EFFECT OF SPINACH (*SPINACIA OLERACEA*) LEAVES POWDER AND VITAMIN E ON GROWTH PERFORMANCE AND NON-SPECIFIC IMMUNE RESPONSE OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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Abstract

The present study was conducted to evaluate the effect of Spinach leaves powder, vitamin E and their mixture on non-specific immunity and the growth performance of *Oreochromis niloticus*. A total of 180 apparently healthy *O. niloticus* were randomly divided into four equal groups. Fish fed on a basal diet with no additive and served as the control group (T1), fish fed a basal diet supplemented with spinach 1% (T2), fish fed a basal diet supplemented with 100 mg/kg vitamin E (T3) and fish fed a basal diet with their mixture (T4) for consecutive 12 weeks. Growth performance parameters and the body composition of the tested fish were examined. After the end of the experimental period, blood was sampled for determination of total proteins, serum Superoxide Dismutase enzyme (SOD) and serum lysozyme activity. All fish groups were then challenged intraperitoneally with a live virulent strain of *Aeromonas hydrophila* and the Relative percent of survival (RPS) were evaluated. The results revealed a significant difference was observed in the growth performance of *O. niloticus* within the treated groups when compared to the control group. The best results of growth performance and feed utilization were obtained in spinach and mixture diet groups .It was found that both Spinach and Vitamin E caused a substantial improvement of serum protein profile, lysozyme activity, Hematocrit percent, and SOD enzyme. Additionally, they caused reduction in the mortality in all groups compared to control one. It can be concluded that the incorporation of spinach *(Spinacia oleracea)* and vitamin E mixture in the diets of Nile tilapia can improve growth performance and non-specific immune response toward the emerging diseases.

Keywords: Medicinal herbs. Spinach. *Spinacia oleracea***.** Nile tilapia. Growth performance. Non - specific immunity. *Aeromonas hydrophila*.

INTRODUCTION

Fish farming is the principal form of aquaculture in the world and is considered one of the important food animal-producing sector (FAO, 2002). Immunostimulants are substances (drugs and nutrients) that stimulate the immune system by inducing activation or increasing activity of any of its components. The immunostimulants could increase the resistance of fish to infectious diseases by enhancing non-specific defense mechanisms (Sakai, 1999 and Yin *et al*., 2006). Many plant materials are widely used in aquaculture to preventing diseases by controlling the pathogenic bacteria and enhancing the immunity of fish (Nya and Austin, 2007).

In recent years, natural herbs have gained much attention to improving growth performance and immunity. Vitamin E is among the most important a nutrient enhancing the fish immune system, and the supply of vitamin E can reduce mortality and improve fish performance while increasing non-specific immune response (Ortuno *et al.,* 2001). In addition, vitamin E is a potent antioxidant that offers protection against oxidative damage to various fish tissues (Ortuno *et al*., 2001). Spinach (*Spinacia oleracea*) is an edible flowering plant in the family of Amaranthaceae (LeStrange *et al*., 1999).

Considerable evidence exists for the role of antioxidative constituents of fruits and vegetables in the maintenance of health and disease prevention (Ames *et al.,* 1993). Spinach (*Spinacia oleracea*) is one of the most important antioxidative vegetables and spinach leaves contain approximately 1,000 mg of total flavonoids per kilogram. The possible presence of flavonoid-like compounds in spinach was firstly reported by Weatherby and Cheng (1943). The use of vitamins as immunostimulant has been used in many fish including Atlantic salmon (Waagbo *et al*., 1992), rainbow trout (VerIhac *et al*., 1998).

Since Tilapia are the most economically important farmed fish species, it is necessary to recognize their dietary requirements and useful additives to improve growth performance and non-specific immunity. The aim of this study was to investigate the effect of dietary spinach leaves powder, vitamin E and

their mixture on the growth, immune response and protection of Nile tilapia against *Aeromonas hydrophila*.

MATERIALS AND METHODS

Plant powder and diet preparation.

Fresh spinach leaves were collected from the local market and identified according to (Arabshahi-Delouee and Urooj, 2007). Leaves washed, shed dried at 70°C for 3 days and well-ground, and stored at 4 °C until use. Four iso-nitrogenous (26% crude protein) diets were formulated to contain 0.0 (control), basic diet mixed with 1% of spinach leaves powder (Zaki *et al*., 2012), basic diet with 100 mg /kg of vitamin E (Chhorn Lim *et al*., 2009) and mixture diet from spinach leaves powder and vitamin E with the same concentration as mentioned before. The diets were reformed into pellets, air dry and stored at 4˚C for the feeding experiment.

