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Synergistic effects of dried *Padina* sp. and prebiotic on growth, histology, and growth-related gene expression in European seabass (*Dicentrarchus labrax*)

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Abstract: This study evaluated the effects of feeding European seabass (*Dicentrarchus labrax*) that developed from the fingerling to juvenile stage a diet supplemented with a blend of *Padina* sp. and GroBiotic®-A over a 12-week period. A total of 480 fish (initial weight: 2.08 ± 0.06 g) were randomly allocated to four groups, each comprising three replicates. The fish were fed to satiation with diets containing varying proportions of *Padina* sp. and GroBiotic®-A: 0% *Padina* sp. and 0% GroBiotic®-A (0P0G), 1% *Padina* sp. and 1% GroBiotic®-A (1P1G), 2% *Padina* sp. and 1% GroBiotic®-A (2P1G), and 4% *Padina* sp. and 1% GroBiotic®-A (4P1G). The 4P1G group demonstrated significant improvements in growth performance parameters, including final weight, weight gain, and specific growth rate ($P < 0.05$). However, no significant differences were found in other parameters including feed conversion ratio, visceral somatic index, and survival rate ($P > 0.05$). Histological analysis of liver and intestinal tissues showed no pathological alterations ($P > 0.05$); more likely, only adaptive and non-pathological morphological changes such as moderate lipid vacuolisation and preserved mucosal structure were noted. Furthermore, dietary supplementation of the *Padina sp.* and GroBiotic®-A blend significantly upregulated the expression of growth-related genes, specifically *growth hormone* and *insulin-like growth factor 1* ($P < 0.05$). These results suggest that the combination of *Padina* sp. and GroBiotic®-A has a synergistic potential to enhance the growth performance without inducing digestive disturbances. Nevertheless, further studies are recommended to assess its effects on fish grown to market size before commercial application.

Keywords: brown macroalgae; growth hormone; gut health; nutritional synergy

The European seabass (*Dicentrarchus labrax*), a carnivorous fish species, holds substantial economic importance in the Mediterranean region due to its rapid growth, efficient feed conversion, and high market demand (Rimoldi et al. 2020; Yazici et al. 2020). Türkiye is a leading producer of seabass in the Mediterranean region, contributing 160 802 tons annually, which represents 29% of the country's total aquaculture production (TUIK 2024). However, as a carnivorous species, seabass require high protein content, making plant-based alternatives to fishmeal and fish oil less suitable due to their potential negative impacts on growth, health, and digestibility (Rimoldi et al. 2020).

The rapid expansion of aquaculture to meet the growing global demand for seafood has highlighted the ecological and economic challenges associated with the reliance on fish-based feeds, underscoring the need for sustainable feed alternatives (Azeredo et al. 2017; Lokesh et al. 2022). The limited

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availability and high costs of fishmeal and fish oil necessitate innovative strategies to promote a circular bioeconomy in aquaculture (Kok et al. 2020; Colombo et al. 2023; Mota et al. 2023). Identifying cost-effective and environmentally friendly feed ingredients is crucial for reducing dependence on traditional resources while maintaining fish health and production efficiency (Anguiano et al. 2013; Mahmoudi et al. 2022).

Among the potential alternatives, algae have gained significant attention due to their nutrient-rich profiles, including proteins, lipids, and carbohydrates, as well as their pharmaceutical and nutraceutical properties (Morshedi 2025). Macroalgae, when included at low dietary levels, have demonstrated functional feed additive properties such as enhancing growth performance, improving immunity, and increasing resistance to stress and diseases (Hoseinifar et al. 2022). Within this category, *Padina* sp., a brown macroalga, stands out for its bioactive compounds, such as fatty acids, dietary fibre, and antioxidants. Its antimicrobial and growth-enhancing properties make it a promising feed ingredient (Monier et al. 2022; Mota et al. 2023). However, the use of *Padina* sp. in aquafeeds, particularly for carnivorous species, remains underexplored, as the anti-nutritional factors present in macroalgae can adversely affect digestibility, growth, and immunity (Rossi et al. 2021; Colombo et al. 2023). Ensuring fish health and welfare is therefore pivotal for sustainable aquaculture practices (Mahmoudi et al. 2022).

Prebiotics, such as GroBiotic®-A, offer a potential strategy to mitigate these challenges. GroBiotic®-A, a commercial prebiotic blend containing partially autolysed brewer's yeast, milk derivatives, and fermentation by-products, has shown promise in enhancing gut health, nutrient absorption, and growth in various aquatic species (Yazici et al. 2020; Rossi et al. 2021). Furthermore, prebiotics have proven effective in mitigating the adverse effects of plant-based ingredients, such as soybean meal, on intestinal health and immune function (Rossi et al. 2021; Mahmoudi et al. 2022). Combining *Padina* sp. with GroBiotic®-A as a synbiotic could potentially yield synergistic benefits, enhancing nutrient digestibility, intestinal health, and overall growth performance in European seabass.

In carnivorous fish, the liver, stomach, and intestines are particularly sensitive to dietary changes, making their histological evaluation critical for assessing the impact of alternative feed ingredients (Colombo et al. 2023). The effects of plant-based feed components, which are increasingly included as substitutes for fishmeal and fish oil, are often most evident in these organs (Rimoldi et al. 2020; Hossain et al. 2024). Evaluating these regions provides valuable insights into the efficacy and safety of novel feed formulations (Anguiano et al. 2013; Abdelrhman et al. 2022).

This study aims to investigate the combined effects of *Padina* sp. and GroBiotic®-A on growth performance, histological features of the liver, stomach, and intestines, and the expression of growth-related genes in European seabass. By exploring the potential benefits of this dietary combination, the research seeks to address the challenges associated with plant-based feed ingredients in carnivorous fish diets. Successful outcomes could pave the way for more sustainable and resilient aquafeeds, ensuring both economic and environmental benefits for the aquaculture industry.

MATERIAL AND METHODS

Ethical approval

The experiment was performed by scientists trained in animal experiments (Certificate No. 2013/24). All experimental studies were carried out in accordance with the European Directive 2010/63/EU of the European Parliament and the Council of the European Union on the protection of animals used for scientific purposes.

