Effect of Some Immunostimulents on the Immune Statuse in Cultured Marin Fish

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Abstract: The present work was designed to investigate the effects of immunostimulants *(Algae*® & *Aquabacteriae*®) on the immune status of cultured marine fish. There was a significant differences effect of the used immunostimulants on *Sea bass* and *Sea bream* serum enzymes. The levels of serum proteins (total protein, Albumin, globulin and albumin /globulin ratio) were increased in case of groups received diet with *Algae*® followed by that received diet with *Aquabacteriae*® in both types of fish. The lymphocyte number and The phagocytic activity and index increased progressively from 0-day to 8th week in *Sea bream* more than in *Sea bass* and commonly the groups received *Algae*® and finally the groups of *Aquabacteriae*®. The antibody titers differ significantly among different groups and different weeks (P< 0.01). The higher antibody titers observed in the first weeks in the groups of *Sea bream* fed *Algae*® and *Sea bream* fed *Aquabacteriae*®. In the 4th week the higher antibody titer was observed in case of *Sea bream* fed *Algae*®. The RLP was higher in group treated with *Algae*® followed by *Aquabacteriae*® than control groups especially in *Sea bream*. The effects of the two tested immunostimulants (*Algae*® and *Aquabacteriae*®) were obvious in both species (*Sea bass* and *Sea bream*). In addition, the immunostimulation was more marked in *Sea bass* than in *Sea bream*. Moreover, upon comparing this immunostimulant effect among the two tested immunostimulants, *Algae*® was the best followed by *Aquabacteriae*®. Histopathologically, the immunopromoting effect on heamopiotic organs were similar in nature either in both species upon the use of any drug, but, it was variable in degree and distribution.
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1. Introduction

Utilization of immunostimulants in fish culture offers a wide range of attractive methods for including and building protection against diseases.

It was stated that the interaction between the microbiota, including probiotics, and the host is not limited to the intestinal tract. Probiotic bacteria could also be active on the gills or the skin of the host but also in its ambient environment. ***(Geert et al., 2002). Sandlheath Industrial State (2003),*** reported that, vaccination and treatment have been able to control many diseases that affect farmed fish.However, circumstances change and new diseases appear for which specific treatments may not be available. This coupled with consumer concerns over food safety and protection of the environment has meant that new strategies for disease control have to be developed through the introduction of immunostimulant in aquaculture industry.

Use of Algae® and Aquabacteria® one know to use as immunostimulants in fish, broilers and large animals ***(Yoshida et al., 1995 and Safinaze, 2001).***

Dietary enrichment with ArA not only improved growth and survival in larval seabream (**Bessonart et al., 1999**), but also reduced the stress-related mortality that occurred after handling and tank transfer (**Koven et al., 2001**).

Due to the importance of fish Industry in our country the effect of these immunostimulants on fish enforced us to study their effects. Therefore, the present work was designed to investigate the effects of some immunostimulants on the immune response of both ***Sea bream*** and ***Sea bass***.

2. Material and Methods

Fish:

A total of 320 ***Marin fish*** (30 ± 5 g body weight) 160 from these were ***Sea bream*** and other 160 were ordinary ***Sea bass***, both were obtained from Private fish farm in Alexandria province (Borg El-Arab). Each ten fish were kept in an artificial airy glass aquaria measuring (90 X 50 X 35 Cm) containing saline water at a temperature of 20 - 23 C, pH 7.1 - 7.3.

Fish diets:

Fish were fed on an commercial fish food containing 40% crude protein (obtained from Barseek fish clture factory) the diet was daily provided at 3% of body weight as described by ***Eurell et al (1978)***.

The daily amount of food was offered on two occasions over the day at 9 AM and 5 PM

Probiotics:

Aqua Grow® DHA and Aqua Grow® (ARA) (Arachidonic acid):

These two types of Aqua bacteria were mixed with each other and added to the glass aquaria of 50 liters capacity by the ratio of 1.5 ml and spontaneously mixed with 0.5spoonful from raw carbon in each aquarium.

Composition of *Algae*®:

Artemia enrichment:

- Blend 2 portions of 0.3 g / 200.000 nauplii / L.
- Feed at time zero hr. & 8 hr.
- Harvest after 16 hr.

Rotifer enrichment:

- Blend 2 portions of 0.5 g / 500.000 cysts/liter.
- Feed at time zero hr. & 4 hr.
- Harvest after 8 hr.

