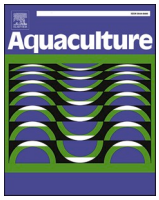
Aquaculture 573 (2023) 739571

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Towards a free wild-caught fishmeal, fish oil and soy protein in European sea bass diet using by-products from fishery and aquaculture

Arianna Marchi [a], Alessio Bonaldo [a], Andrea Di Biase [c], Roberto Cerri [c], Daniel Scicchitano [b], Enrico Nanetti [b], Marco Candela [b], Gianfranco Picone [d], Francesco Capozzi [d,e], Francesco Dondi [a], Pier Paolo Gatta [a], Luca Parma [a,*]

[a] Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia, Italy
[b] Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy
[c] AIA – Agricola Italiana Alimentare S.p.A., Via Valpantena 18/G, 37142 Quinto di Valpantena, Verona, Italy
[d] Department of Agricultural and Food Sciences DISTAL, University of Bologna, Piazza Goidanich 60, 47521 Cesena, Italy
[e] Interdepartmental Centre for Industrial Agrofood Research - CIRI Agrofood, University of Bologna, Via Quinto Bucci 336, 47521 Cesena, Italy

ARTICLE INFO

Keywords:
European sea bass
By-products
Growth
Metabolomics
Gut microbiota

ABSTRACT

One of the main strategies to implement the sustainability of aquaculture is by reducing its dependence on feed raw materials derived from wild marine resources, unsustainable land and freshwater use. To totally replace wild-caught fishmeal (FM), fish oil (FO) and soy protein (SP) in European sea bass, five experimental diets were formulated including increasing levels of fishery and aquaculture by-products (control C, 0FM100FO, 0FM0FO, 0FM0FO-50SP, 0FM0FO-0SP). Diets were administered to triplicate fish groups of 50 individuals reared in a recirculation system for 119 days. No major differences ($p > 0.05$) between C and 0FM100FO were detected in growth (final body weight) and feed efficiency parameters (feed conversion rate, protein efficiency ratio, gross protein efficiency, lipid efficiency ratio, gross lipid efficiency) while they were reduced ($p < 0.05$) in 0FM0FO, 0FM0FO-50SP, 0FM0FO-0SP compared to C diet. At the end of the growth trial the overall metabolomic profiles of fish muscle were impacted by the diets. In particular, the increase in glycine and phosphocreatine in the muscle of fish fed with diets containing FM and FO by-products, may suggest changes or adaptation in metabolic pattern for energy production at muscular level; results which were also supported by the higher level in plasma creatinine, uric acid and lactate found in C diets. Concerning welfare indicators, overall plasma parameters were in line with the normal range for this species indicating a general optimal welfare condition under all dietary regimes. No effect of diets on overall gut microbiota layout was observed. However, the inclusion of the FM and FO by-product increase the relative abundance of several taxa such as *Weissela*, *Enterococcus*, *Streptococcus and Bacillus* which could potentially support immune system and disease resistance. Overall this study highlighted the possibility of totally replacing wild-caught FM and FO using by-products from fisheries and aquaculture with an only marginal reduction of the overall performance.

1. Introduction

The increase in world population and the improvement of living conditions have changed world food habits with a shift towards products of animal origin (FAO, 2017). Over the last two decades the aquaculture sector has been increasingly recognized for its essential contribution to global food security and nutrition (FAO, 2022). However, the rapid growth of aquaculture has led to an overexploitation of natural resources (wild fish populations, land and freshwater uses) with implication on ecological issues such as biodiversity concerns and biotic depletion (Ahmed and Thompson, 2019). The main challenge of aquaculture is to become more sustainable using nutritional resources that are produced through a circular bioeconomy approach (Colombo et al., 2022). In the last twenty years, several studies on soy derivates as alternative protein sources to fishmeal (FM) have reported positive results on the growth and health of several aquatic species (Zhou et al., 2018), such as salmonids (Collins et al., 2013) and Mediterranean marine species, including gilthead sea bream (Parma et al., 2016) and

* Corresponding author at: Department of Veterinary Medical Sciences, University of Bologna, Viale Vespucci 2, 47042 Cesenatico, FC, Italy.
E-mail address: luca.parma@unibo.it (L. Parma).

https://doi.org/10.1016/j.aquaculture.2023.739571
Received 21 November 2022; Received in revised form 5 April 2023; Accepted 8 April 2023
Available online 10 April 2023

European sea bass (Bonvini et al., 2018a). However, plant ingredients may pose contrasts in sustainability and resource efficiency. In fact, several studies on life cycle assessment demonstrated that the substitution of marine ingredients with vegetable ingredients have shifted resource demand from the oceans onto the land with an overall environmental impacts of feed production expected to increase (Newton and Little, 2018; Malcorps et al., 2019). Besides being competing resources in the human food chain, vegetable ingredients when included at high dietary level may also inducing nutritional specification issues (nutrient limitation and antinutritional factor) (Glencross, 2009), may affect fish welfare (Saito et al., 2020) and could also alter the level of micro- and macro nutrient in the final fish product (Nichols et al., 2014; Sprague et al., 2016). Soy management, including growing, processing, transporting, and disposal is directly and indirectly associated with overexploitation of soil, deforestation, and high levels of carbon footprint (Eranki et al., 2019). Imported soybeans for aquafeed use are responsible for 75% of aquaculture greenhouse gas (GHG) emission estimated between 2.9 and 3.8 kg CO_2e kg^{-1} LW of fish at the farm gate (Ghosh et al., 2020). Moreover, the expansion of the aquaculture sector often requires inputs from wild fish stocks for feed formulation, exploiting a sector already overused both for animal and human consumption (Wang et al., 2015). In response to this, the marine ingredients industry is undergoing a period of change as increasing amounts of raw materials are sourced from the by-products of fisheries and aquaculture which now account for as much as 35% of FM worldwide (Newton and Little, 2018).

Fishery and aquaculture by-products are now widespread as promising alternative feed ingredients for fish farming due to their availability and nutritional quality (Stevens et al., 2018). The utilization of different fish and crustaceans species with the inclusion of every kind of animal body part (from bone to skin) with a high content of micro and macronutrients such as protein, DHA and EPA, is what makes these ingredients highly attractive for the sector (Stevens et al., 2018; Malcorps et al., 2021).

Fishery and aquaculture by-products can also be considered as sustainable ingredients because of their potential to reduce environmental impact, improve the efficiency of the industry, and reduce waste production (Stevens et al., 2018; Gasco et al., 2020; Munekata et al., 2020). The inclusion of low economic value fish by-products also showed the potential to lower the Fish in - Fish-out (FIFO) ratio (Kok et al., 2020) and the need for commonly used marine ingredient substitutes, such as plant ingredients, which affect EPA + DHA aquafeed content (Malcorps et al., 2021).

Also, some vegetable by-products of the agriculture processing industry are a rich resource still greatly undervalued (Glencross et al., 2020). It was estimated that approximately 1.3 billion tons per year of food for human consumption is lost or wasted during and after processing, and about half of this is represented by vegetable waste (Ran et al., 2019). However, most agri-food-derived by-products are a promising source of lipids, carbohydrates, proteins with nutritionally and functionally important compounds such as gluten, polyphenols, pectin and many others (Dhillon et al., 2016; Leonard et al., 2020). In recent years, due to emerging health concerns, gluten meal has become a significant cereal by-product of agriculture and is becoming widespread on account of its high protein content (Tapia-Hernandez et al., 2019). In aquaculture, it is used for its nutritional characteristics, safety, ease, cost-effectiveness of purification and, in comparison to soybean products, its production is less associated with unsustainable deforestation (Newton et al., 2023).

Previous studies on the effect of this ingredient on rainbow trout (Tusche et al., 2012), salmon (Glencross et al., 2021), European sea bass (Fountoulaki et al., 2010) and gilthead sea bream (Aragao et al., 2020) have shown positive results on growth performance. Therefore, the present research aims to evaluate the effects of total replacement of wild-caught FM and FO by using fishery and aquaculture by-products and glutens as a valid alternative to soy protein (SP). Growth performance and gut health through gut microbiota investigation of European sea bass juveniles were evaluated. Furthermore, the effects of the different diets on muscle metabolome were evaluated by an NMR-based metabolomics approach and chemometrics analysis which proved to be an ideal tool for analyzing complex samples such as fish muscle (Picone et al., 2011; Laghi et al., 2014; Ciampa et al., 2022).

2. Materials and methods

2.1. Experimental diets

Five experimental diets were formulated to totally replace wild-caught FM, FO and SP using fisheries and aquaculture by-products, and alternative vegetable protein. Wild-caught FM and krill meal (FM prime, krill meal) were totally replaced by adding a combination of FM trimming and shrimp meal (0%FM,100%FO, 0FM100FO). Wild-caught FO (FO Extra) was totally replaced with FO trimming (0 %FM, 0%FO, 0FM0FO). SP (soybean meal and soy protein concentrate) were half (0% FM, 0%FO, 50%SP, 0FM0FO-50SP) and totally replaced (0%FM, 0%FO, 0%SP, 0FM0FO-0SP) with a blend of corn gluten and wheat gluten. Diets were extruded at industrial level with a diameter of 3 mm. Feeds were produced by AIA – Agricola Italiana Alimentare S.p.A. (Verona, Italy). Ingredients, proximate composition and fatty acid composition of the experimental diets are shown in Table 1 and Table 2.

2.2. Fish and rearing trial

The experiment was carried out at the Laboratory of Aquaculture, Department of Veterinary Medical Sciences of the University of Bologna, Cesenatico, Italy. European sea bass juveniles were obtained from an Italian hatchery. At the beginning of the trial, 50 fish (initial average weight: 75.96 ± 6.99 g) per tank were randomly distributed into 15 square tanks with a capacity of 800 L. Each diet was administered to triplicate groups, with random assignment, over 119 days. Tanks were provided with natural seawater and connected to a closed recirculation system (overall water volume: 20 m^3). The rearing system consisted of a mechanical sand filter (PTK 1200, Astralpool, Barcelona, Spain), ultraviolet lights (PE 25 mJ/cm^2: 32m^3 h^{-1}, Blaufish, Barcelona, Spain) and a biofilter (PTK 1200, Astralpool, Barcelona, Spain). The water exchange rate was 100% every hour, while the overall water renewal amount in the system was 5% daily. During the trial, the temperature was kept at 24 ± 0.5 °C and the photoperiod was maintained at 12 h light and 12 h dark through artificial light. The oxygen level was kept constant (8.0 ± 1.0 mg L^{-1}) by means of a liquid oxygen system regulated by a software programme (B&G Sinergia snc, Chioggia, Italy). Each day, ammonia (total ammonia nitrogen ≤0.1 mg L^{-1}) and nitrite (≤ 0.2 mg L^{-1}) were monitored by spectrophotometer (Spectroquant Nova 60, Merck, Lab business, Darmstadt, Germany) and salinity (25 g L^{-1}) was measured by a refractometer (106 ATC, Giorgio Bormac S.r.l., Carpi, Italy). Sodium bicarbonate was added if needed to keep pH constant at 7.8–8.0. Animals were fed to satiation with automatic feeders twice a day, set to release pellets gradually for one and a half hours. The uneaten pellets of each tank were collected, dried overnight at 105 °C, and weighed for feed intake (FI) calculation (Parma et al., 2019).

2.3. Sampling

At the beginning and at the end of the experiment, all animals in each tank were anaesthetised by MS222 at 100 mg L^{-1} and weighed. Specific growth rate (SGR) and feed conversion rate (FCR) were calculated. Moreover, wet body weight, length, viscera and liver were individually evaluated from five fish per tank to determine viscerosomatic (VSI), hepatosomatic indexes (HSI) and condition factor (CF). Proximate composition of the carcasses was determined on a pooled sample of 10 fish per tank at the beginning and on a pooled sample of 5 fish per tank at the end of the trial. For gut microbiota analysis, at the end of the trial, 12 h post meal, digesta content from the posterior intestine of five fish

Table 1
Ingredients and proximate composition of the experimental diets.

