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ORIGINAL RESEARCH

Utilization of fish waste biomass as a fishmeal alternative in European seabass (*Dicentrarchus labrax*) diets: effects on immuno-competence and liver and intestinal histomorphology

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Abstract The potentiality of using two novel aqua-derived meals from fish waste biomass in either non-fermented (FW) or fermented (FFW) form as dietary partial fishmeal (FM) substitutes for juvenile European seabass, *Dicentrarchus labrax*, (IBW, 29 g) was investigated. FW meal (40% crude protein, 20% lipids, and 7.5% ash) was fermented using the marine fungus, *Beauveria bassiana*. The influence of partial substitution of dietary FM using either FW or FFW meal at, 15%, 30% and 45% levels, on fish immunity and liver and intestinal histomorphology was examined to determine the appropriate form and optimal inclusion level of each. Fish were fed a basal control diet and six test diets containing either FW or FFW, each at three FM substitution levels, for 90 days. Results showed that the total serum protein, albumin, globulin, phagocytic activity of leucocyte, respiratory burst, lysozyme activities, and immunoglobulin IgM were all significantly improved in fish consumed the FW diet, at all incorporation levels, relative to the other diets. Intestinal and liver histology were also examined for any morphological alteration. Normal fish liver architecture was recorded in all experimental fish groups, with no signs of inflammation. Inspection of the proximal, mid, and distal intestine via the light and electron microscopy illustrated that seabass fed the FW30 diet had normal gut structure. Meanwhile, administration of the highest dietary FW/FFW level (45%) induced some inflammatory signs in the proximal intestinal mucosa of seabass. These findings suggest that FW meal is a feasible alternative for replacing 30% of FM in the seabass diet without compromising fish immunity and liver / intestinal integrity.

Keywords Fish waste . European seabass . Immunity . Histomorphology . Liver . Intestine . Ultrastructure

Introduction

With the annual increase in the aquaculture production, providing farmed fish and shrimp with high-quality feed is crucial. However, the aquafeed industry has relied largely on fishmeal (FM) and fish oil as the main protein and lipid sources, particularly for carnivorous fish species. Accordingly, there is a rising gap between the supply and demand of FM, prompting the search for viable alternative ingredients to reduce dependency on this commodity and consequently lower the cost of aquaculture production. Finding readily available and economically viable alternative protein sources to replace FM has been identified as a key component of establishing a long-term feed/aquaculture sector (Naylor et al. 2009; Mo et al. 2018). Several studies have been conducted, mostly on plant proteins, to reduce the dependency on FM in fish feed production, particularly for carnivorous species (Kaushik et al. 2004; Wassef et al. 2005; Gatlin et al. 2007; da

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Silva et al. 2014; Baeza-Ariño et al. 2016; Pelletier et al. 2018; Wassef et al. 2019; Wassef et al. 2021). The high dietary inclusion of plant protein meals in carnivore feeds, on the other hand, causes enteritis (Kousoulaki et al. 2015; Rimoldi et al. 2016; Leduc et al. 2018) and immunodepression (Wassef et al. 2021).

As a result of the fish processing, fish waste biomass accounts for more than half of the fish processed in many cases, and these low-cost products have a high nutritional value (Saleh et al. 2022; Khiari 2022). Furthermore, fisheries and aquaculture wastes have been highlighted in several papers as possible sources of biomolecules such as proteins, bioactive peptides, omega-3-rich oils, enzymes, and chitin (Mo et al. 2018; Khiari 2022). Accordingly, various fish wastes biomass are considered promising novel protein components in the production of aquafeeds, and the industry has commenced processing these wastes into FM, which could become a sustainable and economic FM source (Saleh et al. 2022). The efficient management of fish and shrimp processing wastes and their conversion into value-added feedstuffs, through proper treatment, will result in better resource utilization and profit maximization (Setyahadi, 2014; Saleh et al. 2022). Applying heat to the raw mixture is one of the treatments approach, and fermentation is another method suggested among the feasible practices for converting these seafood wastes into useful products with appropriate nutritive value for use in aquafeeds (Nor et al. 2011).

European seabass (*Dicentrarchus labrax*) is a strict carnivorous marine fish species of great commercial importance, particularly in the Mediterranean aquaculture (Vásquez and Muñoz-Cueto 2014). Alternative protein sources in the fish diets, particularly those of plant origin, have been proven to have a considerable effect on the immune system of farmed seabass (Kousoulaki et al. 2015; Leduc et al. 2018; Campos et al. 2019; Wassef et al. 2019). Few studies have used the discarded aquatic protein sources as FM surrogates for seabass such as the fermented prawn waste liquor which replaced 30% of dietary FM for juveniles (Nor et al. 2011), tilapia and shrimp protein hydrolysate, either alone or in combination (Leduc et al. 2018). For more evaluation of the novel fish waste meals, it is also necessary to demonstrate the impact on fish health and immunity (Maita 2007). The current research aimed to test the potential of using the two forms of fish waste meals (non-fermented, FW, and fermented fish waste, FFW, meals), each at three FM substitution levels (15%, 30%, and 45%) in juvenile seabass diet and to determine their effects on immuno-competence and liver and intestinal histomorphological characteristics.

Materials and methods

Processing of fish-waste meals

A feeding trial with juveniles' seabass was carried out in the Marine Fish Hatchery, NIOF, Alexandria Branch, Egypt and lasted for 90 days. The fish-wastes biomass, used in this experiment, were those non-edible parts of tilapias, *Oreochromis spp.*, including in the majority: viscera, raw fish heads, fins, and scales. Raw fish-waste biomass was gathered fresh (usually discarded), and treated in Fish Nutrition Laboratory to develop two novel dried proteinous meals, that can be utilized as fish meal substitutes in fish diets. Firstly, the biomass was steamed for 20 minutes to kill pathogens and decrease fat content then dried into the oven at 60°C, homogenized and sieved to obtain the non-fermented fish waste meal (FW).

The marine fungus, *Beauveria bassiana* (Bals.) (ATCC®) was isolated from Alexandria shoreline silt and chosen for its capability to generate distinct lytic zones surrounding colonies on a colloidal chitin agar media (Suresh and Chandrasekaran 1998). In the microbiology Lab (NIOF), a solid state medium was prepared to contain 5g of dried non-fermented fish waste meal, to act as a substrate, was mixed with sea water to a solid/liquid ratio of 5:2 (w/v) and autoclaved for 20 min at 121°C. Then the solid state fermentation medium (SSF) was inoculated with 2 ml of the prepared *B.bassiana* inoculum and incubated for 7 days at 27°C and 90% relative humidity. After fermentation, the product was filtered, dried (60°C) and then re-grounded, to produce the final fermented fish wastes meal (FFW) to be examined as a FM-replacer. FW and FFW, tested in this experiment, were incorporated into six experimental diets at three substitution levels each (15, 30 and 45%) of the fish meal portion of the control diet (CTRL). Proximate composition analyses (% DM) of the two tested meals, i.e. non-fermented (FW) and fermented (FFW), in comparison with FM showed that the crude protein content was 40.0% and 41.0% vs 69.0% for FM, crude lipids was 20.5% and 19.0% vs 7.24% for FM and ash content was

Table 1 Composition (g/kg) and proximate analysis (%DM) of the experimental diets

Ingredient	Control (CTRL)	Non-fermented fish-waste meal (FW)			Fermented fish-waste meal (FFW)		
		FW15	FW30	FW45	FFW15	FFW30	FFW45
Fish meal (FM) [1]	550	468	385	303	468	385	303
Fish-waste meal[2]	-	82	165	247	82	165	247
Soybean meal (SBM) [3]	150	150	150	150	150	150	150
Corn gluten[4]	110	140	160	200	140	160	200
Wheat flour[5]	30	20	20	20	20	20	20
Wheat bran[6]	40	50	50	30	50	50	30
Fish oil (FO)[7]	90	60	40	20	60	40	20
Vitamins & minerals mix[8]	30	30	30	30	30	30	30
Proximate analyses (% DM)							
Crud protein (CP)	48.54	47.86	47.80	47.74	48.11	48.00	47. 45
Lipids	16.75	16.00	18.00	18.05	16.17	15.20	14.48
Ash	13.94	13.75	14.27	14.70	13.23	13.10	14.53
Crude fiber	1.99	2.01	2.06	2. 42	2.01	2.15	2.18
Nitrogen Free Extract (NFE)[9]	17.66	19.86	18. 57	18.49	20.77	21.55	21.06
Gross Energy (MJ /Kg)	1.20	1.22	1.25	1.26	1.23	1.22	1.19

