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GROWTH AND SURVIVAL OF POST-LARVAE OF SURUVI BOCUDO (STEINDACHNERIDION SCRIPTUM) FED WITH FOOD ENRICHED WITH ESSENTIAL THYME OIL (THYMUS VULGARIS)

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Abstract

The study aims to assess the zootechnical performance of post-larvae of *Steindachneridion scriptum*, fed with feed enriched with thyme essential oil (*Thymus vulgaris*). The experiment employed a control group (T1; control – 0.0%) and feed enriched with varying concentrations of thyme essential oil: T2 (0.1%), T3 (0.5%), T4 (1.0%), T5 (2.0%), and T6 (3.0%). A total of 960 *S. scriptum* larvae were utilized in experimental units over a 15-day period. Zootechnical performance and histomorphometric analysis were carried out to evaluate the effects of thyme on larval feeding. The increase in villi surface area suggests an increased nutrient absorption capacity in *S. scriptum* larvae, improving zootechnical results at the 0.5% thyme essential oil concentration. In conclusion, the inclusion of thyme essential oil at a concentration of 0.5% proved effective in enhancing zootechnical outcomes in *S. scriptum* larvae. However, at higher concentrations, thyme essential oil inclusion demonstrated potential lethality to *S. scriptum* larvae.

Keywords **Essencial Oils, Larval Survival, Ration Enriched, Zootechnical Development.**

INTRODUCTION

The leather fish, commonly known as "Surubins," "Suruvis," or "bocudo," belongs to the Siluriformes order, within the Pimelodidae family and the *Steindachneridion* genus (PRADO et al., 2020). Its appearance features a dark gray-brown color adorned with small, irregular black spots. The species possesses upper mouth teeth and lacks intramuscular spines. With a carnivorous dietary preference, these fish inhabit the serene and deeper waters of the upper Uruguay River basin and upper Paraná River (PRADO et al., 2020; COUTO et al., 2023). The "bocudo" species, scientifically designated as *Steindachneridion scriptum* (MIRANDA RIBEIRO 1918) is characterized by engaging in reproductive migrations (BRITTO et al., 2008). However, these migrations face potential disruptions due to habitat fragmentation, primarily induced by reservoir construction (REZENDE et al., 2023). Additionally, overfishing emerges as a significant contributor to population declines (COUTO et al., 2023) prompting the classification of *S. scriptum* as "endangered" (EN) on Brazil's red list of endangered species (MMA, 2022). The convergence of these factors underscores the significance of studies focused on the captive development of *S. scriptum*. Such research is crucial not only to bolster aquaculture fish production (VALENTI et al., 2021), but also to augment native fish stocks through the controlled release of juveniles (BARROS et al., 2024).

The larval phase of fish poses one of the most significant challenges in fish farming, primarily due to the heightened fragility exhibited during larvae farm, which renders them susceptible to detrimental effects from fungi and bacteria. These factors contribute to a decrease in larval survival rates (BARROS et al., 2024). Consequently, extensive research has been conducted on the biology and cultivation of the *Steindachneridion* genus, with particular emphasis on *S. scriptum*, to enhance survival rates (MAGHELLY et al., 2014; VIANA et al., 2020). The larval stage of *S. scriptum* is particularly sensitive to fluctuations in water quality and is notably vulnerable to fungal infestations stemming from temperature variations, contrasting with the robustness displayed by individuals in the adult stage (SCHUTZ and WEINGARTNER, 2008).

