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Fish protein hydrolysates from rainbow trout processing in replacement of feed protein sources: Effects on growth performances, liver status and body composition of gilthead sea bream, *Sparus aurata* L., juveniles

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Abstract

The use of processing by-products may reduce pressure on fish stocks as well as diminish wastes and negative environmental impact. Different studies investigated fish by-products used as alternative nutritional source to conventional feedstuffs. Fish protein hydrolysates (FPH), as a protein source derived from discards of rainbow trout processing, were included in feeds and effects were evaluated on productive performances, liver status, and body composition in gilthead sea bream, *Sparus aurata* L., juveniles. Three groups of 170 juveniles each (initial weight 37.8 ± 0.5 g), in triplicate indoor tanks of 2 m^3 volume each, were fed including FPH in L1 (7.5 g/kg) and L2 (15 g/kg) feeds in replacement of fishmeal. LC diet was used as control having fish meal and soybean meal as main protein sources. At the end of the trial (85 days), satisfactory productive performances were obtained in all the groups with similar performances. The final mean weight ranged from 76.6 to 78.0 g. The two FPH diets exhibited high

palatability equal to LC. Liver histology did not differ although fat accumulation was detected in all fish. Body composition and fatty acids were similar. FPH as substitute of protein source is suitable in the feeding of gilthead sea bream juveniles.

KEYWORDS
body composition, fish by-products, fish protein hydrolysates, gilthead sea bream, growth performance

1 | INTRODUCTION

Recently, FAO has emphasized the importance of aquatic food systems as drivers of employment, economic growth, social development, and environmental recovery as indicated in the 2030 Agenda as "Blue Transformation" (FAO, 2022). To increase the sustainability of production chains, waste prevention and minimization take high priority together with reuse, recycling, and energy recovery (Boyd et al., 2020; Mair et al., 2023). According to the European Union (EU) Commission Council Directive 2008/98/EC (Directive 2008/98/EC), "waste" is defined as "any substance or object which the holder discards or intends or is required to discard". The use of processing by-products may reduce pressure on fish stocks as well as diminish the waste and negative environmental impact. Recovering wastes from fish processing proximate composition was investigated (Munekata et al., 2020). Amino acids, fatty acids, and other valuable compounds such as collagen and minerals were shown in high amounts (Gerhing et al., 2011; Messina et al., 2013; Nawaz et al., 2020). For this reason, fish waste has been also defined with the term "fish co-products" coming both from fisheries and aquaculture (Samuel-Fitwi et al., 2013). Contribution of by-products to the whole fish can range from 30 to 80% in unprocessed fish body weight and are composed of muscle cuts (15%–20%), skin and fins (1%–3%), bones (9%–15%), heads (9%–12%), viscera (12%–18%), and scales (5%) (Bruni et al., 2021; Ferraro et al., 2010; Gasco et al., 2020; Pateiro et al., 2020; Pinotti et al., 2019; Villamil et al., 2017). Currently, fishmeal and fish oil are produced from trimmings of white fish and pelagic fish destined to human food. A comprehensive analysis (Malcorps et al., 2021) of the nutritional value of separated by-products (skin, heads, frames, trimmings, viscera) was documented in five fish species farmed in Europe, showing crude protein, lipid, and EPA and DHA in different rate content. As consequence, separation of by-product fractions could be interesting to add economic value to fish processing (Malcorps et al., 2021).

In aquafeed, the dietary inclusion of these discards was studied as meal (Benitez-Hernández et al., 2017; Falch et al., 2007; García-Romero et al., 2014; Kim & Kim, 2014), paste (Samarakoon et al., 2021; Wijayanti et al., 2021; Zhu et al., 2022), and hydrolysates (García-Romero et al., 2014; Quinto et al., 2018; Villamil et al., 2017). These compounds offer the advantage they also have as a better amino acid profile than plant-based feedstuffs because they do not have antinutritional factors with favorable economic impact. In case of hydrolysates, the process of enzymatic hydrolysis guarantees the availability of amino acid and bioactive peptides essential for aquatic organisms (Chalamaiah et al., 2012; Siddik et al., 2021; Wald et al., 2016). Fish protein hydrolysates (FPH) appear to prevent intestinal dysbiosis and muscular atrophy induced by-products of animal origin (no aquatic) (Chaklader et al., 2023). The effects of FPH were investigated in salmonids (Aksnes et al., 2006), tilapia (Bae et al., 2019), European sea bass (Parma et al., 2023; Velasco et al., 2023), grouper (Mamauag & Ragaza, 2017), and sparids (Khosravi et al., 2015). In gilthead sea bream, *Sparus aurata*, the dietary substitution of fish meal with FPH was studied at low amount (<5%) (Fronte et al., 2019; Gisbert et al., 2021) or as additive (Rimoldi et al., 2020), but few studies investigated the total replacement during the growing phase of juveniles.

