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CONTROLE PARASITÁRIO EM ALEVINOS DE TILÁPIA DO NILO (*OREOCHROMIS NILOTICUS*) TRATADOS COM LEVAMISOL E ALLIPLUS EM GAIOLAS

PARASITIC CONTROL IN NILE TILAPIA FINGERLINGS (*OREOCHROMIS NILOTICUS*) TREATED WITH LEVAMISOLE AND ALLIPLUS IN CAGES

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RESUMO

Em pisciculturas brasileiras, diferentes protocolos são utilizados para aumentar os índices zootécnicos, sendo utilizados produtos como levamisol e alliplus. O presente estudo testou diferentes protocolos de alimentação em alevinos de *Oreochromis niloticus*. Um total de 105.000 alevinos foram alojados em gaiolas de malha 19x19 mm com bolsas de malha 12 mm, no reservatório de Três Marias e subdivididos em 5 grupos, que foram submetidos a diferentes protocolos: grupo 1 = controle com ração sem aditivo e grupos 2, 3, 4 e 5 submetidos a vários tratamentos com adição de levamisol e alliplus na ração. Amostras de brânquias, nadadeiras e raspados de mucosa foram coletadas no início e no final do experimento para avaliar a taxa de infestação por *Trichodina* spp. e monogenéticos. A maior conversão alimentar foi registrada em alevinos submetidos aos tratamentos com levamisol e alliplus com 6 refeições por dia, quando comparados aos alevinos de peixes, grupo 5, que receberam apenas 3 refeições por dia. No presente estudo, os grupos tratados com levamisol e alliplus apresentaram níveis moderados ou baixos de monogenéticos e Trichodina spp., enquanto o grupo controle apresentou alta taxa de infestação e níveis de monogenéticos presentes. Os resultados mostraram que o uso de levamisol e alliplus e o aumento no número de refeições diminuíram a taxa de infestação e o tempo de cultivo, além de aumentar a lucratividade do piscicultor.

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Palavras-chave: Aquicultura, alimentação, piscicultura, taxa de infestação.

ABSTRACT

In Brazilian fish farms, different protocols are used to increase zootechnical indices, and products such as levamisole and alliplus are used. The present study tested different feeding protocols in *Oreochromis niloticus* fingerlings. A total of 105,000 fingerlings were housed in 19x19 mm mesh cages with pockets of 12 mm mesh, in the Três Marias reservoir and subdivided into 5 groups, which were submitted to different protocols: group 1 = control with feed without additive and groups 2, 3, 4, and 5 submitted to various treatments with the addition of levamisole and alliplus in the feed. Samples of gills, fins, and mucosal scrapings were collected at the beginning and end of the experiment to evaluate the infestation rate by *Trichodina* spp. and monogeneans. Higher feed conversion was recorded in fingerlings submitted to treatments with levamisole and alliplus with 6 feeds per day, when compared to fish fingerlings, group 5, which received only 3 feeds per day. In the present study, the groups treated with levamisole and alliplus did present moderate or low monogenean and *Trichodina* spp. levels, while the control group had a high infestation rate and monogeneans levels present. The results showed that the use of levamisole and alliplus and the increase in the number of feeds, decreased the infestation rate and the cultivation time, in addition to increasing the profitability of the fish farmer.

Keywords: Aquaculture, feeding, fish farming, infestation rate.

INTRODUCTION

Tilapia (*Oreochromis niloticus*) farming in reservoirs and dams has been leading fish production in recent years in Brazil (Solto Cavalli et al., 2021). In the cages at the Três Marias dam, in the country of Felixlândia, Minas Gerais, there has been losses of zootechnical indexes, making it necessary to determine new protocols, especially in cold seasons, because the fingerlings are very sensitive at this time, in addition to the management on arrival and allocation of them (Abdel-Ghany et al., 2021).

Parasite control is crucial to ensure good zootechnical indices in fish raised in cages. Among the main parasitic diseases are the monogenean and protozoan groups. These parasites lodge in fish, causing micro lesions in the mucous membranes and leading

to secondary infections by bacteriosis, resulting in yield loss and even death (Pimentel-Acosta et al., 2019; Abd-Elrahman et al., 2023).

