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Growth and Immune Response of *Pangasius hypophthalmus* Fed Diets Containing Seaweed Extracts as Immunostimulant

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HIGHLIGHTS

- *S. oligocystum* HWE increased growth performance of *P. hypophthalmus.*
- HWE of *S. oligocystum* improved immune response of *P. hypophthalmus.*
- HWE of *S. oligocystum* have potential as natural immunostimulant for finfish.

Abstract: Growth and immune response of *Pangasius hypophthalmus* were evaluated after feeding the fish with diets containing hot-water extracts (HWE) of *Sargassum oligocystum* as immunostimulant at 100, 300, and 500 mg kg-^{1} diet. Basal diet for *P. hypophthalmus* served as the control. The experimental diets were administered for 12 weeks. At the end of the feeding experiment, growth and haematological profile of fish were evaluated. Result showed that final weight, weight gain, daily growth rate and feed conversion ratio were significantly increased in the fish that received 300 and 500 mg kg-^{1} HWE of *S. oligocystum*. Evaluation of the haematological profile showed that white blood cells red blood cells, hemoglobin, hematocrit and platelet of *P. hypophthalmus* that received the HWE of *S. oligocystum* were significantly higher than the control group. Overall, our results indicate that the use of *S. oligocystum* HWE improves growth and haematological profile in *P. hypophthalmus*.

Keywords: Growth performance; Hot water extract; *Pangasius hypophthalmus*; *Sargassum oligocystum.*

INTRODUCTION

Disease outbreaks were recently identified as a result of intensification and expansion in aquaculture production [1], with consequent harmful effect on the industry's economic development and serious economic losses [2]. Commercial approaches were used to control fish diseases, such as the use of anti-microbial agents and vaccination, however, they have limited success in the prevention of/or treatment of aquatic diseases. Antibiotics and other chemotherapeutics have been used to control fish and shellfish diseases can result in the development of drug-resistant pathogens [3], environmental treat [4], and accumulation of residues in fish. Therefore, looking for less-harmful approaches and more environment-friendly treatments became of premium importance in aquaculture management. Immunostimulation, or enhancement of the immune system of an organism, seems to be the most promising methods in increasing host immunity and disease prevention in culture fish [5].

Riverine Catfish *Pangasius hypophthalmus* is considered as one of the most successful aquaculture species due to its relative ease in culture, fast-growing fish, high-market demand, and suitability to local climate conditions [6]. Although, *P. hypophthalmus* is highly tolerant to adverse environmental conditions [7]. However, the occurrence of diseases in *P. hypophthalmus* farming has become a major problem causing significant losses to the farmers [8], due to lack of diagnostic support and appropriate therapeutants [9]. Therefore, the maintenance of fish in health, and enhancement of the fish innate immunity are the primary concern.

The use of seaweeds extracts is inexpensive than chemicals and antibiotics and have little effects on nature, humans, and fish and containing properties that modulate the fish immune system. Brown seaweeds such as *Sargassum* spp. have been studied and has potential antimicrobial agents and as natural immunostimulant in aquaculture industry for treated microbial diseases in infected finfish and crustaceans [10-14]. Several studies demonstrated that natural extracts from seaweeds can modify immune response against infectious diseases in fish. Therefore, the aim of this study was to determine the effects of hot-water extract (HWE) of *Sargassum oligocystum* on growth performance, and immune response using *P. hypophthalmus* as an experimental model. Specifically, we tested the hypothesis that seaweed extract could be used to enhance the growth and immune response in fish.

MATERIAL AND METHODS

Experimental animals

Around 4,000 *P. hypophthalmus* fingerlings were obtained from Frame farm at Muñoz, Nueva, Ecija and were shipped to the aquaculture experimental laboratory room of Institute of Fisheries, Isabela State University, Echague, Isabela. The experimental animals were placed in plastic container (100 L) capacity and were acclimatized under laboratory condition for two weeks prior to the conduct of the experiments. During the acclimatization period, the experimental animals received formulated diets to satiation twice daily (1000 H and 1600 H). The experimental animals were screened for the presence of pathogen before the start of the experiments.

