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Immunogenicity Associated with use of Chitozinc in Competing of *Vibrio* Algenolyticus in Cultured Sea Bream *(Sparus Aurata,* L.)

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ABSTRACT

Improved resistance against infectious diseases can be achieved by the use of Chitozinc ®. The objective of the present study was to evaluate the influence of Chitozinc ® on the immune response of cultured ***sea bream***. The experimental fish were divided into four groups, the first group was fed on diet supplemented with 0.1 gm/kg Chitozinc ® probiotic, the second group was fed on diet supplemented with 0.2 g/kg feed Chitozinc ® probiotic, the third group was fed on diet supplemented with 0.3 gm/kg feed Chitozinc ® probiotic, the 4th group was served as control fed on probiotic-free diet. Eight weeks later the results indicated that, the fish groups which received diet supplemented with 0.3 gm/kg feed Chitozinc ® probiotic revealed significant increase in non specific immune response as detected in vitro differential leucocytic count, phagocytic activity test, serum lysozomal, bactericidal activity, total protein and globulin levels than the control groups. Moreover, the fish groups which received Chitozinc ® increase in antibody titration and relative level of protection against *Vibrio alginolyticus* than the group control fed on probiotic- free diet . In addition to that ***sea bream*** received 0.3 gm/kg feed Chitozinc ® probiotic showed significant decrease in cortisol hormone levels and bacterial counts in gastrointestinal tract than ***sea bream*** received control fed on probiotic- free diet in all weeks.

Keywords: Chitozinc ® seabream, bactericidal activity, Cortisol, *Vibrio alginolyticus*

INTRODUCTION

The intensive rearing of fish species in aquaculture generates a potentially stressful environment to the fish, with the possible suppression of the immune system, rendering the fish more susceptible to different diseases *(Austin and Austin, 1999)*. The routine use of antibiotics during fish culture to minimize the risk of disease is not advisable since it may adversely affect the indigenous microflora of juveniles or adult fish and may increase the risk of promoting antibiotic- resistant microorganisms *(Alderman and Hastings, 1998)*. Thus, the use of probiotics, in the culture of aquatic organisms, is increasing with the demand for more environment-friendly aquaculture practices *(Gatesoupe, 1999)*. Probiotics are usually members of the healthy intestinal microbiota; therefore, they may provide an alternative way to reduce the use of antibiotics in aquaculture, since their addition can assist in returning a disturbed microbiota to its normal beneficial

composition. In the past 10 years there has been a growing interest in fish farming to control diseases through alternative methods, such as probiotics *Irianto and Austin (2002).* A probiotic is generally defined as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" *(Fuller, 1989)*. *Gatesoupe (1999)* redefined probiotics for aquaculture as "microbial cells that are administered in such a way as to enter the gastrointestinal tract and to be kept alive, with the aim of improving health". The definition provided by *Verschuere et al. (2000)* extended the concept of probiotic as "a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment. Probiotics in aquaculture shown to have several modes of action; competitive exclusion of pathogenic bacteria through the production of inhibitory compounds *(Servin, 2004);* improvement of water quality *(Verschuere et al., 2000);* enhancement of immune response of host species *(Balcázar et al., 2007);* and enhancement of nutrition of host species through the production of supplemental digestive enzymes *(Ziaei-Nejad et al., 2006).*

Rengpipat et al. (2000) and Vine (2004) all concluded that probiotics have the ability to improve fish health and prevent bacterial diseases in fish. Consequently, the use of probiotics as a new technique to confer protection in the host fish against pathogenic bacteria in the most economic and environment-friendly manner is certainly worth evaluating in aquaculture.

Probiotics are widely used in poultry and swine rearing farms but little has been done to incorporate them into aquaculture. Thus, the current study aimed to immunologically evaluate the efficiency of Chitozinc® on the culture sea bream.

MATERIALS AND METHODS

I- Materials

1. Fish

A total number of 120 apparently healthy seabream**,** with average body weight of (80±5g/fish) were obtained from El-Wafaa fish farm at Ismailia Governorate. Fish were transported a live to the laboratory of the department of poultry and fish diseases, Faculty of Veterinary Medicine, Alexandria university in large plastic bags containing water enriched by oxygen (2/3) according to (Innes, 1966).

Fish were kept in prepared glass aquaria (90×50×35 cm) supplied with brackish water (salinity ranged between 16-20 ppt).

2. Yeast strain

The *Candida albicans* strain *and Vibrio alginolyticus* were kindly supplied by the department of poultry and fish diseases Fac. Vet. Med., Alexandria University which is used for the serum bactericidal activity study, bacterin preparation and challenge test.

Bacterial strain lyophilized *Micrococcus lysodekticus* which is used for serum lysozomal activity (Sigma M 3770).

3. Media used

Sabuaroud's dextrose broth (Oxoid, 1982) for candida**.**

Thiasulphate citrate, Bile salt sucrose agar (TCBS), BBL,Cat. No. 13543.

MacConkey broth and agar media which is used for isolation and propagation of the number of *Micrococcus lysodekticus* ***(Oxoid, 1982)*** for bacterial growth**.**

4. Kits for clinico- biochemical analysis

Kits for total protein and serum albumin ***(Pasteur, Lab, France).***

Kits for cortisol:

1- ^{125}1- Labeled ACTH 1RMA kit. A Nichols Allergo HS-ACTH kit (ref. CA2194) purchased from Mallinekrodit-Diagnostic-France (Evry, france) was used to assay ACTH

2- ^{125}1- Labeled ALDOSTERONE RIA KIT. Aldosterone was assayed with DPC coat –a- count kit (TKAL 20) obtained from SAPB Hoechst Behring (Rueil-malmaison, France).

3- ^{125}1- Labeled F RIA kit.

4- Angiotensin 1, RIA.**5. Commercial probiotics Chitozinc® used**:

Chitozinc

Composition: Each 1kg contains:

Zinc methionine	200gm
Selenium yeast	200gm
Chitosan	30gm

Dose:

Fish:	1kg/10 ton
Origin :	Korea

Manufactured by:

Samyang

Sole agent:

SOGGY PHARM

II- Methods

1- The experimental design

The experiment was carried out from March 2013 till the end of June 2013. All fish were apparently healthy from any pathogenic bacteria and free from parasitic infestation.

Prior to the experimental use, fish were acclimated to the laboratory conditions for two weeks in glass aquaria each of which with 100 liters capacity and 30 fish density. The aquaria were aerated with air stones attached to air compressor to maintain constant aeration. The water temperature was kept at 23 ± 2^{o}C. The PH was 8.3 ± 0.3 and was monitored with a PH meter. Fish were fed on a commercial fish diet containing 30 % crude protein. The diet was daily provided at fixed feeding ratio 3% of body weight of fish as described by ***Eurell et al. (1978).***

The daily amount of food was offered as two equal meals / day on two occasions over the day (At 9 AM and 12 PM). Moreover, the fish mortality was recorded daily and so, the quantity of food was decided. The water quality parameters contained the following concentrations: 144.5 ± 8 mg $CaCo_3$ /l total hardness , 8.2± 0.3 ppm dissolved oxygen, 0.01mg/ l Nitrite (No_2), 0.02 mg /l Nitrate (No_3), 20± 7 mg/ l H_2s and 0.01 mg/l un ionized ammonia. Two thirds of water was changed daily. Feces and food debris were siphoned out routinely. Following acclimation to the laboratory conditions, the twelve aquaria divided into four groups. Each group consists of three aquaria. The 1st group fed on basal diet and 1 gm/ kg feed Chitozinc ® probiotic. The 2nd group fed on basal diet and 2 gm / kg feed Chitozinc® probiotic. The 3rd group fed on basal diet and 0.3 gm/kg Chitozinc®probiotic. The 4th group fed on basal diet only without any treatment.

2- Blood sampling

At zero days, 2nd, 4th, 6th, 8th weeks during the experimental period, 2 ml blood samples were collected from different groups via the caudal vessels from 2 fish**.**

One ml of blood was collected with syringe containing anticoagulant (0.1 ml of 4% sodium citrate solution / 1ml blood) and used for phagocytic assay according to ***Kawahara et al. (1991)*** and differential leucocytic count and the other ml used for collection of the serum. The collected serum was used for biochemical determination ***(Lied et al., 1975).***

3- Differential leucocytic count: according to Schalm (1986).