	C	0FM100FO	0FM0FO	0FM0FO-50SP	0FM0FO-0SP
Ingredients, % of the diet					
[1]Fish meal Prime	25.0	–	–	–	–
[2]Fish meal Trimming	–	25.0	25.0	25.0	25.0
[3]Krill meal	2.50	–	–	–	–
[4]Shrimps meal	–	2.50	2.50	8.50	8.50
[5]Fish oil extra	14.6	14.25	–	–	–
[6]Fish oil trimming	–	–	14.25	12.5	12.5
Wheat meal	14.5	14.3	14.4	14.0	18.2
[7]Soybean meal	19.2	19.2	19.2	9.6	–
[8]Soy protein concentrate	8.00	8.00	8.00	4.00	–
[9]Corn gluten	4.00	4.00	4.00	10.0	15.5
[10]Wheat gluten	–	2.60	2.50	2.75	9.50
Sunflower seed meal feed, dehulled	5.00	4.00	4.00	5.00	5.00
Guar germ meal	6.00	5.00	5.00	5.00	–
[11]Yeasts	–	–	–	–	2.00
[12]Microalgae				2.50	2.50
[13]Vitamins/ mineral premix	1.20	1.20	1.20	1.20	1.20
Proximate composition, % on a wet weight basis					
Moisture	3.80	1.90	1.81	1.77	2.27
Protein	42.9	42.2	43.5	43.3	42.2
Lipid	18.1	17.5	16.5	17.0	17.7
Starch	11.5	11.5	11.5	11.6	15.1
Ash	7.02	8.91	9.08	9.84	9.02

[1] Origin: Chile; Composition: protein 67%, lipid 10%, ash 14%. Antioxidant: butylated hydroxyanisole, BHA, 40 ppm; Butylated hydroxytoluene BHT, 200 ppm.
[2] Obtained from Atlantic mackerel (*Scomber scombrus*) and sardine (*Sardina pilchardus*). Origin: Morocco; Composition: protein 63%, lipid 10%, ash 20%. BHA, 80 ppm; BHT, 80 ppm.
[3] Composition: protein 54%, lipid, 18%, ash 13%.
[4] Obtained from whiteleg shrimp (*Litopenaeus vannamei*) heads; Composition: protein 52%, lipid 8%, ash 20%.
[5] Origin: Chile. Antioxidant: BHA 90 ppm, BHT 80 ppm, propyl gallate, PG 40 ppm.
[6] Obtained from Atlantic salmon (*Salmo salar*). Origin: EU. Antioxidant: BHA 70 ppm, BHT 145 ppm, PG 40 ppm.
[7] Origin: Italy (gmo-free). Composition: protein 49%, lipid, 1%.
[8] Origin: Serbia. Composition: protein, 61%, lipid, 0.3%.
[9] Origin: Italy.
[10] Origin: Italy.
[11] *Saccaromices cerevisiae.*
[12] Obtained from processed *Schizochytrium* (Corbion, Brazil).
[13] Vitamins/mineral premix (IU or mg kg^{-1} diet): vitamin A: 12000 IU; vitamin D: 2000 IU; vitamin E: 160 mg; vitamin C: 160 mg; Manganese: 40 mg; Zinc: 55 mg; Iron 20 mg; Copper: 8 mg; Iodine: 2 mg; selenium: 0.15 mg; BHA, 150 mg; PG, 75 mg.

per tank was collected and immediately stored at −80 °C (Parma et al., 2020). For the assessment of plasma biochemistry, blood from 5 fish per tank was collected from the caudal vein. Samples were then centrifuged (3000 xg, 10 min, 4 °C) and plasma aliquots were stored at −80 °C until analysis (Pelusio et al., 2021). At the same time 20 fish per treatment were sampled for metabolomic analyses of muscle.

Overall experimental procedures were evaluated and approved by the Ethical-Scientific Committee for Animal Experimentation of the University of Bologna (ID 1136/2019), under European directive 2010/63/UE relating to the protection of animals used for scientific purposes.

2.4. Calculations

Following, the illustration of employed formulae:

Specific growth rate (SGR) (% day^{-1}) = 100*(ln FBW- ln IBW) / days (where FBW represent the final body weight and IBW and the initial

Table 2
Fatty acid composition (% of total fatty acid methyl esters, FAME) of the experimental diets.

	C	0FM100FO	0FM0FO	0FM0FO-50SP	0FM0FO-0SP
12:0	0.71 ± 0.18	0.92 ± 0.23	–	0.65 ± 0.17	–
14:0	6.37 ± 0.92	7.3 ± 1.0	3.25 ± 0.51	3.00 ± 0.48	2.78 ± 0.43
15:0	0.53 ± 0.13	0.66 ± 0.16	0.25 ± 0.06	0.25 ± 0.06	0.22 ± 0.05
16:0	21.0 ± 2.5	23.6 ± 2.7	15.4 ± 2.0	12.8 ± 1.8	13.8 ± 1.7
16:1n-7	7.00 ± 1.33	8.20 ± 1.62	3.59 ± 0.72	3.50 ± 0.65	3.19 ± 0.56
17:0	0.50 ± 0.12	0.58 ± 0.14	0.27 ± 0.06	0.24 ± 0.06	0.25 ± 0.06
17:1n-7	0.20 ± 0.05	0.22 ± 0.05	0.12 ± 0.0	0.11 ± 0.03	0.11 ± 0.03
18:0	4.32 ± 0.63	4.69 ± 0.68	3.41 ± 0.52	3.37 ± 0.52	3.38 ± 0.50
18:1n-9	15.9 ± 2.1	14.2 ± 1.9	30.6 ± 3.4	31.0 ± 3.4	29.6 ±. 3.2
18:1n-7	3.13 ± 0.49	3.16 ± 0.49	2.76 ± 0.44	2.82 ± 0.45	2.67 ± 0.42
18:1n-6	0.12 ± 0.03	0.13 ± 0.03	0.09 ± 0.02	0.09 ± 0.02	0.08 ± 0.02
18:2n-6	7.80 ± 1.2	7.60 ± 1.1	16.2 ± 2.0	16.1 ± 2.0	15.7 ± 1.9
18:3n-6	0.16 ± 0.04	0.17 ± 0.04	0.11 ± 0.03	0.11 ± 0.03	0.11 ± 0.03
18:3n-3	1.72 ± 0.35	1.32 ± 0.29	5.39 ± 0.80	5.71 ± 0.84	5.24 ± 0.72
18:4n-3	1.80 ± 0.35	1.77 ± 0.34	0.77 ± 0.18	0.82 ± 0.20	0.74 ± 0.17
20:0	0.31 ± 0.08	0.31 ± 0.07	0.29 ± 0.07	0.35 ± 0.09	0.36 ± 0.09
20:1n-9	1.66 ± 0.33	1.40 ± 0.30	2.22 ± 0.37	2.84 ± 0.44	2.54 ± 0.40
20:2n-6	0.28 ± 0.06	0.25 ± 0.06	0.50 ± 0.12	0.59 ± 0.14	0.55 ± 0.13
20:3n-6	0.12 ± 0.03	0.11 ± 0.03	0.15 ± 0.04	0.17 ± 0.04	0.17 ± 0.04
20:3n-3	0.13 ± 0.03	0.01 ± 0.02	0.28 ± 0.07	0.44 ± 0.10	0.34 ± 0.08
20:4n-6 (ARA)	0.97 ± 0.23	1.02 ± 0.24	0.38 ± 0.1	0.41 ± 0.10	0.42 ± 0.01
20:5n-3 (EPA)	11.3 ± 1.6	10.7 ± 1.5	3.46 ± 0.52	4.16 ± 0.61	3.85 ± 0.55
22:0	0.12 ± 0.03	0.09 ± 0.02	0.11 ± 0.03	0.15 ± 0.04	0.16 ± 0.04
22:1n-11	1.26 ± 0.30	0.94 ± 0.22	1.15 ± 0.27	1.91 ± 0.34	1.61 ± 0.29
22:1n-9	0.20 ± 0.05	0.12 ± 0.03	0.22 ± 0.05	0.42 ± 0.09	0.33 ± 0.08
22:2n-6	0.03 ± 0.00	–	0.05 ± 0.01	0.08 ± 0.02	0.07 ± 0.02
22:4n-6	0.09 ± 0.02	0.07 ± 0.02	0.04 ± 0.00	0.05 ± 0.01	0.05 ± 0.01
22:3n-3	0.01 ± 0.00	0.02	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
22:5n-6	0.38 ± 0.09	0.32 ± 0.08	0.84 ± 0.20	0.18 ± 0.04	1.13 ± 0.25
22:5n-3	1.26 ± 0.30	1.00 ± 0.24	0.75 ± 0.18	1.17 ± 0.27	1.03 ± 0.24
22:6n-3 (DHA)	7.30 ± 1.0	5.65 ± 0.83	5.46 ± 0.78	4.22 ± 0.62	7.40 ± 1.1
23:0	0.02 ± 0.00	0.09 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
24:0	0.07 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.01 ± 0.02	0.10 ± 0.02
24:1n-9	0.22 ± 0.05	0.19 ± 0.04	0.17 ± 0.04	0.33 ± 0.08	0.30 ± 0.07
Σ SFA	34.8 ± 2.8	39.3 ± 3.0	23.5 ± 2.2	21.5 ± 2.0	21.5 ± 1.8
Σ MUFA	31.2 ± 2.8	30.1 ± 3.7	41.7 ± 4.4	43.7 ± 4.5	41.2 ± 4.1

(*continued on next page*)

Table 2 (*continued*)

	C	0FM100FO	0FM0FO	0FM0FO-50SP	0FM0FO-0SP
Σ PUFA	34.0 ± 2.4	30.6 ± 2.2	34.8 ± 2.4	34.8 ± 2.3	37.3 ± 2.4
Σ n-6	10.3 ± 1.2	10.1 ± 1.2	18.5 ± 2.0	17.9 ± 2.0	18.4 ± 1.9
Σ n-3	23.8 ± 2.0	20.7 ± 1.8	16.4 ± 1.3	16.9 ± 1.3	19.0 ± 1.5
n-3/n-6	2.31 ± 0.33	2.05 ± 0.30	0.89 ± 0.12	0.94 ± 0.13	1.03 ± 0.14
EPA/ARA	11.6	10.5	9.1	10.1	9.2
DHA/EPA	0.65	0.53	1.58	1.01	1.92

SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; ARA: arachidonic acid.

body weights).

Feed intake (FI, g kg ΔBW^{-1} day^{-1}) = ((100*total feed ingestion)/(ΔBW))/days.

Feed conversion ratio (FCR) = feed intake / weight gain.

Protein efficiency ratio (PER) = (FBW – IBW) / protein intake.

Gross protein efficiency (GPE) (%) = 100*[(% final body protein*FBW) - (% initial body protein*IBW)] / total protein intake fish.

Lipid efficiency ratio (LER) = (FBW - IBW)/lipid intake.

Gross lipid efficiency (GLE) (%) = 100*[(% final body lipid*FBW) - (% initial body lipid*IBW)]/total lipid intake fish.

VSI = Viscerosomatic index (%) = 100*(viscera weight/FBW).

HSI = Hepatosomatic index (%) = 100*(liver weight/FBW).

CF = condition factor = 100 × (body weight, g)/(body length, cm)^3.

2.5. Proximate composition analysis

Diets and whole bodies of sampled fish were analysed for proximate composition. Moisture content was obtained by weight loss after drying samples in a stove at 105 °C until a constant weight. Crude protein was determined as total nitrogen (N) by using the Kjeldahl method, multiplying N by 6.25, according to AOAC International (AOAC, 2010). Total lipids were determined according to Bligh and Dyer's (1959) extraction method. Samples were incinerated to a constant weight in a muffle oven at 450 °C to estimate ash content (AOAC, 2010). Fatty acid analyses composition of diets was performed according to ISO16958:2015.

2.6. Metabolomic analysis

Samples were prepared according to Picone et al. (2011): for each diet named "DA", "DB", "DC", "DD" and "DE", 10 aliquots of 4 g of white dorsal muscle from 20 different sea bass specimens for each treatment were homogenized with 8 mL of 7% perchloric acid (1:2 *w/v*). The acidic mixtures were first centrifuged at 4 °C, 10000 rpm (Hermle z366 K) for 10 min and then neutralized to pH 7.80 ± 0.05 using 9 M KOH. Further centrifugation at 14000 rpm for 10 min at 4 °C (Scilogex D30243) was needed to remove potassium perchlorate precipitate. Supernatant (720 μL) was aliquoted and placed in eppendorf microfuge tube adding 80 μL of 3-(trimethylsilyl)-propionic-2,2,3,3-d4 acid sodium salt (TSP) 10 mM as inner standard and then centrifuged again at 14000 rpm for 10 min at 4 °C. Clear sample (690 μL) was placed into a standard 5 mm NMR tube with a TSP final concentration of 0.1 mM and measurements were performed.

All the 300 ^{1}H NMR spectra were recorded at 300 K on a Bruker US+ Avance III spectrometer operating at 600 MHz, equipped with a BBI-z probe and a 24 SampleCase™ cooled for sample storage and automation (Bruker BioSpin, Karlsruhe, Germany). Each spectrum was acquired using 32 K data points over a 7211.54 Hz spectral width and adding 256 transients. A recycle delay of 5 s and a 90° pulse of 11.4 μs were set up. Acquisition time (2.27 s) and recycle delay were adjusted to be 5 times longer than the T1 of the protons under investigation, which has been considered to be no longer than 1.4 s. The saturation of the residual water signal was achieved by irradiating it during the recycle delay at δ equal to 4.703 ppm. For each sample, 128 scans were collected into 32 K data points covering a 12 ppm spectral width and requiring 22 min of measurement time. The phase correction and baseline were adjusted with TOPSPIN software version 3.5 pls (Bruker BioSpin, Karlsruhe, Germany) and successively the spectra were calibrated taking the chemical shift of the TSP signal to 0.000 ppm. For each sample, the analysis was performed in triplicate (Rocculi et al., 2019).

Before statistical analysis, the NMR spectra underwent several preprocessing procedures, such as spectra alignment, removal of some peaks, normalization and a final binning. The alignment of all spectra is led on the internal standard (TSP) peak at 0.00 ppm, then some parts of spectra lacking metabolic information are removed: i) from 9.00 to 20.00 ppm and from −20.00 to 0.50 where there is only noise; ii) the part from 4.60 to 5.10 ppm where water peak may produce a high interference and iii) the part from 6.00 to 6.10 ppm where there is the TCA peak. The new dataset was normalized by the application of the Probabilistic Quotient Normalization (PQN) (Dieterle et al., 2006) which is based on the calculation of a most probable dilution factor by looking at the distribution of quotients of amplitudes of a test spectrum by those of a reference one. Another further crucial data reduction is performed by using a binning also called bucketing algorithm (Craig et al., 2006). In this work, spectra were reduced to 431 bins of 100 points each which correspond to 0.0183 ppm of spectrum length. Bins with a loading value >1% of the overall standard deviation of all loading values were selected to determine the spectral regions encompassing most of the discriminative information (Picone et al., 2018).

