



## The synergy effect of *Spirulina platensis* and/or chitosan nanoparticles on the growth performance, hematological, biochemical parameters, and body composition of the European seabass (*Dicentrarchus labrax*)

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### ABSTRACT

This research was conducted to assess the potential synergy impacts of *Spirulina platensis* (SPs) and chitosan nanoparticles (CHns) on the growth rates, hematological, biochemical parameters, and body chemical composition of the European seabass (*Dicentrarchus labrax*). Seabass with 2.34g initial weight was distributed in 8 hapas (15 fish/ m<sup>3</sup>) and fed twice a daily at a 5% feeding rate for 16 weeks. Four diets containing 45% crude protein were formulated and supplemented by SPs and CHns: T1: control, T2: 1% SPs, T3: 1% CHns, T4: 1% SPs and 1% CHns. Results showed that those fed additives of both *S. platensis* and chitosan nanoparticles observed significant variation and enhanced growth performance such as final body weight (FBW), weight gain (WG%), and specific growth rate (FCR), compared to the groups treated with the control. The results of hematological parameters showed a significant increase in the red blood cells (RBCs) count, hemoglobin (Hb) count, hematocrit (Hct) value, and white blood cells (WBCs) count. In the same trend, biochemical parameters, for instance, the concentration of the total protein, albumin, globulin, glucose, cholesterol, lactate, triglyceride, urea, creatinine, uric acid, and some minerals such as calcium, magnesium, chloride, and inorganic phosphorous showed a significant improvement in *D. labrax* fed a diet containing SPs or/and CHns as feed additives. In the same way, body analysis data recorded a significant difference in protein, lipid, and ash contents in fish fed SPs or/and CHns compared to the control group. More defined experiments are necessary to determine the maximum levels of these microalgae and nanoparticles in *D. labrax* diets.

### INTRODUCTION

Nowadays, the demand for fish culture has improved and is considered one of the essential protein sources necessary for human nutrition (FAO, 2018). Thus, it is crucial to increase aquatic production by intensive culture, which is rich in the amount of harvest per m<sup>3</sup> (Herrera et al., 2019). *S. platensis* (SPs) relieves physiological, immunological, and antioxidant status in farmed fish (Adel et al., 2016). SP comprises various functional

and bioactive products, such as vitamins, minerals, carotenoids, polysaccharides, and  $\alpha$ -linolenic acid (**Victor *et al.*, 2019**). Therefore, SPs play a preventive and stimulatory function in the Nile tilapia exposed to deltamethrin against lipid peroxidation and oxidative stress (**Abdelkhalek *et al.*, 2015**). Many investigations have used dried *S. platensis* as a feed additive (**Sung-Sam *et al.*, 2013; Radhakrishnan *et al.*, 2014**). Moreover, it was examined as a partial supplementation (**Ghaeni *et al.*, 2011**). For example, *Peneaus semisulcatus* feed was used as a dietary supplementation in the guppy fish diet (**Seval *et al.*, 2010**) and as a feed additive to improve the growth performance and the immune response of *Oreochromis niloticus* (**Mahmoud *et al.*, 2018; Abdel-Warith & Elsayed, 2019**).

It is worthy to mention that, the supercritical fluid from *S. platensis* exhibits antioxidants and antimicrobials (**Mendiola *et al.*, 2007**). It is safe and replaces commercially available artificial antioxidants. It contains biological properties, including biosafety, biodegradability and biocompatibility (**Fouad, 2008**).

Chitin, chitosan, oligosaccharides, and derivatives exert many biological activities including antitumoral, antimicrobial, antioxidant, and anti-inflammatory activities, which could be used as therapeutic polymers. It is remarkable that, up till today chitosan and chitosan hydrochloride are only accepted as excipients by the regulatory agencies and not as a drug for the treatment of diseases (**Aranaz *et al.*, 2021**). On the other hand, bacterial resistance to antibiotics is a critical public health concern, and therefore, there is an urgency to find alternatives to antibiotics. Notably, chitosan, chitosan derivatives, and chito-oligosaccharides exert antimicrobial activity against different microorganisms, including bacteria, filamentous fungi, and yeast (**Raafat & Sahl, 2009**).

These properties make chitosan, a natural polymer, dominant over artificial polymers, and promising for complete application (**Fouad, 2008; Muzzarelli, 2010**). One of these biological properties of chitosan is biosafety. For example, in aquaculture, numerous standards should be considered when including any fish food supplement obviating any unwanted effects on fish (**Abdel-Warith *et al.*, 2020; Fath El-Bab *et al.*, 2020a, b**). One of the different attractive features of chitosan nanoparticles was their lower toxicity than other natural polysaccharides (**Abdel-Ghany & Salem, 2020**). Besides, **Qi *et al.*, (2004)** conveyed that chitosan nanoparticles significantly affect antimicrobial activities compared to chitosan particles of big size (**Atay & Çelik, 2017**). In addition, chitosan nanoparticles have antibacterial action against Gram-positive bacteria (*S. auerus*) and Gram-negative bacteria (*E. coli*) (**Hosseinnejad & Jafari, 2017**). The European seabass (*D. labrax*) is presently one of the maximum significant marine fish types produced in Europe and the Mediterranean Sea. It is one of the seven full productions of culture fish in the EU (**Commission, 2014**). Therefore, seabass can be farmed in marine, brackish water ponds and lagoons. It can be reared giving high production in sea cage farms, which confirms that it will be close to land, in the open sea, and even inside protective bays. Usually, juveniles are obtained from hatcheries, with

weights about 1.5 to 2.5g and can reach a final weight of 400 – 450g within 18 to 24 months, depending on water temperature, type of culture and feeding procedures (**FAO, 2016**). This study aimed to evaluate the effectiveness of utilizing SPs and CHns as dietary enhancements in (*D. labrax*) food.

## MATERIALS AND METHODS

### Experimental fish

The European seabass (*D. labrax*) were collected from Burullus and appropriately transported in water and oxygen-filled plastic bags to hapas (1\*2.5\*1 m) placed in 2 cages. All treatments were fed on a basal diet (control) for about fourteen days to acclimatize. Fish with an initial weight of about  $2.34 \pm 0.28$ g were equally allocated into eight hapas (15 fish / m<sup>3</sup>). At the end of the experimental period (16 weeks), nine *D. labrax* individuals were collected from each replicate (hapa) to estimate the hematology and biochemical parameters. At the start and the end of the experiment, five fish were chosen from each hapa to determine the chemical composition of the whole fish chemically analyzed according to the method of **Cunniff (1995)**.

