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EFFECT OF DIETARY OREGANO *Origanum vulgare* ESSENTIAL OIL CONTAINED WITH CARVACROL AND THYMOL ON THE GROWTH PERFORMANCE AND RESISTANCE OF STRIPED CATFISH *Pangasionodon hypophthalmus* AGAINST *Aeromonas hydrophila* INFECTION

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ABSTRACT

The production efficiency of striped catfish Pangasianodon hypophthalmus is very dependent on the quality of feed, which can increase the fish's growth rate and health condition. Thus, the aimed of this study was to investigate the effects of different levels of Oregano Essential Oil (OEO) contained with thymol and carvacrol, at the concentration level of 0.1, 0.2, and 0.4% OEO, which is applied using top-dressing method and included in feed formulation on the growth rate, body composition, digestive health and resistance of striped catfish P. hypophthalmus against the pathogenic bacteria Aeromonas hydrophila. The results of growth trials using top-dressing method for 122 days using formulated feed method for 70 days showed that the application of OEO yielded better growth than the control, with 0.2% OEO providing better growth performance than 0.1 and 0.4% OEO. The body composition analysis results also showed a better level of nutrient absorption in fish fed the OEO-based diet compared to the control. For the number of bacteria in the digestive tract, OEO supplementation can also significantly reduce the number of bacteria compared to fish-fed control feed. The challenge test results after the fish were given treatment feed for 24 days showed that the application of 0.1% OEO provided better protection against Aeromonas hydrophila. However, there was no significant difference with 0.2% OEO. The application of 0.2% OEO can be recommended to increase the efficiency and productivity of catfish cultivation.

KEYWORDS: *Pangasionodon hypophthalmus*; growth performance; health; thymol; carvacrol; Origanum vulgare

INTRODUCTION

Striped catfish or Pangasius, *Pangasianodon hypophthalmus*, is currently the most widely traded fish product globally (Phan *et al.*, 2009). The characteristics of Pangasius, including the great acceptability by the consumer, affordable cost, and white meat make it a popular choice, often replacing the consumption of other expensive white fishes, such as cod (Đ·ng *et al.*, 2021; Guimarães *et al.*, 2016). Currently, Vietnam is the leading producer, representing for more than 75% of the global production and exporting to more than 100 countries with a total economic value of $US 2.2 billion (Hai & Speelman, 2023;

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Nguyen *et al*., 2018). Meanwhile, although Pangasius production in Indonesia also shows an increasing trend (Poernomo & Suryanto, 2020; Thong *et al*., 2016), the production still focused on meeting the domestic market, and only a small portion is processed for export (Tran *et al*., 2017).

The challenge in aquaculture production in countries where the fish is not a native species is the possibility of inbreeding, which decreases genetic diversity (Patta *et al*., 2024), and eventually reduces growth rate performance, lowers viability and survival, increases the number of abnormalities, and shows lower resistance to diseases or pathogen infection (Fessehaye *et al*., 2007; Smallbone *et al*., 2016; Thrower & Hard, 2009). Therefore, other than importing broodstock from the Mekong River Basin as their native area, the Indonesian government has begun using genetic improvement and engineering to maintain the fish's optimum growth performance and health condition (Maryeni & Fitrini, 2023; Nirmala, 2021). Additionally, the optimum growth rate of aquatic organisms can also be maintained by providing feed that can meet the specific nutrient requirements (Khan *et al*., 2018; Peter *et al*., 2022; Sayeed *et al*., 2008), and this is become the main strategy to increase pangasius productivity in Indonesia. In order to fill the nutritional deficiencies in farm made feed that is commonly used, supplements or additives can be included in the diet formulation (Bai *et al*., 2015; Dawood *et al*., 2018). One of the additives that can be used is essential oils (EOs) as a complex mixtures containing volatile compounds of low molecular weights belonging to various chemical families, especially from the terpene family (Ezzat Abd El-Hack *et al*., 2016; Sutili *et al*., 2018), which provides various benefits such as improved growth performance, disease resistance, gut health, and stress reduction (Ezzat Abd El-Hack *et al*., 2016). In the aquaculture context, antioxidant and antimicrobial activities contained in EOs could represent a promising option since numerous diseases have been reported to cause massive mortalities and economic losses to the industry (Behringer *et al*., 2020; Lafferty *et al*., 2015; Meyer, 1991; Novriadi, 2016).

One of the plants that can be used to obtain EOs is Oregano, which is a member of the Labiatae family of plants, indigenous to the Mediterranean region (Alekseeva *et al*., 2020). Oregano essential oil (OEO), with its high content of phenolic compounds, particularly carvacrol and thymol, stands out for its unique anti-bacterial characteristics (Prapti *et al*., 2022). The high content of carvacrol, thymol, and their precursors, ã-terpinene and ñ-cymene, characterizes all "oregano" types of essential oils (Azimzadeh *et al*., 2023; De Mastro *et al*., 2017; Kokkini *et al*., 1997). Currently, there is very little information regarding the implementation of EOs in Pangasius, especially regarding the effectiveness of using EOs both mixed homogeneously in making feed and top-coating process. Therefore, the aim of this study was to evaluate the effect of natural oregano essential oil (OEO, Regavit AquaTM, Ecopharm Hellas, Greece), which is a polyphenol consisting of carvacrol (78-82%) and thymol (1.4-2.4%) as mentioned in Novriadi *et al*. (2023), to the growth performance, body composition and number of bacteria in the digestive tract of pangasius both using top-dressing method or included in the diet formulation. The survival rate of pangasius against *Aeromonas hydrophila* bacteria was also observed to determine the optimum dose of top-dressing application in feed to enhance the immune system of Pangasius juvenile.

