Effect of Ropadiar (*Origanum vulgare* L.) on the Growth Performance and Biochemical Parameters of *Oreochromis niloticus*

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Abstract

This study provided a preliminary framework for the effects of *Origanum vulgare* L. essential oil (EO) supplementation on growth performance, serum biochemical parameters as well as liver and kidney function of Nile tilapia (*Oreochromis niloticus*) throughout a feeding study of 60 days. A total of 60 *O. niloticus* with an average body weight of 14.2-15.1 g were split into two groups for the trial (10 fish/aquarium/3 replicate). The first (control) group was fed a basal diet without any supplementation, and the second (Ropadiar) group was fed a basal diet including 2% Ropadiar. The body weight, weight gain, liver enzymes, total protein, albumin, globulin, and creatinine levels were estimated. A significant improvement in body weight, weight gain, liver enzymes, total protein, albumin, creatinine, and globulin levels was seen in the 2% Ropadiar-treated group than in the control group. Thus, the incorporation of dietary oreganum essential oil 2% in the diet of Nile tilapia is recommended to improve growth performance, serum biochemical parameters, as well as liver and kidney function biomarkers.

Key words: Essential oil, Nile tilapia, Growth performance, Biochemical parameters.

Introduction

Nearly half of the fish consumed by humans globally today is produced by the aquaculture industry. From the hatchery to the final commercial stage, the aquaculture sector works with a variety of stressful circumstances that may jeopardise the welfare of the target species, including handling, confinement,

transport, and other processes ***(El Basuini et al., 2020; Sánchez-Muros et al., 2017)***. The primary method used in Egypt to improve the aquaculture sector has been vertical growth, which involves maximizing the output of planted areas through intensification of farming. However, vertical development has a number of disadvantages, including stress factors that lead to infectious diseases and epidemics, such as high densities, poor water quality, and elevated ammonia levels ***(Ali et al., 2018).***

Antibiotic use in animal production, notably in aquaculture, has become more cautious due to bacterial resistance and antibiotic residues ***(Cabello, 2006; Navarrete et al., 2009).*** This sparked investigation into antibiotic substitutes. In aquaculture, phytogenic extracts stand out as potential replacements for synthetic drugs because, when administered properly, they deliver beneficial biologically active metabolites with a range of advantages, including immune modulation ***(Sevdan Yilmaz, 2019; Yılmaz & Ergün, 2018; Zanuzzo et al., 2015),*** growth promotion, antioxidant enhancement, digestive enhancement, appetite stimulation, ***(Gabriel et al., 2015; P. Zhang et al., 2010)*** and hepatoprotective effects as well ***(S. Yilmaz et al., 2014)***, if properly administered.

Highly concentrated, fragrant volatile oils of plants that are derived from aromatic plants appear to be a natural substitute for antibiotics **(*Calsamiglia et al., 2007),*** highly concentrated, aromatic volatile oils of plant origin are extracted via steam distillation, hydro-diffusion, or pressure. These oils contain a variety of chemical components ***(Manion & Widder, 2017).*** Essential oils' antimicrobial properties can be applied to the food sector, pharmaceuticals, and alternative medicine ***(Tarek et al., 2014) .***

The primary source of oregano essential oil is *Origanum vulgare* L., and it has a high level of functionality as a therapeutic extract in aquaculture ***(Alagawany et al., 2020)***. In aquatic animals, oregano essential oil plays antibacterial, anti-inflammatory, antioxidative, and immunological modulating activities due to its high content of carvacrol and thymol ***(Hernández-Nava et al., 2020).*** Aquaculture Koi carp (*Cyprinus carpio*) showed better growth performance, immune system, and anti-oxidative reactions in response to dietary oregano essential oil ***(R. Zhang et al., 2020*)*,*** as well as in Nile tilapia (*O. niloticus*) ***(Heluy et al., 2020; Shourbela et al., 2021).***

Significantly, consumption of oregano essential oil increased resistance to farming stressors such high ammonia accumulation, intensive stocking density, and bacterial infection ***(Shourbela et al., 2021).***

The purpose of this study is to examine any potential effects of

oreganum essential oil on fish body weight and biochemical parameters such as total protein (TP), albumin (ALB), creatinine, and serum transaminases such as glutamate pyruvate transaminase (GPT) and glutamic oxaloacetic transaminase (GOT).

