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AQUICULTURE

Supplementation with microencapsulated lemongrass essential oil improves protein deposition and carcass yield in silver catfish (*Rhamdia quelen*)

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ABSTRACT. The aim of this study was to investigate the effect of dietary supplementation with *Cymbopogon flexuosus* essential oil (EO) on productive performance, metabolic parameters and body and fillet composition of adult silver catfish, *Rhamdia quelen*. Total length, condition factor, weight gain, specific growth rate, hepatosomatic index, visceral fat content and fillet composition were not affected by EO supplementation. Plasma total protein, globulin and triglycerides were reduced by EO supplementation. Supplementation with 1 mL EO kg^{-1} diet increased carcass yield and protein deposition in the carcass, while reducing gonadosomatic index and fat deposition in the carcass when compared to control group. In addition, 1 mL EO kg^{-1} diet also improved feed intake when compared to 3 mL EO kg^{-1} diet. Our results suggest that EO up to 1 mL kg^{-1} diet can be used in fish feed to improve protein deposition and carcass yield of silver catfish.

Keywords: *Cymbopogon flexuosus*, citral, growth performance, gonads, body composition.

A suplementação com óleo essencial de capim-limão microencapsulado melhora a deposição de proteína e o rendimento de carcaça de jundiás (*Rhamdia quelen*)

RESUMO. O objetivo deste estudo foi investigar o efeito da suplementação na dieta com óleo essencial de *Cymbopogon flexuosus* (EO) sobre o desempenho produtivo, os parâmetros metabólicos e a composição corporal e de filés de jundiás (*Rhamdia quelen*) adultos. O comprimento total, o fator de condição, o ganho de peso, a taxa de crescimento específico, o índice hepatossomático, o teor de gordura visceral e a composição dos filés não foram afetados pela suplementação com EO. Os níveis plasmáticos de proteína total, globulina e triglicérides foram reduzidos pela suplementação com EO. A suplementação com 1 mL de EO kg^{-1} de dieta aumentou o rendimento de carcaça e a deposição de proteína na carcaça, mas reduziu o índice gonadossomático e a deposição de gordura em comparação ao grupo controle. Além disso, 1 mL de EO kg^{-1} de dieta também melhorou o consumo de ração em comparação com 3 mL de EO kg^{-1} de dieta. Os resultados sugerem que se pode utilizar até 1 mL de EO kg^{-1} de dieta na alimentação de peixes para melhorar a deposição de proteína e a produtividade de carcaça de jundiás em fase de engorda.

Palavras-chave: *Cymbopogon flexuosus*, citral, desempenho produtivo, gônadas, composição corporal.

Introduction

Aquaculture plays an important role in economic and social development as it provides high biological value food for human nutrition and generates job opportunities that contribute to economic growth (Subasinghe, Soto, & Jia, 2009). Catfish (Siluriformes) comprises numerous fish species, mostly freshwater fish (Ferraris, 2007), widely distributed with a key role in world aquaculture (The World Bank, 2013). Silver catfish, *Rhamdia quelen*, is endemic from southern Mexico to central Argentina (Valladão, Gallani, & Oilarski, 2016). This freshwater species is well adapted to the low winter temperatures in southern Brazil (Garcia, Copatti, Wachholz, Pereira Filho, & Baldisserotto, 2008), being a valuable species for aquaculture in temperate climate.

Plant extracts and derivatives, including essential oils (EOs), emerged as an alternative to antibiotics and chemotherapy in aquaculture since they have antimicrobial activity and stimulate fish immunity (Vaseeharan & Thaya, 2013). EOs have also been prospected for use as anaesthetic and sedative for fish, and have been shown to reduce the stress during transportation (Parodi et al., 2012; 2014;

Salbego et al., 2017) and increase the shelf-life of refrigerated and frozen fish (Veeck et al., 2013; Daniel et al., 2014; 2016; Veit et al., 2016).

