-effective sustainable diets and ensure a high balanced nutrition, a blend/combination of alternate complementary protein sources was suggested as a suitable solution (e.g., [21–24]). This is facilitated by recent developments in biotechnology [25–27], such as fermentation technology, which lowers the antinutritional elements of plant ingredients and raises their protein content [28]. Before formulating, several criteria need to be taken into account to select new fish meal substitutes, including the quantity and quality of these substitutes, their acceptability for consumers, but also, and importantly, their competitiveness in terms of price [8, 29]. This latter requires the use of alternative raw ingredients that can be produced on an industrial scale, ensuring year-round availability. Additionally, the final cost of the feed must be sustainable and acceptable to producers, while still meeting the nutritional needs of the fish. Because it directly affects output and profit, the use of effective, environmentally, and economically sustainable ingredients for aquaculture feeds is a crucial issue for European fish farming. Additionally, ensuring a high immunity level, as well as a high level of welfare for the farmed species, is crucial when including such innovative components in the fish diet [10, 30]. Indeed, decreasing the contribution of fish meal in aquafeeds appears relatively simple, but the difficulty can lie in not negatively affecting fish growth and health/welfare [9].

In addition to economically important traits related to growth and nutrition, fish health and welfare are thus now major concerns for consumers and regulatory bodies [4, 31]. It is widely acknowledged that fish will grow more efficiently if they are in good health [4], often related to good fish welfare. Recently, methods and tools for measuring health and welfare have greatly developed in farmed fish species, providing a comprehensive evaluation from molecular to behavioral and physiological endpoints, in addition to biological performances [32–35]. Furthermore, in the context of precision fish farming, remote real-time monitoring of behavior and fish physiological state using sensors may provide high-throughput insights into fish responses to different aquaculture conditions [36–38]. For instance, swimming activity and/or acceleration measured by such sensors have been proven to be correlated with heart rate and metabolic rate (indicators of stress and energy expenditure) and affected by acute stressors (e.g., water quality) or rearing conditions (e.g., stocking density, diet group) [39–46]. The use of such sensors is thus providing valuable real-time indicators related to fish welfare without disturbing fish and the daily routines of farmers.

Gilthead seabream is a key species in European marine aquaculture [47]. Forty-five percent of the production cost of gilthead seabream is attributed to the diet. Therefore, extensive research has been conducted to find cost-effective raw materials to replace fish meal and oil. Particular attention has been given to plant-derived ingredients (e.g., [48, 49]), but replacing fish meal with a combination of various protein sources has also been highlighted as promising [50, 51]. In this study, we investigated the effects of a cost-effective and tailor-made low ecological footprint innovative diet, with 60% replacement of fish meal by a blend of plant, yeast, and, in lower proportion, krill meal, on the health and welfare of seabream. To do so, we monitored the growth performance of the fish fed the innovative diet over a 4-month period and measured the levels of physiological blood parameters related to stress, immunity, health, and welfare (e.g., hematocrit, cortisol, lactate, and total proteins), as well as the levels of a stress molecular indicator (*Hsp70* levels). In addition, an accelerometer-tag was implanted in some fish to track their acceleration continuously, acting as a stand-in for energy expenditure. Overall, this study provides a thorough evaluation of the physiological state of seabream under innovative nutrition,

Table 1: Formulation of the diets (%)

Ingredients	Control diet	Innovative diet
Fish meal	25	10
Corn gluten meal	22	12
Soy protein concentrate	5	12
Soybean meal	11	—
Fermented soy	—	7.5
Fish oil	12	11.5
Sunflower meal	12	10
Wheat	10	9
Yeast	—	6
Pea protein	—	6
Wheat gluten meal	—	6
Krill meal	—	5
Monocalcium phosphate	—	1.8
Lecithin	—	1
Synergen	—	0.05
Premix	3	2.2

addressing the environmental sustainability challenges of European aquaculture.

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. All of the experiments were carried out with the approval of the Health Ministry (number 488/2021-PR) and in compliance with EU recommended Directive 2010/63/EU.

2.1. Fish, Feeding, and Experimental Protocol. Gilthead seabreams were obtained from the commercial farm REHOMARE (Gallipoli, Italy) and transferred to the facilities of Fondazione COISPA ETS (Bari, Italy). The fish were kept undisturbed for 2 weeks in seawater (pH = 7.30 ± 0.05, temperature of 18.5 ± 1°C, oxygen saturation of ~80%, and salinity of 35 practical salinity unit [PSU]) at a stocking density of about 20 kg/m^3 and a photoperiod of 12L : 12D (Light:Dark; light from 6 am to 6 pm). The fish were placed in a flow-through system with a water replacement rate of 25 L/min and were fed a commercial feed (Skretting Marine 3P, Italy) amounting to approximately 1% of their body mass daily during this period. After this 2-week period, fish were individually passive integrated transponders (pit)-tagged with ID100 radio frequency identification (RFID) tags (DORSET ID, Trovan, Netherlands) under anesthesia conditions using hydroalcoholic clove oil solution (30 ppm, Erbofarmosan, Bari, Italy), and then randomly distributed into four fiberglass tanks of 1.2 m^3 ($n = \sim 70$ per tank; stocking density: 12 kg/m^3) with the same water quality parameters and photoperiod specified before.

Two weeks post pit-tagging, the fish started to be fed with either a commercial diet, serving as a control (two tanks), and with an innovative diet (two tanks) (Table 1). Both feeds were produced by the Greek fish feed company IRIDA S.A. The innovative diet used in this study was formulated to be more environmentally friendly and contains fishmeal replacers such as protein yeast derived from *Saccharomyces cerevisiae*, plant and krill meals that allowed to decrease the total amount of fishmeal by 60%. The innovative feed was nutritionally balanced with other ingredients commonly used in aquafeeds as well as with macro- and micronutrients. The formulation of the diets and their chemical composition are shown in Table 1 and Supporting Information 1: Table S1, respectively. The amino acid and fatty acid profiles of the two diets are presented in Supporting Information 2: Table S2 and Supporting Information 3: Table S3, respectively.

Fish were fed 6 to 7 days per week with the experimental feeds, amounting to 1% of their body mass, during the whole experiment using automated feeders distributing feed from 8 am to 12 pm. The feeding started on day 0 (July 11, 2022) and lasted for 135 days until November 23, 2022. For the evaluation of various biochemical blood parameters, three sampling times were chosen: T0, 5 days before the start of the experiment to serve as a control; T1, at 72–73 experimental days; and T2, corresponding to the end of the experiment at 134–135 experimental days.

2.2. Growth Measurements, Blood and Organs Sampling. At each sampling time (i.e., T0, T1, and T2), fish were gently caught from the rearing tanks and slightly anesthetized with a hydroalcoholic clove oil solution (15 ppm) for measuring standard length (cm) and weight (g). Mortalities were recorded daily, along with feed provided per tank. Specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were estimated between T0 and T2. The SGR was calculated using the following equation: $SGR = 100 \times (\ln(W_0) - \ln(W_2))/T$, where W is the total weight of the fish (g), respectively, at T0 (W_0) and at T2 (W_2), and T is the number of feeding days. FCR was calculated as the ratio of the feed ingested (kilogram of dry weight) per biomass of weight gained (kg). PER was calculated by dividing fish weight gain by the total amount of protein ingested during the experiment.

At T1 and T2, a subsample of fish ($n = 8$ per tank; i.e., $n = 16$ per diet) was randomly selected for blood collection. Twelve fish were also sampled at T0 (before the start of the feeding experiment) to establish basal values. Before beginning the blood sampling procedure, fish were carefully removed from the rearing tanks and given a 2 to 3 min soak in an anesthetic solution (clove oil, 30 ppm). Blood samples were drawn from the caudal vein. The levels of several physiological markers of fish health and welfare were then measured using these blood samples, as detailed in Section 2.3. At T2, the fish used for blood sampling were then euthanized using an overdose of anesthetics. To perform a quantitative real-time polymerase chain reaction (qPCR) analysis, 100 mg of spleen, kidney, gill, liver, and brain were collected and stored in tubes containing 1 mL of RNAlater (QIAGEN). Samples were kept for 48 h at 4°C and then maintained at −80°C until further use.

2.3. Analysis of Hematological and Biochemical Indicators. Hematological indicators were analyzed in the same way as Alfonso et al. [52]. Briefly, the hematocrit was determined shortly after sampling, and the red blood cell count (RBCC) was evaluated using a Bürker counting chamber under a light microscope (Nikon 400E, Japan), and a commercial kit

(H7379; Sigma, United States) was used to measure hemoglobin. The remaining blood was placed in a tube containing K3EDTA (VACUMED, Torreglia, Italy) and centrifuged to obtain plasma samples, which were stored at −20°C until further analysis. Following the manufacturer's instructions, plasma cortisol was measured in an automatic analyzer Cobas E601 (Roche Diagnostics GmbH, Mannheim, Germany) using a commercial competitive electrochemiluminescent immunoassay kit (Elecsys Cortisol II Gen, Roche Diagnostics). Using commercial kits (Lactate Gen.2, Glucose HK Gen.3, and Total Protein Gen.2, respectively; Roche Diagnostics), the concentrations of plasma glucose, lactate, and total protein were determined in accordance with the manufacturer's instructions in the Biochemical analyzer Cobas C501 (Roche Diagnostics). The enzyme-linked immunosorbent assay kit (BT LABTM, China) was used to measure the levels of total serum immunoglobulin M (IgM) in accordance with the manufacturer's instructions.