### Experimental diets

The diet formulation containing about 45 % protein is illustrated in Table (1). The first diet (T1) was controlled, and the other three diets included: (T2), 1 g/kg diets SPs (*S. platensis*); (T3) 1 g/kg of chitosan nanoparticles (CHns), and (T4), containing both SPs and CHns in the same ration with 1g/kg diet. The goals of the additives of these two materials were to enhance diet utilization, SPs (*S. platensis*), was obtained from the unit of algal biotechnology, NRC, Dokki, Cairo, Egypt CH<sub>ns</sub>, with molecular weight of (MW) = 50,000–190,000 deacetylation degree (Da). In addition, sodium tripolyphosphate (TPP) with MW = 367.86 Da was used as a crosslinking agent; both were obtained from Sigma-Aldrich (St. Louis, MO, USA). CH<sub>ns</sub> was prepared using ionic gelation (**Masarudin et al., 2015; Desai, 2016**). The dose of SP<sub>s</sub> was chosen following the recommendations of **Al-Deriny et al. (2020)**, while the CH<sub>ns</sub> dose was specified by following the manufacturer's approval.

The average water temperature ranged between 26.13 and 26.33 °C, while the pH was 6.6 to 7.03; nitrite NO<sub>2</sub> ranged between 0.037 and 0.039 mg/L; nitrate NO<sub>3</sub> ranged from 0.035 to 0.037 mg/L; ammonia NH<sub>3</sub> was 0.038 mg/L, and dissolved oxygen (DO) ranged from 3.9 to 4.4 mg/L.

**Table 1.** The formulation and chemical composition of experimental diets (g/kg dry weight) containing *S. platensis* or/and chitosan nanoparticles fed to *D. labrax*

Feed ingredient	T1	T $\gamma$	T $\gamma$	T $\zeta$
Fish meal (72%)	485.00	485.00	485.00	485.00
Soybean meal (44%)	200.00	200.00	200.00	200.00
Yellow corn	136.00	136.00	136.00	136.00
Rice bran	115.00	115.00	115.00	115.00
Chitosan (g/kg)	0.00	0.00	1.00	1.00
<i>S. platensis</i> (g/kg)	0.00	1.00	0.00	1.00
Fish oil	60.00	60.00	60.00	60.00
Vit. & Min. mixture*	4.00	4.00	4.00	4.00
Total	1000	1000	1000	1000
Moisture	92.16	93.07	92.55	91.62
Crude protein (CP)	43.79	43.91	43.22	43.57
Ether extract (EE)	13.29	13.91	13.43	13.58
Ash	7.09	7.02	6.97	6.94
Metabolizable energy (Kcal/100g)	99.2	492.4	489.9	494.7

\* Vitamin and minerals mix (mg/kg premix):

vit A 200000 I. U.; vit B1 15 mg; vit D3 30000 I. U; vit B2 12mg; vit E 250 mg; vit B12 250 mg; vit B6 20 mg; vit K3 50 mg; Niacin 15 mg; Folic Acid 2 mg; Bantothonic 80 mg; Biotin 100 mg; Fe 1200mg; Selenium 10 mg; Mn. 2400 mg; Sodium 100 mg; Copper 200 mg; Phosphorus1000 mg.

### Hematology and serum biochemical parameters

All hematological parameters, including red blood cells (RBCs) and white blood cells (WBCs) counts were checked quickly with a hemocytometer after weakening with Natt and Herrick's solution following the methods of **Stoskopf, (1993)**. Moreover, hemoglobin (Hb) concentration was determined utilizing the cyanometer hemoglobin strategy using Drabkin's solution (**Stoskopf, 1993**). As indicated by **Catowsky, (1991)** the smaller scale hematocrit (Hct) strategy was used for the estimation of the packed cell volume (Hct) assurance.

The total serum protein, albumin, globulins, glucose, cholesterol, lactate, triglyceride, urea, creatinine substance, and uric acid were specified by the maker's guidelines (RA-50 science analyzer; (Bayer) utilizing readymade synthetic compounds (kits) provided by Spinreact Co. Spain). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) activities and malonaldehyde (MDA) level in fish serum were estimated utilizing the demonstrative reagent packs following the maker's technique (Cusabio Biotech Co., Ltd; China).

Serum calcium (Ca) was colorimetrically determined with o-cresolphthalein (**Morin, 1974**); magnesium (Mg) was colorimetrically determined with xylydine blue (**McCarthy, 1975**), and chloride (Cl) was determined colorimetrically with mercury thiocyanate (**McCarthy, 1975**). Inorganic phosphorus (IP) was colorimetrically

determined with phosphomolybdate (**Rubino, 1989**); tests were solidified at 20°C, and the accompanying analyses were performed with UV/VIS Spectrometer Lambda EZ 150 (PerkinElmer) utilizing Sclavo Diagnostics Inc. kits.

### Statistical analysis

Data were analyzed by the SAS ANOVA procedure (**SAS, 1988**), and Duncan's numerous range tests were used to look at variations among singular methods (**Duncan, 1955**). The significant difference in treatment was detected at  $P < 0.05$ .

## RESULTS

### Growth performance

Averages of the initial (IW) and final weights (FW), weight gain (WG%), specific growth rate (SGR%), and condition factor (K) of seabass fed diets at distinct levels of SPs or /and CHns are shown in Table (2). The ranges of initial weight were  $2.28 \pm 0.08$ ,  $2.40 \pm 0.29$ ,  $2.36 \pm 0.27$ , and  $2.30 \pm 0.26$ g, and the differences were insignificant, indicating that the experimental groups at the beginning of the experiment were randomly distributed. While, the final weights were  $15.93 \pm 0.38$ ,  $17.89 \pm 0.37$ ,  $17.93 \pm 0.32$ , and  $20.33 \pm 0.49$ g in the control, T1, T2, T3, and T4 groups, respectively. The means of total weight gain (%) were  $610.85 \pm 15.19$ ,  $682.84 \pm 13.26$ ,  $671.55 \pm 12.30$ , and  $762.51 \pm 16.26$ g, in the T1, T2, T3, and T4, respectively. The differences among the treatments with respect to the final weight and weight gain% of seabass were substantially increased ( $P < 0.05$ ) in fish fed both *S. platensis* and chitosan nanoparticles, and the highest FW and WG were recorded by fish treated in T4 ( $20.33 \pm 0.49$ g and  $762.51 \pm 16.26\%$ ), respectively. While, the lowest ones ( $15.93 \pm 0.38$ g and  $610.85 \pm 15.19\%$ ) were recorded in fish treated in T1 (control). *S. platensis* or/and chitosan nanoparticles' effect on specific growth rate (SGR) were illustrated in Table (2). Treatment 4 recorded the highest SGR value ( $1.92 \pm 0.02\%/\text{day}$ ). In contrast, those of T1 (control) showed the lowest SGR ( $1.74 \pm 0.03\%/\text{day}$ ), and the differences among different treatments were significant ( $P < 0.05$ ). As shown in Table (2), The highest final condition factor (K) was registered in fish of T3 ( $0.87 \pm 0.07$ ), and the lowest one ( $0.78 \pm 0.03$ ) was recorded in T1. The analysis of variance among treatments for the condition factor showed no significance ( $P < 0.05$ ). Findings observed that seabass's development performance was significantly affected by including both *S. platensis* or/ and chitosan nanoparticles in diets. The incorporation of SPs and CH<sub>ns</sub> in the diets is related to the improvement of the growth rate, physiological case, and disease resistance of seabass. The results of this study coincide with those of **Niu et al., (2011)**. They reported that chitosan is an active growth promoter and an essential element for aquatic species' growth. **Zaki et al. (2015)** found that seabass (*D. labrax*) diets containing 1 and 2g/kg diets improved the morphological structure of the small intestine, which could be the critical effect of chitosan, which could boost nutrient utilization and growth efficiency. They also reported that the supplemented diets by chitosan of seabass (*D. labrax*) at these levels enhanced the growth performance, feed