2.7. Blood plasma analyses

The levels of glucose (GLU), urea, creatine, uric acid, total bilirubin, cholesterol (CHOL), triglycerides (TRIG), total protein (TP), albumin (ALB), aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), lactate dehydrogenase (LDH), calcium (Ca^{+2}), phosphorus (P), potassium (K^+) sodium (Na^+), iron (Fe), chloride (Cl), magnesium (Mg) were determined in the plasma using samples of 500 μL on an automated analyser AU 480; 220 Olympus/Beckman Coulter, Brea, CA, United States) using specific methods (Olympus system 221 reagent, OSR) and according to Parma et al. (2023). The Albumine/Globulin (ALB/GLO) ratio, lactate (LAC) and Current Calcium (Cur.Ca) were calculated.

2.8. Gut bacterial community DNA extraction, sequencing and analysis

Total DNA was extracted from individual distal gut content obtained from 5 fish per tank (300 mg per fish) at the end of the trial (for a total of 15 samples per experimental diet), as previously reported in Pelusio et al. (2021). DNA was then quantified with NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE) and stored at −20 °C until further processing. The amplification of the V3-V4 hypervariable regions of the 16S rRNA bacterial gene was carried out using the 341F and 785R primers with overhang sequencing adapters attached and 2 x KAPA HiFi HotStart ReadyMix (KAPA Byosystems). As already described by Pelusio et al. (2021), the thermal cycle consists of 30 amplification cycle, at the end the PCR products were purified, and indexed libraries were prepared following Illumina protocol "16S Metagenomic Sequencing Library Preparation". Libraries were normalized to 4 nM and pooled, the resulting pool was denatured with 0.2 N NaOH and diluted to 6 pM with 20% Phix control. Sequencing was performed on Illumina MiSeq platform using 2 × 250 bp paired-end protocol according to the manufacturer's instructions (Illumina, San Diego, CA). At the end of the sequencing process, raw sequences were processed combining PANDAseq and QIIME2 pipelines (Bolyen et al., 2019; https://qiime2.org). High-quality reads, obtained after a filtering step for length (min/max = 350/550 bp) and quality with default parameters, were cleaned using

DADA2 (Callahan et al., 2016) and clustered into amplicon sequence variants (ASVs) using VSEARCH algorithm (Rognes et al., 2016). Taxonomy was assigned using RDP classifier against SILVA database (Quast et al., 2013). Three different metrics were used to evaluate internal ecosystem diversity (alpha-diversity) – Faith's Phylogenetic Diversity (faith_pd), Shannon_entropy index, and number of observed ASVs (observed features). Unweighted UniFrac distances were computed to estimate inter-sample ecosystem diversity (beta-diversity) and used as input for Principal Coordinates Analysis (PCoA).

2.9. Statistical analysis

Growth and plasma biochemistry data are presented as mean ± standard deviation (SD). A single tank was used as the experimental unit for analyzing growth performance and a pool of ten fish was considered the experimental unit for the analysis of carcass composition and nutritional indices. The homogeneity of variance assumptions was validated for all data preceding ANOVA. Tukey's post hoc test was performed. All statistical analyses for growth and plasma biochemistry were performed using GraphPad 8.0.1. The differences among treatments were considered significant at $P \leq 0.05$.

NMR data underwent univariate and multivariate analyses according to Hatzakis (2019). In all pre-processing steps, univariate (ANOVA and *T*-Test) and multivariate data analyses (PCA) were implemented and conducted using the R free software environment for statistical computing (version 4.1.0). Essential bins responsible for group differentiation were chosen by applying the one-way analysis of variance (ANOVA) followed by Tukey's post hoc-test considering $P < 0.05$ as significant and by the Student's *t*-test. The chemical components from ANOVA were identified according to the literature, and by comparing their chemical shift and multiplicity with Chenomx software data bank, version 8.1 (Edmonton, AB, Canada).

Microbiota analysis and respective plots were produced using R software (https://www.r-project.org/) with "vegan" (http://www.cran.r-project.org/package-vegan/), "Made4" (Culhane et al., 2005) and "stats" packages (https://stat.ethz.ch/R-manual/R-devel/library/stats/html/00Index.html). Data separation was tested by a permutation test with pseudo-F ratios (function "Adonis" in "vegan" package). When required, Wilcoxon and Kruskal–Wallis tests were used to assess significant differences in alpha diversity and taxon relative abundance between groups. *P*-value ≤0.05 was considered statistically significant, while a *p*-value between 0.05 and 0.1 was considered as a trend.

3. Results

3.1. Growth

Results of growth performance and FI are summarized in Table 3. FBW was significantly higher in diet C and 0FM100FO compared to other treatments. SGR and WG were higher in C compared to other treatments, at the same time 0FM100FO was lower than C. FI values were significantly higher in 0FM0FO then 0FM0FO-0SP. FCR was statistically lower in C compared to 0FM0FO, 0FM0FO-50SP and 0FM0FO-0SP.

3.2. Proximate composition

Results of body proximate composition, nutritional indices and somatic indices are summarized in Table 4. No significant differences were observed for moisture, lipid and ash. Proteins in 0FM100FO were significantly higher compared to 0FM0FO. PER was higher in C than 0FM0FO, 0FM0FO-50SP and 0FM0FO-0SP, while 0FM0F0-0SP was higher than 0FM0FO and 0FM0FO-50SP. GPE was lower in 0FM0FO and 0FM0FO-50SP compared to C and 0FM100FO. Results of LER shown a *p*-value lower than 0.05 but non-specific differences among treatments were evaluated by multiple comparison Tukey's test. No effects were shown in GLE and CF. Results of VSI were statistically lower in diet C compared to 0FM0FO-0SP. 0FM0FO-0S*P* values of HSI were higher compared to 0FM0FO-50SP, 0FM0FO and C, at the same time 0FM0FO-50SP was lower than 0FM0FO-0SP.

3.3. Metabolomic analysis

The PCA obtained from the final data set together with the relative loadings plot for PC1 is shown in Fig. 1. PC1 and PC2 explain, respectively, 60% and 15% of the total variance, and the separation according to the diets occurs along PC1. The main metabolites which are involved along PC1 in the differentiation of samples, are shown by the loadings plot in Fig. 1C. In Table 5 are listed the main bins contributing to the second principal component. Lactate was higher in C and 0FM0FO-0SP compared to the other treatments (Fig. 2A). Creatine-p was higher in C compared to the other treatments while 0FM0FO-0SP was higher than 0FM100FO and 0FM0FO (Fig. 2B). Glycine was lower in C compared to the other treatments except than 0FM0FO-0SP (Fig. 2C). Taurine was higher in C compared to the other treatments while 0FM0FO-0SP was higher than 0FM100FO, 0FM0FO and 0FM0FO-50SP (Fig. 2D).

Table 3
Growth performance and feed intake of European sea bass fed experimental diets over 119 days.

		Experimental diets				
	C	0FM100FO	0FM0FO	0FM0FO-50SP	0FM0FO-0SP	*P value*
IBW	75.9 ± 1.06	75.8 ± 1.80	76.4 ± 0.79	75.8 ± 2.13	75.8 ± 1.45	*0.9875*
FBW	247 ± 4.75^{b}	239 ± 9.89^{b}	221 ± 5.89^{a}	222 ± 5.85^{a}	217 ± 9.24^{a}	***0.0021***
SGR	0.99 ± 0.01^{c}	0.97 ± 0.02^{b}	0.89 ± 0.04^{a}	0.90 ± 0.02^{a}	0.88 ± 0.02^{a}	***0.0013***
FI	1.19 ± 0.02^{ab}	1.22 ± 0.03^{ab}	1.24 ± 0.03^{b}	1.22 ± 0.02^{ab}	1.16 ± 0.04^{a}	***0.0294***
FCR	1.34 ± 0.02^{a}	1.40 ± 0.05^{ab}	1.50 ± 0.04^{b}	1.48 ± 0.05^{b}	1.43 ± 0.05^{ab}	***0.0038***
WG	171 ± 3.97^{c}	164 ± 8.10^{b}	145 ± 5.57^{a}	146 ± 7.89^{a}	142 ± 7.84^{a}	***0.0012***
Survival	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	*1.00*

Data are given as the mean ($n = 3$) ± SD. In each line, different superscript letters indicate significant differences among treatments ($P \leq 0.05$).
IBW = Initial body weight.
FBW = Final body weight.
SGR = Specific growth rate (% day-1) = 100 * (ln FBW- ln IBW) / days.
Feed intake (FI, g kg ΔBW–1 day–1) = ((1000*total feed ingestion)/(ΔBW))/days.
FCR = Feed conversion rate = feed intake / weight gain.
WG = Weight gain (g).
Survival = Survival (%).

Table 4
Body composition, nutritional indices, somatic indices measured in European sea bass.

	C	0FM100FO	0FM0FO	0FM0FO-50SP	0FM0FO-0SP	*P-value*
Whole body composition, %						
Moisture	60.9 ± 3.79	60.2 ± 8.55	60.3 ± 1.43	60.1 ± 0.48	60.8 ± 10.2	*0.4667*
Protein	17.0 ± 0.97^{ab}	17.2 ± 0.23^{b}	16.5 ± 0.52^{a}	16.8 ± 1.27^{ab}	16.9 ± 0.14^{ab}	***0.0253***
Lipid	18.6 ± 1.99	18.5 ± 5.6	19.4 ± 2.93	19.3 ± 0.47	18.7 ± 14.1	*0.1418*
Ash	3.67 ± 0.5	3.5 ± 1.99	3.25 ± 0.22	3.73 ± 0.57	3.57 ± 0.53	*0.0985*
Nutritional indices						
PER	1.74 ± 0.02^{c}	1.69 ± 0.05^{bc}	1.51 ± 0.05^{a}	1.55 ± 0.05^{a}	1.66 ± 0.05^{b}	***<0.0001***
GPE	30.5 ± 1.51^{b}	29.9 ± 1.09^{b}	25.3 ± 0.54^{a}	26.6 ± 1.72^{a}	28.6 ± 1.09^{ab}	***0.0024***
LER	4.13 ± 0.05	4.07 ± 0.13	3.99 ± 0.02	3.97 ± 0.12	3.95 ± 0.13	***0.0376***
GLE	82.4 ± 3.52	80.6 ± 5.98	85.9 ± 5.26	85.2 ± 2.87	83.6 ± 4.56	*0.6476*
Somatic indices						
CF	1.31 ± 0.01	1.36 ± 0,02	1.24 ± 0.01	1.15 ± 0.00	1.24 ± 0.01	*0.1004*
VSI	11.9 ± 0.06^{a}	12.7 ± 0.18^{ab}	12.9 ± 0.12^{ab}	12.8 ± 0.01^{ab}	13.7 ± 0.22^{b}	***0.0226***
HSI	1.93 ± 0.59^{a}	2.14 ± 0.47^{ab}	1.97 ± 0.74^{a}	2.35 ± 0.24^{b}	2.82 ± 0.42^{c}	***<0.0001***

Data are given as the mean ($n = 3 \pm$ SD). In each line, different superscript letters indicate significant differences among treatments ($P \leq 0.05$).
PER = Protein efficiency ratio = ((FBW-IBW)/protein intake).
GPE = Gross protein efficiency = 100*[(%final body protein*FBW) - (%initial body protein*IBW)]/total protein intake fish.
LER = Lipid efficiency ratio = (FBW - IBW)/lipid intake.
GLE = Gross lipid efficiency = 100*[(% final body lipid*FBW) - (% initial body lipid*IBW)]/total lipid intake fish.
CF = condition factor = 100 × (body weight, g)/(body length, cm)^3.
VSI = Viscerosomatic index (%) = 100*(viscera weight/FBW).
HSI = Hepatosomatic index (%) = 100*(liver weight/FBW).
SD = Standard deviation.

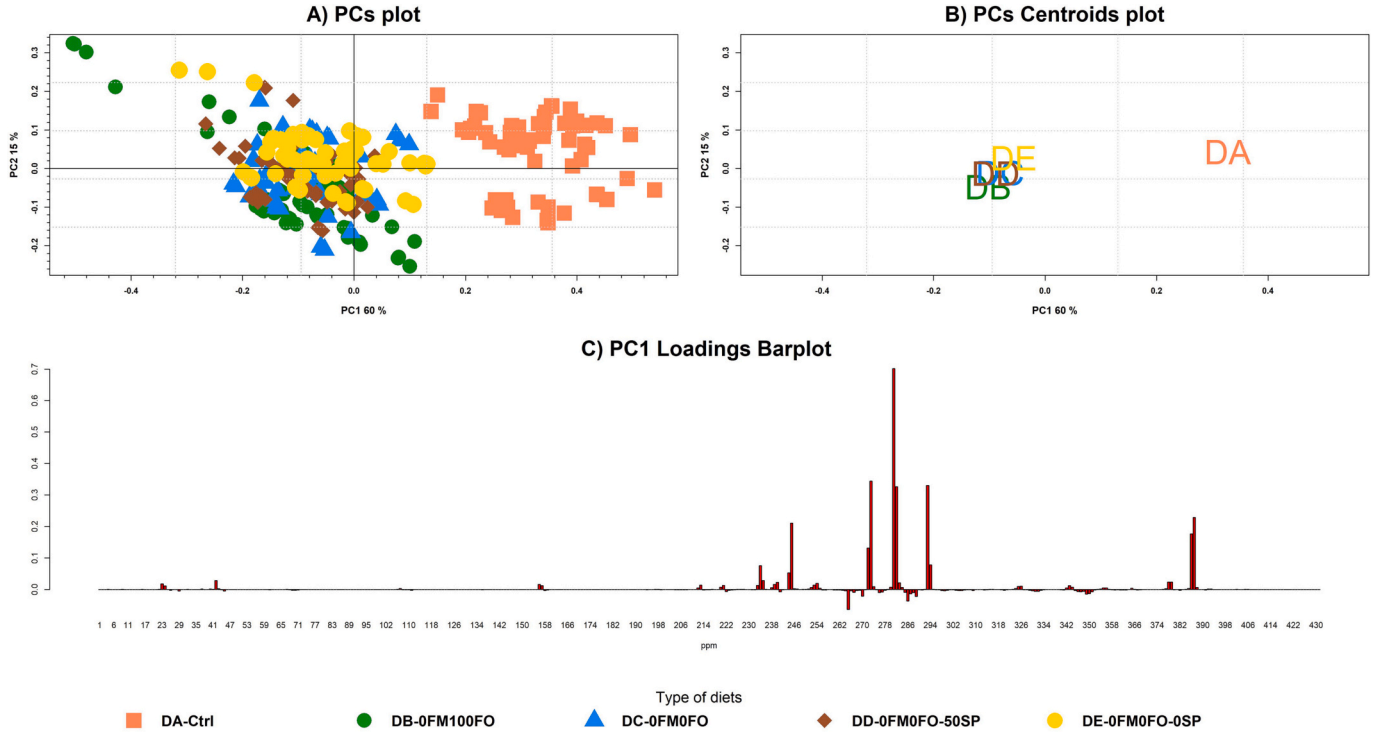


Fig. 1. A) PCA and loading plot of sea bass samples reared with 5 different diets B) PCs centroids plot and C) Loadings plot from PC1.

