**Effects of Oregano Essential Oil in Fish**



***Εικ. 1. Dicentrarchus labrax (λαβράκι)***

**Θεσσαλονίκη 2021**

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# **Τίτλος μελέτης: *The role of oregano herb and its derivatives as immunomodulators in fish***

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**Reviews in aquaculture, 1-12**

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**Περίληψη μελέτης:** Πολλά φυτικά πρόσθετα έχουν θετικές επιδράσεις στα ψάρια όταν τοποθετούνται στο νερό ή στην τροφή τους. Πιο συγκεκριμένα, το αιθέριο έλαιο ρίγανης, με τα βιοενεργά συστατικά του, δρα συνεργιστικά ως **διουρητικός**, **αντισπασμωδικός**, **στομαχικός**, **αντιπαρασιτικός**, **ανοσορρυθμιστικός** και **αντιμικροβιακός παράγοντας**. Γενικά, έχουν προσδιοριστεί **περισσότερα από 60 βιοενεργά μόρια** στη ρίγανη. Επιπροσθέτως, πολλές μελέτες έχουν δείξει πως το ριγανέλαιο προσδίδει στα ψάρια **αντοχή σε σημαντικές ασθένειες**. Ακόμη, φαίνεται πως οι αντιοξειδωτικές ιδιότητες του αιθέριου ελαίου ρίγανης **βελτιώνουν την υγεία των ψαριών** και **αυξάνουν την παραγωγικότητα (**Dabrowski et al. 2004; Gao et al. 2012; Diler et al. 2017). Συμπερασματικά, **οι ερευνητές θεωρούν πως η προσθήκη ρίγανης και των παραγώγων της, ως συμπλήρωμα διατροφής, σε βασικές δίαιτες μπορεί να θεωρηθεί ως ο καλύτερος τρόπος για την ενίσχυση της ανοσίας και της υγείας των ψαριών**.

**Abstract:** The motivation behind this article is to give point-by-point data about the beneficial applications of oregano feed supplement in fish diets as immunomodulators, antioxidant, antiviral, antifungal and antiparasitic. Use of this plant as feed additive plays an important role in the fish diet when compared to other synthetic feed additives. Oregano is rich in phytochemical compounds including carvacrol and thymol in addition to other phenolic compounds with antioxidant and immune enhancing activities. Origanum vulgare extract improved the immunological responses and enhanced non-specific immunity. Also, non-specific immunity and the lysosomal activity were significantly increased in rainbow trout fed diet enriched with 3.0 mL essential oil of Origanum onites L kg-1 diet for 60 days. Furthermore, non-specific immune stimulant, antioxidant and nitric oxide activities were improved due to O. vulgare oil supplementation. In some recent studies, Origanum heracleoticum L essential oil as a growth enhancer increased the antioxidant status. In rainbow trout, the hepatic levels of antioxidant enzymes and the total antioxidant capacity increased by feeding diet enriched with 6 and 10 g kg-1 diet of O. vulgare extract. Therefore, the addition of oregano and/or derivatives as a dietary supplement in fish diet may promote growth and enhance the immunity and health of fish and this will be useful for nutritionists, physiologists and veterinarians.

**Conclusions:** The aforementioned literature and explanations pointed out that oregano feed additive can be used as natural growth promoters (non-antibiotic type), antimicrobial and immunostimulant. Additionally, there are significant positive effects of oregano oil on the increasing absorptive area of the fish intestine. So, adding oregano and its derivatives as dietary supplementation to basal diets may consider the best way to enhance the fish immunity and health.

# **Τίτλος μελέτης: *Antimicrobial effectiveness of gelatin-alginate film containing oregano essential oil for fish preservation***

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**Περίληψη μελέτης:** Στην παρούσα έρευνα χρησιμοποιήθηκαν τεμάχια ιριδίζουσας πέστροφας, τα οποία τυλίχθηκαν με φιλμ ζελατίνης-αλγινικού άλατος. Στο φιλμ προστέθηκε ποσότητα 1.5% αιθέριου ελαίου ρίγανης και η συντήρηση των φιλέτων διήρκησε 15 ημέρες. Επίσης, χρησιμοποιήθηκε δείγμα μάρτυρας, το οποίο δεν τυλίχθηκε με το φιλμ. Διαπιστώθηκε από τους μελετητές ότι το φιλμ με το ριγανέλαιο λειτούργησε ως **βακτηριοστατικός παράγοντας**, προκαλώντας μια πιο αργή ανάπτυξη των αλλοιογόνων βακτηρίων. Επομένως, το αιθέριο έλαιο ρίγανης, λόγω της **αντιμικροβιακής** του **δράσης**, μπορεί να χρησιμοποιηθεί αποτελεσματικά ως πρόσθετο σε συστήματα συσκευασίας ψαριών. Όπως αποδείχθηκε από την έρευνα, το ριγανέλαιο **βοήθησε** **στην** **αύξηση του χρόνου συντήρησης** και **στην τελική ποιότητα** των τεμαχίων της πέστροφας.

