

## Nitrogen and phosphorus flux in wastewater from three productive stages in a hyperintensive tilapia culture

Leticia Félix-Cuencas, Juan Fernando García-Trejo, Samuel López-Tejeda, Jesús Josafat de León-Ramírez, Claudia Gutiérrez-Antonio and Ana Angélica Feregrino-Pérez

### ABSTRACT

In this research, effect of productive stages in nitrogen and phosphorus excretion in wastewater from hyperintensive tilapia (*Oreochromis niloticus*) culture was evaluated. Fish were cultivated considering three development stages (fingerling of 1.79 g, juvenile of 36.13 g, and adult of 72.96 g). Nitrite, nitrate, ammonium, and phosphorus concentration were determined in order to know the amount of nutrients excreted per productive stage of the fish at a high stocking density. Biometric data were recorded during the experiment with the purpose of determining the growth behavior of fish, as well as the measurement of the aerobic metabolism. Results showed that survival, growth, and health of fish are not affected by hyperdensity of culture; as well, combined catabolism of proteins and lipids was presented as substrates for energy with value for O:N ratio ranging between 20 and 60. In addition, higher concentration in excretion of nitrogen compounds and phosphorus per gram of fish was recorded in wastewater from a hyperintensive culture in fingerlings than in juveniles and adults. These results suggest the use of this wastewater in the early stages of fish growth, aiming to enhance sustainable systems with maximum use of the resources, such as aquaponics systems.

**Key words** | hyperintensive, nitrogen and phosphorus, productive stages, recirculation aquaculture systems (RAS), wastewater

Leticia Félix-Cuencas  
Juan Fernando García-Trejo (corresponding author)  
Samuel López-Tejeda  
Jesús Josafat de León-Ramírez  
Claudia Gutiérrez-Antonio  
Ana Angélica Feregrino-Pérez  
Universidad Autónoma de Querétaro, Facultad de Ingeniería,  
Cerro de las Campanas s/n Col. Las Campanas,  
Querétaro, Querétaro, 76010,  
México  
E-mail: fernando.garcia@uaq.mx

### HIGHLIGHTS

- The effect of productive stages in NP excretion in wastewater from hyperintensive tilapia culture are evaluated.
- Survival, growth and health is not affected by hyperdensity of culture.
- Concentration in NP excretion per gram of fish is higher in fingerlings.
- It is suggested the use of aquicultural wastewater in the early productive stages for the use in sustainable systems such as aquaponics systems.

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## INTRODUCTION

Human society faces the immense challenge of increasing food production; for this reason, work has been done for the production of food that optimizes natural resources and provides essential elements for human nutrition, an example is the production of fish (Avnimelech & Kochba 2009). Among the most cultured fish is the Nile tilapia (*Oreochromis niloticus*), which is the second largest group of cultured fish in the world with almost 5.6 million metric tons produced in 2015 (Fitzsimmons 2016). In recent years, there has been a great demand for Nile tilapia due to its high quality of protein, mainly due to its protein and carbohydrate digestibility, because of the species own metabolisms (Barreto-Curiel *et al.* 2015). To meet this demand, tilapia has been cultured in aquaculture production systems that are among the fastest growing animal food-producing sectors, accounting for almost half of the total food fish supply (FAO 2016).

One of the main problems of aquaculture production systems is their wastewater; these chemical-laden waters that are discarded by aquaculture operations have become a problem throughout the world due to their potential for environmental contamination (Chowdhury *et al.* 2013). Aquaculture effluents are rich in dissolved solids and suspension containing mainly nitrogen and phosphorus generated from the excretion of fish, feces, and uneaten food (Summerfelt & Clayton 2003). Works reported in the literature show that 36% of the fish feed is excreted as organic waste, about 75% of the nitrogen and phosphorus consumed is unused and remains as a residue in the water. Depending on the species and the type of crop, more than 85% of the phosphorus, 80–88% of the carbon, and 52–95% of the nitrogen that enters a fish culture system are lost in the environment (Zou *et al.* 2016). According to studies carried out (Zhang 2014; Hu *et al.* 2015; Endut *et al.* 2016; Vilhelm *et al.* 2017), the amount of dissolved nutrients that are released in a system depends on species, and quality of the food and management in the culture system. Moreover, most recently, it was detected that fish growth rate and increasing feed input with water exchange rates resulted in highly variable proportions of the macro-nutrients during the aquatic production cycle (Jia *et al.* 2017).

Until now, few studies have been carried out where the balance of nutrients and energy is recorded, as well as the use of water in highly productive tilapia systems. Thus, the works reported in the literature have been focused on flow of nitrogen and phosphorus, mainly. Schneider *et al.* (2005) studied nutrient conversions in intensive integrated aquaculture systems; the evaluation was made based on the balance of nitrogen and phosphorus with tilapia from 54 to 128 g. They concluded that integrated systems have their specific limitations, which are related to uptake kinetics, nutrient preference, unwanted conversion processes, and abiotic factors. Wahyuningsih *et al.* (2015) presented a study about an aquaculture nitrogen waste reduction in an aquaponic system and indicated that integration of tilapia fish farming with organisms of 20 g, romaine lettuce, and bacteria can reduce inorganic nitrogen with the best removal efficiency.

