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Effect of Some Feed Additives on Fish Immunity

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ABSTRACT

This study aimed to throw the light on the importance of Algae-flora as a feed additives on the fish immunity and diseases prevention through study its effect on, differential leucocytic counts growth performance Its effects of (TWBCs and TRBCs) (Lymphocyte, monocyte), , its effects on Phagocytic activity and Phagocytic index, its effects on antibody titer against some pathogenic ***Aeromonas hydrophila*** . Mortality rate and relative level of protection as well as some pathological alterations were recorded. By addition of the Algae-flora at a higher concentration 0.3 Algae-flora/Kg improve the immunity of the fish against different stress condition that may causes infection of the fish by different diseases. It improves the differential leucocytic counts (Lymphocyte and monocyte,) in the blood of the treated fish with a higher doses of Algae-flora. Improving the level of (TWBCs and TRBCs) by higher doses of Algae-flora than the lower dose of Algae-flora. Improving the Phagocytic activity and Phagocytic index at a higher level of Algae-flora. The antibody titer against some pathogenic diseases at a higher level of Algae-flora feed additives. Decrease the indince of mortality rate and improving the relative level of protection against different fish diseases.

Keywords: ***Probiotics, Algae-flora, TWBCs, TRBCs and challenge test, Phagocytic activity and Phagocytic index***

INTRODUCTION

The algae are photosynthetic macro-algae or microalgae growing in aquatic environments. Macro-algae or “seaweeds” are multicellular plants growing in salt or fresh water. They are often fast growing and can reach sizes of up to 60 m in length ***(***Mc Hugh, 2003). They are classified into three broad groups based on their pigmentation:

Brown seaweed (Phaeophyceae); Red seaweed (Rhodophyceae) and Green seaweed (Chlorophyceae). Seaweeds are mainly utilized for the production of food and the extraction of hydrocolloids. Microalgae are microscopic organisms that grow in salt or fresh water. The three most important classes of micro-algae in terms of abundance are the diatoms (*Bacillariophyceae*), the green algae (*Chlorophyceae*), and the golden algae (*Chrysophyceae*).

MATERIALS AND METHODS

Materials

Fish

A total number of 250 healthy fish (average weight 30 ± 5 gm) *Oreochromis niloticus*, were collected from private fish farm at Behera Province. Fish were transported alive to the laboratory in plastic bags containing water enriched by air (2/3).

Aquaria

Fish were kept in full prepared glass aquaria (90 X 50 X 35 Cm). These aquaria were used for holding the experimental fish throughout the period of the present study (one month), supplied with chlorine free tap water according to Innes (1966).

Fish diets

Fish were fed on commercial fish food containing 25% crude protein. The diet was daily provided at 3% of body weight as described by Eurell et al. (1978). The daily amount of food was offered on two occasssions over the day at 9 AM and 3 PM.

Bacterial and Yeast strains

The *A. hydrophila* standard strain *and Candida albicans* strain was kindly supplied by the Dept. of Poultry and Fish Diseases Dept., Fac. of Vet. Med., Alexandria University.

Immunostimulant used

Algae-flora**:** It is used as a kind of feed additives (Growth promotor) which characterized by a high content of protein, carbohydrate and minerals in addition to its high energy sources.

The experimental design

The experiment extended to 4 weeks, the fish were about 120 *Oreochromis niloticus* (30 ± 5 g body weight) divided into 4 groups of fish:

Table (1): Experimental design of the experiment.

Treatment group	No.of fish
0.1 Algae-flora/Kg	30
0.2 Algae-flora/Kg	30
0.3 Algae-flora/Kg	30
Control	30

Blood sampling

At zero day, 1st, 2nd, 3rd and 4th week during the experimental period, 2 ml blood samples were collected from different groups via the caudal vessels from 2 fish using disposable syringe (Hawk et al., 1965).

Some parameter of whole blood

The estimated parameters of whole blood include RBCs count, Total WBCs count.

Total RBCs and total WBCs count They were determined by haemocytometer according to Lucky (1977).