Hence, the utilization of natural antimicrobials and antioxidants, such as essential oils, holds the potential to empower animals to navigate adverse conditions

in a manner consistent with nature's mechanisms (TOMAZELLI JÚNIOR et al., 2018). Essential oils offer the promise of fostering enhanced survival rates without inducing harm to the encompassing environment (TOMAZELLI JÚNIOR et al., 2018). These essential oils are derived from various plants, each possessing unique characteristics and applications. Noteworthy examples include thyme (*Thymus vulgaris* and *Thymus zygis*), cloves (*Syzygium aromaticum*), laurel (*Laurus nobilis*), and sandalwood (*Santalum album*), all of which showcase antioxidant and antimicrobial properties (FERREIRA et al., 2016). Furthermore, in the context of larval fish development, the application of essential oils, contributing to improved survival and growth during critical early life stages (COSTA et al., 2022; DAWOOD et al., 2022). Larval fish are particularly vulnerable to environmental stressors, disease, and suboptimal water quality, which can significantly impact their survival (MUGWANYA et al., 2022; CANOSA and BERTUCCI, 2023). The use of essential oils, with their antioxidant and antimicrobial properties, can mitigate these challenges by reducing oxidative stress and inhibiting pathogenic organisms (SOUZA et al., 2019). Studies have shown that the incorporation of these natural compounds into fish diets or rearing environments not only enhances immunity but also supports the overall physiological development of larvae, ultimately leading to higher survival rates and more robust individuals capable of transitioning successfully to later life stages (DAWOOD et al., 2022).

Specific foods have the capacity to induce morphological alterations within the intestine, subsequently fostering advancements in the absorption of essential nutrients. MELLO et al. (2013) investigated revealing consequential morphological transformations in the intestines of fingerlings and juveniles of Nile tilapias (*Oreochromis niloticus*) following the consumption of diets enriched with probiotics derived from *Bacillus cereus* and *Bacillus subtilis*. Noteworthy observations encompassed heightened intestinal villus height and augmented mucosal epithelium thickness, indicative of an amplified count of epithelial cells, comprising enterocytes, goblet cells, and enteroendocrine cells (MELLO et al., 2013). Drawing upon insights gleaned from previous research concerning the therapeutic attributes of essential oils, there emerges the potential proposition for their application in enhancing the zootechnical efficacy of fish larvae. This study aimed to evaluate the growth and development of *S. scriptum* post-larvae, focusing on the effects of adding thyme essential oil (*T. vulgaris*) to their diet.

MATERIALS AND METHODS

Place of study development and Origin Biological material

The experiments were conducted in Goio-En Institute (São Carlos – Santa Catarina State) following the International Guidelines for Care and Use of Laboratory Animals, and the experimental protocol received approval from the local Ethics Committee on Animal Use (CEUA- Unochapecó Number 010/2018). *Steindachneridion scriptum* larvae were obtained through laboratory-induced reproduction by the Goio-En Institute team, following the method outlined by WOYNAROVICH and HORVÁTH (1983). The selected breeding individuals were meticulously identified using alphanumeric transponders to prevent any potential crossbreeding. Initially, matrices with appropriate reproductive characteristics were chosen, weighed, and then placed in a breeding tank for a 12-hour resting period. Subsequently, they were administered two doses of crude pituitary extract from carp: females received doses of 0.5 and 5.0 mg, while males received doses of 0.3 and 3.0 mg, all administered within a 12-hour timeframe. The larvae hatched under controlled conditions at 24.5°C.

Throughout the larval development phase, a continuous system of artificial aeration was maintained, ensuring a consistent supply of oxygen to facilitate optimal growth and survival. To further support these conditions, 50% of the water volume was replaced daily. Post-larvae of the Suruvi bocudo (*S. scriptum*), each just one day old, were subsequently transferred to the designated experimental units. These individuals remained within these units for a span of 15 days, encompassing the duration of the experiment. The experimental framework employed followed a fully randomized design, encompassing six distinct treatment groups, each replicated four times. This design led to the utilization of a total of 24 experimental units. The experiment revolved around the utilization of a powdered feed boasting a 56% crude protein composition. This feed was enriched with varying concentrations of thyme essential oil, denoted as T1 (control - 0.0%), T2 (0.1%), T3 (0.5%), T4 (1.0%), T5 (2.0%), and T6 (3.0%). Over the course of 15 days, the enriched feed was consistently administered to the post-larvae three times per day. Each morning, a comprehensive set of environmental parameters were assessed. These included measurements of temperature, oxygen levels, and pH, carried out using a multi parameter probe known as the Orion start. Furthermore, concentrations of ammonia and nitrite within the

water were quantified using specific reagents. A meticulous approach was taken towards the post-larvae's nourishment. Prior to each feeding instance, the provided food was carefully weighed with the assistance of a precision scale. Simultaneously, the water within the experimental units underwent renewal in conjunction with unit cleaning procedures. These practices were guided by criteria established through a prior experiment (SCHUTZ and WEINGARTNER 2008).