Levamisole is an important antiparasitic agent, which in fish that have just arrived in cages, increases immunity and decreases mortality in this critical phase for fingerlings, thus reducing the feed conversion factor (FCF) and their survival, allowing the exploitation of the full genetic potential of the fish (Alves et al., 2019; Loures et al., 2008; Nogueira et al., 2019).

Alliplus is a natural additive based on essential oils and organic acids and has organic principles, such as allicin. Despite the lack of studies, alliplus is associated with an increase of the palatability of the feed, in addition to being excellent against protozoa and bacteria. The fish exposed to allicin increase the feed intake and consequently the FCF due to the stimulation of palatability and ingestion of pellets (Huang et al., 2020).

Tilapia has become one of the most produced fish in Brazil due to its zootechnical qualities such as hardiness, tolerance to low oxygen levels, and great market acceptance. It is a fish that has lineages that seek the potential of the species, such as the Genetically Improved Farmed Tilapia (GIFT) lineage (Santos, 2012). The fingerlings of tilapia of the GIFT strain have better zootechnical conditions, with higher growth rate, better feed conversion and higher fillet yield. This strain is one of the best for cultivation due to its long process of genetic improvement in recent decades (Santos, 2012). Tilapia, cichlid fish, have diurnal habits, since their stomach repletion is greater during the day and feeding management should be carried out during the sunny part of the day, with correct intervals, routine and frequency (Fiod, 2010).

The present study was to verify different treatments with the addition of levamisole and alliplus to food under monitored conditions to evaluate the infestation rate in samples of gills, fins, and mucosal scrapings. The research was conducted in the Três Marias dam, with the objective of exploring the potential of this dam for tilapia farming. Over the years, research and strategic decisions have led the dam to become one of the largest tilapia producers in Brazil, allowing rearing densities of 30 to 50 kg per m^3 of water.

MATERIAL AND METHODS

The study was carried out at Estância da Tilápia, located in the municipality of Felixlândia, Minas Gerais, on the banks of the Três Marias reservoir (18º41'19"S,

45º11'40"W). São Francisco River basin, in the period for 30 days between the months of July and August. A total of 104,994 fingerlings of tilapia O*reochromis niloticus* of the GIFT strain were used, originating from a fish farm located in the municipality of Morada Nova de Minas, MG, Brazil.

The fingerlings had two weight ranges, one with an average of 2.5 grams and the other with an average of 3.5 grams, which were submitted to sanitary protocols to avoid prior contamination. They were analyzed upon arrival at Fazenda Santa Felicidade to check for possible infections. The level of infestation was determined using 5 fingerlings from each transport tank, which were anesthetized with 80% eugenol and euthanized by cross-section of the cervical cord following the ethical principles established by the National Council for the Control of Animal Experimentation (CONCEA). The research was approved by the ethics committee on the use of animals CEUA PUC Minas, protocol n 03/2021.

The 105,000 fingerlings were divided into 19 cages containing 5,526 fish in each tank. Randomly established 5 groups containing the following number of tanks: group 1 = 4 tanks, total of 22,105 fingerlings, group 2 = 4 tanks, total of 22,105 fingerlings, group 3 = 4 tanks, total of 22,105 fingerlings, group 4 = 4 tanks, total of 22,105 fingerlings, and group 5 = 3 tanks, total of 16,578 fingerlings. The water temperature of the tanks was determined daily in the morning and afternoon at fixed times at 7:30 a.m. and 3:30 p.m.

The 19 cages were lined with 19 mm mesh screens and with internal pockets with dimensions of 3x3x2 meters, with 12 mm mesh, capable of holding 5526 fingerlings for a period of 30 days until a final biomass of 20 to 25 kg/m3 per cage.

The diet used in the experiment had the following levels of guarantee: Crude Protein - minimum of 45%; Ether Extract - maximum of 9% (or 8%); Mineral Matter - maximum of 14% (or 15%); Crude Fiber - maximum of 4%; Calcium - minimum of 20g/kg and maximum of 30g/kg; Phosphorus - minimum of 10g/kg; Vitamin C - minimum of 1500mg/kg (or 1000mg/kg); and Vitamin E - minimum of 400mg/kg (or 300mg/kg).

The alliplus used in this study was Alliplus® conventional, a specific mixture of essential oils and organic acids with high content of allicin and it is a liquid product, ideal for mixing on feed in fish farming systems.