Dose of probiotics of Aqua-Grow:

For each material we put 5 g / 1 kg feed, then mix with oil to make pellets by using Carboxy Methyl Cellulose by ratio of 0.2 %

Table (1): Composition of the probiotics used:

Proximate	Composition	
	Aqua Grow® DHA (spray dried C-Type microalgae)	**Aqua Grow (ARA) (spray dried, high Arachidonic acid content)**
Fat	23.0 %	26 %
Protein	40.0 %	40 %
Carbohydrate	19.0 %	20 %
Moisture	4.0 %	5 %
Fiber	5.0 %	1 %
Ash	9.0 %	8 %

Composition of aquagrow:

AquaGrow DHA is a spray dried algal extract of heterotrophically grown alga, *Crypthecodinium*, an aquatic food source high in Omega-3 DHA. The alga is cultivated in a GMP regulated facility to ensure high quality, safety and consistency.

A clean, naturally sustainable source of Omega-3 DHA that blends well with other ingredients. It has a long shelf life at ambient temperatures with an average 100 micro-particle size and minimum 15% DHA by weight. Made in the USA.

AquaGrow ARA is a spray dried nutrition product for animal feeds designed to provide a high level of arachidonic acid. Produced by a GMP-regulated microbial fermentation process, AquaGrow ARA is an ideal component in broodstock maturation and larval diets for increasing hatch rates, decreasing larval mortality while reducing foaming, leaching and residue. Minimum 12% ARA by weight. Made in the USA.

Vaccine preparation:

A virulent strain of ***Pseudomonas flourescence*** (Kindly provided by Fish diseases dep. Fac. of Vet. Med. Alex. Univ.) was inactivated by formalin according to (Sakai et al., 1984). The inactivated ***Pseudomonas flourescence*** was tested for safety and sterility according to (Anderson et al., 1992).

Table (2): Design of the experiment:

Fish species	Sea bream	Sea bass
Treatment	Algae with aqua-bacteria	Algae with aqua-bacteria
	With Algae only	With Algae only
	With aqua-bacteria only	With aqua-bacteria only
	With-out (Algae and aqua-bacteria) = (Control)	With-out (Algae and aqua-bacteria)) = (Control)

Sampling and the Analytical Methods:

Blood samples were collected from the caudal vessels using disposable tuberculin syringe ***(Hawk et al., 1965)*** for estimation of total erythrocytic count (TEC), total leucocytic count (TLC) ***Stoskopf (1993)***. Differential leucocytic count (DLC) was determined according to ***Lucky (1977).*** Both phagocytic activity and index were also determined according to ***Hawk et al. (1965)***. Similarly, blood was collected without anticoagulant for serum separation as described by ***Leid et al. (1975)***. The obtained sera were used for colorimetric determination of the activities serum total protein, albumin, globulin values were determined calorimetrically as implied by the methods of ***Doumas et al. (1981),and, Coles (1974)*** *respectively.*

Experiment I:

A total of 120 ***Sea bream*** and 120 ***Sea bass*** each was divided into 2 sets. Each was subdivided into 4 groups. Each group of 3 replicates 10 fish each. Group **I** received algae® **with aquabacteria®**, group **II** received **algae®** only, group **III** received **aquabacteria®** while group **IV** served as a control feed only with the basal diet.

Experiment II:

The Experiment was carried out for determination of antibody titers and challenge against ***Pseudomonas fluorescence*** and designed in two groups (40 ***Sea bream*** and 40 ***Sea bass***). Each group was subdivided into two subgroup, each of 20 fish.

Group **I** was injected with inactivated ***Pseudomonas fluorescence,*** antibody titration was determined and daily morbidity and mortality were recorded. the survival fish were IP challenged with 0.1 ml / fish containing 9 X 107c.f.u. of the virulent ***Pseudomonas fluoresce*** (Local isolate)after 8 weeks according to (Safinaz, 2001).

The inactivated ***Pseudomonas flourescence*** was tested for safety and sterility according to (Anderson et al., 1992). The potency of bacterin was evaluated by calculating the relative level of protection (RLP) by the following formula which described by ***Newman and Majnarich (1982):***

$$RLP = 1 - \frac{\%\ mortality\ \ of\ \ vaccinated\ \ \ fish}{\%\ mortality\ \ of\ \ control}$$

Detection of immune response:

Pseudomonas flourescence was evaluated by micro-agglutination (MA) test. Agglutination titers were expressed as Log2 of the highest serum dilution still giving a clear agglutination according to (***Eurell et al., 1978***).

HistoPathological studies:

Following complete necropsy in the first experiment, fresh specimens from liver, spleen and kidneys of both ***Sea bass and Sea bream*** were collected and rapidly fixed in 10% neutral buffered formalin. Paraffin blocks were cut at 5 microns thick sections which stained with hematoxylin and eosin (H & E) according to the method described by ***Culling (1983)***.

Statistical analysis: Statistical analysis of the obtained data was performed using the Statistical Analysis System (***SAS, 1987***).

3. Results

The results were tabulated in tables 3, 4, 5, 6.

