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Some Studies on the Effect of Protexin on Immune Status of Cultured Seabass Fingerlings

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Key words

Protexin- *Enterococcus faecium* - sea bass - fingerlings- growth - immune status

ABSTRACT:

The present study aimed to investigate the effect of different concentrations of protexin probiotic (*Enterococcus faecium*) on growth performance, differential leucocytes count, phagocytic activity, phagocytic index, serum total protein, albumin, globulin, Total bacterial count, Total enterobacteriaceae count and Total coliform count of intestinal microbial flora of seabass and disease resistance against challenge with *Vibrio alginolyticus*. Fish were separated in to four experimental groups of 0.0 g/kg feed (control), 0.1 g/kg feed, 0.2 g/kg feed, 0.3 g/kg feed of commercial probiotic protexin concentrate. Fish fed at 3% body weight per day. Results showed that protexin supplementation have significant improvement in growth performance, significantly increasing in lymphocytes, monocytes, phagocytic activity, phagocytic index, Serum total protein and globulin in all treated groups compared to control Contrary neutrophils and albumin/globulin ratio significantly decreased in all treated groups comparing to the control as well as decreasing in Total bacterial count, Total enterobacteriaceae count and Total coliform count of all treated groups comparing to the control. The mortality rates after challenging with Vibrio alginolyticus were significantly lower in all treated groups than the control. Results indicated that by increasing the concentration of the protexin probiotic results getting better in all examined parameters comparing to control. The present study clearly indicated that inclusion of 0.3gm / kg feed of protexin probiotic in fish diet for not less than 6th weeks can improve the immune status of seabass fingerlings to the favor of resistance to diseases.

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1. INTRODUCTION

In recent years aquaculture has been one of the most fast-growing parts of food production. However this industry has faced with problems. Outbreak of disease is the major problem in aquaculture industry affects economic expansion of this part in many countries in the world (Ziyaeiynejad, 2004). Researches clearly show that a healthy immune system comes mainly from a healthy gut. 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 micro-organisms (Alderman and Hastings, 1998) and this result in trade restrictions in export markets. As an alternative strategy to these antimicrobial compounds, the prophylactic use of beneficial bacteria (probiotics) has emerged to improve health and zoo technical performance such as survival, production, and feed conversion and growth rates of cultured aquatic species. 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 defined as microbial dietary adjuvant that beneficially affect the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract (Villamil *et al*., 2002). Reduced mortality, improved growth and quality of fish larvae are among the beneficial effects that have been obtained by the use of probiotics. This likely occur through enhanced immunological responses and reduced adherence of pathogenic strains or other modulation of the gut microbiota at specific locations, as has been previously reviewed Wang *et al*. (2008a). It is preferable to give probiotics to the fish in larval stage, because the larval forms of most fish and shellfish are released in the external environment at an early ontogenetic stage, these larvae are highly exposed to gastro- intestinal-associated disorders, because they start feeding even though the digestive tract is not yet fully developed and immune system is still incomplete (Marzouk *et al*., 2008). Probiotics in aquaculture shown to have several modes of action; competitive exclusion, source of nutrients and enzymatic contribution to digestion, influence on water quality and enhancement of the immune response (José *et al*., 2006). Vine (2004) concluded that probiotics have the ability to improve fish health and prevent bacterial diseases in fish. These biotic can be applied through external bathing or dietary supplementation and have been demonstrated to improve growth performance, feed utilization, digestibility of dietary ingredients, disease resistance and stimulate the immune response of aquatic animals Merrifield *et al* (2010 a). Protexin concentrate contains beneficial probiotics microorganisms (*Enterococcus faecium)* which occur naturally in the gut of animals and birds. These microorganisms colonize the immature gut or re-establish the disrupted gut, thus promoting the mechanism of competitive exclusion against potential pathogenic bacteria. This product is designed for continuous use to promote efficient digestion and immunity or at times of stress when digestive upsets occur. Thus the current study aimed to immunologically evaluate the efficiency of protexin concentrate on the culture of seabass.

