ACTA
AMAZONICA

http://dx.doi.org/10.1590/1809-4392202102442

ORIGINAL ARTICLE

Effect of *Lippia grata* essential oil as a feed additive on the performance of tambatinga juveniles

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ABSTRACT

Lippia grata (formerly known as *Lippia gracillis*) is an aromatic plant native to Brazil, with leaves rich in essential oils that possess significant biological activities. We evaluated the effect of essential oil of *L. grata* (EOLG) as a dietary additive on the growth, somatic indices, and biochemical parameters of juveniles (5.25 ± 0.26 g) of tambatinga, a hybrid fish obtained by crossing tambaqui (*Colossoma macropomum*) with pirapitinga (*Piaractus brachypomum*) of great economic importance in north and northeastern Brazil. We evaluated four dietary treatments, consisting of EOLG supplemented at 0.0, 0.5, 1.0, and 2.0 mL kg^{-1}, over 60 days. Carcass yield was significantly higher in fish fed all EOLG diets compared to those fed the control diet (0.0 mL kg^{-1}). Animals that received the 0.5 mL kg^{-1} treatment gained significantly more weight and showed a higher specific growth rate than those treated with 1.0 and 2.0 ml kg^{-1} EOLG, although none differed significantly from the control. The feed conversion rate was significantly lower in the 0.5 than in the 1.0 mL kg^{-1} treatment. Compared with higher concentrations, the diet containing 0.5 mL kg^{-1} EOLG increased the use of muscle glycogen, glucose, and lactate to meet energy demands, avoiding the use of muscle protein. Our results suggest that dietary supplementation with EOLG significantly improves carcass yield in tambatinga juveniles but that concentrations above 0.5 mL kg^{-1} may compromise growth rates and carbohydrate metabolism in this fish.

KEYWORDS: diets; growth; *Lippia gracillis*; *Colossoma macropomum*; *Piaractus brachypomum*

Efeito do óleo essencial de *Lippia grata* como aditivo alimentar sobre o desempenho de juvenis de tambatinga

RESUMO

Lippia grata (previamente conhecida como *Lippia gracillis*) é uma planta aromática nativa do Brasil, com folhas ricas em óleos essenciais que possuem atividades biológicas significativas. Avaliamos o efeito do óleo essencial de *L. grata* (OELG) como aditivo alimentar sobre o crescimento, índices somáticos e parâmetros bioquímicos de juvenis (5,25 ± 0,26 g) de tambatinga, um hibrido obtido do cruzamento de tambaqui (*Colossoma macropomum*) com pirapitinga (*Piaractus brachypomum)* com grande importância econômica no norte e nordeste do Brazil. Foram avaliados quatro tratamentos dietéticos consistindo na suplementação com OELG em 0,0; 0,5; 1,0 e 2,0 mL kg^{-1} durante 60 dias. O rendimento de carcaça foi significativamente maior nos peixes alimentados com todas as dietas contendo OELG em comparação à dieta controle. Os animais do tratamento 0,5 mL kg^{-1} ganharam significativamente mais peso e apresentaram maior taxa de crescimento específico do que aqueles tratados com 1,0 e 2,0 mL kg^{-1} OELG, embora nenhum tenha diferido significativamente do controle. A taxa de conversão alimentar foi significativamente menor no tratamento 0,5 mL kg^{-1} do que no tratamento 1,0 mL kg^{-1}. Comparada com as concentrações mais altas, a dieta contendo 0,5 mL kg^{-1} OELG aumentou o uso de glicogênio muscular, glicose e lactato para suprir as demandas energéticas, evitando o uso de proteína muscular. Nossos resultados sugerem que a suplementação dietética com OELG melhora significativamente o rendimento de carcaça de juvenis de tambatinga, mas concentrações acima de 0,5 mL kg^{-1} podem comprometer as taxas de crescimento e metabolismo de carboidratos desses peixes.

PALAVRAS-CHAVE: dietas; crescimento; *Lippia gracillis; Colossoma macropomum*; *Piaractus brachypomum*

CITE AS: Costa, T.S.; Silva, R.C.; Pretto, A.; Monteiro, O.S.; Siqueira, J.C.; Baldisserotto, B.; Lopes, J.M. 2022. Effect of *Lippia grata* essential oil as a feed additive on the performance of tambatinga juveniles. *Acta Amazonica* 52: 122-130.