4- Determination of phagocytic activity and phagocytic index

Phagocytic activity was determined according to ***kawahara et al. (1991)***. Results were expressed as means ± S.E. and differences were evaluated by Student's t-test.

Phagocytic activity (PA) = percentage of phagocytic cells containing yeast cells.

$$\text{Phagocytic index (PI)} = \frac{\text{Number of yeast cells phagocytized}}{\text{Number of phagocytic cells}}$$

5- Clinico-biochemical analysis

5.1. Determination of serum lysozyme activity

Serum lysozyme activity was measured with the turbidimetric method described by ***Engstad et al. (1992)***. The result was expressed as one unit of lysozyme activity was defined as a reduction in absorbency of 0.001/min.

$$\text{Lysozyme activity} = \frac{(A0 - A)}{A}$$

5.2. Determination of serum bactericidal activity

Serum bactericidal activity to *Vibrio alginolyticus* strain was determined according to ***Rainger and Rowley (1993)***. The results were recorded as survival index (SI) ***(Word Low and Unlles, 1978).*** Values were calculated as follows**:**

$$SI = \frac{\text{CFU at end}}{\text{CFU at start}} \times 100$$

5.3. Determination of serum total protein

Serum total protein was determined according to ***Doumas et al. (1981)*** using commercial kits produced by Pasteur Lab.

5.4. Determination of serum albumin

Serum albumin was determined according to ***Reinhold (1953)*** using commercially available kits of Chemroy.

5.5. Determination of serum globulin:

Serum globulin was determined by subtract the total serum albumin from total serum protein according to ***(Coles, 1974 and Khalil, 2000).***

5.6. Determination of serum albumin/globulin ratio

Determined by division of serum albumin value on serum globulin value. According to the method implied by ***(Saffinaz, 2001).***

5.7. Determination of cortisol

Radioimmunoassay of cortisone in serum, urine, and saliva to asses the status of the cortisol- cortisone shuttle ***(Gilles et al., 1997).***

These assays were carried out after extraction and Celite chromatography ***(Gilles et al., 1997).***

5.8. Determination of total bacterial, total enterobacteriaceae and total coliform counts (APHA, 1992)

One gram of mucous from gastro intestinal tract was collected from the all treated groups. The all plates incubated at 28 C° for 24-48hrs then counted of the all growth colonies (APHA, 1992).

5.9. Antibody titration against Vibrio alginolyticus:

Detection of immune response to *Vibrio alginolyticus* was evaluated by microagglutination (MA) test according to the method described by ***Badran (1990) and Khalil (2000).***

Agglutination titers were expressed as log 2 of the highest serum dilution still giving a clear agglutination ***(Badran, 1990).*** The negative controls consisted of:

i. one drop of sterile physiological saline and one drop of tested serum.

ii. one drop of sterile physiological saline and one drop of stained antigen.

The positive controls were carried out using collected positive antisera.

5.10- Challenge test:

At the end of 7^{th} week ten fish from each treatment group and from the control were clinically examined and blood samples bacteriologically tested and determined to be free from bacterial infection, were then artificially infected by intraperitoneal injection with 0.2 ml of culture suspension of pathogenic *Vibrio alginolyticus* previously adjusted to 10^4 specificity of death was determined by reisolation of injected bacteria

from freshly dead fish during the period of observation. (One week) according to ***Soliman (1988).*** The relative level of protection (RLP), among the challenged fish was determined according to ***Ruangroupan et al., (1986)*** using the following equation.

$$\text{RLP} = \frac{\text{Percentage of immunized mortality}}{\text{Percentage of control mortality}} \times 100$$

$$\text{Mortality \%} = \frac{\text{NO of death in a specified period}}{\text{Total population during that period}}$$

6-Statistical analysis

The data of hematological and biochemical examinations of exposed fish were statistically analyzed using t-test, Duncan- test after ANOVA and simple correlation according to ***(SAS, 1987)*** to examine the significant effect of the main variables on the studied parameters. After that the results presented in the form of figures according to Harvard graphics (HGW-4) computer program.

RESULTS

1- Effects of different treatments of Chitozinc on differential leucocytic count in cultured sea bream

Table (1) explained the significant ($P<0.05$) effect of different treatments among different weeks on differential leucocytic count in ***sea bream*** blood. In zero days (at the beginning of the experiment) results of differential leucocytic count revealed no significant value in all groups.

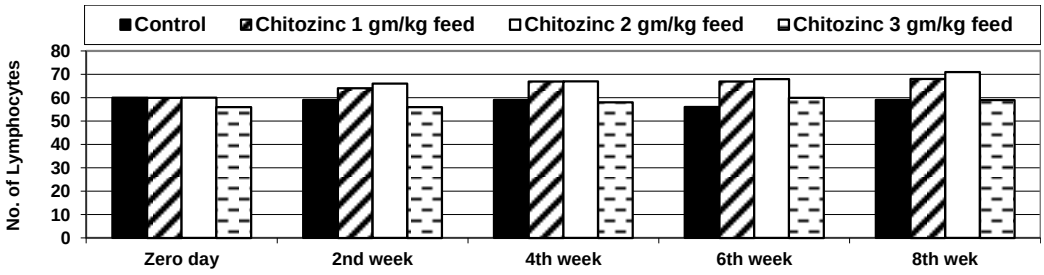
The lymphocytic count increased progressively in ***sea bream*** from the 2nd week till 8th week (end of the experiment) in the groups treated with Chitozinc ® probiotic (higher dose 0.2 gm/kg feed) showed higher value than smaller dose 0.1 gm/kg feed) than the groups treated with Chitozinc ® probiotic and the control group fed on basal diet only as shown in Table (1). Monocyte count showed no significant value in the 2nd, 4th, and 6th week but increased significantly in 8th week in the groups treated with Chitozinc ®probiotic (higher dose 0.2 gm/kg feed showed higher value 3.67 ± 0.33^{a} than smaller dose 0.1 gm/kg feed 2.00 ± 0.58^{b}) than the groups treated with Chitozinc ® probiotic (1.67 ± 0.67^{b} in the groups received 0.3 gm/kg feed and 1.67 ± 0.33^{b} in the groups received 0.1 gm/kg feed) and the control group fed on basal diet only showed increased significant value (2.33 ± 0.33^{ab}) than the groups treated with Chitozinc ® probiotic and the groups received small dose of Chitozinc ®probiotic (2.00 ± 0.58^{b}) as shown in Table (1).

Meanwhile eosinophil and basophils counts revealed no significant value ($P<0.05$) among all treated groups in all weeks as shown in Table (1). Thrombocyte count also considered not significant as shown in Table (1). Concerning neutrophils count the groups treated with Chitozinc ®probiotic (both doses) and the control group showed high significant value ($P<0.05$) than the groups treated with Chitozinc ® probiotic in all weeks. In general the groups treated with Chitozinc ®probiotic (high dose then small dose) is revealed the best results in differential leucocytic count then the groups treated with Chitozinc ® probiotic (both doses).