Table 5
Significant bins and their corresponding metabolites from PC1 loadings plot.

Bin number	PC	ppm_s	ppm_f	Metabolite	Multiplicity*
234	1	4.146	4.110	Lactate	m
244–245	1	3.964	3.909	Creatine-p	s
265	1	3.579	3.543	Glycine	s
272–273	1	3.451	3.396	Taurine	t
281–282	1	3.287	3.232	Taurine	t
293–294	1	3.067	3.012	Creatine-p	s
386–387	1	1.366	1.311	Lactate	d

* s = singlet; d: doublet; m = multiplet.

3.4. Plasma biochemistry

Plasma parameter results are shown in Table 6. Among all data considered, statistical difference was highlighted in CREA, Uric Acid, ALP, P, Mg, HDL, TP, ALB/GLOB and LAC values. In animals fed with C diet, CREA value was higher compared to other treatments. Results of Uric Acid showed higher values in diet C compared to 0FM0FO, 0FM0FO-50SP and 0FM0FO-0SP. ALP and TP were both lower in diet 0FM100FO compared to 0FM0FO-0SP. P shown a *P*-value lower than 0.05 but non-specific difference among treatments was evaluated by multiple comparison Tukey's test. Diet C showed higher value of Mg

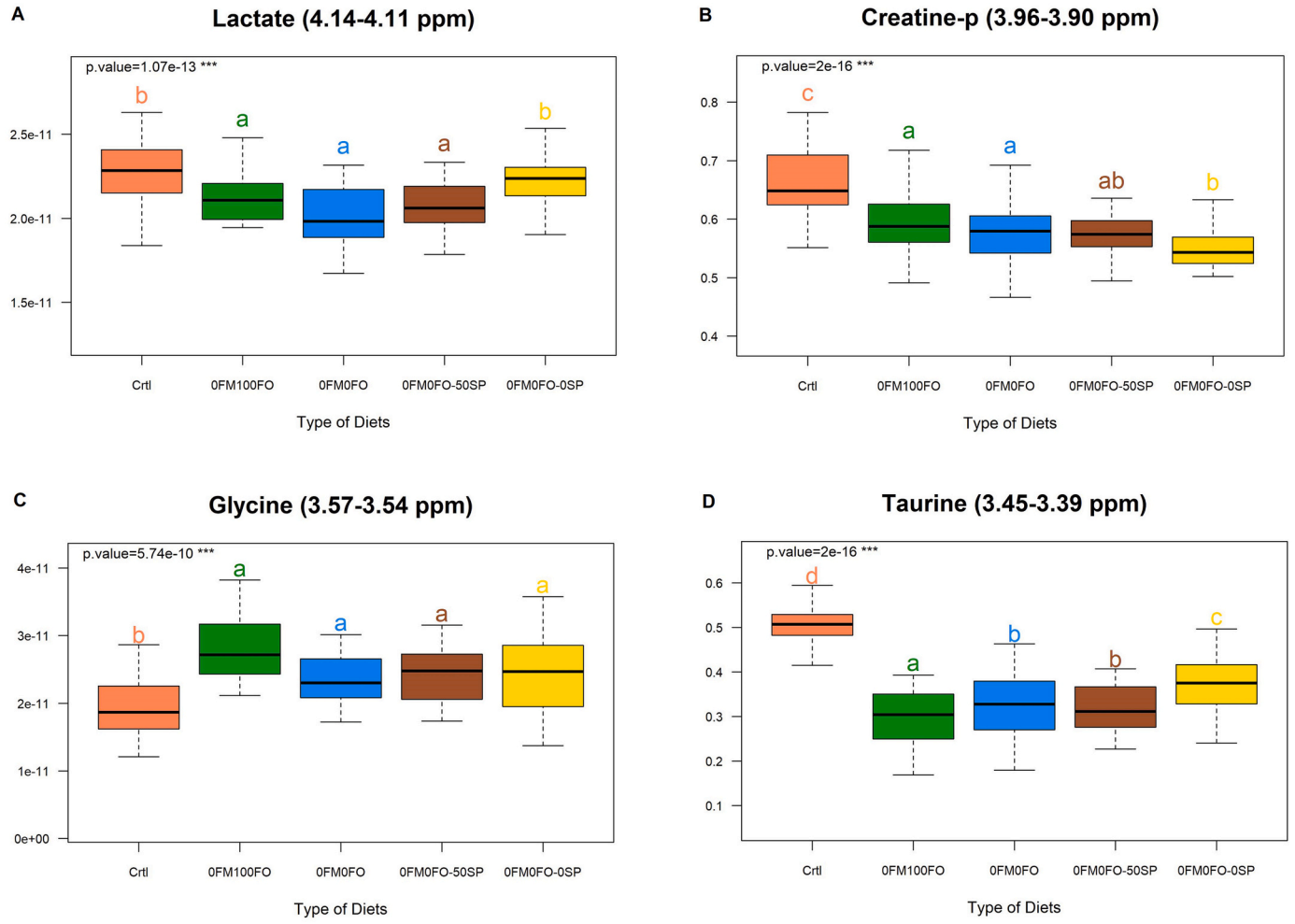


Fig. 2. (A-D). Boxplot of the main metabolites identified by PC analysis. Metabolites' concentrations were considered in area arbitrary units (normalized integrals). Statistical analysis was by one-way ANOVA, using Tukey's post-hoc test. Different letters in the same row indicate significant differences (at least $p < 0.05$).

compared to 0FM0FO-0SP. HDL value was lower in 0FM0FO compared to 0FM0FO-0SP. ALB/GLO was higher in 0FM0FO compared to 0FM0FO-0SP. Values of LAC were lower in 0FM100FO, 0FM0FO-50SP and 0FM0FO-0SP compared to other treatments.

3.5. Gut microbiota

The 16S rRNA gene sequencing was performed on a total of 75 distal intestine content samples, yielding 593′045 high-quality reads (mean ± SD, 7′907 ± 2′938) and clustered into a total of 8′596 ASVs. In order to assess the effects of replacement of FM, FO and SP on the gut bacteria community during the growth process of sea bass, the gut microbiota (GM) was analysed for each dietary group at the end of the trial. Principal Coordinates Analysis (PCoA) based on Unweighted UniFrac distances evaluated the GM variations between samples (beta-diversity). Moreover, the gut microbial community diversity, within each dietary group, was represented with faith-PD, Shannon_entropy and observed-features. According to our results (Fig. 3), in terms of overall GM composition, not all dietary groups showed a significant variation compared to the control group, regarding both alpha and beta diversity ($p > 0.05$). The overall GM composition at different phylogenetic levels was investigated, as reported in Fig. 4, at phylum, family (Fig. 5) and genus level (Fig. 6). More specifically, at phylum level the most abundant taxa observed was Firmicutes (with an overall relative abundance mean of 71%), but no difference was evaluated among treatments. The most represented family was *Lactobacillaceae* (overall r.ab. mean 55%), followed by a much lower abundance of *Staphylococcaceae*, *Planctomycetaceae*, *Bacillaceae*, *Streptococcaceae* and *Enterococcaceae* (overall r.ab. mean 3%, 2%, 2%, 1% and 1%), all belonging to Firmicutes phylum except for *Planctomycetaceae*. Those last family groups all showed statistical differences among groups in terms of relative abundance, with lower values in Ctrl group compared to the others experimental diets (Wilcoxon rank-sum test $p < 0.05$) (Fig. 5).

4. Discussion

Several studies have investigated the inclusion of plant derivates as alternative ingredients for FM and FO replacement in Mediterranean species, while less attention was paid to the application of fish by-products. The use of discarded fish by-products reduces the demand for FM from whole fish, may reduce pressure on fish stocks and is associated with sustainable farming of aquatic animals (Colombo et al., 2022). According to the present study the total replacement of FM with FM by-products did not affect the overall growth performance, feed utilization and FI. Previous studies showed good potential to replace wild-caught FM using FM by-products in several carnivorous marine fish species (Li et al., 2004; Uyan et al., 2006; Benitez-Hernández et al., 2018; Kim et al., 2022) even if most of these studies did not exceed 50% of the FM replacement. If by-products can satisfy fish nutrient requirements in terms of protein, or the amino acids profile of the by-product is similar to standard FM, no negative effect can be expected. However, FM by-products may contain considerably high levels of bones

Table 6
Plasma biochemistry in European sea bass fed with experimental diets.

	C	0FM100FO	0FM0FO	0FM0FO-50SP	0FM0FO-0SP	*P-value*
GLUC	211 ± 86.5	194 ± 62.8	157 ± 37.4	196 ± 53.1	186 ± 55.2	*0.1591*
Urea	8.69 ± 2.35	8.98 ± 1.63	9.32 ± 2.05	8.77 ± 1.49	7.87 ± 1.43	*0.2596*
CREA	0.88 ± 0.15^{b}	0.41 ± 0.08^{a}	0.38 ± 0.08^{a}	0.41 ± 0.09^{a}	0.42 ± 0.09^{a}	***<0.0001***
Uric.Acid	0.54 ± 0.56^{b}	0.29 ± 0.26^{ab}	0.19 ± 0.18^{a}	0.19 ± 0.14^{a}	0.13 ± 0.1^{a}	***0.0018***
Tot Bil	0.03 ± 0.02	0.03 ± 0.03	0.04 ± 0.03	0.04 ± 0.03	0.04 ± 0.03	*0.7198*
Ast	109 ± 84.7	101 ± 106.9	116 ± 93.2	347 ± 837.0	157 ± 80.2	*0.3507*
Alt	18.6 ± 33.1	6.28 ± 4.73	8.18 ± 3.85	117.0 ± 417.7	17.3 ± 23.6	*0.4219*
Alp	65.4 ± 11.6^{ab}	61.7 ± 13.2^{a}	67.2 ± 8.04^{ab}	71.1 ± 14.3 ^{ab}	77.3 ± 13.2^{b}	***0.0093***
CK	1998 ± 1098	1755 ± 2515	2770 ± 2672	2649 ± 2096	2224 ± 1430	*0.6194*
LDH	148 ± 55.4	143 ± 75.3	185 ± 127	180 ± 142	136 ± 51.8	*0.5062*
Ca^{2+}	15.4 ± 1.51	14.1 ± 1.62	15.1 ± 1.49	15.2 ± 2.23	15.3 ± 1.3	*0.1937*
P	12.1 ± 1.26	11.3 ± 1.78	12.6 ± 1.48	12.7 ± 1.57	12.8 ± 1.25	***0.0483***
Mg	4.15 ± 0.76^{b}	3.51 ± 0.44^{ab}	3.72 ± 0.68^{ab}	3.77 ± 0.73^{ab}	3.45 ± 0.44^{a}	***0.0222***
CHOL	301 ± 64.4	265 ± 41.9	250 ± 42.8	262 ± 47.3	275 ± 45.4	*0.0569*
HDL	83.8 ± 20.3^{ab}	74.1 ± 19.3^{ab}	69.4 ± 18.1^{a}	76.2 ± 17.9^{ab}	88.5 ± 18.8^{b}	***0.0431***
TRIG	1370 ± 465	1184 ± 282	1378 ± 346	1505 ± 348	1215 ± 358	*0.1215*
TP	5.04 ± 0.72^{ab}	4.45 ± 0.58^{a}	4.82 ± 0.63^{ab}	4.73 ± 0.63^{ab}	5.22 ± 0.54^{b}	***0.0154***
Alb	1.43 ± 0.20	1.27 ± 0.16	1.39 ± 0.18	1.35 ± 0.18	1.41 ± 0.15	*0.0966*
Cur. Ca	17.5 ± 1.35	16.3 ± 1.50	17.2 ± 1.37	17.4 ± 2.06	17.4 ± 1.26	*0.2472*
ALB/GLO	0.39 ± 0.02^{ab}	0.39 ± 0.03^{ab}	0.41 ± 0.03^{b}	0.40 ± 0.03^{ab}	0.37 ± 0.03^{a}	***0.0477***
LAC	62.7 ± 19.5^{b}	47.9 ± 8.9^{a}	58.2 ± 8.44^{b}	49.0 ± 14.6^{a}	43.3 ± 9.30^{a}	***0.0004***
Fe	131 ± 42.3	112 ± 21.8	111 ± 26.8	108 ± 15.9	111 ± 20.5	*0.1330*
Na^{+}	181 ± 12.0	176 ± 13.8	177 ± 5.21	183 ± 16.6	179 ± 10.6	*0.5775*
K	2.18 ± 0.66	2.16 ± 0.86	2.20 ± 1.27	2.87 ± 1.21	2.67 ± 1.12	*0.1788*
Cl	145 ± 12.0	143 ± 11.2	143 ± 4.44	148 ± 13.6	145 ± 9.02	*0.6398*