Differential leucocytic count

Blood film was taken and prepared according the method described by *Lucky (1977)*. Determination of phagocytic activity and phagocytic index:

Phagocytic activity was determined according to Kawahara *et al.* (1991).

Antibody titration against Aeromonas hydrophila

Detection of immune response to *A. hydrophila* was evaluated by microagglutination (MA) test according to the method described by Badran (1990) .

Challenge test

After 28 days post-immunization both of injected with bacterin and control groups were injected with 0.2 ml of virulent strain of *A. hydrophila* 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) .

The potency of bacterin was examined by calculating the relative level of protection (RLP) by the following formula According to Newman and Majnarich (1982).

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

Pathological studies

Following complete necropsy after 8 weeks of chronic experiment, fresh specimens from liver, spleen and kidneys of both *O. niloticus* and monosex tilapia were harvested and rapidly fixed in 10% neutral buffered formalin. according to the method described by Culling (1983).

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) .***

RESULTS

Effect of different treatments of Algae-flora on differential leucocytic counts

The results in Table (2) showed that there is a significant differences of the level of Lymphocyte and monocyte, among different Algae-flora levels at different weeks.

Table (2): ***Effect of different treatments of Algae-flora on differential leucocytic counts at different weeks of experiment.***

`	Treatment group	N	Lymphocyte	Monocyte	TWBCs
			Mean Std. Error	**Mean Std. Error**	**Mean Std. Error**
1st Week	0.1 Algae-flora/Kg	3	A 46.67±0.33	B 1.33±0.33	C 20.00±0.58
	0.2 Algae-flora/Kg	3	B 45.00±0.58	A 1.67±0.33	B 20.33±0.33
	0.3 Algae-flora/Kg	3	A 46.67±0.33	A 1.67±0.33	A 21.33±0.33
	Control	3	C 43.67±0.33	B 1.33±0.33	D 19.67±0.33
2nd Week	0.1 Algae-flora/Kg	3	C 41.67±0.33	C 1.33±0.33	A 21.33±0.33
	0.2 Algae-flora/Kg	3	A 43.67±0.33	A 2.33±0.33	B 19.67±1.20
	0.3 Algae-flora/Kg	3	B 42.67±1.76	B 1.67±0.33	A 21.33±0.88
	Control	3	D 40.00±0.58	C 1.33±0.33	B 19.67±0.33
3rd Week	0.1 Algae-flora/Kg	3	C 43.00±0.58	B 1.67±0.33	D 19.00±0.58
	0.2 Algae-flora/Kg	3	B 43.33±0.88	B 1.67±0.33	C 19.33±0.33
	0.3 Algae-flora/Kg	3	A 44.00±1.53	A 2.00±0.58	A 20.67±0.33
	Control	3	D 41.00±0.58	C 1.33±0.33	B 19.67±0.88
4th Week	0.1 Algae-flora/Kg	3	B 42.67±0.33	A 2.33±0.33	B 20.67±0.33
	0.2 Algae-flora/Kg	3	A 44.33±0.88	A 2.33±0.33	C 20.00±0.58
	0.3 Algae-flora/Kg	3	B 42.67±1.45	A 2.33±0.33	A 22.33±0.33
	Control	3	C 40.67±0.33	B 1.67±0.33	C 20.00±0.58

For each week: Means within the same column of different letters are significantly different at (P < 0.01).

The level of lymphocyte showed progressive decrease from the first week to the 2nd and 3rd week of the experiment but its level improved at the 4th weeks.

