An Acad Bras Cienc (2020) 92(suppl.1): e20181374 DOI 10.1590/0001-3765202020181374
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

AGRARIAN SCIENCES

Essential oil from *Ocimum basilicum* improves growth performance and does not alter biochemical variables related to stress in pirarucu *(Arapaima gigas)*

SAMANTHA CHUNG, CARLOS H. DA P. LEMOS, DANIEL V. TEIXEIRA, RODRIGO FORTES-SILVA & CARLOS E. COPATTI

Abstract: Diet supplementation with essential oil from sweet basil *Ocimum basilicum* (EOOB) can increase fish growth. So, this study aimed to evaluate the effect of EOOB in the diet on growth performance and plasmatic variables of pirarucu juveniles (*Arapaima gigas*) submitted to stressful condition (stocking density of 7.56 kg m^{-3}per tank and limited space). Four diets (in triplicates) were evaluated with increasing levels of EOOB (0.0 control; 0.5; 1.0; and 2.0 mL kg $diet^{-1}$) over 48 days. Linalool was the major constituent of EOOB (54.19%). The addition of 2.0 mL EOOB kg $diet^{-1}$ improved final weight, weight gain, specific growth rate, condition factor and feed conversion ratio; it also decreased plasma urea levels and increased plasma albumin and total proteins levels. Plasma glucose, cortisol, and acid uric levels were not influenced by the addition of EOOB to the fish diet. In conclusion, the addition of 2.0 mL EOOBkg $diet^{-1}$ is recommended for pirarucu juveniles, due to improved growth performance, and this supplementation did not compromise the homeostasis of fish rearing in a high stocking density.

Key words: fish diet, glucose, growth promoter, high stocking density, linalool, metabolic response.

INTRODUCTION

Pirarucu (*Arapaima gigas*) is one of the largest freshwater fish species in the Amazon basin, an it is a new and promising species for cultivation in fish farms. This is due to the high marketability of its fillet, as well as its high rate of growth, rusticity and adjustment to artificial feeding (de Andrade et al. 2007, Drumond et al. 2010). In addition, pirarucu is an obligate air-breather (Baldisserotto et al. 2008), which can facilitate its existence in environments with low oxygen availability.

Stress can be caused by common farming practices, such as feeding, handling and stocking (Barcellos et al. 2003, 2004, Sena et al. 2016). Fish farmers tend to increase stocking density as a strategy to increase fish production in intensive fish farming (Lemos et al. 2018). Stocking density is one important biological factor in aquaculture because it influences survival, growth, health and production costs (Copatti et al. 2008). In addition, high stocking density is commonly associated with an increase in the concentration of nitrogen compounds in the water, which can impair water quality and fish development and causes stress (Lemos et al. 2018). In previous study, the economic analysis performed by De Oliveira et al. (2012) indicated that the aquaculture of pirarucu in cages at moderated stocking densities (0.26 kg m^{-3}) and without space limitation showed better zootechnical

indices. Similarly, Cavero et al. (2003) verified that stocking densities of 0.15, 0.20 or 0.25 kg m^{-3} can be used for pirarucu reared in confined environments. A high stocking density (1.00 kg m^{-3}) induced stress responses in pirarucu, and the magnitude of stress of the densification was even greater than that caused by transport (Brandão et al. 2006). However, these authors conducted their research with pirarucu juveniles around 10 or 100 g without space limitations.

In the present study, pirarucu juveniles were raised at a very high stocking density (7.56 kg m^{-3} per tank) and limited space (0.5 m^{-3}), which can be considered stressful for fish (Barcellos et al. 2004). In previous study, Souza et al. (2015) evaluated the effect of the essential oil of *Lippia alba* as a feed additive in silver catfish (*Rhamdia quelen*), submitted to a very high stocking density of 10.6 kg m^{-3} and limited space, and they indicated the use of 0.25 mL of essential oil of *L. alba* kg $food^{-1}$ for this species was effective. In addition, a proper diet may improve the response of aquatic animals to stressful farming conditions (Zheng et al. 2009). With the increasing demand for pirarucu outgrowing current operations, the determination of an appropriate diet that contributes to its growth and health is required.

In addition, diet supplementation with essential oils can improve growth performance of fish (Ngugi et al. 2017, Baba et al. 2018, De Souza et al. 2019a). In a recent study, De Souza et al. (2019b) found that essential oil from sweet basil *Ocimum basilicum* (EOOB) (2 mL kg $diet^{-1}$) is a growth promoter for Nile tilapia. This can be due the presence of linalool as the main chemical compound (53.35%) studied by these authors, because linalool is known to have antioxidant, antimicrobial, anti-inflammatory, and digestive-stimulant mechanisms of action (Moghaddam et al. 2011, Heldwein et al. 2014, De Souza et al. 2017). On the other hand, a diet with essential oils of *L. Alba* (55.25% of linalool) and *Ocimum americanum* (33.54% of linalool) did not alter the growth of silver catfish and red drum (*Sciaenops ocellatus*), respectively (Sacco et al. 2013, Sutili et al. 2016).

