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Evaluation of Immunomodulatory Effects of Some Probiotics on Cultured *Oreochromis niloticus*

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ABSTRACT

Improved resistance against infectious diseases can be achieved by the use of probiotics. The objective of the present study was to evaluate the influence of a probiotic AG Flow on the immune response of *O. niloticus*. The experimental fish were divided into three groups, the first group was fed on diet supplemented with dead Saccharomyces cerevisae yeast 1kg/ton feed, the second group was fed on diet supplemented with Saccharomyces Cerevisae 2kg /ton and the third group was served as control fed on probiotic-free diet. Sex weeks later the results indicated that, the fish groups which received diet supplemented with 2 kg probiotic revealed significant increase in non specific immune response as detected in vitro phagocytic activity test. Histologically, the spleen and liver showed great activation of Melano-macrophage centers and kupffer cells. The probiotic fed fish groups showed high resistance to the challenged pathogenic *Aeromonas hydrophila* . *Saccharomyces cerevisiae (yeast)* is a good source for feed (protein, carbohydrate and minerals) and good energy source . Ag flo is a derivatives of *Saccharomyces cerevisiae* and used mainly as a feed additives. *S. cerevisiae* improving the level of (TWBCs, TRBCs) by higher doses 2 kg/ton of Saccharomyces cerevisiae (yeast) than the lower dose 1 kg/ton of *S. cerevisiae. S. cerevisiae* improves the differential leucocytic counts (Lymphocyte, monocyte, basophils, eosinophils and neutrophils) in the blood of the treated fish with a higher doses of *S. cerevisiae* . The antibody titer against some pathogenic diseases at a higher level of *S. cerevisiae* feed additives. Decrease the incidence of mortality rate and improving the relative level of protection against different fish diseases at a higher level of *S. cerevisiae* feed additives. Improving the immunological producing cells in different organs at a higher level of *S. cerevisiae* feed additives.

Keywords: Probiotics, phagocytic assay, phagocytic index and challenge test

INTRODUCTION

The most widely quoted definition was made by ***Fuller (1989)*** He defined a probiotic as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance". This definition is still widely referred to, despite continual content ion with regard to the correct definition of the term. ***Verschuere et al. (2000)*** defined that probiotics 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.

MATERIALS AND METHODS

III. I. Materials and Methods

Fish

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

Aquaria

Fish were kept in 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, supplied with cholrine 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.

Immunostimulant used

AG flo (*Saccharomyces cerevisiae* (yeast)) it is a product of Agresearch company – American company: 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 6 weeks, the fish were about 120 *Oreochromis niloticus* (30 ± 5 g body weight) divided into 3 groups of fish:

Table (1): ***Experimental design of the experiment.***

Weeks	Treatment group
1st Week	Ag flo 1Kg/Ton Ag flo2 Kg/Ton Control
2nd Week	Ag flo 1Kg/Ton Ag flo 2 Kg/Ton Control
3rd Week	Ag flo 1Kg/Ton Ag flo2 Kg/Ton Control
4th Week	Ag flo 1Kg/Ton Ag flo2 Kg/Ton Control
5th Week	Ag flo 1Kg/Ton Ag flo2 Kg/Ton Control
6th week	Ag flo 1Kg/Ton Ag flo2 Kg/Ton Control

Blood sampling

1st, 2nd, 3rd, 4th , 5th and 6th 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).

Differential leucocytic count

1. Blood film was taken and prepared according the method described by Lucky (1977).

2. Total RBCs and total WBCs count : They were determined by haemocytometer and counted in cupic milimeter according to Lucky (1977).

