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The Potential Effects of Chitosan Nanoparticles Biosynthesis using *Spirulina platensis* on the Growth Performance, Hematological and Biochemical Parameters of European Seabass (*Dicentrarchus labrax*)

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ABSTRACT

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The aim of the present study was to investigate the effect of chitosan nanoparticles (CsNPs) synthesis using *Spirulina platensis* for European seabass (*D. labrax*) on the growth performance, blood hematological and biochemical parameters. Therefore, Six experimental diets were formulated to provide 45% crude protein and 499k cal./100 g diet and supplemented by *S. platensis* and chitosan as following: T1: control, T2: 0.05% *S. platensis*, T3: 0.1% *S. platensis*, T4: 1 ml chitosan, T5: 1 ml chitosan and 0.05% *S. platensis*, T6: 1 ml chitosan and 0.1% *S. platensis.* Each diet was offered twice daily at 5% feeding rate in two replicate fish groups (22.6-22.9 g/fish) stocked at 15 fingerlings per m^3 for each of 12 ($1\times2.5\times1m^3$) hapas for 16 weeks. Diet supplementation by each of spirulina and chitosan nanoparticles (T5 and T6) significantly improved ($P<0.05$) growth performance and survival rate values compared to the control group. *Fish fed diets* T5 and T6 showed the highest blood hematological and biochemical parameters compared to the control treatment ($P<0.05$). The results suggested that that diet supplementation by spirulina and chitosan nanoparticles improves the growth performance, blood hematological and biochemical parameters of the European seabass (*Dicentrarchus labrax*).

INTRODUCTION

Studies that incorporated microalgal biomass in the diets for juveniles of marine finfish species are scarce (Shah *et al*. 2018), and those using processed microalgae from biorefineries are even more limited. Although de-fatted microalgal biomass have reduced levels of omega-3 (n-^{3}) polyunsaturated fatty acids compared to the whole algae, they still retain many valuable nutrients and are able to partially replace conventional protein sources like fish meal (FM), corn and soybean meal in diets for European seabass (*Dicentrarchus labrax*), shrimp (*Litopenaeus vannamei*), carp *(Cyprinus carpio)* red drum *(Sciaenops ocellatus)*, Nile tilapia *(Oreochromis niloticus)*, and Atlantic salmon *(Salmo salar)* (Ju *et al*.,2012; Kiron *et al*.,2012; Hussein *et al*.,2013; Patterson and Gatlin 2013; Sørensen *et al*.,2017). Besides the limited availability and high price of microalgae biomass, one of the main challenges associated with its inclusion in feeds for aquaculture species is the high variability in nutrient composition (Lum *et al*.,2013; Shah *et al*.,2018). Replacement of corn gluten

meal by a biofuel algae product (Algamaxx) up to 50% positively affected fish growth and feed consumption in diets for Nile tilapia (Hussein *et al*.,2013).

European seabass (*Dicentrarchus labrax*) is one of the most important marine fish species farmed in Europe and in Mediterranean countries. It is a carnivorous fish species with a high protein requirement, 450 g/kg (Fournier *et al*.,2002), that reinforces the need for searching for alternative and sustainable protein sources able to replace the traditionally used FM assuring fish welfare. Microalgae are natural sources of several biomolecules recognized to offer health-promoting benefits, and in spite of its still high price, they may contribute to the development of functional feeds able to support a sustainable aquaculture industry.

Nanoparticles (NPs) are gaining importance due to their unique characteristics, such as electronic, mechanical, optical, magnetic, and chemical properties (Mukherjee *et al*.,2001; Kajbafvala *et al*.,2012). Microalgae have been shown to produce not only silver nanoparticles but also of other metal and polysaccharides ions, such as silver, gold, cadmium, platinum and chitosan (Brayner *et al*.,2007; Parial *et al*.,2012).

This study aimed to investigate the effects of Chitosan nanoparticles biosynthesis by *S. platensis* on growth performance of European seabass (*D. labrax*) in order to determine the maximum benefit of *Spirulina platensis* and chitosan nanoparticles diets of European seabass (*D. labrax*) reared in cages under intensive culture conditions.

MATERIALS AND METHODS

Location:

This work was conducted in the region of Bourg Magaza-Ibeana, Al Gazirah Al Khadraa, Metobas, Kafr El Sheikh Governorate to the effect of chitosan nanoparticles biosynthesis by *S. platensis* for European seabass (*D. labrax*) reared in hapas placed in 3 different cages (4 hapas per cage).