MATERIALS AND METHODS

Experimental diets

For the top-coating process of the commercial diet (31% crude protein and 5 % crude fat), the coating solutions was prepared by providing 10 g xantham gum as the binder, 100 mL hot water, 1 kg of commercial feed and EOS as much as 1, 2 and 4 g of Regavit Aqua™, (RA, Ecopharm Hellas, Greece). The protocol was initiated by mixing 100 L hot water with xantham gum until reaching a slurry condition. Then, 1, 2 and 4 g of RA were added to the slurry solution and mixed properly. The slurry solution containing OEO was spread homogenously into the commercial feed until it covered the surface of the feed evenly. The feed was then left 10 - 15 minutes under room temperature prior to use, and labeled as 0.1; 0.2; and 0.4% OEO, respectively.

In parallel, three experimental diets were formulated to be isonitrogenous and iso-lipidic practical diet (Table 1, 30% crude protein and 5 % crude lipid). The control diet was prepared by using 12.5 % poultry by-product meal (PBM), 34.5% soybean meal (SBM), 10% double distiller's dried grain soluble (DDGS), and 10% cassava meal (CM) without OEO (Regavit Aqua™, Ecopharm Hellas, Greece). The experimental diets were formulated to contain increasing levels of OEO, as much as 0.1; 0.2 and 0.4 %, and labeled as 0.1; 0.2; and 0.4% OEO, respectively (Table 1). Prior to production, all ingredients including all additives and functional ingredients were mixed in a paddle mixer (Marion Mixers, Inc., Marion, IA, USA) in a 100 kg batch followed by grinding to a particle size of <200 µm using a disk mill (Jinan Shengrun, China). The cooking-extrusion diets were exposed to an average of 110°C for approximately 14 seconds in five-barrel sections and the last section was maintained at 62°C.

Pressure at the die head was approximately 50 bars, and screw speed was maintained at 423 rpm. Portions of feeds were extruded through 2-mm die to produce the experimental feed. Diets were dried in a pulse bed dryer (Jinan Shengrun, China) until moisture readings were below 10%. Pellets were dried at approximately 107°C with an upper limit outflow air temperature of approximately 88°C for 8 – 10 h. All finished diets were bagged and stored in a temperature-controlled room until further use.

Growth Trial and feeding program

The growth trials were conducted at the Jakarta Technical University of Fisheries located in Jakarta, Indonesia. The juvenile of *Pangasius hypophthalmus* were obtained from Cinta Fish Farm (Bogor, West Java, Indonesia) and acclimatized to the culture system. The acclimatized fish were then randomly distributed into 16 concrete tanks for trial 1 and plastic ponds for trial 2. For the growth trial 1, fish with mean initial size of 2.5 ± 0.3 g were stocked into 16 tanks with the size of 1.34 x 1.32 x 1.10 m and density of 50 fish m^{-3}. Meanwhile for the trial 2, 240 fish (mean initial weight 6.5 ± 0.2 g) were stocked into plastic ponds with size of 8 x 10 x 1.5 m and density of 15 fish m^{-3} per culture pond. For both trials, four replicate groups of fish were administered different types of experimental diets for 122 days (trial 1) and 70 days (trial 2) and fed by hand four times daily, at 07:00, 11:00, 15:00, and 20:00h. Based on our historic results, feed inputs were pre-programmed assuming the average growth of pangasius and feed conversion ratio of 1.5. Daily allowances of feed were adjusted based on observed feed consumption, weekly counts, and mortality. Water quality during the culture period, including for physical parameters: pH. Temperature (ºC), and dissolved oxygen (mg L^{-1}) as well as the chemical parameters: nitrite (NO_2-N), nitrate (NO_3-N), ammonia (NH_3-N), and total dissolved solid were summarized in Table 2.

Growth performance analysis

At the termination of the feeding period, fish in each treatment were group counted and individually weighed, and the final biomass, final weight, percentage weight gain (PWG), feed conversion ratio (FCR), percentage survival (SR) and thermal unit growth coefficient (TGC), were calculated as follows:

$$\text{PWG} = \frac{(average\ individual\ final\ weight - average\ individual\ initial\ weight)}{(average\ individual\ initial\ weight)} \times 100$$

$$\text{FCR} = \frac{feed\ given\ (g)}{alive\ weigh\ gain\ of\ fish\ (g)}$$

$$\text{SR} = \frac{final\ number\ of\ fish}{initial\ number\ of\ fish} \times 100$$

$$\text{TGC} = \frac{FBW^{1/3} - IBW^{1/3}}{\sum TD} \times 100$$

where FBW is the final body weight, IBW is the initial body weight, T is the temperature (^{0}C) and D is the number of feeding days.

Total bacteria analysis in fish

Total plate count (TPC) analysis was used to count the total bacteria in fish that was carried out aseptically to prevent unwanted contamination and was done in duplicate to increase the accuracy of the results. Before conducting the TPC test, all tools and mediums were sterilized. After the sterilization, the media temperature was maintained at 45-55°C in a water bath to prevent the media from freezing. The diluent solution was prepared by dissolving 8.5 grams of NaCl in 1 liter of distilled water and sterilized in an autoclave at 121 °C for 15 minutes. The test began with weighing 10 grams of Pangasius digestive tract, grinding it, and dissolving it in a sterile diluent solution until it reached a volume of 100 mL to obtain a dilution of 10^1. Next, 1 mL of the solution was put into a test tube containing 9 mL of sterile dilution solution to obtain a dilution of 10^2. The process continued with the same procedure until a dilution of 10^5 was obtained. From each dilution test tube, 1 mL was taken using a pipette and put into a sterile Petri dish; this was done using Duplo. A total of 15 mL of PCA (Difco) was poured into each Petri dish. Afterward, the Petri dish was shaken in a circle on the table to distribute the PCA media and sample evenly. After the PCA in the Petri dish was frozen, the Petri dish was placed in an inverted position in an incubator at 35 °C for 48 hours. After incubation, bacterial colonies in each Petri dish were counted. The number of bacteria that could be counted in a Petri dish was 30 - 300 colonies.