1, 999 LT Denmark (69% CP)
2, Processed fish-wastes meal: (non-fermented, FW; or fermented FFW)
3, local product, Alexandria Company
4, imported, USA
5& 6, Local products, El Nasr Mill, Egypt
7, Sardine Oil, INDIA
8, local Premix; (AGRE–VET, Co,) Each 3 kg contains: Vit A (12,000000 IU.), Vit D (2500000 IU), Vit E (10000 mg) Vit K3 (500 mg) Vit B1 (1000 mg), Vit B2 (5000 mg), Vit B6 (1500 mg), Vit B12 (50 mg), Biotin (150), Folic acid (1000 mg), Pantothenic acid (10000 mg), nicotinic acid (30000 mg). Magnesium (60000 mg), Copper (4000 mg), Iron (30000 mg), Zinc (4000 mg), Cobalt (200 mg), Iodine (300 mg).
9, NFE: calculated by difference: 100 - (crude protein⁾+ lipids +⁾ crude fiber + ash).

25.0% and 25.2% vs 12.0% for FM respectively.

Experimental fish and facilities

One thousand two hundred healthy juvenile's seabass (initial body weight, IBW = 29.0±1.0 g) were distributed into 21 fiberglass tanks, each of 3 m^3 capacity, supplied with a continuous aerated seawater. Fish were distributed in tanks at a rate of 50 fish per tank, on triplicate basis for each treatment. The salinity was 39‰, water temperature maintained at 24.0±1.9°C, pH was 8.28±0.28, dissolved oxygen was 6.4±0.85 mg/l, and the ambient light regime was 13 L:11 D.

Feeding regimen

Mainly, three major dietary groups of fish were established, one served as control (CTRL) and the other two were test groups. For the CTRL group, seabass were fed on 100% FM-based diet, whereas in the second dietary group fish were fed on a non-fermented fish-waste meal (FW) as a replacer of FM at 15, 30 and 45% levels and assigned as: FW15, FW30, FW45, respectively. Finally, in the third dietary group fish were fed on a fermented fish-waste meal (FFW), with the same levels of FM-replacement, and diets designated as: FFW15, FFW30 and FFW45, respectively. The seven diets were iso-nitrogenous (47- 48% CP), iso-lipidic (14-15.5% L) and were developed to meet the species' dietary requirements. Fish were fed the experimental diets three times a day for 90 days to apparent visual satiation. Table 1 shows the composition and the biochemical analyses of the experimental diets.

Immunity bio-indicators assays

Five fish were randomly selected from each tank (15 fish per treatment) 24 hours after the last feed and mildly anaesthetized with clove oil (20 mg/L) at the end of the experiment. Blood was sampled immediately from the caudal vein of fish, pooled for each dietary group, and was left to coagulate at 4°C for 10 minutes before being centrifuged at 4000 g for 10 minutes and the serum was separated and stored at –80°C

for subsequent use in the immunity tests. Serum total proteins and albumin were estimated by using commercial kits (Chemroy and Pasteur Labs, France) according to Doumas et al. (1981) and Reinhold (1953) methods respectively. Serum globulin was calculated by subtracting total serum albumin from total serum proteins. Serum bactericidal activity was determined by adopting Rainger and Rowley (1993) technique. Immunoglobulin M (IgM) level was determined by ELISA assay using a commercial kit (Cusabio, Wuhan, Hubei, China) and following the method of Sun et al. (2010). The nitro blue tetrazolium (NBT) assay was adopted to estimate the respiratory burst activity according to Anderson (1992) method. Leucocytes phagocytosis activity was estimated by Kawahara et al. (1991) method, (Phagocytic activity = percentage of phagocytic cells containing yeast cells). The amount of serum generating a 0.001 /min reduction in absorbance was quantified spectrophotometrically (Stat Lab, Germany) using the turbidometric assay (Ellis 1990).

Morphology of liver and intestine

Histological examination and measurements of the liver and intestine samples were randomly obtained at the end of the feeding trial (10 per treatment). Subsamples of the liver and the proximal, middle and distal intestinal sections were carefully separated, fixed in 10% phosphate buffered formalin solution (pH 7.4). Samples were sectioned at 5µm and then stained with haematoxylin and eosin (H&E). Histological examination was conducted by using light microscopy (Nikon Phase Contrast Dry, Tokyo, Japan. Liver sections were investigated for any major alterations and the diameters of 25 randomly selected hepatocytes (C) and their nuclei (N±0.01 µm) in one liver section were measured and this procedure was repeated for ten liver sections per treatment. The degree of hepatocytes vacuolization was also noticed.

Intestinal measurements

The intestinal histomorphology was evaluated according to the integrity of the intestinal mucosa, occurrence of supranuclear vacuolization in the enterocytes, appearance of lamina propria within the intestinal folds and leucocyte infiltration in the lamina propria and submucosa layer (Baeza-Ariño et al. 2016; Wang et al. 2017). Furthermore, the following parameters were measured: muscular layer (ML), submusosa layer (SML), lamina propria (LP), villi length (VL), and villi width (VW). For each parameter six measurements per section were recorded to establish the mean value. Besides, goblet cells (GC) were approximately quantified by counting in each section to determine their mean number. All measurements were taken using the Multi Scan Base version 8.08 (Computer Scanning System Ltd., Warsaw, Poland) and NIS-Elements F2.30 version2.21 (Nikon, Tokyo, Japan) computer programs.

Transmission electron microscopy (TEM) examination

At the end of the experiment, the intestine of six fish per diet for only CTRL, FW30 and FFW30 groups were sampled (two fish/tank) and examined by Transmission Electron Microscopy, to detect any alteration that may result after feeding seabass the fish-wastes meals. Fish were euthanized with a clove oil overdose, and placed immediately on an ice block, and a small piece of proximal intestine (≤1 cm) was cut and then placed immediately into a fixative solution (2.5% glutaraldehyde with 0.1M sodium cacodylate buffer (1:1 v/v, pH 7.2, 3% NaCl), then post-fixed in OsO_4 (1% cacodylate buffer, pH 7.2, 0.1M) for 1 hr. Samples were dehydrated through a graded alcohol series, then cleared with propylene oxide, and embedded in predried gelatin capsules. Ultrathin/semi thin sections (1µm) were cut off with a Leica Ultra-microtome (with a diatome diamond knife). Firstly, many sections were stained with Toluidine blue and examined by light microscopy to study some features of the proximal intestine. The rest of sections were then stained using a saturated solution of uranyl acetate (15 min) and Reynolds lead citrate (15 min). Samples were screened using a JOEL 1200 EX TEM (Tokyo, Japan). TEM sampling and processing was conducted after Dimitroglou et al. (2009). Ten well oriented microvilli were measured (villus length and width) within three different images of each sample with a total of four samples per dietary group.

Consent to participate

Fish maintenance and experimental procedures were approved by the Research Committee of the NIOF, Egypt, and in accordance with the Guide for Use and Care of Laboratory Animals (European Communities Council Directive (2010/63/EU).

Statistical analysis

Data are expressed as means ± SE (n=3 per treatment, unless otherwise stated) and differences between dietary treatments for any studied parameter are analyzed by two-ways analysis of variance (ANOVA), with two dietary fish-waste meal forms (FW and FFW) and three FM-substitution levels each as a variable, followed by Duncan's multiple range test. Statistical significance between means was tested at the 0.05 probability level. All statistical tests were performed using the Standard Version of SAS 2009 software package for Windows.

Results

Effect of feeding seabass fish waste meals on serum immunity bio-indicators

At the end of the growth trial, serum immunological parameters, namely, phagocytic activity, respiratory burst activity, bactericidal activity, lysozyme activity, total immunoglobulin, total proteins, albumin and globulin were all measured for the seven dietary groups (Fig. 1). Overall, feeding seabass FW diets at all inclusion levels significantly increased the all estimated immunological parameters relative to their corresponding levels in fish fed with FFW diet. The highest phagocytic activity, respiratory burst , bactericidal and lysozyme activities, total proteins, and globulin levels were registered at 30% FM replacement level (FW30-fed fish), then these values were significantly decreased ($P < 0.05$) with the highest FW inclusion level (45% of FM).