2. MATERIALS and METHODS

2.1. Fish and experimental design: A total of 200 apparently healthy sea bass fingerlings with an average body weight of 40+ 10 gram were obtained from private farm. Fish were kept in full glass aquaria measuring (90 X 45X 45 cm) and maintained in aerated water at 27^{o}c ± 1^{o}c ,pH 8.3 ± 0.3 and salinity 32 for 7 days prior to use in experiments. The health status was examined throughout the acclimation period during the acclimation fish fed on the pelleted basic diet only contained 45% protein twice daily. Fish were randomly divided to four experimental groups. Protexin probiotic were used and mixed thoroughly with the prepared basal fish diet during its preparation. Fish growth was measured in weight by weighing fish at the zero-day and at the 8th week. Half of the water was changed daily.

2.2. Blood collection : At the zero day, 2nd, 4th, 6th and 8th week of the experiment, 2ml blood samples/fish via the caudal vessels were collected from 3 fish from each group of the experiment according to (Hawak et al., 1965). One ml of blood was collected with syringe containing anticoagulant (Heparin) and used for differential leucocytes count Lucky (1977) and Schalm (1986) as well as phagocytic assay (Kawahara et al.,1991) and the another ml of blood used for serum collection for biochemical determination(Lied et al., 1975). Serum total protein was determined according to Doumas et al .(1981) .Serum albumin was determined according to Reinhold (1953).

Table (1): Outline of the experimental design:

Group	Diet	Protexin g/kg
1	Basal diet	0.0
2	Basal diet	0.1
3	Basal diet	0.2
4	Basal diet	0.3

*Protexin: Commercial probiotic manufactured by International Ltd UK contain per kg :*Enterococcus faecium* (NCIMB 11181 E1708). Total Viable Count 2x10^{12} CFU. Ingredients: Dextrose Monohydrate. Protein 0.5%. Oil 2.0 %. Fiber 1.0 %. Ash Trace

Serum globulin was determined by subtract the total serum albumin from total serum protein according to (Coles, 1974 and Khalil, 2000). Albumin/ globulin ratio was determined by division of serum albumin value on serum globulin value according to (Saffinaz, 2001)

2.3. Determination of total bacterial, total enterobacteriaceae and total coliform counts: One gram of mucous was collected from the all groups. The all plates incubated at 28 ^{o}C for 24-48hrs then counted of the all growth colonies (APHA, 1992).

2.4.Challenge test: At the 9th week ten fish from each group were bacteriologically tested and determined to be free from bacterial infection, were artificially infected by I/p injection with 0.2ml/fish of culture suspension of pathogenic *Vibrio alginolyticus* previously adjusted to 10^4 . Specificity of death was determined by re-isolation of injected bacteria from freshly dead fish during the period of observation (one week) according to Soliman (1988).

2.5. Statistical analysis: The data were statistically analyzed according to (SAS, 1987).

3. RESULTS

The analysis of variance indicated that protexin significantly improve live body weight, body weight gain and feed conversion ratio in all treatments compared to control (Table 2). Growth performance measurements showed best results in group fed on diet contain protexin 0.3 gm./kg feed followed by group fed diet contain 0.2 gm./kg feed and group fed on diet contain 0.1 gm. /kg feed respectively.

Regarding the effect of protexin on differential leucocytic count of seabass revealed to significant increase of lymphocytes and monocytes Contrary neutrophils significantly decreased in all treated groups comparing to the control specially in group fed on diet contain protexin 0.3 gm./kg feed but eosinophil's and basophiles had no significant (Table 3).
Phagocytic activity as well as phagocytic index was significantly higher in all treated groups than the control (Table 4). Examination of serum indicated increasing in serum total protein and globulin in all protexin treated groups comparing to the control one. This increasing became significantly from the 4th week in total protein and from the 6th week in globulin. Albumin /globulin ratio decreased in all protexin treated groups in comparison to control. Best results showed in group fed on diet contain protexin 0.3 gm. /kg feed (Table 5).