INTRODUCTION

Plant-derived feed additives present beneficial effects on fish health, growth, and feeding efficiency in cultured fish due to the high quantity and variety of secondary bioactive metabolites (phytochemicals) they contain. The active properties of phytochemicals are due to their synthesis by secondary metabolism in plants (Sutili *et al.* 2018; Souza *et al.* 2019; Heluy *et al.* 2020; Kuralkar and Kuralkar 2021; Faehnrich *et al*. 2021). Phytochemicals act differently depending on the concentration of the active or major ingredients, the amount used in the diet, and the application form (Sutili *et al*. 2018; Souza *et al*. 2019; Chung *et al.* 2020). Dietary addition of essential oils from plants such as *Citrus sinensis* (L.) Osbeck (Acar et *al.* 2015), *Citrus limon* (L.) Burm (Ngugi *et al.* 2016), *Aloysia citriodora* Palau (formerly *A. triphylla*) (Zeppenfeld *et al.* 2016), *Citrus x auranticus* L. (Lopes *et al.* 2019), and *Citrus x latifolia* (Yu. Tanaka) Tanaka (Lopes *et al*. 2020) increase growth rates in fish and are viable alternatives to increase the productivity of intensive fish farming. Yet, essential oils of *Lippia alba* (Mill.) N. E. Brown, *Lippia sinoides* Cham, *Ocimum gratissimum L.*, or *Zingiber officinale* Roscoe do not influence fish growth; rather, they can reduce lipid peroxidation and increase the tissue antioxidant response (Saccol *et al*. 2013) or improve the nonspecific immune response and survival (Monteiro *et al*. 2021).

Several mechanisms have been proposed to explain the beneficial effects of essential oils on fish performance, such as changes in the gastrointestinal tract and its microbiota that improve digestibility, nutrient absorption, and the immune response (Talpur 2014; Acar *et al.* 2015); increased antioxidant activity (Saccol *et al.* 2013; Lopes *et al.* 2019) and resistance to stress (Souza *et al.* 2019) and direct effects on the development of pathogenic organisms that reduce their ability to colonize the digestive tract, thus preventing disorders that affect digestion and nutrient absorption (Harikrishnan *et al.* 2011; Campagnolo *et al.* 2013). The interaction among the intestinal microflora, the morphology of the gastrointestinal mucosa, blood biochemical levels, the immune system, and the absorption of nutrients has a direct influence on the health and productive performance of fish (Ahmadifar *et al.* 2011; Campagnolo *et al.* 2013; Lopes *et al.* 2020).

Lippia grata Schauer (Verbenaceae), formerly designated as *Lippia gracillis* Schauer, is an aromatic shrub up to 1.5 m in height, growing wild in areas of northern and northeastern Brazil (Albuquerque *at al*. 2007; Franco *et al*. 2014). Its aerial parts have antibacterial, antioxidant, and anti-inflammatory properties (Takeuchi and Cafe 2016; Mendes *et al*. 2020) and are used to treat gastrointestinal, respiratory, and cutaneous infections (Albuquerque *et al*. 2006). The main active components of the essential oil of *L. gracillis* are thymol, carvacrol, *p*-cymene, and γ-terpinene, which are molecules with antimicrobial properties (Gomes *et al.* 2011). Although it is used routinely in popular human medicine to treat infections, there are few studies on the action of this essential oil in animal production and as a performance enhancer (Cardoso Junior 2017; Rocha *et al.* 2020). Considering other species of the genus *Lippia*, there are studies evaluating its anesthetic or growth effects in fish (Silva *et al*. 2013; Toni *et al*. 2014; Ventura *et al*. 2019; Monteiro *et al*. 2021). However, to date, there are no published studies on the effect of *L. grata* essential oil on fish.

Tambatinga is a hybrid fish produced from the female of the tambaqui (*Colossoma macropomum* Cuvier) and the male of the pirapitinga (*Piaractus brachypomus* Cuvier) that achieves higher weight gain than its parent species (Hasimoto *et al.* 2012). It is also omnivorous and very resilient to rearing in intensive systems (Alencar Araripe *et al.* 2011). Its culture is of great economic importance in northern and northeastern Brazil (Ribeiro *et al.* 2019); therefore, the aim of this study was to evaluate the potential effect of dietary supplementation with essential oil of *L. grata* on performance and health indicators in tambatinga juveniles.