Feed enrichment took place at Unochapecó using the "direct method." A solution of thyme essential oil, previously prepared, was incorporated into the feed for the post-larvae of *S. scriptum*, following specific quantitative guidelines. The process involved precise steps: the essential oil was carefully weighed to achieve the desired percentage, then dissolved in acetone. This solution was meticulously combined with the feed, creating a homogeneous mixture. Subsequently, the blend was subjected to a rotary evaporator under reduced pressure at a temperature of 40ºC until the solvent was completely evaporated. The thyme essential oil employed in this experiment, categorized as white and conforming to the Food Chemicals Codex, possessed the CAS number 8007-46-3 and was sourced from SIGMA-ALDRICH. The composition of this oil, as determined by Tomazelli et al. (2018), consisted of thymol (60.45%), p-cymene (19.58%), γ-terpinene (4.12%), carvacrol (3.08%), and β-caryophyllene (2.4%).

To evaluate the zootechnical performance, a comprehensive set of assessments was conducted. The process began with the quantification of the fish population to determine the survival rate. Following this initial step, each individual was subjected to measurement using a caliper, enabling accurate size determination. Additionally, the weights of the fish were meticulously recorded utilizing an analytical balance. These combined evaluations facilitated a thorough and all-encompassing analysis of the development and growth of the fish population under study.

Histological analysis of organs and tissues

Histological analyses were conducted by collecting two fractions of the intestines and livers from three randomly selected animals per replicate. The histological processing occurred at the Animal Pathology Laboratory within the Veterinary Hospital of UNIBAVE, located in Orleans, SC. This processing followed the methodology outlined by NUNES and CINSA (2016). After collection, the samples were immersed in Bouin's solution for a duration of 24 hours, subsequently being

preserved in 10% formaldehyde. Following this initial fixation, the samples were sectioned into segments measuring 0.3 cm and then subjected to a clearing process. Subsequently, the sections were embedded in paraffin, facilitating the subsequent sectioning of 3 µm slices utilizing a semi-automatic microtome. This sectioning process adhered to the technology methodology adapted from MELLO et al. (2013). The obtained sections were then stained using the hematoxylin and eosin (HE) method (PROPHER et al., 1995). This staining approach was employed to measure the dimensions of the intestinal villi and the average area of hepatocytes.

In the case of goblet cell enumeration, slides were stained using the periodic acid-Schiff (PAS) technique (BEHMER et al., 1976). Upon completing these staining procedures, the samples were digitized using a microscope (Bioval / L2000[a]) equipped with an attached camera. This digitization process was facilitated by the "TCapture" software (Laborana®). A total of six villi per field and per animal (slides stained with HE at 10x magnification) were selected for measurement based on predefined integrity criteria, including: a) the presence of intact epithelial cells lining the villi; b) uniform villus architecture without signs of damage or disruption (such as detachment from the lamina propria); c) a well-defined and continuous basement membrane; and d) absence of inflammatory infiltration in the surrounding tissue. These measurements encompassed various aspects of the villi morphology, including a) villus height (ASE); b) total villus height (ATV); c) villus width (LT); and d) villus width (LEV). These measurements were conducted in accordance with the adapted methodology from MELLO et al. (2013).

Statistical analysis

To assess the zootechnical and histological performance of *S. scriptum* larvae, an analysis of variance (ANOVA-One Way) was employed. The Shapiro-Wilk test was used to assess data normality, while Levene's test evaluated homoscedasticity, confirming that the assumptions for parametric analysis were satisfied. In the case of zootechnical performance results, prior logarithmic transformation was applied using the natural base. Subsequently, upon identifying statistical differences, the means underwent further examination via the Fisher Least Significant Difference (LSD) test at a significance level of 5%. This statistical approach allowed for a robust assessment of the observed data, aiding in the identification of meaningful differences among the

studied variables.