Later, Vilhelm *et al.* (2017) analyzed the influence of ration size on nitrogen retention in tilapia with an average body mass of  $77.9 \pm 1.7$  g. The lowest rates of nitrogen excretion were observed for fish receiving meal sizes corresponding to 3% of their body mass. In contrast, fish fed ration sizes of 1% displayed a reduction in apparent digestibility of protein, nitrogen-free extract and dry matter, in addition to excreting a disproportionate amount of ingested nitrogen as ammonia and urea. In the same year, Cerozi & Fitzsimmons (2017) quantified a phosphorus flow, phosphorus mass balance and evaluated phosphorus removal efficiency by hydroponic lettuce integrated with tilapia aquaculture of 20 g of weight. These authors report a high excretion of phosphorus by fish, which can be used by plant species, demonstrating that aquaponics is an excellent tool for the reuse of this nutrient to obtain a high quality culture.

In Osti *et al.* (2018), utilized environmental indicators as a quantitative method to evaluate and discuss the nitrogen and phosphorus flux in a tilapia system with fish initial average weight of 191 g. They proposed management oriented to a better use of feed to prevent waste production, making adaptations in food management to avoid loss of nutrients.

As observed in previous reports, studies have focused on adult fish, so the amount of nutrients in the wastewater of

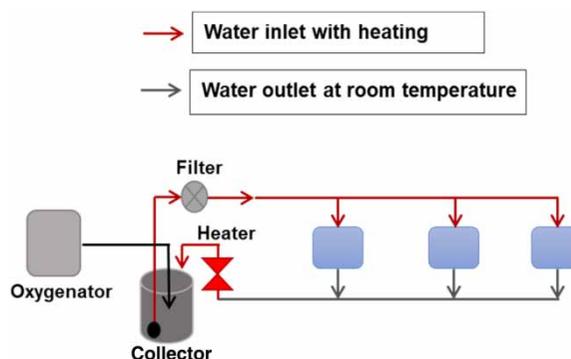
aquaculture systems is unknown according to the stage of development of the organism. Thus, the improvement of aquaculture crop efficiency requires detailed knowledge of the nutrient cycle (Adhikari *et al.* 2014). For this, it is necessary to understand the flow of nutrients in the aquaculture system, from its inputs to the outputs, as well as throughout the production cycle (Cerozi & Fitzsimmons 2017), from the sowing of the fingerlings, to the harvesting of the adult fish. This allows us to identify the destination of nutrients to achieve waste reduction and to promote the use of them. Thus, the present study was carried out to quantify nitrogen and phosphorus proportion in a hyperintensive tilapia aquaculture system through its life cycle to be a decision support system for nitrogen and phosphorus management in sustainable systems.

## MATERIALS AND METHODS

### System design

In order to provide uniform environmental conditions for the culture of tilapia, the system design was located inside a polyethylene greenhouse of 720 m<sup>2</sup> (16 × 45 m). Three treatments were realized considering three productive stages of fish corresponding to fingerlings, juveniles, and adults, under a culture density of 270 fish/m<sup>3</sup> of each stage. The experiments for all the treatments were carried out in triplicate.

The recirculation aquaculture system (RAS) was composed of nine polypropylene tanks of 50 cm width, 120 cm length, and 60 cm depth, with a volume of 300 liters. Water collectors of 1,000 liters were used to maintain the level of the experimental ponds. From collectors, water was transported to experimental tanks with a submersible pump, Boyu brand, for 10,000 L/h. For mechanical and biological filtration, a commercial filter of brand Boyu EFU-13500 pond filter with UV light, of 115 V, with a capacity of 8,000 to 15,000 L/h was used. To control the temperature an Electro EVO 230 V Electric Engineering system was employed for a water flow of 1,000 to 17,000 L/h. To oxygenate water in all the tanks, a Topaz Airsep oxygen generator was used; this generator can produce at 6 LPM, at 9 psig with a purity of 93% (Figure 1).



**Figure 1** | Scheme of the recirculation aquaculture system (RAS) of tilapia culture in triplicate for each of the treatments (fingerlings, juveniles, and adults).

### Fish culture conditions

The culture of tilapia consisted of 240 fingerlings, 240 juveniles, and 240 adults, with an initial average body weight of 1.79 g (15 days), 36.13 g (60 days), and 72.96 g (90 days), respectively, distributed in nine ponds. Fish were fed three times a day with commercial balanced food according to their stage at approximately 10% (fingerlings), 5% (juveniles), and 3% (adults) of body weight per day. A temperature of 28 °C and an oxygenation range of 4–6 mg/L of O<sub>2</sub> were maintained. Additionally, handling of tanks involved the removal of feces, along with a weekly partial water exchange (30%).

In order to monitor physical water quality, dissolved oxygen, pH, and temperature were measured daily during the experiment; this measurement was realized with a multi-parameter instrument (Hach series HQ40d).

