

Original Article

Essential oil and aqueous extract of basil (*Ocimum basilicum*) in the diet of pacu (*Piaractus mesopotamicus*)

Suplementação com óleo essencial e extrato aquoso de manjeriço (*Ocimum basilicum*) na dieta do pacu (*Piaractus mesopotamicus*)

R. M. Sousa^{a*} , A. S. Ventura^b , C. A. L. Cardoso^c , C. E. Fernandes^d , R. A. C. Corrêa-Filho^a , Y. M. Martins^a , L. V. Salve^a , L. S. Roque^a , L. O. Brasileiro^a , L. N. Spica^a , F. I. Kreutz^a , G. R. Stringhetta^a  and J. A. Povh^a 

^aUniversidade Federal de Mato Grosso do Sul – UFMS, Faculdade de Medicina Veterinária e Zootecnia – FAMEZ, Campo Grande, MS, Brasil

^bUniversidade Federal da Grande Dourados – UFGD, Faculdade de Ciências Agrárias – FCA, Dourados, MS, Brasil

^cUniversidade Estadual de Mato Grosso do Sul, Centro de Estudos em Recursos Naturais, Dourados, MS, Brasil

^dUniversidade Federal de Mato Grosso do Sul – UFMS, Instituto de Biociências, Laboratório de Patologia Experimental– INBIO, Campo Grande, MS, Brasil

Abstract

This study aimed to evaluate the effects of *Ocimum basilicum* supplementation in the diet for *Piaractus mesopotamicus* regarding productive performance, intestinal morphology, muscle residue, and hematological changes after simulated transport stress. Juvenile (23 g±0.08, n = 180) were stocked into 300-L tanks in three treatments and six replicates each: a control diet (not supplemented), a diet with 0.5% essential oil of *O. basilicum* and a diet with 0.5% aqueous extract of *O. basilicum*. After 45 days, weight and length were measured, and the fish were submitted to a 4-hour simulated transport to assess the stress responses and the possible effects of the experimental diets on them. The main components of the essential oil were methyl chavicol (70.81%) and linalool (22.04%), while the aqueous extract contained tannin levels of 262.59±24 mg kg⁻¹. The retention rate of the essential oil was about 50%, while the aqueous extract retention varied between 77 and 82%. The results showed no significant effect of basil on the productive performance of the fish. Fish supplemented with the aqueous extract exhibited a lower intestinal mucosal height compared to other groups. Additionally, transported fish showed increased blood glucose concentrations even higher in fish supplemented with the aqueous extract. It was concluded that supplementation with 0.5% basil, either as essential oil or aqueous extract, did not benefit productive performance, intestinal morphology, or reduce transport stress in pacu.

Keywords: aquaculture additives, immunostimulants, Neotropical fish, plant extracts.

Resumo

Este estudo teve como objetivo avaliar os efeitos da suplementação de *Ocimum basilicum* em *Piaractus mesopotamicus* em relação ao desempenho produtivo, morfologia intestinal, resíduo muscular e alterações hematológicas após estresse de transporte simulado. Juvenis (23 g ± 0,08, n = 180) foram distribuídos em tanques de 300 L em três tratamentos e seis repetições cada: uma dieta controle, uma dieta com 0,5% de óleo essencial de *O. basilicum* e uma dieta com 0,5% de extrato aquoso de *O. basilicum*. Após 45 dias, o peso e comprimento foram medidos, e os peixes foram submetidos a um transporte simulado de 4 horas para avaliar as respostas ao estresse e os possíveis efeitos das dietas. Os principais componentes do óleo essencial foram metil chavicol (70,81%) e linalol (22,04%), enquanto o extrato aquoso continha níveis de tanino de 262,59 ± 24 mg kg⁻¹. A taxa de retenção do óleo essencial foi de cerca de 50%, enquanto a retenção do extrato aquoso variou entre 77 e 82%. Os resultados não mostraram efeito significativo do manjeriço no desempenho produtivo dos peixes. Os peixes suplementados com o extrato aquoso exibiram uma altura da mucosa intestinal menor em comparação aos outros grupos. Além disso, os peixes transportados apresentaram concentrações de glicose no sangue, ainda maiores em peixes suplementados com o extrato aquoso. Concluiu-se que a suplementação com 0,5% de manjeriço, seja como óleo essencial ou extrato aquoso, não beneficiou o desempenho produtivo, a morfologia intestinal ou reduziu o estresse de transporte em pacu.

Palavras-chave: aditivos para aquicultura, imunostimulantes, peixes neotrópicos, extratos vegetais.

*e-mail: rebecca_sousa31@hotmail.com

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1. Introduction

Aquaculture plays a fundamental role in providing source of protein to many countries under development. In 2020, 178 million tons of aquatic animals were produced, of which 49% were attributed to aquaculture (FAO, 2022). Among the various fish species, pacu (*Piaractus mesopotamicus*) is distinguished as one of the primary South American Neotropical species (Urbinati and Takahashi, 2020), noted for its easy adaptation, rapid growth, and desirable consumers acceptance. This species is native to the La Plata river basin, that corresponds parts of Paraguay, Uruguay, Argentina, and Brazil (Jomori et al., 2005).

Intensifying farming systems is necessary to increase aquaculture production, which demands attention to the health and well-being of the fish. The inclusion of antibiotics and chemotherapeutics in fish diets has been a common approach to improve immune responses and avoid the negative impacts as cronic mortalities or diseases; however, their uses can lead to bacterial resistance and decreased of fish immunity (Dawood et al., 2022).

