The effect of diets containing different concentrations of niosomal nanocarriers of oregano essential oil on lipid peroxidation in *Cyprinus carpio* fingerling

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Abstract

Oregano is an aromatic plant native to the Mediterranean region of Europe and Asia which is rich in plant chemical compounds including carvacrol and thymol, as well as phenolic compounds with antioxidant properties. In this study, the effects of using oregano essential oil niosomal nanocarriers on the lipid peroxidation and the plasma antioxidant defense of Cyprinus fingerlings were investigated. A total of 240 common carp fingerling were fed in four groups, i.e., basic diet (control group), basic diet+0.05 (T1),+0.1 (T2),+1.5 g/Kg (T3) nano-niosomal oregano powder. On day 30 and day 60, the amount of plasma malondialdehyde (MDA) was significantly reduced in the treatments that had oregano added to their diet compared to the control group ($p<0.0001$). By ferric reducing antioxidant power (FRAP) method, a significant increase in the antioxidant capacity of plasma was observed on day 60 in the T2 ($p=0.0003$) and T3 ($p<0.0001$) compared to the control group.

Keywords: Oregano, Niosomal nanocarriers, Comman carp, Lipd peroxidation, FRAP

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Introduction

In recent decades, with the increase of human population in the world and the need to supply healthy food, more attention has been paid to the increase of aquatic production. Aquatic ecosystems are continuously polluted with different toxic compounds from agricultural, domestic, industrial, aquaculture and animal husbandry wastewater. In recent years, along with the increase in carp breeding, various types of pathogenic stress have been observed in this important breeding species. Diets containing antioxidants are effective in improving fish health and increasing productivity. Herbs and medicinal plants have gained special attention for their potential to improve antioxidant defenses (Galina *et al*., 2009). Due to the dangers of aquaculture and the production of free radicals in adverse conditions, fish always need a strong antioxidant defense to face the negative effects of these factors. One promising approach is the use of natural compounds, such as oregano extract, as a dietary supplement for fish. Oregano is a Mediterranean aromatic plant, and its most important species is Origanum vulgare, which is rich in plant chemical compounds, including carvacrol and thymol, as well as phenolic compounds with antioxidant activity (Alagawany *et al*., 2020). The oil of oregano extract contains phenolic, poly phenolic compounds, flavonoids and alkaloids (Chishti *et al*., 2013). Thymol and carvacrol have strong antioxidant properties (Yanishlieva *et al*., 1999) and the antioxidant effects of oregano are due to the high amount of carvacrol and thymol (Botsoglou *et al*., 2003). During the research of recent years, the beneficial effects of food diets containing oregano in fish have been determined in order to antioxidant activity (Zheng *et al*., 2009; El-Hawarry *et al*., 2018; Abdel-Latif *et al*., 2020). Antioxidant defense is another essential factor to consider, as it plays a critical role in protecting cells from oxidative damage. One of the major problems in the use of plant compounds is the volatility and hydrophobicity of plant essential oils, which limits their use in free form due to the reduction of stability and loss of their properties. In this regard, encapsulation methods can be used in order to increase the stability and prevent the oxidation of plant essential oil compounds in aqueous environments. This research has been conducted to investigate the effects of using oregano essential oil niosomal nanocarriers on the plasma antioxidant defense of common carp fingerlings.

Materials and methods

Preparation of experimental diets

The nano-niosomal oregano (Origanum vulgar) powder was made in laboratory (Sirati *et al*., 2024). A commercial feed of common carp (growing stage I) prepared and were well-mixed with some corn oil and the addition of the variously of nano nisomal oregano powder in each treatment and were kept in refrigerator at -19 ℃ until further use (Table 1). The basic diet containing 32-38% protein, 9-11% fat, 8-10% ash, 8-

10% have been reported from Gilak Daneh Navid Feed Mill Company.

Table 1: Dosage of administration of nano-niosimal oregano powder

Groups	Dosage of administration of nano niosimal oregano powder
Control	Basic diet
Treatment1	Basic diet + 0.05 g/Kg
Treatment2	Basic diet + 0.1 g/Kg
Treatment3	Basic diet + 1.5 g/Kg