Fish and rearing conditions

The experiment was conducted at the marine fish laboratory of a research institution using European seabass fingerlings and diets sourced from a commercial hatchery. Initially, the fish were placed in two 1 000-l tanks and fed a control diet (48% protein, 18% lipid, 6% ash, 2% fibre) for two weeks to acclimatise. Following the health status confirmation, 480 fish (2.08 ± 0.06 g) were randomly distributed across 12 tanks (500 l each, 40 fish per tank) and assigned to four treatment groups with three replicates. Fish were fed to satiation three times daily for 84 days and supplied with sand-filtered seawater (1 l min^{-1} flow rate) with tempera-

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ture, salinity, dissolved oxygen, and pH maintained within optimal ranges for seabass (22–23 °C, 34 ppt, 5.5–6.5 mg $l^{-1}O_2$, and pH 7.2–7.8) under a natural photoperiod.

Harvesting and preparing macroalgae for feed ingredient

Padina sp., a brown macroalga, was collected along the Mediterranean coast of Türkiye, between April and June. The samples were transferred to the Algal Biotechnology Laboratory at a research institution, where they were thoroughly washed with tap and distilled water to eliminate sand, epiphytes, and mud, as described by Rouhani et al. (2022). After air-drying in a shaded area, *Padina* sp. was identified based on morphological features using a binocular light microscope (CKX41SF; Olympus, Japan), without species-level differentiation. The dried samples were ground, sieved to 0.5 mm, and stored in opaque plastic bags in a cool, dry environment.

Chemical composition of *Padina* sp.

The chemical composition of *Padina* sp. was analysed for its fatty acid profile and proximate composition. Fatty acid analysis was performed by preparing fatty acid methyl esters (FAME) and analysing them via gas chromatography (GC) using an HP-INNOWAX capillary column. Hydrogen served as the carrier gas, and fatty acids were identified by comparing retention times with standard mixtures.

For proximate analysis, protein content was determined by AOAC (2005) methods, involving combustion, distillation, and titration with 0.1 N HCl. Lipid content was extracted using a chloroform/methanol (1 : 1 : 0.9) method, followed by centrifugation and weighing the evaporated lipid layer. Ash content was measured by incinerating the samples at 550 °C for four hours, then cooling and weighing the residue. The fatty acid profile and proximate composition details are summarised in Table 1.

Table 1. The fatty acid profile and proximate composition of *Padina* sp.

Code	Name	Content (%)
C4:0	Butyric acid	3.36
C6:0	Caproic acid	1.77
C8:0	Caprylic acid	1.53
C10:0	Capric acid	0.58
C11:0	Undecanoic acid	3.12
C12:0	Lauric acid	3.35
C14:0	Myristic acid	4.79
C15:0	Pentadecanoic acid	0.74
C16:0	Palmitic acid	30.9
C18:0	Stearic acid	3.79
C20:0	Arachidic acid	3.91
C14:1	Myristoleic acid	2.60
C16:1	Palmitoleic acid	6.98
C17:1	*Cis*-10-heptadecanoic acid	57.9
C18:1n9t	*Trans*-oleic acid	1.50
C18:1n9c	Oleic acid	22.3
C20:1n9	*Cis* 11 eicosanoic acid	1.04
∑ MUFA	Mono unsaturated fatty acids	34.4
C18:2n6c	Linoleic acid	5.94
∑ PUFA	Poli unsaturated fatty acids	5.94
∑ SFA	Saturated fatty acids	57.9
Proximat composition of *Padina* sp.		
Protein		7.84
Lipid		1.01
Humidity		3.19
Ash		49.7

Diets and experimental design

This study included four treatment groups with varying levels of *Padina* sp. powder (P) and GroBiotic®-A (G): 0 g P + 0 g G (0P0G), 1 g P + 1 g G (1P1G), 2 g P + 1 g G (2P1G), and 4 g P + 1 g G (4P1G). The feed compositions are detailed in Table 2. Ingredients were blended with a 3D-Mixer Alphi 1 (Hexagon Product Development Pvt. Ltd., India) as outlined in the methodology by Yazici et al. (2020). Prepared diets were stored in polyethylene bottles until use. During the 84-day trial, fish were fed three times daily (09:00, 13:00, and 17:00) until satiated. The feed amount and pellet size were adjusted every 14 days according to the growth of fish and their consumption to ensure optimal nutrition.

Evaluation of growth parameters

European seabass juveniles were fasted for 24 h and then anaesthetised with clove oil (50 mg l^{-1}).

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Table 2. Chemical compositions of diet

Chemical compositions	Percentage (%)
Crude protein*	48.0
Crude fat**	18.0
Crude fibre	1.75
Ash	9.00
Macro elements	% dry weight
Calcium	1.80
Phosphorus	1.20
Sodium	0.38
Vitamin and mineral premix	(IU/kg)
Vitamin A	24 000
Vitamin D3	5 000
Vitamin E	500
Vitamin C	400

*Protein sources: dehulled, extracted, toasted soybean meal, fish meal, corn gluten meal, wheat gluten, soy protein concentrate; **Crude fat sources: fish oil, soy oil

Every 14 days, fish were counted and weighed in groups with 0.01 g precision. Daily mortalities were recorded throughout the trial. Growth performance and survival rate were calculated using standard formulas:

$$Weight\ gain(WG,g) = FWF - IWF \quad (1)$$

$$Specific\ growth\ rate(SGR)(\%) = 100\frac{(\ln FWF - \ln IWF)}{feeding\ days} \quad (2)$$

$$Feed\ conversion\ rate(FCR) = \frac{feed\ intake(g)}{weight\ gain(g)} \quad (3)$$

where:

IWF – initial weight of fish (g);

FWF – final weight of fish (g);

Feed intake – the total amount of feed given to the fish during the trial (g).

$$Visceral\ somatic\ index(\%) = \frac{viscera\ weight}{fish\ weight} \times 100 \quad (4)$$

$$Survival(\%) = \frac{\frac{number\ of\ fish\ that\ survived}{at\ the\ end\ of\ the\ experiment}}{initial\ number\ of\ fish\ stocked} \times 100 \quad (5)$$

Histomorphological examinations of intestine and liver

Histomorphological analysis was performed at the end of the 12-week trial, following the method described by Yazici et al. (2020) with slight modifications. Three fish from each tank were sacrificed after a 24-hour fast, and their intestines and liver were collected for examination. The intestines were divided into anterior, mid, and posterior sections, and the liver and stomach were cut into approximately 0.5 cm^3 pieces. Samples were fixed in 10% neutral buffered formaldehyde for 24 h, followed by standard dehydration, clearing, and paraffin embedding procedures. Tissue sections of 4–5 µm were stained with haematoxylin-eosin and examined for morphological changes using an Olympus BX51 light microscope with a DP72 digital camera. Observations were focused on general tissue morphology, presence of goblet cells, lipid vacuolisation, and any signs of inflammatory or degenerative changes.