Histopathological alterations:

Generally, from histopathologic point of view, the effects of the two tested immunostimulants (***Algae®*** and ***Aquabacteriae)*** were obvious on haematopoitic organs in both species (***Sea bass*** and ***Sea bream***). In addition, this effect was more marked in ***Sea bream*** than in ***Sea bass***. Histopathologically, the immuno-promoting effect was similar in nature either in both species upon the use of any drug, but, it was variable in degree and distribution (The splenic reaction in all experimental groups was hyper activation and enlargement of the melanomacrophage centers. This reaction was the greatest in ***Sea bass*** treated with ***Algae®*** and it was the least in ***Sea bream*** treated with ***Aquabacteriae)***.

1- Kidney:

The immunostimulant effect on the kidney tissue was manifested by moderate to severe (according to species and/or drug) proliferation of the lymphoid elements in the anterior kidney **Fig. (1**). Furthermore, the posterior kidney showed focal to multifocal proliferation of the interstitial lymphoid elements. In addition, the renal melano-macrophage centers were variably enlarged and hyper-activated. Fig. (2).

2- Spleen:

The lymphoid elements of the splenic parenchyma had increased in size upon the extent of the stromal tissue. Additionally, the splenic melanomacrophage centers were markedly proliferated, enlarged and hyperactivated to the extent that the melanin pigment itself was concentrated inside the macrophages and appeared dark brown to black granules. Fig. (3).

3- Hepatopancrease:

It was the least reacted organ against the tested immunostimulants. The microscopic examination revealed that the hepatic melano-macrophage centers appeared slightly activated Fig. (4) However, the livers of ***Sea bass*** treated with ***Algae®*** exhibited more lymphocytic cell proliferation within the hepatic parenchyma. Fig.(5).The specimen from control group organs revealed normal histological structures.(more or less).

Table (3): Effect of different treatments among different weeks on (Lymphocyte, monocyte, Phagocytic activity and Phagocytic index).

Week	Group	N	Lymphocyte	Monocyte	PA	PI
			Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error
1st Week	1	3	52.00±0.58B	2.33±0.33AB	23.67±1.76C	2.67±0.15B
	2	3	50.33±0.33C	2.00±0.58AB	24.00±0.58C	2.33±0.13B
	3	3	51.67±1.45BC	1.33±0.33B	25.00±0.58B	2.60±0.40B
	4	3	55.00±0.58A	1.33±0.33B	27.67±0.33A	2.60±0.12B
	5	3	55.33±1.20A	1.67±0.33B	27.67±0.88A	2.17±0.03B
	6	3	54.33±0.88A	2.00±0.58AB	25.00±0.58B	3.33±0.15A
	7	3	55.33±2.33A	3.00±0.58A	27.33±0.33A	3.23±0.09A
	8	3	50.67±0.33C	2.00±0.58AB	25.00±0.58B	3.43±0.09A
2nd Week	1	3	50.00±1.15B	2.33±0.33AB	28.00±0.58B	2.23±0.28A
	2	3	52.00±0.58AB	1.67±0.33B	29.67±0.88A	1.93±0.12B
	3	3	48.67±0.33C	3.33±0.33A	28.00±0.58B	2.27±0.03A
	4	3	50.67±3.76B	2.33±0.88AB	26.00±0.58C	2.00±0.06A
	5	3	47.67±2.91C	2.00±1.15B	24.33±0.88D	1.70±0.06B
	6	3	46.67±3.18C	2.33±0.88AB	25.67±0.88CD	2.30±0.06A
	7	3	51.33±0.88B	2.00±0.58B	24.33±0.88D	2.93±0.12A
	8	3	53.00±1.73A	3.33±0.33A	23.00±0.58E	2.10±0.10A
3rd Week	1	3	55.33±0.88A	2.00±0.58AB	25.33±0.88AB	3.03±0.20A
	2	3	54.33±0.88AB	1.33±0.33B	21.00±0.58C	3.08±0.06A
	3	3	53.33±0.33B	1.33±0.33B	26.00±0.58A	3.23±0.45A
	4	3	53.00±1.15B	3.00±0.58A	25.00±0.58AB	2.17±0.27AB
	5	3	49.33±0.33C	1.33±0.33B	21.67±0.33C	1.50±0.12B
	6	3	49.33±0.88C	1.00±0.58B	26.67±0.33A	2.17±0.22AB
	7	3	55.00±1.15A	1.67±0.88B	26.67±0.88A	2.23±0.09AB
	8	3	53.00±1.15B	2.00±0.58AB	24.67±0.67B	2.40±0.06AB
4th Week	1	3	53.67±0.33B	1.33±0.33B	26.33±1.20B	2.73±0.09AB
	2	3	55.00±0.58A	2.33±0.33AB	30.00±0.58A	2.83±0.38AB
	3	3	55.33±0.33A	1.67±0.88B	24.00±1.73C	3.63±0.18A
	4	3	49.33±0.88C	2.67±0.88AB	26.00±1.15B	2.33±0.12AB
	5	3	52.00±1.15B	3.00±0.58A	30.00±0.00A	1.80±0.06B
	6	3	55.00±1.15A	2.33±0.33AB	29.33±0.88A	2.23±0.03AB
	7	3	50.00±0.58C	1.33±0.33B	23.33±1.45C	2.57±0.03AB
	8	3	45.00±1.15D	2.00±0.58AB	25.33±0.88C	3.37±0.15A