Regarding to The effect of protexin supplemented diet on logarithmic transformation of Total bacterial count, Total enterobacteriaceae count and Total coliform count among different groups of seabass during experimental period revealed decrease in their count in all protexin treated groups comparing to the control one. This decreasing became significantly from the 6th week especially in group fed on diet contain protexin 0.3 gm. /kg feed (Table 6).

Mortalities of seabass challenged with *Vibrio alginolyticus* were significantly lower in all treated groups than the control moreover the lowest mortality percent was recorded in group fed on diet contain 0.3 gm./kg feed of protexin followed by group fed diet contain 0.2 gm./kg feed and group fed on diet contain 0.1 gm./kg feed respectively (Table 7).

Table 2. Effect of protexin supplementation on growth performance of seabass during experimental period:

Studied groups	Body weight gain (g) Mean + SE			Feed conversion ratio (FCR) Mean + SE
	Initial weight	Final weight	Weight gain	
G 1	50. 00+1.15	81. 00+1.15 c	31.00+1.7 c	4.53+0.02 a
G 2	48. 00+1.52	93.30+1.2 c	45.3+0.33 c	3.79+0.05 b
G3	48. 00+1.52	122.66+2.9 a	74.66+1.66 b	2.32+0.04 c
G 4	47.66+1.2	143.66+6.06 b	96. 00+7.23 a	1.82+0.14 d

Means within the same column of different letters are significantly different at (P < 0.05)

Table 3. Effect of protexin supplementation on differential leucocytic counts of seabass during experimental period:

