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EFFECT OF *ALOE VERA* ON GROWTH RATES AND IMMUNE RESPONSE OF *OREOCHROMIS NILOTICUS*

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ABSTRACT

In the present study, the growth parameters and immunostimulation were investigated in *Oreochromis niloticus* receiving diet supplemented with 1% crude ethanol *Aloe vera* extract and 1% *Aloe vera* gel compared with control fed on basal diet without any additives for 8 weeks. The results indicated significant increase in growth parameters namely weight gain (WG), Absolute growth rate (AGR), Specific growth rate (SGR), Feed conversion rate (FCR) and Feed efficiency rate (FER). Also an increase in non-specific immune response was detected in vitro phagocytic activity test with significant increase in total protein and globulin. The results also indicated an increase in anti-oxidant effect detected by catalase activity.

INTRODUCTION

Tilapia is one of the most productive and internationally traded food fish in the world **(Modadugu and Belen, 2004)**. They constitute the major protein source in many of the developing countries. It is the second most important farmed fish globally (next to carp) and described as the most important aquaculture species of the 21st century **(Shelton, 2002)**. From the production point of view the high stocking density in intensive fish culture systems has led to many drawbacks and among them is stress **(Gabriel and Akinrotimi, 2011)** which results in conditions such as, poor fish performance, alteration of physiological functions, poor digestion and feed utilization **(Pankhurst *et al.,* 2008) (Santos *et.al.,* 2010),** increased susceptibility to diseases **(Wu *et al.,* 2013)** poor fish meat quality **(Jittinandana *et al.,* 2003)**, and in extreme cases lead to mortality **(Akinrotimi *et.al.,* 2007) (McKenzie *et.al.,* 2012).** Fish pathogenic microorganisms are very serious to the economy of any aquaculture practice. The use of antibiotics for the prophylaxis and treatment of fish diseases leads to the development of antibiotic resistant for some bacterial strains, accumulation of residue in cultured fish and environmental problems. Therefore, immunotherapy is recently used to

prevent or treat fish diseases. Various immunostimulants including medicinal plants which they have found to be effective in fish. It has been found that the use of medicinal herbs in fish diets enhances the immune system against infections with various bacteria. Dietary medicinal plant extracts as immunostimulants increase non-specific defenses against pathogens during period of stress **(Masoud and Mostafa, 2014).** Some plants have anti-stress, growth promotion, appetite stimulation, tonic and immune-stimulation, and antimicrobial properties **(Citarasu, 2010; Chakraborty and Hancz, 2011; Ghosal and Chakraborty, 2014)**. *Aloe vera* (synonym: *Aloe barbadensis* Miller) belonging to the botanic family Liliaceae which is widely distributed in the tropical and subtropical regions of the world (**Mahdavi *et al.,* 2013).** *Aloe vera* is an amazing mixture of more than 200 constituents, including polysaccharides, enzymes, glycoproteins, amino acids, vitamins and minerals. The active polysaccharide fractions in aloe are called galacto-mannans or beta-glucomannans (also known as acemannans). Acemannan and the other constituents of *Aloe vera* have been found to improve macrophage activity as much as tenfold, to enhance macrophage effectiveness in modulating the entire immune system, in stimulating, producing, and releasing antibodies **(Jon Barron, 2002)**. Therefore, medicinal plants are getting importance in aquaculture diet due to their positive effects **(Masoud and Mostafa, 2014)**. The objective of this study is to clarify the effect of *Aloe vera* plant on growth and immune response of cultured *Oreochomis niloticus*.

MATERIAL AND METHODS

Fish:

Seventy-eight healthy *Oreochomis niloticus* with an average body weight of 26.97 ± 0.62 g was obtained from Ismailia. The fish were transported in tank supported with oxygen supply according to **(Jensen, 1990)**. Fish were acclimated in cylindrical plastic tanks (0.6m2 x 0.85m), supplied with 300 L of chlore-free tap water with continuous aeration. During the adaptation period, fish were fed twice daily with a commercial diet (30% crude protein). This work was done in Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Cairo University.

Glass aquaria:

Six fully prepared glass aquaria are used sized (100x50x30cm) and cylindrical plastic tank.

