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Impacts of Dietary Marine Synbiotic Solid State Fermentation and Supplemention on European Seabass Larvae Weaning, Growth and Oxidative Stress

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ABSTRACT

A European seabass (Dicentrarchus labrax) weaning trial was conducted from the 30th to the 45th day post-hatching (dph). Larvae aged 3 to 30 dph were reared and fed in the same 2m² tank. In this experiment, several hepatic enzymes and antioxidant parameters were examined, including catalase (CAT), superoxide dismutase (SOD), and total antioxidant capacity (TAC) in larvae subjected to five treatments: control (G); solid-state fermented control (GF); marine synbiotic supplemented (MS); marine synbiotic solid-state fermented (MSF); and marine synbiotic solid-state fermented and resupplemented (MSSF) microdiets. The early weaned larvae fed MS and MSF exhibited significantly greater growth in length during both the 5-45 dph and 30-45 dph periods compared to the other weaning microdiets. Furthermore, larvae fed the MSF microdiet showed significantly better weight growth performance during both periods than those fed the other microdiets. Larvae consuming MS demonstrated the highest activities of glucose, albumin, alkaline phosphatase (ALP), and acid phosphatase (AP). In contrast, larvae consuming the MSF microdiet exhibited notably higher globulin activity. The larvae fed G and GF showed elevated CAT and SOD total activities, while those fed G presented significantly high specific activities of CAT and SOD. Notably, larvae consuming MS revealed the highest total TAC, while those fed MS and MSF displayed the greatest specific TAC activities. In conclusion, synbiotics supplemented or solid-state fermented microdiets enhanced the growth, survival, and antioxidant capacity of the European seabass larvae.

INTRODUCTION

Worldwide, a major bottleneck in aquaculture development remains the scarcity of fish fry in both quantity and quality **(Giebichenstein *et al.*, 2022)**. Significant progress has been made in understanding fish larval nutrition and physiology **(Rønnestad *et al.*, 2013; Yúfera, 2018; Izquierdo *et al.*, 2019; Salem *et al.*, 2021; Giebichenstein *et al.*, 2022; Salem & Ibrahim, 2022)**. Many additives have been developed for aquaculture, including synbiotics, to reduce the use of antibiotics and mitigate the risks of antimicrobial resistance in humans and animals **(Ringø *et al.*, 2020; Villumsen *et al.*, 2020; Knipe *et al.*, 2021; Hong *et al.*, 2022; Monzón-Atienza *et al.*, 2023)**. Synbiotics have been applied to several aquaculture species, including the European seabass

(Hassan *et al.*, 2014; Salem *et al.*, 2015; Huynh *et al.*, 2017; Villumsen *et al.*, 2020; Salem & Ibrahim, 2022; Salem *et al.*, 2022). Research has shown that synbiotics enhance growth, disease resistance, survival, microbiological health, and immune and antioxidative functions in aquatic animals **(Hassan *et al.*, 2014; Lamari *et al.*, 2016; Ringø & Seong, 2016; Huynh *et al.*, 2017; Ohtani *et al.*, 2020; Knipe *et al.*, 2021; Hong *et al.*, 2022; Salem & Ibrahim, 2022; Salem *et al.*, 2022)**.

Solid-state fermentation (SSF) improves the nutritional quality of plant feedstuffs by reducing antinutritional factors, enhancing palatability, and balancing amino acid content **(Vieira *et al.*, 2023)**. SSF of seaweeds and plant feedstuffs has been shown to improve growth, nutritional utilization, and physiology in the turbot (*Scophthalmus maximus*) **(Li *et al.*, 2019)**, the Rohu (*Labeo rohita*) **(Das *et al.*, 2021)**, the rainbow trout (*Oncorhynchus mykiss*) **(Davies *et al.*, 2021)**, the European seabass **(Fernandes *et al.*, 2021; Fernandes *et al.*, 2022a, b; Amaral *et al.*, 2023; Vieira *et al.*, 2023)**, the European seabass (*D. labrax*) **(Fernandes *et al.*, 2022a; Amaral *et al.*, 2023)**, and the Nile tilapia (*Oreochromis niloticus*) **(Magouz *et al.*, 2022)**.

Marine fish larvae have high metabolic activity, and the enrichment of live feeds with polyunsaturated fatty acids creates a pro-oxidant environment that can compromise the adequate supply of these fatty acids **(Viciano *et al.*, 2017; Galindo *et al.*, 2024)**. Antioxidant enzymes reduce lipid peroxidation and halt oxidation reactions **(Betancor *et al.*, 2012; Hamed *et al.*, 2020; Salem & Ibrahim, 2022)**. Recent research has discussed the impacts of marine synbiotics (MS), marine synbiotic solid-state fermentation (MSF), and marine synbiotic solid-state fermentation and resupplementation (MSSF) on oxidative stress, larval growth, survival, and enzyme activity in the European seabass.

MATERIALS AND METHODS

Experimental design

The European seabass larvae were collected from farmed broodstock induced to spawn at the Fish Reproduction and Spawning Lab, Marine Hatchery (N: 31°12'46.2", E: 29°53'06.1"), Aquaculture Division, NIOF. The larvae were reared in a greenwater flow system within the larval rearing unit and fed enriched rotifers and Artemia from 3 dph to 30 dph in a 2m² tank. On the 30th dph, triplicate groups of 100 larvae each were transferred to 30L aquariums within fifteen glass tanks, where a weaning trial was conducted from 30th to 45th dph. The larval feeding and rearing procedures were described by **Salem and Ibrahim (2022)**.