RESULTS

The water limnological data collected from the experimental units revealed no significant differences among the treatments ($p > 0.05$). Throughout the study period, the temperature averaged 22.65°C (± 0.20°C), with a range from 22.58°C to 22.71°C. The oxygen concentration showed an average of 7.70 mg/L (± 0.045 mg/L), with a range from 7.65 mg/L to 7.75 mg/L. The pH values averaged 7.31 (± 0.115), with a range from 7.26 to 7.35. For water quality indicators, ammonia levels had an average of 0.27 mg/L (± 0.05 mg/L; range from 0.24 mg/L to 0.30 mg/L), while nitrite concentrations averaged 0.195 mg/L (± 0.03 mg/L; range from 0.18 mg/L to 0.21 mg/L). The overall stability of these limnological parameters suggests that the experimental conditions were well-maintained and conducive for the well-being of the study species.

The concentration of 0.5% thyme essential oil exhibited notable efficacy in promoting the survival of *S. scriptum*, with markedly high survival rates observed. These rates were statistically distinct from the outcomes observed in the treatments involving 2.0% and 3.0% concentrations of thyme essential oil (as shown in Table 1). Regarding survival rates, the 0.5% concentration yielded the most favorable results, although these results did not display significant differences in comparison to treatments with 1.0%, 0.1%, or the control. Conversely, concentrations exceeding 1.0% of thyme essential oil exhibited potential toxicity, leading to a reduction in survival rates. In terms of weight gain, the treatment involving 0.5% thyme essential oil demonstrated the highest increase when compared to the other treatments ($p < 0.05$). This finding underscores the positive impact of the 0.5% concentration on the growth

Table 1- The survival, weight, and size of *S. scriptum* post-larvae, nourished with varying concentrations of thyme essential oil, are presented alongside their corresponding standard deviations (±) in the following table. Values sharing the same letters within a column are statistically indistinguishable. The data underwent analysis of variance, followed by the Fisher Least Significant Difference (LSD) test with a significance level of 5%.

Concentration	Survival	Weight (mg)	Size (mm)
T1 (0.0%)	14.50±13.38 ab	0.30±0.27 a	13.93±1.99 a
T2 (0.1%)	17.75±10.28 ab	1.57±0.69 ab	13.22±2.05 a
T3 (0.5%)	25.00±40.08 a	81.22±23.64 c	17.30±0.62 a
T4 (1.0%)	14.75±10.31 ab	2.78±1.37 b	15.50±3.18 a
T5 (2.0%)	7.25±8.77 b	1.30±0.38 ab	15.27±5.78 a
T6 (3.0%)	9.50±7.05 b	2.51±2.04 b	15.38±5.35 a

and development of *S. scriptum* individuals, showcasing its potential as an effective approach for enhancing weight gain in this species.

The results of the histomorphometry analysis of *S. scriptum* post-larvae's intestinal villi are summarized in Table 2. This includes measurements of total villus height (TVH), villus height (VH), total villus width (TVW), and width of the villus epithelium (WVE). These findings provide valuable insights into the structural characteristics of the intestinal villi and their variations in response to different experimental conditions.

Table 2- Values ± standard deviation of histomorphometric data of intestinal villi of *S. scriptum* post-larvae among the control (T1) and different thyme essential oil concentrations (T2 to T6). Values followed by same letters in column do not differ. TVH= Total villus height, VH= Villus height, TVW= Total villus width, WVE= Width of the villus epithelium. Data submitted to analysis of variance followed by the Fisher LSD at 5% probability.