Experimental design:

Twelve hapas ($1\times2.5\times1m^3$) were placed in cages, divided into six treatments. The European seabass fingerlings were stocked in these hapas at 15 fingerlings per m^3 to investigate effect of biosynthesis of chitosan nanoparticles by *Spirulina platensis* on growth performance, hematological and biochemical parameters of *D. labrax*.

S. platensis, chitosan nanoparticles and *P. monodon* source:

S. platensis meal was obtained from algae unit of National Research Center was used. The condition of cultivation *S. platensis* cells in Zarrouk's medium (Zarrouk, 1966).

Chitosan (CS) with molecular weight (MW) = 50,000–190,000 De acetylation degree (Da) was used as carrier and sodium tri polyphosphate (TPP) with MW= 367.86 Da was used as crosslinking agent, both were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chitosan nanoparticles (CsNPs) was prepared by using ionic gelation method as reported by Masarudin *et al*. (2015).

The European seabass (*D. labrax*) fingerlings were obtained by one of the fishermen from Burullus. The experimental fingerlings were transported in plastic bags filled with water and oxygen to the cages. The European seabass fingerlings (ranged between 22.60 and 22.9g/fingerling) were adapted and distributed randomly

into 12 hapas. Fish were weighed and the initial weight for each hapa was recorded. Each hapa was stocked with 15 seabass per m^3.

Estimation Preparation of diets and feeding practices:

The experimental European seabass (*D. labrax*) were conducted to 6 treatments in 12 hapas ($1\times2.5\times1m^3$) placed in 3 cages according to adding rate of chitosan nanoparticles and *S. platensis* algae to the diets as found in Table 1 during the experimental periods (16 weeks). Fish were fed a commercial sinking diet to be available for fish (as pellets 3 mm in diameter) at a daily rate of 5% of total biomass for 6 days / week twice daily at 9.00 am and 3.00 pm (Table 1).

Every 14 days, *D. labrax* groups were randomly obtained from each hapa, then weighted and the amount of feed was adjusted according to the changes in body weight throughout the experimental period.

Six isonitrogenous and isocaloric diets were formulated to provide 45% protein and 499 kcal/100 g diet and supplemented by *S. platensis* and chitosan as follow:

T1: control.
T2: 0.05% *S. platensis*.
T3: 0.1% *S. platensis*.
T4: 1 ml chitosan nanoparticles.
T5: 1 ml chitosan nanoparticles and 0.05 % *S. platensis*.
T6: 1 ml chitosan nanoparticles and 0.1 % *S. platensis*.

Table 1: Composition of groups diets used during the experimental period.

Feed ingredients	Experimental diets					
	T1	T2	T3	T4	T5	T6
Fish meal (72%)	48.5	48.5	48.5	48.5	48.5	48.5
Soybean meal (44%)	20	20	20	20	20	20
Yellow corn	10	9.95	9.9	9.9	9.85	9.8
Rice bran	11.5	11.5	11.5	11.5	11.5	11.5
Chitosan (g/kg)	0	0	0	0.1	0.1	0.1
S. platensis (g/kg)	0	0.05	0.1	0	0.05	0.1
Fish oil	6	6	6	6	6	6
Vit. & Min. mixture	4	4	4	4	4	4
Total	100	100	100	100	100	100
Proximate analysis (dry matter basis)						
Crude protein (CP)	92.16	91.98	93.07	92.55	91.62	91.619
Ether extract (EE)	43.79	43.65	43.91	43.22	43.19	43.57
Crude fiber (CF)	13.29	13.77	13.91	13.43	13.39	13.58
Ash	10.88	10.79	10.91	10.77	10.82	10.74
Metabolizable energy (Kcal/100g)	7.09	7.12	7.02	6.97	7.18	6.94

Water quality:

Water temperature was recorded daily at 9:00 am using a mercury thermometer. Dissolved oxygen (DO) was measured at 07:00 am using YSI model 56 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA). Total ammonia, nitrate and nitrite were measured weekly using a DREL, 2000 spectrophotometer (Hash Company, Loveland, CO, USA). pH values were estimated on morning using using a pH meter (Orion pH meter, Abilene, Texas, USA). Water temperature ranged from 16.7 to 17.4°C; dissolved oxygen (DO) ranged between 4.7

and 5.9 mg/l and pH values it was about a neutral ranged between 6.7:7.4 for the different treatments during the experimental period (84 days) of this study.
Generally, all tested water quality criteria (temperature, pH value DO) were suitable and within the acceptable limits for rearing European seabass as reported by (Boyd, 1990).