Five treatments of Inve Orange® weaning microdiets were applied: control greenwater without treatment (G), solid-state fermented (GF), a marine synbiotic developed by **Salem and Ibrahim (2022)** (1×10^7 CFU *Bacillus (B.) subtilis* HS1 + 1mg marine chitosan per gram of microdiet) supplemented (MS), marine synbiotic solid-state

fermented (MSF), and marine synbiotic solid-state fermented and resupplemented (MSSF) microdiets (Fig. 1). The marine synbiotic used in the current investigation showed effective outcomes as reported by **Salem and Ibrahim (2022)**. The SSF of the MSF and MSSF treatments involved mixing the synbiotic with the microdiet and incubating it at 30°C for 24 hours **(Imelda Joseph *et al.*, 2008)**.

Fish growth and survival measurements

Length, weight growth performance, condition factor and survival % were measured following the method of **Salem *et al.* (2022)**.

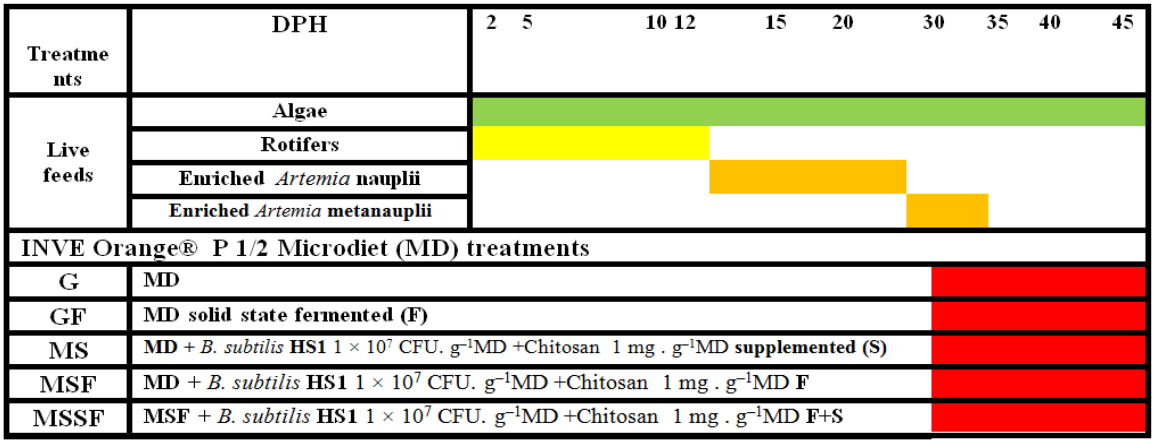

Fig. 1. The European seabass (*D. labrax*) larvae rearing and weaning treatments.
G: Greenwater control non treated; GF: Control solid state fermented; MS: Marine synbiotic supplemented; MSF: Marine synbiotic solid state fermented; and MSSF: Marine synbiotic solid state fermented and resupplemented microdiets.

Water quality measurement

Water parameters were weekly quantified at 2 PM using a Hanna® HI9828 portable electric device. The measured volumes were as follows: dissolved oxygen (%): 91.38 - 104.28; temperature (°C): 17.71 - 19.11; pH: 7.28 - 8.47; total dissolved solids (ppm): 76.96 - 144.08; conductivity (mS/cm): 169.32 - 221.87; and salinity (ppt): 36.02 - 39.48.

Microbiological counts

B. bacterial count (BBC) and *Vibrio* bacterial count (VBC) colony forming unit (CFU) were performed in the Microbiology Lab., Marine Environment Division, NIOF following the method described by **Salem *et al.* (2022)**.

Antioxidants biomarkers and enzymes

Larvae CAT, SOD, TAC antioxidants, total protein, albumin, globulin, glucose, ALP, AP, AST, ALT, and TAG enzymes total and specific activities were assessed using

Spectrophotometer model: 01102, LAXCO, Inc., USA and Biodiagnostic Company kits, Cairo, Egypt, following the method of **Salem *et al.* (2022)**.

Statistical analysis

The one-way analysis of variance (ANOVA) was conducted at a 95% confidence level, with means compared using Duncan's test ($P< 0.05$) to evaluate all data using SPSS software (SPSS for Windows 16; SPSS Inc., Chicago, IL, USA).

RESULTS

Larval growth and survival

The larvae consumed MS and MSF demonstrated greater meaningful ($P< 0.05$) length growth performance of 30-45 dph larvae, including final total length (FTL), standard total length (STL), total length gain (TLG) and standard total length gain (STLG) in mm, total length average daily gain (TLADG) and standard length average daily gain (STLADG) in mm day-1, total length specific growth rate (TLSGR)% day-1 and standard length specific growth rate (STLSGR)% day-1 and standard length gain % (STLG%) than other weaning microdiets. Larvae fed MSF revealed greater considerable ($P< 0.05$) total length gain% (TLG%). Larvae fed MS and MSF exhibited greater substantial ($P< 0.05$) length growth performances of 5-45 dph larvae including, TLG in mm, TLADG in mm day-1, TLSGR% day-1 and TLG% than other weaning microdiets. Larvae consumed G exhibited greater noteworthy ($P < 0.05$) total length condition factor (KTL) and standard length condition factor (KSTL) than other weaning microdiets (Fig. 2).

