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Effect of Levamisole Supplemented Diet on European Sea Bass (*Dicentrarchus labrax*) Larvae in Respect to Survival, Growth Performance, Disease Resistance and Intestinal Histology

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Abstract

Information on the use of levamisole as feed additive in the production of marine fish larvae is scarce and no data is available for sea bass larvae. The current contribution highlights the effects of levamisole incorporation in sea bass larval diet on its survival rate, growth performance, proximate analysis, intestinal histology and disease resistance against the pathogenic *Vibrio anguillarum*. Larvae were assigned to four treatments (L_0, L_1, L_2, L_3) with different levels of levamisole (0, 100, 200 and 300 mg kg^{-1} diet), respectively. Fish were allocated into four triplicated groups of larvae per treatment. The diets were fed to sea bass post larvae (0.18±0.003 g) for 56 days. During the experimental period, no deaths were reported. Then, larvae were challenged with *V. anguillarum,* two weeks post-treatment. Results demonstrated a significant enhancement ($P<0.05$) in growth performance, feed efficiency, crude protein and lipid contents of larvae fed 200 mg kg^{-1} levamisole supplemented diet. The highest larval survival rate (80%) was also reported in L_2 after *V. anguillarum* challenge. Histological sections of the intestinal tract in fish fed L_2 demonstrated the greatest improvement in the length and width of the villi with the highest incidence of scattered goblet cells in the lamina propria and the most marked increase in the branching of mucosal folds and muscularis thickness than their corresponding in the other groups. Thus, the current results demonstrated that administration of 200 mg levamisole kg^{-1} diet would be optimum for enhancing survival rate, growth performance, proximate analysis, improving the intestinal architecture, and immunity of sea bass larvae.

Introduction

The aquaculture industry has globally increased in recent decades, nevertheless, many diseases have emerged and become a major constraint facing the industry (Hoseinifar et al., 2024). The sea bass is one of the most significant species in Mediterranean aquaculture, whereas its industry operations and profitability are very sensitive to different factors (El-Zaeem et al., 2024). Raising marine fish species for aquaculture, disease resistance and growth performance are two of the main issues (Radwan et al., 2023a; Fadel et al., 2024). Using antibiotics and chemotherapeutics for treatment of fish diseases was found of low potential due to the appearance of antibiotic-resistant bacteria which also has a negative effect on juveniles and adult's indigenous microflora (Sakai,1999). It appears that different developing countries and areas use different amounts of antibiotics in agriculture. In fact, the majority of developing nations continue to use antibiotics that have been outlawed in other nations, including the industrialized ones

(Adebowale et al., 2016). The production of vaccines has been essential to the control of infectious diseases in aquaculture for many years, which has led to a reduction in the usage of antibiotics in aquatic species (Assefa and Abunna, 2018). Because herd immunity protects the minority of unvaccinated animals and there is no chance of drug resistance developing in vaccinated animals, vaccines are widely accepted (Gudding, 2014).

Since a solitary approach is not successful alone in the prevention and control of aquaculture health, the combination of different strategies seems more effective. Immunotherapy approach was found to be an active method which is not targeting certain antigen or having non-specific immune response to a pathogen but increasing phagocytic activity (Secombes, 1994). Immunostimulants used for fish are natural or synthetic substances which can be used by injection as immune modulator (Vijayaram et al., 2023), as feed additive (Vijayaram et al., 2022), immersion (Siwicki, 1989) or *in vitro* (Siwicki et al., 1994). Immunostimulant injections have been given to sea bream brooders, which has increased the absorptive surface area by encouraging prolonged mucosal folding and boosted the immune defense system (Pahor-Filho et al., 2017). It was documented that levamisole has the ability to enhance respiratory burst and phagocytosis (Ispir and Yonar, 2007), modulate T-cell function and leucocytes cytotoxic activity (Cuesta et al., 2002), as well as activate the macrophage-activating factor (Kumari and Sahoo, 2006). Further research has shown that levamisole is beneficial for various fish species, involving hybrid striped bass (Li et al., 2006), catfish (Aly et al., 2011), and mrigal carp (Bhatnagar and Lamba, 2016).

Moreover, levamisole is used for improving growth performance and nutrient digestibility and enhancing resistance to pathogenic bacteria and parasitic infections. *Vibrio anguillarum* is a universal marine bacterium found in numerous aquatic environments worldwide, strongly concerned with fish infection and colonization. The term 'vibriosis' used to describe *V. anguillarum* infections, has become broadly spread in different wild and cultured fish, crustaceans and bivalves in brackish or salt water, causing a lethal hemorrhagic septicemic disease (Ghittio et al., 2003; Toranzo et al., 2005). A significant economic loss was reported in farming of European sea bass due to infection with *V. anguillarum* (Čolak and Zrnčic, 2015). In Croatia, Zupičić et al. (2022) indicated vibriosis clinical signs and diagnostics in the farming of European sea bass caused by *V. harveyi* and *V. anguillarum*.

The optimal dose, sustainable duration, and effects of levamisole consumption as an immunostimulant on European sea bass post larvae in terms of survival rate, growth performance, proximate analysis, disease resistance, and intestinal histology are the aim of the present study. Following the administration of various doses of the immunostimulant, the immune response and resistance to disease against the pathogenic bacterium, *V. anguillarum*, were examined.

Material and Methods

Spawning

The experiment was conducted at the National Institute of Oceanography and Fisheries' marine hatchery in Alexandria, Egypt. The newly hatched larvae and eggs of sea bass were obtained by means of induced broodstock reproduction. Hormonal injection of a combination of LHRH and HCG at a dose of 200 mg kg^{-1} for male fish and 1000 UI for female fish was used to stimulate spawning. 48 hours following the hormone induction, ovulation took place. Fertilized eggs were collected by putting them in a funnel incubator with 2 m^3 fiber glass tanks and running water from the sea water reservoir tank through an open water system. During incubation, the average water temperature was 16°C.After fertilization, *D. labrax* larvae hatched 36–48 hours later. Following hatching, the larvae were moved and kept in a 2 m^3 fiber glass tank that was mechanically aerated and contained sea water at a pressure of 35 ppt. Every day, these tanks' water volumes were replenished by at least 30% after the debris was removed by siphoning. Ten larvae were taken from these tanks at least twice a day at this point in order to examine them under a microscope in order to ascertain their developmental stage, the extent of the yolk sac's absorption, and the mouth's formation.