Fish, culture condition, and experimental setup

A total of 300 Common carp fingerlings weighing 15±0.50 g was purchased from a private hatchery aquaculture center in Guilan province, located in the north of Iran. After transferring the fish to the research site, they were bathed with in a 500-liter aquarium containing 30 ppm virkomix® for 30 min and transferred to a quarantine aquarium. Each fish was examined individually by observing its body surface, gills, and fins for clinical symptoms of diseases, erosions, wounds, superficial fungi, and parasites. The fish were fed a basic commercial diet for two weeks. After two weeks, a total of 240 healthy fish (18±0.50 g) were randomly stocked into 12 glass aquariums with a capacity of 200 liters, divided into four groups with three replicates per group. Over the course of 60 days, each group was fed 2% of their body weight using their specific diets. The de-chlorinated water in the aquariums was aerated with continuous aeration via compressed air. Sixty percent of the rearing water in each aquarium was exchanged every other day by pump to maintain water quality. During the quarantine and experimental periods water's physicochemical parameters, including dissolved oxygen level, pH, and temperature, were measured weekly and adjusted to 4.9-5.5 mg/L, 7.2-7.6, and 26.8-27.2ºC respectively. The experiment was conducted under fluorescent light tubes with a 12-hour light and 12-hour dark cycle.

Blood sampling and measured parameters

At day 0, 30 and 60 of the study, blood samples were taken from six fish in each aquarium. After making sure that the fish were anesthetized, blood sampling was collected from the caudal vein using a heparin-coated syringe to prevent clotting. The collected blood was then centrifuged at 3000 × g for 15 min at 4ºC using a Hettich centrifuge from Germany to separate the plasma from the cellular components. Plasma samples were carefully transferred to new tubes and stored at -20ºC until analysis. Samples were transported on ice to the laboratory.

The levels of malondialdehyde (MDA) were measured in the plasma samples using a diagnostic kit from ZellBio GmbH, Germany (Kei, 1978; Armstrong and Browne, 1994; Yagi, 1998).

The antioxidant capacity of plasma was measured using the FRAP (ferric reducing antioxidant power) assay. Briefly, 10 microliters of plasma were added to 300 microliters of FRAP solution at 37°C. After centrifugation of the sample, the supernatant solution was measured against a control at a wavelength of 593 nm after a 5 min incubation period. The antioxidant capacity was determined using a standard curve. The FRAP solution consisted of 10 mL of acetate buffer (300 mM concentration, pH 3.6), 1 mL of TPTZ solution (10 mM concentration), and 1 mL of a 20 mM ferric chloride solution. The Fe^{2+} ions produced in the reaction form a blue-colored complex with TPTZ, which absorb light at 593 nm. The amount of Fe^{2+} ions produced is proportional to the antioxidant capacity of the plasma sample (Benzie and Strain, 1996).

Ethical approval was obtained from the Science and Research Branch, Islamic Azad University Ethics Committee (Cod number: IR.IAU.SRB.REC.1400.314).

Statistical analysis

All the tests of this study were repeated 3 times. All parameters were expressed as means±standard deviation (SD). Kolmogorov-Smirnov test was used to check the normal distribution of continuous numerical variables. The statistical analysis was carried out using the one-way analysis of variance (ANOVA) followed by Tukey's test to determine whether there are any significant differences between the means of the various measured parameters using GraphPad Prism software (version 8.0.2, https://www.graphpad.com). The statistically significant difference was defined as $p<0.05$.

Results

In this study, on day 30 and day 60, the amount of plasma MDA was significantly reduced in the groups that had oregano added to their diet compared to the control group ($p<0.0001$) (Fig. 1).

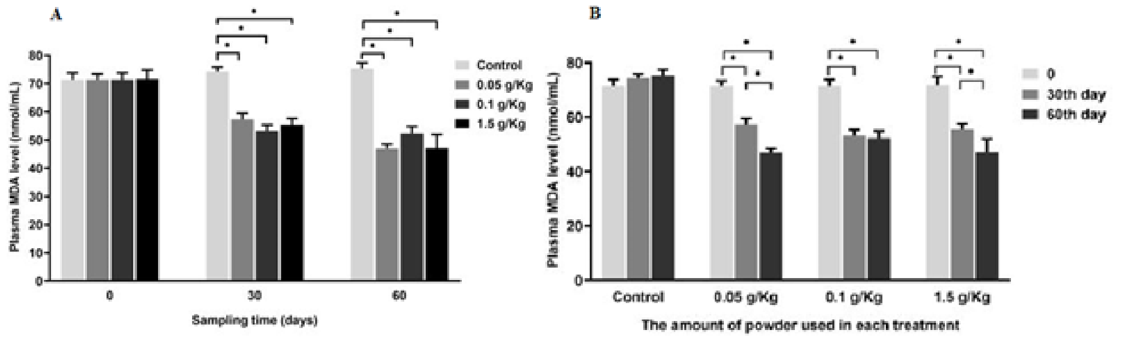

Figure 1: Effect of dietary oregano essential oil on the concentrations of MDA (A & B) in plasma. Data are expressed as means±SD. Nano niosimal oregano powder was supplemented at a concentration 0.05 g kg^{-1}; 0.1 g kg^{-1}; and 1.5 g kg^{-1} of basal diet, respectively. * $p<0.05$.

