Aquaculture 609 (2025) 742820

Contents lists available at ScienceDirect

Aquaculture

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

Nutritional enhancement of lupin meal (*Lupinus albus*), through fermentation with *Saccharomyces cerevisiae, as plant protein ingredient* in aquafeeds for the European sea bass (*Dicentrarchus labrax*).

Antigoni Vasilaki [a,b,*], Ioannis Nengas [b], Eleni Fountoulaki [b], Morgane Henry [b], Dimitra Kogiannou [b], Chrysanthi Nikoloudaki [b], Petros Chronopoulos [b], Ioannis T. Karapanagiotidis [a], Elena Mente [c]

[a] School of Agricultural Sciences, Department of Ichthyology and Aquatic Environment, Aquaculture Laboratory, University of Thessaly, Volos, Greece
[b] Hellenic Centre for Marine Research (HCMR), Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Anavyssos, Attika, Greece
[c] School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

ARTICLE INFO

Keywords:
Sustainable alternative
Fermented lupin
Legumes
Aquaculture
Plant seeds

ABSTRACT

Legumes contain antinutritional factors that restrict their use in fish nutrition, however, biotechnological methods such as solid-state fermentation can improve their nutritional profile, positioning them as a sustainable alternative for aquafeed formulation. This research evaluated the feasibility of replacing soybean meal with *Saccharomyces cerevisiae*-fermented lupin meal (*Lupinus albus*) in feed formulations for European sea bass (*Dicentrarchus labrax*). Four diets were produced; one commercial type diet (FRL0) and three diets with partial (FRL10, FRL12.5) or complete (FRL15) replacement of soybean meal by fermented lupin meal. European sea bass juveniles, with an initial average weight: 18.9 g, were fed the experimental diets for 71 days in triplicate groups. Results indicated no adverse effects on key production indices when soybean meal was replaced partially or totally. Fermented lupin meal linearly improved feed utilization. Apparent digestibility coefficient of protein significantly increased in groups fed 12.5 % and 15 % fermented lupin, as did apparent digestibility coefficient of fat, compared to the FRL0 group. Trypsin activity in pyloric caeca was statistically elevated in the fermented lupin groups compare to FRL0 group. Whole-body composition, amino acid content, and deposition showed no significant differences among groups. Fermented lupin did not suppress the immune system, and a slight immunostimulatory effect was observed at a 12.5 % inclusion level. No histopathological alterations were detected. Overall, the results demonstrated the efficacy of solid-state fermentation process in enhancing lupin meal, highlighting its potential as a sustainable replacement for soybean meal in feed formulations for Mediterranean marine aquaculture species such us *D. labrax*.

1. Introduction

Intensification of aquaculture has become essential in meeting the rising global seafood demand, which has become a challenge amid overfishing and environmental deterioration. This is leading to an increasing demand for aquafeeds which raises major sustainability concerns, as common raw materials of marine origin are derived from wild fish stocks (Mitra, 2021). Equilibrating the impact of feed production with aquaculture's contribution to global food security has become a key challenge. Ongoing research into alternative feed ingredients, with higher feed efficiency, and green practices show promises in reducing these pressures (Aragão et al., 2022).

Abbreviations: FM, Fishmeal; FO, Fish oil; EU, European Union; GMO, genetically modified organism; NSP, Non starch polysaccharides; ANF, Antinutritional factors; SSF, Solid state fermentation; DO, Dissolved oxygen; ADC, Apparent digestibility coefficient; YGC, Yeast Glucose Chloramphenicol; WG, Weight gain; SGR, Specific growth rate; DFI, Daily feed intake; FCR, Feed conversion ratio; PER, Protein efficiency ratio; HSI, Hepatosomatic index; LSI, Liposomatic index; AA, Amino acids; PBS, Phosphate-buffered saline; TUI, Trypsin units inhibited; CVs, Coefficient of variability; MuF, mucosal fold; EP, eosinophil; LamP, lamina propria; SubM, submucosa; LV, lipid vacuoles; GC, Goblet cells; KPI, Key production indices..

* Corresponding author at: Hellenic Centre for Marine Research (HCMR), Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Anavyssos, 46.7 Km Athens Sounio Avenue, Mavro Lithari, 19013 Anavyssos, Attika, Greece.
E-mail address: avasilaki@hcmr.gr (A. Vasilaki).

https://doi.org/10.1016/j.aquaculture.2025.742820
Received 9 April 2025; Received in revised form 2 June 2025; Accepted 6 June 2025
Available online 6 June 2025

Feed producers have limited the incorporation of fishmeal (FM) and fish oil (FO) from wild sources to address both declining availability and increasing prices, as well as to promote environmentally sustainable feed production (Ababouch and Vannuccini, 2024; Fry et al., 2016; Majluf et al., 2024). Several plant-origin products have been tested as alternatives to marine sources with controversial results (Aziza and El-Wahab, 2019; Bonvini et al., 2018; Collins et al., 2013; Gatlin III et al., 2007; Kader et al., 2012; Kokou et al., 2012; Yaghoubi et al., 2016; Zhang et al., 2018). Soybean constitutes a pivotal source of protein in aquafeeds with concentrate protein of soya and soybean meal estimated to be incorporated at levels up to 15 % and 10 %, respectively (Kuepper and Stravens, 2022).

Soybeans represent the largest agricultural commodity worldwide, with over 80 % of production coming from the American continent, establishing it as the primary soybean exporter (Cattelan, and Dall'Agnol, A., 2018). However, there are concerns that the expansion of soybean cultivation in Brazil, main exporter toward Europe, is associated with environmental side effects (Zalles et al., 2019). Escobar and Tizado (2020) noted that 'the European Union (EU) has the highest carbon footprint (0.77 t t^{-1}) in global soybean trade', with associated concerns regarding deforestation, carbon emissions, and the limited availability of non-GMO soybeans (Abraham et al., 2019). This makes the incorporation of soybean meal in aquafeeds a contentious issue (Wilfart et al., 2023).

The EU has taken measures to reduce its dependency on soy imports and encourages domestic production of protein-rich crops (Commission, E, 2018). Legumes, such as lupins, contribute to sustainable farming and environmental protection by fixing nitrogen and reducing the need for fertilizers (Mathesius, 2022). Lupin, a high-yielding legume, is recognized as an excellent protein source, with certain varieties reaching up to 40 % crude protein (Ferchichi et al., 2021). Lupins contain higher levels of certain amino acids (AA) compared to soybean, with the exception of sulfur-containing AA such as methionine and cysteine (Szczepański et al., 2022). Deficiency of those AA is limiting in diets for carnivorous fish species (Kaushik, 2002). Moreover, lupin seeds' non-starch polysaccharides (NSPs) may still impact digestion, nutrient absorption, and metabolism by affecting digesta viscosity, gut health, and intestinal microbiota (Sinha et al., 2011). Other antinutritional factors (ANFs), such as trypsin inhibitors, interfere with protein digestibility by forming stable complexes with trypsin, blocking its activity through its reduced availability and consequently reducing the uptake of proteins and AA (Morais et al., 2019; Zhang et al., 2021).

Solid-state fermentation (SSF) is an approach to diminish the ANFs and elevate the nutritional profile of feed ingredients. During fermentation, a variety of biochemical transformations occur that modify both nutritional and antinutritional components of the substrate, resulting in improving nutrient availability (Chandra-Hioe et al., 2016). Past research has highlighted the enhancement of protein quality and bioavailability by diminishing of ANFs, resulting in improved nutrient digestibility (Rayaprolu et al., 2013; Terefe et al., 2021). Protein yield of SSF processed ingredients can vary widely and is related to several factors, including the species of microorganism used, carbon and other nutrients availability, and the applied conditions of fermentation and specifically temperature, humidity and duration (Shi et al., 2021). Yeast-based fermentation, particularly with *Saccharomyces cerevisiae*, has been found to enhance the protein concentration of several raw materials such as guar and copra meal, soybean meal, cassava meal and maize flour (Dileep et al., 2021; Santos et al., 2018; Terefe et al., 2022; Terefe et al., 2021). Moreover, several authors have reported the ability of SSF to reduce trypsin inhibitor levels in plant ingredients(Dileep et al., 2021; Teng et al., 2012).

The objective of this research study was to assess the viability of replacing soybean meal with lupin meal processed by SSF with *S. cerevisiae* in fish feed formulations for the marine Mediterranean aquaculture industry. In particular, the feasibility of replacing soybean using fermented lupin meal was investigated in feed formulation for European sea bass (*Dicentrarchus labrax*).

2. Materials and methods

2.1. Animal ethics

The EU policies for the animal welfare intended for scientific investigation (Directive 2010/63/EU) were followed for all procedures involving fish. All the operations and experiment were approved by the General Directorate for Regional Agricultural Economy and Veterinary Affairs, Department of Animal Protection, Pharmaceuticals and Veterinary Applications, Greece (114,130/2021) at the registered facilities (EL-43BIO/exp-01) of the Department of Ichthyology and Aquatic Environment, University of Thessaly.

2.2. Fish husbandry

Six hundred and fifty juvenile's European sea bass of 18.9 ± 0.0 g initial average body weight were purchased and transferred from a fish hatchery to the rearing unit of the Department of Ichthyology and Aquatic Environment, University of Thessaly. The acclimation of the experimental fish lasted for 15 days before being distributed in 12 cylinder-shaped tanks of 350 L capacity each, in triplicate groups for each of the four diets, with a total of 50 fish per tank. Settlement column faecal traps were fitted to the tanks according to Cho et al. (1982). A total of fifteen fish were erratic selected from the initial population, euthanized with a lethal overdose of anaesthetic (a 1:10 clove oil and ethanol solution), homogenized, freeze-dried, and analysed to determine their initial body gross nutrient and AA profiles. Throughout the trial, all groups were exposed to a 12 L:12D photoperiod, 30 ppt water salinity and 22 ± 1 °C temperature. Daily monitoring was performed for total ammonia ($NH3/NH_4^+$), nitrate (NO_3^-) and nitrite (NO_2^-) using aquarium pharmaceutical (API) tests and always maintained below 0.25, 0.25 and 1.0 mg/L, respectively. Dissolved oxygen (DO) was recorded daily. For the entire period of the experimental trial (71 days), the feeding of the population was performed by hand, three times daily, until apparent satiety. Apparent Digestibility Coefficients (ADC) of protein and fat were determined in faecal samples collected for 15 days. Faeces were collected daily. Briefly, the experimental population was fed in the evening, following thorough cleaning of the feeding tanks and faecal traps, which were then covered with ice to prevent bacterial degradation of the collected faeces. The following morning, faeces were collected into 50 mL Falcon tubes, centrifuged (1100 ×*g*, 10 min, 4 °C), and stored at −20 °C until freeze-drying and analysis. Daily records were kept for mortality and feed consumption. Before samplings, all fish groups underwent a 1-day fasting period. Fish were sedated using clove oil mixed with ethanol at a 1:10 ratio, and their individual weights were measured at the beginning, halfway, and at the end of the experiment. The number of fish used for the experimental rearing was determined through statistical analysis using the G*Power 3.1.9.4 software (Universität Düsseldorf). In each group, 90 individuals are required to ensure statistical significance for a minimum difference of 3.8 units between any pair of groups, assuming there are 7 groups (a = 0.05 and b = 0.2). The common deviation was assumed to be 7.5. A rejection rate of 0 % has been anticipated. To the required number of individuals per group (90), an additional 60 individuals were added for the assessment of nutrient ADC, which were subsequently reused in another experimental trial.