MATERIAL AND METHODS

Fish and culture conditions

The experiment was conducted at Universidade Federal do Maranhão (UFMA), Maranhão state, Brazil. We used 160 juveniles of tambatinga with an initial weight of 5.25 ± 0.26 g and a length of 7.13 ± 0.15 cm. The fish were purchased from a local fish farm and remained in an outdoor tank for 10 days, fed with commercial feed (45% crude protein) three times a day. After this period, the fish were placed at random in twenty 150-L tanks (eight fish per tank) in a water recirculation system, fed with the control diet (Table 1), and acclimated to laboratory conditions for 10 days. The closed water recirculation system was also equipped with an air blower (pressure 1300 mm H_2O), a peripheral water pump (maximum flow (Q) L h 2400), a decantation box (250 L), and a biofilter (250 L) to perform biological control of nitrogenous waste. The study was authorized by the Ethics Committee on Animal Experimentation of UFMA (process # 23115.004974/2016-46 CEUA/UFMA).

Source material, extraction, and characterization of the essential oil

The essential oil of *L. grata* (EOLG) was extracted from leaves collected in Chapada das Mesas National Park (07°07′47.1″S, 4°25′36.8″W), municipality of Carolina, Maranhão state, Brazil, in February 2016. The plant was identified, and a voucher specimen was deposited at the herbarium of Museu Emilio Goeldi, Belém, Pará state, Brazil (MG 230155). Collection was authorized under Brazilian legislation for the protection of biodiversity (SISGEN license # AD7DF67).

Table 1. Composition and proximate analysis of the experimental diets with of the essential oil of *Lippia grata* (EOLG).

Ingredients (g kg^{-1})	EOLG concentration in diet (mL kg^{-1})			
	0	0.5	1.0	2.0
Soybean meal	300	300	300	300
Meat and bone meal	350	350	350	350
Rice bran	120	120	120	120
Corn grain	150	150	150	150
Canola oil	30	30	30	30
Salt	10	10	10	10
Vitamin/mineral mixture[a]	30	30	30	30
Dicalcium phosphate	10	10	10	10
Essential oil (mL kg^{-1})	0	0.5	1.0	2.0
Proximate analysis (%)[b]				
Dry matter	90.0	91.9	90.9	91.9
Crude protein	29.9	31.4	30.6	30.5
Lipids	8.7	9.2	9.0	9.1
Ash	16.2	15.4	14.4	15.4
Neutral detergente fiber	25.1	26.6	25.6	26.5

[a]Vitamin and mineral mixture (minimum levels per kilogram of diet): folic acid, 250 mg; pantothenic acid, 5,000 mg; antioxidant, 0. 60 g; biotin, 125 mg; cobalt, 25 mg; copper, 2,000 mg; iron, 820 mg; iodine, 100 mg; manganese, 3,750 mg; niacina, 5,000 mg; selenium, 75 mg; vitamin A, 1,000,000 UI; vitamin B1, 1,250 mg; vitamin B12, 3,750 mg; vitamin B2, 2,500 mg; vitamin B6, 2,485 mg; vitamin C, 28,000 mg; vitamin D3, 500,000 UI; vitamin E, 20,000 UI; vitamin K, 500 mg; zinc, 17,500 mg. [b]Analyzed proximate composition.

The leaves were dried for five days at room temperature, ground, and then submitted to hydrodistillation (3 h) using a Clevenger-type apparatus (Figueiredo *et al.* 2019). The essential oil was dried over anhydrous sodium sulfate (Merck-Millipore, São Paulo, Brazil), and the yield per dry weight of plant material was calculated. The moisture content was calculated in duplicate using an Infrared Moisture Balance (Genaka, São Paulo, Brazil) for water loss measurement.

The EOLG was analyzed on a gas chromatograph coupled to a mass spectrometer (GCMS)QP2010 Ultra system (Shimadzu Corporation, Tokyo, Japan), equipped with the GCMS-Solution software containing the NIST 11, FFNSC 2, and Adams libraries, as described in detail by Fernandes *et al.* (2021).