**Abstract:** This study aimed to evaluate the effect of gelatin–alginate film containing 1.5% oregano essential oil (OEO) on the shelf life of rainbow trout (Oncorhynchus mykiss) slices during refrigerated storage over a period of 15 days. The blend film was prepared at 75% fish gelatin to 25% sodium alginate ratio and OEO was added to the film formulation. All the treatments (the control and wrapped slices) were analyzed periodically in terms of microbiological factors (total viable count, psychrotrophic count and spoilage microorganism, such as lactic acid bacteria, Pseudomonas spp. and Enterobacteriaceae). In addition, the samples were analyzed in terms of total volatile base nitrogen (TVB-N) and pH. Use of the OEO–blend film delayed bacterial growth throughout 15 days of storage compared with the control and slices treated with blend films without OEO (P < 0.05). The lowest TVB-N and pH levels were 59.98 and 6.75, respectively, in OEO film at the end of the storage. Therefore, this study showed that OEO–blend film was an effective antimicrobial suitable for the potential food packaging applications.

**Conclusions:** The results of the present study indicated that the application of OEO–blend film treatment maintained the quality of the rainbow trout slices better than the control and slices wrapped with the neat blend film. Application of the blend film-enriched OEO had the highest antimicrobial effect on psychrotrophic bacteria, TVC and Enterobacteriaceae. The blend film without OEO, due to the restricted access to oxygen, significantly (P < 0.05) reduced the growth of Pseudomonas spp. counts. Also, lower increasing rate of the TVB-N and pH values in the slices treated with OEO–blend film, compared with the other treatments, confirmed its good antimicrobial properties.

# **Τίτλος μελέτης: *Dietary oregano essential oil improved the growth performance via enhancing the intestinal morphometry and hepato-renal functions of common carp (Cyprinus carpio L.) fingerlings***

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**Περίληψη μελέτης:** Στη συγκεκριμένη μελέτη χρησιμοποιήθηκε ριγανέλαιο ως πρόσθετο σε δίαιτα κυπρίνων (Cyprinus carpio L.) για δύο μήνες, σε διαφορετικά ποσοστά, προκειμένου να ελεγχθεί η αποτελεσματικότητα του και οι επιδράσεις του στα ψάρια. Οι δίαιτες περιείχαν δείγματα με ποσότητες 0, 5, 15 και 20 *g ριγανελαίου/kg δίαιτας.* Τα καλύτερα αποτελέσματα παρουσιάστηκαν σε **ποσότητα 15 γραμμαρίων** **ανά κιλό τροφής**. Το αιθέριο έλαιο ρίγανης επηρέασε θετικά τις παραμέτρους ανάπτυξης των ψαριών. Ειδικότερα, η προσθήκη του στην δίαιτα των ψαριών έδωσε **υψηλότερο τελικό σωματικό βάρος**, **βελτιωμένη αύξηση βάρους**, **αυξημένο ειδικό ρυθμό ανάπτυξης** και **καλύτερη πρόσληψη της τροφής**. Τέλος, η προσθήκη ριγανελαίου βοήθησε σημαντικά στην **καλύτερη λειτουργία του εντέρου** των ψαριών, το οποίο προκαλεί πολλά **σημαντικά οφέλη** στην γενικότερη λειτουργία του οργανισμού τους και στην βελτιωμένη τελική ποιότητα του κρέατος.