The concentrations of the different protein fractions content in plasma (albumin, alpha 1, alpha 2, beta 1, beta 2, and gamma) were obtained through electrophoresis following a method described in Alfonso et al.'s [53] study.

2.4. Quantification of Hsp70 Using qPCR. The absolute quantification of *Hsp70* (heat shock protein) was conducted using qPCR analysis according to the method developed by Fiocchi et al. [54] using specific plasmid for seabream *Hsp70* [40]. All details regarding previous optimization and qPCR analysis are available in Fiocchi et al.'s [54] study and Supporting Information 4: Annex S1.

2.5. Implantation of Accelerometer Tags and Recording of Swimming Activity. A subsample of fish ($n = 5$ fish per tank; $n = 10$ per diet; 328 ± 53.7 and 304.1 ± 32.4 g for the control and innovative diet, respectively) was implanted on the 44–45th experimental days (corresponding to August 24, 2022 and August 25, 2022) with accelerometer tags VEMCO V9A-2x (AMIRIX Systems Inc., Nova Scotia, Canada) following the protocol described in Alfonso et al.'s [55] study, and fish were then released into their original tank. Feeding and swimming behaviors were monitored daily in the days following the surgical implantation to ensure a good recovery. All fish started to feed 2–3 days following the surgery and displayed normal swimming behavior, testifying that all tagged fish recovered well. While all tagged fish displayed normal behavior following surgery, three tagged fish, however, died over the experimental period and were, as a precaution, excluded from the final analysis.

Data recording started on September 5, 2022 (i.e., 12 days after tag implantation), to ensure full recovery before data acquisition [55], and lasted until the end of the experiment (T2), resulting in a total monitoring of 79 days. All details regarding tag settings and data storage and acquisition using acoustic receivers are available in Supporting Information 4: Annex S1.

2.6. Statistical Analyses. Statistical analyses were conducted using R software Version 4.0.4 [56] at the 95% level of significance. Data are presented as mean ± SD (standard deviation) and the fish was used as a statistical unit. Initially, the tank (duplicates per diet) was included as a random factor in all mixed models, but it was removed when the variance attributed to the tank was null. All conditions for applying linear models (absence of outliers, linearity, homogeneity of variance, normality of residuals, and normality of the random effects) were checked graphically using the *performance* package [57]. All details regarding specific statistical tests and models used are available in Supporting Information 4: Annex S1.

TABLE 2: Growth variables in the control ($n = 135$ fish) and innovative diet ($n = 134$ fish) groups: total weight (mean ± SD; in grams) measured at the three sampling points (T0, T1, and T2), SGR (%/day), FCR, and PER.

Variable	Control diet	Innovative diet
Total weight at T0 (g)	219.7 ± 50.0	220.8 ± 47.8
Total weight at T1 (g)	301.1 ± 59.0	308.2 ± 59.9
Total weight at T2 (g)	359.7 ± 76.9	378.4 ± 84.3
SGR (%/day)	0.46 ± 0.20^{a}	0.49 ± 0.17^{b}
FCR	2.28 ± 2.09	2.09 ± 1.18
PER	1.08 ± 0.47	1.18 ± 0.46

Note: Different letters between the two diet groups indicate statistical differences between diets ($p < 0.05$).
Abbreviations: FCR, feed conversion ratio; PER, protein efficiency ratio; SD, standard deviation; SGR, specific growth rate.

3. Results

3.1. Growth. Overall, the mortality during the experiment was similar between the two diet groups (3.57 ± 1.01% for the control group vs., 3.58 ± 3.02% for the innovative diet group). Regarding weight gain, fish did grow over time in both groups ($F = 1389.33$, $p < 0.001$; Table 2). Over the experimental period, fish fed the control and innovative diets grew from 219.7 ± 50.0 g to 359.7 ± 76.9 g and from 220.8 ± 47.8 g to 378.4 ± 84.3 g, respectively. The diet did not significantly affect weight when considered alone ($F = 1.40$, $p = 0.24$) but a significant interaction between time and diet was found to affect fish weight ($F = 5.05$, $p = 0.007$). In more details, in T0 and T1, there were no significant weight differences between diets, but a difference emerged in T2 (Table 2). There was a slight statistical difference in SGR between diets ($W = 7782.5$, $p = 0.04$), with SGR being slightly higher for fish fed the innovative diet compared to the control diet (Table 2). However, there was no significant difference between diets in FCR ($W = 10091$, $p = 0.08$) or PER ($W = 7864$, $p = 0.08$) (Table 2).

3.2. Hematological and Biochemical Indicators

3.2.1. Stress, Health, and Welfare Parameters. There was no impact of the diet or sampling time on cortisol (diet: likelihood ratio (LR) = 0.01, $p = 0.91$; sampling time: LR = 0.02, $p = 0.89$), hematocrit (diet: $F = 3.73$, $p = 0.19$; sampling time: $F = 3.32$, $p = 0.07$) and RBCC (diet: $F = 1.12$, $p = 0.40$; sampling time: $F = 1.14$, $p = 0.29$) (Figure 1). Glucose was affected by both the diet ($F = 22.78$, $p < 0.001$) and time ($F = 10.16$, $p = 0.002$), with higher values at T2 compared to T1 and

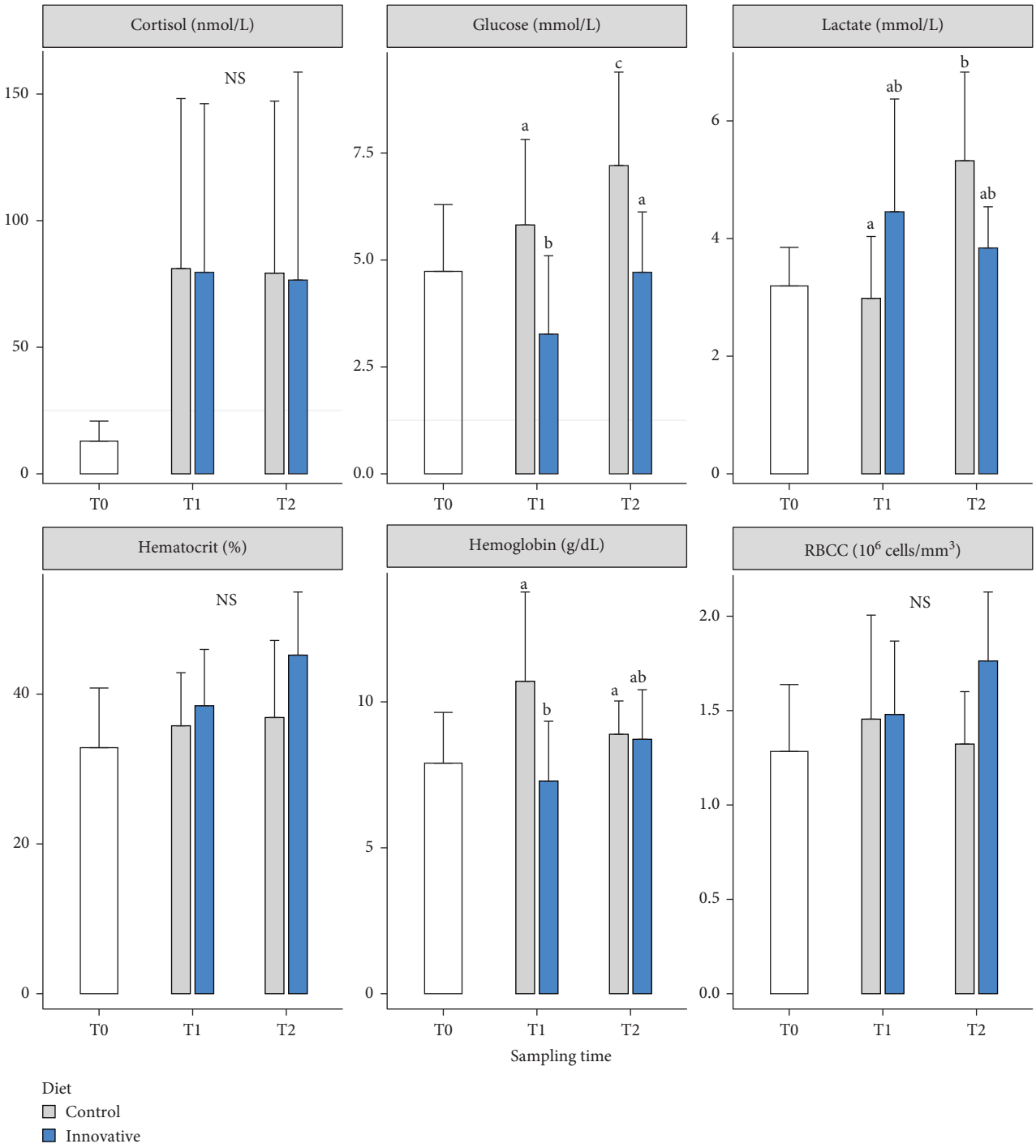

FIGURE 1: Stress, health, and welfare blood physiological parameters (mean ± SD) measured at the two different sampling points (T1 and T2) in the control (gray; $n = 16$) and innovative diet (blue; $n = 16$) groups of seabream. Parameters monitored are cortisol (nmol/L), glucose (nmol/L), lactate (nmol/L), hematocrit (%), hemoglobin (g/dL), and red blood cell count (RBCC; 10^6 cells/mm^3). Significant differences between sampling times and/or or diets are indicated in the figure for each parameter; different letters indicate significant differences ($p < 0.05$), otherwise NS is indicated. T0 was added to the graph as a point of comparison for initial values. NS, not significant; SD, standard deviation.

