REGULAR ARTICLE

# Bioprocess for mass production and feed utilization of *Azolla pinnata* in aquaculture ponds: a perspective of bioeconomy and eco-friendly technology for small farms

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The authors declare no conflict of interest.

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***Autor contribution***
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## Abstract

Aquatic plants have a high potential to be used as eco-friendly technology in fish farming effluent treatment systems. However, there is still a reduced use of the vegetable biomass produced in these treatment systems. Thus, the aim of this study was to develop an alternative feed with the aquatic plant Azolla pinnata to take advantage of plant biomass, reusing the plant to develop a new product and promote a circular economy. *A. pinnata* was implemented in decantation pond that receives effluents from fish farming. Samples were collected weekly at four times (0, 7, 14, and 21 days). Posteriorly, *A. pinnata* was processed to obtain the meal. Azolla meal was offered along with three commercial feed (24%, 28% and 32% of the crude protein) in an experiment to evaluate the zootechnical performance of tilapia in small tanks. The results indicated that *A. pinnata* was efficient to retention of phosphorus and nitrogen after seven days. In the experiment with animals, the treatment using commercial feed with 28% of the crude protein + Azolla showed the best efficiency rates for using the diet. Thus, the use of the *A. pinnata* meal was a viable alternative in the search for sustainable products to promote a bioeconomy in the small fish farms.

## Keywords
bioeconomy, circular economy, reuse effluents, sustainable aquaculture



## Introduction

In the last decades, the demand for healthy food has resulted in an increase in the global demand for protein from aquaculture. In 2020, the global apparent per capita consumption was 20.2 kg, a rate almost twice that of annual world population growth for the same period, and following this market demand the total world aquaculture production registered 178 million tons of aquatic animals (FAO, 2022). Brazilian fish farming produced 841.005 tons of fish, 4.7% more than the previous year, tilapia represented 61% of all Brazilian fish farming, with a production of ~324 thousand tonnes, consolidating Brazil in the 4th position among the largest tilapia producers in the world (Valenti *et al*., 2021).

Despite the expressive growth of aquaculture, one of the limiting factors for the expansion of the activity are effluents (Boyd *et al*., 2020; Pizato *et al*., 2012; Turcios and Papenbrock, 2014; Zhao *et al*., 2019). In aquaculture is important to formulate highly digestible diets, because part of fish feed released into the water as suspended organic solids, ammonia, phosphates, and others (Baccarin and Camargo, 2005; Henry-Silva and Camargo, 2006). A production of one ton of tilapia in excavated nurseries generates about 300 kg daily of dissolved and solid effluents, which are generally discarded in water bodies (rivers, springs, streams) close to rural properties (Pizato *et al*., 2012). In this way, the effluent has a high impact power on water resources (Turcios and Papenbrock, 2014; Zhao *et al*., 2019).

The effluents from fish production are largely made up of components rich in nitrogen and phosphorus, essential elements for the growth of plant organisms, which in excess can cause eutrophication of aquatic environments (Pizato *et al*., 2012; Zhao *et al*., 2019). Eutrophication cause low dissolved oxygen in receiving waters, result of the disintegration of phytoplankton blooms (Milhazes-Cunha and Otero, 2017). Thus, efforts have been made to find an effective treatment to minimize the effects of effluents in aquaculture activities and to attend the legislation (Bueno *et al*., 2019).

Alternatives for the treatment and reuse of this effluent is a trend towards the sustainable development of the segment. The treatment process includes methods of physical, methods chemical, natural sedimentation, macroalgae adsorption, and

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other (Henry-Silva and Camargo, 2006; Zhao *et al.*, 2019). Nevertheless, aquatic plants have a high potential for their use in fish farming effluent treatment systems (Mohd Nizam *et al*., 2020; Osti *et al.,* 2018; Vasconcelos *et al*., 2020; Zhao *et al*., 2019). The fish farming integrated with the cultivation of aquatic plants is an excellent alternative for the treatment of effluents generated in production and the aquatic plant *Azolla pinnata*, a species of floating fern, is a species with great potential for this purpose (Boyd *et al*., 2020; Cherryl *et al*., 2014; Kumar *et al*., 2020).