In this context, the aim of the present study is to evaluate the suitability of fish by-products, obtained after rainbow trout, *Oncorhynchus mykiss*, filleting process, to replace dietary protein source in the feeding of gilthead sea

bream juveniles. A hydrolyzation process was performed using the discards from different body regions of rainbow trout at slaughtering. In order to understand whether the FPH inclusion, as a substitute of a conventional protein source feedstuff (fishmeal), affected growth and liver health status, productive performances, liver histology, and body composition were assessed at the end of a pre-growing trial.

2 | MATERIALS AND METHODS

All experimental procedures were evaluated by the Ethical-Scientific Committee for Animal Experimentation of the University of Camerino in accordance with the European directive 2010/63/UE on the protection of animals used for scientific purposes (Protocol no. 5/2023).

2.1 | FPH employed and experimental diets

For the current trail, the by-products were obtained from a trout slaughterhouse recovering head, skin, viscera, fins, and trimmings of the lateral and ventral part of the fillet of rainbow trout. All the rendered portions were transferred to a controlled laboratory suitable to process manufacturing ingredients. Microbiological risk assessment was previously performed on samples and no biological hazards were showed. Biomass had a 50% moisture and was homogenized in a meat grinder (Hobart Texas, USA) and dried inside a forced air oven at 60°C for 24 h. The dried mass was ground in a mill strained through a 0.25-mm mesh sieve and stored in vessels under refrigeration for 24 h. The procedure was in agreement with the literature (Greyling, 2017; Swanepoel & Goosen, 2018) and based on the employment of alcalase (126,741 Alcalase Enzyme, Sigma Aldrich, Merck Life Science Milan, Italy) added to the biomass at 45°C for 2 h. Then, the temperature was held at 100°C for 10 min in order to get enzymatic deactivation. Biomass was filtered and undigested parts were removed. The FPH was transported by truck, in refrigerated tanks, to the feed mill where feeds were manufactured. The feed plant belonged to the same fish Company involved in the trial. A dedicated line of extruder was used for the preparation of the experimental diets. All the ingredients were mixed according to the target. Three diets (LC, L1, L2) were essayed with the same protein (47.1%) and lipid (16%) content. LC was employed without FPH, as control diet in which the protein source was mainly provided by fish meal, vegetable-origin feedstuffs, and hemoglobin. FPH was included in L1 diet (7.5 g/kg) and in L2 diet (15 g/kg) in replacement of conventional protein source feedstuff (fishmeal). Feeds formulation is reported in Table 1.

The feeds were manufactured in 3.0 mm size using a twin-screw extruder (100 rpm, 110°C, 50 atm). After the coating, the feeds were stocked in buckets and kept in an aerated room. The samples of each diet were taken for proximate composition analysis. Dried feed was shifted, packaged in bags, and stored at −20°C until transport to the Porto Conte Ricerche hatchery. Proximate composition and amino acid content of the experimental diets are reported in Table 2.

2.2 | Experimental design, fish employed, and sampling

The trial was performed at the Porto Conte Ricerche hatchery located in Tramariglio (Alghero, Italy). Three groups of 170 gilthead sea bream juveniles each (initial body weight 37.8 ± 0.5 g) were reared in triplicate indoor tanks of 2 m^3 of volume, supplied by recirculating aquaculture system. Fish were fed diets including FPH in L1 (7.5 g/kg) and L2 (15 g/kg) as substitute for fishmeal, the conventional protein source feedstuff. Through the trial, in all the tanks, the main water physicochemical parameters (water temperature, salinity, dissolved oxygen, pH, nitrogen compounds) were monitored in situ using portable electronic devices (YSI mod. 55 and 60, Yellow Springs, OH, USA) and in laboratory. Nitrogen ammonia, nitrites, and nitrates were weekly analyzed following APHA standard methods

TABLE 1 Formulations of the experimental diets (g/100 g diet).