Collection site and preparation of the hot-water extract of *S. oligocystum*

S. oligocystum was collected by scuba diving and handpicking from the rocky substratum at depth of 1-3 m along the subtidal areas at Diora-Zinungan, Santa Ana (16˚ 46'79" N lat, 121˚ 23'00.48" E long) Cagayan, Philippines. The hot-water extract (HWE) of *S. oligocystum* was prepared based on the method described by [15,19] with some modifications. Briefly 50 g of dried powdered seaweeds were mixed with 500 ml of deionized distilled water and cover with plastic wrap. The suspension was boiled for three (3) hours in a water bath. After boiling, the solid particles were removed by straining and squeezing using cheese cloth and the suspension was filtered using nylon mesh and the filtrate was kept frozen at -80°C for 24 h and lyophilized under reduced pressure for 48 h.

Diet preparation

The HWE of *S. oligocystum* was dissolved in distilled water at concentrations of 10, 30, 50 g/L of water. The solvent of distilled water and HWE of *S. oligocystum* was sprayed at a rate of 100, 300, 500 ml kg-1 of commercial feed. The four experimental diets were air-dried at room temperature for 24 h. The prepared diets were packed, labeled and stored in a refrigerator (4°C) until use.

Experimental treatment and set-up

The experiment was conducted following Completely Randomized Design (CRD). All the treatments of the different experiments have three replicates. Survival and growth of each experimental animal were monitored every two weeks. The length and weight of each experimental animal in each treatment were recorded and served as a basis for the adjustment of feed rate.

Growth trial experiments

There were four experimental treatments in the study and each treatment was replicated three times. The fish were stock in 100 l capacity plastic containers. Feeding trials were carried out under the laboratory condition for 12 weeks. One group for each set-up served control (commercial feeding), the remaining three groups in each set-up were fed with experimental diets containing of 100, 300, 500 mg kg^{-1} HWE of *S. oligocystum* for the dietary administration at 5% of their total body weight. Water management was done thrice weekly. Growth of each experimental animals were monitored every two weeks. At the end of the feeding trial, the growth parameters such as weight gain (WG), survival (S), daily growth rate (DGR), feed conversion ratio (FCR), were calculated by using following formulae:

Weight Gain (WG) = Final weight (g) – Initial Weight
Survival Rate (SR) % = Total no. of live animals / Total no. of initial animals × 100
Daily growth rate (DGR) % = Weight Gained/ No. of days × 100
Feed Conversion Ratio (FCR) = Total feed intake/ Total weight gained (g)

Collection of blood

Six fish was randomly collected from each treatment at days 1, 3, 5, and 7 post-administration of hot water extract. To minimize handling stress, the fish was anesthetized by immersion in water containing 100 mg l^{-1} tricaine-s methane sulfonate (MS 222). Whole blood (1ml) was collected from the caudal vein of each fish using syringes (1 ml) with 25-gauge needles that was rinsed with 0.2 M Ethylenediaminetetraacetic acid (EDTA) as anticoagulant. A portion of a dorsal fin of *P. hypophthalmus* was cut after blood collection for identification purposes. The collected blood was transferred in 0.5 ml heparin vacutainer tube with EDTA and maintained at low temperature and immediately transferred to Diagnostic Prime Laboratory, San Fabian, Echague, Isabela Philippines using Rayto Auto Hematology Analyzer (RT-7600) for further analyses.

Statistical analysis

The effect of treatment on growth, and immune parameter was evaluated using a one-way analysis of variance (ANOVA) with treatment, weeks as variables. A multiple-comparison using (Tukey's) test was determine significant differences among the treatment using the (SPSS, 21.0). Data are reported as mean ± standard deviations. Statistical significant of differences required that $p<0.05$.

RESULTS AND DISCUSSION

Growth performance

Dietary administration seaweeds as immunostimulant is a safe, eco-friendly alternative approach against diseases for the aquaculture industry. Hot-water extract administration from several species of *Sargassum* spp*.* has been reported to promote growth performance immunity and disease resistance of various finfish against selected aquaculture pathogens [16-19]. The immunostimulatory effects of the hot-water seaweed extracts of *Sargassum oligocystum* via oral administration were investigated in this study. The growth performance of *P. hypophthalmus* fed with the different diets containing HWE of *S. oligocystum* in terms of mean initial and final length (cm), initial and final weight (g), WG (g), SR (%), DGR (%) and FCR is shown in Table 1.

