Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(5): 1119 – 1138 (2022)
www.ejabf.journals.ekb.eg

Effects of Dietary Marine *Bacillus subtilis* HS1 Probiotic, Chitosan Prebiotic and Two Marine Synbiotics Mixtures on the Growth and Oxidative Stress of the European Seabass (*Dicentrarchus labrax*) Larvae

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ARTICLE INFO

Article History:
Received: Aug. 30, 2022
Accepted: Sep. 29, 2022
Online: Oct. 27, 2022

Keywords:
Synbiotic,
Probiotic,
Prebiotic,
Oxidative stress,
Sea bass,
Larvae,
Weaning

ABSTRACT

A European sea bass [*Dicentrarchus (D.) labrax*] weaning trial was conducted on the 30- 45 day- post-hatching (dph) reared larvae. Enriched rotifers and *Artemia* were offered as feed in the same 2 m^2 tank from the 3^{rd} to the 30^{th} dphin NIOF hatchery larval rearing unit. In the present study, the total antioxidant capacity (TAC), catalase (CAT) and superoxide dismutase (SOD) antioxidant parameters, and some hepatic enzymes at early life stages were investigated. Four treatments were conducted in triplicates: control mircodiet (G), dietary marine *Bacillus subtilis* SH1 probiotic (Mpro), marine chitosan prebiotic (Mpre), and two marine synbiotics mixtures (MS1 and MS2) treated microdiets. The impact of the five microdiets cofeeding and weaning treatments on the 45^{th} dph early weaned *D. labrax* larvae fed MPro, MPre, MS1, and MS2 exhibited significant ($P < 0.05$) length growth similar to that recorded in G. The MS1 and MS2 showed the best length growth performance, survival, SOD and CAT activities, as well as TAC improvement after treatments, compared to G. The larvae fed MS2 and MS1 recorded the highest considerable ($P < 0.05$) alkaline phosphatase (ALP) and acid phosphatase (AP) enzymes total and specific activities, respectively. The larvae fed Mpre recorded the highest significant ($P < 0.05$) aspartate aminotransferase (AST) total activity; while, larvae fed Mpro recorded the highest substantial ($P < 0.05$) AST-specific activity. The larvae fed G recorded the highest meaningful ($P < 0.05$) alanine aminotransferase (ALT) total and specific activities. In conclusion, mixing marine probiotics and prebiotics in synbiotics treatments improved the European sea bass larval growth, survival, and antioxidant capacity.

INTRODUCTION

In the Mediterranean Sea, the European seabass (*D. labrax*) represents one of the greatest valuable marine aquaculture and fisheries fish species **(FAO, 2022)**. The lack of fry is still the barrier hindering aquaculture development **(Verhaegen, 2012)**. Worldwide, the use of antibiotics in aquaculture threatens the health of human and farmed animals and affected their antimicrobial resistance (AMR) **(Okocha *et al.*, 2018; Knipe *et al.*,**

2021; Hong *et al*., 2022). Hong *et al*. (2022) reported that the use of antibiotics substitution or reducing its use in aquaculture has led to the emergence of many additives, including synbiotics **(Salem *et al*., 2015; Ringø & Seong, 2016; Azimirad & Meshkini, 2017; Li *et al*., 2018; Okay *et al*., 2018; Villumsen *et al*., 2020; Knipe *et al*., 2021)**, probiotics **(Salem *et al*., 2015; Lamari *et al*., 2016; Banerjee & Ray, 2017; Hoseinifar *et al*., 2018; Wang *et al*., 2019; Ringø *et al*., 2020; Serradell *et al*., 2020),** and prebiotics **(Zaki *et al*., 2015; Widanarni *et al*., 2018)**. Synbiotics have shown ameliorated growth, immune- and antioxidative effects on aquatic animals **(Lamari *et al*., 2016; Ringø & Seong, 2016; Huynh *et al*., 2017; Salem *et al*., 2022)**. The synbiotics (*Bacillus* spp. and *Enterococcus* spp.) probiotics, prebiotics such as chitosan, fructooligosaccharide (FOS) and mannan oligosaccharide (MOS)) have been studied on yellow croaker, Japanese flounder, rainbow trout, cobia and the European sea bass **(Salem *et al*., 2015; Zaki *et al*., 2015; Huynh *et al*., 2017; Villumsen *et al*., 2020; Salem *et al*., 2022)**. Synbiotic improved disease resistance, survival and host microbial ecology **(Ohtani *et al*., 2020; Knipe *et al*., 2021; Hong *et al*., 2022; Salem *et al*., 2022)**. Larval oxidation risks are remarkably high, due to its high water, long-chain PUFA tissue contents, oxygen demand and metabolic rate. Therefore, to avoid larvae lipid peroxidation, dietary antioxidant element is highly demanded **(Mourente *et al*., 1999; Betancor *et al*., 2012)**. Free radicals and/or oxygen derivatives are constantly produced through regular cellular metabolism. At low concentrations, these ROS may be beneficial in the defence against microorganisms. However, oxidative stress happens in case of imbalanced ROS generation and removal **(Betancor *et al*., 2012)**. **Betancor *et al*. (2012)** reported that, antioxidant enzymes [CAT, glutathione peroxidase (GPX) and SOD] stop oxidation reactions and close lipid peroxidation. Fish interactive antioxidant system through SOD, CAT, GPx and glutathione S-transferase (GST) encounter the oxidative damage of ROS **(Hamed *et al*., 2020)**.

The current experiment addressed the effects of oxidative stress due to MPro probiotic, MPre and two MS on the European sea bass larvae, and investigated their subsequent influences on larval growth, survival and enzymes. The current research is the first study to estimate oxidative stress and synbiotics' consequences on the alteration of antioxidant parameters.

MATERIALS AND METHODS

Larval rearing and experimental design

Newly hatched larvae of the European seabass (*D. labrax*) obtained from farmed broodstock induced spawning at the Fish Reproduction and Spawning lab. Marine hatchery (N: 31°12'46.2" E: 29°53'06.1"), Aquaculture Division, NIOF. They were stocked in greenwater flowing through a larval rearing unit. Staring from the 3rd dph to the 30th dph reared larvae, enriched rotifers and *artemia* were introduced as feed in the

same 2 m^2 tank in the hatchery larval rearing unit. At 30 dph, triplicate groups of larvae were stocked randomly and equally as 100 larvae/30 L^3 aquaria in the fifteen glass aquariums; the weaning experiment began at 30 to 45 dph with different dietary treatments. For each experimental tank, 30% of water was partially exchanged, using a hose through the plankton net covering the tank. Aeration was performed for 24 hours/day using an electric air blower. Photoperiod was 16: 8 hours light to darkness, and light was 50-100 lux at water surface.