An alternative to antibiotics and chemotherapeutics could be the use of food additives obtained from medicinal herbs and their natural plant extracts in fish diets. These additives can fulfill important roles and contribute to dietary supplementation (Chao et al., 2016), such as: (i) appetite enhancers; (ii) growth promoters (Citarasu 2010; Vaseeharan and Thaya, 2014); (iii) palatability enhancers (Aydin and Barbas, 2020); (iv) facilitators of nutrient absorption (Zeng et al., 2015); (v) stress mitigators; and (vi) prophylactics. These functions can boost immunity and prevent infections, thereby supporting the production of healthy fish (Dawood et al., 2022).

Essential oils are secondary plant constituents formed by the metabolization of active ingredients, with therapeutic properties due to the presence of monoterpenes and other volatile phenolic compounds (Nakatsu et al., 2000). Various solvents, including water and ethanol, are used to extract these active substances from plant materials (Nawaz et al., 2020; Nguyen et al., 2021). In this context, basil has been incorporated in various forms into animal diets (Mansour et al., 2023).

The use of basil (*Ocimum basilicum*) as a dietary inclusion in fish feed has shown beneficial effects in recent studies, including beneficial effects in Nile tilapia (*Oreochromis niloticus*; Souza et al., 2019) and pirarucu (*Arapaima gigas*; Chung et al., 2020), acting as antioxidants and immune protectors in Nile tilapia (Abdel-Rahman et al., 2022), and improving digestive enzyme activity and physiological responses in Nile tilapia (Mansour et al., 2023). Essential oil of basil contains volatile compounds such as linalool, methyl chavicol, eugenol, bergamotene, and methyl cinnamate as its main components (Klimánková et al., 2008; Sonmezdag et al., 2018).

Research using basil essential oil as an anesthetic for pacu has proven effective (Ventura et al., 2021a, 2024). However, no studies have yet explored basil as a food additive for this species. Therefore, this study aims to evaluate the use of basil, in the form of essential oil and aqueous extract, as a food supplement in the diet of pacu (*P. mesopotamicus*), investigating its effects on growth

performance, intestinal morphology, muscle residue, and hematological changes following simulated transport stress.

2. Material and Methods

2.1. Location and animals

The experiment was done at the Fish Farming Experimental Station of the School of Veterinary Medicine and Animal Science of the Federal University of Mato Grosso do Sul (UFMS), located in Campo Grande, MS (20°25'57" S and 55°17'11" W). Fish (n=180) with an average weight of 23.04±0.08 g and length of 10.00±0.02 cm were commercially purchased in Campo Grande, MS, Brazil. The methodology used in this study was previously approved by the Ethics Committee for the Use of Animals of the Federal University of Mato Grosso do Sul (approval no. 1.073/2019).

2.2. Experimental design

A completely randomized design was employed with three treatments: (i) control, in which the fish received feed free of any additives; (ii) essential oil, in which the fish received feed containing 0.5% *O. basilicum* essential oil (5 mL kg⁻¹ of feed); and (iii) aqueous extract, in which the fish received feed containing 0.5% *O. basilicum* aqueous extract (5 g kg⁻¹ of feed). In all three treatments, an extruded commercial feed for omnivorous fish was used (2–3 mm in size with 36% crude protein, 70% ether extract, 7.44% moisture, and 9.77% ash; Comipeixe®). Six replicates were used for each treatment.

The experimental units consisted of 300-L tanks filled with a usable volume of 280-L. Eighteen tanks (six for each treatment) were used, each containing 10 fish. The experiment was conducted using a recirculation system maintained with constant aeration. The recirculating aquaculture system (RAS) was interconnected by a filtration system comprising a 200-L decanter, a chemical/biological filter with a 200-L capacity featuring an acrylic filter mat, shade cloth, expanded clay for nitrifying bacteria, and a sump (200 L) to return water to the tanks. The system was kept aerated by a 2.04 HP blower, which incorporated oxygenation through 80-cm-long porous hoses (Aquadrop®).

2.3. Acquisition and chemical composition of *O. basilicum* essential oil

The essential oil of *O. basilicum* was commercially purchased (Phytoterápica®). For chromatographic analyses, the oil was prepared at a concentration of 100 µg mL⁻¹ in hexane. The chemical composition of the essential oil was analyzed using gas chromatography coupled to mass spectrometry (GC-MS), following the methodology of Ventura et al. (2024), with data interpretation according to Adams (2007).

2.4. Production and chemical composition of *O. basilicum* aqueous extract

Basil (*O. basilicum*) leaves were harvested from the Medicinal Plant Garden of the Federal University of Grande

Dourados - UFGD. The leaves were identified, deposited in the UFGD herbarium, and registered in SisGen, the Brazilian national genetic heritage system, under code A055721. To prepare the aqueous extract, a ratio of 5% plant mass to water volume was used. Fresh basil leaves were crushed, and the extract was prepared by placing the plant material in a closed container with distilled water at room temperature for 24 h. After extraction, the extract was filtered and frozen for subsequent lyophilization (Christ, Alpha 1-2 LD Plus). The chemical composition of the aqueous extract was analyzed by solubilizing it in ultrapure water at a concentration of 1 mg mL⁻¹ and performing spectrophotometric methods and high-performance liquid chromatography (HPLC) analyses in triplicate at the Instrumental Analysis Laboratory of the Center for Studies in Natural Resources – CERNA at the State University of Mato Grosso do Sul – UEMS.