Period	Groups	N	Lymphocytes	Monocytes	Basophils	Eosinophil	Neutrophils	Thrombocytes
Zero day	Control	3	59.33 ± 0.58 ^{b}	1.00± 0.0 ^{ab}	5.67 ± 0.58^{a}	7.67 ± 0.58^{a}	21.33±0.58^{b}	3.0 ±0.0
	Protexin 0.1 gm. /kg feed	3	60 ± 0 ^{a}	1.00± 0.0 ^{ab}	4.33± 0.58 ^{b}	6.67 ± 0.58^{c}	23.67 ±0.58 ^{ab}	3.33±0.58
	Protexin 0. 2 gm./kg feed	3	59.66 ± 0.58 ^{b}	1.67± 0.58 ^{a}	4.00± 0 ^{b}	7.00 ±0.0 ^{ab}	24.33 ±0.58^{a}	3.33 ±0.58
	Protexin 0.3 gm./kg feed	3	56.66±0.58 ^{bc}	1.67± 0.58 ^{a}	5.67 ± 0.58^{a}	6.67±0.58 ^{c}	22.67 ±0.58 ^{ab}	3.33 ±0.58
2nd week	Control	3	58.33±0.58 ^{d}	1.67± 0.58^{b}	5.67± 0.58 ^{ab}	7.67 ±0.58 ^{b}	20.33 ±0.58^{a}	2.67 ±0.58 ^{ab}
	Protexin 0.1 gm. /kg feed	3	63.33± 0.58 ^{c}	1.67 ± 0.58^{b}	5.67± 0.58 ^{ab}	7.33 ±0.58^{c}	19.67 ±0.58^{b}	3.67 ±0.58^{a}
	Protexin 0. 2 gm./kg feed	3	65.33±0.58 ^{b}	1.67 ± 0.58 ^{b}	5.0 ± 0.0 ^{b}	8.0 ±0.0 ^{a}	15.33± 0.58^{c}	2.33 ±0.58 ^{b}
	Protexin 0.3 gm./kg feed	3	67.33± 0.58 ^{a}	2.0 ± 0.0 ^{a}	6.0± 0.0 ^{a}	8.0 ±0.0^{a}	11.67±0.58^{d}	3.67 ±0.58 ^{a}
4th week	Control	3	59.67± 0.58	1.67 ± 0.58 ^{ab}	5.33 ± 0.58 ^{a}	5.33±0.58 ^{c}	25.0 ± 0.0^{a}	2.0 ±0.0 ^{b}
	Protexin 0.1 gm. /kg feed	3	67.0± 0.0	1.0± 0.0 ^{b}	5.33 ± 0.58^{a}	5.33 ±0.58 ^{c}	21.67±0.58 ^{b}	2.33± 0.58 ^{b}
	Protexin 0. 2 gm./kg feed	3	67.67± 0.58	1.0 ± 0.0 ^{b}	4.33± 0.58 ^{b}	6.33 ± 0.58^{b}	15.0 ±.00$^{.c}$	2.33± 0.58 ^{b}
	Protexin 0.3 gm./kg feed	3	69.0 ± 0.0	2.67± 0.58 ^{a}	5.0 ± 0.0 ^{ab}	7.0 ±0.0 ^{a}	13.67 ±0.58^{d}	3.33 ±0.58 ^{a}
6th week	Control	3	56.67± 0.58 ^{b}	1.0± 0.0 ^{b}	5.33 ± 0.58 ^{b}	7.33±0.58 ^{a}	23.0 ±0.0^{a}	3.67 ±0.58 ^{a}
	Protexin 0.1 gm. /kg feed	3	67.0± 1.0 ^{a}	1.0 ± 0.0 ^{b}	5.33± 0.58^{b}	6.33 ±0.58 ^{b}	13.33 ±0.58^{b}	3.67 ±0.58 ^{a}
	Protexin 0. 2 gm./kg feed	3	68.0± 0.0 ^{a}	1.33 ± 0.58^{b}	6.0 ±0.0 ^{a}	6.33 ±0.58 ^{b}	13.67±0.58 ^{b}	3.0 ±0.0 ^{bc}
	Protexin 0.3 gm./kg feed	3	69.33± 0.58 ^{a}	2.0 ± 0.0 ^{a}	5.67 ± 1.16 ^{ab}	6.67 ±1.16^{ab}	12.67 ±0.58^{bc}	2.0 ±0.0 ^{c}
8th week	Control	3	59.67± 0.58 ^{b}	2.33± 0.58 b	5.33 ± 0.58 b	6.0 ±0.0 ^{a}	21.0 ±1.0 ^{a}	3.0 ±0.0 b
	Protexin 0.1 gm. /kg feed	3	70.33 ± 0.58^{ab}	2.67± 0.58 ^{a}	5.67 ± 0.58 ^{a}	5.67±0.58 ^{ab}	11.67 ±0.58 ^{b}	3.0 ±0.0 b
	Protexin 0. 2 gm./kg feed	3	72.33± 1.16 ^{a}	2.67± 0.58 ^{a}	5.33 ± 0.58 b	5.0 ±0.0 ^{b}	9.0± 0.0 ^{c}	4.0 ±0.0 ^{a}b
	Protexin 0.3 gm./kg feed	3	74.67 ± 0.58 ^{a}	2.67± 0.58 ^{a}	5.33± 0.58 b	5.0 ±0.0 ^{b}	6.67± 0.58 ^{d}	4.33±0.58 ^{a}

Means ± SE within the same column of different letters are significantly different at ($P < 0.05$)

Table 4. Effect of protexin supplemented diet on phagocytic activity and phagocytic index of seabass during experimental period:

	Groups	N	Phagocytic activity	Phagocytic index
Zero day	Control	3	21.0 ± 0.0 [a]	2.0 ± 0.0
	Protexin 0.1 gm. /kg feed	3	20.67 ± 0.58 [ab]	2.33±0.58
	Protexin 0. 2 gm./kg feed	3	20.67 ± 0.58 [ab]	2.33 ±0.58
	Protexin 0.3 gm./kg feed	3	21.0 ± 1.0 [a]	2.0 ± 0.0
2 nd week	Control	3	20.67± 0.58 [c]	2.0 ± 0.0 [a]b
	Protexin 0.1 gm. /kg feed	3	22.67 ± 0.58[b]	2.6 7± 0.58 [a]
	Protexin 0. 2 gm./kg feed	3	23.33 ± 0.58[b]	2.67 ± 0.58 [a]
	Protexin 0.3 gm./kg feed	3	24.0 ± 0.0 [a]	2.67 ± 0.58 [a]
4th week	Control	3	20.0 ± 0.0 [a]	2.67 ±0.58 [bc]
	Protexin 0.1 gm. /kg feed	3	23.67 ± 0.58 [bc]	3.33 ± 0.58 [b]
	Protexin 0. 2 gm./kg feed	3	25.33 ± 0.58 [d]	3.33 ± 0.58 [b]
	Protexin 0.3 gm./kg feed	3	26.0 ± 0.0 [d]	4.0 ± 0.0 [a]
6th week	Control	3	20.0 ± 0.0 [c]	2.0 ± 0.0 [c]
	Protexin 0.1 gm. /kg feed	3	25.0 ±0. 0 [bc]	3.67 ± 0.58 [b]
	Protexin 0. 2 gm./kg feed	3	26.33 ± 0.58[ab]	3.67 ±0.58 [b]
	Protexin 0.3 gm./kg feed	3	27.6 ± 0.58 [a]	4.67 ± 0.58 [a]
8h week	Control	3	21.0 ± 0.0 [c]	2.0 ± 0.0 [c]
	Protexin 0.1 gm. /kg feed	3	26.33 ± 0.58 [bc]	4.67± 0.58 [b]
	Protexin 0. 2 gm./kg feed	3	29.33 ± 0.58 [ab]	5.67 ± 0.58 [ab]
	Protexin 0.3 gm./kg feed	3	31.67 ±1.53 [a]	6.33 ± 0.58 [a]

Means ± SE within the same column of different letters are significantly different at (P < 0.05)

Table 5. Effect of protexin supplemented diet on total serum protein, albumin, and globulin and A/G ratio of seabass during experimental period.