overall higher values in the control diet than in the innovative one at both T1 and T2 (Figure 1). Similarly, lactate was influenced by time ($F = 12.22$, $p < 0.001$) and the interaction between diet and time ($F = 18.23$, $p < 0.001$), with control individuals presenting higher values at T2 than T1, while no difference could be seen across times for fish fed with the innovative diet (Figure 1). The diet ($F = 10.68$, $p = 0.002$) and the interaction between diet and time ($F = 8.48$, $p = 0.01$) also

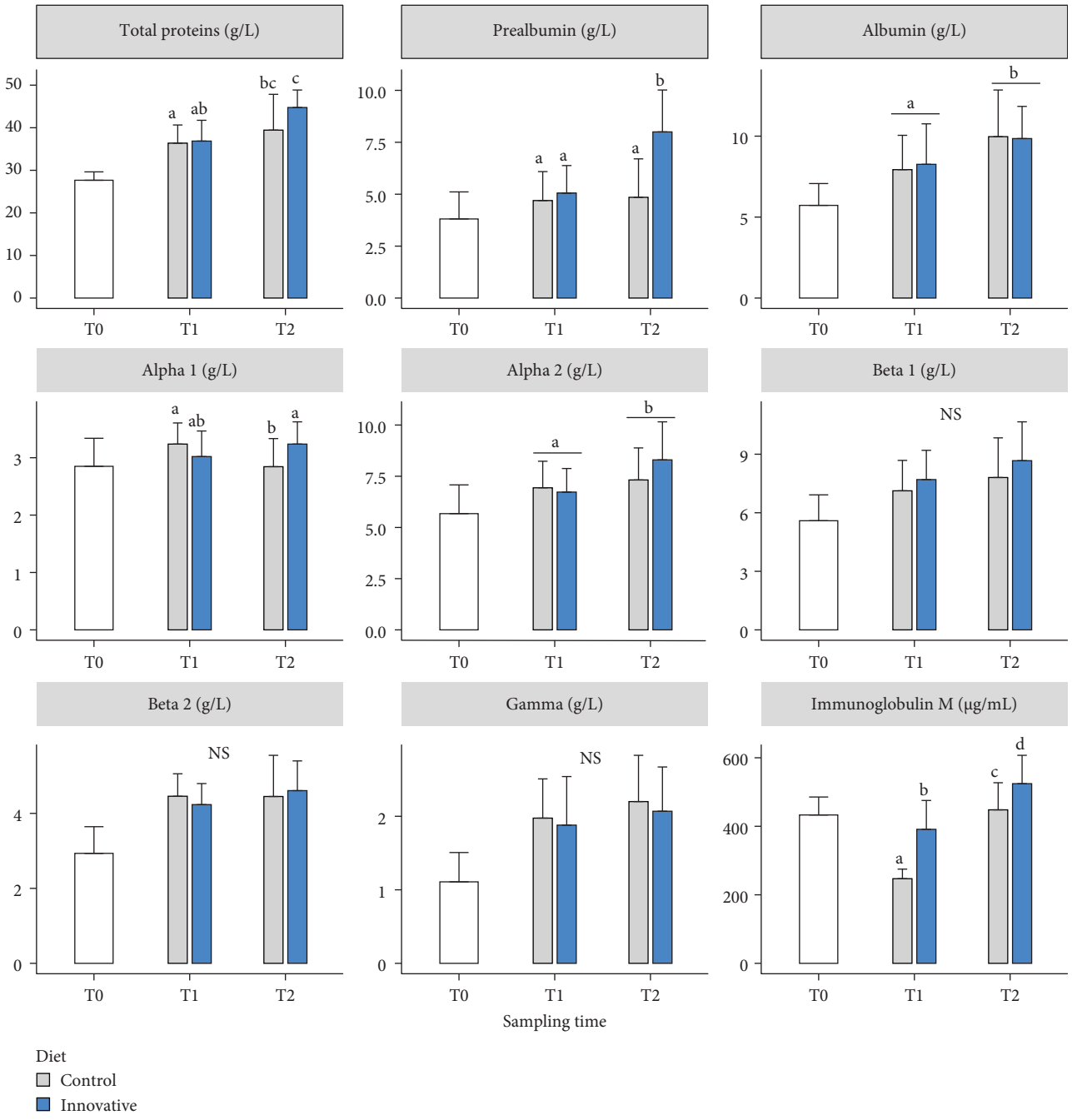

FIGURE 2: Immune parameters and protein content (mean ± SD) measured at two different sampling points (T1 and T2) in the control (gray; $n = 16$) and innovative (blue; $n = 16$) diet groups of seabream. Parameters monitored are total protein (g/L), prealbumin (g/L), albumin (g/L), alpha 1 (g/L), alpha 2 (g/L), beta 1 (g/L), beta 2 (g/L), gamma (g/L), and immunoglobulin M (µg/mL). Significant differences between sampling times and/or or diets are indicated in the figure for each parameter; different letters indicate significant differences ($p < 0.05$), otherwise NS is indicated. T0 was added to the graph as a point of comparison for initial values. NS, not significant; SD, standard deviation.

had an impact on hemoglobin concentration, with lower values in fish fed with the innovative diet compared to control fish at T1 but with no difference between diets at T2 (Figure 1). Finally, there was no significant difference in the expression of *Hsp70* in the targeted organs between the two diet groups at the end of the trial (T2; $t = -1.31$, $p = 0.2$; Supporting Information 5: Figure S1).

3.2.2. Immune Parameters and Protein Blood Content. Total protein levels were significantly influenced by the diet ($F = 4.31$, $p = 0.04$), but the post hoc tests showed no differences in fish fed with the innovative diet compared to control fish in specific sampling times, and by sampling time ($F = 13.86$, $p < 0.001$) with higher values at T2 compared to T1 (Figure 2). There were significant effects of diet ($F = 18.16$, $p < 0.001$),

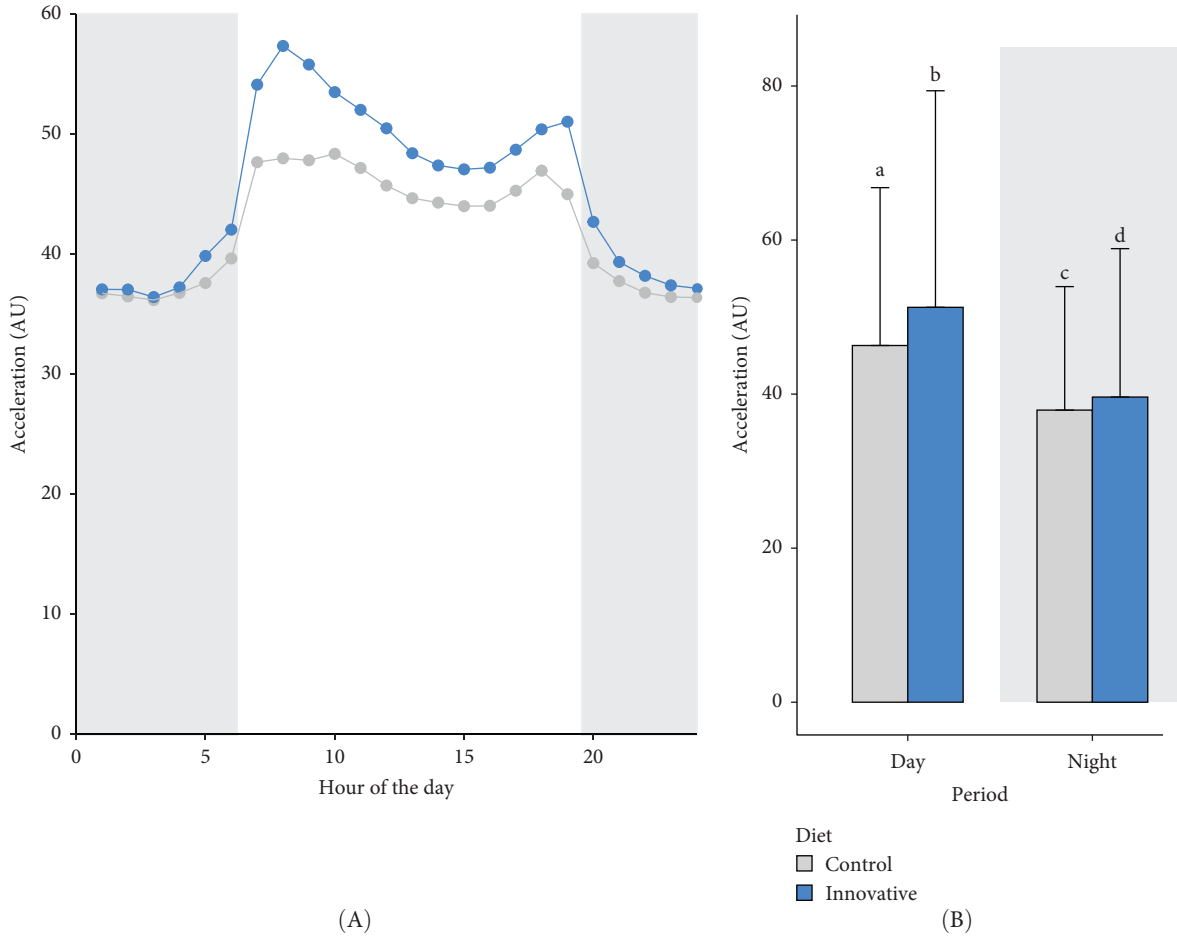

FIGURE 3: Acceleration (mean ± SD; AU) as a function of (A) hours and (B) period of the day (i.e., day and night) for the two diet groups: control diet (in gray; $n = 10$) and innovative diet (in blue; $n = 7$) in seabream. Gray shapes indicate the night period of the photoperiod. Different letters indicate significant differences between diet groups (statistical significance assigned at $\alpha = 0.05$). AU, arbitrary unit; SD, standard deviation.