2.3. Lupin meal fermentation process

Seeds of *Lupinus albus*, variety Tennis, were provided by the Cooperative Farmers of Thessaly (THES-GI) and a sourdough-isolated strain of *S. cerevisiae* (ACA-DC 5036) was derived from the ACA-DC Collection, Laboratory of Dairy Research, Agricultural University of Athens, Greece,

Table 1
Composition of soybean meal and lupin meal prior and after fermentation with *Saccharomyces cerevisiae* (g/100 g, dry sample).

	Soybean meal	Lupin meal	Fermented Lupin meal
Ash	5.7	3.9	4.2
Protein	44.5	41.3	44.9
Fat	1.1	8.0	7.9
Total P	0.40	0.10	0.37
Phytic acid	0.20	1.31	0.77
NSP	28.1	29.2	18.3
TUI/mg sample	8.96	1.50	0.13

for the fermentation process. A tray bioreactor was constructed by GenusX (Cyprus), which met the essential requirements for optimal growth of *S. cerevisiae*. The microorganism strain was cultured on Yeast Glucose Chloramphenicol (YGC) agar in slant cultures at 28 °C for 72 h. The concentration of yeast cells in the cultures was determined by light microscopy using a Neubauer chamber. Lupin seeds were crushed using a hammer mill, moisturised to 70 % and inoculation of the substrate was performed at a concentration of 1×10^6 *S. cerevisiae* cells per gram of lupin meal substrate. Fermentation was performed at 30 °C for 24 h. Thereafter, the product was autoclaved at 121 °C for 20 min to inactivate the *S. cerevisiae* cells and prevent further fermentation. Finally, the product was dried overnight at 40 °C, ground and incorporated into the experimental diets. Enhancement of lupin meal post fermentation with *S. cerevisiae* is provided in Table 1.

2.4. Experimental diets

Four diets, with similar nitrogen and lipid content (Table 2), were developed and manufactured at the Institute of Marine Biology, Biotechnology and Aquaculture in Athens, Greece. These diets were produced using a twin-screw extruder (model EV025A107FAA, CLEXTRAL, France), and oil was incorporated using a small-scale vacuum coater (DINISSEN, the Netherlands). A commercial type FRL0 diet was formulated to satisfy the nutritional needs of the experimental population. The FRL (Fermented Lupin) diets were formulated to replace soybean meal at 66 %, 83 % and 100 %, corresponding to inclusion levels of 10 %, 12.5 % and 15 %, respectively in the diet (Table 2). Fish oil was added to meet the essential fatty acid requirements and all diets were enriched with identical amounts of vitamins and minerals. To assess ADC, chromium oxide was added into each experimental diet as an inert marker. The nutritional profile of the diets is shown in Table 3.

2.5. Sampling, fish growth and dietary digestibility

At the end of the growth period, all individuals were weighed separately, after being anaesthetized, for the assessment of key production indices:

Weight gain : WG = final weight (g)–initial weight (g)

Specific growth rate : SGR (%/day) = [(Ln final weight)

−(Ln initial weight)]*100/trial days

Daily feed intake : DFI (%) = 100* F/W

Where, F represents the average daily feed intake per fish (g), and W refers to the average fish weight, determined as the mean of the initial and final weights (g).

Feed conversion ratio : FCR = feed intake g/WG g

Table 2
Diet formulation of the experimental diets (as fed basis, %).

	FRL0	FRL10	FRL12.5	FRL15
Fishmeal	20.0	20.0	20.0	20.0
Krill meal	3.0	3.0	3.0	3.0
Blood meal	8.0	8.0	8.0	8.0
Wheat meal	12.0	12.4	12.6	12.8
Wheat gluten	3.0	3.0	3.0	3.0
Corn gluten	15.1	15.2	15.0	15.0
Soy protein concentrate	7.0	7.0	7.0	7.0
Soybean meal	**15.0**	**5.0**	**2.5**	–
Fermented Lupin meal[1]	**–**	**10.0**	**12.5**	**15.0**
Fish oil	13.3	12.7	12.6	12.4
Monocalcium Phosphate	1.2	1.2	1.2	1.2
Mineral premix[2]	0.2	0.2	0.2	0.2
Vitamin premix[3]	0.2	0.2	0.2	0.2
Lysine	0.8	0.9	0.9	0.9
Methionine	0.2	0.25	0.26	0.28
Chromium oxide	1.0	1.0	1.0	1.0

[1] *Lupinus albus* fermented with *Saccharomyces cerevisiae.*
[2] Per kilogram of mineral premix: 28 g Fe, 14 g Mn, 2.4 g I, 2.8 g Cu, 24 g Zn.
[3] Per kilogram of vitamin premix: 1200 mg retinol; 20 mg cholecalciferol; 400 mg biotin; 1.6 g folic acid; 60 g niacin; 24 g pantothenic acid; 8 g pyridoxine; 8 g riboflavin; 8 g thiamin; 80 mg vitamin B12; 80 g ascorbic acid; 100 g tocopherol acetate; 4 g vitamin K; 160 mg BHA; 160 mg BHT.

Table 3
Proximate composition and amino acid profile of the experimental diets (g/100 g, dry basis).

	FRL0	FRL10	FRL12.5	FRL15
Dry matter	92.9	93.1	93.5	92.8
Protein	46.3	46.7	46.4	45.9
Fat	16.2	16.5	17.1	17.0
Ash	7.1	6.9	6.5	7.2
Essential amino acids				
Arginine	2.38	2.58	2.67	2.66
Histidine	1.46	1.32	1.35	1.29
Isoleucine	2.17	2.03	1.97	2.03
Leucine	4.75	4.59	4.55	4.68
Lysine	3.40	3.35	3.32	3.40
Methionine	1.16	1.18	1.19	1.22
Phenylalanine	2.54	2.42	2.42	2.57
Threonine	1.81	1.86	1.91	1.93
Valine	2.54	2.46	2.48	2.51
Non-essential amino acids				
Alanine	3.05	3.03	3.03	3.10
Asparagine	4.28	4.13	4.14	4.18
Glutamine	8.46	8.40	8.68	8.51
Glycine	2.37	2.26	2.31	2.31
Proline	2.84	2.89	2.81	2.86
Serine	2.27	2.24	2.29	2.27
Tyrosine	1.70	1.67	1.69	1.75

Protein efficiency ratio : PER = Wet weight gain/Total protein intake

Blood samples were collected by caudal vein puncture of ten fish per tank (n = 30/group) for immunological analysis. Samples left to clot at 4 °C for 3 h and serum were collected after centrifugation (16,000 xg, 10 min, 4 °C) and stored at −80 °C until immunological analyses.

Hepatosomatic and liposomatic indices were calculated as follows:

Hepatosomatic index : HSI (%) = liver weight (g)*100/fish weight (g)

Liposomatic index : LSI (%)
= visceral fat weight (g)*100/fish weight (g)

Ten fish per tank were anesthetised, killed and analysed for whole-

body, AA composition and AA deposition:

$$\text{Amino acid deposition\%} : AA\% = 100^{*}(\text{final AA quantity (g, wet basis)} - \text{initial AA quantity (g, wet basis)}) / \text{AA consumed during experiment (g, dry basis)}$$

Samples of the pyloric caeca and digestive tract were removed from five fish per tank, rinsed with cold PBS, and frozen at −80 °C for trypsin activity determination.

The ADC of crude protein and crude fat, was indirectly calculated using chromium oxide as the inert marker as described by (Cho and Kaushik, 1990):

$$ADC\% = 100^{*}\left[1 - (Fnutr^{*}DCr)/(Dnutr^{*}FCr)\right]$$

Where, Fnutr = faecal nutrient concentration Dnutr = Dietary nutrient concentration, DCr = Dietary chromium oxide, and FCr = Feacal chromium oxide.

2.6. Biochemical analysis

The nutritional profile of raw and fermented lupin, diets, fish tissues, and faeces was assessed according to AOAC (2005); Dry matter (DM), ash content, crude protein and total fat were determined as described by Vasilaki et al. (2023). The phosphovanillin method (Nengas et al., 1995) was followed to determine levels of total lipids in faeces. Chromium oxide concentration was quantified according to Bolin et al. (1952). Phytic acid (phytate) and free phosphorus in raw materials were analysed using a quantitative approach (K-PHYT test kit, Megazyme, Ireland). The determination of Trypsin units inhibited (TUI) followed the method outlined by Kakade et al. (1974), improved by Liu et al. (2021) to obtain repeatable results. The NSP contents of the samples were chromatographically measured in accordance to Englyst et al. (1994) and Ma et al. (2017). Amino acid analysis was conducted following acid hydrolysis (6 N HCL, 110 °C, 24 h) and subsequent derivatization using AccQ-Fluor™ following the protocol outlined in the AA analysis application solution (Waters Corporation, Milford, MA, USA). Alpha-aminobutyric acid (2.5 mM in 0.1 M HCl) was used as an internal standard. For all biochemical analyses, a standard sample (STD) was included in every running set to ensure assay precision and maintain consistency between measurements.

2.7. Trypsin activity in pyloric caeca

For assessing trypsin activity, the pyloric caeca of five individuals per tank (n = 15 / group) were singly homogenized with cold double-distilled water at a 1:10 (w/v) ratio and centrifuged at 12,000 xg for 10 min at 4 °C. The supernatants were split into small aliquots and stored at −80 °C until further analysis. BAPNA (N-benzoyl-DL-arginine-p-nitroanilide) (B4875 Sigma-Aldrich) served as substrate to evaluate trypsin activity. In detail, 40 mg of BAPNA were dissolved in 1 mL dimethyl-sulfoxide (DMSO) and then diluted to 100 mL with Tris-HCl 50 mM (pH 8.2) containing 20 mM $CaCl_2$. The reaction was initiated in a 96-well microplate by mixing 20 μL of the enzyme extract with 300 μL of the substrate solution (Erlanger et al., 1961; Yufera et al., 2019). The release of p-nitroaniline was monitored kinetically at 405 nm using a microplate reader (FLUOstar OMEGA, BMG Labtech, Germany) for 4 min at 25 °C. Enzyme activity was defined as one unit (U), corresponding to the amount of enzyme required for the production of 1 μmol of product per minute under standardized assay conditions. Activities were normalized and expressed as U per 100 g wet body weight of fish. All samples were assayed simultaneously in the same plate and acceptable values were considered with Coefficient of variability CVs < 10 %.

2.8. Immunological analysis

Fish sera were analysed for antibacterial activity against a Gram-positive bacterium, *Micrococcus luteus* (lysozyme activity) and against a luminescent strain of a Gram-negative bacterium, *Escherichia coli* (complement activity) as previously described (Henry et al., 2022). The serum myeloperoxidase activity (Henry et al., 2015; Kokou et al., 2012), and the trypsin inhibition (Henry and Fountoulaki, 2014) were assessed as previously described. The alkaline phosphatase (ALK) activity was adapted from the method described for ALK analysis in fish mucus (Ross et al., 2000). It was determined in 5 μL of serum samples diluted with 75 μL of 100 mM ammonium bicarbonate buffer, 1 mM $MgCl_2$, pH 7.8 incubated in triplicate wells of a 384 well microplate with 20 μL of 8 mM p-nitrophenol phosphate (Sigma) diluted in the same buffer. The increase in OD was measured continuously over 30 min at 405 nm using a microplate reader (Fluostar, BMG Labtech, Germany). ΔOD data were converted in Units/mL serum using 18.75 mM of absorptivity of p-nitrophenol.