Dietary Habits of Larvae

Incubated larvae were fed *Artemia* nauplii grade (AF 430, INVE Aquaculture, Ghent, Belgium) at a ratio of one to two individuals starting on day eight.ml^{-1} and starting on day 12 with 1-2 individuals of *Artemia* nauplii grade (AF 480, INVE Aquaculture, Ghent, Belgium). *Artemia* metanauplii at 2–4 individuals/ ml (EG, *Artemia* Systems SA), supplemented with DHA–Selco (*Artemia* Systems SA, Ghent, Belgium), were seen to be present from day 15 to day 40.

Larval Rearing

In 12 cylinder-conical-shaped, larvae were reared (3 m^3 tanks per system). At a density of 20 individuals l^{-1}, Larvae were stocked in dark grey tanks. Daily measurements were made of the water physicochemical parameters (temperature, salinity, dissolved oxygen, pH, nitrite levels and ammonia). Throughout the larval culture stage, the water's temperature was kept at a constant 18±2°C. Additionally, the salinity, pH, and oxygen levels were kept at >85%, 35, and 7.8 mg l^{-1}, respectively. Both nitrite and ammonia were maintained at or below 0.01 mg l^{-1}. As the larvae grew older, the rate of water exchange steadily increased. Fluorescent light tubes were used to provide 50–100 lux of light at the surface of water. Every day, the photoperiod was adjusted to a 16-hour light: 8-hr dark cycle until the end of the larval rearing stage.

Experimental Design

Larvae of sea bass (*Dicentrarcus labrax*) were sorted into four duplicate groups and placed in 200-liter tanks, each with an initial weight of 0.18±0.003 g fish on average. The stocking density per tank was 50 fish. Fish were given a basal diet for seven days to get used to the experimental setup. Fish individuals were fed 3 times a day, seven days a week, at 09.00, 13.00, and 17.00 hr., till satiation. A total of 180 g kg^{-1} of crude fat and 475 g kg^{-1} of crude protein were used in the formulation of four diets. Following the complete grinding, sieving, and blending of all dry materials, oil was put on. After the mixture was moisturized, it was cold-pelleted using a lab mincer and dried for 24 hours at 60ºC in a convection oven, then stored at -20°C for further need. The test diets included three different amounts of levamisole, which were 100 (L_1), 200 (L_2), and 300 (L_3) mg kg^{-1}, in addition to a control, levamisole-free diet (L_0), and three other test diets. The doses used in this study were selected according to Li et al. (2006); Maqsood et al. (2009) and de Azevedo et al. (2021) studies. Table 1 displays the test diet's proximate analysis.

Evaluation of Feed Consumption Efficiency and Growth Performance

The weight gain (WG, g), percent weight gain (WG %), specific growth rate (SGR, % day^{-1}), feed conversion ratio (FCR), and protein efficiency ratio (PER) were used to examine fishes growth performance and their feed utilization. Additionally noted were the condition factor and survival rate (S%). The following equations were applied:

$$WG\ (g) = FW\ (g) - IW(g)$$

$$WG\ (\%) = 100 \times [(FW - IW) / IW]$$

$$SGR = 100 \times [(\ln FW) - (\ln IW)] / \text{experimental days}$$

$$FCR = \text{Feed fed (g)} / \text{Weight gain (g)}$$

$$PER = \text{Weight gain (g)} / \text{Protein fed (g)}$$

$$K = W / L^3 \times 100$$

$$S\ (\%) = (\text{Final fish count} / \text{Initial fish count}) \times 100$$

Where, FW is final fish weight, IW is initial fish weight, K is condition factor, W is fish weight, L is fish length, S is survival.

Proximate Analysis

At the start and finish of the experiment, thirty sea bass were chosen at random from each aquarium and homogenized to be examined for fish proximate composition. We used the standard approach of AOAC (2000) to determine the moisture, protein, ash, and total lipid contents. For proximate analysis, food sample duplicates were utilized.

Challenge and Microbiological Examination

V. anguillarum was kindly provided by the Microbiology Lab, NIOF. According to Kobayashi et al. (1963), Thiosulfate Citrate Bile Salts Sucrose (TCBS, DM218D) agar was utilized to preserve it. Bacterial suspension was inoculated onto nutrient broth medium containing 2% NaCl and allowed to incubate for the entire night at 28°C. After 24 hours, the culture medium was centrifuged at 10,000 g for 20 min. Afterwards, the pellet that had precipitated was scraped off and put back into phosphate buffered saline (PBS; pH 7.4). A spectrophotometer (UNICO UV-2000) was used to measure the turbidity of the bacteria, which is

Table 1. The formula of the experimental diets and the proximate analyses

Ingredient	g/Kg
Fish meal	450
Squid meal	150
Shrimp meal	50
Soybean meal	100
Starch	56
Soybean lecithin	41
Fish oil	88
Vitamin's premix	30
Mineral premix	30
Vitamin C	5
Sum	1000
Proximate composition	% (Dry weight basis)
Crude protein	47.44
Crude lipid	18.22
Ash	11.36
Fiber	2.5
Moisture	8.09
[1]NFE	20.48
[2]Gross energy (KJ/g)	22.02

[1]Nitrogen-free extracts (NFE)=100–[% ash+% lipid+ % protein+% fiber]

[2]Gross energy (kJ/g)=(protein content 23.6)+(Lipid content 39.5)+(carbohydrate content 17.2)

represented as absorbance (A) at OD_{610}. A set of sea bass larvae were housed in four tanks, each with two replicas and varying immune stimulant feeding amounts. The tanks contained 0.1 ml of *V. anguillarum* life cells, assuming 1 x 10^5cfu ml^{-1} at ambient conditions. To count *V. anguillarum*, one milliliter of water was taken from each treatment tank, inoculated onto plates of TCBs agar, and then incubated for 24 hr at 28°C. To determine how dietary immunostimulant affects the pathogen's ability to cause disease, bacterial enumeration was conducted.

Histological Examination

Following Zhao et al. (2021) protocol, the excised sea bass gut of 5 fish samples was dehydrated in graded ethyl alcohol, cleaned in xylol, blocked by paraffin wax, and fixed in 10% neutral formaldehyde for 24 hours. 4-5 µm thickness of intestinal sections were cut with a rotatory microtome, stained with hematoxylin-eosin, then the slides were viewed with an Olympus light microscope (Japan).

Analytical Statistics

The mean±Standard Error (SE) is used to represent each registered parameter. The comparison was among all the dietary groups. One-way analysis of variance (ANOVA) was used to test significant differences between the treated and the control groups, then the Tukey-HSD test was applied for multiple comparisons. The analysis was performed using IBM- SPSS package version 23.0 (Snedecor and Cochran, 1967). Probability of a significant difference was detected at $P<0.05$.