sampling time ($F = 13.06$, $p < 0.001$), and the interactions between these two factors ($F = 10.88$, $p = 0.002$) on prealbumin levels (Figure 2). More specifically, there was a significant increase in prealbumin between T1 and T2 for fish fed with the innovative diet, and at T2, prealbumin levels were significantly higher in fish fed with the innovative diet compared to control fish, while no difference between the two diets could be seen at T1 (Figure 2). No diet effect but a time effect was detected for albumin (diet: $F = 0.05$, $p = 0.83$; time: $F = 9.19$, $p = 0.004$) and alpha 2 levels (diet: $F = 1.16$, $p = 0.29$; time: $F = 6.42$, $p = 0.01$), with higher values at T2 compared to T1 (Figure 2). Alpha 1 was significantly influenced by the interaction between diet and sampling time ($F = 8.93$, $p = 0.004$), with a significant decrease in alpha 1 level between T1 and T2 in control fish resulting in a significant difference between diets at T2 with innovative fish presenting higher values than control fish (Figure 2). No diet or time effect was observed for beta 1 (diet: $F = 1.03$, $p = 0.42$; time: $F = 3.29$, $p = 0.07$), beta 2 (diet: $F = 0.02$, $p = 0.89$; time: $F = 0.81$, $p = 0.37$), or gamma (diet: $F = 0.54$, $p = 0.54$; time: $F = 1.87$, $p = 0.18$) levels (Figure 2). Finally, IgM levels were significantly influenced by both the diet ($F = 45.04$, $p < 0.001$) and the sampling time ($F = 77.90$, $p < 0.001$), with higher values in the innovative diet compared to control and higher values at T2 compared to T1 (Figure 2).

3.3. Acceleration Data Recorded by Tags. Overall, the acceleration recorded by tags was affected by the period of the day ($t = -161.81$; $p < 0.001$), the diet ($t = 7.64$; $p < 0.001$), and the interaction effect between diet and period ($t = -19.87$; $p < 0.001$). In more details, in both experimental groups, fish displayed higher acceleration during the daytime than night-time and fish fed the innovative diet overall displayed higher acceleration values than fish fed the control diet, even if this difference was more pronounced during the daytime than night-time (Figure 3).

The frequency distribution of data registered by tag differed across diets ($\chi^2 = 7223.80$; $p < 0.001$). There was a higher peak of values ranging from 21 to 50 AU (arbitrary unit) in fish fed the control diet while a higher number of values for fish fed the innovative diet were overall found in values <20 and >61 AU (Figure 4).

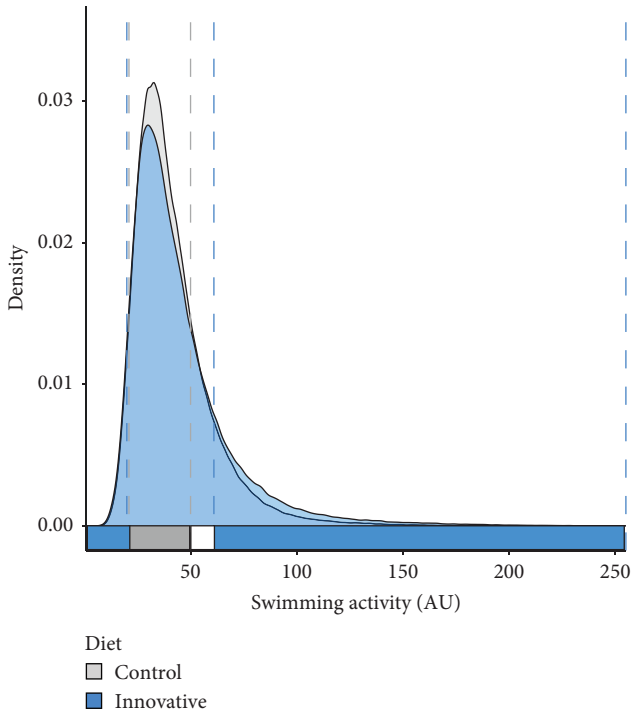

FIGURE 4: Frequency distribution of swimming activity values (in arbitrary unit: AU) as a function of diet (control diet in gray and innovative diet in blue) in seabream (*S. aurata*). Rectangles at the bottom of the graph indicate swimming value intervals for which there is a diet difference: blue rectangles indicate when fish fed the innovative diet are more representative of these swimming values, gray rectangles indicate when the frequency is significantly higher in control fish, and white rectangles indicate when there is no significant difference in the frequency distribution between the two diets.

4. Discussion

4.1. Growth Performance. Irrespective of the diet group, fish exhibited growth consistent with the species' expectations under comparable experimental rearing conditions (i.e., temperature, salinity, and fish size [40]). While no diet effect was seen on survival, FCR or PER, fish fed the innovative diet displayed a slight but significantly greater SGR than fish fed the control diet, resulting in a slightly higher average weight at the end of the experimental monitoring (18.7 g of difference between diets). In previous studies, plant proteins, such as soybean meal, have been widely used as potential substitutes for fishmeal and fish oil in aquafeeds due to their abundant supply, high protein content, and well-balanced amino acid profile. However, antinutritional factors in soybean meal can be problematic, leading to reduced growth [10, 58]. Using soy protein concentrates instead of soybean meal, as done in the present study, may help overcome these issues and avoid detrimental effects on growth [13, 59, 60]. It is worth noting that substituting fishmeal with plant proteins like soy protein concentrates can, in some cases, reduce palatability, ultimately affecting feed intake and growth [60, 61], which was not observed in our study. One way to prevent such a reduction in feed intake is to partially replace fishmeal with ingredients that enhance palatability, such as krill [19, 20], as performed in this study. In addition to increasing palatability, recent studies have shown that including krill meal in feed contributes to better growth performance in seabream juveniles [62] and related farmed fish species like European sea bass [20]. These findings align with the results observed in the present study, although it is important to note that it is difficult to isolate the specific effects of the different ingredients in the innovative diet on growth improvement due to the combination of novel ingredients used (i.e., soy protein concentrates, yeast, krill meal). Overall, the growth performance results support the potential for future use of such innovative diet in seabream aquaculture farming. However, the innovative diet needs to be formally tested in real farming conditions (i.e., in sea cages at higher stocking density) to fully validate its use at larger scale.

4.2. Hematological and Biochemical Indicators of Health, Stress, and Immunity. All hematological and biochemical indicators here monitored are commonly used metrics for assessing fish health and welfare, and the values observed in this study were consistent with those reported in other studies for seabream [40, 63]. Most of these indicators of stress and welfare were not significantly affected by the innovative diet tested.

Concerning hematological parameters, the only notable difference among diets was observed for hemoglobin at T1, but this discrepancy disappeared at T2. Interestingly, a similar change was observed in sea bass fed an innovative diet that included both soy protein concentrate and fermented soy to replace soybean meal, along with yeast proteins to replace fish meal [64], similar to the present study. It is worth noting that an opposite pattern was observed in both red seabream (*Pagrus major*) and olive flounder (*Paralichthys olivaceus*), which displayed increased hemoglobin concentration and hematocrit when fed a krill meal-supplemented diet [65, 66]. Regarding biochemical parameters, lower stress levels were suggested by lower plasma glucose levels [67] in seabream fed the innovative diet compared to control fish. Such changes in glucose levels were not observed in sea bass fed an innovative diet with similar properties to the one formulated in the current study [64], nor in red seabream fed a krill meal-supplemented diet [66]. Additionally, these variations in glucose levels were not corroborated by other stress indicators (e.g., lactate, cortisol, *Hsp70*) in the present study. It is worth mentioning that for cortisol, the levels measured in both T1 and T2 are higher than those expected at basal level for seabream (mean ± standard error: 15.35 ± 2.32 ng/mL [52]), suggesting an acute stress condition induced by handling which may mask some differences in cortisol level due to diet group. However, given that the fish were handled in the same way across experimental groups, even if the cortisol level was influenced by handling (e.g., capture, anesthesia, and sampling) difference in cortisol levels between the two diet groups could still be highlighted. The large inclusion of plant ingredients in the feed of carnivorous fish species can affect basal stress levels and stress responses, as observed in previous studies [68–70]. This may be detrimental to fish when coping

with environmental variations and could impact their overall fitness. However, the inclusion of novel ingredients such as yeast in the feed may help reduce basal cortisol levels and prevent or even enhance the stress response, enabling fish to better cope with environmental challenges and ultimately improving growth [71, 72], which could be what we observed in this study. Overall, based on the different markers evaluated, the innovative diet tested in the present study did not induce any significant negative impact on fish stress, health, and welfare. These findings could be complemented by other parameters interacting with the physiological stress response, such as brain monoamine levels, oxidative stress, and associated stress molecular markers [67, 73–75]. It would be particularly interesting to further evaluate the oxidative stress response in fish fed the innovative diet, as recent studies suggest an enhanced response in fish, such as seabream, fed with krill-inclusive diets [76].