Larvae were fed enriched *B. plactilis* and *B. routindiformis* rotifers from the 2^{nd} to the 12^{th} dph at 20 rotifers/ml and enriched *A. franciscana* (GSL) nauplii from the 12^{th} dph, starting with 1.0 nauplii/ml and increased to 2 nauplii/ml until 29 dph. While, at 30 dph, larvae started cofeeding on artemia metanauplii and treated Orange® microdiets, with 100-200 micron 6 times/day. Beginning from the 35^{th} dph, larvae were fed 4 metanauplii/ml and treated O.range®, with 15gm/m^3 until 38 dph. While larvae were fed 2 metanauplii/ml and treated O.range®, with 30 g/m^3 until 41 dph. Moreover, larvae were fed only on O.range® with 45 gm/m^3 (artemia metanapulii & cofeeding stopped) until the 45^{th} dph. DHA SELCO® (INVE, Belgium) enrichment of rotifers and artemia were conducted for 4 and 6 hours at 28ºC, respectively (Fig. 1). The tanks bottoms were siphoned every day. Five weaning microdiets treatments were: the control greenwater (Inve O.Range® microdiet without treatment) (G), Suez Gulf locally isolated marine bacterial probiotic *Bacillus subtilis* HS1 1 × 10^7 CFU g^{-1} treated Inve O.Range® microdiet (Mpro), locally extracted marine chitosan prebiotic of 1.0 mg g^{-1} treated Inve O.Range® microdiet (Mpre), marine synbiotic 1 (*B. subtilis* HS1 probiotic bacteria 1 × 10^7 CFU + 1 mg chitosan . gm-1microdiet) treated Inve O.Range® microdiet (MS1), and marine synbiotic 2 (*B. subtilis* HS1 probiotic bacteria 1 × 10^7 CFU + 2 mg chitosan . g^{-1} microdiet) treated Inve O.Range® microdiet (MS2) (Fig. 1). Both probiotic, prebiotic and synbiotic involved in the present study had effective results on the European seabass larvae and fry **(Salem *et al*, 2015; Zaki *et al*., 2015; Salem *et al*., 2022)**.

Calculation of fish growth performance

Larvae measured growth length for total length (TL) and standard length (SL) were calculated using a binocular light research microscope with graded eye piece as follows:

Length gain (LG in mm) = Lf – Li.
Length average daily gain (LADG in mm d^{-1}) = Lf – Li/t.
Length specific growth rate % (LSGR % d^{-1}) = (Lin Lf – Lin Li) 100/t.
Length gain % (LG %) = LG/ Li x 100, where Li and Lf are initial and final lengths (mm) and t is the time of experiment (days).
Survival (S %) = (final fish count/initial fish count) × 100

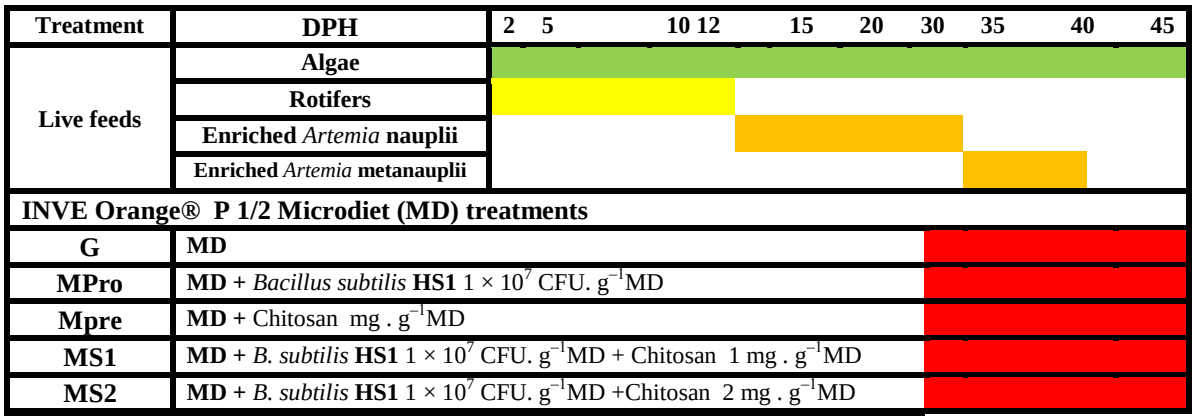

Fig. 1. Weaning and rearing strategies for European seabass (*D. labrax*) larvae using five weaning treatments.
G: Greenwater control non treated; Mpro: Marine probiotic; Mpre: Marine prebiotic; MS1: Marine synbiotic 1 treated, and MS2: Marine synbiotic 2 treated microdiets.

Measurement of water quality

Water quality was evaluated using Hanna® HI9828 portable electric device on a weekly basis, from the beginning till the end of the experiment at 2 pm. Different parameters were measured during the experiments; namely, temperature (17.61 - 19.21 ºC), dissolved oxygen % (91.35 - 104.38%), pH (7.18 - 8.49), conductivity (169.12 - 221.93 Ms/cm), total dissolved solids (77.50 - 143.60 ppm) and salinity (35.45 - 39.54 ppt).

Microbiological measurements

B. (BBC) and *Vibrio (V.)* (VBC) colony forming unit (CFU) were done in the Microbiology Lab., Marine Environment Division, NIOF. Serial dilutions of $10^{-2} \pm 10^{-4}$ were made using filtered sterilized sea water. For each water sample; 100µl was inoculated on sterile plates and incubated at 30^{o}C for 24 - 72 h. Plates of the selective media of sea water agar for BBC and thiosulfate citrate bile salt sucrose agar (TCBS) for VBC were inoculated with 0.1 ml of the diluted samples, and the different bacterial genera were counted following the study of **Salem *et al*. (2015)**.

Measurement of antioxidants biomarkers and enzymes

Biodiagnostic Company kits, Cairo, Egypt purchased a spectrophotometer model: 01102, LAXCO, Inc., USA for measuring antioxidants and enzymes. Larvae CAT, SOD, and TAC were measured according to the protocol of **Aebi (1984), Nishikimi *et al*. (1972),** and **Koracevic *et al*. (2001)**, respectively. Albumin, globulin, and total protein in g/l according to biuret method for protein **(Gornall *et al*., 1949)**, using the modified method of bromocresol green **(Doumas *et al*., 1971)** for albumin, and GOD-PAP enzymatic colorimetric method **(Weissman & Klien, 1958)** for glucose. ALP activity was established with the modified procedure of **Befield and Goldberg (1971)**. Acid

phosphatase (AP) activity was determined using the modified method of **Kind and King (1954),** while AST and ALT activities were measured according to the methods of **Murray (1984)**. TAG was analyzed using modified method of **Fossati and Prencipe (1982)**. Specific activities of enzymes were calculated by dividing the total enzyme activity over the total protein content.

Histological methodology, samples preparation and fixation

Five larvae from each tank were collected at the end of the weaning trial, fixed in 10% formalin saline for 24 – 48 hours, dehydrated through graded alcohols followed by xylene and finally embedded in paraffin wax. Two paraffin blocks containing two larvae from two different tanks were sectioned at 5μm, and sections were stained with hematoxylin and eosin (H&E) for histopathological evaluations **(Martoja & Martoja-Pearson, 1970)**.

Statistical analysis

All data were subjected to a one-way analysis of variance (ANOVA) at a 95% confidence limit, and means were compared by Duncan's test ($P < 0.05$) using a SPSS software (SPSS for Windows 16; SPSS Inc., Chicago, IL, USA).