Larvae consumed MSF disclosed better expressive ($P< 0.05$) final weight (FW) in mg, weight growth performances (Fig. 3) including weight gain (WG) in mg and weight average daily gain (WADG) in mg day-1 of both 5-45 and 30-45 dph larvae and also for 30-45 dph weight gain% (WG%) in % than other weaning microdiets. Larvae fed MS and MSF showed better significant ($P< 0.05$) survival % and weight specific growth rate (WSGR)% day-1 of both 5-45 and 30- 45dph larvae and also for 5- 45dph weight gain% (WG%) in % than other weaning microdiets.

Enzymes activities

The larvae fed the MS diet exhibited the highest significant ($P< 0.05$) total and specific activities of glucose, albumin, alkaline phosphatase (ALP), and acid phosphatase (AP). Also, MSF treatment exhibited the best noteworthy ($P< 0.05$) globulin specific activity. Larvae consumed G showed the topmost noteworthy ($P< 0.05$) activities of AST and ALT. G and GF treatments demonstrated the greatest noteworthy ($P< 0.05$) triglycrides specific activity. G, GF and MS treatments exposed the top significant ($P<$

0.05) triglycrides total activity. Larvae fed G, GF, MSF and MSSF showed the highest significant ($P< 0.05$) globulin total activitiy compared to other microdiets (Figs. 4, 5).

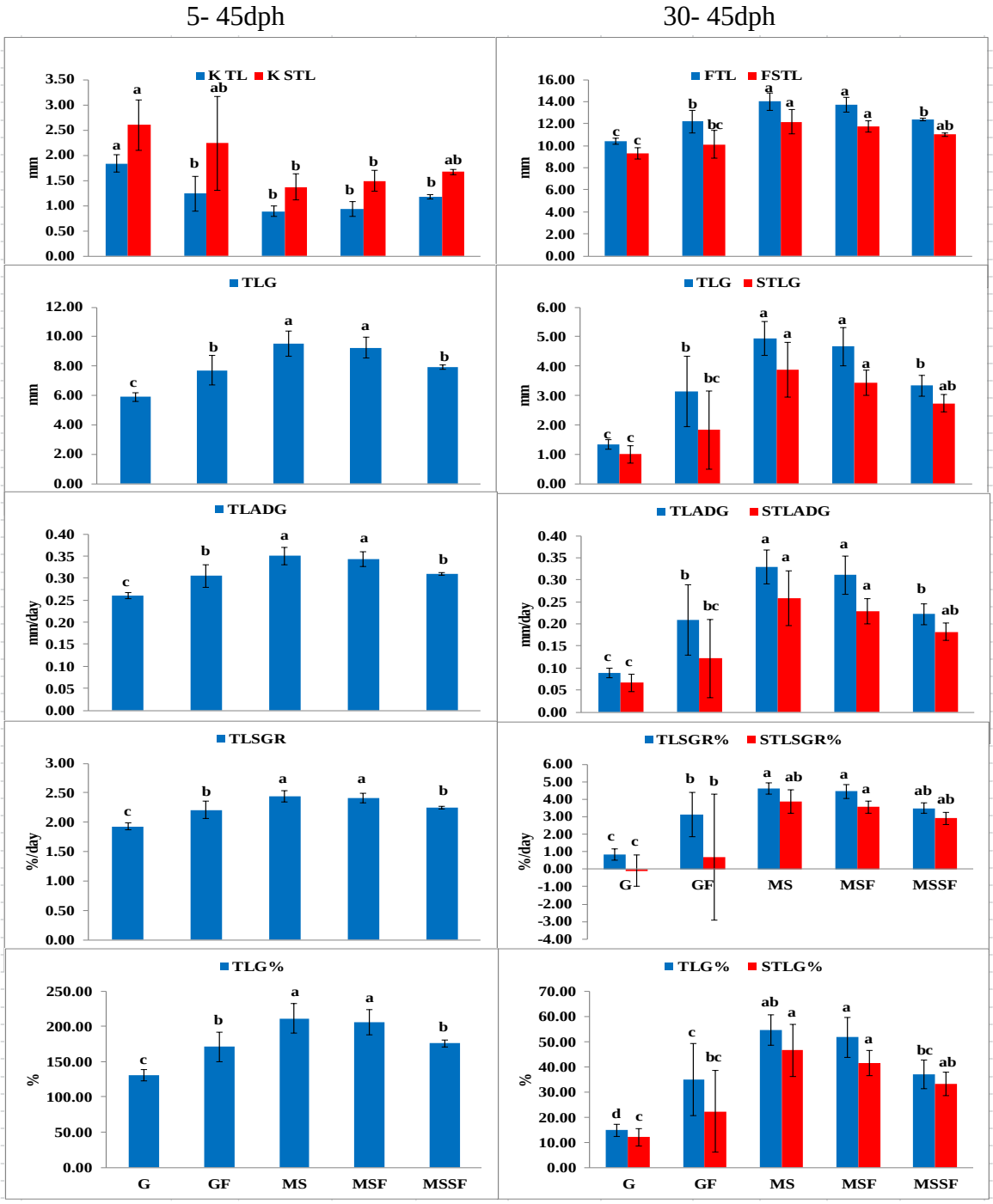

Fig. 2. European Seabass (*D. labrax*) larval length growth performance. G: Greenwater control non treated; GF: Control solid state fermented; MS: Marine synbiotic supplenented; MSf: Marine synbiotic solid state fermented; MSSF: Marine synbiotic solid state fermented and resupplenented microdiets. Letters refer to treatments effects dissimilar significance ($P< 0.05$).