Strategies to improve fish growth performance may accelerate the development of the aquaculture sector and EOs have been investigated as feed additive for different fish species but results are conflicting. In Nile tilapia, *Oreochromis niloticus*, sweet orange peel EO, rich in limonene, improved weight gain and blood parameters (Acar, Kesbiç, Yılmaz, Gültepe, & Türker, 2015), while lemon peel EO, also rich in limonene, had no effect on growth performance and had little effect on blood parameters (Baba, Acar, Öntas, Kesbiç, & Y1lmaz, 2016). However, both EOs increased resistance against pathogens (Acar et al., 2015; Baba et al., 2016). In silver catfish, feed supplementation with EO from *Lippia alba* (linalool chemotype) leaves (0.25 - 2.0 mL EO kg^{-1} diet) did not influence growth performance or blood parameters but increased antioxidant enzymes and reduced lipid oxidation (Saccol et al., 2013). On the other hand, diet containing citral-rich EOs as from *Aloysia triphylla* leaves (2.0 mL EO kg^{-1} diet) and *Cymbopogon flexuosus* increased the number of intestinal folds and/or promoted fish growth (Zeppenfeld et al., 2016; Baldisserotto et al., 2015).

Cymbopogon flexuosus is a highly productive and vigorous plant that has good yield of dry mass (7-25 t ha^{-1}) and higher yield in the production of EO (100 L of EO ha^{-1}) (Zheljazkov, Cantrell, Astatkie, & Cannon, 2011) compared to other citral-rich herbs like *L. alba* (citral chemotype) (~26-67 L of EO ha^{-1}) (Zambrano, Buitrago, Durán, Sanchez, & Bonilla, 2013) and *A. triphylla* (10 L of EO ha^{-1}) (Al-Mefleh, Abu Salah, & Abandeh, 2012). These characteristics increase the economic viability for the production of *C. flexuosus* EO.

Beyond chemical differences between the EOs tested in fish, loss of part of their components during feed production and storage may have contributed to some negative results on animal growth. EOs are known to undergo loss by volatilization and degradation when exposed to high temperature, light or oxidants. Therefore, microencapsulation appears as an important strategy to protect and preserve EOs and their bioactive properties (Asbahani et al., 2015).

The aim of this study was to investigate the effect of dietary supplementation with microencapsulated EO from *C. flexuosus* on productive performance, metabolic parameters and body composition of adult silver catfish.

Material and methods

Essential oil

The EO from *C. flexuosus* was donated by Bio Natural Essenciais (Três Passos, Rio Grande do Sul, Brazil) and had 0.907 g cm^{-3} density. To prevent degradation, EO was microencapsulated by complex coacervation (Alvim & Grosso, 2010) before incorporation into fish feed. The amount of EO contained in the microcapsules, assessed by hydrodistillation (Clevenger apparatus), was 365 mL kg^{-1}.

The identification of EO composition was determined using an Agilent 6890 gas chromatograph (GC) coupled to a mass spectrometry detector (MS) Agilent 5973 with a HP5-MS column (5% phenyl, 95% methylsiloxane, 30 m x 0.25 mm ID × 0.25 μM). The EO chemical constituents were identified by comparison of their retention indices, which were determined by a calibration curve of n-alkanes injected under the same chromatographic conditions as the samples and mass fragmentation patterns described in the literature (Garlet et al., 2016). The chemical constituents of the EO were quantified by GC coupled to flame ionization detector (FID) on an Agilent 7890A. The percentage of the constituents was based on peak area normalization. Ninety-three percent of EO composition was identified and the major constituents (> 1.5%) were: geranial or α-citral (45.7%), neral or β-citral (32.1%), *Z*-verbenol (2.4%), citronellol (2.0%), *Z*-geraniol (2.0%) and caryophyllene (1.7%).

Diets

Three diets were formulated (Table 1): a control diet (without EO), a diet containing 1 mL $kg^{-1}$of *C. flexuosus* EO and a diet containing 3 mL kg^{-1} of *C. flexuosus* EO. Diets had the same formulation except for the presence of EO. The ingredients were manually homogenized, pelleted (6 mm) and then dried at 45°C in an air flow oven for 24 hours. After drying, diets were stored at −18°C.

Table 1. Ingredients and proximate composition of experimental diets.

