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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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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 ($P<0.05$) 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.

1. Introduction

Oregano is a member of the Labiatae family of plants, indigenous to the Mediterranean region. The bacteriostatic effects found in oregano are due to its high content of phenolic compounds, particularly carvacrol. Recent studies have shown that oregano essential oils (OEO; *Origanum heracleoticum* L.) have great antimicrobial properties (Hammer et al., 1999; Dorman and Deans, 2000). Chemical analysis of OEO has shown its constituents to be principally carvacrol and thymol (Salzer, 1977; Charai and Mosaddak, 1996; Burt et al., 2005), although OEO composition from a particular plant species can differ between harvesting seasons (McGimpsey et al., 1994) and geographical sources (Cosentino et al., 1999). A number of the OEO components exhibit significant antimicrobial properties when tested separately (Ultee et al., 1998; Lambert et al., 2001). However, there is evidence that the antimicrobial activity of OEO is greater than the additive effect of its major antimicrobial components; minor components appear, therefore, to play a significant role (Lattaoui and Tantaoui-Elaraki, 1994).

Many authors have attempted to correlate or compare the functional properties of essential oils with those of their individual components (Cox et al., 1998; Dorman and Deans, 2000; Lambert et al., 2001). Others examined various essential oil components (Kim et al., 1995; Pattnaik et al., 1995) and their combinations in order to find their possible synergistic effects (Economou et al., 1991; Didry et al., 1993; Papageorgiou et al., 2003). The most interesting of these studies correlated the structure and molecular properties of 60 terpenoids with their antimicrobial activity (Griffin et al., 1999). The majority of these studies were conducted on monoterpenes, while a few examined sesquiterpenes and diterpenes (Demetzos et al., 1999). However, there were even fewer studies conducted on non-terpenic compounds (Avato et al., 1997). OEO obtained from *O. heracleoticum L.* plants (commercially known as Greek oregano and synonymous with *O. vulgare* subsp. *hirtum*) is characterized by a high phenolic content (carvacrol and thymol comprising 78.27% of the total oil). Other main oregano oil constituents are the two monoterpene

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hydrocarbons, γ-terpinene and ρ-cymene (5.54 and 7.35% of the total oil, respectively). The high content of carvacrol, thymol and their precursors, γ-terpinene and ρ-cymene (Poulose and Croteau, 1978) characterized all "oregano" types of essential oils (Kokkini et al., 1997). Orego-Stim® (OS) contains natural oregano oil and is generally used as a feed additive in livestock production worldwide. In recent years, OS has also been used in aquaculture production, primarily for better growth, disease control and as a replacement for antibiotic growth promoters.

In the study reported here, channel catfish were fed with fish feed containing thymol extract, carvacrol extract, or the combination of the two extracts (all extracted from *O. heracleoticum* L.) or OS (which comprises 5% of natural OEO). Key factors of growth performance and antioxidant ability were measured during the experiment and subsequently the fish were challenged with *A. hydrophila* to determine disease resistance.

2. Materials and methods

2.1. Fish and culture conditions

Channel catfish (*Ictalurus punctatus*) propagated and reared by the Research Institute for Fisheries (Chongqing, China) were used for the experiment. Experiments were conducted in a recirculating aquaculture system at Southwest University, Chongqing, China. Channel catfish with an average initial weight of 50 g were held in 200 L fibreglass tanks. Fish in randomly assigned triplicate aquaria (40 fish per aquaria) were fed one of the five experimental diets to apparent satiation six times daily for 8 weeks. Water temperature and pH were constant (23–24 °C; pH 8.5) during the experimental period and dissolved oxygen was maintained at 80–90% of saturation. Water flow was maintained at 4.5 L/min.

2.2. Herbal extracts

Origanum vulgare L. extracts containing 98% carvacrol and *O. vulgare L.* extracts containing 98% thymol were commercial products of Jinkang Natural Perfume Co., Ltd., China. OS for aquaculture (5% OEO) extracted from *O. heracleoticum* L. was the commercial product of Meriden Animal Health Ltd., UK.

Carvacrol and thymol were both diluted with defatted rice bran to 5% in this experiment, and the combination of carvacrol and thymol was also diluted to 5%, but the ratio of carvacrol and thymol was 33:1, according to the natural combination of OS. Diluted products were used in this experiment.