The final length, final weight, weight gain, daily growth rate, feed conversion ratio significantly increased in the experimental fish fed with HWE of *S. oligocystum* containing diet 300 and 500 mg kg^{-1} when compared with control diets after 12 weeks of feeding trials. However, no significant differences on the FCR and SR were found among treatment. Grouper, *E. coioides* fed *Sargassum cristaefolium* containing diets at 0.5, 1 and 2 g/kg significantly improved growth performance of the fish [18]. Moreover, [20], reported that a significant improvement in growth performance was found in the snakehead *C. striatus* fry fed diets containing 5% *Ulva* spp. which conform to the present investigation. The use of *Porphyra purpurea* at high inclusion levels (16% and 33%), as an ingredient for mullet (*Chelon labrosus*) diets suppressed growth performance and feed utilization efficiency [21]. In another study by [22], revealed that the dietary supplementation of selected microalgae such as *Gracilaria* spp., *Ulva* spp., and *Fucus* spp., at 2.5 and 7.5% levels, in diets for European seabass had no effect on final body weight, DGI, FCR and PER which in contrast to our result. This difference in result might be due to types of fish species used, age, feeding duration, source of the immunostimulant, stocking densities, water quality or other environmental factors [23-24].

Table 1. Growth parameters of Riverine catfish *P. hypophthalmus* fed with experimental diets containing 100, 300 or 500 mg kg -^{1} HWE *S. oligocystum.*

Parameters	**Feeds**			
	Control	**Experimental Treatments**		
	Feed 1 (0 mg kg^{-1} HWE)	Feed 2 (100 mg kg^{-1} HWE)	Feed 3 (300 mg kg^{-1} HWE)	Feed 4 (500 mg kg^{-1} HWE)
Length (Initial) cm	6.66±0.90^{a}	7.53±0.08^{a}	7.43±0.26^{a}	7.41±0.33^{a}
Length (Final) cm	22.13±1.46^{b}	23.37±0.12^{ab}	24.13±0.72^{ab}	24.52±0.78^{a}
Weight (Initial) g	5.3±0.26^{a}	5.56±0.15^{a}	5.46±0.15^{a}	5.38±0.82^{a}
Weight (Final) g	38.53±0.88^{c}	40.43±1.66^{bc}	42.37±1.14^{ab}	44.23±1.51^{a}
Weight gain (g)	33.22±0.95^{c}	34.87±1.51^{bc}	36.9±1.22^{ab}	37.85±2.09^{a}
Survival rate (%)	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}
Daily growth rate (%)	39.56±1.13^{c}	41.51±1.80^{bc}	43.93±1.46^{ab}	46.25±2.48^{a}
Feed conversion ratio	2.26±0.23^{a}	2.25±0.07^{a}	2.19±0.33^{a}	2.18±0.11^{a}

Each value is mean ± of triplicate observations. Mean values having the same superscript are not significantly different ($p > 0.05$).

Haematological profile

Hematological parameters were evaluated to determine the immune response of *Pangasius hypophthalmus*. Blood of fish may be one of the indicators for diagnosis of fish diseases. Thus haematological parameters are an important tool for determining health status [25-26], and fish health diagnosis [27]. In the study, the white blood cell (WBC) count of *P. hypophthalmus* that fed diets containing of HWE of *S. oligocystum* at 500 mg kg-^{1} was significantly higher than the fish fed the control diet over 1-7 days (Figure 1), that confirms the previous findings [17]. WBC is important cells and plays a great role in the immune system of the fish, because of their main defensive function to overcome the toxic stress [28], and defense against infection [29]. The increase in WBC of fish was suggested to indicate an alteration in defense mechanism against the action of the highly toxic and the bioaccumulated heavy metals in fish tissues [30]. In addition, a measurable increase in WBC of fish is a function of immunity and response to vulnerable illness and disease [31].

