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Essential Oregano Oil as a Growth Promoter for the Yellowtail Tetra, *Astyanax altiparanae*

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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.

Owing to the increasing demand for fish and declining fish stocks worldwide, aquaculture has become increasingly necessary and profitable. To achieve higher productivity and thus meet world demand, it is essential to use complete and balanced diets with additives that help maintain health and improve fish growth (Goda 2008).

The growth promoters are natural or synthetic substances, or living organisms that act beneficially in improving the animal body weight gain, feed efficiency ratio, reproductive performance and decreasing mortality (Andreotti and Nicodemo 2004). Among the growth promoters, essential oils are commonly recognized as safe for animals, consumers and the environment for being natural products that have decreased side effects or toxicity and better biodegradability (Kalemba and Kunicka 2003), besides good market availability.

Among the natural products with potential to be used as growth promoters is oregano, *Origanum* sp. (Fukayama et al. 2005), because of its antibacterial (Sivropoulou et al. 1996; Burt and Reinders 2003; Souza et al. 2006; Oliveira et al. 2009), antifungal (Sartoratto et al. 2004; Cleff et al. 2010), antioxidant (Simitzis et al. 2008; Zheng et al. 2009), anti-inflammatory (Azuma et al. 1986; Ocaña-Fuentes et al. 2010), antihelminthic (Force et al. 2000) and

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digestive properties (Hernández et al. 2004; Platel and Srinivasan 2004). These properties are attributed to the presence of phenolic carvacrol and thymol and monoterpenes γ-terpinene and ρ-cymene (Bampidis et al. 2005; Zheng et al. 2009), among others.

The yellowtail tetra, *Astyanax altiparanae* is distributed from the Brazilian Northeast to the La Plata basin (Vilela and Hayashi 2001), located between Brazil, Paraguay, Argentina, Bolivia and Uruguay. This species has a great potential for aquaculture as a result of its high reproductive rate, short production cycle and omnivorous nature (Hayashi et al. 2004; Cotan et al. 2006). This species has been commercially raised for use as live bait for sport fishing and fried as a snack, with potential for canned marketing.

Thus, the aim of this study is to evaluate the potential of oregano oil as a growth promoter for the yellowtail tetra.

TABLE 1. *Chemical composition of the commercial product organic oregano essential oil (LASZLO).*[a]

Chemical composition	g/100 g
α-Thujone	0.4
α-Pinene	1.6
Camphene	0.7
Sabinene	0.8
Myrcene 1.4	1.4
α-Terpinene	1.1
ρ-Cymene	12.8
γ-Terpinene	8.4
1,8 Cineole	0.3
Cis-sabinene hydrate	1.6
Thymol	4.7
Carvacrol	63.0
β-Caryophyllene	1.4

[a]Method of analysis: High-Resolution Gas Chromatography. Gas Chromatograph HP 5890 (Hewlett Packard, Palo Alto, CA, USA). Column: 25 m × 0.25 mm BP1 (SGE). Temperatures: Column: 60 C (3 min), 3 C/min to 200 C. Injector: 250 C Split: 1/200. Detector FID: 250 C. Injection volume: 1 μL (0.5% concentration in chloroform).

Materials and Methods

Animals and Experimental Conditions

The experiment was conducted in a completely randomized design with six treatments and four replicates. Treatments consisted of 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.

The commercial product organic oregano essential oil (LASZLO, Brazil) was used; its chemical composition is shown in Table 1. The commercial product was obtained by steam distillation of the leaves of the plant *Origanum vulgare*.

The oregano oil was mixed with soybean oil and then mixed with the other ingredients. The mixture was pelleted, kiln dried with forced ventilation (30 C for 48 h), crushed, sieved and stored in a freezer at −20 C. The chemical composition of experimental diets (Table 2) was evaluated for dry matter, crude protein, ether extract and total ash. The crude protein content was assessed using the semi-micro Kjeldahl method according to the protocol described by Silva and Queiroz (2002), and dry matter, ether extracts and total ash of the diets were measured according to AOAC (1990).

The yellowtail tetra, *A. altiparanae*, juveniles (initial average weight 1.46 ± 0.09 g) were kept into 60-L aquaria, provided with aeration, biological filters, temperature controlled by heaters and thermostats (27 ± 0.5 C), in a stocking density of 30 fish/aquarium (0.5 fish/L of water). Fish were fed to satiation three times daily for 90 d. At the end of the experimental period, the fish were euthanized with excess anesthetic (clove oil 400 mg/L) for the sample collections.

