



## Monogenoidea parasites of *Oreochromis niloticus* submitted to ractopamine supplemented diet from cultivated system

[*Monogenoidea* parasitos de *Oreochromis niloticus* provenientes de sistema de cultivo submetidos a uma dieta suplementada com ractopamina]

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### ABSTRACT

*Oreochromis niloticus* (Linnaeus) (Perciformes: Cichlidae), commonly named “tilapia”, is the second most cultivated species in the world. Among the diseases that affect tilapia are parasitic diseases caused by parasites of the class Monogenoidea. The aim of the present study was to analyze parasitism rates among specimens of *O. niloticus* in farming systems, after dietary supplementation with ractopamine, a growth promoter that is used for fattening fish. The action of this substance on the quantitative parameters of infestation by Monogenoidea was evaluated. Samples of *O. niloticus* were obtained from a fish farm in Brasília, Federal District, Brazil. Their gills were removed and fixed. Four species of monogenoideans were collected: *Cichlidogyrus halli*, *C. sclerosus*, *C. thurstonae* and *Scutogyrus longicornis*. The results from this study indicate that the monogenoidean parasites of tilapias examined here presented different behaviors in relation to the ractopamine concentrations that were added to the food. The parasitological indexes did not present significant reductions through using ractopamine, thus leading to the conclusion that administration of ractopamine is not efficient as a method for controlling infestations of monogenoidean parasites.

Keywords: Monogenoidea, *Oreochromis niloticus*, ractopamine, cultivated systems

### RESUMO

*Oreochromis niloticus* (Linnaeus) (Perciformes: Cichlidae), tilápia, é a segunda espécie mais cultivada no mundo. Entre outras doenças que afetam a tilápia, estão as doenças provocadas por parasitos da classe Monogenoidea. O presente estudo teve como objetivo analisar os índices de parasitismo em espécies de *O. niloticus* provenientes de sistemas de cultivo, submetidos a uma dieta com suplementação de ractopamina, usada como promotor de crescimento no estágio de engorda dos peixes, para avaliar as ações dessa substância nos parâmetros quantitativos da infestação por Monogenoidea. Exemplares de *O. niloticus* obtidos na piscicultura “Agropecuária do Buriti Perdido”, em Brasília, Distrito Federal, Brasil, foram examinados; as brânquias foram removidas e fixadas. Quatro espécies foram coletadas: *Cichlidogyrus halli*, *C. sclerosus*, *C. thurstonae* e *Scutogyrus longicornis*. Os resultados obtidos no presente estudo indicaram que as espécies de Monogenoidea parasitos de tilápia examinadas apresentam diferentes comportamentos relacionados à concentração de ractopamina adicionada ao alimento. Os índices parasitológicos não apresentaram redução significativa pelo uso da ractopamina, levando à conclusão de que a ractopamina não é eficiente como método de controle parasitário em infestações por Monogenoidea.

Palavra-chave: Monogenoidea, *Oreochromis niloticus*, ractopamina, sistema de cultivo

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Recebido em 6 de setembro de 2019

Aceito em 5 de março de 2020

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## INTRODUCTION

*Oreochromis niloticus* (Linnaeus, 1758) (Perciformes: Cichlidae), commonly named “tilapia”, is a member of the family Cichlidae and is native to Africa and the southwestern Middle East. Since the 1930s, tilapias have been intentionally dispersed worldwide (Canónico *et al.*, 2005) such that they are now present on all continents (FAO, 2014). The hardiness of this species, its ease of adaptation and its omnivorous feeding habits, together with the accelerated growth and easily obtained larvae in intensive rearing are important characteristics that have favored the development of tilapia farming (Coward and Bromage, 2000).

*Oreochromis niloticus* is the second most frequently farmed species in the world, following carps (FAO, 2014), and is the first in Brazil (Portal Brasil, 2017). As one of the main producers of tilapias, Brazil has a very different market orientation compared to its Latin American counterparts, since 99% of its national production is consumed domestically (FAO, 2014).

Tilapias are used for biological control of aquatic weeds and insects, as baitfish in capture fisheries, for aquaria and as a food fish. They have most recently been promoted as an important source of protein that could provide food security for developing countries without the environmental problems associated with terrestrial agriculture (Canónico *et al.*, 2005). On the other hand, it has been documented that they exert negative impacts on native biodiversity, given that these cichlids compete with native fish species for resources and habitat use. They sometimes consume the eggs, larvae and small individuals of other fish species; and have frequently co-introduced their parasites, some of which have switched host to indigenous fish species (García-Vásquez *et al.*, 2017).