Treatment	TVH (µm)	VH (µm)	TVW (µm)	WVE (µm)
T1 (0.0%)	217.13±24.33 a	194.40±248 a	64.98±19.31 b	33.74±8.79 b
T2 (0.1%)	190.17±28.73 a	170.89±28.09 a	72.38±3.81 b	36.68±1.30 ab
T3 (0.5%)	179.04±43.27 a	160.12±40.48 a	91.72±18.62 a	44.64±9.01 a
T4 (1.0%)	167.07±36.75 a	148.56±29.18 a	60.67±13.19 b	30.83±6.11 b
T5 (2.0%)	154.35±18.11 a	140.12±19.90 a	66.23±9.00 b	33.29±4.64 b
T6 (3.0%)	212.91±64.33 a	186.16±55.78 a	72.42±5.54 b	34.96±3.17 b

The concentration of 0.5% exhibited statistical significance in relation to the total villus width (TVW) ($p < 0.05$). Furthermore, the width of the villus epithelium (WVE) at the 0.5% concentration also yielded a favorable outcome, although it did not demonstrate a significant difference when compared to the treatment involving a concentration of 0.1%. Conversely, both the height from the base of the villus to the tip (TVH) and the villus height excluding the external portion (VH) did not exhibit any significant differences across the various treatments (as detailed in Table 2). These findings collectively contribute to a comprehensive understanding of how different thyme essential oil concentrations impact the morphological aspects of intestinal villi in *S. scriptum* post-larvae.

The provided images in Figure 1 depict the outcomes of a histological analysis conducted on intestinal villi. These images offer insight into the observed changes specifically within the villi of treatment three (T3), where a 0.5% concentration of essential thyme oil was applied. The black line in image = TVH - total height of the

villus; Red line = VH - villus height without epithelium; Green line = TVW - villus width; Blue line = WVE - width of the villus epithelium. The arrows show the different types of villi, the black line showing the total height of the villus, the red line showing the height of the villus without the epithelium, the blue line showing the total width of the villus and the green line showing the width of the lateral epithelium of the villus. To measure the height, width and thickness of the intestinal villi of the *S. scriptum* fry, the histomorphometry method and the unit of measurement in micrometers (µm) were used.