Diet components	Essential oil level (mL kg^{-1})		
	0	1	3
Ingredients (g kg^{-1})			
Meat and bone meal	350	350	350
Soybean meal	300	300	300
Broken corn	150	150	150
Rice bran	120	120	120
Canola oil	30	30	30
Vitamin and mineral Premix	30	30	30
Sodium chloride	10	10	10
Dicalcium phosphate	10	10	10
Essential oil microcapsules*	0	2.74	8.22

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Proximate composition			
Moisture (g kg^{-1})	57.3	57.0	58.2
Protein (g kg^{-1})	336.9	343.4	349.3
Fat (g kg^{-1})	85.0	91.6	89.4
Ash (g kg^{-1})	215.7	224.0	219.4
NFE (g kg^{-1})	275.9	254.1	252.3
Energetic value (kJ kg^{-1})	13461	13456	13443

Notes: NFE: Nitrogen free extract. * Microcapsules contained 365 mL of EO kg^{-1}.

Fish treatment

One hundred twenty-six silver catfish (*Rhamdia quelen*) from both sex (average initial body weight = 403.1 ± 8.5 g) were obtained from a local fish farm and transported to the Laboratory of Fisheries in oxygenated water. Fish were randomized into 21 tanks (270 L) with six fish per tank. Seven independent replicates (seven tanks) were conducted for each treatment. The tanks were connected to a water recirculating system with two biological filters, an activated carbon filter and a 2000-L water reservoir.

Animals were given the experimental diets twice a day (8:00 a.m. and 4:00 p.m.) to apparent satiation for 20 days. During the experimental period, water quality was maintained as follows: 21.3 ± 0.3°C; 8.5 ± 0.5 mg dissolved oxygen L^{-1}; 0.32 ± 0.03 mg NH_3+NH_4^+ L^{-1} and 0.17 ± 0.03 mg NO_2 L^{-1}.At the end of the experimental period, fish were fasted for 24 hours and euthanized by spinal cord section. Blood was collected from the caudal vein of each fish using heparinized 5-mL syringes. Fish were eviscerated to collect liver and intestine that were weighed and stored at -20°C until analysis. All procedures were approved by the Ethics Committee on Animal Use, Federal University of Santa Maria (protocol number 120/2014).

Productive performance

Fish total length (cm) and weight (g) were determined individually using digital callipers and scales and used to calculate following indices:

$$\text{Condition factor (g/cm}^3\text{)} = 100 \times \left(\frac{\text{Final weight}}{\text{Final length}^3}\right)$$

$$\text{Weight gain (g)} = (\text{Final body weight} - \text{Initial body weight})$$

$$\text{Specific growth rate (\%)} = 100 \times \left(\frac{ln\ \text{final body weight} - ln\ \text{initial body weight}}{\text{Days of experiment}}\right)$$

Somatic indices and body yield

Seven fish per treatment were eviscerated to assess the digestive tract length and the weight of eviscerated fish, digestive tract, liver, visceral fat and gonads that were used to calculate the following indices:

$$\text{Carcass yield (\%)} = \left(\frac{\text{Eviscerated fish weight}}{\text{Whole fish weight}}\right) \times 100$$

$$\text{Digestive somatic index (\%)} = \left(\frac{\text{Digestive tract weight}}{\text{Whole fish weight}}\right) \times 100$$

$$\text{Visceral fat index (\%)} = \left(\frac{\text{Visceral fat weight}}{\text{Whole fish weight}}\right) \times 100$$

$$\text{Hepatosomatic index (\%)} = \left(\frac{\text{Liver weight}}{\text{Whole fish weight}}\right) \times 100$$

$$\text{Gonadossomatic index (\%)} = \left(\frac{\text{Gonad weight}}{\text{Whole fish weight}}\right) \times 100$$

Proximate composition of diet, whole fish and fish fillet

Moisture, ash and crude protein of experimental diets, whole fish and fillets were analyzed following the methods of Association of Official Agricultural Chemists [AOAC] (2005). Fat content was determined by gravimetric method (Bligh & Dyer, 1959). Dietary crude fiber was determined by method 978.10 of Association of Official Agricultural Chemists [AOAC] (2005) and nitrogen-free extract (NFE) was calculated as:

$$\text{NFE} = 100 - (\%\ \text{moisture} + \%\ \text{protein} + \%\ \text{lipid} + \%\ \text{crude fiber} + \%\ \text{total ash})$$

Plasma assays

Plasma was obtained after blood centrifugation (3000 x *g*, 10 minutes, 4°C) and was stored at -20°C before assessment of the following metabolic markers using commercial kits: alkaline phosphatase (LabTest, Minas Gerais State, Brazil), creatinine, albumin (Bioclin, Minas Gerais State, Brazil), total protein, triglycerides, cholesterol, glucose, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Doles, Goias State, Brazil). Globulins were calculated using the following equation:

$$\text{Globulins (g } dL^{-1}\text{)} = \text{total protein (g } dL^{-1}\text{)} - \text{albumin (g } dL^{-1}\text{)}$$

Free amino acids were determined following the methodology of Spies (1957).