Sweet basil (*O. basilicum* Lamiaceae) is one of the most frequently grown aromatic herbs in the world. It is rich in natural antioxidants such as flavonoids (quercetin, kaempferol, rutin), phenolic acids (p-coumaric acid, caffeic acid, caftaric acid), steroids and vitamins (A, C, E, K) (Mustafa & Usman 2011); these substances could contribute to the growth and health of fish. The EOOB has been shown to be effective in aquaculture as an antioxidant (Lee et al. 2005), antimicrobial (Moghaddam et al. 2011, De Souza et al. 2018), and anesthetic agent (Limma-Neto et al. 2016, 2017). However, there is no previous study verifying the efficacy of adding EOOB to the pirarucu diet.

So, in light of its potential, EOOB may be an alternative for improving growth of pirarucu raised at very high stocking densities and limited space in artificial environments. Therefore, the objective of this study was to evaluate the effect including EOOB into a commercial diet on growth performance and plasmatic variables related to health in pirarucu juveniles reared in artificial environments.

MATERIALS AND METHODS

Essential oil from *Ocimum basilicum*

Leaves from *O. basilicum* cultivated in Três Passos, Rio Grande do Sul, Brazil, were collected in August 2016. EOOB was extracted from fresh leaves from the plants by hydrodistillation using a Clevenger-type apparatus for 2 hours according to guidelines set out by the European Pharmacopoeia (2007). Determination of the chemical compounds in the essential oils was carried out by gas chromatography/mass

spectrometry according to De Souza et al. (2017). The yield was calculated as w/w (%); the EOOB constituents (Table I) were identified by comparing the mass spectra with a mass spectral library (NIST 2008). The GC-MS analysis was performed using an Agilent- 6890 gas chromatograph coupled to an Agilent 5973 mass selective detector and helium was used as the gas carrier. The capillary column choose was HP5-MS (Hewlett Packard, 5% fenilmetilsiloxane, 30 m x 0.25 mm, film thickness: 0.25 µm) and 70 eV of ionization energy. The experimental protocol was approved by the Ethical Committee of the School of Veterinary Medicine and Animal Science of the Universidade Federal da Bahia, Salvador, Bahia, Brazil (number 71/2017).

Animals

Pirarucu juveniles were purchased from Agua Vale Fish Farming (Ituberá, Bahia, Brazil) and the experiments were performed at the Fish Feeding Behavior and Nutrition Laboratory of the Universidade Federal do Recôncavo da Bahia, Cruz das Almas, Bahia, Brazil. Pirarucu juveniles (945.40 ± 18.06 g; 54.60 ± 0.30 cm, n = 4 per tank; stocking density of 7.56 kg m^{-3} per tank) were housed in 12 continuously aerated, 500 L (0.5 m^3) tanks with a recirculation aquaculture systems and chemical and biological filters and were fed with commercial extruded fish food (400 g kg^{-1} crude protein, 9% ethereal extract, 13.40 MJ kg^{-1} digestible energy and 14-16 mm-grain-size pellets; Pratigi Alimentos Company©, Castro Alves, Bahia, Brazil). Prior to the experiment, the animals underwent a fast for 24 h.

Experimental procedure

The experimental design was completely randomized, with four treatments and three replicates. Different concentrations of EOOB (0.0

Table I. Chemical composition of essential oil of *Ocimum basilicum*. IKc: retention índex calculated. IKt: retention index reference (NIST 2008). %: relative percentage.

Chemical compound	IK^c	IK^t	%
β-Myrcene	990	985	0.59
Eucalyptol	1031	1031	4.91
β-trans-ocimene	1048	1048	0.80
Linalool	1099	1101	54.19
Camphor	1142	1146	0.86
(-)-Bornyl acetate	1285	1284	1.45
p-Eugenol	1356	1356	4.96
β-Elemen	1386	1394	0.54
α-bergamotene	1437	1436	5.24
Germacrene D	1455	1453	1.07
α-amorphene	1483	1490	2.45
δ-Guaiene (α-bulnesene)	1507	1505	2.08
γ-Cadinene	1515	1514	2.60
T-Cadinol	1640	1640	3.24
Identified Compounds			**85.02**

– control, 0.5, 1.0, or 2.0 mL EOOB kg diet^{-1}) were added to the commercial extruded fish food (Pratigi Alimentos Company©). The EOOB was diluted with cereal alcohol 1:7.5 (EOOB: alcohol). These solutions were sprayed manually with the aid of a hand sprayer. The food was then dried at room temperature for 24 h before its use in feeding the fish, and it was kept under refrigeration (–20°C) until use, following the methodology suggested by Dairiki et al. (2013). The groups (0.0, 0.5, 1.0, and 2.0), with the same initial stocking density described above (7.56 kg m^{-3}),were fed until apparent satiety into three meals (9:00 a.m., 12:30 p.m. and 5:00 p.m.) for 48 days. This stoking density was based on previously study (Pedrosa et al. 2018), which considered6.0 kg m^{-3} as high density. Feed consumption was measured every three days, and biometry was performed every 15 days by weighing all fish in each tank.