For each week means within the same column of different letters are significantly different at ($P < 0.01$)
Gp.1 = *Sea bass* + Algae + Aqua bacteria Gp. 2 = *Sea bass* + Aqua bacteria only. Gp.3 = *Sea bass* + Algae only Gp. 4 = *Sea bass* only. Gp.5 = *Sea bream* + Algae + Aqua bacteria Gp. 6 = *Sea bream* + Aqua bacteria only. Gp.7 = *Sea bream* + Algae only
Gp. 8 = *Sea bream* only.

Table (4): Effect-of-Algae-and- aqua bacterial-supplementation-on protection of Sea bream and Sea bass against virulent strain of *Pseudomonas flourescence* after vaccination by intramuscular injection of Pseudomonas flourescence vaccine (n = 10).

Parameter	Algae + Aqua bac		Algae only		Fish with Aquabacteria only		Fish without Algae and aquabacteria	
	Sea bass	Sea bream	Sea bass	Sea bream	Sea bass	Sea bream	Sea bass	Sea bream
Dead	7	5	7	6	8	7	10	10
Survival	3	5	3	4	2	3	0	0
Mortality %	70	50	70	60	80	70	100	100
R.L.P	0.3	0.5	0.3	0.4	0.2	0.3	0.0	0.0

Table (5): Effect of different treatments among different weeks on (WBCs, RBCs, Albumin, Globulin and Total protein).

Week	Group	N	WBCs	RBCs	Albumin	Globulin	Total protein
			Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error
1st -Week	1	3	21.00±1.15C	2.00±0.06BC	2.77±0.26C	2.07±0.12A	4.83±0.15C
	2	3	22.33±0.88B	2.03±0.15BC	2.20±0.25C	2.30±0.36A	4.50±0.12C
	3	3	19.00±0.58D	3.23±0.09A	3.00±0.35B	2.17±0.17A	5.17±0.22B
	4	3	23.00±0.58AB	2.40±0.17BC	3.17±0.12B	1.57±0.34B	4.73±0.23C
	5	3	22.00±0.58B	2.27±0.32BC	3.70±0.26B	2.30±0.21A	6.00±0.06A
	6	3	23.33±0.33A	2.70±0.51BC	3.77±0.26B	2.30±0.15A	6.07±0.12A
	7	3	24.00±0.58A	1.80±0.06C	4.03±0.38A	1.77±0.20B	5.80±0.17B
	8	3	22.33±0.88B	1.90±0.32C	3.70±0.26B	2.07±0.07A	5.77±0.20AB
2nd -Week	1	3	24.33±0.33A	2.17±0.15AB	2.47±0.12C	0.97±1.38D	3.43±1.27D
	2	3	22.00±0.58C	2.37±0.18AB	3.20±0.06B	2.10±0.00B	5.30±0.06B
	3	3	24.67±0.33A	3.07±0.18A	4.40±0.12A	1.90±0.00B	6.30±0.12A
	4	3	23.00±1.15BC	2.77±0.20AB	3.80±0.23B	2.07±0.20AB	5.87±0.03AB
	5	3	23.67±0.33B	2.40±0.06AB	2.27±0.20C	2.60±0.23A	4.87±0.03C
	6	3	23.00±1.15B	1.97±0.09B	2.33±0.15C	2.70±0.17A	5.03±0.32B
	7	3	23.67±0.33B	3.07±0.18A	3.13±0.15B	2.60±0.17A	5.73±0.03AB
	8	3	22.67±0.88C	3.00±0.06A	2.30±0.26C	2.73±0.09A	5.03±0.20B
3rd –Week	1	3	22.67±1.20B	2.83±0.15AB	3.80±1.60A	0.20±1.62C	4.00±0.35A
	2	3	25.00±1.15A	2.73±0.35AB	2.00±0.06B	1.47±0.38B	3.47±0.43B
	3	3	24.33±0.33A	2.60±0.21AB	1.97±0.20C	1.60±0.00B	3.57±0.20B
	4	3	23.67±1.20B	3.03±0.17A	1.87±0.03C	1.53±0.15B	3.40±0.17B
	5	3	24.67±0.33A	2.37±0.18AB	1.77±0.03C	1.73±0.03B	3.50±0.06B
	6	3	25.00±1.15A	1.77±0.03B	2.17±0.15B	2.00±0.36A	4.17±0.50A
	7	3	25.00±0.58A	2.33±0.20AB	2.40±0.17B	1.60±0.23B	4.00±0.06A
	8	3	22.33±0.33B	2.50±0.15AB	2.30±0.12B	1.17±0.09B	3.47±0.09B
4th -Week	1	3	23.00±0.58C	2.17±0.07A	2.37±0.18A	2.17±0.20A	4.53±0.03A
	2	3	23.33±1.45C	3.17±0.09A	2.87±0.35A	2.00±0.25A	4.87±0.12A
	3	3	23.33±0.33C	2.77±0.23A	2.77±0.20A	2.13±0.23A	4.90±0.44A
	4	3	22.33±0.88C	2.33±0.23A	2.23±0.07AB	2.10±0.10A	4.33±0.15A
	5	3	24.33±0.88B	2.10±0.06A	1.90±0.12B	2.07±0.32A	3.97±0.43B
	6	3	25.33±0.88B	2.30±0.06AA	1.80±0.06B	1.83±0.03B	3.63±0.03B
	7	3	26.00±1.15A	2.40±0.25A	2.23±0.09A	2.20±0.00A	4.43±0.09A
	8	3	20.33±0.88D	2.47±0.09A	1.93±0.09B	2.33±0.48A	4.27±0.56A