	LC	L1	L2
Fish meal	30.0	22.0	15.0
FPH	**0**	**7.5**	**15.0**
Soybean meal	18.5	18.5	18.5
Wheat meal	12.2	10.2	7.5
Gluten corn	10.0	10.0	10.0
Gluten wheat	8.0	10.3	12.5
Hemoglobin	5.4	5.4	5.4
Fish oil	8.0	8.0	8.0
Soybean oil	4.8	5.0	5.0
L-Lysine	0.5	0.5	0.5
Choline powder	0.4	0.4	0.4
DL-Methionine	0.2	0.2	0.2
Phosphate monocalcium	1.0	1.0	1.0
Vitamin premix	1.0	1.0	1.0

TABLE 2 Proximate composition (% wet weight basis) and amino acid profile of the three experimental diets.

	LC	L1	L2
Moisture	7.0	6.9	6.8
Protein	46.7	47.1	47.1
Lipids	16.5	16.5	16.2
Fiber	2.0	2.0	2.3
Ash	8.41	9.41	8.84
Amino acid profile (g/100 g)			
Arginine	2.37	2.50	2.44
Aspartic acid	3.99	3.81	3.69
Cysteine	0.69	0.66	0.69
Glutamic acid	7.18	7.24	7.15
Histidine	1.04	1.14	1.07
Isoleucine	1.55	1.54	1.53
Leucine	3.86	3.90	3.82
Lysine	2.96	2.72	2.70
Methionine	0.82	0.84	0.85
Phenylalanine	1.93	2.12	2.01
Threonine	1.55	1.60	1.55
Tryptophan	0.49	0.49	0.48
Tyrosine	1.20	1.29	1.25

(APHA, 1995) by means of spectrophotometer (mod. DR 2000, Hach, Milan, Italy). In all the tanks, water quality parameters showed that temperature was maintained at 20°C; salinity was 37 ppt ± 1, dissolved oxygen was between 6.8 and 7.2 mg/L, pH ranged between 7.6 and 7.11, ammonia nitrogen was absent, nitrites were absent, and nitrates stayed under 10 mg/L.

During the experiment, which lasted 85 days, fish were fed twice a day (8 a.m. and 3 p.m.) until the satiation level, receiving the feeding through 56 days (no feed was administered on the weekend and festivity). The unconsumed feed was collected. Palatability was determined in terms of pellet consumption (Pc, %) = (consumed feed/administered feed) × 100 (Alves et al., 2019; Alves et al., 2020). For handling events, all the fish were anesthetized using tricaine methanesulfonate (MS222, Pharmaq, Fordindbridge, UK) bath (20 mg/L).

The following zootechnical performances were evaluated in different groups: Specific Growth Rate (SGR, %/day) = {Ln (final weight) − Ln (initial weight)/duration} × 100; Feed Conversion Rate (FCR) = live weight gain (g)/feed administered (g); Survival Rate (SR, %) = final number of fish/initial number of fish × 100 (Steffens, 1989); Condition index (KI) = (fish weight fish/length^3) × 100 (Bagenal & Tesch, 1978); Perivisceral Fat Index (PFI) = (perivisceral fat/body weight) × 100; Hepatosomatic index (HSI) = (liver weight/body weight) × 100 (EPA, 2000).

2.3 | Histological analysis of fish

At the end of the trial, to investigate differences in the hepatic structure among the considered groups, the liver of five gilthead sea bream per diet was sampled and fixed in 10% buffered formalin then processed for histological examination. Tissues were serially sectioned at 4 μm using a rotary microtome (Leica RM2235, Leica Microsystems, Wetzlar, Germany). Histological sections were stained with hematoxylin and eosin (HE) and evaluated under a light microscope in a blinded fashion. An optical microscope (Leica DM 2500, Leica Microsystems Srl, Buccinasco, Italy) equipped with a camera (Leica DFC 7000 T, Leica Microsystems Srl, Buccinasco, Italy) was used to acquire images. Every section was entirely analyzed at low (4–10×) and medium (20×) magnification considering the following pathologic traits, namely circulatory changes, regressive change (presence of degeneration and/or necrosis of hepatocytes and/or bile ducts), progressive change (presence of hypertrophy and/or hyperplasia of hepatocytes and/or bile ducts, and infiltration of adipose cells in the peripancreatic fat), and inflammatory changes (i.e., presence of inflammatory cells). For each histopathologic change detected, a score was assigned as follow: 0 (not observed), 1 (presence of 1–3 foci), 2 (presence of 4–10 foci); 3 (more than 10 foci). Thus, the score of 0 represented the minimum level of fat infiltration in the hepatocytes, while the score of 3 represented the maximum.

