

**Óleo de pimenta rosa *Schinus terebinthifolius* raddi usado como aditivo em dietas de
Hyphessobrycon eques, Steindachner**

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steindachner fish diet**

**Aceite de pimenta rosa *Schinus terebinthifolius* raddi utilizado como aditivo em dietas de
Hyphessobrycon eques steindachner**

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Resumo

O óleo de pimenta possui propriedades antioxidantes e antifúngicas que podem beneficiar a saúde e o bem-estar dos peixes. O objetivo deste estudo foi avaliar os benefícios da inclusão do óleo de pimenta no desenvolvimento e coloração de peixes do Mato Grosso (*Hyphessobrycon eques* Steindachner, 1882). Um total de 68 espécimes de mato foi distribuído em quatro unidades de 10L em um sistema de recirculação individual e água termostaticada a 28° C. Durante o teste de 60 dias, os peixes foram alimentados com dieta comercial (CD) e dieta comercial suplementada com pimenta do Brasil essencial óleo (5g kg⁻¹) (DP). Ao final do período experimental, foram avaliadas a sobrevivência, crescimento, coloração e termogenicidade. Os dados foram analisados pelo teste de Tukey (P>0,05). A adição de óleo de pimenta brasileiro promoveu maior sobrevivência dos peixes (94,11%) e melhor fator de condição (1,87). Não houve diferença no consumo de dieta dos peixes. No entanto, os fatores de crescimento foram menores em comparação ao grupo controle, exceto pelo ganho de comprimento. O grupo controle apresentou maior peso homogêneo, enquanto a melhor uniformidade de tamanho foi obtida para o grupo DP. Em destaque, o DP proporcionou um aumento na termogenicidade dos peixes e também aumentou a coloração nos índices L^* e a^* . Conclui-se que essa dose de óleo de pimenta rosa não é recomendada como complemento para otimizar a coloração em peixes ornamentais, no entanto, a substância é mostrada como um bioativo promissor para o desenvolvimento do sistema imunológico desses peixes.

Palavras-chave: Peixes ornamentais; Pigmentação; *Serpae tetra*.

Abstract

Pepper fruit oil has antioxidant and antifungal properties that can benefit the health and well-being of fish. The objective of this study was to evaluate the benefits of the inclusion of brazillian pepper crude oil in the development and staining of Mato Grosso fish (*Hyphessobrycon eques* Steindachner, 1882). A total of 68 specimens of bush were distributed in four 10 L units in an individual recirculation system and thermostat water at 28° C. During the 60-day test, the fish were fed commercial diet (DC) and commercial diet supplemented with brazillian pepper essential oil (5g.kg⁻¹) (DP). At the end of the experimental period, survival, growth, staining and thermogenicity were evaluated. Data were analyzed by the Tukey test (P>0.05). The addition of brazillian pepper oil promoted higher fish survival (94.11%) and better condition factor (1.87). There was no difference in diet consumption by fish. However, the growth factors were smaller compared to the control group, except for the gain in length. The control group presented more homogenous weight, while the best size uniformity was obtained for the DP group. In highlight the DP provided an increase in fish thermogenicity and also

increased staining in the L^* and a^* indices. It is concluded that this dose of pink pepper oil is not recommended as a complement to optimize color in ornamental fish, however, the substance is shown as a promising bioactive for the development of the immune system of these fish.

Keywords: Ornamental fish; Pigmentation; *Serpae tetra*.

Resumen

El aceite de pimienta tiene propiedades antioxidantes y antifúngicas que pueden beneficiar la salud y el bienestar de los peces. El objetivo de este estudio fue evaluar los beneficios de incluir el aceite de pimienta en el desarrollo y la coloración de peces en Mato Grosso (*Hyphessobrycon eques* Steindachner, 1882). Se distribuyeron un total de 68 especímenes de arbustos en cuatro unidades de 10 litros en un sistema de recirculación individual y agua termostatazada a 28° C. Durante la prueba de 60 días, los peces fueron alimentados con una dieta comercial (CD) y una dieta comercial complementada con Aceite esencial de pimienta brasileña (5g kg⁻¹) (DP). Al final del período experimental, se evaluaron la supervivencia, el crecimiento, el color y la termogenicidad. Los datos se analizaron utilizando la prueba de Tukey (P>0.05). La adición de aceite de pimienta brasileña promovió una mayor supervivencia de los peces (94.11%) y un mejor factor de condición (1.87). No hubo diferencia en la ingesta de dieta de pescado. Sin embargo, los factores de crecimiento fueron más bajos en comparación con el grupo control, excepto por la ganancia de longitud. El grupo control tuvo un mayor peso homogéneo, mientras que la mejor uniformidad de tamaño se obtuvo para el grupo DP. Destacado, el DP proporcionó un aumento en la termogenicidad de los peces y también aumentó el color en los índices L^* y a^* . Se concluye que esta dosis de aceite de pimienta rosa no se recomienda como complemento para optimizar el color en los peces ornamentales, sin embargo, la sustancia se muestra como un bioactivo prometedor para el desarrollo del sistema inmune de estos peces.