According to Vicente *et al.* (2014), tilapia production is growing year-by-year in Brazil. Consequently, concern about proper management and prophylaxis against various infectious agents is increasing. Over the last few decades, the parasites of introduced fish species have received attention because of their economic implications, especially in situations of intensive fish-farming (Martins *et al.*, 2010). A great number of parasite species can cause mortality in fish farms and

proper treatment can be a difficult and expensive process for producers to implement (Pavanelli *et al.*, 2008).

The losses caused by parasites can be seen through reductions in assimilation and growth rates, along with diminished final value of the product (Eiras, 1994). Among the diseases that affect tilapia are parasitic diseases caused by parasites of the class Monogenoidea, that have been widely reported parasitizing the gills and body surface of *O. niloticus* in Brazil. Dactylogyridae of the genera *Cichlidogyrus* Paperna, 1960, *Onchobdella* Paperna, 1968, and *Scutogyrus* Pariselle & Euzet, 1955; and Gyrodactylidae of the genus *Gyrodactylus* Normdmann, 1832, are the main species that inhabit these farmed fish (Ranzani-Paiva *et al.*, 2005; Ghiraldelli *et al.*, 2006; Graça and Machado, 2007; Lizama *et al.*, 2007; Marengoni *et al.*, 2009; Jeronimo *et al.*, 2011; Pantoja *et al.*, 2012; Bittencourt *et al.*, 2014; Zago *et al.*, 2014).

In 1964, in a study on the relationship between carps and protozoan and monogenoidean populations, Paperna & Kohn observed that fish that were in a better nutritional condition presented lower infestation by monogenoideans (Paperna & Kohn, 1964). Nutritional factors support physiological processes, stimulate host immunity and increase the presence of defense cells (Rotta, 2003; Fujimoto *et al.*, 2010; Biller-Takahashi, 2014). According to Fujimoto *et al.* (2010), vitamins and minerals are important in the physiological processes of fish species, through minimizing the effects of stressor agents.

Despite numerous studies within aquiculture, knowledge relating to nutrition in fish is scarce, in comparison with the amount of knowledge available on other species of zootechnical importance. Nonetheless, technology is widely applied in the stages of formulation and manipulation of nutritionally efficient diet (Pezzato *et al.*, 2004). One of these examples is the use of food additives such as ractopamine (Mundim *et al.*, 2016), which is known to improve food efficiency through reducing body fat content and increasing muscle growth in animals (Wendelaar Bonga, 1997).

However, supplementation with ractopamine may have a negative effect on animal welfare, such that typical stress alterations are generated, that may

reflect negatively in the quality of the final product (Marchant-Forde *et al.*, 2003). The stress within fish-farming may originate from various sources, such as fish handling, high stocking density, transportation, biological interactions, water quality and food management, including the use of nutritional additives (Mundim *et al.*, 2016) and is known that is one of the triggers of monogenoidean infestations in farming systems.

The aim of the present study was to analyze parasitism rates among specimens of *O. niloticus* in farming systems, after dietary supplementation with ractopamine, a growth promoter that is used for fattening fish. The action of this substance on the quantitative parameters of infestation by Monogenoidea was evaluated.

## MATERIAL AND METHODS

Samples of *O. niloticus* were obtained from the farm “Agropecuária do Buriti Perdido”, in Brasília, Federal District, Brazil. For this study on the effects of dietary supplementation with ractopamine, only male fish were selected, considering that they present better growth performance than females. Forty-one specimens of *O. niloticus* that were submitted to the treatment were analyzed.

The experiment was conducted in the “Centro de Tecnologia em Piscicultura da Secretaria de Agricultura do Distrito Federal,” in Brasília, Brazil. The study was done in a closed system of recirculation, with mechanical and biological filters with a volume of 14.8m<sup>3</sup> and maximum filtration capacity of 20L/min. A total of 112 specimens of *O. niloticus* were distributed randomly in 16 tanks of 500L that were installed in a closed and covered environment. Their initial weight was 519.37±114.3g and they were individually identified using PIT-tags.

There was a pre-experimental period of 20 days for the fish to become acclimatized to the tanks. Over the first 10 days of this period, they were fed twice a day with commercial pellets (32% crude protein, 5% lipids and 3,000kcal/kg of energy), i.e. the same food that they had been consuming under fish-farming conditions. Over the second 10 days of this period, they were fed with baseline pellets of the control diet.