Antibody titration against A. hydrophila

Detection of immune response to *A. hydrophyla* 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:

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

According to *Newman and Majnarich (1982).*

RESULTS

1-Effect of different treatments of ag flow on differential leucocytic counts

Table (2) : ***Effect of different treatment groups on differential leucocytic count and leucocytic transformation test among different weeks of experiment.***

Weeks	Treatment group	N	Lymphocyte%	Monocyte%	Basophils %	Eosinophils %
			Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error
1st Week	Ag flo 1Kg/Ton	3	B% 36.33±0.33	A% 1.67±0.33	A% 2.33±0.33	A% 6.33±0.33
	Ag flo2 Kg/Ton	3	A 39.33±0.33	B 3.33±0.33	A 2.33±0.33	A 6.33±0.33
	Control	3	C 35.33±0.33	B 1.33±0.33	B 1.33±0.33	A 6.33±0.33
2nd Week	Ag flo 1Kg/Ton	3	B 36.67±0.33	B 2.33±0.33	A 1.67±0.33	C 5.33±0.33
	Ag flo 2 Kg/Ton	3	A 40.67±0.33	A 3.00±0.58	B 1.00±0.58	A 6.33±0.33
	Control	3	B 36.33±0.33	B 2.33±0.33	A 1.67±0.67	B 6.00±0.58
3rd Week	Ag flo 1Kg/Ton	3	C 36.33±0.33	B 1.67±0.33	A 2.33±0.33	B 6.00±0.58
	Ag flo 2 Kg/Ton	3	A 40.33±0.33	A 3.33±0.33	B 2.00±0.58	B 6.00±0.58
	Control	3	B 38.67±0.33	C 1.00±0.58	C 1.67±0.33	A 6.33±0.33
4th Week	Ag flo 1Kg/Ton	3	A 40.00±0.58	B 2.33±0.33	A 2.67±0.33	C 5.67±0.33
	Ag flo 2 Kg/Ton	3	A 40.67±0.33	A 3.33±0.33	C 1.67±0.33	B 6.00±0.58
	Control	3	B 35.33±0.33	C 1.67±0.33	B 2.33±0.33	A 6.67±0.33
5th Week	Ag flo 1Kg/Ton	3	B 38.67±0.33	B 2.33±0.33	A 2.67±0.33	A 6.33±0.67
	Ag flo 2 Kg/Ton	3	A 41.33±0.33	A 3.33±0.33	C 2.00±0.58	A 6.33±0.33
	Control	3	B 37.00±0.58	C 2.00±0.58	B 2.33±0.33	A 6.33±0.33
6th week	Ag flo 1Kg/Ton	3	B 38.00±0.58	A 3.67±0.33	B 2.00±0.58	C 5.33±0.88
	Ag flo 2 Kg/Ton	3	A 42.33±0.33	A 3.67±0.33	C 1.67±0.33	A 6.00±0.58
	Control	3	C 36.67±2.33	B 1.67±0.33	A 2.33±0.33	B 5.67±0.33

For each week means within the same column of different letters are significantly different at ($P < 0.05$).

The results in Table (2) showed that there is a significant differences of the level of differential leucocytic counts of (Lymphocyte, monocyte, basophils, eosinophils, neutrophils) among different probiotics flow levels at different weeks.

The level of **lymphocyte** showed progressive increase from the

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

Weeks	Treatment group	N	Neutrophils %	TWBCs10^3	TRBCs10^6
			Mean Std. Error	Mean Std. Error	Mean Std. Error
1st Week	Ag flo 1Kg/Ton	3	B% 53.33±0.33	B 20.67±0.33	B 1.43±0.03
	Ag flo2 Kg/Ton	3	C 48.67±0.88	A 21.67±0.33	A 1.63±0.03
	Control	3	A 55.67±0.67	C 19.33±0.33	C 1.27±0.03
2nd Week	Ag flo 1Kg/Ton	3	A 54.00±0.00	B 21.00±0.58	B 1.57±0.03
	Ag flo 2 Kg/Ton	3	C 49.00±0.58	A 23.00±0.58	A 1.80±0.06
	Control	3	B 53.67±0.33	C 19.00±0.58	C 1.33±0.03
3rd Week	Ag flo 1Kg/Ton	3	A 53.67±0.33	B 20.67±0.88	B 1.77±0.03
	Ag flo 2 Kg/Ton	3	C 48.33±0.33	A 21.33±0.33	A 2.00±0.06
	Control	3	B 52.33±0.33	C 18.33±0.33	C 1.63±0.03
4th Week	Ag flo 1Kg/Ton	3	B 49.33±0.67	B 20.67±0.33	B 1.77±0.03
	Ag flo 2 Kg/Ton	3	C 48.33±1.20	A 22.00±0.58	A 1.93±0.03
	Control	3	A 54.00±0.00	C 19.00±0.58	C 1.57±0.03
5th Week	Ag flo 1Kg/Ton	3	B 50.00±1.00	B 19.00±0.58	B 2.00±0.06
	Ag flo 2 Kg/Ton	3	C 47.00±1.53	A 21.33±0.33	A 2.03±0.03
	Control	3	A 52.33±1.20	C 17.67±0.33	C 1.80±0.15
6th week	Ag flo 1Kg/Ton	3	B 51.00±0.58	B 21.33±0.33	C 1.70±0.06
	Ag flo 2 Kg/Ton	3	C 46.33±0.33	A 22.00±1.15	A 1.90±0.12
	Control	3	A 53.67±2.96	C 19.67±0.33	B 1.70±0.15