2.9. Histopathological analysis

Liver and posterior intestine samples were maintained in 10 % formalin solution, dehydrated through a graded series of methanol/ethanol concentrations up to 100 %, embedded in paraffin, and sectioned into 2–4 μm-thick slices using a Leica 2055-Autocut microtome (Leica Instruments GmbH, Nussloch, Germany). The sections were stained with hematoxylin and eosin using a Leica Autostainer XL (Nussloch, Germany) for microscopic evaluation (Bullock, 1978). Micrographs were obtained at magnifications of 10× and 20× using an Olympus VANOX-T microscope (Olympus, NJ, USA) equipped with a Lumenera Infinity camera (Ontario, Canada). Image processing was performed using Digital Image Systems software (Athens, Greece).

Histomorphology of liver was analysed with particular attention to lipid accumulation accompanied by nucleus displacement (LA). Scoring was applied as indicated: 1 for normal histomorphology, 2 for moderate changes, and 3 for severe alterations indicative of steatosis (Kokou et al., 2019).

Intestinal sections were examined using a semiquantitative approach based on criteria for assessing SBM-induced enteritis in Atlantic salmon (Urán et al., 2008) and gilthead sea bream (Kokou et al., 2017). The evaluation included six criteria: (1) mucosal fold (MuF) appearance and length; (2) eosinophil (EP) infiltration in the lamina propria; (3) lamina propria (LamP) widening; (4) submucosa (SubM) widening; (5) the presence of lipid vacuoles (LV) in enterocytes; and (6) the number of Goblet cells (GC). Each criterion was scored on a scale of 1 to 3, with higher scores representing more severe morphological alterations (Table 10).

2.10. Statistical analysis

Software IBM SPSS Statistics 25 was used for all statistical analyses except immunology. Data are reported as means ± SEM of triplicates. One-Way ANOVA followed by Tukey's post hoc test was conducted. Correlation analyses were applied using the Spearman correlation method. Immunological statistical analyses were carried out using software JASP 0.19.1.0. The normality and homogeneity of variances were assessed via Shapiro-Wilk and Levene's tests, respectively. One-Way ANOVA with simple contrast analysis was used to compare each treatment with the FRL0 (control). The linear and quadratic effects of dietary fermented lupin were assessed using regression analysis. Statistical significance was determined at $P < 0.05$. The term 'trend' was used for results near the significance threshold ($0.05 < P < 0.1$), where findings approached but did not reach the conventional threshold of $P < 0.05$. These results were interpreted as suggestive of potential effects

Table 4
Key performance and somatometric indices of European sea bass fed the four experimental diets.

	FRL0	FRL10	FRL12.5	FRL15	SEM	T	L	Q
Initial body weight (g) [1]	18.9	18.9	18.9	18.9	0.029	0.715	0.895	0.987
Final body weight (g) [1]	61.8	63.8	63.1	62.5	1.452	0.560	0.415	0.340
Weight gain (g) [1]	42.8	44.9	44.2	43.6	1.455	0.563	0.417	0.341
FCR[1]	1.19	1.17	1.15	1.16	0.015	0.127	0.040	0.092
SGR (%/day) [1]	1.67	1.71	1.70	1.68	0.033	0.578	0.444	0.351
DFI %[1]	2.15	2.14	2.10	2.11	0.041	0.625	0.307	0.577
PER[1]	1.96	1.98	2.02	2.03	0.028	0.111	0.031	0.066
HSI[2]	1.43	1.46	1.35	1.70	0.162	0.165	0.335	0.239
LSI[2]	6.67	6.53	6.49	7.65	0.618	0.268	0.352	0.186

Different letters in the same line denote statistically significant differences ($P < 0.05$), SEM = standard error of the mean, *P*-value (T = treatment; L = linear; Q = quadratic). FCR = feed conversion ratio, SGR = specific growth rate, DFI = daily feed intake, HIS = hepatosomatic index, LSI = liposomatic index.
[1] The data are the means of 3 replicates of 50 fish in each group ($n = 3$).
[2] The data are the means of 3 replicates of 17 fish in each group ($n = 3$).

that may warrant further investigation (Amrhein et al., 2019; Bender and Lange, 2001; Gelman and Stern, 2006; Wasserstein and Lazar, 2016).

3. Results

3.1. Fermentation influence on lupin meal

The influence of SSF on lupin meal composition is presented in Table 1. After the fermentation process with *S. cerevisiae* at 70 % moisture for 24 h at 30 °C, protein was increase by 3.6 %, phytic acid levels were reduced from 1.31 to 0.77 g/100 g and total phosphorus increased from 0.10 to 0.37 g/100 g. The levels of NSP were also decreased from 29.2 to 18.3 g/100 g, while an approximately 11-fold decline in TUI was observed (from 1.50 to 0.13).

3.2. Growth parameters and feed utilization

The Key Performance Indices (KPIs) obtained are presented in Table 4. All fish that received the fermented lupin meal (FRL10, FRL12.5, FRL15) had a higher final body weight than the group fed only with soybean meal (FRL0), but with no significant differences ($P = 0.56$). No mortalities were detected throughout the experimental trial. In addition, weight gain was recorded at higher levels for the groups fed the FRL10, FRL12.5 and FRL15 diets compared to the FRL0 group. Although no significant differences were detected in FCR, a trend was noticed with $P = 0.09$ for lower FCR for the group fed with FRL12.5 diet compared to the FRL0 group. Similar SGR and PER values were found in all the experimental groups. Higher liposomatic (LSI) and hepatosomatic indices (HSI) were observed in the group fed solely with fermented lupin meal (FRL15), while the aforementioned indices were lower for the group fed FRL12.5 diet; however, these observations were not significant ($P = 0.26$, $P = 0.16$). The substitution of soybean meal with fermented lupin meal resulted in a linear reduction in FCR ($P = 0.04$) and raised PER ($P = 0.03$) which also tended to be increased quadratically ($P = 0.06$).

3.3. Nutrient apparent digestibility coefficients

The values for the ADC are shown in Table 5. The groups fed the FRL12.5 and FRL15 diets resulted in significantly higher protein ADC ($P = 0.002$) in contrast to the group fed exclusively with soybean meal (FRL0). In addition, fat ADC diverse significantly ($P = 0.002$). The FRL0 group had the lowest fat ADC levels along with the group fed the FRL10 diet. Fish fed the diet containing solely fermented lupin meal (FRL15) and the diet with high inclusion level 12.5 % of fermented lupin meal (FRL12.5) demonstrated a significantly higher ADC of fat ($P = 0.002$) than the group fed with the FRL0 diet. The same observation, of lower ADC of fat, was also recorded for the group treated with low inclusion levels of fermented lupin meal (FRL10) relative to those fed the high and sole inclusion of fermented lupin meal ($P = 0.002$). Inclusion of fermented lupin meal instead of soybean meal increased ADC of protein and fat in linear manner ($P < 0.001$ and $P = 0.001$, respectively).

3.4. Whole body and amino acid composition

The total body composition of the tested fish is displayed in Table 6. Moisture, protein, fat and ash levels were comparable among the fish groups with no significant differences ($P = 0.28$, $P = 0.57$, $P = 0.08$, $P = 0.49$). Protein and fat levels were elevated in the group fed solely with fermented lupin meal, but not significantly differentiated. However, a trend toward lower fat content ($P = 0.08$) was observed for the group fed the high inclusion levels of fermented lupin meal (FRL12.5) relative to those fed the sole inclusion of fermented lupin meal (FRL15). The same pattern was observed also in whole body AA levels, but with no significant differences in any of the essential and non-essential AA among the different groups (Table 6). Replacement of soybean by fermented lupin meal significantly increased isoleucine in linear manner ($P = 0.01$). Histidine content tended to increased linearly with the inclusion of fermented lupin meal ($P = 0.08$).

3.5. Amino acid deposition

The AA deposition is shown in Table 7. Inclusion of fermented lupin meal at all levels of incorporation resulted in similar or higher deposition of AA in fish tissue relative to the FRL0 group. However, no significant differences were observed among the experimental groups. A tendency for higher deposition of histidine and isoleucine was observed for the group fed solely with fermented lupin (FRL15) compared to the group treated exclusively with soybean meal (FRL0) ($P = 0.09$ and $P = 0.05$, respectively). This was also observed linearly with significant difference for isoleucine ($P = 0.01$) and with a tendency for histidine (*P*

Table 5
Apparent digestibility coefficient of nutrients (%).

	FRL0	FRL10	FRL12.5	FRL15	SEM	T	L	Q
Protein	91.1[a]	92.0[ab]	92.5[b]	92.9[b]	0.307	0.002	<0.001	<0.001
Fat	95.2[a]	95.5[a]	96.3[b]	96.4[b]	0.234	0.002	0.001	0.002

Different letters in the same line denote statistically significant differences ($P < 0.05$), SEM = standard error of the mean, *P*-value (T = treatment; L = linear; Q = quadratic). The data are the means of 3 replicates of 20 fish in each group (n = 3).

Table 6
Whole body and amino acid composition of European sea bass (g/100 g wet weight basis).

	Initial Population	FRL0	FRL10	FRL12.5	FRL15	SEM	T	L	Q
Moisture	71.6	63.8	63.1	64.5	62.5	0.979	0.281	0.530	0.706
Protein	16.0	16.4	16.6	16.3	17.0	0.538	0.567	0.414	0.598
Fat	6.9	15.4	16.2	14.9	16.3	0.503	0.076	0.482	0.748
Ash	4.4	4.1	3.9	3.9	4.0	0.147	0.492	0.232	0.344
Essential amino acids									
Arginine	0.85	1.16	1.15	1.15	1.20	0.054	0.783	0.301	0.405
Histidine	0.33	0.38	0.38	0.36	0.41	0.025	0.399	0.078	0.169
Isoleucine	0.62	0.77	0.78	0.74	0.79	0.022	0.173	0.012	0.051
Leucine	1.03	1.25	1.27	1.20	1.29	0.037	0.175	0.121	0.318
Lysine	1.16	1.43	1.44	1.37	1.47	0.040	0.175	0.351	0.615
Methionine	0.43	0.53	0.53	0.50	0.54	0.016	0.137	0.490	0.753
Phenylalanine	0.60	0.69	0.71	0.67	0.72	0.025	0.269	0.280	0.309
Threonine	0.63	0.79	0.75	0.77	0.77	0.038	0.791	0.278	0.504
Valine	0.69	0.83	0.84	0.79	0.86	0.024	0.136	0.261	0.545
Non-essential amino acids									
Alanine	0.92	1.07	1.07	1.05	1.09	0.027	0.547	0.408	0.711
Asparagine	1.39	1.60	1.65	1.56	1.66	0.055	0.310	0.105	0.282
Glutamine	1.99	2.27	2.32	2.20	2.35	0.075	0.293	0.493	0.781
Glycine	1.06	1.00	1.01	1.01	1.03	0.046	0.929	0.127	0.328
Proline	0.60	0.69	0.69	0.67	0.70	0.030	0.753	0.500	0.584
Serine	0.63	0.69	0.71	0.67	0.72	0.024	0.255	0.186	0.399
Tyrosine	0.43	0.56	0.56	0.52	0.58	0.017	0.075	0.816	0.891

Different letters in the same line denote statistically significant differences ($P < 0.05$), SEM = standard error of the mean, P-value (T = treatment; L = linear; Q = quadratic). The data are the means of 3 replicates of 10 fish in each group (n = 3).