Data are given as the mean ($n = 15$ diet^{-1}) ± SD. Different letters indicate significant difference (One-way ANOVA P ≤ 0.05) between treatments.
GLU, glucose, (mg dL^{-1}); Urea, (mg dL^{-1}); CREA, creatinine, (mg dL^{-1}); Uric Ac, uric acid, (mg dL^{-1}); Tot Bil, total bilirubin, (mg dL^{-1}); Ast, aspartate aminotransferase, (U L^{-1}); Alt, alanine aminotransferase (U L^{-1}); Alp, alkaline phosphatase, (U L^{-1}); CK, creatine kinase, (U L^{-1}); LDH, lactate dehydrogenase, (U L^{-1}); Ca^{+2}, calcium, (mg dL^{-1}); P, inorganic phosphorus;, (mg dL^{-1}); Mg, magnesium, (mg dL^{-1}); CHOL, cholesterol, (mg dL^{-1}); HDL, high density lipoprotein; TRIG, triglycerides, (mg dL^{-1}); TP, total protein, (mg dL^{-1}); Alb, albumin, (g dL^{-1}); Cur Ca^{2+}, current calcium (mg dL^{-1}); ALB/GLO, albumin/globulin; LAC, lactate (mmol L^{-1}); Fe, iron, (μg dL^{-1}); Na^{+}, sodium, (mEq L^{-1}); K^{+}, potassium, (mEq L^{-1}); Cl, chloride, (mEq L^{-1}); SD, standard deviation.

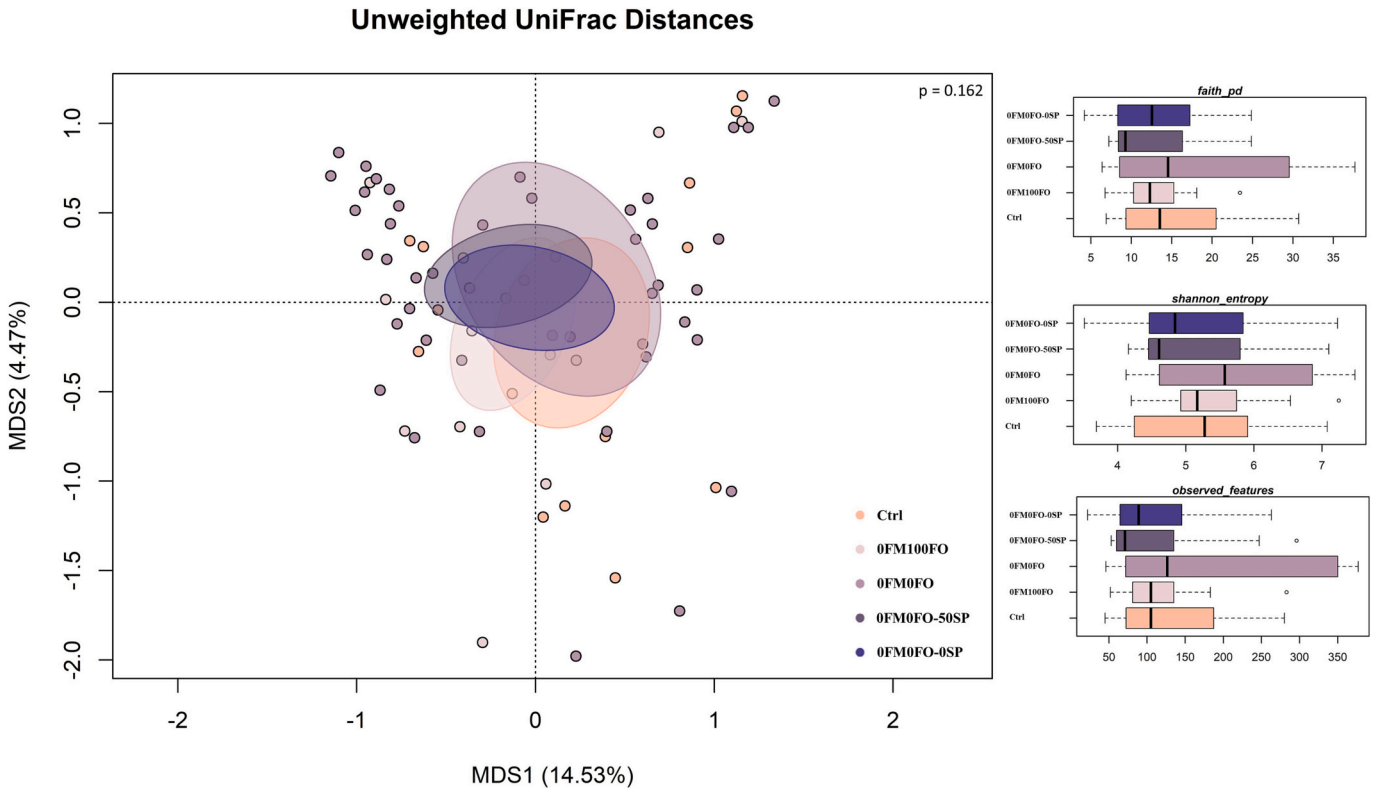


Fig. 3. Beta diversity and alpha diversity of gut microbiota of sea bass fed with experimental diets over 119 days. On the left, Principal Coordinates Analysis (PCoA) based on unweighted UniFrac distances between gut microbiota composition of animales fed with experimental diets. No significant separations were highlighted (permutation test with pseudo-F ratios Adonis; $p = 0.162$). On the right, Boxplots of alpha diversity values with 3 metrics, *faith_pd*, *shannon_entropy* and *observed_features* (ASVs). All metrics did not highlight any significant variations (Kruskal-Wallis test $p > 0.05$) of alpha diversity among dietary groups.

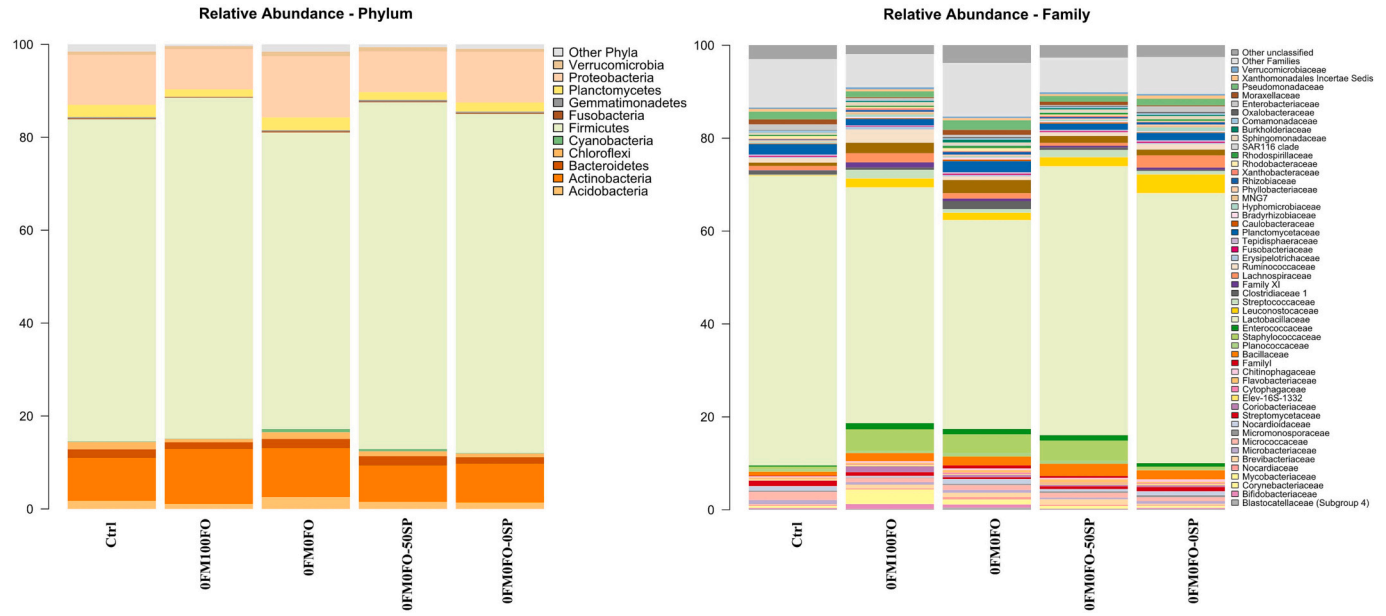


Fig. 4. Microbiota composition of distal gut content of sea bass fed with experimental diets. Bar plot summarizing the microbiota composition at Phylum (left) and family (right) of fish intestinal content. Only phyla and families with a relative abundance ≥1.0% in at least 3 samples are shown.

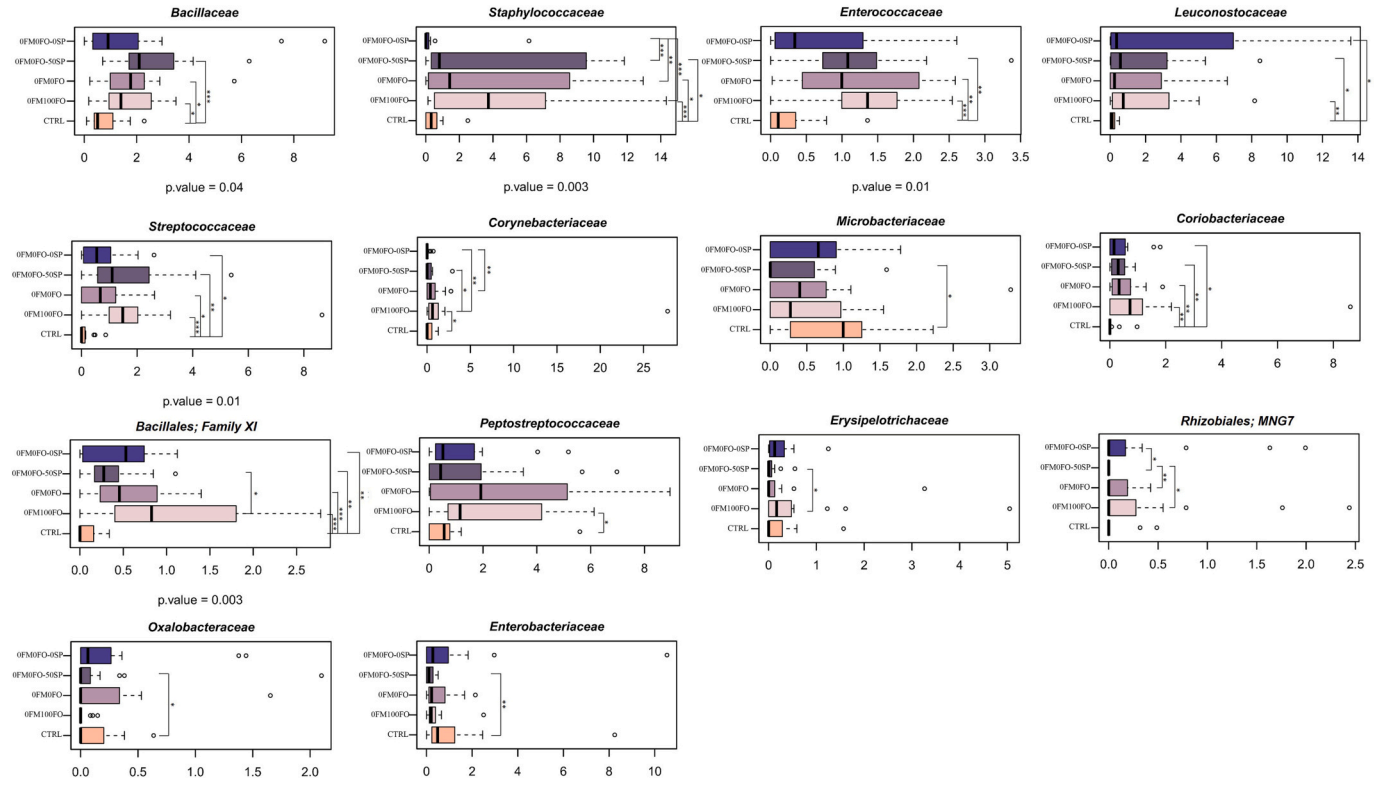


Fig. 5. Taxonomic composition of bacterial communities of distal gut content of sea bass fed experimental diets. Distributions of relative abundance of families that showed a significant variation between groups fed with different diets (Wilcoxon rank-sum test, *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$). Only families with a mean relative abundance ≥1.0% in at least 3 samples were represented. The central box of each dataset represents the distance between the 25th and the 75th percentiles. The median between them is marked with a black line.

with lower availability of nutrients such as phosphorus and calcium which can affect overall performance (Albrektsen et al., 2022). Further replacement of wild FO with FO from by-products resulted in reduced growth as observed in 0FM0FO. In fact, the total replacement of both wild FMFO leads to a ~ 10% reduction in SGR mainly due to a reduced FCR and specifically a reduced protein efficiency. Several studies have found reduction in performance of this species with the decreasing of marine FO in favour of vegetable oils. The reduction of LC-PUFA could affect growth performance as previously described on this species (Torrecillas et al., 2017a, 2018; Pelusio et al., 2022). However, a recent