In this study, in measuring the antioxidant capacity of plasma by FRAP method, a significant increase in the antioxidant capacity of plasma was observed on days 30 in the T2 ($p=0.0129$) and T3 ($p<0.0001$), and on day 60th in the T2 ($p=0.0003$) and T3 ($p<0.0001$) compared to the control group. Also, on both days, the increase in plasma antioxidant capacity was significantly higher in the group that had the highest amount of oregano in their diet than in the first (day 30: p=0.0002, day 60: p<0.0001) and T2 (day 30: p=0.0103, day 60: p=0.0056). On the 60th day, the antioxidant capacity of the plasma of the T2 was significantly higher than that of the T1 (p=0.0184). There was no significant difference between the T1 and the control group. The antioxidant capacity of plasma was significantly higher on the 30th and 60th days in the treatment that had the highest amount of oregano added to their diet compared to the first day (p<0.0001), while there was no significant difference between the 30th and 60th days in this treatment. In the T2, only the antioxidant capacity on the 60th day had a significant increase compared to the first day of the experiment (p=0.0018) (Fig.2).

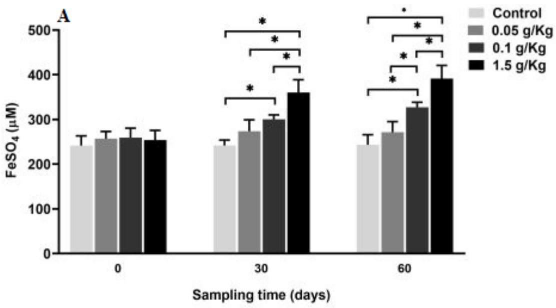

Figure 2: Effect of dietary oregano essential oil on the antioxidant capacity of plasma (A & B). Data are expressed as means±SD. Nano niosimal oregano powder was supplemented at a concentration 0.05 g kg^{-1}; 0.1 g kg^{-1}; and 1.5 g kg^{-1} of basal diet, respectively. *p<0.05.

Discussion

In this study, we used niosomal nanocarriers of oregano essential oil (OEO) in the diet of treatment groups compared to the control group of common carp fingerling. Plant extracts and phytobiotics have gained considerable attention in the field of aquaculture because of their potential benefits in displaying antioxidant activity in fish. Phytobiotics refer to bioactive compounds derived from plants that are used as feed additives or supplements in aquaculture. These compounds can be extracted from various parts of plants, including leaves, stems, roots, and seeds. Plant extracts and phytobiotics are rich in bioactive compounds such as phenolic compounds, flavonoids, alkaloids, terpenoids, and essential oils, which contribute to their beneficial effects. However, it's important to note that the efficacy of specific plant extracts and phytobiotics can vary depending on factors such as species of fish, dosage, duration of administration, and the specific bioactive compounds present in the extract. Niosomes can be an effective carrier system for OEO, providing improved stability and sustained release properties. This could potentially enhance the therapeutic efficacy of the oil and reduce its side effects. Nanonisomes are drug carriers that protect medicinal compounds against degradation, increase their effectiveness, and prevent the volatilization and oxidation of plant essential compounds (Severino *et al.*, 2019). Due to the volatility and hydrophobicity of the compounds of plant essential oils, using them in free form will reduce their stability and lose their properties. Encapsulation preserves active plant compounds against heat, humidity and pH and releases them when needed. By using new formulations and encapsulating plant essences in nano form, their bioavailability and therapeutic effect increases. Encapsulation could be used in order to increase the stability and prevent the oxidation of plant essential oil compounds in aqueous environments (Haghiralsadat *et al*., 2017).

Lipid peroxidation (LPO) is a well-recognized outcome of oxidative stress. Specific reactive oxygen species (ROS), including hydroxyl and hydroperoxyl radicals, can remove electrons from polyunsaturated fatty acids (PUFAs), leading to deprotonation at the double bond of these fatty acids and the formation of a lipid radical ($L^\bullet$). This is subsequently followed by the introduction of molecular oxygen. As a result, a lipid peroxide radical ($LOO^\bullet$) is formed, which can oxidize an adjacent PUFA, yielding a lipid hydroperoxide (LOOH) along with a new $L^\bullet$ (Halliwell and Chirico, 1993). The LOOH generated from LPO may further decompose into a multitude of secondary products through mechanisms such as beta-scission and hock cleavage, with aldehydes being the most prominent harmful secondary products associated with LPO (Fritz and Petersen, 2013).