Body composition analysis

Upon termination of both growth trials, three fish were randomly selected from each tank or twelve fish per dietary treatment and stored at - 80^0 C for body

composition analysis. Prior to proximate analysis, dried whole fish were rigorously blended and chopped in a mixer according to methods described by Association of Official Analytical Chemist (AOAC). All parameters were analyzed at the accredited laboratory Saraswanti Indo Genetech (SIG, Bogor, West Java, Indonesia) and the mean of each value were taken.

Challenge test

Bacterial culture

The bacteria used for the challenge studies were obtained from the Main Center of Freshwater Aquaculture Development, Sukabumi (West Java, Indonesia). The pure bacterial species were confirmed as *Aeromonas hydrophila* following the identification methods described by Frerichs & Millar (1993). The identification tests included Gram stain, oxidase, oxidation/ fermentation (O/F), motility, hemolysis on 5% sheep blood agar, and biochemical profiles obtained using the commercially available API 20E kit (Biomerieux). The Gram stain, O/F, motility, and oxidase tests were performed as described in Frerichs & Millar (1993). The biochemical tests were conducted as described in the manufacturer's instructions and read after 24 and 48 hours. A bacterial suspension of early log-phase growth was prepared overnight with a culture of 1–2 colony-forming units (cfu) in 50 mL of tryptone soya broth (TSB, Oxoid) in an orbital shaking incubator at 28ºC at 120 rpm. The cultures were then centrifuged and washed twice in sterile phosphate buffer saline (PBS 0.02 m phosphate, 0.15 m NaCl), and turbidity was measured to give an OD 600 nm value of 1. This was expected to be 1×10^9 cfu mL^{-1}.

Bacterial infection

The infection begins by stocking fish into each container, which is 60 × 40 × 40 cm in size and has a density of 15 fish per container. Each treatment has four replicates. The fish (initial mean weight 10 g) were then given different feeds using a top-dressing process as described in growth trial-1 for 28 days. After 28 days, the fish were challenged using the immersion method with a concentration of *A. hydrophila* 1×10^8 cfu mL^{-1}, which is confirmed as the dose that gave 50% total mortality for fish. The control group received no bacterial challenge but was given an injection of PBS at 0.1 mL per fish. After the challenge, fish were monitored for two weeks, and the monitoring campaign was performed at least twice per day. Dead and moribund fish were immediately removed and weighed, and their lengths were measured. Fish were monitored until 14 days post-infection. During the challenge period, fish still receive experimental and control feed. All remaining fish at the end of observation period were examined for clinical symptoms and the number of survival rate.

Statistical analysis

Data on the growth parameters and total bacteria were analyzed using one-way analysis of variance (ANOVA) to determine significant differences among treatments, followed by Tukey's multiple comparison tests to determine the difference between the means among the treatments. All statistical analyses were conducted using SAS system (V9.4. SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Plant extracts can mitigate the challenges in an effort to reduce feed prices by substituting expensive raw materials, such as fish meal, with cheaper alternative protein sources (Bae *et al*., 2020; Rimoldi *et al*., 2020; Torrecillas *et al*., 2021). Plant extracts containing antioxidants have been widely observed and have the potential to be used in the aquaculture industry (Kulisic *et al*., 2004). Among plant extracts, oregano essential oil (OEO), which is rich in thymol and carvacrol, has been observed as able to stimulate growth and improve health conditions of Pacific white shrimp *Litopenaeus vannamei* (Novriadi *et al*., 2023), channel catfish *Ictalurus punctatus* (Zheng *et al*., 2009), and zebrafish *Danio rerio* (Rashidian *et al*., 2021). However, it is important to note that the effectiveness of OEO can vary depending on factors such as species, dosage, extraction method, and the specific challenges associated with low fishmeal diets.

In this research, from the data presented in Table 3 for the growth rate of *P. hypophthalmus* in concrete tanks for 122 days, it is shown that fish fed without OEO significantly had the lowest growth rate and level of feed efficiency compared to the group of fish given feed treated with OEO ($P < 0.05$). In general, the addition of OEO in feed using top-dressing process increases the growth rate of fish significantly, whereas the use of 0.2% OEO provides better final weight, thermal growth coefficient (TGC), percentage weight gain (PWG), and a better survival rate compared to other dietary treatments. Giving a higher dose (0.4% OEO) using the top-dressing method also provided significant growth compared to the control. All the water quality data as shown in Figure 2, were still within the acceptable range for catfish to be cultured in the concrete tank. Meanwhile, the growth of fish *P. hypophthalmus* kept for 70 days in plastic ponds using specially formulated feed with the several inclusion levels of OEO has the same growth tendency as fish kept in the concrete tanks. As presented in Table 4, the inclusion of OEO

improved growth performance of fish compared to the control treatment. Fish fed with 0.2% OEO provided better biomass, FBW, PWG, and FCR than other treatments, including those treated with 0.1 and 0.4% OEO. For survival rate, the addition of OEO provided a better survival rate compared to the control group, with no significant difference between the 0.1 and 0.2% OEO treatments.