Liver metabolic parameters

Liver samples were deproteinized with trichloroacetic acid using an ultra Turrax-type homogenizer (Marconi, Brazil) to measure glucose (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), lactate (Harrower & Brown, 1972) and ammonia (Verdouw, Van Echteld, & Dekkers, 1978) levels. Liver samples were extracted with KOH and the extracts were used to assess glycogen (Dubois et al., 1956) and total protein (Lowry, Rosebrough, Lewis Farr, & Randall, 1951). Liver samples were homogenized with 20 mM phosphate buffer, pH

7.5, centrifuged at 1000 x *g* for 10 minutes and the supernatant was used to determine free amino acids (Spies, 1957) and AST and ALT activity using commercial laboratory kits (Doles, Goiás State, Brazil).

Digestive enzymes

Intestine was homogenized (1:20, w v^{-1}) in 0.02 M Tris / 0.01 M phosphate / 50% glycerol, pH 7.5 buffer using an ultra Turrax-type homogenizer (Marconi, Brazil) and centrifuged at 1200 x *g,* at 4°C for 10 minutes and the supernatant was collected for enzyme analysis.

Trypsin activity was determined at 247 nm after incubating supernatants with Nα-p-tosyl-L-arginine methyl ester hydrochloride (TAME) at 25°C, pH 8.1 (Hummel, 1959). Chymotrypsin activity was determined at 256 nm after incubating supernatants with N-benzoyl-L-tyrosine ethyl ester (BTEE) at pH 7.8 (Hummel, 1959).

Statistical analysis

Levene's test was used to evaluate the homogeneity of variances. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test, applied to determine differences between groups. Gonadosomatic index data did not met ANOVA assumptions and were analyzed by Kruskal-Wallis test followed by multiple comparisons of mean ranks. Spearman's test was used to evaluate the correlation between gonadosomatic index and other variables. The significance level was set at $p < 0.05$. Analyses were run with SPSS 16.0 for Windows (Chicago, IL, USA).

Results

At the end of the experimental period, total length, condition factor, weight gain and specific growth rate did not differ among treatments. Fish fed 1 mL EO kg^{-1} diet had feed intake similar to the control group but higher than fish fed 3 mL EO kg^{-1} diet ($p < 0.05$). Despite the lower feed intake by fish fed 3 mL EO kg^{-1} diet, the estimated consumption of EO by this group was ~3.6-fold higher compared to fish fed 1 mL EO kg^{-1} diet (Table 2).

EO supplementation did not affect hepatosomatic index or visceral fat mass but reduced the digestive somatic (1 and 3 mL EO kg^{-1} diet) and gonadosomatic indices (1 mL EO kg^{-1} diet) and increased carcass yield (1 mL EO kg^{-1} diet) ($p < 0.05$; Table 3) compared to control diet.

Table 2. Productive performance of silver catfish (*Rhamdia quelen*) fed different concentrations of *Cymbopogon flexuosus* EO.

Variable	Essential oil level (mL kg^{-1})		
	0	1	3
Total diet consumption (g kg^{-1} body weight per day)	8.8 ± 0.5^{ab}	10.2 ± 0.5^{a}	7.8 ± 0.7^{b}
EO consumption (μL kg^{-1} body weight per day)★	0	7.9	28.3
Total length (cm)	35.1 ± 1.1	34.9 ± 1.2	34.4 ± 1.1
Condition factor (g per cm^3)	1.04 ± 0.07	1.06 ± 0.07	1.03 ± 0.07
Weight gain (g)	47.6 ± 10.8	47.1 ± 12.8	40.5 ± 17.2
Specific growth rate (% per day)	0.49 ± 0.12	0.68 ± 0.17	0.46 ± 0.16

Notes: Values are expressed as mean ± SEM (n = 7). Values that have no common superscript letter within the same row are different by Tukey's test ($p < 0.05$). ★Calculated based on the amount of EO in diet and the apparent consumption of diet.