Results

Responses of Sea Bass to Levamisole

Table 2 displays the growth performance and feed utilization metrics. Addition of levamisole produced favorable effects, as indicated by the values of WG, WG%, and SGR. The results of WG, WG%, and SGR indicated a noteworthy rise in diet L_2. Furthermore, the feed utilization indices showed the same tendency as results indicated that values of FCR, PER and K were significantly increased ($P<0.05$) due to levamisole supplementation.

Relative Percentage Survival After Challenge Assay with *V. anguillarum*

Following the feeding trial, a two-week challenge test was conducted. The results showed that levamisole added to the food over an extended period of time increased the resistance of sea bass larvae to bacterial infection (Figure 1). Throughout the challenge trial, water quality measures were examined every five days, and no appreciable changes were found. Following a *V.anguillarum* challenge, the fish groups that fed 200 mg levamisole kg^{-1} diet (80%) had a considerably higher ($P<0.05$) post-challenge survival rate, followed by L_1 (70%) and L_3 (68%). However, the highest mortality rate (40%) was seen in fish fed basal diet in L_0. Conversely, during the feeding study, a high pre-challenge survival rate (%) was recorded in all treated groups. Besides, a little mortality was only noticed in the early stages of the trial.

Effect of Levamisole on the Body Composition% of Sea Bass Larvae

The analysis of the body composition of sea bass larvae is illustrated in Table 3. At the end of the feeding trial, fish fed L_2 and L_3 diets had a significantly higher fish proximate composition ($P<0.05$) than fish fed L_0 diet; nonetheless, there was no significant difference in moisture, lipid, or ash contents between any of larvae fed groups.

***V. anguillarum* Count in Water**

Dietary levamisole with different concentrations showed a significant effect on the pathogenic *V. anguillarum* counts infected sea bass larvae. Reduction in *V. anguillarum* count from 84.67±7.572 to 3.33±2.08 x10^5 cfu ml^{-1} was recorded in the larvae group fed on L_2 ($P<0.05$) within 10 days after hosting. One week after,

Table 2. Growth performance and feed utilization indices

Parameters	Diets			
	L_0	L_1	L_2	L_3
Initial Weight (g)	0.18±0.003	0.17±.003	0.17±.003	0.19±.003
Final Weight (g)	0.85±0.24^b	1.41±0.20^{ab}	1.55±0.11^a	1.33±0.10^{ab}
Weight Gain (g)	0.67±0.03^b	1.23±0.20^{ab}	1.38±0.11^a	1.14±0.10^{ab}
%Weight Gain	366.9±17.49^b	713.62±122.9^{ab}	831.13±82.77^a	588.25±41.33^{ab}
SGR (% day^{-1})	2.56±0.06^b	3.46±0.24^a	3.71±0.15^a	3.21±0.10^{ab}
FI/Fish	1.55±0.03	1.54±0.12	1.56±0.22	1.49±0.15
FCR	2.33±0.12^a	1.29±0.10^b	1.12±0.07^b	1.32±0.15^b
PER	0.88±0.08^a	1.62±0.22^b	1.83±0.18^b	1.59±0.29^b
K	1.48±0.21	1.24±0.12	1.12±0.07	1.42±0.14
Survival (%)	60	70	80	68

Different superscript letters are significantly different (P≤0.05).
Specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and the condition factor (K).

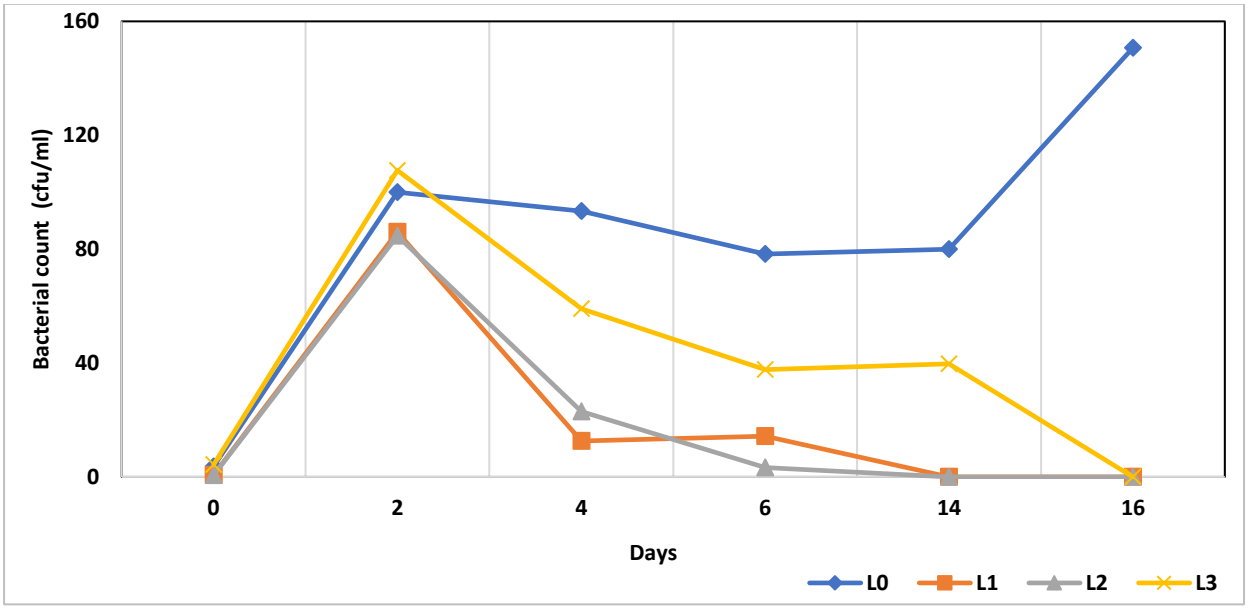

Figure 1. *V. anguillarum* count in cfu ml^{-1} collected from the sea bass rearing water of the infected tanks treated with different levamisole levels.

Table 3. Fish biochemical composition at the end of the feeding trial

Parameter	L_0	L_1	L_2	L_3
Moisture	73.75±2.39	74.61±5.04	74.32±3.45	76.00±3.77
Crude protein	15.04±1.60^{b}	15.76±2.43^{ab}	16.75±2.77^{a}	16.47±1.33^{a}
Crude lipid	7.1±0.51	6.41±0.97	6.77±0.24	6.65±0.44
Ash	1.29±0.10	1.22±0.17	1.25±0.02	0.99±0.06

Different superscript letters are significantly different (P≤0.05).

no bacterial count was detected compared with control tanks. This was followed by L_1 showing the same significant effect within the two weeks of treatments at P<0.05. It was also observed that the highest *V. anguillarum* count was 39.67±5.51 x10^{5}cfu ml^{-1} of larvae fed on L_3within the two weeks of the treated tanks compared to the control ones (P<0.05) (Figure 1).