Table 1. Composition (% *as is*) of diets consisting of several inclusion levels of oregano essential oil (OEO, Regavit Aqua™, Ecopharm Hellas, Greece) powder and fed to *Pangasius hypophthalmus* over 70 days of culture period

Ingredients (% *as is*)	Diet code			
	Control	0.1% OEO	0.2% OEO	0.4% OEO
Poultry by-product meal[1]	12.50	12.50	12.50	12.50
Soybean meal[1]	34.50	34.50	34.50	34.50
DDGS[1]	10.00	10.00	10.00	10.00
Menhaden fish oil[1]	2.00	2.00	2.00	2.00
Cassava meal[2]	10.00	10.00	10.00	10.00
Powder of oregano polyphenols[3]	0.00	0.10	0.20	0.40
Corn starch[2]	10.90	10.80	10.70	10.90
Wheat products[4]	15.00	15.00	15.00	15.00
Mineral premix[5]	2.50	2.50	2.50	2.50
Vitamin premix[6]	2.50	2.50	2.50	2.50
Rovimix Stay C-35%[2]	0.10	0.10	0.10	0.10
Proximate analysis				
Ash Content (%)	9.55	9.40	9.32	9.31
Total Fat (%)	5.35	5.33	5.33	5.34
Moisture Content (%)	9.12	9.08	9.14	9.11
Protein Content (%)	30.02	30.04	30.12	30.14

[1] PT FKS Multi Agro, Tbk. Jakarta, Indonesia

[2] PT Rajawali Mitra Pakanindo, Banten, Indonesia

[3] Regavit aqua™, RA, Ecopharm Hellas, Greece

[4] PT Pundi Kencana, Cilegon, Banten, Indonesia

[5] Contained (as g/kg premix): cobalt chloride, 0.04; cupric sulfate pentahydrate, 2.50; ferrous sulfate, 40.00; magnesium sulfate anhydrous, 138.62; manganous sulfate monohydrate, 6.50; potassium iodide, 0.67; sodium selenite, 0.10; zinc sulfate heptahydrate, 131.93; and cellulose, 679.64.

[6] Contained (as g/kg premix): thiamin-HCl, 0.438; riboflavin, 0.632; pyridoxine-HCl, 0.908; D-pantothenic acid, hemicalcium salt, 1.724; nicotinic acid, 4.583; biotin, 0.211; folic acid, 0.549; vitamin B12, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; vitamin A acetate (500,000 IU/g), 0.677; vitamin D3 (1,000,000 IU/g), 0.116; DLalpha-tocopheryl acetate (250 IU/g), 12.632; and alpha-cellulose, 955.589.

Table 2. Water quality analysis for physical and chemical parameters for both growth trials

No	Parameters	Unit	Growth trial 1		Growth trial 2	
			AM	PM	AM	PM
Physical parameters						
1	pH		7.58 ± 0.44	7.95 ± 0.77	7.63 ± 0.22	7.74 ± 0.54
2	Dissolved oxygen	mg L^{-1}	4.18 ± 1.18	6.04 ± 1.09	5.12 ± 0.29	6.01 ± 0.72
3	Temperature	°C	27.18 ± 2.14	29.22 ± 0.68	29.12 ± 0.42	29.89 ± 0.33
Biological parameters						
1	Nitrite-nitrogen (NO_2-N)	mgL^{-1}	0.085 ± 0.012		0.036 ± 0.008	
2	Nitrate-nitrogen (NO_3-N)	mgL^{-1}	8.274 ± 1.833		6.188 ± 0.112	
3	Ammonia (NH_3)	mgL^{-1}	0.024 ± 0.019		0.018 ± 0.007	

Table 3. Growth performance of *Pangasius hypophthalmus* (~ 2.5 ± 0.3 g as the initial mean weight) fed experimental diets for 122 d fed with top-dressing diets with several levels of OEO (Ecopharm Hellas, Greece) in liquid form. Values represent the mean of four replicates. Results in the same columns with different superscript letter are significantly different (*P<0.05*) based on analysis of variance followed by Tukey's multiple comparison test.

Treatment	FBW[1] (g)	FCR[2]	TGC[3]	PWG (%)[4]	SR (%)[5]
Control	242.45[b]	1.43[a]	0.3827[b]	11176.74[b]	81.92
0.1% OEO[7]	311.29[a]	1.29[ab]	0.4250[a]	14378.49[a]	81.85
0.2% OEO	340.28[a]	1.11[b]	0.4408[a]	15726.86[a]	83.54
0.4% OEO	332.62[a]	1.19[b]	0.4366[a]	15370.58[a]	83.75
P-value	<0.0001	0.0020	<0.0001	<0.0001	0.7844
PSE[6]	9.1453	0.05255	0.0055	425.3622	1.9658

Note: [1]FBW = Final body weight; [2]FCR = Feed conversion ratio; [3]TGC = Thermal growth coefficient; [4]PWG = Percentage weight gain; [5]SR = survival rate; [6]PSE = Pooled standard error, and [7]OEO = Oregano Essential Oils.

Table 4. Growth performance of *Pangasius hypophthalmus* (Mean initial weight ~ 7 g) fed with several inclusion levels OEO (Ecopharm Hellas, Greece) in dry form for 70 d. Values represent the mean of four replicates. Results in the same columns with different superscript letter are significantly different (*P<0.05*) based on analysis of variance followed by Tukey's multiple comparison test.

Treatment	Biomass	FBW[1] (g)	PWG (%)[2]	FCR[3]	SR (%)[4]
Control	1479.33[a]	116.87[c]	1569.52[a]	1.66[b]	84.44[a]
0.1% OEO[7]	2320.00[ab]	161.83[b]	2211.90[ab]	1.32[a]	95.56[b]
0.2% OEO	2636.67[b]	183.87[a]	2526.67[a]	1.29[a]	95.56[b]
0.4% OEO	2397.33[ab]	179.73[a]	2467.62[ab]	1.34[a]	88.89[ab]
P-value	<0.0001	<0.0001	<0.0001	<0.0001	0.0192
PSE[5]	67.7777	2.0259	28.9426	0.0112	2.2224

Note: [1]FBW = Final body weight; [2]PWG = Percentage weight gain; [3]FCR = Feed conversion ratio; [4]SR = survival rate; and [5]PSE = Pooled standard error.

