Research, Society and Development, v. 9, n. 11, e1319119699, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i11.9699

Nitrogen and phosphorus dynamics in Nile tilapia farming in excavated rearing ponds

Dinâmica de nitrogênio e fósforo no cultivo de tilápia do Nilo em tanques escavados

Dinámica del nitrógeno y el fósforo en el cultivo de tilapia del Nilo en estanques de cría excavados

Received: 10/28/2020 | Reviewed: 11/02/2020 | Accept: 11/03/2020 | Published: 11/08/2020

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Abstract

The purpose of this study was to determine the total nitrogen (TN) and total phosphorus (TP) balance during intensive tilapia farming in excavated ponds. To quantify TN and TP released into the environment, the supply water, effluents at the harvest time, fish composition, feed, and sediment were analysed. The mass balance between the amount of nutrients that is inserted through the feed, which is transformed into biomass by the fish and is retained in the sediment was calculated based on dry matter. The nutrient load arriving from the supply water was calculated as a function of the concentration of TN and TP. The TN and TP dynamics during the harvesting process in three different pond sizes identified that, on average, 2.37% of TN and 2.05% of TP inserted into the system during rearing is eliminated with 10.64% TN and 37.01% TP are retained in the sediment. The TN and TP input into the system occurs through the water supply, young fish, and the feed, the latter being responsible for about 92.87% TN and 96.05% TP. The feed composition indicates that the P level of the food is above the nutritional recommendations for the species. The amount of TP accumulated in the sediments indicates that there is a need for good management practices for water quality during the rearing and sediment management period before the beginning of a new production cycle.

Keywords: Excavated ponds; Effluent; Harvest; Water quality.

Resumo

O objetivo deste estudo foi determinar o balanço de nitrogênio total (TN) e fósforo total (TP) durante o cultivo intensivo de tilápia em tanques escavados. Para quantificar o TN e o TP liberados no meio ambiente, foram analisados a água de abastecimento, os efluentes na época da colheita, a composição dos peixes, a ração e os sedimentos. O balanço de massa entre a quantidade de nutrientes que é inserida na ração, que é transformada em biomassa pelos peixes e fica retida no sedimento foi calculada com base na matéria seca. A carga de nutrientes proveniente da água de abastecimento foi calculada em função da concentração de TN e TP. A dinâmica de TN e TP durante o processo de colheita em três diferentes tamanhos de tanques identificou que, em média, 2,37% de TN e 2,05% de TP inseridos no sistema durante a criação são eliminados com 10,64% de TN e 37,01% de TP são retidos no sedimento. A entrada de TN e TP no sistema ocorre por meio do abastecimento de água, peixes jovens e ração, sendo esta última responsável por cerca de 92,87% TN e 96,05% TP. A composição da ração indica que o nível de P do alimento está acima das recomendações nutricionais para a espécie. A quantidade de TP acumulada nos sedimentos indica que há necessidade de boas práticas de manejo da qualidade da água durante o período de criação e manejo dos sedimentos antes do início de um novo ciclo de produção.

Palavras-chave: Tamques escavadas; Efluente; Despesca; Qualidade de água.

Resumen

El propósito de este estudio fue determinar el balance de nitrógeno total (TN) y fósforo total (TP) durante el cultivo intensivo de tilapia en estanques excavados. Para cuantificar TN y TP liberados al medio ambiente, el suministro de agua, efluentes en el momento de la cosecha, peces Se analizaron la composición, la alimentación y los sedimentos. El balance de masa entre la cantidad de nutrientes que se inserta a través del alimento, que es transformado en biomasa por los peces y es retenido en el sedimento se calculó en base a la materia seca. La carga de nutrientes proveniente del suministro de agua se calculó en función de la concentración de TN y TP. La dinámica de TN y TP durante el proceso de cosecha en tres tamaños de estanque diferentes identificó que, en promedio, el 2,37% de TN y el 2,05% de TP insertados en el sistema durante la cría se eliminan con 10,64% de TN y el 37,01% de TP se retienen en el sedimento . La entrada de TN y TP al sistema se produce a través del suministro de agua, los peces jóvenes y el alimento, siendo este último responsable de aproximadamente el 92,87% de TN y el 96,05% de TP. La composición del pienso indica que el nivel de P del alimento está por encima de las recomendaciones nutricionales para la especie. La cantidad de TP acumulada en los sedimentos indica que existe la necesidad de buenas prácticas de manejo de la calidad del agua durante el período de cría y manejo de sedimentos antes del inicio de un nuevo ciclo de producción.

Palabras clave: Estanques excavados; Efluente; Cosecha; Calidad del agua.

1. Introduction

Aquaculture and fishing are important sources of food and income for millions of people worldwide (FAO, 2018). Aquaculture activity has shown significant growth in recent years in the production of a healthy and nutrient-rich food (Osti et al., 2017). As is the case with any agricultural activity, the search for environmental sustainability is ongoing. Irrespective of the form of production, the environmental impact exists and may be of greater or lesser intensity due to the rearing system used (Boyd, 1999).

For intensive fish farming in excavated ponds, the food provided to the fish in the form of feed is rich in nutrients and consumed in large quantities. Intensive farms are preferably established as a monoculture, and unused food waste or residues are not utilised by other fish species, generating an accumulation of organic matter that can be physically, chemically, or biologically assimilated into the system or disposed of through the effluents (Boyd et al., 2007).

The main environmental impact of aquaculture occurs through the loss to the environment of effluents that are rich in nitrogen (N) and phosphorus (P), generally considered the main causes of eutrophication (GREEN et al., 2002; Tucker & Hargreaves, 2009; Boyd & McNevin, 2015). Thus, the release of aquaculture effluents into natural aquatic environments, when rich in N and P, may have impacts on the environment, as they are directly related to the primary production of algae (Cyrino et al., 2010). One of the main causes of eutrophication and degradation of water quality worldwide is the discharge of phosphorus (P) into the environment (Sibrell & Tucker, 2012; Sibrell & Kehler, 2016).

Studies have shown that some of the nutrients in food are converted into biomass by fish. In tilapia farms, 23-26% total nitrogen (TN) and 40-45% total phosphorus (TP) in the feed are converted by fish into biomass (Boyd et al., 2007; Osti et al., 2017). For Norwegian salmon, these percentages are equivalent to 38% and 30%, respectively, for TN and TP (Wang et al., 2012). The recovery of nutrients offered in the feed in fish biomass is related to the quality of the food provided and the good practices applied during rearing.

In this sense, intensive tilapia farming in excavated ponds should be associated with good management practices so that nutrients do not accumulate in the ponds with ultimate release into the environment. A critical management stage carried out during farming is the harvest. This process, due to high fish densities and sediment suspension, promotes a significant increase in TN and TP, which worsen the quality of the effluent from the environmental discharge (Coldebella et al., 2018).