Productive Performance

At the end of the experimental period the fish were counted, weighed and measured the standard length for calculation of the following productive performance:

$$\text{Survival rate (SR)} = \text{initial number of fish/final number of fish} \times 100;$$

$$\text{Weight gain (WG)} = \text{average final weight} - \text{average initial weight};$$

TABLE 2. *Composition (g/kg) of the experimental diets.*

	Oregano oil levels in experimental diets (g/kg)					
Ingredient	0.0	0.5	1.0	1.5	2.0	2.5
Soybean meal	584.8	584.8	584.8	584.8	584.8	584.8
Corn gluten	40.0	40.0	40.0	40.0	40.0	40.0
Corn meal	100.0	100.0	100.0	100.0	100.0	100.0
Wheat bran	180.0	180.0	180.0	180.0	180.0	180.0
L-lysine	2.3	2.3	2.3	2.3	2.3	2.3
DL-methionine	3.2	3.2	3.2	3.2	3.2	3.2
Soybean oil	42.0	41.5	41.0	40.5	40.0	39.5
Oregano oil	0.0	0.5	1.0	1.5	2.0	2.5
Dicalcium phosphate	37.0	37.0	37.0	37.0	37.0	37.0
Table salt	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin and mineral supplement[a]	8.0	8.0	8.0	8.0	8.0	8.0
BHT[b]	0.2	0.2	0.2	0.2	0.2	0.2
Chemical composition of the diets (g/kg)						
Dry matter	882.70	887.80	881.40	887.20	885.80	897.70
Gross energy[c]	4.272.11	4.272.11	4.272.11	4.272.11	4.272.11	4.272.11
Crude protein	358.10	350.50	365.50	355.20	352.30	353.90
Ether extract	60.10	57.30	53.00	53.00	57.20	59.00
Ash	80.00	81.80	84.00	83.30	81.70	83.30
Crude fiber[c]	54.60	54.60	54.60	54.60	54.60	54.60
Total calcium[c]	12.40	12.40	12.40	12.40	12.40	12.40
Available P[d]	7.10	7.10	7.10	7.10	7.10	7.10
Carvacrol[e]	0.00	0.32	0.63	0.95	1.26	1.58
Thymol [e]	0.00	0.02	0.05	0.07	0.09	0.12
ρ-Cymene[e]	0.00	0.06	0.13	0.19	0.26	0.32
γ-Terpinene[e]	0.00	0.04	0.08	0.13	0.17	0.21

[a]Assurance levels per kilogram of product: Vit. A^1, 200,000 UI; Vit. D_3, 200,000 UI; Vit. E, 12,000 mg; Vit. K_3, 2400 mg; Vit. B_1, 4800 mg; Vit. B_2, 4800 mg; Vit. B_6, 4000 mg; Vit. B_{12}, 4800 mg; folic acid, 1200 mg; Ca pantothenate, 12,000 mg; Vit. C, 48,000 mg; biotin, 48 mg; choline, 65,000 mg; niacin, 24,000 mg; ferro, 10,000 mg; copper, 6000 mg, manganese, 4000 mg, zinc, 6000 mg, iodine, 20 mg, cobalt, 2 mg, selenium, 20 mg.

[b]Butyl hydroxy toluene (antioxidant).

[c]Values calculated according to the chemical composition of foods given by Rostagno et al. (2005).

[d]Values calculated for Nile tilapia according to Miranda et al. (2000).

[e]Values calculated based on the chemical composition of the oregano oil provided by the manufacturer LASZLO.

$$\text{Feed intake (FI)} = (\text{weight of initial feed} - \text{weight of finished feed}) / \text{number of fish};$$

$$\text{Feed conversion ratio (FCR)} = \text{FI}/\text{WG};$$

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

Specific growth rate (SGR), using the equation shown below:

$$\text{SGR} = \frac{\ln \text{FW (g)} - \ln \text{IW (g)}}{\text{time (days)}} \times 100$$

wherein IW is the initial average weight of fish (g); FW, final average weight of fish (g) and body condition factor (K), using the equation shown below:

$$K = \frac{\text{FW}}{\text{FL}^3}$$

wherein FW, final average weight (g); FL, final average standard length (cm);

$$\text{Carcass Yield (CY)} = (\text{carcass weight}/\text{final weight}) \times 100$$

$$\text{Viscero-somatic index (VSI)} = (\text{weight of viscera}/\text{body weight}) \times 100$$

