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Effects of dietary lysophospholipids and butyrate supplementation on growth, feed utilization, fillet fatty acids, intestinal enzymes, innate immunity and liver and gut histology in European Sea bass, *Dicentrarchus labrax*

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ABSTRACT

Lysophospholipids (LPLs) and butyrate, when added in fish diets, are known to have positive effects such as better growth rates, feed utilization, lipid absorption, antimicrobial properties and improvement of the intestine morphology and immune function, in animal nutrition. This study investigated the potential role of the commercial product Aquatria™ LQ, which contains LPLs, glyceryl mono-oleate, synthetic emulsifier and a butyrate source to formulate high and low-fat functional diets and characterise their effects on growth performance, feed utilization, intestinal enzymes, gut and liver morphology and immune parameters of European Sea Bass (*Dicentrarchus labrax*). Initial body weight of experimental fish was 18.0 ± 1.9 g and trial duration was 13 weeks. The experimental diets were formulated as follows; Diet 19/0: Positive control high-fat (19%) commercial formula, Diet 19/0.05: Diet 19/0 with the addition of 0.05% Aquatria™ LQ, Diet 16/0: Low-fat (16%) control, Diet 16/0.025 and Diet 16/0.05: Diet 16/0 supplemented with 0.025% and 0.05% Aquatria™ LQ, respectively. The dietary addition of Aquatria™ LQ enhanced fish growth parameters. LPLs supplementation showed a positive effect on high fat diets and no effect in low-fat diets in the fillet fatty acid profiles. Intestinal lipase activity values were improved in all treatments with the inclusion of Aquatria™ LQ and the antioxidant activity was slightly enhanced, without statistically significant differences between the treatments. The effects of reducing dietary fat on the fish innate immunity did not showed significant differences with the addition of Aquatria™ LQ. Positive effects of the functional low and high-fat diets were also evidenced in the liver histology analysis with significant differences at inclusion levels of 0.05%. In conclusion, these findings show that incorporating Aquatria™ LQ at 0.025% allows for a 3% reduction in dietary fat content without negative impacts on growth, feed efficiency, or the health status of juvenile European sea bass within the tested conditions.

1. Introduction

It is projected that the global population will exceed 9.2 billion in 2050, with total food demand expected to increase by 35% to 56% between 2010 and 2050 (van Dijk et al., 2021) Consequently, the global demand for seafood will continue to rise. Aquaculture is expanding to meet the growing need for high-quality protein and lipid derived from seafood. For instance, total aquaculture production in 2021 reached 126 million tonnes of live weight, with an estimated value of $296.5 billion and farmed fish accounted for 47.1% of production (Mair et al., 2023). As the aquaculture industry continuously grows, there is an increasing demand for high quality aquafeeds. Historically, aquafeeds for carnivorous fish have relied heavily on fishmeal and fish oil to meet the nutritional requirements. However, in recent years, there has been increasing research into alternative and sustainable sources to replace fishmeal and fish oil (Panteli et al., 2025). This shift is driven not only by

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rising prices of marine ingredients but also by the need to reduce the environmental impact of the industry and prevent the overfishing of crucial fish stocks (Oliva-Teles et al., 2022).

As a replacement for fish oil, vegetable oils are the most widely studied and used alternative sources (Naylor et al., 2021, Sáez-Royuela et al., 2022, Ofori-Mensah et al., 2022, Álvarez et al., 2020). However, vegetable oils lack highly unsaturated fatty acids (LC-PUFA) (Miller et al., 2008). LC-PUFAs, particularly eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) are critical for the nutritional value of marine fish. Consequently, the use of vegetable oils may reduce the nutritional quality of fish fillets and compromise the health benefits of the product. Salmon oil derived from by-products is another alternative source that is rich in essential fatty acids, including EPA and DHA (Rodrigues et al., 2024). Salmon oil from by-products has been successfully used as a partial replacement for fish oil in European sea bass diets (Marques et al., 2023). In the context of a circular economy, this approach can also help reduce the carbon footprint of fish oil production (De La Fuente et al., 2022).

Simultaneously, the total protein content in diets has been reduced and substituted with oils. Not all dietary protein contributes to fish growth; a portion is used as an energy source and can be replaced with lipids. This so-called protein-sparing effect of lipids reduces both diet cost and nitrogenous pollution in the water (Thirunavukkarasar et al., 2022). However, high lipid levels in feed can lead to lipid accumulation in the liver, which can adversely affect liver health (Zhou et al., 2024). Therefore, the appropriate lipid inclusion must be determined to ensure that all the essential fatty acids are provided without compromising fish health. In low-fat diets it is crucial that the fats are efficiently utilized, ensuring optimal fish performance and fillets yield.

Lysophospholipids (LPLs), also known as lysolecithins, are derivatives of phospholipids that have one fatty acid chain removed (D'Arrigo and Servi, 2010). These compounds possess unique properties that make them useful in various applications including aquaculture. LPLs can improve the palatability of aquafeeds which is particularly important during the early stages of fish and shrimp development when ensuring feed acceptance is crucial for the survival and growth of larvae (Tocher et al., 2008). Similar to phospholipids, LPLs also contribute to cell membrane formation and support the growth and survival of larvae in aquaculture hatcheries (Ukwela et al., 2024). LPLs also improve the digestive process and nutrient absorption including dietary lipids in the intestines of aquatic organisms (Che et al., 2023), leading to improved nutrient utilization, higher growth rates and increased feed efficiency (Liu et al., 2020; Adhami et al., 2021; Ibarz et al., 2023; Bao et al., 2024; Wang et al., 2024) and modulation of the fatty acid composition of fish and shrimp tissues (Xu et al., 2022, Li et al., 2022a, Jafari et al., 2024, Xu et al., 2012, Khan et al., 2018, Song et al., 2024). Furthermore, LPLs exhibit antimicrobial properties, potentially contributing to the prevention of bacterial infections in aquaculture systems. By disrupting the cell membranes of certain bacteria, LPLs can help control the spread of pathogens in aquatic environments (Munsch-Alatossava et al., 2018; Alves et al., 2020; Kim et al., 2020). Finally, LPLs have been investigated for their potential role in enhancing the immune response of aquatic organisms (Elsayed Sallam et al., 2024; Limwachirakhom et al., 2025; Taghavizadeh et al., 2020). By modulating immune functions, they may contribute to increased resistance against diseases in fish and shrimp (Taghavizadeh et al., 2020, Weng et al., 2022, Marques et al., 2022, Elsayed Sallam et al., 2024, Wang et al., 2024). It is important to note that the specific effects of LPLs can vary depending on the species of aquatic organisms, the stage of development, and the overall nutritional composition of the diet. Research in this area is ongoing, and the application of LPLs in aquaculture remains an area of continued interest and exploration.

The impact of dietary butyric acid and butyrate on aquatic animals has been extensively studied (Abdel-Latif et al., 2020). The inclusion of butyric acid, butyrate or their protected forms, enhances weight gain, reduces the feed conversion ratio, and improves intestinal immune function, epithelial barrier function and gut permeability (Liu et al., 2014; Chen et al., 2025). Dietary butyrate can upregulate genes involved in intestinal cell proliferation and nutrient absorption while mitigating the negative effects of high soybean meal inclusion in the diet (Zhang et al., 2020, Ullah et al., 2025). It can alleviate enteropathy in fish fed a high soybean meal diet, increase the absorption surface and reduce infiltration of mixed leucocytes into the lamina propria (Liu et al., 2019).