utilization, and the intestinal villi height (**Zaki *et al.*, 2015**). This might be attributed to the use of chitosan nanoparticles as a feed additive to seabass, enhancing digestion, and food digestibility. In addition, they increased nutrient use/absorption, enhanced feed intake and fish performance. Therefore, *O. niloticus* fed diets supplemented with chitosan nanoparticles may be protected from common diseases, enhancing the number of inhabitants in a helpful microorganism, as well increasing the microbial enzyme activities in fish intestine, which would affect feed digestibility and subsequently the nutrient absorption/assimilation (**Abd El-Naby *et al.*, 2019; Abdel-Tawwab *et al.*, 2019**).

**Table 2.** Growth performance of *D. labrax* fed *S. plantensis* or/and chitosan nanoparticles

Treatments	No.	T1	T2	T3	T4
IBW <sup>1</sup>	20	2.28±0.08 <sup>a</sup>	2.40± 0.29 <sup>a</sup>	2.36± 0.27 <sup>a</sup>	2.30± 0.26 <sup>a</sup>
FBW <sup>2</sup>	20	15.93±0.38 <sup>c</sup>	17.89±0.37 <sup>b</sup>	17.93±0.32 <sup>b</sup>	20.33±0.49 <sup>a</sup>
IBL <sup>3</sup>	20	4.42±0.12 <sup>a</sup>	4.42±0.10 <sup>a</sup>	4.70±0.08 <sup>a</sup>	4.55±0.09 <sup>a</sup>
FBL <sup>4</sup>	20	12.76±0.18 <sup>a</sup>	12.98± 0.24 <sup>a</sup>	13.09±0.38 <sup>a</sup>	13.43±0.32 <sup>a</sup>
WG% <sup>5</sup>	20	610.85±15.19 <sup>c</sup>	682.84±13.26 <sup>b</sup>	671.55±12.3 <sup>b</sup>	762.51±16.26 <sup>a</sup>
DWG	20	0.12±0.002 <sup>c</sup>	0.14± 0.003 <sup>b</sup>	0.14±0.003 <sup>b</sup>	0.16±0.002 <sup>a</sup>
SGR <sup>6</sup>	20	1.74±0.03 <sup>c</sup>	1.83±0.02 <sup>b</sup>	1.82±0.03 <sup>b</sup>	1.92± 0.02 <sup>a</sup>
K <sup>7</sup>	20	0.78±0.03 <sup>a</sup>	0.85±0.05 <sup>a</sup>	0.87±0.07 <sup>a</sup>	0.85±0.022 <sup>a</sup>

Means in the same line not sharing a common superscript letter were significantly different ( $P<0.05$ ).

<sup>1</sup> IBW: initial body weight (g);

<sup>2</sup> FBW: final body weight (g);

<sup>3</sup> IBL: initial body length (cm<sup>3</sup>);

<sup>4</sup> FBL: final body length (cm<sup>3</sup>);

<sup>5</sup> WG (%) = final body weight - initial body weight / initial weight (g) × 100;

<sup>6</sup> SGR = [Ln final body weight (g) - Ln initial body weight (g)]/experimental period ×100;

<sup>7</sup> K: Condition factor = body weight (g)/body length (cm<sup>3</sup>) × 100.

### Blood hematology indices

Table (3) showed that the supplementation of *D. labrax* diets containing SPs or/and CHns affected the contents of hemoglobin (Hb), red blood cells (RBCs) count, hematocrit (Ht) value, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and white blood cells (WBCs) count existing in table 3. The values of hemoglobin (Hb) of *D. labrax* were increased from 8.68±0.07 in T1 to 9.79±0.015 g/dL in T4 ( $P<0.05$ ), while the values of red blood cells (RBCs) were varied between 2.79±0.041 ( $\times 10^6/\text{mm}^3$ ) in T1 and 3.26±0.012 ( $\times 10^6/\text{mm}^3$ ) in T4 ( $P<0.05$ ). Meanwhile, hematocrit values ranged from 27.00±0.58% in T1 to 31.33±0.67 % in T4 ( $P<0.05$ ). The analysis of variance among treatments was significant ( $P<0.05$ ).

As presented in Table (3), analysis of variance showed that the MCV, and MCHC did not affect significantly ( $P<0.05$ ) by SPs and CHns while the differences among treatments of MCH (pg) were significant ( $P<0.05$ ) and the averages were 31.17±0.36, 30.73±0.10, 30.12±0.07 and 30.01±0.09 for T1, T2, T3, and T4, respectively. The present results indicated that there were significant variations for WBCs constituents among

different treatments. The averages were  $3.64 \pm 0.02$ ,  $3.75 \pm 0.02$ ,  $3.83 \pm 0.026$ , and  $3.93 \pm 0.02$  (103/mm) for T1, T2, T3, and T4, respectively (Table 3).

This data is consistent with the data obtained by **Raji et al., (2018)**. They stated that the inclusion of the two microalgae *S. vlatensis* and *C. Vulgaris* enhanced the oxidative stress, hematological parameters, enzyme activities, and growth efficiency potential antioxidants in African catfish. Furthermore, they found that hematological parameters observed a substantial surge in the value of red and white blood cells in catfish fed supplemented diets but slightly decreased when replacing increased. Also, **Ibrahem et al., (2013)** when studied the role of *S. platensis* in growth and immunity of *O. niloticus*. They found that data obtained of white shrimp *Litopenaeus vannamei* fed diet having 10% hot-water extract of *S. platensis* showed enhancing in its hematocrit values, also improved innate immunity (lysozyme), and reduction the repeatedly *Vibrio alginolyticus* infection (**Tayag et al., 2010**).

The data in the current analysis has been following **Taufek et al., (2016)** who reported that level of RBCs and WBCs varying suggestively in all the experimental diets compared to control, which might be due to the existence of physiological properties, and raises the number of antigens in the circulating system. Besides, increasing red blood cells also enhances the fish respiratory by enabling oxygen transportation capability. Moreover, WBC's rise implies the immunostimulatory impacts of *S. platensis* and *C. Vulgaris* (**Yeganeh et al., 2015**).