Palabras clave: Peces ornamentales; Pigmentación; *Serpae tetra*.

1. Introduction

The Pink pepper or Brazilian pepper it's a Brazilian native fruit from the *Schinus terebinthifolius* Raddi tree from Anacardiaceae R. Br family, also known as “Aroeira-vermelha”, these specific fruits occur in Alagoas, Bahia, Espírito Santo, Mato Grosso do Sul, Minas Gerais, Pernambuco, Paraná, Rio de Janeiro, Rio Grande do Norte, Rio Grande do Sul, Santa Catarina, São Paulo, and Sergipe states. Another common name includes Brazilian peppertree, rose pepper, Christmas-berry tree and Florida holly, they are natively used as a food seasoning because of your soft pepper taste and some native knowledges of which it has medicinal properties.

The oil extracted from the leaves, flowers and fruits has antioxidant, wound healing, antitumor and antimicrobial activities and revealed α -pinene as the major component, Other major constituents were α -phellandrene, elixene, germacrene D, limonene and p-cymene (Barbosa et al., 2007), these components can't be synthesized by vertebrates, however can be found in photosynthetic organisms (Sefc et al., 2014).

So, thinking in a possible additive to stimulate coloration, possibly healthiness and well-being to ornamental fishes the Pink pepper oil was chosen for a test in one most common and high appreciated ornamental fish species, the *Serpaes-tetra* or natively known "Mato Grosso fish" in Brazil, from Characidae family. One of the most popular hobby's is to keep ornamental fishes in an aquarium, people who wants to has an entire saltwater or freshwater aquatic ecosystem in a glass tank and keep beautiful fishes and another aquatic organisms, like plants for example, for that the fishes needs to be healthy and colorful but to provide this the fishes needs an excellent water and food quality, the food manufacturers produces a specialized diet for a various species of ornamental fishes, and for that they use additives in theirs formula to maximize fish coloration and also increase the fish development (Sampaio et al., 2001), so evaluating the positive healthy properties of pink pepper they were tested as a possibly potential food additive to a specific ornamental fish.

The objective from this study is evaluate the possible benefits to the inclusion of pink pepper oil in commercial fish diet and measures the gains in coloration and fish development with the *Hyphessobrycon eques*.

2. Materials and Methods

The experiment was conducted at the aquaculture laboratory of the Federal University of Grande Dourados (UFGD). Sixty-eight specimens of *H. eques* fish with initial mean weight of 0.39 ± 0.01 grams (g) and average length of 3.17 ± 0.01 cm was used, the fish underwent a period of acclimatization of 7 days before beginning the experiment. For the tests an individual filtration system was used. The water temperature was $28.73 \pm 0.53^\circ$ C maintained by thermostat. pH remained at 7.22 ± 0.14 , and dissolved oxygen at 6.05 ± 0.26 mg L⁻¹, measured with YSI Model 6920 V2 multi-parameter probe (YSI Inc., Yellow Springs, OH, USA). Ammonia - at 0.10 ± 0.16 mg L⁻¹ - was measured by the colorimetric method (Alfakit, Florianópolis, SC, Brazil).

For the feeding trial of 60 days the fishes were stored at a density of 1.7 fish per L, using a two-treatment design consisting of a control diet (DC) and 5g.kg⁻¹ of pink pepper oil

(DP). The method of extracting the oil is by Clevenger (hydrodistillation technique). For the extraction of the essential oil, the hydrodistillation method was used, where 200 g of dehydrated pink pepper were introduced in a 2000 ml volumetric flask and 1400 ml of distilled water were added. The flask was attached to the Clevenger apparatus under the heating mantle. Five replicates were performed with a 2 h extraction cycle. At the end of each process, the obtained oil was collected, stored in a sealed glass bottle and labeled, stored in a freezer at a low temperature.