Glyceryl mono-oleate is an emulsifier used in animal nutrition to improve nutrient absorption, gut health and performance and is a mild antimicrobial with anti-inflammatory effects (Xuanni, 2024). It has been shown to improve growth, gut morphology and microbiota, lipid utilization and fat-soluble vitamins absorption crucial for immune function, bone health and growth of fish and shrimp (Ullah et al., 2025; Xu et al., 2024).

European sea bass (*Dicentrarchus labrax*) is a key species in Mediterranean aquaculture, accounting for 13% of the total economic value of European aquaculture production (Zoli et al., 2024). Given its importance, it is essential to find ways to reduce the percentage of fish oil in the diet while maximising nutrient utilization and growth. In the current study, we evaluated the impact of adding Aquatria™ LQ to a lipid-reduced diet on growth performance, feed utilization, lipase and antioxidant enzyme activity, liver and intestinal morphology, and immune function in European sea bass. The liquid additive combines lysophospholipids, glyceryl mono-oleate, a synthetic emulsifier and a butyrate source. Aquatria™ LQ has been tested in Largemouth bass (*Micropterus salmoides*) diets, where supplementation of 0.1% alleviated liver lipid accumulation and fibrosis caused by high soybean meal levels (Cao et al., 2024). In European sea bass, dietary phospholipids have been evaluated for larval development, with inclusion levels ranging from 1.1% to 4.8% and up to 11.6% (Gisbert et al., 2005; Cahu et al., 2003). Sodium butyrate has also been investigated in this species, both for its effects on immune responses at dietary levels of 0.2% and 0.5% (Terova et al., 2016, Fontinha et al., 2025) and for its role in mitigating the impacts of high soybean meal diets at an inclusion level of 0.2% (Rimoldi et al., 2016). Finaly, an emulsifier containing soy lecithin and LPLs has been tested in combination with terrestrial animal fat sources (Marques et al., 2022). To the best of our knowledge this is the first time that Aquatria™ LQ has been evaluated for its effects in a low-fat diet for European sea bass.

2. Materials and methods

2.1. Experimental diets and design

Five diets were evaluated through an in vivo trial. The experimental diets were formulated as follows; Diet 19/0: Positive control high-fat commercial formula, Diet 19/0.05: Diet 19/0 with the addition of 0.05% Aquatria™ LQ, Diet 16/0: Low-fat commercial formula, Diet 16/0.025: Diet 16/0 with the addition of 0.025% Aquatria™ LQ, Diet 16/0.05: Diet 16/0 with the addition of 0.05% Aquatria™ LQ (Table 1). The experimental diets were formulated to be isonitrogenous (~44% crude protein), with varying total lipid and energy content. Micronutrients (essential amino acids, phosphorus, essential vitamins and minerals) were balanced across the experimental diets (Table 1). The diets were produced by extrusion at the Applied Fish Nutrition Laboratory of the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC) at the Hellenic Centre for Marine Research (HCMR, Athens, Greece) using an experimental twin-screw extruder (model EV025A107FAA, CLEXTRAL). Fish oil was incorporated via vacuum coating (DINISSEN). The study design followed the principle of the 3Rs (Factorial Trial design/Mixture design) for animal research, aiming to minimize the number of animals used. The trial was conducted at the IMBBC facilities at HCMR (Crete, Greece) and was authorised by the ethics committee of the Region of Crete, Greece (License No 255340).

Twenty-five sea bass individuals, with an initial mean weight of 18.0 ± 1.9 g were distributed in 15 tanks of 250 L capacity each (375 total

Table 1
Diet formulation and nutrient composition of the experimental diets (as fed basis, %).

Raw materials	Diet 19/0	Diet 19/ 0.05	Diet 16/0	Diet 16/ 0.025	Diet 16/ 0.05
Fish meal 67	20.00	20.00	20.00	20.00	20.00
Blood meal	7.00	7.00	7.00	7.00	7.00
Poultry byproduct meal	12.00	12.00	12.00	12.00	12.00
Wheat meal	19.60	19.55	22.60	22.58	22.55
Corn gluten	12.00	12.00	12.00	12.00	12.00
Soybean meal 47	6.00	6.00	6.00	6.00	6.00
Sunflower meal	8.00	8.00	8.00	8.00	8.00
Salmon Oil	14.00	14.00	11.00	11.00	11.00
Vitamin premix[1]	0.20	0.20	0.20	0.20	0.20
Vit C – 35	0.10	0.10	0.10	0.10	0.10
Monocalcium Phosphate	0.50	0.50	0.50	0.50	0.50
Taurine	0.05	0.05	0.05	0.05	0.05
Lysine	0.30	0.30	0.30	0.30	0.30
Methionine	0.15	0.15	0.15	0.15	0.15
Mineral pack[2]	0.10	0.10	0.10	0.10	0.10
Aquatria		0.05		0.025	0.05
Nutrients	Diet 19/0	Diet 19/ 0.05	Diet 16/0	Diet 16/ 0.025	Diet 16/ 0.05
Moisture %	8.27	8.89	9.14	8.70	8.93
Protein %	44.47	45.10	44.37	45.10	45.12
Fat %	19.5	19.40	15.80	16.02	15.72
Ash %	7.24	7.33	7.40	7.30	7.34
Fiber %	2.67	2.71	2.80	2.76	2.78
Starch	14.67	14.82	16.89	16.66	16.72
Gross Energy (kj/g)	21.00	20.98	20.17	20.27	20.20
EPA %	0.61	0.60	0.59	0.59	0.58
DHA %	0.67	0.64	0.61	0.64	0.64
Lysine %	2.72	2.83	2.71	2.76	2.65
Methionine %	1.08	1.12	1.08	1.10	1.05
Calcium %	1.20	1.25	1.19	1.21	1.16
Phosphorus %	1.11	1.16	1.11	1.14	1.09

[1] 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.

[2] Per kilogram of mineral premix: 28 g Fe, 14 g Mn, 2.4 g I, 2.8 g Cu, 24 g Zn.

fish). The diets were tested in triplicate groups. Fish were fed ad libitum, twice per day, for 13 weeks in an open flow system supplied with borehole sea water. Water parameters were controlled and monitored daily. The temperature was maintained between 19 and 21 °C, oxygen levels were kept at 96% and feed consumed (g) was recorded daily. Fish were weighed individually at the beginning, mid- and end of the experimental trial under anaesthesia with clove oil (1:10, clove oil: ethanol-5 ppm). At the end of the 91-day growth period, samples were collected for fatty acid profiles, immune indices, enzyme activities and gut histology. Eight fish per tank were sampled for the aforementioned analyses. Growth and feed utilization parameters, as well as somatometric indices were calculated using the following equations:

Specific growth rate (SGR) %: 100 × [lnWf-lnWi]/rearing days.

Where Wf is the final weight and Wi is the initial weight.

Feed conversion ratio (FCR): total feed intake /(Wf-Wi).

Daily feed intake: DFI (%) = 100 × F: daily feed intake per fish (g) /W: the mean weight per fish in each tank (g).

F represents the average daily feed intake per fish (g), while W corresponds to the mean weight per fish in each tank, determined as the average of the initial and final weights (g).

Feed intake: FI (g) = feed consumed (g) total period / fish.

Hepatosomatic index: HIS (%) = (liver weight/fish weight) × 100.

Liposomatic index: LSI (%) = fat weight/fish weight × 100.

Protein efficiency ratio: PER (%) = Wf-Wi / protein consumed × 100.