RESULTS

Larval growth and survival

The 45 dph weaned *D. labrax* larvae fed MPro, MPre, MS1 and MS2 exhibited similar significant ($P < 0.05$) final TL and SL growth higher than G. The MS1 exhibited the best TLG and SLG in mm, TLADG and SLADG in mm/day, and TLSGR and SLSGR in %/day. MS1 and MS2 recorded the best significant ($P<0.05$) S% of 45 dph sea bass larvae (Tables 1, 2).

Larval body protein, glucose and enzymes activities

The larvae fed Mpre showed the highest considerable ($P< 0.05$) total protein in g/l. While, larvae fed Mpro revealed an excessive noticeable ($P< 0.05$) glucose in g/l. Larvae fed MS1 revealed considerably higher ($P< 0.05$) total and specific albumin activities, and larvae fed Mpro recorded a remarkable high value ($P< 0.05$) of total globulin; whereas, larvae fed MS1 verified the lowest substantial ($P < 0.05$) specific globulin activity. Larvae fed Mpro displayed the highest noteworthy ($P< 0.05$) total TAG activity; however, larvae fed MS1 and Mpro exhibited the highest considerable ($P< 0.05$) specific TAG activity (Fig. 2).

On the other hand, the larvae fed MS2 and MS1 recoded the highest noteworthy ($P< 0.05$) ALP and AP enzymes total and specific activities, respectively. The larvae fed

Mpre recoded the greatest substantial (P< 0.05) AST total activity; while, the larvae fed Mpro recoded the topmost considerable (P< 0.05) AST specific activity. The larvae fed G recoded the supreme meaningful (P< 0.05) ALT total and specific activities (Fig. 3).

Antioxidants biomarkers

It was observed that the larvae fed G showed high significant effect (P < 0.05) on body CAT, SOD total and specific activities. The larvae fed MS1 showed the highest considerable (P < 0.05) of body TAC total and specific activities (Fig. 5).

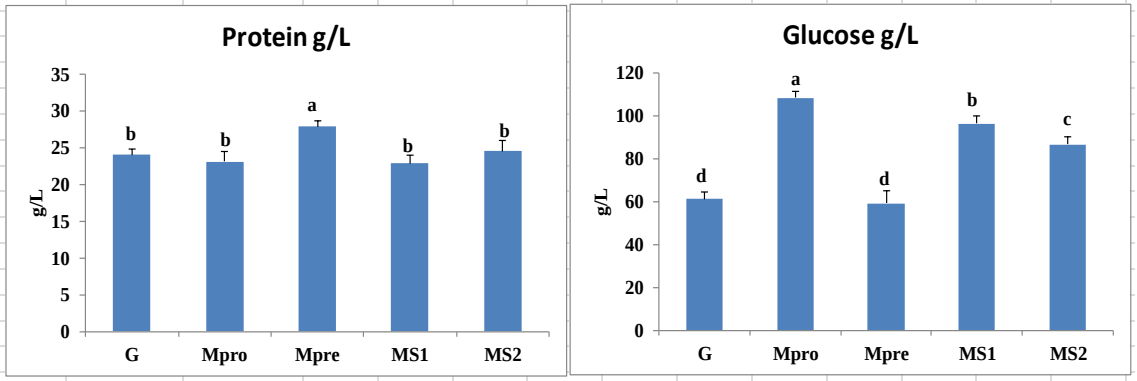

Fig. 2. Effect of marine synbiotic enriched microdiets on the 45 dph European seabass (*D. labrax*) larval protein and glucose content in g/L.

Different letters are for significance of treatments' effects (P < 0.05).

G: Greenwater control non treated; Mpro: Marine probiotic; Mpre: Marine prebiotic; MS1: Marine synbiotic 1 treated, and MS2: Marine synbiotic 2 treated microdiets.

Bacterial counts

VBC was neither found in water nor in larvae samples. BBC in water samples at the 45 dph was considerably (P<0.05) higher in MS1 and MS2. While, BBC in larvae samples at the 45 dph were considerably (P<0.05) higher in Mpro, MS1& MS2 (Fig. 6).

Histological examinations

The effect of 45dph *D. labrax* larval weaning using G, Mpro, Mpre, MS1 and MS2 treated microdiets on the histological sections in comparison with 31 dph larvae, which was the starting point of cofeeding in microdiets and weaning, as shown in Fig. (7). The effect of larval weaning using G, Mpro, Mpre, MS1 and MS2 treated microdiets on the histological sections in liver was explained in details. The liver of G control larvae showed homogenous hepatic parenchyma; the hepatocytes were polyhedral shaped cells with a cord-like arrangement of two or more hepatocytes thick. Hepatocytes have spherical centrally located nucleus, with densely staining chromatin margins and eosinophilic cytoplasm. Hepatocytes showed irregular vacuolization. Sinusoids appearing throughout in the interstitial connective tissue between the hepatic plates. The sinusoidal capillaries were narrow and irregularly shaped. Abundant red blood cells are found in the

liver sinus of G control larvae (Fig. 7aB). In the liver of the control fish as well as MS treated larvae, (Fig. 7Be and F) and 35 dph larvae, (Fig. 7aA), the tissue structure was clear and hepatic cells were regularly arranged. The hepatic cord and the hepatic sinusoid were connected to each other forming a net. The effect of larval weaning using G, Mpro, Mpre, MS1 and MS2 treated microdiets on the histological sections in kidney was determined. Kidney of G control larvae (Fig. 7aB) showed urineferous tubules with distal and proximal tubular types. Each tubule consists of a layer of columnar epithelial cells resting on a basement membrane with a wide lumen and hemopoietic tissue between tubules. No structural differences were detected in kidney structure of 35 dph larvae (Fig. 7aA) or G control (Fig. 7aB). In MS treated larvae, some epithelial cells of proximal tubules showed vacuolization (Figs. 7B, F).