### Nutrients' content analysis

Chemical determinations were carried out in three phases to know the behavior of nutrients over 24 hours, 5 days, and 60 days; each of the phases of chemical determinations was achieved for the three stages of fish development. All samples were taken in triplicate for each treatment. In the 24-hour phase, samples were collected every 4 hours; for the 5-day phase, samples were taken once a day; after that, samples were taken weekly until the 60 days of experimentation were completed. The criterion for the period of creation of the determinations was based on the need to know the behavior

during a 24-hour cycle of the fish in each of the stages. After that, it was necessary to know the moment in which the nutrients reached toxic levels for the fish and to establish the periods and percentages of water changes. Finally, the duration of 60 days of the experiment was considered so that each of the development stages reached the stage of the next treatment, in order to cover most of the development of the fish. Determinations of different forms of nitrogen present in water: ammonia ( $\text{NH}_3\text{N}$ ), nitrates ( $\text{NO}_3^-$ ), and nitrites ( $\text{NO}_2^-$ ), as well as phosphorus ( $\text{PO}_4^-$ ), were made.

To carry out chemical determinations in water, 250 mL of water sample was taken manually from each tank. Water was collected, in triplicate, directly from the tanks in plastic containers, trying to obtain the sample from the same place to minimize variations. Analyses were carried out immediately after collection of the sample.

Ammonia ( $\text{NH}_3\text{N}$ ) concentration was determined according to HACH method 8038 for DR6000 spectrophotometer called Nessler method with an interval of 0.02–2.50 mg/L  $\text{NH}_3\text{N}$ . Nitrite concentration  $\text{NO}_2^-$  was determined using HACH method 8057 for DR6000 spectrophotometer called diazotation method with an interval of 0.002–0.300 mg/L  $\text{NO}_2\text{N}$ . Nitrate concentration  $\text{NO}_3^-$  was determined according to HACH method 8039 for DR6000 spectrophotometer called cadmium reduction method with an interval of 0.3–30.0 mg/L  $\text{NO}_3\text{N}$ . Analytical determination for phosphorus was carried out using HACH method 8048 known as ascorbic acid for orthophosphates ( $\text{PO}_4^{3-}$ ).

In addition to the concentration of each of the nitrogen compounds and orthophosphates, the flow of nutrients was calculated, in the following equation:

$$\text{Nutrient load } (L) = [ ] * WF \quad (1)$$

where  $L$  = load of N and P- $\text{PO}_4$  ( $\text{g day}^{-1}$ ),  $[ ]$  = nutrient concentrations ( $\mu\text{g L}^{-1}$ ) and  $WF$  = water flow ( $\text{L s}^{-1}$ ) (Valenti *et al.* 2018).

### Metabolism analysis

Three individuals from each treatment were randomly selected to measure aerobic metabolism (oxygen

consumption =  $\text{QO}_2$ ) at the beginning and end of the experiment, using closed respirometric chamber technique with constant temperature and a known water volume (Timmons *et al.* 2002; Soto-Zarazúa *et al.* 2010). Measurements of dissolved oxygen and ammonium were taken every 4 hours (14:00, 18:00, 22:00, 02:00, 06:00, and 10:00 h) during a 24 h cycle. All the fish used to determine  $\text{QO}_2$  were euthanized on ice immediately after finishing the experiment in order to determine their dry weight and to get the relation of the oxygen consumed by biomass (Steffensen 1989). The ethical procedures for the sacrifice of the fish were reviewed and endorsed by the bioethics committee of the Universidad Autónoma de Querétaro with registration number 9463.

### Growth performance

The following parameters were used to evaluate tilapia growth performance according to Kumar & Garg (1995):

$$\text{Weight gain } (WG) = 100 (W1 - W0) \quad (2)$$

$$\text{Weight gain } (WG) = \frac{(W1 - W0)}{t} \quad (3)$$

$$\text{Specific growth rate } (SGR) = \frac{(\ln W1 - \ln W0) \times 100}{t} \quad (4)$$

$$\text{Survival rate } (\%)(SR) = \frac{Ni \times 100}{N0} \quad (5)$$

where  $W1$  = final wet weight,  $W0$  = initial wet weight,  $t$  = time interval in days,  $Ni$  = number of fish at the end,  $N0$  = number of fish initially stocked. As well, total mortality and survival, for each treatment, were obtained at the end of the experiment.

### Statistical analysis

Non-parametric Kruskal–Wallis test was done to compare means between treatments. Moreover, correlations were performed using the Pearson correlation coefficient and probability (Walpole *et al.* 1999). Statistical analysis was made using STATGRAPHICS software (Stat Point Inc. 2006).

## RESULTS AND DISCUSSION

### Nutrient content analysis

The determinations of nutrients (nitrogen and phosphorus) present in wastewater of the hyperintensive aquaculture of tilapia is reported in its entirety present in water, as well as the concentration of nutrients excreted per gram of fish, in order to know the exact amount of nitrogen compounds and phosphorus excreted in each of the stages. The same trend was found in all the nutrients examined, the amount of total nutrients present in water was greater in adults; however, when the relationship was made per gram of weight of fish, there is a greater amount in the concentration of nutrients excreted by fingerlings (Tables 1 and 2).