Histological Findings

Photomicrographs of intestinal histology of sea bass larvae fed various concentrations of levamisole supplementation for 56 days are illustrated in Figure 2. The intestinal wall of the sea bass larvae in the control group, which is made up of the outermost serosa, the lamina propria-submucosa, the tunica muscularis, and the inner mucosa layer, was arranged normally in histological sections. Intestinal villi or mucosal folds appeared with uniform columnar epithelium possessing a number of goblet cells scattered along the mucosal surface (Figure 2A). In addition, the different concentration levels of the dietary immunostimulant improved the intestinal histology of sea bass larvae with a marked rise in the length and width of the intestinal villi, goblet cells number, the mucosal folds as well as the muscularis thickness (Figure 2B-2D). Compared to other experimental groups, fish fed 200 mg levamisole kg^{-1} supplemental diet in L_2 had the most effective and the greatest enhancement in overall intestine with the most branching, the widest and longest intestinal villi as well as the highest incidence of uniformed goblet cells in the epithelium (Figure 2C).

Discussion

Fish larval production is frequently hampered by high rates of mortality in the aquaculture sector due to infectious diseases (Hoseinifar et al., 2024). Nowadays, utilization of feed additives as alternative strategies to stimulate immunity, improve growth and welfare, and control the pathogen load in farmed species is applied (Radwan et al.,2023b; Eissa et al., 2024 a, b ; El-Sayed et al., 2024). Several reports recommended the utilization of levamisole, as feed additive in cultured fish since it maintains fish health, improves its overall growth and increases its resistance against diseases (Kumar et al., 2022; Nogueira et al., 2019; Woo et al., 2023). Elumalai et al. (2023) reported that levamisole incorporation in the diet of fish larvae boosted its innate responses until its adaptive immune response will be fully developed improving the larval survival rate.

In the current study, the growth trial revealed a significant (P<0.05) enhancement in growth performance, feed efficiency (WG%, SGR, PER), as well as crude protein content of sea bass (*Dicentrarchus labrax*) larvae after 56 days of consuming 200 mg

levamisole kg^{-1}diet. This pointed out that growth performance was influenced by the gain in body weight, which in turn relied on food palatability and the increase in the feed utilization efficiency (Amiri and Bahrekazemi, 2017). In addition, the assessment of the proximate profiles of an organism is crucial to evaluate its energy value (Ahmed 2017). Our findings revealed a high significant increase in the crude protein content of L_2 ($P<0.05$) than their corresponding in L_0. In the current experiment, the feed conversion ratio (FCR) of L_2 fish was significantly lower than that of fish fed a baseline diet. Therefore, this result indicates better nutrient digestibility and feed efficiency of larvae diet since the immunostimulant enhances the active transportation of oxygen molecules across fish cell membrane. Consequently, cells metabolism will perform at a higher level improving FCR levels and food consumption (Alishahi et al., 2012). Similar findings were informed in mrigal (*Cirrhinus mrigala)* fed a supplementation of 125, 250 and 500 mg kg^{-1} levamisole diet (Bhatnagar and Lamba, 2016). Moreover, Maqsood et al. (2009) showed that the growth parameters were significantly enhanced in common carp (*Cyprinus carpio*) upon its dietary supplementation with 250 mg levamisole kg^{-1} for 70 days when compared with their corresponding in the control group. Our results also concur with Mulero et al. (1998) who observed a significant increase in the weight and size of gilthead sea bream (*Sparus aurata*) during the experimental trial due to the levamisole dietary intake when compared with the untreated group. This is consistent with Li et al. (2006) who found that juvenile hybrid striped bass (*Morone chrysops× Morone saxatilis*) were significantly improved in development and feed efficiency after three weeks of low dose (<500 mg kg^{-1}) levamisole diet treatment as compared to fish fed the basal diet. Although, in the group of fish given a supplementation diet containing 1000 mg of levamisole kg^{-1} for 3 weeks, they saw indications of chronic toxicity, including growth retardation, a decrease in feed efficiency, and a fall in feed intake. This suggests that fish growth and health are negatively impacted by large doses of levamisole (Li et al*.,* 2006). Further, Eslami and Bahrekazemi (2019) indicated that 0.1% Levamisole dietary supplementation had no effects on feed conversion ratio and protein efficiency of beluga (*Huso huso*). It is clear from the above divergence that the efficacy of levamisole as one of the immunostimulants is species-specific, related to dose, time, life stage, administration route, fish physiological and immunological status (Sink and Lochmann, 2014). In addition, an increase in the growth of the L_2 group was observed in the current experiment indicating that

