Effect of Echinacea on body gain, survival and some hematological and immunological parameters of *Oreochrmis niloticus* and their response to challenge infection

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Abstract

Six hundred and forty *O. niloticus* were equally distributed in 16 hapas (each of 1.5 m^3) to be used for 4 treatments in 4 replicates. Fish of groups 1-3 were fed on basal diet mixed with Echnicacea (0.25 ppt) for $_1^{st}$, $_2^{nd}$ and $_3^{rd}$ summer months; respectively (1^{st} phase). Fish of group 4 were fed basal diet only as a control. By the end of the 3rd months, some fish were used to measure hematological, immunological parameters. The remaining fish in the 4 hapas of each treated group were fed with the basal diet without Echinacea for 6 months (winter season, 2^{nd} phase). The same growth parameters, survival and relative protection against challenge infection were determined by the end of 1^{st} and 2^{nd} phase of the experiment. At end of 1^{st} phase, a marked increase in the neutrophil adherence, hematocrit values and total leukocytic count was seen in most treated groups. Also an increase in the body gain, specific growth and survival rates were recorded. At end of 2^{nd} phase, no significant changes were seen in the body gain, specific growth rate and coefficient factor but a significant increase in the survival rate was noticed.

Challenge of experimented tilapia resulted in low mortality rate of all treated groups in comparison with the control. The level of protection was high in both seasons; however it was higher in winter than summer season. As a general observation, one month administration of 0.25 ppt of Echinacea induced remarkable effect on the survival and protect against challenge infection. The prolonged addition of 0.25 ppt Echinacea (2 & 3 months) showed similar effect on survival and more protection against challenge.

Introduction

Historical tradition for the use of Echinacea species is definitely related to Native Americans since several American Indian tribes. Major disorder targets of these treatments were related to: sore mouth, toothache, colds, tonsillitis, septic diseases, snakebite, coughs and general inflammatory conditions (Hobbs, 1989; Foster, 1991).

During the European colonization, Echinacea started to be known also among settlers, it became one of the most popular remedy of the following 50 years. Echinacea was described in the National Formulary of the United States from 1916 to 1950, and then due to

the incoming prevalence of synthetic chemistry in the pharmaceutical field, it declined in the medical interest. Nevertheless, the use of Echinacea still remained popular. In particular, it exploded as a dietary supplement with a peak of estimated market of about 300 millions USD (Brevoort,1998) after the approval of the Dietary Supplement Health and Education Act (1994) in the USA. A huge number of scientific data are available in literature and they are related to several kinds of preparations obtained from the three most relevant species of *Echinacea spp. (E. angustifolia, E. pallida, E. purpurea).*

On the other hand, hundreds of commercial preparations are now available in pharmacies, groceries and health-food stores of most of the developed countries and consequently a rigorous parallelism between the composition of the products used in the available literature and the products on the market is an absolute need in order to avoid misleading information to the consumer. Independently from the kind of preparation, the main indications of Echinacea are the prevention and treatment of common cold, flu and upper respiratory infections.

A good level of evidence is available on the role played by the polysaccharidic fraction in the immunostimulatory effect of Echinacea preparations. In particular, the polysaccharides (heteroglycans) isolated from *E. purpurea* have been deeply investigated for their capacity to activate macrophages in mice, rats and humans along with other immunological functions (Bauer,1999; Emmendorffer et al.,1999; Wagner et al.,1988).

Data available on the lipophilic components present in Echinacea preparations are less consistent even if some of them such as alkylamides and in particular isobutylamides have been described to produce a strong stimulating effect on phagocyte functions (Bauer, 1998) and on lipoxygenase-inhibiting activity (Wagner, 1989).

Immunostimulants is a natural compound that modulates the immune system by increasing the host's resistance against diseases. Rapid growth and disease resistance of aquacultured organisms are two of the most important concern.

The present study aimed to evaluate the effect of Echinacea as a feed additive on some hematological and immunological parameters as well as body gain, survival and response to the challenge infection.