Table 7
Amino acid deposition (%) in European sea bass.

	FRL0	FRL10	FRL12.5	FRL15	SEM	r	T	L	Q
Essential Amino Acids									
Arginine	49.4	45.9	44.9	47.3	3.333	−0.259	0.583	0.301	0.405
Histidine	25.0	28.6	25.9	31.8	2.459	−0.713**	0.089	0.078	0.169
Isoleucine	34.9	38.4	37.3	39.5	1.436	−0.273	0.061	0.012	0.051
Leucine	25.7	27.6	26.2	27.8	1.044	−0.065	0.190	0.121	0.318
Lysine	41.6	43.1	41.4	44.0	1.730	0.228	0.439	0.351	0.615
Methionine	45.3	45.2	41.9	45.0	2.036	−0.086	0.341	0.490	0.753
Phenylalanine	26.4	29.1	27.1	27.9	1.267	−0.171	0.245	0.280	0.309
Threonine	43.0	39.4	40.7	39.9	3.162	−0.367	0.687	0.278	0.504
Valine	32.1	34.1	31.8	34.5	1.277	−0.259	0.149	0.261	0.545
Non-essential Amino Acids									
Alanine	34.0	34.8	34.3	35.2	1.243	0.171	0.803	0.408	0.711
Asparagine	35.9	39.2	36.9	39.5	1.647	−0.302	0.167	0.105	0.282
Glutamine	25.7	27.1	24.8	27.4	1.133	−0.259	0.151	0.493	0.781
Glycine	37.4	40.2	40.1	40.9	2.485	−0.239	0.540	0.127	0.328
Proline	23.2	23.2	23.4	24.4	1.360	0.162	0.782	0.500	0.584
Serine	28.5	30.4	28.4	31.4	1.327	−0.341	0.135	0.186	0.399
Tyrosine	33.1	33.9	31.5	34.4	1.599	0.238	0.347	0.816	0.891

Different letters in the same line denote statistically significant differences ($P < 0.05$), SEM = standard error of the mean, P-value (T = treatment; L = linear; Q = quadratic). In the correlation analysis (r), ** asterisk indicates significance at the 0.01 level. The data are the means of 3 replicates of 10 fish in each group (n = 3).

= 0.08). In most cases of essential AA, the group fed solely with fermented lupin meal (FRL15) exhibited higher values compared to the FRL0 group. The experimental groups treated with the FRL0 and FRL12.5 diets exhibited lower deposition of AA. Unlike other essential and all non-essential AA, only histidine deposition in the European seabass body was negatively correlated with the dietary AA content (i.e. the higher the AA content, the lower the deposition).

3.6. Trypsin activity in pyloric caeca

The trypsin activity in the pyloric caeca of European seabass fed the four diets is provided in Table 8. Fish fed on fermented lupin meal exhibited increased trypsin activity in the examined tissue. Specifically, significantly higher trypsin activity in the pyloric caeca was observed in FRL10 and FRL12.5 groups ($P < 0.001$) in relation to the FRL0 group. In addition, the group fed the FRL15 diet exhibited the highest trypsin activity in the pyloric caeca among all groups ($P < 0.001$).

3.7. Immunology

All data related to the immunological parameters are displayed in Table 9. The antibacterial activity of the lysozyme in the fish sera tended

Table 8
Trypsin activity in pyloric caeca of European sea bass (U/100 g fish).

	FRL0	FRL10	FRL12.5	FRL15	SEM	T	L	Q
Pyloric	1.77^{a}	2.18^{b}	2.39^{b}	2.73^{c}	0.120	<0.001	<0.001	<0.001

Different letters in the same line denote statistically significant differences ($P < 0.05$), SEM = standard error of the mean, P-value (T = treatment; L = linear; Q = quadratic). The data are the means of 3 replicates of 5 fish in each group (n = 3).

Table 9
Immune parameters in European sea bass.

	FRL0	FRL10	FRL12.5	FRL15	SEM	T	L	Q
Lysozyme activity (U/ml)	489.3	433.0	430.8*	447.9	10.273	0.151	0.050	0.072
E. coli growth inhibition (%)	83.73	83.77	85.52	83.07	0.602	0.148	0.805	0.556
Serum maximal *E. coli* killing capacity (%)	98.13	97.75	98.24	97.01	0.204	0.143	0.207	0.247
Myeloperoxidase activity (U/ml)	0.16	0.14	0.16	0.13*	0.006	0.066	0.110	0.192
Trypsin inhibition (%)	85.67	85.39	83.88	81.54	0.850	0.281	0.131	0.146
Alkaline Phosphatase activity (U/ml)	2.09^{a}	2.44^{ab}	2.68^{b}*	2.40^{ab}	0.074	0.043	0.030	0.043

Different letters in the same row represent significant differences between diets (One-Way ANOVA, T = treatment followed by Tukey's *t*-test, and polynomial contrast analysis, L = linear, Q = quadratic), * the asterisk represent a significant difference compared to the control diet by the simple contrast analysis. $P < 0.05$). The data are the means of 3 replicates of 10 fish in each group (n = 3).

to be lower in all fish fed fermented lupin compared to soybean meal, significantly so in fish fed FRL12.5 compared to the FRL0 fish (simple contrast analysis, $P = 0.04$). This activity followed a linear ($P = 0.05$) and to a lesser level a quadratic effect ($P = 0.07$). The opposite tendency was observed concerning the complement antibacterial activity (*E. coli* growth inhibition), with the highest activity obtained in fish fed FRL12.5 although no significant difference was present ($P = 0.15$). The myeloperoxidase activity tended to be decreased in fish fed FRL15 compared to FRL0 fish (One-Way ANOVA, P = 0.07, simple contrast analysis, $P = 0.02$). Trypsin inhibition by the fish sera was not significantly affected by dietary lupin ($P = 0.28$). Alkaline phosphatase activity in the fish sera was increased in all fish fed fermented lupin in a linear ($P = 0.03$) but also quadratic manner ($P = 0.043$), with a significant increase at dietary level of 12.5 % (FRL12.5) compared to FRL0 fish ($P = 0.043$).

3.8. Histopathology

Liver morphology of the different groups did not show significant differences upon the end of the trial (Fig. 1, Table 10), whether fermented lupin was included or not. A moderate level of hepatocyte vacuolization due to fat accumulation was observed in all liver sections. The cytoplasmic vacuole was clear and visually empty, affecting the shape of the nucleus or its position in some cases. Bile ducts appeared normal and no signs of inflammation in the pancreatic tissue were present in any group.

The overall image of the posterior part of the gut in the sea bass samples showed no significant differences between the experimental diets (Fig. 2). In all 6–9 fish/group sections, epithelial integrity without signs of hypertrophy or hyperplasia was observed. The nuclei of the enterocytes were located either at the base or in the middle position of the cell and were accompanied by a supranuclear vacuole normal in size and number.

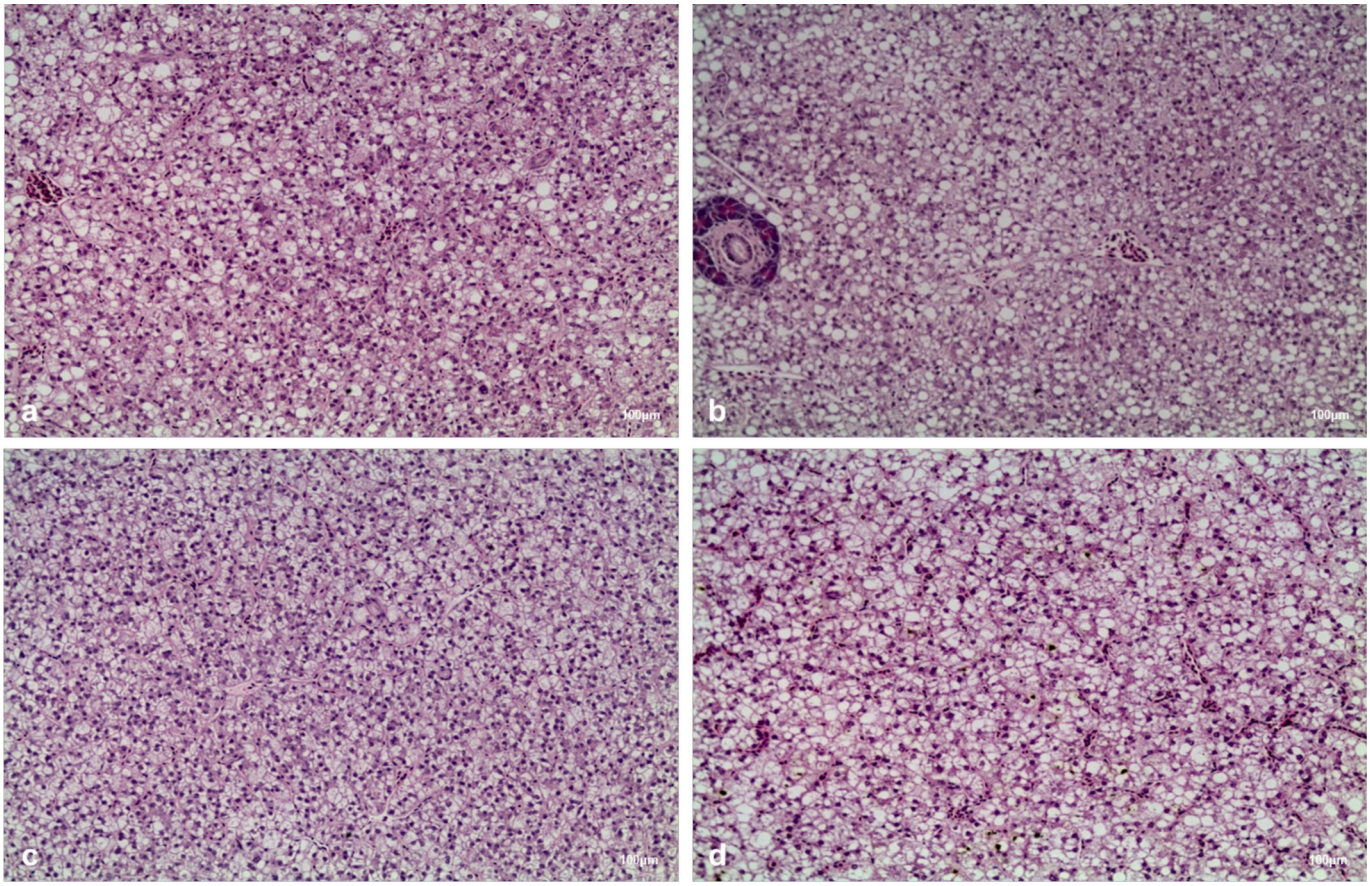

Fig. 1. Liver sections at the end of the growth period a: FRL0, b: FRL10, c: FRL12.5 and d: FRL15, (10×).

Table 10
Severity score (expressed as Grade 0–3) for the observed histopathological alterations of *Dicentrarchus labrax* posterior gut and liver, where 1 = normal morphology, 2 = moderate alterations, 3 = severe alterations. No statistical differences were detected.