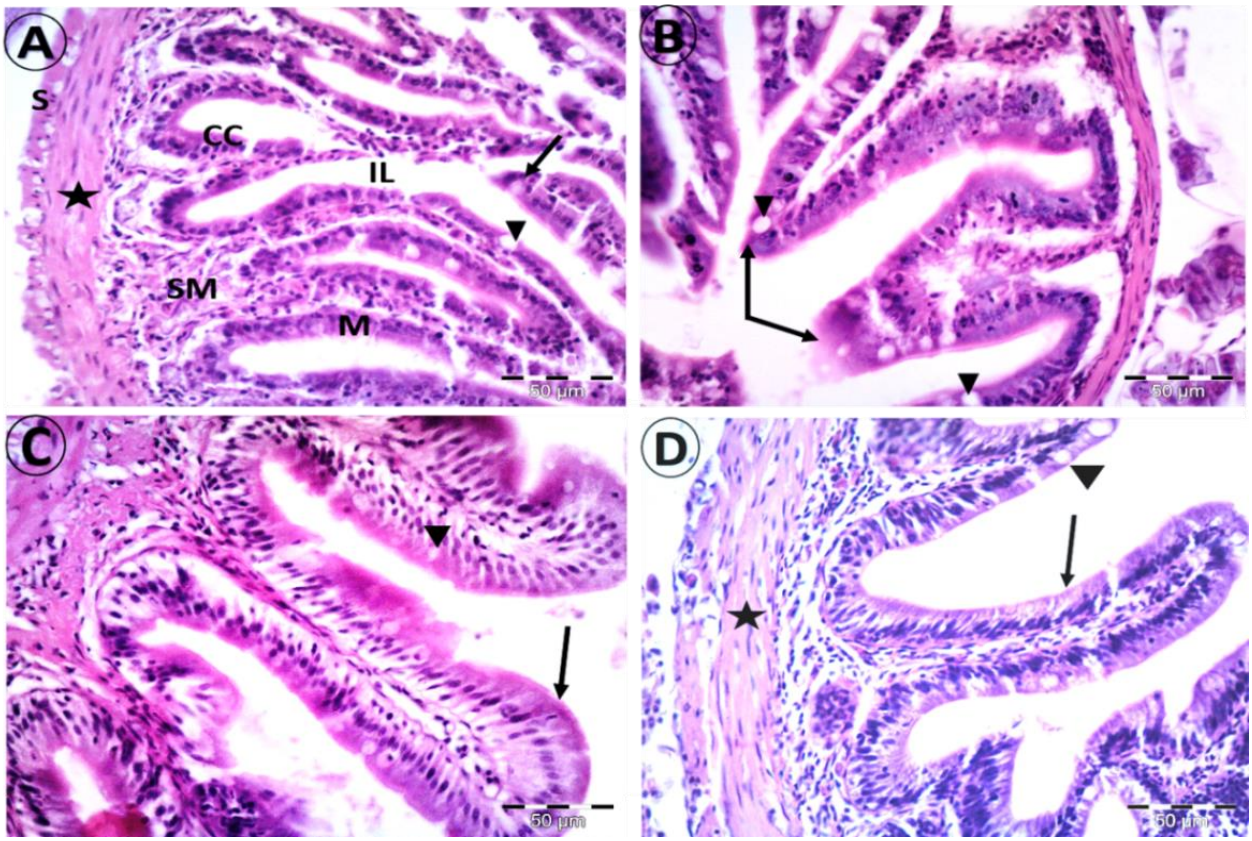

Figure 2. Microscopic sections of intestine of sea bass post larvae fed levamisole incorporated dietof the four experimental groups: L0 group (control group): (A), L1 group: (B), L2 group: (C), and L3 group: (D). intestine of the control group looked intact. It is formed of villi (arrows) that filled the intestinal lumen (IL), mucosa (M), submucosa (SM), muscularis (black star), and the outer serosa (S). Note also, goblet cells (arrowhead) and columnar cells (CC). H&E stain, Bar = 50 µm.

levamisole has a potent efficiency as a growth promoter and may serve as an alternative low-cost strategy in the culture of sea bass to improve its health status. Reduction of survival rate in the early developmental stages of fish is a major concern that encounters fish breeders especially during larval production (Amiri and Bahrekazemi, 2017).

The major prevention approaches and the modern control strategies of *Vibriosis* that provoke inordinate damage in aquaculture, comprising probiotics, antibiotics, vaccines, bacteriophages as well as antimicrobials from plants and other natural sources are reviewed (Xu et al., 2022; Defoirdt, 2023). In the current experiment, a noteworthy high survival rate was noted in the fish group fed diets containing 200 mg levamisole kg^{-1} following a 14-day challenge with *V. anguillarum*. This information clarified how the immune booster that was utilized could offset the immunosuppression brought on by an infection with *Vibrio* spp. Feyera et al. (2021) explained levamisole mechanism of action against infectious disease revealing that it inhibits fumarase activity in the parasite by producing a stable S-S chain which will block the reduction of fumaric acid into succinic acid. As a result, this obstruction will impair the metabolism of sugar and lower the parasite's ATP levels, paralyzing and killing it. Our results also concur with Woo et al. (2023) who demonstrated the effectiveness and safety of levamisole orally administrated as a therapeutic agent against the infection of Korean rockfish (*Sebastes schlegelii*) with *Microcotyle sebastis*. Maqsood et al. (2009) also revealed a high survival rate and an increase resistance to infection with *Aeromonas hydrophila* in the group of common carp fingerlings fed 250 mg levamisole kg^{-1}. Ispir and Yonar (2007) also suggested that Levamisole incorporation in rainbow trout (*Oncorhynchus mykis*) diet can prevent its mortality due to pathogens since this immunostimulant increased the non-specific immunity, phagocytic activity and resistance of fish to infection with *Yersinia ruckeri* after 14 days of exposure. Furthermore, a number of studies supported levamisole's beneficial effects as a possible immunostimulant and efficient immune response modulator by boosting resistance following an experimental challenge, as seen in *Sparus aurata* (Kumari and Sahoo,2006), *Labeo rohita* (Misra et al.,2009), *Cirrhinus mrigala* (Bhatnagar and Lamba,2016) and *Lutjanus jocu* (Oliveira et al.,2019).This means that dietary intake of levamisole is effective in reducing mortality rate in infected fish which in turn elicits its disease resistance (Li et al., 2006). Interestingly, the values of the recorded growth and feed utility indices in L_1 and L_2 experimental groups were relatively close, however, these values were numerically superior in L_2 group relative to L_1. Furthermore, the highest survival rate and the lowest pathogenic effect were in L_2 group. From an economic view, the growth and the survival rate are the two limiting factors in marine hatcheries (Şahin and Üstündağ, 2003). In contrast, some other studies pointed out that the employed immunostimulant had no effect on the survival rate of fish (Alishahi et al., 2012; Amiri and Bahrekazemi, 2017). Borges et al. (2004) explained that immunostimulants can provoke significant changes in fish survival after six months from its administration. Kumari and Sahoo (2006) also added that the effect of levamisole to enhance fish immune response is a dose and time- dependent. Although, there is no available information found indicating levamisole effect on sea bass larvae.

Histological examination is a suitable means to evaluate the nutritional state and health conditions of aquatic species (Eissa et al., 2024c). The current study revealed that providing levamisole to sea bass larvae diet for 56 days improved the histological architecture of their intestine. The present histological findings of the untreated group indicated that the description of the intestinal layers formerly described by Wassef et al. (2020) and Fadel et al. (2024) are consistent with the intestinal structure of sea bass larvae. The intestinal histology of sea bass larvae was improved by adding dietary immunostimulant at different concentration levels during the experimental trial. There was a noticeable increase in the width and length of the intestinal villi, goblet cells number, mucosal folds, and thickness of the muscularis. Compared to other experimental groups, fish fed 200 mg levamisole kg^{-1}supplemental diet in L_2 had the greatest enhancement in overall intestine with the most branching, the epithelium with the longest and largest villi and the highest frequency of uniformed goblet cells. The dietary levamisole's stimulating impact may be the cause of the intestinal improvement. The current results are at the same line with those obtained in turbot (*Scophthalmus maximus*) study where the dietary immunostimulant supplementation activated fish cellular immunity as one of the defense mechanisms of levamisole well-described in mammals (Alvarez-Pellitero et al., 2006). Aly et al. (2011) found similar results, explaining that adding 150 mg of levamisole kg^{-1} to the diet of catfish (*Clarias gariepenus*) boosted the humoral immune response against *Aeromonas hydrophila*, particularly macrophages, which will improve the antigen's processing and entrapment. The histological pictures of L_2 were supporting the recorded survival rate percentage and the growth parameters data indicating that sea bass larvae were protected against challenge with *V. anguillarum*. This point concerned the efficacious role of the applied immunostimulant in enhancing the immunological response of sea bass larvae against *V. anguillarum* infection, as well as the safety of the dose that was chosen. The present data also dealt with Pahor-Filho et al. (2017) who revealed that the dietary 300 mg levamisole kg^{-1} caused mild histopathological alterations in the hepatic tissue of juvenile pacu (*Piaractus mesopotamicus*) and could control fish infection by *Rondonia rondoni*. Moreover, the addition

of 0.1% levamisole in the diet of beluga (*Huso huso)* was recommended as a safe immunostimulant since no injury was detected in its internal body organs (Eslami and Bahrekazemi, 2019).