For each week means within the same column of different letters are significantly different at (P < 0.01)
Gp.1 = Sea bass + Algae + Aqua bacteria Gp. 2 = Sea bass + Aqua bacteria only. Gp.3 = Sea bass + Algae only Gp. 4 = Sea bass only. Gp.5 = Sea bream + Algae + Aqua bacteria Gp. 6 = Sea bream + Aqua bacteria only.
Gp.7 = Sea bream + Algae only Gp. 8 = Sea bream only.

Table (6): Antibody titers (Log.2) in different experimental groups.

Group	N	Antibody titration			
		Weeks			
		1st	2nd	3rd	4th
		X ± SD	X± SD	X± SD	X± SD
1	3	3 ± 1.2Ec	3 ± 1.1Dc	5 ± 2.3Ba	4 ± 1.5Eb
2	3	4 ± 1.5Db	4 ± 1.5Cb	5 ± 2.3Ba	5 ± 2.3Ca
3	3	4 ± 1.3DB	2 ± 0.5Ec	5 ± 2.3Ba	5 ± 2.3Ca
4	3	3 ± 1.4Eb	5 ± 2.3Ba	2 ± 0.5Ec	3 ± 1.3Fb
5	3	3 ± 1.5Ec	4 ± 1.4Ca	4 ± 1.4Ca	3 ± 1.3Fc
6	3	5 ± 2.3Ca	4 ± 1.4Cb	4 ± 1.4Cb	2 ± 0.5Gc
7	3	6 ± 1.5Ba	3 ± 1.1Db	2 ± 0.5Ec	2 ± 0.5Gc
8	3	4 ± 1.4Db	3 ± 1.1Dc	6 ± 3.3Aa	3 ± 1.2Fc
Total mean	**48**	**4.87± 2.5**	**3.75 ± 1.3**	**4.12 ± 2.3**	**4.50 ± 1.5**

For each week means within the same column of different letters are significantly different at (P < 0.01)
Gp.1 = Sea bass + Algae + Aqua bacteria Gp. 2 = Sea bass + Aqua bacteria only.
Gp.3 = Sea bass + Algae only Gp. 4 = Sea bass only.
Gp.5 = Sea bream + Algae + Aqua bacteria Gp. 6 = Sea bream + Aqua bacteria only.
Gp.7 = Sea bream + Algae only Gp. 8 = Sea bream only.

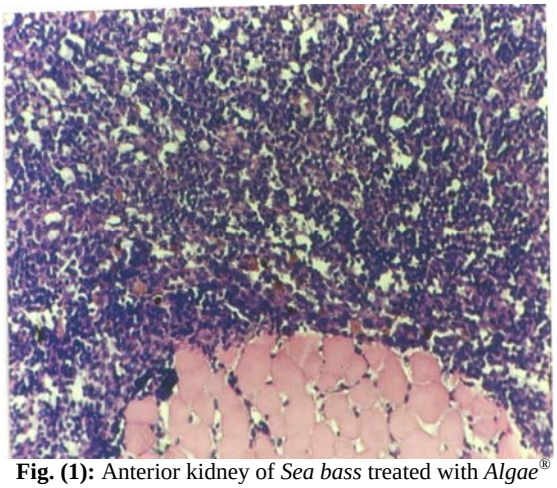


Fig. (1): Anterior kidney of *Sea bass* treated with *Algae*® *and Aquabacteria:* showing marked proliferation of the lymphoid elements. H, E. (X 250).