	FRL0	FRL10	FRL12.5	FRL15	SEM	T	L	Q
Posterior gut								
MF	2.00	1.56	1.67	1.67	0.232	0.321	0.105	0.170
EG	2.44	2.00	2.00	2.11	0.174	0.103	0.033	0.038
LP	2.22	1.67	2.00	2.22	0.222	0.111	0.664	0.051
SM	2.00	1.67	2.33	2.33	0.226	0.053	0.291	0.104
LV	1.33	1.00	1.00	1.44	0.158	0.047	0.735	0.031
GC	2.56	2.33	2.17	2.67	0.463	0.713	0.842	0.642
Liver								
LA	2.44	2.44	2.56	2.22	0.318	0.509	0.350	0.294

MF mucosal fold, EG eosinophilic granulocytes, LP lamina propria, SM submucosa, LV lipid vacuoles, GC goblet cells, LA lipid accumulation. The data are the means of 3 replicates of 3 fish in each group (n = 3). SEM = standard error of the mean, P-value (T = treatment; L = linear; Q = quadratic).

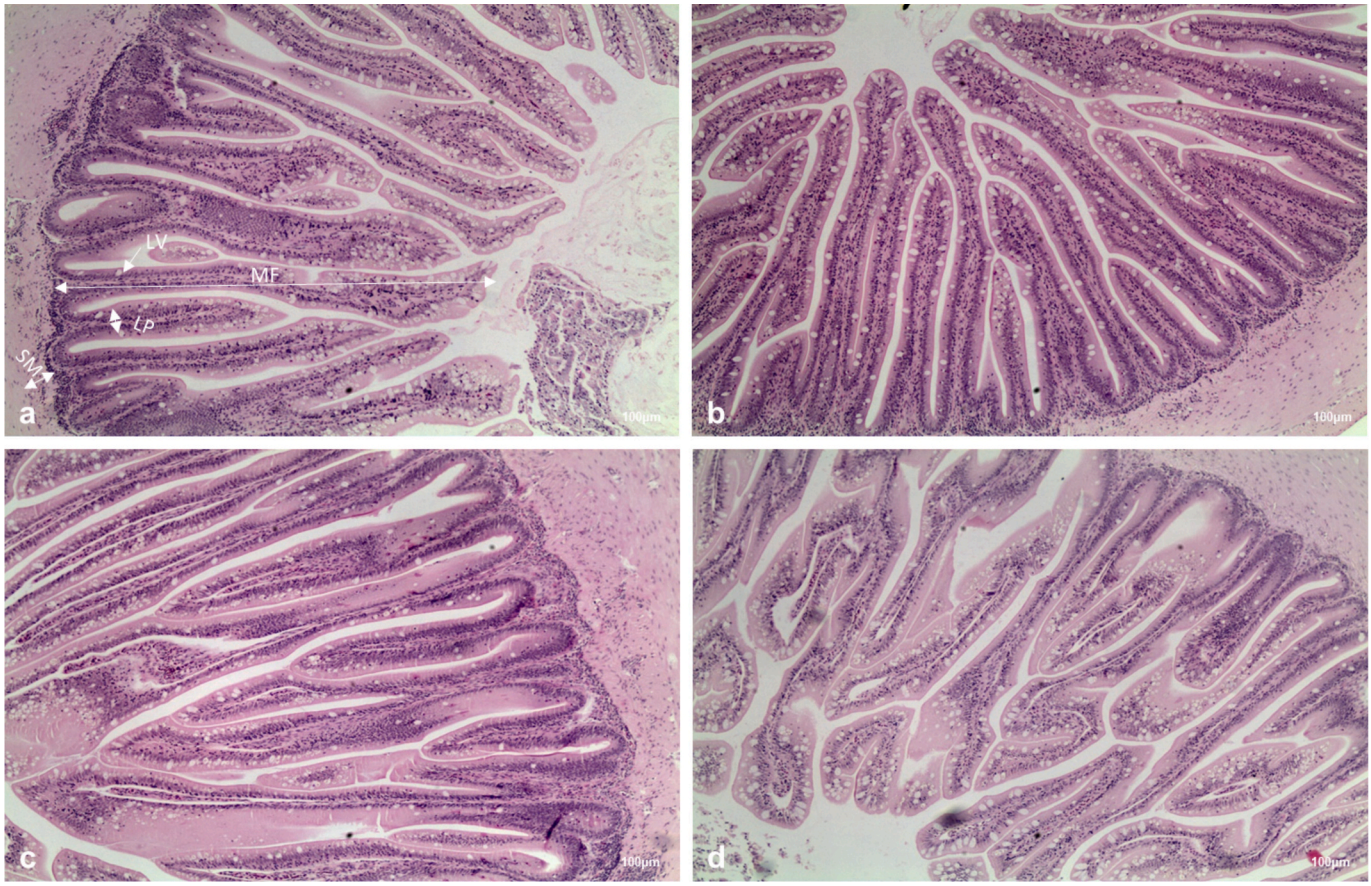

Fig. 2. Posterior gut sections at the end of the growth period a: FRL0, b: FRL10, c: FRL12.5 and d: FRL15 (10×) MF = mucosal folds, LP = lamina propria, SM = submucosa, LV = lipidic vacuoles.

A small number of leukocytes and eosinophilic granulocytes were detected in the intestinal mucosa similar to all experimental groups. The number of mucocytes (GC) was slightly increased in the FRL0 diet and also in the diet with the highest inclusion of fermented lupin in complete replacement of soybean (Table 10) but without significant difference between groups.

4. Discussion

Is well documented that FM remains vital for the health and welfare of carnivorous farmed fish, even at low inclusion levels (Pelusio et al., 2022). In parallel, the incorporation of plant protein ingredients has been extensively investigated over the past several decades, yielding diverse findings (Acar et al., 2018; Huang et al., 2018; Liang et al., 2017; Rodríguez-Estrada et al., 2020). The majority of related aquaculture research has been directed at substituting FM with plant protein sources, while the potential of locally produced ingredients as substitutes for soybean meal or other imported plant-based components in low-FM diets has not yet been thoroughly explored.

Legumes have not yet been a primary focus in the quest for plant-based protein options for marine aquaculture species. Although early research has produced promising results (Adamidou et al., 2009; Adamidou et al., 2011), to our knowledge, no additional research has been carried out for many years. Some research efforts have been undertaken to investigate different varieties of lupin meal as a protein source, in diets with high FM inclusion levels, in a broad number of farmed species in aquaculture, and the outcomes have been generally encouraging (Hoerterer et al., 2016; Molina-Poveda et al., 2013; Zhang et al., 2012).

The effectiveness of such ingredients could be further improved through the use of modern biotechnology, as ANFs, which are naturally abundant in most plant-based ingredients, can adversely affect nutrient utilization, fish performance and health (Kortner et al., 2025). Numerous studies have explored the inclusion of fermented vegetable ingredients into fish feed, yielding promising outcomes (Dossou et al., 2018a; Dossou et al., 2018b; Hassaan et al., 2017; Hassaan et al., 2018; Ilham and Fotedar, 2017; Moniruzzaman et al., 2018; Plaipetch and Yakupitiyage, 2012). In a meta-analysis conducted by Mugwanya et al. (2023), the authors reported that 50.75 % (n = 34 studies) of the research focused on fermented soybean meal, making it the most extensively studied plant protein source. Additional fermented plant materials explored as alternatives to FM in aquaculture diets have not been widely studied for Mediterranean marine species, as the European sea bass and gilthead sea bream.

When a mixture of plant raw ingredients (soybean, rapeseed, sunflower, and rice bran) fermented with *Aspergillus niger* was incorporated at 20 % in the diets of European sea bass, an improvement in overall feed utilization was observed without compromising key production indices (Vieira et al., 2023). Moreover, fermentation of corn distillers' dried grains with solubles by *Aspergillus Ibericus* showed increased nutrient ADC for European sea bass when incorporated at 30 % (Filipe et al., 2023). In red sea bream diets, the replacement of fish meal with yeast-fermented rapeseed meal had no adverse effects on key production indices and, in some cases, resulted in superior performance among other groups (Dossou et al., 2018a). Similarly, when *Aspergillus niger* was used to ferment rapeseed meal in diets for red sea bream, the results showed improvements in selected key performance indicators (KPIs) (Dossou et al., 2018b). These findings suggest a high receptiveness in fermented plant proteins by carnivorous marine species. The process of fermentation can improve the solubility of proteins, enhance the palatability of feedstuffs, and increase the ADC of nitrogen, AA and fat since the decrease of ANFs results in an increase of enzyme activity (Nkhata et al., 2018). In this investigation, KPIs were not jeopardized and the linear regression revealed a better feed conversion and protein efficiency with higher inclusion of dietary lupin meal. This indicates that the fermented lupin meal could totally replace soybean meal in the diet of *D. labrax*. Furthermore, the fat and protein ADC were increased when lupin meal was incorporated at 12.5 % or 15 %. It is known that the produced metabolites of microorganisms involved in fermentation, such as enzymes, organic acids, pigments and polyphenols support intestinal health and enhance digestive efficiency (Tamang et al., 2016). Specifically, there is evidence that a lipid source in the substrate increases lipase production after fermentation with *Beauveria bassiana* (Hegedus and Khachatourians, 1988), while *S. cerevisiae* has been shown to produce extracellular lipases (Shirazi et al., 1998). Therefore, it is likely that the level of fat in the lupin substrate (8 %) promoted lipase production during fermentation process, thus leading to higher fat ADC for the groups fed fermented lupin meal. Moreover, during fermentation, *S. cerevisiae* secretes proteolytic enzymes that break down complex storage proteins into simpler peptides and AA, making them more digestible (Takalloo et al., 2020). In addition, *S. cerevisiae* reduces protease inhibitors and phytic acid resulting in enhanced protein availability (Kasprowicz-Potocka et al., 2018). These facts justify the higher protein ADC in the groups fed the moderate and high levels of fermented lupin meal.

The current diets tested were designed to fulfil the AA requirements of European sea bass. This was reflected in the whole-body AA profile, where all essential AA were present in comparable concentrations across all experimental groups. Regarding AA deposition, histidine and isoleucine exhibited a tendency for increased accumulation in the group exclusively fed with fermented lupin meal compared to the group treated with soybean meal. This finding could be attributed to the presence of protease produced by *S. cerevisiae* during fermentation process, and the ability of this enzyme to increase the hydrolysis of proteins, nevertheless, this hypothesis demands further validation. Moreover, probably the presence of other enzymes produced by *S. cerevisiae*, such us amylases and alpha-glucosidases may have facilitated the digestion of carbohydrates to meet energy demands, thereby reducing the utilization of AAs in catabolic pathways (Castillo and Gatlin III, 2015). The analysis of the relationship between specific AA body deposition and the AA profile of the test diets showed an inverse correlation where elevated levels of certain dietary amino acids were predominantly broken down for energy, resulting in lower retention of those AA in the body.