Fish rearing and feeding regime.

One hundred and eighty healthy *Oreochromis niloticus*, 24.5 ± 0.37 g mean body weight were obtained from fish hatchery at the CLAR, Abbassa, Abo-Hammad, Sharkia, Egypt. Fish were transported to a laboratory then the health status of the experimental fish was inspected, and both fish and tank water were disinfected. Before the feeding trial, fish were acclimatized to laboratory conditions for 3 weeks. Fish were randomly distributed into a 120 L glass aquarium (15 fish per aquarium in triplicates). The first group (T1) fish fed with the control diet (without any additive). The second group (T2) fish fed a basic diet supplemented with spinach 1%. The third group (T3) fish fed basic diet supplemented with 100 mg/kg of vitamin E. The fourth group (T4) fish fed a basic diet with their mixture. Fish were fed on the treated diets up to apparent satiation twice a day at 9.00 and 14.00 h for 12 weeks. The aquaria were cleaned, and excreta of fish were siphoned daily by remove half of the aquarium's water and replaced by fresh water from a storage tank. Fish were weighed individually at the beginning and every two weeks to adjust the feeding ratio and at the end of the experimental period using a digital scale with a precision of 0.1 g. Water quality parameters were monitored weekly throughout the experiment.

The chemical analysis of diets.

Experimental diets were formulated to meet the nutritional requirement of fish according to the standard methods of AOAC (1990). The composition of the experimental diets represented in table 1. The chemical analysis of the experimental diets according to NRC (2011) Table 1.

Table 1. Ingredients formulation and chemical composition % on dry matter basis of the experimental diets.

Ingredient (%)	Treatments			
	T1	T2	T3	T4
Fish meal	90	90	90	90
Soybean meal	370	370	370	370
Starch	20	10	19.9	9.9
Yellow Corn	480	480	480	480
Vegetable oil	20	20	20	20
Vitamin permix[1]	10	10	10	10
Minerals permix[2]	10	10	10	10
Spinach		10		10
Vit. E			0.1	0.1
Total	1000	1000	1000	1000
Chemical analysis of the experimental diets.				
Crude protein (CP%)	25.22	25.82	25.77	25.81
Ether extract	11.12	10.32	11.81	11.64
Crude fiber (CF) %	3.59	4.41	3.64	4.48
Ash%	5.33	4. 23	5.48	5.30
NFE%[3]	54.74	55.22	53. 30	53.77
GE (Kcal/100 gm) [4]	472.55	469.17	476.26	476.8
P/E %ratio (mg/ Kcal) [5]	5.33	5.50	5.41	5.41

1-Vitamin premix (per kg of premix): thiamine, 2.5g; riboflavin, 2.5g; pyridoxine, 2.0g; inositol, 100.0g; biotin, 0.3g; pantothenic acid, 100.0g; folic acid, 0.75g; para-aminobenzoic acid, 2.5g; choline, 200.0g; $FeC_6H_5O_7.3H_2O$, 25.0; $ZnCO_3$, 5.5; $MnCl_2.4H_2O$, 2.5; $CuCl_2$, 0.785; $CoCl_3.6H_2O$, 0.477; $CaIO_3.6H_2O$, 0.295; $CrCl_3.6H_2O$, 0.128; $AlCl_3.6H_2O$, 0.54; Na_2SeO_3, 0.3 g nicotinic acid, 10.0g; cyanocobalamine, 0.005g; a-tocopherol acetate, 20.1g; retinol palmitate, 100.000 IU; cholecalciferol, 500.000 IU.

2-Mineral premix (g/kg of premix): $CaHPO_4.2H_2O$, 727.2; $MgCO_3.7H_2O$, 127.5; K.Cl, 50.0; NaCl, 60.0;

3- Nitrogen free extract (NFE) = 100- (protein + lipid + ash + fiber)

4- Gross energy was calculated according to NRC (2011) as 5.65, 9.45, and 4.11 kcal/g for protein, lipid, and carbohydrates, respectively.

5- Protein efficiency ratio = Body weight gain (gm) / protein intake (DM) gm .

Proximate body composition.