Table 3. Body yield and somatic indices of silver catfish (*Rhamdia quelen*) fed different concentrations of *Cymbopogon flexuosus* EO.

Variable (%)	Essential oil level (mL kg^{-1})		
	0	1	3
Carcass yield	80.4 ± 3.4^{b}	84.7 ± 1.7^{a}	83.5 ± 1.6^{ab}
Digestivesomatic index	3.1 ± 0.1^{a}	2.5 ± 0.1^{b}	2.5 ± 0.1^{b}
Hepatosomatic index	1.62 ± 0.14	1.50 ± 0.24	1.29 ± 0.07
Visceral fat index	1.05 ± 0.27	1.27 ± 0.24	0.84 ± 0.18
Gonadosomatic index★	5.4(3.4-9.3)^{a}	1.8(0.9-2.7)^{b}	3.1(2.4-5.9)^{ab}

Notes: Values are expressed as mean ± SEM. Values that have no common superscript letter within the same row are different by Tukey's test ($p < 0.05$). ★Gonadosomatic index is expressed as median followed by interquartile range (n = 7 per group) and submitted to *post hoc* multiple comparison of mean ranks.

Body composition was also affected by EO supplementation, but it did not affect fillet composition (Table 4). EO supplementation reduced fat content and increased moisture of whole fish ($p < 0.05$). Protein content of whole fish was increased only by supplementation with 1 mL EO kg^{-1} diet.

Table 4. Proximate composition of whole fish and fish fillet of silver catfish (*Rhamdia quelen*) fed different concentrations of *Cymbopogon flexuosus* EO.

Composition (%)	Essential oil level (mL kg^{-1})		
	0	1	3
	Whole fish		
Moisture	66.3 ± 0.8^{b}	70.1 ± 0.5^{a}	71.2 ± 0.3^{a}
Fat	14.1 ± 0.3^{a}	11.0 ± 0.2^{b}	10.6 ± 0.2^{b}
Protein	14.9 ± 0.2^{b}	16.4 ± 0.3^{a}	14.8 ± 0.6^{b}
Ash	2.9 ± 0.3	3.4 ± 0.4	3.8 ± 0.3
	Fillet		
Moisture	76.7 ± 0.5	75.9 ± 0.8	77.8 ± 0.4
Fat	3.7 ± 0.4	4.5 ± 0.6	2.9 ± 0.4
Protein	18.1 ± 0.5	18.8 ± 0.6	19.0 ± 0.2
Ash	1.1 ± 0.1	1.1 ± 0.0	1.2 ± 0.1

Notes: Values are expressed as mean ± SEM (n = 7 per group). Values that have no common superscript letter within the same row are different by Tukey's test ($p < 0.05$).

Plasma total protein was reduced by EO supplementation compared to control group, whereas globulin and triglycerides levels were reduced only in fish fed 1 mL EO kg^{-1} diet ($p < 0.05$). Other plasmatic variables, namely albumin, creatinine, alkaline phosphatase, free aminoacids, cholesterol, glucose, AST and ALT were not affected by EO supplementation (Table 5).

Table 5. Plasmatic variables of silver catfish (*Rhamdia quelen*) fed different concentrations of *Cymbopogon flexuosus* EO.

Variable	Essential oil level (mL kg^{-1})		
	0	1	3
Total protein (g dL^{-1})	4.4 ± 0.1^{a}	3.6 ± 0.2^{b}	3.7 ± 0.3^{b}
Albumin (g dL^{-1})	1.4 ± 0.1	1.4 ± 0.2	1.3 ± 0.1
Globulin (g dL^{-1})	3.0 ± 0.1^{a}	2.1 ± 0.2^{b}	2.3 ± 0.3^{ab}
Creatinine (mg dL^{-1})	0.18 ± 0.04	0.26 ± 0.04	0.27 ± 0.08
Alkaline phosphatase (U L^{-1})	47.6 ± 4.6	45.5 ± 5.6	51.1 ± 6.4
Free amino acids (nmol dL^{-1})	11.8 ± 0.7	9.6 ± 0.8	10.4 ± 0.9
Triglycerides (mg dL^{-1})	243.0 ± 36.1^{a}	114.5 ± 25.5^{b}	173.9 ± 24.3^{ab}
Cholesterol (mg dL^{-1})	98.3 ± 7.1	106.5 ± 8.1	102.1 ± 4.8
Glucose (mg dL^{-1})	23.8 ± 1.8	24.9 ± 3.4	22.3 ± 3.0
ALT (U L^{-1})	23.9 ± 6.9	23.3 ± 4.5	26.5 ± 8.5
AST (UL^{-1})	21.3 ± 5.1	20.9 ± 3.4	23.3 ± 6.4

