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Effect of propolis extract on the parasite load of Nile tilapias reared in cages

Influência do extrato de própolis na carga parasitária de tilápias do Nilo criadas em tanques-rede

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Abstract

Due to increase in demand for healthy and chemical residue-free products, natural therapeutic substances are being enhanced in fish cultivation. Current study evaluates in an unprecedented way (Teseachoxxygen), the species is he effect of propolis on the parasite charge of the Nile tilapia (*Oreochromis niloticus*) reared in fish cages. Six hundred male Nile tilapia (200g) conditioned in ten 1 m³ cages were used. Two treatments with five replications each were provided: TCON: control (extruded meal without propolis) and TPRO: extruded meal with 4% propolis extract. Parasite collection occurred on 0, 35, 70 and 105 days. At the same time, fillet was weighed and fish standard length measured. Temperature was kept within the comfort range for the species during the experimental period (> 25°C). Trichodinids and Monogenoids (*Dactylogyridae*) were detected in the two treatments. There was no statistical difference ($p > 0.05$) in mean parasite intensity (total parasites/specimens with parasites) and abundance (total parasites/examined specimens) among treatments in the four evaluation periods. Lowest parasite prevalence occurred after 70 days in TPRO (26.66%). There was no statistical difference ($p > 0.05$) among treatments with regard to fillet weight and standard length of fish. Results show that propolis extract 4% did not significantly affect parasite load, fillet weight and standard length of Nile tilapia.

Key words: Aquaculture. *Oreochromis niloticus*. Fish parasites. Natural products.

Resumo

Devido ao aumento da demanda por produtos saudáveis e livres de resíduos químicos, a utilização de terapêuticos naturais na criação de peixes tem sido cada vez mais estimulada. O objetivo do presente trabalho foi avaliar de forma inédita o efeito do extrato de própolis sobre a carga parasitária de tilápias-do-Nilo (*Oreochromis niloticus*) criadas em tanque-rede. Foram utilizados 600 machos revertidos de tilápia-do-Nilo (200g) acondicionados em 10 tanques-rede de 1 m³. Foram utilizados dois tratamentos com cinco repetições: TCON: controle (ração extrusada sem própolis) e TPRO: ração extrusada

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contendo extrato de própolis a 4%. As coletas de parasitas foram realizadas nos dias 0, 35, 70 e 105 dias. Paralelamente, foi realizado a mensuração do peso do filé e comprimento padrão. A temperatura durante o período experimental se manteve dentro da faixa de conforto para a espécie. Foi verificada a presença de tricodinídeos e Monogenídeos (*Dactylogyridae*) em ambos os tratamentos. Não houve diferença estatística ($p > 0,05$) nos valores de intensidade (total de parasitas/número de indivíduos parasitados) e abundância (total de parasitas/total de indivíduos examinados) média parasitária entre os tratamentos nos quatro períodos de avaliação. A menor prevalência parasitária foi aos 70 dias em TPRO (26,66%). Não foi constatada diferença estatística ($p > 0,05$) entre os tratamentos na mensuração do peso do filé e comprimento padrão. Conclui-se que o extrato de própolis a 4% não influenciou significativamente a carga parasitária, no peso do filé e no comprimento padrão em tilápias-do-Nilo.

Palavras-chave: Aquicultura. *Oreochromis niloticus*. Parasitas de peixe. Produtos naturais.

Introduction

Fish production in Brazil in 2015 reached 483,241 tons, totaling R\$ 3,064 billion, with tilapia (*Oreochromis niloticus*) culture ranking first, or rather, 45.4% of total fresh-water fish production and high growth rates during the last decade (IBGE, 2015).

Great progress in tilapia production during the last few years is mainly due to several characteristics (white flesh, firm texture, nice taste, fillets without fish bones) featuring the species as one of the consumers most favorable fish. Since the tilapia adapts itself well to several and different environmental conditions (variations in temperature, pH, salinity and dissolved oxygen), the species is also of interest for producers (EL-SAYED, 2006). However, the intensification of tilapia production has also triggered high density breeding systems with great risks related to diseases caused by stocking stress (KUBITZA, 2011). For instance, infestations by Tricodinids and Monogenoids parasites cause external lesions that become the gateway for bacteria, with significant mortality rates for fish (BRACCINI et al., 2008; JERÔNIMO et al., 2011; DOTTA et al., 2015).