By considering the interaction and comparing the immunological parameters according to fish waste form/dietary groups, the highest values were obtained for fish fed the FW diet followed by FFW, and the lowest, unexpectedly, were those of CTRL fish in most measured parameters. Conversely, based on the incorporation level of fish–wastes meal, regardless of FW form, the highest values were registered up to the 30% FM substitution level, and then these values significantly decreased at the highest FW inclusion level (45% of FM). Surprisingly, the lowest immunity indices were shown in fish fed the CTRL diet (at 0% FW substitution level) (Fig. 1).

Effect of feeding seabass fish waste meals on liver histomorphology

Examination of seabass liver sections, at the end of the trial, revealed no major alterations in the histomorphological characteristics among the different dietary groups (Figs. 2 A–G). In most liver sections, including those of the CTRL group, all hepatocytes maintained their eccentric nuclei, and the cytoplasm vacuolization caused by the storage of lipid droplets was noticeable to a moderate degree. However, these lipid vacuolizations subsequently deformed hepatocytes' shape and displaced the nucleus position in a few sections (Figs. 2B, E). Unexpectedly, with the higher levels of FM substitution in diets (30% and 45%), fish hepatocytes recorded comparatively lower rates of lipid accumulation than in the other dietary groups, CTRL and FW15/FFW15 (Figs. 2 C, D, F, G).

Furthermore, the morphometric measurements on the photomicrographs of seabass liver sections showed slight differences among the tested dietary groups relative to the CTRL group (Table 2). The shape of the hepatocytes was spherical in CTRL fish, oval in FW15- and FFW15-fed fish, polygonal in FW30 fish, and hexagonal in FW45-, FFW30-, and FFW45-fed fish groups. No significant variations in the hepatocyte count (within 1.32 mm^2) were observed among dietary groups (Table 2). However, the hepatocyte length of both fish groups fed either FW or FFW diets, only at 45% substitution level, showed a significant increase ($P < 0.05$) relative to their corresponding in CTRL fish. In contrast, at the same inclusion level, the hepatocyte width recorded a concomitant decrease ($P < 0.05$) compared with that of CTRL fish (Table 2).

Hepatocyte nuclei were eccentric in shape up to the 30% FM replacement level, similar to that of CTRL fish, but appeared centric at the highest inclusion level (45% of FM) (Table 2 and Figs. 2 D, G). Meanwhile, hepatocytes' nuclear count, position, and dimensions were insignificantly varied by diet (Table 2). Briefly, a comparison between seabass fed with FW and those fed with FFW diets at all inclusion levels revealed that fermentation had no significant effect on liver morphology, either positively or negatively. Fish fed the experimental diets showed a regular morphology of hepatic-tissue structure, except for a trivial degree of nuclear displacement, within hepatocytes, noticeable only in fish fed the highest FM replacement level by either FW or FFW.

Light microscopy

Six anatomical measures from the three portions of the seabass intestine, namely, proximal (PI), mid (MI), and distal (DI), were examined via light microscopy. Mean thickness of muscle layer (ML), submucosa layer (SML), length and width of villi (VL and VW), presence and distribution of goblet cells (GC), and lamina propria (LP) (Table 3), in addition to the presence, and extent of any inflammatory infiltrates**.** At the end of the experiment, the major differences among dietary groups were observed in PI and DI segments. The morphometric measures showed a significant ($P < 0.05$) graded increase in the muscle layer thickness (ML) in both PI and DI of fish fed with diets containing FM > FW > FFW. An opposite trend of variation was

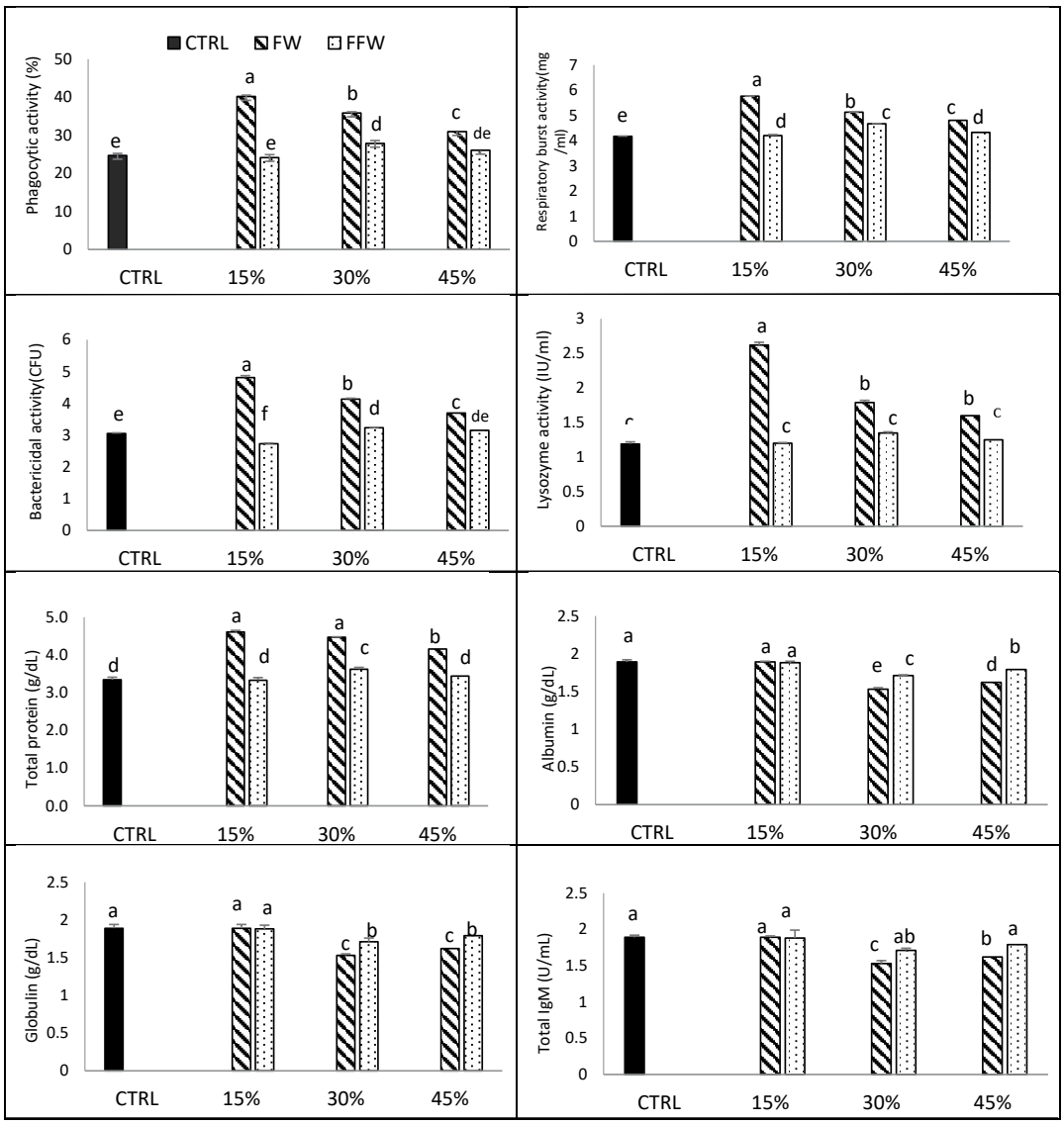

Fig. 1 Immunity parameters in serum of seabass (*D.labrax*) fed two fish-waste meals (FW or FFW) for 90 days.

observed in SML, which recorded the lowest values in the fish fed the FFW diets. Moreover, a significant reduction ($P < 0.05$) in VL and was noticed in only the PI. Additionally, FFW-containing diets induced a significant decrease in the GC number in the lumen for both PI and DI. Furthermore, FFW diets caused a significant increase in LP width in DI compared with the other provided diets (Tables 3 & 4).