The addition of the various substances in each group was in amounts as follows:

Group 1 (Con)	Control (without medicines and herbs)
Group 2 (Thy)	0.05% thymol extract
Group 3 (Car)	0.05% carvacrol extract
Group 4 (Car + Thy)	0.0485% carvacrol extract and 0.0015% thymol extract
Group 5 (OS)	0.05% Orego-Stim®

2.3. Experimental design and diets

Five practical-type diets were formulated to be isocaloric (2.98 kcal/kg diet) and isonitrogenous (34.5% crude protein). All ingredients were finely ground, mixed in a Hobart mixer and pelleted through a 2.4 mm diameter die in a Hobart meat grinder. The pellets were air-dried at room temperature, broken into small pieces and stored in a freezer until used. Ingredients and proximate composition of the experimental diets are presented in Table 1.

The feeding trial lasted 8 weeks. At each feeding, an excess amount of feed was fed to the fish and uneaten feed was collected 1 h after feeding, dried at 70 °C and reweighed. Leaching of uneaten feed was estimated by placing weighed samples of each diet into a tank without fish for 1 h and then recovered, dried and reweighed. The average leaching value was used to correct the amount of uneaten feed.

Table 1
Formulation (%) and proximate composition of the diets.

Ingredients		Proximate composition	
Soybean meal	30.00	Digestible energy (kcal/g)	2.98
Rapeseed meal	26.00	Dry matter (%)	88.79
Corn gluten meal	5.00	Crude protein (%)	34.50
Fish meal	10.00	Crude lipid (%)	3.85
Wheat shorts	22.00	Ash (%)	6.24
Rapeseed oil	2.00	Ca (%)	0.85
$Ca(H_2PO_4)_2$	1.60	Total phosphorus (%)	1.22
Vitamin premix[a]	0.17	Available phosphorus (%)	0.80
Choline chloride	0.20		
Sodium chloride	0.10		
Mineral premix[b]	0.50		
Bentonite	2.43		

Crude protein, crude lipid, ash, crude fibre are expressed on a dry matter basis and given as means ($n = 2$).

[a] Vitamin mix provided the following vitamins (mg/kg diet unless otherwise stated): vitamin A, 4000 IU; vitamin D_3, 2000 IU; vitamin K, 10; vitamin E, 50; thiamine, 10; riboflavin, 12; pyridoxine, 10; panthothenic acid, 32; nicotinic acid, 80; folic acid, 2; biotin, 0.2; vitamin B12, 0.01; L-ascorbyl-2-polyphosphate (25% vitamin C activity), 60.

[b] Trace mineral mix provided the following minerals (mg/kg diet): zinc (as $ZnSO_4 \cdot 7H_2O$), 150; iron (as $FeSO_4 \cdot 7H_2O$), 40; manganese (as $MnSO_4 \cdot H_2O$), 25; copper (as $CuCl_2$), 3; iodine (as KI), 5; cobalt (as $CoCl_2 \cdot 6H_2O$), 0.05; selenium (as Na_2SeO_3), 0.09.

2.4. Sample collection and analytical methods

2.4.1. Growth performance detection

At the termination of the 8-week feeding trial, fish in each tank were individually weighed and sampled for tissue analysis 24 h after the last feeding. Twenty fish at the start were sampled and stored frozen (−18 °C) for analysis of whole body composition. Three to five fish from each tank were used for whole body composition analysis, and the livers and viscera of five fish per tank were weighed for calculation of hepatosomatic index (HSI) and viscerosomatic index (VSI). Dorsal muscles of fish were sampled, sealed in plastic bags and stored frozen (−18 °C) until analysis for muscle nutrient composition.