**Abstract:** The current work was designed to assess the influences of dietary oregano essential oil (OEO) on the indices of growth and feed utilization, proximate composition, hepato-renal functions, and histomorphological criteria of livers, kidneys, and intestines of common carp (Cyprinus carpio L.) fingerlings. Fish (20.3 ± 0.8 g) were randomly divided into five treatments and fed on different levels of OEO at 0, 5, 10, 15, and 20 g/kg diet for 2 months. The results showed that the dietary OEO significantly improved the growth parameters (final body weight, weight gain, weight gain %, specific growth rate, and feed intake) (P < .05) in a dose-dependent regime; meanwhile, the feed conversion ratio was not affected with respect to the control group. The body proximate composition and survival rates were not significantly affected. The serum protein profile (total protein, albumin, and globulin), activities of liver function enzymes (alanine transaminase aspartate transaminase, and alkaline phosphatase), and renal markers (creatinine and urea) were not significantly altered (P < .05) by OEO supplementation. The histomorphology of hepatopancreatic and renal tissues of common carp fed OEO at varying levels were correlated with the serum hepato-renal functions without any noticed pathologic lesions. Moreover, the intestinal villi with associated goblet cells and crypt, tunica muscularis (both internal and external muscular layers) and submucosal tissues appeared free of inflammatory and/or degenerative changes. All intestinal morphometric measurements revealed a significant improvement (P < .05) in OEO-fed fish in comparison with the control one. Conclusively, in common carp, the dietary OEO improved growth performance and intestinal histomorphology with no inflammatory signs, and with potential hepato-protective effects with the optimum level of 15 g/kg diet. These beneficial effects were possibly because of OEO-mediated beneficial improvements in the histomorphometric criteria of the fish intestines.

**Conclusions:** In conclusion, the obtained results herein illustrated that dietary supplementation with OEO considerably improved growth performance indices and feed utilization parameters of common carp fingerlings. Additionally, OEO maintained their normal hepato-renal features (serum markers and histomorphological structures). Moreover, dietary administration of OEO at a dose rate of 15 g/kg diet can beneficially improve the intestinal histomorphological and morphometrical criteria. The beneficial effects of dietary OEO were possibly accredited to its hepatoprotective function and its vital role in improving fish guts. However, further research is required for better understanding of the mechanisms of dietary OEO in improving fish health and productivity.

**Extra information taken from the paper**

Μελέτες που έχουν δείξει θετικές επιδράσεις της ρίγανης σε συγκεκριμένα είδη ψαριών: *“In fish, several researchers examined the beneficial effects of the whole leaves of oregano, O. vulgare extract meal or its essential oil (OEO) as dietary supplements to improve fish health and promote its growth rate. In this concern, it was found that dietary OEO-supplementation can considerably improve the growth and immune responses in several species of fish; for instance, channel catfish (Ictalurus punctatus) (Zheng et al., 2009), sturgeon (Huso huso) (Ahmadifar et al., 2014), Nile tilapia (Oreochromis niloticus) (Abdel-Latif and Khalil, 2014; El-Hawarry et al., 2018), rainbow trout (Oncorhynchus mykiss) (Ahmadifar et al., 2011), Tilapia zillii (Mabrok and Wahdan, 2018), and Yellowtail Tetra (Astyanax altiparanae) (Ferreira et al., 2014). Additionally, the oregano leaves can significantly improve the immune and antioxidant status of gilthead seabream (Sparus aurata) (Beltrán et al., 2018; Beltrán et al., 2020) and increased protection of Nile tilapia against Streptococcus agalactiae (Santo et al., 2019).”*

# **Τίτλος μελέτης: *The immune modulatory effect of oregano (Origanum vulgare L.) essential oil on Tilapia zillii following intraperitoneal infection with Vibrio anguillarum***

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**Περίληψη μελέτης:** Στην παρούσα εργασία μελετήθηκε το κατά πόσο μπορεί το αιθέριο έλαιο ρίγανης να προστατέψει τα ψάρια από πιθανές μολύνσεις. Το είδος των βακτηρίων Vibrio anguillarum αποτελεί μια συνηθισμένη απειλή για τους «κατοίκους» του θαλάσσιου κόσμου. Τα ψάρια που είχαν στη διατροφή τους συμπλήρωμα ριγανελαίου εμφάνισαν σχεδόν **μηδαμινά συμπτώματα συγκριτικά με τα υπόλοιπα δείγματα**. Η μελέτη έδειξε πως το αιθέριο έλαιο ρίγανης μπορεί να βοηθήσει στην **πρόληψη ασθενειών από προβλέψιμα απειλητικά παθογόνα στα ψάρια**.

