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Research Articles

Nitrogen excretion and oxygen consumption on hyper intensive tilapia *Oreochromis niloticus* culture using three different commercial diets

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ABSTRACT. The fish feed used in aquaculture systems has many nutritional factors like protein, fat, fiber, ash, and humidity; from these factors, one of great importance is protein. The protein of fish feed is obtained from fish meal, and the demand for fish meal has increased; this induces to increase the price. Many pieces of research have been done about this topic to mitigate this problem. This research shows the differences between three commercial foods of the different processes with the same quantity of protein, using tilapia *Oreochromis niloticus*, to prove any difference between NO_2^-, NO_3^-, and NH_{4+}. Nitrogen compounds were determined with HACH methods for the DR6000 spectrophotometer. Biometric data were registered throughout the growth of fish, along with measure aerobic metabolism. Results have shown that has better outcomes related to low excretion of nitrogen compound was medium-cost fish feed (MCFF). It also has sound effects associated with the length-weight relationship; that could be connected to these fish feed's protein sources.

Keywords: *Oreochromis niloticus*; nitrogen excretion; oxygen consumption; hyper intensive; aquaculture

INTRODUCTION

Aquaculture is the animal production industry with the highest growth rate in the last 20 years (Dantas *et al.*, 2017); in 2016, the production was 80 million tons (FAO, 2018). The aquaculture is also widely considered an option to fight hunger and poverty in developing countries (Lithgow *et al.*, 2017). One of the most important activities in aquaculture is fish nutrition. Hence 40% of the total production costs are inverted in fish feed; besides, if this task has inefficiencies will be a loss, especially for intensive aquaculture systems (Zhou *et al.*, 2017). Fish meal is a high quality, very digestible feed ingredient that is preferred to use on the fish diet; it carries large quantities of energy per unit weight. It is an excellent source of protein, lipids (oils), minerals, and vitamins (Hyacinth *et al.*, 2019). Meanwhile, fish feed is made of some sub-products with restricted use because it contains antinutrients; these compounds affect protein and mineralization; another disadvantage is that it decreases palatability, digestibility, or metabolism. It may even exert a toxic effect resulting in liver damage (Duodu *et al.*, 2018).

The ingredients used in fish feed are small fishes, chopped scrap fish, meat scraps, grain, plant meals, and fish meal. Another important characteristic to consider is that depending on the kind of fish will be the nutritional requirements (Boyd, 2013). However, the main ingredient of aquaculture feeds is fish meal. This ingredient's principal characteristic is digestibility and high protein (Kokou & Fountoulaki, 2018). Generally, proteins are used for hormone synthesis, cellular homeostasis, and activity to synthesize new tissue or simply grow (Vilhelm *et al.*, 2017).

The diet adds protein, and all the ingredients cannot be assimilated by the fishes and are throw away to the ambient in the form of waste (Valenti *et al.*, 2018). The primary compound found in protein is nitrogen. When the fish uptake this nitrogen-containing compound, the fish metabolize them and are excreted in ammonium (NH_4^+), the principal nitrogenous waste in the water.

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The nitrogen ingests also discharged as waste within 24 h (Ip & Chew, 2018). The ammonia can be found in two forms, (un-ionized form) and molecular NH_3 form, sometimes called non-dissociated, and the other form is the ammonium (ionic form) NH_4^+ ion. The two forms mentioned before coexisting in the water, and the proportion depends on pH and water temperature (Svobodova *et al.*, 2017).

There are two other nitrogen compounds, which are nitrite (NO_2^-) and nitrate (NO_3^-); nitrite principally comes from the oxidation metabolism of ammonia and gets accumulated because of the imbalance of nitrifying bacterial activity (Yun *et al.*, 2019). High concentrations of nitrite can cause nitrite accumulation in blood plasma (Thi *et al.*, 2019). Also, the nitrite can interfere with the physiological function like oxygen capacity of cell hemoglobin, immune system health, physiological metabolism, and endocrine regulation (Xie *et al.*, 2019). Fishes have various mechanisms to detoxify nitrites, such as methemoglobin reductase secretion or nitrite to nitrate oxidation as this compound is less toxic (Kim *et al.*, 2019). Nitrate is often considered nontoxic in comparison with ammonia and nitrite.

Moreover, nitrate is the most abundant form of inorganic nitrogen in freshwater (Isaza *et al.*, 2018). The high concentration of nitrate causes methemoglobinemia that reduces the capacity of hemoglobin to bind oxygen and carry it through the body (Freitag *et al.*, 2015); other adverse effects are decreased like reproductive maturation, abnormal skin coloration, and tissue damage (Yang *et al.*, 2019). Lastly, nitrogen is essential for animal nutrition and to control environmental pollution. To achieve waste reduction is vital to understand the quantitative nitrogen budget (David & Proença, 2017).