Material and methods:

1- Fish:

Six hundred and forty *O. niloticus* were taken from WorldFish Center Hatchery by 1st of July, 2004 and stocked in quarantine tank for the period of 15 day to assess their health status and

to reach size 0.8- 1.0 gm. After that, the fish were distributed in 16 hapas (40 fish / hapa, each of 1.5 m^3) to be used for 4 treatment groups (1-4) in 4 replicates that distributed randomly in 4 race ways as shown in Table (1).

II- ***Feeding and formulation:***

A balanced ration was prepared as shown in Table (2). The ingredients of the diet were obtained from many specialized factories and prepared locally in our Center in the form of pellets. Fish were fed 5 % of body weight 3 time / day using clean plastic feeders (1 feeder / hapa). The basal diets were prepared by grinding of the corn to granules of 0.5 mm mesh (Thomes-Willey Laboratory Mill Model 4). The ingredients were mixed mechanically by horizontal mixture (Hobarts model D300T) at lower speed for 30 minutes. The oil was added gradually to assure the homogeneity of ingredients. The mixing speed was increased for 5 minutes during the addition of water (600 ml water) and at beginning to clump formulation of the diet, then by using pellet machine (CPM California pellet mill Co.); the pellet size was 0.5 cm which left for 24 hrs for air drying.

III-<u>First Phase (summer season):</u>

Echinacea was added to balanced ration in 0.25ppt. Fish of 1^{st}, 2^{nd}, and 3^{rd} groups were fed on basal diet contained Echinacea (0.25ppt) as a feed additive for 1, 2 and 3 months; respectively. Fish of 4^{th} group served as a control and received basal diet only along the 3 months of the experiment (15 July – 15 October 2004). The formulated feed was added to the fish with 3% of the total fish weight. The daily ration was subdivided twice and each was fed to the fish at 9.00 am and 3.00 pm.

By the end of the first phase (three months), the survival and growth parameters as well as hematocrit value, total and differential leukocytic count and neutrophil adherence of experimented fish were calculated. Mortality and relative level of protection after challenge infection was also estimated.

IV-<u>Second Phase (winter season):</u>

1-Experimental fish:

The total number of the remaining fish in the 4 hapas of each group were collected, pooled and 80 random fish from each treatment were redistributed in 4 hapas (20 fish/ hapa). At the beginning of the 2^{nd} phase, all fish were individually weighted. Fish were fed with the basal diet for 6 months (16 October 04 – 16 April, 2005). The daily ration was subdivided twice and fed at 9.00 am and 3.00 pm daily.

By the end of the second phase (six months), the survival and growth parameters of experimented fish were calculated. Mortality and relative level of protection after challenge infection was also counted.

V- Laboratory tests:

1- Survival and growth performance:

Fish of all replicates were weighted individually and their body gain was measured (Innes, 1982). *S*pecific growth rate (SGR) and condition factor (CF) were calculated (Laird and Needham, 1988) and the mortality was recorded along the period of experiment.

SGR=In[final mean body weight (g)]-In[initial mean body weight (g)] ÷ time interval (days) X 100.

CF = weight (g) ÷ [length (cm)]^3

2- Hematological analysis and immunological tests:

10 fish from each replicate of echinacea treated and control groups were anaesthetized with MS-222, taken for blood collection. Blood was collected from caudal vein and used for the preparation of whole blood and serum. The blood used for the estimation of total and differential leukocytic count (Stoskoph, 1993), hematocrit value (Wintrobe, 1974) and neutrophil adherence (Anderson et a., 1992)**.**

3- Challenge experiment:

20 fish from each treatment (5 fish from each replicate) were challenged intraperitoneally with 0.5 ml of culture suspension of pathogenic *Aeromonas hydrophila* containing 10^8 bacteria ml^{-1} that were previously isolated from morbid fish and studied for pathogenicity, transferred to one aquaria (50X60X60) and observed for 7 days for mortality.