Research conducted by YILMAZ *et al*. (2017) on *P. hypophthalmus* also demonstrated the positive effects of thymol and carvacrol when added separately to commercial feed at a dose of 0.1, 0.3, and 0.5% to the growth performance of fish compared to the control group. In this research, YILMAZ *et al*. (2017) highlighted that the higher the dose of thymol and carvacrol added to the diet, the better the growth, including final weight and weight gain of the fish. The improvement in growth rate is possible because thymol and carvacrol can prevent free radicals and improve the gut microflora condition of the fish, which eventually improves the digestion coefficient and absorption of nutrient compounds to optimize the growth (Alagawany *et al*., 2015; Alagawany *et al*., 2021). However, slightly different from YILMAZ *et al*. (2017), our results showed that the addition of OEO contain with thymol and carvacrol at doses higher than 0.2% did not have a significant impact on the growth of pangasius fish, whether fed with the top-dressing method or fed with experimental formulated feed. The difference might be due to the different types of purity of thymol and carvacrol used and the application of thymol and carvacrol, which was carried out separately in the study conducted by YILMAZ *et al*. (2017).

The proximate analysis of fish shows that fish fed thymol and carvacrol have better crude protein and fat level than the control group (Table 5). According to Ferreira *et al*. (2016), Oregano EO increases the height and width of intestinal folds due to its antimicrobial activity and can also improve the digestive and absorptive processes by increasing the surface area of the pleats. In a study with Nile tilapia, Abd El-Naby *et al*. (2020) indicated that feeding Nile tilapia fingerlings with dietary thymol significantly enhanced the intestinal villi length in fish. Therefore, the increase in protein and fat retention and greater energy availability in the fish group fed with OEO-based diet can be correlated with improvements in the intestinal villi of fish, which facilitate better levels of nutrient absorption compared to the control treatments.

Table 5. Proximate composition and energy (wet weight basis) of whole body of pangasius fed experimental diets for 122 d using top-dressing method and 70 d using formulated experimental diet. The value represented duplicate analysis per treatment.

No	Parameter	Unit	Top dressing method				Formulated experimental diet			
			Control	0.1% OEO	0.2% OEO	0.4% OEO	Control	0.1% OEO	0.2% OEO	0.4% OEO
1	Protein content	%	17.48	17.79	17.88	17.65	15.55	15.49	15.85	15.82
2	Ash content	%	1.22	1.18	1.09	1.16	1.12	1.03	1.08	1.05
3	Moisture content	%	78.92	78.84	78.85	78.62	80.23	80.12	80.11	79.67
4	Total calories	Kcal/100 g	79.14	79.47	79.89	79.42	71.14	72.12	72.19	72.18
5	Total fat	%	0.63	0.78	0.79	0.72	0.52	0.54	0.55	0.56
6	Crude fiber	%	0.14	0.13	0.14	0.14	0.18	0.17	0.17	0.19

Many studies have demonstrated that carvacrol and thymol are potent antibacterial agents, especially against pathogenic bacteria (HNKS *et al.*, 2021; Menezes *et al.*, 2018; Zhou *et al.*, 2007). This capability might be due to the ability of carvacrol and thymol, precisely because of their hydrophobic nature, to inhibit the synthesis of cell membranes (HNKS *et al.*, 2021). In our recent findings, the number of bacteria (10^7 cells mL^{-1}) shown at the end of the growth trial, either at 122 days for the top-dressing method or 70 days using the experimentally formulated feed, had lower number of bacteria in fish digestive tract compared to the control treatment. The consistent results, even though using different methods and durations of observation, show that thymol and carvacrol can be used as active ingredients to act as antibacterial agents in the aquaculture industry.

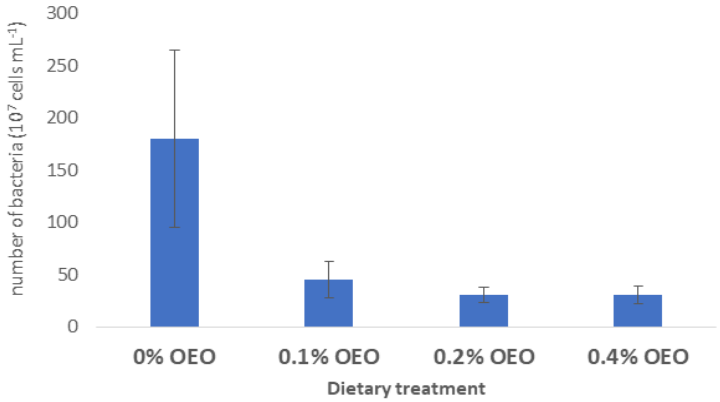

Figure 1. Number of bacteria (10^7 cells mL^{-1}) after 122 d of the feeding period with top-dressing diets with several levels of OEO (Ecopharm Hellas, Greece). Values represent the mean of four replicates (*P= 0.0233).*

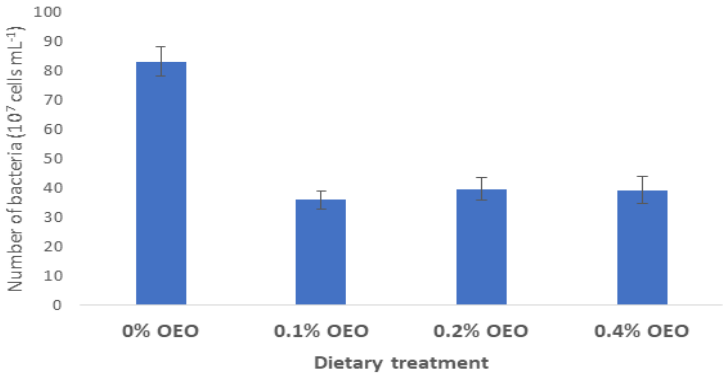

Figure 2. Number of bacteria (10^7 cells mL^{-1}) after 70 d of the feeding period with formulated feed contained with several inclusion levels of OEO (Ecopharm Hellas, Greece). Values represent the mean of four replicates (*P<0.0001*).