The levels of phenolic compounds and flavonoids were analyzed using the colorimetric method described by Djeridane et al. (2006). Results were expressed as milligrams of gallic acid equivalent (GAE) per kilogram of feed for flavonoids and as rutin equivalent (RE) per kilogram of feed for phenolic compounds. Tannin content was determined by the Folin-Denis spectrophotometric method, following the methodology of Pansera et al. (2003). Results were expressed as milligrams of tannic acid equivalent (TAE) per kilogram of feed.

High-performance liquid chromatography (HPLC) analysis was conducted using a Shimadzu LC-20A Prominence liquid chromatograph (Shimadzu Co., Kyoto, Japan) and a Shim-pack XR-ODS column (2.0 × 75 mm × 2.2 µm) (Shimadzu Co., Kyoto, Japan). The gradient employed was as follows: 0 min, 3% B (acetonitrile); 8 min, 3% B; 30 min, 25% B; 60 min, 80% B; and 70 min, 3% B. The water was acidified with formic acid (0.1% v/v). The flow rate was set at 0.3 mL/min, the column temperature at 40 °C, and the injection volume at 5 µL. The chromatograph was coupled to a quadrupole time-of-flight mass spectrometer (micrOTOF-Q™, Bruker Daltonik GmbH, Bremen, Germany) with electrospray ionization (ESI) in negative mode. Spectra were acquired in the mass range *m/z* 50–1200. The adjusted ESI-MS parameters were as follows: capillary voltage 4.5 kV, drying gas temperature 200 °C, drying gas flow rate 9.0 L/min, and nebulizer pressure 4 bar. Compounds were identified by comparing spectra with the literature (Brito et al., 2014; Chen et al., 2015; Grayer et al., 2000; Peter et al., 2015; Piccinelli et al., 2008; Pikulski and Brodbelt, 2003; Safarov, 2020; Yuan et al., 2008).

2.5. Incorporation of additives into the feed

The incorporation of additives was adapted from Dairiki et al. (2013): a solution was prepared by diluting *O. basilicum* essential oil in 92.8% ethyl alcohol at a 1:10 ratio (1 mL of essential oil and 10 mL of alcohol); subsequently, 100 mL of cereal alcohol (96%) were added per 1000 g of feed. To incorporate the aqueous extract, 50 mL of 92.8% ethyl alcohol were used to dissolve the extract (5 g), followed by 100 mL of cereal alcohol (96%) for 1000 g of commercial feed. The control treatment feed also included the addition of 50 mL of 92.8% ethyl alcohol and 100 mL of

cereal alcohol (96%) for 1000 g of feed, ensuring the same incorporation process across all treatments.

The diet for each treatment was added to a plastic bag and manually shaken for 10 min. The feed was then dried at room temperature for 24 h and subsequently packaged and stored in a freezer (Dairiki et al., 2013; Menezes 2019). These procedures aimed at achieving maximum incorporation of the additives into the feed. Chemical composition analysis was performed using NIR (Near Infrared Reflectance Spectroscopy; Dardenne et al., 2000), quantifying crude protein (36.73% control; 35.91% essential oil; and 36.70% aqueous extract), moisture (7.08% control; 8.02% essential oil; and 8.28% aqueous extract), and ash (7.33% control; 7.63% essential oil; and 7.60% aqueous extract). The diets were supplied to the animals of the respective treatments twice daily (09:00 and 16:00 h) until apparent satiety (limited to 5% of the biomass in each tank) throughout the 45-day experimental period.

2.6. Analysis of the chemical composition of additive-incorporated feed samples

To analyze the feeds from the treatments, each feed was separated into hexane and aqueous extracts. The hexane extract was prepared to isolate constituents of lower polarity, whereas the aqueous extract targeted more polar constituents. Ten grams of feed were weighed and exposed to 25 mL of hexane for 30 min in an ultrasonic bath. The mixture was then filtered and dried under nitrogen to obtain the hexane extract. The sludge from this extraction was subsequently placed in contact with 25 mL of distilled water for 30 min in an ultrasonic bath. Following filtration and lyophilization, the aqueous extract was obtained. Two extracts - hexane and aqueous - were derived from each incorporated feed to comprehensively determine the composition and to assess the similarity to the essential oil and aqueous extract treatments. The extracts were analyzed using the same techniques employed for the essential oil and crude aqueous extract. Spectrophotometric methods and HPLC were used for analyzing the aqueous extract, while GC-MS was employed for the hexane extract.

For the GC-MS analyses, an analytical curve was constructed to quantify the residuals of methyl chavicol and linalool. The linearity of the method was determined by a linear regression curve, using concentrations ranging from 0.40 to 0.02 µg kg⁻¹ for methyl chavicol, and from 2.40 to 0.080 µg kg⁻¹ for linalool. The detection and quantification limits were ascertained at various concentrations through the signal-to-noise ratio.

2.7. Productive performance and water quality

Data from biometric measurements were used to calculate indices related to productive performance: Average weight (g) = The mean of individual weights of the fish in the tank; Final biomass (kg) = Final number of fish × Final average weight; Apparent feed conversion = Feed intake/Weight gain; Average weight gain (g) = Average final weight – Average initial weight; Daily weight gain (g day⁻¹) = Average weight gain/Period (days); Daily feed intake (kg) = Total weight of feed consumed daily; Specific growth rate (weight, % day⁻¹) = [(ln Final Weight – ln Initial Weight) /

Period] x 100; Fulton's condition factor (K factor = [Weight / (Total length)³] x 100); Hepatosomatic index (HSI) = [(Liver weight / Body weight) x 100]; and Survival rate (%) = (Initial number of fish / Final number of fish) x 100.