4- Statistical analysis:

Statistical analysis was performed using the one way and two ways analysis of variance (ANOVA) and Dauncan (1955). Multiple Range Test was done to determine differences between treatment (mean at significance level of $P<0.05$). Standard errors were also estimated. All analysis was run on the computer using the SAS program ***(SAS, 2000)****.*

Results and Discussion

The administration of Echinacea (0.25 ppt) for 1, 2 and 3 months, in this study, revealed a significant (E_2) to non significant (E_1 & E_3) increase in the neutrophil adherence in comparison with the control (Table 3, Graph 1). Similar studies shown that, Echinacea stimulate phgocytosis in vitro and in vivo, and enhance the production of oxygen redicals by macrophages in a dose-dependant manner (Stimpel et al., 1984). Phagocytosis enhancement

in vitro has also been reported for non volatile sesquiterpene esters isolated from *E. purpurea* (Bauer et al., 1985) and tissue culture experiments have yielded immunologically active polysaccharides from *E. purpurea* (Wagner et al., 1988).

The percent volume of erythrocytes in fish blood is an indication of the health status and can be helpful in detecting any abnormal changes through the use of immunostimulants. Anemic fish may have hematocrit values as low as 10 %. Reduced hematocrit values may indicate that fish are not eating properly or are suffering from infections (Blaxhall, 1972). The hematocrit values in the experimented tilapia of all treated groups showed a non significant increase in comparison with the control. That could indicate the safety of the used product by the three doses.

In the present study, all treatments in comparison with the control revealed a non significant increase in the total leukocytic count as well as lymphocytes, while monocytes showed significant (E_2) to non significant (E_1 & E_3) increase (Table 3, Graph 1). Changes in the number and activities of T and B cell leukocytes in response to Echinacea exposure have been reported; also increased white cell counts in peripheral blood have been noted following injection of *E. purpurea* extracts (Lorenze et al., 1972). Bauer (1996) stated that relevant pharmacological effects have been demonstrated in vitro and in vivo for the expressed juice of the aerial parts of *E. purpurea* and for alcoholic extracts of the roots of *E. angustifolia, E. purpurea* and *E. pallida*. According to the author, the effects are mainly linked to a modulation of nonspecific cellular immune system and the compounds responsible for such an effect are: polysaccharides, glycoproteins, caffeic acid derivatives and alkylamides. Later on, stimulation of various immune cells such as macrophages, other monocytes and natural killer cells have also been demonstrated in vitro (Bauer ,1998 and 1999; Rininger et al., 2000; Sun LZ-Y et al., 2001).

Also a non significant increase in the body gain and significant increase in the specific growth and survival rates were recorded in all treatments in comparison to the control group (Table 4, Graph 2). Maass (2005) mentioned that supplementation of echinacea tend to improve feed conversion and other botanicals (herbs and/or spices) administered as feed additives showed similar improvement of feed conversion. The mode of action of the herb mixtures as enhancement of digestive functions (Przybilla and Weib 1998).

Feeding of fish on a basal diet for 6 months during winter after addition of Echinacea (0.25 ppt) as a feed additive for 1, 2 and 3 months resulted no significant changes (E_1) to non significant increase (E_2 & E_3) in the body gain, specific growth rate and coefficient factor, but

a significant increase in the survival rate was noticed in all treated groups in comparison with the control (Table 5, Graph 3). In that phase, its noticed that low dose of Echinacea (E_1) induced high survival rate while higher doses (E_2 & E_3) improve survival rate also and have positive impact on body gain and specific growth rate.