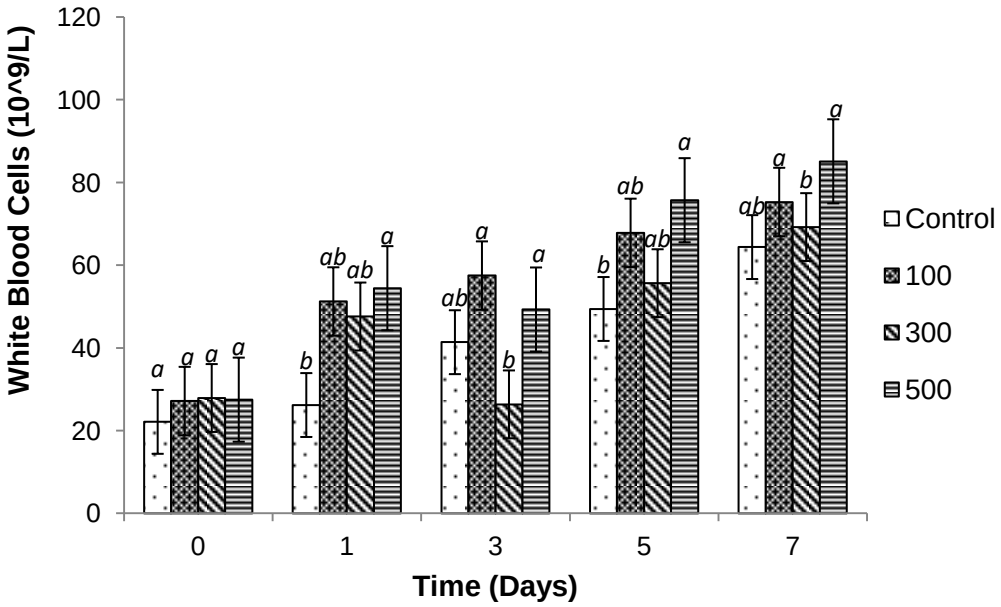

Figure 1. Mean (± S.E.) white blood cell count of *P. hypophthalmus* that received HWE *S. oligocystum* at 100, 300 or 500 mg kg -^{1}. Each bar represents mean value from six determinations with standard error. Data at the same exposure time with different letters are significantly different (p<0.05) among treatments.

The red blood cell (RBC) count of *P. hypophthalmus* that fed diets containing of HWE of *S. oligocystum* at was significantly higher than the fish fed the control diet over 1, 3 and 7 days, (Figure 2), which is similar to the study of [32]. The increase of erythrocyte or RBC count is an indication of high oxygen carrying capacity of blood which is the characteristic of fishes capable of aerial respiration and with high activity [33]. However, a decline in hemoglobin content and red blood cells showed anemic characters of the fish which leads to hemodilution and immunosuppression due to which infection grows faster resulting in fish mortality [34].

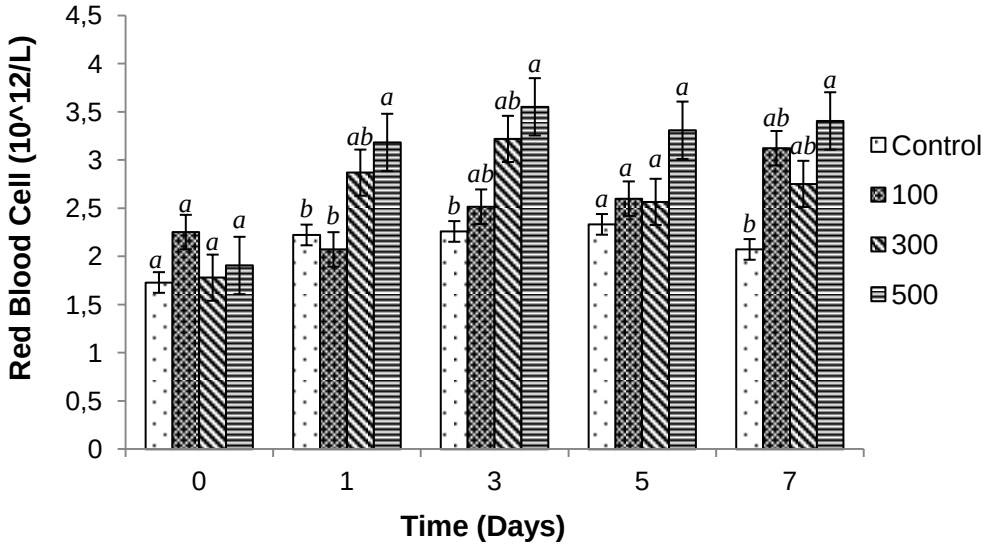

Figure 2. Mean (± S.E.) red blood cell count of *P. hypophthalmus* that received HWE *S. oligocystum* at 100, 300 or 500 mg kg $^{-1}$. Each bar represents mean value from six determinations with standard error. Data at the same exposure time with different letters are significantly different (p<0.05) among treatments.