The higher level of Algae-flora 0.3 Algae-flora/Kg showed a higher level of lymphocyte among all periods followed by 0.2 Algae-flora and the lower level of lymphocyte showed in

Table (2): ***Continued.***

`	Treatment group	N	TRBCs	PA	PI
			Mean Std. Error	Mean±Std. Error	Mean Std. Error
1st Week	0.1 Algae-flora/Kg	3	B 1.77±0.03	C 21.00±0.58	A 2.00±0.06
	0.2 Algae-flora/Kg	3	A 2.07±0.03	B 21.33±0.88	B 1.97±0.03
	0.3 Algae-flora/Kg	3	A 2.03±0.09	A 23.33±0.33	A 2.07±0.07
	Control	3	B 1.67±0.03	D 20.00±0.58	B 1.73±0.09
2nd Week	0.1 Algae-flora/Kg	3	B 1.70±0.06	C 20.00±0.58	B 1.80±0.06
	0.2 Algae-flora/Kg	3	A 2.00±0.06	C 20.00±0.58	B 1.87±0.03
	0.3 Algae-flora/Kg	3	B 1.67±0.03	A 21.33±0.33	A 2.07±0.03
	Control	3	B 1.60±0.06	B 20.33±0.33	C 1.57±0.03
3rd Week	0.1 Algae-flora/Kg	3	A 1.90±0.15	D 18.33±0.33	B 1.63±0.03
	0.2 Algae-flora/Kg	3	B 1.77±0.03	B 19.67±0.33	A 1.70±0.00
	0.3 Algae-flora/Kg	3	C 1.63±0.03	A 20.33±0.33	A 1.73±0.03
	Control	3	C 1.57±0.03	C 18.67±0.33	B 1.57±0.03
4th Week	0.1 Algae-flora/Kg	3	AB 1.83±0.07	B 21.33±0.33	BC 1.87±0.12
	0.2 Algae-flora/Kg	3	B 1.73±0.27	A 21.67±0.67	B 1.93±0.07
	0.3 Algae-flora/Kg	3	A 1.93±0.12	B 21.33±0.33	A 2.30±0.06
	Control	3	B 1.67±0.03	C 19.67±0.33	C 1.80±0.06

For each week: Means within the same column of different letters are significantly different at (P < 0.01).

0.1 Algae-flora and all of them improve the lymphocyte level than the control group that not take any Algae-flora in the diet.

The monocyte level showed a higher level in the groups treated with Algae-flora than that of the control groups at all periods of the experiment, and its level in the 1st week of the experiments lower than of the last weeks (3rd and 4th weeks) and its level increased with the 0.3 Algae-flora followed by 0.2 Algae-flora then 0.1 Algae-flora and all of them higher than the control group.

Effect of different treatments of lgae-flora on WBCs and TRBCs

Table (2) showed that, there is a significant ($P < 0.01$) effect of different treatments with Algae-flora at different weeks of experiment on TWBCs and TRBCs.

The level of TWBCs showed a higher level in the 1st week then decreased in the 2nd and 3rd week then it increased at the 4th week of the experiment.

The level of TRBCs showed a higher level at the 1st weeks then it decreased progressively but at the 4th week it increased . The level of TRBCs in the groups take 0.3 Algae-flora followed by 0.1 Algae-flora, then 0.2 Algae-flora and all of them higher than that of the control group.

Effect of different treatments of Algae-flora on Phagocytic activity and phagocytic index

Table (2) showed that, there is a significant ($P < 0.01$) effect of different treatments with Algae-flora at different weeks of experiment on Phagocytic activity (PA) and Phagocytic index (PI).

The phagocytic activity showed increasing of its level at the 1st and 4th week which showed the maximum level of Phagocytic activity while, at the 2nd and 3rd weeks showed the lower level of PA.

The phagocytic index showed a higher level at the 1st week and decreased rapidely till the 4th week which showed the lowest level of phagocytic index.

Effect of different treatments of Algae-flora on antibody titer

Table (3) showed that, there is a significant ($P < 0.01$) effect of different treatments with Algae-flora at different weeks of experiment on antibody titer level. The results indicated that, the antibody titer increased progressively from the 1st week to the 4th week of the experiment. The maximum antibody

Table (3): Effect of different treatments of Algae-flora on antibody titer (value= log 10 antibody titer) at different weeks of experiment.