Notes: Values are expressed as mean ± SEM (n = 7). Values that have no common superscript letter within the same row are different by Tukey's test ($p < 0.05$). ALT = alanine aminotransferase; AST = aspartate aminotransferase.

In liver, fish fed 3 mL EO kg^{-1} diet had higher content of free amino acids than control group ($p < 0.05$). However, EO treatment did not affect total protein content, the activity of transaminases, glucose, glycogen, ammonia or lactate levels in liver neither the activity of digestive enzymes (Table 6).

Table 6. Metabolic parameters in liver and digestive enzymes of silver catfish (*Rhamdia quelen*) fed diets containing different concentrations of *Cymbopogon flexuosus* EO.

Variable	Essential oil level (mL kg^{-1})		
	0	1	3
Protein (mg g^{-1})	68.5 ± 4.4	69.6 ± 6.6	63.0 ± 6.0
Free aminoacids (mmol g^{-1})	128.5 ± 7.3^{b}	138.3 ± 11.8^{ab}	161.4 ± 6.7^{a}
Ammonia (mmol g^{-1})	6.6 ± 1.1	6.4 ± 0.6	7.1 ± 0.4
Glucose (μmol g^{-1})	302.5 ± 29.3	293.2 ± 15.3	343.7 ± 19.0
Glycogen (μmol g^{-1})	174.0 ± 51.8	118.7 ± 25.7	140.8 ± 44.5
Lactate (μmol g^{-1})	4.3 ± 0.3	4.8 ± 0.3	4.7 ± 0.3
ALT (UI g^{-1})	35.2 ± 8.4	30.7 ± 1.9	31.1 ± 3.8
AST (UI g^{-1})	1166.1 ± 105.1	1189.7 ± 73.6	1104.0 ± 99.4
	Digestive enzymes		
Trypsin (μmol of TAME min.$^{-1}$ mg of protein^{-1})	4.8 ± 1.0	5.0 ± 1.5	5.1 ± 0.9
Chymotrypsin (μmol of BTEE min.$^{-1}$ mg of protein^{-1})	3987.0 ± 294.7	4396.8 ± 439.4	4425.3 ± 297.3

Notes: Values are expressed as mean ± SEM (n = 7 per group). Values that have no common superscript letter within the same row are different by Tukey's test ($p < 0.05$). ALT = alanine aminotransferase; AST = aspartate aminotransferase.

Discussion

Dietary *C. flexuosus* EO supplementation favored muscle deposition instead of gonadal tissue development as the lowest dose of EO increased carcass yield and protein content of carcass and reduced gonadosomatic index and fat content in the carcass. The gonadosomatic index suffers little influence from dietary components like lipids (Coldebella et al., 2013) or energy content (Tessaro et al., 2014). In silver catfish, the gonadosomatic index is strongly influenced by seasonality and, in both sexes, the gonadosomatic peak coincides with testosterone peak (Barcellos et al., 2002). We propose that the decrease in gonadosomatic index induced by the lowest dose of EO is likely associated to changes in the distribution of testosterone or other sexual hormone caused by the decrease in blood globulin levels, which are known to be responsible for regulating the bioavailability and concentration of steroid hormones in blood (Hammond, 2016). In fact, we detected a significant positive association between blood globulin levels and the gonadosomatic index ($R = 0.448$; $p < 0.05$). EO had no effect on the activity of intestinal trypsin and chymotrypsin suggesting that changes in protein content of carcass were not due to an improvement in protein digestibility. Thus, the decrease in gonadosomatic index is probably responsible for lower fat and higher protein content of carcass and also for the higher carcass yield. These data demonstrate that supplementation of EO in the finisher diet for farmed catfish can increase the proportion of tissues of commercial interest, improving the commercial value of fish.