Discharges of nutrients into the environment from aquaculture are being studied. Bouwman et al. (2013), developed in 2010 an estimate and projections of nutrients released into the environment through aquaculture and found that global freshwater aquaculture alone released 5 million tonnes of N and 0.9 million tonnes of P. Despite concluding that the contribution of freshwater aquaculture to the accumulation of nutrients in rivers is still small, they point out that the rapid growth of the activity is a major anthropogenic source of N and P for rivers in various parts of the world. The continued growth of aquaculture activity depends on control strategies to minimise environmental impacts (Alexander et al., 2016).

The geometric and fluid mechanical aspects of ponds may also influence the accumulation of nutrients during rearing. Flow and speed of the water were evaluated in the elimination of residues produced in trout farming (Watten et al., 2000), the length-to-width ratio was evaluated in rectangular ponds with central discharge (Oca & Masaló, 2007), and different points of water entry used to avoid dead zone formation (Oca et al., 2004). The size and shape of ponds used in tilapia farming are not standardised, and specific studies on the influence of pond size on nutrient cycling during production have not been found.

Fish harvesting is the process of removing the fish from the breeding environment when they reach the appropriate slaughter weight, which can be total or partial. A trawler is used manually when the water level of the pond is 50% of its total water capacity, the net is passed through the pond several times while the water is drained, this process stirs the sediment at the bottom of the pond, rich in N and P, and therefore causes the worsening of the quality of the effluent released into the environment (Mardini & Mardini, 2006).

Due to the impacts N and P on the enviroment, it is necessary to quantify these elements released into the environment by Nile tilapia (Oreochromis niloticus) in a pond excavated for farming. The objective of this work was to characterize the water supply, the wastewater effluents, the sediment, feed, and fish in three pond size classes on how production practices affect the release of nitrogen and phosphorus into the environment.

2. Materials and Methods

2.1 Study area

The study was conducted in excavated ponds in the western region of the state of Paraná, Brazil, characterised as a hub for tilapia production (*Oreochromis niloticus*) in excavated ponds (Sussel, 2013). In this region, several tilapia processing units are installed, operating with an integration system.

Twelve excavated ponds from nine rural farms belonging to an integrated production system were evaluated from June to October 2016 where, as well, are shown geographic position (please see Figure 1). All ponds were filled with water from near creeks and the inlet water quality average is shown in Table 1. The integrated production system is standardized for all producers and established as conditions to start the creation of the absence of chemical or organic fertilization to stimulate primary production. Young fish were stocked with a mean weight of 20 ±0.08 g, stocking density of 5.0 fish m^{-2}, average slaughter weight of

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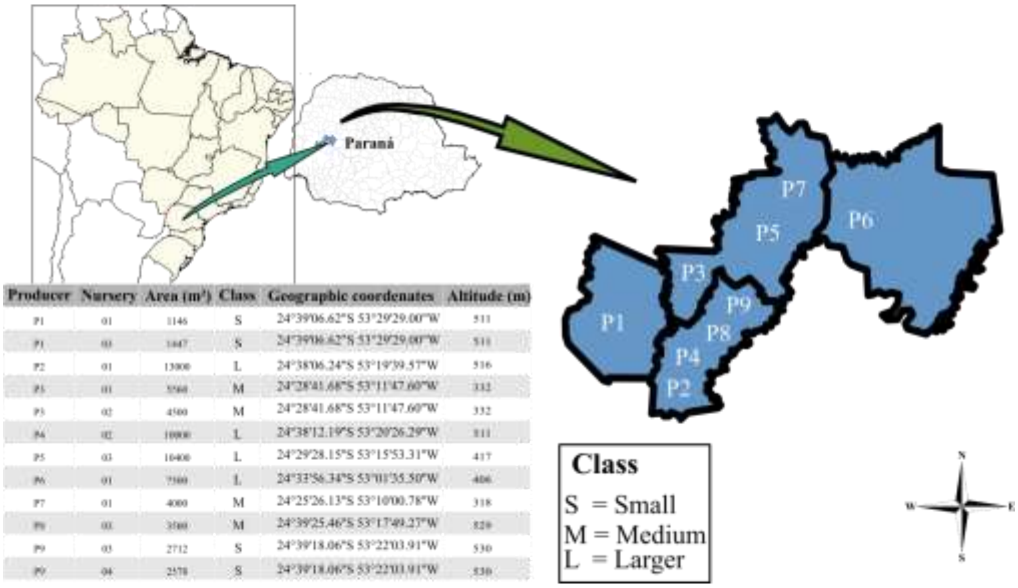
approximately 0.9 kg, and cycle duration of 200 days. Feeding was carried out with balanced feed supplied by the integration system with 32% crude protein (CP) supplied to the fish 3 times a day. Mechanical aeration was used mainly at night with paddle or fountain aerators in the proportion of 1.0 horsepower per 4,500 fish, water renewal rate of 1-10% per day and average pond depth of 1.5 m. The fishing process varies from 24 to 36 hours depending on the size of the pond. This time is counted from the beginning of the drainage to the total removal of the fish.

This study took into account an area of one ha, corresponding to 10,000 m^2 and depth average of 1.5 m, being the standard depth in the region. In this way, it was possible to calculate the eutrophic nutrients loads released by the fish farming effluent in one hectare, and it may later be possible to estimate values for a small or larger area.

Figure 1. Map of Brazil showing the location and area of the ponds and the locations where water was sampled.



Producer	Nursery	Area (m^2)	Class	Geographic coordenates	Altitude (m)
P1	01	1146	S	24°39'06.62"S 53°29'29.00"W	511
P1	02	1447	S	24°39'06.62"S 53°29'29.00"W	511
P2	01	13000	L	24°38'06.24"S 53°19'39.57"W	516
P3	01	5500	M	24°28'41.68"S 53°11'47.60"W	332
P3	02	4500	M	24°28'41.68"S 53°11'47.60"W	332
P4	02	10000	L	24°38'12.19"S 53°20'26.29"W	511
P5	03	10400	L	24°29'28.15"S 53°15'53.31"W	417
P6	01	7500	L	24°33'56.34"S 53°01'35.50"W	406
P7	01	4000	M	24°25'26.13"S 53°10'00.78"W	318
P8	02	3500	M	24°39'25.46"S 53°17'49.27"W	520
P9	03	2712	S	24°39'18.06"S 53°22'03.91"W	530
P9	04	2578	S	24°39'18.06"S 53°22'03.91"W	530

Source: Prepared by the authors (2020).