Conclusion

In summary, incorporation of levamisole with a dose of 200 mg kg^{-1} in sea bass (*Dicentrarchus labrax*) larvae diet in the present study can enhance its survival rate, growth performance, proximate analysis, disease resistance and intestinal architecture. Hence, oral administration of levamisole acts as a therapeutic agent against *Vibrio* infection in the farming of sea bass larvae. Nevertheless, more subcellular and molecular research is needed to validate levamisole's beneficial benefits on aquatic fish health and parasite management.

Ethical Statement

All experiments were approved by the authority of the NIOF (National Institute of Oceanography and Fisheries) Committee in Cairo, Egypt for Institutional Care of Aquatic Organisms and Experimental Animals.

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Author Contribution

Sara F. Ghanem: Conceptualization, Writing - review and editing;

Heba S. Elsayed: Data Curation, Formal Analysis, Investigation, Methodology, Visualization and Writing - original draft;

Norhan E. Saleh: Resources, Writing -review and editing;

Khouloud M. Barakat: Supervision, Writing - review and editing,

Nevine M. Abou Shabana: Data curation, Conceptualization. All authors Approved the submitted manuscript.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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References

Adebowale, O.O., Adeyemo, O.K., Awoyomi, O., Dada, R.& Adebowale, O. (2016). Antibiotic use and practices in commercial poultry laying hens in Ogun State Nigeria. *Revue d'élevage et de médecine vétérinaire des pays tropicaux,* 69, 41–45. https://doi.org/10.19182/remvt.31170

Ahmed, E.O., Ahmed, A.M., Ebrahim, S.J.& Adm, H.H. (2017). Proximate and mineral composition of some commercially important fishes in Jebl Awlia reservoir, Sudan. I*nternational Journal of Fisheries and Aquaculture Research,*3,51–57. https://doi.org/10.12816/0033512

Alishahi, M., Mesbah, M., Namjouyan, F., Sabzevarizadeh, M.& Razi Jalali, M. (2012). Comparison the effects of some chemical and natural immune stimulants on Oscar fish (*Astronotus ocellatus*). *Iranian Veterinary Journal*, 8 (2), 58-69. https://doi.org/10.1155/2024/6957201

Alvarez-Pellitero, P., Sitjà-Bobadilla, A., Bermúdez, R.& Quiroga, M.I. (2006). Levamisole activates several innate immune factors in *Scophthalmus maximus* (L.) (Teleostei). I*nternational Journal of Immunopathology and Pharmacology,* 19(4),727-738. https://doi.org/10.1177/039463200601900403

Aly, S., Abd-Allah, O., Mahmoud, A.& Gafer, H. (2011). Efficiency of Levamisole in improving the immune response of catfish (*Clarias gariepenus*) to *Aeromonas hydrophila* vaccine: clinico-pathological studies. *Mediterranean Aquaculture Journal,*4(1), 1826. https://doi.org/10.21608/MAJ.2010.2670

Amiri, Z.& Bahrekazemi, M. (2017). Effect of oral administration of Levamisole, Quil-A and Cinnamon in growth amount, hematological and immune parameters of Marmalade cichlid, *Labeotrophus fuelleborni* (Ahl, 1926). *Sustainable Aquaculture and Health Management Journal*, 3(2), 86-97.

AOAC (2000). Association of Official Analytical Chemists.15th ed. Arlington: AOAC International.

Assefa, A.& Abunna, F. (2018). Maintenance of Fish Health in Aquaculture: Review of Epidemiological Approaches for Prevention and Control of Infectious Disease of Fish. *Veterinary medicine international*, 1, p. 5432497. https://doi.org/10.1155/2018/54

Bhatnagar, A.& Lamba, R. (2016). Immunostimulating and growth promoting activity of dietary levamisole on *Cirrhinus mrigala* fingerlings. *Journal of Aquaculture Marine Biology*, 4,1-9. https://doi.org/10.15406/jamb.2016.04.00102

Borges, A., Scotti, L.V., Siqueira, D.R., Jurinitz, D.F. & Wassermann, G.F. (2004). Hematological and serum biochemical *values forjundia* (*Rhamdi aquelen*). Fish Physiology and Biochemistry, 30, 21-25. https://doi.org/10.1007/s10695-004-5000-1

Čolak, S.& Zrnčić, S. (2015). Health situation in Croatian mariculture. In: Atti del XXI Convegno Nazionale S.I.P.I. Società Italiana Patologia Ittica. (Della Pozza, M., A. Manfrin, Eds.), Chioggia, Italy, pp. 68–68.

Cuesta, A., Esteban, M.A.& Meseguer, J. (2002). Levamisole is potent enhancer of gilthead sea bream natural cytotoxic activity. *Veterinary Immunology and Immunopathology*, 89(3-4),169-174. https://doi.org/10.1016/S0165-2427(02)00220-9

de Azevedo, P.F.O., Martins, M.A., Blank, M., Cardoso, L., de Aguiar Bertaglia, E., Lisboa, T.R., Jerônimo, G.T., Furtado,

W.E. & Martins, M.L. (2021). Dietary supplementation of levamisole modulates protein and lipid MALDI-TOF MS profiles of Nile tilapia without causing negative histological alterations. *Aquaculture*, *533*, p.736177. https://doi.org/10.1016/j.aquaculture.2020.736177

Defoirdt, T. (2023). Indole signaling, a promising target to control vibriosis in aquaculture. *Aquaculture*, *574 p.*, 739692. https://doi.org/10.1016/j.aquaculture.2023.739692