This study aimed to compare three commercial fish feed with the same quantity protein, using tilapia *Oreochromis niloticus*, to prove any difference between NO_2^-, NO_3^-, and NH_4^+. Also, the metabolic rate was measured to relate the consumption of dissolved oxygen between the fish feed. Additionally, to determine the growth and health of organisms, length-weight relationship and growth between treatments are shown. Lastly, the fish feed had the difference relating to the price; for that reason, the fish feed was differentiated for the price: low-cost fish feed (LCFF), medium cost fish feed (MCFF), and high-cost fish feed (HCFF).

MATERIALS AND METHODS

Rack system

Three treatments were realized considering three commercial fish feed, corresponding to LCFF, MCFF and HCFF, in a rack system to *Oreochromis niloticus* (Fig. 1).

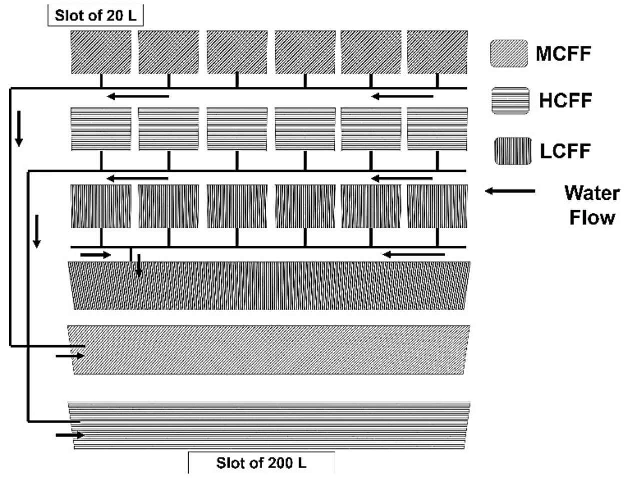

Figure 1. Placement of the three treatments and triplicates in the rack system to compare three commercial fish feed in *Oreochromis niloticus*. LCFF: low-cost fish feed, MCFF: medium cost fish feed, HCFF: high-cost fish feed.

A device was designed to culture fish with a structure made with 1" stainless steel rectangular tube. The system had 24 fishbowls, each of 20 L, made of acrylic glass (30 cm long × 25 cm wide × 20 cm high). Besides, the system had three rows of six fishbowls, each row had a tank also made of acrylic glass with a capacity of 80 L (150 cm long × 32 cm wide × 30 cm high), which works like water reservoir and avoid that the concentrations of nitrogen compounds get dangerous values for fishes. A Topaz Airsep oxygen generator was used to oxygenate the water in all tanks; this generator can produce an oxygen flow of 0.31 $Nm^3 h^{-1}$, at 62 KPa (9 psi) with a purity of 93%.

Experimental design

The experiment had three treatments and six replicates with an experimental unit of six fish, under a culture density of 270 ind m^{-3}. An amount of 108 individuals were randomly selected and cultured in the system for one week to get adapted. After adaptation week, biometry was done using a digital Mitutoyo® Vernier to get the length and a digital Sartorius® AY303 scale to gain weight. This first biometry was considered as an initial fish condition. During all experiments, the fish feed was supplied four times per day, with 10% of their total biomass. Treatments were ordered by fish feed; treatment 1 was MCFF, treatment 2 was HCFF, and treatment 3 was LCFF. Table 1 shows the nutritional aspects of each treatment.

Respirometry and metabolic rate measurements

After two weeks that fishes stayed in the rack system and were fed with the different fish feed, a respirometry was done to measure the differences between metabolisms due to the fish feed. The metabolism of freshwater fish may vary depending on the physical and

Table 1. Percent of nutritional aspects of fish feed used. LCFF: low-cost fish feed, MCFF: medium cost fish feed, HCFF: high-cost fish feed.

Fish feed	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Humidity (%)
LCFF	45	16	2.5	12	12
MCFF	45	16	2.5	12	11
HCFF	44	15	2.5	12	12

chemical variables of the water. In pH, the optimal range is 6.5-9.0. This range may vary depending on the carbonated compounds present in water (Mohammadi *et al*., 2019). Optimal temperature ranges may vary depending on the species that are being cultivated, but this can be 24-30°C (Li, 2017). Dissolved oxygen (DO) is considered on 6 mg L^{-1} (Ma *et al*., 2019). Abdel-Tawwab *et al*. (2019) and Wang *et al*. (2019) mentioned the rate of oxygen consumption could be used as a primary parameter to evaluate metabolic activities. The oxygen consumption can be used to measure the respiration rate of fish; for this reason, the respiration rate is used according to a measure for the metabolic rate (Magnhagen *et al*., 2018). For this research, a stop-flow respirometry was made, were used 17 plastic containers of 10 L as a chamber, five chambers were used for each treatment, and the remaining two were used as a control.

Five individuals from each treatment were randomly selected to measure aerobic metabolism (oxygen consumption = QO_2), using a 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 four hours (14:00, 18:00, 22:00, 02:00, 06:00, and 10:00 h) during a 24 h cycle. The fish feed was added to each chamber per treatment, considering the 10% of fish biomass, and a water sample was taken. All the fish used to determine QO_2 were euthanized on ice immediately after finishing the experiment to determine its dry weight and get the relation of the oxygen consumed by biomass (Steffensen, 1989).