**Abstract:** The current study aimed to evaluate the possible effect of Origanum essential oil on innate immune parameters as well as the hematological profiles of Tilapia zillii following challenge with Vibrio anguillarum. Fifty-four of Tilapia zillii weighing 180 ± 10.2 g were randomly distributed into three identical closed recirculating seawater systems. The study included three groups (G1, G2, and G3) repeated in triplicates. Fish of the first two groups were fed on a basal diet without herbs, whereas fish of the last group were fed on a basal diet supplemented with Origanum essential oil at concentration 1 g kg−1 for 15 days. Subsequently, fish of G2 and G3 subjected to a peritoneal inflammation by intraperitoneally injecting V. anguillarum (5.5 × 105 CFU mL−1), whereas fish of G1 injected with saline and served as control. Fish of all groups were then sampled at 4, 12, and 24 h post injection. No mortalities were observed in both basal and Origanum fed groups. However, some specimens of fish fed basal diet showed dorsal fin erosions, eroded mouth, and detached skin. Although the kinetics of RBCs, WBCs, Hb, and differential leukocyte values remained unchanged in fish fed different diets at the beginning of the trial, significant increases in those values were observed in fish fed Origanum essential oil at 12 and 24 h post injection. Similarly, an augmentation of plasma proteases, antiproteases, and lysozyme activities were recorded in fish fed Origanum essential oil at the same particular sampling points. A significant enhancement in plasma bactericidal capacity was only recorded in fish fed Origanum essential oil at 12 and 24 h post challenge compared to those fed basal diet. In conclusion, Origanum essential oil had a pronounced influence on the innate immunity and increased the fish resistance to V. anguillarum. These data gave insight into the potential use of Origanum in prophylactic strategies against threatening pathogens.

**Conclusions:** In conclusion, Origanum essential oil at a concentration of 1 g kg−1 appears to exert positive effects on T. zillii immune status via improving the peripheral leucocyte response and plasma bactericidal capacity. Among acute inflammation, this exerted immunity is translated to an increase of both cellular and humoral responses. The current data gave insight into the potential use of Origanum essential oil in prophylactic strategies against predictable threatening pathogens, hopefully, to cope to the problem of antibiotic resistant. Further detailed immunological and molecular studies are still required to expand the application of these results in aquaculture as a prophylactic measure.

# **Τίτλος μελέτης: *Effect of feed supplementation with Origanum vulgare L. essential oil on sea bass (Dicentrarchus labrax): A preliminary framework on metabolic status and growth performances***

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**Aquaculture Reports**

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**Περίληψη μελέτης:** Στην μελέτη αυτή παρατηρήθηκε η επίδραση του συμπληρώματος διατροφής αιθέριου ελαίου ρίγανης σε λαβράκια**. Η καλύτερη επίδοση εμφανίστηκε σε ποσότητα 100 ppm ριγανελαίου**. Η διατροφή με συμπλήρωμα **100 ppm** είχε ως αποτέλεσμα **υψηλές επιδόσεις ανάπτυξης**, **καλύτερη αξιοποίηση της τροφής** (*food conversion*) και **υψηλότερο συντελεστή πρωτεϊνικής αποτελεσματικότητας** (Protein Efficiency Ration-PER). Ακόμη, το αιθέριο έλαιο ρίγανης **βελτίωσε την υγεία των ψαριών** και λειτούργησε ως **αντιοξειδωτικός παράγοντας** στα ψάρια. Οι ερευνητές θεωρούν πως αυτή του η δράση οφείλεται στην **καρβακρόλη**, ένα από τα κύρια συστατικά της ρίγανης.

**Abstract:** This study provided a preliminary framework for the effects of Origanum vulgare L. essential oil (EO) on sea bass (Dicentrarchus labrax) health status over a 60-day feeding trial. Fish were fed twice a day until apparent satiety with three different diets: a control diet (CD), and two experimental diets supplemented with 100 (D100) and 200 (D200) ppm of oregano EO. No mortality was observed in each treatment. Feeding on D100 diet resulted in high growth performances and better food conversion and protein efficiency ratios. Additionally, the supplementation of 100 ppm EO diet also improved (P < 0.05) hepatosomatic and viscerosomatic indices, compared both to control and D200 diets. EO feeding positively affected (P < 0.05) several serum biochemical indices (amylase activity and total proteins, glucose, triglycerides, and cholesterol levels). Focusing on the antioxidant potential of blood, D100 led to the highest (P < 0.05) ferric reducing antioxidant power values and the lowest (P < 0.05) thiobarbituric acid-reactive substances levels in blood.

**Conclusions:** Summarizing, the preliminary framework we constructed clearly shows that feed supplementation with oregano EO may differently affect several biomarkers of sea bass wellness depending on the level of feed supplementation. In particular, low doses of oregano EO hold a promising potential as health promoter for sea bass. Several biometric indices, blood parameters, and oxidative stress biomarkers benefited from the administration of oregano EO. One mechanism underlying the metabolic effects of oregano EO might be imputable to the biological properties of its main constituent, carvacrol.