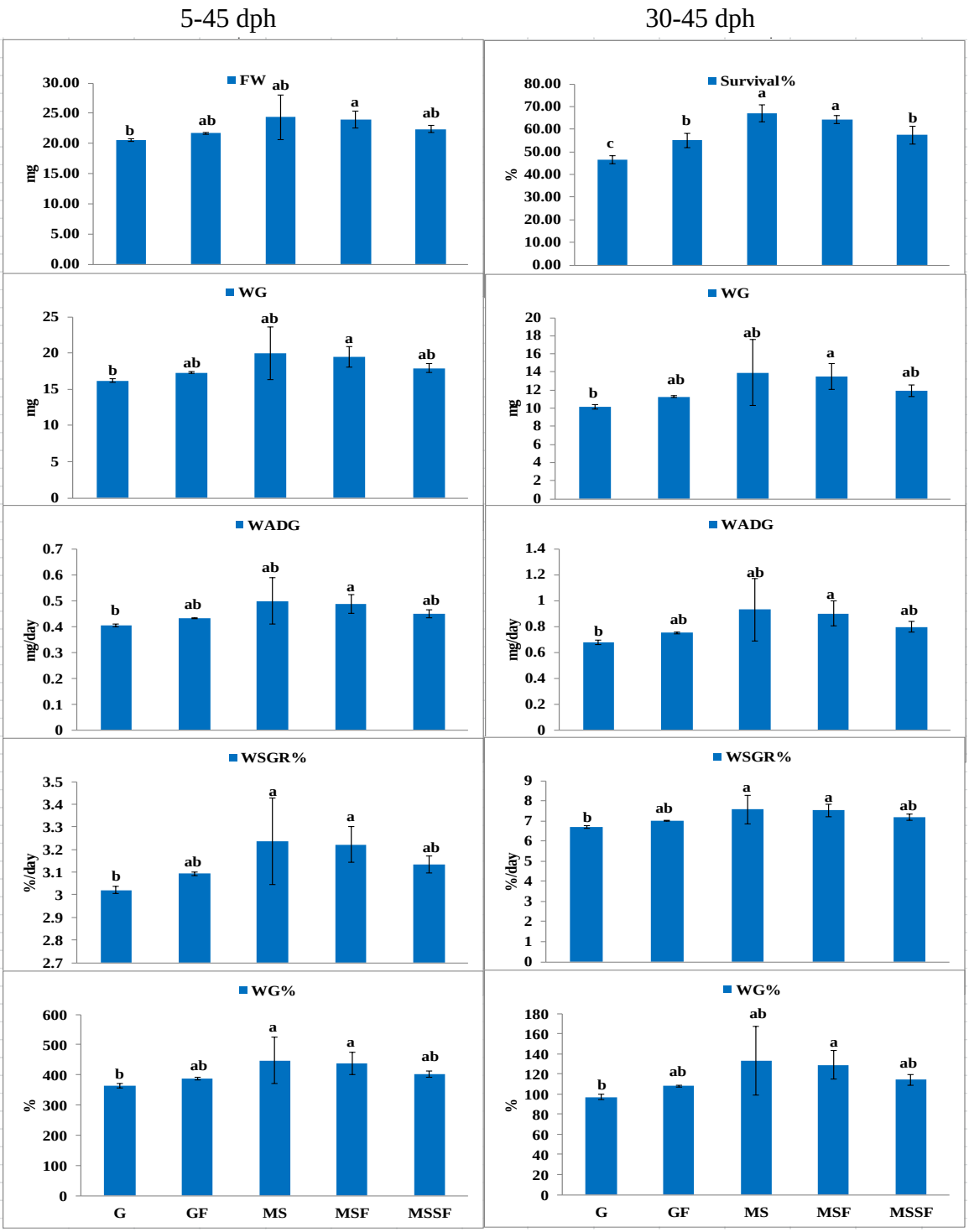

Fig. 3. European seabass (*D. labrax*) larval survival% and weight growth performance. G: Greenwater control non treated; GF: Control solid state fermented; MS: Marine synbiotic supplemented; MSf: Marine synbiotic solid state fermented; MSSF: Marine synbiotic solid state fermented and resupplenented microdiets. Letters refer to treatments effects dissimilar significance ($P< 0.05$).

Antioxidants biomarkers

The current results indicate that larvae fed the MS diet exhibited the highest significant ($P< 0.05$) total antioxidant capacity (TAC) in their bodies, while both MS and MSF treatments showed the greatest specific TAC activities ($P< 0.05$). Additionally, larvae fed the G and GF diets demonstrated elevated total activities of catalase (CAT) and superoxide dismutase (SOD) ($P< 0.05$), with those fed the G diet exhibiting significantly higher specific activities of CAT and SOD ($P< 0.05$) (Fig. 6).

Bacterial counts

In both the water and larvae samples, VBC were not observed. While, BBC in water and larvae at 45 dph were greatly ($P< 0.05$) upper in MSF and MSSF (Fig.7).