Several antibiotics and anti-parasites agents have been employed to minimize the effects of disease on tilapia production and to decrease production liabilities. On the other hand, several products used are prohibited in countries where consumers give priority to high quality meat, food safety and

products free from chemical residues (TELLI et al., 2014). Further, concern on the contamination of the environment is also present and research for alternative products is stimulated to lessen liabilities. Propolis, a natural product with therapeutic capacity (FISCHER, 2008), has been suggested to minimize the negative effects in fish production.

Propolis, an adhesive substance with a characteristic smell, is a resin collected by bees from fissures in tree barks, leaf buds and shoots (BANKOVA, 2005). Among its several chemical components, propolis may be highlighted for anti-parasite compounds, such as phenolic and flavonoid substances (HEINZEN et al., 2012), anti-inflammatory and antimicrobial compounds (FISCHER, 2008) and other active chemical ingredients (MARCUCCI, 1995). Although the employment of propolis in animals has had positive results against parasites (HEINZEN et al., 2012; MELLO-PEIXOTO et al., 2013), few studies have been undertaken to evaluate the effect of propolis against pathogens.

Since propolis extract as a diet supplement had provided positive results in growth performance, antimicrobial potential and immune-stimulant in fish (ABD-EL-RHMAN, 2009; DENG et al., 2011) and due to the growing demand by consumers for healthier and residue-free meat, the current study assesses (Teseachoxxygen), the species is he effect of propolis on the parasite charge of the Nile tilapia reared in fish cages.

Materials and Methods

Place and experimental conditions

Current assay was performed on the Regional Campus of the Universidade Estadual de Maringá in Diamante do Norte PR Brazil, in the Rio do Corvo, an affluent of the River Paranapanema. The Rio do Corvo is a tributary of the Rosana Hydroelectric Reservoir, delimiting the municipalities of Diamante do Norte and Terra Rica, both in the state of Paraná, Brazil. Current research was approved by the Committee for Ethics in the Use of Animals of the Universidade Estadual de Londrina (Process 27941.2012.79).

Six hundred reversed male Nile tilapias (strain GIFT), initial weight $200 \text{ g} \pm 25 \text{ g}$ were used. Two treatments were evaluated: TCON - diet without propolis, with five replications (five 1 m^3 cages; 60 fish per tank) and TPRO - diet with propolis with five replications (five 1 m^3 cages; 60 fish per tank). Fish were acclimated for 10 days and four parasite samples were collected (0, 35, 70 and 105 days) in November, December (2013), January and February (2014), respectively. Mortality rate was calculated every day by individual observation for each cage and for each treatment.

Diet comprised an extruded commercial meal without propolis and a meal with 4% propolis extract (30% crude protein; 5 mm diameter). Propolis extract was prepared with alcohol 4% by the Laboratory for the Development and Quality Control of Phytotherapies and Apitherapies of the Department of Pharmacy and Pharmacology of the Universidade Estadual de Maringá, Maringá PR Brazil, following protocol (adapted) by Franco and Bueno (1999). Propolis extract was added to the diet by direct aspersion and dried in the open air to remove alcohol excess. The product was placed in a dry and aired placed till use. Diet was provided three times a day at alternate periods.

Temperature

Mean water temperature was registered twice a day (09:00 and 18:00 h). Measurements were taken at three sites of the cage lines and at alternate lines.

Analysis of parasites

Procedures followed Braccini et al. (2008). Ectoparasites were determined by the scratching test involving the first branchial arc and left dorsal region of each fish previously anesthetized with benzocaine (0.1 g mL^{-1} alcohol 96° into 10 L water). Each treatment comprised 15 animals (totaling 30 fish per harvest) in four harvests (totaling 60 fish per treatment). Scratching, visualized between the lamina and the cover slip ($22 \times 22 \text{ mm}$) was performed by scalpel and magnified 100 x under an optic microscope Olympus CBB. Material was disinfected with an iodine-based product (2.6 % iodine - 1000 ml alcohol 96°) after each harvest.

Weight of fillet and standard length

Standard length (the distance from the tip of the lip till the caudal peduncle) was measured by caliper ruler. The fillet weight (g) and standard length (cm) were also measured. These measurements were performed at 0, 35, 70 and 105 days. Animals were then insensitized with benzocaine (0.1 g mL^{-1} alcohol 96° in 10 L of water); filleting was performed by technique involving the cutting of the head (dorsum-ventral direction), close to the opercula, and evisceration. Fillets were removed from the skeleton within a cranium-caudal and dorsum-ventral direction, one side at a time. The skin was removed in a caudal-cranial direction by tweezers and the fillets were cleaned by removing skin residues and fish bones in the cranial section. After cleaning, the fillets were weighed on a digital scale. Filleting was performed by the same researcher to avoid handling mistakes.