Table (1): ***Showing results of differential leucocytic count among different groups in different weeks.***

	Groups	N	Lymphocytes	Monocytes	Basophils	Eosinophil	Neutrophils	Thrombocytes
Zero day	**Control**	**3**	**60±0.58 a**	**1±0.34 a**	**5±0.56 ab**	**8±0.56 a**	**21±0.61 a**	**3±0.54 a**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**60±0.54 a**	**1± 0.35 a**	**5±0.57 ab**	**7±0.5.4 a**	**19±0.57 b**	**3±0.53 a**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**60±0.57 a**	**1±0.33 a**	**4±0.56 b**	**7±0.56 a**	**24±0.57 c**	**3±0.55 a**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**56±0.56 b**	**2±0.34 a**	**6±0.56 a**	**7.55 a**	**23±0.56 b**	**30.57 a**
2nd week	**Control**	**3**	**59±0.56 a**	**1±0.33 a**	**6±0.57 a**	**8±0.56 a**	**20±0.56 b**	**2±0.54 a**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**64±0.57 b**	**1±0.33 a**	**5±0.56 a**	**8±0.56 a**	**19±0.57 a**	**3±0.57 ab**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**66±0.57 c**	**1±0.34 a**	**5±0.54 a**	**8±0.57 a**	**11±0.57 c**	**3±0.56 ab**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**56±0.54 d**	**1±0.35 a**	**6±0.58 a**	**8±0.57 a**	**21±0.56 b**	**4±0.53 b**
4th week	**Control**	**3**	**59±0.57 a**	**1±0.34 a**	**6±0.56 a**	**6±0.56 a**	**25±0.54 a**	**2±0.57 a**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**67±0.58 b**	**1±0.34 a**	**6±0.57 a**	**6±0.61 a**	**15±0.58 b**	**3±0.57 a**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**67±0.57 b**	**1±0.34 a**	**5±0.54 a**	**7±0.57 a**	**13±0.56 c**	**3±0.58 a**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**58±0.57 a**	**2±0.33 a**	**6±0.58 a**	**6±0.56 a**	**23±0.58 d**	**3±0.57 a**
6th week	**Control**	**3**	**56±0.58 a**	**1±0.32 a**	**6±0.58 a**	**8±0.57 a**	**23±0.59 a**	**3±0.56 a**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**67±0.57 b**	**2±0.33 a**	**6±0.54 a**	**7±0.57 a**	**14±0.58 b**	**3±0.59 a**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**68±0.57 b**	**1±0.35 a**	**6±0.57 a**	**7±0.58 a**	**12±0.57 c**	**3±0.58 a**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**60±0.58 c**	**2±0.34 a**	**7±0.57 a**	**7±0.58 a**	**18±0.56 d**	**4±0.57 a**
8th week	**Control**	**3**	**59±0.58 a**	**2±0.32 a**	**6±0.58 a**	**7±0.57 a**	**22±0.58 a**	**3±0.58 a**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**68±0.57 b**	**2±0.33 a**	**7±0.58 a**	**7±0.57 a**	**11±0.58 b**	**3±0.57 a**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**71±0.57 c**	**3±0.34 a**	**6±0.54 a**	**8±0.55 a**	**7±0.57 c**	**4±0.58 a**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**59±0.59 a**	**1±0.34 b**	**6±0.57 a**	**7±0.58 a**	**22±0.59 c**	**4±0.57 a**

Fig. (1): ***Results of differential leucocytic count among different groups in different weeks***



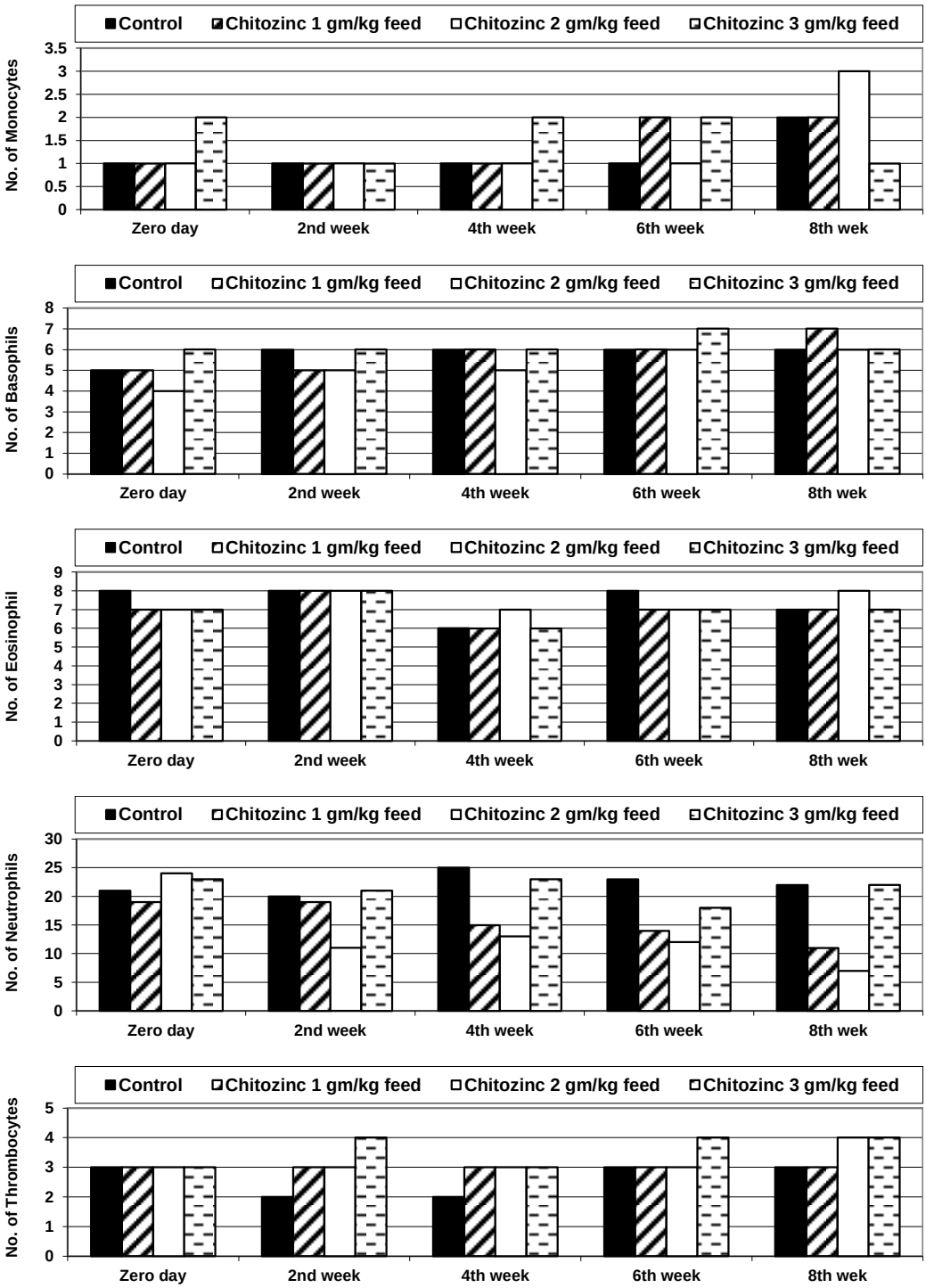


Fig. (1): ***Results of differential leucocytic count among different groups in different weeks***

2. Effects of different treatments of chitozinc® on phagocytic activity and phagocytic index in. cultured sea bream blood

We can notice that there was progressive increasing in phagocytic activity and phagocytic index in the groups treated with Chitozinc ® probiotic (high dose then small dose) than the control group from the 2nd week till 8th week as indicated in Table (2).

Table (2): ***Showing results of phagocytic activity and phagocytic index among different groups in different weeks.***

	Groups	N	Phagocytic activity	Phagocytic index
Zero day	**Control**	**3**	**21±0.57 a**	**2**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**20±0.58 a**	**2**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**20±0.57 a**	**2**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**20±0.57 a**	**2**
2 nd week	**Control**	**3**	**20±0.59 a**	**2**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**23±0.56 b**	**2**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**24±0.58 b**	**2**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**19±0.56 a**	**2**
4th week	**Control**	**3**	**20±0.57 a**	**2**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**26±0.59 b**	**2**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**29±0.58 c**	**3**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**21±0.57 a**	**2**
6th week	**Control**	**3**	**20±0.58 a**	**2**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**27±0.57 b**	**3**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**33±0.57 c**	**3**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**22±0.58 d**	**2**
8th week	**control**	**3**	**21±0.59 a**	**2**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**30±0.57 b**	**3**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**38±0.57 a**	**4**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**23±0.58 b**	**2**