The proximate composition of the diets and fish fillets was determined according to AOAC (2005); The determination of dry matter (DM) was conducted by drying pre-weighed samples in porcelain cups at 104 °C for 24 h, following Method 950.46. Ash content was quantified through incineration at 500 °C for 12 h, based on Method 920.153. Crude protein content (calculated as Nx6.25) was analyzed using the Kjeldahl method (Method 988.05). The total fat content in extruded feeds was assessed via acid hydrolysis with HCl, followed by ether extraction using a Soxhlet apparatus (SOXTEC SYSTEM HT, 1043 Extraction Unit, Foss Tecator) in compliance with ISO Method 6492:1999 for animal feeding stuffs. The fatty acid profile in fish fillet was determined according to Alexi et al. (2019).

2.2. Intestinal lipase activity

At the end of the experiment, three fish per tank (from the total eight sampled) were weighed and the digestive track was dissected for determination of the lipase activity. Each tissue was cleaned with phosphate buffered saline (PBS), pH 7.4, to remove red blood cells and clots. Each sample was homogenised on ice with cold PBS buffer (1× phosphate buffered saline with protease inhibitors) at a 1/7 (*w/v*) ratio. The samples were centrifuged at 10000 x*g* for 10 min at 4 °C. The supernatant was stored at −80 °C until assayed. Samples were stable for at least 1 month. Analysis was performed using a Lipase Activity Assay Kit (Cayman, USA) based on a fluorescence-based method. Briefly, lipase hydrolyses arachidonoyl-1-thioglycerol to arachidonic acid and thioglycerol. Thioglycerol reacts with the thiol fluorometric detector to give a highly fluorescent product. A standard curve with a known concentration of thioglycerol was used and samples were diluted 1:20 with 50 mM sodium phosphate, pH 7.2. Fluorescence was read every 30 s for 15 min at 37 °C using an excitation wavelength of 380 nm and an emission wavelength of 520 nm on a FLUOstar OMEGA microplate reader (BMG Labtech, Germany).

2.3. Liver anti-oxidative enzymes

At the end of the experiment, three fish per tank were weighed and the livers were dissected for the determination of catalase (CAT) and superoxide dismutase (SOD) activity. Each tissue was cleaned with phosphate buffered saline (PBS), pH 7.4, to remove red blood cells and clots.

For catalase (CAT) determination, each liver sample was homogenised on ice with cold buffer (50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA) at a 1/7 (*w/v*) ratio. Samples were centrifuged at 10000 x*g* for 15 min at 4 °C. The supernatant was stored at −80 °C until analysis. The samples were stable for at least 1 month. Analysis was performed using a CAT Assay Kit (Cayman, USA). The method is based on the reaction of the enzyme with methanol in the presence of an optimum concentration of H_2O_2. The formaldehyde is measured colorimetrically using 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as a chromogen. Purpald specifically forms a bicyclic heterocycle with aldehydes, which turns from colourless to purple upon oxidation. A standard curve with a known concentration of formaldehyde was used to determine the concentration of formaldehyde in the samples. A positive control (bovine liver CAT) was used in the assay. Samples were diluted to a concentration that resulted in an activity between 2 and 35 nmol/min/ml in the well. The activity of CAT was measured at 540 nm using a microplate reader (Fluostar omega, BMG Labtech, Germany).

For superoxide dismutase (SOD) determination, each liver sample was homogenised on ice with cold 20 mM HEPES buffer, pH 7.2, containing 1 mM EGTA, 210 mM mannitol and 70 mM sucrose in a 1/7 (*w/v*) ratio. The samples were centrifuged at 1500 x*g* for 5 min at 4 °C. The supernatant was stored at −80 °C until analysis. The samples were stable for at least 1 month. Analysis was performed using a SOD Assay kit (Cayman, USA). The method uses tetrazolium salt to detect superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme required to show 50% dismutation of the superoxide radical, measured as the change in

absorbance per minute at 25 °C and pH 8.0. The SOD assay measures all three types of SOD (Cu/Zn, Mn and FeSOD). The standard curve was used to determine the activity of SOD in the samples. Samples were diluted to a concentration giving an activity of 0.005–0.05 units/mL in the well. The activity of SOD was measured at 450 nm using a microplate reader (Fluostar omega, BMG Labtech, Germany).

2.4. Immunology

At the end of the growth trial, unheparinised blood was collected by caudal vein puncture and kept overnight at 4 °C. After clotting, blood samples were centrifuged at 10000 *g* for 10 min and sera kept at −80 °C until immunology analyses were performed. The serum lysozyme activity was assessed as described by Kokou et al., 2012 but miniaturized. In brief, 5 μl of serum or Hen Egg White Lysozyme (HEWL) standard (0–3000 u/ml) were placed in triplicate wells of a transparent 384 wells microplate and incubated with 80 μl of *Micrococcus luteus* at 0.5 mg/ml in 0.1 sodium phosphate buffer pH 5.8 and the kinetic decrease of OD was followed every minute for 10 min. The serum complements antibacterial activity and the nitric oxide concentration were assessed according to Henry et al., 2022. The myeloperoxidase activity was determined as described by Kokou et al. (2012) but adapted for a 384 wells microplate. In brief, 10 μl of serum diluted with 30 μl of Hank's Buffered Saline Solution (HBSS) without Ca^{2+} or Mg^{2+} were incubated for 10 min at RT with 30 μl of 2.5 mM 3,3′,5,5′-tetramethylbenzidine hydrochloride (TMB, Sigma) and 5 mM hydrogen peroxide. The reaction was stopped by the addition of 30 μl of 1 N sulfuric acid and OD was read at 450 nm in a photometer. The alkaline phosphatase activity was assessed using *p*-nitrophenyl phosphate as substrate (Guardiola et al., 2014) miniaturized to be used in 384 well microplates. In brief, 5 μl of serum samples diluted with 75 μl of 100 mM ammonium bicarbonate buffer with 1 mM $MgCl_2$, pH 7.8 were incubated in a 384 well microplate with 20 μl of 8 mM p-nitrophenol phosphate (Sigma) 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). OD data were converted in units/ml serum using 18.75 mM of absorptivity of p-nitrophenol. The anti-protease activity was determined using azocasein as substrate and trypsin as standard (Henry and Fountoulaki, 2014). All immunological parameters were measured in triplicate wells of 384-wells transparent microplate (or white microplate concerning the complement antibacterial activity against the luminescent strain of *E.coli*) using a spectrophoto-lumino-fluoro-meter (Fluostar omega, BMG Labtech, Germany). (Ernst and Zor, 2010).

2.5. Histology

Liver samples ($n = 3$) were fixed in 4% neutral-buffered formalin, embedded in paraffin, cut in sections (5 μm-thick) in a Leica 2055-Autocut microtome (Leica Instruments GmbH, Nussloch, Germany) and stained with H & E (hematoxylin and eosin) for optical examination. Micrographs of each individual section were taken at a final magnification of 10× and 20× using an Olympus Cx41 microscope and an Olympus XC50 camera. Liver samples were evaluated for general histomorphology, giving particular attention to the cytoplasm vacuolation of hepatocytes and any signs of inflammation. Scores were attributed as follows: 1 — normal histomorphology; 2 — moderate alterations; 3 — severe alterations. (steatosis) (Kokou et al., 2019).