Regarding the inclusion levels in PI and DI, the ML thickness has recorded a significantly high value for fish fed on diets with the highest FW inclusion level (15% < 30% < 45% FM) (Table 5). The SML remained unaltered in all intestinal portions with the three inclusion levels, indicating that the FM substitution level did not affect this layer. However, a significant reduction in VL was noticeable in PI and DI, and a similar reduction in VW in only DI of fish fed the highest substitution level (Table 5). Regardless of the substitution level, the VL and VW decreased significantly in only the DI portion (Table 3&4). Goblet cell count (GC) count varied significantly in the same manner in both PI and MI portions of seabass intestine. In PI, no significant differences were existed in GC count among dietary groups, except for fish fed the highest FM substitution diets (Table 3). In MI, fish fed the FW30 diet showed a higher goblet cell number than those in the CTRL group. A statistically significant effect on lamina properia measure (LP) was appeared only in the DI, and fish

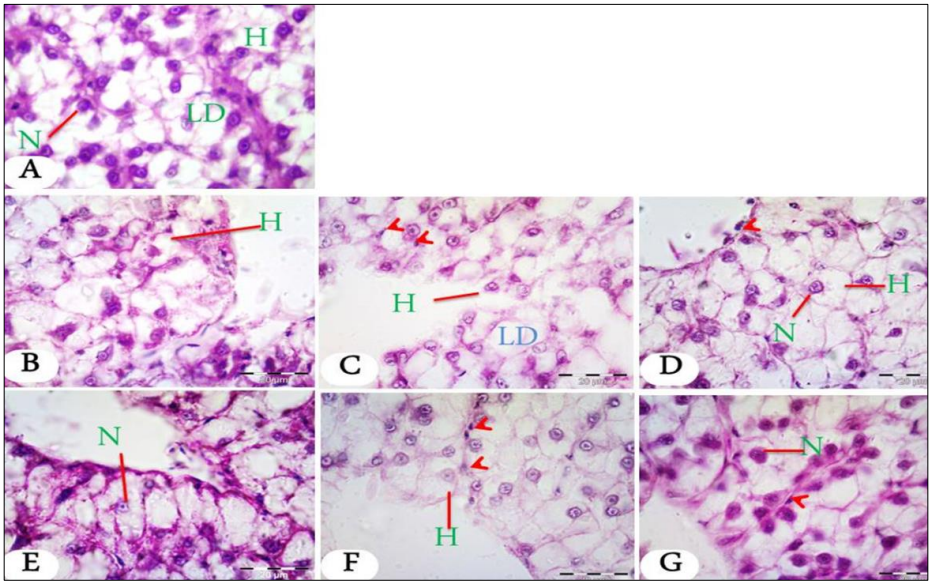

Fig. 2 (A-G) Light photomicrographs of TS in liver of seabass fed fish-waste containing diets with three FM-substitution levels for 90d. (A) Fish fed CTRL diet, showing almost rounded hepatocytes (H), each contains one terminal nucleus (N), and lipid droplets (LD) which may cover the nucleus. (B) fish fed FW15 diet, showing hepatocytes (H), some nucleus disappearance and presence of Kupffer cells (arrows heads); (C) fish fed FW30 diet showing polygonal hepatocytes (H) with few lipid droplets, many Kupffer cells (arrows heads) and low nuclear displacement, (D) fish fed FW45 diet, showing hexagonal hepatocytes of the same size, contained central eu-chromatic nucleus (N) and Kupffer cells (arrows heads). (E) fish fed FFW15 diet, showing hepatocytes with some nucleus (N) disappearance. (F) Fish fed FFW30 diet, showing polygonal hepatocytes (H) with no lipid vacuoles, low nuclear displacement and few Kupffer cells (arrows heads). (G) Fish fed FFW45 diet, showing hexagonal hepatocytes contained central eu-chromatic nucleus and Kupffer cells (arrows heads). (H&E, X 100).

Table 2 Measures of hepatocyte morphology (mean ± SE, n=10) of European seabass (*D. labrax*) fed fish waste meal (FW or FFW) diets for 90 days. (Data are presented in one decimal for simplification)

Parameter		Dietary groups							P value
			FW			FFW			
		CTRL	FW15	FW30	FW45	FFW15	FFW30	FFW45	
Hepatocytes shape		Spherical	Oval	Polygonl	Hexagonal	Oval	Hexagonal	Hexagonal	
Hepatocyets count (in 1.32 mm^2)		55.5± 0.3^{ab}	46.0± 1.0^{b}	46.0±0.7^{b}	57.0±1.9^{a}	54.0±4.0^{ab}	53.5±0.1^{b}	58.8±3.0^{a}	<.001
Hepatocyte	Length (HL)	6.9±0.0^{b}	7.0±0.0^{b}	7.4±0.0^{ab}	8.2±0.0^{a}	7.5±0.0^{b}	7.7±0.0^{b}	8.1±0.1^{a}	0001
dimension (µm)	Width (HW)	6.8±0.1^{a}	6.5±0.1^{ab}	6.4±0.^{ab}	5.8±0.1^{c}	7.3±0.0^{a}	6.4±0.1 ^{ab}	5.5±0.1^{c}	0.003
Nucleus count (in 1.32 mm^2)		38.0±0.0	29.0±1.0	40.0±0.0	49.9±0.0	29.5±9.5	39.5±1.5	43.5±0.0	0.6
Nuclear position		Eccentric	Eccentric	Eccentric	Centric	Eccentric	Eccentric	Centric	
Hepatocyte-nucleus	Length (NL)	1.6±0.0	0.8±0.5	1.9±0.4	1.4±1.2	2.5±0.0	1.9±0.0	0.9±0.3	0.51
dimension (µm)	Width (NW)	2.2±0.0^{ab}	1.8±0.0^{b}	1.9±0.1^{b}	2.5±0.1^{a}	1.9±0.1^{b}	2.5±0.1^{a}	1.8±0.2^{b}	<.001

Means in the same row with different letters are significantly different ($P < 0.05$)

fed the highest fish waste inclusion level (FW45 diet) showed the greatest values of LP among all dietary treatments (Table 5).

Histo-morphology of seabass proximal intestine (PI)

To study some features of seabass PI intestine, semi-thin sections of the three dietary treatments: CTRL, FW30-fed, and FFW30-fed fish samples were selected for comparison. Based on the light photomicrographs, the morphometric measures of some dimensions of specific cells (epithelial cells, nucleus of epi-

Table 3 Intestinal measures (mean ±SE, n=10) of seabass fed the experimental diets for 90 days