Gene expression related to growth performance

RNA ISOLATION

For RNA isolation, 25 mg of tissue was excised from each fish in the experimental group, and extraction was conducted using the RNeasy Mini Kit (Qiagen, Germany) on a QIAcube automated platform. The QIAcube protocol was optimised for RNA elution. First, 350 µl of RTL buffer was added to the tissue and homogenised in a Tissue Lyser LT (Qiagen). The homogenate was transferred to a 2 ml tube, combined with 600 µl of 70% ethanol, and processed through a series of centrifugation steps. Buffers RW1 and RPE were added in sequence, with centrifugation at 8 000 *g* after each addition. Finally, 30 µl of RNase-free water was added to elute RNA (Onalan 2019).

cDNA SYNTHESIS

cDNA synthesis was performed on ice using the RT2 First Strand cDNA Synthesis Kit (Qiagen). RNA (1 µl) was adjusted to 100 ng and diluted to a 10 µl volume.

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Table 3. Sequences of primers used in the study and their properties

Gene	Primer	Fragment length (bp)	TM (°C)
GH	F: GTG ATC AGT CGG GTT CAG GT	20	60
	G: CGT TGT GTC TCG TGC TTG TC	20	60
IGF	F: TAC AGG CTA TGG CCC CAA T	19	59
	G: TTG GCA GGT GCA CAG TAC AT	20	59
β-*actin*	F: GAG CGT GGC TAC TCC TTC ACC	21	59
	G: GGT ACC CATCTC CTG CTC G	19	59

Buffer GE (2 µl) was added, bringing the total to 12 µl, and the mixture was incubated at 42 °C for five minutes. Then, 4 µl 5X reaction buffer, 2 µl primer, and 2 µl reverse transcriptase mix were added, making a final volume of 20 µl. The reaction mixture was incubated at 42 °C for 15 min, followed by 95 °C for 5 min to inactivate the enzyme (Onalan 2019).

GENE EXPRESSION ANALYSIS

Gene expression analysis was performed using the RotorGene Q 9000 (Qiagen) and RT2 SYBRGreen qPCR Master Mix (Qiagen). β-*actin* was used as the reference gene, while *GH* and *IGF-1* were target genes, with primers synthesised based on NCBI gene bank sequences, and their sequences are presented in Table 3.

The qPCR mixture consisted of 12.5 µl of SYBR Green qPCR Master Mix, 1 µl each of forward and reverse primers, 6.5 µl of nuclease-free water, and 5 µl of cDNA, for a total volume of 25 µl.

The thermal cycling protocol began with an initial denaturation step at 95 °C for 30 sec, followed by 40 cycles of denaturation at 95 °C for 5 sec and annealing/extension at 60 °C for 30 seconds (Onalan 2019).

Statistical analyses

Growth performance parameters and histological data were analysed using one-way analysis of variance (ANOVA) after confirming normality and homogeneity of variances by the Kolmogorov-Smirnov and Levene's tests, respectively.

Statistical analyses were performed with SPSS software (v22.0), applying Duncan's new multiple range test at a 5% significance level. Mean values were expressed as mean ± standard deviation (SD). For gene expression analysis, relative quantification was performed.

The $2^{\Delta\Delta Ct}$- values were compared across treatment groups using one-way ANOVA followed by Duncan's test. $P < 0.05$ was considered statistically significant (Onalan 2019).

RESULTS

Evaluation of growth parameters

Table 4 summarises the 12-week growth performance and survival rates of European seabass juveniles fed the experimental diets. All groups demonstrated active feeding behaviour throughout the trial, including the control and those receiving macroalgae-prebiotic supplementation. Survival rates ranged from 93.3% to 100% with no significant differences between the treatments ($P > 0.05$). The *Padina* sp. and GroBiotic®-A mixture did not significantly affect the FCR or VSI. However, growth parameters (FWF, WG, SGR) were significantly higher in the 4P1G group compared to all other treatment groups ($P < 0.05$). While the 1P1G and 2P1G groups exhibited slightly higher values than the control, these differences were not statistically significant ($P > 0.05$).

Histological results

Histological examinations revealed changes in the liver, intestine, and stomach of European seabass fed the *Padina* sp. and GroBiotic®-A mixtures.

LIVER HISTOLOGY

Liver tissues across all groups, including the control, displayed intense fat vacuolisation, causing he-

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Table 4. Growth performance of European seabass was fed with 0% *Padina* sp. and 0% GroBiotic®-A (0P0G), 1% *Padina* sp. and 1% GroBiotic®-A (1P1G), 2% *Padina* sp. and 1% GroBiotic®-A (2P1G), and 4% *Padina* sp. and 1% GroBiotic®-A (4P1G) for 12 weeks

Growth parameters	0P0G	1P1G	2P1G	4P1G
IWF	2.16 ± 0.11	2.13 ± 0.02	2.05 ± 0.03	2.08 ± 0.02
FWF	24.6 ± 0.62^{a}	25.1 ± 0.56^{a}	25.7 ± 0.62^{a}	27.3 ± 0.31^{b}
WG	22.4 ± 0.62^{a}	23.0 ± 0.58^{a}	23.6 ± 0.60^{a}	25.3 ± 0.29^{b}
SGR	3.48 ± 0.08^{a}	3.53 ± 0.05^{a}	3.61 ± 0.03^{ab}	3.68 ± 0.00^{b}
FCR	1.18 ± 0.03	1.19 ± 0.07	1.13 ± 0.11	1.09 ± 0.06
VSI	8.68 ± 0.40	10.10 ± 0.59	9.11 ± 0.57	9.67 ± 0.82
SR	97.5 ± 2.50	95.8 ± 5.20	95.0 ± 0.00	93.3 ± 1.44