The hematocrit count of *P. hypophthalmus* that fed diets containing of HWE of *S. oligocystum* at was significantly higher than the fish fed the control diet over 1, 5 and 7 days, respectively (Figure 3). Similar results were observed by [35]. A decline in RBC and hematocrit combined with signs of anaemia and cases of proliferative kidney disease [36]. In addition, decreased RBC counts and hematocrit indicated that erythrocytes were being affected or destroyed with the infection [37]. The normal hematocrit values usually fall within the range of 20-35% and are rarely greater than 50% for fish. However, since transport of metals in fish occurs through the blood where the ions are usually bound to proteins and pollutants generally produce relatively rapid changes in blood characteristics of fish [38].

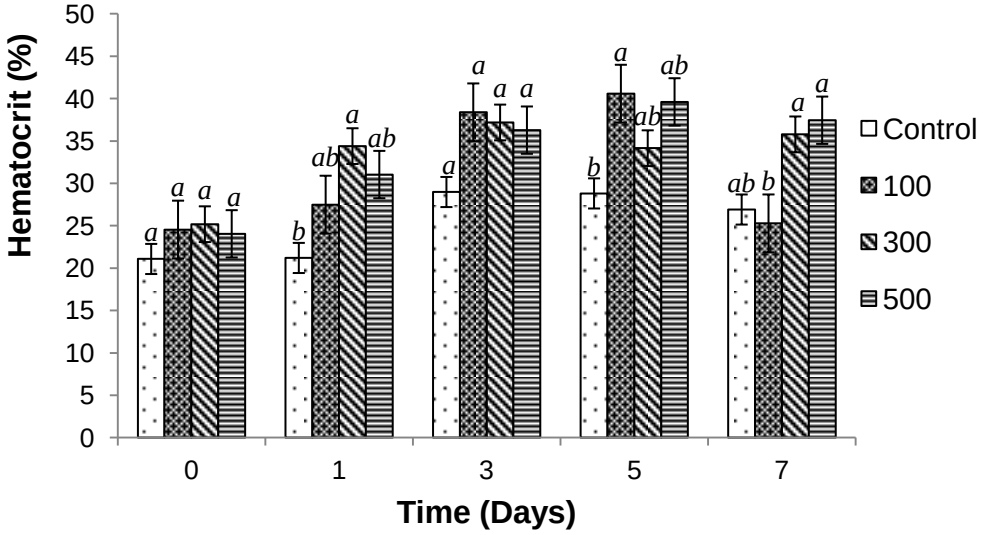

Figure 3. Mean (± S.E.) hematocrit count of *P. hypophthalmus* that received HWE *S. oligocystum* at 100, 300 or 500 mg kg $^{-1}$. Each bar represents mean value from six determinations with standard error. Data at the same exposure time with different letters are significantly different (p<0.05) among treatments.

The Hemoglobin count of *P. hypophthalmus* that fed diets containing of HWE of *S. oligocystum* at 100 mg kg-^{1} and 500 mg kg-^{1} was significantly higher than the fish fed the control diet over 1-7 days (Figure 4). The Hemoglobin count from the present study was more or less similar to the findings of [39]. Decrease in hemoglobin trend may be a result of swelling of RBCs as well as poor mobilization of hemoglobin from spleen [34]. Prolonged reduction in hemoglobin content is deleterious to oxygen transport and degeneration of the erythrocytes could be due to pathological condition in fish exposed to toxicants. The decrease in hemoglobin corresponds with the decrease in dissolved oxygen; an indication that the decrease in hemoglobin resulted in hemodulation [40].