Intestinal Portion	CTRL	Dietary groups						
		FW15	FW30	FW45	FFW15	FFW30	FFW45	P Value
Muscular layer (ML, µm)								
Proximal (PI)	2586.1±130.4^{cd}	2313.6±14.8^{cd}	2365.9±810.3^{cd}	6410±329.8^{c}	4501.4±49.4^{cd}	7738±584.0^{b}	9049±407.7^{a}	0.001
Mid (MI)	1259.4±58.7^{c}	1395.4±76.6^{bc}	2469.0±45.2^{b}	3962±108.0^{a}	1306.4±17.7^{bc}	2617.0±78.1^{b}	4456±194.0^{a}	0.001
Distal (DI)	2776.2±75.5^{bc}	2612.6±220.0^{bc}	3109.5±340.0^{b}	4071.7±75.0^{a}	2781.7±165.6^{bc}	3975±146.8^{a}	4125±189.8^{a}	0.001
Submucosa layer (SML, µm)								
PI	898.1±47.0	911.3±85.9	925.8±110.0	690.3±76.3	816.5±36.8	763.8±21.3	616.5±104.4	0.91
MI	807.0±23.9	851.2±61.0	846.0±94.0	707.1±15.6	722.8±42.0	678.6±30.3	626.5±42.65	0.91
DI	1334.4±59.0	1364.7±116.8	1412.6±220.0	1264.8±75.0	731.9±253.6	785.1±55.2	746.4± 12.54	0.08
Villus length (VL, µm)								
PI	5076.1±51.4^{c}	7909.50±99^{ab}	8540.1±135.7^{a}	4435.8±81.4^{d}	7753.7±96.8^{ab}	4392.1±88.5^{d}	4156.4±12.4^{d}	0.02
MI	7469.8±294.4	7986.50±132	8594.7±935.0	5152.8±10.1	699.9±145.9	6736.0±401.9	5061.4±150.0	0.22
DI	1263.8±37.3^{c}	1662.05±115^{ab}	1773.0±105.0^{a}	1247.6±23.1^{c}	1765.3±152.0^{a}	1182±806.0^{d}	1173±259.0^{d}	0.03
Villus width (VW, µm)								
PI	1339.4±27.7^{b}	1470.3±112.1^{b}	1549.95±98.1^{a}	1311.0±18.0^{b}	1263.1±63.6^{b}	1146.2±28.3^{b}	1113.8±7.8^{b}	0.03
MI	2070.1±73.4^{ab}	2574.5±244.5^{a}	2188±315.6^{ab}	1791.0±37.5^{b}	2049±160.7^{ab}	1780±166.3^{b}	1666.3±9.3^{b}	0.01
DI	1899.5±83.5	2156.5±154.5	2591.3±198.0	1876.5±54.7	1750.2±118.0	1688.3±169.0	1337.1±90.0	0.36
Goblet cells count (GC)								
PI	99.5±16.5^{a}	79.5±1.5^{ab}	87.5±7.5^{a}	64.5±30.0^{b}	51.0±3.0^{b}	22.5±2.5^{c}	26.0±4.0^{c}	0.022
MI	27.5±2.5^{b}	29.5±0.5^{a}	34.0±1.0^{a}	18.0±7.0^{b}	33.0±3.0^{a}	15.0±1.0^{b}	26.0±4.0^{b}	0.002
DI	25.5±1.5	24.0±1.0	30.0±2.0	21.5±3.5	12.5±2.5	10.0±1.0	8.0±1.0	0.15
Lamina propria (LP, µm)								
PI	751.4±14.6	697.0±68.5	759.15±119.0	950.6±110.0	629.1±124.5	998.1±183.4	898.1±113.4	0.23
MI	500.4±93.4	449.1±169.0	557.0±118.0	564.7±95.0	599.0±7.0	586.3±48.4	915.9±103.0	0.09
DI	514.4±25.4^{c}	526.5±4.5^{c}	516.4±23.5^{c}	587.5±1.1^{c}	619.8±7.8^{b}	679.4±36.7^{ab}	874.8±65.1^{a}	0.001

Means in the same row followed by different letters are significantly different (P <0.05)

Table 4 Intestinal measures (mean ±SE, n=10) of seabass fed two forms of fish-waste (FW or FFW) diets for 90 days

Intestinal portion	Fish waste Form			P Value
	CTRL	FW	FFW	
Muscular layer (ML, µm)				
Proximal Intestine (PI)	2986.1±33.9^{b}	3439.1±15.2^{b}	9253.5±97.0^{a}	<.001
Mid intestine (MI)	1259.4±58.7	1271.8±26.5	1804.4±77.8	0.61
Distal Intestine (DI)	2776.2±75.5^{b}	3128.4±70.4^{b}	4515.7±68.1^{a}	<.001
Submucosa layer (SML, µm)				
PI	898.9±47.0^{b}	1653.8± 59.5^{a}	812.1±63.3^{b}	0.001
MI	807.0±23.9	875.2±15.2	568.7± 66.7	0.32
DI	1334.4±59.0^{ab}	1646.8± 44.3^{a}	754.4±74.7^{b}	0.03
Villus length (VL, µm)				
PI	5078.5±42.3^{a}	6161.4±94.0^{a}	1467.8±67.3^{b}	<.001
MI	7469.8±94.6	9244.7±60.3	6199.1± 90.4	0.14
DI	1264.0±60.1	1403.6±30.0	1105.8±37.3	0.21
Villus width (VW, µm)				
PI	1339.4± 27.7^{b}	2159.07±36.43^{a}	1452.0±17.2^{ab}	0.02
MI	2070.1±73.4	2006.97±96.15	1989.4±11.8	0.99
DI	1899.5±81.5	1385.4±22.8	1254.3±39.7	0.34
Goblet cells count (GC)				
PI	99.5±16.5^{a}	97.5±29.5^{a}	69.8±19.2^{b}	0.04
MI	27.5±2.5	27.2±3.5	21.3±5.5	0.08
DI	25.2±1.9^{b}	31.5±1.5^{a}	10.2±1.1^{c}	<.001
Lamina propria (LP, µm)				
PI	751.4±14.6	786.6±79.5	876.1±13.4	0.62
MI	514.4±25.4	610.5±125.3	798.2±99.5	0.72
DI	500.4±13.4^{b}	483.6±14.2^{b}	633.7±10.7^{a}	0.04

Means in the same row followed by different letters are significantly different (P <0.05)

thelial cells, GC, and cilia thickness) from the PI of seabass of these dietary groups are shown in Table 6 and Figs. 3A-C.

In PI of the CTRL fish, the length of the epithelial cells was slightly less ($P < 0.05$) than that of FFW30-fed fish but comparable with that of FW30-fed fish. Meanwhile, epithelial cells-width was comparable ($P > 0.05$) in all dietary treatments. Therefore, fermentation of FW had no appreciable effect ($P > 0.05$) on either epithelial cell length, up to 30% FM-substitution level, or width of the PI of seabass. Moreover, no significant variations were noted for the nucleus dimensions of the epithelial cells among all dietary treatments. In the meantime, fish fed the FW30 diet recorded the highest significant length (5.7 µm) of GC among the three dietary groups, whereas those fed on the FFW30 diet registered the lowest numerical GC length (1.64 µm) but were comparable ($P > 0.05$) with that of CTRL fish (2.62 µm). Although FFW diets have recorded significantly ($P < 0.05$) higher GC width (2.42 µm) than that of CTRL fish (1.66 µm), values for FW30 and FFW30 (2.42 and 2.27 µm, respectively) were similar. Furthermore, fish fed the FFW30 diet showed the highest cilia thickness among the three dietary groups, with no significant ($P > 0.05$) differences

Table 5 Intestinal measures (mean ±SE, n=10) of seabass fed fish waste meals (FW or FFW) at the three fish meal-substitution levels for 90 days.

Intestine Portion	Fish Meal-substitution level (%)				
	0	15	30	45	P Value
			Muscular layer (ML, µm)		
Proximal Intestine (PI)	2986.1±130.4^{b}	5361.9±176.1^{c}	3883.6±127.1^{bc}	9793.5±193.3^{a}	0.0
Mid intestine (MI)	1259.4±58.7	1300.9±402.2	1246.8±195.3	3462.9±195.9	0.6
Distal Intestine (DI)	2776.2±75.5^{b}	28097.1±24.1^{b}	2940.5±60.0^{b}	4300.2±102.8^{a}	0.0
			Submucosa layer (SML, µm)		
PI	898.1±47.0	1070.9±208.1	1104.9±317.3	653.38±21.7	0.11
MI	807.0±23.9	929.7±165.1	861.6±61.2	574.6±16.3	0.33
DI	1334.4±59.0	1848.3±808.5	1128.1±2.1	625.6±9.1	0.08
			Villus length (VL, µm)		
PI	5078.5±122.3^{b}	7350.8±114.8^{b}	10387.9±23.4^{a}	4705.0±129.6^{c}	0.00
MI	7469.8±194.6	8361.2±548.3	8397.4±146.4	5407.1±449.6	0.24
DI	1263.8±37.3^{b}	1427.38±1627^{a}	1456.4±1499.6^{a}	1117.5±35.5^{c}	0.001
			Villus width (VW, µm)		
PI	1339.4±27.7	1733.2±201.2	1566.6±129.2	1116.9±73.3	0.20
MI	2070.1±73.4	2312.0±193.0	1984.0±187.2	1728.6±39.3	0.046
DI	1899.5±81.5^{b}	2016.6±133.59^{a}	1512.7±153.9^{b}	1170.7±71.1^{b}	0.014
			Goblet cells count (GC)		
PI	99.5±16.5^{b}	94.3±16.7^{b}	95.1±20.7^{b}	45.8±8.9^{c}	0.001
MI	27.5±2.5^{a}	31.3±1.6^{a}	19.5±8.4^{b}	12.0±4.0^{b}	0.023
DI	25.2±1.9	19.3±3.5	20.0±5.9	17.8±4.2	0.081
			Lamina propria (LP, µm)		
PI	751.37±14.6	795.53±165.2	720.6±88.2	940.8±78.9	0.39
MI	500.4±93.4	524.1±81.6	392.9±135.6	680.3±142.7	0.06
DI	514.4±25.4^{b}	539.6±30.3^{b}	592.9±135.6^{b}	835.7±140.6^{a}	0.001

