

New Approach to use *Origanium Vulgare* Extract as Immunostimulant to Increase Resistance to *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*

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Abstract

The aim of the present study to assess the use of ethanol extracts of *Origanium vulgare* as a growth and immunity promoter for Nile tilapia (*Oreochromis niloticus* L) fingerlings. Fish (Average 12.27 g) were randomly distributed into four treatments; three replicates each at a rate of 20 fish per 100-L aquarium. Fish were fed one of the tested diets containing similar crude protein (30%) and gross energy (4.40 kcal/g). In addition, 0.0, 0.5%, 1.0%, or 1.5% *Origanium vulgare* extract. Diets were given twice daily at a rate of 3% of live body weight, for six days a week during 10 weeks. After the feeding trial, fish of each treatment were challenged by pathogenic *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*, which was given by intraperitoneal (I/P) injection and they were kept under observation for 10 days to follow up any abnormal clinical signs and the daily mortality rate. The growth promoting influence of *Origanium vulgare* extract was observed on fish. The maximum growth was observed at 0.5% *Origanium vulgare* extract as compared to the control. No significant differences in fish survival were reported among the experimental treatments, falling within the range of 93.3-100%. The control fish consumed less diet and gave a higher Feed conversion ratio (FCR), while fish fed diet supplemented with 0.5% *Origanium vulgare* extract demonstrated the highest protein efficiency ratio (PER), apparent protein utilization (APU), and energy utilization (EU). The supplementation of *Origanium vulgare* extract had no significant effect on the fish body composition (dry matter, crude protein, fat, and ash), mean while total protein, albumin, and globulin increased significantly to the highest values at 0.5% *Origanium vulgare* extract, as compared to the control. However, supplementation of *Origanium vulgare* extract did not significantly affect the albumin/globulin ratio (A/G). In conclusion, 0.5% *Origanium vulgare* extract in Nile tilapia diets increased the fish resistance to *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*, indicating the effective role of *Origanium vulgare* extract.

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$$E = \frac{a}{b} \times 100$$

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At the start of the study, the proximate chemical composition of Nile tilapia fed diets containing different levels of *Origanum vulgare* extract (0.00%, 1.50%, 1%, and 0.50%) was 75.22 ± 0.30% moisture; 59.04 ± 0.82% protein; 18.11 ± 0.76% lipid and 21.42 ± 0.57% ash. The results showed that there were no significant differences ($P > 0.05$) in the proximate chemical composition of fish at the start of this study between the control and the experimental groups. The results also showed that the survival rate of Nile tilapia fed diets containing different levels of *Origanum vulgare* extract (0.00%, 1.50%, 1%, and 0.50%) was 93.30 ± 3.85%, 100 ± 0.0%, 100 ± 0.0%, and 96.03 ± 0.33% respectively. The results showed that there were no significant differences ($P > 0.05$) in the survival rate of Nile tilapia fed diets containing different levels of *Origanum vulgare* extract (0.00%, 1.50%, 1%, and 0.50%) compared to the control group.

Items	0.00%	At <i>Origanum vulgare</i> extract % of the diets		
	(control)	1.50%	1%	0.50%
Moisture	74.64 ± 0.68 ^a	73.77 ± 0.14 ^a	73.75 ± 0.27 ^a	73.84 ± 0.46 ^a
Crude protein	60.00 ± 0.06 ^a	60.79 ± 0.32 ^a	60.59 ± 0.37 ^a	60.68 ± 0.39 ^a
Total lipids	19.72 ± 0.22 ^a	19.79 ± 0.36 ^a	19.67 ± 0.18 ^a	19.97 ± 0.49 ^a
Ash	19.81 ± 0.31 ^a	19.24 ± 0.55 ^a	19.11 ± 0.61 ^a	1.31 ± 0.60 ^a

a-no significant different in the same row from the table at $P < 0.05$. Proximate chemical composition of fish at start of this study was 75.22 ± 0.30% moisture; 59.04 ± 0.82% protein; 18.11 ± 0.76% lipid and 21.42 ± 0.57% ash.

Table 2: Proximate chemical analysis on dry matter basis (mean ± SE) of Nile tilapia fed diets containing different levels of *Origanum vulgare* extract.