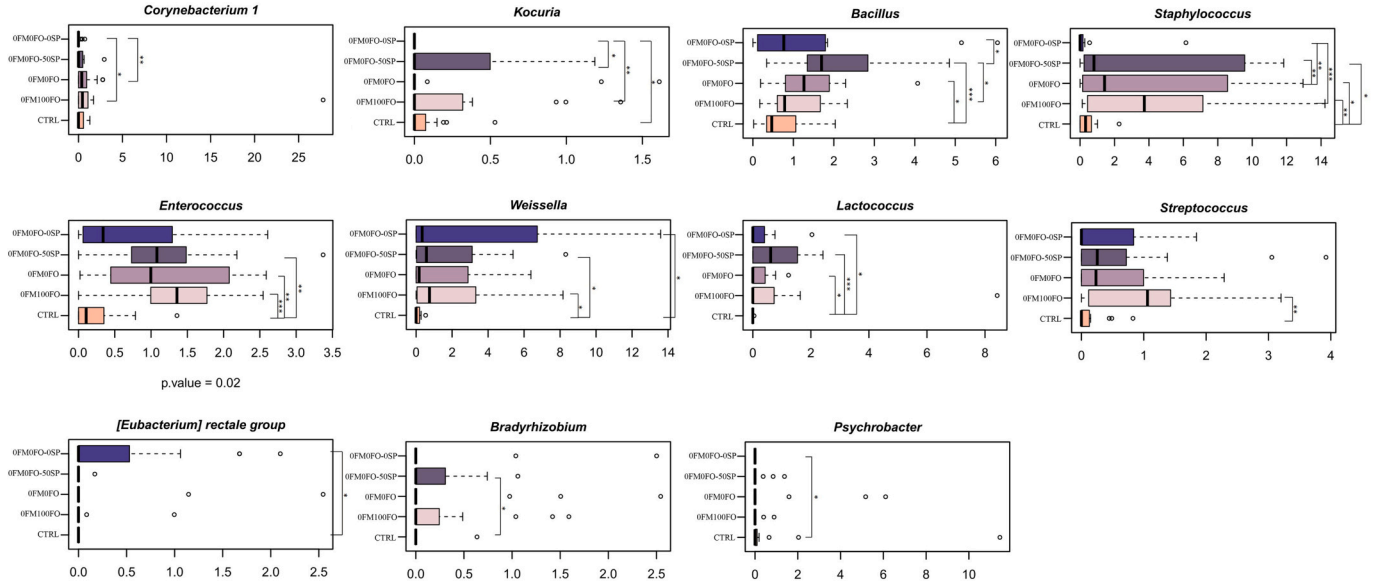


Fig. 6. Taxonomic composition of bacterial communities of distal gut content of sea bass fed experimental diets. Distributions of relative abundance of genera that showed a significant variation between groups fed with different diets (Wilcoxon rank-sum test, *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$). Only genera with a mean relative abundance ≥1.0% in at least 3 samples were represented. The central box of each dataset represents the distance between the 25th and the 75th percentiles. The median between them is marked with a black line.

finding denotes that 0.9% dietary LC-PUFA was effective to cover the requirements for European sea bass from juveniles to adults (Castro et al., 2022). This finding is in line with the 0.7% LC-PUFA requirement established by Skalli and Robin (2004) for this species. Dietary Σn-3/Σn-6 are also known to affect growth performance and stress response in fish species (Montero and Izquierdo, 2010). At this regard, the dietary Σn-3/Σn-6 reported in the present study are in line with previous studies on this species which provided a successfully nearly complete replacement of FO by vegetable oil when a suitable source of LC-PUFA was present (Torrecillas et al., 2017b; Castro et al., 2022). In the light of the above-mentioned studies we may exclude a dietary deficiency in LC-PUFA or altered Σn-3/Σn-6 able to affect the growth performance. However, it should be mentioned that different raw materials could have different lipid and energy availability for fish growth, which could have been responsible for the growth impairments observed. The replacement of soy products (SBM and SPC) with more sustainable vegetable protein ingredients (wheat gluten and corn gluten, 0FM0FO-50SP and 0FM0FO-0SP) in terms of land and water uses (Newton and Little, 2018, Newton et al., 2023) did not further alter growth except for a slight reduction in FI in comparison to 0FM0FO. Despite dietary SBM being well known to possibly induce inflammation processes in the distal intestine of some fish species (Baeverfjord and Krogdahl, 1996; Knudsen et al., 2008), the SBM level employed in the present study is within the tolerance range for European sea bass (Bonvini et al., 2018b). Previous studies on this and other Mediterranean species, have shown that 70% FM protein can be successfully replaced by wheat glutens in diets supplemented with the most limiting amino acids (Messina et al., 2013). However, reduction in palatability was also observed in gilthead sea bream fed 42% glutens in comparison to a blend of vegetable protein ingredients including also soybean meal (SBM, Parma et al., 2016). The application of plant protein mixture in aquafeed formulation is known to reduce the inhibition effect of feed intake given by a specific effect of a single ingredient (Bonaldo et al., 2015). On the other hand, the inclusion of yeast in 0FM0FO-0SP was probably responsible for restoring the FCR to that of the control group as also indicated by an increase in protein efficiency. In addition to an optimal protein profile (equivalent to SBM), yeasts are known to promote feed efficiency via the action of bioactive and prebiotic compounds such as vitamins B, β-glucans, mannoprotein and nucleotides (Agboola et al., 2021).

The application of metabolomics tools to fish nutrition is in its early stages, but recently some studies have demonstrated that it can provide a global insight into muscle metabolism by the identification of multiple metabolites involved in the biological responses of individuals exposed to different dietary treatments (Roques et al., 2020a). In the present study, the overall metabolomic profiles of fish muscle were clearly impacted by the diets. The PCA reveals a cluster of the control diet separated by the other diets which otherwise showed complete overlap. This finding agrees with previous studies where the substitution of FM with alternative protein sources such as soybean meal, insect meal, and feather meal (Jasour et al., 2017; Casu et al., 2017; Roques et al., 2020b) impacted the metabolic profiles of fish muscle. In the present study, a deep investigation reveals the main metabolites which were responsible for the separation in the PC. Specifically, phosphocreatine and lactate tended to be lower in the muscle of fish fed with FM by-products compared to those fed wild FM diet. These metabolites have a key role on muscle energy metabolism both in energy store and energy production; thus, the changes of these metabolites could indicate a disturbance of energy metabolism or (as in the case of creatine) can also be attributed to a reduced supply due to plant-based diets or other alternative ingredients to FM (Roques et al., 2020b). Interestingly, the higher level of glycine found at increasing FM replacement may also explain changes in metabolic pattern for energy production. In fact, increasing muscle level of glycine was previously found in fish fed a plant-based diet and may indicate an adaptation of energy metabolism towards an increased protein catabolism for the production of glucogenic amino acids into energy to counterbalance an energy-deficient state (Casu et al., 2017; Wei et al., 2017). Another explanation of the increase in the glycine could be in relation to the creatine synthesis since glycine together with arginine, is transformed into its precursor, the guanidinoacetate. Creatine seems to be synthesized in the fish muscle but its uptake can also occurs from the feed (Wuertz and Reiser, 2022; Borchel et al., 2014). Therefore, exogenous creatine seems to regulate the endogenous creatine synthesis. The presence of both metabolites in the muscle (glycine and p-creatine) may then suggest a difference in creatine metabolism when wild FM was replaced, probably due to a lower creatine content in the FM by-product. Although it has not been measured, a lower creatine content in fishmeal by-products is expected since 95% of the creatine is stored in fish muscle which is usually a minor component of FM derived

from trimming. This is also supported by the higher creatinine level found in the blood of fish fed wild FMFO indicating the higher creatine metabolism of fish fed this diet since creatinine formation occurs spontaneously during the conversion of creatine to phosphocreatine. Creatine metabolism and requirements in fish species are still poorly investigated; however, creatine is a key factor in the development of skeletal muscle and its inclusion seems advisable in carnivorous fish fed plant-based diets (Wuertz and Reiser, 2022). The higher level of taurine in the muscle of fish fed with the control diet could also explain the differences in growth performance. In turbot, a metabolomic study revealed that a high plant protein diet reduced taurine level in muscle in comparison to a FM control diet indicating that dietary taurine level may affect taurine metabolism in the muscle (Wei et al., 2017). Among its several physiological functions, dietary taurine promotes proliferation of muscle fibrils and elevates quantities of myosin and actin myofilaments to increase muscle growth of European sea bass (Saleh et al., 2020; Wassef et al., 2021). To evaluate welfare indicators, plasma biochemistry and gut microbiota were performed. Most of the plasma parameters analysed were in line with the normal range for this species, indicating a general optimal welfare condition for all the treatments (Pelusio et al., 2022; Bonvini et al., 2018b; Peres et al., 2014). The most significant differences occurred in the level of creatinine, uric acid and lactate with higher values in C diet compared to the other treatments. Interestingly, this value seems to be strictly correlated with the higher phosphocreatine and lactate found in the muscle of the fish of the same treatment, supporting higher energy muscle metabolism in specimens fed C diet. In gilthead sea bream, a high level of plasma creatinine was previously associated to high FMFO diet in combination to a single meal during a feeding frequency trial or during different rearing density, indicating higher protein metabolism in comparison to a low FMFO diet (Busti et al., 2020a; Parma et al., 2020). On the other hand, in European sea bass the observed higher plasma lactate after stress exposure only in fish fed high FM, FO level (30% FM, 15% FO) indicates a stronger metabolic muscle response to crowding in comparison to a low FMFO level (10% FM, 3% FO) (Pelusio et al., 2022). It should also be noted that plasma Mg tended to be higher under this treatment. It is well known that Mg participates in muscle energy metabolism and Mg plasma level is positively associated with muscle performance in both human and animals (Zhang et al., 2017).

Gut microbiota is a valid method to assess digestive condition and gut health especially concerning dietary formulation, which can drive gut microbiome towards potential consequences on metabolism and host immune response. Up to now several studies have addressed the effect of novel ingredients/additives including insect meal (Rangel et al., 2022; Pérez-Pascual et al., 2020), oligosaccharides (Rimoldi et al., 2020), organic acid (Busti et al., 2020b), glycerol (Louvado et al., 2020); and vegetable ingredients (Parma et al., 2019; Serra et al., 2021). However, no studies have assessed the gut microbiota layout in response to the total replacement of wild marine feedstuff using by-products from fisheries and aquaculture. GM was dominated by Firmicutes phylum, while *Lactobacillaceae, Staphylococcaceae, Bacillaceae, Streptococcaceae* and *Enterococceae* were the most represented bacterial families. These data are in accordance with previous studies on this species, recognizing bacteria within Firmicutes phylum as promoters of a healthy intestinal epithelium and generally of a good fish health status in European sea bass and other Mediterranean species (Parma et al., 2019; Parma et al., 2020; Busti et al., 2020b; Pérez-Pascual et al., 2020). No effect of diets on overall gut microbiota composition were observed as highlighted by Alpha and Beta diversities indices. Data that agreed with previous observations where slight differences in the growth performance followed by FMFO replacement with alternative non-marine ingredients did not result in gut microbiome alteration (Pérez-Pascual et al., 2020). Indeed, in the present study a greater abundance of some specific taxa was observed at the increase of fish by-product inclusion (i.e. *Bacillus*, *Staphylococcus*, *Streptococcus, Enterococcus, Weissela, Lactococcus, Leuconostocacea*). Specifically, it was observed that the inclusion of the FM by trimming (0FM100FO) was mainly responsible for promoting taxa such as *Streptococcus, Weissela* and *Enterococcus* while changes in *Bacillus* abundance were enhanced only after the FO by-product inclusion. These data support a general gut health status of fish fed under FM and FO by-product; in fact most of these families and genera belonging to Firmicutes phylum, such as *Bacillus, Weissela, Leuconostaceae, Streptococcus*, *Lactococcus*, *Enterococcus* are considered beneficial taxa for fish species with several positive contributions to nutrition, the immune system, and disease resistance (Ringø et al., 2020a, 2020b).

5. Conclusion

This study highlighted the potential of totally replacing wild-caught FM and FO and soy protein using by-products from fisheries and aquaculture and glutens, with only a marginal reduction of overall performance. In particular, when only wild-caught FM was totally replaced by FM by-products no differences were recorded, while the combined replacement of wild-caught FM and FO resulted in a performance reduction. The replacement of soy products (SBM and SPC) with more sustainable vegetable protein ingredients (wheat and corn gluten) in terms of land and water uses did not further alter growth. On the other hand, the inclusion of 2% of yeast in zero wild-caught FMFO and soy protein was probably responsible for restoring the FCR and the protein efficiency to that of the control group.

Muscle metabolomic profiles suggest changes or adaptation in the metabolic pattern for energy production at the muscular level when fish were fed FM and FO by-products. These findings deserve further investigation in regard to creatine and taurine metabolism or dietary supplementation for this species. The absence of differences in gut microbiota layout suggests no major implication of FM and FO by-products on gut health. Rather, the inclusion of FM and FO by-products promoted several taxa such as *Weissela, Enterococcus, Streptococcus* and *Bacillus* which can potentially support the immune system and disease resistance.