Data analysis

Occurrence of ectoparasites was assessed qualitatively (presence or absence) and quantitatively (the sum of the occurrence of parasites in the branchial filaments, skin or in both) of Trichodinids and Monogenoids (*Dactylogyridae*) in the two treatments. The number of fish with and without parasites was counted, coupled to the number of parasites in the branchiae and in the skin for each treatment to determine prevalence, intensity and abundance, following Bush et al. (1997). Results generated efficaciousness, according to Dotta et al. (2015) by formula: $E = \frac{MNPCG - MNPTG}{MNPCG} \times 100$ (E: efficaciousness; MNPCG: number of parasites in control group; and MNPTG: number of parasites in the group with treatment). Results on parasite intensity and abundance underwent

Student's *t* test ($p > 0.05$). Results were compared according to treatment and cultivation days. Means of fillet weight and standard length were similarly analyzed, as those between treatments.

Results

Low mortality was reported in the assay with the two treatments (TCON: four fish; TPRO: two fish). Mean temperature rates of water were within the comfort zone for Nile tilapia (KUBITZA, 2000), above 25°C in most of the experimental period, except during the mornings in November (24.8°C). Table 1 shows that mean general temperature of water ranged between 25.1°C (November) and 29.3°C (February).

Table 1. Mean water temperature (°C) during the experimental period.

| Date | Water temperature (°C) | | |
|----------|------------------------|-----------|----------|
| | Morning | Afternoon | Mean |
| Nov/2013 | 24.8±0.8 | 25.3±1 | 25.1±4.6 |
| Dec/2013 | 27.2±1.0 | 27.7±1.0 | 27.4±0.9 |
| Jan/2014 | 27.3±2.0 | 27.8±1.1 | 27.5±6.9 |
| Feb/2014 | 28.7±0.4 | 29.8±0.4 | 29.3±0.3 |

Monogenoids in the branchiae and Trichodines in the tegument mucus occurred at the onset of the assay (day 0). As a rule, the two types of parasites during the four periods studied were restricted to a single tissue, or rather, Monogenoids in the branchial filaments and Trichodines in the tegument. The only exception occurred for the evaluation on the 35th day with Trichodines on two sites in the two treatments. Highest prevalence rates (P%) for parasite Trichodina were identified on the 35th day in TCON and on the 105th day in TPRO (26.67%) (Table 2).

When total prevalence rates were analyzed (for the two parasites together), higher percentages were reported after 35 days (46.66%) for the two treatments. There was no difference ($p > 0.05$) in Mean Intensity (MI) between TCON and TPRO during the four periods analyzed. However, when only the evaluation days were taken into account, a significant rise in intensity rate occurred between the 70th and 105th days in both treatments. There was no difference ($p > 0.05$) in mean abundance (MA) between treatments and between the evaluation periods (Table 3).

Table 2. Prevalence of ectoparasites in the Nile tilapia (strain GIFT) for control treatment (TCON) and for treatment with propolis (TPRO) on 0, 35, 70 and 105 days.

| Treatment (day) | Parasite | Branchiae | | Tegument | |
|-----------------|-------------|-----------|-------|----------|-------|
| | | PP/PE | P (%) | PP/PE | P (%) |
| TCON (0) | Trichodina | 0/15 | 0 | 2/15 | 13.33 |
| | Monogenoida | 2/15 | 13.33 | 0/15 | 0 |
| TPRO (0) | Trichodina | 0/15 | 0 | 3/15 | 20 |
| | Monogenoida | 2/15 | 13.33 | 0/15 | 0 |
| TCON (35) | Trichodina | 1/15 | 6.67 | 4/15 | 26.67 |
| | Monogenoida | 2/15 | 13.33 | 0/15 | 0 |
| TPRO (35) | Trichodina | 1/15 | 6.67 | 3/15 | 20 |
| | Monogenoida | 3/15 | 20 | 0/15 | 0 |
| TCON (70) | Trichodina | 0/15 | 0 | 2/15 | 13.33 |
| | Monogenoida | 3/15 | 20 | 0/15 | 0 |
| TPRO (70) | Trichodina | 0/15 | 0 | 2/15 | 13.33 |
| | Monogenoida | 2/15 | 13.33 | 0/15 | 0 |
| TCON (105) | Trichodina | 0/15 | 0 | 3/15 | 20 |
| | Monogenoida | 2/15 | 13.33 | 0/15 | 0 |
| TPRO (105) | Trichodina | 0/15 | 0 | 4/15 | 26.67 |
| | Monogenoida | 2/15 | 13.33 | 0/15 | 0 |

PP: fish with parasites; PE: examined fish; P(%): Prevalence.

