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Short Communication

EFFECT OF POULTRY LITTER AS AN ORGANIC FERTILIZER ON WATER QUALITY, PARASITIC ABUNDANCE, AND GROWTH OF NILE TILAPIA*

Lidiany Doreto CAVALCANTI[1]*
Élida Jeronimo GOUVEIA[1]
Emanuelly Costa Ventura SOUZA[1]
Juliana Rosa CARRIJO-MAUAD[1]
Márcia Regina RUSSO[1]

[1] Universidade Federal da Grande Dourados – UFGD, Faculdade de Ciências Biológicas e Ambientais, Núcleo de Pesquisa em Aquicultura - NUPAQ. Rodovia Dourados-Itahum km 12, Caixa Postal 364, 79.804-970, Dourados, MS, Brazil. lidianydoretto@hotmail.com (*corresponding author).

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ABSTRACT

Nile tilapia feed on plankton in natural environments. This food source can be increased in ponds through fertilization and can reduce feed expenses or improve fish performance when used as supplementary food. Organic fertilization is an alternative to commercial fertilization; however, its use increases concerns regarding water quality and sanitary aspects. This study aimed to evaluate the effects of the use of poultry litter as organic fertilizer on the physical and chemical parameters of water, gill ectoparasite metazoan abundance, and growth of Nile tilapia (*Oreochromis niloticus*) during the culture cycle lasting 240 days. Four earthen ponds, two fertilized with poultry litter and two non-fertilized ponds, as fertilized and non-fertilized treatments, respectively, were used. Stocking density was 3 fish per m^2 with an average initial weight of 0.64 ± 0.15 g. The mean water quality values and the growth performance parameters of Nile tilapia did not show significant differences between the two treatments. The gill parasites found in the fish belonged to the class Monogenea, comprising two genera, *Cichlidogyrus* and *Scutogyrus*, with significant differences between treatments. The non-fertilized treatment showed a high abundance of parasites throughout the culture cycle months, with peak abundance in the months with low concentrations of dissolved oxygen in the water.

Keywords: ectoparasite; immunonutrition; integrated farming; plankton; fish farming.

EFEITO DA CAMA DE FRANGO COMO FERTILIZANTE ORGÂNICO NA QUALIDADE DA ÁGUA, ABUNDÂNCIA PARASITÁRIA E CRESCIMENTO DE TILÁPIA DO NILO

RESUMO

A tilápia do Nilo se alimenta de plâncton em ambientes naturais. Esta fonte de alimento pode ser aumentada em tanques por meio da fertilização e pode reduzir os gastos com alimentação ou melhorar o desempenho dos peixes quando usada como alimento suplementar. A fertilização orgânica é uma alternativa ao uso de fertilizantes comerciais, porém aumenta a preocupação com a qualidade da água e aspectos sanitários. O estudo teve como objetivo avaliar os efeitos do uso de cama de frango como fertilizante orgânico nos parâmetros físicos e químicos da água, na abundância de metazoários ectoparasitos branquiais e no crescimento da tilápia do Nilo (*Oreochromis niloticus*) durante o ciclo de cultivo, com duração de 240 dias. O experimento foi realizado em quatro tanques escavados, dois fertilizados com cama de frango e dois tanques não fertilizados, denominados: tratamento fertilizado e não fertilizado, com densidade de estocagem de 3 peixes por m^2 e peso inicial médio de 0,64 ± 0,15 g. As médias dos parâmetros da qualidade da água e o desempenho da tilápia do Nilo não apresentaram diferenças significativas entre os tratamentos. Os parasitos branquiais encontrados nos peixes pertenciam à classe Monogenea, composta por dois gêneros, *Cichlidogyrus* e *Scutogyrus*, mostrando diferenças significativas entre os tratamentos. O tratamento não fertilizado apresentou maior abundância de parasitos ao longo dos meses do ciclo de cultivo, com pico de abundância nos meses de menor concentração de oxigênio dissolvido na água.