Blood Sampling:

At the end of the experimental period, ten of European seabass (*Dicentrarchus labrax*) were chosen at random from each replicate (hapa). Blood samples of five European seabass fish were collected from the vein in clean tube with 10% EDTA solution to determine hematocrit (Ht), hemoglobin (Hb). Blood samples of the other five European seabass were collected also from the vein in clean dry centrifuge tubes, kept for 15 minutes and centrifuged at 3000 rpm for 10 minutes, then kept frozen at -20°C for determination of glucose. Total protein was determined by the method of Bradford (1976), albumin content by the method of Doumas *et al*. (1971). Serum cholesterol and Lactate were measured by the method of Trinder (1969) method. Hematocrit (Ht) was determined by the microhematocrit method as described by Reitman and Frankel (1957).

Growth performance parameters:

Records of live body weight (g) of individual European seabass were measured in 20 fish from each hapa and registered every 14 days (two weeks) during the experiment period.
Type the formula to calculate:

- Weight gain (WG),
- Specific growth rate (SGR)
- and Survival rate%

Statistical analysis:

The statistical analysis of data carried out using applying the computer program SAS (1996). Differences among means were tested for significance according to Duncan (1955) using using the following model: -

$$\mathbf{xij = \mu.. + \alpha i + \Sigma i.}$$

Where:
μ = Overall mean.
αi = The effect of treatments.
ei= Random error.

RESULTS AND DISCUSSION

Growth performance:

The effect of chitosan nanoparticles biosynthesis using *S. platensis* on body weight (BW), daily weight gain (WG), specific growth rate (SGR) and survival rate% of *D. labrax* are summarized in Table 2. At the start of the experiment, the means of initial BW of *D. labrax* within all treatment groups were nearly similar (ranged between 22.60 and 22.90 g) and the differences among different treatments were insignificant ($P>0.05$) indicating that the experimental groups at the start of the experiment were randomly distributed. But the significant differences ($P<0.05$) were observed at the end of experiment (after 112 days), The highest final BW (129.50 and 129.90 g) were recorded in T5 and T6 (1ml chitosan and 0.05% *S. platensis*; 1ml chitosan and 0.1% *S. platensis*, respectively). While the lowest one (107.70 g) was found in T1 (control). These results were in agreement with the findings of Shata

(2020) who found that the highest final BW of black tiger shrimp (18.49 g) was recorded in T6 (1ml chitosan and 0.1% *S. platensis*) while the lowest one (12.62 g) was observed in T1 (control).

In connection to weight gain (WG) of European seabass (*D. labrax*), the highest WG (107.12, g) was recorded in T6 (1ml chitosan and 0.1% *S. platensis*), while the lowest one (84.86, g) was recorded in T1 (P<0.05). The present results corresponded with Shata (2020) who found that the highest values of WG were recorded in T6 (1ml chitosan and 0.1% *S. platensis*), while the lowest one were shown in the control. Moreover, Carnevali *et al*., (2006), reported that weight gain for black tiger shrimp was significantly better for the treated groups than the control when *S. platensis* treated by chitosan nanoparticles was used for 70 days. Al-Dohail *et al*., (2009) also indicated that African catfish,*Clarias gariepinus* fed the combination of *S. platensis* with chitosan showed better growth performance than the control fish group. Similarly, application of *S. platensis* and chitosan nanoparticles was found to enhance the growth performance of Nile tilapia, *Oreochromis niloticus* (Wang *et al*., 2008) and *Siluris glanis* (Bogut *et al*., 2000). In addition, Badawy *et al.* (2008) investigated the algae mixture (*S. platensis* and chitosan nanoparticles) as feed supplements and indicated that the growth performance of Nile tilapia (final body weight, BWG, and SGR) increased significantly (P<0.05) with increasing any or both of *S. platensis* then chitosan.

Table 2: Effect of chitosan nanoparticles biosynthesis using *S. platensis* on body weight of European seabass (*D. labrax*).