The physicochemical variables of the water, including pH (6.31 ± 0.41) (pH meter Hanna - HI 98130), temperature (26.92 ± 1.03°C) and dissolved oxygen (7.24 ± 0.79 mgL^{-1} O_2) (oximeter Politerm-POL 60) were monitored daily and total ammonia (0.91 ± 0.72 mg L^{-1} N-NH_3), nitrite (0.66 ± 0.21 mg L^{-1} N-NO_2) and alkalinity (24.89 ± 12.75 mg L^{-1} $CaCO_3$) (kit Alfatecnoquímica, Florianópolis, SC) were monitored twice a week. In order to remove excess feces and other dirt, the tanks were cleaned by siphoning daily.

Growth performance

After 48 days, the following animal performance variables were analyzed at the end of the experiment: final weight (g), final length (cm), weight gain (WG) (g) = final body weight – initial body weight; specific growth rate (SGR) (% per day) = 100 * (ln final weight – ln initial weight)/ days of experiment; feed conversion ratio (FCR) = consumed feed/weight gain; condition factor (CF) (g cm^{-3} *100) = 100 * (final weight/ final length^3); and survival (SR) (%) = (final fish number/initial fish number)*100.

Sample collection and analysis

At the end of the experiment, the animals underwent a fast of 24 h and then blood was collected from all fish. Blood (2.0 mL per fish) was drawn from the caudal vasculature with the aid of pre-heparinized syringes (heparin sodium 5,000 I.U. mL^{-1}). The blood was centrifuged at 10000 x g for 5 min (4°C), and the plasma was carefully pipetted out. The samples were stored under refrigeration at –20°C.

The cortisol S kit (Bio Mérieux, France) was used for the determination of plasma cortisol levels (ng mL^{-1}) in the mini-VIDAS® equipment from the enzyme-linked fluorescent assay. The volume of plasma used was 200 µL. Both analyzers were cleaned, calibrated, and operated in accordance with the manufacturer's instructions. The measurement values of the Vidas cortisol S kit range from 2 to 650 ng mL^{-1}. The repeatability, inter-run reproducibility and the inter-lot reproducibility were all calculated according to Tholen et al. (2004). The observed values of total precision, dependent from serum concentration, ranged from 7.42 to 12.98 for the coefficient of variance (%). The coefficient of variation for the fish ranged from 8.68 to 13.59%, and the detection limit of the assay was 2 ng mL^{-1}.

Plasma glucose levels (mg dL^{-1}) were determined enzymatically using glucose oxidase/glucose peroxidase according to the protocol by Sena et al. (2016). Determinations of the plasma urea (mg dL^{-1}), albumin (g L^{-1}), total proteins (g L^{-1}), and uric acid (mg dL^{-1}) were performed using commercial Kits (Labtest ® kits; Vista Alegre, MG, Brazil) in a semi-automatic biochemical analyzer (Doles®, model D-250).

Statistical analysis

The results are expressed as means ± standard error of the mean (S.E.M.), with tanks as the statistical units. Levene's test was performed to evaluate the homogeneity of data variances. The data showing homogeneous variances were compared using one-way analysis of variance (ANOVA) ($p< 0.05$). The effects of EOOB on the growth performance and biochemical variables were evaluated based on linear regression. In addition, significant differences among treatments were determined by post-hoc Tukey tests ($p< 0.05$).

RESULTS

A linear effect ($p< 0.05$) was observed between the EOOB and thefinal weight (y = 1385.304 + 43.504x, R^2 = 0.75), WG (y = 437.990 + 43.883x, R^2 = 0.89), SGR (y = 0.784 + 0.069x, R^2 = 0.98), CF (y = 0.184 + 0.027x, R^2=0.94) and FCR (y = 1.940 – 0.223x, R^2 = 0.90).Therefore, according to the linear effect, pirarucus fed with 2.0 mL EOOB kg diet^{-1} increased their Final W, WG, SGR, and CF, and they decreased their FCR (Table II). In addition, the inclusion of 2.0 mL EOOB kg diet^{-1} significantly increased the WG and SGR and decreased the FCR in comparison with the control group (0.0 mL EOOB kg diet^{-1}; $p< 0.05$) (Table II). Experimental conditions did not influence survival since there was no mortality during the experimental period for either the control or treatment group.

Another linear effect ($p< 0.05$) was observed between the EOOB and the plasma albumin (y = 0.630 + 0.049x, R^2 = 0.82), total proteins (y = 3.058 + 0.179x, R^2=0.74) and urea levels (y = 7.744 – 0.713x, R^2 = 0.86). So, according to the linear effect, an increase in EOOB concentration in the diet proportionally decreased plasma urea levels and increased plasma albumin and total proteins levels in juveniles (Table III). Additionally, juveniles fed with the 2.0 mL EOOB kg diet^{-1} presented significantly higher plasma albumin than those feed with 0.0 (control group) or 1.0 mL EOOB kg diet^{-1} and total proteins than those receiving other concentrations of EOOB in the diet ($p< 0.05$). Plasma glucose, cortisol, and uric acid levels were not influenced by the addition of EOOB to the pirarucu diet (Table III).

Table II. Growth performance (mean ± SEM) of pirarucu fed with diets containing different concentrations of the essential oil of *Ocimum basilicum* (EOOB).