# **Τίτλος μελέτης: *Essential Oregano Oil as a Growth Promoter for the Yellowtail Tetra, Astyanax altiparanae***

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**Περίληψη μελέτης:** Στην παρούσα μελέτη, οι ερευνητές εξέτασαν τη δράση του ριγανελαίου ως προαγωγό της ανάπτυξης του ψαριού *Astyanax altiparane*. Εφάρμοσαν δίαιτες με ποσότητα 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 g ΟΕΟ/kg τροφής. Μετά τις 90 ημέρες ελέγχου βρέθηκε ότι το αιθέριο έλαιο ρίγανης είχε **θετική επίδραση στην αύξηση του βάρους των ψαριών**, **στον ειδικό ρυθμό ανάπτυξης**, **στον λόγο απόδοσης της πρωτεΐνης** και **στο βάρος του σφαγίου**. Η καλύτερη απόδοση των παραπάνω παρουσιάστηκε σε ποσότητες μεταξύ 0.2 και 0.6 g OEO/kg τροφής. Τα αποτελέσματα της έρευνας δείχνουν ότι **το αιθέριο έλαιο ρίγανης σε ποσότητα 0.5 γραμμάρια ανά κιλό τροφής βοηθά στην αύξηση του βάρους των ψαριών**.

**Abstract:** This study aimed to evaluate the potential of oregano oil as a growth promoter for the yellowtail tetra, Astyanax altiparanae. The fish (1.46 ± 0.09 g) were distributed into twenty-four 60-L aquaria at a stocking density of 0.5 fish/L. Six isonitrogenous (350 g crude protein/kg) and isocaloric (4272 kcal gross energy/kg) diets containing 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 g/kg of oregano oil were evaluated. At the end of 90 d there was a quadratic effect of oregano oil levels for weight gain, specific growth rate, protein efficiency ratio and carcass weight, and the estimated values to maximize these variables were between 0.2 and 0.6 g/kg. There was also a quadratic effect on the feed conversion ratio, and the estimated value to improve this variable was 0.62 g/kg. A positive linear effect of the treatments was observed for muscle glycogen. With increased levels of oregano oil in the diet, there was a reduction in dry matter and ether extract and an increase in the protein content of the carcass. Thus, it can be concluded that oregano oil, at the level of 0.5 g/kg, acts as a growth promoter for A. altiparanae by improving growth performance and carcass composition.

**Conclusions:** The results of this study demonstrate that the essential oregano oil, at the level of 0.5 g/kg, acts as a growth promoter for the yellowtail tetra, A. altiparanae.

# **Τίτλος μελέτης: *The Effects of Some Phytoadditives on Growth, Health and Meat Quality on Different Species of Fish***

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**Περίληψη μελέτης: Τα φυτικά πρόσθετα**, όπως το αιθέριο έλαιο ρίγανης, **πλεονεκτούν** συγκριτικά με άλλες μεθόδους **γιατί** είναι φυσικές ουσίες και «***δεν αποτελούν απειλή για τα ψάρια, τον άνθρωπο ή το περιβάλλον***». Δείγματα-groups ψαριών, των οποίων η τροφή εμπεριείχε ποσότητα ριγανελαίου παρουσίασαν **βελτιωμένη ανάπτυξη** συγκριτικά με τα δείγματα-groups ελέγχου. Επίσης, μεταξύ άλλων, παρατηρήθηκε **ενισχυμένη αντιοξειδωτική ικανότητα**. Ακόμη, το αιθέριο έλαιο ρίγανης έδωσε τα **χαμηλότερα ποσοστά θνησιμότητας** σε groups που είχαν μολυνθεί πειραματικά με το παθογόνο βακτήριο Aeromonas h. Οι ερευνητές συμπεραίνουν ότι τα φυτικά πρόσθετα, όπως το ριγανέλαιο, μπορούν να χρησιμοποιηθούν στην καλλιέργεια ψαριών ως **βελτιωτές ανάπτυξης**, ως **ανοσοδιεγερτικά**, ως **ανοσορρυθμιστές**, ως **αντιμικροβιακά** και **αντιοξειδωτικά** πρόσθετα.

**Abstract:** The growing tendency for food safety led to the ban of antibiotics. To replace their effects, the search for natural alternatives has begun. Thus, strong candidates to replace antibiotics are phytoadditives. Phytoadditives are fodder additives obtained from medicinal plants or plants extracts. Through their use it can be hoped to achieve the same results as in the use of antibiotics. Although they have a large spectrum of use, even today their mechanisms of action are not fully understood. Only recent, the study on the possibility of phytoadditives usage in aquaculture has begun. The present study is a review of the use of some plants as phytoadditives (garlic, onion, oregano, etc.) and of the researches made to reveal their effects on fish. The researches conducted in this field have shown the diverse effects of these phytoadditives used in fish as immunomodulators, immunostimulants, bioproductives, antioxidants, antimicrobials, stimulants of the enzymatic equipment, stimulants of nitrogen absorption. A major advantage in the use of phytoadditives is the fact that they are natural substances and do not pose any threat to fish, man or environment.