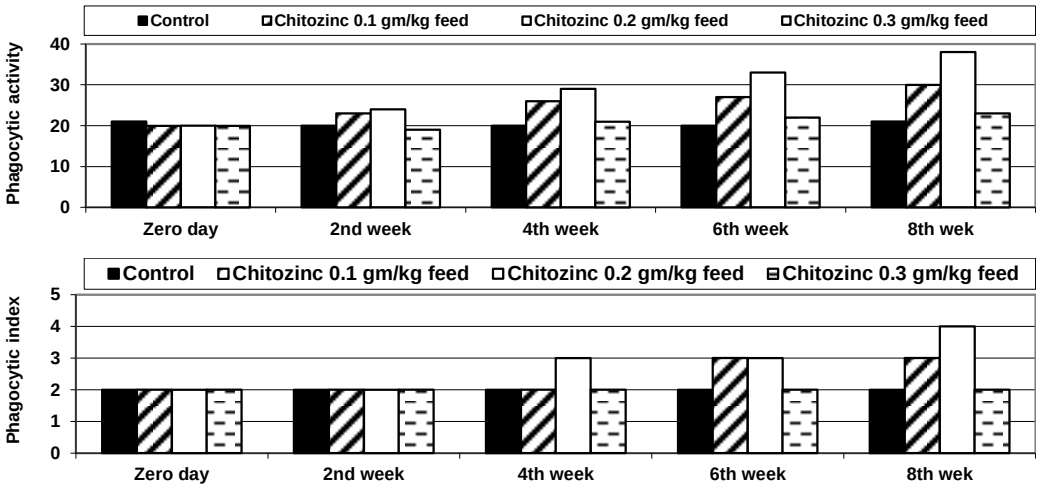


Fig. (2): ***Phagocytic activity and phagocytic index among different groups in different weeks.***

3. Effects of different treatments on serum lysozyme and bactericidal activity

The serum lysozyme and bactericidal activity were significantly elevated progressively in the groups treated with Chitozinc ® probiotic (high dose then small dose) than the groups treated with Chitozinc ® probiotic (both doses) and also than the control one from the 2nd week till 8th week as indicated in Table (3).

Table (3): Showing results of serum lysozyme and bactericidal activity among different groups in different weeks.

	Groups	N	Lysozome activity	Bactericidal activity
Zero day	**Control**	**3**	**0.02±0.006 a**	**4.30±0.54 a**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**0.03±0.006 a**	**3.70±0.57 a**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**0.02±0.007 a**	**3.60±0.56 a**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**0.02±0.006 a**	**3.60±0.58 a**
2nd week	**Control**	**3**	**0.02±0.007 a**	**3.93±0.58 a**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**0.04±0.007 b**	**4.47±0.59 a**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**0.05±0.006 b**	**5.03±0.56 a**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**0.02±0.006 a**	**3.77±0.57 a**
4th week	**Control**	**3**	**0.02±0.006 a**	**3.87±0.57 a**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**0.05±0.006 b**	**4.87±0.57 a**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**0.08±0.007 c**	**5.53±0.58 a**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**0.03±0.006 a**	**3.87±0.58 a**
6th week	**Control**	**3**	**0.01±0.006 a**	**3.67±0.57 a**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**0.05±0.007 b**	**4.87±0.61 ab**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**0.11±0.006 c**	**5.57±0.59 b**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**0.02±0.006 a**	**3.83±0.58 ab**
8th week	**control**	**3**	**0.01±0.006 a**	**3.70±0.57 a**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**0.07±0.008 b**	**5.27±0.58 ab**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**0.17±0.007 c**	**6.30±0.57 b**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**0.04±0.006 d**	**4.07±0.57 a**

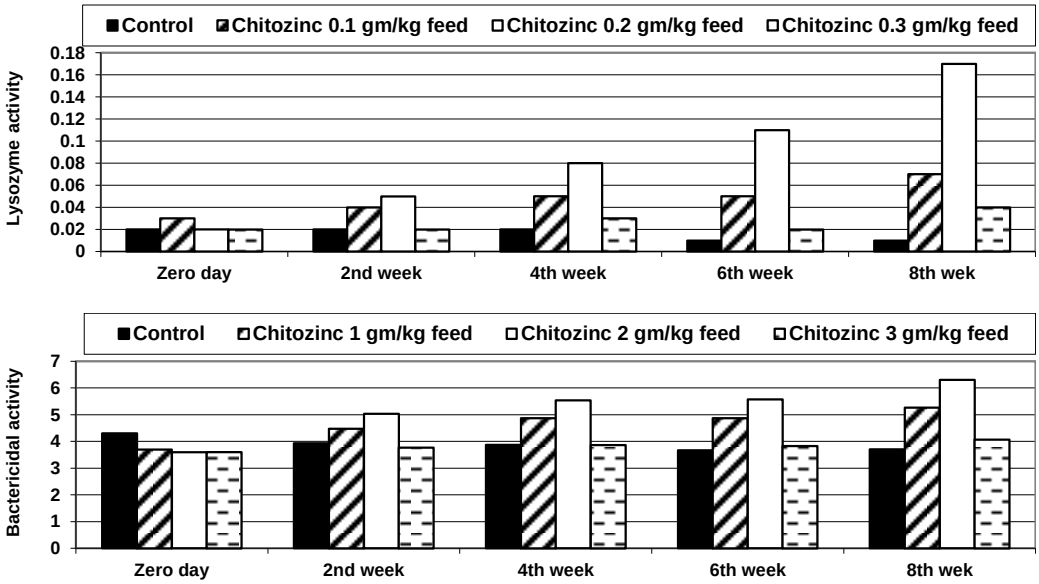


Fig. (3): ***Serum lysozyme and bactericidal activity among different groups in different weeks***

4- Effects of different treatments of Chitozinc® on (Total proteins, albumin, globulin and albumin/globulin ratio) on cultured sea bream

In zero day (at the beginning of the experiment) results showed no significant value in total proteins, albumin, globulin and albumin/globulin ratio in all groups of *sea bream* Otherwise the serum total proteins, and globulin were significantly elevated progressively in the groups treated with Chitozinc ®probiotic (high dose then small dose) than the groups treated with Chitozinc® probiotic and also than the control from the 2nd week till 8th week. The groups treated with Chitozinc ®probiotic (high dose then small dose) revealed also decreased values in serum albumin and albumin/globulin ratio. Concerning the groups treated with Chitozinc® probiotic showed decreased values in serum total proteins, and globulin and increased significant values in serum albumin and albumin/globulin ratio than other treated groups in all weeks as shown in Table (4).

5- Effects of different treatments of Chitozinc® on (Antibody titer)

The antibody titration differed significantly among different treated groups at different weeks according to the effect of probiotic used. The higher antibody titers in general observed in the groups treated with Chitozinc ® probiotic (high dose then small dose) than the groups treated with Chitozinc ® probiotic and also than the control group as shown in Table (5).

Table (4): Showing results of serum total protein, albumin, globulin and albumin/globulin ratio among different groups in different weeks.

	Groups	N	Total protein	Albumin	Globulin	A/G ratio
Zero day	**Control**	**3**	**5.33±0.58 a**	**2.67±0.57 a**	**2.67±0.56 a**	**1.06±0.02**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**4.67±0.57 a**	**2.33±0.56 a**	**2.33±0.58 a**	**1.06±0.02**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**4.67±0 a**	**1.67±0 a**	**3.00±0 a**	**0.56±0.12**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**5.33±0.56 a**	**2.67±0.58 a**	**2.67±0.57 a**	**1.06±0.02**
2 nd week	**Control**	**3**	**4.33±0 a**	**1.67±0 a**	**2.67±0 ab**	**0.72±0**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**6.00±0.57 b**	**2.67±0.57 ab**	**3.33±0.58 ab**	**0.81±0.058**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**6.67±0.56 b**	**3.00±0.57 ab**	**3.67±0.58 a**	**0.81±0.031**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**5.33±0.56 ab**	**3.33±0.57 b**	**2.00±0.56 b**	**2.39±0.306**
4th week	**Control**	**3**	**4.67±0.57 a**	**2.33±0.57 a**	**2.33±0.58 a**	**1.06±0.02**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**6.00±0.57 ab**	**2.33±0.57 a**	**3.67±0.59 ab**	**0.64±0.027**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**6.97±0.58 b**	**2.33±0.56 a**	**4.63±0.577 b**	**0.53±0.031**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**5.33±0.577 ab**	**2.33±0.563 a**	**3.00±0.577 ab**	**0.88±0.306**
6th week	**Control**	**3**	**4.50±0.578 a**	**2.33±0.586 a**	**1.83±0.579 a**	**1.28±0.02**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**6.77±0.578 bc**	**1.87±0.577 a**	**4.90±0.579 b**	**0.39±0.063**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**7.43±0.576 c**	**1.60±0.577 a**	**5.83±0.579 c**	**0.27±0.067**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**5.33±0.579 ab**	**3.67±0.578 b**	**1.67±0.567 a**	**2.27±0.065**
8th week	**Control**	**3**	**4.40±0.578 a**	**3.00±0.577 a**	**1.40±0.592 a**	**2.84±0.387**
	Chitozinc ® **0.1 gm/kg feed**	**3**	**7.50±0.577 b**	**2.13±0.579 a**	**5.37±0.577 b**	**0.40±0.069**
	Chitozinc ® **0.2 gm/kg feed**	**3**	**7.97±0.05 b**	**1.83±0.097 a**	**6.13±0.143 b**	**0.30±0.1**
	Chitozinc ® **0.3 gm/kg feed**	**3**	**5.03±0.576 a**	**2.87±0.567 a**	**2.17±0.577 a**	**1.37±0.11**