Preparation of Aloe Vera Extract:

The leaves were collected, confirmed by botanist and washed in sterile distilled water and cut into pieces. The leaves were separately shade-dried for 10 days till weight constancy was

achieved. The sample was powdered in an electric blender. The extract was prepared with the standard method of percolation according to **Haghighi *et al.*, (2014).**

Preparation of diets:

Experimental diets were formulated, of which, one was a control and two were supplemented with *Aloe vera* gel (it is the inner part of the *Aloe vera* plant) which can be obtained by cutting the external green rind of the plant carefully without contamination with yellow latex) and crude ethanol extract. 1% *Aloe vera* gel and 1% crude *Aloe vera* ethanol extract were added to the commercial diet.

Experimental design:

Seventy-eight *Oreochromis niloticus* were distributed into 6 glass aquaria in 2 dublicate (2 glass aquaria for each treated diet) at a stocking density of 13 fish /glass aquarium (Table 1). The fish were fed experimental diets as follow: Group 1 was fed on diet supplemented with 1% crude ethanol *Aloe vera* extract, Group 2 was fed 1% *Aloe vera* gel and group 3 were fed basal commercial food (control) for 2 months (60 days) at a rate of 3% of biomass. Dietary *Aloe vera* addition levels used in this study is a modification of those previously used according to available literature data. **(Mahdavi *et al.*, 2013 and Heidarieh *et al.*, 2013).** Throughout the experimental period continuous aeration Furthermore, the water in all 6 glass aquaria was exchanged with de-chlorinated freshwater to maintain the water quality during the study.

Table (1): Showing Oreochromis Nilotic us studied groups

Fish group	Fish No.	Treatment	Concentration	Feeding% /fish biomass
control	**26**	**Basal diet**	**-**	**3%**
Group I	**26**	**Crude ethanol *Aloe vera* extract**	**1%**	**3%**
Group II	**26**	***Aloe vera* gel**	**1%**	**3%**

Blood sampling:

At the end of the experimental period (60 days), blood samples were collected from the caudal blood vessels according to **(Black, 2000)**. The collected blood was divided into two portion the first portion was taken on 100 IU/ml Heparin for measuring of blood parameters and the phagocytic activity tests. The second portion was collected without anti-coagulant for serum separation to be used in measuring blood chemistry.

Fish growth and feed utilization performance:

Fish growth was evaluated in terms of weight gain (WG), absolute growth rate (AGR), specific growth rate (SGR), whereas feed utilization parameters included, food conversion ratio (FCR) and Feed efficiency ratio (FER). Body weight of all the fish in each tank is measured. Throughout the experiment, the amount of feed consumed in each replicate was noted. Calculations are carried out using the formulas according to **(Haghighi *et al.,* 2014).**

Haematological examinations:

A. Hematogram

a) Hemoglobin concentration (g/dl): Hemoglobin concentration was determined using the cyanomet-hemoglobin method according to **Stoskopf (1993)**.

b) PackedCellVolume(PCV%): Packed cellvolume was estimated by the micro-haematocrite method described by **Decie and Lewis (1991).**

c) Erythrocyte and leukocyte count: A manual method for counting using a hemocytometer counting chamber and Natt-Herrik solution was carried out according to **Stoskopf (1993)**.

d) Differential leukocytic count: The stained blood film was prepared. The relative and absolute count was estimated according to **Thrall (2004).**

B. Biochemical parameters

a) Alanine aminotransferase activity (ALT) and Aspartate aminotransferase activity (AST): Colorimetric determination of ALT and AST activity were performed according to **Reitman and Frankel (1957).**

b) Total proteins: Assay of total proteins was carried by a test kit according to biuret method described by **Weichselbaum (1946).**

c) Albumin: Serum samples from all experimental groups were estimated for albumin by a colorimetric method at wave length 550 nm according to **Dumas and Biggs (1972).**

d) Globulin: Globulin was calculated by mathematical subtraction of albumin value from total proteins.

e) Albumin / Globulin (A/G) Ratio: Albumin: Globulin ratio was calculated from data of albumin and globulin.

f) Creatinine: Serum samples from all experimental groups were estimated for creatinine by a colorimetric method at wave length 495 nm according to **Bartles *et al.* (1972)**.

Phagocytic activity test: according to Weeks and Warinner (1984).

Catalase (Serum catalase activity): according to **Goth (1991).**

Statistical analysis:

The data is analyzed by one-way analysis of variance (ANOVA) to determine the significant variations among the various parameters in the experimental groups. All of analysis is performed using the statistical package for the social sciences (SAS) computer software (version 6.2). **SAS (2000).**

RESULTS

Growth performance:

Growth and feed utilization of the fish fed dietary crude *Aloe vera* ethanol extract and *Aloe vera* gel were significantly improved compared to the control (Table 2). WG, AGR, and SGR significantly increased ($P < 0.05$) in both crude *Aloe vera* ethanol extract and *Aloe vera* gel compared to the control. Fish fed crude *Aloe vera* ethanol extract supplemented diet presented better FCR and ultimately high FER values; significant differences ($p < 0.05$) when compared to un-supplemented ones.