Means in the same row followed by different letters are significantly different ($P < 0.05$)

Table 6 Dimensions of specific cells from the proximal intestine of seabass fed fish-waste (FW or FFW) diets at 30% of fish meal for 90 days

Dimension of cells (µm)		Dietary groups		
		CTRL	FW30	FFW30
		18.48±0.77^{a}	16.84±0.95^{ab}	15.90±0.41^{b}
Epithelial cells	width	2.77±0.26	2.57±0.17	3.03±0.19
	Number	8	8	8
	length	3.03±0.11	2.57±0.09	2.96±0.41
Epithelial nucleus	width	1.87±0.22^{a}	1.47±0.10^{ab}	1.46±0.12^{ab}
	Number	7	7	7
	length	2.62±0.26^{b}	5.70± 0.3^{a}	1.64±0..55^{b}
Goblet cells	width	1.66±0.75^{b}	2.42±0.52^{a}	2.27±0.45^{a}
	Number	3	3	3
Cillia	Thickness	1.66±0.12^{b}	2.19±0.17^{ab}	2.42±0.31^{a}
	Number	7	7	7

Means in the same row followed by different letters are significantly different ($P < 0.05$)

between FW30 and FFW30 fish groups. Briefly, these results point to that the replacement of dietary FM with 30% FW (FW30 diet) has no major effect on the measured dimensions of epithelial cells, GC, or cilia thickness. The slight the slight variations in the length of epithelial cells or thickness of cilia, can be considered trivial alterations in the PI of juvenile seabass.

Transmission electron microscopy (TEM) examination `of seabass proximal intestine (PI)

Morphology

Seabass PI from the selected three dietary groups (CTRL, FW30, and FFW30) were further examined, at the end of the experiment, via TEM to evaluate any cellular changes that may be induced by feeding fish with the provided fish waste diets (Figs. 4A–C). Investigation of seabass PI sections illustrated normal intestinal appearance for FW30-fed and CTRL fish (Figs. 4 A, B). Enterocytes are tall and narrow, with elon-

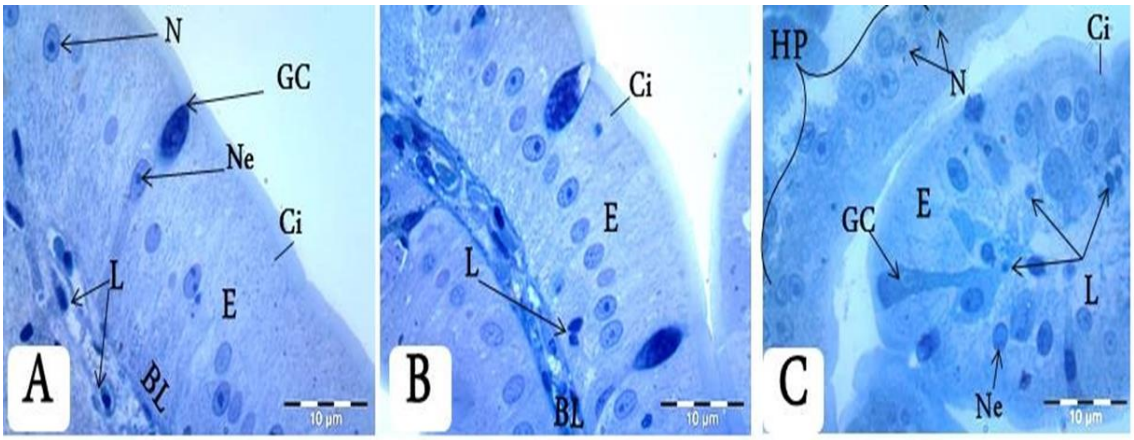

Fig. 3 A-C. Semi-thin section for proximal intestine of European seabass; (A) Fish fed CTRL diet, showing normal enterocytes (E) with one elongated nuclei (N) located below the middle of the cell on the same level, have one centric nucleoli (Ne) and aligned on basal lamina (Bl), normal long cilia at the top (Ci), and goblet cell (GC) amongst enterocytes..(B) Fish fed the non-fermented fish-waste diet (FW30), showing normal appearance of enterocytes (E) with obvious tight junction in-betweens; and aligned on basal lamina (BL) seemed nearly one level nucleus with one centric nucleoli (NE), normal cilia (Ci), very large goblet cells (GC), and few intraepithelial leucocytes infiltration (L). (C) Fish fed the fermented fish-waste diet (FFW30), showing abnormal enterocytes possess columnar shape (E) with hyperplasia appearing (HP), have many nuclei (N) arranged randomly in many levels with many nucleoli (Ne), necrosis and sloughed for intestinal cilia (Ci), small goblet cells (GC), and many intra-epithelial leucocytes infiltration (L) [Specimen were fixed with ($_4F_1G$) stained with Toluidine blue), Scale bar = 10 µm].

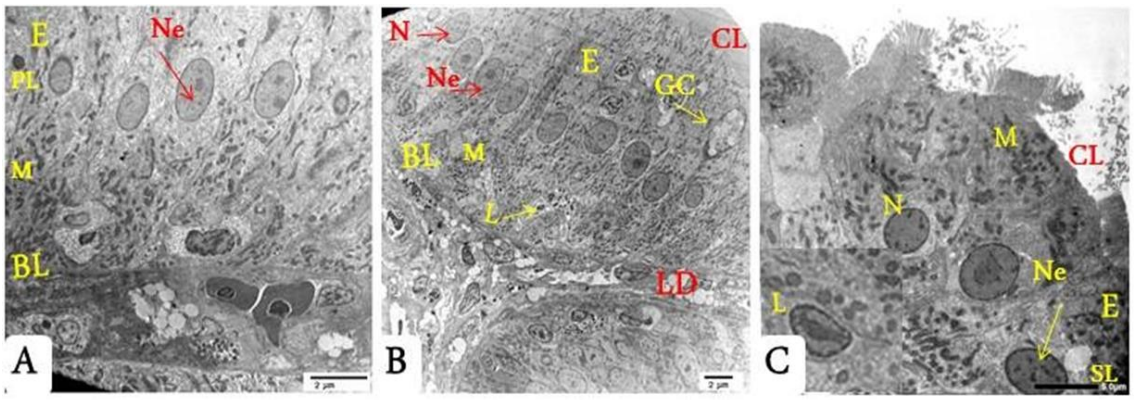

Fig. 4 A-C. TEM photomicrographs of seabass proximal intestine. (A) Fish fed CTRL diet, showing normal structure of enterocytes (E) with closed level nucleus (N); which contains centric nucleoli (Ne); cells are rich in mitochondria metamorphosis (M), primary lysosome (PL) and normal cilia (Ci). (B): fish fed FW30 diet, showing enterocytes (E) apparent with almost normal shape, with nearly one level nucleus (N) which contains one or two nucleoli (Ne); metamorphic mitochondria (M) by great number in basal lamina, goblet cells (GC), intraepithelial infiltrated leucocytes (L) amongst enterocytes and lipid droplets (LD). (C) Fish fed FFW30 diet, showing abnormal entrecotes (E) contains aside nucleus (N); with un-obvious tight junction in between, irregular distribution of mitochondria (M); many secondary lysosome (SL) and crushing parts of cilia (Ci).[Specimen were fixed with ($_4F_1G$) Scale bar = shown on each picture]

gated nuclei right below the cell's center and one centric nucleolus in each. Additionally, enterocytes have mitochondria, a well-developed brush border (ciliated cells), numerous zymogen cells/granules, as well as Golgi apparatus concentrated in the lower portion of the cell. Furthermore, mature Rodlet cells (RC), large rod-shaped cytoplasmic granules, many primary and secondary lysosomes (PL, SL), as well as numerous leukocytes and lymphocytes with many lipid droplets were revealed (Fig. 4A). GCs, the dominant mucous cells, were visible among columnar cells, from which mucus is discharged, in addition to zymogen cells/granules in the apical region (Fig. 4B).