The amount of feed used was distributed as follows: 59.5 kg of 1.0 mm granules, 400.4 kg of 1.5 mm granules and 1,346 kg of 1.8 mm granules. These quantities were determined based on the weight of the fingerlings and considering the feed table provided by the feed manufacturer. Food was available at 7:30 a.m., 9:00 a.m., 11:00 a.m., 12:30

p.m., 2:00 p.m. and 4:00 p.m. for groups 1, 2, 3 and 4. The treatments of the 5 groups were established as follows:

Group 1: Control, only the diet without additives, distributed in 6 rations per day;

Group 2: single treatment in the diet with 18.8% levamisole in the amount of 250 ml per 25kg of feed. The product was mixed manually during the first 5 days of the experiment, distributed in 6 rations per day;

Group 3: single treatment in the diet with 18.8% levamisole in the amount of 250 ml during the first 5 days of the experiment, with daily addition of alliplus in the amount of 50 ml for every 25kg of feed. The product was mixed manually daily, distributed in 6 rations per day;

Group 4: daily addition of alliplus in the amount of 50 ml per 25kg of feed. The product was mixed manually daily, distributed in 6 rations per day;

Group 5: submitted to routine feeding of the fish farm, which had alliplus in the amount of 50 ml per 25kg of feed, being offered 3 feeds during the day at the hours of 8:30, 12:00 and 15:00 h.

For initial follow-up of the experiment, the amount of feed provided was weighed daily and recorded in the database to calculate the next day's feed supply. On the eighth day of the experiment, a biometric weighing 100 fingerlings from each tank was performed to calculate the average weight of fingerlings/tank, to control the ratio and to calculate the growth curve.

At the end of the experiment, the rate of infestation by monogeneans and protozoa was determined, sacrificing 5 fingerlings from each tank, randomly selected and obeying the ethical principles already mentioned, to analyze mucosal scrapings, gill fragments and fin fragments. Monogenean analysis was performed to detect positive (+) and negative (-) lesions. For the protozoan *Trichodina* spp., an occurrence scale was established, where a detection greater than 5 per field was considered high, between 2 and 5 per field was considered moderate, and less than 2 per field was considered low. On the last day of the experiment, another biometric was performed using 50 fingerlings from each tank, using an ichthyometer and a precision scale to determine the total length in centimeters and the body weight in grams.

To evaluate statistical differences between the results obtained, data normality test Shapiro–Wilk for was performed. For variables that met the assumptions of normality and homogeneity, differences between groups were assessed using one-way ANOVA and when data were not normally distributed the Kruskal-Wallis test was used to analyze

independent samples of equal or different sizes and levels of infestation. The significance level was set at $p < 0.05$. Statistical analyses were performed using GraphPad Prism software.

RESULTS

The average water temperature of the cages during the experiment was 23.64 °C ± 0.74 in the morning and 24.69 °C ± 0.92 in the afternoon.

Biometric parameters were performed by individually weighing and measuring 50 fingerlings from each tank with the aid of a precision scale and ruler. Initial weight, final weight, grams/day, difference in biomass and feed conversion factor FCF of fingerlings are shown in Figure 1 and Table 1.

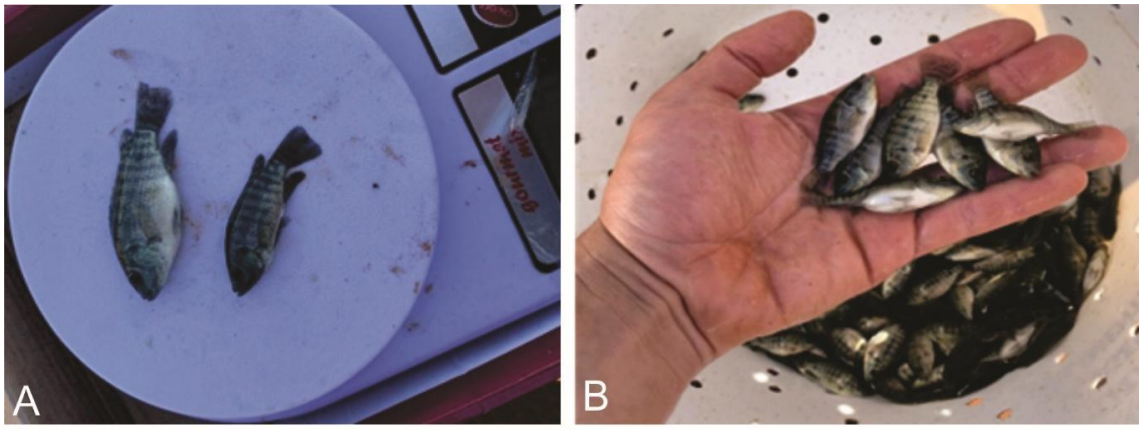

Figure 1- (A) Heterogeneous growth of fingerlings in group 5; (B) Standardization of fingerling size in groups with correct management.