Experimental design and treatments

The experimental design was randomized with four treatments (test diets) and five replicates per treatment. The diet formulations were based on Zeppenfeld *et al.* (2016), with a control diet and three diets with increasing concentrations of EOLG (0.5, 1.0, and 2.0 mL kg^{-1}) (Table 1). The ingredients of the base diet were ground, weighed, and mixed to complete homogenization. The EOLG was added with canola oil for adequate homogenization. Water was added to aid mixing with the dried ingredients, and the mixtures were pelletized. The pellets were dried for 24 h at 40 ºC in a forced-air circulation oven. The diets were stored at -18 ºC until use. The analysis of diet composition followed AOAC (1995) for crude protein, dry matter, and ash; Bligh and Dyer (1959) for fat; and Van Soest *et al.* (1991) for neutral detergent fiber.

Feed management and water quality

Fish were fed with the experimental diets to apparent satiation three times a day (8:00, 12:00, and 17:00) for 60 days. Thirty minutes after the first meal, fecal residues were removed from the tanks by siphoning, and the water level was replaced.

The temperature (28.0 ± 1.5 °C) and dissolved oxygen levels (6.94 ± 0.92 mg L^{-1}) were monitored daily with an oximeter (HANNA, T160). Weekly measures of pH (6.9 ± 0.2 units) were taken with a digital (DMPH-2) pH meter, and those of total ammonia (0.25 ± 0.03 mg L^{-1}) and non-ionized ammonia (0.08 ± 0.02 mg L^{-1}) using a commercial ammonia kit (LabconTest toxic ammonia). All water quality parameters were within the limits considered suitable for the species (Moro *et al.* 2013; Ribeiro *et al.* 2019).

Performance parameters

At the end of the feeding trial, all fish were fasted for 24 h, anesthetized with eugenol (40 µL L^{-1}) (Moraes *et al.* 2017), and then weighed and measured to determine growth variables. Two fish per replicate (tank) were randomly selected and euthanized by spinal cord sectioning behind the operculum, and their livers were removed, weighed, quickly placed on ice, and frozen at –20 ºC for biochemical analysis. After complete dissection, each carcass was weighed, the fillets were removed and weighed, and a sample of white muscle was taken and stored at -20 ºC for biochemical analysis.

We determined the following performance parameters: final weight (FW, g); final total length (FL, cm); condition factor (CF) = 100 x (body weight; g)/(body length; cm)^3; feed conversion ratio (FCR) = feed intake/weight gain; specific growth rate (SGR, %/day) = (ln (final weight) – ln (initial weight)/period x 100; weight gain (WG, g) = final weight – initial weight; survival (S, %) = live animals at day 60/initial number of animals in the tank (Ribeiro *et al.* 2019; Lopes *et al.* 2020); hepatic somatic index (HSI, %) = (liver weight / whole fish weight) x 100; carcass yield (CY, %) = (eviscerated fish weight/whole fish weight) x 100 (Rampelotto *et al.* 2018); fillet yield (FY, %) = (fillet weight/whole fish weight) x 100 (Geraldo *et al.* 2015).

Biochemical parameters

Glycogen levels in the liver and muscle were determined following Bidinotto *et al.* (1997). Subsamples of 50 mg were added to 1 mL potassium hydroxide (KOH) and 3 mL ethanol for hydrolysis and precipitation of glycogen. Tissue

subsamples of 50 mg were heated at 100 ºC with KOH, and the supernatant was used to determine the total protein level following Lowry *et al.* (1951), with bovine serum albumin as the standard. Other tissue subsamples of 50 mg were homogenized with 10% trichloroacetic acid (1:20 dilution) using a motor-driven Teflon pestle and centrifuged at 1,000 g for 10 min to flocculate the proteins. The supernatant was used for glucose and lactate determination following Dubois *et al.* (1956) and Harrower and Brown (1972), respectively.

Statistical analysis

All variables showed homogeneity of variances (Levene test) and a normal distribution (Shapiro-Wilk test). The variables were compared among treatments using one-way analysis of variance and Duncan's test ($P < 0.05$), with the statistical program SPSS 21.0.

RESULTS

Characterization of the essential oil

The major constituents of the EOLG were α-pinene (24.47%), 1,8-cineole (16.18%), β-pinene (11.89%), and limonene (9.64%) (Table 2).

Performance parameters

Overall, performance parameters in the EOLG treatments did not differ significantly from those of the control, except for carcass yield, which was significantly higher in all EOLG treatments (Table 3). The final weight, final total length,

Table 2. Chemical composition of the essential oil of *Lippia grata* leaves collected in Chapada das Mesas National Park (Maranhão, Brazil). IRC[a] = calculated retention index (on Rxi-5ms column); IRC[b] = retention index from literature (Adams, 2001).

