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EFFECT OF PEPPERMINT EXTRACT ON GROWTH,HAEMATOLOGICAL AND BIOCHEMICALPARAMETERSOF*OREOCHROMIS NILOTICUS*

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ABSTRACT

A total of three treatments, i.e., negative control group (group A), 4% Mentha piperita powder (group B) and 4% ethanol extract of mentha piperita (group C) were added to the diets of Nile tilapia, Oreochromis niloticus to investigate the effects of the respective treatments on the growth, haematological and biochemical indices. Fish (30 ± 10 g) were distributed at a rate of 13 fish per glass aquarium and two aquaria have been assigned for each treatment. At the end of the experiment (2 months), results indicated that there was significant increase ($p<.05$) in the weight gain and specific growth rate of groups which fed diet enriched with 4% mentha piperita powder and groups which fed on diet enriched with 4% ethanol extract of mentha piperita when compared with control group. The recorded results of haematological and biochemical parameters included Hb, pcv, RBCs count, WBCs count, albumin, glucose, ALT, AST, uric acid, urea and creatinine and indicated no significant difference between different groups.

INTRODUCTION

Fish are considered an important source for protein with high nutritive value **(Khoshkhoo *et al*., 2012).** Because of depletion of fisheries and increase market demand in many developed and developing countries, there is a very rapid growth in Industrial aquaculture **(Gold burg and Naylor, 2005).** However, increasing aquaculture fish production in a stressful environment leads to suppression of the fish immune system, increasing the susceptibility to infectious diseases; which lead to many economic losses.**(Agarwal and singh,(1999).** So Fish producers use a large quantity of antibiotics and chemicals to prevent and control diseases to maintain fish health as well as to improve fish performance **(Harikrishnan *et al*.,2011),**but massive use of antibiotics in fish farming is restricted in many countries due to increasing development of antibiotic resistant bacterial strains **(Citarasu, 2010)**, Also, the occurrence of antibiotic residues in aquaculture products threatens human

health **(WMO, 2006)**.Therefore, there are increasing interest about testing effects of natural additives on health status and growth effects in fish **(Citarasu, 2010)**.The use of medicinal plants have advantages of low/minimum cost, potency and efficiency, enhanced tolerance, more protection, fewer side-effects, complete accessibility, and they are recyclable **(Parveen and Shrivastava, 2012).** Nile tilapia *(Oreochromis niloticus*) is the most cultured freshwater species among the farmed tilapias and contributes about 71% of the world total tilapia production **FAO, (2002)**.*Oreochromis niloticus* is preferred in aquaculture as it can tolerate a wide range of environmental conditions, fast growth, successful reproductive strategies, and ability to feed at different trophic levels. These traits allow them to be an extremely successful invasive species in subtropical and temperate environments **(Peterson *et al.,* 2005).** The genus Mentha is one of the most important sources for the production of some of the most economically essential oil throughout the world **(Pauli, 2006).** Peppermint (Mentha piperita) is commonly used in many pharmaceutical and industrial products due to their wide range of pharmacological properties including antioxidant, antitumor, antiallergenic, anti-viral, fungicide, insecticide and antibacterial activities**(McKayand Blumberg,(2006);Yadegarinia *et al*.,(2006); Mimica-Dukic *et al*.,(2003); Lin *et al*.,(2008) and Tung *et al*.,(2008).**The aim of this study is to evaluate the effect of dietary supplementation of mentha piperita on the growth,haematological and biochemical indices of *Oreochrmis niloticus* and finding new natural growth promoters can be used in aquaculture.

MATERIAL AND METHODS

Fish:

A total of 78 apparently healthy *Oreochromis niloticus* with an average body weight 30±10 g, were collected alive from a private fish farm at Ismailia governorate. The Fish were acclimated for two weeks in de-chlorinated tap water. Continuous aeration was maintained in aquaria using electric air pumps. The water temperature was adjusted to 25ºC according to **Innes, (1996).**

Feed:

a. Diet.

Commercial fish diet 30% crude protein, 4.5 % fiber and 5% fat as well as vitamins and minerals from Aller aqua Company.

b- Mentha piperita powder.