	Groups	N	Total protein	Albumin	Globulin	A/G ratio
Zero day	Control	3	4.30 ± 0.23	2.63 ± 0.05	1.67 ± 0.09 [ab]	1.58 ± 0.11 [a]
	Protexin 0.1 gm. /kg feed	3	4.78 ± 0.18	2.5 ± 0.15	2.28 ± 0.12	1.09 ± 0.09 [ab]
	Protexin 0. 2 gm./kg feed	3	4.72 ± 0.11	2.59 ±0.07	2.14 ± 0.15	1.22 ± 0.11[ab]
	Protexin 0.3 gm./kg feed	3	4.49 ± 0.11	2.44 ±0.11	2.05 ± 0.21	1.21 ± 0.15 [ab]
2 nd week	Control	3	4.39 ± 0.21 [ab]	2.53 ± 0.16 [b]	1.86 ± 0.36 [b]	1.39± 0.34 [ab]
	Protexin 0.1 gm. /kg feed	3	4.94 ± 0.05 [ab]	2.86 ± 0.05 [b]	2.08 ± 0.06 [a]	1.43 ± 0.04 [ab]
	Protexin 0. 2 gm./kg feed	3	5.09 ± 0.03 [a]	3.04 ± 0.06 [a]	2.05 ± 0.04 [a]	1.49 ± 0.06 [ab]
	Protexin 0.3 gm./kg feed	3	5.32 ± 0.29 [a]	3.18 ± 0.13 [a]	2.15 ± 0.35 [a]	1.51 ± 0.28 [a]
4th week	Control	3	4.61 ± 0.05 [b]	2.35 ± 0.02 [b]	2.26 ± 0.04	1.04 ± 0.01 [a]
	Protexin 0.1 gm. /kg feed	3	5.17 ± 0.06 [ab]	2.55 ± 0.12 [b]	2.61 ± 0.18	1.11 ± 0.11 [a]
	Protexin 0. 2 gm./kg feed	3	5.58 ± 0.04 [a]	2.93 ± 0.04 [ab]	2.65 ± 0.05	1.11 ± 0.03 [a]
	Protexin 0.3 gm./kg feed	3	5.95 ± 0.03 [a]	3.13 ± 0.13 [a]	2.82 ± 0.16	0.98 ± 0.11 [b]
6th week	Control	3	4.72 ± 0.22 [b]	3.01 ± 0.17	1.70 ± 0.36 [c]	1.42± 0.21 [a]
	Protexin 0.1 gm. /kg feed	3	5.59 ± 0.16 [ab]	2.91 ± 0.19	2.68 ± 0.04 [b]	1.08± 0.08 [ab]
	Protexin 0. 2 gm./kg feed	3	5.88 ±0.03 [ab]	2.97 ± 0.09	2.91 ±0.10 [ab]	1.02 ± 0.07 [ab]
	Protexin 0.3 gm./kg feed	3	6.18 ± 0.08 [a]	2.96 ± 0.14	3.21 ± 0.07 [a]	0.92 ± 0.06 [b]
8th week	Control	3	4.75 ± 0.14 [c]	2.89 ± 0.04 [b]	1.86 ± 0.17 [c]	1.56± 0.16 [a]
	Protexin 0.1 gm. /kg feed	3	5.29 ± 0.36 [b]	2.71 ± 0.17 [b]	2.58 ± 0.45 [b]	1.08 ± 0.23 [ab]
	Protexin 0. 2 gm./kg feed	3	6.22 ± 0.08 [a]	2.68 ± 0.02 [b]	3.54 ± 0.08 [a]	0.76 ± 0.02 [b]
	Protexin 0.3 gm./kg feed	3	7.00 ± 0.13 [a]	3.07± 0.17 [a]	3.93 ± 0.08 [a]	0.78 ± 0.06 [b]

Means ± SE within the same column of different letters are significantly different at ($P < 0.05$)

4. DISCUSSION:

Stimulation of the non-specific defense mechanisms by using specific biological compounds, called immunostimulants, enhances the disease resistance and growth of the host (Skjermo *et al*., 2006). In this study, immunostimulants supplemented improved growth and immune response of seabass.

Protexin concentrate contains beneficial probiotic microorganisms (*Enterococcus faecium*) which promote efficient digestion and immunity by improving intestinal microbial balance Fuller (1989) .Stimulating the immune system and decreasing pH as well as release of bacteriocins Rolfe (2000) and inhibition of pathogenic bacteria like *Vibrio spp.*, *Yersinia spp.* and *Aeromonas spp.* Rosskopf (2010).

Table 6. Effect of protexin supplemented diet on logarithmic transformation of Total bacterial count, Total enterobacteriaceae count and Total coliform count among different groups of seabass during experimental period.