The gut sections (n = 3) were evaluated according to a semi-quantitative method. The applied criteria in the present study were based on criteria used to assess the degree of SBM-induced enteritis in the distal intestine of Atlantic salmon (Urán et al., 2008), and gilthead sea bream (Kokou et al., 2017). The chosen criteria were the following: 1. the appearance and length of the mucosal folds (MF); 2. the degree of infiltration and abundance of eosinophils (EP) into the lamina propria; 3. the degree of widening of the lamina propria (LP); 4. the degree of widening of the submucosa (SM); and 5. the number of lipidic vacuoles (LV) within the enterocytes. Each of these parameters was scored on a scale from 1 to 3. Higher scores represent more severe morphological alterations. All histological slides were evaluated by a single trained observer to ensure consistency in scoring. The same person performed all observations under identical conditions.

2.6. Statistics

Normality and homogeneity of variances were checked using the Kolmogorov-Smirnov and Levene tests respectively. All data were subjected to One-Way ANOVA. When significant differences among groups were identified, multiple comparisons among means were performed using the Tukey tests. Alternatively, when data did not comply with the normality assumption, the non-parametric Kruskal-Wallis test was followed by the Tamhane *t*-test. Values with common letters were statistically similar. Treatment effects were considered at a significance level of $P < 0.05$. Histomorphology data were analyzed using parametric tests (one-way ANOVA followed by Tukey's post hoc test), as the data satisfied assumptions of normality and homogeneity of variances.

3. Results

3.1. Growth and feed utilization

The results showed statistically significant differences ($P < 0.05$) among specific experimental diets on growth and feed utilization indices (Table 2). Specifically, the final weight for Diet 16/0.025 and Diet 16/0.05 was significantly higher ($P < 0.05$) compared to Diet 16/0. These values were also higher than those for Diet 19/0 and Diet 19/0.05, although no significant difference was observed. Similar trends were determined for SGR for the same diets ($P < 0.05$). FCR and feed consumption was similar for all experimental diets with no significant differences. A statistically significant improvement in the PER was observed in fish fed Diet 16/0.025 relative to the corresponding control group. No statistically significant differences were determined for both HSI and VSI indicating similar fat accumulation in liver and visceral across all experimental groups.

3.2. Feeds and fillets fatty acids profile

The fatty acid profile of the experimental diets and fish fillets are presented in Table 3. Saturated fatty acids were comparable among

Table 2
Key performance and somatometric indices of European sea bass fed the experimental diets.

	Diet 19/0	Diet 19/0.05	Diet 16/0	Diet 16/0.025	Diet 16/0.05
Final Weight (g)[1]	49.62 ± 1.19^{ab}	48.82 ± 0.86^{ab}	48.00 ± 0.60^{b}	51.79 ± 1.71^{a}	51.17 ± 1.11^{a}
SGR (%/day)[1]	1.12 ± 0.03^{ab}	1.10 ± 0.02^{ab}	1.08 ± 0.03^{a}	1.16 ± 0.02^{b}	1.15 ± 0.02^{b}
Daily feed intake[1]	2.32 ± 0.46	2.59 ± 0.08	2.66 ± 0.01	2.57 ± 0.04	2.63 ± 0.06
FCR[1]	1.48 ± 0.14	1.51 ± 0.07	1.56 ± 0.04	1.41 ± 0.05	1.41 ± 0.08
PER (%)[1]	1.58 ± 0.10^{ab}	1.55 ± 0.11^{ab}	1.43 ± 0.06^{a}	1.61 ± 0.05^{b}	1.56 ± 0.06^{ab}
HSI (%)[2]	1.84 ± 0.16	1.95 ± 0.33	1.96 ± 0.28	1.80 ± 0.29	2.11 ± 0.62
LSI (%)[2]	11.01 ± 1.55	10.46 ± 1.16	10.01 ± 0.81	10.24 ± 1.35	10.67 ± 1.59

Data are presented as means ± standard deviation of the means. Different letters in the same line denote statistically significant differences ($P < 0.05$). SGR = specific growth rate, FCR = feed conversion ratio, PER = Protein efficiency ratio, HSI = hepatosomatic index, LSI = liposomatic index.

[1] The data are the means of 3 replicates of 25 fish in each group ($n = 3$).

[2] The data are the means of 3 replicates of 5 fish in each group (n = 3).

Table 3
Fatty acid profile of experimental diets and fish fillet (% of total fatty acid).

	Diet 19/0	Diet 19/0.05	Diet 16/0	Diet 16/0.025	Diet 16/0.05
	Feeds (% of FA)				
Saturated	19.68	19.56	19.88	19.86	19.86
Monounsaturated	49.08	49.64	47.05	46.95	47.38
∑n9	42.02	42.51	40.20	40.08	40.45
∑n6	17.33	17.26	18.37	18.32	17.98
∑n3	13.09	12.71	13.90	14.08	13.97
ARA	0.39	0.33	0.51	0.47	0.45
EPA	3.19	3.14	3.70	3.69	3.65
DHA	3.52	3.35	3.81	3.98	3.99
HUFA	6.71	6.49	7.51	7.67	7.64
n3/n6	0.76	0.74	0.76	0.77	0.78
	Fish fillet (% of FA)				
Saturated[1]	23.71 ± 0.57[b]	23.84 ± 0.36[b]	24.57 ± 0.20[ab]	24.80 ± 0.34[a]	24.77 ± 0.26[a]
Monounsaturated[1]	48.34 ± 0.88	48.31 ± 0.63	47.59 ± 0.64	47.62 ± 0.52	47.89 ± 0.33
∑n9[1]	41.35 ± 0.61	41.21 ± 0.43	40.55 ± 0.57	40.61 ± 0.44	40.74 ± 0.28
∑n6[1]	13.20 ± 0.43	13.24 ± 0.25	13.25 ± 0.33	13.14 ± 0.38	12.92 ± 0.26
∑n3[1]	13.91 ± 0.98	13.77 ± 0.61	13.76 ± 0.49	13.62 ± 0.49	13.60 ± 0.30
ARA[1]	0.50 ± 0.06	0.53 ± 0.03	0.53 ± 0.04	0.52 ± 0.04	0.53 ± 0.03
EPA[1]	3.53 ± 0.20	3.53 ± 0.12	3.61 ± 0.14	3.62 ± 0.11	3.59 ± 0.10
DHA[1]	5.72 ± 0.60	5.64 ± 0.40	5.71 ± 0.28	5.66 ± 0.28	5.61 ± 0.17
HUFA[1]	9.25 ± 0.79	9.17 ± 0.52	9.32 ± 0.41	9.28 ± 0.40	9.20 ± 0.25
n3/n6[1]	1.05 ± 0.04	1.04 ± 0.03	1.04 ± 0.02	1.04 ± 0.02	1.05 ± 0.01

Data are presented as means ± standard deviation of the means. Different letters in the same line denote statistically significant differences ($P < 0.05$).
[1] The data are the means of 3 replicates of 8 fish in each group (n = 3).

diets, whereas monounsaturated fatty acids represented the predominant fraction. Total n-9 fatty acids ranged from 40.1 to 42.5%, n-6 from 17.3 to 18.4%, and n-3 from 12.7 to 14.1%. ARA, EPA and DHA contents ranged from 0.33 to 0.51%, 3.14–3.70% and 3.35–3.99%, respectively. The n-3/n-6 ratio remained consistent across all diets.

In fish fillets saturated fatty acids ranged from 23.7 to 24.8% of total fatty acids, with significantly higher values observed in fish fed Diet 16/0.025 and Diet 16/0.05. Monounsaturated fatty acids constituted the major fraction of fillet lipids. Total n-9, n-6 and n-3 fatty acids did not differ significantly among dietary treatments, while EPA and DHA levels remained comparable across experimental groups. Consequently, the n-3/n-6 ratio was stable in fish fillets among all groups.