2.4 | Diets, body composition, and fatty acids analyses

Diets and whole body of gilthead sea bream from each group were analyzed in order to determine proximate composition according to the Association of Official Analytical Chemists procedure for moisture, protein, and ash (AOAC, 1990). The chemical composition and the amino acid profile of the three feeds was determined before starting the trial according the procedures previously adopted (Addeo et al., 2021). Total lipids were measured using a modification of the chloroform:methanol procedure described by Folch et al. (1957). Regarding fish, three fish per group were analyzed at the end of the trial. After having determined the total lipid content, fatty acids were converted to methyl esters following the method described by Christopherson and Glass (1969) and performed as described in a previous paper (Coco et al., 2022).

2.5 | Statistical data analyses

Data collected were submitted to one-way analysis of variance (ANOVA) using SPSS 25 (IBM, 2017) to show significant differences in productive performance and composition of body composition of gilthead sea bream fed with the different experimental diets. Means were considered significant with a value of $p < 0.05$ and compared using the Student–Newman–Keuls (SNK) test. The histological analysis of liver was also considered and histopathological

differences were analyzed among the groups using Kruskal–Wallis's test followed by Dunn's multiple comparison test. The level of significance was set at $p < 0.05$.

3 | RESULTS

At the end of the growing trial, similar performances were observed among L1, L2, and LC (Table 3). The final mean weight ranged from 76.6 to 78.0 g without significant differences. SGR varied from 1.26% to 1.31%. FCR was included between 1.24 and 1.26. SR was high in all the groups, with the rate ranging between 98.83% and 100%. All the three diets exhibited 100% pellet consumption. Somatic indices did not show differences in terms of KI (1.50–1.52), PFI (7.5–8.1), and HSI (1.62–1.66), which were similar among the groups.

The results of the histological scoring concerning the fat infiltration in the tissue performed on the liver of fish fed with L1, L2, and LC diet are reported in Table 4. In all the fish fed with different diets (L1, L2, LC), a diffuse cytoplasmic vacuolization of the hepatocytes was observed, with nuclei frequently located at the periphery (Figures 1–3). In all the groups, fish liver also showed the presence of adipocyte infiltrates associated with intrahepatic pancreatic tissue. No statistical difference was observed among the three groups for both hepatocyte vacuolization and peripancreatic fat infiltration.

No significant differences were found in body composition (Table 5). Regarding the macronutrients, the protein fraction amounted at around 19% in all the groups and emerged with respect to the lipid fraction, stable at 16%. No notable variations were observed among the main categories of fatty acids, with SFA being the most prevalent, followed by MUFA, n-6 PUFA, and n-3 PUFA (Figure 4).

TABLE 3 Growth performances at the end of the trial (mean ± standard deviation).

	LC	L1	L2	*p*
Initial body weight (g)	37.8 ± 0.5	37.8 ± 0.5	37.8 ± 0.5	-
Final body weight (g)	78.8 ± 12.2	76.6 ± 11.2	78.0 ± 12	>0.05
Average weight gain (g)	41.10 ± 2.46	39.13 ± 0.32	39.50 ± 1.56	>0.05
Weight gain (%)	108.91 ± 5.77	104.64 ± 0.45	102.93 ± 5.80	>0.05
SGR (%)	1.31 ± 0.05	1.28 ± 0.01	1.26 ± 0.05	>0.05
FCR	1.25 ± 0.03	1.26 ± 0.01	1.24 ± 0.04	>0.05
SR (%)	100 ± 0.0	99.40 ± 1.01	98.83 ± 2.02	>0.05
Pc (%)	100 ± 0.0	100 ± 0.0	100 ± 0.0	>0.05
KI	1.51 ± 0.01	1.52 ± 0.01	1.50 ± 0.02	>0.05
PFI	7.5 ± 1.12	8.0 ± 1.23	8.1 ± 1.36	>0.05
HSI	1.62 ± 0.24	1.63 ± 0.31	1.66 ± 0.41	>0.05

Note: No significant difference ($p < 0.05$) was found among the means.