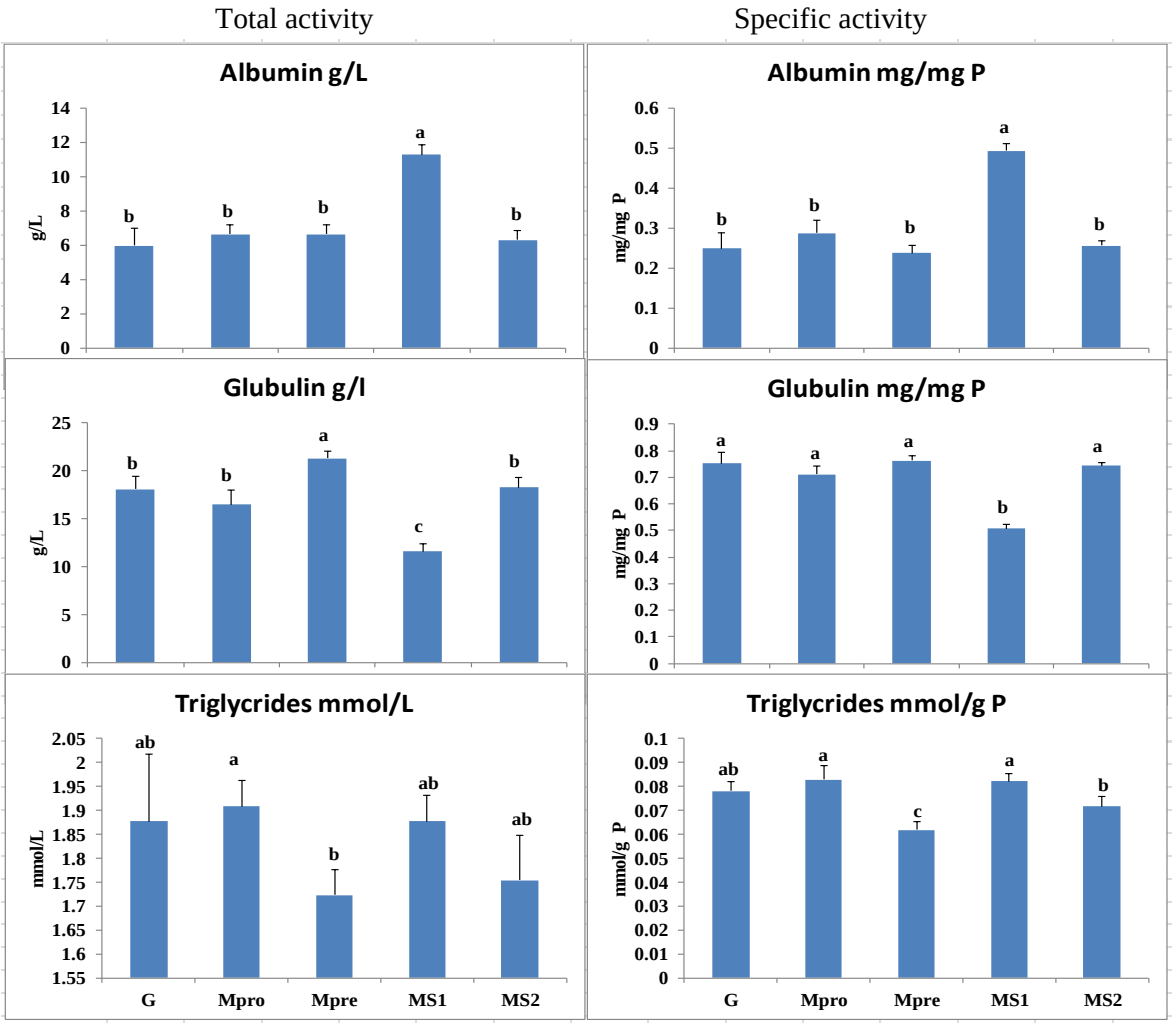

Fig. 3. Effect of marine synbiotic enriched microdiets on the 45 dph European seabass (*D. labrax*) larval albumin, globulin and triglycerides total and specific activities

Different letters are for significance of treatments effects ($P < 0.05$).

G: Greenwater control non treated; Mpro: Marine probiotic; Mpre: Marine prebiotic; MS1: Marine synbiotic 1 treated, and MS2: Marine synbiotic 2 treated microdiets.

Table 1. The effects of greenwater, marine probiotic, marine prebiotic, marine synbiotic 1 and marine synbiotic 2 on the European Seabass larvae final total length and final standard length growth performance between 30 and 45 dph

Treatment	G	Mpro	MPre	MS1	MS2
Initial total length (mm)	9.27 ± 0.12	9.07 ± 0.42	9.07 ± 0.42	9.07 ± 0.42	9.07 ± 0.42
Initial standard length (mm)	8.43 ± 0.06	8.30 ± 0.26	8.30 ± 0.26	8.30 ± 0.26	8.30 ± 0.26
Initial body width mm	1.37 ± 0.06	1.37 ± 0.06	1.37 ± 0.06	1.37 ± 0.06	1.37 ± 0.06
Final total length (mm)	10.50^{b} ± 0.10	13.17^{a} ± 0.59	13.00^{a} ± 1.21	14.00^{a} ± 0.80	13.10^{a} ± 0.56
Final standard length (mm)	9.60^{b} ± 0.10	11.10^{a} ± 0.26	11.40^{a} ± 1.20	12.17^{a} ± 1.10	11.50^{a} ± 0.62
Final body width (mm)	1.60^{b} ± 0.00	1.97^{a} ± 0.06	2.10^{a} ± 0.26	2.03^{a} ± 0.15	2.03^{a} ± 0.12
Final Survival%	46.59^{c} ± 1.84	55.02^{b} ± 3.03	54.22^{b} ± 2.41	66.27^{a} ± 5.25	65.06^{a} ± 4.34
Total length gain (mm)	1.23^{b} ± 0.06	4.10^{a} ± 0.95	3.93^{a} ± 1.12	4.93^{a} ± 0.58	4.03^{a} ± 0.45
Standard length gain (mm)	1.17^{b} ± 0.15	2.80^{a} ± 0.26	3.10^{a} ± 1.18	3.87^{a} ± 0.93	3.20^{a} ± 0.53
body width gain (mm)	0.23^{b} ± 0.06	0.60^{a} ± 0.10	0.73^{a} ± 0.25	0.67^{a} ± 0.12	0.67^{a} ± 0.06
Total length average daily gain (mm/day)	0.08^{b} ± 0.01	0.27^{a} ± 0.06	0.26^{a} ± 0.07	0.33^{a} ± 0.04	0.27^{a} ± 0.03
Standard length average daily gain (mm/day)	0.08^{b} ± 0.01	0.19^{a} ± 0.02	0.21^{a} ± 0.08	0.26^{a} ± 0.06	0.21^{a} ± 0.04
body width average daily gain (mm/day)	0.02^{b} ± 0.00	0.04^{a} ± 0.01	0.05^{a} ± 0.02	0.04^{a} ± 0.01	0.04^{a} ± 0.00
Total length specific growth rate (%/day)	0.61^{b} ± 0.13	4.03^{a} ± 0.70	3.88^{a} ± 0.90	4.61^{a} ± 0.33	4.03^{a} ± 0.32
Standard length specific growth rate (%/day)	0.43^{b} ± 0.39	2.97^{a} ± 0.28	3.11^{a} ± 1.25	3.86^{a} ± 0.68	3.34^{a} ± 0.46
Total length gain %	13.31^{b} ± 0.72	45.59^{a} ± 12.58	43.44^{a} ± 12.31	54.43^{a} ± 6.02	44.57^{a} ± 5.57
Standard length gain %	13.84^{b} ± 1.90	33.79^{a} ± 3.80	37.39^{a} ± 14.12	46.48^{a} ± 10.42	38.56^{a} ± 6.22
body width gain %	17.22^{b} ± 5.08	44.14^{a} ± 9.13	53.66^{a} ± 17.86	48.72^{a} ± 7.48	48.72^{a} ± 2.22

Different letters in the same row are for significance of treatments effects ($P < 0.05$).
G: Greenwater control non-treated; Mpro: Marine probiotic; Mpre: Marine prebiotic; MS1: Marine synbiotic 1, and MS2: Marine synbiotic 2 treated microdiets.