Variables	EOOB (mL kg diet^{-1})				*p*
	0.0	0.5	1.0	2.0	
Initial W	955.00±14.42	944.10±22.49	936.10±7.72	951.00±11.17	NS
Final W	1401.25±26.35	1406.00±59.28	1398.50±19.90	1515.00±5.07	NS
WG	446.25±23.86^{b}	461.90±38.16^{ab}	462.40±12.75^{ab}	564.00±12.94^{a}	0.037
Final L	61.00±1.13	61.80±0.88	60.70±0.67	61.60±0.68	NS
SGR	0.79±0.04^{b}	0.82±0.08^{ab}	0.84±0.02^{ab}	0.97±0.03^{a}	0.025
CF	0.19±0.01	0.19±0.02	0.21±0.01	0.24±0.01	NS
FCR	1.93±0.04^{a}	1.78±0.12^{ab}	1.81±0.08^{ab}	1.46±0.11^{b}	0.029

Initial W (initial weight), Final W (final weight) and WG (weight gain) are expressed in g. Final L (final length) is expressed in cm, SGR (specific growth rate) is expressed as % per day and, and CF (condition factor) is expressed as g cm^{-3}*100. FCR = feed conversion ratio. Different letters indicate statistical difference between treatments (Tukey's test, $p< 0.05$; n = 3 tanks per treatment). NS = No significant.

Table III. Plasma biochemical variables (mean ± SEM) of pirarucu fed with diets containing different concentrations of the essential oil of *Ocimum basilicum* (EOOB).

Variables	EOOB (mL kg diet^{-1})				*p*
	0.0	0.5	1.0	2.0	
Cortisol	42.70±3.73	53.03±4.04	46.69±3.75	45.82±3.92	NS
Glucose	46.64±1.68	52.11±2.42	46.56±3.02	52.73±2.99	NS
Albumin	0.64±0.02^{b}	0.66±0.04^{ab}	0.65±0.03^{b}	0.74±0.02^{a}	0.045
Total proteins	3.12±0.03^{b}	3.13±0.05^{b}	3.11±0.11^{b}	3.48±0.06^{a}	0.009
Urea	8.01±0.94	7.12±0.81	6.90±0.64	6.45±0.02	NS
Uric acid	0.15±0.01	0.17±0.02	0.17±0.02	0.16±0.02	NS

Cortisol is expressed in ng mL^{-1}, glucose, urea and uric acid are expressed in mg dL^{-1} and albumin and total proteins are expressed in g L^{-1}. Different letters indicate statistical difference between treatments (Tukey's test, $p< 0.05$; n = 3 tanks per treatment). NS = No significant.

DISCUSSION

The present study showed that dietary supplementation of EOOB (2.0 mL kg diet^{-1}) exerted a positive effect on growth performance (final weight, WG, SGR, CF, and FCR) in pirarucus. In our study, pirarucus' weight gain occurred within the range expected for juveniles, with initial weight above 500 g (Gandra et al. 2007, Pedrosa et al. 2018). The growth promotion effect of essential oils could be attributed to their distinctive aromatic flavor, which makes them strong appetite stimulants, that causes increased voluntary feed intake and results in improved weight gain (Abdel-Latif & Khalil 2014). According to El-Dakar et al. (2015), the olfactory feed ingredients of *O. basilicum* could enhance fish growth through their ability to act as feeding enhancers.

In addition, the mechanism attributed to the essential oil's effect has been postulated to be a digestibility enhancer which balances the intestinal microbiota (Zheng et al. 2009, Reverter et al. 2014). Its consequence could be increase food consumption and better nutrient utilization and absorption (Radhakrishnan et al. 2015), which could improve growth performance. Another model that could explain the essential oil coumponds' mode of action to promote growth could be the strong antibacterial effect. In this sense, De Souza et al. (2018) showed that EOOB showed an inhibitory effect on *Aeromonas* spp.

Previous studies reported similar results in which a diet with *O. basilicum* extract was a growth promoter for common carp (*Cyprinus carpio*) (400 mg kg^{-1}) (Amirkhani & Frouzbakhsh 2015) and gilthead sea bream (*Sparus aurata*) (20 g kg^{-1}) (El-Dakar et al. 2015). Supplementation with *O. basilicum* dried leaves improved the growth rate in hybrid tilapia (20 g kg^{-1}) (*Oreochromis niloticus* X *Oreochromis aureus*) (El-Dakar et al. 2008), and EOOB was effective at a concentration of 2.0 mL kg diet^{-1} for improving growth performance in Nile tilapia (De Souza et al. 2019b).