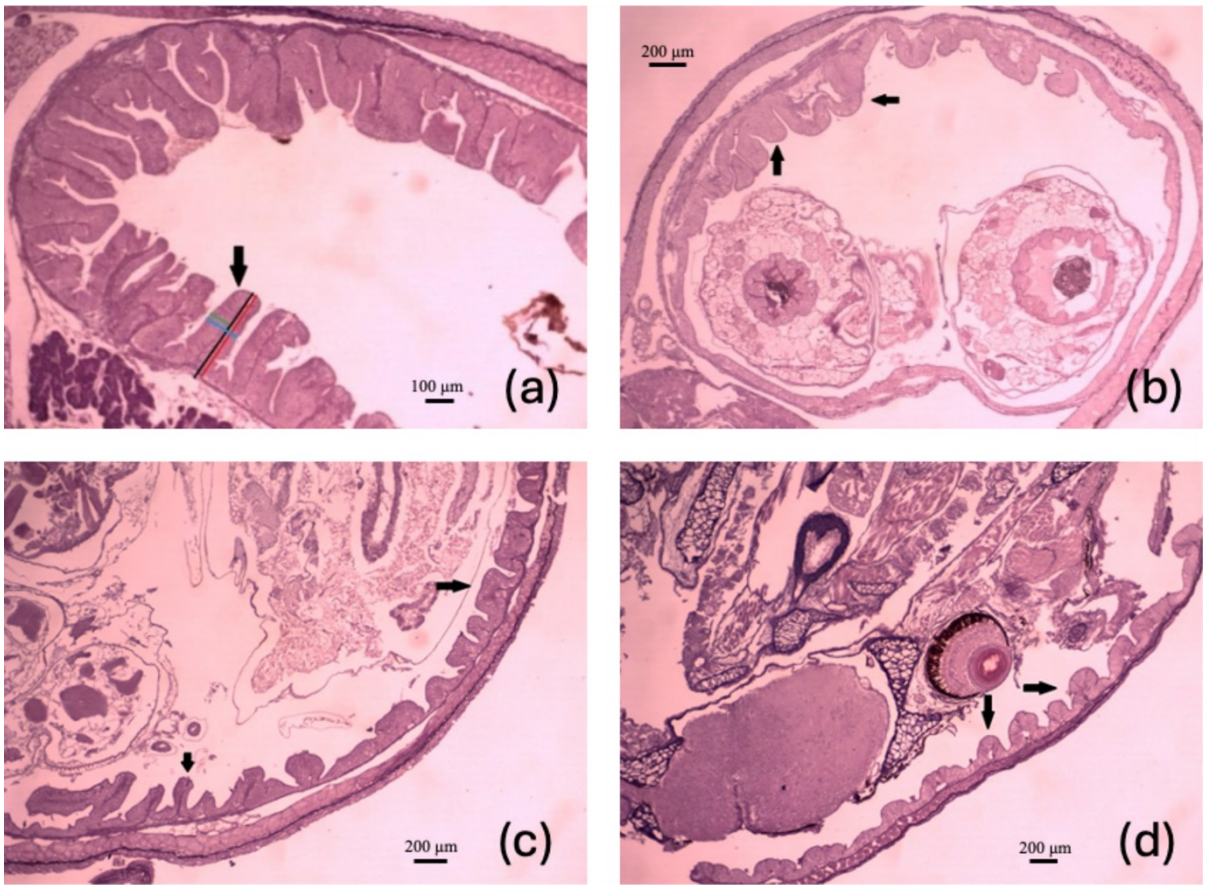

Figure 1- Images of the intestinal villi of *Steindachneridion scriptum* post-larvae. Black line = ATV - total height of the villus; Red line = ASE - villus height without epithelium; Green line = LT - villus width; Blue line = LEV - width of the villus epithelium. Arrows point to intestinal villi of *S. scriptum* in 0.5% treatment (T3). The arrows show the different types of villi, the black line showing the total height of the villus, the red line showing the height of the villus without the epithelium, the blue line showing the total width of the villus and the green line showing the width of the lateral epithelium of the villus.

DISCUSSION

The findings of this study highlight the significant effects of thyme essential oil concentrations on the survival and growth of *S. scriptum* larvae. Specifically, the

0.5% concentration increased the survival rates of *S. scriptum* larvae. This result may be attributed to the fact that lower doses can act as immune system stimulants, enhancing the resilience of fish during the larval stage (JASIM et al., 2023). Another mechanism that explains this result is the enhanced digestive capacity of fish larvae due to the addition of thyme oil to their diet (GHAFARIFARSANI et al., 2022). The bioactive components of thyme oil, such as phenolics, may possess antioxidant and anti-inflammatory properties, which, at moderate concentrations, optimize metabolism and digestion, leading to improved zootechnical performance (ZARGAR et al., 2019; GHAFARIFARSANI et al., 2022). Conversely, higher concentrations may result in increased toxicity, negatively impacting the health, growth, and survival of fish larvae (DA SILVA Jr. et al., 2023). This may occur because bioactive compounds at elevated levels can induce oxidative stress or interfere with physiological processes such as nutrient absorption (FIRMINO et al., 2021). Thus, we can infer that lower concentrations of thyme essential oil may provide a more favorable balance, promoting larval growth and survival while minimizing potential toxicity associated with higher doses.

The thyme essential oil employed in this experiment exhibits a notable concentration of monoterpenes, with the phenolic compound thymol serving as the principal constituent (60.45%). Alongside thymol, other significant components can be discerned, including p-cymene (19.58%), γ-terpinene (4.12%), carvacrol (3.08%), and β-caryophyllene (2.4%) (TOMAZELLI JÚNIOR et al., 2018). Thymol (2-isopropyl-5-methylphenol) is the predominant element within thyme essential oil. It holds prominence as a phenolic compound widely employed as an antiseptic within medical applications (BELATO, 2014). In this context, thymol demonstrates robust fungicidal, bactericidal, and antioxidant properties (MARCHESE et al., 2016; HOU et al., 2022). Thus, we suggest that the presence of thymol in thyme oil likely played a key role in improving the growth performance of *S. scriptum* larvae.