Data are presented as mean ± SD; n = 40

^{a,b}Data in the same row with different superscript are significantly different (P < 0.05)

FCR = feed conversion ratio; FWF = final weight of fish (g); IWF = initial weight of fish (g); SGR = specific growth rate; SR = survival rate; VSI = visceral somatic index; WG = weight gain

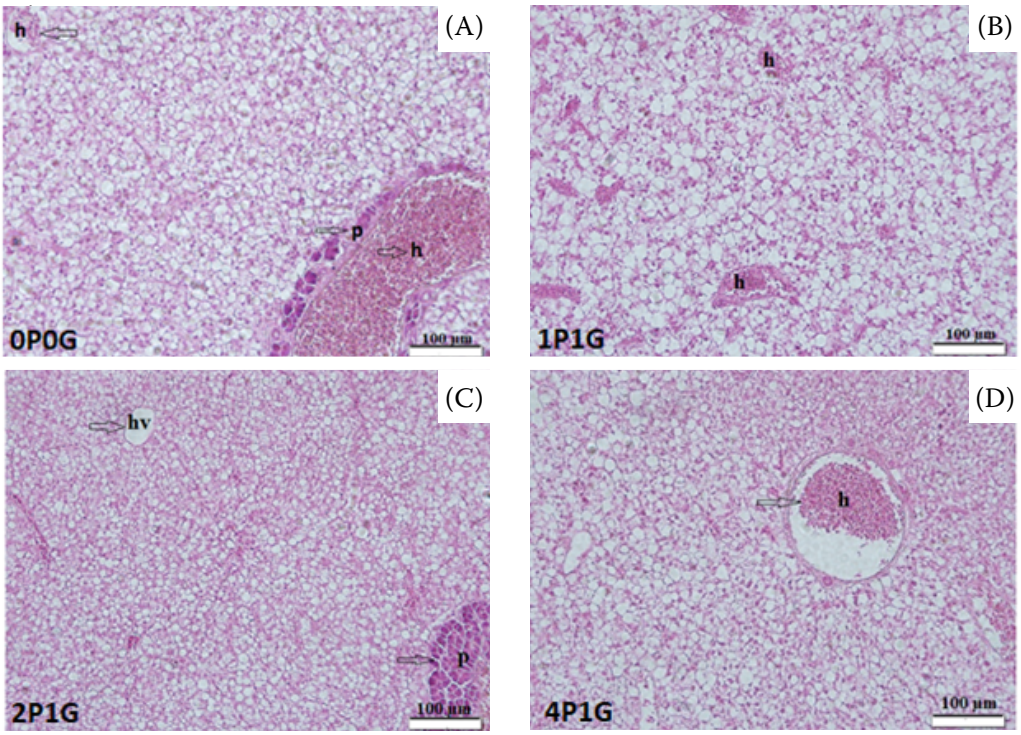

Figure 1. (A–D) Histological changes in the liver of European seabass was fed with 0% *Padina* sp. and 0% GroBiotic®-A (0P0G), 1% *Padina* sp. and 1% GroBiotic®-A (1P1G), 2% *Padina* sp. and 1% GroBiotic®-A (2P1G), and 4% *Padina* sp. and 1% GroBiotic®-A (4P1G) for 12 weeks

Arrows indicate nuclear displacement in hepatocytes due to lipid vacuolisation and hyperemia in blood vessels surrounding hepatocytes; H&E staining

h = hyperemia; hv = hepatic vein; p = pancreas

patocyte nuclei to shift towards the cell periphery (Figure 1A–D; P > 0.05). Hyperaemia was evident in all treatments, with the 1P1G group showing small, dispersed hyperaemic regions throughout the liver (Figure 1B; P > 0.05).

INTESTINAL HISTOLOGY

Table 5 presents villus lengths measured in the foregut and midgut of the fish. In the foregut, villus lengths were significantly longer in the 2P1G

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Table 5. Intestinal villus lengths of European seabass was fed with 0% *Padina* sp. and 0% GroBiotic®-A (0P0G), 1% *Padina* sp. and 1% GroBiotic®-A (1P1G), 2% *Padina* sp. and 1% GroBiotic®-A (2P1G), and 4% *Padina* sp. and 1% GroBiotic®-A (4P1G) for 12 weeks

Intestinal segment	0P0G	1P1G	2P1G	4P1G
Foregut	592 ± 26.7[a]	632 ± 26.8[ab]	685 ± 41.2[b]	680 ± 19.1[b]
Midgut	419 ± 26.7[a]	449 ± 64.8[a]	367 ± 59.4[a]	400 ± 29.0[a]

Data are presented as mean ± SD; $n = 12$

[a,b]Data in the same row with different superscript are significantly different ($P < 0.05$)

and 4P1G groups compared to the control (0P0G) ($P < 0.05$).

No significant differences were found between the groups supplemented with the macroalgae-prebiotic mix ($P > 0.05$). Although the 1P1G group displayed numerically longer villi than the control, the difference was not statistically significant ($P > 0.05$). In the midgut, villus lengths were similar across all groups.

FOREGUT HISTOLOGY

Dense lipid deposits were observed in the foregut villi of the 0P0G, 2P1G, and 4P1G groups, though these deposits did not cause any significant pathological changes in the enterocytes (Figure 2A,C,D; $P > 0.05$).