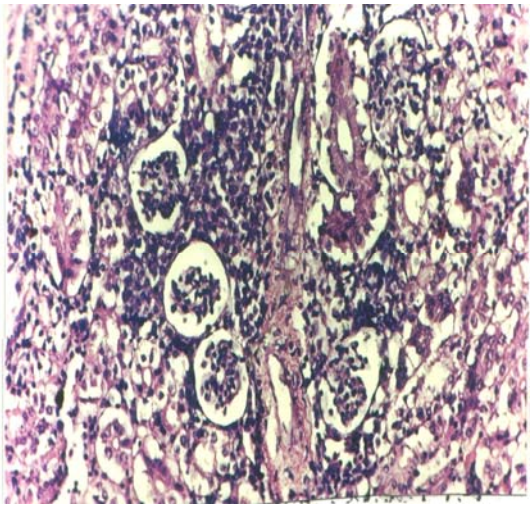
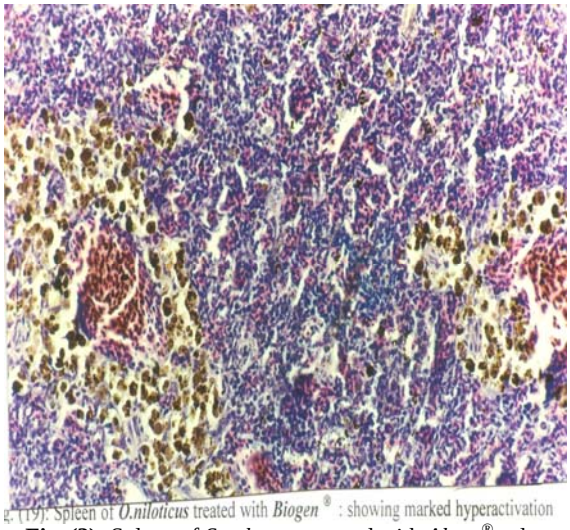


Fig. (2): Posterior kidney of *Sea bass* treated with *Aquabacteriae only:* showing Proliferation of the interstitial lymphoid elements. H, E. (X 160).



g. (19): Spleen of *O.niloticus* treated with *Biogen* ® : showing marked hyperactivation

Fig. (3): Spleen of *Sea bream* treated with *Algae*® only: showing marked hyperactivation of melanomacrophage center with intensive concentration of melanin (black) pigment. H, E. (X 160).

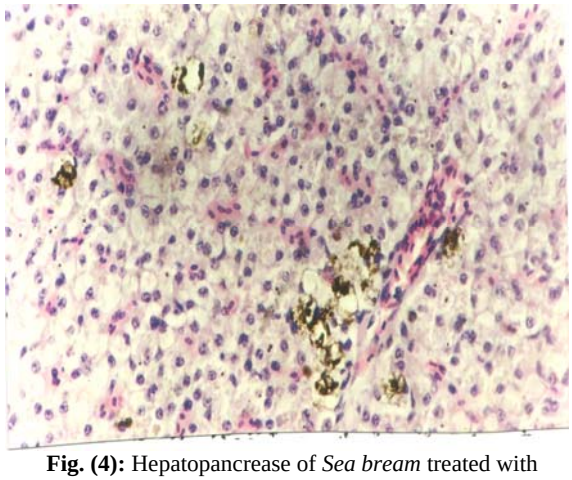


Fig. (4): Hepatopancrease of *Sea bream* treated with *Aquabacteriae only*: there is activated melanomacrophage centers. H, E. (X 160).

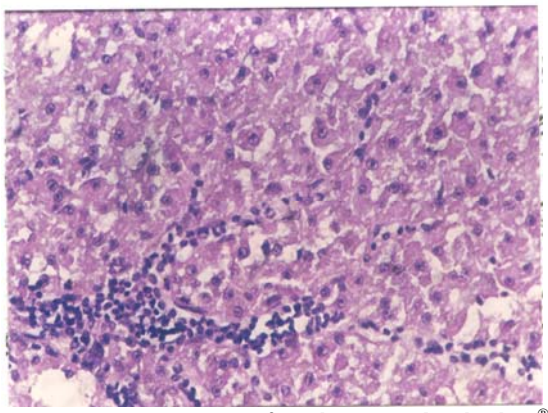


Fig. (5): Hepatopancrease of *Sea bass* treated with *Algae*® *and Aquabacteria*: showing lymphocytic cell proliferation (arrow). H, E X 250).