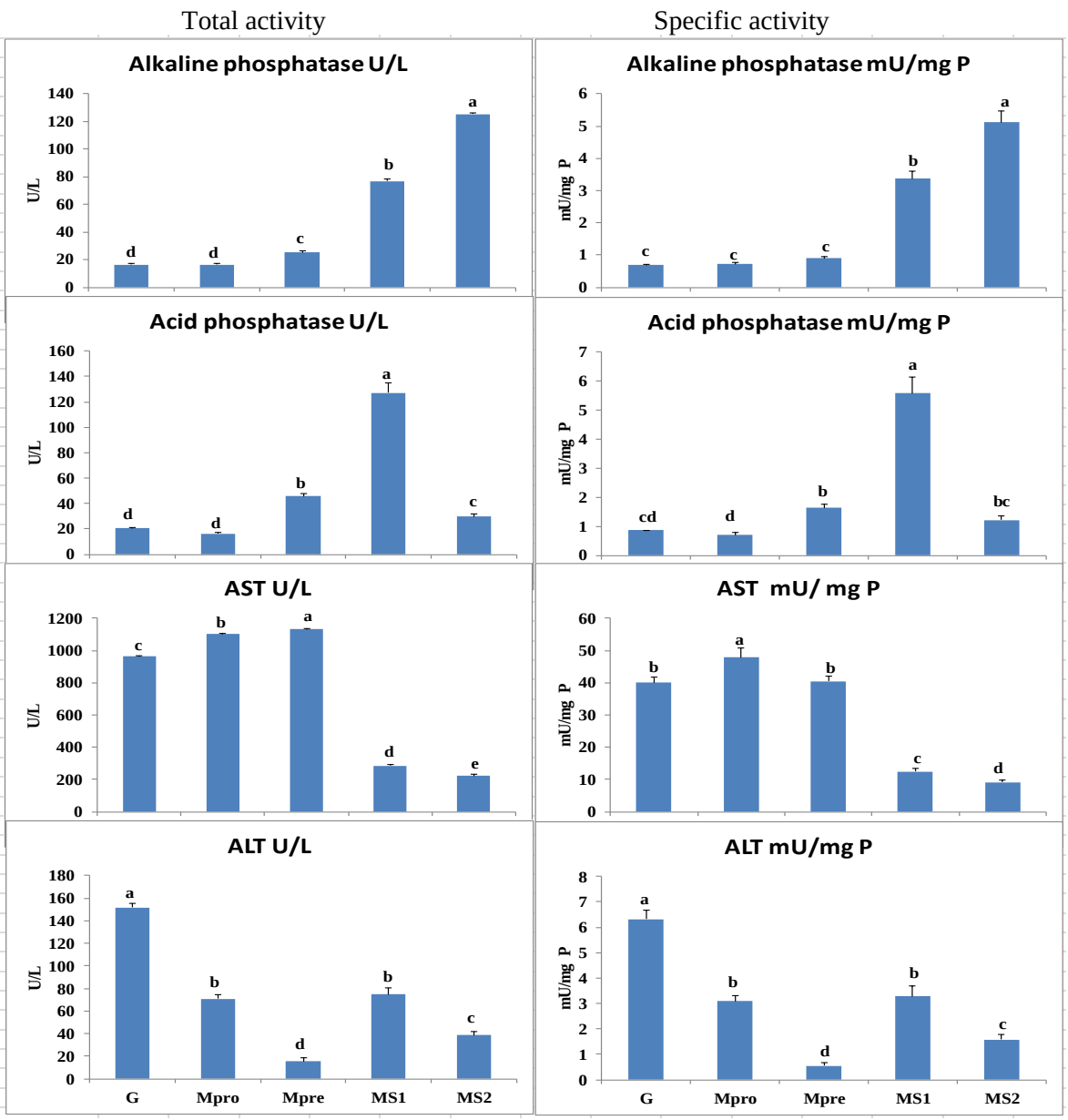

Fig. 4. Effect of marine synbiotic enriched microdiets on the 45 dph European seabass (*D. labrax*) larval ALP, AP, ALT and AST total and specific activities
Different letters are for significance of treatments effects ($P < 0.05$).
G: Greenwater control non treated; Mpro: Marine probiotic; Mpre: Marine prebiotic; MS1: Marine synbiotic 1 treated, and MS2: Marine synbiotic 2 treated microdiets.

DISCUSSION

The present results of the 45 dph weaned *D. labrax* larvae fed MPro, MPre, MS1 and MS2 exhibited significant length growth higher than G. MS1 and MS2, and recorded the best significant survival %. **Salem *et al.* (2015)** recorded that *Bacillus subtilis* HS1 marine probiotic (the same Mpro strain and dose included in the present study MS) and commercial synbiotic treated rotifers, enriched *Artemia*, sea bass yolk sac larvae and first

fed larvae were added to weaning tanks until weaning showed significant improvement of larval growth and survival. **Zaki *et al*. (2015)** suggested that chitosan marine prebiotic (the same source used in the present study) combined with sea bass fish fry diets improved the survival and fish growth performance. Synbiotics have revealed ameliorated growth, immune and antioxidative influences on aquatic animals **(Lamari *et al*., 2016; Ringø & Seong, 2016; Huynh *et al*., 2017)**. In addition, synbiotic improved the disease resistance, survival, and host microbial ecology **(Ohtani *et al*., 2020; Knipe *et al*., 2021; Hong *et al*., 2022; Salem *et al*., 2022).**