Table 3. Mean rates of prevalence (P%), medium intensity (IM) and medium abundance (AM) of ectoparasites of the Nile tilapia for control treatment (TCON) and treatment with propolis (TPRO) on 0, 35, 70 and 105 days.

| | TCON | | | TPRO | | |
|----------|-------|----------------|--------------|--------|---------------|---------------|
| | P% | IM | AM | P% | IM | AM |
| 0 Days | 26.66 | 4.5 (4.94) Aab | 0.6 (0.7) Aa | 33.33 | 3.92 (2) Aab | 0.7 (0.59) Aa |
| 35 Days | 46.66 | 1.8 (1.13) Aab | 0.5 (0.5) Aa | 46.66 | 1.8 (0.6) Aab | 0.43(0.24) Aa |
| 70 Days | 33.33 | 1.66 (0.47) Ab | 0.27 (0) Aa | 26.66 | 1.5 (0.7) Ab | 0.2 (0.09) Aa |
| 105 Days | 33.33 | 4.66 (5.19) Aa | 0.9(1.1) Aa | 40 | 4.62 (3.4) Aa | 0.77(0.24) Aa |
| Mean | 35 A | 3.15(1.64) A | 0.57(0.26) A | 36.6 A | 2.96(1.54) A | 0.525(0.26) A |

Capital letters indicate significant difference between treatments (TCON and TPRO); small letters indicate significant difference between different cultivation days by Student's *t* test at $p < 0.05$.

Propolis-supplemented diet had the best efficaciousness on the 70th day (25%) when compared to evaluation rates on the 35th and 105th days (13.33 and 14.81%, respectively) (Figure 1). There was no difference ($p > 0.05$) between fillet

weight (g) and standard length (cm) between treatments after 35, 70 and 105 days. On the 70th day, the two parameters were numerically higher in TPRO, although at the end of the assay (105 days), rates were higher in TCON (Table 4).

Table 4. Fillet weight (g) and standard length (CP) of Nile tilapia for control treatment (TCON) and treatment with propolis (TPRO) on 35, 70 and 105 days.

| | TCON | | TPRO | |
|----------|---------------|----------------------|----------------|----------------------|
| | Fillet (g) | Standard length (cm) | Fillet (g) | Standard length (cm) |
| 35 Days | 126(27.37) | 21.28(1.14) | 126.27(18.01) | 21.29(0.98) |
| 70 Days | 197.53(46.24) | 24.27(1.56) | 217.6(35.23) | 24.94(1.09) |
| 105 Days | 292.53(71.86) | 28.01(1.65) | 259.33 (73.86) | 27.05(1.68) |

Discussion

Occurrence and intensity of ectoparasites in fish are correlated with eutrophication rates, diet management and adopted technology in productive systems. Conditions worsened when production intensified through high densities (TAVARES-DIAS; MARIANO, 2015). Trichodines and monogenenoids in the Nile tilapia have already been detected in several fish farms with different stocking densities, cultivation phases and at different periods of the year (BRACCINI et al., 2008; JERÔNIMO et al., 2011; ZAGO et al., 2014; DOTTA et al., 2015; PAREDES-TRUJILLO et al., 2016). The above reveals that parasites always occur in culture conditions but are only relevant when there is an imbalance between host, parasite and environment.

High temperature during spring and summer enhances parasite infestation due to the improvement of proliferation conditions of fish pathogens (SCHALCH et al., 2005). On the other hand, sub-optimal temperature conditions may reduce immune response and fish's capacity with regard to their response to antigens. Consequently, the animals are greatly susceptible to parasites. Braccini et al. (2008) analyzed the occurrence of ectoparasites of the Nile tilapia in the Rio do Corvo (the same river as that in current study) during May, June, July and August (mean water temperature at approximately 23°C) and detected a slightly higher mean prevalence (38.2%) when compared to that in current assay. The same authors also detected high ectoparasite prevalence at the start of the assay in May, although infestations decreased as fish grew up, with an increase of more than 25% in ectoparasites prevalence during the last

month (August). Similarly, Marengoni et al. (2009) failed to report the relationship with monogenoids in the tilapias bred in cages between February and June, even though there was a greater temperature oscillation throughout the period. Contrastingly, Zago et al. (2014) reported an increase in mean intensity of ectoparasites according to the cultivation phase (initial, intermediate and final).