Growth performance was calculated as follows:

$$\text{Weight gain (WG\%)} = 100 \times (\text{final body weight} - \text{initial body weight}) / \text{initial body weight}$$

$$\text{Specific growth ratio (SGR)} = 100 \times \ln(\text{final weight}/\text{initial weight}) / \text{days of the experiment}$$

$$\text{Feed conversion ratio (FCR)} = \text{feed consumed (g, dry weight)} / \text{weight gain (g)}$$

$$\text{Protein efficiency ratio (PER)} = \text{weight gain (g)} / \text{protein intake (g)}$$

$$\text{Hepatosomatic index (HSI)} = 100 \times (\text{liver weight, g}) / (\text{whole body weight, g})$$

$$\text{Viscerosomatic index (VSI)} = 100 \times (\text{visceral weight, g}) / (\text{whole body weight, g})$$

$$\text{Condition factor (CF)} = 100 \times (\text{body weight; g}) / (\text{body length, cm})^3.$$

Crude protein, crude lipid, moisture and ash in diets, and dorsal muscle samples, were determined following standard methods (AOAC, 1995). Crude protein ($N \times 6.25$) was determined by the Kjeldahl method

Table 2
Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival of channel catfish fed different diets of *Origanum heracleoticum* L. extracts.

Diets	Con	Thy	Car	Car + Thy	OS
WG	102.63 ± 2.22^{a}	104.97 ± 2.41^{a}	111.90 ± 0.62^{b}	116.77 ± 2.49^{b}	127.53 ± 6.40^{c}
FCR	1.94 ± 0.09^{a}	1.91 ± 0.04^{ab}	1.82 ± 0.04^{bc}	1.84 ± 0.03^{ac}	1.64 ± 0.04^{d}
Survival	95.83 ± 2.89^{ab}	93.33 ± 1.44^{a}	95.00 ± 4.33^{ab}	95.00 ± 2.50^{ab}	98.33 ± 1.44^{b}

Data were presented as mean ± SD ($n = 3$). Values within the same row having different superscripts are significantly different ($P<0.05$).

after acid digestion using an Auto Kjeldahl System (1030-Auto-analyzer, Tecator, Hoganos, Sweden). Crude lipid was determined by the ether-extraction method using a Soxtec System HT (Soxtec System HT 6, Tecator, Sweden). Moisture was determined by oven-drying at 105 °C until a constant weight was achieved. Ash content was measured after placing the samples in a muffle furnace at 550 °C for 24 h.

2.4.2. Antioxidant activity measurement

Blood samples (6 fish/group) were collected from the caudal vein 8 weeks after the start of feeding. Heparin was used as an anticoagulant. Individual fish were sampled only once to avoid the influence on the assays due to multiple bleeding and handling stress on the fish. Sample preparations were centrifuged at 700 g for 30 min at 4 °C. After centrifugation, plasma was collected and stored at −20 °C for future analysis.

Lysozyme activity was determined using a modification of the methods described by Chen et al. (1998). Briefly, 100 μl of fish serum was added to a 3-ml suspension of *M. lysodeikticus*. The reaction was carried out at 25 ± 1 °C, and an absorbance at 540 nm was measured after 0.5 and 4.5 min. One unit of lysozyme activity was defined as the amount of lysozyme producing a decrease in absorbance of 0.001/min.

Plasma superoxide dismutase (SOD) activity was assayed according to the method of Misra and Fridovich (1972), which is based on the oxidation of epinephrine to adrenochrome by the enzyme. 0.1 ml of tissue homogenate was added to the tubes containing 0.75 ml of ethanol and 0.15 ml of chloroform (chilled in ice) and centrifuged. 0.5 ml of EDTA solution and 1 ml of buffer were added to 0.5 ml of supernatant. The reaction was initiated by the addition of 0.5 ml of epinephrine and the increase in absorbance at 480 nm was monitored at 30-second intervals for 3 min. Enzyme activity was expressed as 50% inhibition of epinephrine auto-oxidation/min/mg protein.

Plasma catalase (CAT) activity was assayed according to the method of Aebi (1984). The reaction mixture (2 ml) contained 11 mM H_2O_2 in a 50 mM phosphate buffer, pH 7.0, then 10 μL of 50-times diluted assay solution or 10 μl of CAT standards (2.5, 5.0, 7.5 and 10.0 U/10 μl) were added and the mixture rapidly agitated. CAT activity was measured at the end of the agitation and 30 s later, by the decrease in absorbance at 240 nm (H_2O_2) and 25 °C. One CAT unit was defined as the enzyme activity necessary to convert 1 μmol of H_2O_2 to $H_2O + O_2$ at 25 °C and pH 7 in 1 min.