Plasma immunological parameters were also within the range of values previously reported for seabream (e.g., [77–79]). Some variations in the immunological parameters measured in the plasma of seabream were observed between the two diets, notably a higher level of total proteins with a specific higher level of prealbumin and alpha 1 at T2 and higher values of IgM throughout the entire experimental period in fish fed the innovative diet compared to fish fed the control diet. Overall, regarding total protein levels, a similar increase in plasma has been observed in sea bass fed an innovative diet with properties similar to the one formulated in the present study (as detailed above) [64]. However, no changes in total protein levels were noted in red seabream or walleye pollock (*Gadus chalcogrammus*) when fed a krill meal-supplemented diet [66, 80]. As for the specific increase in prealbumin levels, prealbumin is a protein known to help carry thyroid hormones and vitamin A through the bloodstream. Low levels of prealbumin are generally indicative of malnutrition [81]. However, the prealbumin levels measured in fish fed the control diet were close to previously reported values for seabream and related species [79] and no signs of malnutrition were observed during the experiment, as supported by the increased growth in fish fed the innovative diet. Prealbumin is also known to help controlling how the body uses energy [81]. The higher level observed at T2 may be more linked to higher metabolism resulting in greater energy expenditure and hence be consistent with the greater acceleration values measured in the fish fed innovative diet using accelerometer tags (discussed in the following section). Additionally, the higher level of alpha 1 at T2 in fish fed the innovative diet may suggest a potential immunostimulant effect of the innovative diet, as alpha globulins are acute-phase proteins typically associated with inflammation and illness [82]. This increase in alpha 1 was not observed in the plasma of sea bass fed a similar innovative diet, where a decrease in alpha 1 levels was measured instead [64]. Although yeast inclusion is generally used to boost fish immunity, including in seabream [83–85], this effect was not seen in the sea bass study mentioned. This discrepancy could suggest that the krill included in the feed of the present study might be responsible for the observed immune enhancement, aligning with the higher plasma IgM levels in fish fed the innovative diet compared to those on the control diet, although the effects of other ingredients are unknown. In olive flounder, krill meal in the diet is known to increase plasma total immunoglobulin levels at an inclusion rate of 6%, compared to diets with low fish meal content [65]. Other nonspecific immunity indicators, such as antiprotease and lysozyme, were also enhanced following krill meal inclusion [65]. Immunoglobulins are a major component of the vertebrate humoral immune system and a good indicator of the nonspecific immunological system [78, 86]. IgM levels in blood are generally higher in response to immunostimulants administration in seabream [78]. This overall suggests a potential improvement in the immune defences of fish fed the innovative diet. In the case of olive flounder, Tharaka et al. [65] demonstrated that fish fed a krill-inclusive diet had lower mortality rates compared to those on a low fish meal diet following injection with the *Edwardsiella tarda* pathogen. However, both in vivo and in vitro studies will be further needed in seabream to evaluate whether this increase is nonspecific or directed specifically against some pathogens, also because no further variations of globulins (alpha 2, beta 1, beta 2, and gamma) were observed between fish fed the two different diets.

4.3. Acceleration Recorded by Tag. In the present study, 17 out of the 20 tagged fish survived the entire experiment, representing a mortality rate of 15%. While this falls within the range expected in tanks according to Macaulay et al. [87] (mean: 2.5%, range = 0%–17%), it is toward the upper end of the observed data. It is, however, crucial to note that due to the low number of tagged individuals in the trial ($n = 20$), one individual represents a relatively high percentage (5%), and the high percentage of 15% must be considered in the context of the low sample size. Additionally, all three deceased fish during the trial were from the innovative group. Still, when considering all fish, the overall mortality was similar between the diet groups over the trial (3.6% for both diet groups), indicating that this higher mortality in tagged fish could not be directly linked to the diet. Finally, the tagged fish died long time after the tagging (26, 34, and 71 days later) so it is quite plausible to consider that these fish deaths fall within the moderate mortality of such a long rearing period (135 days), also considering that such tagging practice is already known to not induce chronic stress and/or negatively affect growth [88, 89].

Swimming activity is considered a sensitive marker of welfare in fish [35, 90]. Especially, acceleration measured using accelerometer tags is a reliable indicator of oxygen consumption and energy expenditure in fish [91–94], including seabream [55]. Generally, a higher level of muscle activity (i.e., acceleration recorded by tag) implies that energetic reserves are going to be lower, reflecting a reduced ability for the fish to cope with future stress energy-consuming events. Overall swimming activity displayed by seabream individuals in this study is consistent with values reported in the literature [40, 95–97]. A higher activity was shown,

regardless of the diet, during the day compared to the night. This diurnal pattern was previously reported for seabream [40, 98], as well as other species [46, 64, 99, 100] and is overall linked with feeding during the morning.

Regarding the specific diet effect, fish fed the innovative diet displayed a higher acceleration level than fish fed the control diet, confirming what prealbumin levels were previously suggesting. This difference was consistent during both the day and night times, although the difference was greater between the two diet groups during the daytime. This would suggest an overall higher energy consumption for fish fed the innovative diet than fish fed the control diet over the experimental trial [55]. Furthermore, it was observed that fish fed the innovative diet exhibited a greater data frequency for high swimming activity values (>61 AU) compared to fish fed the control diet (higher frequency for low and medium swimming activity values, i.e., between 21 and 50 AU). The analysis of red and white muscle activities during seabream swimming, carried out in previous studies using electromyograms, revealed that when the swimming activity values recorded by the tag are less than 80 AU, the fish swims using energy from aerobic metabolism [55]. However, when the values exceed 80 AU, the fish gradually use energy from anaerobic metabolism until they reach 255 AU, which is the limit of tag measurement [55]. In the current study, the swimming of fish fed the control diet is hence more fueled by aerobic metabolism than fish fed the innovative diet, while, conversely, fish fed the innovative diet consume more energy from anaerobic metabolism than fish fed the control diet. In this way, fish from the control diet group would have more energy available to cope with potential future stressful conditions [55]. Indeed, our results suggest that fish fed the innovative diet overall expend more energy swimming, leaving them with less energy to handle potential future stressors. This indicator does not, therefore, favor the use of the innovative diet. However, it appears that the acceleration differences between diets are not significant enough to compromise the general welfare of the fish fed the innovative diet as, overall, this higher energy expenditure did not translate into impaired growth. Longer term experiments are required to verify that the higher energy expenditure in fish fed the innovative diet does not have long-term consequences on fish growth, health, and welfare.

5. Conclusion

In conclusion, the tested innovative diet, incorporating protein yeast from *S. cerevisiae*, plant, and krill meals as replacements for fish meal (60% replacement), did not adversely affect the survival of seabream but slightly enhanced the growth performance. The overall biochemical blood indicators suggest the absence of strong negative effects on the health and welfare of seabream due to the innovative diet, and, on the contrary, may suggest an enhancement of humoral immunity, potentially linked to krill inclusion. Furthermore, although a greater acceleration was recorded by tags in fish fed the innovative diet compared to the control diet, this did not translate into impaired growth. This suggests that higher energy metabolism in fish fed the innovative diet might be compensated by the diet's content which may boost humoral immunity. The latter may hence help the fish to cope with viral and bacterial load present in the rearing environment, ensuring overall better growth in fish fed the innovative diet. Longer term monitoring experiments are needed in real farming conditions (i.e., sea cages at higher density), considering additional parameters (e.g., oxidative stress indicators, pathogen challenge), to draw more definitive conclusions on the use of this promising innovative diet.

Data Availability Statement

All raw datasets were deposited in Figshare (doi: 10.6084/m9.figshare.25360225).

Conflicts of Interest

Phelly Vasilaki is an employee of IRIDA S.A., and the company is a partner in the FutureEUAqua project. The other coauthors have no relevant financial or nonfinancial interests to disclose.

Author Contributions

Conceptualization: Sébastien Alfonso, Amedeo Manfrin, Elena Mente, Walter Zupa, Giuseppe Lembo, and Pierluigi Carbonara. Methodology: Sébastien Alfonso, Eleonora Fiocchi, Amedeo Manfrin, Phelly Vasilaki, Elena Mente, Giuseppe Lembo, and Pierluigi Carbonara. Investigation, data curation, and formal analysis: Sébastien Alfonso, Lola Toomey, Eleonora Fiocchi, Amedeo Manfrin, Marilena Boscarato, Phelly Vasilaki, Ioannis Nengas, Walter Zupa, Marie-Laure Bégout, Valentina Bertazzo, Elena Mente, and Pierluigi Carbonara. Writing–original draft preparation and visualization: Sébastien Alfonso and Lola Toomey. Writing–review and editing: all authors. Project administration: Sébastien Alfonso, Amedeo Manfrin, Elena Mente, and Giuseppe Lembo. Funding acquisition: Amedeo Manfrin, Maria Teresa Spedicato, Giuseppe Lembo, Elena Mente, and Pierluigi Carbonara. All authors have read and agreed to the submitted version of the manuscript. Sébastien Alfonso and Lola Toomey contributed equally to this work.