In spite of increase in mean temperature throughout the cultivation period detected in current assay, there was no relationship with parasite load, probably due to the fact that mean temperature in all periods remained within the species's comfort zone and management provided an adequate immunological response for pathogens. According to Kubitz (2000), tilapias cultivated at a temperature between 23 and 32°C tended to be less harmed by parasites since they lay between the thermal comfort boundaries for the species and, consequently, less susceptible to immunity decrease.

Due to its different therapeutic factors, such as anti-parasite (mainly phenolic and flavonoid compounds) (HEINZEN et al., 2012) and anti-inflammatory substances (FISCHER, 2008), propolis added to diets provided good results against parasites in several animal species, such as mammals (MELLO-PEIXOTO et al., 2013) and fish. Dotta et al. (2015) analyzed the effect of diet supplementation composed of a mixture of propolis and *Aloe barbadensis*, between 15 and 21 days, and detected significant decrease in mean parasite intensity and prevalence in tilapias. Different from the above experiments, results in current analysis revealed that diet supplemented with propolis extract

4% failed to affect mean prevalence, occurrence and abundance of ectoparasites.

Lack of influence may be associated with the cultivation medium. Adequate temperature interval during the assay did not affect anti-parasite conditions of propolis contents since, within the best cultivation conditions (best temperature; low density), fish had sufficient immunological defense and probably responded naturally to the agents. It has been reported that propolis extract may increase bactericide activity in tilapias confronted with *Aeromonas hydrophila* by stimulating the defense cells (ABD-EL-RHMAN, 2009). Probably, in more challenging conditions within the environment, the therapeutic components of propolis act more significantly and efficaciously.

Efficaciousness should be underscored. Its rate was higher on the 70th day when compared to that on the 105th day. Dotta et al. (2015) found the same pattern between the 15th and the 21st day and attributed decrease to possible stress caused by animal density and product accumulation. However, in current research, the animal's age and immunity, environmental variations or pharmacological reactions due to the period in which propolis was employed may have decreased efficaciousness during the final period.

It seems that there was no difference between TCON and TPRO with regard to animals' age and immunity since mortality rate was low and animals grew homogeneously, when standard length was analyzed. According to Saha et al. (1999), the chronic use of phenolic compounds may reduce fish growth and development. The authors underscore that compounds enhance excessively mucus secretion on the skin and in the branchiae, causing difficulty in oxygen transport and, consequently, respiration capacity. Further, high concentration of these compounds may decrease the nutritional rates of the food when they interact with proteins, carbohydrates and minerals (SHAHIDI; NACZK, 1995). Consequently, the dispensation of propolis

may have either triggered a chronic toxicity reaction or it interfered in nutrient absorption causing difficulties in the assimilation of propolis components after 70 days. Future studies are required to elucidate toxic concentrations and the manner the compounds' concentration affects the performance of the species.

Several researches on the effect of propolis in the diet have given satisfactory results on growth performance in fish (ABD-EL-RHMAN, 2009; DENG et al., 2011). However, no study has yet related the use of therapeutic products with biometric rates and the occurrence of parasites. Current assay failed to identify any statistical difference in intensity and abundance of ectoparasites, in fillet weight and in standard length between treatments on days 35, 70 and 105.

Stress is a crucial factor in animal production with regard to animals zootechnical performance. Research with mammals have already underscored that propolis may have a positive effect on stress-related biochemical responses (MISSIMA; SFORCIN, 2008). Since propolis decreases stress and parasite load, it may have affected fillet weight rates and standard length after 70 days. Further studies should be undertaken to elucidate this effect on fish and verify the mechanisms involved in anti-parasite processes, performance and stress in cage conditions.

Conclusions

Propolis extract 4% did not affect mean parasite prevalence, intensity and abundance, fillet weight and standard length. A greater efficiency occurred on the parasite load after 70 days. Although a probable effect of the metabolic assimilation of propolis may have occur after 70 days of cultivation, further studies should be undertaken to elucidate other effects of the product on the cultivation of tilapias in cages.

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