The system was oxygenated through silicone hoses of 4mm in diameter, with a porous stone at one end, connected to a PVC tube fed with a 0.75HP compressor. The water temperature was controlled through 16 500W thermostats that were installed individually inside each tank. The fishes were kept under natural photoperiod conditions (12h of light and 12h of darkness).

The experimental design was completely randomized, with four treatments with ractopamine incorporated into the food at different concentrations: 0ppm of ractopamine, 10ppm of ractopamine, 20ppm of ractopamine and 40ppm of ractopamine. Thus, among the 16 tanks, there were four tanks per treatment (i.e. four replications). At the end of the experimental period, fish specimens were chosen randomly and anesthetized with clove oil at a dosage of 50mg L<sup>-1</sup> (Inoue *et al.*, 2003).

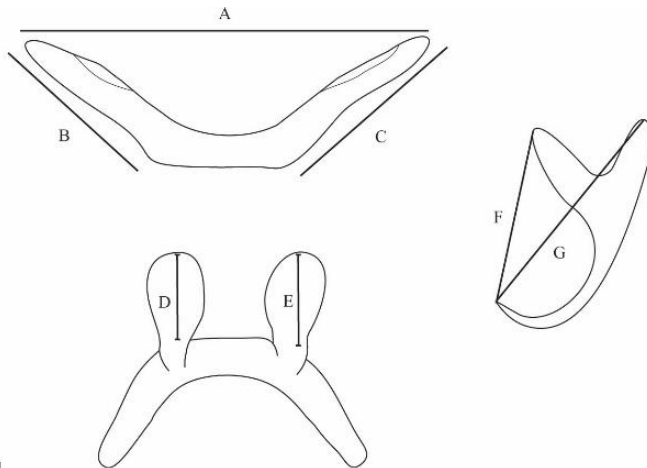
The gills were removed and placed in vials containing 1:4,000 formalin solution and shaken vigorously and the formalin solution of each vial was then increased to about 5%. The samples were then sent to the “Laboratório de Helmintos Parasitos de Peixes – LHPP, Instituto Oswaldo Cruz”, in Rio de Janeiro, to be examined. In the laboratory, parasites were collected with the aid of a stereoscopic microscope and were stored in the same fixative. Some specimens were mounted unstained in Hoyer’s mounting medium in order to study the sclerotized parts. The remainder were stained with Gomori’s trichrome, cleared in beechwood creosote and mounted on slides in Canada balsam.

All the specimens were observed under a Zeiss Axioskop bright-field microscope, equipped with a millimeter-grid eyepiece and with differential interference contrast (DIC) optics, and were photographed using a Sony MPEGEX digital camera that was coupled to this microscope. All measurements were expressed in micrometers, taking the scheme shown in Figure 1, and are presented here as the mean±standard deviation. The mean is followed by the range and the number of specimens measured (when greater than two) in parentheses. Representative specimens were deposited in the Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC) in Brazil.

The data were compared using one-way analysis of variance (ANOVA) and treatment means were

compared using the Tukey's test. The *p* values less than or equal to 0.05 were considered statistically significant. The prevalence (P), mean abundance (MA) and mean intensity (MI) of the

parasites were calculated assuming binominal distribution, using the Quantitative Parasitology 3.0 statistical software (Bush *et al.* 1997; Rózsa *et al.* 2000).



**1**  
Figure 1. Measurement protocol for some haptor structures.

## RESULTS AND DISCUSSION

Forty-one specimens of *O. niloticus* of total weight 478-727g ( $582.05 \pm 74.83$ g) were examined for the presence of monogenoideans and 36 (88%) were found to be parasitized by at least one species of Monogenoidea. Four species were collected: *Cichlidogyrus halli* Price & Kirk, 1967 (Figure 2a-c), *Cichlidogyrus sclerosus* Paperna & Thurston, 1969 (Figure 2d-f), *Cichlidogyrus thurstonae* Ergens, 1981 (Figure 2g-i), and *Scutogyrus longicornis* (Paperna & Thurston, 1969) (Figure 2j-l).

The morphometric analysis on the four species collected in the gills of these *O. niloticus* specimens from the commercial fish-farm "Agropecuária do Buriti Perdido", Brasília (Table 1) showed that they were compatible with species that had previously been described and were known to be parasites of tilapias.