2.4.3. Challenge test with A. hydrophila

At the end of the experiment, a challenge test was performed on each experimental group with *A. hydrophila*. The BYK-038 strain of *A. hydrophila*, which had originally been isolated in Shanghai, China, was used for the test. This strain was chosen because in our preliminary experiments, it proved to be a very virulent strain. Bacteria were inoculated into 10 ml of liquid tryptic soy broth (TSB, Sigma) medium and were grown overnight at 28 °C. Cultures were centrifuged at 1000 g for 10 min. Supernatant was removed and the pelleted bacteria were washed twice in sterile phosphate buffered saline (PBS) solution. The concentration of bacteria was adjusted to 5×10^7 by the optical density of suspension.

0.1 ml of suspended bacteria was injected into the peritoneal cavity of fish. Mortality was recorded for 6 days following infection.

2.4.4. Statistical analysis

Results are expressed as mean ± SD. All data were subjected to one-way ANOVA. When significant differences occurred, the group means were further compared with Duncan's multiple range tests. All statistical analyses were performed using SPSS 11.5 (SPSS, IL, USA).

3. Results

3.1. Growth performance

Survival at the end of the experiment was high (about 95%) and unrelated to dietary treatment, the survival of fish fed the OS diet was significantly higher than that of the Thy diet (93.33%), but no significant differences were noted among other treatments (Table 2).

Data on the growth performance of channel catfish, including weight gain (WG) and feed conversion ratio (FCR) are shown in Table 2. Weight gain of fish fed the OS diet was significantly higher than those of fish fed the Con diet, Thy diet, Car diet and Car + Thy diet ($P<0.05$). Weight gain of fish fed the Car diet and the Car + Thy diets was significantly higher than those of fish fed the Con diet ($P<0.05$). There were no significant differences in weight gain between fish fed the Car diet and fish fed the Car + Thy diet and also between fish fed the Con diet and those fed the Thy diet ($P>0.05$).

Feed conversion ratio was also affected by the feed additives, and the effect on them had the opposite trend. Lowest FCR and highest PER values were observed for catfish fed the OS diet compared to catfish fed the other four diets ($P<0.05$). The FCR value was significantly different between the Con diet and the Car diet ($P<0.05$), but not between fish fed the Con diet, Thy diet and Car + Thy diet ($P>0.05$).

3.2. Hepatosomatic index, viscerosomatic index and condition factor

The hepatosomatic index of fish fed the OS diet was significantly lower than fish fed with the Car diet ($P<0.05$), but not significantly different compared to fish fed the Con diet, Thy diet and Car + Thy diet ($P>0.05$; Table 3).

Except for a significant difference between the OS diet and the Con diet ($P<0.05$), the differences of viscerosomatic index among the other treatments were also not significant ($P>0.05$).

After eight weeks of feeding, fish fed the OS diet had a significantly higher condition factor (CF) compared to the other four treatments (Con diet, Thy diet, Car diet and Car + Thy diet) while no significant differences were noted between the four treatments ($P>0.05$).

Table 3
Hepatosomatic index (HSI), viscerosomatic index (VSI) and condition factor (CF) of channel catfish fed different diets containing *Origanum heracleoticum* L. extracts.

Diets	Con	Thy	Car	Car + Thy	OS
HSI	3.96 ± 0.30^{ab}	3.92 ± 0.21^{ab}	4.03 ± 0.16^{a}	3.98 ± 0.12^{ab}	3.67 ± 0.03^{b}
VSI	10.26 ± 0.20^{a}	10.12 ± 0.11^{ab}	10.19 ± 0.04^{ab}	10.14 ± 0.08^{ab}	10.03 ± 0.07^{b}
CF	2.31 ± 0.04^{a}	2.32 ± 0.07^{a}	2.40 ± 0.07^{a}	2.37 ± 0.08^{a}	2.65 ± 0.08^{b}

Data were presented as mean ± SD ($n = 3$). Values within the same row having different superscripts are significantly different ($P<0.05$).

Table 4
Moisture, crude protein, crude lipid and ash of dorsal muscle samples.