The analysis of trypsin activity in the pyloric caeca revealed increased enzymatic activity in groups fed with fermented lupin meal. These findings are in accordance with observations reported in a study on rainbow trout (*Oncorhynchus mykiss*), where the inclusion of *S. cerevisiae* as a probiotic for immunostimulation led to enhanced trypsin activity (Heidarieh et al., 2013). The increased trypsin activity might justify the increased protein ADC observed in the groups fed fermented lupin meal. This supports the hypothesis that *S. cerevisiae* contributes to protease production during the fermentation process, thereby facilitating enhanced protein hydrolysis (Gopalraaj et al., 2024). The presence of proteases in fermented lupin meal likely promotes the breakdown of plant proteins into smaller polypeptides or free AA, improving the overall efficiency of protein digestion. Additionally, protease supplementation has been reported to enhance lipase activity in the digestive tract (Maryam et al., 2022; Wiszniewski et al., 2019; Wiszniewski et al., 2022). This phenomenon may account for the significantly higher fat ADC observed in fish receiving diets that include fermented lupin meal, despite all experimental diets utilizing identical FO sources and inclusion levels.

Replacing dietary soybean with fermented lupin significantly modulated fish immunity, exerting a negative effect on lysozyme antibacterial activity ($P = 0.15$). In contrast, complement antibacterial activity showed a tendency to be positively influenced by dietary lupin at a 12.5 % inclusion level. The same trend was observed, but in a significant manner, for alkaline phosphatase in fish sera ($P = 0.04$). This enzyme is often associated with antibacterial activity, suggesting a correlation between dietary lupin and the antibacterial activity of fish serum, with an optimal effect observed at a 12.5 % inclusion level. The total replacement of soybean meal with fermented lupin tended to reduced myeloperoxidase activity (MPO) in fish sera ($P = 0.06$). However, the low MPO levels observed across all fish suggest that this reduction would not pose a problem in vivo. In contrast to the present findings, juvenile Atlantic salmon (*Salmo salar*) fed a diet containing 15 % fermented lupin meal exhibited increased lysozyme activity and enhanced leucocyte respiratory burst, indicating improved immune function (Rodríguez-Estrada et al., 2020). However, another study on salmon reported no significant effects of 40 % dietary lupin on lysozyme activity, trypsin inhibition, respiratory burst activity and immunoglobulin titers (Bransden et al., 2001). In rainbow trout, *O. mykiss* the expression of immune-regulatory genes and immune parameters remained unaffected by lupin meal inclusion, indicating no adverse effects on innate immunity (Farhangi and Carter, 2001; Hernández et al., 2013). Overall, the dietary fermented lupin did not suppress immune system of European sea bass and an indication of marginal immunostimulatory effects was observed at 12.5 % inclusion.

The hepatic tissue was unaffected by the inclusion of fermented lupin meal compared to the FRL0 group, which contrasts with the findings reported for carp by Anwar and Wan (2020) fed on white lupin supplemented with a SSF supplement. An analogous finding was reported by Borquez et al. (2011), who noted lipid infiltration into enterocytes and hepatocytes at a 40 % inclusion level of unprocessed whole grain white lupin in rainbow trout (*O. mykiss*) diets. However, a contrasting study found no differences in lipid or glycogen storage in the hepatocytes of gilthead seabream (*Sparus aurata*) fed with 30 % dehulled blue lupin (*L. angustifolius*) seed meal (Robaina et al., 1995). The histological examination of the distal part of the intestine which is responsible for the absorption (endocytosis) of large molecules such as proteins didn't

reveal any negative effect of the use of fermented lupin meal on its morphology in any of the examined fish groups. All sections showed no signs of enteropathies in any of the lupin-based diets. This suggests that replacing soybean meal with fermented lupin meal did not induce histopathological changes in European sea bass. In the contrary, Farhangi and Carter (2001) and Borquez et al. (2011) observed histological changes in the mid-intestine with the dietary inclusion of lupin in rainbow trout (*O. mykiss*) diets. These discrepancies may be attributed not only to the higher inclusion levels lupin used in those studies, but also to differences in lupin processing. Specifically, those studies employed dehulled and whole grain lupin, which may retain higher levels of ANFs, while in the present study, fermented lupin meal was used processed with *Saccharomyces cerevisiae*, which has been shown to reduce such compounds (Oboh, 2006). *Saccharomyces cerevisiae* fermentation may contribute to maintaining intestinal morphology by reducing ANFs and producing bioactive compounds with immunomodulatory and prebiotic properties. These effects can enhance gut health by minimizing inflammation and supporting the integrity of intestinal tissues (El-Bab et al., 2022).

5. Conclusions

In conclusion, this study marks the first investigation into replacing soybean meal with *S. cerevisiae*-fermented lupin meal in the diets of European sea bass. The findings highlight the efficacy of this locally sourced alternative as an effective raw material for diet formulation in this species. Under the specific experimental conditions, soybean meal can be entirely replaced with *S. cerevisiae*-fermented lupin meal without negatively impacting growth performance, nutrient ADC or the welfare of farmed European sea bass. At levels up to 83 %, soybean meal can be substituted with fermented lupin meal, leading to improved performance across multiple parameters. However, further studies are necessary in order to verify the results of the current study. Moreover, is important to assess the potential of incorporating fermented lupin meal at different life stages. Utilizing locally produced legumes, such as those of the genus *Lupinus,* coupling with fermentation processes, may be key to enhancing the sustainability of the aquaculture industry. This approach could reduce reliance on imported plant ingredients, lowering the environmental footprint, while fostering the growth of a resilient and valuable agricultural sector in the Mediterranean region.

CRediT authorship contribution statement

Antigoni Vasilaki: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ioannis Nengas:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Eleni Fountoulaki:** Writing – review & editing, Investigation, Formal analysis. **Morgane Henry:** Writing – review & editing, Investigation, Formal analysis. **Dimitra Kogiannou:** Writing – review & editing, Investigation, Formal analysis. **Chrysanthi Nikoloudaki:** Writing – review & editing, Investigation, Formal analysis. **Petros Chronopoulos:** Writing – review & editing, Investigation, Formal analysis. **Ioannis T. Karapanagiotidis:** Writing – review & editing, Supervision, Resources, Methodology. **Elena Mente:** Writing – review & editing, Validation, Supervision, Investigation, Funding acquisition, Conceptualization.

Funding

The project entitled “Improvement of the nutritional quality of forage legumes through biotechnological processes for the nutrition of Mediterranean aquaculture species” [MIS 5067490] have been funded from the European Union, European Maritime and Fisheries Fund, in the context of the Operational Program “Maritime and Fisheries 2014–2020”. This output reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.

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.

Acknowledgements

The authors wish to thank Eugenia Manolopoulou, Georgia Zoumpopoulou and Effie Tsakalidou from Laboratory of Dairy Research, Agricultural University of Athens, Iera Odos 75 Athens, Greece for providing the *Saccharomyces cerevisiae* strain ACA-DC 5036 for the fermentation process and Cooperative Farmers of Thessaly (THES-GI) for providing lupin seeds. The authors would also like to thank Anna Tampou, Spyros Panagiotis Andreou, Katerina Katouni, Pier Psofakis, Christina Papadouli and Mytro Maniaki, for their contribution to the feeding trial.

Data availability

Data will be made available on request.

References

Ababouch, L., Vannuccini, S., 2024. FAO the State of World Fisheries and Aquaculture 2024—Blue Transformation in Action. FAO Geneva, Switzerland.

Abraham, E.M., Ganopoulos, I., Madesis, P., Mavromatis, A., Mylona, P., Nianiou-Obeidat, I., Parissi, Z., Polidoros, A., Tani, E., Vlachostergios, D., 2019. The use of lupin as a source of protein in animal feeding: genomic tools and breeding approaches. Int. J. Mol. Sci. 20 (4), 851.

Acar, Ü., Kesbiç, O.S., Yılmaz, S., Karabayır, A., 2018. Growth performance, haematological and serum biochemical profiles in rainbow trout (Oncorhynchus mykiss) fed diets with varying levels of lupin (Lupinus albus) meal. Aquac. Res. 49 (7), 2579–2586. https://doi.org/10.1111/are.13724.

Adamidou, S., Nengas, I., Henry, M., Grigorakis, K., Rigos, G., Nikolopoulou, D., Kotzamanis, Y., Bell, G.J., Jauncey, K., 2009. Growth, feed utilization, health and organoleptic characteristics of European seabass (Dicentrarchus labrax) fed extruded diets including low and high levels of three different legumes. Aquaculture 293 (3–4), 263–271.

Adamidou, S., Nengas, I., Henry, M., Ioakei Midoy, N., Rigos, G., Bell, G., Jauncey, K., 2011. Effects of dietary inclusion of peas, chickpeas and faba beans on growth, feed utilization and health of gilthead seabream (Sparus aurata). Aquac. Nutr. 17 (2), e288–e296. https://doi.org/10.1111/j.1365-2095.2010.00762.x.

Amrhein, V., Greenland, S., McShane, B., 2019. Scientists rise up against statistical significance. Nature 567 (7748), 305–307.

Anwar, A., Wan, A.H., Omar, S.e.a., 2020. The potential of a solid-state fermentation supplement to augment white lupin (Lupinus albus) meal incorporation in diets for farmed common carp (Cyprinus carpio). Aquacult. Rep. 17, 100348. https://doi.org/10.1016/j.aqrep.2020.100348.

AOAC, 2005. Association of Official Analytical Chemists (Official methods of analysis of AOAC International).

Aragão, C., Gonçalves, A.T., Costas, B., Azeredo, R., Xavier, M.J., Engrola, S., 2022. Alternative proteins for fish diets: implications beyond growth. Animals 12 (9), 1211. https://doi.org/10.3390/ani12091211.

Aziza, A., El-Wahab, A.A., 2019. Impact of partial replacing of dietary fish meal by different protein sources on the growth performance of Nile tilapia (Oreochromis niloticus) and whole body composition. J. Appl. Sci. 19 (5), 384–391. https://doi.org/10.3923/jas.2019.384.391.

Bender, R., Lange, S., 2001. Adjusting for multiple testing—when and how? J. Clin. Epidemiol. 54 (4), 343–349.

Bolin, D.W., King, R.P., Klosterman, E.W., 1952. A simplified method for the determination of chromic oxide (Cr2O3) when used as an index substance. Science 116 (3023), 634–635. https://doi.org/10.1126/science.116.3023.634.

Bonvini, E., Bonaldo, A., Mandrioli, L., Sirri, R., Dondi, F., Bianco, C., Fontanillas, R., Mongile, F., Gatta, P., Parma, L., 2018. 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. https://doi.org/10.1017/S1751731117002683.

Borquez, A., Serrano, E., Dantagnan, P., Carrasco, J., Hernandez, A., 2011. Feeding high inclusion of whole grain white lupin (Lupinus albus) to rainbow trout (Oncorhynchus mykiss): effects on growth, nutrient digestibility, liver and intestine histology and muscle fatty acid composition. Aquac. Res. 42 (8), 1067–1078. https://doi.org/10.1111/j.1365-2109.2010.02690.x.