Materials and Methods

Experimental diet

Commercial basal diet was obtained from SKRETTING®, Egypt has 30% crude protein, 6.1% Crude fat % and 4.8 % Crude fibers with energy 4000 Kcal where grind into fine powder and supplemented with 2% Ropadiar (*Origanum vulgare* L.) containing 20% oreganum oil in diet. The dough was electronically minced into pellets, and then it was air dried. Until they were consumed, the prepared diets were kept in the refrigerator at 4^{o}C until used.

Experimental design

A total number of 60 apparently healthy of *O. niloticus* with an average body weight 14.2-15.1 gm were divided into two groups and reared in 6 glass aquaria each contained 10 fish with triplicate (10 fish/ aquarium/3 replicate) where the first group was fed the basal diet as control (C) and the second group was fed basal diet supplemented with 2% Ropadiar® (R). For 60 days, the fish were fed at a rate of 3% of their body weight. Fish were weighed at the beginning of the experiment, after 30 days, and after 60 days. Subtracting the end weight from the baseline weight allowed us to determine the weight gain at 30 and 60 days.

Sampling

At 30 and 60 days of experiment, blood samples were collected from the caudal vein of anesthetized fish with clove oil solution (12.5 mg/L) ***(Javahery et al., 2012)*** and was left in a plain centrifuge tube without anticoagulant in order to clot and get sera. The clotted blood was kept in refrigerator for 30 minutes then centrifuged at 2000 *rpm* for 15 min at room temperature, the supernatant serum was collected and stored at -80℃ in screw capped vials until used for biochemical tests.

Serum biochemical examinations

Serum transaminases; glutamate pyruvate transaminase (GPT), and glutamic oxaloacetic transaminase (GOT) were measured using Biodiagnostic Co., Egypt kits. Both total protein (TP) and albumin (ALB) were measured using Diamond-diagnostic kits, Egypt. Creatinine was measured using calorimetric kit purchased from Biodiagnostic Co., Egypt.

Statistical analysis

T-tests were used to evaluate the data from the current study in order to assess for significance among the groups that were being studied ***(Snedecor & Cochran, 1989)*.** Software such as Statistical Analysis System (SAS Institute 2003) and SPSS for Windows (SPSS version 20) were used to conduct the statistical analysis

Results

Body weight and weight gain

Growth performance parameters of tilapia fed with 2% Ropadiar during feeding period (60 days) were shown in **table (1).** The body weights at 30 and 60 days of experimental period exhibited significant ($P \leq 0.05$) increase in Ropadiar group than control. The body weight gains at 30 and 60 days of experimental period exhibited significant ($P \leq 0.05$) increase in Ropadiar group than control.

Serum biochemical parameters

Serum biochemical parameters of Nile tilapia fed 2% Ropadiar during feeding period (60 days) were displayed in **table (2).** After 30 and 60 days of feeding period, GPT level was significantly ($P \leq 0.05$) decreased in Ropadiar group than the control group. On the same trend, GOT was significantly ($P \leq 0.05$) decreased in Ropadiar group than the control at 30 and 60 days of experiment. On the other side, both serum TP and ALB were increased significantly ($P \leq 0.05$) as compared to the control at 30 and 60 days of experiment. Serum creatinine level, revealed non-significant variation at 30 and 60 days of feeding period.

Table 1. *Effect of Ropadiar on body weight and weight gain of Nile tilapia.*

Parameter	Duration	Control group	Ropadiar group
Initial weight	0	14.2±0.05	14.05±0.13
Body weight (g)	30 days	42.43±0.66	46.55±0.70*
	60 days	64.30±0.86	72.65±0.38*
Weight gain (g)	30 days	28.23±0.61	32.50±0.57*
	60 days	50.10±0.59	58.60±0.25*

The data was represented as mean ±SE. Superscripts * was statistically significant at *($P \leq 0.05$)*.

Table 2. *Effect of Ropadiar on serum biochemical parameters of Nile tilapia*

Parameters	Days	Control group	Ropadiar group
Creatinine (mg/dL)	30	0.61 ± 0.01	0.62 ± 0.08
	60	0.43 ± 0.02	0.44 ± 0.03
GPT(U/L)	30	19.09 ± 0.43	16.44 ± 0.58*
	60	19.77 ± 0.73	16.82 ± 0.26*
GOT(U/L)	30	42.39 ± 3.89	34.15 ± 1.02
	60	41.21 ± 2.38	25.63 ± 2.17*
ALB(g/dL)	30	1.44 ± 0.04	1.98 ± 0.03*
	60	1.32 ± 0.04	2.29 ± 0.09*
T.P(g/dL)	30	2.29 ± 0.04	3.95 ± 0.18*
	60	2.40 ± 0.07	3.86 ± 0.21*
GLO(g/dL)	30	0.97 ± 0.07	1.97 ± 0.19*
	60	1.09 ± 0.11	1.57 ± 0.18*

The data was represented as mean ±SE. superscripts * was statistically significant at *($P \leq 0.05$)*.