Metabolic Variables

Blood sampling was performed with an incision along the caudal peduncle of two fish of each experimental unit (8 fish/treatment). Blood glucose was evaluated using glucose test strips in a digital glucometer (Accu-Chek Active, Roche, Mannheim, Germany). For glycogen analysis, the liver was collected from about 15 fish in each experimental unit, to obtain 206 ± 22 mg. Muscle samples were also obtained from two fish in each experimental unit for obtaining 339.00 ± 103.00 mg. The liver and muscle of animals were collected in centrifuge tubes containing 30% KOH, were hydrolyzed in a boiling water bath for an hour, adding five drops of saturated Na_2SO_4 to remove the bath. It was added 4.5 mL of absolute alcohol to make a 70% alcohol solution and the samples were heated in a water bath for 15 sec. The tubes were then centrifuged at 670.8 *g* for 10 min. The supernatant was discarded, and the precipitate was dissolved in distilled water (to complete 10 mL). Samples of 2 mL were kept refrigerated during the addition of 4.0 mL of anthrone reactive and again were heated in a water bath for 10 min. The concentrations of liver and muscle glycogen were determined in a spectrophotometer at 620 nm wavelength (Sjörgren et al. 1938).

Carcass Chemical Composition

The carcass was considered as the animal gutted and without scales. For the analysis of the chemical composition of the carcass, 10 fish were collected from each experimental unit (40 fish/treatment). The crude protein content was assessed by the semi-micro Kjeldahl method, according to the protocol described by Silva and Queiroz (2002), and dry matter, ether extract and total ash carcasses were carried out according to AOAC (1990).

Statistical Analysis

The evaluation of the effects of oregano oil levels in the diet on productive performance, metabolic variables and carcass composition was performed by analysis of variance and polynomial regression at 10% probability. The most appropriate regression model was chosen by considering the significance of the regression coefficients, the magnitude of the coefficients of determination as well as the behavior of the variables under analysis.

Results and Discussion

A quadratic effect of oregano oil was observed on weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER) and carcass weight (CW) (Table 3). The estimated values of oregano oil that show the higher values of these variables were 0.58, 0.48, 0.18 and 0.50 g/kg, respectively. For the feed conversion ratio (FCR), a quadratic effect was also observed, with the estimated value to improve this variable as 0.62 g/kg. The effect of oregano oil on productive performance might be caused by increased digestibility (Hernández et al. 2004) and the absorption of nutrients (Michiels et al. 2010), improving the feed efficiency. Similarly, positive effects were observed on the growth performance of channel catfish, *Ictalurus punctatus*, fed Orego-Stim (Bedford, UK), a commercial product containing natural oil of *Origanum heracleoticum* L. (Zheng et al. 2009). The use of thymol and carvacrol, substances found in oregano oil, also improved the growth performance of the rainbow trout, *Oncorhynchus mykiss* (Ahmadifar et al. 2011).

There was no significant effect of oregano oil on blood glucose and liver glycogen levels. For muscle glycogen, a positive linear effect of the treatments was observed (Table 4). However, a negative linear effect of oregano oil levels was observed on the dry matter and ether extract of the carcass. A quadratic effect was observed for the carcass crude protein. The highest value for carcass crude protein was observed for fish fed the diet containing the highest level of oregano oil (2.5 g/kg). There was no effect of the treatments on the ash content of the carcass (Table 5).

The improvement in carcass quality probably occurred due to two effects: the activation of digestive enzymes and/or activation of metabolic enzymes. The increase in muscle glycogen content, concomitant with the reduction of fat in the carcass, indicated that

TABLE 3. *Productive performance of* Astyanax altiparanae *fed diets containing oregano oil as a growth promoter.*

	Oregano oil levels in experimental diets (g/kg)						
Productive performance	0.0	0.5	1.0	1.5	2.0	2.5	CV (%)
Survival rate (%)	87.5	95.0	95.0	95.8	95.8	90.8	7.48
Weight gain (g)[a]	1.85	2.05	2.20	1.72	1.45	1.52	22.82
Feed intake (g)	3.48	3.55	4.05	3.33	3.03	3.25	16.41
Feed conversion ratio[b]	1.93	1.77	1.85	1.96	2.13	2.25	16.69
Protein efficiency ratio[c]	1.54	1.64	1.55	1.47	1.35	1.32	14.59
Specific growth rate (%/d)[d]	0.91	0.97	1.01	0.86	0.75	0.78	14.75
Body condition factor	2.52	2.55	2.55	2.68	2.46	2.49	4.48
Carcass weight (g)[e]	2.61	2.73	2.85	2.47	2.32	2.33	13.26
Carcass yield (g/kg)	789.9	778.1	778.2	775.6	797.2	781.9	21.0
Viscero-somatic index (g/kg)	197.8	199.4	201.3	207.8	188.1	201.3	71.2