During the experiment, nitrogen compounds were also measures like NO_2^-, NO_3^- and NH_4^+ in wastewater. A total water change was made in each treatment one day before starting the experiment. The next day fish feed was given at 8:00, 12:00 and 16:00 h; the samples were taken at 9:00, 13:00, 17:00, 21:00, 1:00 and 9:00 h, avoiding to take bubble airs fish feed or excretions in the sample.

Fish metabolism behavior in seven days

An important fact in the aquaculture is the feeding. Vilhelm *et al*. (2017) mention that meanwhile, the largest ration size led to a high content of lipids and reduced the protein retention efficiency (PER); also, the increase of fat deposition involves the destination of ingested protein N and can be fecal excretion or branchial excretion. Considering the results from 24 h behavior, the next part of this research was to obtain the NO_2^-, NO_3^- and NH_4^+ behavior in fish water of each fish feed during one week. The experiment began after 24 h. The fishes were fed three times per day (08:00, 12:00 and 16:00 h), and samples were taken after one hour of the first feeding for analysis.

Fish metabolism in seven days with 10% of water replacement

This part aimed to study the nitrogen compounds present in the water, considering daily 10% of water changes for seven days. Some research has made a replacement of 15-20% were the systems had continuous water flow. Hassaan *et al*. (2018) and Nguyen *et al*. (2019) mention that one-third of water volume in each tank was replaced daily by aerated freshwater after removing the accumulated excreta. The experiment began with feeding the fish, and after one hour, the sample was taken, and then 10% of water replacement was done. Uneaten food, feces, and sediments were also removed every day. The samples were taken after one hour of the first feeding for analysis of nitrogen compounds.

Determination of nutrients in the water

The measurement of nitrogen compounds was made using an analytical method. The importance of water analysis lies in the fact that concentrations must be suitable for the organisms. Also, Groenveld *et al*. (2019) mention that aquaculture's nitrogen emissions must have more attention because the effects of reactive nitrogen; lead to acidification because of NH_3^- and eutrophication because of NH_4^+ and NO_3^-. The levels recommend for the organisms are NH_3^- 0.23 ± 0.02 mg L^{-1}, NO_2^- 0.39 ± 0.22 mg L^{-1}, NO_3^- 0.89 ± 0.37 mg L^{-1} (Estim *et al*., 2018).

Analysis of the samples was determined using a Hach® DR6000 spectrophotometer; an analysis of NH_4^+ was done using the 8038 Hach® method called Nessler with an interval of 0.02-250 mg L^{-1}. This method uses a mineral stabilizer, which reduces the sample hardness. A dispersing agent contributes to forming coloration with the Nessler with the ammonium ions; this reaction can be measured at 425 nm. The NO_2^- analysis was done with the 8507 Hach® method called diazotization with an interval of 0.02-2.50 mg L^{-1}. The sample reacts with the sulfanilic acid to form diatonic salt that reacts with the chromotropic acid to produce a pink color proportional to the quantity of nitrite. It can be measured at 507 nm. The NO_3^- analysis was determined with the 8039 Hach® method called cadmium reduction with an interval of 0.3-30 mg L^{-1}; in this procedure, the metallic cadmium reduces nitrite to nitrate present in the sample. The nitrite ion reacts in the medium acid with the sulfanilic acid to form an intermediate diazonium salt; this salt reacts with the gentisic acid to form a solution with amber color that can be measured at 500 nm.

Length-weight relationship

Length-weight relationships are used for estimating the condition factor; this condition helps to know the fatness or well-being of fishes (Rodriguez *et al*., 2017). An equation ($W = a\ L^b$) is used to estimate the relationship between weight (g) and length (cm), the above mentioned can be transformed to:

$$\log(W) = \log(a) + b \log(L)$$

When this formula is applied, b may deviate from the ideal value of 3.0, representing an isometric growth; on the other side, if b is less than 3.0 means that fish is becoming slimmer with increasing length, and growth will be negatively allometric. In the other case, when b is greater than 3.0 indicate that the fish is becoming heavier, showing positive allometric growth and reflecting optimum condition for depending on the kind of fish feed; this is related to the content of protein (Jisr *et al*., 2018).

Statistical analysis

Water data analysis were processed using OriginLab® 8 and Statgraphics® Centurion XV. A single factor ANOVA with Tukey HSD was used to test the significance ($P < 0.05$) of differences between treatments based on the mean values of data collected. Graphs were done with the means of using the standard deviations.