Challenge of experimented tilapia in all treated groups after the 1st and 2nd phases of experiment using 0.5 ml of culture suspension of pathogenic *Aeromonas hydrophila* containing 10^8 bacteria ml^{-1} via I/P route resulted in low mortality rate of all treated groups in comparison with the control. At 1st phase the protection against challenge increased by the increase in the dose of Echinacea. The level of protection in winter season was high than the corresponding treatment after summer season although fish fed on basal diet without Echinacea during winter and the virulence of used *Aeromonas hydrophila* during challenge was the same(6 months) (Table 6, Graph 4). This finding could be due to the improvement in the immune status and resistance ability of experimented tilapia as a result of eitherusing Echinacea during summer or exposure to cold stress during winter (Roesler et al., 1991) noticed that *Echinacea purpurea* could induce increased proliferation of phagocytes and migration of granulocytes to the peripheral blood that resulted in excellent protection against predominantly dependant pathogen.

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Table (1): Experimental design for the random distribution of the tested immunostimulants (cells within columns represented hapas).

Raceway I	Raceway II	Raceway III	Raceway IV
E_1	E_3	C	E_1
E_2	C	E_1	E_3
˜C	EI	E_2	C
E_3	E_2I	E_3	E_2

Table (2) Analysis and ingredient of the basal diet used along the experiment.

Ingredients (Ing.)	% in diet	% protein (in ing.)	% protein in feed	ME* (in Ing.)	ME* in Feed
Fish meal	8.00	0.72	5.76	4000	32000
Soybean meal	52.9	0.48	25.392	2870	151823
Ground corn	29.1	0.109	3.1719	1240	36084
Wheat flour	5.00	0.134	0.67	2700	13500
Vegetable oil	2.00	0.00	0.00	9100	18200
Cod liver oil	2.00	0.00	0.00	9100	18200
Dicalcium phosphate	1.00	0.00	0.00	0.00	0000
Mineral mix.	0.07	0.00	0.00	0.00	0000
Vitamin mix.	0.05	0.00	0.00	0.00	0000
Vitamin C	0.03	0.00	0.00	0.00	0000

Total	100.15	0.00	34.9939	0.00	269807
Dietary requirement			35		280000

**ME= metabolic energy*

Table (3): Some immunological and hematological parameters in experimented fish by the end of the summer season (1st Phase).

Group. Treatment	N. adherence (mg/ml)	HCV (%)	TLC (10^3/µl)	Neutrophils (10^3/µl)	Lymphocytes (10^3/µl)	Eosinophils (10^3/µl)	Basophils (10^3/µl)	Monocytes (10^3/µl)
1. E_1	10.6^{B}±1.01	27.33^{A}± .41	41.5^{A} ± 0.89	8.65^{AB}± .71	31.06^{A}± 0.73	0.29^{A}±0.06	0.08^{A}±0.05	1.43^{AB}± 0.10
2. E_2	15.2^{A}±1.92	29.1^{A} ± 1.33	40.7^{A} ± 0.84	8.75^{B} ± 0.58	31.05^{A}± 0.58	0.40^{A}±0.06	0.12^{A}±0.06	1.2^{A}± 0.17
3. E_3	11.8^{AB}±1.03	28.33^{A}±0.67	42.4^{A}± 0.72	9.32^{A} ± 0.49	31.39^{A}±0.45	0.21^{A}±0.07	0.09^{A}±0.06	1.39^{AB}± 0.16
4. C	9.1^{B}± 0.94	26.33^{A}± .99	40.9^{A} ± 1.08	9.67^{A} ± 0.24	29.89^{A} ± 0.94	0.24^{A}±0.07	0.08^{A}±0.06	1.01^{B}± 0.16

Table (4): Growth performance and survival rate of experimented fish by the end of the summer season (1st Phase).