Similarly, seabass fed the FW30 diet showed intestinal appearance almost similar to that of those in the CTRL fish group. By contrast, seabass fed the FFW30 diet demonstrated an almost abnormal intestinal appearance, such as divided enterocytes, circular, narrow, and obvious less-tight junction in-betweens with clear appearance, having many forms of elongated nuclei in the middle or side of cells that contain one or more nucleoli. The mitochondria are located in apical and basal regions and normally appear around the brush border (ciliated cells). Only one view of GC, from which mucus is discharged, is visible among columnar cells, and no zymogen or RC are shown in sections (Fig. 4C).

Discussion

Fish and shrimp waste biomass, generally, has proven to be a great source of protein, lipids, minerals, and specific fatty acids (monounsaturated fatty acids), palmitic and oleic acids (Caruso 2016). In this context, local FW meals used herein for feeding seabass contained higher monounsaturated fatty acids (approximately 46% *vs.* 32% for FM) and eicosapentaenoic acid (EPA, 4.0% ***vs.*** 1.5% for FM) contents and lower total saturated fatty acids (approximately 36.5% *vs.* 38.3% for FM), than those of the control FM ingredient (analyses are not given herein). Furthermore, all fish meals have significant protein and iron content, making them effective and affordable sources of essential nutrients for aquafeeds (Abbey et al. 2017, Hlordzi et al. 2022). Studies on seabass have evaluated different aquatic-derived meals alone or combined with other rendered animal proteins, as FM replacers and demonstrated several results. The current study results agree with those of Nor et al. (2011), who reported that 30% of dietary FM could be substituted by fermented prawn waste liquor in juvenile seabass feeds. Furthermore, combining tilapia and shrimp hydrolysates improved the effects of hydrolysate inclusion in seabass diets, since the combined hydrolysates regulated more genes and metabolic pathways than each separately evaluated hydrolysate (Leduc et al. 2018). Robert (2014) concluded that protein hydrolysates produced from the aquaculture byproducts are promising applicants to aid substituting FM in aquaculture feeds without disrupting the fish metabolism and performance. In another trial, shrimp- and tilapia-based protein hydrolysates associated with a mixture of poultry-byproduct meal (PBM) were used to replace 15% of dietary FM.

In agreement with our findings, earlier studies conducted with other marine fish species have shown that 30% of dietary FM can be substituted using a tuna-byproduct meal or 50% by tuna-muscle byproduct powder for olive flounder without negatively affecting the growth performance (Kim et al. 2014; Uyan et al. 2006, respectively). In this context, a combination of various rendered animal protein components (PBM, shrimp meal, and spray-dried blood meal) and/or fish silage could be potential feedstuffs for hybrid grouper to replace a larger amount of their dietary FM (Ye et al. 2019), rainbow trout (El-Haroun et al. 2009), and Nile tilapia (Wassef et al. 2005) among others. More recently, partial replacement of FM with two kinds of fermented animal byproduct, namely fish offal silage (35.2% CP) and poultry offal silage (41.7% CP), fermented with *Lactobacillus acidophilus*, was successful for African catfish (*Clarias gariepinus*) (El Sayed et al. 2020). By contrast, this study highlights that the application of fermentation by *Beauveria bassiana*, as a suggested technique for the recovery of valuable biomolecules from fish processing biomass, was not that beneficial, particularly at the highest FW meal inclusion level. The authors have expected that the bioconversion of fish waste biomass through fungal treatment would improve the FW meal quality and facilitate nutrient utilization by breaking down proteins, such as amino acids and peptides, as well as chitin to a simpler form; however, it did not, even though *B. bassiana* secretes extracellular enzymes, such as chitinase, lipase, and L-glutaminase, and is considered a source of protease as reported by Dhawan and Joshi (2017). The immunomodulatory effects of dietary chitin present in FW meal (originated from shrimp carapace/exoskeleton) on the innate immune system were evidenced for gilthead seabream (*Sparus aurata*) (Esteban et al. 2001) and common carp (*Cyprinus carpio*) (Gopalakannan and Arul 2006). The antioxidant property

of chitosan has been recently demonstrated (Muthu et al. 2021). To the author's knowledge, there are no available studies on the effect of *B. bassiana* metabolites on fish health, except for a recent preliminary 30-d test to assess the chronic toxicity and pathogenicity of *B. bassiana* strain (ANT-03), which reported no toxic or pathogenic effects on rainbow trout (*Oncorhynchus mykiss*) (Nadeau 2021). Furthermore, Haas-Costa et al. (2010) have provided significant evidence for the safety of *B. bassiana* in chicken. As a result of these findings, it appears that the relationship between this fungus and fish performance is complicated, inconsistent, and context-dependent. Therefore, more research on *B. bassiana* metabolites and their impacts on farmed fish performance and overall health are needed.

The link between fish nutrition and immunity has long been a research focus. Herein, no obvious suppression in the immunity of juvenile seabass was recorded when fish were fed the FFW diets for 90 d where the mean survival rates (98 % –100 %) were not significantly different among treatments, and no negative symptoms were detected. The interplay between nutrition and immunity of fish is well recognized (Villagas and Mulero 2014), and blood serum parameters are susceptible to dietary manipulations and are considered useful tools for evaluating the health status and physiological condition of fish (Buscaino et al. 2010). The nutritional and physiological health of fish is frequently linked to serum protein levels (Maita 2007, Buchmann and Secombes 2022). Amino acid oxidation or peripheral proteolysis common causes of altered plasma total protein levels in malnourished or stressed individuals (Di Marco et al. 2008). The measured immunity indices in this study (Fig. 1) showed no pathological signs in seabass fed the fish waste-containing diets, indicating the fish good general health status. Furthermore, Biller-Takahashi et al. (2013) mentioned that the presence of the protective proteins in fish blood can be assessed by the serum bactericidal activity, which is a useful tool for analyzing the innate immune system. The antibacterial activity is regarded a nonspecific response to hinder the growth of pathogenic microorganisms, and lysozyme is a protein involved in the defense mechanism against bacteria (Yano 1996). The role of blood neutrophils is to eliminate any pathogen by producing antibacterial molecules, such as lysozyme, myeloperoxidase, and cathepsins (Buscaino et al. 2010; Uribe et al. 2011). In this study, seabass fed with FW diets recorded the highest values of the measured immunity parameters among all dietary groups, indicating a positive effect of the tested FW on the general health status of seabass. Moreover, the highest globulin concentration recorded for the dietary group, FW30, suggests an enhancement in the humoral immunity of fish, as indicated by Villagas and Mulero (2014). These findings support the immune-stimulating effect of combined tilapia and shrimp hydrolysates, which has functional immune-stimulatory properties in seabass and promotes the fish health by regulating immuno-stimulatory genes (Leduc et al. 2018). Our results agree with those for other carnivorous fish species, i.e., in the serum of hybrid grouper, superoxide dismutase activity was significantly higher in fish fed 3% or 4% hydrolyzed fish protein (HFP) powder, replacing 15% or 20% of FM, respectively, whereas malondialdehyde activity was lower compared with other treatments (Hlordzi et al. 2022). Detailed information on the fish immunity, generally, is given by Uribe et al. (2011) and more recently by Buchmann and Secombes (2022), and, particularly, of seabass by Scapigliati et al. (2002) and Villagas and Mulero (2014).

Evaluation of the histomorphological structure of the fish liver is an important tool for detecting the influence of different unconventional feedstuffs on the physiological status of fish (Maita 2007). Either FW or FFW did not cause an inflammatory reaction in the liver of seabass in this investigation, regardless of dietary inclusion amount. Furthermore, the absence of tumors, lesions, or parenchymal inflammation in all dietary groups fed the fish waste-containing diets was evidenced (Figs. 2A–G). Hence, this indicates a normal seabass liver structure and function for fish of all dietary groups fed the provided formulae.