	Treatment group	N	Antibody titer (Log 10) Mean Std. Error
1st Week	0.1 Algae-flora/Kg	3.00	C 0.42±0.06
	0.2 Algae-flora/Kg	3.00	B 0.52±0.04
	0.3 Algae-flora/Kg	3.00	A 0.63±0.03
	Control	3.00	D 0
2nd Week	0.1 Algae-flora/Kg	3.00	B 0.52±0.04
	0.2 Algae-flora/Kg	3.00	B 0.52±0.04
	0.3 Algae-flora/Kg	3.00	A 0.56±0.04
	Control	3.00	C 0
3rd Week	0.1 Algae-flora/Kg	3.00	B 0.56±0.04
	0.2 Algae-flora/Kg	3.00	C 0.52±0.04
	0.3 Algae-flora/Kg	3.00	A 0.63±0.03
	Control	3.00	D 0
4th Week	0.1 Algae-flora/Kg	3.00	A 0.63±0.03
	0.2 Algae-flora/Kg	3.00	B 0.42±0.06
	0.3 Algae-flora/Kg	3.00	A 0.63±0.03
	Control	3.00	C 0.00

The Anti-body titer of control group = zero

titer observed at the 4th week of the experiment.

The groups that treated with 0.3 Algae-flora/kg followed by 0.1 and 0.2 Algae-flora/kg , while the minimum level of antibody titer observed in the control group of the experiment.

Effect of different treatments of Algae-flora on mortality percentage and relative level of protection

Table (4) showed that, there is a significant ($P < 0.01$) effect of different treatments with Algae-flora at different weeks of experiment on antibody titer level.

The results indicated that, the mortality percentage reached to its maximum percentage in control non-vaccinated groups followed by vaccinated group and control vaccinated group. While, the vaccinated groups showed lower level of mortality percentage.

The RPL showed that maximum protection observed in the vaccinated groups followed by control vaccinated group and the lower protection level observed in control-non-vaccinated groups.

The Histopathological findings indicated that there was hyper activation of melanomacrophage center in kidney, and spleen in case of fish groups fed on Algae-flora supplemented feed than fish groups fed on basal diet and the administration ofimmunostimulants to fish showed activation of melanomacrophage centers in spleen and kidney. Also, by histopatholgoical study in the liver and kidney sections showed the normal

Table (4): Relative level of protection (R.L.P) among vaccinated and non vaccinated fish groups.

Treatment group	Mortality percentage	R.P.L
30 fish with 0.1 Algae-flora/Kg Vaccinated group	33.33±5.8 B	1- 3/9 = 6/9 = 66.67 %
30 fish with 0.2 Algae-flora/Kg Vaccinated group	22.23±7.9 C	1-2/9 = 7/9 = 77.77 %
30 fish with 0.3 Algae-flora/Kg Vaccinated group	22.23±6.8 C	1-2/9 = 7/9 = 77.77 %
20 fish with-out Algae-flora Control vaccinated	33.34±9.11 B	1 – 3/9 = 6/9 = 66.67 %
20 fish Control non-vaccinated	100±10.11A	0
130 fish		

Means within the same column carrying different letters are significantly different at (P < 0.01).

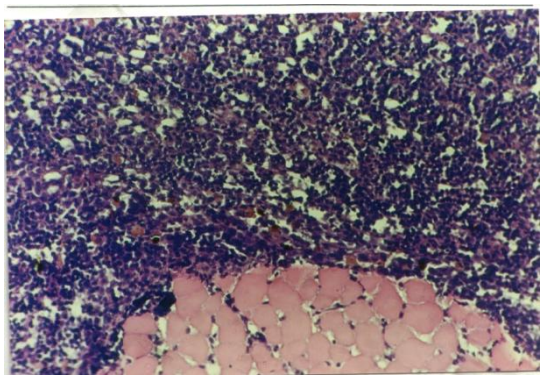

Fig. (1): Anterior kidney of ***O.niloticus*** treated with ***Aquagrow*** ® 0.1gm/kg at 1st wk.: showing marked proliferation of the lymphoid elements. H, E. (X 250).