The efficiency of 0.5% thyme oil on survival improvment and weight gain is possibly also related to intestinal development. The intestine is the primary site for food digestion and nutrient absorption, where the most important physiological digestive functions occur, enabling nutrient uptake (WANG et al., 2021). The higher TVW and WVE values recorded at the 0.5% thyme oil concentration likely explain the enhanced growth performance of *S. scriptum* larvae at this concentration. Intestinal

nutrient absorption relies on the presence of villi, and larger villi imply an expanded surface area for more efficient nutrient absorption (WALTON et al., 2016). In fish, the secretory cells within the intestine possess extensive folds along their walls (BJØRGEN et al., 2020). Nevertheless, these secretory cells migrate to the crests of these folds to release their secretions. The cells that form the lining of the intestinal walls are equipped with minute folds on their apical membrane, known as microvilli (MINGHETTI et al., 2017). These microvilli serve to establish continuous contact with the intestinal lumen, thereby enhancing the surface area for nutrient absorption (BJØRGEN et al., 2020). The length of these microvilli is also subject to modification based on the fish's nutritional condition, decreasing during prolonged periods of fasting (WANG et al., 2021). Therefore, an increase in villi length directly translates to a greater surface area available for enhanced nutrient uptake (FERREIRA et al., 2014). Thus, we can infer that dietary supplementation with thyme oil appears to be a viable strategy for enhancing larval development by promoting intestinal maturation and functionality, ultimately leading to improved overall performance.

The present study reinforces the efficiency of thyme essential oil in improving the fish zootechnical performance, particularly during the larval stage. The use of moderate concentrations of thyme oil supports findings in the literature that various essential oils can enhance growth and survival rates in fish, especially in larvae. A similar pattern was observed in a study with carp, where a 0.5% concentration of essential oil led to higher growth rates and improved feed conversion efficiency. However, higher concentrations negatively impacted these parameters (FRECCIA et al., 2014). Additionally, the link between improved zootechnical performance and intestinal development was also observed following the addition of thyme essential oil to diets of tilapia and lambari. These additives created a more homeostatic environment for the fish, contributing to better survival rates (FERREIRA et al., 2014; PICOLI et al., 2019). Similarly, in rainbow trout, diets enriched with thyme essential oil led to better growth and survival rates, further substantiating the potential of thyme oil in fish feed (CIHANGIR AND DILER, 2016). Based on the results observed in *S. scriptum* larvae and consistent with patterns described in the literature, it is clear that the moderate incorporation of thyme essential oil into fish diets is a promising strategy for improving zootechnical performance across various fish species.

Finally, the incorporation of other dietary additives may yield similar results,

particularly through positive effects on hepato-intestinal histomorphology. For instance, PICOLI et al. (2019) reported similar improvements in villus development after incorporating bee pollen (up to 2.5%) into commercial Nile tilapia diets. This approach increased villus height, goblet cell abundance, and nutrient storage in liver cells without negatively affecting zootechnical performance. Another noteworthy example involves Nile tilapia (*Oreochromis niloticus*), where the inclusion of 50 ppm of a compound containing essential oils during the reproductive season improved hepatosomatic function, providing valuable insights into the physiological and reproductive health of the fish.

CONCLUSIONS

The results reported in this work indicate inclusion efficiency of thyme essential oil in larvae feeding of *Steindachneridion scriptum*. For concentrations up to 0.5%, thyme essential oil showed to improve larvae weight gain without affecting its survival. It can also be concluded that dosages lower 0.5% were not significant for survival while doses greater than 1.0% demonstrate higher larvae mortality in comparison to 0.5% treatment of thyme essential oil, possibly due to the toxicity of the essential oil. Furthermore, it was experimentally observed that the thyme essential oil did not affect the water quality. Histological tests demonstrated increased intestinal development, providing better nutrients absorption together with weight gain of *S. scriptum*, for doses of 0.5% of thyme essential oil in the initial feeding of post-larvae.

Conflict of interest

The authors declare no conflict of interest.

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Authors contributions

All authors wrote, edited, commented on and approved the manuscript.

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