Table (2): Growth performance and feed utilization of *O. niloticus* fed dietary Aloe vera for 60 days.

Group / parameters	crude ethanol *Aloe vera* extract group	*Aloe vera* gel group	Control
WG	**22.12 ±1.93***	**19.42 ±1.85***	**13.65 ±0.80**
AGR	**0.37 ± 0.03***	**0.32 ± 0.03***	**0.23 ± 0.01**
SGR	**0.97 ± 0.05***	**0.94 ± 0.06***	**0.64 ± 0.04**
FCR	**2.69 ± 0.27***	**3.21 ± 0.48**	**4.5 ± 0.40**
FER	**0.47 ± 0.04***	**0.46 ± 0.04*.**	**0.25 ± 0.01**

Data are expressed as mean ± standard error (M ± SE). Values with * is significantly different (P < 0.05) from the control. Where, WG =weight gain, SGR = specific growth rate, AGR = absolute growth rate, FCR = food conversion ratio, and FER = Feed efficiency ratio.

Haematological Findings

Hematogram:

Dietary crude *Aloe vera* ethanol extract and *Aloe vera* gel supplemented diets revealed no significant ($p <0.05$) effect on red blood cell count (RBC), white blood cell count (WBC), differential leukocytes count (monocytes, lymphocytes and neutrophils), hematocrit (HCt), hemoglobin (Hb). All the values of red blood cell indices, the mean values of cell hemoglobin (MCH pg.), cell hemoglobin concentration (MCHC %), and cell hemoglobin volume (MCV

FL) except significant increase of PCV% and MCV of fish fed on crude ethanol extract (Table 3).

Table (3): The hematological parameters,WBC,RBC, Hct,Hb,MCH,MCV,MCHC, neutrophil, monocyte, and lymphocyte of O. niloticus fed with 1% crude Aloe Vera ethanol extract and 1% Aloe Vera gel in feed for 8 weeks.

	Crude ethanol extract group mean ± SE	A.gel group mean ± SE	Control group mean ± SE
HB (g/100ml)	**6.21 ± 0.10**	**6.67 ± 0.41**	**6.64 ± 0.30**
Htc %	**25.83 ± 1.91***	**24 ± 0.91**	**20.17 ± 0.98**
RBCs (X10^6 /mm3)	**1.33 ± 0.10**	**1.50 ± 0.09**	**1.39 ± 0.11**
MCV	**201.82 ± 23.74***	**165.03 ±16.06**	**148.83 ± 9.03**
MCH	**48.24 ± 3.62**	**45.37 ± 4.44**	**48.80 ± 2.21**
MCHC	**24.61 ± 1.29**	**27.79 ± 1.51**	**33.01 ± 0.82**
WBCs (X 10^3 /mm3)	**53.67 ± 6.08**	**65 ± 2.17**	**53.5± 2.14**
Heterophils %	**38 ± 6.04**	**40.67 ± 5.65**	**30 ± 4.27**
Heterophils (X 10^3 /mm3)	**20.91 ± 4.18**	**25.91 ± 3.10**	**28.13 ± 10.33**
Lymphocyte %	**60.33 ± 5.36**	**59.67 ± 5.00**	**69.33 ± 4.39**
Lymphocyte (X 10^3 /mm3)	**31.99 ± 3.78**	**39.17 ± 4.18**	**57.02 ± 11.02**
Monocyte %	**1.67 ± 0.87**	**1.33 ± 0.38**	**1.33 ± 0.38**
Monocytes (X 10^3 /mm3)	**0.76 ± 0.35**	**0.84 ± 0.24**	**1.35 ± 0.54**

Data are expressed as mean±SE (n=6).Values with * is significantly different (P < 0.05) from the control.

Biochemical parameters:

Protein profile, liver and kidney enzymes:

Crude *Aloe vera* ethanol extract and *Aloe vera* gel showed significant ($p<0.05$) effect in increase of total protein (TP), albumin (AL), and globulin (GL) compared to control group (Table 4). However, albumin/globulin ratio, liver enzymes (ALT and AST) and kidney function tests (urea and creatinine) were not exhibited significant differences in compared to control group ($p>0.05$; Table 4).