3.3. Intestinal lipase activity and anti-oxidative enzymes

Activity of intestinal lipase was significantly higher for the groups fed Diet 19/0.05 and Diet 16/0.05 ($P < 0.05$) compared to Diet 19/0 (Table 4). Furthermore, groups fed Diet 16/0.025 and Diet 16/0.05 showed similar activity with group fed with the Diet 19/0.05.

Superoxide dismutase (SOD) and catalase (CAT) activities were measured as indicators of antioxidant status and were found to be similar among all experimental groups, with no statistically significant differences detected between dietary treatments (Table 4).

Table 4
Intestinal lipase activity (nmol/min/ml) and liver antioxidative enzymes. CAT (nmol/min/ml), SOD (U/ml).

	Diet 19/0	Diet 19/0.05	Diet 16/0	Diet 16/0.025	Diet 16/0.05
Lipase	190.1 ± 9.4[a]	284.0 ± 60.9[b]	144.0 ± 61.9[a]	210.0 ± 26.8[ab]	259.6 ± 34.5[b]
CAT	3845 ± 351	4268 ± 405	3210 ± 336	5182 ± 402	4569 ± 339
SOD	0.256 ± 0.060	0.302 ± 0.041	0.256 ± 0.068	0.300 ± 0.065	0.294 ± 0.014

Data are presented as means ± standard deviation of the means. Different letters in the same line denote statistically significant differences ($P < 0.05$). The data are the means of 3 replicates of 5 fish in each group ($n = 15$).

3.4. Immunology

No significant differences were detected among dietary treatments for lysozyme activity, myeloperoxidase activity, alkaline phosphatase activity, or trypsin inhibition, indicating that these humoral immune parameters were not influenced by dietary treatment. *E. coli* growth inhibition showed statistically significant differences, with fish fed Diet 16/0 exhibiting the highest inhibition percentage, while Diet 19/0.05 recorded the lowest. Overall, the tested dietary treatments did not induce major alterations in innate humoral immune responses of juvenile European sea bass under the conditions of the present study (Table 5).

3.5. Histology

At the end of the growth period liver morphology of the different groups showed specific differences (Table 6a). The effects of Aquatria™ LQ (Diet 19/0.05, Diet 16/0.025 and Diet 16/0.05) were evident in the attenuation of liver lipid deposition, and nucleus displacement. Diet 19/0 and Diet 16/0 showed the highest score, 2.3 and 2.1 respectively with moderate and severe alterations in some sections, while Diet 19/0.05, Diet 16/0.025 and Diet 16/0.05 containing the Aquatria™ LQ showed scores below two (1.6, 1.6 and 1.3 respectively). As a general observation liver sections of these diets showed a normal organization of the hepatic cell and blood capillaries, optically empty cytoplasmatic vacuoles in most of the sections, normal nuclei shape and position and no signs of inflammation. In Diet 19/0 and Diet 16/0, some fat vacuoles of variable size were observed in many sections, with some large vacuoles (Fig. 1A and C black arrow) present within some hepatocytes and relatively smaller vacuoles in other hepatocytes. Due to the level of vacuolation in some hepatocytes, there was some distortion.

The overall histological appearance in the distal intestine of sea bass showed differences between dietary groups, such as the lamina propria

Table 5
Immunological parameters in the sera of treated fish.

	Diet 19/0	Diet 19/0.05	Diet 16/0	Diet 16/0.025	Diet 16/0.05
Lysozyme antibacterial activity (HEWL equivalent Units/ml)	2277.74 ± 393.78	2188.55 ± 406.42	1581.14 ± 121.82	1622.00 ± 298.75	2152.59 ± 387.94
E. coli growth inhibition (%)	70 ± 0.89[ab]	69.44 ± 0.74[a]	72.64 ± 0.65[b]	71.63 ± 0.59[ab]	71.75 ± 0.84[ab]
Myeloperoxidase activity (Units/ml)	0.36 ± 0.06	0.38 ± 0.07	0.42 ± 0.07	0.23 ± 0.03	0.31 ± 0.06
Nitric Oxide concentration (μM)	58.63 ± 12.23	74.60 ± 16.83	69.43 ± 15.86	35.26 ± 6.29	50.79 ± 14.15
Alkaline Phosphatase activity (Units/ml)	245.04 ± 18.93	210.90 ± 14.03	213.74 ± 13.27	218.04 ± 11.90	199.63 ± 15.01
Trypsin inhibition (%)	98.41 ± 0.13	98.41 ± 0.13	98.56 ± 0.10	98.73 ± 0.04	98.65 ± 0.06

Data are presented as means ± standard error of the mean. Different letters in the same line denote statistically significant differences ($P < 0.05$). The data are the means of 3 replicates of 8 fish in each group ($n = 24$).

Table 6a
Scoring system to evaluate histomorphology of liver tissue.

	Diet 19/0			Diet 19/0.05			Diet 16/0			Diet 16/0.025			Diet 16/0.05		
Tanks	A3	A12	B1	A1	A5	B3	A2	A8	B2	A6	A7	A10	A4	A9	A11
	2	3	2	2	2	1	2	2	2	2	2	1	2	1	1
	2	3	2	1	2	1	1	2	2	2	1	1	2	1	1
	2	2	3	2	1	2	2	3	3	2	2	1	1	1	2
Avg/tank	2	2.7	2.3	1.7	1.7	1.3	1.7	2.3	2.3	2	1.7	1	1.7	1	1.3
Avg/diet			2.3c			1.6ab			2.1bc			1.6ab			1.3a

*1 normal morphology = Lightly granular, small and distinct nuclei, 2 moderate alterations = Nuclei with abundant dark granules and cytoplasm homogeneous, vacuolized only to a very limited degree, 3 severe alterations = pyknotic nuclei, large lipidic vacuole within the cytoplasm, displacement of the nucleus. Data are presented as mean scores ± standard deviation of the means. Different letters in the same column denote statistically significant differences ($P < 0.05$). The data are the means of 3 replicates of 3 fish in each group ($n = 9$).