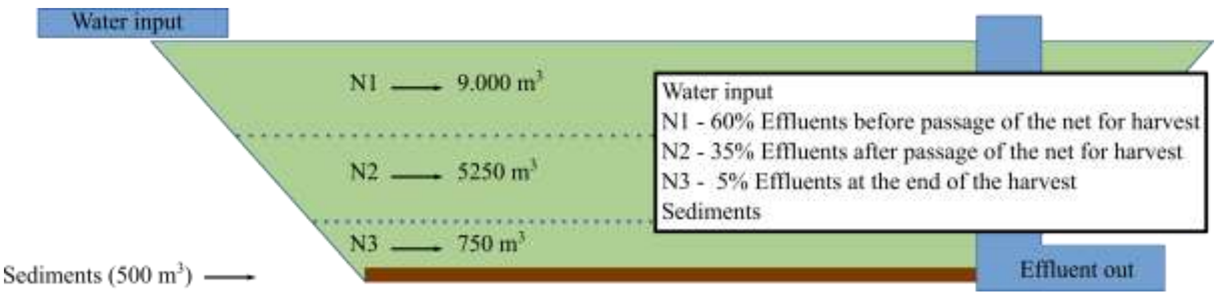
2.2 Sampling and analysis of water supply and effluent

For the study, sampled ponds were divided into three size classes with 4 replicates each. Class I ponds had an area of up to 3,000 m^2 (P), Class II ponds had an area of 3,001-7,000 m^2 (M); and Class III ponds had an area greater than 7,000 m^2 (G) (Figure 1). Size classes were defined to assess whether pond size interferes with the dynamics of nitrogen and phosphorus behaviour within ponds. All evaluated properties used spring water as source of supply for the ponds. Samples of this water was collected, and the same analyses were carried out for the effluents.

The harvesting system consists of draining 40% of the water from the pond and starts the passage of the fishing net to catch the fish. To assess how much the sediments are moved by the passage of the fishing net, the analyses were performed at levels N1, N2 and N3 (Figure 2). In this way, it is possible to evaluate the quality of the effluents discharged into the environment as the volume of water inside the pond decreases.

The effluent samples for quantification of phosphorus and nitrogen compounds were collected during the spreading process at three different times, defined as “levels” within the same collection point, thus being considered as repeated measurements within the same site. The levels were characterised as N1, N2, and N3 (Figure 2). Among the samples in the N1 level, there was no interaction with nets, only effluent drainage; N2 level collection was performed after the first pass of the fishing net; and the N3 level corresponded to the end of the harvest, with approximately 5% of the total remaining water volume in the pond.

Figure 2. Scheme for collection of effluents from the harvest, sediment at the pond bottom and volume (L) in each effluent collection scenario, considering 1.0 hectare of water area and an average depth of 1.5 m.



Source: Prepared by the authors (2020).

The effluent volumes (in litres), represented in Figure 2, considering one hectare of the water level of 1.5 m (Equation 1) were considered for the calculation of TP and TN released through the effluent at the L1 level. The mean concentration of the compounds from the beginning of the drainage and then again when it reached 60% of the drained volume were collected for sampling. In the same way the nutrient load for the L2 and L3 levels (Equation 2) were calculated.

$$V = A \times D \quad (1)$$

Where, V is the volume of water (m3); A, the pond area (m2); and D, the mean depth (m).

$$C_N = V \times C_n \tag{2}$$

Where, C_N is the nutrient load at a given level (L1, L2, or L3); V is the volume of water (L) of the evaluated level; and C_n, the nutrient concentration in mg L^{-1} (converted into kg).

In order to characterise the nitrogen and phosphorus forms present in the water supply and in the effluents, the following analyses were carried out in the laboratory: Total Phosphorus (TP mg L^{-1}), Soluble Orthophosphate (PO_4^{3-} mg L^{-1}), Nitrite ($NO_2^{\ -}$ mg L^{-1}), Nitrate ($NO_3^{\ -}$ mg L−1), Ammonia Nitrogen (NH_3 mg L^{-1}), and Kjeldahl Nitrogen (TN mg L^{-1}). The analyses were performed as described by the Standard Methods for the Examination of Water and Wastewater (APHA, 2017).

At the time of collection of the effluent samples, the parameters of dissolved oxygen (DO mg L^{-1}), pH, electrical conductivity (EC µS cm^{-1}) and air and water temperature (T°C) were measured using portable YSI Pro20, YSI F-1010PH and YSI F-1030A devices, respectively.

2.3 Collection and analysis of sediment from pond bottoms

After the total drainage of the ponds, a sample of the sediment accumulated at the bottom of the ponds (Figure 2) was collected to quantify the total nitrogen and total phosphorus present in the sediment accumulated during the production cycle. To enable the analysis sample to be representative, a composite sample was obtained by collecting six single samples from the bottom area of the ponds, two near the water inlet, two in the middle of the pond, and two near the water box. The six samples were homogenised in a plastic tray and 500 g of the material were subsequently withdrawn. Sediment collection was superficial from 0-5 cm in depth and was performed using a cutting shovel.

To characterise the sludge, Total Phosphorus (g kg^{-1}) and Kjeldahl Nitrogen (g kg^{-1}) analyses were carried out according to the methodology of APHA (2017), as well as Dry Matter analysis, according to the methodology proposed by AOAC (2016).

To quantify the nutrients that are present in the sediment, an accumulation equivalent to 5 cm of sediment was considered in each cycle, totaling 500 m^3 ha^{-1}, this value was estimated in the mass balance proposed by Boyd & Queiroz (2004) who considers that 79% of the dry matter inserted as feed becomes waste at the bottom of the nursery.

2.4 Collection and analysis of the fish and feed

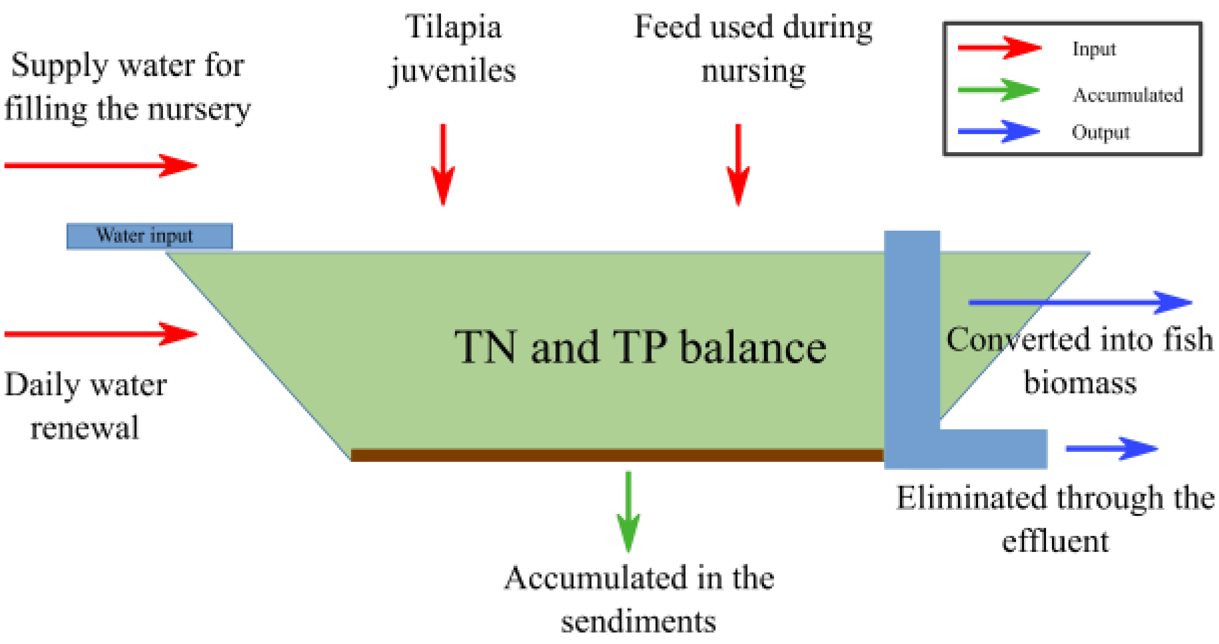
From the time of the first trawl to catch the fish, five specimens of tilapia were collected in each studied pond. The fish were identified by producer, packaged in ice and later analysed at the Food Quality Laboratory (LQA) of the Aquaculture Study and Management Group (GEMAq), of the State University of Western Paraná. Fish samples were also collected along with the feed used for fattening. Both fish and ration were stored in a freezer (-6°C) and subsequently subjected to moisture, crude protein, total lipid and mineral analyses and the percentage of phosphorus, following the methodology proposed by AOAC (2016). At the time of the analysis, the fish were crushed whole without evisceration for the preparation of the samples.