Items	0.0 %	At <i>Origanum vulgare</i> extract % in the diets		
	(control)	0.50%	1.00%	1.50%
Initial weight (g)	12.27 ± 0.46 ^a	12.17 ± 0.15 ^a	12.37 ± 0.50 ^a	12.23 ± 0.36 ^a
Final weight (g)	29.30 ± 0.67 ^c	36.00 ± 0.85 ^a	34.40 ± 0.55 ^{ab}	32.37 ± 0.74 ^b
Weight Gain (g)	17.03 ± 0.52 ^c	23.83 ± 0.97 ^a	22.03 ± 0.33 ^{ab}	20.14 ± 0.87 ^{ab}
Weight Gain %	138.79 ± 7.03 ^c	195.81 ± 9.99 ^a	178.09 ± 9.13 ^{ab}	164.68 ± 6.02 ^{bc}
SGR (% day)	1.24 ± 0.03 ^c	1.55 ± 0.05 ^a	1.46 ± 0.05 ^{ab}	1.39 ± 0.03 ^b
Survival rate (%)	93.30 ± 3.85 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a	96.03 ± 0.33 ^{ab}

Means the same letter (a/b/c) in the same row is not significantly different at $P < 0.05$.

Table 3: Growth performance (means ± SE) of Nile tilapia fed diets containing different levels of *Origanum vulgare* extract.

Items	0.0 % (control)	At <i>Origanum vulgare</i> extract % in the diet		
		0.50%	1.00%	1.50%
Feed intake (g feed/fsh)	34.10 ± 0.34 ^c	39.74 ± 0.22 ^a	38.75 ± 0.36 ^a	37.29 ± 0.52 ^b
FCR	2.01 ± 0.05 ^a	1.67 ± 0.07 ^b	1.72 ± 0.06 ^b	1.86 ± 0.04 ^{ab}
PER	1.81 ± 0.05 ^c	2.17 ± 0.08 ^a	2.05 ± 0.05 ^{ab}	1.94 ± 0.04 ^{bc}
APU %	28.37 ± 0.28 ^d	35.73 ± 0.20 ^a	34.08 ± 0.32 ^b	32.40 ± 0.45 ^c
AFU %	41.36 ± 0.40 ^d	50.76 ± 0.28 ^a	46.56 ± 0.42 ^b	44.14 ± 0.60 ^c
EU %	17.32 ± 0.17 ^c	21.84 ± 0.11 ^a	20.63 ± 0.22 ^b	20.40 ± 0.21 ^b

Feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER), apparent protein utilization (APU), apparent fat utilization (AFU) and energy utilization (EU) of Nile tilapia fed diets containing different levels of *Origanum vulgare* extract. Means the same letter (a/b/c/d) in the same row is not significantly different at $P < 0.05$.

The results showed that there were no significant differences ($P > 0.05$) in the proximate chemical composition of fish at the start of this study between the control and the experimental groups. The results also showed that the survival rate of Nile tilapia fed diets containing different levels of *Origanum vulgare* extract (0.00%, 1.50%, 1%, and 0.50%) was 93.30 ± 3.85%, 100 ± 0.0%, 100 ± 0.0%, and 96.03 ± 0.33% respectively. The results showed that there were no significant differences ($P > 0.05$) in the survival rate of Nile tilapia fed diets containing different levels of *Origanum vulgare* extract (0.00%, 1.50%, 1%, and 0.50%) compared to the control group. The results also showed that the weight gain of Nile tilapia fed diets containing different levels of *Origanum vulgare* extract (0.00%, 1.50%, 1%, and 0.50%) was 17.03 ± 0.52 g, 23.83 ± 0.97 g, 22.03 ± 0.33 g, and 20.14 ± 0.87 g respectively. The results showed that there were no significant differences ($P > 0.05$) in the weight gain of Nile tilapia fed diets containing different levels of *Origanum vulgare* extract (0.00%, 1.50%, 1%, and 0.50%) compared to the control group. The results also showed that the survival rate of Nile tilapia fed diets containing different levels of *Origanum vulgare* extract (0.00%, 1.50%, 1%, and 0.50%) was 93.30 ± 3.85%, 100 ± 0.0%, 100 ± 0.0%, and 96.03 ± 0.33% respectively. The results showed that there were no significant differences ($P > 0.05$) in the survival rate of Nile tilapia fed diets containing different levels of *Origanum vulgare* extract (0.00%, 1.50%, 1%, and 0.50%) compared to the control group. The results also showed that the weight gain of Nile tilapia fed diets containing different levels of *Origanum vulgare* extract (0.00%, 1.50%, 1%, and 0.50%) was 17.03 ± 0.52 g, 23.83 ± 0.97 g, 22.03 ± 0.33 g, and 20.14 ± 0.87 g respectively. The results showed that there were no significant differences ($P > 0.05$) in the weight gain of Nile tilapia fed diets containing different levels of *Origanum vulgare* extract (0.00%, 1.50%, 1%, and 0.50%) compared to the control group.

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