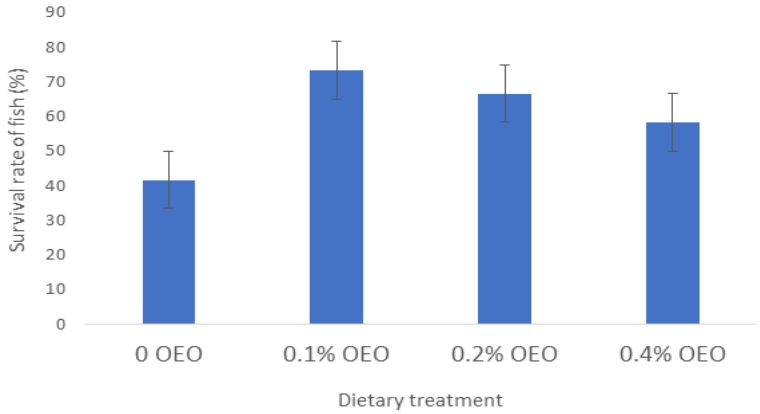

Figure 3. Cumulative survival of fish *Pangasius hypophthalmus* after challenged by *Aeromonas hydrophila* at final dose of 10^9 cfu mL^{-1} and observed for 14 d post-challenged ($P<0.05$).

The antibacterial ability of thymol and carvacrol can also be seen in the results of the challenge test carried out in this study against *Aeromonas hydrophila* at a dose of 1 x 10^8 cfu mL^{-1}, where the survival rate of pangasius fish fed with the top-dressing method for 24 days was better than that of the control treatment. It is very interesting to see that the 0.1% OEO treatment has a survival rate that is numerically better than that of the 0.2 and 0.4% OEO treatments. The use of thymol has been proven effective in increasing the resistance of Nile tilapia against *Aeromonas hydrophila* (Khalil *et al.*, 2023). In addition, the application of OEO, where thymol and carvacrol are one of the main components, can increase the resistance of rainbow trout, *Oncorhynchus mykiss* (Walbaum) against *Lactococcus garvieae* (Diler *et al.*, 2017). To our knowledge, this is the first time that a challenge test and analysis of the number of bacteria in the digestive tract have been performed in *P. hypophthalmus*. This provides a link between survival rate in both growth trials, digestive health, and resistance to pathogenic bacteria.

CONCLUSIONS

The present study showed that using the top-dressing method and formulated feed, thymol, and carvacrol can induce better growth performance of Pangasius fish. Thymol and carvacrol also possess antibacterial solid activity against *Aeromonas hydrophila* and can reduce the number of bacteria in the digestive tract of fish. Based on the results, the use of 0.2% OEO is proven to be effective for increasing growth rate, body composition, digestive health, and resistance against pathogenic bacteria in *P. hypophthalmus.*

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REFERENCES

Abd El-Naby, A. S., Al-Sagheer, A. A., Negm, S. S., & Naiel, M. A. (2020). Dietary combination of chitosan nanoparticle and thymol affects feed utilization, digestive enzymes, antioxidant status, and intestinal morphology of *Oreochromis niloticus*. *Aquaculture*, *515*, 734577.

Alagawany, M., El-Hack, M., Farag, M. R., Tiwari, R., & Dhama, K. (2015). Biological effects and modes of action of carvacrol in animal and poultry production and health-a review. *Advances in Animal and Veterinary Sciences*, *3*(2s), 73-84.

Alagawany, M., Farag, M. R., Abdelnour, S. A., & Elnesr, S. S. (2021). A review on the beneficial effect of thymol on health and production of fish. *Reviews in Aquaculture*, *13*(1), 632-641.

Alekseeva, M., Zagorcheva, T., Atanassov, I., & Rusanov, K. (2020). *Origanum vulgare* L.-a review on genetic diversity, cultivation, biological activities and perspectives for molecular breeding. *Bulgarian Journal of Agricultural Science*, *26*(6).

Azimzadeh, Z., Hassani, A., Mandoulakani, B. A., Sepehr, E., & Morshedloo, M. R. (2023). Intraspecific divergence in essential oil content, composition and genes expression patterns of monoterpene synthesis in *Origanum vulgare* subsp. vulgare and subsp. gracile under salinity stress. *BMC plant biology*, *23*(1), 380.

Bae, J., Hamidoghli, A., Won, S., Choi, W., Lim, S.-G., Kim, K.-W., Lee, B.-J., Hur, S.-W., & Bai, S. C. (2020). Evaluation of seven different functional feed additives in a low fish meal diet for olive flounder, P*aralichthys olivaceus*. *Aquaculture*, *525*, 735333.

Bai, S., Katya, K., & Yun, H. (2015). Additives in aquafeed: An overview. *Feed and feeding practices in aquaculture*, 171-202.

Behringer, D. C., Wood, C. L., Krkošek, M., & Bushek, D. (2020). Disease in fisheries and aquaculture. *In* Behringer, D. C., Silliman, B. R., & Lafferty, K. D. (Eds.), *Marine Disease Ecology*, (pp. 183-212). New York, NY: Oxford University Press.