Table (4): Changes in the serum total protein, albumin, globulin, albumin/globulin ratio, creatinine, urea, and ALT and AST of O. niloticus after feeding with 1% crude *Aloe vera* ethanol extract and *Aloe vera* gel for 60 days.

	Crude *Aloe vera* ethanol extract group mean ± SE	***Aloe vera* gel group mean ± SE**	**Control group mean ± SE**
Total protein (g/100ml)	**3.63 ± 0.26***	**3.42 ± 0.17***	**2.57 ± 0.15**
Albumin(g/100ml)	**1.32 ± 0.13**	**1.38 ± 0.09***	**1.10 ± 0.05**
Globulin (g/100ml)	**2.31 ± 0.25***	**2.03 ± 0.20**	**1.47 ± 0.12**
A/G ratio	**0.63 ± 0.11**	**0.74 ± 0.11**	**0.78 ± 0.06**
Creatinine (mg/dl)	**0.47 ± 0.10**	**0.38 ±0.06**	**0.42 ± 0.02**
Urea (mg/dl)	**5.41 ± 0.51**	**5.01 ± 0.63**	**4.54 ± 0.06**
AST (U/L)	**71.83 ± 3.55**	**69.58 ± 4.17**	**60.17 ± 2.41**
ALT(U/L)	**70.5 ± 3.45**	**70.67 ± 2.74**	**62.83 ± 0.60**

Data are expressed as mean ±SE (n=6). *: P<0.05 is significant compared with the control.

Phagocytic assay:

The phagocytosis activity and phagocytic index were significantly increased in the fish groups fed on diet supplemented with crude *Aloe vera* ethanol extract and *Aloe vera* gel in comparison with the control group. These values are demonstrated in (Table 5) and the Fig. (1) Shows the phagocytic activity of blood monocytes to *Candida albican*.

Table (5): mean and standard error of phagocytosis and phagocytic index of different *O. niloticus* groups

	Crude *Aloe vera* ethanol extract group mean ± SE	***Aloe vera* gel group mean ± SE**	**Control group mean ± SE**
Phagocytic activity	**39.53 ± 0.58***	**39.55 ± 0.71***	**33.37 ± 0.22**
Phagocytic index	**1.84 ± 0.03***	**1.78 ± 0.04***	**1.44 ± 0.04**

Data are represented as means of six samples ± SE. Means. *: P<0.05 is significant compared with the control.

Catalase activity:

Catalase activity for fish supplemented with crude *Aloe vera* ethanol extract and *Aloe vera* gel shows significant increase compared to control (Table 6).

Table (6): Catalase activity for *O.niloticus* supplemented with 1% crude ethanol *Aloe vera* extract and *Aloe vera* gel.

	Crude *Aloe vera* ethanol extract group	*Aloe vera* gel group	Control
Catalase activity	**26.5 ± 0.06***	**26.25 ± 0.16***	**18.95 ± 0.07**

Data are expressed as mean ± standard error (M ± SE). *: P<0.05 is significant compared to control.

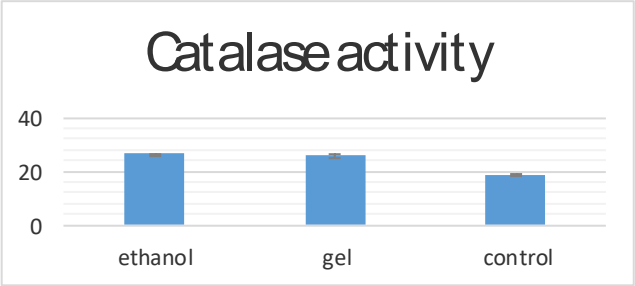

Fig. (2): Showing catalase activity of both fish supplemented with crude *Aloe vera* ethanol extract and *Aloe vera* gel compared to control.

DISCUSSION

Aloe vera is as old as civilization and throughout history it has been used as a popular folk medicine. Recently, it's potential to serve as an alternative growth promoter, anti-stressor, immunostimulant, appetizer and digestion stimulant have been reported in fish farming **Mehdi, (2010); Heidarieh *et al.*, (2013)**. The current study demonstrated that, the addition of crude *Aloe vera* ethanol extract and *Aloe vera* gel in tilapia diet markedly affect feed utilization, and ultimately growth performance as well as immunity compared to the control. From the results (Table 2) feed utilization parameters such as WG, AGR, SGR, FCR and FER are significantly increased for both fish fed on crude *Aloe vera* ethanol extract and *Aloe vera* gel when compared with the control. These results are in accordance with the findings of **Heidarieh *et al.* (2013)** who showed that *Aloe vera* inclusion level of 0.1 and 1% increased growth performance in rainbow trout (O. mykiss). That may be attributed to the wide range of immuno-nutrional ingredients such as proteins, lipids, vitamins, enzymes, minerals, sugar, lignin, saponin and salicylic acids **(Kemper and Chiou, 1999), (Adesuyi and Awosanya,**