*Cichlidogyrus halli* presented the highest prevalence rate (63.4%) (MI: 9.19; MA: 5.83; IR: 1-43, with a total of 239 specimens), followed by *C. sclerosus* (58.5%) (MI: 18.08; MA: 10.59; IR: 1-137, with a total of 434 specimens), *S. longicornis* (22%) (MI: 2.44; MA: 0.54; IR: 1-5,

with a total of 22 specimens) and *C. thurstonae* (14.06%) (MI: 2; MA: 0.29; IR: 1-4, with a total of 12 specimens).

After administration of the ractopamine-supplemented food, it was observed that the prevalence of three of the monogenean species increased. The exception was *C. thurstonae*, which was absent in the control and presented prevalence of 10% at the concentration of 10ppm and 40% at 20ppm, but only 14.3% at 40ppm, i.e. a decrease.

According to Marchant-Forde *et al.* (2003), supplementation with ractopamine may have a negative effect on animal welfare, thus generating typical alterations of stress. In this light, increased stress in fish may lead to higher parasitological indexes. There were no statistically significant differences ( $P > 0.05$ ) in prevalence between the control group and the experimental groups after exposure to the monogenean parasites or to the different concentrations of ractopamine that were added to the food. Similarly, for all other parasitological index parameters tested between species and treatment groups, no statistically significant differences were observed ( $P > 0.05$ ).

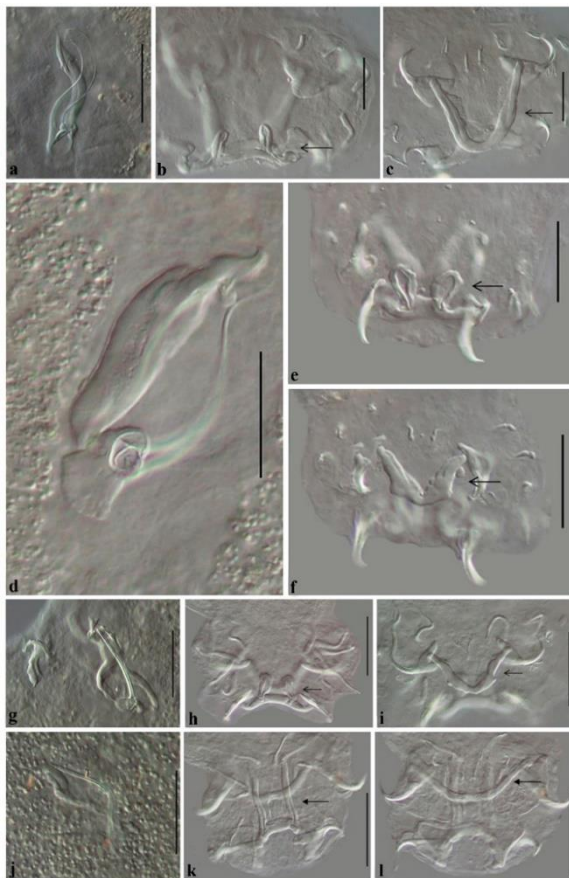


Figure 2. Photomicrographies of sclerotized parts of Monogenoidea parasites of *Oreochromis niloticus* obtained from the farm “Agropecuária do Buriti Perdido”, in Brasília, Federal District, Brazil. a-c *Cichlidogyrus halli* (Price & Kirk, 1967). a. Copulatory complex. b. Haptor showing dorsal bar with projections (arrow). c. Haptor showing ventral bar (arrow). d-f *Cichlidogyrus sclerosus* Paperna & Thurston, 1969. d. Copulatory complex. e. Haptor showing dorsal bar with projections (arrow). f. Haptor showing ventral bar (arrow). g-i *Cichlidogyrus thurstonae* Ergens, 1981. g. Copulatory complex. h. Haptor showing dorsal bar with projections (arrow). i. Haptor showing ventral bar (arrow). j-l *Scutogyrus longicornis* (Paperna & Thurston, 1969). j. Copulatory complex. k. Haptor showing dorsal bar with projections (arrow). l. Haptor showing ventral bar (arrow). Scale-bars: Figure a, d, g, j: 30µm; Figure b-c, e-f, h-i, k-l: 50µm.