**Conclusions:** Phytoadditives represent alternative solutions to substitute antibiotics used in aquaculture. They can be used as growth promoters, recent studies showing their effects as immunostimulants, immunomodulators, antimicrobials and antioxidants. However, further researches are necessary, including total commercial cost and benefit analysis, before they can be used on a large scale in aquaculture.

# **Τίτλος μελέτης: *Evaluation of oregano essential oil (Origanum heracleoticum L.) on growth, antioxidant effect and resistance against Aeromonas hydrophila in channel catfish (Ictalurus punctatus)***

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**Περίληψη μελέτης:** Στην παρούσα εργασία, οι μελετητές παρατήρησαν την επίδραση αιθέριου ελαίου ρίγανης σε γατόψαρα. Έλεγξαν την επίδραση της θυμόλης, της καρβακρόλης, του συνδυασμού τους, όπως υπάρχει φυσικά στο ριγανέλαιο, αλλά και την απουσία τους. Ο διαχωρισμός των group έδειξε ότι το δείγμα με τον συνδυασμό της θυμόλης και της καρβακρόλης είχε τα καλύτερα αποτελέσματα. Επιβεβαίωσαν ότι ο συνδυασμός αυτός **βελτίωσε την ανάπτυξη** των γατόψαρων. Οι ερευνητές κατέληξαν στο συμπέρασμα ότι το ριγανέλαιο **προάγει την καλύτερη ανάπτυξη** των ψαριών και δρα ως **αντιοξειδωτικός παράγοντας**.

**Abstract:** Carvacrol and thymol are the two main active components of oregano essential oil (OEO). In this study, the effect of carvacrol and thymol was evaluated separately, in combination and in their natural composition as natural OEO. A total of five treatments, i.e., negative control group, carvacrol, thymol, a combination of carvacrol and thymol and Orego-Stim® (OS, commercial product containing natural OEO from Origanum heracleoticum L.) were added to the diets of channel catfish (Ictalurus punctatus) to investigate the effects of the respective treatments on the growth performance and antioxidant activity. After eight weeks of feeding, fishes were infected with Aeromonas hydrophila and mortality was recorded. Results of this study showed that channel catfish fed with natural OEO (OS), containing a combination of carvacrol, thymol and other minor constituents, significantly enhanced growth performance, which was the highest amongst all treatments (P<0.05). The addition of OS also effectively enhanced hepatosomatic index (HSI), viscerosomatic index (VSI) and condition factor compared to the control diet (Pb0.05) and distinctly promoted the sedimentation of muscle protein. OS also remarkably enhanced the antioxidant activity of channel catfish. Both the combination of carvacrol and thymol and OS reduced fish mortality following A. hydrophila infection, but the lowest mortality was observed in the group fed with OS. It can be concluded that OS, which contains natural OEO extracted from O. heracleoticum L., can act as a growth promoter, increase antioxidant activity, enhance muscle protein sedimentation and also improve disease resistance to pathogens when added to channel catfish feed.

**Conclusions:** Results of our study showed that although carvacrol extract and a combination of carvacrol and thymol extract in catfish diets could increase growth performance, still it was OS extracted from O. heracleoticum L. that showed the best performance as a growth promoter. Feed or protein utilisation following the addition of OS in the diet was also the highest. The addition of OS could effectively reduce hepatosomatic index, viscerosomatic index and enhance condition factor. However, none of the treatments showed any effect on the moisture, protein, lipid or ash content of muscle samples, although the addition of the combination of carvacrol and thymol extract, and OS, could both promote the sedimentation of protein in muscle. OS was able to significantly enhance the activity of lysozymes, superoxide dismutase and catalase. Although the survival rates in the feeding trial were not related to the addition of Origanum extracts, survival rates of fish in OS treatment group were greatly enhanced following the challenge with A. hydrophila. The addition of carvacrol extract, or the combination of carvacrol and thymol extracts, both had some effect on the survival rates, but it was not significant. Thus, it can be concluded that the addition of OS in feed can act as a growth promoter and an antioxidant in channel catfish aquaculture.