The aquatic plants accumulate high amounts of nitrogen and phosphorus in their body composition (Kumar *et al*., 2020; Zhao *et al*., 2019). However, there is still a reduced use of the vegetable biomass produced in these treatment systems, where the plants need to be removed periodically to optimize the removal of nutrients, having no alternatives for the use and destination of these aquatic plants (Kumar *et al*., 2020; Pizato *et al*., 2012). Thus, alternatives to take advantage of this excess biomass can be successfully implemented (Pizato *et al*., 2012; Vasconcelos *et al*., 2020; Zhao *et al*., 2019).

In this context, the aim of the present study was to use *A. pinnata* to treated the effluents generated in tilapia production and reused the aquatic plant to develop an alternative meal to Nile Tilapia. The use of the meal of *A. pinnata* to develop a new product can promote sustainability and improvements in the yield of the fish produced and new marketing strategies for the small producer.

## Materials and methods

### *Location*

The study was carried out in fish farm Agropecuária do Buriti Perdido located 45 km from the center of Brasília / DF, Brazil. Buriti Perdido is a commercial fish farm, that raising fish of the tilapia species (*Oreochromis niloticus*), targeting the promising consumer market in Brasília, Federal District, which has a high demand for fish that is supplied through imports from other regions and countries.

### *Treating effluents*

Before the experiment starts, the aquatic plant *A. pinnata* was grown in water without organic matter for three months (control). Subsequently, a PVC structure was developed which had a dimension of 5 x 5m. The installation was placed in the decanting pond with a 20 x 20m dimension, which receives all the effluent generated from the intensive production of tilapia from five earth pond, which add up to 0.8 ha of area and an average annual production of 60 tons. It was used 50 kg of *A. pinnata* in four quadrants of 4 $m^2$ of the structure and samples were collected weekly that allowed the identification of the composition and rate of nutrient retention performed by the plant at times 0, 7, 14 and 21 days. At each time the plants' collection to biomass analysis and nutrient retention, 20% of the wet biomass volume was removed from the aquatic plant in each quadrant, which was identified, weighed, and stored in a freezer at -10 ºC for later bromatological analysis. At the end of the plant's exposure to the affluent, the evolution of the concentration of nitrogen, phosphorus, and in the plant's biomass was verified over the experimental time.

### *Determination of daily growth rate*

The daily growth rate (DGR) values were evaluated weekly from the ratio of the fresh mass variation (Δm: ln [mfinal / minitial]) and the time in days (Δt:tfinal − tinitial): DGR = Δ(m / t).

### *Processing and composition of the Azolla meal*

The aquatic plants were wash in freshwater and weigh. The drying was carried out at the Laboratory of Analytical Processing and Animal Welfare Studies of the University of Brasília (UNB) in a forced air circulation oven where the plants were left for 8 hours at 35 °C until the plant was dried. After this procedure, the dry material was crushed and sieved in a 2 mm sieve, a process that allowed the preparation of Azolla meal, which was stored in a freezer at -10 ºC for posterior analysis of the composition. Subsequently, the Azolla meal was evaluated and the crude protein content (% PB =% N × 6.25) was determined using the Kjeldahl method, with the aid of the Kjeltech 1030 autoanalyser (Tecator, Hoganas, Sweden) and fat, by the hydrolysis acid method, both following the one proposed by AOAC (2000). The ashes were determined by incineration at 550 ºC.

The phosphorus content was determined according to the metavanadate colorimetry method, in which the samples were previously incinerated in a muffle furnace for four hours at 550 °C (Michelsen, 1957). The energy was determined using an IKA® C20000 calorimetric pump. Qualitative analysis of amino acids in the Azolla meal were applied using high performance liquid chromatography (HPLC) as proposed by Appenroth et al. (2017).

### *Experimental design*

After preparing the Azolla meal, it was realized an experiment to evaluate the zootechnical performance of tilapia in small tanks. Azolla meal was offered along with three commercial feed (Table 1) and also was offered to fish only Azolla meal. The treatments were: 1 - Feed with 24% crude protein (24% P); 2 - Feed with 24% crude protein and Azolla (24% PA); 3 - Feed with 28% crude protein (28% P); 4 - Feed with 28% crude protein and Azolla (28% PA); 5 - Feed with 32% crude protein (32% P); 6 - Feed with 32% crude protein and Azolla (32% PA); 7 - Pure *Azolla* (A).