For each week means within the same column of different letters are significantly different at ($P < 0.05$).

first week to the 6th weeks of the experiment.

The higher level of lymphocyte of probiotics ag flo 2 Kg/ ton showed a higher level of lymphocyte among the all periods followed by 1 Kg/ton of Ag flo and both of them showed a higher level of lymphocytes than that of the control group.

The monocyte level showed a

higher level in the groups treated with 2 Kg/ton followed by 1 kg/ton of Ag flo and the level of the monocytes in both treatment groups higher than that of the control group. The results also showed progressive increase of the monocytes level from the first week to the 6th week of the experiment.

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

The level of TWBCs showed a higher level in the end week in the group that treated with 2 Kg/ton probiotics flow. In general the level of TWBCs increased in the 1st three weeks and after that decreased in the 3rd and 4th weeks then it increased and returned to its normal level.

The level of TWBCs increased with 2 Kg/ton probiotics flow and, followed by 1 Kg/ton and both of them higher than that of the control group.

The level of TRBCs showed a lower level at the 1st , 2nd 3rd weeks, but at the 5th and 6th weeks of experiment it increased and the level of TRBCs showed a higher level in the groups treated with 2 Kg/ton , followed by 1 Kg/ton and all of the higher than the control groups.

2-Effect of different treatments of Ag flo on antibody titer.

Table (3) showed that, there is a significant ($P < 0.01$) effect of different treatments with Ag flo at different weeks of experiment on antibody titer level.

Table (3): ***Effect of different treatment on antibody titer among different weeks of experiment.***

Week	Treatment group	Mean ± Std. Error
1st week	**Ag flo 1Kg/Ton**	**3.33±0.33 F**
	Ag flo 2 Kg/Ton	**4.67±0.33 C**
2nd Week	**Ag flo 1Kg/Ton**	**3.33±0.33 F**
	Ag flo 2 Kg/Ton	**5.33±0.33 B**
3rd Week	**Ag flo 1Kg/Ton**	**4.33±0.33 D**
	Ag flo 2 Kg/Ton	**5.33±0.33 B**
4th Week	**Ag flo 1Kg/Ton**	**4.00±0.58 E**
	Ag flo 2 Kg/Ton	**5.67±0.33 A**

For each week means within the same column of different letters are significantly different at (P < 0.05).

The results indicated that 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 2 Kg/ton Ag flo showed a higher antibody titer than the groups treated with 1 Kg/ton Ag flo and both of them higher than that of the control group.

8- Effect of different treatments of Ag flo on mortality percentage and relative level of protecti.

The results in Table (4) 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 level. 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 of immunostimulants to fish showed activation of melanomacrophage centers in spleen and kidney.