TABLE 4 Histological scores of the liver of fish fed with LC, L1, L2 diets (mean ± dev.st.).

	LC	L1	L2
Hepatocyte vacuolization	3 ± 0	3 ± 0	3 ± 0
Peripancreatic fat infiltration	2.4 ± 0.5	1.6 ± 1.1	2 ± 1.4

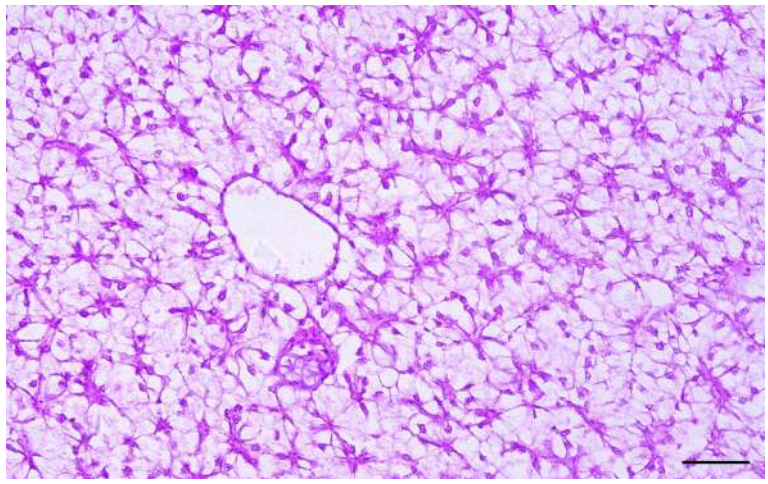

FIGURE 1 Liver from fish fed with LC diet. Diffuse hepatocellular degeneration with intracytoplasmic lipid accumulation (HE, bar 100 μm).

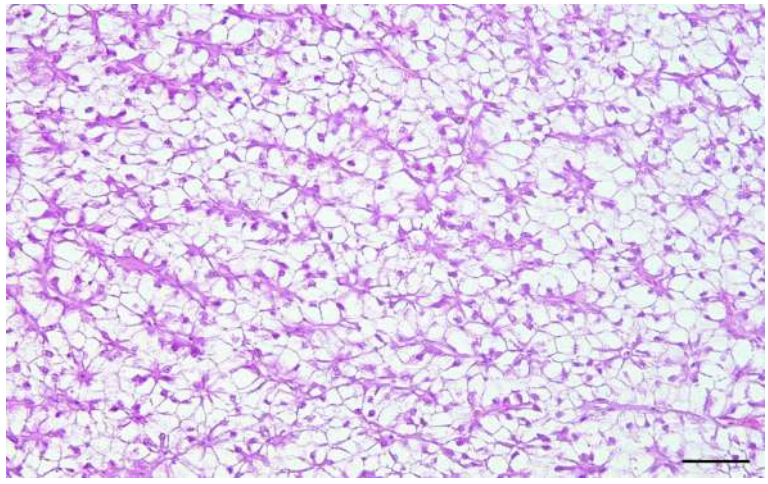

FIGURE 2 Liver from fish fed with L1 diet. Diffuse hepatocellular degeneration with intracytoplasmic lipid accumulation (HE, bar 100 μm).

4 | DISCUSSION

The aim of the present study was to assess the suitability of fish by-products from rainbow trout as a protein source, as an alternative to conventional protein sources (fish meal, hemoglobin), for feeding gilthead sea bream juveniles at the end of the pre-growing phase, evaluating growth performance, liver status, and body composition traits. For this study, we used the by-products of the filleting process from rainbow trout (>1.1 kg) in which the discards incidence was roughly 50% of the whole-body weight. The by-products were represented by head, skin, viscera, fins, and trimmings of the fillet and were used in a mixture balanced to obtain essential nutrients, mainly protein at high availability after the FPH process, as requested by the gilthead sea bream juveniles to sustain optimal growth and reduce nitrogenous losses (Davis & Hardy, 2022). As regards water quality, the monitored physicochemical parameters were