In contrast, the 1P1G group lacked these lipid deposits and showed goblet cells in the foregut tis-

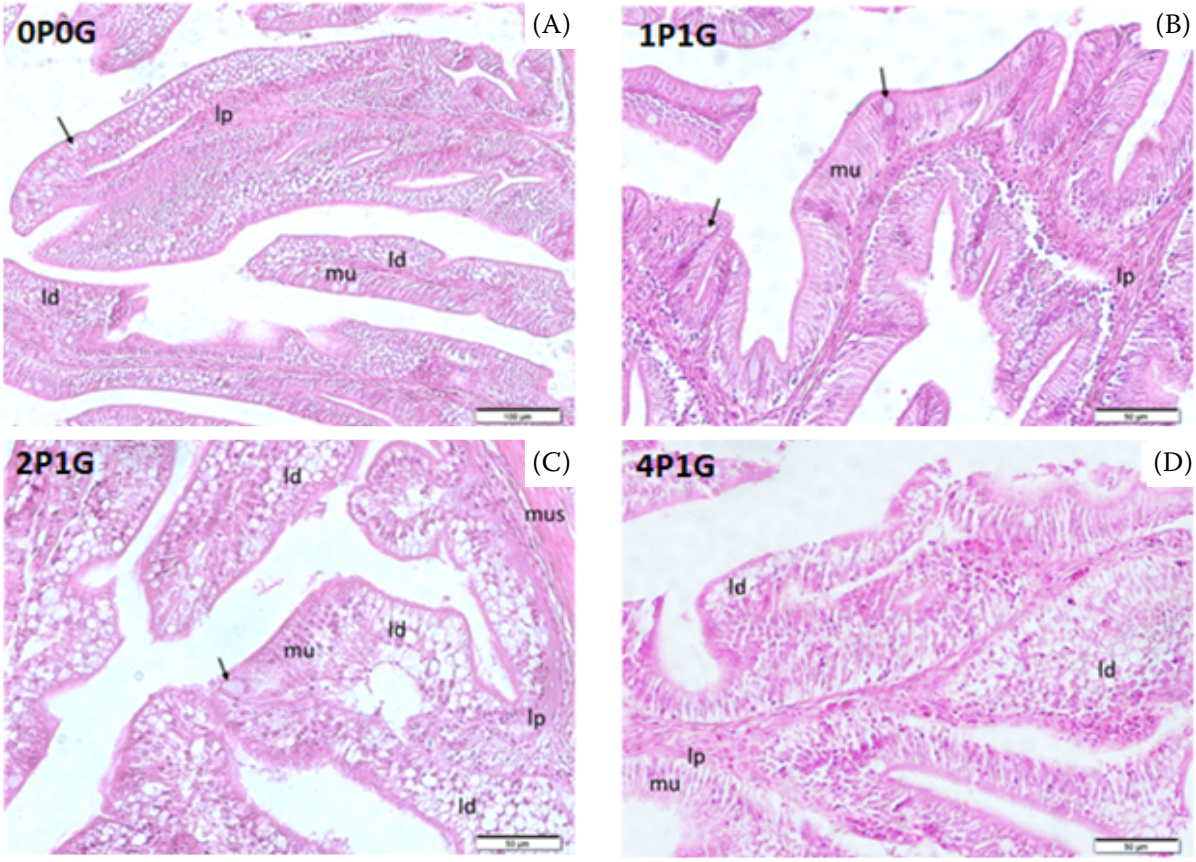

Figure 2. (A–D) Histological sections of foregut tissue of European seabass fed diets containing 0% *Padina* sp. and 0% GroBiotic®-A (0P0G), 1% *Padina* sp. and 1% GroBiotic®-A (1P1G), 2% *Padina* sp. and 1% GroBiotic®-A (2P1G), and 4% *Padina* sp. and 1% GroBiotic®-A (4P1G) for 12 weeks

Dense lipid deposits (ld) are visible along the intestinal villi in the foregut tissue of fish from the 2P1G and 4P1G groups, along with branching of the intestinal villi in these groups. Goblet cells are indicated by arrows; H&E staining

lp = lamina propria; mu = mucosa; mus = muscularis mucosa

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sue (Figure 2B; $P < 0.05$). Villus branching was also noted in the 2P1G and 4P1G groups (Figure 2C,D; $P > 0.05$).

MIDGUT HISTOLOGY

Midgut tissues across all groups demonstrated a dense presence of goblet cells within the lumen-facing enterocytes. Villus branching was more pronounced in the 1P1G group, while the villus tips and the *lamina propria* were more expanded in the 4P1G group (Figure 3D; $P > 0.05$). No pathological changes were observed in the midgut lumen-facing enterocytes in any group (Figure 3A–D; $P > 0.05$).

STOMACH HISTOLOGY

No histological alterations were observed in the stomach tissues of any treatment group (Figure 4A–D; $P > 0.05$). The mucosal epithelial cells, gastric glands, and the *muscularis mucosae* appeared unaffected by the macroalgae-prebiotic mixture (Figure 4A–D; $P > 0.05$).

Gene expression related to growth performance – Expression patterns of growth-related genes

The housekeeping gene β-*actin*, used as a reference in the real-time PCR analysis, had a threshold value of 0.02, and all samples produced Ct values above this threshold. The GH and IGF-1 primers were optimised, with the device setting their threshold at 0.02. All samples yielded results with clear sigmoidal amplification curves.

The expression of the *GH* gene showed significant upregulation in all experimental groups compared to the control. The 4P1G group exhibited the highest upregulation, with a 7.31-fold increase, fol-