At the end of the feeding trial, three fish from each aquarium were randomly collected for subsequent proximate analysis. Proximate composition was done according to (AOAC, 1990). Moisture content was determined by drying samples in an oven at 100 °C until a constant weight was reached. Samples used for dry matter were digested with nitric acid and incinerated in a muffle furnace at 600 °C (6 hour) for measurement of ash contents. Protein was measured by the combustion method using an FP-2000 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Lipid content of samples was determined by petroleum ether extraction using a Soxtec System (2055 Soxtec Avanti; Foss Tecator, Höganäs, Sweden).

Fish growth and feed efficiency.

After 12 weeks, all fish experimental groups were measured individually for weight and length. Growth parameters such as specific growth rate (SGR) and weight gain (WG) were calculated (Pechsiri and Yakupitiyage, 2005) as follows:

SGR (%body weight gain/day) = [(ln final weight–ln initial weight) / time (days)] × 100

WG (g) = mean final weight (g) – mean initial weight (g).

The feed conversion ratio (FCR) = Feed intake (g) / weight gain (g).

Protein efficiency ratio (PER) = Weight gain (g) / Protein intake (g)

Feed efficiency (FE %) = [Weight gain (g) / Feed intake (g)] /100

Protein productive value (PPV %) = [PR1– PR0 / PI] 100

Where: PR1 = the total fish body protein at the end of the experiment. (On dry matter basis)

PR0 = the total fish body protein at the start of the experiment. (On dry matter basis)

PI = Protein intake.

Energy retention (ER %) = E-E0 / EF X 100

Where: E= the energy in the fish carcass (kcal) at the end of the experiment.

E0= the energy in the fish carcass (kcal) at the start of the experiment.

EF = the energy (kcal) in feed intake.

Blood sampling.

At the end of experiment**,** blood samples were drawn from the caudal blood vessels from five fish per aquarium (Lied *et al*., 1975) into clean dry Eppendorf with anticoagulant for hematocrit test and another samples of blood without anticoagulant, centrifuged at 3000 rpm for 15 minutes for serum separation and stored at -20^{o}C for total protein, lysozyme and antioxidant enzyme examination.

Serum protein profile.

Total protein (g/dL) (Lowry *et al*., 1951) and albumin (g/dL) (Doumas *et al*., 1971) were assayed. As well, globulin (g/dL) was calculated by subtraction of albumin from total protein.

Hematocrit level.

Hematocrit capillary tubes previously rinsed in heparin (15 unit/ml) were filled 2/3 with whole blood and centrifuged in hematocrit centrifuge for 5 minutes. The percentage of erythrocyte volume is measured by a hematocrit tube reader (Schaperclaus *et al.,* 1992).

Serum lysozyme activity.

The lysozyme activity was measured using a photoelectric colorimeter with an attachment for turbidity measurement. A series of dilution was prepared by diluting the standard lysozyme from hen egg white (Fluka, Switzerland) and mixed with *Micrococcus lysodeikticus* (ATCC No. 1698 Sigma) suspension for establishing the calibration curve. Ten µl of standard solution or serum were added to 200 µl of *Micrococcus* suspension (35 mg of *Micrococcus* dry powder/95 ml of 1/15 M phosphate buffer and 5.0 ml of NaCl solution). The changes in the extinction were measured at 546 nm by measuring the extinction immediately after adding the solution which contained the lysozyme (start of the reaction) and after a 20 min incubation of the preparation under investigation at 40˚C (end of the reaction). The lysozyme content is determined based on the calibration curve and the extinction measured (Schaperclaus *et al.*, 1992).

Antioxidant enzyme.

Superoxide Dismutase (SOD) enzyme activity in serum was estimated using the colorimetric method (Nishikimi *et al*., 1972).

Bacterial challenge.

At the end of the experiment, all treated groups were intraperitoneally challenged with 24 hours live of virulent *A. hydrophila* (0.1 ml per fish of 1.5x 10^8cells / ml) which previously isolated from moribund fish from Abbassa farms and identified according to Bergey's Manual of determinative Bacteriology (1994).

Daily mortalities were recorded for 10 consecutive days. The mortality rate was calculated for all replicates for different groups. Also, the relative percent of survival (RPS) was calculated according to Amend (1981) as follow:

RPS = 1 - (% mortality in challenged fish / % mortality in control fish) x 100.

Statistical analysis.