Compound	Composition (%)	IRC[a]	IRC[b]
α-Pinene	24.47	934	932
1,8-Cineole	16.18	1031	1026
β-Pinene	11.89	978	974
Limonene	9.64	1029	1024
E-Caryophyllene	4.28	1421	1417
Canphene	3.25	948	946
Monoterpene hydrocarbons	55.95		
Oxygenated monoterpenes	23.80		
Sesquiterpenes	6.69		
Oxygenated sesquiterpenes	8.09		
Total	**94.53**		

Table 3. Growth parameters and somatic indices of tambatinga juveniles fed diets supplemented with different concentrations of the essential oil of *Lippia grata* (EOLG). IW: initial weight; IL: initial length; FW: final weight; FL: final length; CF: condition factor; FCR: feed conversion rate; SGR: specific growth rate; WG: weight gain; HSI: hepatic somatic index; CY: carcass yield; FY: fillet yield.

Parameter	EOLG concentration in diet (mL kg^{-1})			
	0	0.5	1.0	2.0
IW (g)	5.35 ± 0.22	5.17 ± 0.37	5.34 ± 0.27	5.13 ± 0.17
IL (cm)	7.23 ± 0.14	7.07 ± 0.17	7.16 ± 0.14	7.05 ± 0.09
FW (g)	42.67 ± 5.65^{ab}	47.55 ± 4.23^{a}	36.58 ± 4.11^{b}	37.58 ± 6.08^{b}
FL (cm)	13.90 ± 0.65^{ab}	14.50 ± 0.30^{a}	13.51 ± 0.36^{b}	13.56 ± 0.57^{b}
CF	1.58 ± 0.04^{a}	1.56 ± 0.05^{ab}	1.48 ± 0.07^{b}	1.50 ± 0.08^{ab}
FCR	1.86 ± 0.19^{ab}	1.71 ± 0.14^{b}	2.09 ± 0.17^{a}	1.94 ± 0.30^{ab}
SGR (%/day)	3.45 ± 0.24^{ab}	3.70 ± 0.12^{a}	3.20 ± 0.22^{b}	3.30 ± 0.28^{b}
WG (g)	37.32 ± 5.72^{ab}	42.38 ± 4.01^{a}	31.24 ± 4.16^{b}	32.45 ± 6.14^{b}
HSI	1.62 ± 0.21^{a}	1.41 ± 0.34^{ab}	1.46 ± 0.19^{ab}	1.44 ± 0.26^{ab}
CY (%)	87.16 ± 1.00^{b}	88.55 ± 1.16^{a}	88.73 ± 0.54^{a}	89.72 ± 1.21^{a}
FY (%)	29.52 ± 1.95^{a}	27.46 ± 3.07^{a}	30.24 ± 4.24^{a}	29.94 ± 2.06^{a}

Values are the mean ± SD (n = 5). For all variables, except survival, data were analyzed as average per tank (replicate). Different letters in the rows indicate significant difference by the Duncan test ($P < 0.05$).

specific growth rate, and weight gain were significantly higher in the 0.5 mL kg^{-1} EOLG treatment than in the 1.0 or 2.0 mL kg^{-1} treatments, but no EOLG treatment differed significantly from the control. The condition factor was significantly lower in the 1.0 mL kg^{-1} treatment than in the control. The feed conversion rate was significantly lower in the 0.5 mL kg^{-1} EOLG treatment than in the 1.0 mL kg^{-1} treatment but did not differ significantly from the control. The EOLG treatments tended to produce a lower HSI than the control. Fillet yield was not affected significantly by dietary EOLG, and no mortality was observed in any treatments during the experimental period.

Biochemical parameters

Biochemical parameters tended to vary more in the EOLG treatments than in the control (Table 4). Lactate in muscle was significantly higher in all EOLG treatments than in the control, while protein levels were significantly lower in the 1.0 and 2.0 mL kg^{-1} EOLG treatments. Glycogen levels in muscle were significantly higher in the 1.0 and 2.0 mL kg^{-1} than in the 0.5 mL kg^{-1} EOLG treatment but did not differ from the control. Muscle glucose levels were significantly lower in the 0.5 and 2.0 mL kg^{-1} EOLG treatments than in the other two treatments.