The reason for immunity capacity might be related to cphycocyanin in *Spirulina* alga (**Abd-El Alim et al., 2018**). The finding of this study was similar to (**Abdel-Warith and Elsayed, 2019**), who reported that *O. niloticus* fed diets containing *A. platensis* observed significantly higher RBCs, WBCs, Hb, and Hct values when compared with fish fed the control diet. These high counts of WBCs and RBCs might also be due to the existence of cphycocyanin in *Spirulina* that can improve immunity (**Tayag et al., 2010**). Also, **Bermejo-Bescós et al., (2008)** reported that *Spirulina* has high amounts of antioxidants and the protein extract in this microalga, including biliproteins, such as phycocyanin. Therefore, this results were in agreement with **Meshkini et al., (2012)** who reported that chitosan as a natural material had improved the hematological parameters and stress resistance in rainbow trout *Oncorhynchus mykiss* that fed diets containing 0.25, 0.50, and 1%. Also, rainbow trout fed a chitosan diet containing 0.25% has increased significantly the number of white blood cells count, the authors concluded that dietary supplementation of 0.25% chitosan for 56 days could enhance *O. mykiss* hematological parameters and resistance against low oxygen, salinity, and temperature stresses (**Meshkini et al., 2012**). These findings might be related to the cooperation reaction of these mixes, and the activity of microflora in the intestine may be accountable for such a beneficial effect. **Abd El-Naby et al., (2020)** when studied the effects of dietary combination of chitosan nanoparticle and thymol for *Oreochromis niloticus*. They found that mixture diets of CHnp with thymol affected feed, digestive enzyme activities,

antioxidant, and intestinal morphology in *O. niloticus*. Also, it improved the values of Hb, Hct, RBCs, MCV, and MCHC and enhanced enzymes such as catalase, lipase, and protease activities. Besides, the improvement of WBCs when diets containing CH<sub>np</sub> or/and SP might due to the monocyte and granulocytes are involved in non-specific For instance, they destroy pathogens with their enzymes and active oxygen; they also take part in phagocytic action (**Dalmo, 1997**).

**Table 3: Haematological parameters of *D. labrax* fed or/and chitosan nanoparticles.**

Treatments	T1	T2	T3	T4
Hb (g/DL)	8.68±0.07 <sup>d</sup>	8.92±0.035 <sup>c</sup>	9.29±0.032 <sup>b</sup>	9.79±0.015 <sup>a</sup>
RBCs (count×10 <sup>6</sup> /mm <sup>3</sup> )	2.79±0.041 <sup>d</sup>	2.90±0.020 <sup>c</sup>	3.08±0.018 <sup>b</sup>	3.26±0.012 <sup>a</sup>
Hct (%)	27.00±0.58 <sup>d</sup>	28.33±0.33b	30.00±0.09 <sup>a</sup>	31.33±0.67 <sup>a</sup>
MCV(μ <sup>3</sup> )	96.87±0.70	97.58±0.60	97.30±0.56	96.03±2.30
MCH(pg)	31.17±0.36 <sup>a</sup>	30.73±0.10 <sup>ab</sup>	30.12±0.07 <sup>bc</sup>	30.01±0.09 <sup>c</sup>
MCHC(%)	32.18±0.53	31.50±0.27	30.96±0.10	31.28±0.67
WBCs (10 <sup>3</sup> /mm)	3.64±0.02 <sup>d</sup>	3.75±0.02 <sup>c</sup>	3.83±0.026 <sup>b</sup>	3.93±0.02 <sup>a</sup>

Means in the same line not sharing a common superscript letter were significantly different (P<0.05).

Hemoglobin (Hb), Red blood cells (RBCs), Hematocrit (Ht), Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and White blood cells (WBCs).

### Biochemical parameters:

The effect of supplementation of *D. labrax* diets containing *S. platensis* or/and chitosan nanoparticles on biochemical parameters (Table 4). As shown in Table (4) the blood serum total protein, albumin, and globulin improved considerably in fish fed with *S. platensis* or/and chitosan nanoparticles additions related to the control group (P < 0.05). The values of glucose were 82.01±1.20, 108.75±0.76 111.97±0.39, and 115.56±0.68 mg/dL in the different diets. The highest amount of cholesterol was recorded in T4, being 117.20±5.08, while the lowest value was 84.26±5.65 mg/dL in control (P<0.05). The averages of lactate were 7.05±0.02, 8.78±0.03, 8.90±0.04, and 9.88±0.012 mmol/l for the four diets T1, T2, T3, and T4, respectively, with Significant differences among treatments (P<0.05). Meanwhile, triglycerides' values increased from 208.34±8.39 in the control group to be 225.21±6.32mg/dL in T4, with no significant differences were shown between treatments (P>0.05).

In connection to kidney functions, presented data shown that the averages of urea were 1.98±0.006, 1.97±0.006, 1.99±0.006, and 1.96±0.006 mg/dL. While, creatinine values were 0.22±0.006, 0.18±0.017, 0.18±0.006, and 0.17±0.017 mg/dL. However, the values of uric acid were 0.50±0.009, 0.43±0.009, 0.39±0.012, and 0.43±0.006 mg/dL. Both creatinine and uric acid values decreased significantly with a diet supplemented by SPs and/or CHns.

Also, Table (4) shown that, averages of serum content of calcium (Ca), magnesium (Mg), chloride (Cl) and inorganic phosphorus (Ip). The results indicated that using *S. platensis* or chitosan nanoparticles increased Ca, Mg, Cl, and Ip. The averages of Ca were  $2.28 \pm 0.015$ ,  $2.84 \pm 0.021$ ,  $3.47 \pm 0.024$ , and  $2.86 \pm 0.01$  mmol/l; averages of Mg were  $0.93 \pm 0.006$ ,  $1.11 \pm 0.012$ ,  $0.75 \pm 0.005$ , and  $0.94 \pm 0.003$  mmol/l, averages of Cl were  $85.50 \pm 0.97$ ,  $107.46 \pm 3.84$ ,  $99.60 \pm 0.44$ , and  $105.70 \pm 0.95$  mmol/l. The values of Ip were  $2.11 \pm 0.035$ ,  $2.35 \pm 0.031$ ,  $2.10 \pm 0.03$ , and  $2.55 \pm 0.034$  mmol/l for T1, T2, T3, and T4, respectively, with significant differences between control and other groups.

The results of the biochemical analysis showed that *D. Labrax* fingerlings fed to *S. Platensis*, or/and chitosan nanoparticles showed typical biochemical values considered within the average range for healthy fish (**Fazio, 2019**). However, the biochemical indices were markedly affected by adding both *S. platensis* and chitosan nanoparticles in seabass diets. Also, the combination of *S. platensis* / chitosan nanoparticles in *D. labrax* are associated with improving the blood properties, physiological status, immune response, and growth performance (**Fath El-Bab et al., 2020**).

Results in the current study were in agreement with **Raji et al., (2018)** who found that data of *C. gariepinus* fed diets contained *S. platensis* observed the level of albumin slightly increased with different supplementation levels. Albumin and globulins help tolerate the osmotic pressure to preserve the immunity health and serve as a plasma transporter (**Nya and Austin, 2009**). The increasing level of albumin and total protein for the *D. labrax* fed *S. platensis* diet in this study verifies with that stated by **Yeganeh et al., (2015)** in rainbow trout diets. Therefore, data in the current study is in agreement with **Simanjuntak, (2009)** who found that increasing dietary supplementation of *S. platensis* observed high results in total protein, albumin, and globulin compared to controls in Gouramy (*Oosphronemus gouramy*). Therefore, results in this study were by data obtained by **Yu et al., (2018)**, who stated that coral trout *Plectropomus leopardus* fed diets containing *S. platensis* observed increase of the blood glucose with increasing the levels of *S. platensis* in the diets up to 8% but decreased at level 10% that could be related to the reduced level of cortisol in biochemical indices of *P. leopardus*, which indicated that high level of *S. platensis* supplementation did not cause any damage response to *P. leopardus*.