Data were analyzed (means ± SD) using a one-way analysis of variance (ANOVA) using (software SPSS version 17) (SPSS Inc., Chicago, IL, USA). Differences between means were determined and compared using Duncan's test and considered significant at $p < 0.05$.

RESULTS

Fish growth and feed utilization.

The growth performance of Nile tilapia was affected significantly by dietary spinach and Vitamin E and their mixture supplementation ($P<0.05$) (Table 2).

The growth performance of Nile tilapia fed on tested diets shows increase growth performance. However, fish fed with control diet exhibited the lowest final weight, weight gain, and SGR as compared with experimental diets ($P<0.05$). The best weight gain was obtained in fish fed a mixture of spinach and vitamin E (53.99±0.67) when compared to the other treated groups and control one. FCR values were observed among the treated diets and ranged from 1.46±0.11 to 1.83±0.13 ($P>0.05$). During the feeding period, fish in all

treated groups appeared in good health as observed from their general activity. No significant difference in fish survival between the experimental fish groups and it ranged from 97.8% in the control group to 100% spinach and mixture of spinach and Vitamin E fed fish groups (P>0.05). This result suggests that dietary spinach leaves powder are safe additives for fish.

Table 2. Growth performance and feed utilization (means ± SE) of Nile tilapia for 12 weeks.

Items	T1	T2	T3	T4
Initial. Fish weight (g)	25.20±0.47[a]	24.63±0.23[a]	24.93±0.24[a]	24.43±0.29[a]
Final fish weight (g)	52.33±1.45[d]	73.00±0.57[b]	59.33±1.76[c]	78.66±0.18[a]
weight gain (g)	27.16±1.01[d]	48.10 ±0.66[b]	34.40±1.98[c]	53.99±0.27[a]
AV. Daily gain (g)	0.32±0.11[d]	0.58±0.01[b]	0.41±0.26[c]	0.63±0.15[a]
SGR %/d	0.87±0.11[d]	1.29±0.15[b]	1.03±0.46 [c]	1.38±0.12[a]
Feed conversion ratio (FCR)	1.97±0.04[a]	1.53±0.11[c]	1.83±0.13[b]	1.46±0.11[d]
protein efficiency ratio (PER)	20.06±0.36[d]	25.37±0.22[b]	21.44±0.27[c]	26.33±0.40[a]
Feed efficiency (FE)	0.50±0.01[d]	0.65±0.01[b]	0.45±0.33[c]	0.67±0.01[a]
Protein productive value (PPV)	2.64±0.01[d]	7.39±0.01[b]	4. 99±0.02[c]	8.54±0.44[a]
Fish survival (%)	97.8±0.2[a]	100±0.0[a]	100±0.0[a]	100±0.0[a]

[a–d] Means followed by different letters in the same row are significantly different at $P < 0.05$

Fish whole-body proximate composition.

The carcass proximate composition (Table 3) was not significantly different ($P > 0.05$) in the percent of dry matter (DM), crude protein, fat and ash content of the whole body of fish between the control group and other groups. However, the ash content of the groups of fish fed diets supplemented with spinach 1% or a mixture of Spinach 1% and Vitamin E (100 mg/kg) was higher than the content in the control group.

Table 3. Body composition (%) on dry matter basis of Nile tilapia fed the tested diets.

	Moisture	Crude protein	Total lipids	Ash
Initial	76.81±0.34^{b}	49.19±0.12^{e}	25.72±0.15^{a}	24.25±0.22^{a}
T1	78.17±0.13^{a}	52.20±0.34^{d}	23.92±0.2^{b}	22.92±0.26^{b}
T2	71.59±0.81^{d}	63.10±0.72^{b}	19.11±0.27^{d}	17.10±0.28^{d}
T3	73.21±0.09^{c}	56.99±0.11^{c}	22.17±0.24^{c}	20.10±0.26^{c}
T4	72.99±0.71 ^{e}	67.00±0.65 ^{a}	17.10±0.42 ^{e}	15.71±0.26 ^{e}

$^{a-e}$ Means followed by different letters in the same column are significantly different at $P < 0.05$.

Serum protein profile.

Serum protein (total protein, albumin, and globulin) values in all treated groups were significantly elevated than control one (Table 4).

Besides**,** there was a substantial elevation ($P< 0.05$) in serum total protein values (g/dL) in T4 followed by T2 and T3 groups when compared with the control group (5.15±0.02, 4.89±0.01, 4.41±0.04, and 3.48±0.04 respectively).