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 Ag flo 1Kg/Ton Vaccinated group	**3**	**1- 3/8 = 5/8 = 72.67 %**
30 fish with Ag flo 2Kg/Ton Vaccinated group	**2**	**1-2/8 = 6/8 = 77.77 %**
20 fish with-out Ag flo Control vaccinated	**4**	**1 – 4/8 = 4/8 = 50 %**
20 fish Control non-vaccinated	**8**	**0**
100 fish		

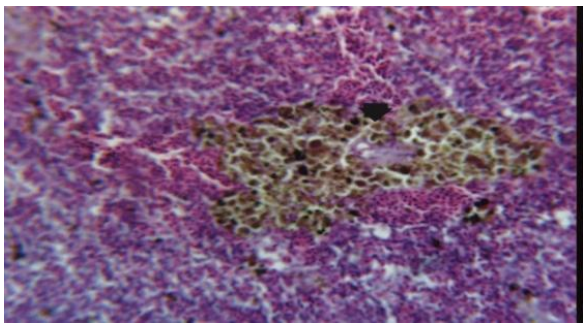

Photo. (1): Spleen of O.niloticus treated with Algae flow® 1kg/ton at 1st wk. showing excessive lymphpoiesis and hyperactivation of melanomacrophage centers (arrow). H , E. (X 160).

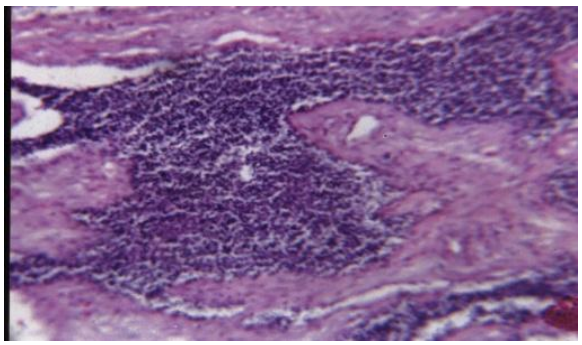

Photo. (2): Anterior kidney, O.niloticus treated with Alg flo® 1kg/ton at 2nd wk. showing marked dilatation of renal sinusoid with leukocytesprimary lymphocytes. H, E. (X 250).

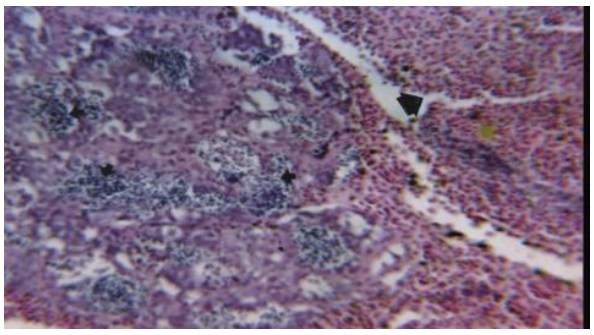

Photo. (3): Posterior kidney, O.niloticus treated with Alg flo® 1kg/ton at 3rd wk.showing lymphopoiesis (small arrows) and erythropoiesis (big arrow). H , E. (X 160).

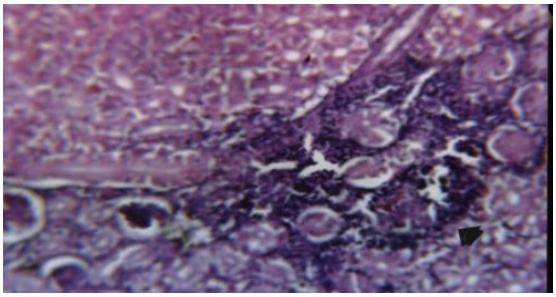

Photo. (4): Posterior kidney, O.niloticus treated with Alg flo® 1kg/ton at 4th wk.showing excessive intertubular lymphoid tissue (arrow). H, E. (X 160).