Funding

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

Acknowledgments

The authors are also grateful to COISPA team (Pasquale Gallo, Stefano Fazi, Piero Antonio Pinto, and Cosmidano Neglia) for running the experiments and valuable help during sampling.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting Information 1. Table S1: chemical composition (%) of the two feeds used in the experiment (control and innovative) according to AOAC 1995 analysis protocols.

Supporting Information 2. Table S2: amino acid profiles for the control and the innovative feeds used in the experiment (in percent).

Supporting Information 3. Table S3: fatty acid composition (percentage of total fatty acids) for the control and the innovative feeds used in the experiment.

Supporting Information 4. Annex S1: detailed Material and Methods.

Supporting Information 5. Figure S1: number of HSP70 copies per microliter measured at the end of the experiment (T2) in the two diets (control in gray and innovative in blue). No significant difference was found between the two diets (statistical significance assigned at $\alpha = 0.05$).

References

[1] FAO, *The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation* (2022), FAO, Rome.

[2] R. L. Naylor, R. J. Goldburg, J. H. Primavera, et al., "Effect of Aquaculture on World Fish Supplies," *Nature* 405, no. 6790 (2000): 1017–1024.

[3] F. A. Huntingford, C. Adams, V. A. Braithwaite, et al., "Current Issues in Fish Welfare," *Journal of Fish Biology* 68, no. 2 (2006): 332–372.

[4] P. J. Ashley, "Fish Welfare: Current Issues in Aquaculture," *Applied Animal Behaviour Science* 104, no. 3-4 (2007): 199–235.

[5] S. M. Hixson, "Fish Nutrition and Current Issues in Aquaculture: The Balance in Providing Safe and Nutritious Seafood, in an Environmentally Sustainable Manner," *Journal of Aquaculture Research & Development* 5, no. 3 (2014).

[6] R. L. Naylor, R. W. Hardy, A. H. Buschmann, et al., "A 20-Year Retrospective Review of Global Aquaculture," *Nature* 591, no. 7851 (2021): 551–563.

[7] R. Jannathulla, V. Rajaram, R. Kalanjiam, et al., "Fishmeal Availability in the Scenarios of Climate Change: Inevitability of Fishmeal Replacement in Aquafeeds and Approaches for the Utilization of Plant Protein Sources," *Aquaculture Research* 50, no. 12 (2019): 3493–3506.

[8] R. Luthada-Raswiswi, S. Mukaratirwa, and G. O'brien, "Animal Protein Sources as a Substitute for Fishmeal in Aquaculture Diets: A Systematic Review and Meta-Analysis," *Applied Sciences* 11, no. 9 (2021): 3854.

[9] A. Oliva-Teles, P. Enes, A. Couto, and H. Peres, "Replacing Fish Meal and Fish Oil in Industrial Fish Feeds," in *Feed and Feeding Practices in Aquaculture*, (Woodhead Publishing Series in Food Science, Technology and Nutrition, Second edition, 2022): 231–268.

[10] C. Aragão, A. T. Gonçalves, B. Costas, et al., "Alternative Proteins for Fish Diets: Implications Beyond Growth," *Animals* 12, no. 9 (2022): 1211.

[11] A. Oliva-Teles, P. Enes, and H. Peres, "Replacing Fishmeal and Fish Oil in Industrial Aquafeeds for Carnivorous Fish," in *Feed and Feeding Practices in Aquaculture*, (Woodhead Publishing, 2015): 203–233.

[12] B. Randazzo, P. Di Marco, M. Zarantoniello, et al., "Effects of Supplementing a Plant Protein-Rich Diet With Insect, Crayfish or Microalgae Meals on Gilthead Sea Bream (*Sparus aurata*) and European Seabass (*Dicentrarchus labrax*) Growth, Physiological Status and Gut Health," *Aquaculture* 575 (2023): 739811.

[13] Q. C. Zhou, K. S. Mai, B. P. Tan, and Y. J. Liu, "Partial Replacement of Fishmeal by Soybean Meal in Diets for Juvenile Cobia (*Rachycentron canadum*)," *Aquaculture Nutrition* 11, no. 3 (2005): 175–182.

[14] S. A. Collins, M. Øverland, A. Skrede, and M. D. Drew, "Effect of Plant Protein Sources on Growth Rate in Salmonids: Meta-Analysis of Dietary Inclusion of Soybean, Pea and Canola/Rapeseed Meals and Protein Concentrates," *Aquaculture* 400-401 (2013): 85–100.

[15] T. Bandara, "Alternative Feed Ingredients in Aquaculture: Opportunities and Challenges," *Journal of Entomology and Zoology Studies* 6 (2018): 3087–3094.

[16] E. D. Macusi, M. A. Cayacay, E. Q. Borazon, et al., "Protein Fishmeal Replacement in Aquaculture: A Systematic Review and Implications on Growth and Adoption Viability," *Sustainability* 15, no. 16 (2023): 12500.

[17] A. R. Hodar, R. J. Vasava, D. R. Mahavadiya, and N. H. Joshi, "Fish Meal and Fish Oil Replacement for Aqua Feed Formulation by Using Alternative Sources: A Review," *Journal of Experimental Zoology-India* 23 (2020): 13–21.

[18] B. Randazzo, M. Zarantoniello, G. Gioacchini, et al., "Physiological Response of Rainbow Trout (*Oncorhynchus mykiss*) to Graded Levels of *Hermetia illucens* or Poultry by-Product Meals as Single or Combined Substitute Ingredients to Dietary Plant Proteins," *Aquaculture* 538 (2021): 736550.

[19] Y. Wei, H. Shen, W. Xu, et al., "Replacement of Dietary Fishmeal by Antarctic Krill Meal on Growth Performance, Intestinal Morphology, Body Composition and Organoleptic Quality of Large Yellow Croaker *Larimichthys crocea*," *Aquaculture* 512 (2019): 734281.

[20] S. Torrecillas, D. Montero, M. Carvalho, T. Benitez-Santana, and M. Izquierdo, "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 (2021): 737166.

[21] D. Jirsa, F. T. Barrows, R. W. Hardy, and M. Drawbridge, "Alternative Protein Blends as a Replacement for Fish Meal in Diets for White Seabass, *Atractoscion nobilis*," *Aquaculture Nutrition* 21, no. 6 (2015): 861–867.

[22] R. Monge-Ortiz, A. Tomás-Vidal, F. J. Gallardo-Álvarez, et al., "Partial and Total Replacement of Fishmeal by a Blend of Animal and Plant Proteins in Diets for *Seriola dumerili*: Effects on Performance and Nutrient Efficiency," *Aquaculture Nutrition* 24, no. 4 (2018): 1163–1174.

[23] E. Villanueva-Gutiérrez, M. L. González-Félix, D. M. Gatlin, and M. Perez-Velazquez, "Use of Alternative Plant and Animal Protein Blends, in Place of Fishmeal, in Diets for Juvenile Totoaba, *Totoaba macdonaldi*," *Aquaculture* 529 (2020): 735698.

[24] G. M. Turchini, J. T. Trushenski, and B. D. Glencross, "Thoughts for the Future of Aquaculture Nutrition: Realigning Perspectives to Reflect Contemporary Issues Related to Judicious Use of Marine Resources in Aquafeeds," *North American Journal of Aquaculture* 81, no. 1 (2019): 13–39.

[25] K. Ghosh and A. K. Ray, "Aquafeed Formulation Using Plant Feedstuffs: Prospective Application of Fish-Gut Microorganisms and Microbial Biotechnology," in *Soft Chemistry and Food Fermentation*, (Elsevier Inc, 2017): 109–144.