Table 4. Serum protein profile of different groups for Nile tilapia.

Treatment	Total protein g/dl	Albumin g/dl	Globulin g/dl
T1	3.48±0.04^{d}	1.27±0.04^{d}	2.21±0.03^{d}
T2	4.89±0.01^{b}	1.51±0.04^{b}	3.38±0.02^{b}
T3	4.41±0.04^{c}	1.31±0.01^{c}	3.10±0.03^{c}
T4	5.15±0.02^{a}	1.62±0.01^{a}	3.53±0.01^{a}

$^{a-d}$Means followed by different letters in the same column are significantly different at $P < 0.05$

Hematocrit level.

Hematocrit values were significantly increased in treated groups T2, T3 and T4 (55, 45 and 58% respectively) than T1, control group (30%). The best results obtained in T4 followed by T2 and T3 (Table 5).

Lysozyme and SOD enzyme activities.

The results from the Table (5) revealed that T2, T3, and T4 groups enhanced the lysozyme activity in relation to control one. T4 was the best

treatment enhanced lysozyme activity (3.91±0.02) compared with the other groups T1, T2, and T3 (1.23±0.03, 3.82±0.03 and 2.81±0.02 respectively).

The results of the SOD enzyme revealed that T2, T3, and T4 groups elevated the level of SOD enzyme in comparison of the control group (14.23±0.01, 13.64±0.03, 16.13±0.04 and 6.12±0.02 respectively). T4 showed the elevation of the SOD enzyme when compared to T2 and T3.

Table 5. Lysozyme activity, hematocrit % and SOD enzyme of different groups for Nile tilapia.

Treatment	Lysozyme µg/ml	Ht%	SOD U/ml
T1	1.23±0.03[c]	30±0.02[d]	6.12±0.02[d]
T2	3.82±0.03[a]	55±0.02[b]	14.23±0.01[b]
T3	2.81±0.02[b]	45±0.01[c]	13.64±0.03[c]
T4	3.91±0.02[a]	58±0.02[a]	16.13±0.04[a]

[a-d] Means followed by different letters in the same column are significantly different at $P < 0.05$.

Bacterial challenge.

From Figure (1), the Relative percent of survival was higher in all treated groups (T2, T3 and T4) than control one (T1), 70, 60, 85 and 0% respectively.

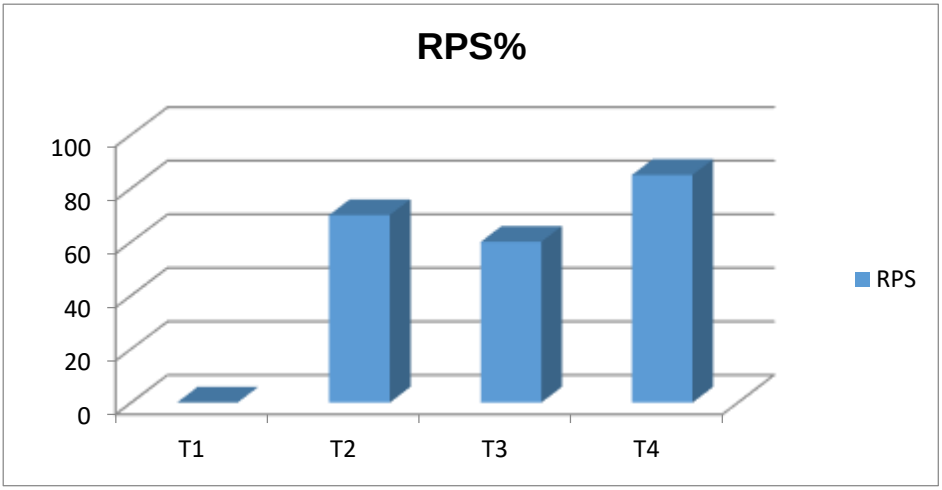

Fig. 1. Relative percent of survival of *O. niloticus*, fed diets containing spinach leaves powder, vitamin E and their mixture for 12 weeks.