DISCUSSION

Solid-state fermentation (SSF) has been shown to improve the nutritional value of plant feed meals **(Chebaibi *et al*., 2019; Vandenberghe *et al.,* 2021)**. It positively affects the growth performance of several species, including the Nile tilapia (*Oreochromis niloticus*), rainbow trout (*Oncorhynchus mykiss*), Rohu (*Labeo rohita*), turbot (*Scophthalmus maximus*), and European seabass (*D. labrax*) **(Ghosh & Mandal, 2015; Hassaan *et al.,* 2017; Li *et al.,* 2019; Das *et al.,* 2021; Davies *et al.,* 2021; Fernandes *et al.,* 2022a; Amaral *et al*., 2023)**. The current outcomes of the early weaned *D. labrax* larvae consumed MS and MSF displayed greater substantial growth performances than other weaning microdiets including MSSF that have double dose treatment fermented and resupplemented, which may caused over stimulation and over dose that resulted in a reduced growth than single treatments supplemented or fermented. **Ringø and Seong (2016)** and **Huynh *et al*. (2017)** verified that synbiotics enhanced aquatic animals growth, immune- and antioxidative impacts. **Moniruzzaman *et al.* (2018)** disclosed that the rainbow trout considerably reduced growth when fed 22% *B. subtilis* SSF diet substitution, while 13% substitution did not affect growth. Similaly, **Amaral *et al.* (2023)** indicated that feed consumption and growth was decreased in fish consumed the *Aspergillus niger* SSF diet. These may be due to the *A. niger* and inclusion levels reduced palatability. **Amaral *et al.* (2023)** suggested that the 20% *A. niger* fermented plants meals included in seabass diet risked the diet palatability and reduced voluntary feed intake, causing lower growth performance. The present study larvae survival % notably improved when fed MS and MSF compared to other weaning microdiets. Conversely, **Salem *et al.* (2022)** revealed that the marine synbiotic (MS) showed positive effects, while **Salem and Ibrahim (2022)** demonstrated that *B. subtilis* HS1 and chitosan, along with MS1 and MS2, contributed to improvements in seabass larval length, weight growth, and survival percentage. Additionally, chitosan was found to benefit the seabass fry **(Zaki *et al.,* 2015)**.

Larvae fed MSF microdiet exhibited the best noteworthy globulin specific activity. Also, glucose, albumin, ALP and AP activities considerably increased when larvae consumed MS. Correspondingly, **Salem and Ibrahim (2022)** postulated that MS1 displayed expressively upper albumin and globulin activities. Similarly, synbiotics dramatically enhanced food conversion ratio, cholesterol, lipid, total protein content, albumin and globulin **(Mehrabi *et al.*, 2011; Hassan *et al.*, 2014; Ringø & Song, 2016)**.