**Table 1:** Nutritional composition of commercial diets using in the experiments[1].

| Proximate Analyses | 24%CB | 26%CB | 32%CB |
|---|---|---|---|
| Total Dry Matter (%) | 89.33 | 89.70 | 90.27 |
| Digestible Dry Matter (%) | 63.10 | 61.80 | 59.93 |
| Gross Energy (kcal / kg) | 3.840 | 3.800 | 4.138 |
| Digestible Energy (kcal / kg) | 2.643 | 2.687 | 3.096 |
| Crude Protein (%) | 23.57 | 26.77 | 31.89 |
| Digestible Protein (%) | 19.59 | 22.38 | 25.25 |
| Total Phosphorus (%) | 8.27 | 8.15 | 8.69 |
| Digestible phosphorus (%) | 4.96 | 4.64 | 4.89 |

[1]24% CP - Feed with 24% crude protein; 28% CP - Feed with 28% crude protein; 32% CP - Feed with 32% crude protein.

Seven tanks structures were constructed with dimensions of 2 x 2m, divided into four quadrants of 1 x 1m each, or generated up to four final repetitions (4x4) to perform the feeding tests with seven treatments. The experiment started with an average body weight of 129 g and which were randomly distributed in 40 units for each quadrant, totaling 160 fish per repetition and 480 throughout the experiment.

The fish were feed for 30 days, with a quantity restricted to 3% in relation to body biomass. Feeding was performed three times a day: 9 am, 2 pm and 6 pm. At the end of the experiment, all fish were harvested for later biometry, morphometric analysis and filleting to determine the fish body performance. The water temperature (ºC), pH, dissolved oxygen (OD), transparency (Secchi disk) and saturation (μS.cm$^{-1}$) were measured weekly near the pond surface using a multi-parameter YSI probe (Model 6820).

Parameters of fish growth performance were calculated: mortality (%), final weight (g), biomass gain (kg), consumption fresh *Azolla* (kg), apparent feed conversion (total feed consumption / total biomass gain were analyzed), specific growth rate (SGR) (% / day): (final weight - initial weight / days of cultivation) x 100) and yield of fillets (%).

### *Data analysis*

The descriptive analysis of the evaluated characteristics considered the mean and standard deviation values within each treatment. Values greater than 3 times the standard deviation in relation to the mean were considered outliers and removed from the analysis. The effect of the treatment on the variables evaluated was obtained through one-way analysis of variance (ANOVA One-Way), followed by Tukey post-hoc test to compare the means of each treatment level. The statistical model used in the analysis of variance is represented by the equation:

$$y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

In which yij is the response variable j from treatment i; μ is the general average; τi is the fixed effect of treatment i; and εij is the random residue. The residual of the regression model was considered normal according to the Shapiro-Wilk test and its homoscedastic variance by the Levene test. The SGR variable did not present homoscedasticity, for this reason, the Box-cox transformation was applied (Box and Cox, 1964). The Consumption fresh *Azolla* variable did not meet the assumptions for carrying out an ANOVA. Therefore, the Kruskal-Wallis non-parametric test was applied to this variable, followed by Dunn's post-hoc test.

Principal component analysis (PCA) was performed using the variables that met the assumptions of ANOVA, always based on the correlation matrix between the variables. The first two eigenvectors with the highest percentage of accumulated variance were considered for the construction of the graphs. The *confidence ellipses* indicate the degree of grouping of the treatments evaluated based on a coefficient of 0.95. All analyzes were performed using Software R (R Core Team, 2020), with a significance level of 5%.

## Results

### *Treating effluents*

The main nutrients in the eutrophication process, phosphorus and nitrogen, had similar behaviors up to the 7th day of exposure of *Azolla* in decantation tanks, retaining significant amounts of these nutrients (Table 2). There was a retention of 8.2% (2.3t/ha) of phosphorus, while the plant retained 6.3% (3.1t / ha) of nitrogen. However, from the 14th day, the nitrogen followed a linear behavior regarding the retention of this nutrient, reaching 16.5% (5.1 t / ha), while the phosphorus decreased by -26.3% (-9.6t / ha). The results demonstrate that, when leaving the aquatic plants for a time above 7 days, the expected effects of water purification are not achieved (Table 2). Visually, the absorption of nutrients by *Azolla* also was observed and the color gives evidence to the producer of the period in which the plant must be removed. When the plant is colored brownish-green, it is time to remove the plant (Figure 1).