Physicochemical parameters of the water, including dissolved oxygen, temperature (measured using an Alfakit AT-160 digital oximeter), and pH (measured using a pH-1500 Intelligent Meter), were monitored daily at 08:00 h and 16:00 h. Weekly measurements included total ammonia nitrogen (TAN), toxic ammonia (NH₃), nitrite (NO₂⁻), nitrate (NO₃⁻) using colorimetric tests, and alkalinity by titration. During the 45-day experiment, water quality parameters were recorded as follows: morning temperature averaged 24.59 ± 1.63 °C and afternoon temperature 27.58 ± 1.94 °C; dissolved oxygen levels in the morning were 7.51 ± 0.92 mg L⁻¹ and in the afternoon 7.06 ± 0.82 mg L⁻¹; pH levels in the morning were 7.31 ± 0.66 and in the afternoon 7.24 ± 0.67; total ammonia nitrogen was 0.3 ± 0.11 mg L⁻¹; toxic ammonia 0.01 ± 0.01 mg L⁻¹; nitrite 0.4 ± 0.32 mg L⁻¹; nitrate 18.81 ± 9.41 mg L⁻¹; and alkalinity was 17.5 ± 6.45 mg L⁻¹.

2.8. Histological analyses

At the end of the experimental period (45 days), the fish were fasted for 24 h and subsequently anesthetized with eugenol at 50 mg L⁻¹ (Inoue and Moraes, 2007) for the collection of final biometric data on weight (g) and length (cm). Afterwards, three fish from each replicate (n=18) were euthanized via spinal cord sectioning for the harvesting of liver, intestine, and muscle tissues. The liver was analyzed to calculate the hepatosomatic index and assessed visually on a color scale ranging from 1 to 5, where a score of 1 indicates pale/yellowish and 5 denotes dark brown (Morkore et al., 2020). Intestinal tissues were processed for histological analyses, while muscle samples were preserved and frozen for subsequent analysis of residual basil content in the fillet.

Histological slides for histomorphometry of the midgut epithelium were prepared at the Experimental Pathology Laboratory, Lapex/InBio Biosciences Institute, Federal University of Mato Grosso do Sul. The fish were longitudinally sectioned ventrally to access the internal organs. Approximately 3-cm segments of the midgut were excised, fixed in 10% formalin, dehydrated through a graded alcohol series, cleared in xylene, and embedded in paraffin. Cross-sections of 5-μm thickness were produced using a microtome. Three slides, each containing three serial sections, were prepared from each intestine sample, stained with hematoxylin-eosin (HE), and examined under a light microscope. Images were captured using a Zeiss optical microscope (Optican LOPT14001®) connected to a microcomputer with ImageJ software (adapted from Nunes et al. 2020). For the histomorphometric analysis of the intestines, measurements of 15 villi per animal were taken, resulting in a total of 270 villi measured per treatment (Silva et al., 2010). Measurements included villus height (VH), villus width (VW), mucosal height (MH), and muscle width (MW¹) (Figure 1).

2.9. Residue analysis in the muscle

Residual compound analysis in the fillet was conducted at the CERN Laboratory at UEMS. Muscle samples (n =

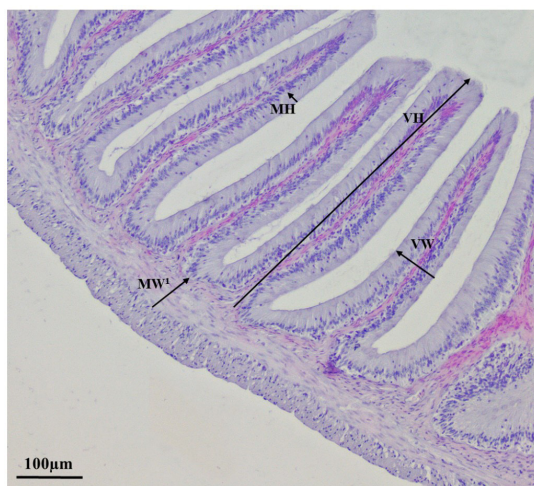


Figure 1. Histological section (hematoxylin and eosin) of the medial portion of the intestine of pacu (*Piaractus mesopotamicus*) receiving feed containing essential oil and aqueous extract of basil (*Ocimum basilicum*) for 45 days. VH: villus height; VW: villus width; MH: mucosal height; MW¹: muscle width.

18 per treatment, three per experimental unit) were thawed at a controlled temperature of 20 °C. One gram of muscle was weighed, and 3 mL of chromatographic grade hexane was added. The samples were homogenized and agitated in an ultrasonic bath (L 100 - Schuster) with a timer set for 30 min. The hexane extract was then filtered, and the residue was subjected to three consecutive extractions using the same sample volume; the hexane fractions were pooled and evaporated under an exhaust hood. The evaporated extracts were weighed and redissolved in 1 mL of hexane for GC-MS analysis. The solid residue remaining after hexane extraction was subjected to extraction using a 1:1 v/v ethanol mixture of chromatographic grade.

Subsequently, the samples were homogenized and subjected to ultrasonic agitation for 30 min. The ethanol:water fraction was filtered, and the residue was re-extracted again three times consecutively using the same sample and solvent. The combined fractions were dried under a nitrogen atmosphere to remove moisture, then redissolved in 1 mL of methanol for analysis by spectrophotometry and HPLC. All analyses were performed in triplicate using the Adams (2007) methodology.