Discussion

Aquaculture has made extensive use of phytogenic feed additives because of their beneficial effects on fish development rates. Additionally, these additives don't harm fish health in any way **(Menanteau-Ledouble et al., 2015).**The current study examined the effects of oreganum essential oil

2%, a commercial product sold as Ropadiar, as a feed supplement on the growth performance, liver function, and kidney function of Nile tilapia.

The current study found that Ropadiar has beneficial effects on body weight and weight increase in terms of both weight loss and weight gain. These findings were in line with those of ***Ayoub et al. (2021) and Shourbela et al. (2021)*** who suggested that Oreganum essential oil may regulate the secretion of hormones and digestive enzymes, stimulate the immune system, interact synergistically with the gut microbiota, and have antibacterial and antioxidant properties. Increased digestibility, vitamin absorption, and protein conversion may be the results of all these effects ***(Castañeda-Monsalve et al., 2019).***

Contrary findings were reported by ***Cararo et al. (2017)*** who claimed that food supplementation with oregano essential oil was ineffective for enhancing the growth performance of juvenile Silver catfish. Similarly, feeding oregano essential oil to juvenile Nile tilapia (*Oreochromis niloticus*) had no impact on growth metrics **(*Campagnolo et al., 2013).***Additionally, essential oils' capacity to stimulate appetite due to their distinct scent and flavor, which results in greater voluntary feed intake and improved weight gain, may be the cause of their growth-promoting effects ***(Abdel-Latif & Khalil, 2014).***

Additionally, the bioactive phytochemicals carcacrol and thymol present in oreganum essential oil may increase the secretion of digestive enzymes, resulting in an increase in food intake ***(R. Zhang et al., 2020),*** improved enzymes vital activity ***(Puvača, Stanaćev, Glamočić, Lević, Perić, & Milić, 2013),*** a decrease in the number of dangerous microorganisms in the gastrointestinal tract ***(Dawood et al., 2021),*** protected the mucosal layer and enhanced the permeability of digested nutrients in the gastrointestinal tract ***(Heluy et al., 2020).***

As several fish species are supplemented with herbal compounds, information about the health of the fish is vital to know from their biochemical profiles ***(Abdel-Tawwab et al., 2015).***The analysis of biochemical blood indicators has an emphasis on physiological state, health status, response to stressors and external stimuli, and disease resistance. To assess potential harm to the hepatocytes or liver tissue, the GPT and GOT enzymes are frequently elevated in response to exposure to pollution or hazardous substances ***(Zadmajid & Mohammadi, 2017).*** The breakdown of mitochondria caused by swollen hepatic tissue and muscle injury is what is responsible for the elevated GOT

enzyme activity in the plasma ***(Eslami & Bahrekazemi, 2019).***
While GOT can be found in the liver, heart, muscle, brain, and kidney, GPT is a particular hepatic enzyme ***(Petricevich, 2010).***The present findings, which were in agreement with those of ***Fawzy I Magouz et al. (2022a)*** and ***Shourbela et al. (2021)*** who showed a considerable reduction in GOT and GPT following Ropadiar feeding. The active components of oreganum oil, thymol and carvacrol, which enhance antioxidant reserve and reduce oxidative load in several body organs, may be responsible for Ropadiar's lower GPT ***(Abdel-Latif & Khalil, 2014).*** This outcome was consistent with the growth that was encouraged by Oreganum EO, which may also stimulate the release of digestive enzymes and other enzymatic processes ***(Hernandez et al., 2004; Puvača, Stanaćev, Glamočić, Lević, Perić, Stanaćev, et al., 2013).*** On the other hand, ***Abdel-Latif et al. (2020)*** reported that supplementing common carp (*Cyprinuscarpio* L.) fingerlings with Oreganum oil for two months did not significantly change the activity of liver enzymes.
Serum TP is a critical sign of the humoral defense system since it serves as a broad biomarker for all protective enzymes, stress hormones, and metabolites circulating in the body's essential fluid ***(Shiry et al., 2019).***Any stress that creates this scenario may change the total protein levels because changes in plasmatic volume are what primarily cause changes in serum TP concentration ***(Melo et al., 2009).*** Low quantities of TP, a marker of protein metabolism, may be present in conditions affecting the liver ***(John, 2007).*** According to our findings, serum TP levels significantly increased in treated group as compared to the control group. Our results concerning Ropadiar influence on TP were got by Haghighi et al. (2018) who reported that the dietary supplementation of dried *Oreganum vulgare* extract in fish diets significantly enhanced TP values with respect to the control group.
TP elevation may be due to improving liver and other organs functions, which synthesized serum protein ***(Metwally, 2009)*** as well as the contribution of important liver defense protein molecules ***(Hoseini & Yousefi, 2019)*** and antibodies ***(Devi et al., 2019)*** like agglutinins, lecithins, and immunoglobulins which are important defense molecules.
By conveying numerous external substances, endogenous metabolites, and some immunological parameters against infections, including ALB and GLO aids in maintaining the osmotic balance ***(Jha et al., 2007).*** The most significant protein in plasma, ALB, has been implicated in regulating osmotic pressure and reducing the effects of medicines, toxic metals,