All studies carried out by other researchers concerning the monitoring of nutrients derived from the

wastewater of aquaculture worked with data of the total excretion of a specific development state of the organism in culture, as well as working with lower densities than those shown in the present study. Cao *et al.* (2020) studied tilapia fingerlings with an initial average weight of  $3.54 \pm 2.82$ g and a culture density of  $5.12 \pm 0.04$ kg/m<sup>3</sup>; the concentrations of NH<sub>4</sub><sup>+</sup> in RAS were less than 2.0 mg/L, NO<sub>2</sub>-N was less than 1.12 mg/L, and the maximum concentration of NO<sub>3</sub>-N was 118.4 mg/L. In general, the aforementioned values were lower than those obtained in the present study. The differences presented in the excretion in both studies may be due to the difference in the physical conditions of water, as well as the type of food provided.

Higher concentrations of nitrogen compounds and phosphorus were obtained for juvenile tilapia with respect to fingerlings in the present research, with concentrations of NH<sub>4</sub><sup>+</sup> of 1.142 mg/L, NO<sub>2</sub>-N of 1.081 mg/L, NO<sub>3</sub>-N of 19.182 mg/L, and PO<sub>4</sub> of 0.99 mg/L. Nonetheless, Obirikorang *et al.* (2019) indicated data of juvenile fish with initial mean mass size of 27.44 g and with a stocking density of 3.6 kg/m<sup>3</sup>; they obtained lower nutrient concentrations (NH<sub>3</sub> 0.63 mg/L, PO<sub>4</sub> 39 mg/L, NO<sub>3</sub> 2.89 mg/L, NO<sub>2</sub> 0.06 mg/L) than those reported in the present study. This is explained by the difference in stocking density that was used for both researches.

The maximum values of nitrogenous and phosphorus excretion presented by adults indicated the amount and frequency of water exchange in the system. In this case, ammonia presented very high levels that could affect the development and survival of organisms; therefore, partial water changes were scheduled to avoid damage to fish. In the case of adult tilapia, this study used a lower average initial weight (72.96 g) than that used by Gichana *et al.* (2019) in their research (110.5 g), who reported lower values than those obtained in the present study (2.4 mg/L of NH<sub>4</sub><sup>+</sup>, 2.73 mg/L of NO<sub>3</sub>-N, 0.6 mg/L of NO<sub>2</sub>-N, and 2.65 mg/L of PO<sub>4</sub>); this may be due to the difference in the population density of the culture. For larger fish (160 g), values of 0.20 mg/L of NH<sub>4</sub>, 0.13 mg/L NO<sub>2</sub>, 0.65 mg/L NO<sub>3</sub>, and 0.09 mg/L of orthophosphate, in a stocking density of 1 kg/m<sup>3</sup>, was shown (Moustafa *et al.* 2020). Nevertheless, the amount of nutrient excreted in an aquaculture production system depends on the stocking

**Table 1** | Concentration of total nutrients (ammonia, nitrite, nitrate, and phosphorus) present in wastewater of a hyperintensive culture of tilapia

Nutrients	Total excretion in water		
	Fingerling	Juvenile	Adult
NH <sub>4</sub> <sup>+</sup> (mg/L)	1.746 ± 0.004 <sup>a</sup>	1.142 ± 0.037 <sup>b</sup>	3.079 ± 2.221 <sup>c</sup>
NO <sub>2</sub> -N (mg/L)	0.520 ± 0.014 <sup>a</sup>	1.081 ± 0.081 <sup>b</sup>	1.942 ± 0.083 <sup>c</sup>
NO <sub>3</sub> -N (mg/L)	12.689 ± 0.590 <sup>a</sup>	19.182 ± 2.173 <sup>b</sup>	11.804 ± 0.532 <sup>a</sup>
PO <sub>4</sub> (mg/L)	0.33 ± 0.001 <sup>a</sup>	0.99 ± 0.01 <sup>b</sup>	2.466 ± 0.172 <sup>c</sup>

Average values for each treatment followed by a superscript letter indicates that there is a significant difference ( $P < 0.05$ ).

**Table 2** | Concentration of nutrients (ammonia, nitrite, nitrate, and phosphorus) per gram of fish present in wastewater of a hyperintensive culture of tilapia

Nutrients	Excretion per gram of fish		
	Fingerling	Juvenile	Adult
NH <sub>4</sub> <sup>+</sup> (mg/L)	1.014 ± 0.181 <sup>a</sup>	0.022 ± 0.001 <sup>b</sup>	0.062 ± 0.0006 <sup>b</sup>
NO <sub>2</sub> -N (mg/L)	0.302 ± 0.056 <sup>a</sup>	0.020 ± 0.001 <sup>b</sup>	0.017 ± 0.0009 <sup>c</sup>
NO <sub>3</sub> -N (mg/L)	7.309 ± 1.247 <sup>a</sup>	0.370 ± 0.039 <sup>b</sup>	0.108 ± 0.002 <sup>b</sup>
PO <sub>4</sub> (mg/L)	0.191 ± 0.033 <sup>a</sup>	0.024 ± 0.004 <sup>b</sup>	0.022 ± 0.001 <sup>c</sup>

Average values for each treatment followed by a superscript letter indicates that there is a significant difference ( $P < 0.05$ ).

density, as well as the size of organisms and the quantity and quality of feed provided, among other factors; thus, making a comparison with other studies was difficult due to the difference in the density of the culture.