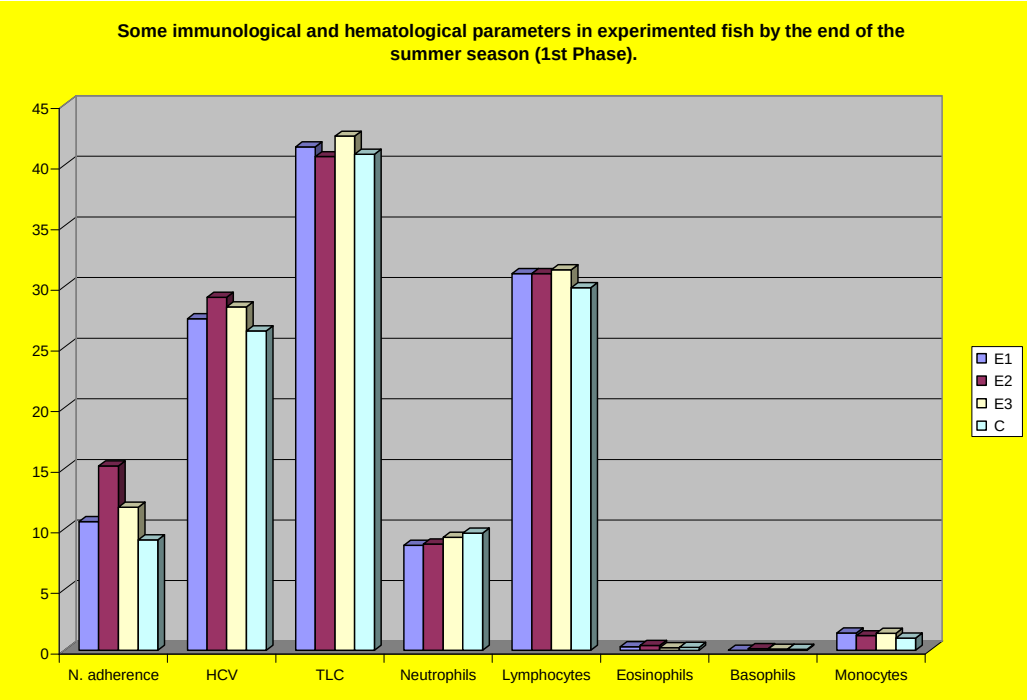
Group/Treatment	BG	SGR	CF	Survival rate
1. E_1	11.59^{A}± 0.38	2.85^{A}± 0.03	0.038^{A} ±0.001	90.00^{A}± 2.28
2. E_2	11.59^{A}± 0.4	2.86^{A}±0.03	0.036^{B}±0.001	93.75^{A}± 2.17
3. E_3	11.53^{A}± 0.41	2.84^{A}±0.04	0.036^{B} ±0.001	88.75^{A}± 3.61
4. C	10.45^{A}± 0.40	2.73^{B} ±0.05	0.037^{AB}±0.001	75.63^{B}±2.13

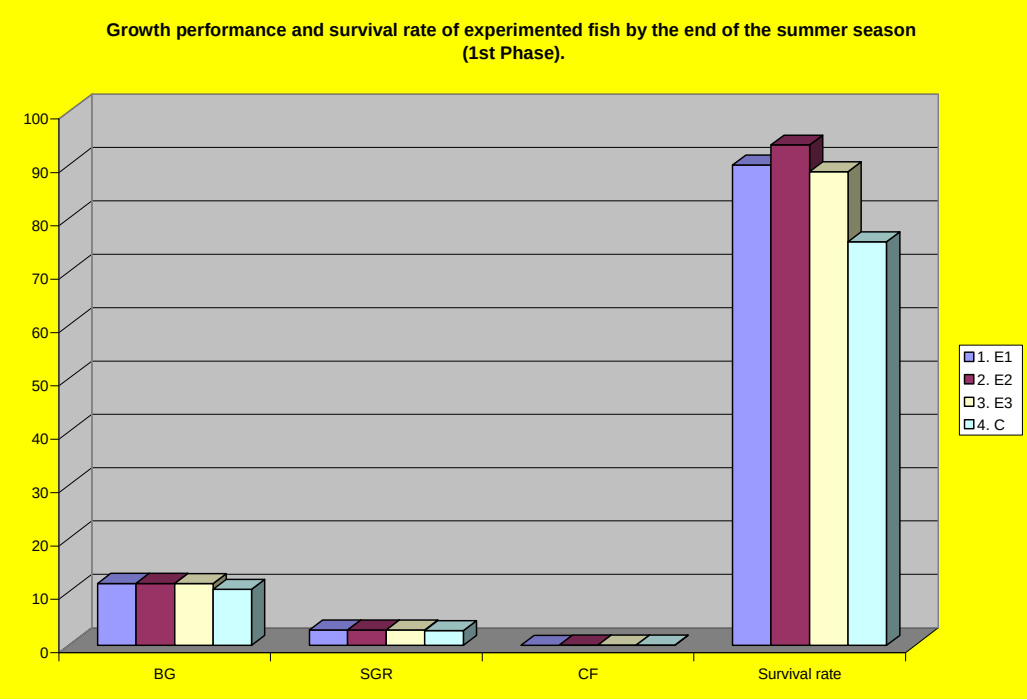
Table (5): Growth performance and survival rate of experimented fish by the end of the winter season (2nd Phase).