[26] S. W. Jones, A. Karpol, S. Friedman, B. T. Maru, and B. P. Tracy, "Recent Advances in Single Cell Protein Use as a Feed Ingredient in Aquaculture," *Current Opinion in Biotechnology* 61 (2020): 189–197.
[27] J. O. Agboola, M. Øverland, A. Skrede, and J.Ø. Hansen, "Yeast as Major Protein-Rich Ingredient in Aquafeeds: A Review of the Implications for Aquaculture Production," *Reviews in Aquaculture* 13, no. 2 (2021): 949–970.
[28] M. A. O. Dawood and S. Koshio, "Application of Fermentation Strategy in Aquafeed for Sustainable Aquaculture," *Reviews in Aquaculture* 12, no. 2 (2020): 987–1002.
[29] R. L. Naylor, R. W. Hardy, D. P. Bureau, et al., "Feeding Aquaculture in an Era of Finite Resources," *Proceedings of the National Academy of Sciences* 106, no. 36 (2009): 15103–15110.
[30] B. D. Glencross, J. Baily, M. H. G. Berntssen, R. Hardy, S. MacKenzie, and D. R. Tocher, "Risk Assessment of the Use of Alternative Animal and Plant Raw Material Resources in Aquaculture Feeds," *Reviews in Aquaculture* 12, no. 2 (2020): 703–758.
[31] European Food Safety Authority (EFSA), "General Approach to Fish Welfare and to the Concept of Sentience in Fish - Scientific Opinion of the Panel on Animal Health and Welfare," *EFSA Journal* 7, no. 2 (2009): 1–27.
[32] L. U. Sneddon, D. C. C. Wolfenden, and J. S. Thomson, "Stress Management and Welfare," *Fish Physiology* 35 (2016): 463–539.
[33] C. S. F. Raposo de Magalhães, M. A. C. Cerqueira, D. Schrama, et al., "A Proteomics and Other Omics Approach in the Context of Farmed Fish Welfare and Biomarker Discovery," *Reviews in Aquaculture* 12, no. 1 (2020): 122–144.
[34] B. Sadoul, S. Alfonso, X. Cousin, et al., "Global Assessment of the Response to Chronic Stress in European Sea Bass," *Aquaculture* 544 (2021): 737072.
[35] M. Dara, P. Carbonara, Corte C. La, et al., "Fish Welfare in Aquaculture: Physiological and Immunological Activities for Diets, Social and Spatial Stress on Mediterranean Aqua Cultured Species," *Fishes* 8, no. 8 (2023): 414.
[36] M. Føre, K. Frank, T. Norton, et al., "Precision Fish Farming: A New Framework to Improve Production in Aquaculture," *Biosystems Engineering* 173 (2018): 176–193.
[37] I. Halachmi, M. Guarino, J. Bewley, and M. Pastell, "Smart Animal Agriculture: Application of Real-Time Sensors to Improve Animal Well-Being and Production," *Annual Review of Animal Biosciences* 7 (2019): 403–425.
[38] J. Brijs, M. Føre, A. Gräns, T. D. Clark, M. Axelsson, and J. L. Johansen, "Bio-Sensing Technologies in Aquaculture: How Remote Monitoring Can Bring Us Closer to Our Farm Animals," *Philosophical Transactions of the Royal Society B: Biological Sciences* 376, no. 1830 (2021): 20200218.
[39] J. Brijs, E. Sandblom, M. Axelsson, et al., "The Final Countdown: Continuous Physiological Welfare Evaluation of Farmed Fish During Common Aquaculture Practices Before and During Harvest," *Aquaculture* 495 (2018): 903–911.
[40] P. Carbonara, S. Alfonso, W. Zupa, et al., "Behavioral and Physiological Responses to Stocking Density in Sea Bream (*Sparus aurata*): Do Coping Styles Matter?" *Physiology & Behavior* 212 (2019): 112698.
[41] P. Carbonara, S. Alfonso, F. Gai, et al., "Moderate Stocking Density Does Not Influence the Behavioural and Physiological Responses of Rainbow Trout (*Oncorhynchus mykiss*) in Organic Aquaculture," *Aquaculture Research* 51, no. 7 (2020): 3007–3016.
[42] P. Carbonara, W. Zupa, I. Bitetto, et al., "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, no. 11 (2020): 1–23.
[43] M. Gesto, W. Zupa, S. Alfonso, M. T. Spedicato, G. Lembo, and P. Carbonara, "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 (2020): 105084.
[44] M. Hvas, O. Folkedal, and F. Oppedal, "Heart Rates of Atlantic Salmon *Salmo sala*r During a Critical Swim Speed Test and Subsequent Recovery," *Journal of Fish Biology* 98, no. 1 (2021): 102–111.
[45] E. Rosell-Moll, M. C. Piazzon, J. Sosa, et al., "Use of Accelerometer Technology for Individual Tracking of Activity Patterns, Metabolic Rates and Welfare in Farmed Gilthead Sea Bream (*Sparus aurata*) Facing a Wide Range of Stressors," *Aquaculture* 539 (2021): 736609.
[46] L. Toomey, M. Gesto, S. Alfonso, et al., "Monitoring Welfare Indicators of Rainbow Trout (*Oncorhynchus mykiss*) in a Commercial Organic Farm: Effects of an Innovative Diet and Accelerometer Tag Implantation," *Aquaculture* 582 (2024): 740549.
[47] FEAP, "Federation of the European Association of Producers," Annual Report 2023, 2023.
[48] S. Martínez-Llorens, A. V. Moñino, A. T. Vidal, et al., "Soybean Meal as a Protein Source in Gilthead Sea Bream (*Sparus aurata* L.) Diets: Effects on Growth and Nutrient Utilization," *Aquaculture Research* 38, no. 1 (2007): 82–90.
[49] M. De Francesco, G. Parisi, J. Pérez-Sanchez, et al., "Effect of High-Level Fish Meal Replacement by Plant Proteins in Gilthead Sea Bream (*Sparus aurata*) on Growth and Body/Fillet Quality Traits," *Aquaculture Nutrition* 13, no. 5 (2007): 361–372.
[50] M. Carvalho, D. Montero, G. Rosenlund, R. Fontanillas, R. Ginés, and M. Izquierdo, "Effective Complete Replacement of Fish Oil by Combining Poultry and Microalgae Oils in Practical Diets for Gilthead Sea Bream (*Sparus aurata*) Fingerlings," *Aquaculture* 529 (2020): 735696.
[51] N. Porcino and L. Genovese, "Review on Alternative Meals for Gilthead Seabream, *Sparus aurata*," *Aquaculture Research* 53, no. 6 (2022): 2109–2145.
[52] S. Alfonso, C. Houdelet, E. Bessa, et al., "Water Temperature Explains Part of the Variation in Basal Plasma Cortisol Level, Within and Between Fish Species," *Journal of Fish Biology* 103, no. 4 (2023): 828–838.
[53] S. Alfonso, E. Fiocchi, L. Toomey, et al., "Comparative Analysis of Blood Protein Fractions in Two Mediterranean Farmed Fish: *Dicentrarchus labrax* and *Sparus aurata*," *BMC Veterinary Research* 20, no. 1 (2024): 322.
[54] E. Fiocchi, M. Civettini, P. Carbonara, et al., "Development of Molecular and Histological Methods to Evaluate Stress Oxidative Biomarkers in Sea Bass (*Dicentrarchus labrax*)," *Fish Physiology and Biochemistry* 46, no. 4 (2020): 1577–1588.
[55] S. Alfonso, W. Zupa, M. T. Spedicato, et al., "Mapping the Energetic Costs of Free-Swimming Gilthead Sea Bream (*Sparus aurata*), a Key Species in European Marine Aquaculture," *Biology* 10, no. 12 (2021): 1357.
[56] R Core Team, Team RC, *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna, Austria, 2023).