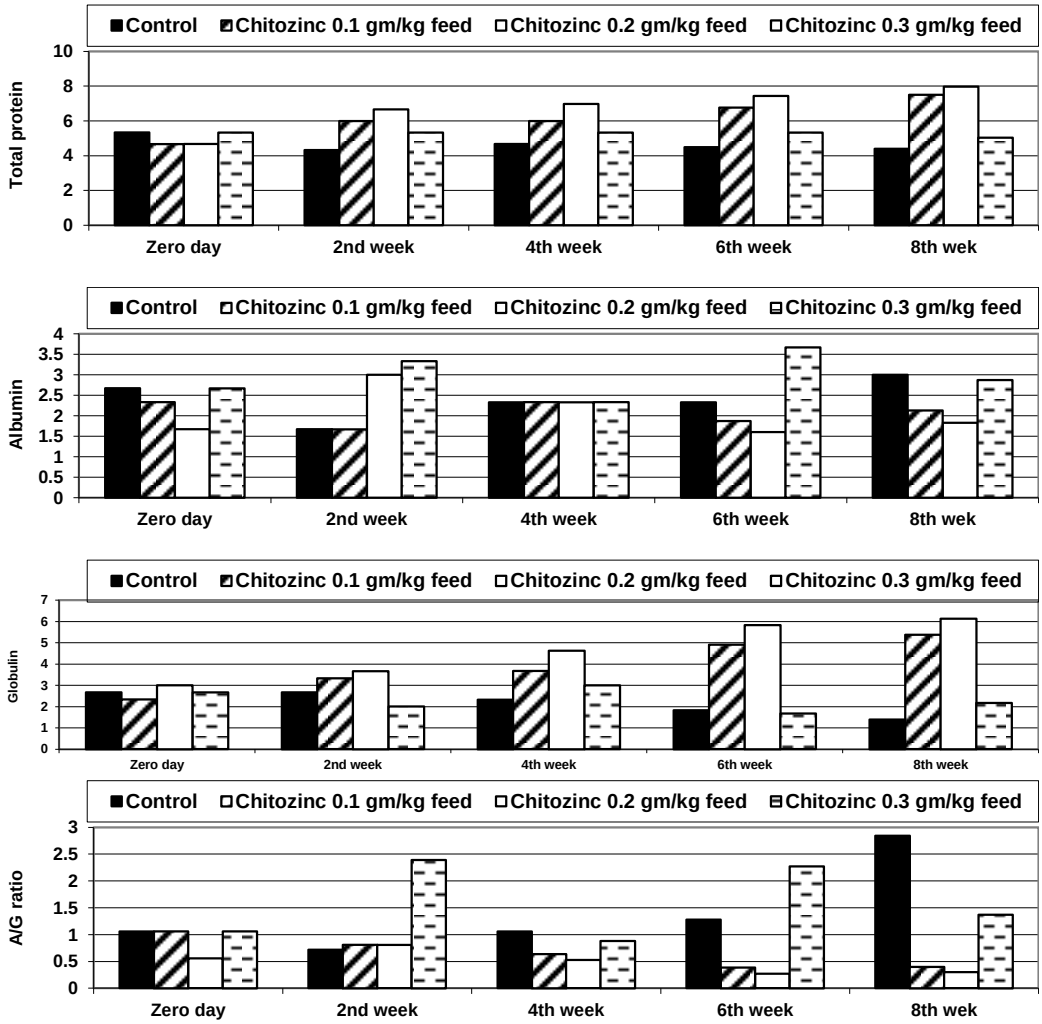


Fig. (4): ***Results of serum total protein, albumin, globulin and albumin/globulin ratio among different groups in different weeks.***

6- Effects of different treatments of Chitozinc® on cortisol level of on cultured sea bream:

Cortisol hormone levels were significantly decreased progressively in the groups treated with Chitozinc ® probiotic (high dose then small dose) revealed decreased values in cortisol levels in zero day, 2nd, 4th, 6th and 8thweeks. Concerning the groups treated with Chitozinc ® probiotic, these groups showed increased significant values in cortisol levels than other treated groups in zero day, 2nd, 4th, 6th and 8th weeks as shown in Table (6).

Table (5): Showing results of antibody titer (log_2) among different groups in different weeks.

	Groups	N	Antibody titer
			Mean± Std. Error
first week	Control	3	2. 3333±.3333[d]
	Chitozinc® 0.1 gm/kg feed	3	5.0000±.3333[a]
	Chitozinc® 0.2 gm/kg feed	3	5.6667±.3333[a]
	Chitozinc® 0.3 gm/kg feed	3	2.6667±.3333[c]
2nd week	Control	3	2.6667±.3333[d]
	Chitozinc® 0.1 gm/kg feed	3	4.6667±.3333[ac]
	Chitozinc® 0.2 gm/kg feed	3	6.6667±.3333[a]
	Chitozinc® 0.3 gm/kg feed	3	2.3333±.3333[d]
3rd week	Control	3	3.3333±.3333[c]
	Chitozinc® 0.1 gm/kg feed	3	5.3333±.3333[ab]
	Chitozinc® 0.2 gm/kg feed	3	6.3333±.6667[a]
	Chitozinc® 0.3 gm/kg feed	3	3.6667±.3333[bc]
4th week	Control	3	3.6667±.3333[c]
	Chitozinc® 0.1 gm/kg feed	3	5.6667±.3333[a]
	Chitozinc® 0.2 gm/kg feed	3	5.6667±.6667[a]
	Chitozinc® 0.3 gm/kg feed	3	4.0000±.5774[bc]

Table (6): Showing results of cortisol level, among different groups in different weeks.

	Groups	N	Cortisol level
			Mean ± Std. Error
Zero day	Control	3	547.98±8.66 a
	Chitozinc® 0.1 gm/kg feed	3	531.20±6.928 a
	Chitozinc® 0.2 gm/kg feed	3	521.84±9.238 a
	Chitozinc® 0.3 gm/kg feed	3	548.67±8.083 a
2nd week	Control	3	540.93±8.66 a
	Chitozinc® 0.1 gm/kg feed	3	484.03±4.333 b
	Chitozinc® 0.2 gm/kg feed	3	479.48±4.041 b
	Chitozinc® 0.3 gm/kg feed	3	510.61±2.309 c
4th week	Control	3	542.30±2.887 a
	Chitozinc® 0.1 gm/kg feed	3	486.75±1.732 b
	Chitozinc® 0.2 gm/kg feed	3	449.34±2.309 c
	Chitozinc® 0.3 gm/kg feed	3	606.45±3.464 d
6th week	Control	3	534.13±4.041 a
	Chitozinc® 0.1 gm/kg feed	3	455.17±4.619 b
	Chitozinc® 0.2 gm/kg feed	3	425.23±3.464 c
	Chitozinc® 0.3 gm/kg feed	3	572.13±5.196 d
8th week	Control	3	548.40±4.619 a
	Chitozinc® 0.1 gm/kg feed	3	423.90±2.887 b
	Chitozinc® 0.2 gm/kg feed	3	404.00±3.467 c
	Chitozinc® 0.3 gm/kg feed	3	513.40±6.928 d