# **Τίτλος μελέτης: *Oregano essential oil addition in rice starch films and its effects on the chilled fish storage***

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**Journal of Food Science and Technology**

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**Περίληψη μελέτης:** Στη συγκεκριμένη μελέτη, ερευνήθηκε η επίδραση της προσθήκης αιθέριου ελαίου ρίγανης σε ενεργές συσκευασίες ψαριών. Πιο συγκεκριμένα, έγινε προσθήκη ριγανελαίου σε φιλμ από άμυλο ρυζιού. Στα φιλμ με το ριγανέλαιο παρατηρήθηκε **μεγαλύτερη αντοχή**, **μικρότερα ποσοστά οξείδωσης** και **πιο χαμηλά επίπεδα μικροβιολογικής ανάπτυξης** από το κανονικό φιλμ. Τέλος, η ενεργή συσκευασία με το αιθέριο έλαιο ρίγανης **αύξησε τη διάρκεια ζωής τους προϊόντος**, γεγονός που αποδεικνύει την αποτελεσματικότητα του ριγανελαίου, όταν αυτό προστίθεται σε σύνθετα συστήματα συσκευασίας ψαριών.

**Abstract:** Active packaging produced from biodegradable polymers and essential oil could have a great value to food industries. This study aimed to evaluate the effect of the addition of oregano essential oil (OEO) on rice starch films and its application as packaging for fish fillets. Several concentrations of OEO were added to the films, the film added with 4.5% OEO showed lower permeability to water vapor 3.7 g mm kPa-1 m-2 day-1 , intermediate solubility of 24% and high tensile strength (4.4 MPa) while the standard film (without the addition of OEO) presented 8.8 g mm kPa-1 m-2 day-1 , 25.8% and 2.2 MPa, respectively. Packed fish fillets with the active film showed an increase in its shelf life when compared to the standard film. Packaged fish fillets in OEO films showed greater resistance (13.4 N), less oxidation (1.65 mg malonaldehyde/kg of sample) and less microbiological growth 107 CFU/g in 6 days of storage, while packaged fish fillets in standard film showed lower resistance (10.4 N), higher oxidation (1.88 mg malonaldehyde/kg of sample) and higher microbiological growth 108 CFU/g. Thus, the active packaging developed had the capacity to increase the shelf life of a perishable product that has great interest of food industries.

**Conclusions:** The addition of OEO promoted improvements in the characteristics of the films. The films showed an increase in the tensile strength and elasticity and reduced the solubility in water and water vapor permeability. The film with better properties was produced with 3% (w/v) of starch, 30% of glycerol (in relation of the starch weight) and 4.5% of OEO (in relation to the solids weight). The shelf life study of packed fish fillet showed few differences when comparing to the product packed with standard film and with OEO film, but indicated the trend that OEO film can protected for a longer time the packed fish fillets.

# **Τίτλος μελέτης: *Dietary oregano essential oil improved the immune response, activity of digestive enzymes, and intestinal microbiota of the koi carp, Cyprinus carpio***

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**Aquaculture**

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**Περίληψη μελέτης:** Η παρούσα μελέτη έγινε σε ασιατικούς κυρπίνους. Οι ερευνητές προσπάθησαν να επιβεβαιώσουν την πιθανή επίδραση του αιθέριου ελαίου ρίγανης στους κυπρίνους, όταν αυτό γίνεται μέρος της δίαιτάς τους. Τα αποτελέσματα έδειξαν ότι **το ριγανέλαιο επηρεάζει θετικά τα ψάρια**. Ειδικότερα, **αυξάνει τη δραστηριότητα των πεπτικών ένζυμων** των ψαριών και **την** **αντιοξειδωτική ικανότητα**. Ακόμη, το αιθέριο έλαιο ρίγανης δρα ως **ανοσορρυθμιστικός παράγοντας** και **αυξάνει την αντοχή σε συνήθεις ασθένειες** που μπορεί να παρουσιαστούν στα ψάρια. Οι ερευνητές θεωρούν ότι **όλες αυτές οι ευεργετικές επιδράσεις μπορεί να οφείλονται στις αλλαγές της δομής της μικροχλωρίδας του εντέρου των ψαριών**.