Bransden, M., Carter, C., Nowak, B., 2001. Effects of dietary protein source on growth, immune function, blood chemistry and disease resistance of Atlantic salmon (Salmo salar L.) parr. Anim. Sci. 73 (1), 105–113.
Bullock, A., 1978. Laboratory Methods in Fish Pathology. By Roberts RJ, pp. 235–267.
Castillo, S., Gatlin III, D.M., 2015. Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: A review. Aquaculture 435, 286–292. https://doi.org/10.1016/j.aquaculture.2014.10.011.
Cattelan, A.J., Dall'Agnol, A., 2018. The rapid soybean growth in Brazil. OCL 25 (1), D102.
Chandra-Hioe, M.V., Wong, C.H., Arcot, J., 2016. The potential use of fermented chickpea and faba bean flour as food ingredients. Plant Foods Hum. Nutr. 71, 90–95.
Cho, C.Y., Kaushik, S.J., 1990. Nutritional energetics in fish: energy and protein utilization in rainbow trout (Salmo gairdneri). Aspects of food production, consumptionand energy values 61, 132–172.
Cho, C., Slinger, S., Bayley, H., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comp. Biochem. Physiol. B: Comp. Biochem. 73 (1), 25–41.
Collins, S.A., Øverland, 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.
Commission, E, 2018. Report from the Commission to the Council and the European Parliament on the Development of Plant Proteins in the European Union.
Dileep, N., Pradhan, C., Peter, N., Kaippilly, D., Sashidharan, A., Sankar, T., 2021. Nutritive value of guar and copra meal after fermentation with yeast Saccharomyces cerevisiae in the diet of Nile tilapia, Oreochromis niloticus. Trop. Anim. Health Prod. 53, 1–13.
Dossou, S., Koshio, S., Ishikawa, M., Yokoyama, S., Dawood, M.A., El Basuini, M.F., El-Hais, A.M., Olivier, A., 2018a. Effect of partial replacement of fish meal by fermented rapeseed meal on growth, immune response and oxidative condition of red sea bream juvenile, Pagrus major. Aquaculture 490, 228–235.
Dossou, S., Koshio, S., Ishikawa, M., Yokoyama, S., Dawood, M.A., El Basuini, M.F., Olivier, A., Zaineldin, A.I., 2018b. Growth performance, blood health, antioxidant status and immune response in red sea bream (Pagrus major) fed aspergillus oryzae fermented rapeseed meal (RM-Koji). Fish Shellfish Immunol. 75, 253–262.
El-Bab, A.F.F., Saghir, S.A., El-Naser, I.A.A., El-Kheir, S.M.A., Abdel-Kader, M.F., Alruhaimi, R.S., Alqhtani, H.A., Mahmoud, A.M., Naiel, M.A., El-Raghi, A.A., 2022. The effect of dietary Saccharomyces cerevisiae on growth performance, oxidative status, and immune response of sea bream (Sparus aurata). Life 12 (7), 1013.
Englyst, H.N., Quigley, M.E., Hudson, G.J., 1994. Determination of dietary fibre as non-starch polysaccharides with gas–liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. Analyst 119 (7), 1497–1509.
Erlanger, B.F., Kokowsky, N., Cohen, W., 1961. The preparation and properties of two new chromogenic substrates of trypsin. Arch. Biochem. Biophys. 95 (2), 271–278. https://doi.org/10.1016/0003-9861(61)90145-X.
Escobar, N., Tizado, E.J., zu Ermgassen, E.K., Löfgren, P., Börner, J., Godar, J., 2020. Spatially-explicit footprints of agricultural commodities: mapping carbon emissions embodied in Brazil's soy exports. Glob. Environ. Chang. 62, 102067.
Farhangi, M., Carter, C., 2001. Growth, physiological and immunological responses of rainbow trout (Oncorhynchus mykiss) to different dietary inclusion levels of dehulled lupin (Lupinus angustifolius). Aquac. Res. 32, 329–340.
Ferchichi, N., Toukabri, W., Vrhovsek, U., Nouairi, I., Angeli, A., Masuero, D., Mhamdi, R., Trabelsi, D., 2021. Proximate composition, lipid and phenolic profiles, and antioxidant activity of different ecotypes of Lupinus albus, Lupinus luteus and Lupinus angustifolius. J. Food Meas. Charact. 15, 1241–1257. https://doi.org/10.1007/s11694-020-00722-8.
Filipe, D., Dias, M., Magalhães, R., Fernandes, H., Salgado, J., Belo, I., Oliva-Teles, A., Peres, H., 2023. Solid-state fermentation of distiller's dried grains with Solubles improves digestibility for European seabass (Dicentrarchus labrax) juveniles. Fishes 8 (2), 90.
Fry, J.P., Love, D.C., MacDonald, G.K., West, P.C., Engstrom, P.M., Nachman, K.E., Lawrence, R.S., 2016. Environmental health impacts of feeding crops to farmed fish. Environ. Int. 91, 201–214.
Gatlin III, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, Å., Nelson, R., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac. Res. 38 (6), 551–579. https://doi.org/10.1111/j.1365-2109.2007.01704.x.
Gelman, A., Stern, H., 2006. The difference between "significant" and "not significant" is not itself statistically significant. Am. Stat. 60 (4), 328–331.
Gopalraaj, J., Velayudhannair, K., Arockiasamy, J.P., Radhakrishnan, D.K., 2024. The effect of dietary supplementation of proteases on growth, digestive enzymes, oxidative stress, and intestinal morphology in fishes–A review. Aquac. Int. 32 (1), 745–765. https://doi.org/10.1007/s10499-023-01191-8.
Hassaan, M., Goda, A.S., Kumar, V., 2017. Evaluation of nutritive value of fermented de-oiled physic nut, Jatropha curcas, seed meal for Nile tilapia Oreochromis niloticus fingerlings. Aquac. Nutr. 23 (3), 571–584.
Hassaan, M.S., Soltan, M.A., Mohammady, E.Y., Elashry, M.A., El-Haroun, E.R., Davies, S.J., 2018. Growth and physiological responses of Nile tilapia, Oreochromis niloticus fed dietary fermented sunflower meal inoculated with Saccharomyces cerevisiae and Bacillus subtilis. Aquaculture 495, 592–601.
Hegedus, D.D., Khachatourians, G.G., 1988. Production of an extracellular lipase by Beauveria bassiana. Biotechnol. Lett. 10, 637–642.
Heidarieh, M., Mirvaghefi, A., Akbari, M., Sheikhzadeh, N., Kamyabi-Moghaddam, Z., Askari, H., Shahbazfar, A., 2013. Evaluations of H ilyses™, fermented S accharomyces cerevisiae, on rainbow trout (O ncorhynchus mykiss) growth performance, enzymatic activities and gastrointestinal structure. Aquac. Nutr. 19 (3), 343–348.
Henry, M., Fountoulaki, E., 2014. Optimal dietary protein/lipid ratio for improved immune status of a newly cultivated Mediterranean fish species, the shi drum Umbrina cirrosa, L. Fish Shellfish Immunol. 37 (2), 215–219. https://doi.org/10.1016/j.fsi.2014.02.005.
Henry, M., Nikoloudaki, C., Tsigenopoulos, C., Rigos, G., 2015. Strong effect of long-term Sparicotyle chrysophrii infection on the cellular and innate immune responses of gilthead sea bream, *Sparus aurata*. Dev. Comp. Immunol. 51 (1), 185–193.
Henry, M., Golomazou, E., Asimaki, A., Psofakis, P., Fountoulaki, E., Mente, E., Rumbos, C., Athanassiou, C., Karapanagiotidis, I., 2022. Partial dietary fishmeal replacement with full-fat or defatted superworm (Zophobas morio) larvae meals modulates the innate immune system of gilthead seabream, *Sparus aurata*. Aquacult. Rep. 27, 101347. https://doi.org/10.1016/j.aqrep.2022.101347.
Hernández, A., Román, D., Hooft, J., Cofre, C., Cepeda, V., Vidal, R., 2013. Growth performance and expression of immune-regulatory genes in rainbow trout (O ncorhynchus mykiss) juveniles fed extruded diets with varying levels of lupin (L upinus albus), peas (P isum sativum) and rapeseed (B rassica napus). Aquac. Nutr. 19 (3), 321–332.
Hoerterer, C., Zeytin, S., Weiss, M., Slater, M.J., 2016. Lupine Meal as Primary Protein Source in Diets for European Sea Bass *Dicentrarchus labrax*. Aquaculture Europe 16, Edinburgh, Scotland.
Huang, Y.-J., Zhang, N.-N., Fan, W.-J., Cui, Y.-Y., Limbu, S.M., Qiao, F., Zhao, Y.-L., Chen, L.-Q., Du, Z.-Y., Li, D.-L., 2018. Soybean and cottonseed meals are good candidates for fishmeal replacement in the diet of juvenile Macrobrachium nipponense. Aquac. Int. 26, 309–324.
Ilham, I., Fotedar, R., 2017. Growth, enzymatic glutathione peroxidase activity and biochemical status of juvenile barramundi (Lates calcarifer) fed dietary fermented soybean meal and organic selenium. Fish Physiol. Biochem. 43, 775–790.
Kader, M.A., Bulbul, M., Koshio, S., Ishikawa, M., Yokoyama, S., Nguyen, B.T., Komilus, C.F., 2012. Effect of complete replacement of fishmeal by dehulled soybean meal with crude attractants supplementation in diets for red sea bream, Pagrus major. Aquaculture 350, 109–116.
Kakade, M., Rackis, J., McGhee, J., Puski, G., 1974. Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure. Cereal Chem. 376–382, 3.
Kasprowicz-Potocka, M., Zaworska, A., Gulewicz, P., Nowak, P., Frankiewicz, A., 2018. The effect of fermentation of high alkaloid seeds of Lupinus angustifolius var. Karo by Saccharomyces cerevisieae, Kluyveromyces lactis, and Candida utilis on the chemical and microbial composition of products. J. Food Process. Preserv. 42 (2), e13487.
Kaushik, S., 2002. European sea bass. Nutr. Requir. Feeding Finfish Aquacult. 28.
Kokou, F., Rigos, G., Henry, M., Kentouri, M., Alexis, M., 2012. Growth performance, feed utilization and non-specific immune response of gilthead sea bream (Sparus aurata L.) fed graded levels of a bioprocessed soybean meal. Aquaculture 364, 74–81.
Kokou, F., Sarropoulou, E., Cotou, E., Kentouri, M., Alexis, M., Rigos, G., 2017. Effects of graded dietary levels of soy protein concentrate supplemented with methionine and phosphate on the immune and antioxidant responses of gilthead sea bream (Sparus aurata L.). Fish Shellfish Immunol. 64, 111–121.
Kokou, F., Henry, M., Nikoloudaki, C., Kounna, C., Vasilaki, A., Fountoulaki, E., 2019. Optimum protein-to-lipid ratio requirement of the juvenile shi drum (Umbrina cirrosa) as estimated by nutritional and histological parameters. Aquac. Nutr. 25 (2), 444–455.
Kortner, T.M., Løkka, G., Francis, G., Becker, K., Krogdahl, Å., 2025. Antinutritional factors and adventitious toxins. In: Feed and Feeding for Fish and Shellfish. Elsevier, pp. 153–172.
Kuepper, B., Stravens, M., 2022. Mapping the European Soy Supply Chain. Amsterdam, The Netherlands, Profundo, Commissioned by WWF European Policy Office.
Liang, X., Hu, L., Dong, Y., Wu, X., Qin, Y., Zheng, Y., Shi, D., Xue, M., 2017. Substitution of fish meal by fermented soybean meal affects the growth performance and flesh quality of Japanese seabass (Lateolabrax japonicus). Anim. Feed Sci. Technol. 229, 1–12.
Liu, K., Seegers, S., Cao, W., Wanasundara, J., Chen, J., da Silva, A.E., Ross, K., Franco, A. L., Vrijenhoek, T., Bhowmik, P., 2021. An international collaborative study on trypsin inhibitor assay for legumes, cereals, and related products. J. Am. Oil Chem. Soc. 98 (4), 375–390.
Ma, J., Adler, L., Srzednicki, G., Arcot, J., 2017. Quantitative determination of non-starch polysaccharides in foods using gas chromatography with flame ionization detection. Food Chem. 220, 100–107. https://doi.org/10.1016/j.foodchem.2016.09.206.
Majluf, P., Matthews, K., Pauly, D., Skerritt, D.J., Palomares, M.L.D., 2024. A review of the global use of fishmeal and fish oil and the fish in: fish out metric. Sci. Adv. 10 (42) eadn5650.
Maryam, Shah, S.Z.H., Fatima, M., Hussain, S.M., Nadeem, H., Hussain, M., 2022. The effectiveness of protease supplemented poultry by-product meal-based diet on growth, nutrient digestibility and digestive enzyme activities of rohu (Labeo rohita). Aquac. Res. 53 (10), 3841–3852.
Mathesius, U., 2022. Are legumes different? Origins and consequences of evolving nitrogen fixing symbioses. J. Plant Physiol. 276, 153765. https://doi.org/10.1016/j.jplph.2022.153765.
Mitra, A., 2021. Thought of alternate aquafeed: conundrum in aquaculture sustainability?. In: Proceedings of the Zoological Society. Springer, pp. 1–18.
Molina-Poveda, C., Lucas, M., Jover, M., 2013. Evaluation of the potential of Andean lupin meal (Lupinus mutabilis sweet) as an alternative to fish meal in juvenile Litopenaeus vannamei diets. Aquaculture 410, 148–156.