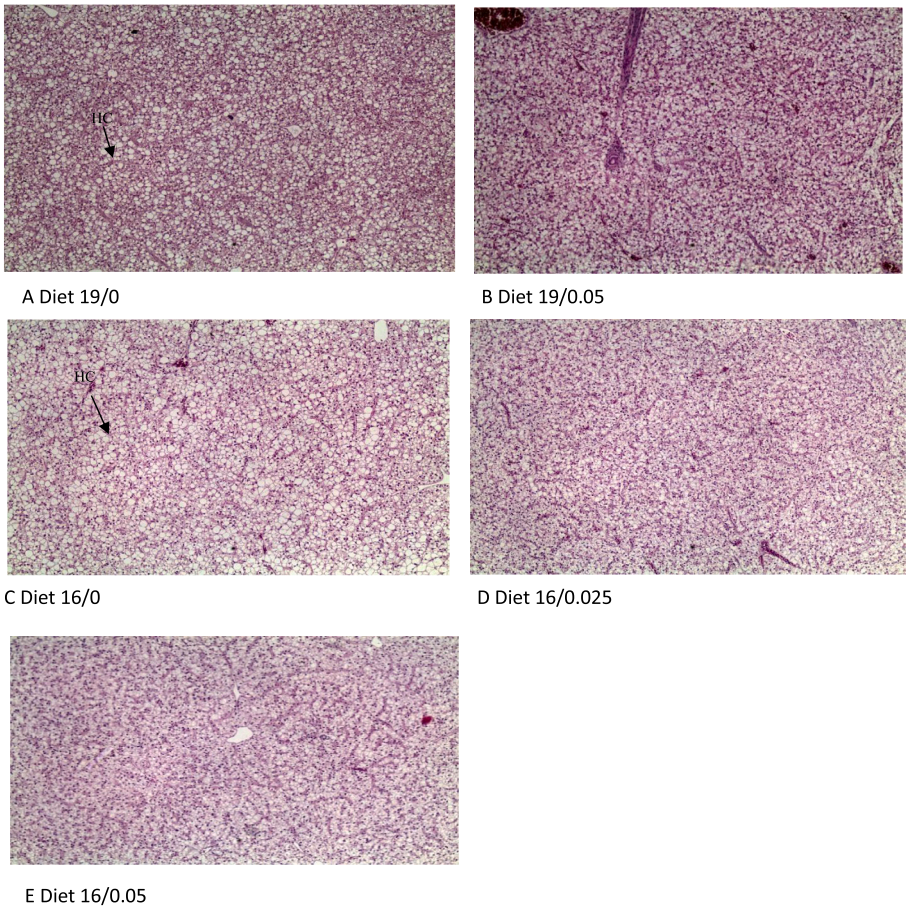


Fig. 1. Histomorphological features of liver tissue of European sea bass fed on different experimental diets 10× (A: Diet 19/0, B: Diet 19/0.05, C: Diet 16/0 D: Diet 16/0.025 and E: Diet 16/0.05) HC: hepatocytes.

width, the lamina propria leucocytes infiltration, lipid vacuoles although not significant (Table 6b, Fig. 2). Diet 19/0.05, Diet 16/0.025 and Diet 16/0.05 exhibited the lowest score 1.2, 1.4 and 1.3 respectively. Enterocyte exhibited reduced to absent vacuolization and apical displacement of nuclei in all diets. The lamina propria tended to be thicker in Diet 19/0 while leukocyte infiltration was higher in Diet 16/0. Submucosa scoring for width and cellularity was highest in Diet 19/0. A mixed population of leucocytes and eosinophilic granulocytes was observed in the intestinal mucosa, with no signs of inflammation and differences were not profound between experimental groups.

4. Discussion

Aquatria™ LQ supplementation overall showed positive effects on fish growth parameters. The group fed the positive control diet (Diet 19/0, high-fat) exhibited no significant FCR values compared to Diet 16/0. Higher-lipid diets provide more calories per gram, meaning fish need less protein for energy. This allows them to convert protein more efficiently for growth and reduces FCR. This effect has been observed in European sea bass studies (Peres and Oliva-Teles, 1999, Cardoso et al., 2023). Lower FCR was observed for supplemented Diets 16/0.025 and 16/0.05 compared to the low fat control diet (16/0), demonstrating the possible positive impact of the incorporation of Aquatria™ LQ on feed utilization. This is likely due to the enhanced emulsification and digestion of lipids facilitated by LPLs and/or glyceryl mono-oleate. LPLs can improve micelle formation in the intestinal lumen, which leads to increased lipid absorption and more efficient energy utilization (Kinh et al., 2022; Ibarz et al., 2023). Additionally, Diets 16/0.025 and 16/

Table 6b
Scoring system to evaluate histomorphology of posterior intestine.

	Diet 19/0	Diet 19/0.05	Diet 16/0	Diet 16/0.025	Diet 16/0.05
MF	1.3	1	1	1	1
	1	1	1.3	1	1
	1	1	1.3	1.3	1
Avg/diet	**1.1**	**1**	**1.2**	**1.1**	**1**
EP	2	1	1.7	1	1.7
	1	1	1.7	2	1.3
	1.3	1.7	2.3	2	1.7
Avg/diet	**1.4**	**1.2**	**1.9**	**1.7**	**1.6**
LP	1.7	1	1	1	1.7
	1.5	1.3	1.3	1.7	1
	1.7	1.7	1.7	1.7	1.7
Avg/diet	**1.6**	**1.3**	**1.3**	**1.4**	**1.4**
SM	1.5	1	1	1	1.3
	1	1	1	1	1
	1.3	1	1	1	1
Avg/diet	**1.3**	**1**	**1**	**1**	**1.1**
LV	1.7	1.3	1.3	1.3	2
	1.5	1.3	2	2	1.3
	1.7	1.3	2.3	1.7	1.3
Avg/diet	**1.6**	**1.3**	**1.9**	**1.7**	**1.6**
Total score	**1.4**	**1.2**	**1.5**	**1.4**	**1.3**

MF: mucosal folds, EP: Eosinophils, LP: lamina propria, SM: submucosa, LV: lipid vacuoles. Data are presented as mean scores ± standard deviation of the means. Different letters in the same column denote statistically significant differences ($P < 0.05$). The data are the means of 3 replicates of 3 fish in each group ($n = 9$).

0.05 exhibited improved final weight and SGR values compared to the other experimental groups. Statistical analysis indicated significant differences when compared to Diet 16/0. These findings are consistent with the growth-enhancing and feed-utilization effects of LPLs reported in other aquaculture species, including rainbow trout (0.9% and 0.1–0.5%), largemouth bass (0.03–0.10%), Atlantic salmon (0.10%), and Pacific white shrimp *Litopenaeus vannamei* (0.10%) (Adhami et al., 2021, Li et al., 2022b, Xu et al., 2022, Che et al., 2023 Ibarz et al., 2023, Bao et al., 2024, Wang et al., 2024, Cao et al., 2024). Similarly, dietary butyrate has been reported to improve growth parameters in species such as large yellow croaker (*Larimichthys crocea*) (Xu et al., 2021), turbot (Yang et al., 2021), common carp (*Cyprinus carpio*) (Xie et al., 2021) and Pacific white shrimp (Liu et al., 2020). Butyrate is an important energy substrate for intestinal epithelial cells (enterocytes), playing a key role in maintaining gut integrity, enhancing nutrient absorption efficiency, and supporting more effective energy allocation toward growth (Salvi and Cowles, 2021).

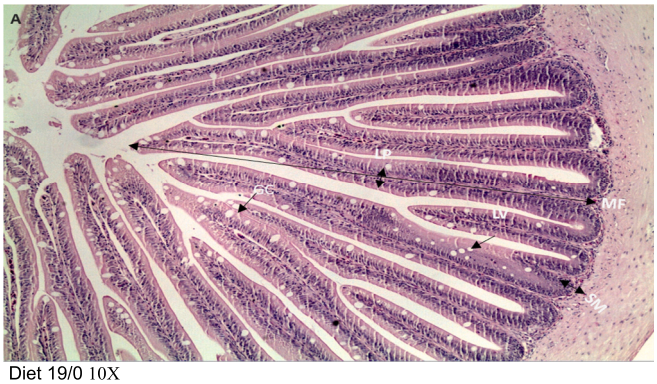
Daily feed intake for the low fat diets diet 16/0, 16/0.025 and 16/0.05 showed no significant differences despite the lower energy content of the latter diets compared to the higher fat content in Diet 19/0. European sea bass voluntarily reduce their feed intake as the energy density of their diet increases, especially when sufficient protein is present (Peres and Oliva-Teles, 2001, Boujard et al., 2004). Similar results have been obtained for gilthead sea bream (*Sparus aurata*) (Pelusio et al., 2021). Diet 19/0.05, which had a high fat content and the tested dietary supplement, also showed no significant differences in feed intake compared to Diet 19/0. This may be due to the appetite-stimulating effects of LPLs and/or butyrate. In mammals, butyrate influences gut hormone signaling, stimulating the secretion of peptides such as ghrelin and neuropeptide Y, which may increase feed intake (Romaní-Pérez et al., 2021, Leeuwendaal et al., 2021, Zhang et al., 2023). LPLs have been reported to increase appetite and feed consumption in pigs and poultry (Zhang et al., 2022b). In Largemouth bass (*Micropterus salmoides*) the addition of 0.1% LPLs to the diet resulted in a higher feeding rate than the control (Che et al., 2023). Similarly, sodium butyrate in fish diets has been shown to improve feed intake (Arciuch-Rutkowska et al., 2024). Thus, it is expected that the addition of Aquatria™ LQ can enhance feed consumption in some cases, although this effect was not observed with Diets 16/0.025 and 16/0.05 compared to their control (Diet 16/0).