2.5 Nitrogen and Phosphorus flow during the production cycle

The flow of TN and TP was estimated through the input and output balance of nutrients within the system are shown in Figure 3, where are shown the flux of these nutrients. The TN and TP input was considered as the quantity present in the water supply, considering the following as the initial contribution: filling of the ponds; quantity entered through water renewal, with a daily renewal rate of 10% and a production cycle of 200 days, totalling 20 complete renovations during one cycle; quantity of nutrients present in the feed calculated as a function of the dry matter; and the amount present in the young tilapia stored to during fattening. The concentrations found in the analyses carried out on the fish at the time of the harvest were considered for calculation.

The TN and TP outputs considered were the amount eliminated in the effluents during drainage for the harvest, considering each moment of effluent collection as previously described; the TN and TP amount converted into fish biomass at the time of harvest and the amount that accumulates in the bottom sludge of the fish ponds. Fish and sludge TN and TP calculations were performed as a function of the dry matter. The TN and TP mass balance that determines how much of the nutrients inserted into the system is eliminated and/or transformed during farming was calculated by Equations 3 and 4, respectively.

Figure 3. Scheme of total nitrogen and total phosphorus mass balance in the fish pond.



Source: Prepared by the authors (2020).

$$TN = (N_A + N_R + N_{RA} + N_J) - (N_E + N_P + N_S) \qquad (3)$$

Where TN is the total Nitrogen eliminated and/or transformed during fattening (kg); NA, total water supply nitrogen (kg); NRe, total renewal water nitrogen (kg); NRA, total nitrogen introduced through feed; NJ , total nitrogen introduced through the young fish (kg); NE , total nitrogen from the harvest effluents (kg); NP , total nitrogen recovered in fish biomass (kg); and NS , total nitrogen accumulated in the sediment (kg). In this calculation, the increments of atmospheric N or droppings of birds and other animals that could be attracted to the farming ponds were not considered.

$$TP = (P_A + P_R + P_{RA} + P_J) - (P_E + P_P + P_S) \qquad (4)$$

Where, TP is the total phosphorus eliminated or transformed during fattening (kg); PA , total phosphorus in the supply water (kg); P_{Re}, total phosphorus in renewal water (kg); P_{Ra} , total phosphorus inserted through the feed; P_J , total phosphorus inserted through the young fish (kg); P_E , total phosphorus of the harvest effluents (kg); P_P , total phosphorus recovered in the fish biomass (kg); and P_S , total phosphorus accumulated in the sediment (kg). In this calculation, the increments of atmospheric P or droppings of birds and other animals that could be attracted to the farming ponds were not considered.

In order to calculate the TN and TP amount inserted through the dry matter of the the methodology proposed by Boyd & Queiroz (2004) was used, in which the quantity of nutrients inserted through the feed is subtracted from that accumulated in the fish, and the remainder accumulates in the sediment or is lost in the effluents during the farming period or at harvest. The amount of feed used in the production cycle was calculated by Equation 5.

$$Q_R = (B_d - B_i) \times FC \tag{5}$$

Where QR is the amount of feed consumed in fattening (kg); Bd, fish biomass at harvest (kg); Bi, initial biomass of the young fish (kg); and FC, feed conversion (kg feed per kg fish biomass, feed dry weight basis).

2.6 Statistical analyses

To evaluate the differences in nitrogen and phosphorus fluxes in the mass balance for pond sizes, the data were submitted to analysis of variance (ANOVA) and comparison of means test (Tukey's test) with a 5% of significance level, with a p-value < 0.05 being considered significant. The analyses were performed using the software R (R Core Team, 2019).

3. Results and Discussion

Table 1 shows the mean results of the water characterization of the different properties evaluated. The variability of the results expressed by the standard deviation refers to the differences between the springs of each property.

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Table 1. Characterization of water supply*.

Parameter	Concentration
TP (mg/L)	0.034 ± 0.014
NO_3^- (mg/L)	0.129 ± 0.075
NO_2^- (mg/L)	0.008 ± 0.004
NH_3 (mg/L)	0.089 ± 0.068
TN (mg/L)	0.540 ± 0.050
DO (mg/L)	6.330 ± 0.950
EC (µ S/cm)	49.100 ± 31.580
pH	6.840 ± 0.450
T°C ef	22.230 ± 0.980
T°C air	28.800± 4.680

* Results expressed by mean ± standard deviation. The presence of PO43- in the supply water samples were below the minimum detection concentration (0.001 mg/L) and were not presented in this Table, where TP: Total phosphorus; NO3-: Nitrate; NO2-: Nitrite; NH3: ammoniacal nitrogen; TN: total nitrogen; DO: Dissolved oxygen; EC: Electrical conductivity; pH: Hydrogen ionization potential; T°C ef: Effluent temperature; and T°C air: Air temperature. Source: Prepared by the authors (2020).

The quality of the water in the production environment is directly influenced by the characteristics of the water supply, being related to the presence of organic and/or inorganic substances, primary productivity and microorganisms (Macedo & Sipaúba-Tavares, 2018).