2.10. Stress challenge – transport simulation

After 45 days of experimentation, the fish were anesthetized with eugenol (50 mg L⁻¹, Inoue and Moraes, 2007) for basal blood collection. Following this, the fish were placed in a tank with clean, aerated water until they regained their normal swimming position and ability (Woody et al., 2002). They were then transferred to bags corresponding to each treatment for the transport simulation.

The stress challenge involved a transport simulation adapted from Ferreira et al. (2022). Prior to the simulation, the fish were fasted for 24 h. A total of 126 fish (57.26±0.29 g and 13.80±0.06 cm) from the 18 experimental units (control,

essential oil, and aqueous extract of *O. basilicum*) were used. For the simulation, seven fish from each unit were placed in 18 plastic bags (70 x 90 cm), each containing 20 L of water and 2/3 pure oxygen. Each plastic bag represented an experimental unit. These bags were then placed in 1000-L tanks and subjected to four hours of constant aeration and intermittent manual agitation every 30 min to mimic transportation disturbances. Following the simulation, the respective bags were opened and the fish were anesthetized again for post-transport blood collection (post-stress).

Following the simulation, the fish were returned to their original tanks (each treatment) for a seven-day recovery period. During recovery, water quality parameters - temperature, dissolved oxygen, and pH - were monitored. The water quality over the seven days post-stress showed an average temperature of 24.52 ± 0.76 °C in the morning and 29.02 ± 1.08 °C in the afternoon; dissolved oxygen levels of 7.22 ± 1.46 mg L⁻¹ in the morning; morning pH of 6.40 ± 0.22 and afternoon pH of 6.51 ± 0.16 ; total ammonia nitrogen at 0.28 ± 0.07 mg L⁻¹; toxic ammonia (NH₃) at 0.00 ± 0.17 mg L⁻¹; nitrite at 0.44 ± 0.00 mg L⁻¹; nitrate at 24.50 ± 0.13 mg L⁻¹; and alkalinity at 18.89 ± 0.83 mg L⁻¹. After the recovery period, the fish were anesthetized once more for a final blood collection to assess hematological parameters post-recovery. No fish mortality occurred during this period.

2.11. Hematological and biochemical analyses

Blood was collected at three time points: prior to the stress challenge (baseline), immediately following the challenge (post-stress), and on day seven post-challenge (recovery). Blood samples were drawn from seven fish from each treatment, from the caudal vessel, using 1 mL syringes and needles pre-coated with 5000 IU of sodium heparin. A pooled sample from the seven fish in each experimental unit was created to ensure sufficient volume for analysis. The analyses, conducted at the Clinical Pathology Laboratory of the Federal University of Mato Grosso do Sul, included hemoglobin concentration (g dL⁻¹), packed cell volume ($\times 10^6$ µL⁻¹), total plasma protein levels, and glucose concentration (mg dL⁻¹) as described by Ranzani-Paiva et al. (2013). All analyses were performed in duplicate.

2.12. Statistical analyses

Growth performance variables were tested for normality and homogeneity of variances using the Shapiro-Wilk and Levene tests, respectively. Variables that met these underwent analysis of variance with a model with one independent variable (One-Way ANOVA), followed by the Student's t-test. For variables that did not meet these criteria, nonparametric Kruskal-Wallis and Dunn tests were applied.

Hematological, biochemical, and histological variables underwent Repeated Measures Analysis (Kaps and Lamberson, 2017) using mixed models (SAS MIXED procedure), accounting for treatment, time, and their interaction as sources of variation. The error associated with subplots was considered within each treatment replicate.

The analyses followed the protocols outlined by Kaps and Lamberson (2017), Littell et al. (2006), Littell et al. (1998), and Wolfinger and Chang (1995). The best covariance matrix structure was selected based on the -2 Res Log Likelihood (RLL), Akaike's Information Criterion (AIC), and Schwarz's Bayesian Criterion (BIC) as recommended by Kincaid (2005) and Littell et al. (2006). Lower values of these statistics indicate a better structure. The least squares mean were compared using the Tukey-Kramer test at a significance level of 0.05. All analyses were performed using the Statistical Analysis System (SAS, 2002).

3. Results

3.1. Chemical composition analysis of *O. basilicum* essential oil and aqueous extract

The chemical composition of the essential oil was characterized by major constituents: methyl chavicol (70.81%) and linalool (22.04%), with the remaining 7.15% comprising minor constituents. In the aqueous extract, the identified constituents included luteolin-4'-glucoside, apigenin 6,8-di-C-glucoside, kaempferol-3-O-glucoside, rutin, quercetin-3-O-glucoside, rosmarinic acid, cirsimaritin, and baicalein-7-O-glucoside. Additionally, the extract contained significant levels of tannins (262.59 ± 6.24 mg kg⁻¹), phenolic compounds (2542.22 ± 50.92 mg kg⁻¹), and flavonoids (918.08 ± 5.66 mg kg⁻¹).

3.2. Chemical composition analysis of feed after *O. basilicum* inclusion

Tannins were detected solely in the feed containing the aqueous extract. Both phenolic compounds and flavonoids were found in higher concentrations in this feed compared to the commercial feed and the feed supplemented with essential oil (Table 1).

3.3. Incorporation rate of *O. basilicum* into the commercial feed

The incorporation rate of the aqueous extract into the feed ranged from 77 to 82%, while for the essential oil, this rate was 50%. These values were derived by calculating the concentration of compounds in the feed samples after inclusion relative to the quantities present in the extract and crude essential oil. In the analysis of the hexane fraction compounds, only basil essential oil demonstrated incorporation of the major components: methyl chavicol (191.47 ± 0.42 mg kg⁻¹) and linalool (44.00 ± 0.06 mg kg⁻¹), as well as minor components (15.09 ± 0.67 mg kg⁻¹).