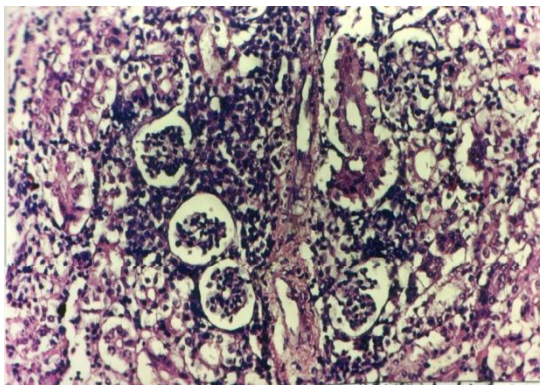

Fig. (2): Posterior kidney of ***O.niloticus*** treated with ***Aquagrow*** ® 0.1gm/kg at 2nd wk. ***:*** showing Proliferation of the interstitial lymphoid elements. H, E. (X 160).

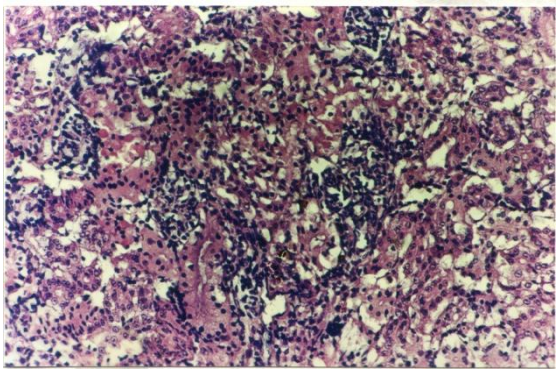

Fig. (3): Posterior kidney of ***O.niloticus*** treated with ***Aquagrow*** ® 0.1gm/kg at 2nd wk.: showing slight proliferation of the interstatial lymphoid elements. H, E. (X 160).

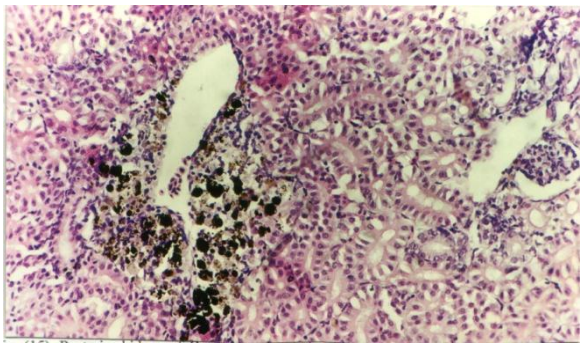

Fig. (4): Posterior kidney of ***O. niloticus*** treated with ***Aquagrow*** ® 0.2 gm/kg at 1st wk.: showing hyperactivation of the melanomacrophage centers. Note that the melanin pigment was dark black due to great concentration of the pigment.H, E. (arrows). (X 160).

architecture with mild residual microvesicular steatosis and also, the normal hepatocytes and kidney cells with reduced of hepatic damage.

DISCUSSION

Various algae are receiving attention as possible alternative protein sources for cultured fish, particularly in tropical countries and Egypt, because of their relatively high protein content and production rate ***(Nakagawa and Montgomery, 2007).*** Seaweeds are considered as rich sources of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities not only against human pathogens but also against fish pathogens ***(Liao et al., 2003).***

The level of **TRBCs** showed a higher level at the 1st weeks then it decreased progressively but at the 4th week it increased . The level of TRBCs in the groups take 0.3 aqua followed by 0.1 Algae-flora, then 0.2 Algae-flora and all of them higher than that of the control group.

This results agreed with those of ***(El-Tawil, 2010),***where they reported that the algae contain a different minerals and vitamins that improve the growth and immunity of the fish.

The level of **TWBCs** showed a higher level in the 1st week then decreased in the 2nd and 3rd week then it increased at the 4th week of the experiment. The level of TWBCs showed a higher level at 0.3 Algae-flora/Kg , then 0.2 Algae-flora/kg and 0.1 Algae-flora/kg and all of them of

higher TWBCs than the control groups. Our results showed that, the level of **lymphocyte** decreased progressively from the first week to the 2nd and 3rd week of the experiment but its level improved at the 4th weeks. The higher level of Algae-flora 0.3 Algae-flora/Kg showed a higher level of lymphocyte among the all periods followed by 0.2 Algae-flora and the lower level of lymphocyte showed in 0.1 Algae-flora and all of them improve the lymphocyte level than the control group that not take any Algae-flora in the diet.