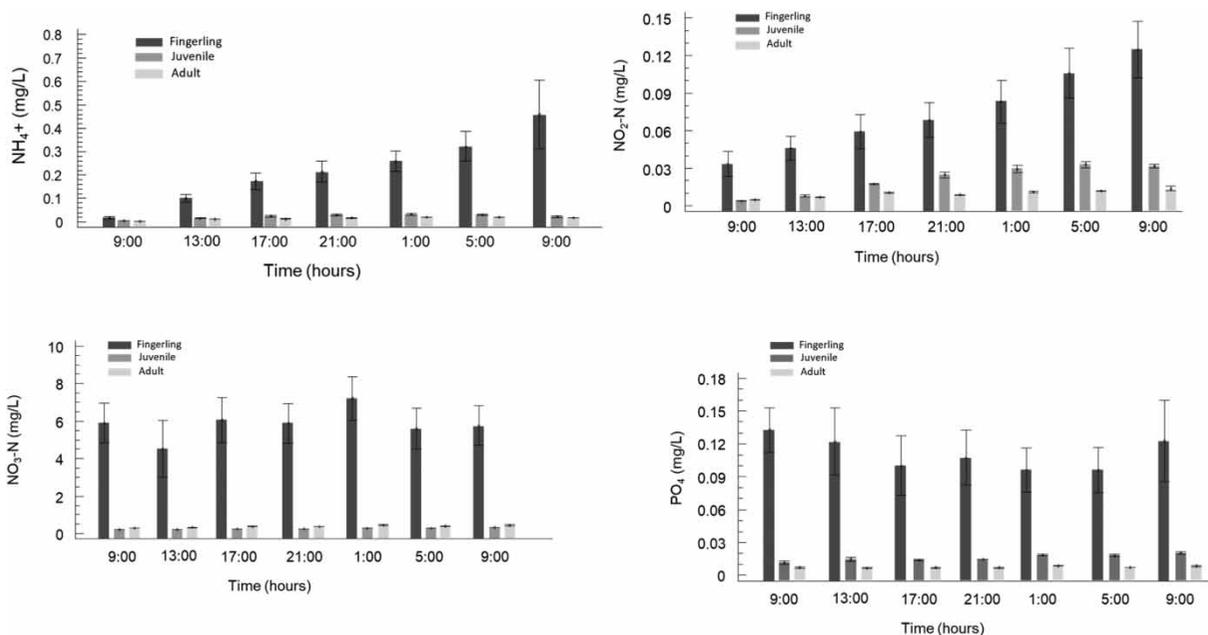
Calculation of the nutrient flow (Table 3) was carried out in order to obtain information that would allow contribution of elements for the evaluation of the amount of resources that are available in the aquaculture wastewater, which could be harmful to the environment in the case of being discarded, or used in the case of being reused. This generates a support instrument to evaluate the sustainability of the system (Valenti et al. 2018).

**Table 3** | Flow of total nutrients (ammonia, nitrite, nitrate and phosphorus) in wastewater of a hyperintensive culture of tilapia

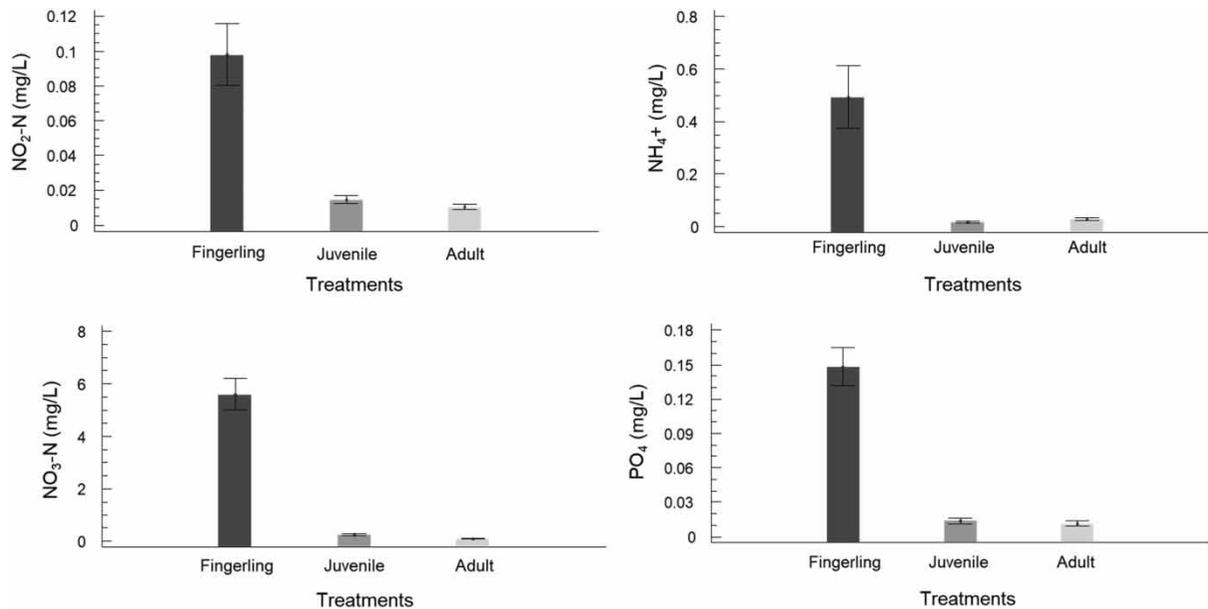
Nutrients	Flow of nutrients		
	Fingerling	Juvenile	Adult
NH <sub>4</sub> <sup>+</sup> (g day <sup>-1</sup> )	13.6188	8.9076	24.0162
NO <sub>2</sub> -N (g day <sup>-1</sup> )	4.056	8.4318	15.1476
NO <sub>3</sub> -N (g day <sup>-1</sup> )	98.9742	149.6196	92.0712
PO <sub>4</sub> (g day <sup>-1</sup> )	2.574	7.722	19.2348