According to Conama Resolution No. 430/2011, the quality conditions of supply water from established Class II rivers are pH between 6 and 9; dissolved oxygen greater than 5 mg L^{-1} ; total phosphorus for lotic environments of less than 0.05 mg L^{-1} ; nitrate and nitrite of less than 10 and 1 mg L^{-1} , respectively, and total ammoniacal nitrogen of less than 3.7 mg L^{-1} for environments with pH below 7.5 (Brasil, 2011). Thus, the results found for the supply water are within the standards established in the legislation (Table 1). These waters present TN and TP loading, which, according to Hu et al. (2012) and Palácio et al. (2016), is probably due to percolation of nutrients from intense agricultural activities around the properties. Additionally, the sites of the springs that supply water to the ponds are protected by a ciliary forest, in which the accumulation and decomposition of leaves can also be considered as sources for nutrient production, mainly in nitrogenous form.

The ideal conditions of the supply water for Nile tilapia farming may vary. The ideal water temperature is 29-31°C, with dissolved oxygen of 5-6 mg L^{-1}, pH of 4-9, and total ammoniacal nitrogen levels below 0.43 mg L^{-1} (Colt & Kroeger, 2013). In this study, although the average water supply temperature was lower than the recommended temperature, the region presents a high ambient temperature during almost the entire year, water temperature during the fattening period tends to remain, most of the time, in the range considered ideal.

Electrical conductivity (EC) indicates the presence of dissolved ions, such as nitrites, nitrates, bicarbonates, carbonates, phosphates, and ammonia, reflecting on the water's capacity to conduct electricity (Leira et al., 2017). Figueiró et al. (2018) suggest that, for fish farms in excavated ponds, EC values close to 70 μS cm^{-1} are considered ideal. The values found for EC in the supply water are considered adequate for the use of water in fish farming.

3.1 Characterisation of the effluents in the harvesting process

The results of the characterisation of the effluents evaluated at the different moments of the harvesting process (Table 2) show that at the N1 level, which can also be considered as rearing water at the end of the fattening cycle, the TP and TN values presented values higher than those found in the supply water, with emphasis on the TN level, which is in a concentration 9 and 11 times higher in medium-sized and large ponds, respectively.

It can be observed that the concentrations of TP, TN and NH_3 increased at each harvesting stage, with higher concentrations in N3 (end of the harvest) due to the reduction in water volume in the ponds. This behaviour can be explained by the decrease in water volume, which causes a higher concentration of fish, with higher water movement and suspension of the organic matter at the bottom of the pond, helping liberate these compounds. In the case of NH_3, despite the high concentrations found, they are not present in their toxic form for the fish due to the pH of the water, which remained stable throughout the harvesting process and ideal for the rearing of this species.

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Table 2. Characterisation of effluents during the harvesting process, in different nursery sizes*.

Parameter	Small			Medium			Large		
	N1	N2	N3	N1	N2	N3	N1	N2	N3
TP (mg L^{-1})	0.32 ± 0.22	2.06 ± 1.78	3.67 ± 4.92	0.91 ± 0.49	2.20 ± 1.21	8.56 ± 8.54	0.62 ± 0.30	1.07 ± 0.74	1.30 ± 0.31
PO_4^{3-} (mg L^{-1})	0.02 ± 0.02	0.01 ± 0.00	0.03 ± 0.03	0.02 ± 0.03	0.01 ± 0.01	0.07 ± 0.10	0.01 ± 0.02	0.01 ± 0.01	0.02 ± 0.01
NO_3^- (mg L^{-1})	0.44 ± 0.11	0.42 ± 0.15	0.36 ± 0.21	0.76 ± 0.39	0.79 ± 0.61	0.47 ± 0.41	1.20 ± 0.93	1.01 ± 0.79	0.47 ± 0.53
NO_2^- (mg L^{-1})	0.12 ± 0.08	0.03 ± 0.02	0.04 ± 0.03	0.45 ± 0.37	0.43 ± 0.37	0.19 ± 0.14	0.24 ± 0.16	0.21 ± 0.24	0.20 ± 0.26
NH_3 (mg L^{-1})	0.67 ± 0.46	1.03 ± 0.36	1.06 ± 0.71	2.05 ± 1.14	2.49 ± 1.37	3.23 ± 1.79	1.06 ± 1.10	1.09 ± 1.10	1.78 ± 0.70
TN (mg L^{-1})	2.27 ± 1.05	5.18 ± 2.90	9.25 ± 11.87	6.03 ± 1.95	9.08 ± 2.40	24.68±16.91	4.86 ±1.44	6.30 ± 2.16	6.25 ± 1.85
DO (mg L^{-1})	6.23 ± 0.48	1.64 ± 0.32	0.86 ± 0.74	5.53 ± 1.62	2.99 ± 1.81	1.31 ± 1.27	6.73 ± 2.58	3.17 ± 0.79	1.97 ± 2.48
EC (µ.S cm^{-1})	71.87±27.18	152.5±27.54	118.7± 8.54	82.50±29.86	117.5±42.52	133.8 ± 14.4	93,12 ± 17.95	123.75±49.6	91.25±20.16
pH	6.88 ± 0.43	6.35 ± 0.12	6.29 ± 0.12	6.81 ± 0.17	6.63 ± 0.23	6.59 ± 0.20	6.64 ± 0.26	6.43 ± 0.37	6.34 ± 0.21
T°C$_{ef}$	20.50 ± 1.70	18.00 ± 5.35	22.88 ± 8.19	21.10 ± 2.00	14.50 ± 1.73	18.25 ± 4.57	21.70 ± 1.60	16.75 ± 0.96	26.75 ± 6.65
T°C$_{air}$	20.50 ± 1.80	18.55 ± 2.74	21.33 ± 5.81	19.60 ± 2.00	19.90 ± 1.07	19.18 ± 1.14	21.10 ± 0.80	21.68 ± 1.80	24.50 ± 5.13

* Result expressed by the mean ± standard deviation, where L1: Level 1; L2: Level 2; L3: Level 3; TP: Total phosphorus; PO_4^{3-}: Orthophosphate; NO_3^-: Nitrate; NO_2^-: Nitrite; NH_3: Ammoniacal nitrogen; TN: Total nitrogen; DO: Dissolved oxygen; EC: Electrical conductivity; pH: Hydrogen ionization potential; T°C ef: Effluent temperature; and T°C air: Air temperature. Source: Prepared by the authors (2020).

Higher values were observed for medium-sized ponds compared to small and large ponds. A probable cause of the difference in the concentration of these parameters in relation to the size of the pond is due to their geometric characteristics, such as the differences in shape and slope, which may cause differences in drainage speeds.

Regarding the parameters PO_4^{3-}, NO_2^- and NO^{3-}, no relevant changes were observed during the expenditure process. These parameters can be considered products resulting from biochemical transformations, in which they do not occur in a significant way during the harvesting process, but rather throughout the rearing process. For NO_2^- and NO^{3-}, all pond sizes showed a decrease in concentration during the harvesting process, which indicates that these nitrogen forms are more dissolved in the effluents.