**Table 2:** Relative percentage of nutrient retention in *Azolla*'s wet biomass during the experiment bioacumlation[1]

| | Percentage Relative to Time (Days) | | | |
|---|---|---|---|---|
| Nutrient | 0 | 7 | 14 | 21 |
| Phosphorus (%) | 7.8 | 8.2 | -26.3 | -22.7 |
| Nitrogen (%) | 5.5 | 6.3 | 16.5 | 15.1 |
| Calcium (%) | -6.2 | -6.7 | -61.0 | -69.5 |
| | Kg ha/day | | | |
| Nutrient | 0 | 7 | 14 | 21 |
| Phosphorus | 1.8 | 2.3 | -9.6 | 1.0 |
| Nitrogen | 2.6 | 3.1 | 5.1 | -0.7 |
| Calcium | -1.0 | -1.0 | -8.1 | -1.3 |

[1]mean values (n=12) per treatment.

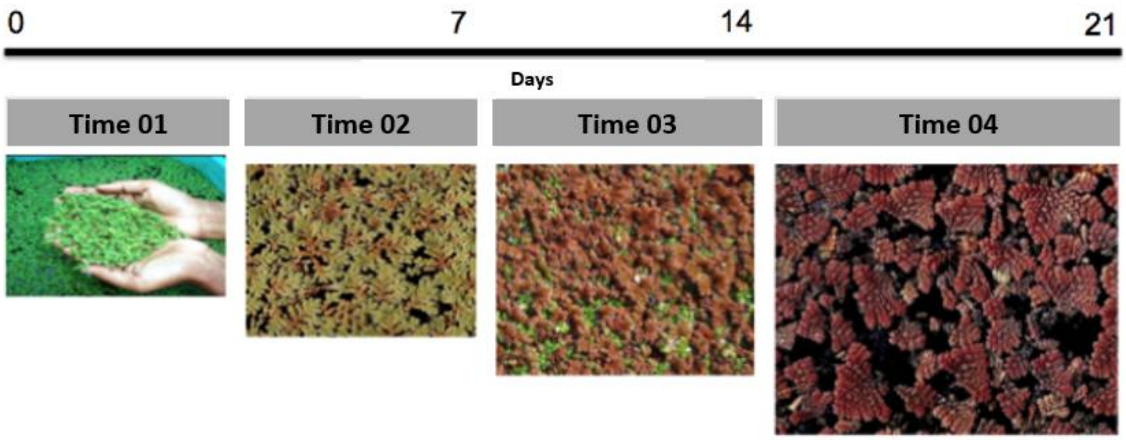


**Figure 1:** Flowchart illustrating the temporal monitoring of nutrient retention from fish production. The brownish-green coloring of the plant indicates the time the environmental management.

***Composition of Azolla meal***

The bromatological parameters, minerals and amino acids content of the dry matter *Azolla* meal was determined (Table 3). The *Azolla* meal showed 22.31% of the crude protein, 15.22% of the ash content and 13.75% of the crude fiber. The meal also showed low lipid content (3.32%) and a high value of the fiber in acid detergent (41.50%) and neutral detergent fiber (44.10%). In relation to amino acids content, the glutamic acid presented the highest value (10.50%) (Table 3).

**Table 3.** Bromatological parameters, minerals and amino acids content of the dry matter *Azolla* meal.

| **Chemical composition (%)** | ***Azolla* meal** |
|---|---|
| Dry matter (%) | 95.12 |
| Moisture (%) | 4.88 |
| Ash content (%) | 15.22 |
| Protein content (%) | 22.31 |
| Crude fiber (%) | 13.75 |
| Lipid contente (%) | 3.32 |
| Gross energy (MJ/kg) | 12.60 |
| Lignin (%) | 23.75 |
| Cellulose (%) | 10.13 |
| Hemicellulose (%) | 8.35 |
| Fiber in acid detergent (%) | 41.50 |
| Neutral detergent fiber (%) | 44.10 |
| Calcium (%) | 1.05 |
| Phosphorus (%) | 1.94 |
| Magnesium (%) | 0.34 |
| Potassium (%) | 1.65 |
| Arginine (%) | 1.24 |
| Histidine (%) | 0.46 |
| Isoleucine (%) | 1.12 |
| Leucine (%) | 1.91 |
| Lysine (%) | 1.15 |
| Methionine (%) | 0.35 |
| Phenylalanine (%) | 1.33 |
| Threonine (%) | 1.10 |
| Tryptophan (%) | 0.40 |
| Valina (%) | 1.22 |
| Aspartamic acid (%) | 8.40 |
| Glutamic acid (%) | 10.20 |
| Cystine (%) | 0.20 |
| Glycine (%) | 1.10 |
| Serina (%) | 1.00 |
| Tyrosine (%) | 0.72 |