3.4. Productive performance

The weight and length (standard and total) of the fish, both at the beginning and at the end of the experiment (45 days of culture), did not differ significantly between the treatments and the control. Similarly, at the conclusion of the experiment, the pacu juveniles showed no improvements ($p > 0.05$) in weight gain, daily weight gain, or final biomass compared to fish fed with additive-free feed. The other productive performance variables also

showed no significant effects when additives were included. No fish mortality was observed during the experimental period (Table 2).

3.5. Intestinal morphology

Histological analyses performed on the medial portion of the intestine of the sampled fish (n=3) among the different groups revealed a significant result (p<0.05) for mucosal height, with the lowest value observed in the group tested with aqueous extract (16.28 µm), followed by the groups fed essential oil (19.09 µm) and control (20.44 µm) (Table 3).

3.6. Muscle analysis

Chemical analysis of the muscle tissue of fish fed for 45 days with basil revealed deposition of the major constituents of the essential oil, methyl chavicol

(9.42±2.19 mg kg⁻¹) and linalool (1.99±0.05 mg kg⁻¹). In fish fed with the aqueous extract, 0.53±0.02 mg kg⁻¹ of phenolic compounds were identified in the muscle tissue. The other compounds from the essential oil and aqueous extract were not detected in the muscle tissue of the fish.

3.7. Stress challenge – transport simulation

Following the 4-h transport simulation, no interactions (p>0.05) were observed between the evaluation times (baseline, post-stress, and recovery) and the treatments assessed (control, essential process, and aqueous extract) for biochemical and hematological variables. An increase (p<0.05) in glucose concentration was observed in fish subjected to treatment with aqueous extract, which remained elevated at all evaluated time points: baseline, post-stress, and recovery, compared to those in the control group. The other hematological variables only differed between the evaluation time points (Table 4).

Table 1. Phytochemical analysis of different treatments corresponding to *Ocimum basilicum*-enriched feeds provided to pacu (*Piaractus mesopotamicus*) juveniles over 45 days.

Treatment	Content (mg kg ⁻¹)		
	Tannins	Phenolic compounds	Flavonoids
Commercial feed	-	1.81±0.01	0.52±0.02
Feed with essential oil	-	1.96±0.02	0.58±0.02
Feed with aqueous extract	1.39±0.02	9.77±0.05	3.42±0.02

-: not detected.

Table 2. Growth performance of pacu (*Piaractus mesopotamicus*) receiving feed supplemented with basil (*Ocimum basilicum*) over 45 days.

Variable	Control	Incorporation of 0.5% <i>Ocimum basilicum</i> per kg of feed		¹ CV %	² P-value
		Essential oil (5 mL kg ⁻¹)	Powdered extract (5 g kg ⁻¹)		
Initial weight (g)	23.26	22.80	23.07	2.06	0.2688
Final weight (g)	58.48	55.49	57.81	4.11	0.1013
Initial std. length (cm)	8.72	8.66	8.70	0.56	0.1541
Final std. length (cm)	11.85	11.76	11.94	2.24	0.5669
Initial total length (cm)	10.08	9.92	9.92	3.16	0.6353
Final total length (cm)	13.87	13.88	13.96	2.29	0.8600
Average weight gain (g)	35.22	35.69	34.74	6.59	0.1526
Daily feed intake (g)	7.15	7.03	6.99	2.16	0.2114
Daily weight gain (g/day)	0.78	0.73	0.77	6.63	0.1662
Final biomass (g)	584.83	554.87	578.10	4.11	0.1013
Apparent feed conversion	0.92	0.97	0.91	5.28	0.1026
Specific growth rate (%)	0.89	0.86	0.89	4.40	0.3234
Hepatosomatic index (%)	1.20	1.26	1.36	13.41	0.3050
Fulton's condition factor	2.28	2.08	2.22	9.72	0.2483
Liver color (1-5) ³	3.78	3.72	3.78	7.04	0.9153
Survival (%)	100	100	100	-	-

¹Coefficient of Variation. ²P-value from analysis of variance using Student's t-test, considered significant at p<0.05; ³On a scale of 1 to 5, in which 1 denotes pale/yellowish and 5 represents dark brown.

Table 3. Median values (mean ranks) for histomorphometric variables of the midgut of pacu (*Piaractus mesopotamicus*) receiving feed supplemented with basil (*Ocimum basilicum*) over 45 days.

Variable (μm)	Control	Incorporation of 0.5% <i>Ocimum basilicum</i> per kg of feed		¹ P-value
		Essential oil (5 mL kg ⁻¹)	Aqueous extract (5 g kg ⁻¹)	
Villus height	345.67 (13.17)	309.43 (9.67)	272.68 (5.67)	0.0516
Villus width	81.79 (8.33)	87.05 (11.17)	85.05 (9.00)	0.6300
Mucosal height	20.44 (12.83) a	19.09 (10.83) a	16.28 (4.23) b	0.0260
Muscle width	27.72 (10.67)	26.78 (8.33)	28.12 (9.50)	0.7508

¹P-value from the Kruskal-Wallis test, considered significant at p<0.05. Mean ranks followed by different letters in the same row differ statistically according to Dunn's test (p<0.05).

Table 4. Biochemical and hematological variables post-simulated transport stress (4 h) in pacu (*Piaractus mesopotamicus*) receiving feed supplemented with basil (*Ocimum basilicum*) over 45 days.