*Cichlidogyrus* is the most species-rich genus of Monogenoidea, and its species almost exclusively parasitizing Cichlidae (a few representatives of *Cichlidogyrus* also occur in Cyprinodontidae and Nandidae) (Rahmouni et al., 2017). In South America, this genus was first reported by Thatcher (1991) parasitizing *O. mossambicus* (Peters, 1852) (= *Tilapia mossambica*) in Colombia. Later, many reports of this genus parasitizing *O. niloticus* in different states of Brazil have been published. *Cichlidogyrus* sp. was reported parasitizing farmed fishes in Paraná by Graça and Machado (2007); in Santa Catarina by Azevedo et

al. (2006), Ghiraldelli et al. (2006) and Martins et al. (2010); and in São Paulo by Ranzani-Paiva et al. (2005), Lizama et al. (2007) and Zago et al. (2014). *Cichlidogyrus halli* and *Cichlidogyrus thurstonae* were recorded in Santa Catarina by Jeronimo et al. (2011) and in São Paulo by Zago et al. (2014). *Cichlidogyrus sclerosus* was reported in Santa Catarina by Ghiraldelli et al. (2006) and Jeronimo et al. (2011); in São Paulo by Lizama et al. (2007); in Paraná by Graça and Machado (2007); in Santa Catarina by Azevedo et al. (2006) and Martins et al. (2010); and in Rio de Janeiro by Silva et al. (2015).

Table 1. Main measurements of species of Monogenoidea found parasitizing *Oreochromis niloticus* coming from the farming “Agropecuária do Buriti Perdido”, in Brasília, Federal District, Brazil

	<i>Cichlidogyrus sclerosus</i>	<i>Cichlidogyrus thurstonae</i>	<i>Scutogyrus longicornis</i>	<i>Cichlidogyrus halli</i>
Body length	659(400–850)	387(300–470)	604(510–720)	759(700–860)
Body width	238(200–280)	110(100–220)	149(100–200)	266(200–330)
Pharynx length	61(55–66)			
Pharynx width	59(47–74)			
Haptor length	122(105–155)	115(91–150)		
Haptor width	92(70–110)	77(53–110)	130(108–145)	
Ventral bar				
A	41(40–45)	42(25–56)	70(45–90)	95(75–120)
B	41(37–45)	36(25–43)	41(40–50)	77(57–87)
C	41(35–44)	35(30–43)	35(30–50)	77(57–87)
Dorsal bar				
D	24(17–31)	19(13–23)	51(40–75)	21(15–32)
E	25(18–30)	19(15–22)	49(40–75)	21(15–25)
F	53(35–63)	47(35–55)	48(32–82)	84(75–100)
Ventral anchor				
G	30(26–36)	29(25–34)	31(17–40)	48(37–55)
H	33(27–36)	34(31–40)	35(27–60)	46(42–50)
Dorsal anchor				
G	30(20–33)	26(15–32)	27(15–35)	40(32–50)
H	32(28–40)	29(20–34)	32(22–50)	41(35–47)
Male copulatory organ (I)	75(63–86)	47(37–60)		81(67–93)
Accessory piece (J)	61(50–67)	51(29–71)		64(59–75)
Deposited specimens	CHIOC 39269 a-e	CHIOC 39270, 39271 a-c	CHIOC 39272, 39273a-c, 39274	CHIOC 39265a-b, 39266, 39267, 39268

Letters in parenthesis refers to measurements given in Figure 1.

*Scutogyrus longicornis* was originally described as belonging to the genus *Cichlidogyrus*, and was observed parasitizing tilapias in Uganda, Africa, by Paperna and Thurston in 1969. Pariselle and Euzet (1995) erected *Scutogyrus* and transferred the species to this genus. *Scutogyrus longicornis* was recorded in Santa Catarina by Jeronimo *et al.* (2011); in Paraná by Graça and Machado (2007) and in São Paulo by Zago *et al.* (2014). *Cichlidogyrus tilapiae*, which was not found in *O. niloticus* in the present study, had been previously reported in Amapá by Pantoja *et al.* (2012) and Bittencourt *et al.* (2014); and in Rio de Janeiro by Silva *et al.* (2015).

Lizama *et al.* (2007) examined specimens of *O. niloticus* from fish farms in Assis, São Paulo, and found *C. sclerosus* and another four different unidentified *Cichlidogyrus* species. These authors reported that the highest prevalence was of *Cichlidogyrus* sp. 1 and *C. sclerosus* (43.8% and 40.4%, respectively), followed by the other species, with similar mean intensity and mean abundance values for all the five species found. The quantitative parameter values observed for *C.*

*sclerosus* in the present study were higher than those observed by Lizama *et al.* (2007) for this species (40.4%; MI: 6.4; MA: 2.6; and IR: 1-24 in their study, versus 58.5%; MI: 18.08; MA: 10.59; and IR: 1-137 in the present study).