Taking as the reference what is stipulated by Timmons et al. (2002), nitrates and phosphorus were found within the established tolerant ranges for tilapia development in a RAS; however, the ammonium and nitrites exceeded the reference standards (NH<sub>3</sub>-N < 1 mg/L, NO<sub>2</sub> < 1 mg/L, NO<sub>3</sub> 0–400 mg/L, and phosphorus 0.01–3 mg/L). Despite this, the fish were not affected in their growth and survival, as long as the partial replacement of water was carried out when the levels were excessive.

Additionally, in order to know the amount of nutrients excreted per gram of fish in each of its productive stages at high cultivation densities, data of the concentration per gram of fish are presented. Figure 2 shows the nutrient concentration per gram of fish for three productive stages of tilapia in 24 hours, observing the same trend in all nutrients analyzed. Fingerlings have a higher concentration in nitrogen and phosphorus excretion, considered per gram of fish, followed by juveniles, and the lowest concentration of nutrient excretion is by adults. This shows that metabolism of fish in its early stages is more accelerated; hence, the concentration of excreted nutrients was higher. That condition may be a factor for the use of these nutrients in sustainable production systems, such as



**Figure 2** | Nutrient concentration graphs per gram of fish weight for three productive stages of tilapia *Oreochromis niloticus* in 24 hours.



**Figure 3** | Nutrient concentration graphs per gram of fish weight for three productive stages of tilapia *Oreochromis niloticus*.

aquaponics. Figure 3 shows the same pattern for each of the nutrients analyzed in this research per day.

The importance of knowing the concentration of nitrogen and phosphorus in aquaculture production systems is based on data reported in several investigations (Hu *et al.* 2015; Cerozi & Fitzsimmons 2017; Gichana *et al.* 2019; Cao *et al.* 2020), as they are considered to be the elements that generate the greatest pollution in aquaculture wastewater. When carrying out a culture with a high population density, the generation of highly polluting wastewater increases, so it is important to know the flow of these nutrients in all stages of development of farmed fish, in order to avoid increasing the negative effects of these production systems. By knowing the flow of nutrients, controlled-environment agriculture (CEA) (Chen *et al.* 2020) can be developed where food is produced in a closed structure under conditions optimized for the maximum performance of plant and animal crops, with the maximum yield of resources, such as aquaponic systems (Gomez *et al.* 2019). With the information provided in the present study, data are contributed for the evaluation and comparison of the environmental performance of the cradle-to-grave life cycle (LCA) of intensive production systems (Ghamkhar *et al.* 2021), in order to operate sustainable systems at reduced environmental cost with high production.

### Metabolism analysis

O:N ratio is an indicator of the main substrate that is used by organisms aerobically to obtain energy, where values from 3 to 16 are associated with protein catabolism, values from 16 to 60 are related to the combined use of proteins and lipids as substrates, and values greater than 60 are associated with the predominance of lipids as an energy resource (Tseng & Hwang 2008; Barreto-Curiel *et al.* 2015). In the present study, it was found that the atomic ratio O:N did not present a significant difference between culture treatments ( $P < 0.05$ ), ranging between 20 and 60. This indicates that tilapia in its three productive stages, and under the established cultivation conditions, presented combined catabolism of proteins and lipids as substrates for energy. Results presented here coincide with those obtained by Barreto-Curiel *et al.* (2015), who compared O:N ratio in juveniles of tilapia cultivated in fresh and saltwater, and reported a value of  $18.2 \pm 5.3$  for those cultivated in freshwater; however, the culture density administered in this research was higher than the Barreto-Curiel *et al.* (2015) study, indicating that there is no effect on aerobic metabolism with increasing density. The difference in stages did not have a significant effect on the type of

metabolic substrate used as the main source of energy (Figure 4).