In the current study, linalool was the major constituent of EOOB (54.19%), and linalool (or an interaction of the different components of EOOB with linalool) may have acted as a growth promoter (De Souza et al. 2019a). Linalool is known to have antioxidant, antimicrobial, anti-inflammatory, and digestion-stimulating mechanisms of action (Moghaddam et al. 2011,

Heldwein et al. 2014, De Souza et al. 2017). Similarly, a higher growth performance was found in Nile tilapias fed with an added of 2.0 mL essential oil of *L. alba* kg diet^{-1} or 2.0 mL EOOB kg diet^{-1}; where linalool was the major compound (81.64 and 53.35%, respectively) was found in these components (De Souza et al. 2019a, b). In addition, the specific growth rate of *O. mossambicus* was also greater when fed with lipid extract from ginger (*Zingiber officinale*) (Immanuel et al. 2009). Ginger is a rich source of volatile oil, and linalool is an important constituent of its oil (Shakya 2015), as it is in EOOB. However, dietary addition of other essential oils containing linalool as the main compound did not affect silver catfish or red drum growth (Saccol et al. 2013, Sutili et al. 2016).

Accordingly, it was found that dietary addition of EOOB possibly did not change metabolism in the pirarucu, since plasma glucose and cortisol levels were not affected (Lemos et al. 2018). Plasma cortisol is a modulator of various physiological processes that rise in response to stress (Lemos et al. 2018). An elevation in plasma cortisol levels induces secondary physiological responses, such as increased plasma glucose levels (Pankhurst 2011). In addition, the values found in the present study for plasma cortisol and glucose levels are close to or slightly above baseline values found in previous studies with non-stressed pirarucus (Brandão et al. 2006, Gomes 2007). Therefore, these results allow us to state that the addition of EOOB to the pirarucu did not impair the homeostasis of the fish during 48 days of rearing in a very high stocking density.

Similar results were record in pirarucu juveniles subjected to different feeding strategies (Pedrosa et al. 2018). Linalool has anti-inflammatory and antioxidant properties (Heldwein et al. 2014), but the exact mechanism of the linalool and/or EOOB effect on plasma cortisol has not been established. Accordingly, Souza et al. (2015) found that silver catfish that were fed diets supplemented with essential oil of *L. alba* (55.25% of linalool) also did not alter plasma cortisol and glucose levels. Similarly, Nile tilapia fed a 1.0 or 2.0 mL kg diet^{-1} of essential oil of *L. alba* (81.64% of linalool) did not present with metabolic changes (De Souza et al. 2019a). However, Nile tilapia which received diets supplemented with 1.0 mL EOOB kg diet^{-1} (53.35% of linalool) had reduced plasma glucose levels and experienced benefits in growth, intestinal enzymes, lysozyme, and hematological variables (De Souza et al. 2019b).

On the other hand, the plasma total proteins and albumin levels were high in fish that were fed a diet with 2.0 mL of EOOBkg diet^{-1}. Plasma total proteins levels area result of synthesis and degradation of whole-body nitrogen. In some cases, higher plasma protein levels could indicate a better nutritional status in these fish (Higuchi et al. 2011) which promotes protein deposition. For example, high plasma total proteins concentrations were related with growth performance and protein utilization in rainbow trout (Rumsey et al. 1994). Similarly, previous studies have found an increase in total proteins levels with the use of essential oils or plant extracts in fish diets (Dugenci et al. 2003, Gulec et al. 2013, El-Dakar et al. 2015, De Souza et al. 2019b). In addition, the metabolic importance of plasma albuminis limited, and the increase of plasma albumin levels can be considered a nonspecific immune response in fish (Amirkhani & Firouzbakhsh 2015, Reverter et al. 2014). Furthermore, the values found in the present study for plasma albumin and total proteins levels are close to the baseline values found in previous studies (Tavares-Dias et al. 2007, Hoshino et al. 2017) in pirarucu.

Finally, stress-induced protein mobilization may cause an increase in the amount of ammonia

excreted by fish and consequently elevation in plasma uric acid and urea levels (Barcellos et al. 2003). However, this was not verified in the present study. Plasma urea levels decreased with an increase in the concentration of EOOB in the diet of pirarucu juveniles, and their levels remained within the values described as normal for pirarucu by Tavares-Dias et al. (2007). In addition, urea production does not reflect direct oxidative deamination of amino acids and, consequently, does not reflect increased metabolic cost (Wood et al. 2017).

CONCLUSIONS

The supplementation of 2.0 mL EOOB kg diet^{-1} is the best indicated concentration for pirarucu juveniles reared in a very stocking density of 7.56 kg m^{-3}, since it improved growth performance, guaranteed health, and did not evoke changes related to stress in the metabolic profile.

Acknowledgments

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil (CAPES) through a research grant awarded to the first author.

REFERENCES

ABDEL-LATIF HMR & KHALIL RH. 2014. Evaluation of two phytobiotics, *Spirulina platensis* and *Origanum vulgare* extract on growth, serum antioxidant activities and resistance of nile tilapia (*Oreochromis niloticus*) to pathogenic *Vibrio alginolyticus*. Int J Fish and Aquat Stud 1: 250-255.

AMIRKHANI N & FIROUZBAKHSH F. 2015. Protective effects of basil (*Ocimum basilicum*) ethanolic extract supplementation diets against experimental *Aeromonas hydrophila* infection in common carp (*Cyprinus carpio*). Aquacult Res 46: 716-724.

BABA B, ACAR Ü, ÖNTAŞ C, KESBIÇ OS & YILMAZ S. 2018. Evaluation of *Citrus limon* peels essential oil on growth performance, immune response of Mozambique tilapia *Oreochromis mossambicus* challenged with *Edwardsiella tarda*. Aquaculture 465: 13-18.