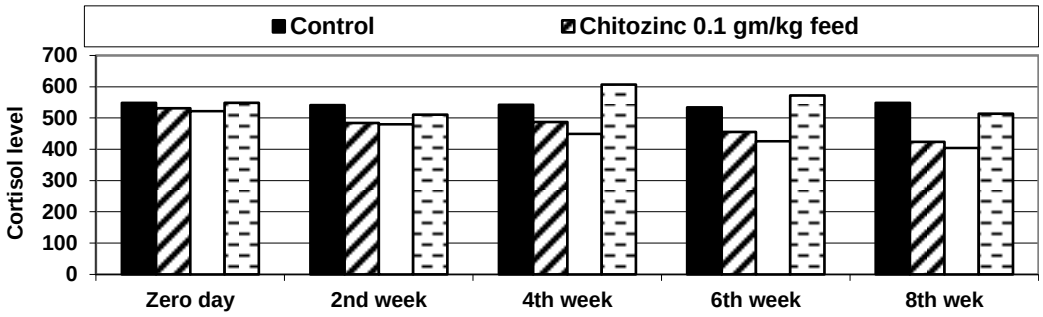


Fig. (5): ***Results of cortisol level, among different groups in different weeks***

7- Effects of different treatments of Chitozinc® on Total bacterial count, Total Enterobacteriaceae count and Total Coliform count present in gut of cultured sea bream:

Data of Total bacterial, Total Enterobacteriaceae and Total Coliform counts were transformed to logarithmic transformation and analysis were take place on this logarithmic transformation. In zero day and 2nd week these bacterial counts showed no significant value. In 4th, 6th, and 8th weeks these bacterial counts decreased progressively in the groups treated with Chitozinc ® probiotic (high dose then small dose). Concerning the groups treated with Chitozinc ® probiotic showed increased significant values in all bacterial counts at 4th, 6th, and 8th weeks than other treated groups as shown in Table (7).

Table (7): ***Showing results of logarithmic transformation of Total bacterial count, Total enterobacteriaceae count and Total coliform count among different groups in different weeks.***

	Groups	N	Total bacterial count	Total enterobacteriaceae count	Total coli form count
Zero day	Control	3	3.48±0.058 a	3.41±0.058 a	3.11±0.056 a
	Chitozinc ® 0.1 gm/kg feed	3	3.36±0.069 a	3.26±0.067 a	2.850.068 a
	Chitozinc ® 0.2 gm/kg feed	3	3.30±0.115 a	3.23±0.069 a	2.78±0.065 a
	Chitozinc ® 0.3 gm/kg feed	3	3.34±0.121 a	3.49±0.111 a	2.95±0.096 a
2 nd week	Control	3	3.51±0.064 ab	3.41±0.068 ab	3.11±0.072 a
	Chitozinc ® 0.1 gm/kg feed	3	3.43±0.087 ab	3.26±0.079 a	2.85±0.067 b
	Chitozinc ® 0.2 gm/kg feed	3	3.36±0.058 a	3.23±0.063 a	2.78±0.055 b
	Chitozinc ® 0.3 gm/kg feed	3	3.58±0.065 b	3.49±0.066 b	2.95±0.068 ab
4th week	Control	3	3.50±0.069 a	3.44±0.071 a	2.58±0.073 a
	Chitozinc ® 0.1 gm/kg feed	3	3.36±0.059 ab	3.18±0.056 b	2.63±0.059 a
	Chitozinc ® 0.2 gm/kg feed	3	3.27±0.067 b	3.19±0.069 b	2.48±0.057 a
	Chitozinc ® 0.3 gm/kg feed	3	3.55±0.064 a	3.50±0.069 a	2.90±0.065 b
6th week	Control	3	3.45±0.075 a	3.41±0.068 ab	2.26±0.076 a
	Chitozinc ® 0.1 gm/kg feed	3	3.38±0.087 a	3.18±0.076 bc	2.48±0.075 a
	Chitozinc ® 0.2 gm/kg feed	3	3.25±0.087 a	3.17±0.067 c	2.46±0.069 a
	Chitozinc ® 0.3 gm/kg feed	3	3.48±0.069 a	3.43±0.082 a	2.91±0.076 b
8 week	control	3	3.47±0.068 a	3.44±0.059 a	2.36±0.067 a
	Chitozinc ® 0.1 gm/kg feed	3	3.24±0.068 ab	2.92±0.069 b	2.52±0.064 a
	Chitozinc ® 0.2 gm/kg feed	3	3.10±0.087 b	3.0±0.086 b	2.36±0.089 a
	Chitozinc ® 0.3 gm/kg feed	3	3.44±0.064 a	3.38±0.064 a	2.85±0.066 b

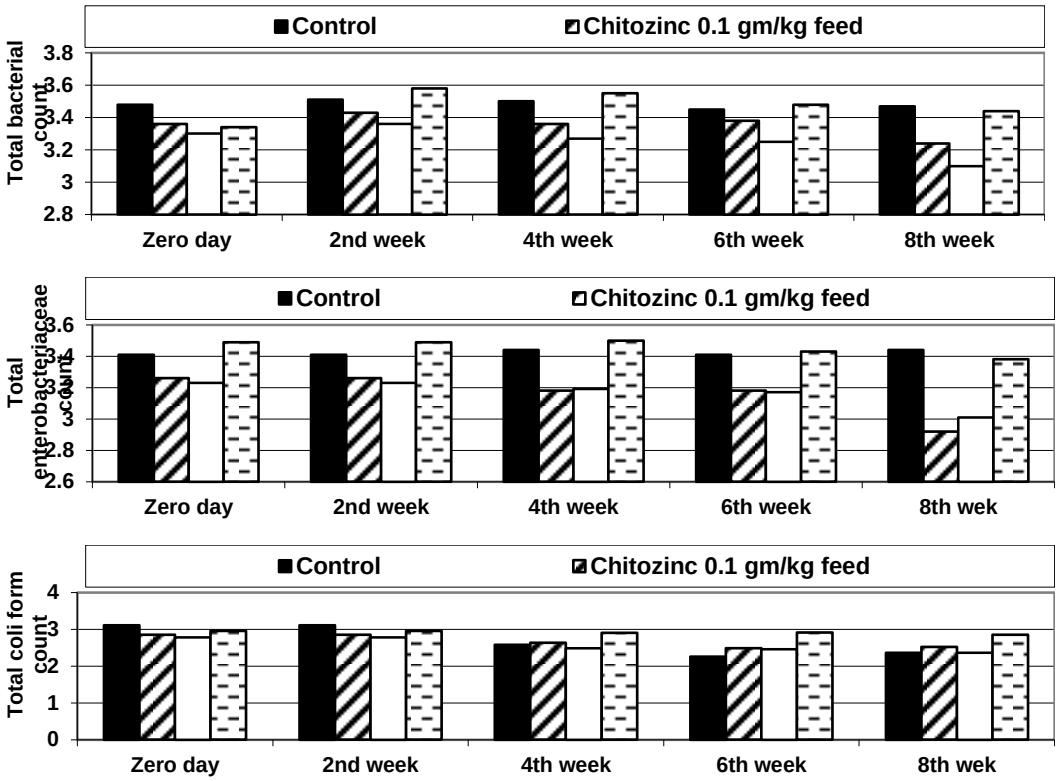


Fig. (7): ***Results of logarithmic transformation of Total bacterial count, Total enterobacteriaceae count and Total coliform count among different groups in different weeks.***

8- Effects of different treatments of Chitozinc on mortality after challenge with Vibrio alginolyticus and relative level of protection:

The mortality level decreased in the groups treated with Chitozinc ® than the groups treated with Chitozinc ®probiotic. Meanwhile, the relative level of protection showed higher level in Chitozinc ® treated groups (70% in high dose and 50% in small dose) than Chitozinc ® treated groups (20% in both doses). Chi square analysis revealed high significant value ($P<0.01$) in mortality and protection percent between the treated groups as indicated in Table (8).