CV = Coefficient of variation.
[a]Weight gain = $-0.1691x^2 + 0.1976x + 1.9363$, $R^2 = 66.06\%$ ($P = 0.06$).
[b]Feed conversion ratio = $0.1262x^2 - 0.1561x + 1.8861$, $R^2 = 93.19\%$ ($P = 0.07$).
[c]Protein efficiency ratio = $-0.0541x^2 + 0.0181x + 1.5793$, $R^2 = 87.49\%$ ($P = 0.06$).
[d]Specific growth rate = $-0.0532x^2 + 0.0506x + 0.9389$, $R^2 = 69.9$ ($P = 0.03$).
[e]Carcass weight = $-0.1148x^2 + 0.1145x + 2.6692$, $R^2 = 69.27\%$ ($P = 0.08$).

TABLE 4. *Metabolic variables of* Astyanax altiparanae *fed diets containing oregano oil as a growth promoter.*

	Oregano oil levels in experimental diets (g/kg)						
Metabolic variables	0.0	0.5	1.0	1.5	2.0	2.5	CV (%)
Glycemia (mg/dL)	76.38	71.88	72.25	66.50	66.38	75.41	17.37
Hepatic glycogen (g/100 g)	2.13	3.14	2.60	2.49	2.67	2.22	31.84
Muscle glycogen (g/100 g)[a]	0.04	0.02	0.08	0.11	0.10	0.08	85.52

CV = Coefficient of variation.
[a]Muscle glycogen = $0.0256x + 0.0393$, $R^2 = 53.09\%$ ($P = 0.09$).

the fish fed on higher levels of oregano oil probably used lipids as a main source of energy, thus, saving the muscle glycogen. However, the oregano oil, carvacrol and thymol did not affect the deposition of fat in the carcass of channel catfish (Zheng et al. 2009) and rainbow trout (Ahmadifar et al. 2011).

The reduction in the carcass fat content of the fish in this study may still have been caused by inhibiting enzymes related to fatty acid synthesis and activating enzymes related to fatty acid oxidation, this was observed by Kim et al. (2013) in the liver of mice fed a carvacrol-supplemented high-fat diet.

TABLE 5. *Chemical composition (g/kg) of the carcass of* Astyanax altiparanae *fed diets containing oregano oil as a growth promoter.*

	Oregano oil levels in experimental diets (g/kg)						
	0.0	0.5	1.0	1.5	2.0	2.5	CV (%)
Dry matter[a]	233.5	230.0	218.9	204.6	222.8	203.8	11.19
Crude protein[b]	629.0	615.6	622.1	636.0	623.4	649.0	2.60
Ether extract[c]	143.7	164.9	136.3	131.8	139.9	116.1	17.58
Ash	162.8	156.0	167.0	162.2	162.4	168.0	4.00

CV = Coefficient of variation.
[a]Dry matter = $-10.523x + 232.070$, $R^2 = 61.90\%$ ($P = 0.07$).
[b]Crude protein = $8.461x^2 - 13.320x + 626.420$, $R^2 = 61.30\%$ ($P = 0.07$).
[c]Ether extract = $-12.396x + 154.265$, $R^2 = 52.80\%$ ($P = 0.05$).

The preferential use of fat for energy also may have contributed to reduced protein catabolism, which would explain the higher protein content in the carcass of fish fed the highest level of oregano oil (2.5 g/kg). Similarly, an increase in crude protein content was observed in the carcass of rainbow trout fed with 3.0 g timol-carvacrol/kg (Ahmadifar et al. 2011) and in the muscle of channel catfish fed 0.5 g of Orego-Stim/kg (Zheng et al. 2009).

Another possibility to explain the reason why oregano oil increased the protein content of the carcass and glycogen in muscle would be the activation of digestive enzymes (Jamroz et al. 2005); this could improve the digestibility of nutrients in the diet and increase the amount of nutrient deposition in the carcass of the fish. In broilers, Basmacioğlu Malayoğlua et al. (2010) observed that the supplementation of oregano oil increased chymotrypsin activity in the digestive system, and improved crude protein digestibility. Betancourt et al. (2012) observed that broilers fed supplemented with *Oregano majorana* presented higher values of ileal digestibility for energy and fat compared to control group.

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*.

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