BALDISSEROTTO B, COPATTI CE, GOMES LC, CHAGAS EC, BRINN RP & ROUBACH R. 2008. Net ion fluxes in the facultative air-breather *Hoplosternum littorale* (tamoata) and the obligate air-breather *Arapaima gigas* (pirarucu) exposed to different Amazonian waters. Fish Physiol Biochem 34: 405-412.

BARCELLOS LJG ET AL. 2004. Nursery rearing of jundiá, *Rhamdia quelen* (Quoy & Gaimard) in cages: Cage type, stocking density and stress response to confinement. Aquaculture 232: 383-394.

BARCELLOS LJG ET AL. 2003. Haematological and biochemical characteristics of male jundiá (*Rhamdia quelen* Quoy & Gaimard Pimelodidae): Changes after acute stress. Aquacult Res 34: 1465-1469.

BRANDÃO FR, GOMES LC & CHAGAS EC. 2006. Respostas de estresse em pirarucu (*Arapaima gigas*) durante práticas de rotina em piscicultura. Acta Amaz 36: 349-356.

CAVERO BAS, PEREIRA-FILHO M, ROUBACH R, ITUASSÚ DR, GANDRA AL & CRESCÊNCIO R. 2003. Efeito da densidade de estocagem na homogeneidade do crescimento de juvenis de pirarucu em ambiente confinado. Pesq Agropec Bras 38: 103-107.

COPATTI CE, DOS SANTOS TA & GARCIA SFS. 2008. Densidade de estocagem e frequência alimentar de juvenis de piava *Leporinus obtusidens* Valenciennes, 1836 (Characiformes: Anostomidae). R Bras Agrociência 14: 107-111.

DAIRIKI JK, MAJOLO C, CHAGAS EC, CHAVES FCM, OLIVEIRA MR & MORAIS IS. 2013. Procedimentos para inclusão de óleos essenciais em rações para peixes. Bol Pesq Embrapa Amaz Ocid 42: 1-8.

DE ANDRADE JI, ONO EA, MENEZES GC, BRASIL EM, ROUBACH R, URBINATI EC, TAVARES-DIAS M, MARCON JL & AFFONSO EG. 2007. Influence of diets supplemented with vitamin C and E on pirarucu (*Arapaima gigas*) blood parameters. Comp Biochem Physiol A 146: 576-580.

DE OLIVEIRA EG, PINHEIRO AB, DE OLIVEIRA VQ, SILVA JÚNIOR ARM, DE MORAES MG, ROCHA IRCB, DE SOUSA RR & COSTA FHF. 2012. Effects of stocking density on the performance of juvenile pirarucu (*Arapaima gigas*) in cages. Aquaculture 370-371: 96-101.

DE SOUZA EM, DE SOUZA RC, DA COSTA MM, PINHEIRO CG, HEINZMANN BM & COPATTI CE. 2018. Chemical composition and evaluation of the antimicrobial activity of two essential oils. Bol Inst Pesca 44: e321.

DE SOUZA EM, DE SOUZA RC, MELO JFB, DA COSTA MM, SOUZA AM & COPATTI CE. 2019b. Evaluation of the effects of *Ocimum basilicum* essential oil in Nile tilapia diet: Growth, biochemical, intestinal enzymes, haematology, lysozyme and antimicrobial challenges. Aquaculture 504: 7-12.

DE SOUZA RC, DA COSTA MM, BALDISSEROTTO B, HEINZMANN BM, SCHMIDT D, CARON BO & COPATTI CE. 2017. Antimicrobial and synergistic activity of essential oils of *Aloysia triphylla* and *Lippia alba* against *Aeromonas* spp. Microb Pathog 113: 29-33.

DE SOUZA RC, DE SOUZA EM, DA COSTA MM, MELO JFB, BALDISSEROTTO B & COPATTI CE. 2019a. Dietary addition of the essential oil from *Lippia alba* to Nile tilapia and its effect after inoculation with *Aeromonas* spp. Aquacult Nutr 25: 39-45.

DUGENCI SK, ARDA N & CANDAN A. 2003. Some medicinal plants as immunostimulant for fish. J Ethnopharmacol 88: 99-106.

DRUMOND GVF, CAIXEIRO APA, TAVARES-DIAS M, MARCON JL & AFFONSO EG. 2010. Características bioquímicas e hematológicas do pirarucu *Arapaima gigas* Schinz, 1822 (Arapaimidae) de cultivo semi-intensivo na Amazônia. Acta Amaz 40: 591-596.

EL-DAKAR AY, HASSANIEN GD, GAD SS & SAKR SE. 2008. Use of dried basil leaves as a feeding attractant for hybrid tilapia, *Oreochromis niloticus* X *Oreochromis aureus*, fingerlings. Mediter Aquac J 1: 35-44.

EL-DAKAR AY, SHALABY SM, NEMETALLAH BR, SALEH NE, SAKR EM & TOUTOU MM. 2015. Possibility of using basil (*Ocimum basilicum*) supplementation in gilthead sea bream (*Sparus aurata*) diet. Egypt J Aquat Res 41: 203-210.