Palavras chaves: ectoparasito; imunonutrição; agricultura integrada; plâncton; piscicultura.

INTRODUCTION

Aquaculture is an expanding activity in Brazil, and the main species of fish produced in the country is the Nile tilapia *Oreochromis niloticus*, being the second most farm-produced fish in the world (FAO, 2018; PeixeBR, 2019). The preference for this species in fish farming is due to characteristics such as rusticity in relation to climatic and sanitary conditions, easy reproduction, high survival of fry, and good acceptance in the consumer market (Braccini et al., 2013; Kumar et al., 2019).

Furthermore, the omnivorous filtering eating habits of Nile tilapia indicate a wide consumption of plankton, thereby reducing feed expenses, which can vary between 30% and 60% (Figueredo and Giani, 2005; FAO, 2010). The input of natural plankton in fish farming as a complementary food improves fish performance and is a source of vitamins, lipids, and amino acids, which are commonly used additives in semi-intensive systems (Adebayo et al., 2004; Zahid et al., 2013). In this context, a balanced diet has a positive impact on the health and functioning of the immune system (Kiron, 2012; Martin and Król, 2017).

The addition of nutrients (mainly nitrogen and phosphorus), denominated fertilization, increases plankton in the production environment (Dhawan and Toor, 1989; Haobijam and Ghosh, 2018). Fertilizers may originate from inorganic or organic sources (Kumar et al., 2005). Organic fertilization is an alternative to the use of commercial products and uses resources available from other animal farming operations, such as poultry litter from poultry farming (Kaatz et al., 2011). However, its use raises concerns regarding water quality and sanitary aspects (Garg and Bhatnagar, 1999; Deka et al., 2018).

Changes in water quality favor the emergence of pathogens, such as parasites (Hossain et al., 2011; Sathish et al., 2021). Parasites are considered the gateway for other diseases, such as those caused by bacteria and fungi, which may compromise growth and even cause fish death (Xu et al., 2007; Pádua et al., 2012). Therefore, this study aimed to evaluate the effects of the use of poultry litter as an organic fertilizer on the physical and chemical parameters of water, gill ectoparasite metazoan abundance, and growth of Nile tilapia (*O. niloticus*) in earthen ponds.

MATERIALS AND METHODS

Study area and management

The study was conducted in a small fish farm in a semi-intensive aquaculture system, located in Laguna Carapã (22º30'13.6″S - 55º06'53.3″W), Mato Grosso do Sul, Brazil. Organic fertilizer is commonly applied in the cultivation of Nile tilapia on this farm, and this management was followed in the previous production cycles. Four earthen ponds (176 m^2) with independent water inlets from a single stream near the farm were used as experimental units, with average daily water renewal of 30%. Two treatments – fertilized and non-fertilized ponds – with two replicates each were established. The number of earthen ponds used in this study was based on availability at the fish farm.

Before stocking of Nile tilapia fingerlings, the ponds were disinfected with 20 kg of lime. One week later, two ponds were randomly selected and fertilized with 18 kg of poultry litter. The organic fertilizer remained exposed to sunlight for two months before utilization, and all ponds were subsequently filled with water to begin the culture. Notably, both treatments received fingerlings from the same batch stocked at the same density, which was subjected to the same extruded commercial diet (42% to 32% crude protein). Fish were fed up with four times a day, and the quantity offered was according to the biomass in the pond, as recommended by the manufacturer, and the daily handling was adjusted to avoid losses. The only difference between the treatments was the application of organic fertilizer.

The fingerlings were stocked in October 2017, with an average weight of 0.64 ± 0.15 g and a density of 3 individuals per m^2. The duration of the culture was 8 months, from October 2017 to May 2018. In the fertilized treatment, two additional organic fertilizer applications (18 kg of organic fertilizer each) were performed, one in late October and the other in early December. Fertilization occurred only in the initial months of production, as fish consume more plankton in the early stages, as reported by Moreira et al. (2012). According to Mo et al. (2014), fish feces and the remains of uneaten feed help to fertilize the ponds. Therefore, organic fertilizer was not applied in the consecutive months of production to avoid eutrophication of the system.