	Moisture	Protein	Lipid	Ash
Con	72.13 ± 0.18^{a}	17.42 ± 0.20^{a}	8.99 ± 0.58^{a}	1.22 ± 0.05^{a}
Thy	72.31 ± 0.40^{a}	17.56 ± 0.10^{ab}	9.06 ± 0.27^{a}	1.13 ± 0.15^{a}
Car	72.03 ± 0.33^{a}	17.45 ± 0.02^{a}	9.04 ± 0.24^{a}	1.18 ± 0.03^{a}
Car + Thy	72.46 ± 0.36^{a}	17.80 ± 0.18^{bc}	8.84 ± 0.33^{a}	1.07 ± 0.13^{a}
OS	71.90 ± 0.38^{a}	17.95 ± 0.11^{c}	9.12 ± 0.31^{a}	1.07 ± 0.05^{a}

Data were presented as mean ± SD ($n = 3$). Values within the same column having different superscripts are significantly different ($P<0.05$).

3.3. Moisture, crude protein, crude lipid and ash of dorsal muscle samples

In terms of muscle composition, no significant differences among treatments were detected for moisture, ash and lipid content in muscle samples, but muscle protein content was affected by dietary Car + Thy and OS (Table 4). The protein content in muscle of fish fed OS diet was the highest, but it was not significantly different compared to the Car + Thy diet. The protein content in muscle of fish fed the Thy diet and the Car diet was not significantly different from those fed the Con diet ($P<0.05$).

3.4. Lysozyme, superoxide dismutase, catalase

Antioxidant activity was measured by superoxide dismutase (SOD), lysozyme (LSZ) and catalase (CAT) activity (Table 5). Lysozyme and catalase activity in plasma of fish fed with the OS diet were both significantly higher than fish from the other four treatments ($P<0.05$) and no significant differences were noted among fish fed the Con diet, Thy diet, Car diet and Car + Thy diet ($P>0.05$). Superoxide dismutase activity in fish plasma was not significantly different between the Car + Thy diet and the OS diet, but these were significantly higher than the Thy, Car and Con groups.

3.5. Challenge test with A. hydrophila

After 8 weeks of feeding, fish were challenged with *A. hydrophila* and cumulative mortality was recorded for 6 days (Fig. 1). All treated groups showed reduced mortality compared to the Con diet, except for the Thy diet. The Car diet reduced the mortality by 12%, the Car + Thy diet reduced the mortality by 21% and the OS diet was the most effective, with the mortality of the fish reduced by 60% (all data is in comparison to the Con diet).

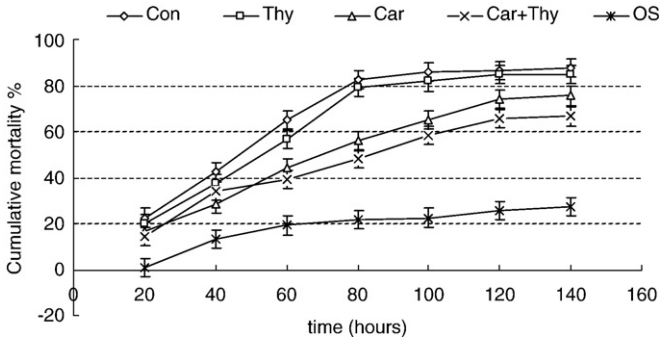

Fig. 1. Cumulative mortality (%) of fish in control group and in groups fed diets containing different *Origanum heracleoticum* L. extracts. The figure shows a six-day period following an artificial infection with *A. hydrophila*. Twenty fish from each group were used for the challenge test.

4. Discussion

Growth promoters, especially those of antibiotic origin, have played a fundamental role in animal production as growth and health enhancers throughout the last fifty years. However, in the past decade, we have seen a characteristic trend of searching for alternatives to these additives due to public concerns related to their residues and subsequent occurrence of resistant bacteria. As growth promoters presumably acting on the intestinal microflora and leading to improved animal performance, most alternative supplements claim to have effects on the microflora either directly or indirectly (MacLennan et al., 2002). Thus, intestinal microflora in fish should not be ignored in relation to their performance. Although fish have only marginal nutritional benefits from intestinal microflora compared to other terrestrial animals such as ruminant and non-ruminant herbivores, microflora can adversely affect the resilience of the host if not properly controlled. Therefore, it is clear that controlling the microflora could positively influence animal performance and OEO, with its known antimicrobial properties, is a good potential alternative to growth promoters in this respect.