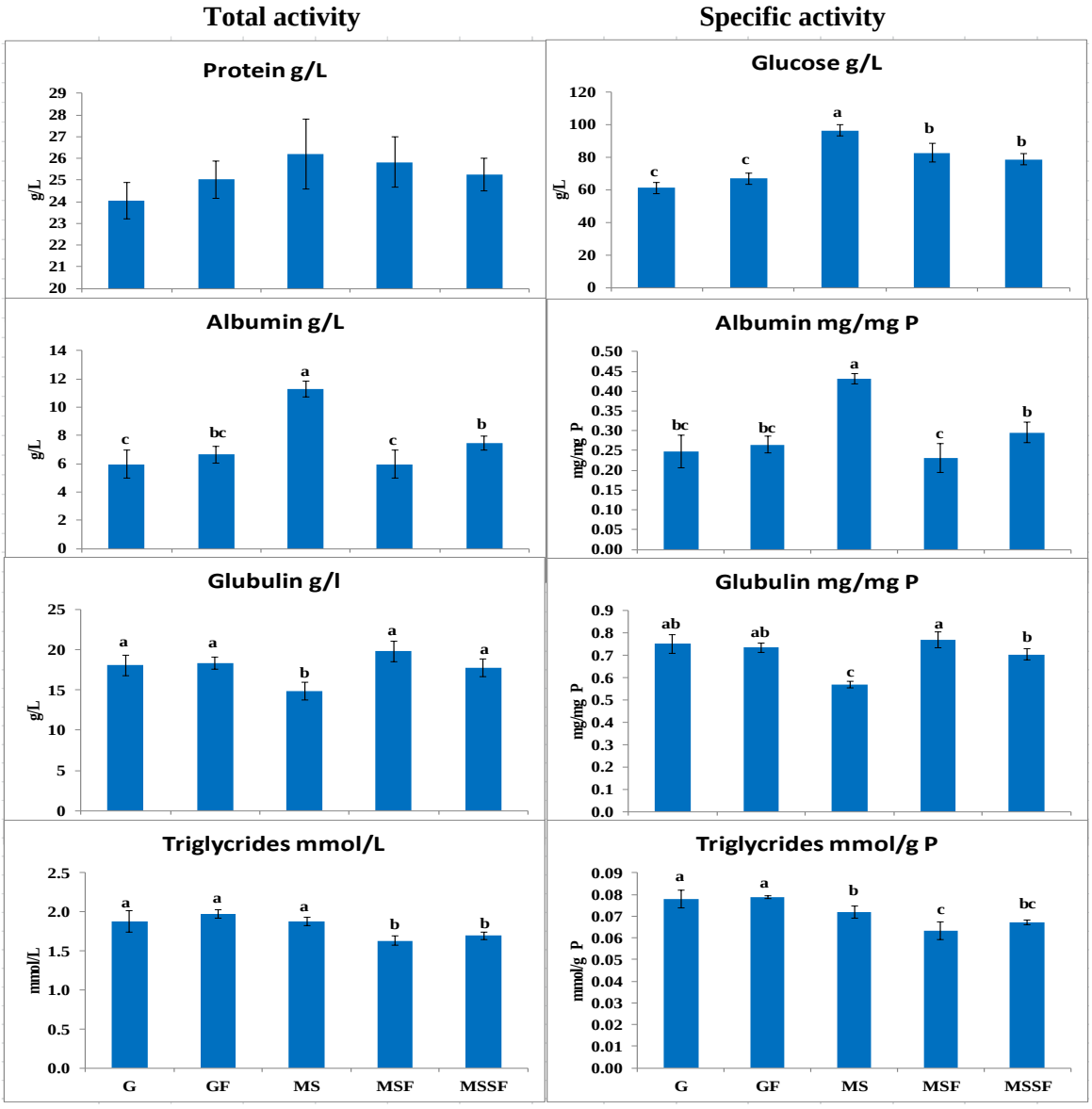

Fig. 4. The European seabass (*D. labrax*) larval protein and glucose content in g/L, albumin, globulin and triglycerides activities. G: Greenwater control non treated; GF: Control solid state fermented; MS: marine synbiotic supplemented; MSf: Marine synbiotic solid state fermented; MSSF: Marine synbiotic solid state fermented and resupplenented microdiets. Letters refer to treatments effects dissimilar significance ($P<$ 0.05)

Larvae fed the G, GF, and MS diets exhibited the highest significant total activity of triacylglycerols (TAG). In contrast, larvae consuming G, GF, MSF, and MSSF demonstrated the greatest total globulin activity compared to the other microdiets. Larvae fed MS1 and Mpro showed the highest significant specific TAG activity **(Salem & Ibrahim, 2022)**. The levels of energetic metabolites (triglycerides and cholesterol) are important indicators of fish health **(Mensinger *et al.*, 2005; Desouky *et al.*, 2020)**.

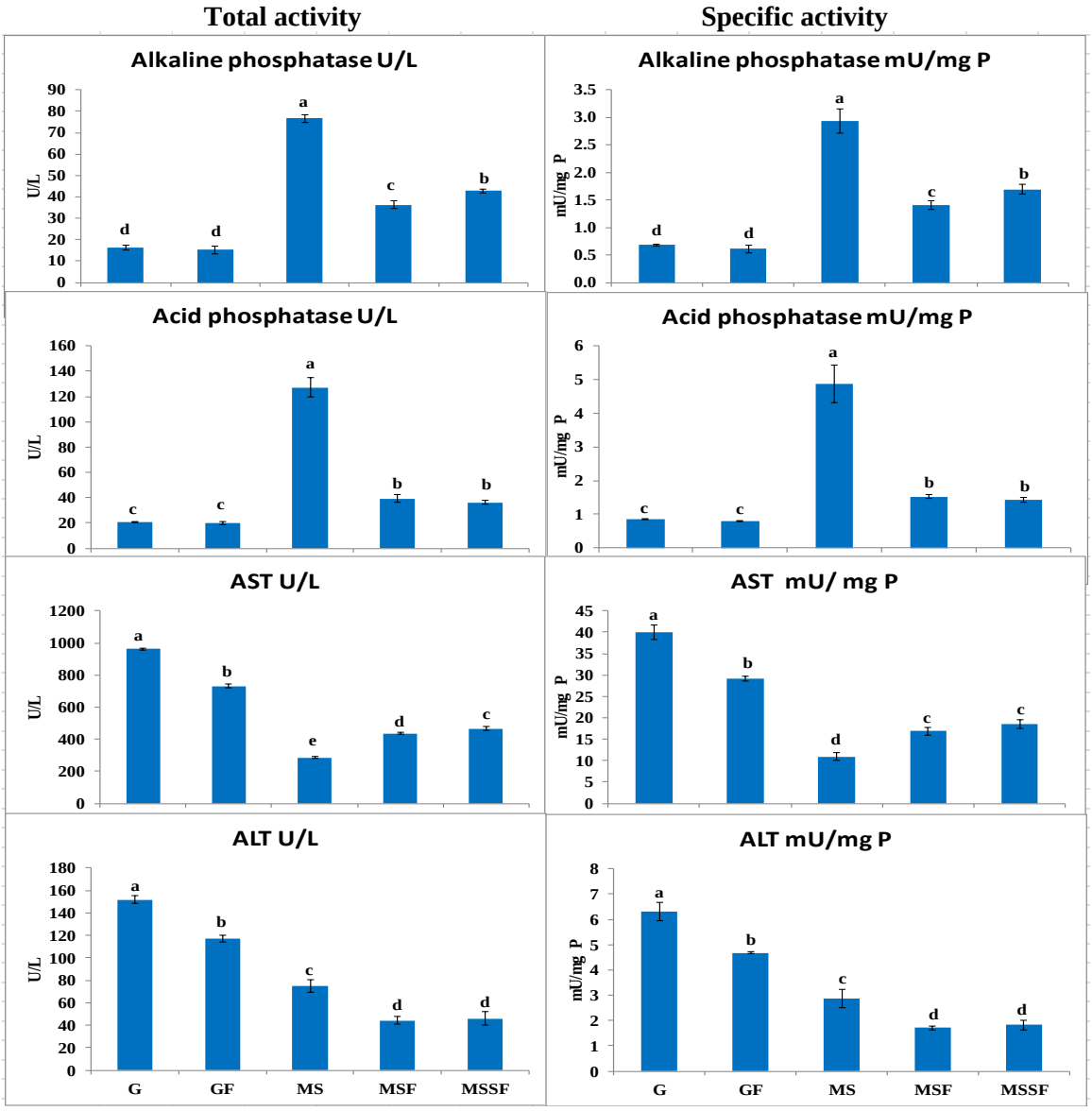

Fig. 5. The European seabass (*D. labrax*) larval liver function activities. G: Greenwater control non treated; GF: Control solid state fermented; MS: Marine synbiotic supplenented; MSF: Marine synbiotic solid state fermented; MSSF: Marine synbiotic solid state fermented and resupplenented microdiets. Letters refer to treatments effects dissimilar significance ($P< 0.05$)