Despite the improvement in carcass yield and carcass composition, EO supplementation did not change fish growth performance compared to the control group but 1 mL EO kg^{-1} diet (equivalent to 0.9 g EO kg^{-1} diet) increased diet intake compared to the highest EO dose (equivalent to 2.7 g EO kg^{-1} diet). Corroborating with these results, Jensen, Provan, Larssen, Bron and Obach (2015) verified that pure citral (0.3 g kg^{-1} diet) had no effect on growth of Atlantic salmon (*Salmo salar*) fed for 30 days. Similarly, dietary supplementation with ginger (*Zingiber officinale*) EO (~40% of citral) at concentrations of 5 g kg^{-1} and 10 g kg^{-1} diet did not affect the weight gain of Nile tilapia fed for 55 days but 15 g kg^{-1} EO decreased weight gain (Brum et al., 2017). These data suggest that high doses of citral-rich EO may impair growth efficiency. Although in the present study both doses of *C. flexuosus* EO did not impair growth performance compared to the control, we did observe lower feed intake in fish feed 3 mL EO kg^{-1} when compared to 1 mL EO kg^{-1}.

Feeding behavior in some teleosts is influenced by the amino acids profile of feed and the olfactory system is dominant in initiating feeding behavior (Bazáes, Olivares, & Schmachtenberg, 2013). EOs are characterized by a strong odor, which may have masked the odor of amino acids and contributed to impair the consumption of the diet containing the highest EO level.

The digestive somatic index was reduced by EO supplementation, contrary to the effect of *L. alba* (Saccol et al., 2013) and *A. triphylla* EOs (Zeppenfeld et al., 2016), which did not affect visceral weight of

silver catfish. Despite this decrease, *C. flexuosus* EO did not impair fish weight gain or specific growth rate.

Dietary supplementation with EOs has shown controversial results on fish plasma markers. Diets containing lemon peel and sweet orange peel EO at doses ranging from 3 to 80 g kg^{-1} of feed tend to increase plasma proteins and decrease cholesterol, triglycerides and glucose in Nile tilapia (Acar et al., 2015, Baba et al., 2016). However, diets containing *L. alba* EO (0.25 – 2 mL kg^{-1}) did not affect plasma variables of silver catfish (Saccol et al., 2013). In the present study, EO supplementation at the lowest dose reduced plasmatic levels of total protein, globulin and triglycerides, whereas the highest dose reduced only total protein levels. The decrease in total protein level in plasma appears to be caused by a decrease in globulin fraction, which includes carrier proteins, enzymes, complement, and immunoglobulins. Most globulins are produced in liver but no impairment in liver function (plasma ALT and AST activity) or kidney function (creatinine levels) was verified. Blood globulin fraction also contains low amount of sex hormone-binding globulin (SHBG), and corticosteroid-binding globulin (CBG), which are proteins responsible for steroid hormones regulation on blood (Hammond, 2016). For a better understanding of EO effect on blood proteins, it is necessary to analyze each fraction in detail.

The only change observed in liver metabolites was the increase in free amino acids in fish that received the highest dietary level of *C. flexuosus* EO. The type of dietary protein promotes changes in free amino acids and liver enzymes and other enzymes involved in protein metabolism are also altered (Cai et al., 2015). In the present study, the increase in liver free amino acids did not seem to be related to changes in dietary protein as all diets had the same protein level and the activity of digestive proteinase enzymes and hepatic transaminases were not affected by EO supplementation, indicating that there was no change in protein digestibility. Some phytoadditives have been shown to improve protein deposition and to promote protein synthesis increasing liver free amino acids and free amino acids uptake by muscle in fish (Gabor, Şara, & Barbu, 2010). Similar mechanism may be responsible for the beneficial effects of *C. flexuosus* EO on protein deposition and carcass yield.

Conclusions

Dietary supplementation of silver catfish with *C. flexuosus* EO for 20 days showed no toxic effects for the animals. In addition, EO supplementation at 1 mL kg^{-1} diet improved carcass yield and protein deposition, and therefore can be indicated for diets for the finisher phase.

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