## Growth performance

Table 4 shows the growth performance parameters of fingerling, juvenile, and adult tilapia in a hyperintensive culture. In general, the stocking density did not affect the survival rate with values of 97.5, 100, and 100% for fingerlings, juvenile, and adult, respectively. Regarding the specific growth rate (SGR), similar values were obtained to studies carried out with the same species, similar weight, but lower stocking density. For fingerlings, a SGR of 4.54 was obtained, a value above that obtained in other researches like Yustiati et al. (2019) who reported an SGR of 2.70% in fish with an initial average weight of 8.75 g. On the other

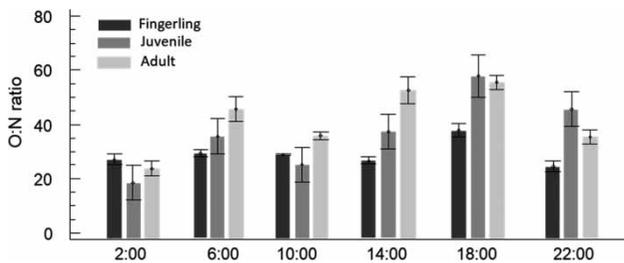


Figure 4 | O:N ratio in three productive stages of *Oreochromis niloticus*.

Table 4 | Growth performance parameters for three productive stages of *Oreochromis niloticus* in a hyperintensive culture (mean  $\pm$  DS)

Treatment	Fingerling	Juvenile	Adult
Initial number (n)	240 <sup>a</sup>	240 <sup>a</sup>	240 <sup>a</sup>
Final mean number (n)	234 <sup>a</sup>	240 <sup>b</sup>	240 <sup>b</sup>
Initial total weight (g)	107.46 <sup>a</sup>	8,672 <sup>b</sup>	17,512 <sup>c</sup>
Final total weight (g)	1,637.74 <sup>a</sup>	16,480 <sup>b</sup>	26,680 <sup>c</sup>
Initial individual mean weight (g)	1.79 <sup>a</sup>	36.13 <sup>b</sup>	72.96 <sup>c</sup>
Final individual mean weight (g)	27.29 <sup>a</sup>	60.33 <sup>b</sup>	102.83 <sup>c</sup>
Weight gain (g)	1,530.28 <sup>a</sup>	7,808 <sup>b</sup>	9,168 <sup>c</sup>
Weight gain (%)	1,424.04 <sup>a</sup>	90.036 <sup>b</sup>	52.352 <sup>c</sup>
Specific growth rate (% day)	4.54 <sup>a</sup>	1.07 <sup>b</sup>	0.70 <sup>c</sup>
Survival rate (%)	97.5 <sup>a</sup>	100 <sup>b</sup>	100 <sup>b</sup>

Average values for each treatment followed by a superscript letter indicates that there is a significant difference ( $P < 0.05$ ).

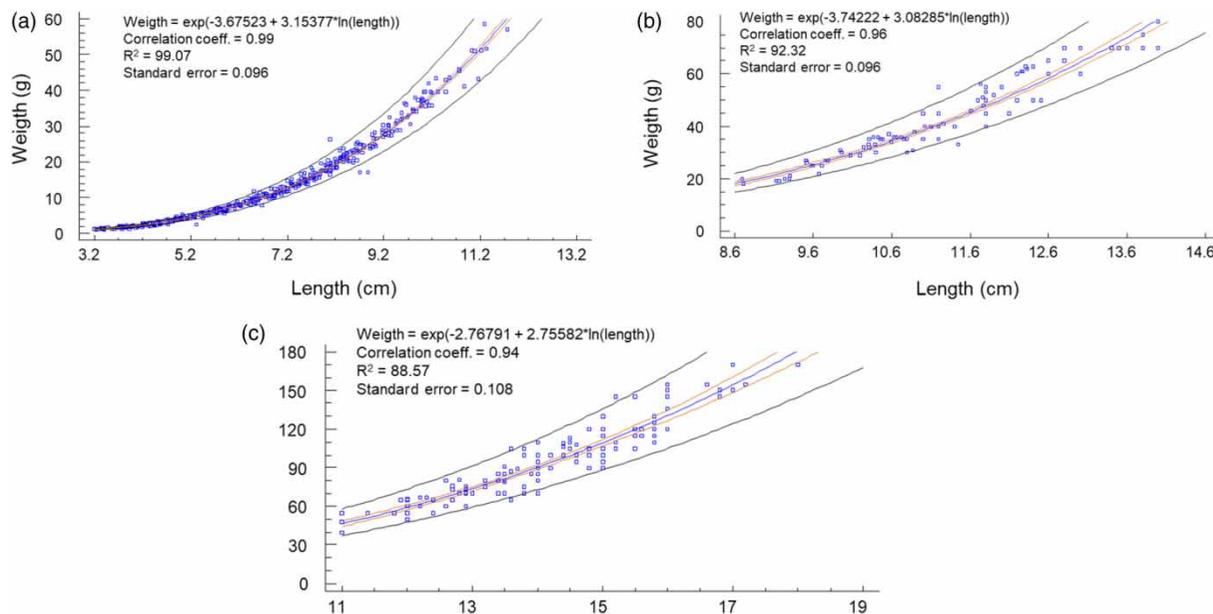
hand, Rahmat et al. (2019) showed a SGR of 1.31% of tilapia with an average initial weight of 9 g.