In Table 2, it can be observed that the EC varied in the harvesting process. This parameter is directly related to the concentration of inorganic salts in the water. In small and large ponds, the EC increased between levels N1 and N2 probably due to the increase in suspended solids and decreases in N3. In medium-sized ponds, in turn, there was a progressive increase in EC between N1 and N3. Notwithstanding, during the rearing period, the EC can be considered as a water eutrophication indicator parameter, in which high values indicate a high degree of organic matter decomposition (Bhatnagar & Devi, 2013). Values close to 70 µS cm^{-1} for fish farming in excavated ponds are considered normal (Figueiró et al., 2018).

3.2 Characterisation of the sediment

The characteristics of the sediment accumulated at the bottom of the ponds are presented in Table 3. It can be verified that the TP contents in the sediment are similar between the different pond sizes. For TN, however, the medium-sized ponds presented greater accumulation of TN of the sludge with 3.98 g kg^{-1}.

Table 3. Characteristics of the sediment present at the bottom of the different sizes of nurseries*.

Nursery size	DM (%)	TP (g kg^{-1})	TN (g kg^{-1})
Small	25.42±0.37	2.23±0,43	2.13±0.54
Medium	26.22±0.60	2.90±0.86	3.98±1.15
Large	26.10±0.20	2.48±0.60	2.80±0.91

* Expressed by the mean ± standard deviation, where DM: Dry Matter; TP: Total phosphorus; and TN: Total nitrogen. Source: Prepared by the authors (2020).

3.3 Percentage characterization of the feed and fish at the end of the fattening period

The production system evaluated in this study is standardized, indicating that both feeding and food management were the same for all pond sizes. This can be confirmed by the results found in the percentage composition of the feed and fish presented in Table 4, which presented no significant differences between the different sizes of ponds.

Table 4. Centesimal composition of the feed and fish sampled during effluent evaluation*.

Variable / Sample and nursery size	Fish			Feed		
	S	M	L	S	M	L
CP (%)	15,94±0,23	15,33±0,58	15,33±0,80	31,86±0,70	33,28±1,18	32,67±1,32
ET (%)	14,17±1,53	12,98±0,67	12,56±1,15	5,99±0,83	5,13±1,03	4,72±0,66
MM (%)	4,12±1,00	3,61±0,61	4,39±0,56	8,48±0,25	8,40±0,99	8,56±1,27
HU (%)	64,93±0,69	67,73±2,03	65,56±1,70	8,10±0,40	8,75±0,70	8,05±0,50
DM (%)	35,07±0,69	32,27±2,03	34,44±1,69	91,90±0,40	91,24±0,70	91,94±0,51
TN (%)	2,55±0,04	2,45±0,09	2,45±0,13	5,10±0,11	5,32±0,19	5,23±0,21
TP (%)	0,70±0,08	0,66±0,06	0,65±0,06	1,31±0,09	1,35±0,12	1,34±0,16

* Expressed by the mean ± standard deviation, where S: Small nursery; M: Medium-sized nursery; L: Large nursery; CP: Crude Protein; ET: Ethereal Extract; MM: Mineral Matter; HU: Humidity; DM: Dry Matter; TN: Total Nitrogen; TP: Total Phosphorus.. Source: Prepared by the authors (2020).

In Table 4, it can also be observed that the TP content found in the fresh feed ranged from 1.31% to 1.35%, being higher than the recommended amount for the growth phase, which according to Boscolo et al. (2003), suggest a variation of 0.35-0.70%, while (Schamber et al., 2014) suggest 0.61% of the food composition.

3.4 Nitrogen and phosphorus dynamics in the tilapia harvesting process

3.4.1 Mass TN and TP balance in the conversion of Tilapia biomass

The dry matter conversion balance of TN and TP (Table 5) present in the food and transformed into dry matter in the form of biomass (fish) represent an overview of the production, irrespective of the size of the ponds, and was calculated according to the feed and fish characterization.

The residual TN and TP accumulated in the ponds was calculated considering the zootechnical production parameters of the evaluated properties, in which the mean productivity for the period from April 2016 to March 2017 was 45,636.0 kg ha^{-1}, mean final weight of 0.9 kg, initial young fish biomass of 1,074.0 kg ha^{-1}, and mean feed conversion rate of 1.438. The productivity and feed intake information were provided by the integration system.

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Table 5. TN and TP mass balance in the conversion of Tilapia (*Oreochromis niloticus*) biomass in excavated nurseries.

Variable	Natural matter	Dry matter	TN	TP
Feed kg ha $^{-1}$	64,043	58,724	3,341	852
Fish kg ha $^{-1}$ live weight	44,536	15,107	1,110	301
Nursery residue load (kg ha $^{-1}$)		43,617	2,230	551
Converted into fish biomass (% of the addition)		25.7	33.2	35.3

Source: Prepared by the authors (2020).

Our findings show that 25.7% of the dry matter supplied as feed was converted into dry matter as fish (Table 5). By the relationship presented, 33.2% TN and 35.3% TP added in the form of feed are withdrawn in the form of fish at the harvest time. The difference between the TN and TP amount inserted in the feed in relation to the biomass converted into fish may have been eliminated in the liquid effluents by the water renewal process, during harvest, accumulated in the sediment, absorbed by the algae, and/or lost during the production cycle. Boyd & Queiroz (2004) studied the dynamics of nitrogen and phosphorus removal in tilapias at harvesting, verifying that 20.7% of the dry matter, 32.5% TN and 46.8% TP, added to the ponds by the feed during the rearing period, were recovered by the fish.

These results were found using feed with 30% of CP and 1.2% of TP. Compared to the data found in Table 5, an improvement of 5% was observed in the dry matter recovery was observed, indicating a lower accumulation of residue. Regarding the recovery of phosphorus by the fish, this can be explained by the composition of the feed studied, which was 1.33% (Table 3).

Considering that the phosphorus requirement for the species varies from 0.35% to 0.70%, the feed evaluated in this study contains on average 1.33% in its formulation, and the recovery in the fish was 35.30%, it can be concluded that approximately 0.47% of the phosphorus in the feed would be sufficient to meet the fishes requirement. Nevertheless, it is known that the rates of absorption and assimilation also vary among species, which explains the use of higher amounts of this nutrient in the formulations. Since the fish needs a smaller amount of phosphorus for its development, it can be seen that the unabsorbed surplus is being excreted into the environment.

In relation to the nitrogen recovery, Osti et al. (2017) studied the TN flux in tilapia farming with a mean final weight of 667 g and found a recovery rate of 26% TN offered by a feed with 28% of this nutrient. In this study, a 33.2% TN recovery rate was observed indicating a higher digestibility which is possibly explained by the better protein quality of the feed.

According to Boscolo et al. (2011), the performance and efficiency of protein utilization of the balanced feeds is directly related to the energy to protein ratio. Feeds with excess energy may provide reduced protein intake, and consequently, amino acids required to maximise body protein deposition, compromising fish performance. On the contrary, in the use of feeds with low energy content, part of the amino acids will be used for energy purposes, reducing their availability for the deposition of body protein and increasing the production of nitrogenous metabolic waste.