Mentha piperita was purchased from a private farm. Leaves were washed well then shade

dried and crushed into powder form using a household electric grinder .it was mixed directly with fish feed to achieve 4% concentration (40g/kg diet) and gelatin was added to feed ingredients as a binder according to **Talpur,(2013)**.

C-Ethanol extract of Mentha piperita.

The extract was prepared with the standard method of percolation. To do this. The powdered leaves in 80% ethanol were percolated for 72 hours. Then the slurry was filtered with Whattman No. 1 filter paper and centrifuged for 5 min at 5000 rpm. The filtrate obtained from ethanol using a rotary device according to **Haghighi *et al*.,(2014)**.

Feed preparation:

Commercial fish diet was mixed with feed additive (Mentha piperita powder, Ethanol extract of Mentha piperita) and 2 experimental rations were prepared.

Fish Group	Fish No.	Treatment	Dose	Feeding% /fish Biomass
Group A	**26**	**Basal diet**	**-**	**3%**
Group B	**26**	**Basal diet + 4% Mentha piperita powder**	**40 g / Kg**	**3%**
Group C	**26**	**Basal diet +4% Ethanol extract of Mentha piperita**	**40 g / Kg**	**3%**

Experimental design:

Seventy eight apparently healthy *Oreochromis niloticus* were stocked in 6 glass aquaria and acclimated for 15 days prior to the experiment, during that period, the fish were fed on control diet (without any additive), water was changed every 3days to maintain good water quality, water temperature was adjusted at 25°C using thermo-stable heater. During the experimental period, fish were fed on diet supplemented with the feed additives at feeding rate 3% of the total biomass of fish twice per day . Every two weeks fish were weighted, and the amount of feed was re-calculated according the new biomass for 2 months. After 2 months, blood samples were collected from the caudal blood vessels of fish according to **Noga, (2010)**, and divided into 2 portions; the first with sodium heparin 100 IU/ml as anticoagulant according to **Jain (1986)** for determination of hematological parameters and the other without anticoagulant for serum separation; for measuring the biochemical parameters.

Growth parameters determination:

a. Body weight gain: Final fish weight (g) - Initial fish weight (g) according to **Annet, (1985)**.

b. Specific Growth Rate %: It was calculated as the percentage increase in weight per fish per day as suggested by **Pouomonge and Mbonglang (1993)**.

Haematological and biochemical indices:

a.Haemgram:

-Determination of packed cell volume (PCV %):

Packed cell volume was determined by microhaematocrit method according to **Decie and Lewis (1991).**

Determination of hemoglobin concentration:

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

Erythrocytes and leukocytes count:

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

b. Biochemical analysis:

Determination of serum Total Protein (g/dl) :

Assay of total protein was carried by a test kit according to biuret method described by **Gornall *et al*., (1949).**

Serum albumin:

This method is based on colorimetric end point method according to modified bromcresol green binding assay **Doumas *et al*., (1971).**

Liver enzymes: serum amino transferases:

Serum Alanine Amino transferase (ALT) and Aspartate Amino transferase (AST) activities were estimated calorimetrically using Vitro bio diagnostic kits as described by **Reitman and Frankel (1957).**

Kidney function tests:

1- Serum creatinine.

Serum creatinine was estimated calorimetrically using Vitro bio diagnostic kits as described by **Bartles *et al*., (1972).**

2- Serum urea.

Serum urea was estimated calorimetrically using Vitro bio diagnostic kits using Urease – Berthelot Method as described by **Fawcett and Scott (1960)**.

3-serum uric acid.

Serum uric acid was estimated calorimetrically using Vitro bio diagnostic kits.According to **Barham and Trinder (1972).**

Serum glucose:

It was determined by the colorimetric method described by **Trinder, (1969).**

Statistical analysis:

Data were represented as means ± standard error (SE) and the significance of differences was evaluated using analysis of variance (ANOVA) and t-student test **SPSS14, (2006)**.