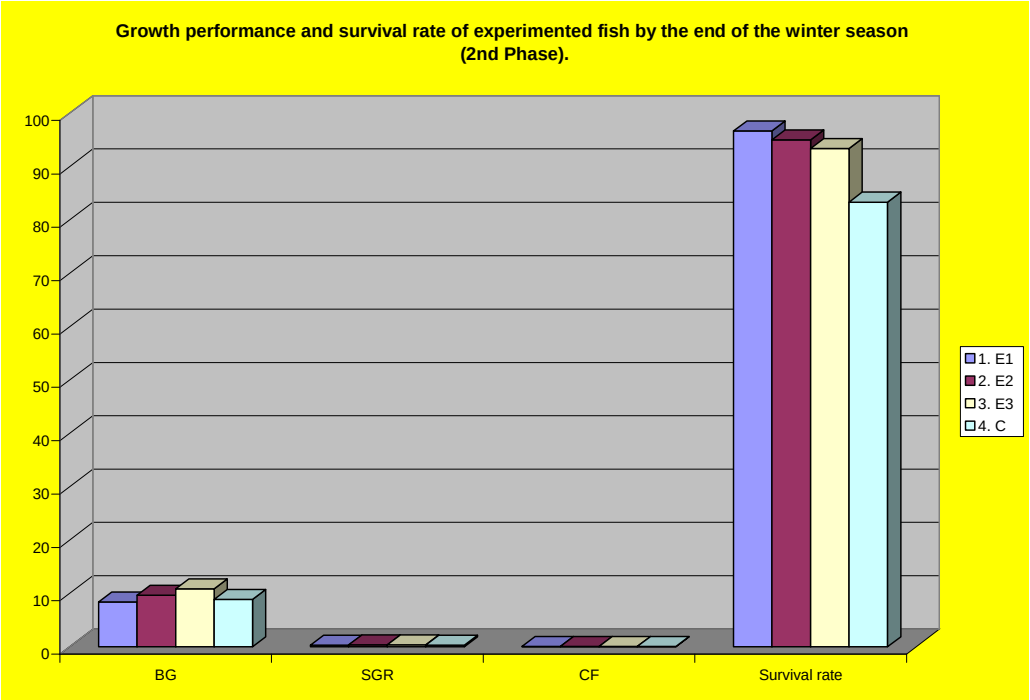
Group.Treatment	BG	SGR	CF	Survival rate
1. E_1	8.38^{A}± 0.47	0.29^{A}±0.001	0.038^{A}±0.001	96.67^{A}±1.67
2. E_2	9.63^{A}± 0.40	0.32^{A}±0.01	0.038^{A}±0.001	95^{A}± 2.80
3. E_3	10.84^{A}±1.05	0.33^{A} ± 0.03	0.039^{A}±0.001	93.33^{A}±3.33
4. C	8.82^{A}± 0.87	0.29^{A}±0.02	0.038^{A}±0.001	83.33^{B} ±1.67