Table 4. Hepatic and muscular metabolic parameters of tambatinga juveniles fed diets supplemented with different concentrations of the essential oil of *Lippia grata*. Glucose and glycogen= µmol glucose g $tissue^{-1}$; Lactate= µmol lactate g $tissue^{-1}$; Protein= mg g $tissue^{-1}$.

Parameter	EOLG concentration in diet (mL kg^{-1})			
	0	0.5	1.0	2.0
Muscle				
Lactate	16.93 ± 2.34^{b}	25.71 ± 1.70^{a}	24.63 ± 5.84^{a}	24.11 ± 2.19^{a}
Glycogen	7.15 ± 1.01^{ab}	6.63 ± 0.30^{b}	7.82 ± 0.55^{a}	7.89 ± 0.58^{a}
Glucose	25.69 ± 1.73^{a}	18.70 ± 2.55^{b}	24.50 ± 4.21^{a}	18.28 ± 2.46^{b}
Protein	177.66 ± 25.58^{a}	170.44 ± 25.71^{ab}	151.02 ± 16.09^{b}	147.80 ± 14.20^{b}
Liver				
Lactate	5.23 ± 0.24^{a}	4.68 ± 0.45^{b}	4.95 ± 0.27^{ab}	5.10 ± 0.49^{ab}
Glycogen	75.00 ± 14.70^{a}	66.87 ± 12.63^{a}	81.52 ± 18.95^{a}	79.44 ± 11.02^{a}
Glucose	573.87 ± 35.22^{a}	465.26 ± 35.64^{b}	493.65 ± 54.56^{b}	498.99 ± 60.72^{b}
Protein	162.62 ± 20.29^{a}	143.43 ± 10.64^{a}	148.21 ± 21.16^{a}	112.60 ± 5.26^{b}

Values presented as mean ± SD (n=8). Different letters in the rows indicate significant difference by Duncan test ($P < 0.05$).

Hepatic lactate was significantly lower in the 0.5 mL kg^{-1} EOLG treatment than in the control. Hepatic glycogen was not affected by dietary supplementation with EOLG, but glucose was significantly lower in all EOLG treatments than in the control. A significantly lower hepatic protein level was observed only in the 2.0 mL kg^{-1} EOLG treatment.

DISCUSSION

Lippia grata varies in the composition of its volatile constituents throughout northeastern Brazil (Franco *et al.* 2014). The major constituents of EOLG from Chapada das Mesas National Park (α-pinene, 1,8-cineole, β-pinene, and limonene) in this study corroborated those described by Monteiro *et al.* (2021) for EOLG from the same region of the state Maranhão but differed markedly from those from other localities. The essential oil of *L. grata* [*L. gracilis*] leaves from other northeastern Brazilian localities has components in common with our sample, even regarding low level constituents. EO of *L. grata* [*L. gracilis*] leaves collected in Crato, Ceará state, contained a minor percentage of α-pinene (Bitu *et al.* 2012), however, the oxygenated monoterpenes thymol and carvacrol were the primary constituents, as in other EOs from the Brazilian northeast (Teles *et al.* 2010; Souza 2013; Santos *et al.* 2014). The chemical composition of our sample suggests the presence of a new *L. grata* chemotype from Maranhão state, with a predominance of α-pinene, β-pinene, 1,8-cineole, and limonene, instead of thymol and carvacrol. In a detailed review, Pascual *et al.* (2001) reported that the compounds most frequently found in essential oils of *Lippia* species are limonene, β-caryophyllene, ρ-cymene, camphor, linalool, α-pinene, and thymol. In fact, essential oil content and composition can differ greatly, even within the same genus, as well as amongs different ripening stage or in different organs (Tirado *et al.*1995; Stashenko *et al.* 1996). The variability in the constitution of *Lippia* essential oils owes to the large number of species in the genus and their wide geographic distribution (Monteiro *et al.* 2021). Gomes *et al.* (2011) reported that the chemical composition of *L. gracilis* oil presents quantitative fluctuations of the major components which are probably owed to genetic variability, depending on where and in which conditions the plant was cultivated.