RESULTS

Growth performance:

The results of growth performance of different fish groups fed with basal diet (group A) , groups fed with diet supplemented with 4% Mentha piperita powder (group B) and groups fed with diet supplemented with 4% ethanol extract of Mentha piperita (group C) represented in (Table1) . There was a significant increase in weight gain and specific growth rate in groups fed with diet supplemented with Mentha piperita powder or ethanol extract of Mentha piperita when compared with control group.

Table (1): Showing the mean values of weight gain and specific growth rate of different fish groups.

Fish groups	Final Weight gain (g)	Specific growth rate (%)
Group A	**6.00 ± 1.00**	**0.12 ± .01**
Group B	**20.00 ± 5.00**	**0.29 ± .06**
Group C	**18 ± 3.39**	**0.32 ± .04**

Data are represented as means of three samples ± SE.

SE=standard error of mean.

Haematological studies:

The results of RBCs count,WBCs count ,Hb and PCV of differnet fish groups fed with basal diet (group A) , groups fed with diet supplemented with 4% Mentha piperita powder (group B) and groups fed with diet supplemented with 4% ethanol extract of Mentha piperita

(group C) represented in (Table2).There were no significant increases in RBCs count,WBCs count, Hb and PCV in different fish groups.

Table (2): The mean values of RBCs count, WBCs count, Hb and pcv of differnet fish groups.

Fish groups	Hb (g/dl)	PCV (%)	RBCs $\times 10^6/mm^3$	WBCs$\times 10^3/mm^3$
Group A	**4.86 ± .39 [a]**	**16.00 ± 1.15 [a]**	**.74 ± .11 [a]**	**56.33 ± 17.40 [a]**
Group B	**4.83 ± .62 [a]**	**16.00 ± 2.30 [a]**	**.71 ± .12[a]**	**51.00 ± 2.30 [a]**
Group C	**4.82 ± .75 [a]**	**18.00 ± 2.30 [a]**	**.60 ± .07 [a]**	**43.33 ±11.39 [a]**

Data are represented as means of three samples ± SE.

SE=standard error of mean.

Total protein, albumin and glucose:

The results of total protein ,albumin and glucose of different fish groups fed with basal diet (group A) , groups fed with diet supplemented with 4% Mentha piperita powder (group B) and groups fed with diet supplemented with 4% ethanol extract of Mentha piperita (group C) represented in (Table 3). There was significant increase in total protein in group B and C than control group but there was no significant increase among group B and C. but there was no significant change in albumin and gluose between different groups.

Table (3): The mean values of total protein, albumin and glucose of different fish groups.

Fish groups	Total protein (g/dl)	Albumin (g/dl)	Glucose (g/dl)
Group A	**1.53 ±. 08 a**	**.90 ±. 04 a**	**5.64 ± 1.25 a**
Group B	**2.66 ± .25 b**	**1.03 ± 11 a**	**6.86 ± 3.88 a**
Group C	**2.84 ± .28 b**	**.93 ± .06 a**	**4.04 ± 1.33 a**

Data are represented as means of three samples ± SE.
SE=standard error of mean.

Serum transferases:

The results of Serum transferases of different fish groups fed with basal diet (group A) , groups fed with diet supplemented with 4% Mentha piperita powder (group B) and groups fed with diet supplemented with 4% ethanol extract of Mentha piperita (group C) represented in (Table 4). There was no significant change in serum transferases among different groups.

Table (4): The mean values of ALT and AST of different fish groups.

Fish groups	ALT (Units/ml)	AST (Units/ml)
Group A	**39.66+-3.71 a**	**29.66+-4.09 a**
Group B	**39.66+-3.71 a**	**31.00+-3.78 a**
Group C	**41.00+-3.00 a**	**42.33+-5.17 a**

Data are represented as means of three samples ± SE.

SE=standard error of mean.

Kidney function tests:

The results of urea, uric acid and creatinine of different fish groups fed with basal diet (group A) , groups fed with diet supplemented with 4% Mentha piperita powder (group B) and groups fed with diet supplemented with 4% ethanol extract of Mentha piperita (group C) represented in (Table 5). There was no significant change in urea, uric acid and creatinine among different groups.

Table (5): Showing the mean values of urea, uric acid and creatinine of different fish groups.