Recently, the effect of incorporating other novel proteins to replace fish meal in aquaculture diets on gut health has been investigated. Preserving the integrity of fish intestine is of critical issue when considering the novel/unconventional fish feed stuff. It is well emphasized that the histological responses of the fish intestine are crucial aspects for assessing the nutritional value of any feed ingredient since the intestine is not only the main site of digestion and nutrient absorption but also has an important function in fish immunity (Villagas and Mulero 2014). Several aspects, such as feed components, stress, and diseases, modify the intestinal morphology (Khojasteh 2012). The gut surface area is determined by many morphological features, such as the villus length (VL) and thickness of the epithelial layer, that affects the ingestion and absorption processes and, subsequently, affect the utilization of the dietary nutrients. The fish PI function is the nutrient digestibility and absorption, while the fish DI is important for the absorption of proteins and peptide

molecules (Krogdahl et al. 2003), furthermore DI may has an immunological function (Khojasteh 2012; Villagas and Mulero 2014). In fish, the health and conditions of the intestinal mucosa can be determined by the overall organization and morphometric characteristics of the intestine, which are indicated by the number of goblet cells (GC) and the villi size. Increased intestinal villi size indicates improved exchange surface, brush border enzyme activity, and nutrient transport systems, all of which aid digestion and absorption. (Caspary 1992), and the reduction in epithelium thickness facilitates absorption. The measures of the three gut portions (PI, MI, and DI) of seabass in relation to dietary FM substitution level (Table 5), indicate a direct correlation between FW inclusion level and PI morphology (Figs. 4A–C). This may be explained by the fact that the FW contains a high content of indigestible material, which has increased the gut dimensions or the need for species to ingest a large volume of these unconventional feedstuffs, increase absorptive surface area and maximize the digestive efficiency (German 2011). Our findings further agree with the study of Leduc et al. (2018), who mentioned that reducing FM in seabass diets negatively affected the various intestinal functions, including food transport, immunological defense, and gut morphogenesis. However, only few reports are available on the effects of dietary FM replacement, mostly using plant-derived proteins, on seabass intestinal histomorphology (Bonaldo et al. 2008; Rimoldi et al. 2016; Wassef et al. 2016, 2019, 2021).

Additionally, our results, based on the inspection of the intestinal sections, revealed some histological alterations in fish fed with FFW diets compared with those fed the CTRL diet. The ML thickness showed significant changes in the three intestinal segments and more pronounced with the highest incorporation level. Fish fed the FFW45-diet recorded the highest ML thickness than those for the other dietary groups. Similarly, feeding seabass (500 g) a high soybean meal diet led to alterations in ML thickness (Rimoldi et al. 2016). By contrast, Bonaldo et al. (2008) observed no morphological changes in the DI of juvenile seabass when fed a 30% soybean meal diet for 89 d. For other marine fish species, Hlordzi et al. (2022) registered that hybrid grouper fed 1.5% hydrolyzed fish protein (HFP) with the low FM diet had thicker intestinal muscle relative to the other dietary groups. Baeza-Ariño et al. (2016) noticed significant differences in PI and MI of gilthead seabream fed a high level of a mixture of plant protein concentrates. Moreover, a similar significant increase in ML thickness was reported in *Diplodus puntazzo* fed a diet with 30% sunflower meal (Nogales-Mèrida 2010). In carnivorous fish, the SML, controls the intestine expansion when food is consumed and is responsible for utmost of the absorption process (Khojasteh 2012). In this study, the SML was unaffected by diet composition. These findings agree with those of earlier studies on the species, which illustrated normal mucosal and submucosal layers in seabass fed with different levels of soybean meal (Bonaldo et al. 2008).

Furthermore, intestinal villus length and width (VL and VW) are regarded as a sign of nutrient absorption ability (Khojasteh 2012). The lowest villi dimensions (VL and VW) recorded in both PI and DI portions of seabass fed the FFW45 diet (Table 3) may be associated with the fermentation process concomitant with the highest FM replacement level. In this context, Leduc et al. (2018) found that adding shrimp and tilapia hydrolysates to the low FM (FM5) for seabass enhanced the histological structure of the intestinal mucosa, with villi heights similar to those in the control group. Hlordzi et al. (2022) reported that, adding HFP powder to the low FM diets of juvenile grouper enhanced the intestinal development and elevated the levels of intestinal digestive enzymes, and fish fed 1.5% HFP had significantly longer villi compared with the other groups, including the CTRL.

The number of goblet cells (GC) and villi size, as well as the general organization and morphometric parameters of the intestine, are good indicators of gut integrity and the condition of the fish intestinal mucosa, i.e., any increase in the intestinal villi area reflects an enhancement of the exchange surface, brush border enzyme activity, and nutrient transportation, with progressive effects on digestion and absorption (Caspary 1992). Additionally, GC regulate proteins or peptides secretion, as well as ion and fluid transport, and provide an effective immune barrier against potentially pathogenic gut bacteria in seabass (Gisbert et al. 2018). This study demonstrates that seabass fed with diet containing the lowest FM level (303 g/kg in FW45/FFW45 diets) revealed an altered intestinal mucosa and a decrease in VL and GC count relative to CTRL fish (Table 3). Similarly, in another seabass (2.2 g) trial, Leduc et al. (2018) reported that the decrease of dietary FM level from 20% to 5% significantly impaired growth performance, intestinal histological architecture, and prompted significant changes in the transcriptomic profile of the intestine. These authors added that the increase in GC count in seabass enhances mucin production, which physically displaced po-

tentially pathogenic organisms. A more diverse microbiota induces a thickening of the mucus layer, which enhances gut microniches occupied by these beneficial bacteria. These results also agree with the previous studies that showed a decrease in VL and GC count in red seabream (*Pagrus major*) fed on diets with high FM replacement levels (Khosravi et al. 2015).

In the present study, only the DI of FFW-fed seabass (Table 3) revealed a diffusely expanded lamina propria (LP) due to the increased leukocyte infiltration (sections are not presented herein), indicating a sign of inflammation. This criterion reduces the capacity of the enterocytes lining the epithelium to absorb nutrients (Rimoldi et al. 2016, Saleh et al. 2021). Similarly, Daprá et al. (2009) observed an infiltration of lymphocytes in the SML with FM substitution up to 35% by rice protein in *Pagellus bagaraveo* but attributed this observation to the individual variations rather than to the plant protein inclusion. By contrast, Nogales-Merida et al. (2010) discovered no significant difference in the thickness of the LP between the different intestinal segments in juvenile sharp-snout seabream (*D. puntazzo*) fed sunflower meal, as a partial substitute of FM with different substitution levels.

In summary, administration of the highest dietary FW/FFW levels has altered some morphometric features of the intestine of juvenile seabass and suggests inflammatory reaction in the DI mucosa after a 90-d feeding period depending on the inspected intestine segment. This information could help a better understanding of the absorption mechanisms in the seabass' intestine and encourage future research in this area. Therefore, in this context, additional scientific research suggests that the most appropriate treatment to better elucidate the benefits and impact of aqua/FW meals on seabass welfare and sustainability would be of high interest to the industry.

Conclusions

Local aquatic fish waste (FW) meals are considered sustainable and feasible protein sources as alternatives to fish meal in seabass diets. Both non-fermented and fermented meals (FW and FFW) are potential candidates to replace 30% of dietary FMs without compromising immunity and liver or gut integrity in seabass aquaculture. The FW30 diet improved all measured immunity bio-indicators compared with the CTRL or FFW30 diets. Furthermore, no pathological signs on liver structure were revealed after feeding seabass either FW or FFW meals for 90 d. Nevertheless, inflammatory signs in the proximal intestine (PI) of fish may compromise its dietary inclusion at high levels (45% of FM). Overall, the current findings suggest that the inclusion of fish waste biomass (FW) in juvenile seabass diets, replacing fish meal as a protein source, is suitable for up to 156 g/kg level. Using these meals obtained from processing locally marketed fish could help in reducing the dependency on imported fish meals for aquafeeds.

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Authors' contributions Elham Wassef: wrote the manuscript and supervised the experiment, Norhan Saleh: shared in the practical section and analyses and responsible for publication. Nabila Abde-Meguid: contributed to the histological section of the study. Heba Abdel-Mohsen was responsible for the practical section, serum and statistical analyses. All authors read and approved the final manuscript. The authors declare that they have no conflicts of interests.

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