EUROPEAN PHARMACOPOEIA. 2007. European Pharmacopoeia, 6th ed., Strasbourg, France. European Directorate for the Quality of Medicines.

GANDRA AL, ITUASSÚ DR, PEREIRA-FILHO M, ROUBACH R, CRESCÊNCIO R & CAVEIRO BAS. 2007. Pirarucu growth under different feeding regimes. Aquac Int 15: 91-96.

GOMES LC. 2007. Physiological responses of pirarucu (*Arapaima gigas*) to acute handling stress. Acta Amaz 37: 629-634.

GULEC AK, DANABAS D, URAL M, SEKER E, ARSLAN A & SERDAR O. 2013. Effect of mixed use of thyme and fennel oils on biochemical properties and electrolytes in rainbow trout as a response to *Yersinia ruckeri* infection. Acta Vet BRNO 82: 297-302.

HELDWEIN CG, SILVA LL, GAI EZ, ROMAN C, PARODI TV, BÜRGER ME, BALDISSEROTO B, FLORES EMM & HEINZMANN BM. 2014. S-(+) -Linalool from *Lippia alba*: Sedative and anesthetic for silver catfish (*Rhamdia quelen*). Vet Anaesth Analg 41: 621-629.

HIGUCHI LH, FEIDEN A, MALUF MLF, DALLAGNOL JM, ZAMINHAN M & BOSCOLO WR. 2011. Avaliação eritrocitária e bioquímica de jundiás (*Rhamdia quelen*) submetidos à dieta com diferentes níveis proteicos e energéticos. Ci Anim Bras 12: 70-75.

HOSHINO MDFG, MARINHO RGB, PEREIRA DF, YOSHIOKA ETO, TAVARES-DIAS M, OZORIO ROA, RODRIGUEZ AFR, RIBERIO RA & FARIA FSEDV. 2017. Hematological and biochemical responses of pirarucu (*Arapaima gigas*, Arapaimidae) fed with diets containing a glucomannan product derived from yeast and algae Acta Amazon 47: 87-94.

IMMANUEL G, UMA RP, IYAPPARAJ P, CITARASU T, PETER SMP, BABU MM & PALAVESAM A. 2009. Dietary medicinal plant extracts improve growth, immune activity and survival of tilapia *Oreochromis mossambicus*. J Fish Biol 74: 1462-1475.

LEE SJ, UMANO K, SHIBAMOTO T & LEE KG. 2005. Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. Food Chem 91: 131-137.

LEMOS CH DA P, RIBEIRO CVDM, OLIVEIRA CPB DE, COUTO RD & COPATTI CE. 2018. Effects of interaction between pH and stocking density on the growth, haematological and biochemical responses of Nile tilapia juveniles. Aquaculture 495: 62-67.

LIMMA-NETTO JD, OLIVEIRA RSM & COPATTI CE. 2017. Efficiency of essential oils of *Ocimum basilicum* and *Cymbopogum flexuosus* in the sedation and anaesthesia of Nile tilapia juveniles. An Acad Bras Cienc 89: 2971-2974.

LIMMA-NETTO JD, SENA AC & COPATTI CE. 2016. Essential oils of *Ocimum basilicum* and *Cymbopogon flexuosus* in the sedation, anesthesia and recovery of tambacu (*piaractus mesopotamicus* male x *colossoma macropomum* female). Bol Inst Pesca 42: 727-733.

MOGHADDAM AMD, SHAYEGH J, MIKAILI P & SHARAF JD. 2011. Antimicrobial activity of essential oil extract of *Ocimum basilicum* L. leaves on a variety of pathogenic bacteria. J Med Plant Res 5: 3453-3456.

MUSTAFA G & USMAN KH. 2011. Phytochemical constituents and pharmacological activities of sweet basil-*Ocimum basilicum* L. (Lamiaceae). Asian J Chem 23: 3773-3782.

NIST. 2008. EPA, NIHmass spectral library and search, analysis programs. Hoboken, NJ: J Wiley & Sons.

NGUGI CC, OYOO-OKOTH E & MUCHIRI M. 2017. Effects of dietary levels of essential oil (EO) extract from bitter limon (*Citrus limon*) fruit peels on growth, biochemical, haematoimmunological parameters and disease

resistance in Juvenile *Labeo victorianus* fingerlings challenged with *Aeromonas hydrophila*. Aquac Res 48: 2253-2265.

PANKHURST NW. 2011. The endocrinology of stress in fish: An environmental perspective. Gen Comp Endocrinol 170: 265-275.

PEDROSA RU, MATTOS BO, PEREIRA DSP, RODRIGUES ML, BRAGA LGT & FORTES-SILVA R. 2018. Effects of feeding strategies on growth, biochemical parameters and waste excretion of juvenile arapaima (*Arapaima gigas*) raised in recirculating aquaculture systems (RAS). Aquaculture 500: 562-568.