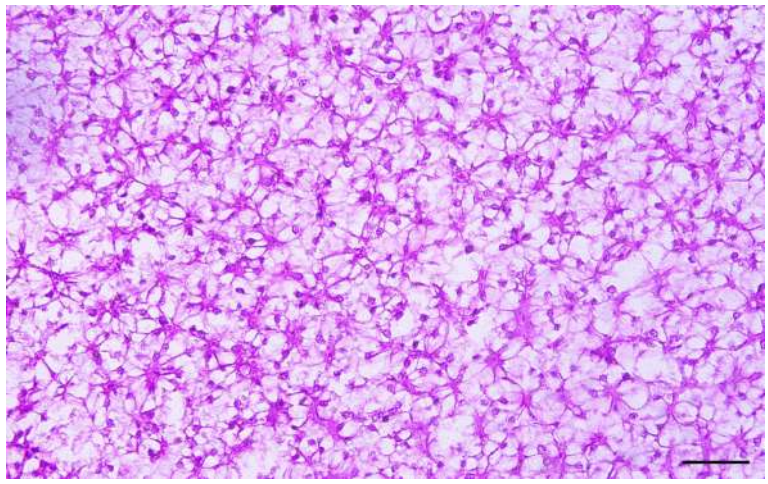

FIGURE 3 Liver from fish fed with L2 diet. Diffuse hepatocellular degeneration with intracytoplasmic lipid accumulation (HE, bar 100 μm).

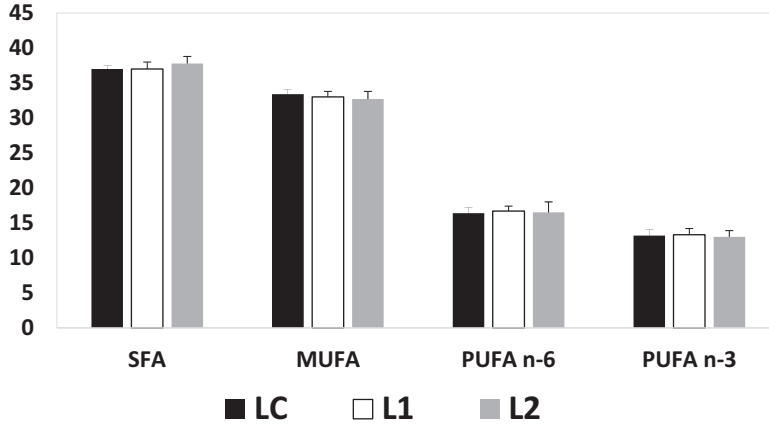

FIGURE 4 Main categories of fatty acids (% total fatty acids) determined in the body of gilthead seabream of the different groups at the end of the trial.

TABLE 5 Proximate composition (% wet weight basis) of the body of gilthead seabream fed the different diets, determined at the end of the trial.

	LC	L1	L2	*p*
Moisture	60.25 ± 0.6	60.86 ± 0.7	60.98 ± 0.5	n.s.
Protein	19.39 ± 0.9	19.47 ± 0.8	19.96 ± 0.8	n.s.
Lipids	16.62 ± 0.7	16.26 ± 0.8	16.14 ± 0.7	n.s.
Ash	6.85 ± 0.9	6.92 ± 0.8	6.98 ± 0.5	n.s.

Abbreviation: n.s., not significant.

within the range considered optimal for the gilthead sea bream in all the fish groups tested throughout the study (Bernabè, 1990).

The L1 and L2 diets, including 50% and 100% of FPH instead of fish meal and hemoglobin, as other protein source of animal origin, demonstrated to be suitable to sustain a good growth of the gilthead sea bream juveniles, without showing significant differences with respect to the control group. The productive results were in agreement with those reported by Kotzamanis et al. (2001) that essayed waste material resulting from trout processing; although they employed juveniles lower (4 g) than those used in the current study (37.8 g), SGR and the other productive performances confirmed similar results. FCR was favorable considering the short feeding period (56 days of feed administration). The present study ascertained the suitability of replacement of fish meal with fish by-products or FPH can be obtained at high level of substitution for juvenile stage growing. In juvenile rainbow trout, the FPH was successfully included up to 20% of fish meal (Güllü et al., 2014). In our case, the level of FPH substitution was significantly higher (177, 354 g/kg) than those used in yellowtail juveniles fed marine by-products in the diet (125 g/kg) (Benitez-Hernández et al., 2017) or in shrimps where the substitution of fish meal with FPH provided satisfactory results at 10% (Hernández et al., 2011). In the three feeds, the amino acid profile was similar, and this confirms the goodness of the hydrolysation technique adopted, which has allowed the amino acids availability for a growth of the young fish similar to the control ones. In juvenile tilapia, the replacement of fish meal with 30% FPH, obtained by means of an enzymatic process similar to that used in the current trial, improved the growth performances compared with the control group (Bae et al., 2019). In juvenile red drum, feeding shrimp processing waste meal exhibited good performances with an average range of protein substitution (Whiteman & Gatlin, 2005) similar to that used in our study. In agreement with the literature on the physicochemical composition of FPH derived from rainbow trout Kvangarsnes et al. (2023), showing an amino acid profile with lysine content and other essential amino acids similar to conventional diets, LC, L1, and L2 diets could completely replace dietary fish meal and hemoglobin, and contribute to the functional properties of feeds. Consequently, the Pc showed a good palatability as proof L1 and L2 diets were not negatively affected by inhibitors of chemical transformation of FPH production, which could generate scarce feed attraction (Suratip et al., 2023). The very high SR was in line with other studies (Bae et al., 2019; Tefal et al., 2023).