Thus, feed formulations should be as close as possible to the requirement of the species. Similarly, the quality of the feed ingredients is an important factor to be considered in order to minimise the amount of waste generated and, consequently, the reduction of nitrogen and phosphorus levels (Bomfim, 2013).

3.4.2 TN and TP mass balance during the harvesting process

One of the moments that may have an impact on the environment in relation to the activity of fish farming is the harvest moment, especially when it is performed in an inadequate way, as a large volume of effluents is drained from the ponds in a short time, with sufficient pollutant loads for localized impacts (Boyd et al., 2007; Santos & Camargo, 2014).

Table 6 shows the TN and TP inflows and outflows and the mass balance according to the size of the ponds and the volume of effluents drained at each harvesting stage. It can be observed that, with respect to the TN and TP inputs in the system, the results are similar due to the standardisation of the production system. Although the water supply is from different sources, the characteristics are similar, and the TN and TP increase at the time of filling of ponds, however, this does not interfere in the quality of the generated effluents.

The same can be said for the amount of nutrients inserted through the water renewal process during the production cycle. The quantity presented in the Table 6 is diluted along the fattening period, and the daily TN and TP load incorporated into the system by the renewal is small. The total nutrient load inserted in the pond filling and in renewal during the production cycle corresponds, on average, to 2.42% TN and 1.45% TP.

Table 6. Mass Balance of Total Nitrogen and Total Phosphorus for different nursery sizes*.

	TN ($kg.ha^{-1}$)			TP ($kg.ha^{-1}$)		
	Small	Medium	Large	Small	Medium	Large
Inputs						
Supply water	8.10±0.17	8.18±1.04	8.45±1.14	0.60±0.33	0.50±0.09	0.42±0.19
Renewal water	162.00±3.34	163.36±20.87	168.83±22.82	12.00±6.61	9.90±1.09	8.40±3.81
Young fish	80.00±2.03	83.86±6.15	78.62±7.41	21.28±2.24	22.68±2.23	21.01±3.13
Feed	3257.37±85.22	3427.11±142.73	3338.75±128.68	836.92±55.56	866.11±81.27	857.31±99.52
Outputs						
L1 effluent	20.49±9.49	54.34±17.60	43.76±12.95	2.90±0.93	8.15±4.42	5.56±2.70
L2 effluent	27.17±15.20	47.64±12.58	33.08±11.36	10.82±9.32	11.55±6.34	5.63±3.87
L3 effluent	6.94±8.90	18.51±12.68	4.69±1.39	2.75±3.69	6.42±6.40	0.98±0.23
Converted into fish	1135.49±16.39	1092.20±41.15	1092.50±57.01	310.75±36.38	295.83±28.21	291.33±28.93
Sediment	269.73±67.17	519.42±142.50	364.71±116.51	282.24±50.37	379.94±113.43	322.79±96.20
Mass balance						
Total inputs	3507.51±86.75	3683.16±139.06	3594.64±144.57	871.35±49.46	899.19±82.99	887.28±99.85
Converted into fish	1135.49±16.39	1092.20±41.15	1092.55±57.01	310.72±36.38	295.84±28.21	291.33±28.93
Harvest effluents **	54.59±25.58[a]	120.49±24.64[b]	81.52±23.85[ab]	16.47±9.32	26.11±10.60	12.18±5.93
Accumulated in the sediment **	269.73±67.17[a]	519.42±142.50[b]	364.71±116.5[ab]	282.24±50.37	379.94±113.43	322.79±77.28
Eliminated during fattening	2047.70±54.90	1951.05±172.49	2055.85±88.53	261.91±34.15	197.30±103.35	260.99±96.20

* Expressed by the mean ± standard deviation. ** By analysis of variance (ANOVA) at the significance level of 5%, there was significant difference. Mean values followed by the same letter do not differ from each other by the Tukey test at the 5% probability level. Prepared by the authors (2020).

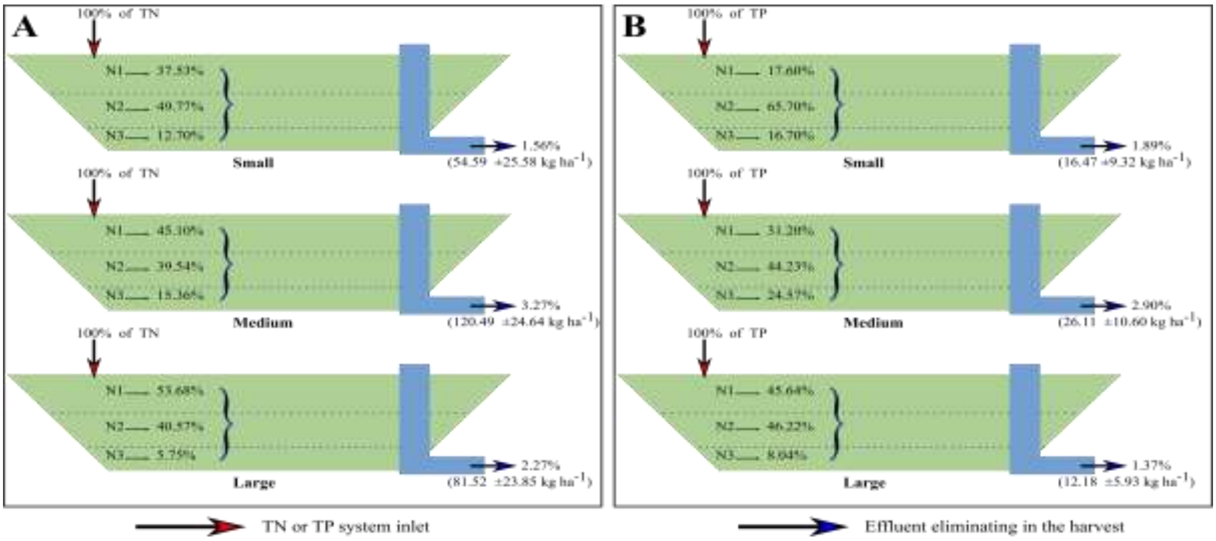
The young tilapia used for pond stocking at the start of rearing period also represent a nutrient load due to their composition, and this biomass was disregarded in the balance sheet presented in Table 5. The young fish account for 2.28% TN and 2 .44% TP. The main source of nutrients in the system is the fish feed. The feed in this evaluation accounted for 92.87% TN and 96.05% TP in the production system.

Statistically, the mass balance shows a significant difference between the pond sizes for the TN eliminated in the harvest effluents and the TN accumulated in the sediments, with medium-sized ponds presenting the highest results, but not differing from the large ponds (Table 6).