Variable	Control	Incorporation of <i>Ocimum basilicum</i> 0.5% per kg of feed		¹ <i>p</i> -value
		Essential oil (5 mL kg ⁻¹)	Aqueous extract (5 g kg ⁻¹)	
Glucose (mg dL ⁻¹)				
Baseline	80.20 Cb	82.83 Cb	94.67 Ca	<0.0001 ^a
Post-stress	102.83 Bb	113.17 Bb	135.17 Ba	<0.0001 ^b
Recovery	124.00 Ab	131.67 Ab	145.82 Aa	0.2664 ^c
Hemoglobin (g dL ⁻¹)				
Baseline	9.22 Aa	8.87 Aa	7.78 Aa	0.7329 ^a
Post-stress	6.97 Ba	7.03 Ba	7.67 Ba	<0.0001 ^b
Recovery	6.40 Ca	5.90 Ca	6.13 Ca	0.1587 ^c
Globular volume (x 10 ⁶ μL ⁻¹)				
Baseline	40.50 Aa	41.58 Aa	40.08 Aa	0.4118 ^a
Post-stress	32.58 Ca	32.33 Ca	32.75 Ca	<0.0001 ^b
Recovery	34.67 Ba	35.50 Ba	33.83 Ba	0.3352 ^c
Total plasma protein (mg dL ⁻¹)				
Baseline	3.22 Ba	3.30 Ba	3.33 Ba	0.9382 ^a
Post-stress	3.12 Ba	3.02 Ba	3.07 Ba	<0.0001 ^b
Recovery	4.07 Aa	4.17 Aa	4.03 Aa	0.2136 ^c

¹P-value indicates the significance of differences for the sources of variation in the model: treatment^a, time^b, and their interaction^c. The best structure for the covariance matrix was determined by -2 Res Log Likelihood (RLL), Akaike's Information Criterion (AIC), and Schwarz's Bayesian Criterion (BIC), following the guidelines of Kincaid (2005) and Littell et al. (2006). Fourteen structures were evaluated. Least-square means were compared by the Tukey-Kramer test with a significance level of 0.05, in which uppercase letters indicate differences between times and treatments and lowercase letters indicate differences between treatments.

4. Discussion

Basil (*O. basilicum*) is an aromatic herb extensively used as a culinary seasoning. It is rich in natural antioxidants such as flavonoids, phenolic acids, steroids, and vitamins (A, C, E, K) (Marwat et al., 2011). The phytochemical composition of *O. basilicum* varies between the essential oil and the extract used in this study. These discrepancies are primarily due to the essential oil extraction method, the process to obtain the aqueous extract, and the cultivation conditions of the herbs (Martins et al., 2000).

Basil essential oil contains several constituents, notably methyl chavicol and linalool, which exhibit anti-

inflammatory, anthelmintic, antioxidant, and antimicrobial properties (Osei Akoto et al., 2020). Analysis of the chemical composition revealed that the essential oil used in this study predominantly contained these constituents, similar to findings by El-Dakar et al. (2008) with 14.99% methyl chavicol and 10.20% linalool; Ventura et al. (2021b) with 66.51% methyl chavicol and 20.90% linalool; and Yigit et al. (2022) with 72.57% methyl chavicol and 21.60% linalool.

The aqueous extract of *O. basilicum* comprises phenolic compounds, flavonoids, and tannins, noted for their therapeutic potential. Saied et al. (2020) confirmed the presence of these compounds in their phytochemical

screening, using an ethanolic extract of *O. basilicum* in the treatment of bovine papillomatosis. These compounds are crucial in aromatic herbs as they help reduce oxidative stress and protect the body from free radicals, thereby enhancing fish health (Chung et al., 2020).

However, the use of *O. basilicum* in the forms of essential oil or aqueous extract was not able to improve fish performance, potentially due to the low percentage of linalool. Souza et al. (2019) suggested that higher linalool concentrations in the essential oil might significantly enhance fish growth performance. This hypothesis is supported by Chung et al. (2020), who reported 54.19% linalool as the major component in their study. In contrast, in our study, linalool constituted only 22.04%, which could explain the low productive performance of fish supplemented with *O. basilicum*.

The positive effects of basil as a feed additive have been reported in several studies. For instance, El-Dakar et al. (2008) evaluated the incorporation of 0.5, 1.0, and 2.0% dried basil leaves in the diet of hybrid tilapia (13 g, *O. niloticus* x *O. aureus*) over 84 days and found a significant improvement in final weight, weight gain, and specific growth rate compared to the control diet. Chung et al. (2020) observed growth effects in juvenile pirarucu (945.40 ± 18.06 g, *Arapaima gigas*) with the use of 2.0 mL kg⁻¹ of essential oil in the diet for 48 days. For Nile tilapia (12.13 ± 0.11 g, *Oreochromis niloticus*), Souza et al. (2019) reported that 1.0 mL kg⁻¹ of essential oil in the diet for 45 days was more efficient in improving productive performance. Shahsavani et al. (2021), in a 60-day study with common carp (63.16 ± 0.72 g, *Cyprinus carpio*), observed greater weight gain (13.58 g) and specific growth rate (14% per day) in the group fed a diet with 6% basil powder. Additionally, Mansour et al. (2023) noted that Nile tilapia (40.0 ± 1.0 g) fed basil aqueous extract (200, 300, and 500 mg kg⁻¹) for eight weeks exhibited greater growth performance compared to the control group.