Treatment	No.	Initia1 weight	Fina1 weight	WG*	Survival rate%
T1	40	22.80±0.90	107.70±1.06d	84.86±1.092d	73.00±1.00c
T2	40	22.60±0.93	116.78±1.05c	94.19±1.09c	80.00±1.70b
T3	40	22.90±0.91	118.28±1.06c	95.40±1.062c	84.00±1.70ab
T4	40	23.50±0.68	123.90±1.04b	100.90±1.04b	85.67±1.50a
T5	40	22.50±0.71	129.50±1.06a	107.00±1.02a	88.00±1.15a
T6	40	22.90±0.91	129.90±1.05a	107.12±1.04a	87.30±0.90a

+ Means with the same letter in each column are not significantly differences (P < 0.05).
* Weight gain (WG)= Final Weight-Initial weight

European seabass (*D. labrax*) of T5 (1ml chitosan and 0.05% *S. platensis*) showed the highest survival rate (SR) values (88.00 %, respectively), whereas, those of T1 (control) showed the lowest value being 73.00% and the differences between the control treatment and the other treatments were significant (P<0.05). These results were in accordance with Shata (2020) who found that, *Penaeus monodon* of T5 (1ml chitosan and 0.05% *S. platensis*) and T6 (1ml chitosan and 0.1% *S. platensis*) were recorded the highest SR values (74.19 and 73.22%, respectively), Whereas, those of T1 (control) showed the lowest value being 57.32%. Furthermore, Moe (2011) found that growth performance and the mean survival rate higher (100%) in *Litopenaeus vannamei* fed 0.05% dietary *S. platensis* and chitosan than that of the control group (80%). Also, Jana *et al*. (2014) found 94% and 80% survival rate for the diet supplemented with spirulina and chitosan at all the 0.05% levels and without spirulina, respectively at the termination of the experiment. So spirulina improved survivability rate of fish.

Biochemical parameter:

Effect of chitosan nanoparticles biosynthesis by *S. platensis* on the total protein g/dl, cholesterol, triglycerides and lactate of European seabass (*D. labrax*) are

summarized in Table 3. Values of total protein increased from 4.15 g/dl in T1 to 5.96 g/dl in T6 (P<0.05). Non-significant differences were showed between T2 and T3 and between T5 and T6 (P>0.05). The highest value of CHO was recorded in T6 being 200.2mg/dl while, the lowest value was 137.26 mg/dl in the control (P<0.05). Non-significant differences were found among T3, T4, T5 and T6 (P>0.05). Meanwhile, the values of triglycerides increased from 321.34 g/dl in the control group to be 378.21 g/dl in T6 (P<0.05). Non-significant differences were found among T4, T5 and T6 (P>0.05). The average lactate values found to remain 9.21, 10.14, 10.96, 11.12, 11.98 and 12.22 mmol/l for T1, T2, T3, T4, T5 and T6, respectively with significant differences among different treatments (P<0.05).

Table 3: Effect of chitosan nanoparticles biosynthesis using *S. platensis* on the total protein (TP)g/dl, cholesterol (CHO), triglycerides (TRIG) and lactate (LACT) of European seabass (*Dicentrarchus labrax*)..

Treatment	No.	Total Protein (g/dL)	Cholesterol (g/dL)	Triglycerides (g/dL)	Lactate (mmol/L)
T1	3	4.15±0.06d	137.26±5.65c	321.34±3.39b	9.21±0.03f
T2	3	4.93±0.06c	179.51±6.52b	341.99±3.39ab	10.14±0.06e
T3	3	5.07±0.06c	185.59±5.95ab	307.81±3.75c	10.96±0.04d
T4	3	4.06±0.02b	191.33±5.95ab	366.95±10.35ab	11.12±0.05c
T5	3	5.66±0.06a	197.51±6.24ab	377.32±6.33a	11.98±0.04b
T6	3	5.96±0.07a	200.20±5.07a	378.21±6.32a	12.22±0.06a

Data are presented as means ± standard error (SE). Mean values with the different superscript along the same column are significantly different (p<0.05).