In this study, data were agreed with **Abdel-Daim et al., (2020)**. They found that renal dysfunction signs, blood creatinine, and urea observed increased tilapia values exposed to chlorpyrifos (CPF), whereas supplementation of *Spirulina platensis* is relieved the toxicity of CPF. Besides, the rising of creatinine and urea can be referred to as the decreasing impact of CPF on glomerular filtration and catabolic protein rates, which can diminution the kidney's capability to release the urea and urine (**Akturk et al., 2006**). Moreover, Nile tilapia exposed to CPF or other pesticides displayed increased serum levels of urea and creatinine (**Abdelkhalek et al., 2017**). Although other studies have shown reduced levels of feed additives in fish diets (**Dawood et al., 2020**). In corroborates with the findings by **Abdel-Daim et al., (2020)**, Nile tilapia subjected to

CPF also increased total cholesterol. When fish fed on SP, the cholesterol levels have been decreased. The blood's high cholesterol levels are customarily attributed to the increased metabolic rates of lipids in body tissues, particularly when fish are exposed to stress circumstances (**Üner *et al.*, 2006**). However, further confirming SPs' role in preserving tilapia's normal healthy condition was subjected to CPF by reducing blood cholesterol (**Nachankar *et al.*, 2005**). Likewise, SP feeding diminishes diazinon's severe impacts on tilapia by decreasing blood cholesterol (**Abdelkhalek *et al.*, 2017**).

Besides, **Ranjan *et al.*, (2014)** found that Asian seabass (*Lates calcarifer*) fed different chitosan levels for 60 days of, 0, 5, 10, and 20 g kg<sup>-1</sup> enhanced the hematology, innate immunity then assayed against *Vibrio anguillarum*. Therefore, the supplementation of 20 g chitosan kg<sup>-1</sup> in diets for the past 30 days and 10 g eating routine past 45 days negatively affects leucocytes (**Ranjan *et al.*, 2014**). **Akbary and Younesi (2017)** found that chitosan's dietary supplementation significantly impacts hematology biochemical parameters and immune response of grey mullet (*Mugil cephalus*) when fish fed on diet inclusion different levels of 0, 5, 10, and 15 g chitosan kg<sup>-1</sup>. **Nicula *et al.*, (2018)** reported that calcium, like other minerals having entry control, Ca has a transporter protein that takes it from the absorption mucosa and whose synthesis is vitamin D dependent. Also, found that *Carassius gibelio* fed diets containing Spirulina Powder improve the calcium Ca and magnesium Mg levels in tissues, mainly in the gills, compared to fish exposed and fed 75 ppm Lead Pb into water+2% lyophilized cilantro in the feed. The Ca and Mg tissue levels were substantially decreased by sublethal lead-induced toxicity (p <0.001). However, *Spirulina* powder was able to relieve much of the lead antagonistic effect concerning calcium absorption and bioavailability; Spirulina was found to be more active when fed to this fish in the case of the gill and kidney tissue. coriander in the case of cardiac tissue.

Furthermore, *Spirulina* powder has enhanced magnesium allocation in all tissue analyses. Meanwhile, this study finding this a first time to establish the effect of Spirulina or/and Chitosan on mineral in the blood biochemical in *D. labrax* such as calcium (Ca), magnesium (Mg), chloride (Cl), and inorganic phosphorus (Ip) all these minerals have been increased significantly when fish fed treated diets. Besides, these feed additives might improve the absorption of minerals because it has high amounts of antioxidant enzymes that might be increasing in fish as it contains antioxidants substance such as carotene, minerals, vitamins, protein, carbohydrates, and lipids (Upasani and Balaraman 2003).

### **Body composition:**

The present results indicated wide variations for the significant body chemical composition constituents among different treatments of *S. platensis* or/and chitosan nanoparticles on *D. labrax* (Table, 5). The moisture content was considerably lower than that. for T4 (66.04±0.51%) than control (69.65±0.50%). On the contrary, the crude protein content of *D. labrax* was higher for T4 (18.57±0.05%), but the lower protein

content ( $17.60\pm0.08\%$ ) was recorded in control. The crude lipid content was also varied between treatments, indicating the higher value of lipid content in T2 ( $7.70\pm0.06\%$ ) matched to other treatments. The total ash content was higher ( $18.59\pm0.10\%$ ) for T4 than in control ( $17.90\pm0.09\%$ ).

**Table 4: Biochemical parameters of *D. labrax* fed *S. platensis* or/and chitosan nanoparticles.**

Treatments	T1	T2	T3	T4
Total protein (mg/dL)	$2.81\pm0.06^d$	$3.72\pm0.06^c$	$4.01\pm0.06^b$	$4.27\pm0.02^a$
Albumin(g/dL)	$1.11\pm0.06^c$	$1.60\pm0.003^b$	$1.68\pm0.006^a$	$1.69\pm0.006^a$
Globulin (g/dL)	$1.70\pm0.06^d$	$2.11\pm0.06^c$	$2.33\pm0.06^b$	$2.58\pm0.028^a$
Glucose (mg/dL)	$82.01\pm1.20^d$	$108.75\pm0.76^c$	$111.97\pm0.39^b$	$115.56\pm0.68^a$
Chol (mg/dL)	$84.26\pm5.65^b$	$102.59\pm5.95^{ab}$	$108.34\pm5.95^a$	$117.20\pm5.08^a$
Lact (mmol/L)	$7.05\pm0.026^d$	$8.78\pm0.030^c$	$8.90\pm0.049^b$	$9.88\pm0.012^a$
Tg (mg/dL)	$208.34\pm8.39$	$213.46\pm7.48$	$213.96\pm10.34$	$225.21\pm6.32$
Urea (mg/dL)	$1.98\pm0.006^{ab}$	$1.97\pm0.006^b$	$1.99\pm0.006^a$	$1.96\pm0.006^c$
Cr (mg/dL)	$0.22\pm0.006^a$	$0.18\pm0.017^{ab}$	$0.18\pm0.006^{ab}$	$0.17\pm0.017^b$
Uric acid (mg/dL)	$0.50\pm0.009^a$	$0.43\pm0.009^b$	$0.39\pm0.012^c$	$0.43\pm0.006^b$
Ca (mmol/L)	$2.28\pm0.015^c$	$2.84\pm0.021^b$	$3.47\pm0.024^b$	$2.86\pm0.01^a$
Mg (mmol/L)	$0.93\pm0.006^b$	$1.11\pm0.012^a$	$0.75\pm0.005^c$	$0.94\pm0.003^b$
Cl (mmol/L)	$85.50\pm0.97^c$	$107.46\pm3.84^a$	$99.60\pm0.44^b$	$105.70\pm0.95^{ab}$
IP (mmol/L)	$2.11\pm0.035^c$	$2.35\pm0.031^b$	$2.10\pm0.03^c$	$2.55\pm0.034^a$

Means in the same line not sharing a common superscript letter were significantly different ( $P<0.05$ ).