Protein Efficiency Ratio (PER) values were found statistically similar for Diet 19/0 in comparison to Diet 16/0, despite the fact that an increase in dietary lipid levels was expected to result in a better protein-sparing effect (Arenas et al., 2021, Thirunavukkarasar et al., 2022). However, Diet 16/0.025 showed significant higher PER than the low-fat diet, providing evidence that the protein sparing effect can be achieved in low fat diets through supplementation with Aquatria™ LQ. Diet 16/0.05 yielded similar results to Diet 16/0.025, however, only Diet 16/0.025 showed a significant difference compared to Diet 16/0, suggesting that the optimal Aquatria™ LQ dose in low fat diets is 0.025%. The incorporation of Aquatria™ LQ did not affect visceral fat accumulation among the experimental groups, which aligns with findings from studies on the use of LPLs and dietary butyrate in Largemouth bass (*Micropterus salmoides*) diets (Che et al., 2023; Bao et al., 2024). The same was true of the LSI which showed no significant differences between the experimental diets.

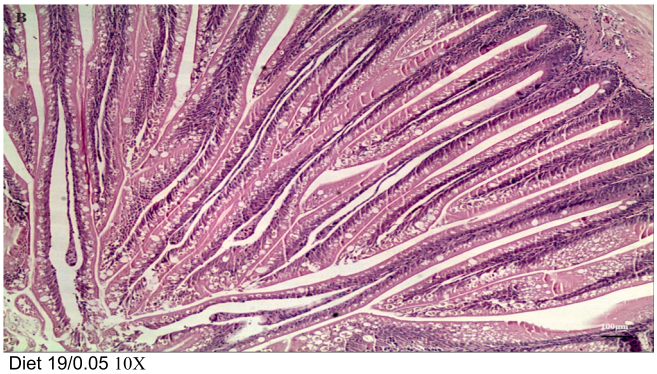
All sums of fatty acids were similar among the experimental groups, with the exception of saturated fatty acids, which were detected at significantly higher levels in fish fed Diet 16/0.025 and Diet 16/0.05. The overall similarity in total fatty acid classes suggests that reducing dietary salmon oil from 14% to 11%, as well as the inclusion of Aquatria, did not markedly influence total lipid deposition in the fillet. However, the significantly higher levels of saturated fatty acids detected in fish fed Diet 16/0.025 and Diet 16/0.05 may be attributed to the combined effect of reduced dietary lipid inclusion and the presence of Aquatria. Lysophospholipids and emulsifiers are known to enhance lipid emulsification, and digestion (Kim et al., 2018; Li et al., 2022a), which may enhance the intestinal absorption and subsequent deposition of saturated fatty acids. Moreover, this observation did not affect the overall balance of mono- and polyunsaturated fatty acids, nor the n-3/n-6 ratio, indicating that fillet fatty acid quality was maintained. In European sea bass diets containing mammalian and poultry fat sources, the inclusion of 0.01% of an emulsifier based on soy lecithin and lysophospholipids increased whole-body lipid and energy content, irrespective of fat source (Marques et al., 2022).

The inclusion of an emulsifier at 0.035% and 0.045% alongside dietary lipid concentrations ranging from 0.7% to 10% may be associated with improved digestion and/or absorption of dietary lipids (El-Sayed et al., 2021; Wangkahart et al., 2022). LPLs have been shown in several studies to enhance lipid metabolism in various fish species: in juvenile turbot when included at 0.1% and 0.25% in diets containing 15.8% and 15.4% fat, respectively (Xu et al., 2022), in Atlantic salmon when incorporated at 0.1% in diets with 28.8% total dietary fat (*Salmo salar*) (Ibarz et al., 2023) and in Pacific white shrimp (*Litopenaeus vannamei*) when added at 0.05% in diets containing 7% fat (Wang et al., 2024). Although no digestibility determination was performed in the current study, intestinal lipase activity can serve as an indicator of improved fat digestibility. Intestinal lipase activity increased in all treatments receiving Aquatria™ LQ at the 0.05% inclusion level, indicating a positive effect of the product on fat utilization under the specific experimental conditions. Similar findings of enhanced lipase activity have been observed on rainbow trout diets supplemented with LPLs (Adhami et al., 2021). An assumption can be made that Aquatria™ LQ facilitates lipid breakdown by increasing lipase secretion or activation. LPLs have the ability to increase the surface area of dietary fat droplets (Zhang et al., 2022a, Zhang et al., 2022b). This improves access for lipases, accelerating the hydrolysis and uptake of fatty acids. This effect leads potentially to improved growth parameters.

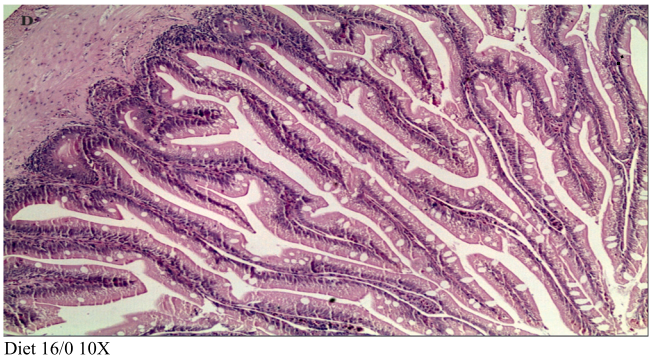
Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify them or repair the resulting damage. Antioxidant enzymes play a crucial role in neutralizing ROS and maintaining cellular homeostasis. In the intestines, antioxidant enzymes such as superoxide dismutase (SOD),



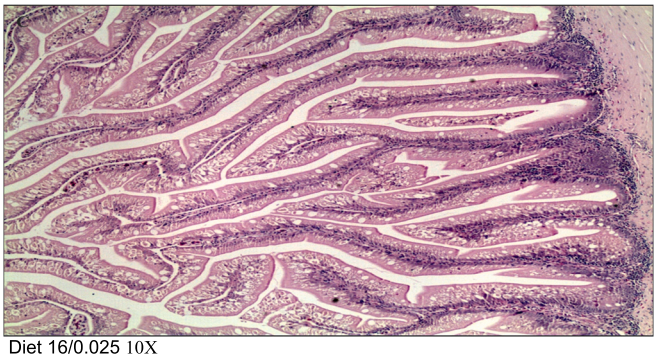
Diet 19/0 10X



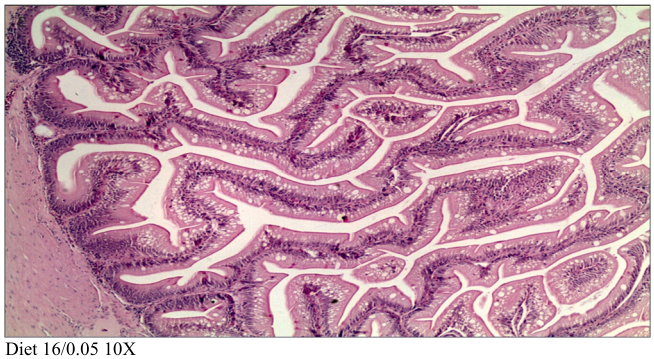
Diet 19/0.05 10X



Diet 16/0 10X



Diet 16/0.025 10X



Diet 16/0.05 10X

Fig. 2. Histomorphology of posterior intestine of European sea bass fed on different supplemented diets (A: Diet 19/0, B: Diet 19/0.05, C: Diet 16/0, D: Diet 16/0.025, E: Diet 16/0.05) MF = Mucosa fold, LP = lamina propria, SM = submucosa, GC = goblet cells, LV = Lipid vacuoles.