Eissa, E.S.H., Dighiesh, H.S., Ghanem, S.F., Bodrul Munir, M.B., Syafaat, M., Eissa, M.E., AL-Farga, A., Abdul Kari, Z. & Abd El-Hamed, N.N. (2024a). Evaluation of hatching mixed probiotics effects on water quality, larval development, thermal, hyposalinity shocks of kuruma shrimp, *Penaeus japonicus* (Bate 1888). *International Aquatic Research*, *16*(3), 257-269. https://doi.org/10.22034/iar.2024.1999915.1541

Eissa, E.S.H., El-Sayed, A.F.M., Hendam, B.M., Ghanem, S.F., Abd Elnabi, H.E., Abd El-Aziz, Y.M., Abdelnour, S.A., Eissa, M.E. & Dighiesh, H.S. (2024b). The regulatory effects of water probiotic supplementation on the blood physiology, reproductive performance, and its related genes in Red Tilapia (*Oreochromis niloticus* X *O. mossambicus*). *BMC Veterinary Research*, *20*(1), p.351. https://doi.org/10.1186/s12917-024-04190-w

Eissa, E.S.H., Hendam, B.M., Dighiesh, H.S., Abd Elnabi, H.E., Abd El-Aziz, Y.M., Eissa, M.E.H., Abdelnour, S.A.& Ghanem, S.F. (2024c). Comparative effects of curcumin, nano curcumin and their combination on reproductive traits and spawning performance of red tilapia (*Oreochromis Niloticus* X *O. Mossambicus*). *BMC Veterinary Research*, 20(1), p.427. https://doi.org/10.1186/s12917-024-04257-8.

El-Sayed, H.S., Ghanem, S.F. & Barakat, K.M. (2024). Role of recent feeding protocols, rearing water systems and microbial trends in improving marine larviculture: insights into water quality and larval performance. *International Aquatic Research*, *16*(3),213-232. https://doi.org/10.22034/iar.2024.2008550.1645

Elumalai, P., Soltani, M. & Lakshmi S. (2023). Immunomodulators in Aquaculture and Fish Health. CRC Press.

El-Zaeem, S.Y., El-Hanafy, A., El-Dahhar, A.A., Elmaghraby, A.M., Ghanem, S.F. &Hendy, A.M. (2024). A novel investigation for early sex determination in alive adult European seabass (*Dicentrarchus labrax*) using *cyp19a1a*, *dmrt1a*, and *dmrt1b* genes expression in tail fin tissues. *Marine Biotechnology*, 26(3),423-431. https://doi.org/10.1007/s10126-024-10313-z

Eslami, M.& Bahrekazemi. M. (2019). Effects of levamisole, echinacea, and thyme oral administration on growth factors, blood parameters, and immunity in beluga (*Huso huso*). *Journal of Applied Aquaculture,* 31(1),68-84. https://doi.org/10.1080/10454438.2018.1493018

Fadel, K.A., El-Sayed, H.S., Barakat, K.M., El-Bermawi, N., Massoud, R.G., Abouelkheir, S.S.& Ghanem, S.F. (2024). Effect of Different Intensities of Magnetized Water on Histological Characteristics and Growth Performance of the Sea Bream (*Sparus aurata*) Juveniles. *Egyptian Journal of Aquatic Biology & Fisheries*, 28(1), 281-300. https://doi.org/10.21608/EJABF.2024.337883

Feyera, T., Ruhnke, I., Sharpe, B., Elliott, T. &Walkden-Brown, S.W. (2021). Comparative therapeutic efficacies of oral and in-water administered levamisole, piperazine and fenbendazole against experimental *Ascaridia galli* infection in chickens. *Veterinary Parasitology*, 298, 109514. https://doi.org/10.1016/j.vetpar.2021.109514

Ghittino, C., Latini, M., Agnetti, F., Panzieri, C., Lauro, L., Ciappelloni, R. & Petracca, G. (2003). Emerging pathologies in aquaculture: effects on production and food safety. *Veterinary Research Communications*, *27*, 471-479. https://doi.org/10.1023/b:verc.0000014204.37722.b6

Gudding, R. (2014). Vaccination as a Preventive Measure," in Fish Vaccination. John Wiley & Sons, Inc, Oxford, UK, 1, 12–21.

Hoseinifar, S.H., Ashouri, G., Marisaldi, L., Candelma, M., Basili, D., Zimbelli, A., Notarstefano, V., Salvini, L., Randazzo, B., Zarantoniello, M.& Pessina, A. (2024). Reducing the Use of Antibiotics in European Aquaculture with Vaccines, Functional Feed Additives and Optimization of the Gut Microbiota. *Journal of Marine Science and Engineering,*12(2),204. https://doi.org/10.3390/jmse12020204

Ispir, U.& Yonar, M.E. (2007). Effects of levamisole on phagocytic activity of rainbow trout (*Oncorhynchus mykiss* W.). *Acta Veterinaria Brno,*76(3),493-497. https://doi.org/10.2754/avb200776030493

Kobayashi, T., Enomoto, S., Sakazaki, R.& Kuwahara, S. (1963). A New Selective Isolation Medium for The Vibrio Group; On A Modified Nakanishi's Medium (Tcbs Agar Medium). *Japan Journal of Bacteriology*,18, 387-92. https://doi.org/10.3412/jsb.18.387

Kumar, S., Choubey, A.K. & Srivastava, P.K. (2022). The effects of dietary immunostimulants on the innate immune response of Indian major carp: A review. *Fish & Shellfish Immunology*,123,36-49. https://doi.org/10.1016/j.fsi.2022.02.039

Kumari, J.& Sahoo, P.K. (2006). Dietary levamisole modulates the immune response and disease resistance of Asian catfish *Clarias batrachus* (Linnaeus). *Aquaculture Research*, 37(5),500-509. https://doi.org/10.1111/j.1365-2109.2006.01456.x

Li, P., Wang, X. &Gatlin III D.M. (2006). Evaluation of levamisole as a feed additive for growth and health management of hybrid striped bass (*Morone chrysops*× *Morone saxatilis*). *Aquaculture,*251(2-4),201-209. https://doi.org/10.1016/j.aquaculture.2005.11.015

Maqsood, S., Samoon, M.H. &Singh, P. (2009). Immunomodulatory and growth promoting effect of dietary levamisole in *Cyprinus carpio* against the challenge of *Aeromonas hydrophila*. *Turkish Journal of Fisheries & Aquatic Sciences*, 9, 111– 120.