Cholesterol (Chol), Lactate (Lactl), Triglyceride (Tg), Urea, Creatinine, Uric acid, Calcium (Ca), Magnesium (Mg), Chloride (Cl) and Inorganic Phosphorus (Ip).

The present study finding was in agreement with **Ranjan et al., (2014)**. They noted that Asian seabass, *Lates calcarifer* fed diets containing *Spirulina* meal up to 5%, showed increases in protein contents when *Spirulina* levels increased in the diets. In the present study, data were incongruous with **Abd El-Naby et al., (2019)**. They reported that *Oreochromis niloticus* fed diets containing thymol (THY) or chitosan nanoparticle (CHnp) as feed additives significantly increased the muscles' lipid contents. This might be due to the efficient nutrient consumption resulting from the appropriate adjustment of the intestinal microbiota. Besides, **Abdel-Warith and Elsayed, (2019)** found that using *Arthrospira platensis* as a diet supplementation up to 20% in the diet has a significant effect on the carcass composition of *Oreochromis niloticus* and enhanced the amounts of protein and lipid in fish. These improvements may be associated with changes in their structure, deposition rates in muscle, and different growth rates; this also might be

referring to *A. platensis* being an excellent substitution for fish meal in *O. niloticus* diets, even at a high level up to 10 and 20%, because it has contained high amounts of essential amino acids, fatty acids, vitamins, and minerals (**Akturk *et al.*, 2006**).

**Table 5: Body composition of *D. labrax* fed *S. platensis* or/and chitosan nanoparticles.**

Treatments	T1	T2	T3	T4
Moisture	69.65±0.50 <sup>a</sup>	68.35±0.54 <sup>ab</sup>	67.78±0.44 <sup>b</sup>	66.04±0.51 <sup>c</sup>
Crude protein %	17.60±0.08 <sup>c</sup>	17.99±0.05 <sup>b</sup>	18.13±0.08 <sup>b</sup>	18.57±0.05 <sup>a</sup>
Crude fat %	5.64±0.04 <sup>c</sup>	7.70±0.060 <sup>a</sup>	7.03±0.03 <sup>b</sup>	5.78±0.05 <sup>c</sup>
Ash %	17.90± 0.09 <sup>c</sup>	17.94±0.11 <sup>b</sup>	18.22±0.10 <sup>b</sup>	18.59±0.10 <sup>a</sup>

Means in the same line not sharing a common superscript

## CONCLUSION

Results of the current study demonstrate that dietary supplementation with *platensis* Spirulina and chitosan nanoparticles positively affected and enhanced growth performance, hematological, biochemical parameters, and body composition of the commercially valuable seabass *D. labrax*. The possibility of using SPs and CHnp inclusion levels in fish diets needs further work to estimate Its desirable effect on growth success, feed production, immune response, and resistance to infection in *D. Labrax*.

## REFERENCES

- Abd El-Naby, A. S; Al-Sagheer, A. A.; Negm, S. S. and Naiel, M. A.E. (2020). Dietary combination of chitosan nanoparticle and thymol affects feed utilization, digestive enzymes, antioxidant status, and intestinal morphology of *Oreochromis niloticus*. Aquaculture, 515: 734577.
- Abd El-Naby, F. S.; Naiel, M. A. E.; Al-Sagheer, A. A. and Negm, S. S. (2019). Dietary chitosan nanoparticles enhance the growth, production performance, and immunity in *Oreochromis niloticus*. Aquaculture. 501: 82-89.
- Abd-El Alim F. A.; Khalifa, H. A.; El-Murr, A. and Magdy, M. (2018). Evaluation of *Spirulina Platensis* Enriched Diet on Growth Performance, Biochemical Parameters and Immune Response of *Oreochromis niloticus*. Veterinary Medicine In-between Health & Economy (VMHE)–16-19 October 2018, 55(20-Suppl).
- Abdel-Daim, M. M.; Dawood, M.; Elbadawy, M.; Lotfi, A. and Alkahtani, S. (2020). *Spirulina platensis* reduced oxidative damage induced by chlorpyrifos toxicity in Nile tilapia (*Oreochromis niloticus*). Animals, 10(3): 473.
- Abdel-Ghany, H. M. and Salem, M. E. S. (2020). Effects of dietary chitosan supplementation on farmed fish; a review. Reviews in Aquaculture. 12(1): 438-452.

- Abdelkhalek, N. K.; Ghazy, E. W. and Abdel-Daim,M. M. (2015). Pharmacodynamic interaction of *Spirulina platensis* and deltamethrin in freshwater fish Nile tilapia, *Oreochromis niloticus*: impact on lipid peroxidation and oxidative stress. Environmental Science and Pollution Research., 22(4): 3023-3031.
- Abdelkhalek, N. K.; Eissa, I. A. M.; Ahmed, E.; E Kilany, O.; El-Adl, M.; Dawood, M.; Hassan, A. O.; Ahmed M. and Abdel-Daim, M. M. (2017). Protective role of dietary *Spirulina platensis* against diazinon-induced Oxidative damage in Nile tilapia; *Oreochromis niloticus*. Environmental Toxicology and Pharmacology. 54: 99-104.
- Abdel-Tawwab, M.; Razek, N. A. and Abdel-Rahman, A. M. (2019). Immunostimulatory effect of dietary chitosan nanoparticles on the performance of Nile tilapia, *Oreochromis niloticus* (L.). Fish & shellfish immunology. 88: 254-258.
- Abdel-Warith, A. and Elsayed, E. (2019). Use of *Arthrospira platensis* as a Feed Additive to Improve Growth Performance, Feed Utilization, Body Composition, and Immune Response of Nile Tilapia, *Oreochromis niloticus*. Journal of Scientific and Industrial Research. 78, 681-686.
- Abdel-Warith A. A.; Fath El-Bab, A. F.; Younis, E. M. I.; Al-Asgah, N. A.; Allam, H. Y.; Abd-Elghany M. F.; Shata, Y. H. M. and Shamlol, F. S. (2020). Using of chitosan nanoparticles (Cs NPs), Spirulina as a feed additive under intensive culture system for black tiger shrimp (*Penaeus monodon*). Journal of King Saud University - 32, 8, 3359-3363. <https://doi.org/10.1016/j.jksus.2020.09.022>.
- Adel, M.; Yeganeh, S.; Dadar, M.; Sakai, M., Dawood, M. A .O., (2016). Effects of dietary *Spirulina platensis* on growth performance, humoral and mucosal immune responses and disease resistance in juvenile great sturgeon (*Huso huso* - Linnaeus, 1754). Fish & shellfish immunology. 56: 436-444.
- Akbary, P. and A. Younesi, (2017). Effect of dietary supplementation of Chitosan on growth, hematology and innate immunity of grey Mullet (*Mugil cephalus*).
- Akturk, O.; Demirin, H.; Sutcu, R.; Yilmaz, N.; Koylu, H. and Altuntas, I. (2006). The effects of diazinon on lipid peroxidation and antioxidant enzymes in rat heart and ameliorating role of vitamin E and vitamin C. Cell biology and toxicology. 22(6): 455.
- Al-Deriny, S.H.; Dawood, M. A. O.; Elbialy, Z. I.; El-Tras, W. F. and Mohamed, R. A. (2020). Selenium Nanoparticles and Spirulina Alleviate Growth Performance, Hemato-Biochemical, Immune-Related Genes, and Heat Shock Protein in Nile Tilapia (*Oreochromis niloticus*). Biological Trace Element Research, 2020: p. 1-8.
- Aranaz, I.; Alcántara A. R.; Civera, M. C.; Arias, C.; Elorza, B.; Caballero A. H. and Acosta, N. (2021). Chitosan: An Overview of Its Properties and Applications. Polymers, 13, 3256. <https://doi.org/10.3390/polym13193256>.
- Atay, H. Y. and Çelik, E. (2017). Investigations of antibacterial activity of Chitosan in the polymeric composite coatings. Progress in Organic Coatings., 102: 194-200.