Regarding liver histology, assigning a score has recently been reported in the literature as a support to the histological examination (Pacorig et al., 2022). The score assigned to the three groups showed a morphological appearance affected by hepatocyte vacuolization and peripancreatic fat infiltration. This status was different from previous studies, which detected no cellular alterations in the livers of gilthead sea bream fed FPH diets (Fronte et al., 2019). The FPH inclusion aimed at increasing the traits of sustainability of the diets for juveniles replacing the conventional raw materials, fish meal, and hemoglobin. L1 and L2 diets maintained the protein source also supplied by a blend of plant-based feedstuffs (soybean, wheat, gluten corn, gluten wheat) as in LC diet. The hepatocellular modification could not be ascribed to FPH inclusion, but to the employment of vegetable oils as responsible of fat accumulation in liver as reported in the literature for euryhaline fish species (Caballero et al., 2004; Martinez-Llorens et al., 2012; Roncarati et al., 2010). In the current trial, soybean oil was included at a rate higher than fish oil, suggesting high fat storage in all the three groups. In Atlantic salmon, a decrease in HSI was noted when dietary fishmeal was replaced with FPH (Espe et al., 2012). Other study (Kotzamanis et al., 2001) reported high fat accumulation in sea bream fed fish by-products and the respective control diet, but also in that experiment, as in our trial, liver fat deposition did not appear to be related to unbalanced fatty acids content in the diet. However, zootechnical performances were not compromised in our study. The lipid source is very important for juvenile growth. In fact, in gilthead sea bream, this liver fat deposition is considered transitory when changing the lipid source in the diet, for example, during the finishing phase of the farming cycle (Caballero et al., 2004).

As regards the whole-body composition, proximate analysis was similar among the three groups of sea bream fed different inclusion of FPH. Dry matter was consistent with that reported in other studies on gilthead sea bream juveniles (around 36%–39%) fed fish meal from organic trout remaining after processing (Tefal et al., 2023), whereas lipids were lower (around 20%) than those reported by the same authors (37.1%). The use of discards from

rainbow trout aimed at including adequate rates of the refilled ventral part of the fillet, particularly rich of high nutritional values as n-3 PUFA. This reflected the fatty acid profile of the fillet of rainbow trout obtained at the end of the productive cycle providing well-balanced extruded feed. In our case, the possibility to submit the rainbow trout discards in an area dedicated to all the FPH production very close to the slaughtering process gave the advantage to avoid long transports or prolonged storage time, preserving biomass from oxidation or loss of hygienic conditions, as reported in the literature (Antonelli et al., 2023; Gasco et al., 2020; Shumilina et al., 2016). Further studies need to be performed.

5 | CONCLUSION

The exploration of alternative sources and the utilization of by-products coming from the food industry are gaining more importance. Fish by-products can be used in the formulation of feeds for the main fish species of interest in aquaculture as a good raw organic material; in this context, this study showed that FPH derived from rainbow trout processing waste is feasible as a source of alternative protein in the feeding of gilthead sea bream juveniles in the pre-growing phase. Further analyses are in progress to determine the suitability of FPH during the second phase of farming cycle (on-growing phase) to produce large-size (>1 kg) gilthead sea bream.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

DATA AVAILABILITY STATEMENT

Data openly available in a public repository that issues datasets with DOIs.

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