The **monocyte** level showed a higher level in the groups treated with Algae-flora than that of the control groups at all periods of the experiment, and its level in the 1st weeks of the experiments lower than of the last weeks (3rd and 4th weeks) and its level increased with the 0.3 Algae-flora followed by 0.2 Algae-flora then 0.1 Algae-flora and all of them higher than the control group. This results indicated that the higher level of Algae-flora increased the protection of the fish against bacterial infection through increasing immunity of the fish via increasing the WBCs, lymphocyte, monocyte, neutrophils. This results attributed to the Algae-flora algae contain protein, fatty acids and the algae is a good source of energy which can used to prevent any stress exposed to it fish . This results agreed with those of ***(Yung-Tse et al., 2010)*** where they reported that, the Algae-flora algae is agood source of energy for the fish that can increased vitality of the fish against any stress. The **phagocytic activity and index** showed increasing of its level at the 1st and 4th week which showed the maximum level of Phagocytic activity while, at the 2nd and 3rd weeks showed the lower level of PA. The 0.3 Algae-flora/kg treated fish showed a maximum phagocytic activity followed by 0.2 and 0.1 which showed the same level of phagocytic activity and the control group showed the lower level of phagocytic activity. ***Buck and Finlay (1979)*** reported that the suppressive effect of corticoids is due to enhanced production of certain factors by the macrophagesthemselves (e.g. α-2 macroglobulin) which suppress other macrophage products. The **antibody titer** increased progressively from the 1st week to the 4th week of the experiment. the maximum antibody titer observed at the 4th week of the experiment. The groups that treated with 0.3 Algae-flora/kg followed by 0.1 and 0.2 Algae-flora/kg , while the minimum level of antibody titer observed in the control group of the experiment.

Our results agreed with those of ***(Evenberg et al., 1986)*** who reported that, liver damage and anorexia and non specific proteolysis decrease

antibody titer against fish diseases ***(Ellis, 1981)***. The results indicated that the **mortality percentage** reached to its maximum percentage in control non-vaccinated groups followed by vaccinated group and control vaccinated group. While, the vaccinated groups showed lower level of mortality percentage. This results attributed to the algae contain a different minerals and vitamins that improve the immunity of the fish. Also contain some bioactive substances that act as antibiotic against different diseases affecting fish. ***(Nakagawa and Montogomry, 2007).*** The **RPL** showed that, maximum protection observed in the vaccinated groups followed by control vaccinated group and the lower protection level observed in control-non-vaccinated groups. This results indicated that, the Algae-flora at a higher level improve the immunity and protection of the fish against different diseases. This results attributed algae contain a substances as bioactive substances that elevated the phagocytosis and protection of the fish against different fish diseases ***(Ergun et al., 2008).***

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تأثير بعض إضافات الأعلاف على الحالة المناعية فى الأسماك

رياض حسن خليل ــ طلعت طلعت سعد ــ طلال عبد اللطيف أبو سليمة

قسم أمراض الدواجن والأسماك ــ كلية الطب البيطرى ــ جامعة الإسكندرية

زادت أهمية الاسماك فى الآونة الاخيرة فى حل الازمة الغذائية التى تعانى منها مصر ولذلك تم إستخداث بعض إضافات الاعلاف التى يدخل فيها بعض النباتات ذات الاصل البحرى ومنها الطحالب البحرية .

لذلك أجريت هذه الدراسة لمعرفة مدى تأثير الطحالب البحرية على الحالة المناعية فى الاسماك وذلك من خلال تاثير تلك الاضافات على كرات الدم ابيضاء و أنواعها (الخلايا الليمفاوية ، و الخلايا أحادية الخلية ، الخلايا القاعدية ، الحامضية و المتعادلة) ، كرات الدم الحمراء ، نسبة الهيموجلوبين ، و معدل الخلايا المنضغطة(، و مدى تأثير تلك الاضافات على معدل النشاط الالتهامى ومدلوله.