The antimicrobial mechanism of OEO is not well defined. It has been suggested that their lipophilic properties (Conner, 1993) and chemical structure (Farag et al., 1989) could play a role. Several studies have also shown the inhibition of growth of different microorganisms by carvacrol (Conner, 1993). Helander et al. (1998) investigated how OEO, carvacrol and thymol exert their antibacterial effects on *Escherichia coli* O_{157} and *Salmonella typhimurium*. These phenols disintegrated the membrane of the bacteria, leading to the release of membrane-associated material from the cells to the external medium. It was thus suggested that terpenoids and phenylpropanoids can penetrate the membrane of the bacteria and reach the inner parts of the cell because of their lipophilicity (Helander et al., 1998). Although both OEO and a combination of carvacrol and thymol are often thought to be similar, it has also been proposed that structural properties, such as the presence of the functional groups (Farag et al., 1989) and aromaticity (Bowles and Miller, 1993) are responsible for their antibacterial activity. This is the first comparison made between the growth performances of channel catfish fed with the monomer, combination or natural mix of *O. heracleoticum* L. extracts. The results indicated that carvacrol, a combination of carvacrol and thymol and OS could enhance the weight gain, but performance of OS was still the best. The feed conversion ratio and protein efficient ratio of the study also showed that fish fed with OS was significantly superior to other treatments.

In the present study, HSI, VSI and CF were positively correlated to dietary OS supplementation and the addition of Thy, Car, and Car + Thy into the feed had no positive effect on the HSI, VSI and CF of channel catfish compared to the control diet. As there are more than 30 different active components found in OS, it is most likely that these other minor constituents played an important role in improving these factors, but the exact mechanism for this needs further and more advanced study.

The proximate analysis data of this study indicated that moisture, lipid and ash content of muscle samples were not affected by the feed additives. However there were differences in muscle protein where

Table 5
Lysozyme, superoxide dismutase, catalase in plasma of channel catfish fed different diets containing *Origanum heracleoticum* L. extracts.

Diets	Con	Thy	Car	Car + Thy	OS
LSZ	50.07 ± 3.12^{a}	47.3 ± 3.84^{a}	48.5 ± 7.59^{a}	53.7 ± 3.95^{a}	73.57 ± 2.00^{b}
SOD	104.7 ± 6.56^{a}	110.80 ± 21.63^{a}	110.30 ± 12.02^{a}	118.60 ± 12.68^{ab}	138.5 ± 9.23^{b}
CAT	20.83 ± 2.62^{a}	21.23 ± 3.52^{a}	22.83 ± 2.51^{a}	21.13 ± 2.37^{a}	30.83 ± 1.71^{b}

Data were presented as mean ± SD ($n = 3$). Values within the same row having different superscripts are significantly different ($P<0.05$).

the addition of carvacrol and thymol or OS resulted in higher protein sedimentation.

Superoxide dismutase (SOD) catalyses the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide. Subsequently, catalase (CAT) activity decomposes this hydrogen peroxide into oxygen and water and this constitutes a crucial part of the cellular antioxidant defence mechanism. In this study, the activities of superoxide dismutase and catalase were assessed in the plasma of channel catfish and these values were markedly enhanced in fish fed the OS diet compared to the control diet and other treatments.

Following challenge with *A. hydrophila*, all treatments showed a reduced mortality compared to the control diet, but the best survival rate was observed in fish fed with the OS diet. Among the other groups, fish treated with the Car + Thy diet had a lower mortality than the fish treated with the Car diet. Fish fed the Thy diet had nearly the same mortality compared to the Con diet, but had no negative effect on survival rate. This result indicated that while thymol had a positive effect on the survival rates, and while carvacrol was the effective component to enhance the survival rate of channel catfish, nevertheless OEO in its natural form (OS) showed the greatest effect in enhancing the survival rate and this can also be attributed to the synergistic effects of the other active components within OEO. Survival rates of infected fish usually increase after treatment with various immunostimulants (Anderson, 1992; Sakai, 1999); feeding common carp with chitosan and levamisole reduced mortality after challenge with *A. hydrophila* (Gopalakkanan and Arul, 2006). Large yellow croaker treated with glucans and subsequently challenged with *Vibrio harveyi* had a similar result (Ai et al., 2007).

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.

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