Author statement

Conceptualization A.B., L.P., A.D.B., M.C., F·C., P·P.G.; Methodology A.B., L.P., A.D.B., A.M., D.S., E.N., G.P., R.C., F·D. Investigation A.B., L. P., A.M., D.S., E.N., G.P., R.C., F.D. Writing-original draft preparation A. M., L.P., D.S., G.P.; Writing-review and Editing A.M., L.P., D.S., G.P., A. B., A.D.B., M.C., R.C. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

This research was undertaken under the NewTechAqua (New technologies Tools and Strategies for a Sustainable, Resilient and Innovative European Aquaculture) project, which has received funding from the European Union's Horizon 2020 Programme under grant agreement No 862658 (https://www.newtechaqua.eu/).

This study represents partial fulfilment of the requirements for the Ph.D. thesis of Daniel Scicchitano at the Ph.D. course of Innovative Technologies and Sustainable Use of Mediterranean Sea Fishery and Biological Resources (FishMed – University of Bologna, Italy). The authors would like to thank Gillian Forlivesi Heywood for English

language editing.

References

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

Ahmed, N., Thompson, S., 2019. The blue dimensions of aquaculture: A global synthesis. Sci. Total Environ. 652, 851–861. https://doi.org/10.1016/j.scitotenv.2018.10.163.

Albrektsen, S., Kortet, R., Skov, P.V., Ytteborg, E., Gitlesen, S., Kleinegris, D., Mydland, L.-T., Hansen, J.Ø., Lock, E.-J., Mørkøre, T., James, P., Wang, X., Whitaker, R.D., Vang, B., Hatlen, B., Daneshvar, E., Bhatnagar, A., Jensen, L.B., Øverland, M., 2022. Future feed resources in sustainable salmonid production: A review. Rev. Aquac. 14, 1790–1812. https://doi.org/10.1111/raq.12673.

AOAC, 2010. Officials Methods of Analysis, 17th edn. Association of Official Analytical Chemists, Washington, DC.

Aragao, C., Cabano, M., Colen, R., Fuentes, J., Dias, J., 2020. Alternative formulations for gilthead seabream diets: towards a more sustainable production. Aquac. Nutr. 26, 444–455. https://doi.org/10.1111/anu.13007.

Baeverfjord, G., Krogdahl, Å., 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: A comparison with the intestines of fasted fish. J. Fish Dis. 19 (5), 375–387.

Benitez-Hernández, A., Jimenez-Barcenas, S.P.L., Sanchez-Gutierrez, E.Y., Perez-Urbiola, J.C., Tovar-Ramirez, D., Palacios, E., Civera-Cerecedo, R., 2018. Use of marine by-product meals in diets for juvenile longfin yellowtail Seriola rivoliana. Aquac. Nutr. 24, 562–570. https://doi.org/10.1111/anu.12588.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917. https://doi.org/10.1139/o59-099.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37 (8), 852–857. https://doi.org/10.1038/s41587-019-0252-6.

Bonaldo, A., Di Marco, P., Petochi, T., Marino, G., Parma, L., Fontanillas, R., Koppe, W., Mongile, F., Finoia, M.G., Gatta, P.P., 2015. Feeding turbot juveniles *Psetta maxima* L. with increasing dietary plant protein levels affects growth performance and fish welfare. Aquac. Nutr. 21, 401–413.

Bonvini, E., Parma, L., Badiani, A., Fontanillas, R., Gatta, P.P., Sirri, F., Nannoni, E., Bonaldo, A., 2018a. Integrated study on production performance and quality traits of European sea bass (*Dicentrarchus labrax*) fed high plant protein diets. Aquaculture 484, 126–132. https://doi.org/10.1016/j.aquaculture.2017.10.041.

Bonvini, E., Bonaldo, A., Mandrioli, L., Sirri, R., Dondi, F., Bianco, C., Fontanillas, R., Mongile, F., Gatta, P.P., Parma, L., 2018b. Effects of feeding low fishmeal diets with increasing soybean meal levels on growth, gut histology and plasma biochemistry of sea bass. Animal 12 (5), 923–930.

Borchel, A., Verleih, M., Rebl, A., Kühn, C., Goldammer, T., 2014. Creatine metabolism differs between mammals and rainbow trout (*Oncorhynchus mykiss*). SpringerPlus 3, 510. https://doi.org/10.1186/2193-1801-3-510.

Busti, S., Bonaldo, A., Dondi, F., Cavallini, D., Yúfera, M., Gilannejad, N., Moyano, F.J., Gatta, P.P., Parma, L., 2020a. Effects of different feeding frequencies on growth, feed utilisation, digestive enzyme activities and plasma biochemistry of gilthead sea bream (*Sparus aurata*) fed with different fishmeal and fish oil dietary levels. Aquaculture 529, 735616. https://doi.org/10.1016/j.aquaculture.2020.735616.

Busti, S., Rossi, B., Volpe, E., Ciulli, S., Piva, A., D'Amico, F., Soverini, M., Candela, M., Gatta, P.P., Bonaldo, A., Grilli, E., Parma, L., 2020b. Effects of dietary organic acids and nature identical compounds on growth, immune parameters and gut microbiota of European sea bass. Sci. Rep. 10, 21321.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Castro, P.L., Torrecillas, S., Montero, D., Izquierdo, M.S., Ginés, R., 2022. Effect of combined fishmeal and fish oil replacement on growth performance, flesh quality and shelf life of European sea bass (*Dicentrarchus labrax*). Aquaculture 560, 738452. https://doi.org/10.1016/j.aquaculture.2022.738452.

Casu, F., Watson, A.M., Yost, J., Leffler, J.W., Gaylord, T.G., Barrows, F.T., Bearden, D. W., 2017. Metabolomics analysis of effects of commercial soy-based protein products in red drum (*Sciaenops ocellatus*). J. Proteome Res. 16, 2481–2494. https://doi.org/10.1021/acs.jproteome.7b00074.

Ciampa, A., Laghi, L., Picone, G., 2022. Validation of a 1H-NMR spectroscopy quantitative method to quantify trimethylamine content and K -index value in different species of fish. J. Food Qual. 3612095.

Collins, S.A., Overland, M., Skrede, A., Drew, M.D., 2013. 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, 85–100. https://doi.org/10.1016/j.aquaculture.2013.03.006.

Colombo, S.M., Roy, K., Mraz, J., Wan, A.H.L., Davies, S.J., Tibbetts, S.M., Øverland, M., Francis, D.S., Rocker, M.M., Gasco, L., Spencer, E., Metian, M., Trushenski, J.T., Turchini, G.M., 2022. Towards achieving circularity and sustainability in feeds for farmed blue foods. Rev. Aquac. 1–27.

Craig, A., Cloarec, O., Holmes, E., Nicholson, J.K., Lindon, J.C., 2006. Scaling and normalization effects in NMR spectroscopic metabonomic data sets. Anal. Chem. 78, 2262–2267. https://doi.org/10.1021/ac0519312.

Culhane, A.C., Thioulouse, J., Perrière, G., Higgins, D.G., 2005. MADE4: an R package for multivariate analysis of gene expression data. Bioinformatics 21, 2789–2790. https://doi.org/10.1093/bioinformatics/bti394.

Dhillon, G.S., Kaur, S., Oberoi, H.S., Spier, M.R., Brar, S.K., 2016. Chapter 2 - agricultural-based protein by-products: Characterization and applications. In: Singh Dhillon, G. (Ed.), Protein Byproducts. Academic Press, pp. 21–36. https://doi.org/10.1016/B978-0-12-802391-4.00002-1.

Dieterle, F., Ross, A., Schlotterbeck, G., Senn, H., 2006. Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures. Application in 1H NMR metabonomics. Anal. Chem. 78, 4281–4290. https://doi.org/10.1021/ac051632c.

Eranki, P.L., Devkota, J., Landis, A.E., 2019. Carbon footprint of corn-soy-oats rotations in the US Midwest using data from real biological farm management practices. J. Clean. Prod. 210, 170–180. https://doi.org/10.1016/j.jclepro.2018.11.002.

FAO (Ed.), 2017. The Future of Food and Agriculture: Trends and Challenges. Food and Agriculture Organization of the United Nations, Rome.

FAO, 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. FAO, Rome. https://doi.org/10.4060/cc0461en.

Fountoulaki, E., Morgane, H., Rigos, G., Antigoni, V., Mente, E., Sweetman, J., Nengas, I., 2010. Evaluation of zinc supplementation in European sea bass (*Dicentrarchus labrax*) juvenile diets: zinc requirements in sea bass. Aquac. Res. 41, e208–e216. https://doi.org/10.1111/j.1365-2109.2010.02503.x9.

Gasco, Acuti G., Bani, P., Zotte, A.D., Danieli, P.P., Angelis, A.D., Fortina, R., Marino, R., Parisi, G., Piccolo, G., Pinotti, L., Prandini, A., Schiavone, A., Terova, G., Tulli, F., Roncarati, A., 2020. Insect and fish by-products as sustainable alternatives to conventional animal proteins in animal nutrition. Ital. J. Anim. Sci. 19, 360–372. https://doi.org/10.1080/1828051X.2020.1743209.

Ghosh, A., Misra, S., Bhattacharyya, R., Sarkar, A., Singh, A.K., Tyagi, V.C., Kumar, R.V., Meena, V.S., 2020. Agriculture, dairy and fishery farming practices and greenhouse gas emission footprint: a strategic appraisal for mitigation. Environ. Sci. Pollut. Res. 27, 10160–10184. https://doi.org/10.1007/s11356-020-07949-4.

Glencross, B.D., 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Aquac. Res. 48 (7), 71–124.

Glencross, B.D., Baily, J., Berntssen, M.H.G., Hardy, R., MacKenzie, S., Tocher, D.R., 2020. Risk assessment of the use of alternative animal and plant raw material resources in aquaculture feeds. Rev. Aquac. 12 (2), 703–758.

Glencross, B., Grobler, T., Huyben, D., 2021. Digestible nutrient and energy values of corn and wheat glutens fed to Atlantic salmon (*Salmo salar*) are affected by feed processing method. Aquaculture 544, 737133. https://doi.org/10.1016/j.aquaculture.2021.737133.

Hatzakis, E., 2019. Nuclear magnetic resonance (NMR) spectroscopy in food science: A comprehensive review. Compr. Rev. Food Sci. Food Saf. 18, 189–220. https://doi.org/10.1111/1541-4337.12408.

Jasour, M.S., Wagner, L., Sundekilde, U.K., Larsen, B.K., Greco, I., Orlien, V., Dalsgaard, T.K., 2017. A comprehensive approach to assess feathermeal as an alternative protein source in aquafeed. J. Agric. Food Chem. 65, 10673–10684. https://doi.org/10.1021/acs.jafc.7b04201.

Kim, J., Baek, S., Cho, S.H., Kim, T., 2022. Evaluating the efficacy of partially substituting fish meal with unfermented tuna by-product meal in diets on the growth, feed utilization, chemical composition and non-specific immune responses of olive flounder (*Paralichthys olivaceus*). Aquacult. Rep. 24, 101150 https://doi.org/10.1016/j.aqrep.2022.101150.

Knudsen, D., Jutfelt, F., Sundh, H., Sundell, K., Koppe, W., Frøkiær, H., 2008. Dietary soya saponins increase gut permeability and play a key role in the onset of soyabean-induced enteritis in Atlantic salmon (*Salmo salar* L.). Br. J. Nutr. 100 (1), 120–129.

Kok, B., Malcorps, W., Tlusty, M.F., Eltholth, M.M., Auchterlonie, N.A., Little, D.C., Harmsen, R., Newton, R.W., Davies, S.J., 2020. Fish as feed: using economic allocation to quantify the fish in - fish-out ratio of major fed aquaculture species. Aquaculture 528, 735474.

Laghi, L., Picone, G., Capozzi, F., 2014. Nuclear magnetic resonance for foodomics beyond food analysis. Trends Anal. Chem. 59, 93–102.

Leonard, W., Zhang, P., Ying, D., Fang, Z., 2020. Application of extrusion technology in plant food processing byproducts: an overview. Compr. Rev. Food Sci. Food Saf. 19, 218–246. https://doi.org/10.1111/1541-4337.12514.

Li, P., Wang, X., Hardy, R.W., Gatlin III, D.M., 2004. Nutritional value of fisheries by-catch and by-product meals in the diet of red drum (*Sciaenops ocellatus*). Aquaculture 236, 485–496. https://doi.org/10.1016/j.aquaculture.2004.02.010.

Louvado, A., Coelho, F.J.R.C., Palma, M., Tavares, L.C., Ozorio, R.O.A., Magnoni, L., Viegas, I., Gomes, N.C.M., 2020. Effect of glycerol feed-supplementation on seabass metabolism and gut microbiota. Appl. Microbiol. Biotechnol. Appl. Microbiol. Biotechnol. 104 (19), 8439–8453.

Malcorps, W., Kok, B., Van't Land, M., Fritz, M., van Doren, D., Servin, K., van der Heijden, P., Palmer, R., Auchterlonie, N.A., Rietkerk, M., Santos, M.J., Davies, S.J., 2019. The sustainability conundrum of fishmeal substitution by plant ingredients in shrimp feeds. Sustainability (Switzerland) 11 (4), 1212.

Malcorps, W., Newton, R.W., Sprague, M., Glencross, B.D., Little, D.C., 2021. Nutritional characterisation of European aquaculture processing by-products to facilitate strategic utilisation. Front. Sustain. Food Syst. 5, 720595.

Messina, M., Piccolo, G., Tulli, F., Messina, C.M., Cardinaletti, G., Tibaldi, E., 2013. Lipid composition and metabolism of European sea bass (Dicentrarchus labrax L.) fed diets containing wheat gluten and legume meals as substitutes for fish meal. Aquaculture 376–379, 6–14. https://doi.org/10.1016/j.aquaculture.2012.11.005.