	Group	N	Total bacterial	Total enterobacteriaceae	Total coli form
Zero day	Control	3	3.41± 0.08 [a]	3.44 ± 0.02 [a]	2.42 ± 0.30
	Protexin 0.1 gm. /kg feed	3	3.31 ± 0.07 [ab]	3.40 ± 0.14 [a]	2.44 ± 0.36
	Protexin 0. 2 gm./kg feed	3	3.22 ± 0.09 [ab]	3.23 ± 0.01 [b]	2.45 ± 0.29
	Protexin 0.3 gm./kg feed	3	3.37± 0.04 [ab]	3.29 ± 0.14 [b]	2.42 ± 0.31
2nd week	Control	3	3.51± 0.14 [a]	3.33 ± 0.16 [ab]	2.35 ± 0.15 [b]
	Protexin 0.1 gm. /kg feed	3	3.4 ± 0.14 [b]	3.37 ± 0.13 [a]	2.45 ± 0.35 [ab]
	Protexin 0. 2 gm./kg feed	3	3.37 ± 0.05 [b]	3.3 ± 0.19 [ab]	2.58 ± 0.18 [ab]
	Protexin 0.3 gm./kg feed	3	3.36 ± 0.16 [b]	3.40 ± 0.17 [a]	2.52 ± 0.28 [ab]
4th week	Control	3	3.5 ± 0.06 [a]	3.46 ± 0.05 [a]	3.58 ± 0.06
	Protexin 0.1 gm. /kg feed	3	3.31± 0.06[b]	3.31 ± 0.19 [b]	3.59 ± 0.06
	Protexin 0. 2 gm./kg feed	3	3.43± 0.14 [a]	3.32 ± 0.27 [b]	3.39 ± 0.12
	Protexin 0.3 gm./kg feed	3	3.37 ± 0.13 [b]	3.39 ± 0.05 [ab]	3.47 ± 0.18
6th week	Control	3	3.49 ± 0.14 [a]	3.47 ± 0.07 [a]	2.28 ± 0.07[b]
	Protexin 0.1 gm. /kg feed	3	3.27 ± 0.09 [b]	3.17 ± 0.05 [b]	2.34 ± 0.13 [b]
	Protexin 0. 2 gm./kg feed	3	3.33 ± 0.16 [a]	3.24 ± 0.08 [ab]	2.51 ± 0.05 [a]
	Protexin 0.3 gm./kg feed	3	3.38 ± 0.13 [a]	3.28 ± 0.10 [b]	2.43 ± 0.18 [ab]
8th week	Control	3	3.41 ± 0.07 [a]	3.30 ± 0.12 [ab]	2.28 ± 0.07
	Protexin 0.1 gm. /kg feed	3	3.33 ± 0.17 [ab]	3.56 ± 0.35 [a]	2.37 ± 0.14
	Protexin 0. 2 gm./kg feed	3	3.22 ± 0.12 [ab]	3.19 ± 0.16 [b]	2.32 ± 0.06
	Protexin 0.3 gm./kg feed	3	3.41 ± 0.07 [a]	3.28 ± 0.07 [b]	2.44 ± 0.25

Means ± SE within the same column of different letters are significantly different at ($P < 0.05$)

Table 7. Effect of protexin supplemented diet on mortality percent of seabass after challenge with *Vibrio alginolyticus*

groups	N	Mortalities		Protected	
		No	%	No	%
Control (- ve)	10	10	100	0	0
Protexin 0.1 gm. /kg feed	10	5	50	5	50
Protexin 0. 2 gm./kg feed	10	4	40	6	60
Protexin 0.3 gm./kg feed	10	2	20	8	80

The present study indicated significant increase in growth performance of all seabass protexin treated groups in comparison to control these results are similar to those observed by Wang *et al*. (2008 b) in tilapia after addition of *E.faecium* ($1×10^7$cfu/ml) in aquaria water and Merrifield *et al*., (2010 b) in rainbow trout when obtained *B. subtilis*, *B. licheniformis*, and *Enterococcus faecium* probiotics for 10 weeks along with the diet as well as Maurilio and Miguel (2013) where Tilapia fry fed with native bacteria (*Enterococcus faecium*) supplemented diets presented significantly higher growth and feeding performance than those fed with control diet. The presented results revealed that best growth performance results were obtained in group fed on diet contain the highest protexin concentration (3 gm./kg feed) Contrary, measurable effects on the growth parameters in the Oscar *A. ocellatus* fingerlings were obtained by protexin, at the smallest level ($0.15\ g\ kg^{-1}$ dry food) Firouzbakhsh *et al.(*2011) and She Ahmadvand *et al.* (2012) where all the groups of rainbow trout fry protexin different levels supplemented diets and control basal diet revealed the same results in growth parameters. This is may be due to different fish species, fish age and different concentrations of the protexin probiotic