Table 1 – Mean ± standard deviation of Initial weight, final weight, grams/day, difference in biomass, and feed conversion factor (FCF).

Groups	Initial weight	Final weight	g/day	Biomass difference	FCF
1 = control	3,00±0,50^{A}	17,81±2,54^{A}	0,51±0,07^{A}	75,23±10,96^{A}	0,89±0,13^{B}
2 = levamisole	2,75±0,43^{A}	17,76±1,77^{A}	0,52±0,05^{A}	76,05±6,97^{A}	0,77±0,07^{C}
3 = levamisol/alliplus	3,00±0,50^{A}	17,33±2,21^{A}	0,49±0,06^{A}	72,96±8,86^{A}	0,87±0,10^{B}
4 = alliplus	2,75±0,43^{A}	16,31±2,12^{A}	0,47±0,06^{A}	68,89±9,29^{A}	0,83±0,11^{BC}
5 = Fishfarm	3,16±0,47^{A}	12,67±2,02^{B}	0,33±0,05^{B}	48,14±5,58^{B}	0,97±0,06^{A}

Different letters denote statistical differences between groups ($p < 0.05$).

The biometric parameters of groups 1, 2, 3 and 4 did not present relevant statistical differences, with the exception of the FCF. The group 5 presents lower final weight and Biomass difference, and higher FCF values in comparison with the other groups.

The infestation rate determined at the arrival of the fingerlings did not show parasites in all the material analyzed, confirming the good origin of the fingerlings used in the experiment, and ruling out some pre-infection of the fingerlings, which may interfere with the result.

In the present study, the groups treated with levamisole plus alliplus and alliplus present low monogenean levels ($P < 0.05$) of *Trichodina* spp., while the control and levamisole groups presented a high infestation rate ($P < 0.05$) and monogenean levels. The fingerlings of group 5 (fish farming), with the lowest amount of feeding, even using alliplus, were susceptible to moderate infestation by *Trichodina* spp. (Table 2).

Table 2 - Mean ± standard deviation of Infestation levels (monogenetic and *Trichodina* spp) and body condition index (BCI) of *O. niloticus* fingerlings from the Gift lineage subjected to different treatments for 30 days.

Groups	Infestation levels		BCI
	Monogeneans	*Trichodina* spp.	
1 - control	+	5,2±0,02^{A} - High	0,2080±0,02
2 - levamisole	-	4,2±0,05^{A} - Moderate	0,2134±0,03
3 - levamisole/alliplus	-	1,25,2±0,30^{B} - Low	0,2046±0,02
4 - alliplus	-	1,30,2±0,50^{B} - Low	0,2147±0,04

5 - fish farm	-	3,30,2±0,50^{B}	- Moderate	0,1907±0,01

Different letters denote statistical differences between groups ($p < 0.05$).

The body condition index (BCI) is measured through the total length (TC) and weight of the fish, and shows us the body condition that these fingerlings are in. Since values greater than 0.16 show us fish with correct nutritional management. The present study shows that the fish of all groups were in good body condition (Table 2).

DISCUSSION

The study was conducted in cages installed in the Três Marias dam. To meet this production pressure at high densities, the GIFT strain was selected. This choice is due to the fact that this strain has a better carcass yield, and is currently one of the most genetically improved tilapia species for production. It demonstrates higher conversion and growth rates than other strains (Santos, 2012).

In order to achieve the expected production and mortality levels within the standard, experimental studies have shown that the survival of fingerlings is higher at temperatures between 28 °C and 30 °C and lower at temperatures between 20 °C and 24 °C (Maciel Junior, 2006). The mean temperatures of 23.64 °C ± 0.74 in the morning and 24.69 ºC ± 0.92 in the afternoon recorded in the present study may explain the mortality rate recorded: 1937 = 8.7% fingerlings in the cages of group 1 (control), 2025 = 9.1% of group 2, 1867 = 8.4% of group 3, 2000 = 9% of group 4 and 1132 = 7.3% of group 5 of fish farming.