Silva *et al.* (2015) studied specimens of *O. niloticus* from an urban lake in the municipality of Itaperuna, Rio de Janeiro, which were obtained at two different collection times. In February 2015, the prevalence was *C. tilapiae* 100% and *C. sclerosus* 0% (zero). In the second collection, in May 2016, the prevalence was *C. tilapiae* 44.44% and *C. sclerosus* 88.88%.

Zago *et al.* (2014) examined farmed fish at three different growth phases, according to their weight, which were classified as initial, intermediate and final: ≤ 150g; 151-400g; and 401-800g, respectively. The prevalence of *Cichlidogyrus halli* found in the initial phase (61.9%) by these authors was similar to what was seen in the present study (63.4%); while the prevalence was higher in the intermediate and final phases (100% and 90.5%). Regarding mean

intensity, the value of the present study (9.19) was higher than the values found by Zago *et al.* (2014) (5.3, 4.5 and 7.2 in the initial, intermediate and final phases, respectively). Regarding mean abundance, which was 3.3, 4.5 and 8.0 in the different phases of the hosts examined by Zago *et al.* (2014), their values are similar than those of the present study. All the parameters found by Zago *et al.* (2014) for *Cichlidogyrus thurstonae* were higher than those in the present study, regarding prevalence, mean intensity and mean abundance.

The parasitological parameters for *Scutogyrus longicornis* observed in the present study (22%) were different from those in the study by Zago *et al.* (2014), that found higher prevalence indexes (42.9%, 85.7% and 76.2%). On the other hand, their intensity values were 0.3, 1.6 and 1.7 in the different phases of growth, i.e. lower than the value found in the present study (2.44). The mean abundances of *S. longicornis* found by Zago *et al.* (2014) were 0.1, 1.4 and 1.3, i.e. values that were higher than what was observed in the present study. For all the parasitological index parameters tested between species and treatment groups in the present study, no statistically significant differences were observed ( $P>0.05$ ).

Monogenoideans are parasites with direct life cycle and they are resistant to elimination when translocated (Pérez-Ponce de León *et al.*, 2000). Thus, species of the genus *Cichlidogyrus* that were translocated via *O. niloticus* were found to have colonized species of endemic cichlids in Mexico (Jiménez-García *et al.*, 2001) and Panama (Roche *et al.*, 2010) and in several records in Brazil (cited above) due to the relative parasite specificity (Bittencourt *et al.*, 2014).

According to Kubitza and Kubitza (2000), the susceptibility of tilapias to parasitosis and other illnesses depends on various factors, including the parasite species or lineages, water quality and organic load in farm production units, nutritional condition of the fish and, especially, water temperature (Marengoni *et al.*, 2009). Records

from monogenoideans that were found in farmed tilapias in Brazil, including in the present study, have shown that these fish are parasitized by monogenoidean fauna that are similar to those of the original locality. It is known that *O. niloticus* in the Nile River hosts the following species: *Cichlidogyrus tilapiae*, *C. aegypticus*, *C. cirratus*, *C. halli*, *C. thurstonae*, *C. arthracanthus*, *Scutogyrus longicornis*, *Gyrodactylus cichlidarum* and *Enterogyrus cichlidarum*, demonstrating that tilapias co-introduced some of their monogenoidean parasites following their invasions.

The results obtained from the present study indicate that monogenoidean parasites of tilapias from the farm "Agropecuária do Buriti Perdido" presented different behaviors relating to the ractopamine concentrations that were added to the food. *Cichlidogyrus halli* presented lower prevalence at the concentration of 10ppm (40%), compared with the control (80%), but lower mean intensity and abundance were observed at concentrations of 40ppm (4.3 for both indexes) and 10ppm (6.0 and 2.4, respectively). *Cichlidogyrus sclerosus* showed lower parameters at the concentration of 10ppm. *Cichlidogyrus thurstonae* was not observed in the control samples but was found parasitizing tilapias fed with different concentrations of ractopamine. Together with *S. longicornis*, *C. thurstonae* presented lower indexes, compared with the other species.

According to Trombeta (2016), use of ractopamine proved to be efficient for decreasing the fat content in tilapia fillets. However, this did not present significant results with regard to improvement of zootechnical performance. Regarding the control of parasites, ractopamine did not prove to be efficient as a method for parasitic control regarding monogenoidean infestations. Considering the increasing levels of tilapia farming in Brazil, further studies combining different substances to define their efficacy for controlling monogenoidean species in farmed fish are necessary.

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