Control diet presented the levels of 35% CP (Crude Protein), 6% LIP (Lipids) and 3800 kcal.kg⁻¹ with two replicates. The animals were fed twice times a day until apparent satiety. The cleaning of the experimental units was performed daily. At the end of the experimental phase the fish were weighed and measured and survival was evaluated by direct counting of the individuals.

The specific growth rate (TCE) was calculated, based on the formula $TCE=100 (\ln Pf - \ln Pi) \Delta t^{-1}$, where: Pi is the initial mass; Pf is the final mass; and Δt is the duration of days between the samplings. All procedures were approved by the Ethics Committee on the Use of Animals, from the University of Grande Dourados (CEUA/Unigran 004/2014). The coloration was evaluated using the method that, according to Leão (2005), is a CIE $L^* a^* b^*$ color model created by the International Lighting Commission to increase the uniformity of colors perceived by the human visual system. L^* represents the brightness value of the color, while a^* can vary from green to red and b^* from yellow to blue, also the thermogenicity was evaluated too.

The condition factor (K) was calculated by the allometric method, from the expression $K = W/L^b$, which W represents the weight and L the standard length of the individuals. To estimate the value of the coefficient b, a single weight/length ratio ($W=aL^b$) equation was set from the set of all individuals collected, according to the methodology proposed by Vazzoler (1996). To evaluate the uniformity of weight, an adaptation of the equation was proposed by (Furuya et al., 1998): $U=(N/N1) \times 100$, where: U=uniformity (%); N=number of animals in the tank; N1=total number of animals with weight or length 20% higher or lower than the average live weight in each experimental unit.

At the end of the experimental period data such as survival and growth were analyzed by Tukey tests. ($p > 0.05$).

3. Results and Discussion

The utilization of bioactive in the aquatic organism's food have been reported as a growth promoter (Safari et al., 2016; Wang et al., 2017) consumption stimulator (Citarasu, 2010) and metabolism (Wang et al., 2017).

The condition factor of the fishes fed with supplemented diet was lower than the observed in common diet (Table 1). That results are correlated with animal wellbeing, proportionated by bioactive presents in the supplement.

Table 1. Growth performance of fish *H. eques* fed with control diet and diet with crude oil of *S. terebinthifolius*.

Parameters	Commercial Diet	Diet plus pepper oil
Wheigth (g)	0,12±0,03	0,07±0,04
Size (cm)	1,42±0,06b	1,70±0,92a
Feed conversion rate (%)	1,84±0,18	1,53±0,46
Growth specific rate (%)	0,80±0,21	0,50±0,32
Protein specific rate (%)	0,009±0,001	0,005±0,002
Survival (%)	88,24±8,32	94,11±6,47
Thermogenicity	30,22±0,81	32,4±0,16
Condition factor	0,4b	1,87a

Statistic differences represented by different letters by Tukey ($p>0,05$). Source: Own Study.

The results demonstrate that the pepper oil influenced the length of the fishes, presenting greater value in the commercial diet. It was also observed that the condition factor was higher when the pink pepper oil was included in the fish diet.

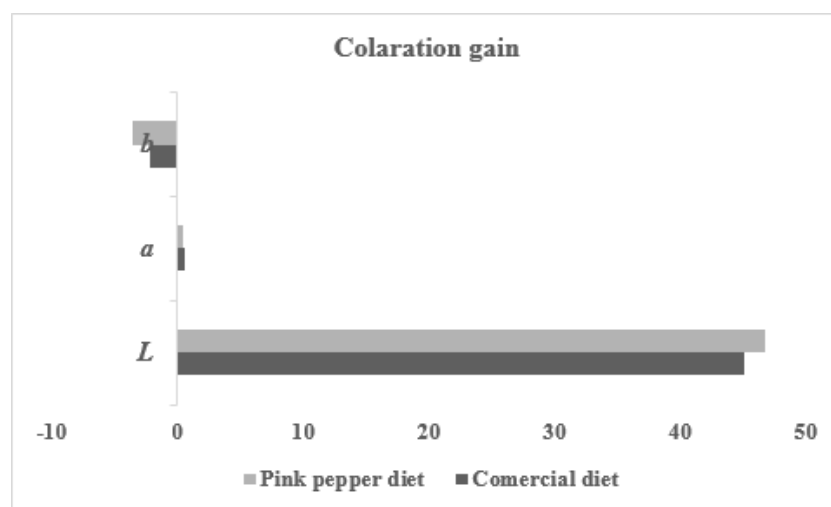
The relation weight/length is used to estimate the general state, both for fish in their habitat and in captivity (Braga, 1997), healthy (Tavares-Dias et al., 2000). The condition factor is also used to estimate feeding conditions, density, climate, degree of food activity (Weatherley and Gill, 1987). The use of the condition factor as an important index to determine the best body shape, it was tested for Nile tilapia (*Oreochromis niloticus*) submitted to microencapsulated diets, demonstrating the best welfare of the fish submitted to a certain food condition (Honorato et al., 2012).