A relative mass balance of TN and TP for the different pond sizes is shown in Table 6. It is observed that the percentage of TN and TP eliminated through the harvesting effluent is small in relation to the percentage eliminated or transformed during the fattening period. Nevertheless, the eliminated load is punctual and capable of generating local impacts. To illustrate how much TN and TP is released into the environment during the expenditure process, Figure 4 presents the different pond sizes and the proportion of nutrients eliminated at each harvesting level.

The amount in kg of the TN and TP eliminated through the effluents at harvest is significant; however, the amount that is inserted into the system during the production cycle is proportionally small, corresponding on average to the different pond sizes to 2.37% TN and 2.05% TP. The medium-sized ponds had the highest percentage of TN elimination at harvest, reaching 3.27%, 45.10% of which was eliminated in N1; 39.54%, in N2; and 15.36%, in N3 (Figure 4A). For TP in medium-sized ponds, this percentage amounted to 2.90%, with 31.20%, 44.23% and 24.57% of this percentage eliminated in N1, N2 and N3, respectively (Figure 4B).

Figure 4. A) Percentage of TN eliminated through the effluent at the time of harvest, with the proportion for each water level in the different pond sizes. B) Percentage of TP eliminated through the effluent at the time of harvest, with the proportion for each water level in the different pond sizes (Prepared by the authors (2020).



Source: Prepared by the authors (2020).

The values referring to the 3.27% TN and 2.90% TP exported to the environment in the medium-sized ponds refer only to the drainage at harvest. Boyd & Queiroz (2001) determined that 16.0% TN and 11.4% TP are exported to the environment during the rearing period. Osti et al. (2017) found similar values following several phases of fattening of tilapias for 128 days. Nevertheless, there is no information regarding the effluent released only at the time of harvest.

In relation to nutrients accumulated in the sediment, medium-sized ponds had significantly higher N accumulation than small ponds but did not differ from large ponds (Table 6). Regarding P accumulation, the size of the pond did not interfere with the accumulated proportion. The values of N accumulated in the sediment are smaller because N may be lost in the denitrification process or volatilized by ammonia diffusion (Gross et al., 2000), as phosphorus can easily be absorbed by the soil (Masuda & Boyd, 1994), which explains the percentage accumulated in the sediment.

Aquaculture conducted in excavated ponds promotes the accumulation of waste at the bottom of the ponds. The organic matter generated by faeces and food leftovers decomposes or accumulates at the bottom of the ponds (Boyd et al., 2007). The amount accumulated in ponds depends on the feed conversion rate as a function of the dry matter of the feed and the fish species. In an evaluation in which a feed conversion rate of 1.7, feed dry matter of 90%

and tilapia dry matter of 25% were considered. Chatvijitkul et al. (2017) calculated a feed conversion rate of 6.12 for the dry matter, concluding that each 200 kg of feed dry matter used in the feeding generates 150 kg of waste.

The dry matter feed conversion rate was 3.89, considering a mean value of 91.7% dry matter for feed and 33.9% for fish (Table 4) and a feed conversion rate of 1.438. These values indicate that for each 200 kg of feed dry matter, 133.2 kg of waste are generated. This shows the continuing importance of care for feed management.

On average, it is observed that 58.55% of N and 29.18% of P (Table 6) inserted into the system are eliminated in the effluent from the harvest or during fattening. Boyd & McNevin (2015), describe that in efficient fish farming, 20-30% N and 5-10% P applied through the feed are eliminated in effluents. Boyd & McNevin (2015) indicate that 40% N and 20% P can be eliminated through effluents. Nevertheless, it should be noted that the study did not evaluate only the nutrient intake via feed or how the losses occurred during the rearing period, as some of these nutrients may have been absorbed by the phytoplankton that also return to the fish.

The input of nutrients into the aquatic environment - whether it is from the industry or from farming activities, including aquaculture - should be monitored and minimized. In intensive aquaculture activity carried out in excavated ponds, the moment of the harvest presents itself as a threat with impacts that are no longer local, as the same watershed may have several areas for aquaculture, and the sum of the daily volume of effluents may be greater than the self-purification capacity of the environment.

The main source of nutrient input in intensive fish farming in excavated ponds observed in this work was the feed offered to the animals, being responsible for approximately 93% of TN and 96% of TP. The volume of nutrients not absorbed by fish is not only concerned with the environmental impact that it can cause but also with problems caused to fish during the development period. The excess of nutrients causes water eutrophication and thus causes a reduction in the quality standards of water considered ideal for fish farming where causing stress can increasing the risk of disease and even mortality.

The results of the feed analysis indicate that the P content present in the feed is above the amount required by Nile tilapia, and corrective measures should be taken to minimize the input of P through the feed. Food management can also be enhanced to improve feed conversion rates that directly influence the amount of waste produced.

Supply waters should remain protected, especially as far as contamination by agricultural areas in the environment is concerned, not only in relation to percolation of nutrients, but also in relation to the risk of contamination by agrochemicals used in agriculture. The supply of nutrients from the water supply and renewal represents 2.42% TN and 1.45% TP but can be reduced through good conservation practices.

Considering the possibility of sediment removal at the end of the fattening period, in this study medium-sized nurseries had higher retention of nutrients in the sediment, which can be used as fertilizer in agriculture, however, studies on the efficacy and/or toxicity of the sediment should be performed. Correct management at the end of the rearing period, such as sun exposure and plowing at the bottom of the nurseries may help in the total decomposition of this organic matter before the start of another productive cycle. Actions taken during fattening may also assist in reducing sediment deposition, such as improving feed quality and improving water conditions during rearing, maintaining adequate levels of dissolved oxygen throughout the water column, and pH and alkalinity correction só that the microorganisms perform the cycling of the nutrients.

The amount of nutrients released into the environment during the harvesting process, despite representing only a small portion of the total amount inserted into the system is released in a short time-span and mitigating measures should be considered. Although, there were not statistical differences, medium-sized ponds had a greater impact on TN during harvesting, it cannot be stated that this pond size is not suitable for production, since good management practices may reverse this situation. The lack of difference between the results of the ponds (small, medium and large) may be explained due to the quality of the inlet water used was similar, the feed comes from the same cooperative industry, and the farmers were trained equally by the company.

4. Conclusion

This study demonstrates the need to assess the balance of nutrients used in the activity of tilapia farming, in excavated pond, for making decisions regarding process improvements, such as the evaluation of water supply sources and animal feed management, as well as evaluating the composition of effluents discharging into the environment. In this work it was verified that the size of the tank influences the retention of nutrients in the sediments which provide benefit for use in other agricultural crops due to its composition in terms of TN and TP.

5. Final Considerations

As future measures, adaptations must be made in the management with regard to better control of the amount of feed and water offered, avoiding the loss of nutrients, and thus the use of feed with better quality allowing greater efficiency in the assimilation of nutrients.

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Research, Society and Development, v. 9, n. 11, e1319119699, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i11.9699

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