2012) in *Aloe vera* supplemented diet could have enhanced the growth performance of the fish. Besides, the polysaccharides molecules such as Acemannan present in *Aloe vera* leaves are believed to possess prebiotic properties **(Ahmed and Hussain 2013)**. The improvement in feed utilization and growth performance could be associated with increased nutrient digestibility, absorption, and assimilation capacity, through improved digestive enzymes and healthy intestinal microflora fostered by the supplemented *Aloe vera*. A study by **Heidarieh *et al.,* (2013)** reported that *Aloe vera* improved gastrointestinal morphology of *Oncorhynchus mykiss* by increasing intestinal villus length and intestinal surface area, for increased food digestion and absorption capacity of the gut. Blood parameter tests (hematological and biochemical) have been used in aquaculture as an important tool to evaluate the health status of fish. Hematological parameters including RBC, Hb, Htc and their derivative indices such as MCV, MCH, and MCHC are particularly known to indicate erythropoiesis and oxygen carrying capability in fish **(Houston, 1997)**. WBC and a number of leukocytes together with biochemical parameters such as serum proteins (especially globulin) play a crucial role in fish innate immune response especially during stressful conditions (e.g. infections, dietary imbalance, high stocking density, and environmental stressors) **(Roberts,1978)**. In the present study, fish fed crude *Aloe vera* ethanol extract and *Aloe vera* gel supplemented diet did not show significant changes in hematological parameters compared to un-suplemented ones. Except there is increase in the Htc and MCV of fish fed crude *Aloe vera* ethanol extract compared to control dietary. *Aloe vera* in other studies elicited the same effects in Nile tilapia **(Dotta *et al.,* 2014**), and rainbow trout (O. mykiss) **(Haghighi *et al.,* 2014)**. While increase of MCV in fish fed diet supplemented with crude *Aloe vera* ethanol extract may be related that Polysaccharides in *Aloe vera* have been associated with increased erythropoiesis and subsequently MCV **(Channa *et al.,* 2014).** In this study there is significant increase in phagocytic activity and phagocytic index of fish supplemented with crude *Aloe vera* ethanol extract and *Aloe vera* gel compared to the control and this is similar to high phagocytic activities that reported in *O. mykiss* fed 1% *Aloe vera* supplemented diet **(Haghighi *et al.,* 2014),** which is an indication that oral administration of *Aloe vera* may provide some non-specific and specific immune response in fish. The present study indicated that serum total protein in dietary crude *Aloe vera* ethanol extract and *Aloe vera* gel supplemented fish significantly increased compared to the control. Similar findings were also reported in *O. mykiss* fed 1% *Aloe vera* supplemented diet **(Haghighi *et al.,* 2014)**. Also in this study fish

supplemented with 1% crude ethanol *Aloe vera* extract and *Aloe vera* gel show significant increase in catalase activity compared with un-supplemented one and that may be due to flavonoids, folic acid, and ascorbic acid present in *Aloe vera* leaves are known to act as antioxidants, which detoxify and eliminate highly unstable and reactive molecules; free radicals, which have the tendency to attack and damage normal cells of the body and cause a variety of health related problems **(Raa, 1996)**. This may help animals to easily recover from external stresses. The improvement of health indicative parameters in fish by medicinal plant extracts owes it to their plenteous bioactive compounds **(Goda, 2008, Stauth, 2007)**. The modes of action for many bioactive compounds to initiate immune responses are yet unknown, however **Davis *et al.* (1989)** explained that probably, they interact with specific receptors on cells surface and enhance the expression of intracellular genes encoding for antimicrobial molecules. This was further supported by **Picchetti *et al.* (2013)** who reported that, Aloe extract of 1.2 mg/mL, acted as a powerful immunostimulant in lipopolysaccharide (LPS), and activated Sparus aurata fibroblast SAF-1 cells, inducing a synergic effect on interconnected genes that involved in different aspects of the immune responses. Overall, the current study concluded good health growth and immune effects of dietary crude *Aloe vera* ethanol extract and *Aloe vera* gel in *O. niloticus*.

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