RESULTS

Nitrogen compounds analyzed in fish feed used

Figure 2 shows NO_2^- concentration of *Oreochromis niloticus* in 24 h, seven days without water exchange, and seven days with water 10% exchange. In 24 h, the high-cost fish feed (HCFF) reached the high concentration, and the medium cost fish feed (MCFF) the lowest, having significant differences. The monitoring during seven days without water exchanges shows an accumulation of nitrites with a toxic amount. That is why partial water exchange is important, as shown in the graph with water exchange, where the behavior begins to stabilize from the fifth day.

Nitrates (NO_3^-) did no show significant differences in their behavior during 24 h for three treatments. The same trend occurred in 7-day monitoring without water exchange; however, significant differences were observed from day five, having a higher concentration in MCFF treatment and a lower concentration in the low-cost fish feed (LCFF) treatment, reaching a maximum amount of 40 mg L^{-1} of NO_3^-. The trend in seven days with water exchange had similar behavior in all treatments (Fig. 3).

Ammonium (NH_4^+) shows the same trend in 24 h and seven days monitoring, an ascending behavior, with a greater amount in HCFF but without showing significant differences (Fig. 4). It reached a maximum value of 5 mg L^{-1} of ammonium in seven days of monitoring, being a toxic amount for fish. The aforementioned is shown in graph with water replacements, where values less than 1 mg L^{-1} of NH_4^+ were achieved, which are tolerable by organisms.

The HCFF has more quantity of nitrogen compounds in the experiments of 24 h. The high amounts of these nitrogen compounds obtained by the HCFF could be considered as energy expenditure.

Fish metabolic rate

Metabolism behavior shows the same trend in the three treatments, showing no significant differences, with a maximum consumption peak at night and less consumption during the day. The daily oxygen consumption per treatment is shown (Fig. 5). The metabolic rates are related to oxygen consumption, and this is directly linked to fish size, feeding rate, activity level, system cleanliness from wastes, and water temperature. Tilapia *O. niloticus* could be better adapted to the occasional low DO levels than cold water species, even though all species can tolerate low DO levels. There is a particular relation between oxygen consumption and the excretion of nitrogen compounds, especially the excretion of NH_4^+, considering the fish weight and time.

The MCFF had less consumption of dissolved oxygen, and excretion of NH_3^+ was less compared with the other two fish feed. The LCFF had low levels related to nitrogen compounds and had low levels of consumed DO, and, in the case of NH_4^+, it had low levels compared with the other two fish feed.

Length-weight relationship

The length-weight relationship was obtained for the three treatments in the present study, getting the same regression coefficient in all of them, with a value of 2.9, which suggests an isometric growth, indicating a good

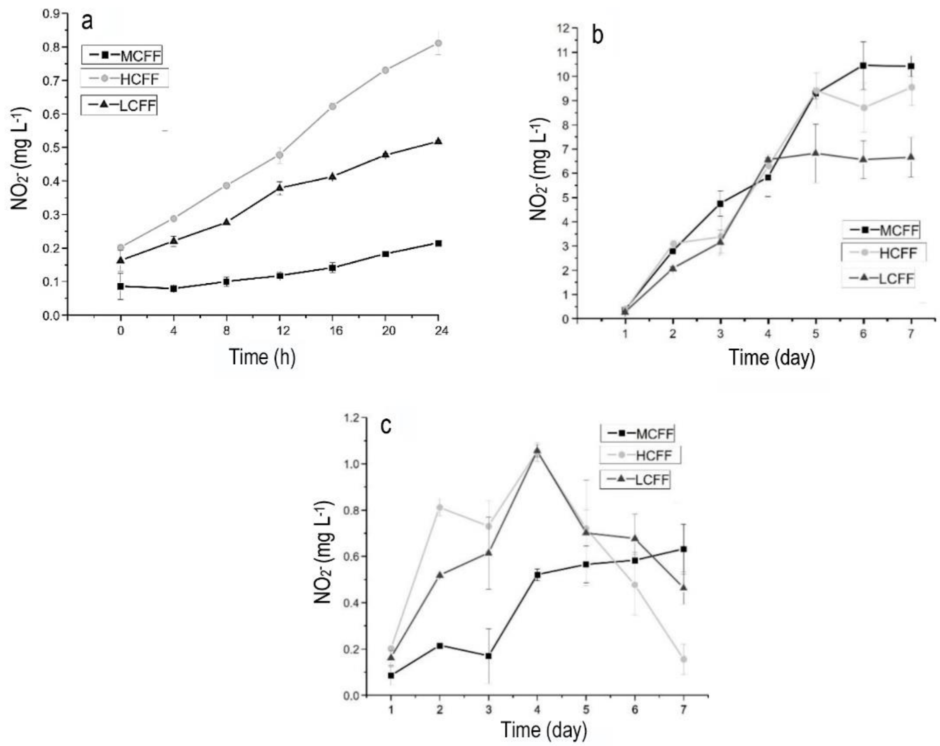

Figure 2. Quantities of NO_2^- obtained to compare three commercial fish feed *Oreochromis niloticus*. a) Experiment of 24 h, b) experiment of seven days without water exchange, c) experiment with 10% water exchange. LCFF: low-cost fish feed, MCFF: medium cost fish feed, HCFF: high-cost fish feed.