and other substances. This may indicate that the active principles of the therapies, which enhanced ALB production, may have potential hepatic-promoting effects ***(Mirghaed et al., 2017).***

The increased production of protective molecules is suggested by the possibility that the high ALB levels will sustain osmotic pressure and neutralize poisons ***(Abdel-Tawwab & El-Araby, 2021).*** According to the current study, the Ropadiar group's ALB and GLO levels were significantly higher than those in the control group. According to researchers, having more GLO in blood serum is an indication of health and immunity ***(Kumar et al., 2005; Misra et al., 2006; Rao et al., 2004).*** It is considered that an increase in GLO in the blood is directly related to the generation of immunoglobulins ***(Rao et al., 2004)***. In fish fed a herbal immunostimulant, past investigations have found elevated TP or immunoglobulin levels ***(Yonar, 2019).***

Serum creatinine is filtered out by kidneys (glomerular filtration) and blood levels rise in severe kidney dysfunction ***(Kulkarni & Pruthviraj, 2016).*** Serum creatinine levels were significantly decreased in Ropadiar 2% fed group. Our results were similar to ***Abdel-Latif et al. (2020)*** who reported that oreganum oil improved serum creatinine in common carp fed oreganum oil. Opposite to these results of ***Fawzy I. Magouz et al. (2022b)*** reported that organum oil had no effect on serum creatinine level of Nile tilapia fed oreganum essential oil. The effectiveness of thymol and carvacrol in Oreganum oil of Ropadiar as potent antioxidants preserving the healthy kidney structure may be responsible for this outcome ***(Abdel-Latif et al. 2020).***

Conclusion

The incorporation of dietary oreganum essential oil 2% in the diet of Nile tilapia is recommended to improve body weight as evidenced by improved biochemical parameters such as decreased GPT, GOT and creatinine while increased TP, ALB and GLO.

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تأثير الروباديار (اوريجانوم فالجارى) على أداء النمو والمعايير البيوكيميائية فى البلطى النيلى

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الملخص العربي

قدمت هذه الدراسة إطارًا أوليًا لتأثيرات مكملات الزيت العطري اوريجانوم فالجارى *Origanum vulgare* L على أداء النمو والكيمياء الحيوية في المصل وكذلك وظائف الكبد والكلى للبلطي النيلي (*Oreochromis niloticus*) خلال دراسة التغذية لمدة 60 يومًا. تم تقسيم عدد 60 من أسماك البلطي بمتوسط وزن جسم 14.2-15.1 جم إلى مجموعتين للتجربة (10 أسماك / حوض سمك / 3 مكررات). تم تغذية المجموعة الأولى (الضابطة) بنظام غذائي أساسي بدون أي مكملات ، بينما تم تغذية المجموعة الثانية (Ropadiar) بنظام غذائي أساسي يتضمن 2٪ Ropadiar. تم تقدير وزن الجسم ، زيادة الوزن ، إنزيمات الكبد ، البروتين الكلي ، الكرياتينين ، الألبومين ، الجلوبيولين ، ومستويات الكرياتينين. لوحظ تحسن كبير في وزن الجسم ، وزيادة الوزن ، وإنزيمات الكبد ، والبروتين الكلي ، والألبومين ، والكرياتينين ، ومستويات الجلوبيولين في المجموعة التي عولجت بـ 2 ٪ من Ropadiar مقارنة بالمجموعة الضابطة. وبالتالي ، يوصى بدمج زيت الأوريجانوم الأساسي 2٪ في النظام الغذائي لبلطي النيل لتحسين أداء النمو ، والمعايير البيوكيميائية في المصل ، وكذلك المؤشرات الحيوية لوظائف الكبد والكلى.