These studies demonstrate that the concentration and evaluation period used in the present study could have produced positive results in the productive performance of pacu, which was not observed. One hypothesis for this outcome may be the composition and/or concentration or even the interaction of the major components in *O. basilicum* in the present study. Moreover, differences in the studies analyzed, including variations in methodologies used to extract both the extract and the essential oil, and their different applications in various fish species, may pose a challenge in comparing the results related to the productive performance of these animals during the evaluations.

An indication of improved intestinal health includes an increase in the absorption area, as well as in the number, height, and width of the villi (Abdel-Latif et al., 2020). However, we observed a reduction in the height of the intestinal mucosa in the group of fish that received feed with aqueous extract compared to the other treatments. This reduction may decrease their ability to absorb nutrients present in the diet (Chung et al., 2021), potentially due to the high content of phytochemicals such as tannins, phenolic compounds, and flavonoids in the feed containing the aqueous extract.

Phenolic compounds can be classified as endogenous antinutritional factors that impair the digestibility or metabolic utilization of proteins (Chubb, 1982). Tannins, being high molecular weight phenolic compounds, when incorporated into the diet of fish, can trigger metabolic changes, hemorrhages, gastroenteritis, and can also bind with digestive enzymes, proteins, and other polymers, forming stable complexes that inhibit efficient nutrient absorption (Pinto et al., 2004). This results in an unfavorable effect on the aqueous extract. In a related study, Nguyen et al. (2021) describe how the ethanolic extract of basil, containing compounds such as flavonoids and tannins, can vary in phytochemical constituents and biological properties depending on the extraction technique used.

After incorporating additives into fish diets, it is recommended to perform residue analysis to ascertain the potential presence of substances not metabolized during the digestion process. Residue analysis involves determining whether a specific component used in fish feed persists in significant quantities in the fillet after processing. According to the guidelines of the World Health Organization (WHO 2006), commercial feeds should not contain residues of chemical compounds, and if present, these residues must be within acceptable limits for human consumption. Our results corroborate those observed by Ventura et al. (2020), who detected a residual concentration of methyl chavicol (20.52 µg kg⁻¹) in Nile tilapia fillets after two hours of transportation. Similarly, we observed that the predominant component, methyl chavicol, has a higher residual concentration in the muscle tissue of fish fed a diet containing the essential oil.

It is important to emphasize that although they are present in fish muscle, these constituents (methyl chavicol and linalool) were below the level recommended by the WHO (2006), which establishes a maximum value of 24 mg kg⁻¹ for the inclusion of basil essential oil (*O. basilicum*), classifying it as generally safe for human consumption. Additionally, Ventura et al. (2024) stated that the main constituents of *O. basilicum* essential oil are rapidly eliminated by pacu after 24 h of anesthetic recovery.

In studies involving the incorporation of plant additives into fish diets, challenging tests that have the potential to induce stress in the animals are usually conducted to evaluate the possible beneficial effects of using essential oils and plant extracts. These challenges may include exposure to bacterial agents (Amirkhani and Firouzbaksh, 2015; Souza et al., 2019), high stocking densities (Chung et al., 2020), or simulated transportation (Ferreira et al., 2022).

Following hematobiochemical evaluation, it was observed that exposure to a stressor agent via simulated transportation led to an increase in glucose levels, indicating that the fish experienced some form of stress (Fazio et al., 2015). Given that glucose is a primary energy reserve metabolite utilized in stress responses, an increase under such conditions was expected and indeed noted in this study as post-stress glucose elevation. Although it was hypothesized that the sedative effects of basil (Mahajan et al., 2013; Uritu et al., 2018) might positively influence glucose reduction after recovery from simulated transport stress, this trend was not observed in our study;

the inclusion of basil in the diet did not mitigate the stressor. Similarly, studies conducted by Ventura et al. (2020) and Chung et al. (2020) also reported no decrease in glucose levels with the use of *O. basilicum* essential oil during the transport of Nile tilapia and in the diet of pirarucu, respectively.

The glucose-increasing effect was also detected in the fish fed aqueous extract, which maintained higher levels at all evaluation times compared to the other treatments. In contrast to our study, Amirkhani and Firouzbakhsh (2015) noted that glucose levels (10.02 g) in common carp (*Cyprinus carpio*) decreased as concentrations (100, 200, 400, 800, 1600 mg kg⁻¹) of the ethanol extract of basil leaves increased in fish challenged with *Aeromonas hydrophila* after 60 days of feeding, indicating a reduction in stress effects. Elevated glucose levels resulting from the use of the aqueous extract of basil have not been reported in other studies, making comparisons challenging. Nonetheless, the substances present in the aqueous extract in our study are attributed to phenolic compounds produced by the secondary metabolism of plants; these chemical compounds can reduce blood glucose. Nonetheless, these active compounds may undergo changes depending on the extraction method, making them easily oxidized and thus variably effective in attenuating the effects related to glucose (Rumengan et al., 2019).

In our study, we observed that the hematological variables after recovery from transport simulation did not return to baseline levels. This behavior may be attributed to the successive collections performed at three different times within a short period, potentially prolonging the stress effect on the fish. Barton (2002) notes that fish can exhibit a cumulative response to repeated stress, which may compromise their physiological responses. In addition to these factors, the efficacy of plant extracts is directly related to the method of extraction and chemical composition. The percentage of these extracts relative to other constituents and/or their interactions, as well as their specific effects on various fish species, can significantly influence productive performance and animal welfare.

5. Conclusion

Basil (*Ocimum basilicum*), both as an essential oil (methylethyl chavicol/linalool chemotype) and an aqueous extract, did not demonstrate beneficial effects on productive performance, intestinal morphology, or the attenuation of simulated transport stress in pacu (*Piaractus mesopotamicus*).

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