DISCUSSION

Tilapia is considered an important economically cultured teleost fish (FAO, 2016). Recently, the use of phytogenic products in practical diets for fish has become a very topical concept in aquaculture (Gatlin *et al*., 2007). Besides the use of plant products in practical diets for fish is a very topical concept in aquaculture and it should be looked at as an immunity promotor and potential growth. The results of the present study showed that dietary supplementation of spinach and vitamin E improved the growth performance of the Nile tilapia better than in the control. These results are in accordance with those reported by (Abdelhamid, 2010) who showed that the diet containing Alpinia (AM) as a medicinal plant produced the best growth performance parameters compared with other different medicinal plants (Ginger, *Cresson,* and *Lpecdcuanha)*. The beneficial effect of spinach (*Spinacia oleracea*) may be due to its contents of nutrients bioactive compounds like Vitamin E, A, C, and K. Besides this, folic and oxalic acids. Moreover, spinach has many important minerals such as magnesium, manganese, calcium, phosphorus, iron, zinc, copper and potassium (Mehta and Belemkar, 2014).

The highest growth performance parameters were obtained in spinach diets. These results agree with Murray *et al*. (1991) and Abdelhamid (2010). The highest SGR % was recorded by the fish fed mixture of spinach and Vit E, these results agree with the results of (Abdel-Latif *et al*., 2004, Abdel-Maksoud *et al*., 2002 and Shalaby, 2004). Also, Khalafalla (2009) reported that fish fed diets contained 1% level of dried marjoram leaves, caraway seed meal, chamomile flower meal, and fennel seed meal was superior in growth performances of Nile tilapia fry as compared to those fed 0.5% level. On the other hand, the present results disagree with the findings of Salem (2008) who reported that the addition of 1 % fenugreek seed as anti-mycotoxins in Nile tilapia diets decreased the growth performance parameters.

The highest feed utilization parameters results agree with Abdelhamid (2010). On the other hand, the present results disagree with the findings of Soltan and El-Laithy (2008) who reported that the addition of 1 % fenugreek seed as anti-mycotoxins in Nile tilapia diets decreased the feed utilization parameters.

In the present study, the improvement in the carcass ash of all treated groups compared to the control group suggests that the spinach, vitamin E and their mixture can stimulate mineral deposition in the fish. these results similar findings of Ahmadifar *et. al*., (2011) that supplementing the diet with thymol-carvacrol can influence some carcass proximate composition in rainbow trout juveniles.

Total serum protein is important to evaluate the nutritional state of the fish health condition (Patriche *et al*., 2011). Some authors reported that the total protein, albumin, and globulin concentration in serum indicate the state of liver function. Therefore, the decrease of serum protein could be attributed to renal excretion or impaired protein synthesis, or liver hypofunction or disorder (Kori-Siakpere *et al*., 2006).

Deficiency of vitamin E has been reported to reduce serum protein, serum globulin and phagocyte activity in rainbow trout (Blazer and Wolke; 1984 and Clerton *et al*., 2001). In the present study, the results of serum total protein in agreement with the findings of Kumar *et al.* (2013); Basha *et al*. (2013) and Sahu *et al*. (2007) in *L. rohita.* Similarly, a significant increase in total protein, albumin and globulin were recorded in *C. carpio* fed diets containing 0.5% and 1% Chinese herbal medicine (Yuan *et al*., 2007).

The elevated hematocrit value was due to the multi nutrients in spinach and improved the fish health status. The increase of hematocrit may attribute to its bioactive compound (Mehta and Belemkar, 2014). The results of hematocrit in group that fed on basal diet and vitamin E (100 mg/ kg) showed similarity to the results of Bai and Lee (1998) who showed in *Sebastesschlegeli*, hematocrit of fish fed control group was lower than that of fish fed on vitamin E. However, the spinach, and the mixture diets group gave better results and this in agreement with (Amar *et al*., 2004, Puangkaew *et al*., 2004 and Cerezuela *et al*., 2009) who reported the addition of different feed additives such as vitamins, carotenoids and herbal remedies to the fish feed in aquaculture reduce the stress response, increasing the activity of innate immune response and improving disease resistance.

The lysozyme is a fish defense element, which makes hydrolysis of the N-acetylmuramic acid and N-acetylglucosamine which are the main constituents of the peptidoglycan layer of the bacterial cell wall and enhances the phagocytic activity (Ellis, 1999 and Magnadóttir, 2006). Vitamin E is abundant in immune cell membranes (Beharka *et al*., 1997) and plays an important role in the fish immune response (Waagbø, 1994). In this study, spinach *(Spinacia oleracea)* 1% and their mixture significantly increased the serum lysozyme activity; therefore, it stimulated the innate immune response in Nile tilapia. The increased lysozyme activity has been reported after supplementing the fish diet with spinach, *Spinacia oleracea* (Zhuang *et al*., 2009).