**Abstract:** The objective of this research was to evaluate the effects of dietary [oregano](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/oregano) [essential oil](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/essential-oils) (OEO) on the non-specific immunity and intestinal [bacterial community](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/bacterial-communities) of [*Cyprinus carpio*](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/mirror-carp) (C. carpio). We randomly assigned 300 fish to one of the four treatments: basal diet (0 mg/kg OEO, control), OEO-L (basal diet plus 500 mg/kg OEO), OEO-M (basal diet plus 1500 mg/kg OEO), and OEO-H (basal diet plus 4500 mg/kg OEO). Blood and intestinal samples were collected at the end of the experiment to investigate the immune response, [digestive enzyme](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/digestive-enzymes) activity levels, and intestinal microbiota. Dietary supplementation with OEO significantly increased the levels of [lysozyme](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/lysozyme) (P < .0001), complement C3 (P < .0001), complement C4 (P < .0001), [superoxide dismutase](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/superoxide-dismutase) activity (SOD; P < .0001), and [glutathione peroxidase](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/glutathione-peroxidase) (GPx; P < .0001), but significantly decreased malonaldehyde (MDA) levels (P < .0001). OEO supplementation also significantly increased the activity levels of protease (P < .0001), [lipase](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/lipase) (P < .0001), and [amylase](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/amylase) (P = .0071), but significantly downregulated the relative expression of [tumor necrosis factor α](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/tumor-necrosis-factors) (TNF-α) (P = .0002) and [transforming growth factor β](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/transforming-growth-factor-beta) (TGF-β) (P = .0064). After [*Aeromonas hydrophila*](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/aeromonas-hydrophila) (A. hydrophila) injection, the 7-d cumulative survival rate of C. carpio was significantly increased by oral OEO administration (P = .005). Bacterial community composition in the OEO-H group was distinct from that in the control (ANOSIM, R = 0.3165, P = .011). We identified 22 taxa, which were differentially abundant between the OEO-H group and the control, as potential biomarkers. The genera [*Propionibacterium*](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/propionibacterium), Brevinema, and Corynebacterium\_1 were enriched in the OEO-H group, whereas [*Vibrio*](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/vibrio) was enriched in the control. Thus, in C. carpio, dietary OEO increased digestive enzyme activity and antioxidant capability, stimulated immunomodulatory effects, and enhanced disease resistance. These beneficial effects were probably due to OEO-mediated alternations in the structure of the C. carpio gut microbiota.

**Conclusions:** In conclusion, our results indicated that, in fish, dietary supplementation with OEO increased digestive enzyme activity and antioxidant capability. OEO also stimulated immunomodulatory effects and enhanced disease resistance. Oral administration of OEO at the concentration of 4,500 mg/kg diet altered the gut bacterial community composition of fish. The beneficial effects of OEO were probably due to alternations in the structure of the fish gut microbiota.

**Γενικά συμπεράσματα**

Το αιθέριο έλαιο ρίγανης αποτελεί έναν ισχυρό σύμμαχο κατά την παραγωγή και τη συντήρηση ψαριών. Οι πολλαπλές μελέτες επιβεβαιώνουν ηχηρά τον ισχυρισμό ότι το ριγανέλαιο αποτελεί ένα από τα αποτελεσματικότερα πρόσθετα που μπορούν να χρησιμοποιηθούν στον τομέα της ζωικής παραγωγής.

Το αιθέριο έλαιο ρίγανης μπορεί να χρησιμοποιηθεί ως πρόσθετο στη διατροφή των ιχθύων. **Επηρεάζει θετικά**, μεταξύ άλλων, **την αντιοξειδωτική και αντιμικροβιακή ικανότητα των ψαριών** και **δρα ανοσορρυθμιστικά**, ενώ ταυτόχρονα **προσδίδει αυξημένη αντοχή κατά των πιθανών ασθενειών** που απειλούν τα ψάρια. Οι μελέτες δείχνουν ότι οι θετικές αυτές επιδράσεις του ριγανελαίου, οφείλονται στις αλλαγές που αυτό προκαλεί στην μικροχλωρίδα του εντέρου των ιχθύων. Γενικότερα, θα μπορούσαμε να πούμε **ότι ευνοεί την καλύτερη υγεία των ψαριών**.

Επιπλέον, πολυάριθμες μελέτες δείχνουν ότι το αιθέριο έλαιο ρίγανης μπορεί να **συμβάλει στην καλύτερη συντήρηση των ψαριών**. Με τη χρήση του σε συστήματα συσκευασίας**, αυξάνει το χρόνο συντήρησης** και **βοηθά στη διατήρηση ενός ποιοτικότερου τελικού προϊόντος**.

Συμπερασματικά, το ριγανέλαιο αποτελεί ένα σημαντικό μέσο στο οπλοστάσιο του σύγχρονου ιχθυοπαραγωγού. Αποτελεί ένα φυσικό προϊόν, το οποίο μπορεί να βελτιώσει ποικιλοτρόπως την ποιότητα αλλά και το χρόνο συντήρησης των ψαριών, διατηρώντας μια φιλική στάση προς τη χλωρίδα και την πανίδα του θαλάσσιου περιβάλλοντος.

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