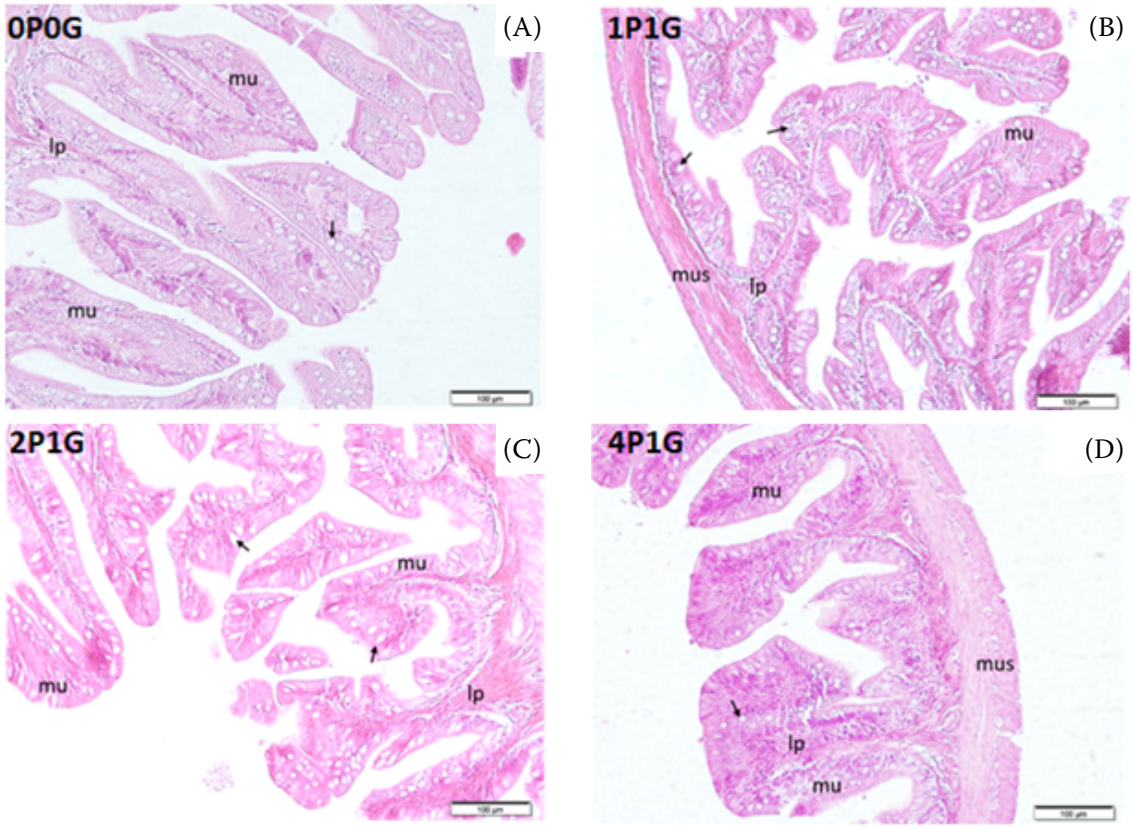

Figure 3. (A–D) Histological changes in the midgut structure of European seabass fed diets containing 0% *Padina* sp. and 0% GroBiotic®-A (0P0G), 1% *Padina* sp. and 1% GroBiotic®-A (1P1G), 2% *Padina* sp. and 1% GroBiotic®-A (2P1G), and 4% *Padina* sp. and 1% GroBiotic®-A (4P1G) for 12 weeks

Goblet cells (indicated by arrows) are positioned among enterocyte cells; H&E staining

lp = lamina propria; mu = mucosa; mus = muscularis mucosa

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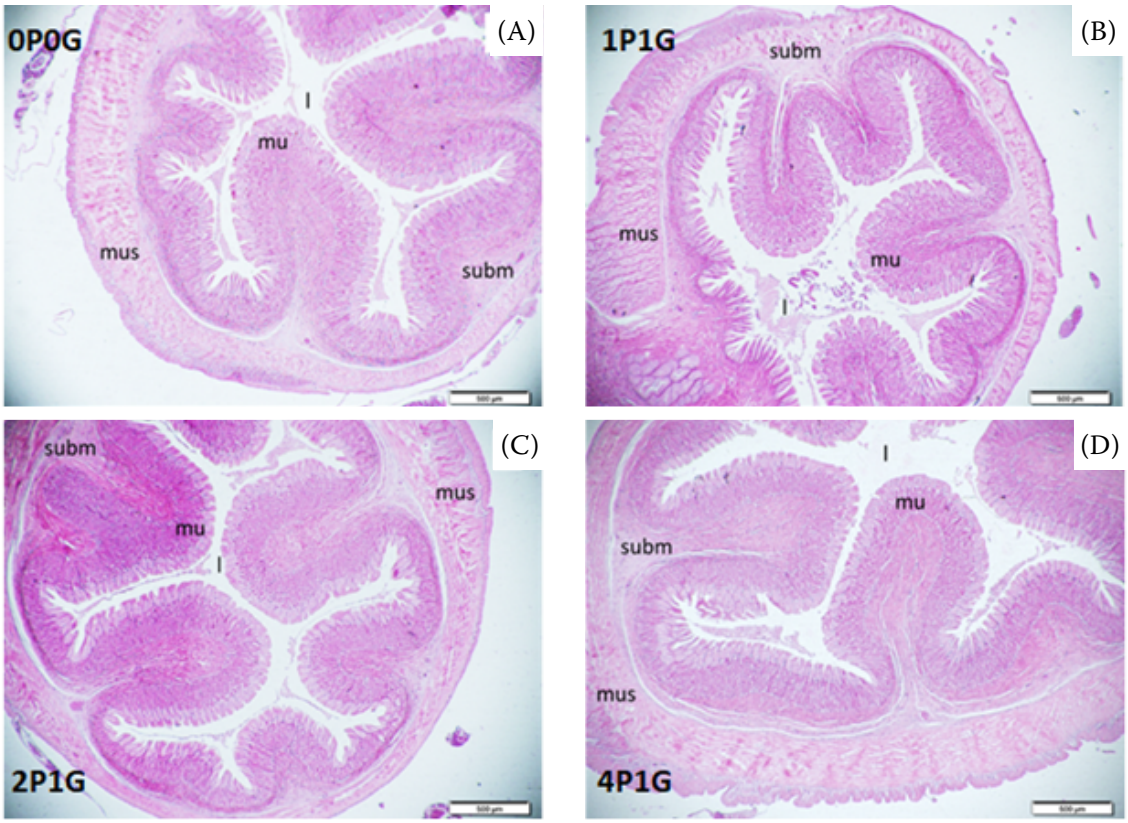