Antioxidants vitamins like vitamin E can scavenge ROS and upregulate the activities of antioxidant enzymes (Kelestemur and Ozdemir, 2013).

The addition of vitamin E to the diet of fish prevents variable unsaturated fatty acids in diets and tissues of aquatic animals from oxidative rancidity and maintains a normal metabolism, thus increasing the survival rate, weight gain as well as feed efficiency of animals. in addition, vitamin E is among the most important nutrients that enhance the immune system and their supply can reduce fish mortality and improve growth performance (Wassef *et al*., 2001). The Superoxide Dismutase enzyme was enhanced in the serum in spinach (*Spinacia oleracea*) diet and their mixture due to the presence of many nutrients that have excellent antioxidant properties (Mehta and Belemkar, 2014).

The mortality rate decreased after challenge with *Aeromonas hydrophila* in treated groups compared to the control one. However, the best survival rate was observed in fish group fed with mixture diet followed by spinach and vitamin E diets groups. The immune parameters, which enhanced could be observed in fish fed on a diet supplemented with spinach or vitamin E and their mixture might be associated with the elevated resistance of the fish against *A. hydrophila*. (Sahu *et al*., 2007 and Basha *et al*., 2013).

CONCLUSIONS

From obtained results, we concluded that using Spinach and a mixture of vitamin E and spinach as immunostimulants for Nile tilapia diets for 12 weeks can improve growth performance parameters and stimulate non-specific immune response and antioxidant activity.

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تأثيراضافه مسحوق نبات السبانخ و فيتامين هـ على معدلات النمو والحاله المناعيه الغير متخصصه عند إضافتهم إلي علائق أسماك البلطي النيلي

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الملخـص العربـى

دراسة تأثير إضافة مسحوق نبات السبانخ وفيتامين هـ فى علائق أسماك البلطى النيلى على معدلات النمو والحاله المناعيه الغير متخصصه ومقاومه الأمراض وقد أجريت هذه الدراسه على عدد 180 سمكه قسمت إلى أربعه مجموعات المجموعه الاولى مجموعه (كنترول) والمجموعه التانيه تم إضافه مسحوق السبانخ بنسبه 1% للكيلو من العليقه والمجموعه الثالثه تم إضافه فيتامين هـ بنسبه 100 ملجم للكيلو للعليقه والمجموعه الرابعه تم إضافه مخلوط السبانخ مع فيتامين هـ بنفس النسب. إستمرت التغذيه لمده 12 إسبوع وتم قياس وتقييم معدلات النمو المختلفه وكانت افضل معدلات النمو فى المجموعه التى تغذت على اضافه السبانخ والمجموعه التى تغذت على المخلوط .وافضل معامل تحويل كان فى مجموعه المخلوط ومجموعه السبانخ وتم سحب عينات الدم وقياس نسب البروتين الكلي والألبيومين والجلوبيولين ونسبه الهيماتو كرايت ونشاط انزيم الليزوزيم (lysozyme) وانزيم سوبر أكسيد داى ميوتيز (SOD) وأيضا تم عمل اختبارعدوى المجموعات بميكروب الأيروموناس هيدروفيلا وتم حساب معامل الحمايه. وقد أسفرت النتائج عن تحسن أداء النمو وكذلك زياده ملحوظه فى نسبه البروتين الكلى والالبيومين والجلوبيولين ونسبه الهيماتو كرايت وزياد نشاط الليزوزيم وايضا زياد انزيم السوبر أوكسيد داى ميوتيز فى المجموعات التى تغذت على فيتامين هـ والسبانخ وقد لوحظ أعلى تحسن فى المجموعات التى تم تغذيتها على مخلوط السبانخ مع الفيتامين هـ وكانت نسبه الحمايه فى مجموعات الكنترول (0%)، (70%) فى المجموعه الثانيه، (60%) فى المجموعه الثالثه، (85%) فى المجموعه الرابعه. من هذه الدراسه يمكن التلخيص ان اضافه مسحوق السبانخ وفيتامين هـ يحسن من اداء النمو والمناعه الغير متخصصه ومقاومه البلطي النيلي للأمراض.