Analysis of the physical and chemical parameters of water

The physical and chemical parameters of the water were measured monthly (samples were collected between 8:00 and 9:00 am). Temperature, dissolved oxygen, pH, and electrical conductivity were measured using the Hanna HI9829® multiparameter probe, and transparency was measured using a Secchi disk.

The amount of organic matter in the ponds was also measured monthly according to the fluorescence intensity (Figueiró et al., 2018). For this, pond water samples were collected and stored at 10°C (Agra et al., 2012). Fluorescence intensity was measured using a Cary Eclipse (Varian) spectrophotometer. Measurements were recorded within an excitation wavelength range of 200-450 nm and an emission wavelength range of 200-700 nm with 5 nm intervals in the excitation domain.

Sampling of fish, parasites, and fish performance parameters

Fish were sampled monthly, totaling eight collections, from stocking to harvest, using a casting net. Forty fish were sampled per collection in the initial months of cultivation (October and November) and 20 in the other months (December to May), totaling 200 fish sampled, 100 per treatment, and 50 per earthen pond. The fish used for the study were additionally stocked at the beginning of the cultivation cycle.

Fish were then transported to the Applied Aquatic Biology Laboratory of the Federal University of Grande Dourados (UFGD) and subjected to a lethal dose of clove oil anesthesia (50 mg L^{-1}), as authorized by the university's ethics committee (Protocol

n. 20/2018 – CEUA/UFGD). Biometry (weight and measurement of total and standard lengths) was performed. The gills were removed to sample the parasites and identification was performed according to Pariselle et al. (2003). Abundance and prevalence were calculated according to the method of Bush et al. (1997).

In the last sampling, the ponds were drained, and all the fish were harvested. They were then counted and weighed, and the following growth performance parameters were calculated: weight gain (final weight - initial weight); feed conversion rate (feed given/weight gain); survival rate (final number of fish/initial number of fish × 100); and feed intake (sum of all the feed given during the culture).

Data analysis

Data were subjected to tests of normality (Shapiro-Wilk) and homoscedasticity (Bartlett). The Student's t-test was applied to verify the differences in the physical and chemical parameters of the water between the fertilized and non-fertilized treatments during the culture cycle. The Kruskal-Wallis test was performed to compare growth performance parameters and mean parasite abundance between treatments during the culture cycle. The posteriori Dunn's test was used where necessary to distinguish between groups. Statistical analyses were performed using R software (R Core Team, 2016).

RESULTS

Effect of organic fertilizer on water quality parameters

The mean values of the physical and chemical water parameters did not differ significantly between the treatments (p >0.05) (Table 1).

In the monthly analysis (Table 2), the pH and electrical conductivity did not show abrupt fluctuations during the culture, with no significant differences between the two treatments (p >0.05). Transparency differed significantly in October (p = 0.01) and November (p = 0.016), and it was lower in the fertilized treatment than in the non-fertilized treatment during the beginning of the culture cycle, which was because of the fertilizer applied to the ponds (Table 2).

The dissolved oxygen values oscillated during the harvesting stage, hence, high values were observed during the early months, subsequently decreasing in January, February, and March, and increasing again in April and May (Table 2). The dissolved oxygen levels in the water were similar in both treatments, with significant differences observed in November (p = 0.002), December (p = 0.03), and January (p = 0.03) (Table 2). The fluorescence intensity of organic matter in the first farming month was significantly higher in the fertilized treatment (p = 0.006) than in the non-fertilized treatment probably because of fertilizer application (poultry litter). Except for the first month, both treatments presented similar values for this variable (Table 2).