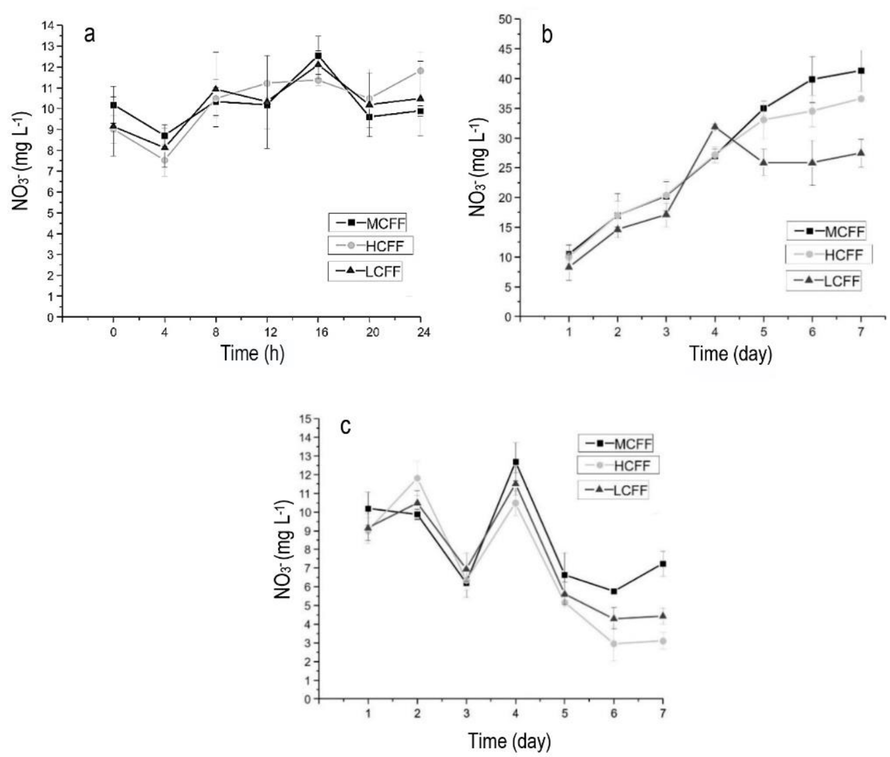

Figure 3. Quantities of NO_3^- to compare three commercial fish feed *Oreochromis niloticus*. a) Experiment of 24 h, b) experiment of seven days without water replacement, c) experiment with 10% water exchange. LCFF: low-cost fish feed, MCFF: medium cost fish feed, HCFF: high-cost fish feed.

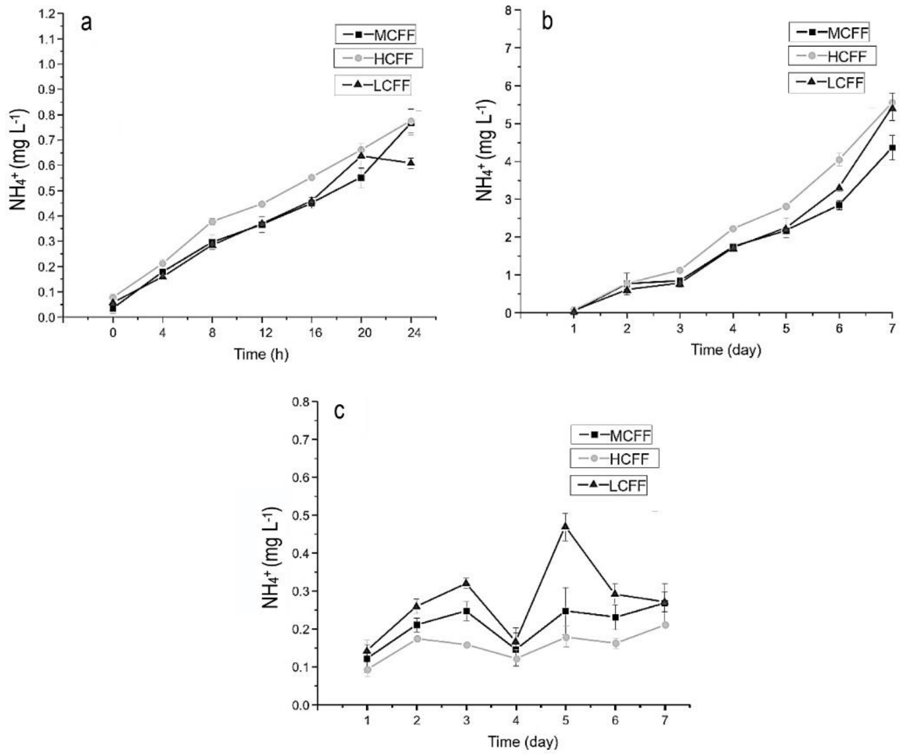

Figure 4. Quantities of NH_4^+ to compare three commercial fish feed *Oreochromis niloticus*. a) Experiment of 24 h, b) experiment of seven days without water replacement, c) experiment with 10% water exchange. LCFF: low-cost fish feed, MCFF: medium cost fish feed, HCFF: high-cost fish feed.

physiological state; in Figures 6-8, are shown the relation length-weight obtained in MCFF, HCFF and LCFF respectively.