Moniruzzaman, M., Bae, J., Won, S., Cho, S., Chang, K., Bai, S., 2018. Evaluation of solid-state fermented protein concentrates as a fish meal replacer in the diets of juvenile rainbow trout, *Oncorhynchus mykiss*. Aquac. Nutr. 24 (4), 1198–1212.
Morais, A.H.d.A., Lima, V.C.O.d., Piuvezam, G., Maciel, B.L.L., 2019. Trypsin inhibitors: promising candidate satietogenic proteins as complementary treatment for obesity and metabolic disorders? J. Enzyme Inhib. Med. Chem. 34.
Mugwanya, M., Dawood, M.A., Kimera, F., Sewilam, H., 2023. Replacement of fish meal with fermented plant proteins in the aquafeed industry: A systematic review and meta-analysis. Rev. Aquac. 15 (1), 62–88.
Nengas, I., Alexis, M., Davies, S., Petichakis, G., 1995. Investigation to determine digestibility coefficients of various raw materials in diets for gilthead sea bream, *Sparus auratus* L. Aquac. Res. 26 (3), 185–194.
Nkhata, S.G., Ayua, E., Kamau, E.H., Shingiro, J.B., 2018. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Sci. Nutr. 6 (8), 2446–2458.
Oboh, G., 2006. Nutrient enrichment of cassava peels using a mixed culture of Saccharomyces cerevisae and Lactobacillus spp solid media fermentation techniques. Electron. J. Biotechnol. 9 (1), 0–0.
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.
Plaipetch, P., Yakupitiyage, A., 2012. Use of yeast-fermented canola meal to replace fishmeal in the diet of Asian sea bass Lates calcarifer (Bloch, 1790). J. Aquac. Res. Dev. 3 (2).
Rayaprolu, S.J., Hettiarachchy, N.S., Chen, P., Kannan, A., Mauromostakos, A., 2013. Peptides derived from high oleic acid soybean meals inhibit colon, liver and lung cancer cell growth. Food Res. Int. 50 (1), 282–288.
Robaina, L., Izquierdo, M., Moyano, F., Socorro, J., Vergara, J., Montero, D., Fernandez-Palacios, H., 1995. Soybean and lupin seed meals as protein sources in diets for gilthead seabream (Sparus aurata): nutritional and histological implications. Aquaculture 130 (2–3), 219–233.
Rodríguez-Estrada, U., González-Alfaro, K., Shene, C., 2020. Replacement of fish meal by solid state fermented lupin (Lupinus albus) meal with Latobacillus plantarum 299v: effect on growth and immune status of juvenile Atlantic salmon (Salmo salar). Ann. Anim. Sci. 20 (3), 991–1009.
Ross, N.W., Firth, K.J., Wang, A., Burka, J.F., Johnson, S.C., 2000. Changes in hydrolytic enzyme activities of naive Atlantic salmon Salmo salar skin mucus due to infection with the salmon louse Lepeophtheirus salmonis and cortisol implantation. Dis. Aquat. Org. 41 (1), 43–51.
Santos, V.A.Q., Nascimento, C.G., Schmidt, C.A., Mantovani, D., Dekker, R.F., da Cunha, M.A.A., 2018. Solid-state fermentation of soybean okara: Isoflavones biotransformation, antioxidant activity and enhancement of nutritional quality. Lwt 92, 509–515.
Shi, H., Yang, E., Li, Y., Chen, X., Zhang, J., 2021. Effect of solid-state fermentation on nutritional quality of leaf flour of the drumstick tree (Moringa oleifera lam.). Front. Bioeng. Biotechnol. 9, 626628.
Shirazi, S., Rahman, S., Rahman, M., 1998. Production of extracellular lipases by Saccharomyces cerevisiae. World J. Microbiol. Biotechnol. 14, 595–597.
Sinha, A.K., Kumar, V., Makkar, H.P., De Boeck, G., Becker, K., 2011. Non-starch polysaccharides and their role in fish nutrition–A review. Food Chem. 127 (4), 1409–1426.
Szczepański, A., Adamek-Urbańska, D., Kasprzak, R., Szudrowicz, H., Śliwiński, J., Kamaszewski, M., 2022. Lupin: A promising alternative protein source for aquaculture feeds? Aquacult. Rep. 26, 101281.
Takalloo, Z., Nikkhah, M., Nemati, R., Jalilian, N., Sajedi, R.H., 2020. Autolysis, plasmolysis and enzymatic hydrolysis of baker's yeast (Saccharomyces cerevisiae): a comparative study. World J. Microbiol. Biotechnol. 36, 1–14.
Tamang, J.P., Shin, D.-H., Jung, S.-J., Chae, S.-W., 2016. Functional properties of microorganisms in fermented foods. Front. Microbiol. 7, 578.
Teng, D., Gao, M., Yang, Y., Liu, B., Tian, Z., Wang, J., 2012. Bio-modification of soybean meal with Bacillus subtilis or aspergillus oryzae. Biocatal. Agric. Biotechnol. 1 (1), 32–38.
Terefe, Z.K., Omwamba, M.N., Nduko, J.M., 2021. Effect of solid state fermentation on proximate composition, antinutritional factors and in vitro protein digestibility of maize flour. Food Sci. Nutr. 9 (11), 6343–6352.
Terefe, Z.K., Omwamba, M., Nduko, J.M., 2022. Effect of microbial fermentation on nutritional and antinutritional contents of cassava leaf. J. Food Saf. 42 (3), e12969.
Urán, P., Gonçalves, A., Taverne-Thiele, J., Schrama, J., Verreth, J., Rombout, J., 2008. Soybean meal induces intestinal inflammation in common carp (Cyprinus carpio L.). Fish Shellfish Immunol. 25 (6), 751–760.
Vasilaki, A., Mente, E., Fountoulaki, E., Henry, M., Nikoloudaki, C., Berillis, P., Kousoulaki, K., Nengas, I., 2023. Fishmeal, plant protein, and fish oil substitution with single-cell ingredients in organic feeds for European sea bass (Dicentrarchus labrax). Front. Physiol. 14, 1199497.
Vieira, L., Filipe, D., Amaral, D., Magalhães, R., Martins, N., Ferreira, M., Ozorio, R., Salgado, J., Belo, I., Oliva-Teles, A., 2023. Solid-state fermentation as green technology to improve the use of plant feedstuffs as ingredients in diets for European sea bass (Dicentrarchus labrax) juveniles. Animals 13 (17), 2692.
Wasserstein, R.L., Lazar, N.A., 2016. The ASA Statement on p-Values: Context, Process, and Purpose. Taylor & Francis, pp. 129–133.
Wilfart, A., Garcia-Launay, F., Terrier, F., Soudé, E., Aguirre, P., Skiba-Cassy, S., 2023. A step towards sustainable aquaculture: multiobjective feed formulation reduces environmental impacts at feed and farm levels for rainbow trout. Aquaculture 562, 738826. https://doi.org/10.1016/j.aquaculture.2022.738826.
Wiszniewski, G., Jarmołowicz, S., Hassaan, M.S., Mohammady, E.Y., Soaudy, M.R., Łuczyńska, J., Tońska, E., Terech-Majewska, E., Ostaszewska, T., Kamaszewski, M., 2019. The use of bromelain as a feed additive in fish diets: growth performance, intestinal morphology, digestive enzyme and immune response of juvenile Sterlet (Acipenser ruthenus). Aquac. Nutr. 25 (6), 1289–1299.
Wiszniewski, G., Jarmołowicz, S., Hassaan, M.S., Soaudy, M.R., Kamaszewski, M., Szudrowicz, H., Terech-Majewska, E., Pajdak-Czaus, J., Wiechetek, W., Siwicki, A.K., 2022. Beneficial effects of dietary papain supplementation in juvenile sterlet (Acipenser ruthenus): growth, intestinal topography, digestive enzymes, antioxidant response, immune response, and response to a challenge test. Aquacult. Rep. 22, 100923.
Yaghoubi, M., Mozanzadeh, M.T., Marammazi, J.G., Safari, O., Gisbert, E., 2016. Dietary replacement of fish meal by soy products (soybean meal and isolated soy protein) in silvery-black porgy juveniles (Sparidentex hasta). Aquaculture 464, 50–59.
Yufera, M., Nguyen, M., Navarro-Guillén, C., Moyano, F.J., Jordal, A.-E., Espe, M., Conceição, L.E., Engrola, S., Le, M.H., Rønnestad, I., 2019. Effect of increased rearing temperature on digestive function in cobia early juvenile. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 230, 71–80.
Zalles, V., Hansen, M.C., Potapov, P.V., Stehman, S.V., Tyukavina, A., Pickens, A., Song, X.-P., Adusei, B., Okpa, C., Aguilar, R., 2019. Near doubling of Brazil's intensive row crop area since 2000. Proc. Natl. Acad. Sci. 116 (2), 428–435.
Zhang, Y., Øverland, M., Xie, S., Dong, Z., Lv, Z., Xu, J., Storebakken, T., 2012. Mixtures of lupin and pea protein concentrates can efficiently replace high-quality fish meal in extruded diets for juvenile black sea bream (Acanthopagrus schlegeli). Aquaculture 354, 68–74.
Zhang, C., Rahimnejad, S., Wang, Y.-r., Lu, K., Song, K., Wang, L., Mai, K., 2018. Substituting fish meal with soybean meal in diets for Japanese seabass (Lateolabrax japonicus): effects on growth, digestive enzymes activity, gut histology, and expression of gut inflammatory and transporter genes. Aquaculture 483, 173–182. https://doi.org/10.1016/j.aquaculture.2017.10.029.
Zhang, W., Tan, B., Deng, J., Dong, X., Yang, Q., Chi, S., Liu, H., Zhang, S., Xie, S., Zhang, H., 2021. Mechanisms by which fermented soybean meal and soybean meal induced enteritis in marine fish juvenile pearl gentian grouper. Front. Physiol. 12, 646853.