Figure 4. (A–D) Histological structure of the stomach in European seabass fed diets containing 0% *Padina* sp. and 0% GroBiotic®-A (0P0G), 1% *Padina* sp. and 1% GroBiotic®-A (1P1G), 2% *Padina* sp. and 1% GroBiotic®-A (2P1G), and 4% *Padina* sp. and 1% GroBiotic®-A (4P1G) for 12 weeks

mu = mucosa; mus = muscularis; subm = submucosa

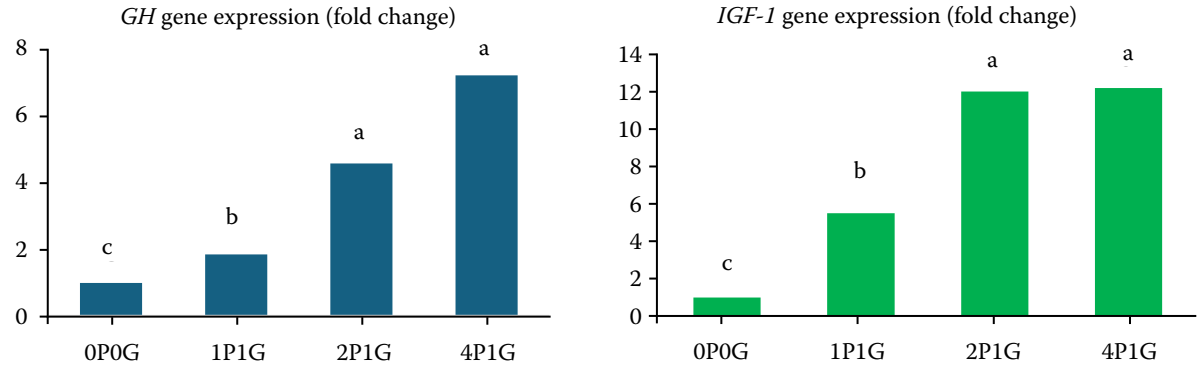

Figure 5. Gene expression levels of *GH* (A) and *IGF-1* (B) in European seabass fed diets containing 0% *Padina* sp. and 0% GroBiotic®-A (0P0G), 1% *Padina* sp. and 1% GroBiotic®-A (1P1G), 2% *Padina* sp. and 1% GroBiotic®-A (2P1G), and 4% *Padina* sp. and 1% GroBiotic®-A (4P1G) for 12 weeks

[a–c]Different letters indicate statistically significant differences ($P < 0.05$)

Expression levels are presented as fold changes relative to the control group, with data normalised to β-*actin*; Bars represent mean ± standard deviation ($n = 3$)

lowed by the 2P1G group (4.63-fold) and the 1P1G group (1.86-fold) ($P < 0.05$) (Figure 5A). Similarly, *IGF-1* gene expression was significantly upregulated in all supplemented groups, with the 4P1G and 2P1G groups showing the highest increases (12.2-fold and 12-fold, respectively), while the 1P1G group exhibited a 5.5-fold increase compared to the control ($P < 0.05$) (Figure 5B).

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DISCUSSION

Addressing challenges in aquaculture feed formulation is crucial for ensuring sustainability within the circular economy (Mota et al. 2023). Aquafeed additives present alternative strategies to alleviate the negative effects of plant-based diets and environmental stress in aquaculture species (Rouhani et al. 2022; Mota et al. 2023). Among these, marine algae are particularly promising due to their biologically active compounds, which offer both nutritional and functional benefits with minimal environmental impact (Batista et al. 2020; Mota et al. 2023). While algae have been extensively studied as sustainable alternatives to fishmeal and fish oil, research on the synergistic effects between macroalgae and prebiotics remains limited.

This study investigated the combined use of *Padina* sp. and GroBiotic®-A in European seabass diets, highlighting synergistic benefits. The combination demonstrated a significant potential, effectively mitigating the growth and digestive challenges typically associated with the inclusion of macroalgae in carnivorous fish diets. The inclusion of 4% macroalgae powder and 1% GroBiotic®-A (4P1G) improved final weight, weight gain, and specific growth rate compared to control and other treatment groups, with no effect on fish survival rates (ranging from 93.75% to 100%).

The fatty acid profile of *Padina* sp., rich particularly in palmitic acid, likely supported energy metabolism, while GroBiotic®-A improved lipid digestibility. These complementary roles may explain the observed improvements in growth performance. Although no studies have directly examined the combined use of macroalgae and prebiotics, findings from research on macroalgae with other feed additives or prebiotics with plant-based diets provide support for these results. For instance, Mir et al. (2017) reported significant improvements in weight gain (WG), specific growth rate (SGR), and protein efficiency ratio in *Labeo rohita* offspring when fed fucoidan from brown algae combined with methionine. In contrast to our study, the feed conversion ratio (FCR) was lower in the control group. Similarly, a 0.5% brown macroalgal polysaccharide and probiotic improved WG and SGR increases in *Lates calcarifer* juveniles, with no impact on survival rate (SR) or viscerosomatic index (VSI), though FCR was lower in the control group (Ashouri et al. 2020). Conversely, Nimalan et al. (2022) observed no significant growth improvements in Atlantic salmon fed a combination of marine and plant ingredients supplemented with probiotics, which partially supports our findings.

The importance of cost-effective feed ingredients for carnivorous species such as European seabass, which heavily rely on fishmeal, cannot be overstated (Kok et al. 2020). Research indicates that increasing algae content in diets can reduce digestibility and growth due to shorter digestive tracts in carnivorous species (Hu et al. 2013; Lokesh et al. 2022; Morshedi 2025). Previous studies on European seabass have reported no growth impairment when fishmeal was replaced with specific plant ingredients and additives (Azeredo et al. 2017). Similarly, the phytogenic-prebiotic combination employed by Rimoldi et al. (2020) was well accepted and it maintained consistent growth performance over a nine-week trial. These findings are consistent with those of Mota et al. (2023), who observed improved growth and digestibility using macroalgae-microalgae blends, although differences in feed conversion and viscerosomatic index were noted.

The synergistic or additive effects of phytogenic compounds can enhance the animal growth beyond what is achieved when these compounds are used individually (Rimoldi et al. 2020). This may explain the observed growth improvements, alongside the fermentative properties of prebiotics that aid digestion. Macroalgae may also exhibit prebiotic effects due to their polysaccharides and oligosaccharides, potentially promoting growth by stimulating beneficial gut bacteria (Sotoudeh and Mardani 2018). The impact of dietary algae varies depending on factors such as algae species, fish species, inclusion levels, experiment duration, and specific nutrient content (Hoseinifar et al. 2022; Mahmoudi et al. 2022; Rouhani et al. 2022).