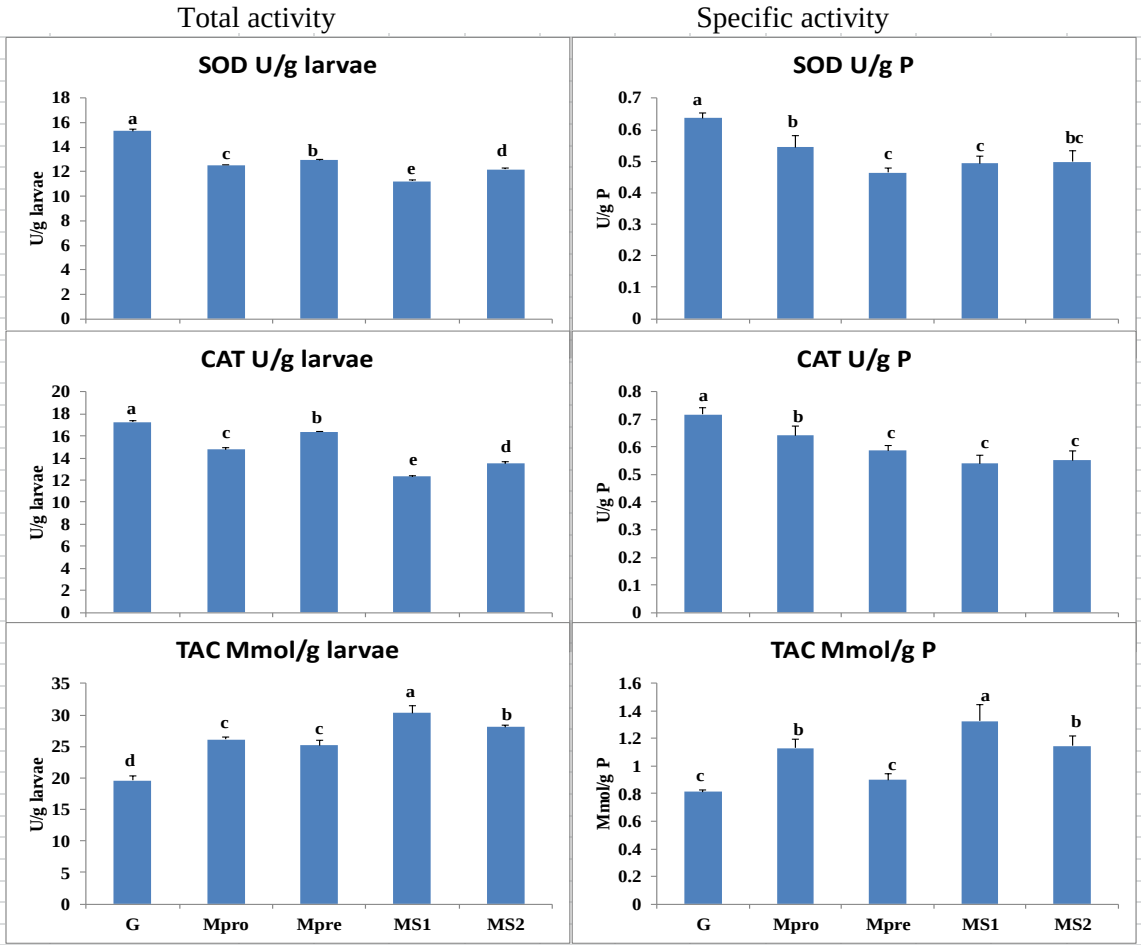

Fig. 5. Effect of marine synbiotic enriched microdiets on the 45 dph European seabass (*D. labrax*) larval antioxidants biomarkers total and specific activities
Different letters are for treatments effects significance ($P < 0.05$).
G: Greenwater control non-treated; Mpro: Marine probiotic; Mpre: Marine prebiotic; MS1: Marine synbiotic 1 treated, and MS2: Marine synbiotic 2 treated microdiets.

In the current investigation, larvae fed Mpre showed the highest significant values for total protein in g/l. While, larvae fed Mpro showed the highest significant total glucose, globulin activities and TAG. On the other hand, larvae fed MS1 showed significantly higher values of total, specific albumin and specific globulin activities, and those fed MS1 and Mpro recorded the highest significant specific TAG activity. It was

noticed that, synbiotic reduced food conversion ratio, carcass lipid, Serum cholesterol, globulin **(Ringø & Song, 2016)**. Moreover, **Hassan *et al.* (2014)** indicated that synbiotic expressively improved total protein content and albumin. Furthermore, synbiotic (Biomin IMBO) improved serum protein, albumin, and globulin of rainbow trout **(Mehrabi *et al.* 2011)**. **Desouky *et al.* (2020)** reported that the fish energetic metabolites (triglycerides and cholesterols) levels are health indications **(Mensinger *et al.* 2005)**. **Desouky *et al.* (2020)** related fish low growth and their high crude lipid content to the high fat diet.

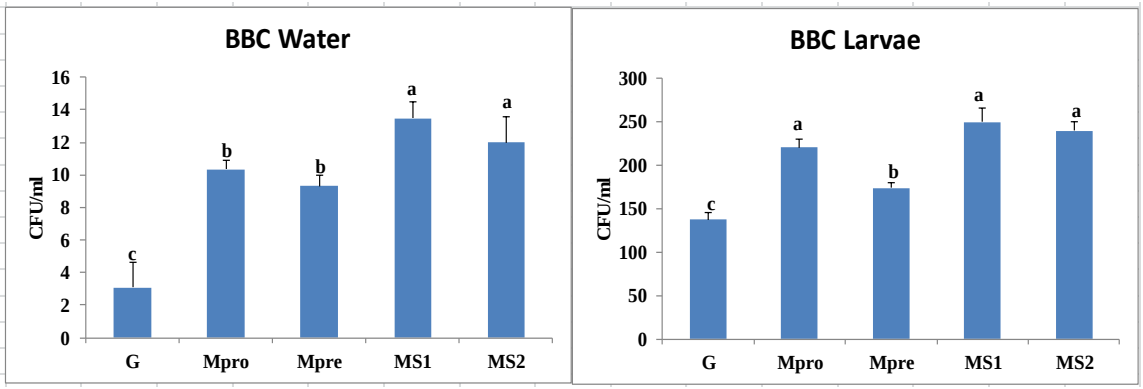

Fig. 6. Effect of marine synbiotic enriched microdiets on the 45 dph European seabass (*D. labrax*) larval water quality, water and larval bacterial counts quality
Different letters are for significance of treatments effects ($P < 0.05$).
G: Greenwater control non-treated; Mpro: Marine probiotic; Mpre: Marine prebiotic; MS1: Marine synbiotic 1 treated, and MS2: Marine synbiotic 2 treated microdiets.

In the present study, larvae fed MS2 and MS1 recoded the highest significant ALP and AP enzymes of total and specific activities, respectively. In addition, larvae fed Mpre recoded the highest significant AST total activity; while, those fed Mpro showed the highest significant AST specific activity. The larvae fed G recoded the highest significant ALT total and specific activities. ALT and AST enzymes are principal liver enzymes. Mostly during liver cell damage, ALT and AST are discharged substantially into animal blood **(Kumar *et al.*, 2011; Hassan *et al.*, 2014)**. Nile tilapia fed probiotics diets considerably reduced ALT and AST levels **(Soltan & El-Laithy, 2008)**.

AKP and AP enzymes had important roles on the digestive process and on bone mineralization, formation and resorption respectively **(Piattelli *et al.*, 1997)**. Also, gilthead seabream (*Sparus (S.) aurata*) larvae AP and AKP increased at late weaning (W43) at 70dph **(Salem *et al.*, 2021)**, white seabream (*Diplodus sargus*) larvae AMP and AKP increased at late weaning (W27) at 48 dph (T3) **(Guerreiro *et al.*, 2010)** and *D. Puntazzo* intestinal enzymes activities increased until 50 dph **(Suzer *et al.*, 2007)**. While, for AKP at 9 dph *D. sargus* **(Cara *et al.*, 2003)** and for *Dentex dentex* AKP and AMP were high at 6 dph **(Gisbert *et al.*, 2009)**. **Salem *et al.* (2019)** exposed that peal prebiotic decreased ALP in sea bream. **Dehaghani *et al.* (2015)** indicated that ALP was considerably higher in the control. **Suzer *et al.* (2008)** revealed that ALP in gilthead sea

bream larvae improved when *Lactobacillus* spp. Bacteria applied. **Song *et al*. (2006)** observed that AP activity and the immune system increased in *Miichtys miicy* fed with *C. butyricum*. **Zhang *et al*. (2013)** revealed that black Amur bream notably improved ALP, plasma AP, lysozyme, total serum protein, globulin, IgM, TAC when fed FOS and *B. licheniformis* synbiotic **(Ringo & song, 2016)**. **Li *et al*. (2009)** revealed that inclusion *Bacillus* and IMOS synbiotic improved growth performance, AP and ALP activities.