Previous reports investigated the effects of dietary inclusion of OEO on the antioxidant and immune responses of common carp. The study found that dietary inclusion of OEO significantly decreased levels of hepatic MDA, indicating improved antioxidant status (Abdel-Latif *et al*., 2020). This was consistent with our results, which showed that after OEO supplementation, the levels of plasma MDA decreased. MDA is a widely used marker for oxidative stress resulting from pollutant and lipid peroxidation in biological samples (Garcia *et al*., 2020). Reduction of MDA is a sign of reduction of oxidative injuries to cell lipids. This indicates an improvement in the antioxidant status of the fish.

The antioxidant activity of a compound is primarily due to the presence of aromatic rings and the number and arrangement of hydroxyl groups, as reported in studies by Brewer. These properties allow the compound to scavenge free radicals and neutralize reactive oxygen species (ROS), protecting cells and tissues from oxidative damage. In the case OEO, its antioxidant activity is attributed to the presence of several phenolic compounds, including carvacrol, thymol, rosmarinic acid, and others. These phenolic compounds have been shown to possess strong antioxidant activity, making OEO a potentially effective natural antioxidant that can improve the health and performance of fish in aquaculture systems (Brewer, 2011). Thymol also exhibits antioxidant and anti-inflammatory effects, which contribute to its potential health benefits. Due to their bioactive properties, carvacrol and thymol have been studied and utilized in various fields, including food preservation, pharmaceuticals, agriculture, and aquaculture. The antioxidant properties of OEO have been attributed to its ability to scavenge free radicals, chelate transition-metal ions, and decompose peroxides (Yanishlieva *et al*., 1999, Su *et al*., 2007). These properties make OEO a potentially effective natural antioxidant that can improve the antioxidant status of fish and prevent oxidative damage caused by free radicals and ROS. In gilthead seabream, *Sparus aurata* L., use of Origanum vulgare leaf extracts improved the antioxidant capacity of the liver (Beltrán *et al*., 2018). By FRAP method, a significant increase in the antioxidant capacity of plasma was observed on days 30th and 60th in the second and third groups compared to the control group. The FRAP assay measures the ability of an antioxidant to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions in an acidic medium. The FRAP method is a sensitive approach for measuring the total antioxidant capacity of biological fluids (Szőllősi and Varga, 2002).

In conclusion, the current study illustrated that dietary niosomal nanocarriers of OEO seems a suitable feed additive in common carp fingerlings that boosted their antioxidant capacity and lipid peroxidation. These findings suggest that OEO can be an effective natural antioxidant that could improve the antioxidant status of fish and protect them from oxidative damage caused by free radicals and ROS.

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References

Abdel-Latif, H.M., Abdel-Tawwab, M., Khafaga, A.F. and Dawood, M.A., 2020. Dietary origanum essential oil improved antioxidative status, immune-related genes, and resistance of common carp (*Cyprinus carpio L.*) to *Aeromonas hydrophila* infection. *Fish and Shellfish Immunology*, 104,1-7. https://doi.org/10.1016/j.fsi.2020.05.056.

Alagawany, M., Farag, M.R., Salah, A.S. and Mahmoud, M.A., 2020. The role of oregano herb and its derivatives as immunomodulators in fish. *Reviews in Aquaculture.* 12(**4**), 2481-2492. https://doi.org/10.1111/raq.12453.

Armstrong, D. and Browne, R., 1994. The analysis of free radicals, lipid peroxides, antioxidant enzymes and compounds related to oxidative stress as applied to the clinical chemistry laboratory. *Free radicals in diagnostic medicine: A systems approach to laboratory technology, clinical correlations, and Antioxidant Therapy*, 43-58.

https://doi.org/10.1007/978-1-4615-1833-4_4.

Beltrán, J.M.G., Espinosa, C., Guardiola, F.A. and Esteban, M.Á., 2018. In vitro effects of Origanum vulgare leaf extracts on gilthead seabream (*Sparus aurata L.*) leucocytes, cytotoxic, bactericidal and antioxidant activities. *Fish and Shellfish Immunology.* 79, 1-10. https://doi.org/10.1016/j.fsi.2018.05.005.

Benzie, I.F. and Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry.* 239(**1**),70-76.

Botsoglou, N., Grigoropoulou, S., Botsoglou, E., Govaris, A. and Papageorgiou, G., 2003. The effects of dietary oregano essential oil and α-tocopheryl acetate on lipid oxidation in raw and cooked turkey during refrigerated storage. *Meat Science.* 65(**3**),1193-1200. https://doi.org/10.1016/S0309-1740(03)00029-9.