Đ·ng, H. A., Đ·ng, C. T., Bùi, M. D., Ph¡m, H. N. H., & Thach, N.B (2021). An analysis of factors affecting the competitiveness of the Vietnamese Pangasius exporting companies to the China market. Bachelor of International Business Thesis. FPT university. Danang, Vietnam.

Dawood, M. A., Koshio, S., & Esteban, M. Á. (2018). Beneficial roles of feed additives as immunostimulants in aquaculture: a review. *Reviews in Aquaculture*, *10*(4), 950-974.

De Mastro, G., Tarraf, W., Verdini, L., Brunetti, G., & Ruta, C. (2017). Essential oil diversity of *Origanum vulgare* L. populations from Southern Italy. *Food Chemistry*, *235*, 1-6.

Diler, O., Gormez, O., Diler, I., & Metin, S. (2017). Effect of oregano (*Origanum onites* L.) essential oil on growth, lysozyme and antioxidant activity and resistance against *Lactococcus garvieae* in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture nutrition*, *23*(4), 844-851.

Ezzat Abd El-Hack, M., Alagawany, M., Ragab Farag, M., Tiwari, R., Karthik, K., Dhama, K., Zorriehzahra, J., & Adel, M. (2016). Beneficial impacts of thymol essential oil on health and production of animals, fish and poultry: a review. *Journal of Essential Oil Research*, *28*(5), 365-382.

Ferreira, P. M., Caldas, D. W., Salaro, A. L., Sartori, S. S., Oliveira, J. M., Cardoso, A. J., & Zuanon, J. A. (2016). Intestinal and liver morphometry of the Yellow Tail Tetra (*Astyanax altiparanae*) fed with oregano oil. *Anais da Academia Brasileira de Ciências*, *88*, 911-922.

Fessehaye, Y., Komen, H., Rezk, M. A., van Arendonk, J. A., & Bovenhuis, H. (2007). Effects of inbreeding on survival, body weight and fluctuating asymmetry (FA) in Nile tilapia, *Oreochromis niloticus*. *Aquaculture*, *264*(1-4), 27-35.

Frerichs, G. N., & Millar, S. D. (1993). *Manual for the isolation and identification of fish bacterial pathogens*. Piscess Press, Scotland

Guimarães, C. F. M., Máralém, E. T., Monteiro, M. L. G., Lemos, M., Mano, S. B., & Conte Junior, C. A. (2016). The chemical quality of frozen Vietnamese *Pangasius hypophthalmus* fillets. *Food science & nutrition*, *4*(3), 398-408.

Hai, A. T. N., & Speelman, S. (2023). Understanding vulnerability and resilience of Vietnamese pangasius farming in the context of the Covid-19 pandemic. *Aquaculture*, 739733.

HNKS, P., SH MP, W., BCJ, D. S., & GJ, H. (2021). Antibacterial activity of oregano (*Origanum vulgare*) essential oil, carvacrol and thymol against fish pathogenic bacteria isolated from cultured olive flounder (*Paralichthys olivaceus*). *Iranian Journal of Fisheries Sciences*, *20*(6), 1822-1834.

Khalil, S. R., Zheng, C., Abou-Zeid, S. M., Farag, M. R., Elsabbagh, H. S., Siddique, M. S., Azzam, M. M., Di Cerbo, A., & Elkhadrawey, B. A. (2023). Modulatory effect of thymol on the immune response and susceptibility to *Aeromonas hydrophila* infection in Nile tilapia fish exposed to zinc oxide nanoparticles. *Aquatic Toxicology*, *259*, 106523.

Khan, N., Atique, U., Ashraf, M., Mustafa, A., Mughal, M. S., Rasool, F., Azmat, H., Tayyab, M., & Iqbal, K. J. (2018). Effect of various protein feeds on the growth, body composition, hematology and endogenous enzymes of catfish (P*angasius hypophthalmus*). *Pakistan Journal of Zoology*, *13*, 112-119.

Kokkini, S., Karousou, R., Dardioti, A., Krigas, N., & Lanaras, T. (1997). Autumn essential oils of Greek oregano. *Phytochemistry*, *44*(5), 883-886.

Kulisic, T., Radonic, A., Katalinic, V., & Milos, M. (2004). Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry*, *85*(4), 633-640.

Lafferty, K. D., Harvell, C. D., Conrad, J. M., Friedman, C. S., Kent, M. L., Kuris, A. M., Powell, E. N., Rondeau, D., & Saksida, S. M. (2015). Infectious diseases affect marine fisheries and aquaculture economics. *Annual review of marine science*, *7*(1), 471-496.

Maryeni, S., & Fitrini, E. (2023). Spawning technique of Siamese catfish (*Pangasionodon hypophthalmus*) Pustina at the Freshwater Aquaculture Center (BPBAT) in Sungai Gelam, Muaro Jambi Regency, Jambi Province. *SEMAH Jurnal Pengelolaan Sumberdaya Perairan*, *7*(2), 85-100.

Menezes, N. M. C., Martins, W. F., Longhi, D. A., & de Aragão, G. M. F. (2018). Modeling the effect of oregano essential oil on shelf-life extension of vacuum-packed cooked sliced ham. *Meat science*, *139*, 113-119.

Meyer, F. P. (1991). Aquaculture disease and health management. *Journal of animal science*, *69*(10), 4201-4208.

Nguyen, L. A., Pham, T. B., Bosma, R., Verreth, J., Leemans, R., De Silva, S., & Lansink, A. O. (2018). Impact of climate change on the technical efficiency of striped catfish, *Pangasianodon hypophthalmus*, farming in the Mekong Delta, Vietnam. *Journal of the World Aquaculture Society*, *49*(3), 570-581.