- Bermejo-Bescós, P.; E. Piñero-Estrada, and del Fresno, Á. M. V. (2008). Neuroprotection by *Spirulina platensis* protean extract and phycocyanin against iron-induced toxicity in SH-SY5Y neuroblastoma cells. *Toxicology in Vitro*, 22(6): 1496-1502.
- Catowsky, D., (1991). Leucocyte cytochemical and immunological techniques. *Practical Hematology*. 7th ed. Churchill Livingstone, London: 143-174.
- Commission, E., (2014). Towards a circular economy: A zero waste programme for Europe. COM. 398.
- Cunniff, P., (1995). Official methods of analysis. Association of Official Analytical Chemists (AOAC). 16<sup>th</sup> ed. Arlington, Virginia, USA.
- Dalmo, R.; Ingebrigtsen, K. and Bøgwald, J. (1997). Non-specific defence mechanisms in fish, with particular reference to the reticuloendothelial system (RES). *Journal of fish diseases*. 20(4): 241-273.
- Dawood, M. A.; Moustafa, E. M.; Gewaily, M. S.; Abdo, S. E.; AbdEl-Kader, M. F.; SaadAllah, M. S. and Hamouda, A. H. (2020). Ameliorative effects of *Lactobacillus plantarum* L-137 on Nile tilapia (*Oreochromis niloticus*) exposed to deltamethrin toxicity in rearing water. *Aquatic Toxicology*. 219: 105377.
- Desai, K. G., (2016). Chitosan nanoparticles prepared by ionotropic gelation: An overview of recent advances. *Critical Reviews™ in Therapeutic Drug Carrier Systems*, 33(2).
- Duncan, M. B. (1955). Multiple ranges and multiple F-tests. *Biometrics*, 11:1-42.
- Fath El-Bab A. F.; Allam, H. Y. and Shata, Y. H. M. (2020). Effect of Chitosan Nanoparticles Biosynthesis Using *Spirulina platensis* on Growth Performance and Blood Parameters of the Black Tiger Shrimp (*Penaeus monodon*). *Egyptian Journal of Aquatic Biology & Fisheries*. 24(1): 421 – 429.
- Fath El-Bab, A. F.; El-Moghazy, M. M. and Marhie R. M., (2020). The Potential Effects of Chitosan Nanoparticles Biosynthesis using *Spirulina platensis* on the Growth Performance, Hematological and Biochemical Parameters of European Seabass (*Dicentrarchus labrax*). *Egyptian Journal of Aquatic Biology and Fisheries*. 24(1): 583-591.
- Fazio, F., (2019). Fish hematology analysis as an important tool of aquaculture: a review. *Aquaculture*, 500: 237-242.
- Food and Agriculture Organization of the United Nations - FAO. (2016). *FishStatJ—software for fishery statistical time series*. Rome.
- Food and Agriculture Organization of the United Nations - FAO. (2018). *The state of world fisheries and aquaculture*. Rome.
- Fouad, D. R. G. (2008). Chitosan as an antimicrobial compound: modes of action and resistance mechanisms. *Mathematisch-Naturwissenschaftliche Fakultät, Universität Bonn*.
- Ghaeni, M.; Matinfar, A.; Soltani, M. and Rabbani, M. (2011). Comparative effects of pure spirulina powder and other diets on larval growth and survival of green tiger

- shrimp, *Peneaus semisulcatus*. Iranian Journal of Fisheries Sciences. 10(2): 208-217.
- Herrera, M.; Mancera, J. M. and Costas, B. (2019). The use of dietary additives in fish stress mitigation: comparative endocrine and physiological responses. Frontiers in Endocrinology. 10: 447.
- Hosseinnejad, M. and Jafari, S. M. (2016). Evaluation of different factors affecting antimicrobial properties of Chitosan. International journal of biological macromolecules. 85: 467-475.
- Ibrahem, M. D.; Mohamed, M. F. and Ibrahim, M. A. (2013). The role of *Spirulina platensis* (*Arthospira platensis*) in growth and immunity of Nile tilapia (*Oreochromis niloticus*) and its resistance to bacterial infection..
- Institute, S., SAS/GRAPH User's Guide: Release 6. 1988: Sas Inst.
- Mahmoud, M .A. M.; El-Lamie, M. M.; E. Kilany, O. and Dessouki A. A. (2018). *Spirulina (Arthospira platensis)* supplementation improves growth performance, feed utilization, immune response, and relieves oxidative stress in Nile tilapia (*Oreochromis niloticus*) challenged with *Pseudomonas fluorescens*. Fish & Shellfish Immunology. 72:291-300.
- Masarudin, M. J.; Cutts S. M.; Evison, B. J.; Phillips, D. R. and Pigram, P. J. (2015). Factors determining the stability, size distribution, and cellular accumulation of small, monodisperse chitosan nanoparticles as candidate vectors for anticancer drug delivery: application to the passive encapsulation of [14C]-doxorubicin. Nanotechnology, science and applications. 8: 67.
- McCarthy, D.; Stevenson, J. and Roberts, M. (1975). Some blood parameters of the rainbow trout (*Salmo gairdneri* Richardson) II. The Shasta variety. Journal of Fish Biology, 7(2): 215-219.
- Mendiola, J. A.; Jaime, L.; Santoyo, S.; Reglero, G.; Cifuentes, A.; Ibañez, E. and Señoráns, F. J. (2007). Screening of functional compounds in supercritical fluid extracts from *Spirulina platensis*. Food chemistry. 102(4): 1357-1367.
- Meshkini, S.; Tafy, A.; Tukmechi, A.; Farhang-Pajuh, F. (2012). Effects of Chitosan on hematological parameters and stress resistance in rainbow trout (*Oncorhynchus mykiss*). in Veterinary Research Forum. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
- Morin, L. G. (1974). Direct colorimetric determination of serum calcium with o-cresolphtha complexion. American journal of clinical pathology, 61(1):114-117.
- Muzzarelli, R.A., Chitins and chitosans as immunoadjuvants and non-allergenic drug carriers. Marine drugs. 8(2): 292-312.
- Nachankar, R.; Juvekar, A. and Sonawane, A. (2005). Prevention of cold stress induced adrenal hypertrophy by *Spirulina platensis*. Acta Hortic. 680: 101-107.
- Nicula, M.; Păcală, N.; Stef, L.; Pet, I.; Bencsik. I.; Iancu, T.; Tulcan, C.; Dragomirescu, M.; Dr, onca D.; Berbecea, A.; Simiz, E.; Ahmady, M.; Marcu A. and Erina S.