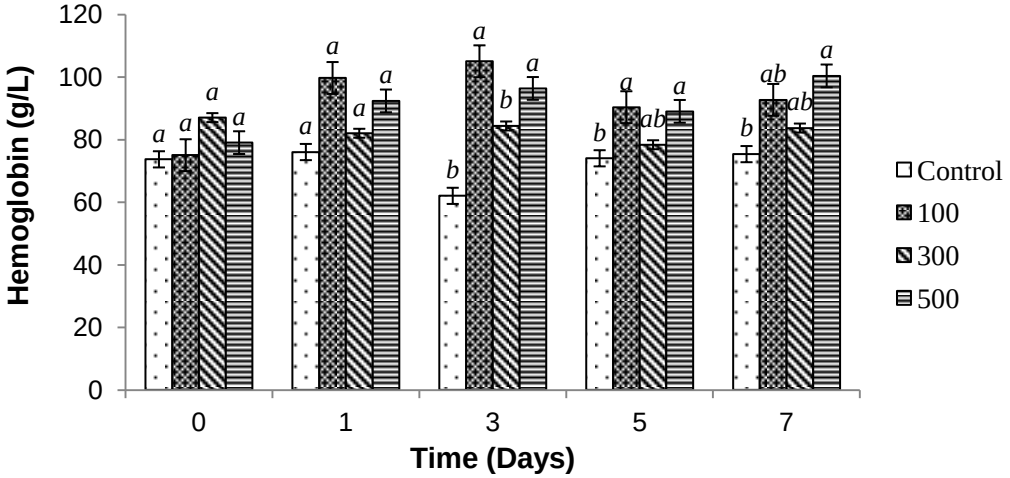

Figure 4. Mean (± S.E.) hemoglobin count of *P. hypophthalmus* that received HWE *S. oligocystum* at 100, 300 or 500 mg kg -^{1}. Each bar represents mean value from six determinations with standard error. Data at the same exposure time with different letters are significantly different (p<0.05) among treatments.

The platelet count of *P. hypophthalmus* that fed diets containing of HWE of *S. oligocystum* at was significantly higher than the fish fed the control diet over 1, 3 and 7 days, (Figure 5). Platelets are also surprisingly multifunctional and are involved in many pathophysiological processes including haemostasis and thrombosis, clot retraction, vessel constriction and repair, including promotion of atherosclerosis, host defense and even tumour growth/metastasis [41].

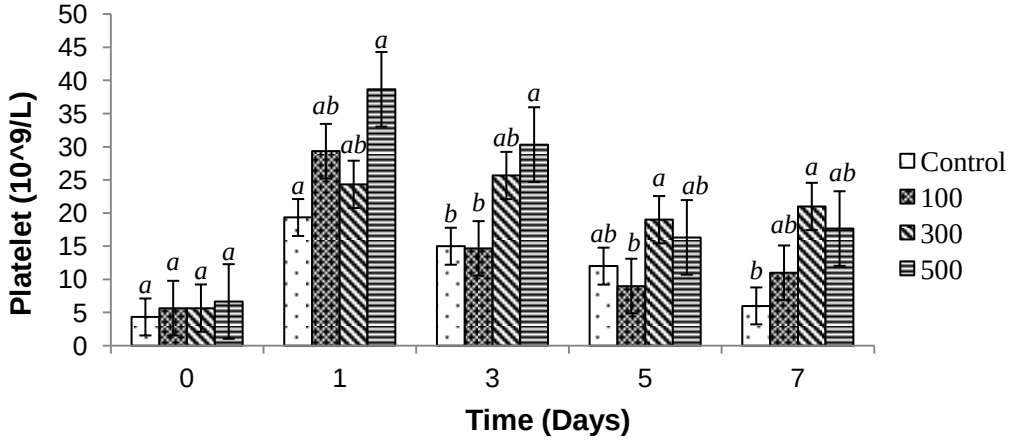

Figure 5. Mean (± S.E.) platelet count of *P. hypophthalmus* that received HWE *S. oligocystum* at 100, 300 or 500 mg kg -^{1}. Each bar represents mean value from six determinations with standard error. Data at the same exposure time with different letters are significantly different (p<0.05) among treatments.

For effective use of immunostimulants, timing, dosage, route of administration, and physiological conditions of fish should always be considered [42]. In addition, polysaccharides present in seaweeds may activate the non-specific immune response in both finfish and crustaceans. The blood composition in fish might be changed by dietary treatment, malnutrition and disease condition [43], and the changes in hematological profile in response to stressing agents are indicators of the stressful stage of fish [44].

CONCLUSION

Overall, our results indicate that the used HWE of *S. oligocystum* improves growth and immune responses in *P. hypophthalmus* and it can be used as a natural immunostimulant and may be a valuable tool to increase the immune-competency of valuable aquaculture fish species.

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Conflicts of Interest: The authors declare no conflict of interest.

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