Table 1. Mean and standard deviation of physical and chemical parameters of the water from fertilized and non-fertilized treatments.

Parameters	Fertilized	Non-fertilized
Temp. (°C)	25.11±2.65	25.24±2.63
pH	7.53±0.46	7.86±2.19
Cond. (µS m^{-1})	0.068±0.010	0.064±0.006
Trans. (cm)	50.85±15.48	55.18±20.42
DO (mg L^{-1})	4.56±1.90	4.84±1.21
OM (nm)	168.62±23.49	148.10±32.31

Temp. = Temperature; Cond. = Electrical conductivity; Trans. = Transparency; DO = Dissolved oxygen; OM = Organic matter fluorescence intensity.

Table 2. Physical and chemical parameters of the water sampled monthly from the fertilized and non-fertilized ponds.

	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Fertilized								
DO (mg L^{-1})	6.5±0.2	**6.6±0.1**	**5.1±0.05**	**3.1±0.08**	2.4±0.1	2.6±0.3	4.1±0.2	4.3±0.2
pH	7.9±0.02	6.9±0.08	6.9±0.03	7.2±0.05	7.6±0.1	7.7±0.05	7.7±0.01	7.6±0.03
Temp. (°C)	26.6±0.1	25.5±0.1	27.1±0.02	28.9±0.05	25.7±0.1	25.6±0.08	21.5±0.2	21.1±0.2
Trans. (cm)	**52.9±0.3**	**69.6±1.1**	74.1±2.6	43.6±0.72	48.6±1.2	51.0±1.1	32.3±0.7	32.2±1.1
Cond. (µS m^{-1})	0.08±0.005	0.06±0.003	0.05±0.003	0.06±0.002	0.06±0.001	0.07±0.002	0.05±0.001	0.05±0.001
OM (nm)	**209±6.4**	142±10.3	159±7.8	144±7.4	180±3.1	149±3.8	181±1.02	-------
Non-fertilized								
DO (mg L^{-1})	7.1±0.1	**7.4±0.06**	**4.1±0.1**	**4.0±0.1**	2.5±0.06	2.8±0.2	4.2±0.1	4.3±0.1
pH	7.9±0.09	7.3±0.06	6.9±0.04	7.0±0.03	7.6±0.02	7.6±0.03	7.7±0.01	7.7±0.07
Temp. (°C)	26.6±0.1	25.9±0.1	27.4±0.06	29.1±0.1	25.5±0.05	25.6±0.2	21.4±0.2	21.5±0.1
Trans. (cm)	**66.7±1.5**	**83.0±0.2**	76.5±1.5	49.3±1.1	50.7±0.5	50.6±1.1	26.5±2.9	26.3±1.1
Cond. (µS m^{-1})	0.07±0.004	0.06±0.002	0.06±0.002	0.06±0.001	0.06±0.001	0.06±0.001	0.05±0.001	0.05±0.001
OM (nm)	**88±8.3**	130±3.5	151±9.5	126±0.2	193±7.2	156±8.9	169±4.8	-------

DO = Dissolved oxygen; Temp. = Temperature; Trans. = Transparency; Cond. = Electrical conductivity; OM = Organic matter fluorescence intensity. Oct = October; Nov = November; Dec = December; Jan = January; Feb = February; Mar = March; Apr = April; May = May. The bold indicates significant differences between treatments ($p \leq 0.05$), (---) not analysed.

Effects of organic fertilizer on parasite indices during culture

Regarding parasites, 8,737 individuals were recorded, 2,945 in the fertilized treatment and 5,792 in the non-fertilized treatment, all belonging to the class Monogenea of the genera *Cichlidogyrus* and *Scutogyrus.* The fertilizer treatment (77%) showed a lower prevalence than the non-fertilized treatment (88%).