Brewer, M., 2011. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Comprehensive Reviews in Food Science and Food Safety*, 10(**4**),221-247. https://doi: 10.1111/j.1541-4337.2011.00156.x.

Chishti, S., Kaloo, Z.A. and Sultan, P., 2013. Medicinal importance of genus Origanum: A review. *J. Pharmacogn. Phytother.* 5(**10**),170-177. https://doi.10.5897/JPP2013.0285.

El-Hawarry, W.N., Mohamed, R.A. and Ibrahim, S.A., 2018. Collaborating effects of rearing density and oregano oil supplementation on growth, behavioral and stress response of Nile tilapia (*Oreochromis niloticus*). *The Egyptian Journal of Aquatic Research.* 44(**2**),173-178. https://doi.org/10.1016/j.ejar.2018.06.008.

Fritz, K.S. and Petersen, D.R., 2013. An overview of the chemistry and biology of reactive aldehydes. *Free Radical Biology and Medicine.* 59,85-91.

Galina, J., Yin, G., Ardo, L. and Jeney, Z., 2009. The use of immunostimulating herbs in fish. An overview of research. *Fish Physiology and Biochemistry.* 35,669-676. https://doi.10.1007/s10695-009-9304-z.

Garcia, D., Lima, D., Da Silva, D.G.H. and De Almeida, E.A., 2020. Decreased malondialdehyde levels in fish (Astyanax altiparanae) exposed to diesel: Evidence of metabolism by aldehyde dehydrogenase in the liver and excretion in water. *Ecotoxicology and Environmental Safety.* 190,110107. https://doi.org/10.1016/j.ecoenv.2019.110107.

Haghiralsadat, F., Amoabediny, G., Sheikhha, M.H., Forouzanfar, T., Helder, M.N. and Zandieh-Doulabi, B., 2017. A novel approach on drug delivery: Investigation of a new nano-formulation of liposomal doxorubicin and biological evaluation of entrapped doxorubicin on various osteosarcoma cell lines. *Cell Journal (Yakhteh),* 19(**Suppl 1**),55. https://doi.org/10.22074%2Fcellj.2017.4502.

Halliwell, B. and Chirico, S., 1993. Lipid peroxidation: Significance and its mechanism. *Am. J. Clin. Nutr.* 57,715-725. https://doi.org/10.1016/j.freeradbiomed.2012.06.025.

Kei, S., 1978. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica chimica acta.* 90(1),37-43. https://doi.org/10.1016/0009-8981(78)90081-5.

Severino, P., Da Silva, C.F., Andrade, L.N., De Lima Oliveira, D., Campos, J. and Souto, E.B., 2019. Alginate nanoparticles for drug delivery and targeting. *Current pharmaceutical design.* 25(**11**),1312-1334. https://doi.org/10.2174/1381612825666190425163424.

Sirati, R., Khajehrahimi, A.E., Kazempoor, R., Kakoolaki, S. and Ghorbanzadeh, A., 2024. Development, physicochemical characterization, and antimicrobial evaluation of niosome-loaded oregano essential oil against fish-borne pathogens. *Heliyon.* https://doi.org/10.1016/j.heliyon.2024.e26486.

Su, L., Yin, J.J., Charles, D., Zhou, K., Moore, J. and Yu, L.L., 2007. Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. *Food Chemistry.* 100(**3**),990-997. https://doi.org/10.1016/j.foodchem.2005.10.058.

Szőllősi, R. and Varga, I.S.I., 2002. Total antioxidant power in some species of Labiatae: Adaptation of FRAP method. *Acta Biologica Szegediensis.* 46(**3-4**),125-127.

Yagi, K., 1998. Simple assay for the level of total lipid peroxides in serum or plasma. *Free Radical and Antioxidant Protocols.*101-106. https://doi.org/10.1385/0-89603-472-0:101.

Yanishlieva, N.V., Marinova, E.M., Gordon, M. H. and Raneva, V.G., 1999. Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. *Food Chemistry.* 64(**1**),59-66. https://doi.org/10.1016/S0308-8146(98)00086-7.

Zheng, Z., Tan, J.Y., Liu, H., Zhou, X., Xiang, X. and Wang, K., 2009. Evaluation of oregano essential oil (Origanum heracleoticum L.) on growth, antioxidant effect and resistance against *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*). *Aquaculture*, 292(3-4),214-218. https://doi.org/10.1016/j.aquaculture.2009.04.025.