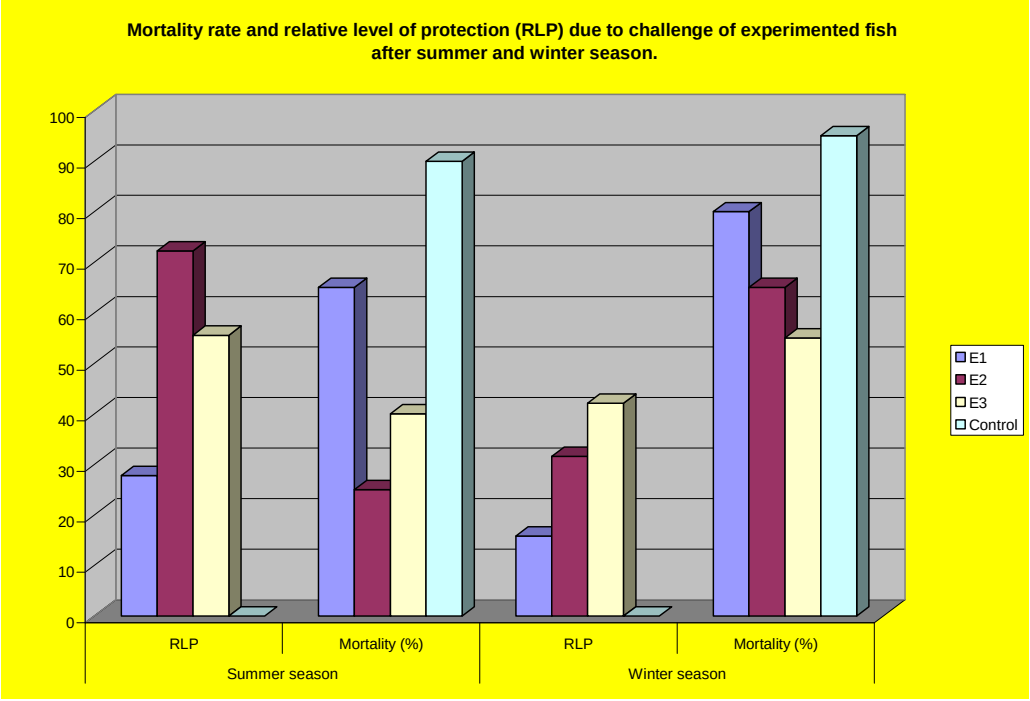
Table (6): Mortality rate and relative level of protection (RLP) due to challenge of experimented fish after summer and winter season.

Treatments	Winter season		Summer season	
	Mortality (%)	RLP	Mortality (%)	RLP
E_1	80	15.78947	65	27.77778
E_2	65	31.57895	25	72.22222
E_3	55	42.10526	40	55.55556
Control	95	00.0000	90	00.0000


Some immunological and hematological parameters in experimented fish by the end of the summer season (1st Phase).
45
40
35
30
25
20
15
10
5
0
N. adherence
HCV
TLC
Neutrophils
Lymphocytes
Eosinophils
Basophils
Monocytes
E1
E2
E3
C


Growth performance and survival rate of experimented fish by the end of the summer season (1st Phase).
100
90
80
70
60
50
40
30
20
10
0
BG
SGR
CF
Survival rate
1. E1
2. E2
3. E3
4. C


Growth performance and survival rate of experimented fish by the end of the winter season (2nd Phase).
100
90
80
70
60
50
40
30
20
10
0
BG
SGR
CF
Survival rate
1. E1
2. E2
3. E3
4. C


Mortality rate and relative level of protection (RLP) due to challenge of experimented fish after summer and winter season.
100
90
80
70
60
50
40
30
20
10
0
RLP
Mortality (%)
RLP
Mortality (%)
Summer season
Winter season
E1
E2
E3
Control