Fish groups	Urea g/dl	uric acid mg/dl	Creatinine mg/dl
Group A	**2.74+-1.90 [a]**	**10.36+-3.41 [a]**	**1.12+-.61 [a]**
Group B	**2.66+-1.92 [a]**	**12.67+-3.23 [a]**	**0.87+-.37 [a]**
Group C	**3.60+-.31 [a]**	**18.98+-1.74 [a]**	**0.62+-.26 [a]**

Data are represented as means of three samples ± SE.

SE=standard error of mean.

DISCUSSION

As many studies showed that herbs have beneficial effects on many farmed animals such as pigs **(Yan *et al*., 2012)**, poultry **(Hashemi and Davoodi, 2010)** and fish **(Galina *et al.,* 2009).** Herbs have gained considerable attention in the feed industry as feed additives.so in this study we aimed to evaluate dietary supplementation of mentha piperita on growth, haematological and biochemical parameters of Oreochromis niloticus. Botanists consider Mentha piperita as an astringent, antiseptic, antipyretic, antispasmodic, anticatarrhal, antimicrobial, rubefacient, stimulant, emmenagogue and anti-aging properties **(Ali *et al* .,(2002)** . Growth enhancement

is a very important factor in aquaculture as it improves the productivity and profitability. The results from the present study indicated that 4% Mentha piperita or 4% ethanol extract of Mentha piperita included in the diet is useful for improving the growth performance as they significantly increase ($p<.05$) weight gain and specific growth rate comparing with control group. This result agree with **Talpur ,(2013)** who reported that feeding of Mintha piperita diet (1, 2, 3, 4 and 5 g/kg) to *L. Calcarifer* triggered appetite and led to a significantly improved weight gain and SGR except fish fed with Mintha piperita diet at 1 g/kg feed . similarly , **Adel *et al* .,(2015)** after adding of 1%, 2% and 3% of peppermint extracts for 8 weeks to fry Caspian white fish (*Rutilus frisii kutum*) , found a Dose-dependent increase of growth parameters (WG and SGR) where the highest increments was found in fish fed 3% peppermint enriched diets. This beneficial effect of mentha piperita on the growth parameters may be attributed to the presence of fatty acids, essential oil, different vitamins (carotenoids and ascorbic acid) ,minerals (K, Ca, Mg and lower amounts of Na, along with smaller amounts of Fe, Mn, Zn and Cu) and also some traces of Cr, I and Se **.(lozak *et al.,* 2002**).Regarding haematological parameters ,we found that there was no significant difference between different groups in Hb , PCV, RBCs count and WBCs count. This result disagree with **Talpur, (2013)** who mentioned that, the number of erythrocytes (RBC) and leucocytes (WBC) was significantly higher ($p <0.05$) in fish that were fed Mintha piperita diet (1, 2, 3, 4 and 5 g/kg) than the controls. There was also a significant ($p<0.05$) increase in haematocrit values in those fish fed Mintha piperita diet at 4 and 5 g/kg feed compared with the control. He reported that A significant ($p<0.05$) increase in haemoglobin (Hb) (g/dl) was determined in those fish fed with Mintha piperita diet 3, 4 and 5 g/kg feed compared with those of controls and no significance difference was seen in fish fed Mintha piperita diet at 1 and 2 g/kg feed .Regarding biochemical parameters in this study, there was no significant difference in albumin, glucose, ALT and AST between different groups. this result agree with **Adel *et al* .,(2016)**,who evaluated the effect of adding of Mentha piperita 0, 1, 2 and 3% to the diet of rainbow trout (Oncorhynchus mykiss) for 8 weeks on the haematological and biochemical parameters and found that dietary mintha piperita plant extract supplements have no significant effect on blood biochemical parameters (glucose, AST and ALT). The difference in this result may be due to the effects of dietary additives on fish may vary depending on fish species, size, the dose of the additive ,fish nutritional/physiological status, and/or ambie nt culturing conditions.

CONCLUSION

From the present study, it was revealed that, the incorporation of 4% Mintha piperita powder or ethanol extract in *Oreochromis niloticus* diet enhance growth performance without any alterations in physiological parameters .

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