Haematological profile of an animal is the reflection of its immunological status. Panigrahi *et al.* (2007) have reported on the immune system modulation of rainbow trout fed an *E.faecium* diet. Examination of differential leucocytic counts characterized by increase of lymphocytes and monocytes Contrary neutrophils significantly decreased in all treated groups comparing to the control. These findings were supported by Manal A. A. Essa *et al*., (2012) after *E.faecium* supplementation in the diet of *Oreochromis niloticus.* Phagocytosis is a primary, non- specific defense mechanism against invasion of pathogenic organisms of hosts Olivier *et al*. (1988). The present study showed, significant increasing in the phagocytic activity as well as phagocytic index in all protexin treated group as compared to the control. The same results were obtained by Irianto and Austin (2002) revealed that feeding with Gram-positive and Gram-negative probiotics at (10^7 cells 1g) of feed led to increase in the number of erythrocytes, macrophages, lymphocytes and enhanced phagocytic and lysozyme activity within 2 weeks of feeding with probiotics in rainbow trout. Nikoskelain *et al*. (2003) they said that oral administration of probiotics can increase immune response such as phagocytosis, respiratory burst, lymphocyte proliferation and cytokine synthetize.

The serum total proteins as well as total globulin levels were significantly higher in all treated groups than the control. Similar results were also obtained after *E.faecium* supplementation in the diet of *O.niloticus* by Manal A. A. Essa *et al*. (2012) but these findings was disagreed with Wang *et al.*, (2008 b) who reported that there was no significant increase in the serum parameters of *O. niloticus* after addition of *E. faecium* in water and these differences might be due to the fish species, fish age and method of the probiotic application

Regarding to The effect of protexin supplementation on total bacterial count, total enterobacteriaceae count and total coliform count revealed significant decrease in their count in all protexin treated groups comparing to the control one. The obtained results supported by Bogut *et al.* (2000) where administration of *E.faecium* to sheet fish positively effects on its intestinal microbiota, reducing harmful bacterial load, and its increased weight gain as well as Chang and Liu(2002) indicated that Feeding of *E.faecium* in eel through a 14-day diet influenced the gut microbiota and increased disease resistance.

Inclusion of protexin in seabass fingerlings feeding have positively impacted the resistance of fish to *Vibrio alginolyticus* infection as was indicated by significantly lower mortality rates of the treated fish challenged by *Vibrio alginolyticus* in comparison to the control. These results were supported by Chang and Liu (2002) who recorded that the survival rate of eels challenged with *E. tarda* after feeding on *E. faecium* supplement was significantly higher (73%) than those of control eels (45%)and Krummenauer *et al*.,(2009) after dietary application of *E.faecium* (strain IMB 52) alone or in combination to shrimp challenged by *Vibrio parahaemolyticus* as well as Swain *et al*. (2009) reported the antagonistic effects of *E.faecium* in shrimp, reducing vibriosis in challenge trials. These results also were nearly obtained by Gopalakannan and Arul (2011) after challenging *E.faecium* MC13 supplemented *Cyprinus carpio* by *Aeromonas hydrophila*

5. CONCLUSION

It could be concluded that addition of protexin (*Enterococcus faecium*) as probiotic in seabass fingerlings diets improve the growth performance and immunity as was indicated by significant increase of lymphocytes, monocytes, total protein, globulin, phagocytic activity and index of phagocytes which enhanced the resistance of challenged fish to *Vibrio alginolyticus* as was indicated by significant decrease in mortalities rates in protexin treated groups than the control. The highest dietary level 0.3 gm. /kg feed of protexin showed best results than 0.2 gm./kg feed and 0.1 gm./kg feed. Protexin should be used for at least 6th weeks to give best immune status results.

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