catalase, and glutathione peroxidase are involved in combating oxidative stress (Jomova et al., 2023). In the present study, liver CAT and SOD activity were similar with no statistical differences with the addition of Aquatria™ LQ in all dietary treatments compared to their respective controls. In contrast, enhanced antioxidant capacity has been reported with the addition of LPLs in shrimp diets (Wang et al., 2024). Furthermore, even though butyrate contains antioxidant properties, which can modulate the expression of antioxidant enzymes via the Nrf2 signaling pathway (Xing et al., 2016; Tang et al., 2022) that was not observed statistically in the present study. It has been shown that butyrate can indirectly reduce oxidative stress by improving nutrient metabolism and gut barrier integrity (Li et al., 2022a).

During the present study a number of immune indices were evaluated, however, only growth inhibition against *E. coli* demonstrated significant differences. In other studies, dietary supplementation of butyrate for 2 months has been shown to increase the lysozyme, complement activity, phagocytosis activities and/or immunoglobulin titers of several species, including grey mullet (*Liza ramada*), especially at 0.2% inclusion level (El-Sharkawy et al., 2023); crucian carp (*Carassius auratus*) at 0.0002% (Fang et al., 2021); and of Nile tilapia (*Oreochromis niloticus*), at 0.00015% (Abdel-Latif et al., 2021) or 0.15–0.2% (Dawood et al., 2020). Furthermore, 3 months of dietary butyrate supplementation significantly increased both lysozyme and bactericidal activities against *Aeromonas hydrophila* at a 0.5% inclusion level and respiratory burst activity at inclusion levels of 0.75–1% (Abd El-Naby et al., 2019). This immunostimulation was accompanied by potent protection against infection by this bacterium as shown by strongly increased survival after an i.p. challenge (Abd El-Naby et al., 2019). In European sea bass (*Dicentrarchus labrax*), feeding fry with 0.2 and 0.3% butyrate for 3 months increased lysozyme, myeloperoxidase, respiratory burst, phagocytic activities and immunoglobulin titers (Abdel-Mohsen et al., 2018). Regarding dietary supplementation with LPLs in aquafeeds, several studies have investigated their impacts on the immune system of fish and shrimp. One month of 0.06% LPLs supplementation did not affect the phenoloxidase or lysozyme activity of Pacific white shrimp *(Litopenaeus vannamei)* (Limwachirakhom et al., 2025). In fish, dietary supplementation of 0.2% LPLs for 2 months increased the lysozyme activity and complement C3 and C4 in rainbow trout (*Oncorhynchus mykiss*), while 0.3% LPLs significantly decreased lysozyme activity, highlighting the quadratic effect of dietary LPLs and the importance of finely adjusting the dietary inclusion level (Taghavizadeh et al., 2020). In red tilapia (*Oreochromis niloticus* x *O. mossambicus*), 0.3% LPLs increased lysozyme activity, while the hemolytic activity of complement was increased by 0.1–0.3% LPLs (Elsayed Sallam et al., 2024). This increase of lysozyme and complement activity was also obtained in LPLs-fed tilapia (El-Sayed et al., 2021). In the present trial, no statistically significant advantage could be underlined with the dietary supplementation of Aquatria™ LQ regarding immunological parameters, and no negative effects were observed in any of the immune parameters tested for Aquatria supplemented diets.

The liver is a sensitive organ, and its function is closely linked to the nutritional condition of the fish. High fat diets can lead to increased lipid accumulation in the fish liver, because high fat diets can overwhelm the fish's ability to process and store lipids (Naiel et al., 2023). Dietary LPLs have been shown to prevent abnormal fat accumulation in the fish liver (Zhu et al., 2025). In the present study, liver histomorphology was significantly improved by supplementing Aquatria™ LQ at the 0.05% inclusion level, resulting in reduced lipid accumulation within hepatocytes. This effect may result from the increased capacity for lipid oxidation and export induced by LPLs and butyrate, as well as improved mitochondrial function and reduced hepatic lipogenesis (Che et al., 2023, Li et al., 2022b). Butyrate is also known to upregulate genes involved in lipid metabolism and antioxidant defence due to its role as a histone deacetylase inhibitor (Canani et al., 2011, Terova et al., 2016). Bao et al. (2024) reported that in Largemouth bass, dietary LPLs supplementation significantly decreased total lipid in the liver compared to the control. Similar improvements in lipid metabolism with LPLs supplementation have been observed in Largemouth bass in several studies (Lu et al., 2022, Che et al., 2023, Cao et al., 2024). In Atlantic salmon, supplementation with LPLs led to improved lipid metabolism in the liver and enhanced overall hepatocyte function (Ibarz et al., 2023). In rainbow trout, replacing dietary fish oil with fat powder produced from sunflower oilseeds resulted in enlarged hepatocyte nuclei, but the addition of LPLs reduced nucleus size (Adhami et al., 2021). Overall, the positive effects of LPLs on liver histology and liver lipid metabolism are consistent across different fish species and varying supplementation levels. Improvements in intestinal health following LPL supplementation have been documented in largemouth bass (Bao et al., 2024) and turbot (Li et al., 2022a), whereas no significant effects were reported in channel catfish (*Ictalurus punctatus*) (Liu et al., 2019). Similarly, dietary butyrate has been shown to enhance both liver and intestinal histology in several fish species (Chen et al., 2023; Qi et al., 2025). Overall, the results of the present study demonstrated beneficial effects of Aquatria™ LQ supplementation on liver although no significant differences were identified for intestinal morphology in both high-fat and low-fat dietary treatments. Feeding sea bass for a longer period with diets supplemented with Aquatria™ is necessary to confirm possible improvements of the gut health.

5. Conclusions

In conclusion, the supplementation of Aquatria™ LQ in both high fat and low-fat diets of juvenile sea bass resulted in improvements of growth performance, feed utilization, lipase activity, liver lipid accumulation and antioxidant capacity. The results suggest that aqua feed mills can reformulate diets to reduce dietary marine oil by 3%, through supplementation with 0.025% of Aquatria™ LQ without negative effect on growth performance, feed utilization or health of juvenile sea bass under the given conditions and feed formulations.

CRediT authorship contribution statement

Katsoulis-Dimitriou Stefanos: Writing – review & editing, Writing – original draft, Data curation. **Vasilaki Antigoni:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Henry Morgane:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Fountoulaki Eleni:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Nikoloudaki Chrysanthi:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Mastoraki Maria:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Chronopoulos Petros:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Alcalde Elvira:** Supervision, Funding acquisition, Conceptualization. **Meynen Koen:** Writing – review & editing, Supervision, Conceptualization. **Mente Elena:** Writing – review & editing, Supervision, Resources. **Nengas Ioannis:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

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