Fish growth

In all the aquaculture systems, fish growth is important because it is one of the aquaculture's objectives to improve fish growth. Figure 9 shows growth performance between treatments having significant differences, resulting in a higher growth MCFF treatment, followed by HCFF treatment and the lowest growth LCFF treatment. Protein is the main component of fish muscle and is the source of nitrogen excretion. The ingested protein by fish is digested in the stomach and absorbed in the intestine as amino acids and peptides. These are used for muscle growth, energy production, or both, depending on the fish condition and feed composition. When the protein is metabolized, the final products are inorganic nitrogen, CO_2, and water. Subsequently, said inorganic nitrogen would be released as either ammonia (NH_3^+). An important characteristic of the protein is that if the fish entirely digested it, everything would be absorbed by the fish, and the feces would not contain proteins. From those above, and related to fish growth, it was observed that protein in MCFF is the most digestible by organisms.

DISCUSSION

Previous research has shown that 60% of fish feed used in aquaculture was in the form of tiny particles. The decomposition of said particles consumes oxygen, produces ammonia, and other toxic substances that affect fish welfare and cause a heavy burden on filtration and oxygenation equipment (Zhou *et al.*, 2018). An element of great importance in biological systems is nitrogen; this is a fundamental component of amino acids, proteins, and nucleic acids. The catabolism of these nitrogen-containing compounds releases ammonia, which is toxic and must be eliminated. When the fish consume the amino acids present in the food, they digest them to obtain proteins. The amount of amino acids that the fish absorbs for growth and development exceeds those it needs; for this reason, it degrades since this excess cannot be stored (Ip & Chew, 2018). Hence suppose the water is disposed of in the water effluents. In that case, this could be a big problem, can cause issues like eutrophication of aquatic bodies, which damages the flora and fauna that grow in the water and disturbs the food chain and food web of the ecosystem (Singh *et al.*, 2019). Recirculating aquaculture systems (RAS) is used to avoid this kind of problem. In this kind of system,

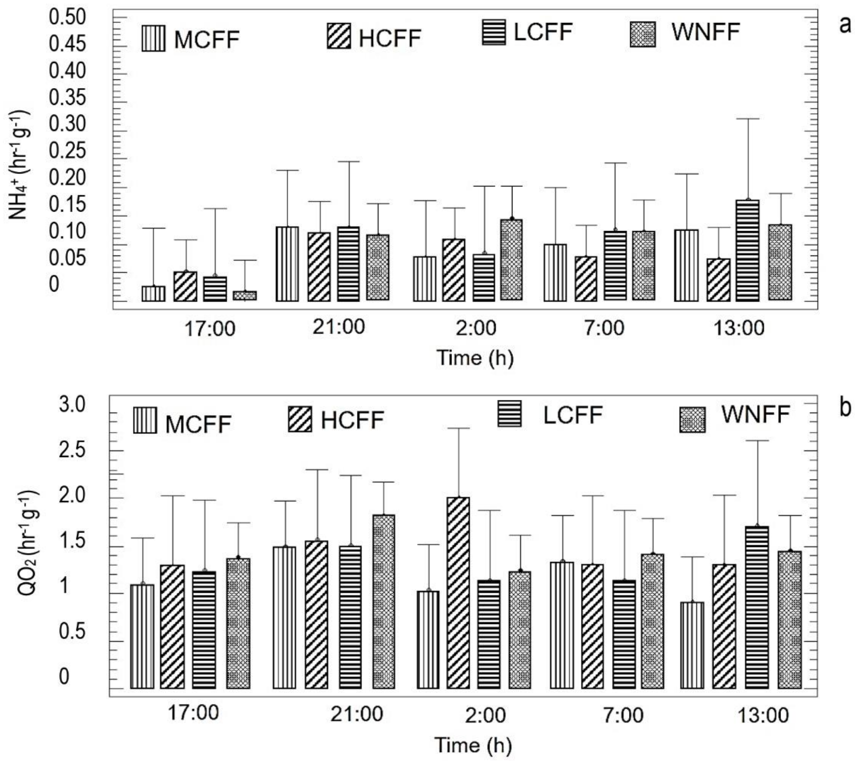

Figure 5. Results obtained from the respirometry by fish feed. a) NH_4^+ excretion during 24 h by fishes in different treatments, b) dissolved oxygen consumption (QO_2) during 24 h by fishes in different treatments. LCFF: low-cost fish feed, MCFF: medium cost fish feed, HCFF: high-cost fish feed.