4. Discussion

The importance of immunostimulants originated from that, the immunostimulants are a group of chemicals or biologicals giving to the human, animals, birds and fish to give them the protection against pathogens via increasing and enhancing the specific and non-specific defenses of the body ***(Bangi et al., 2000 and Smith et al., 2003).***

In the present investigation**,** the two species of fish either ***Sea bass*** and ***Sea bream*** treated with two types of immunostimulants for 4 weeks. The effects of the two immunostimulants on serum proteins and deferential leucocytic counts of WBCs (lymphocyte, Monocyte, as well as phagocytic activity, Index and the level of antibody titer and RPL) were determined.

These results indicated that, under the experimental conditions, there is no any change in serum protein among the two species of fish. This results agreed with those of ***Smith et al.(2003),*** who reported that, the stress conditions causes elevation of the serum enzymes levels and serum proteins than the fish live under the normal conditions or not treated with any treatments

The total serum proteins were useful in diagnosis of fish diseases ***(Mulcathy, 1967).*** In the present work, the significant increase in albumin, globulin, total protein was higher in the groups received ***Algae and Aquabacteriae*** than that treated with ***Alga only, Aquabacteriae*** only and control group.

The levels of albumin, globulin and protein were higher in case of ***Algae®*** treated group than ***Aquabacteriae*** one while the levels in both treatment were higher than control group. This result attributed to the effect of immunostimulants on liver that causes increasing of serum protein concentration ***(Manning and Wyatt, 1984 and Edvington et al., 1994)***. Moreover**,** fish without immunostimulant diet and under stress has the greatest decrease in total protein due to liver affection ***(Saffinaz, 2001).***

The present results showed that decreasing in globulin level in groups treated with ***Aquabacteriae*** than the groups treated with ***Algae®***, or Algae and Aquabacteria but the all higher than the control.

Meanwhile the albumin fraction increased with addition of immunostimulants (***Algae®*** or ***Aquabacteriae*** or both together) than the control groups which not treated with any immunostimulants.

The increase of all serum proteins in case of ***Algae and Aquabacteriae*** than that treated with ***Alga only, Aquabacteriae*** only and control group. Moreover, the increasing of serum proteins in the ***Sea bream*** was higher than that of ***Sea bass*** may be due to higher sensitivity of ***Sea bream*** immune system to immunostimulant than that of ***Sea bass.***

Increase albumin level was also observed in the groups exposed to immunostimulants. ***Kovn et al.,(1989)*** stated that chronic liver disorder is usually accompanied by hypo-albuminaemia. Both hypogammaglobulinaemia and hypoalbuminaemia confirmed the recorded hypoproteinaemia, which was associated with liver damage ***(Manning and Wyatt, 1984).***

The same authors also indicated that the immunostimulant causes increasing of albumin level than groups not treated with any immunostimulants. This result attributed to the using of immunostimulants in fish farms causes regeneration of the liver cells and increasing the immune status of fish body with increasing of serum proteins. ***(Manning and Wyatte, 1984).***

Stressor has a drastic effect on immune system of ***Sea bass*** or ***Sea bream*** in challenge experiments. This stress may activate the hypothalamus hypophysis-adrenal endocrine system and stimulate corticosteroids and catecholamines in fish blood which negatively affect the process of lymphobiosis and interfere with the synthesis of ascorbic acid, thus lowering the resistance of fish and induce immuno-suppression ***(Paulsen et al., 2001 and Sealey and Galtin, 2002).***

The immunostimulants causes increasing the level of serum proteins and so overcome the action of stress conditions or the action of bacterial infections which causes decreasing of the serum proteins level due to liver damage resulted from the bacterial infection. *(Naglaa Mangwood, 2004).*

The blood parameters as leucocytic counts and differential leucocytic count have diagnostic importance and usually readily respond to identical factors such as physical, chemical and biological stressors ***(Hickey, 1976 and Soliman, 1996).***

The results indicated that, the groups received ***Algae and Aquabacteriae*** showed increase in lymphocytes than groups treated with ***Algae only or Aquabacteriae*** only and both (***Aquabacteriae*** & ***Algae®***) revealed increases of D.L.C. than the control fish. These results agreed with those of ***(Edvington et al., 1994)*** which reported that fish not take any immunostimulants or live under stress conditions show decrease D.L.C. No. and increase susceptibility to infection.

Also there was increasing in lymphocyte and monocyte in groups receive immunostimulant (***Algae®*** and ***Aquabacteriae***) than the control groups.