Misra, C.K., Das, B.K.& Mukherjee, S.C. (2009). Immune response, growth and survival of *Labeo rohita* fed with levamisole supplemented diets for longer duration. *Aquaculture Nutrition*,15(4),356–365. https://doi.org/10.1111/j.1365-2095.2008.00600.x

Mulero, V., Esteban, M.A., Munoz, J.& Meseguer, J. (1998). Dietary intake of levamisole enhances the immune response and disease resistance of the marine teleost gilthead seabream (*Sparus aurata* L). Fish& Shellfish Immunology, 8(1),49–62. https://doi.org/10.1006/fsim.1997.0119

Nogueira, J.N., Santos, G.G.& Tavares-Dias, M. (2019). High concentration of levamisole in the diet of *Colosso mamacropomum* (Pisces: Serrasalmidae) is effective for controlling monogenean parasites. *Revista Brasileira de Parasitologia Veterinária*,28(4),708-712.

https://doi.org/10.1590/S1984-29612019095

Oliveira, B.D.L., Souza, R.M.D., Silva, Filho, J.P.D., Ribeiro, H.P., Fioresi, D.B., Gomes, L.D.C.& Fernandes, L.F.L.(2019). Levamisole on the biochemical and hematological responses to parasitism by monogenoids in dog snapper. *Ciência Rural*, 49, p.e20190013. https://doi.org/10.1590/0103-8478cr20190013

Pahor-Filho, E., Pereira, J.Jr., Pilarski, F.& Urbinati, E.C. (2017). Levamisole reduces parasitic infection in juvenile pacu (*Piaractus mesopotamicus*). *Aquaculture*, 470, 123-128. http://dx.doi.org/10.1016/j.aquaculture.2016.12.034.

Radwan, M., Darweesh, K.F., Ghanem, S.F., Abdelhadi, Y., Kareem, Z.H., Christianus, A., Karim, M., Waheed, R.M.& El-Sharkawy, M.A. (2023b). Regulatory roles of Pawpaw (*Carica papaya*) seed extract on growth performance, sexual maturity, and health status with resistance against bacteria and parasites in Nile tilapia (*Oreochromis niloticus*). *Aquaculture International*, 31(5), 2475-2493. https://doi.org/10.1007/s10499-023-01094-8.

Radwan, M., El-Sharkawy, M.A., Alabssawy, A.N., Ghanem, S.F., Mohammadein, A., Al Malki, J.S., Al-Thomali, A.W., Manaa, E.A., Soliman, R.A., Yassir, S.& Mekky, A.E. (2023a). The synergy between serious parasitic pathogens and bacterial infestation in the cultured Nile tilapia (*Oreochromis niloticus*): a severe threat to fish immunity, causing mass mortality and significant economic losses. *Aquaculture International*, 31(5), 2421-2449. https://doi.org/10.1007/s10499-023-01093-9.

Şahin, T. & Üstündağ, C. (2003). Effect of different rearing systems on survival rate of hatchery reared Black Sea turbot, Scophthalmus maximus. *Turkish Journal of Fisheries and Aquatic Sciences*, *3*(1),25-27.

Sakai, M. (1999). Current research status of shrimp immunostimulants. *Aquaculture*, 172, 63-92.

Secombes, C.J. (1994). Enhancement of fish phagocyte activity. *Fish &Shellfish Immunology*,4, 421-436. https://doi.org/10.1006/fsim.1994.1038

Sink, T. & Lochmann, R.T. (2014). The effects of soybean lecithin supplementation to a practical diet formulation on juvenile channel catfish: growth, survival, hematology, innate immune activity and lipid biochemistry. *Journal of the world Aquaculture society*, 45(2), 163–172. https://doi.org/10.1111/jwas.12108.

Siwicki, A.K., Anderson, D.P.& Rumsey, G.L. (1994). Dietary intake of Immunostimulants by rainbow trout affects non-specific immunity and protection against Furunculosis. *Veterinary immunology and immunopathology*, 41(1-2),125-139. https://doi.org/10.1016/0165-2427(94)90062-0

Siwicki, A.K. (1989). Immunostimulating of levamisole on non-specific immunity in carp (*Cyprinus carpio* L). Developmental & Comparative Immunology,13, 87-91.

Snedecor, G.W.& Cochran, W.G. (1967). Statistical Methods. Oxford and IBH, New Delhi, 381-418.

Toranzo, A.E., Magariños, B. & Romalde, J.L. (2005): A review of the main bacterial diseases in mariculture systems. *Aquaculture*, 246, 37-61. DOI: 10.1016/j.aquaculture.2005.01.002

Vijayaram, S., Sun, Y-Z., Zuorro, A., Ghafarifarsani, H., Doan, H.V.& Hoseinifar, S.H. (2022). Bioactive immunostimulants as health-promoting feed additives in aquaculture: A review. *Fish & Shellfish Immunology*, 130, 294-308. https://doi.org/10.1016/j.fsi.2022.09.011

Vijayaram, S., Ringø, E., Zuorro, A., Doan, H. & Sun, Y. (2023). Beneficial roles of nutrients as immunostimulants in aquaculture: A review. *Aquaculture and Fisheries.* https://doi.org/10.1016/j.aaf.2023.02.001

Wassef, E.A., Saleh, N.E., Abdel-Meguid, N.E., Barakat, K.M., Abdel-Mohsen, H.H.& El-bermawy, N.M. (2020). Sodium propionate as a dietary acidifier for European sea bass (*Dicentrarchus labrax*) fry: immune competence, gut microbiome, and intestinal histology benefits. *Aquaculture International*, 28(1),95-111. https://doi.org/10.1007/s10499-019-00446-7

Woo, W.S., Kang, G., Kim, K.H., Son, H.J., Sohn, M.Y., Lee, J.H., Seo, J.S., Kwon, M.G.& Park, C.I. (2023). Exploring the Efficacy and Safety of Levamisole Hydrochloride against *Microcotylese bastis* in Korean Rockfish (*Sebastes schlegelii*): An *In Vitro* and *In Vivo* Approach. *Animals*, 13(11),1791. https://doi.org/10.3390/ani13111791

Xu, K., Wang, Y., Yang, W., Cai, H., Zhang, Y.& Huang, L. (2022). Strategies for prevention and control of vibriosis in Asian fish culture. *Vaccines*, *11*(1), p.98.

Zhao, W., Liu, Z.L. &Niu, J. (2021).Growth performance, intestinal histomorphology, body composition, hematological and antioxidant parameters of *Oncorhynchus mykiss* were not detrimentally affected by replacement of fish meal with concentrated dephenolization cottonseed protein. *Aquaculture Reports*,19, 100557. https://doi.org/10.1016/j.aqrep.2020.100557

Zupičić, I.G., Pavlinec, Ž., Veić, T., Oraić, D. & Zrnčić, S. (2022). Similarities and differences between vibriosis in European sea bass caused by *Vibrio anguillarum* and *Vibrio harveyi*. *Veterinarski Arhiv*, 92 (4), 509-524, DOI: 10.24099/vet. arhiv.1575