The results for FW, FL, WG, and SGR indicated that the use of EOLG concentrations greater than 0.5 mL kg^{-1} does not favor the growth of juvenile tambatinga. Similarly, Brum *et al*. (2017) evaluated the effect of the essential oil of *O. gratissimum* and *Z. officinale* (40 and 16% 1,8-cineole, respectively) in the diet of *Oreochromis niloticus* Linnaeus, 1758, at concentrations of 0.5, 1.0 and 1.5%. The essential oil of *O. gratissimum* at 0.5% significantly improved food conversion in comparison with the control, while no improvements in the growth parameters were observed for *Z. officinale*. Likewise, diets supplemented with essential oil of *O. gratissimum*, *Z. officinale* (28.2% and 15.8% 1,8-cineole, respectively), and *Lippia sidoides* Cham. (0.7% 1.8-cineole) did not significantly affect growth parameters of tambaqui (Monteiro *et al*. 2021).

The concentration and synergy of compounds in an essential oil and the level of dietary inclusion, as well as factors such as the form of administration, target species, and age of the individuals, explain variations in the responses observed to its use (Campagnolo *et al*. 2013). Essential oil components differ in their mechanisms of action, which may result in improved or antagonistic specific activity (Efferth and Koch 2011). The biological effect of essential oil is a result of interactions among its constituent compounds (Sonboli *et al*. 2006).

The evaluation of fish carcasses is of great economic and production importance (Fernandes *et al*. 2010). In this sense, supplementation with EOLG increased the carcass yield in tambatinga juveniles compared to the control. Fish carcass is an important indicator in nutrition studies, as it is mainly related to visceral fat or muscle deposition. In *Salvelinus fontinalis* Mitchill, *Salmo trutta* Linnaeus, 1758, and hybrid trout, differences in carcass yield were approximated by fat content (Şahin *et al*. 2011). The same method was used for *Astronotus ocellatus* Agassiz, *Pellona castelnaeana* Valenciennes, and *Leporinus friderici* Bloch (Barai *et al*. 2022). We did not analyze fat content, but the EOLG may have influenced the fish body composition and, consequently, the carcass yield.

The levels of metabolites in different tissues such as plasma, liver, and muscle can be indicative of dietary nutrient utilization (Saccol *et al.* 2013). In our study, the response of biochemical parameters suggests that metabolic pathways varied with the EOLG concentration. Fish fed with 0.5 mL EOLG kg^{-1} apparently used muscle glycogen and glucose stores and liver glucose and lactate to meet energy demands and showed lower preference for the use of body protein. This strategy did not impair the growth of juveniles. However, fish in the 1 and 2 mL EOLG kg^{-1} treatments may have prioritized the use of body protein (reduced muscle protein concentrations) for conversion to energy and thus meet the need to maintain body tissues, blood glucose levels, and growth rates. In this case, the glycogen stores in the muscle and liver were maintained, but the possible use of amino acids through the gluconeogenic pathway and their conversion into glucose may be reflected in the lower growth rates presented by tambatinga juveniles in these treatments. According to Lehninger *et al.* (2006), the metabolic routes that maintain blood sugar levels in fish are gluconeogenesis, using substrates such as amino acids, lactate, and pyruvate, and the breakdown of glycogen reserves in the muscle and liver, which can occur aerobically or anaerobically. Still, the lactate produced in the muscle and released into the circulation and that available in the fish liver are metabolically converted into glucose (Rito *et al*. 2018).

Different responses to essential oil concentrations were also observed in silver catfish (*Rhamdia quelen* Silfvergrip) fry treated with dietary supplementation of *Citrus* x *aurantium* essential oil (93.8% limonene, 2.6% linalool, and 1.7% β-pinene): fish treated with 2 mL kg^{-1} oil showed changes in carbohydrate and protein metabolism in the liver and muscle that resulted in a higher growth rate, while lower concentrations were associated with metabolic rearrangements to maintain blood sugar and tissue glycogen and lactate levels, impairing zootechnical performance (Lopes *et al.* 2019).

CONCLUSIONS

Our results suggest that dietary supplementation with EOLG significantly improves carcass yield in tambatinga juveniles but that concentrations above 0.5 mL kg^{-1} may compromise growth rates and carbohydrate metabolism in this fish.

ACKNOWLEDGMENTS

The present work was supported in part by Instituto Nacional de Ciência e Tecnologia – Centro de Estudos de Adaptações Aquáticas da Amazônia II (INCT-Adapta II) and a fellowship from Fundação de Amparo à Pesquisa do Estado do Maranhão (FAPEMA, Brazil) for Thaisa Sales Costa.

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RECEIVED: 19/08/2021
ACCEPTED: 23/05/2022
ASSOCIATE EDITOR: Rodrigo do Valle