The mean abundance values of *Cichlidogyrus* and *Scutogyrus* were significantly different between treatments ($p = 0.014$), totaling 29.45 parasites in the fertilized treatment, and 57.92 parasites in the non-fertilized treatment. The monthly abundance data (Figure 1) showed significant differences over time in the culture cycle ($p < 0.05$). From January, there was a sharp increase in the mean abundance of these parasites in the non-fertilized treatment. The peaks occurred in February and March and were significantly different between treatments ($p = 0.02$ February 2018, $p = 0.03$ March 2018). These months also showed the lowest measurements for dissolved oxygen (Table 2).

Effect of organic fertilizer on growth performance parameters

Regarding growth performance parameters (Table 3), both treatments showed survival greater than 70%, and there were no significant differences between treatments for total length ($p = 0.74$), final weight ($p = 0.45$), weight gain ($p = 0.41$), feed conversion rate ($p = 0.39$), and feed given ($p = 0.25$).

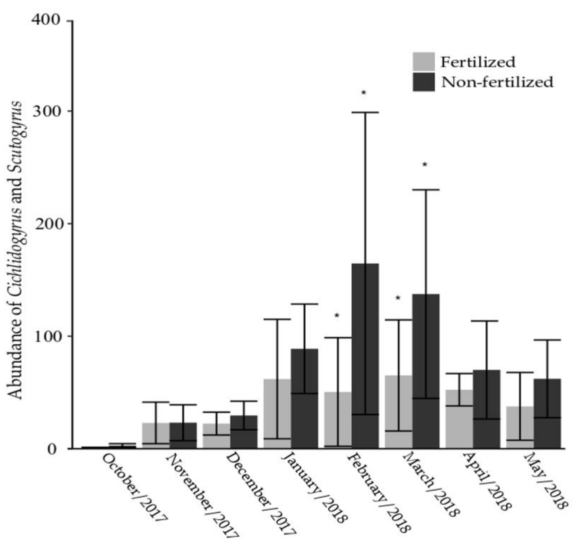

Figure 1. Mean abundance values of *Cichlidogyrus* and *Scutogyrus* in fertilized and non-fertilized treatments, evaluated monthly during the production cycle of the Nile tilapia *Oreochromis niloticus*. Asterisks (*) indicate significant differences between treatments. Number of fish evaluated = 200.

Table 3. Mean and standard deviation of the productive performance of Nile tilapia *Oreochromis niloticus* in fertilized and non-fertilized treatments.

Parameters	Fertilized	Non-fertilized
TL (cm)	30.64±1.98	31.33±1.63
WF (g)	569.15±63.68	557.70±78.74
WG (g)	563.05±62.12	554.26±61.05
S (%)	80.02±0.03	77.52±0.01
FI (kg)	1,037.73	1,128.21
FCR	1.73±0.54	2.04±0.42

TL = total length; WF = final weight; WG = Weight gain; S = Survival rate in %; FI = Feed intake; FCR = Feed conversion rate

DISCUSSION

In this study, the mean values of the physical and chemical parameters of water in both the treatments applied were within the range recommended for tropical fish farming (Boyd and Tucker, 1998). Analysis during the culture showed that the transparency of the water decreased sharply in both treatments during the last two months. This parameter reflects the primary productivity of the aquatic environment, low transparency (below 25 cm) indicates a high level of organic matter, whereas high transparency (above 80 cm) may result in damage to the fish due to solar radiation (Macedo and Sipaúba-Tavares, 2010).

In this study, dissolved oxygen levels fluctuated with similar responses for both treatments, showing high values at the beginning of cultivation, falling in the months of February and March, and increasing in April and May, possibly due to a sharp decrease in temperature (-4°C between March and April). Higher dissolved oxygen concentrations have been reported at lower temperatures (Esteves, 1998; Bhatnagar and Garg, 2000). In the final months of the culture, when there is an increase in biomass, biological activity, excrement (such as feces), and feed leftovers in the ponds, the oxygen demand increases and its level tends to decrease together with transparency (Baccarin and Camargo, 2005; Mungkung et al., 2013).