RADHAKRISHNAN S, SARAVANA BHAVAN P, SEENIVASAN C, MURALISANKAR T & SHANTHI R. 2015. Effects of native medicinal herbs (*Alternanthera sessilis, Eclipta alba* and *Cissus quadrangularis*) on growth performance, digestive enzymes and biochemical constituents of the monsoon river prawn *Macrobrachium malcolmsonii*. Aquacult Nutr 21: 496-506.

REVERTER M, BONTEMPS N, LECCHINI D, BANAIGS B & SASAL P. 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. Aquaculture 433: 50-61.

RUMSEY GL, SIWICKI AK, ANDERSON DP & BOWSER PR. 1994. Effect of soybean protein on serological response, non-specific defense mechanisms, growth, and protein utilization in rainbow trout. Vet Immunol Immunopathol 41: 323-339.

SACCOL EMH ET AL. 2013. Addition of *Lippia alba* (Mill) N. E. Brown essential oil to the diet of the silver catfish: An analysis of growth, metabolic and blood parameters and the antioxidant response. Aquaculture 416-417: 244-254.

SENA AC, TEIXEIRA RR, FERREIRA EL, HEINZMANN BM, BALDISSEROTTO B, CARON BO & COPATTI CE. 2016. Essential oil from *Lippia alba* has anaesthetic activity and is effective in reducing handling and transport stress in tambacu (*Piaractus mesopotamicus* × *Colossoma macropomum*). Aquaculture 465: 374-379.

SHAKYA SR. 2015. Medicinal uses of ginger (*Zingiber officinale* Roscoe) improves growth and enhances immunity in aquaculture. Int J Fish Aquat Stud 3: 83-87.

SOUZA CF ET AL. 2015. Silver catfish submitted to a stressful condition: Effect of dietary addition of the essential oil of *Lippia alba* (Mill.) N. E. Brown on metabolism, osmoregulation and endocrinology. Neotrop Ichthyol 13: 707-714.

SUTILI FJ, VELASQUEZ A, PINHEIRO CG, HEINZMANN BM, GATLIN DM & BALDISSEROTTO B. 2016. Evaluation of *Ocimum americanum* essential oil as an additive in red drum (*Sciaenops ocellatus*) diets. Fish Shellfish Immunol 56: 155-161.

TAVARES-DIAS M, BARCELLOS JFM, MARCON JL, MENEZES GC, ONO EA & AFFONSO EG. 2007. Hematological and biochemical parameters for the pirarucu *Arapaima gigas* Schinz, 1822 (Osteoglossiformes, Arapaimatidae) in net cage culture. Electron J Ichthyol 2: 61-68.

THOLEN DW, KALLNER A, KENNEDY JW, KROUWER JS & MEIER K. 2004. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline, 2nd ed., Pennsylvania: NCCLS document EP5-A2, 39 p.

WOOD CM, GONZALEZ RJ, FERREIRA MS, BRAZ-MOTA S & VAL AL. 2017. The physiology of the Tambaqui (*Colossoma macropomum*) at pH 8.0. J Comp Physiol B 188: 393-408.

ZHENG ZL, TAN JYW, LIU HY, ZHOU XH, XIANG X & WANG KY. 2009. Evaluation of oregano essential oil (*Origanum heracleoticum* L.) on growth, antioxidant effect and resistance against *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*). Aquaculture 292: 214-218.

How to cite

CHUNG S, LEMOS CHP, TEIXEIRA DV, FORTES-SILVA R & COPATTI CE. 2020. Essential oil from *Ocimum basilicum* improves growth performance and does not alter biochemical variables related to stress in pirarucu (*Arapaima gigas*). An Acad Bras Cienc 92: e20181374. DOI 10.159/0001-3765202020181374.

Manuscript received on January 7, 2019; accepted for publication on May 7, 2019

SAMANTHA CHUNG[1]
https://orcid.org/0000-0002-7913-4639

CARLOS H. DA P. LEMOS[1]
https://orcid.org/0000-0003-2187-1131

DANIEL V. TEIXEIRA[2]
https://orcid.org/0000-0002-9809-6121

RODRIGO FORTES-SILVA[1,3]
https://orcid.org/0000-0003-0763-4500

CARLOS E. COPATTI[1]
https://orcid.org/ 0000-0002-0114-0334

[1]Programa de Pós-Graduação em Zootecnia, Universidade Federal da Bahia, Av. Adhemar de Barros, 500, Ondina, 40170-110 Salvador, BA, Brazil

[2]Universidade Federal Rural de Pernambuco, Campus Garanhuns, Av. Bom Pastor, s/n, Boa Vista, 55292-270 Garanhuns, PE, Brazil

[3]Universidade Federal de Viçosa, Departamento de Ciência Animal, Av. PH. Rolfs, s/n, Campus Universitário, 36570-900 Viçosa, MG, Brazil

Correspondence to: **Carlos E. Copatti**
E-mail: carloseduardocopatti@yahoo.com.br

Author contributions

Samantha Chung: carried out the experiments, biometric and metabolic analysis and discussion of the results. Carlos Henrique P. Lemos and Daniel V. Teixeira: collaboration on data sampling and metabolic analysis. Rodrigo F. da Silva: collaboration on statistical analysis, discussion of the results and writing. Carlos Eduardo Copatti: conception and design, statistical analysis, supervised the findings and final text.