Table (8): Showing mortality percent and Relative level of protection after challenge with pathogenic bacteria *(Vibrio alginolyticus)* among different treated groups.

Groups	N=50	Mortalities		Protected	
		No	%	No	%
Control (- ve)	**10**	**10**	**100**	**0**	**0**
Chitozinc ® **0.1 gm/kg feed**	**10**	**5**	**50**	**5**	**50**
Chitozinc ® **0.2 gm/kg feed**	**10**	**3**	**30**	**7**	**70**
Chitozinc ® **0.3 gm/kg feed**	**10**	**8**	**80**	**2**	**20**

DISCUSSION

Chitosan is a polysaccharide biopolymer that combines a unique set of versatile physicochemical and biological characteristics which allow for a wide range of applications. Although its antimicrobial activity is well documented, its mode of action has hitherto remained only vaguely defined. In this work we investigated the antimicrobial mode of action of chitosan using a combination of approaches, including in vitro assays, killing kinetics, cellular leakage measurements, membrane potential estimations, and electron microscopy, in addition to transcriptional response analysis. Chitosan, whose antimicrobial activity was influenced by several factors, exhibited a dose-dependent growth-inhibitory effect. A simultaneous permeabilization of the cell membrane to small cellular components, coupled to a significant membrane depolarization, was detected. A concomitant interference with cell wall biosynthesis was not observed (*Dina Raafat etal. , 2008*).

This study was planned to evoke the differential aspects of using Chitozinc ®probiotics in cultured sea bream from their effects on the immune response of treated fish to pathogenic bacteria, changes in gut microbiota and cortisol hormone level.

The groups treated with Chitozinc ®probiotic showed increased significant value ($P<0.05$) of lymphocytes from 2nd week to 8th week than the groups treated with Chitozinc ®probiotic and control group. Monocytic count showed also increased significant value in the groups treated with Chitozinc ®probiotic in 8th week only than other treated groups with Chitozinc ®probiotic as indicated in table (1), This could be attributed to high non-specific immune response was developed as manifested by increasing the number of lymphocytes and monocytes in the differential leucocytic count. Concerning the neutrophils count the groups treated with Chitozinc ®probiotic in addition to the control group showed higher significant value ($P<0.05$) than the groups treated with Chitozinc ®probiotic in all weeks. These results also are directly proportional with the results of cortisol levels in these groups. This means that leucocyte profiles are particularly useful in the field of conservation physiology because they are altered by stress and can be directly related to stress hormone levels. *Bly et al. (1990) and Harris and Bird (2000)* provide excellent reviews on these responses. In general, acute stress induces both neutrophilia and lymphopenia in fish *(Pulsford et al., 1994),* although sometimes only lymphopenia is reported *(Larsson et al., 1980),* and these stress-induced changes have been shown repeatedly to be related to elevated glucocorticoids. In the present study the increased value of neutrophils in the groups treated with Chitozinc ®probiotic (both doses) and control group, means that this groups have decreased ability in controlling stress conditions than the groups treated with high and small dose of Chitozinc ® (0.2 and 0.1ml/kg feed respectively).

The thrombocytes, basophils and eosinophil's counts in the present study considered not significant in all weeks.

The increased value in leucocytic count in Chitozinc ®probiotic treated groups could be attributed to the fact that, this probiotic used increased the blood parameter values as a result of haemopoietic stimulation. These

results agreed with those obtained by *(Marzouk et al., 2008)* who studied the effect of using two commercial products containing probiotics (Daimond-V Yeast® and Megalo®) on the hematology of cultured *Oreochromis niloticus* and mixed thoroughly with the prepared basal fish diet during its preparation. The results of hematogram revealed a significant increase in RBCs count, H.B. value, PCV%, WBCs and differential leucocytic count in the two groups treated with probiotics.

These results also agreed with those obtained by *(Al-Dohail et al., 2009)* who reported that better concentrations of % haematocrit, ESR, RBC,WBC, were observed in *C. gariepinus* fingerling maintained on the diet supplemented with *L. acidophilus* which showing significant differences (P<0.05) from the control. This observation probably indicates support for the suggestion that fish fed probiotic-supplemented diets were healthier than the controls due probably to the decreased cortisol levels in the blood plasma as reported by *Carnevali et al. (2006) and Rollo et al. (2006)* in Sea bream *(S. aurata).* Also *Taoka et al. (2006a)* confirmed recently that the viability of probiotics affected the immune response of Nile tilapia fed a commercial preparation including *S. cerevisiae*, *Bacillus subtilis*, *Lactobacillus acidophilus*, and *Clostridium butyricum* (Alchem Poseidon®; Alchem-Korea Co. Ltd., Wonju, Korea), but the specific importance of yeast viability was not considered. *Gatesoupe (2007)* revealed that a commercial preparation of live *Saccharomyces cerevisiae and Lactobacillus coagulans*, give better results in Indian carp fry. Our results also agreed with the results of histological examination of liver and spleen through the activation of kupffer's cells and melanomacrophage center.

Probiotics can effectively trigger the phagocytic cells in host and enhancement of phagocytic activity by LAB group of probiotics such as *L. rhamnosus, L. lactis* and *Lactobacillus acidophilus* has already been observed in several animals *Rutherfurd and Gill (2004).* As indicated in table (2) results revealed that the groups received large dose (0.3 gm/kg feed) Chitozinc ® probiotic showed increased significant value (P<0.05) in phagocytic assay than the groups received smaller dose (0.1 gm/kg feed), but also all doses showed increased significant value (P<0.05) in phagocytic activity and phagocytic index, than the control group from the 2nd week till 8th week. Our results agreed also with *Marzouk et al. (2008)* who reported that the percent of phagocytosis and phagocytic index in *O. niloticus* group which received (Megalo®) which composed of *S. cerevisiae* and *Bacillus subtilis (B. subtilis)* was the best 83.1% and 2.63 respectively followed by *O. niloticus* in group received (Diamond®) which composed of *Saccharomyces cerevisiae (S. cerevisiae)* yeast in which the values were 81.7% and 2.27 respectively in comparison to *O. niloticus* kept on a basal diet in which values were 73.9% and 1.9 respectively.

Contrary to our findings the results obtained by *(Robertson, 1999)* and *Tewary and Patra (2011)* who revealed that using Baker's yeast act as an immunostimulant by stimulating the immune response via increasing the phagocytic activity, respiratory burst activity. In our study the Chitozinc® probiotic give good results in differential leucocytic count and phagocytic assay.

Lysozyme, one of the important bactericidal enzymes of innate immunity is an indispensable tool of fish to fight against infectious agents *(Lindsay, 1986)*. the groups received higher dose (0.3 gm/kg feed Chitozinc ®) probiotic showed increased significant value (P<0.05) in serum lysozyme activity and serum bactericidal activity than the groups received smaller dose (1 gm/kg feed), but also all doses showed increased significant value (P<0.05) in serum lysozyme activity and serum bactericidal activity than the control group from the 2nd week till 8th week as indicated in table (3). These results in agreement with *Taoka et al. (2006a)* who cited that combination in vivo between *Bacillus subtilis, Lactobacillus acidophilus, Clostridium butyricum, Saccharomyces cerevisiae* (Commercial probiotics preparation) viable or inactivated enhanced the bactericidal activity and lysozyme activity (mucus and serum). The increase in serum bactericidal activity of cultured *sea bream* against pathogenic bacteria in comparison to the control especially after 2 months may attributed to either the antimicrobial substances that produced by *L. acidophilus (Smoragiewicz et al., 1993)* or to the increased natural complement, serum peroxidase and phagocytic activities *(Salinas et al., 2008).* These findings were also in agreement with *Paturi et al. (2008)* who reported that, the phagocytic activity of the peritoneal macrophages was significantly higher in mice fed either *L. acidophilus or L. paracasei* compared with control mice. Moreover *Nouh et al. (2009)* reported that the serum bactericidal activities against *A. hydrophila, P. fluorescens* and *Strept. iniae* were lowest in the control group and highest in the group that received mixture of the two bacteria *(B. subtilus, L. acidophilus),* after one and two months of experiment and the viable bacterial counts of *A. hydrophila, P. fluorescens and Strept. iniae* were lower in two months than that in one month of experiment and also in all probiotics treated groups in comparison with untreated control group or bacterial control (without serum treated). In addition to that, the viable bacterial counts in the group that received a mixture of the two bacteria *(B. subtilis, L. acidophilus)* were lower than group received either *L. acidophilus or B. subtilis*. The serum bactericidal activity was significantly higher in the groups received a mixture of probiotics compared to those supplemented with single probiotic species or the control groups; this observation was in agreement with *(Salinas et al., 2008).*