This result can be observed by the weight/length rate, the lower condition factor in the Pink pepper oil supplemented diet than the common commercial diet, confirms the worse nutrients management in metabolism, some researches relates some bio substances can positively influence the animal development. (Güroy et al., 2012).

The Phytoterapy has been tested to aquatic organisms with the intuit to promote improvements in the zootechnic indexes and animal welfare, in that context some studies were observed like Passion fruit extract for Nile tilapia (De Oliveira et al., 2010), Herbal extract to *Lateolabrax japonicus* (Wang et al., 2017), Cinnamon extract to *Pyrrhulina brevis* larvae (Abe et al., 2016).

The thermogenicity gains was not observed, but survival of the DP group was higher than the DC, which shows an ability to promote improvements on fish immune system. The gains in coloration was minimal like seen in the graphics below (Figure 1).

Figure 1 – Coloration grain.



(L^* = Brightness (%); a^* = red/green coordinate (+a indicates more red and -a indicates green), b^* = yellow/blue coordinate (+b indicates yellow and -b indicates blue)). Source: Own Study.

The inclusion of pink pepper oil influenced the luminosity of the fish when compared to fish fed the commercial diet, which means that the closer to +100 the more luminous the fish becomes. Already the parameter that indicates the variation from red to green was not influenced by the diet. For parametron b the pink pepper oil diet decreases the yellow/blue scale of the fish. The Mato Grosso fish in its natural habitat has its base color as bright red, it can be seen that the diet did not intensify the red color of the fish, with the bright aspect indicated by parameter L being more influenced.

In this assay, can be see a minimal gain in coloration aspects, however the survival rate was higher. Probably this can be explained by the health protection derived from the consumption of vegetables, according to Gomis et al. (2001), is attributed, to a large extent, to the biological properties of the phenolic content, which is antioxidant, anti-inflammatory, antihistamine, antiviral, antimicrobial, antitumor, anticarcinogenic and antimutagenic. In the

metabolic scenery the intestinal mucosa should appropriate morphofunctional characteristics, since the integrity of the epithelium influence the better processes of absorption. Countless infectious agents or not infections can damage the intestinal mucosa, in addition to compromise the digestive processes.

The is activities anti-inflammatory (Gazzeno et al., 2005) and antimicrobial activities of *Schinus terebinthifolius* raddi (Martinez et al. 1996; Martinez Guerra et al., 2000; Melo-Junior et al., 2000; Sokmen et al., 2004; Schmourlo et al. 2005; Lima et al., 2006), contributed for a healthier metabolism, probably attributed to presence of phenolic substances against a series of microorganisms.

The action of antioxidant enzymes in the body is not able to totally minimize the damage caused by free radicals produced during metabolic processes or by exposing the organism to exogenous factors (Silva et al., 2016). In contrast, the protective potential of some foods has become widespread, increasing interest in substances that may prevent their formation (Fidrianny et al., 2008, Basumatary & Nath, 2018).

This protective action is mainly due to the presence of bioactive compounds, with emphasis on phenolic compounds, produced from the secondary metabolism of fruits and vegetables (Rocha et al., 2016; Silva et al., 2016). The consumption of fruits and vegetables helps to prevent various diseases, such as obesity (Roy et al., 2007). However, the processing of these foods by industries produces tons of by-products with biological potential, including bioactive compounds and dietary fibers (Gawlik-Dziki, 2012; Vetrani et al., 2012), which remain unexploited and some are treated as agricultural waste (Filho & Franco, 2015; Shah et al., 2005), and with that, these residues are being used in the animal industry as substitutes and/or incorporated into the feeds, benefiting the production chain and generating sustainability with the reuse of these residues.

4. Final Considerations

Studies with essential oils as additives for fish feed have shown great potential, however, the Benfica action of these compounds will depend on how the oil was obtained and how it is being used. Fish species can also influence the potential of this compound in feeding these fish.

So, we conclude that dose of pepper oil is not recommended as a supplement to optimize coloration in ornamental fishes, however the substance is shown as a promising bioactive for the fish immune system development.

However, research that can identify the action of the main components present in pink pepper oil is necessary to identify the mode of action on the species of fish used in this study when referring to its influence on the skin color of these fish.

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