The current results detected that the larvae fed G showed high significant effect on body SOD, CAT total and specific activities. The larvae fed MS1 showed the highest significant of body TAC total and specific activities. **Thilagam *et al*. (2010)** revealed that ROS may decrease SOD and CAT activity as a chain reaction, or SOD and CAT reduction might induce additional ROS production. **Salem *et al*. (2019)** indicated that orange peel prebiotic improved oxidative stress resistance (SOD, CAT, GSH-Px and TAC activities) while reduced tissue damage, lipid peroxidation in sea bream. **Lamari *et al*. (2016)** recorded that *Lactobacillus casei* probiotic upregulated CAT at 20 dph sea bass, while there was no considerable difference among the antioxidant enzymes (CAT, SOD and GPX) at 20 and 41 dph sea bass.

Lamari *et al*. (2016) revealed that pathogenic *Vibrio* did not infect Sea bass larvae as well as in the current study as well as **Salem *et al*., (2018)** determined that *Vibrio sp.* was not detected in European sea bass fry and rearing water which was the same source of the present study larvae and water. Synbiotic reduced intestinal total viable bacterial counts and *Vibrio* counts **(Li *et al*., 2009)**. The present study BBC in water samples at 45dph was significantly higher in MS1 and MS2. BBC in larvae samples at 45dph were significantly higher in Mpro, MS1 and MS2. In accordance, **Salem *et al*. (2015)** recorded that *Bacillus subtilis* HS1 marine probiotic (Mpro of the present study) and commercial synbiotic plus enzymes treating rotifers, *Artemia* enrichment and sea bass yolk sac larvae and first fed larvae to weaning tanks showed significant improvement of total bacterial and *Bacillus* sp. counts while significantly decreased the *Vibrio sp., Aeromonas sp.* and *Staphylococcus sp.* counts Vs. control treatment. **Salem *et al*., (2022)** revealed a significant improvement of larval length, weight growth, and survival with MS (MS1 in the present study) dietary supplementation. The ALP enzyme showed a significant increase after MS treatment. The SOD and CAT activities showed a significant decrease after MS treatment. MS treated sea bass larvae improved growth rate and survival, decreased the negative impacts of E2 and increased and ameliorated morphological structure of liver and kidney of ES (MS and E2 mixture) compared with E2 treatments.

Table 2. The effects of treatments on the European Seabass larvae final body parts [head(H), trunk (TR) and tail (TA)] length growth performance between 30 and 45 dph

Treatment	G			MPRO			MPRE			MS1			MS2		
IHL mm	1.67	±	0.15	1.67	±	0.15	1.67	±	0.15	1.67	±	0.15	1.67	±	0.15
ITRL mm	3.80	±	0.10	3.80	±	0.10	3.80	±	0.10	3.80	±	0.10	3.80	±	0.10
ITAL mm	3.57	±	0.15	3.57	±	0.15	3.57	±	0.15	3.57	±	0.15	3.57	±	0.15
FHL mm	2.17^{b}	±	0.15	2.50^{ab}	±	0.26	2.70^{ab}	±	0.50	2.70^{ab}	±	0.26	2.77^{a}	±	0.21
FTRL mm	4.70^{b}	±	0.46	5.50^{a}	±	0.53	5.40^{a}	±	0.30	5.33^{ab}	±	0.15	5.20^{ab}	±	0.35
FTAL mm	3.67^{b}	±	0.35	5.10^{a}	±	0.26	5.43^{a}	±	0.51	5.57^{a}	±	0.06	5.20^{a}	±	0.26
LG HL mm	0.50^{b}	±	0.20	0.83^{ab}	±	0.15	1.03^{a}	±	0.47	1.03^{a}	±	0.15	1.10^{a}	±	0.20
LG TRL mm	0.90^{b}	±	0.40	1.70^{a}	±	0.56	1.60^{a}	±	0.36	1.53^{ab}	±	0.15	1.40^{ab}	±	0.26
LG TAL mm	0.10^{b}	±	0.35	1.53^{a}	±	0.42	1.87^{a}	±	0.45	2.00^{a}	±	0.20	1.63^{a}	±	0.15
ADG HL mm/day	0.03^{b}	±	0.01	0.06^{ab}	±	0.01	0.07^{a}	±	0.03	0.07^{a}	±	0.01	0.07^{a}	±	0.01
ADG TRL mm/day	0.06^{b}	±	0.03	0.11^{a}	±	0.04	0.11^{a}	±	0.02	0.10^{ab}	±	0.01	0.09^{ab}	±	0.02
ADG TAL mm/day	0.01^{b}	±	0.02	0.10^{a}	±	0.03	0.12^{a}	±	0.03	0.13^{a}	±	0.01	0.11^{a}	±	0.01
G% HL %	30.70^{b}	±	14.73	49.98^{ab}	±	7.74	62.40^{a}	±	28.59	62.05^{a}	±	7.72	66.60^{a}	±	14.46
G% TRL %	23.61^{b}	±	10.11	44.85^{a}	±	15.30	42.24^{a}	±	10.14	40.39^{ab}	±	4.42	36.77^{ab}	±	6.17
G% TAL %	2.87^{b}	±	9.71	43.38^{a}	±	13.76	52.31^{a}	±	12.04	56.30^{a}	±	8.05	45.79^{a}	±	3.66

Different letters in the same row are for treatments effects significance ($P < 0.05$).

G: Greenwater control non treated; MS: Marine probiotic; MS: Marine prebiotic; MS1: Marine synbiotic 1, and MS2: Marine synbiotic 2 treated microdiets.