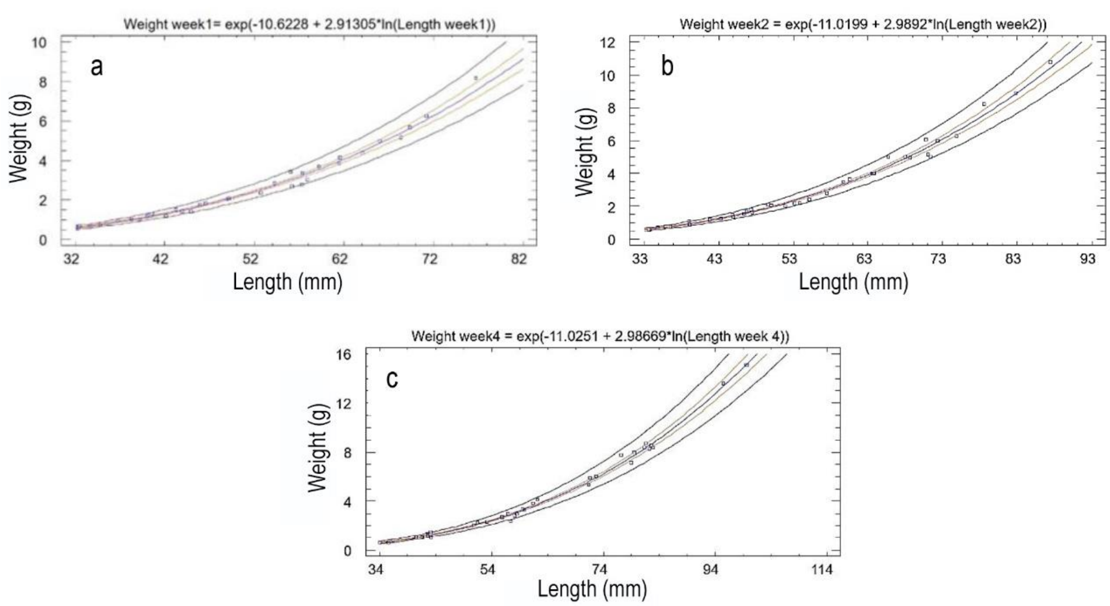

Figure 6. Relation length-weight presented in the treatment of medium-cost fish feed (MCFF). a) Week 1, b) week 2, c) week 4.

the aquaculture wastewater is circulated to an additional treatment unit for purification, and cleaned water is recycled to be used for fish cultivation (Zou *et al.*, 2016).

On the other hand the fish metabolic rate has a great importance because if organism stay a long time with low DO levels, it can potentially cause irreversible damage (Thorarinsdottir, 2015). Nevertheless, this rela-

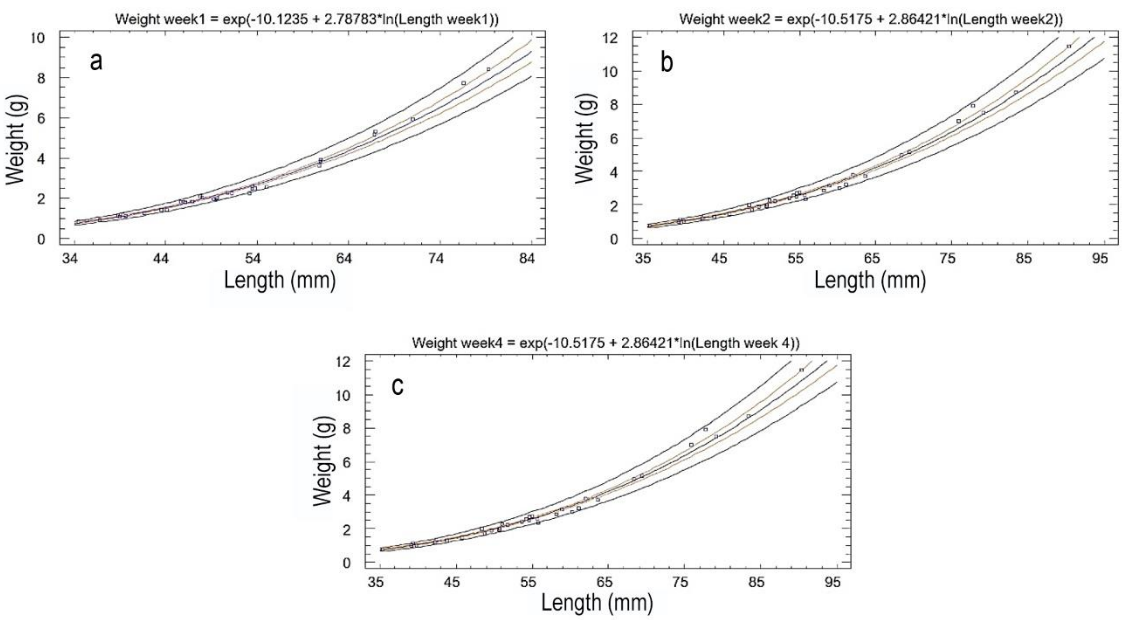

Figure 7. Relation length-weight presented in treatment of high-cost fish feed (HCFF). a) Week 1, b) week 2, c) week 4.