For juveniles, a lower SGR was obtained than for fingerlings, being 1.07%. Yong et al. (2018) worked with tilapia males with a mean initial body weight of 37 g, a stocking density of 35 fish/m<sup>3</sup> and obtained a SGR of 1.60, and Bowyer et al. (2020) with an average initial fish weight of 36.22 g, a stocking density of 20 kg/m<sup>3</sup> obtained an average of SGR of 2.27. Comparing the data from these previously mentioned studies with those obtained in the present study, we can see that the results are similar, indicating that stocking density does not affect considerably the specific growth rate.

Adults of tilapia in this study with a 0.70% specific growth rate were within the range compared to other studies with tilapia of the same size, such as Obirikorang et al. (2019), who studied tilapia with an average body mass of 77.9 g and a stocking density of 74 fish/m<sup>3</sup> and obtained a SGR of 0.65%. Osti et al. (2018), who analyzed tilapia from 191 g obtained a SGR of 1.2% cultivated in a stocking density of 20 fish/m<sup>3</sup>.

On the other hand, the length–weight relationship is an important parameter for assessing the growth pattern, health, and general well-being of fish (Asase et al. 2016). That is why this relationship is shown in the three productive stages of tilapia analyzed in this study (Figure 5). In the length–weight relationship, the regression coefficient  $b$  provides information about the type of growth; if  $b = 3$  the growth is isometric and when  $b \neq 3$  the growth is allometric.

In fingerlings and juveniles of the present study, growth was isometric, indicating a good physiological state. Researchers have carried out studies regarding the weight–length relationship of *O. niloticus* under different conditions, resulting in most isometric growth conditions. This confirms the fact that tilapia is an adaptable species to different conditions. One of the studies was carried out by Gullian-Klanian (2013), who worked with different stocking densities of fingerlings of tilapia and obtained a regression coefficient ( $b$ ) of 3.168 for highest stocking densities which were 1.22 kg/m<sup>3</sup>. On the other hand, Nunoo & Anani (2016) studied juvenile tilapia with an initial mean weight of  $22.8 \pm 2.1$  g and a density of 2 fish/m<sup>2</sup>, and they showed a regression coefficient ( $b$ ) of 3.1.



**Figure 5** | Relation of length–weight relationship of *O. niloticus* in three productive stages: (a) fingerling, (b) juvenile, and (c) adult.

Regarding adults, there have not been as favorable results as with studies carried out with fingerlings and juveniles. Such is the case of Malik *et al.* (2017), who studied male and female tilapia, the female brooders (average weight  $133.9 \pm 15.6$  g) and male brooders (average weight  $143.42 \pm 11.4$  g); they obtained values of  $b$  of 1.20 for females and 1.36 for males. Also, Ondhoro *et al.* (2019) studied adult tilapia of 153 g and showed positive allometry with values of  $b$  of 3.3 which means that the weight of an organism increases more than length ( $b > 3$ ). This is in contrast to what happened in the present study where a value of  $b$  of 2.75 was obtained, indicating a negative allometry which means that length increases more than weight ( $b < 3$ ). Even though the adults had a slight negative allometry, it is considered to be within the acceptable range of fish with good physiological health; thus, values obtained from  $b$  for three productive stages during this experiment tended to an isometric growth (Figure 5), considering that isometric growing species are the ones who fluctuate within the values  $b = 2.5$  and  $b = 3.5$  (Froese 2006).

The difference in length–weight relationship obtained during the three development stages of fish indicates that fish do not normally maintain the same shape or body conformity at different productive stages. This variation can also be attributed to the variation in sample size, growth stage, and

environmental factors. There was no negative effect on physiological health derived from the hyperintensive density of culture that was administered in this study, which is greater than reported in the aforementioned studies.

## CONCLUSION

In this research, a comparison of nitrogenous excretion (nitrites, nitrates, and ammonium) and phosphorus in residual water of a hyperintensive tilapia culture is presented. Based on the results, it might be concluded that a higher concentration per gram of fish is generated in tilapia fingerlings than in juveniles and adults in a hyperintensive culture of 270 fish/m<sup>3</sup>. According to the data presented, the density of the culture did not affect the survival of the organisms in any of the productive stages, as well as the growth or health of the fish; in addition, combined catabolism of proteins and lipids was presented as substrates for energy. This research might contribute to the improvement in the use of wastewater from aquaculture systems towards plant production, with greater use in the fingerling stage. As the nutritive solution of hydroponic vegetable crops is an important economic factor, a way is sought to avoid the supplementation of these fertilizers, which is why the study provides important information on

the flow of nitrogen and phosphorus with a view to their use in the generation of plant products, avoiding the pollution generated by the discharge of aquaculture effluents. In addition, it shows the carrying capacity of a hyperintensive culture, improving fish farming, by obtaining higher production, estimated at 80 kg/m<sup>3</sup>, without compromising the growth and quality of the fish.

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## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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