This increasing mainly in ***Algae and Aquabacteriae*** than that taken ***Alga only, Aquabacteriae*** only and control group due to its effect on haemobiotic organs ***(Change et al., 1979).***

The group fed ***Algae and Aquabacteriae*** characterized by increasing of Phagocytic activity and phagocytic index than the group treated with ***Aquabacteriae*** or Algae than the groups fed regular diet without immunostimulants. This result, attributed to the action of immunostimulants on liver, kidney, spleen and other haemopiotic organs as it activate this organs ***(Fuchs et al., 1986 and Gekle et al., 1998),*** so it causes increases of D.L.C. and increases phagocytic activity and phagocytic index than control groups.

This results may suggests that, the action of ***Algae®*** and ***Aquabacteriae*** on fish leads to decreasing level of serum cortisol and increase fish resistance. The decrease of cortisol level may lead in turn to increase the phagocytosis process.

This results may be due to that ***Algae®*** containing Artemia and rotifier protein which increase the physiological function of fish and immune response with increasing resistance of fish to different stress related disease. ***(Safinaz 2001).***

Artemia and rotifier protein active principle increasing phagocytic activity and phagocytic index with increasing of mitogenesis of T and B lymphocytes, which causes reduction of cell damage via its act directly on body cell promoting DNA , protein synthesis and increasing T-cell mediated immunity with increasing of body resistance due to increasing antibody producing cells***. (Naglaa-Mangood, 2004).***

The antibody titers increased progressively from the 2nd week to 4th week in ***Algae®*** and ***Aquabacteriae*** treated groups. While, the titers were higher in ***Algae®*** and ***Aquabacteriae*** treated groups. The addition of immunostimulants causes raising of the antibody titers than the groups that not received immunostimulants. Also the antibody titer level was higher in ***Sea bream*** than that of ***Sea bass.***

These results attributed to the immunostimulants contain some factors that enhancing the activity of the fish and stimulating the antibody secretion leads to increasing the antibody titers.

The Relative Level of Protection (RLP) was higher in the groups treated with ***Algae®*** and Aquabacteria than the groups treated with ***Algae only or Aquabacteriae*** only and all of them higher in its RLP than the control groups.

These results attributed to the action of immunostimulants on liver, kidney, spleen and other haemopiotic organs as it activate this organs ***(Fuchs et al., 1986 and Gekle et al., 1998),*** which causes increases of D.L.C. counts and the relative level of protection of the fish against any stress conditions, its level in ***Sea bream*** was higher than ***Sea bass*** may be due to hormonal treatment, this results need more study to be clarify).

By bacterine injection and addition of immunostimulants, that affecting lymphoid tissue and increasing the fish immunity and increasing the activity of haemopiotic tissue like spleen and kidney mesonephrons. The immunostimulant causes increasing of serum protein and increasing immunoglobulin formation, also increasing humeral immunity. Moreover, the increasing of RLP was proved by higher titer of antibody in case of groups received immmunostimulant.

Collins et al. (1976) and Faisal et al. (1988), reported that immunostimulant increasing antibody titer in fish exposed to different environmental stressors than fish without immunostimulant.

From the previous results we noticed that the addition of immunostimulants improve the fish immunity and survival than the other groups that not taken any immunostimulants. This results agreed with those of ***Saad (2002)*** who reported that, the immunostimulants causes reduction of the effect of stress condition on blood enzymes, body weight, body weight gain, feed conversion and feed efficiency.

Moreover, this results agreed with those of ***(Saad, 2002)*** who reported that, the ***Algae®*** and Aqua-bacteria immunostimulant contain the active material as protein, charbohydrate and fat which activate and coordinate the function of various glands in body and enable them to work normally with high efficiency.

The protein, carbohydrate and fat active principle increase antibody activity and inhibit RNA synthesis , Also the protein, carbohydrate and fat has antibacterial and antifungal characteristics due to specific interference with sulphohydryl group so, it will increase the fish immunity ***(Nikitina et al., 1995; Angelo et al., 1998 and Feldberge et al., 1998).***

All the previous results explain why fish that fed on the immunostimulants supplemented diets of good characters on blood enzymes, PA, PI and higher level of RLP than groups take diet free from immunostimulants.

Also results indicated that ***Sea bream*** characterized by higher immunogenic characteristics than ***Sea bass*** and gave a good response to the used immunostimulant this point need more research to be clarified.

The Histopathological findings indicated that there was hyperactivation of melanomacrophage center in kidney, liver & spleen in case of fish received immunostimulant than control and this results agreed with those of ***(Easa, 1997 and Saad, 2002)*** which confirmed this results

histopathologically by activation of melanomacrophage centers of spleen.

These results indicated that the immunostimulants induced hyperactivation of haemopiotic organs and MMCs especially in case of ***Sea bream***. These results agreed with those of ***Agrawala et al. (2001).***

These results indicated that the ***Algae®*** and ***Aquabacteriae*** induced hyperactivation of haemopiotic organs and MMCs especially in case of ***Sea bream.***

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