Salem and Ibrahim (2022) found that alkaline phosphatase (ALP) and acid phosphatase (AP) levels were enhanced when the European seabass larvae were fed MS1 and MS2. In the gilthead seabream larvae, late weaning also increased ALP and AP levels **(Salem *et al.*, 2021)**. In addition, *Lactobacillus* spp. was shown to enhance ALP activity **(Suzer *et al.*, 2008)**. Similarly, **Zhang *et al.* (2013)** verified that synbiotics increased ALP, plasma AP, lysozyme, total serum protein, globulin, IgM, and total antioxidant capacity (TAC) in the black Amur bream **(Ringo & Song, 2016)**. **Salem *et al.* (2022)** reported that the MS treatment led to a significant increase in ALP.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are key liver enzymes. They are released into the bloodstream during liver cell damage **(Kumar *et al.*, 2011; Hassan *et al.*, 2014)**. ALT and AST levels significantly decreased in the Nile tilapia fed probiotic diets **(Soltan & El-Laithy, 2008)**. Larvae fed the G diet displayed the highest total and specific activities of AST and ALT. Meanwhile, **Salem and Ibrahim (2022)** showed that larvae consuming the chitosan prebiotic and *B. subtilis* HS1 probiotic exhibited significantly higher total and specific AST activities, respectively; however, larvae fed the G diet had the highest ALT activities.

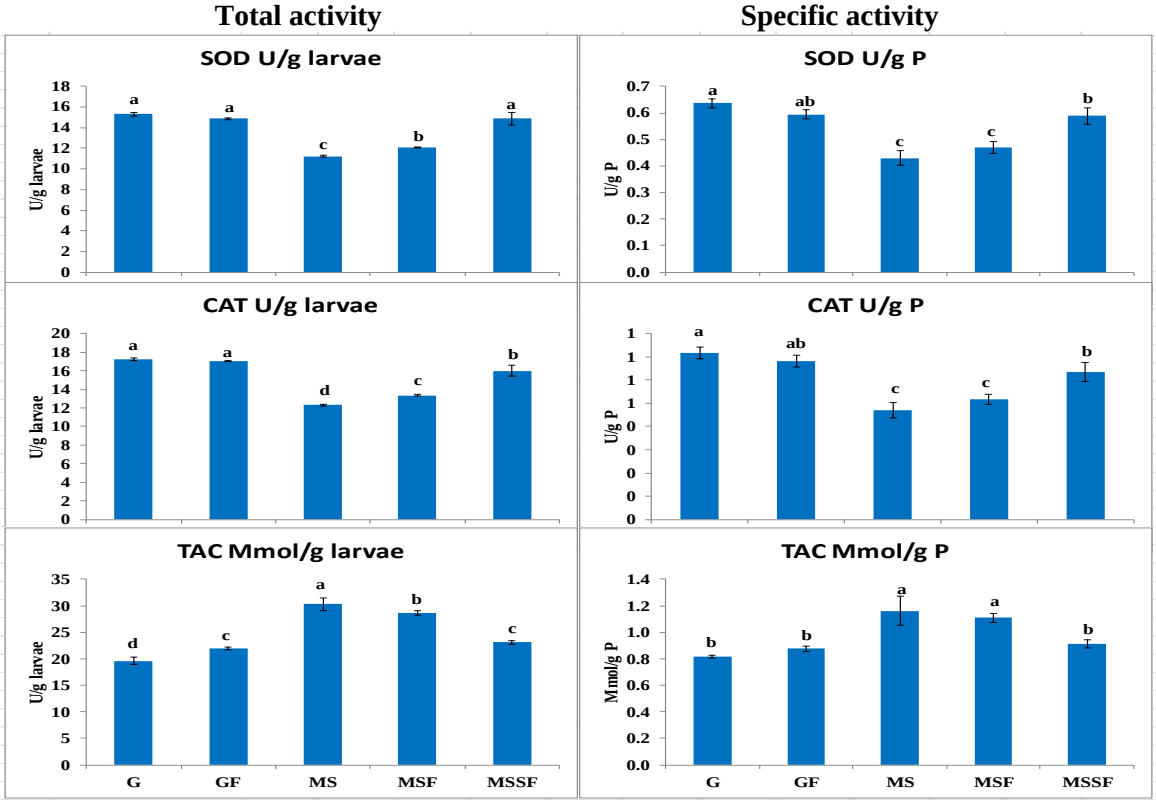

Fig. 6. The European seabass (*D. labrax*) larval antioxidants biomarkers activities. G: Greenwater control non treated; GF: Control solid state fermented; MS: Marine synbiotic supplenented; MSF: Marine synbiotic solid state fermented; MSSF: Marine synbiotic solid state fermented and resupplenented microdiets. Letters refer to treatments effects dissimilar significance ($P< 0.05$)