أيضا مدى تأثير تلك الاضافات على إنزيمات الدم مثل الـــــ GOT , GPT و مدى تأثيرها على جلوكوز ودهون الدم الثلاثية ، أيضا مدى تأثيره على بروتينات الدم مثل (الجلوبيولين ، الالبيومين و إجمالى نسبة البروتين والنسبة بين الالبيومين و الجلوبيولين) أيضا مدى تأثيرها على المضادات البكتيرية و مستواها ومعدل نشاط الانزيمات المضادة للبكتيريا ومدى تأثير تلك الاضافات على معدل الحماية ومدى تأثيرها على تقليل نسبة النفوق فى الاسماك.

أجريت هذه الدراسة على عدد ٢٥٠ سمكة من البلطى النيلى بحالة صحية جيدة (متوسط وزنها (٣٠ جرام ± ٥ جرام) حيث تم تجميعها من مزارع قطاع خاص و تم نقلها الى معمل الاسماك بكلية الطب البيطرى – جامعة الاسكندرية فى أكياس تحتوى على (٣/٢) هواء .

إضافات الاعلاف التى تم إستخدامها تمثلت فى الاكوا فلو (المحفز للنمو) والذى يحتوى على طحالب و بكتريا نافعة و محفزة للنمو و بروتينات و مصادر عالية لامداد الاسماك بالطاقة).

تصميم التجربة :

تم تصميم التجربة بحيث تحتوى على أربعة مجموعات :

١- المجموعة الاولى: تم تغذيتها على العليقة الاساسية مضافا اليها الاكوا فلو ٠,١ /كجم .

٢- المجموعة الثانية : تم تغذيتها على العليقة الاساسية مضافا اليها الاكوا فلـــو ٠,٢ /كجم .
٣- المجموعة الثالثة : تم تغذيتها على العليقة الاساسية مضافا اليها الاكوا فلـــو ٠,٣ /كجم .
٤-المجموعة الرابعة : غذيت على العليقة الاساسية فقط ولـــم تأخـــذ أيـــة إضـــافات أعلاف.
إستمرت التجربة لمدة أربعة أسابيع.

وخلصت الدراسة ألى انتائج الآتية:-

١-أن إضافة الاكوا فلو (مصدر جيد للبروتين ، و الكربوهيدرات و الاملاح) كما أنها مصر جيد للطاقة.
٢-الاكوا فلوا تركيبه الاساسى يعتمد على الطحالب و هى مصدر جيد لزيادة المناعة فى الاسماك حيث تؤدى إلى:

-تحسن فى كرات الدم ابيضاء و أنواعها (الخلايا الليمفاوية ، و الخلايا أحادية الخلية ، الخلايا القاعدية ، الحامضية و المتعادلة).

-تحسن فى كرات الدم الحمراء ، نسبة الهيموجلوبين ، و معدل الخلايا المنضغطة.

-تحسن و زيادة فى معدل النشاط الالتهامى ومدلوله.

-تحسن فى مستوى إنزيمات الدم مثل الـــــ GOT , GPT .

-تحسن فى مستوى جلوكوز ودهون الدم الثلاثية.

-تحسن فى مستوى بروتينات الدم (الجلوبيولين ، الالبيومين و إجمالى نسبة البروتين والنسبة بين الالبيومين و الجلوبيولين).

-تحسن فى مستوى المضادات البكتيرية و مستواها ومعدل نشاط الانزيمات المضادة للبكتيريا.

-تحسن فى معدل الحماية ومدى تأثيرها على تقليل نسبة النفوق فى الاسماك.

٣-أوضحت الدراسة أيضا أن التركيزات العالية من الاكوا فلو (٠,٣ / كجـــم مـــن العليقة) يعطى نتائج أفضل من التركيزات الصغيرة ٠,١ ، ٠,٢ / كجم من العليقة.