As tilapia are diurnal fish (Loures et al., 2008), in order to obtain homogeneity of the fingerlings, the feeding management occurred during the sunny part of the day, with distribution of the diet at different times of the day, obtaining greater homogeneity of the fingerlings and lower feed conversion in groups 1 , 2, 3 and 4 that were fed 6 times a day when compared to fish farms (group 5) that were fed 3 times a day. To avoid losses and increase in the FCF, the use of pockets was necessary since the fingerlings and the feed granules were very small, so that there was no escape of the fish and loss of feed that would pass through the meshes of the cages (Sussel, 2008; Carvalho, 2010).

In commercial fish farming systems, one of the key aspects is proper feeding management. In this study, final weight showed no statistically significant differences between treatments, except for group 5, which had the lowest final weight. Similarly, FCF

showed significant differences between treatments, being higher in group 5. This demonstrates that proper feeding management directly influences the commercial success of the fish farming system, requiring studies to determine the maximum feed intake that the system can support for every species and different life stages (Ali et al., 2010; Omasaki et al., 2017).

The levamisole and alliplus have already known effects on fish species. Fish exposed to levamisole chronically can present hematological and immunological effects (Oliveira et al., 2019). Also, the allicin, presents immunostimulant and antioxidant effects in fish improving rates on survival, growth, antioxidant capacity, innate immunity and expression of inflammatory and appetite related genes (Huang et al., 2020; Hamed et al., 2021).

The use of specific treatments, such as the combination of levamisole plus alliplus and only alliplus, coinciding with our results, contributed to the control of *Trichodina* spp. in fish. Our results showed a decrease in the intensity of parasite infection, resulting in better health and performance indexes in tilapia. On the other hand, the use of only levamisole showed a high infestation rate in our study although it is commonly used in the treatment of *Trichodina* spp. (Athanassopoulou et al., 2009).

Alliplus has been poorly studied for the control of parasitosis in fishes, since it is a potential food supplement based on garlic extract enriched with allicin, a substance known for its antimicrobial and antiparasitic properties (Valenzuela-Gutiérrez et al., 2018). This can be observed since alliplus contains in its formulation allicin, the active ingredient of garlic, and other natural products essential fatty acids that in addition to stimulating the appetite of fish (Silva, 2000), also serve as protectors against bacteria and protozoa, as observed in this study.

Recent research has shown promising results with the use of allicin in combating different parasites that affect fishes (Reda et al., 2024; Rosny et al., 2016). Our study showed that supplementation with allicin resulted in a reduction in parasite load and an improvement in the health status of fish. In addition, it was observed that tilapia fed with allicin showed increased immune activity, which contributed to resistance to parasites. This indicates that alliplus not only acts directly against parasites, but also strengthens the immune system of the fish, making them better able to fight parasitic infections. The use of alliplus can be considered a natural and effective alternative in the control and prevention of parasitosis in tilapia, reducing the need for chemicals that are aggressive to the environment and animal health.

These results show the efficacy of levamisole plus alliplus and alliplus as an antiparasitic agent for tilapia farming, contributing to the reduction of the negative impacts caused by parasitic infestations on fish. The proper use of this drug, following the recommendations of dosage and treatment time, may be a promising strategy in the sanitary management of tilapia in cages. However, the alliplus group showed positive results, even without the use of levamisole, which suggests the efficacy of other measures adopted in this group.

CONCLUSIONS

Lower feed conversion was observed in fingerlings submitted to treatments with levamisole and alliplus with 6 feeds per day when compared to fish fingerlings, group 5, which received only 3 feeds per day.

The fingerlings submitted to treatment with alliplus, 50 ml per 25 kg of feed, showed a lower infestation rate than that of the fingerlings submitted to the other treatments. The feeding management with a higher number of feeds during the day showed promising results in relation to FCF, homogeneity of the flock and growth of the fingerlings and levels of infestation. The results of the present study provide subsidies for the producer to obtain better working conditions with more attractive cost and benefit in the rearing of tilapia fingerlings.

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