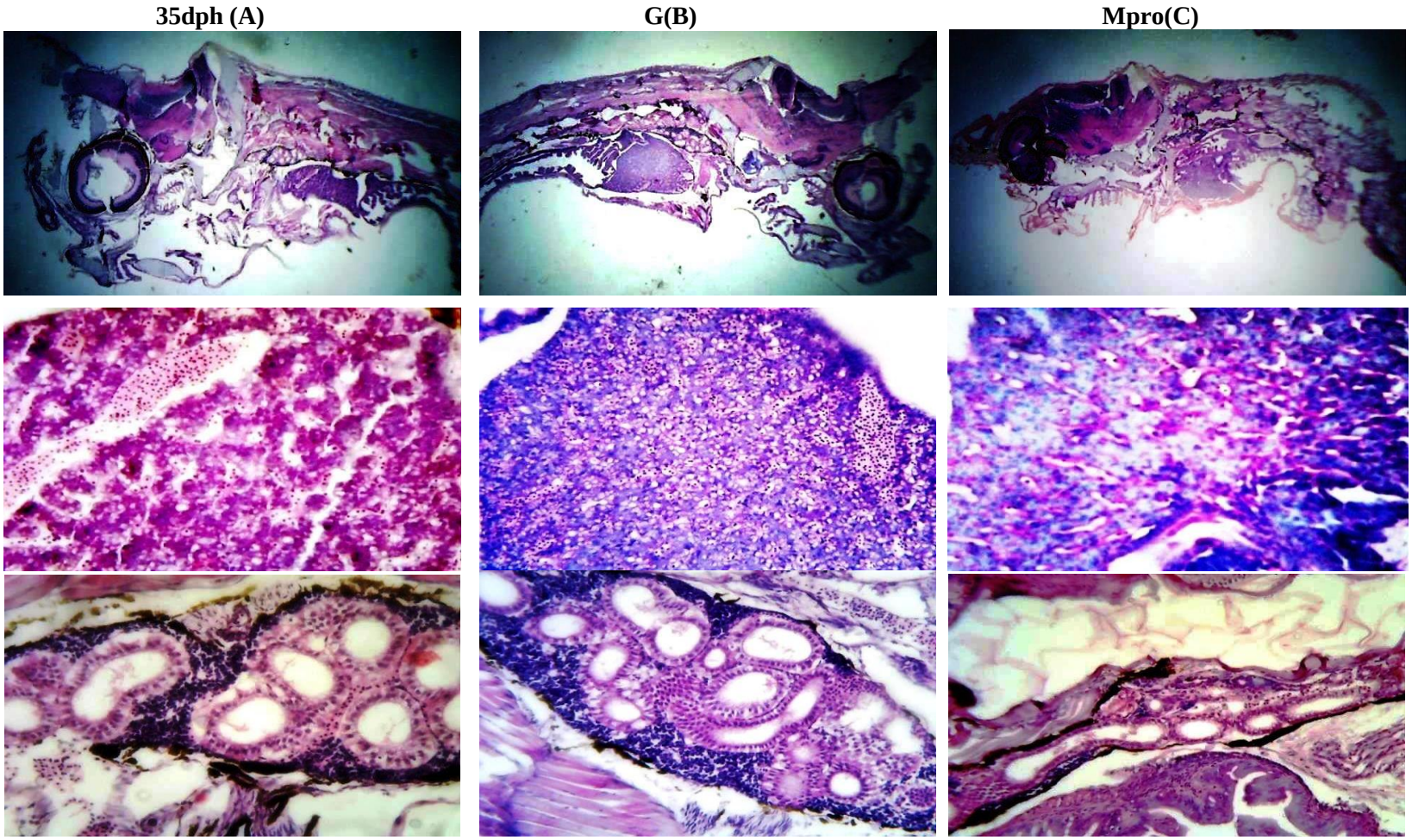

Figure 7a. photomicrograph of *D. labrax* larvae at 31dph(A) and at 45dph G(B), Mpro(C) larvae (H&E X40), liver and kidney larvae (H&E X400).

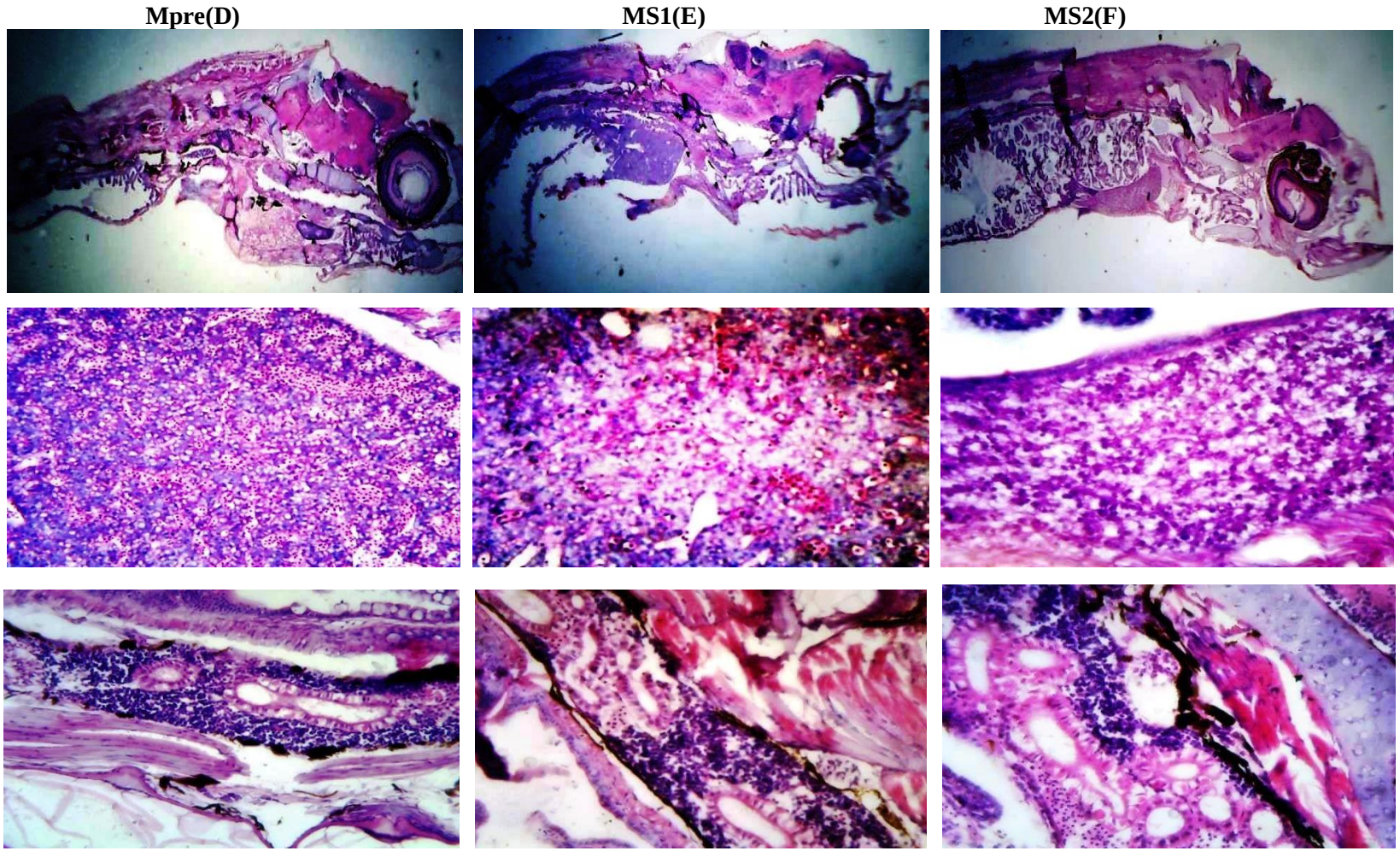

Figure 7b. photomicrograph of 45dph *D. labrax* **larvae at Mpre(D), MS1(E) and MS2(F) larvae (H&E X40), liver and kidney larvae (H&E X400).**

CONCLUSION

The current findings revealed a significant improvement of larval length, weight growth, and survival with MS dietary supplementation than G. The MS1 and MS2 exhibited best length growth performance, survival, SOD and CAT activities, and TAC improvement after treatments compared to G. The larvae fed MS2 and MS1 recoded the highest considerable alkaline phosphatase (ALP) and acid phosphatase (AP) enzymes total and specific activities, respectively. The larvae fed Mpre recoded the highest significant aspartate aminotransferase (AST) total activity; while, larvae fed Mpro recoded the highest substantial AST specific activity. The larvae fed G recoded the highest meaningful alanine aminotransferase (ALT) total and specific activities. In conclusion, mixing marine probiotics and prebiotics in synbiotics treatments improved European sea bass larval growth, survival, and antioxidant capacity.

ACKNOWLEDGMENTS

The authors wish to thank Prof. Dr. Noha Khalil, Dr. Shaimaa M. Hebalah and the team of NIOF marine hatchery for their support and help throughout the experimental work, Dr. Ahmed Abu El-Wafa for his help in fry and water microbiology.

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