DISCUSSION

The use of natural immunostimulants in fish culture for the prevention of diseases is a promising new development and could solve the problems of massive antibiotic use. *Villamil, L.et al. (2003)* The choice of probiotic delivery may be influenced by certain factors related to the biology of the fish species, for example the time required from hatching to the start of exogenous feeding

This study was planned to evaluate the effect of *Saccharomyces cerevisiae* on the blood parameters and immune response of cultured O. niloticus. Concerning the effect of commercial products ag flo on the health status of O. niloticus, There is a significant effect of different treatment with *Saccharomyces cerevisiae* at different weeks of experiment on body weight and body weight gain . The results indicated that, the body weight gain increased progressively from the 1st to last week and the groups treated with 2 Kg/ton showed a higher body weight gain than the other treated groups with 1 Kg/ton and control group ,this contributes to better protein use for growth, a significant quality given that protein is the most expensive nutrient component of feed. Similar results were found by *Lara-Flores et al. (2003)* who reported that supplementing diets with *Saccharomyces cerevisiae* significantly improves protein utilization in tilapia.

The results indicated that the body weight increased progressively from the 1st week to the 6th week of the experiment.

The groups that treated with 2 /Kg *Saccharomyces cerevisiae* showed a higher body weight , followed by 1 Kg/ton and control groups.

Our results agreed with (Andlid et al., 1999) This is advantageous in probiotic preparations used for preventing disturbances in the normal microflora in the presence of antibacterial metabolites

I think this better results found in using supplemented diets suggests that the addition of *Saccharomyces cerevisiae* improved the digestibility of the diet and protein, *Saccharomyces cerevisiae* may stimulate appetite and improve nutrition by the production of vitamins, detoxification of compounds in the diet, and by the breakdown of indigestible components.

Also the results indicated a positive effect represented by significant increase in RBCs count and WBCs and differintial leukocytic count. These may be attributed to the fact that, the *Saccharomyces cerevisiae* used increased the blood parameter values as a result of hemopiotic stimulation. These results supported the results of Rajesh et al. (2006).

Also, the results indicated that, there is a significant effect of different treatment treatments with *Saccharomyces cerevisiae* at different weeks of experiment on TWBCs and TRBCs. The level of TWBCs showed a higher level in the 2nd week in the group that treated with 2 Kg/ton *Saccharomyces cerevisiae*. In general the level of TWBCs increased in the 1st three weeks and after that decreased in the 3rd and 4th weeks then it increased and returned to its normal level. The level of TWBCs increased with 2 Kg/ton *Saccharomyces cerevisiae* and, followed by 1 Kg/ton and both of them higher than that of the control group.

This results indicated that *Saccharomyces cerevisiae* improve immunity and phagocytic acyivity of fish. This results agreed with those of Amar et al. (2004) . The level of TRBCs showed a lower level at the 1st , 2nd 3rd weeks, but at the 5th and 6th weeks of experiment it increased and the level of TRBCs showed a higher level in the groups treated with 2 Kg/ton , followed by 1 Kg/ton and all of the higher than the control groups.

Much less work has been directed at the immunological enhancement of defense mechanisms of fish by probiotic bacteria or the protective mechanisms of probiotic bacteria in fish *(*Rengpipat, S. etal (1998). Also less work has been directed at the blood parameters.

Our results indicated that the level of lymphocyte showed progressive increase from the first week to the 6th weeks of the experiment.

The higher level of lymphocyte of observed in groups treated with *Saccharomyces cerevisiae* 2 Kg/ton among the all periods followed by 1 Kg/ton of *Saccharomyces cerevisiae* and both of them showed a higher level of lymphocytes than that of the control group.

The monocyte level showed a higher level in the groups treated with 2 Kg/ton followed by 1 kg/ton of *Saccharomyces cerevisiae* and the level of the monocytes in both treatment groups higher than that of the control group. The results also showed progressive increase of the monocytes level from the first week to the 6^{th} week of the experiment.