Nirmala, D. (2021). The breeding of Perkasa catfish *(Pangasius hypophthalmus)*. *Diploma Thesis*. Politeknik Negeri Lampung.

Novriadi, R. (2016). Vibriosis in aquaculture. *Omni-Akuatika*, *12*(1).

Novriadi, R., Malahayati, S., & Kuan, S. (2023). Supplementation Effect of Dietary Carvacrol and Thymol Polyphenols from Oregano *Origanum vulgare* on Growth Performance and Health Condition of Pacific White Shrimp *Litopenaeus vannamei*. *Asian Journal of Fisheries and Aquatic Research*, *24*(1), 27-39.

Patta, C., Panthum, T., Thatukan, C., Wongloet, W., Chalermwong, P., Wattanadilokchatkun, P., Thong, T., Srikampa, P., Singchat, W., & Ahmad, S. F. (2024). Questioning inbreeding: Could outbreeding affect productivity in the North African catfish in Thailand? *PloS One*, *19*(5), e0302584.

Peter, N., Pradhan, C., Dileep, N., Musharraf, M., & Thazhakot Vasunambisan, S. (2022). Dietary taurine improved growth performance, nutrient utilization, and antioxidant enzyme activities in pangasius *Pangasianodon hypophthalmus*. *Journal of the World Aquaculture Society*, *53*(1), 106-121.

Phan, L. T., Bui, T. M., Nguyen, T. T., Gooley, G. J., Ingram, B. A., Nguyen, H. V., Nguyen, P. T., & De Silva, S. S. (2009). Current status of farming practices of striped catfish, *Pangasianodon hypophthalmus* in the Mekong Delta, Vietnam. *Aquaculture*, *296*(3-4), 227-236.

Poernomo, A., Suryanto, M., & Sujuliyani (2020). Carbon footprint of frozen pangasius fillet: a case study. IOP Conference Series: *Earth and Environmental Science*, 404, 1-10.

Prapti, J. T., Sumaiya, K., & Maliha, F. (2022). *Antibacterial effect of essential oil extracted from homegrown Origanum vulgare L and Azadirachta indica tree on pathogenic bacteria* Brac University.

Rashidian, G., Boldaji, J. T., Rainis, S., Prokiæ, M. D., & Faggio, C. (2021). Oregano (*Origanum vulgare*) extract enhances zebrafish (*Danio rerio*) growth performance, serum and mucus innate immune responses and resistance against *Aeromonas hydrophila* challenge. *Animals*, *11*(2), 299.

Rimoldi, S., Torrecillas, S., Montero, D., Gini, E., Makol, A., Valdenegro V, V., Izquierdo, M., & Terova, G. (2020). Assessment of dietary supplementation with galactomannan oligosaccharides and phytogenics on gut microbiota of European sea bass (*Dicentrarchus labrax*) fed low fishmeal and fish oil based diet. *PloS One*, *15*(4), e0231494.

Sayeed, M., Hossain, G., Mistry, S., & Huq, K. (2008). Growth performance of thai pangus (*Pangasius hypophthalmus*) in polyculture system using different supplementary feeds. *University Journal of Zoology, Rajshahi University*, *27*, 59-62.

Smallbone, W., Van Oosterhout, C., & Cable, J. (2016). The effects of inbreeding on disease susceptibility: *Gyrodactylus turnbulli* infection of guppies, *Poecilia reticulata*. *Experimental Parasitology*, *167*, 32-37.

Sutili, F. J., Gatlin III, D. M., Heinzmann, B. M., & Baldisserotto, B. (2018). Plant essential oils as fish diet additives: benefits on fish health and stability in feed. *Reviews in Aquaculture*, *10*(3), 716-726.

Thong, N. T., Nielsen, M., Roth, E., Nguyen, G., & Solgaard, H. S. (2016). The estimate of world demand for Pangasius catfish (*Pangasiusianodon hypopthalmus*). *Aquaculture Economics and Management*, *21*(3), 400-417.

Thrower, F. P., & Hard, J. J. (2009). Effects of a single event of close inbreeding on growth and survival in steelhead. *Conservation Genetics*, *10*, 1299-1307.

Torrecillas, S., Terova, G., Makol, A., Serradell, A., Valdenegro-Vega, V., Izquierdo, M., Acosta, F., & Montero, D. (2021). Dietary phytogenics and galactomannan oligosaccharides in low fish meal and fish oil-based diets for European sea bass (*Dicentrarchus labrax*) juveniles: Effects on gill structure and health and implications on oxidative stress status. *Frontiers in Immunology*, *12*, 663106.

Tran, N., Rodriguez, U.-P., Chan, C. Y., Phillips, M. J., Mohan, C. V., Henriksson, P. J. G., Koeshendrajana, S., Suri, S., & Hall, S. (2017). Indonesian aquaculture futures: An analysis of fish supply and demand in Indonesia to 2030 and role of aquaculture using the AsiaFish model. *Marine Policy*, *79*, 25-32.

YILMAZ, E., Çoban, D., KIRIM, B., Güler, M., & Öztürk, S. (2017). The effects of the carvacrol and thymol addition to *Pangasius hypophthalmus* feeds in different amount on the growth performance. *Aquaculture Studies*, *17*(2), 157-162.

Zheng, Z., Tan, J. Y., Liu, H., Zhou, X., Xiang, X., & 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.

Zhou, F., Ji, B., Zhang, H., Jiang, H., Yang, Z., Li, J., Li, J., & Yan, W. (2007). The antibacterial effect of cinnamaldehyde, thymol, carvacrol and their combinations against the foodborne pathogen *Salmonella typhimurium*. *Journal of food safety*, *27*(2), 124-133.