Dissolved organic matter is naturally present in water bodies, varying in intensity and origin, being autochthonous (originating from excreta, and animal and plant remains) or allochthonous (owing to rain carrying nutrients into water bodies) (Mcintyre and Guéguen, 2013). In the case of fish farming, organic matter is also the product of the provision of feed and organic or inorganic fertilizers (Martins et al., 2007). In this study, significant differences in organic matter fluorescence intensity were observed only in the first month, and in the subsequent months, the intensity was very similar between treatments, indicating that the source of autochthonous organic matter was possibly greater than that generated by organic fertilization.

Regarding parasites, *Cichlidogyrus* and *Scutogyrus* are commonly reported to parasitize Nile tilapia, and prevalence values above 70% are also frequently described in fish farms (Akoll et al., 2012; Zhang et al., 2019). In this context, the parasite transmission form is essential for its establishment and success, and characteristics such as a monoxenous life cycle and fish density in the aquaculture system favor the establishment and predominance of the monogeneans, being the most relevant parasite in fish farming (Jerônimo et al., 2011).

In the production system, a decrease in water quality, excessive handling, and nutritional deficiency result in a stress condition for fish, which consequently impairs the immunological system and makes fish prone to disease outbreaks (Lanes et al., 2012; Reynolds et al., 2019). In general, monogeneans infestations in the gills can cause lamellar hyperplasia and difficulty in gas exchange, leaving the host more vulnerable to severe mixed infections (fungi, bacteria, and parasites) affecting growth and can lead to death (Dezfuli et al., 2007; Pádua et al., 2016). Xu et al. (2007) reported that *O. niloticus* parasitized by monogenean *Gyrodactylus niloticus* showed higher mortality following exposure to the bacteria *Streptococcus iniae* than fish without parasites.

Even without significant differences in growth performance parameters between treatments, the low parasitic infestation in the months of low concentration of dissolved oxygen in the fertilized treatment was a satisfactory result. In short, considering the parasite-host relationship, even if immediate damage is not caused, some level of damage is inflicted on the host owing to injuries caused by the fixation of the parasite, or direct competition for nutrients (Jerônimo et al., 2014; Matos et al., 2017).

Thus, diet plays an important role in fish development and may influence hematological parameters (Landolt, 1989; Cyrino et al., 2010). Nutritional deficiency may impair the immune system and response to pathogens (Kiron, 2012). In this sense, plankton are a good nutritional source of proteins, lipids, and minerals, and can be an additional, cost-free food that improves fish performance (Green et al., 1989; Rosa et al., 2014).

Previous studies have assessed the relationship between the immune system and feeding termed as immunonutrition (Adeshina et al., 2021; Noor et al., 2021). For example, Gopalakannan and Arul (2006) showed the positive effects of food supplementation with chitin, chitosan, and levamisole on growth and immune response in *Cyprinus carpio,* against bacterial infections of *Aeromonas hydrophila*, the authors also noted that chitin is present in plankton organisms such as copepods, highlighting the fundamental role of food in the health and well-being of fish.

In this study, the fertilized treatment showed low parasitic abundance without spikes in the months of decreased water quality, as occurred in the non-fertilized treatment. Moreover, it is important to note that the physical and chemical parameters of water did not differ significantly between treatments, excluding being the cause of differences in parasitic abundance. Considering these results, plankton may have played a role in improving the performance of the immune system in the fertilized treatment, however, to verify this, further studies are required on the effects of natural plankton on the immune system and resistance to parasites.

CONCLUSIONS

There were no significant differences between the treatments in growth performance parameters and water quality parameters. However, the low mean abundance and the prevalence of *Cichlidogyrus* and *Scutogyrus* in the fertilized treatment indicate satisfactory results with the use of poultry litter organic fertilizer in the initial phases of production in semi-intensive aquaculture systems, and its use is recommended in similar farming conditions.

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