Montero, D., Izquierdo, M., 2010. Welfare and health of fish fed vegetable oils as alternative lipid sources to fish oil. In: Turchini, G., NG, W., Tocher, D. (Eds.), Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. CRC Press, Cambrige, UK, pp. 439–485.

Munekata, P.E.S., Pateiro, M., Domínguez, R., Zhou, J., Barba, F.J., Lorenzo, J.M., 2020. Nutritional characterization of sea bass processing by-products. Biomolecules 10, 232. https://doi.org/10.3390/biom10020232.

Newton, R.W., Little, D.C., 2018. Mapping the impacts of farmed Scottish salmon from a life cycle perspective. Int. J. Life Cycle Assess. 23 (5), 1018–1029.
Newton, R.W., Maiolo, S., Malcorps, W., Little, D.C., 2023. Life cycle inventories of marine ingredients. Aquaculture 565, 739096.
Nichols, P.D., Glencross, B., Petrie, J.R., Singh, S.P., 2014. Readily available sources of long-chain omega-3 oils: is farmed australian seafood a better source of the good oil than wild-caught seafood? Nutrients 6 (3), 1063–1079.
Parma, L., Candela, M., Soverini, M., Turroni, S., Consolandi, C., Brigidi, P., Mandrioli, L., Sirri, R., Fontanillas, R., Gatta, P.P., Bonaldo, A., 2016. Next-generation sequencing characterization of the gut bacterial community of gilthead sea bream (*Sparus aurata*, L.) fed low fishmeal based diets with increasing soybean meal levels. Anim. Feed Sci. Technol. 222, 204–216. https://doi.org/10.1016/j.anifeedsci.2016.10.022.
Parma, L., Yúfera, M., Navarro-Guillén, C., Moyano, F.J., Soverini, M., D'Amico, F., Candela, M., Fontanillas, R., Gatta, P.P., Bonaldo, A., 2019. Effects of calcium carbonate inclusion in low fishmeal diets on growth, gastrointestinal pH, digestive enzyme activity and gut bacterial community of European sea bass (*Dicentrarchus labrax* L.) juveniles. Aquaculture 510, 283–292. https://doi.org/10.1016/j.aquaculture.2019.05.064.
Parma, L., Pelusio, N.F., Gisbert, E., Esteban, M.A., D'Amico, F., Soverini, M., Candela, M., Dondi, F., Gatta, P.P., Bonaldo, A., 2020. Effects of rearing density on growth, digestive conditions, welfare indicators and gut bacterial community of gilthead sea bream (*Sparus aurata*, L. 1758) fed different fishmeal and fish oil dietary levels. Aquaculture 518, 734854. https://doi.org/10.1016/j.aquaculture.2019.734854.
Parma, L., Busti, S., Ciulli, S., Volpe, E., Errani, F., Oterhals, Å., Romarheim, O.H., Aspevik, T., Dondi, F., Gatta, P.P., Bonaldo, A., 2023. Growth, plasma biochemistry and immune-related gene expression of European sea bass (*Dicentrarchus labrax*) fed bioactive peptides from farmed salmon by-products. Aquaculture 563, 738982.
Pelusio, N.F., Scicchitano, D., Parma, L., Dondi, F., Brini, E., D'Amico, F., Candela, M., Yúfera, M., Gilannejad, N., Moyano, F.J., Gatta, P.P., Bonaldo, A., 2021. Interaction between dietary lipid level and seasonal temperature changes in Gilthead Sea bream *Sparus aurata*: effects on growth, fat deposition, plasma biochemistry, digestive enzyme activity, and gut bacterial community. Front. Mar. Sci. 8 https://doi.org/10.3389/fmars.2021.664701.
Pelusio, N.F., Bonaldo, A., Gisbert, E., Andree, K.B., Esteban, M.A., Dondi, F., Sabetti, M. C., Gatta, P.P., Parma, L., 2022. Different fish meal and fish oil dietary levels in European Sea bass: welfare implications after acute confinement stress. Front. Mar. Sci. 8, 779053 https://doi.org/10.3389/fmars.2021.779053.
Peres, H., Santos, S., Oliva-Teles, A., 2014. Blood chemistry profile as indicator of nutritional status in European seabass (*Dicentrarchus labrax*). Fish Physiol. Biochem. 40, 1339–1347. https://doi.org/10.1007/s10695-014-9928-5.
Pérez-Pascual, D., Estellé, J., Dutto, G., Rodde, C., Bernardet, J.-F., Marchand, Y., Duchaud, E., Przybyla, C., Ghigo, J.-M., 2020. Growth performance and adaptability of european sea bass (*Dicentrarchus labrax*) gut microbiota to alternative diets free of fish products. Microorganisms 8, 1–18.
Picone, G., Balling Engelsen, S., Savorani, F., Testi, S., Badiani, A., Capozzi, F., 2011. Metabolomics as a powerful tool for molecular quality assessment of the fish Sparus aurata. Nutrients 3, 212–227. https://doi.org/10.3390/nu3020212.
Picone, G., Zappaterra, M., Luise, D., Trimigno, A., Capozzi, F., Motta, V., Davoli, R., Nanni Costa, L., Bosi, P., Trevisi, P., 2018. Metabolomics characterization of colostrum in three sow breeds and its influences on piglets' survival and litter growth rates. J. Anim. Sci. Biotechnol. 9, 23. https://doi.org/10.1186/s40104-018-0237-1.
Quast, C., Klindworth, A., Pruesse, E., Schweer, T., Horn, M., Glo, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, 1–11. https://doi.org/10.1093/nar/gks808.
Ran, X., Zhang, M., Wang, Y., Adhikari, B., 2019. Novel technologies applied for recovery and value addition of high value compounds from plant byproducts: A review. Crit. Rev. Food Sci. Nutr. 59, 450–461. https://doi.org/10.1080/10408398.2017.1377149.
Rangel, F., Enes, P., Gasco, L., Gai, F., Hausmann, B., Berry, D., Oliva-Teles, A., Serra, C. R., Pereira, F.C., 2022. Differential modulation of the European sea bass gut microbiota by distinct insect meals. Front. Microbiol. 13 https://doi.org/10.3389/fmicb.2022.83103.
Rimoldi, S., Torrecillas, S., Montero, D., Gini, E., Makol, A., Victoria Valdenegro, V., Izquierdo, M., Terova, G., 2020. Assessment of dietary supplementation with galactomannan oligosaccharides and phytogenics on gut microbiota of European sea bass (*Dicentrarchus Labrax*) fed low fishmeal and fish oil based diet. PLoS One 15. https://doi.org/10.1371/journal.pone.0231494.
Ringø, E., Doan, H.V., Lee, S., Song, S.K., 2020a. Lactic acid bacteria in shellfish: possibilities and challenges. Rev. Fish. Sci. Aquac. 28, 139–169. https://doi.org/10.1080/23308249.2019.1683151.
Ringø, E., Van Doan, H., Lee, S.H., Soltani, M., Hoseinifar, S.H., Harikrishnan, R., et al., 2020b. Probiotics, lactic acid bacteria and bacilli: interesting supplementation for aquaculture. J. Appl. Microbiol. 129, 116–136. https://doi.org/10.1111/jam.14628.
Rocculi, P., Cevoli, C., Tappi, S., Genovese, J., Urbinati, E., Picone, G., Fabbri, A., Capozzi, F., Rosa, M.D., 2019. Freshness assessment of European hake (*Merluccius merluccius*) through the evaluation of eye chromatic and morphological characteristics. Food Res. Int. 115, 234–240. https://doi.org/10.1016/j.foodres.2018.08.091.
Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 2016, e2584. https://doi.org/10.7717/peerj.2584.
Roques, S., Deborde, C., Guimas, L., Marchand, Y., Richard, N., Jacob, D., Fauconneau, B., 2020a. Integrative metabolomics for assessing the effect of insect (*Hermetia illucens*) protein extract on rainbow trout metabolism. Metabolites 10, 83. https://doi.org/10.3390/metabo10030083.
Roques, S., Deborde, C., Richard, N., Skiba-Cassy, S., Moing, A., Fauconneau, B., 2020b. Metabolomics and fish nutrition: a review in the context of sustainable feed development. Rev. Aquac. 12, 261–282. https://doi.org/10.1111/raq.12316.
Saito, T., Whatmore, P., Taylor, J.F., Fernandes, J.M.O., Adam, A.-C., Tocher, D.R., Espe, M., Skjærven, K.H., 2020. Micronutrient supplementation affects transcriptional and epigenetic regulation of lipid metabolism in a dose-dependent manner. Epigenetics 16 (11), 1217–1234.
Saleh, N.E., Wassef, E.A., Ashry, A.M., 2020. Is a taurine supplement necessary in fishmeal-based feeds for juvenile European sea bass (*Dicentrarchus labrax*)? Aquac. Int. 28, 321–333. https://doi.org/10.1007/s10499-019-00464-5.
Serra, C.R., Oliva-Teles, A., Enes, P., Tavares, F., 2021. Gut microbiota dynamics in carnivorous European seabass (*Dicentrarchus labrax*) fed plant-based diets. Sci. Rep. 11 (1), 447.
Skalli, A., Robin, J.H., 2004. Requirement of n-3 long chain polyunsaturated fatty acids for European sea bass (*Dicentrarchus labrax*) juveniles: growth and fatty acid composition. Aquaculture 240, 399–415. https://doi.org/10.1016/j.aquaculture.2004.06.036.
Sprague, M., Dick, J.R., Tocher, D.R., 2016. Impact of sustainable feeds on omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006-2015. Sci. Rep. 6, 21892.
Stevens, J.R., Newton, R.W., Tlusty, M., Little, D.C., 2018. The rise of aquaculture by-products: increasing food production, value, and sustainability through strategic utilisation. Mar. Policy 90, 115–124.
Tapia-Hernandez, A.J., Del-Toro-Sanchez, C.L., Cinco-Moroyoqui, F.J., Juarez-Onofre, J. E., Ruiz-Cruz, S., Carvajal-Millan, E., Lopez-Ahumada, G.A., Castro-Enriquez, D.D., Barreras-Urbina, C.G., Rodriguez-Felix, F., 2019. Prolamins from cereal by-products: classification, extraction, characterization and its applications in micro- and nanofabrication. Trends Food Sci. Technol. 90, 111–132. https://doi.org/10.1016/j.tifs.2019.06.005.
Torrecillas, S., Mompel, D., Caballero, M.J., Montero, D., Merrifield, D., Rodiles, A., et al., 2017a. Effect of fishmeal and fish oil replacement by vegetable meals and oils on gut health of European sea bass (*Dicentrarchus labrax*). Aquaculture 468, 386–398. https://doi.org/10.1016/j.aquaculture.2016.11.005.
Torrecillas, S., Robaina, L., Caballero, M.J., Montero, D., Calandra, G., Mompel, D., Karalazos, V., Kaushik, S., Izquierdo, M.S., 2017b. Combined replacement of fishmeal and fish oil in European sea bass (*Dicentrarchus labrax*): production performance, tissue composition and liver morphology. Aquaculture 474, 101–112.
Torrecillas, S., Betancor, M.B., Caballero, M.J., Rivero, F., Robaina, L., Izquierdo, M., et al., 2018. Supplementation of arachidonic acid rich oil in European sea bass juveniles (*Dicentrarchus labrax*) diets: effects on growth performance, tissue fatty acid profile and lipid metabolism. Fish Physiol. Biochem. 44, 283–300. https://doi.org/10.1007/s10695-017-0433-5.
Tusche, K., Arning, S., Wuertz, S., Susenbeth, A., Schulz, C., 2012. Wheat gluten and potato protein concentrate — promising protein sources for organic farming of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 344–349, 120–125. https://doi.org/10.1016/j.aquaculture.2012.03.009.
Uyan, O., Koshio, S., Teshima, S., Ishikawa, M., Thu, M., Alam, M.S., Michael, F.R., 2006. Growth and phosphorous loading by partially replacing fishmeal with tuna muscle by-product powder in the diet of juvenile Japanese flounder, *Paralichthys olivaceus*. Aquaculture. 257, 437–445. https://doi.org/10.1016/j.aquaculture.2006.02.060.
Wang, Y., Wang, F., Ji, W.-X., Han, H., Li, P., 2015. Optimizing dietary protein sources for Japanese sea bass (*Lateolabrax japonicus*) with an emphasis on using poultry by-product meal to substitute fish meal. Aquac. Res. 46, 874–883. https://doi.org/10.1111/are.12242.
Wassef, E.A., Saleh, N.E., Ashry, A.M., 2021. Effects of taurine or sodium diformate supplementation to a low fishmeal plant-based diet fed to European sea bass (*Dicentrarchus labrax*). Aquac. Res. 52, 1513–1524.
Wei, Y., Liang, M., Mai, K., Zheng, K., Xu, H., 2017. 1H NMR-based metabolomics studies on the effect of size-fractionated fish protein hydrolysate, fish meal and plant protein in diet for juvenile turbot (*Scophthalmus maximus* L.). Aquac. Nutr. 23, 523–536.
Wuertz, S., Reiser, S., 2022. Creatine: A valuable supplement in aquafeeds? Rev. Aquac. https://doi.org/10.1111/raq.12717.
Zhang, Y., Xun, P., Wang, R., Mao, L., He, K., 2017. Can magnesium enhance exercise performance? Nutrients 9, 946. https://doi.org/10.3390/nu9090946.
Zhou, Z., Ringø, E., Olsen, R.E., Song, S.K., 2018. Dietary effects of soybean products on gut microbiota and immunity of aquatic animals: a review. Aquac. Nutr. 24, 644–665. https://doi.org/10.1111/anu.12532.