The present results were in agreement with Shata (2020) who found that averages of total protein g/dl as affected by chitosan nanoparticles biosynthesis using *S. platensis* were increased from 1.8 to 3.43, g/dl for *P. monodon* fed the diets supplemented with chitosan nanoparticles using *S. platensis*. He added that the highest value of CHO was recorded by T4 (0.34 mg/ml) while, the lowest value was 0.30 mg/ml for the *P. monodon* fed diets treated by chitosan nanoparticles biosynthesis using *S. platensis*. The present study supports the conclusion of Pascoli *et al*. (2011), they found that the whole body total protein, cholesterol, triglycerides and lactate contents were a better indicator of chitosan nanoparticles requirement using spirulina than growth response. Hemre *et al*., (2002) found that, the growth response of *D. labrax* indicated that the cholesterol requirement of *D. labrax* could remain met with a dietary cholesterol content of less than 2.3 g kg^{-1}. The growth responses measured suggest that the requirement would remain met with between 0.7 and 1.2 g kg^{-1} of diet. However, the increase for whole body cholesterol content suggests that the requirement was met when the dietary cholesterol content was close to 1.7 g kg^{-1}. As the dietary cholesterol content increased, retention efficiency consistently decreased, with the whole body cholesterol content reaching a maximum when the dietary cholesterol content was 1.7 g kg^{-1}. This maximum level appears to reflect the optimum whole body cholesterol content for structural and metabolic purposes, and so indicates the requirement.

Hematological parameter:

Effect of biosynthesis of chitosan nanoparticles by *S. platensis* on the average blood hemoglobin (Hgb), red blood cells (RBCs), hematocrit and glucose of European seabass (*D. labrax*) were presented in Table 4. The values of hemoglobin (Hgb) as affected by biosynthesis of chitosan nanoparticles using *S. platensis* of *D. labrax* were increased from 10.52 g/dl in T1 to 11.13 in T5 (P<0.05). Non significant

differences were observed among T3, T4, T5 and T6 (P>0.05). The values of red blood cells (RBCs) were varied between 3.95/L in T1 to 4.25 /L in T5 (P<0.05). Non significant differences were observed among T4, T5 and T6 (P>0.05). Meanwhile, the values of hematocrit ranged from 32.39 in T1 to 35.27% in T5 (P<0.05). Non significant differences were observed among T2, T3, T4, T5 and T6 (P>0.05). In connection to glucose values, they were estimated as 2.58, 2.77, 3.21, 3.22, 3.09 and 3.19mg/dl in T1, T2, T3, T4, T5 and T6, respectively. Significant differences were observed between the control treatment and the other five treatments (P<0.05), while non significant differences were detected among T3, T4 and T6 (P>0.05). The present results corresponded with Shata (2020) who found that the averages of hemoglobin (Hgb) were increased from 10.21 to 10.97 g/dl for *P. monodon* fed the diets supplemented with chitosan nanoparticles using *S. platensis*. Also, he outlined the effect of biosynthesis of chitosan nanoparticles using *S. platensis* on hematocrit which was higher for shrimp fed the diet supplemented with chitosan nanoparticles using *S. platensis* than those fed the other diets. Glucose values found to remain 0.51, 0.60, 0.63, 0.64, 0.70 and 0.71 mg/100 ml blood for T1, T2, T3, T4, T5 and T6, respectively Analysis of variance showed that blood glucose was significantly (P<0.05) affected by biosynthesis of chitosan nanoparticles using *S. platensis*.

Table 4: Effect of chitosan nanoparticles biosynthesis using *S. platensis* on The average blood components (hematological parameters) and Glucose of European seabass (*Dicentrarchus labrax*)

Treatment	No.	Hgb (g/dL)	RBC (10^{12}/L)	Hct (%)	Glucose (mg/dL)
T1	3	10.52±0.03c	3.95±0.03c	32.39±0.37b	2.58±0.06d
T2	3	10.96±0.09b	4.01±0.026bc	34.62±0.38a	2.77±0.03c
T3	3	11.09±0.03a	4.06±0.016b	34.91±0.35a	3.21±0.02a
T4	3	11.12±0.04a	4.18±0.036a	35.20±0.32a	3.22±0.05a
T5	3	11.13±0.12a	4.25±0.027a	35.27±0.34a	3.09±0.02b
T6	3	11.12±0.06a	4.24±0.03a	35.26±0.32a	3.19±0.06a

Data are presented as means ± standard error (SE). Mean values with the different superscript along the same column are significantly different (p<0.05).

CONCLUSION

Based on the results obtained in this study, it could be concluded that *Spirulina platensis* biomass may be a precious, cost effective means for the production of chitosan nanoparticles, indicating their potential in the future production of other valuable nanoparticles in the emerging field of nanobiotechnology in an environmentally friendly way. Finally, it could be concluded that, feeding European seabass (*Dicentrarchus labrax*) on diets contained 45% protein and supplemented with chitosan nanoparticles biosynthesis using *S. platensis* lead to increase fish growth performance and improve its blood biochemical and hematological parameters.

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