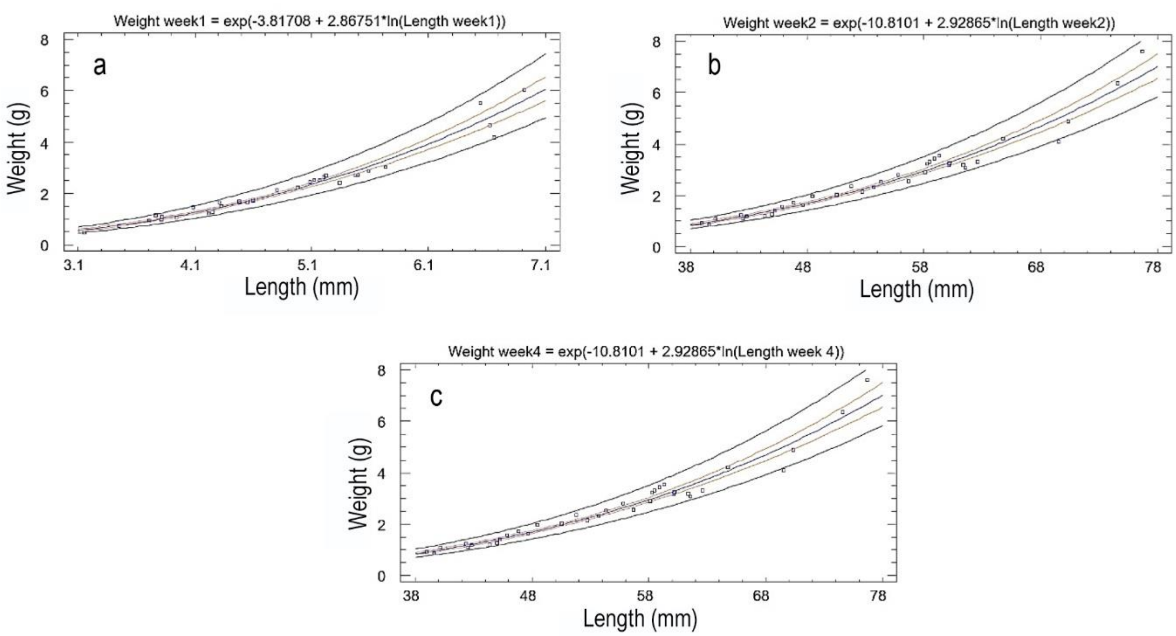

Figure 8. Relation length-weight presented in treatment of low-cost fish feed (LCFF). a) Week 1, b) week 2, c) week 4.

tes that fish had environmentally hypoxia, which induced a reduction in metabolic rate and suppression of ammonia production (Ip & Chew, 2018). Maybe it could be necessary to make a digestibility study because of this kind of study rive due to how much of a given nutrient is digested by fish (Vilhelm *et al.*, 2017). It can also be considered low metabolic waste because the waste production and dissolved oxygen consumption are similar (Mohammadi *et al.*, 2019).

Where as the relation between length and weight has important implications in the first instance for fisheries science and then for the population dynamics (Torres *et al.*, 2012). Additionally, the fish length can be an important parameter for the biologic process related to the absorption of nutrients and the correct metabolism (Sabaly *et al.*, 2019). Nevertheless, in some cases, the food's protein is indigestible, and therefore the fish excretes feces as organic nitrogen (Lekang, 2013).

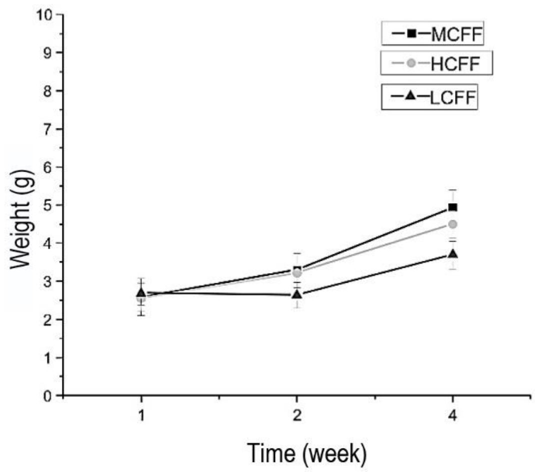

Figure 9. Growth obtained by treatments. LCFF: low-cost fish feed, MCFF: medium cost fish feed, HCFF: high-cost fish feed.

CONCLUSIONS

This study provided data related to three commercial fish feed with three different prices; these fish feed can be found in the research area. The fish feed that has better results on low excretion of nitrogen compound was MCFF and has good results associated with the length-weight relationship. This study fulfilled the aims set for it, and the data presented might constitute a valuable guideline for establishing new experiments related to the use of this fish feed.

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