[57] D. Lüdecke, M. Ben-Shachar, I. Patil, P. Waggoner, and D. Makowski, "Performance: An R Package for Assessment, Comparison and Testing of Statistical Models," *Journal of Open Source Software* 6, no. 60 (2021): 3139.
[58] G. Francis, H. P. S. Makkar, and K. Becker, "Antinutritional Factors Present in Plant-Derived Alternate Fish Feed Ingredients and Their Effects in Fish," *Aquaculture* 199, no. 3-4 (2001): 197–227.
[59] C. Aragão, L. E. C. Conceição, J. Dias, et al., "Soy Protein Concentrate as a Protein Source for Senegalese Sole (*Solea senegalensis* Kaup 1858) Diets: Effects on Growth and Amino Acid Metabolism of Postlarvae," *Aquaculture Research* 34, no. 15 (2003): 1443–1452, 2109.
[60] Y. Chen, J. Ma, H. Huang, H. Zhong, and J. J. Loor, "Effects of the Replacement of Fishmeal by Soy Protein Concentrate on Growth Performance, Apparent Digestibil Ity, and Retention of Protein and Amino Acid in Juvenile Pearl Gentian Grouper," *PLOS ONE* 14, no. 12 (2019): e0222780.
[61] P. Blaufuss and J. Trushenski, "Exploring Soy-Derived Alternatives to Fish Meal: Using Soy Protein Concentrate and Soy Protein Isolate in Hybrid Striped Bass Feeds," *North American Journal of Aquaculture* 74, no. 1 (2012): 8–19.
[62] R. Saleh, L. Burri, T. Benitez-Santana, et al., "Dietary Krill Meal Inclusion Contributes to Better Growth Performance of Gilthead Seabream Juveniles," *Aquaculture Research* 49, no. 10 (2018): 3289–3295.
[63] F. Fazio, C. Saoca, S. Casella, et al., "Relationship Between Blood Parameters and Biometric Indices of *Sparus aurata* and *Dicentrarcus labrax* Cultured in Onshore Tanks," *Marine and Freshwater Behaviour and Physiology* 48 (2015): 289–296.
[64] S. Alfonso, E. Mente, E. Fiocchi, et al., "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, no. 1 (2023): 21269.
[65] K. Tharaka, T. Benitez-Santana, B. E. Gunathilaka, et al., "Evaluation of Antarctic Krill (*Euphausia superba*) Meal Supplementation in Diets for Olive Flounder (*Paralichthys olivaceus*)," *Aquaculture Research* 51, no. 6 (2020): 2291–2302.
[66] B. E. Gunathilaka, S. Khosravi, J. Shin, et al., "Evaluation of Shrimp Protein Hydrolysate and Krill Meal Supplementation in Low Fish Meal Diet for Red Seabream (*Pagrus major*)," *Fisheries and Aquatic Sciences* 24, no. 3 (2021): 109–120.
[67] C. B. Schreck and L. Tort, "The Concept of Stress in Fish," in *Fish Physiology*, (Elsevier, 2016): 1–34.
[68] R. Ganga, J. G. Bell, D. Montero, et al., "Adrenocorticotrophic Hormone-Stimulated Cortisol Release by the Head Kidney Inter-Renal Tissue From Sea Bream (*Sparus aurata*) Fed With Linseed Oil and Soyabean Oil," *British Journal of Nutrition* 105, no. 2 (2011): 238–247.
[69] V. Benítez-Dorta, M. D. Caballero, M. Izquierdo, et al., "Total Substitution of Fish Oil by Vegetable Oils in Senegalese Sole (*Solea senegalensis*) Diets: Effects on Fish Performance, Biochemical Composition, and Expression of Some Glucocorticoid Receptor-Related Genes," *Fish Physiology and Biochemistry* 39, no. 2 (2013): 335–349.
[70] A. Oxley, C. Jolly, T. Eide, A.-E. O. Jordal, A. Svardal, and R.-E. Olsen, "The Combined Impact of Plant-Derived Dietary Ingredients and Acute Stress on the Intestinal Arachidonic Acid Cascade in Atlantic Salmon (*Salmo salar*)," *British Journal of Nutrition* 103, no. 6 (2010): 851–861.
[71] H. Guo, W. Lin, L. Wang, et al., "The Supplementation of Dietary Selenium Yeast and Green Tea-Derived Polyphenols Improves Antioxidant Capacity and Immune Response in Juvenile Wuchang Bream Under Ammonia Stress," *Aquaculture Research* 51, no. 9 (2020): 3790–3803.
[72] J. Sánchez-Velázquez, G. A. Peña-Herrejón, and H. Aguirre-Becerra, "Fish Responses to Alternative Feeding Ingredients under Abiotic Chronic Stress," *Animals* 14, no. 5 (2024): 765.
[73] M. Gesto, M. A. López-Patiño, J. Hernández, J. L. Soengas, and J. M. Míguez, "Gradation of the Stress Response in Rainbow Trout Exposed to Stressors of Different Severity: The Role of Brain Serotonergic and Dopaminergic Systems," *Journal of Neuroendocrinology* 27, no. 2 (2015): 131–141.
[74] M. Gesto, L. Madsen, N. R. Andersen, et al., "Early Performance, Stress- and Disease-Sensitivity in Rainbow Trout Fry (*Oncorhynchus mykiss*) After Total Dietary Replacement of Fish Oil With Rapeseed Oil. Effects of EPA and DHA Supplementation," *Aquaculture* 536 (2021): 736446.
[75] S. Chowdhury and S. K. Saikia, "Oxidative Stress in Fish: A Review," *Journal of Scientific Research* 12, no. 1 (2020): 145–160.
[76] S. Torrecillas, M. Carvalho, D. Montero, et al., "Benefits of Dietary Krill Meal Inclusion Towards Better Utilization of Nutrients, and Response to Oxidative Stress in Gilthead Seabream (Sparus Aurata) Juveniles," *SSRN* (2024).
[77] M. Á. Gallardo, M. Sala-Rabanal, A. Ibarz, et al., "Functional Alterations Associated With, Winter Syndrome, in Gilthead Sea Bream (*Sparus aurata*)," *Aquaculture* 223, no. 1–4 (2003): 15–27.
[78] A. Cuesta, J. Meseguer, and M. A. Esteban, "Total Serum Immunoglobulin M Levels Are Affected by Immunomodulators in Seabream (*Sparus aurata* L.)," *Veterinary Immunology and Immunopathology* 101, no. 3-4 (2004): 203–210.
[79] F. Fazio, C. Saoca, G. Fortino, et al., "Seasonal Biochemical Changes in Two Aquaculture Species, *Sparus aurata*. Linnaeus, 1758 and, *Dicentrarchus labrax*. Linnaeus, 1758," *Iranian Journal of Fisheries Sciences* 19 (2020): 1757–1769.
[80] J. Choi, K. W. Lee, G. S. Han, S.-G. Byun, H. J. Lim, and H. S. Kim, "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 (2020): 100331.
[81] D. M. Power, J. Melo, and C. R. A. Santos, "The Effect of Food Deprivation and Refeeding on the Liver, Thyroid Hormones and Transthyretin in Sea Bream," *Journal of Fish Biology* 56, no. 2 (2000): 374–387.
[82] E. F. Christiansen, C. Cray, G. A. Lewbart, and C. A. Harms, "Plasma Protein Electrophoresis and Acute Phase Proteins in Koi Carp (*Cyprinus carpio*) Following Exploratory Coeliotomy," *Journal of Exotic Pet Medicine* 24, no. 1 (2015): 76–83.
[83] F. J. Gatesoupe, "Live Yeasts in the Gut: Natural Occurrence, Dietary Introduction, and Their Effects on Fish Health and Development," *Aquaculture* 267, no. 1–4 (2007): 20–30.
[84] M. A. Mahdy, M. T. Jamal, M. Al-Harb, B. A. Al-Mur, and M. F. Haque, "Use of Yeasts in Aquaculture Nutrition and Immunostimulation: A Review," *Journal of Applied Biology & Biotechnology* 10 (2022): 59–65.
[85] J. Ortuño, A. Cuesta, A. Rodríguez, et al., "Oral Administration of Yeast, *Saccharomyces cerevisiae*, Enhances the Cellular Innate Immune Response of Gilthead Seabream (*Sparus aurata* L.)," *Veterinary Immunology and Immunopathology* 85, no. 1-2 (2002): 41–50.
[86] A. E. Ellis, "Fish Immune System," in *Encyclopedia of Immunology*, eds. P. J. Delves and I. M. Roitt, (Academic Press, London, 1998): 920–926.

[87] G. Macaulay, F. Warren-Myers, L. T. Barrett, F. Oppedal, M. Føre, and T. Dempster, "Tag Use to Monitor Fish Behaviour in Aquaculture: A Review of Benefits, Problems and Solutions," *Reviews in Aquaculture* 13, no. 3 (2021): 1565–1582.
[88] S. Alfonso, W. Zupa, A. Manfrin, et al., "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, no. 1 (2020): 21.
[89] N. Jepsen, L. E. Davis, C. B. Schreck, and B. Siddens, "The Physiological Response of Chinook Salmon Smolts to Two Methods of Radio-Tagging," *Transactions of the American Fisheries Society* 130, no. 3 (2001): 495–500.
[90] C. I. M. Martins, L. Galhardo, C. Noble, et al., "Behavioural Indicators of Welfare in Farmed Fish," *Fish Physiology and Biochemistry* 38, no. 1 (2012): 17–41.
[91] S. Wright, J. D. Metcalfe, S. Hetherington, and R. Wilson, "Estimating Activity-Specific Energy Expenditure in a Teleost Fish, Using Accelerometer Loggers," *Marine Ecology Progress Series* 496 (2014): 19–32.
[92] J. D. Metcalfe, S. Wright, C. Tudorache, and R. P. Wilson, "Recent Advances in Telemetry for Estimating the Energy Metabolism of Wild Fishes," *Journal of Fish Biology* 88, no. 1 (2016): 284–297.
[93] P. Carbonara, S. Alfonso, M. Dioguardi, et al., "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 (2021): 100817.
[94] W. Zupa, S. Alfonso, F. Gai, et al., "Calibrating Accelerometer Tags With Oxygen Consumption Rate of Rainbow Trout (*Oncorhynchus mykiss*) and Their Use in Aquaculture Facility: A Case Study," *Animals* 11, no. 6 (2021): 1496.
[95] P. Carbonara, S. Alfonso, W. Zupa, et al., "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 (2022): 1–9, 95. 1179.
[96] L. Muñoz, E. Aspillaga, M. Palmer, et al., "Acoustic Telemetry: A Tool to Monitor Fish Swimming Behavior in Sea-Cage Aquaculture," *Frontiers in Marine Science* 7 (2020): 545896.
[97] D. J. McKenzie, A. P. Palstra, J. Planas, et al., "Aerobic Swimming in Intensive Finfish Aquaculture: Applications for Production, Mitigation and Selection," *Reviews in Aquaculture* 13, no. 1 (2021): 138–155.
[98] M.-L. Bégout and J.-P. Lagardère, "An Acoustic Telemetry Study of Seabream (*Sparus aurata* L.): First Results on Activity Rhythm, Effects of Environmental Variables and Space Utilization," in *Space Partition Within Aquatic Ecosystems* (1995): 417–423
[99] M. Føre, E. Svendsen, J. A. Alfredsen, et al., "Using Acoustic Telemetry to Monitor the Effects of Crowding and Delousing Procedures on Farmed Atlantic Salmon (*Salmo salar*)," *Aquaculture* 495 (2018): 757–765.
[100] I.-H. Chen, D. G. Georgopoulou, L. O. E. Ebbesson, D. Voskakis, P. Lal, and N. Papandroulakis, "Food Anticipatory Behaviour on European Seabass in Sea Cages: Activity-, Positioning-, and Density-Based Approaches," *Frontiers in Marine Science* 10 (2023): 1–14.