Reactive oxygen species (ROS) are produced during environmental stress conditions, causing lipid peroxidation, protein denaturation, and DNA hydroxylation molecular damages **(Lushchak & Bagnyukova, 2006; Amaral *et al.*, 2023)**. To avoid these impacts, endogenous antioxidant mechanisms are activated **(Vinagre *et al.*, 2012)**. Fish diet combination performs an essential role in capacity to aggravate oxidative stress influences **(Hoseinifar *et al.*, 2021)**. SSF compounds help enhance the fish oxidative status including the European seabass **(Fernandes *et al.*, 2022a)**. Lignocellulosic enzymes produced through fermentation reduced the bioactive antioxidant composites in SSF diets **(Amaral *et al.*, 2023)**. Correspondingly, **Amaral *et al.* (2023)** noticed that when fish consumed SSF diet, CAT and GPx decreased, demonstrating that SSF diet might reduce the protective impact but without overall oxidative status negative impacts. Likewise, larvae consumed G and GF displayed elevated substantial influence on CAT, SOD total activitiy. While, larvae consumed G flashed excessive meaningful influences on CAT, SOD specific activitiy than GF, MS, MSF and MSSF. Larvae consumed MS demonstrated the greatest substantial TAC total activitiy, while larvae fed MS and MSF displayed the greatest substantial TAC specific activitiy. The fermented diet antioxidant bioactive compounds reduction triggered a redemptive reaction by endogenous antioxidant processes. Fish consumed SSF diet displayed an enhanced ACH50 activity **(Amaral *et al.*, 2023)**. Correspondingly, **Salem and Ibrahim (2022)** demonstrated that larvae consumed G exposed elevated expressive SOD and CAT, while MS revealed great substantial TAC activities. ROS may reduce SOD and CAT, or its decline may generate more ROS production **(Thilagam *et al.*, 2010)**. **Lamari *et al.* (2016)** revealed when sea bass consumed *Lactobacillus casei* probiotic at 20 dph larvae, CAT increased and there was no substantial variance at 20 or 41 dph sea bass larvae antioxidant enzymes (CAT, SOD and GPX). After the MS treatment in seabass larvae, significant reductions in superoxide dismutase (SOD) and catalase (CAT) activities were observed **(Salem *et al.*, 2022)**.

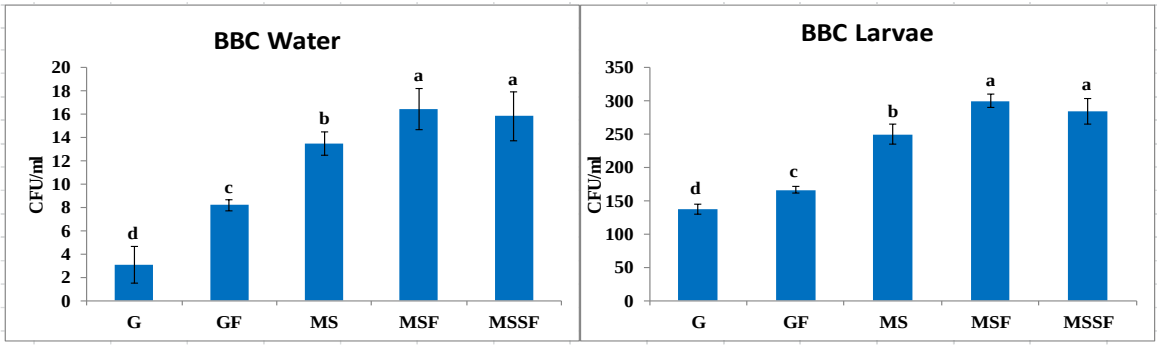

Fig. 7. The European seabass (*D. labrax*) larval water and larval bacterial counts quality. G: Greenwater control non treated; GF: Control solid state fermented; MS: Marine synbiotic supplemented; MSF: Marine synbiotic solid state fermented; MSSF: Marine synbiotic solid state fermented and resupplenented microdiets. Letters refer to treatments effects dissimilar significance ($P< 0.05$)

In the current investigation, Vibrio bacteria (VBC) were not detected in either the water or the European seabass larvae samples. Similarly, **Lamari *et al.* (2016)**, **Salem *et al*. (2018)**, **Salem and Ibrahim (2022)** and **Salem *et al*. (2022)** reported that the seabass larvae, fry, and rearing water were not infected by pathogenic Vibrio. Additionally, synbiotics were found to decrease both total viable bacterial counts and Vibrio counts in the intestines **(Li *et al.*, 2009)**.

Moreover, larvae fed the MSF and MSSF diets showed significantly higher bacterial counts (BBC) in both water and larvae samples at 45dph. Correspondingly, **Salem and Ibrahim (2022)** and **Salem *et al*. (2022)** noted that the MS treatment significantly increased BBC in the water and in the seabass larvae at 45dph. **Salem *et al.* (2015)** clarified that when seabass larvae were treated with *B. subtilis* HS1 and a commercial synbiotic combined with enzymes, there was a substantial increase in BBC and total bacterial counts; however, counts of VBC, *Aeromonas* sp., and *Staphylococcus* sp. were significantly reduced.

CONCLUSION

The results from the MS and MSF treatments showed significant improvements in larval length, weight growth, survival, superoxide dismutase (SOD) and catalase (CAT) activities, as well as specific total antioxidant capacity (TAC) activity. Larvae fed the MS diet recorded enhanced activities of alkaline phosphatase (ALP) and acid phosphatase (AP) enzymes. Additionally, larvae consuming the MSF microdiet exhibited the highest significant specific activity of globulin. The greatest total TAC activity was achieved by larvae fed the MS diet. In conclusion, both MS and MSF treatments enhanced the growth, survival, and antioxidant capacity of the European seabass larvae.

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