Histological analysis of the liver, intestines, and stomach provides valuable insights into the effects of new dietary ingredients on the digestive system (Passos et al. 2021; Rossi et al. 2021; Abdelrhman et al. 2022). Previous studies have highlighted the beneficial effects of macroalgae and GroBiotic®-A on the digestive system morphology of carnivorous fish when used individually (Anguiano et al. 2013; Passos et al. 2021), Furthermore, no pathological changes have been observed in fish such as European seabass and hybrid red tilapia, fed diets containing these additives (Yazici et al. 2020; Abdelrhman et al. 2022).

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The liver, a primary metabolic organ, can reflect the nutritional status alterations through histological changes (Ashouri et al. 2020). In this study, mild hyperaemia with lipid vacuoles was observed in liver tissue across all groups, potentially indicating increased blood flow to transport macrophages to damaged areas for metabolic detoxification (Kliemann et al. 2018). European seabass, known for its sensitivity to stress, may have exhibited these changes as part of a stress response. However, no pathological signs were detected and the liver appeared macroscopically healthy in all groups.

Intestinal morphology is a critical indicator of the nutritional and physiological condition of aquatic organisms in response to novel nutritional sources. Healthy intestinal digestion is strongly associated with enhanced growth performance, making it an essential parameter for evaluating the overall health (Dawood 2021; Passos et al. 2021; Mota et al. 2023).

In this study, the increased foregut villus length in the 2P1G and 4P1G groups likely enhanced nutrient absorption, benefiting carnivorous fish with shorter intestines (Colombo et al. 2023). Goblet cells were abundant in the midgut across all groups, but only the 1P1G group exhibited goblet cells in the foregut, consistent with findings by Batista et al. (2020), where a macroalgae-microalgae mixture increased the goblet cell number in the foregut. No pathological changes were observed in enterocytes in any treatment group. In line with previous studies, algae supplementation has been shown to maintain intestinal integrity and improve the microvillus length in shrimp (Schleder et al. 2018), as well as enhance intestinal morphology and microvillus length in Asian seabass when combined with polysaccharides from brown algae and probiotics (Ashouri et al. 2020). Mota et al. (2023) also highlighted the positive effects of a macroalgae-microalgae mixture on foregut integrity. Structural changes in intestinal tissue are likely attributed to the ability of prebiotics and macroalgae, which may possess prebiotic properties, to regulate the microbiota (Ashouri et al. 2020; Abdelrhman et al. 2022). Therefore, further studies should explore the impact of combining macroalgae and prebiotics on the gut microbiota.

In stomach tissue, no abnormalities were detected in the mucosal epithelium, gastric glands, or in the *muscularis mucosae* across all groups, consistently with findings from studies in which brown algae additions did not alter the stomach histology in tilapia (Abdelrhman et al. 2022). Further research is warranted to assess the combined effects of macroalgae and prebiotics on the gut microbiota (Ashouri et al. 2020; Abdelrhman et al. 2022).

Nutrigenomic applications in aquaculture provide an opportunity to gain deeper insights into the impact of feed ingredients on the physiology of organisms at the molecular level (Zeynali et al. 2020). This study demonstrated a significant upregulation of growth-related genes *GH* and *IGF-1* in the muscle of European seabass. *GH* expression was highest in the 4P1G group (7.31-fold), followed by 2P1G (4.63-fold) and 1P1G (1.86-fold), while *IGF-1* was similarly upregulated in all treatment groups, peaking in 4P1G (12.2-fold) and 2P1G (12.0-fold), with 1P1G also higher than the control.

GH and *IGF-1* are crucial for growth regulation and are frequently studied to assess nutritional impacts on fish (Hoseinifar et al. 2022; Rouhani et al. 2022). Previous studies have shown that macroalgae and prebiotics independently enhance growth-related gene expression in fish, though few studies have investigated their combined effects. For instance, in zebrafish, the addition of macroalgae resulted in increased *GH* and *IGF-1* expression (Rouhani et al. 2022; Mahmoudi et al. 2022). Vazirzadeh et al. (2022) found that feeding to rainbow trout a diet containing 10% *Sargassum* and *Hypnea* macroalgae for 83 days increased *GH* mRNA levels in the pituitary though no effect was observed at 5%. In contrast to our study, *IGF-1* expression was lower than in the control at both 47 and 83 days, suggesting that gene expression may depend on feed quality and duration of supplementation.

The upregulation of growth-related genes observed in European seabass fed a mixture of *Padina* sp. and GroBiotic®-A reflects an increase in growth at the molecular level. Such changes in gene expression, when carnivorous species are fed plant-based diets, are often attributed to an immune response that counteracts anti-nutritional factors (ANFs) in plants, or the species ability to adapt for better utilisation of the new diet (Colombo et al. 2023). Substituting fishmeal with marine-derived ingredients, such as macroalgae, holds promise for sustainability, though challenges related to digestibility remain (Wan et al. 2019). Prebiotics, which may mitigate ANFs, could improve both digestibility and growth outcomes when combined with nutrient-rich macroalgae, offering an effective aquafeed strategy to promote growth while ensuring sustainability.

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CONCLUSION

This study investigated the combined effects of the brown macroalgae *Padina* sp. and the prebiotic GroBiotic®-A on growth, histology, and growth-related gene expression in European seabass during early developmental stages. The findings demonstrate that this combination effectively enhances the growth performance by improving nutrient absorption and utilisation, which is particularly advantageous for carnivorous species such as European seabass. The increased villus length in the 2P1G and 4P1G groups indicates improved digestive efficiency, while the upregulation of *GH* and *IGF-1* genes highlights molecular mechanisms supporting growth. Histological analysis revealed no pathological changes in the liver, intestine, or stomach, suggesting a healthy physiological response to the supplemented diet. These results suggest that combining *Padina* sp. and GroBiotic®-A offers a sustainable and effective strategy for aquafeed formulation with strong commercial potential. Future research should explore optimal inclusion levels and assess the applicability of this approach in other carnivorous fish species to further advance sustainable aquaculture practices.

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Conflict of interest

The author declares no conflict of interest.

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