- (2018). Ameliorative Effect of Active Principles from Cilantro and Spirulina Powder on Lead Antagonism to Calcium and Magnesium in *Carassius gibelio* Bloch. Jurnal Scientific Papers: Animal Science & Biotechnologies. 51: 2.
- Niu, J.; Liu, Y. J.; Lin, H. Z.; Mai, K. S.; Yang, h. J.; Liang, G. Y. And Tian L. X. (2011). Effects of dietary Chitosan on growth, survival and stress tolerance of postlarval shrimp, *Litopenaeus vannamei*. Aquaculture Nutrition. 17(2): e406-e412.
- Nya, E.J. and Austin, B. (2009). Use of dietary ginger, *Zingiber officinale* Roscoe, as an immunostimulant to control *Aeromonas hydrophila* infections in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of fish diseases, 32(11):971-977.
- Qi, L.; Xu, Z.; Jiang, X.; Hu, C. and Zou, X. (2004). Preparation and antibacterial activity of chitosan nanoparticles. Carbohydrate research, 2004. 339(16): 2693-2700.
- Raafat, D. and Sahl, H.G. (2009). Chitosan and its antimicrobial potential—A critical literature survey. Microb. Biotechnol., 2, 186–201.
- Radhakrishnan, S.; Saravana B. P.; Seenivasan, C.; Shanthi, R. and Muralisankar, T. (2014). Replacement of fishmeal with *Spirulina platensis*, *Chlorella vulgaris* and *Azolla pinnata* on non-enzymatic and enzymatic antioxidant activities of *Macrobrachium rosenbergii*. The Journal of Basic & Applied Zoology. 67(2): 25-33.
- Raji, A. A.; Alaba, P. A.; Yusuf, H.; Abu Bakar, N. H.; Taufek, N. M.; Muin, H.; Alias, Z.; Milow, P. and Abdul Razak, S. (2018). Fishmeal replacement with *Spirulina Platensis* and *Chlorella vulgaris* in African catfish (*Clarias gariepinus*) diet: Effect on antioxidant enzyme activities and haematological parameters. Research in veterinary science. 119:67-75.
- Ranjan, R.; Prasad, K. P.; Vani, T. and Kumar, R. (2014). Effect of dietary Chitosan on haematology, innate immunity and disease resistance of Asian seabass *Lates calcarifer* (Bloch). Aquaculture Research. 45(6): 983-993.
- Rubino, L.; Catapano, V. and Guerra, G. (1989). Determination of inorganic phosphorus in serum: Evaluation of three methods applied to the Technicon RA-1000 analyzer. Journal of Automatic Chemistry. 11(4):164-7. doi: 10.1155/S1463924689000350.
- Seval, D.; Hatice, U.; Ismihan, K. and Orhan, A. (2010). Effect of dietary supplementation of different rates of Spirulina (*Spirulina platensis*) on growth and feed conversion in Guppy (*Poecilia reticulata* Peters, 1860). Journal of Animal and Veterinary Advances. 9(9): p. 1395-1399.
- Simanjuntak, S. B. I.; Indarmawan, I. and Wibowo, E. S. (2018). Impact of Fed Containing Different Levels of Diets Supplementation *Spirulina platensis* on Growth, Haematological, Body Composition and Biochemical Parameters, of Gurami (*Osphronemus goramy*). Turkish Journal of Fisheries and Aquatic Sciences. 18(5):681-690.
- Stoskopf, M. (1993). Anatomy and physiology of sharks. Fish Medicine. Stoskopf, MK (Ed), WB Saunders Co., Philadelphia, Pennsylvania: 751.

- Sung-Sam, K.; Samad, R.; Kang-Woong, K. and Kyeong-Jun, L. (2013). Partial replacement of fish meal with *Spirulina pacifica* in diets for parrot fish (*Oplegnathus fasciatus*). Turkish Journal of Fisheries and Aquatic Sciences. 13(2): 197-204.
- Taufek, N. M.; Aspani, F.; Muin, H.; Raji, A. A.; Abdul Razak, S. and Alias, Z. (2016). The effect of dietary cricket meal (*Gryllus bimaculatus*) on growth performance, antioxidant enzyme activities, and haematological response of African catfish (*Clarias gariepinus*). Fish physiology and biochemistry. 42(4): 1143-1155.
- Tayag, C.M.; Yong-Chin, L.; Chang-Che L.; Chyng-Hwa L. and Jiann-Chu C. (2010). Administration of the hot-water extract of *Spirulina platensis* enhanced the immune response of white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. Fish & shellfish immunology. 28(5-6): 764-773.
- Üner, N.; Oruç, E.; Sevgiler, Y.; Sahin, N.; Durmaz, H. and Usta, D. (2006). Effects of diazinon on acetylcholinesterase activity and lipid peroxidation in the brain of *Oreochromis niloticus*. Environmental toxicology and pharmacology. 21(3): 241-245.
- Upasani, C. and Balaraman, R. (2003). Protective effect of Spirulina on lead induced deleterious changes in the lipid peroxidation and endogenous antioxidants in rats. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 17(4): 330-334.
- Victor T. R.; Luís H. P.; Luis A. R. and Marcelo B. T. (2019). Feasibility of the use of Spirulina in aquaculture diets. Reviews in Aquaculture. 11(4): 1367-1378.
- Yeganeh, S.; Teimouri, M. and Amirkolaie, A. K. (2015). Dietary effects of *Spirulina platensis* on hematological and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Research in Veterinary Science. 101: 84-88.
- Yu, W.; Guoliang, W.; Heizhao, L.; Yukai, Y.; Xiaolin H.; Chuanpeng Z.; Zaiwang, Z.; Yafei, D.; Zhong H. and Tao, L. (2018). Effects of dietary *Spirulina platensis* on growth performance, hematological and serum biochemical parameters, hepatic antioxidant status, immune responses and disease resistance of Coral trout *Plectropomus leopardus* (Lacepede, 1802). Fish & shellfish immunology. 74: 649-655.
- Zaki, M., A.; Salem, M. El-S.; Gaber, M. M. and Nour, A. M. (2015). Effect of Chitosan supplemented diet on survival, growth, feed utilization, body composition & histology of sea bass (*Dicentrarchus labrax*). World Journal of Engineering and Technology. 3(04): 38.