This previous results indicated that the higher level of *Saccharomyces cerevisiae* increased the protection of the fish against bacterial infection through increasing immunity of the fish via increasing the WBCs, lymphocyte and monocyte. These could be attributed to the different components of S. crevisiae in particularly the ²-glucan, that activate the phagocytic cells and melanomacrophages in the hemopiotic organs other than increasing the size of hemopiotic organs as confirmed in the histological examination and results of heptosomatic and splenosomatic indices. These results agreed with the results obtained by Jessus et al. (2002),who worked on yeast and reported the similar results and stated that, the activation mechanisms involved are known to be related to the carbohydrates derived from the yeast cell wall and ²-glucans added to the feed stimulated the phagocytic function and protection after challenge with pathogenic bacteria in some fish species .Moreover, the existence of ²-glucan receptor on the macrophage cell surface has been demonstrated in Atlantic salmon. The intake of *Saccharomyces cerevisiae* also increases the cellular immune response, including phagocytosis, repository burst and natural cytotoxic activity. This results attributed to the *Saccharomyces cerevisiae* contain protein, and the *Saccharomyces cerevisiae* is a good source of energy which can used to prevent any stress exposed to it fish.

These the results are in agreement with those of Peters et al (1988) who found elevated of glucose levels in plasma of Salmo *gairdneri* when injected with *Aermonas hydrophila* or add to water of aquarium.

This results indicated that the feeding of *Saccharomyces cerevisiae* to the fish improve the lysozyme activity and lowering the bacterial activity affecting fish. This results attributed to the good nutritive value of

the *Saccharomyces cerevisiae* and it consider as a good energy source to the fish and so it will improve the vitality of the fish. Our results indicated that, the antibody titer improved in fish fed a higher level of *Saccharomyces cerevisiae* than the other fish, this results attributed to *Saccharomyces cerevisiae* improved the activity of the liver and secreations of antibody in blood of the fish. Our results agreed with those of Smith, V. etal (2003). who reported that, liver damage and anorexia and non specific proteolysis decrease antibody titer against fish diseases Ronald, J. etal (2001). Content of *Saccharomyces cerevisiae* help in protection of fish from this dangerous effect of the stress. Also, there is a significant effect of different treatment with *Saccharomyces cerevisiae* at different weeks of experiment on mortality rate and relative level of protection this is advantageous in probiotic preparations used for preventing disturbances in the normal microflora in the presence of antibacterial metabolites. 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 level.this not agree with Waché et al. (2006*)* experiment, mortality was low and the survival within groups fed with *Saccharomyces cerevisiae* enriched food did not show any significant difference.

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تقييم التأثير المناعى لبعض روافع المناعة على البلطى النيلى المستزرع

رياض حسن خليل ــ طلعت طلعت سعد ــ يوسف العبد

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 إستخدام بعض إضافات الاعلاف التى يدخل فيها بعض النباتات ذات الاصل البحرى ومنها الطحالب البحرية .

لذلك أجريت هذه الدراسة لمعرفة مدى تأثير الطحالب البحرية علـــى الحالـــة 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 نشـــاط الانزيمات المضادة للبكتيريا ومدى تأثير تلك الاضافات على معدل الحمايـــة ومـــدى 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 خـــاص و تم نقلها الى معمل الاسماك بكلية الطب البيطرى – جامعة الاسكندرية فـــى أكيـــاس 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 مصـــادر عاليـــة لامداد الاسماك بالطاقة).

تصميم التجربة :

تم تصميم التجربة بحيث تحتوى على أربعة مجموعات :

١ -المجموعة الاولى: تم تغذيتها على العليقة الاساسية مضافا اليها الالجى فلو ١ كجم /طن.
٢ -المجموعة الثانية : تم تغذيتها على العليقة الاساسية مضافا اليها الالجى فلو ٢ كجم /طن.
٣ -المجموعة الثالثة : غذيت على العليقة الاساسية فقط ولم تأخذ أية إضافات أعلاف.

إستمرت التجربة لمدة أربعة أسابيع.

وخلصت الدراسة إلى النتائج الآتية:-

١ - أن إضافة الالجى فلو (مصدر جيد للبروتين ، و الكربوهيدرات و الاملاح) كما أنها مصر جيد للطاقة.

٢ - الالجى 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 الـــ 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 الالجى فلو (٢ كجم / طن من العليقة) يعطى نتائج أفضل من التركيزات الصغيرة ١ كجم / طن العليقة.