The groups treated with Chitozinc ® probiotic showed increased significant value (P<0.05) in serum total protein and globulin level and decreased significant value (P >0.05) in A/G ratio and in plasma cortisol level than the groups treated with Chitozinc ® probiotic which showed decreased significant value (P>0.05) in serum protein and globulin levels and increased significant value (P<0.05) in plasma cortisol level from the 2nd week to 8th week as reported in tables (4) and (5). These results agreed with those obtained by *(Mohamed, 2007 and Eid and Mohamed, 2008)* who reported that an improvement of fish health when fed *L. acidophilus* supplement diets. These results also agreed with *Marzouk et al. (2008*) who reported that a significant increase in total protein and decrease A/G ratio which could be attributed to the immuno- modulatory effect of *S. crevisiae and B. subtilis* than the group treated with dead *saccharomyces cerevisiae* on the liver cells which activate the anabolic capacity of the hepatocytes to produce blood proteins particularly globulin and these results were supported by several authors *ortuno et al.*

(2002) and Nayak et al. (2004) and *Safinaz (2006).* Decreased cortisol levels have been reported by *Carnevali et al. (2006)* when fish was fed a diet supplemented with *L. delbrueckii.* Our results also agreed with those obtained by *(Al-Dohail et al., 2009)* who reported that total serum protein, cholesterol and total immunoglobulin concentrations were also significantly better in fish maintained on the diet supplemented with the probiotic, *L. acidophilus,* than in fish fed the control diet. Albumin/ globulin ratio is a measurable humeral component at the non-specific defenses. The reduction of A/G ratio might be due to the increase of total serum globulin level with significance protective mechanisms for fish *(Sahoo and Mukherjee, 2001).* Contrary to our finding about Chitozinc ® probiotic *Tewary and Patra (2011)* reported that oral administration of Baker's Yeast (*S .cerevisiae*) stimulate the non specific immunity level as measured through enhanced phagocytic activity, leucocytic level, respiratory burst activity and reduced A/G ratio.

The dietary administration of probiotic strain caused a decrease of the bacterial densities in the gut of the fishes. Similarly, *Bogut et al. (2000)* observed reduction in bacterial counts in gut of fishes fed the probiotic preparation. The concentration of probiotic in feed in the present study was significant with regard to reduction in gut bacterial counts, emphasizing that a probiotic dose of 10^6 to 10^7 cfu g^{-1} of feed administered continuously is sufficient to obtain a healthy balance between probiotic micro-organisms and other bacteria in the gut *(Guillot, 2003).* From table (6) results revealed that the groups received higher dose (0.3 gm/kg feed) of (Chitozinc ®) probiotic showed decreased significant value (P>0.05) in bacterial counts than the groups received smaller dose (0.1 gm/kg feed), but also both doses showed decreased significant value (P>0.05) in bacterial counts than the treated groups with (Chitozinc ®) probiotic and the control group in all weeks. Further these results could be attributed to the bactericidal or the bacteriostatic substances produced by *lactobacillus and Saccharomyces* that inhibit the growth of other bacteria as reported by *Pybus (1994)* and the ability of these probiotic bacteria and yeast to bind the intestinal mucosal cell receptors for some members of Enterobacteriaceae and other bacteria.

Concerning the challenge of the cultured *seabream* fish groups the results indicated that the groups treated with 0.2 and 0.3 gm/kg feed (Chitozinc ®) showed high level of protection (50% and 70% respectively) and survival than the groups treated with (Chitozinc ®) probiotic (20% relative level of protection in both doses) as showed in table (8). These results confirmed the immunostimulatory effect of the living *Saccharomyces and lactobacillus, Pediococcus, Glucanobacter* sp present in (Chitozinc ®) probiotic and also their inhibitory effect to *Vibrio alginolyticus.* These results also directly proportional with the results of antibody titer (log_2) in the groups treated with 0.2 and 0.3 gm/kg feed (Chitozinc ®) ($5.6667\pm.3333^a$ and $5.6667\pm.6667^a$ respectively).

Our results also agreed with *Marzouk et al. (2008)* who reported that the *O. niloticus* in the group kept on diet supplemented with Megalo® did not show any mortality within one week post challenge and survival rate was 100% while *O. niloticus* of group kept on diet

contain dead *S. cerevisiae* showed 14.3% mortality and these results confirmed the immune stimulatory effect of the living *S. cerevisiae and B. subtilis* and also their inhibitory effect to *P. fluorescens* also the variation in the mortality ratios in both groups indicated that the living yeast cells and bacteria cells are more potent than dead yeast cell in the protecting the *P. fluorescens* infection.

Our results also agreed with that obtained by *Mohanty et al. (1993)* who introduced a commercial preparation of live *S. cerevisiae* and *Lactobacillus coagulans* (Bioboost Forte®, Lyka Labs. Ltd., Bombay) which used as a growth promoter for Indian carp *(Labeo rohita*) fry then introduced the preparation in an experimental diet, but it was not possible to conclude any effect of the probiotic on *Labeo rohita*, due to the lack of a suitable control diet. The effect of growth promotion was shown in later experiments on *Catla catla* and *Cirrhinus mrigala (Mohanty et al., 1996 and Swain et al., 1996*), though it was impossible to demarcate the respective efficiencies of the yeast and *L. coagulans*.

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زيادة المناعة المصاحبة لاستخدام مركب الشيتوزنك لمقاومة الإصابة ببكتريا الفيبريو الجينوليتكس في اسماك الدنيس البحري المستزرع

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- أجريت هذه الدراسة لتقييم تاثير الشيتوزنك على زيادة المناعة لأسماك الدنيس البحري المستزرع ومقاومة هذه الأسماك للإصابة ببكتريا الفيبريو الجينوليتكس.

- تم تقسيم الأسماك المستخدمة في هذه التجربة إلى أربع مجموعات وتم تغذية هذه المجموعات بعليقه تحتوى على ٠.١ جم شيتوزنك / كجم عليقة ، ٠.٢ جم شيتوزنك / كجم عليقه ، ٠.٣ جم شيتوزنك / كجم عليقه والمجموعة الرابعة تم تغذيتها بعليقه ضابطة لا تحتوى على شيتوزنك وذلك لمدة ٨ أسابيع.
- وكانت النتائج التي تم التوصل إليها تدل على إن مجموعة الأسماك التي تم أضافه الشيتوزنك بتركيز ٠.٣ جم / كجم حصلت على زيادة ملحوظة في المناعة وذلك من خلال قياس نشاط الخلايا البلعومية ، العد النوعي لخلايا الدم البيضاء ، قياس البروتين الكلى والجلوبيولين الكلى والنشاط الليزوزمى لقتل الخلايا البكتيرية .
- كما أوضحت النتائج إن المجموعات التي تغذت على عليقه تحتوى على الشيتوزنك زادت نسبة الأجسام المضادة بها ومقاومة هذه الأسماك لبكتريا الفيبريو الجينوليتكس أكثر من المجموعة الضابطة التي لم تتغذى على الشيتوزنك .
- كما أوضحت النتائج أيضا إن استخدام الشيتوزنك في اسماك الدنيس البحري المستزرع بنسبة ٠.٣ جم / كجم عليقه أدى إلى انخفاض هرمون الكورتيزول وعدد البكتريا في القناة الهضمية للأسماك .

وبصفة عامة أكدت هذه النتائج أن مركب الشيتوزنك لـه أهميـة كإضـافة غذائيـة خاصـة بتركيز ٠.٣ جـم / كجـم وذلـك لزيـادة المناعـة ومقاومـة الأمـراض البكتيريـة خاصـة بكتريـا الفيبريـو الجينوليتكس في اسماك الدنيس البحري المستزرع .