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***Evaluation of zootechnical performance***

It was found that the treatment 28% PA, 32% P and 32% PA showed the best efficiency rates for using the diet with feed conversion of 1.5, 1.4 and 1.5 respective (Table 4). The same treatments has higher values final weight and weight gain. The yield of fillets of treatment only *Azolla* possessed a lower percentage (22.5 %) when compared to others treatment. The thermal growth coefficient showed differences in most of treatment ($P < 0.05$) (Table 4).

No statistical differences ($P < 0.05$) in specific growth rate was observed between the treatment 24% PA, 32% P and 32% PA (Table 4). Mortality was not observed in most treatments, only in the 32% PA treatment 3.3% of the animals died. There was no significant difference ($P < 0.05$) in the consumption fresh *Azolla* (Table 4).

**Table 4.** Principal component analysis (PCA). Weight gain and final weight have a high positive correlation and both have a high negative correlation with apparent feed conversion[1].

| Treatment | IW (g) | FW (g) | YF (%) | SGR | M (%) | CFA (kg) | WG (g) |
|---|---|---|---|---|---|---|---|
| **24% P** | 129.7 (2.7)$^{a}$ | 185.0 (5.0)$^{c}$ | 29.0 (0.5)$^{abc}$ | 1.7 (0.2)$^{b}$ | 0 | 0 (0.0)$^{a}$ | 55.5 (2.5)$^{cd}$ |
| **24% PA** | 129.1 (6.2)$^{a}$ | 190.0 (0.0)$^{bc}$ | 29.0 (0.0)$^{abc}$ | 1.8 (0.0)$^{a}$ | 0 | 150 (0.0)$^{ab}$ | 60.8 (6.2)$^{bc}$ |
| **28% P** | 123.5 (3.5)$^{a}$ | 200.0 (10.0)$^{b}$ | 27.8 (0.7)$^{c}$ | 1.2 (0.0)$^{d}$ | 0 | 0 (0.0)$^{a}$ | 76.5 (13.5)$^{ab}$ |
| **28% PA** | 126.1 (3.7)$^{a}$ | 215.3 (2.0)$^{a}$ | 28.0 (0.0)$^{bc}$ | 1.3 (0.0)$^{c}$ | 0 | 150 (0.0)$^{ab}$ | 89.1 (1.8)$^{a}$ |
| **32% P** | 133.5 (3.5)$^{a}$ | 226.2 (1.2)$^{a}$ | 29.6 (0.5)$^{a}$ | 1.8 (0.0)$^{a}$ | 0 | 0 (0.0)$^{a}$ | 92.7 (4.7)$^{a}$ |
| **32% PA** | 135.0 (0.0) $^{a}$ | 220.6 (8.0)$^{a}$ | 29.3 (0.5)$^{ab}$ | 1.8 (0.0)$^{a}$ | 3.3 (3.0) | 150 (0.0)$^{ab}$ | 72.5 (10.6)$^{abc}$ |
| **A** | 132.3 (2.5)$^{a}$ | 169.6 (1.5)$^{d}$ | 22.5 (0.7)$^{d}$ | 0.8 (0.0)$^{e}$ | 0 | 350 (0.0)$^{b}$ | 37.3 (2.3)$^{d}$ |

[1]Values are expressed as the average and standard error. Values in columns marked with the same letters do not differ significantly ($p < 0.05$). Initial weight (IW); Final weight (FW); Yield of fillets (YF); Specific growth rate (SGR); Mortality (M); Consumption fresh *Azolla* (CFA); Weight gain (WG); Apparent feed conversion (AFC). 24% P - Feed with 24% crude protein; 24% PA - Feed with 24% crude protein and *Azolla*; 28% P - Feed with 28% crude protein; 28% PA - Feed with 28% crude protein and *Azolla*; 32% P - Feed with 32% crude protein; 32% PA - Feed with 32% crude protein and *Azolla*; Pure *Azolla* (A).

These results were supported by the principal component analysis (PCA) (Figure 2). The analysis of PCA shows that variables highly correlated tend to stick together and in the same direction. Weight gain and final weight have a high positive correlation and both have a high negative correlation with apparent feed conversion (Figure 2).

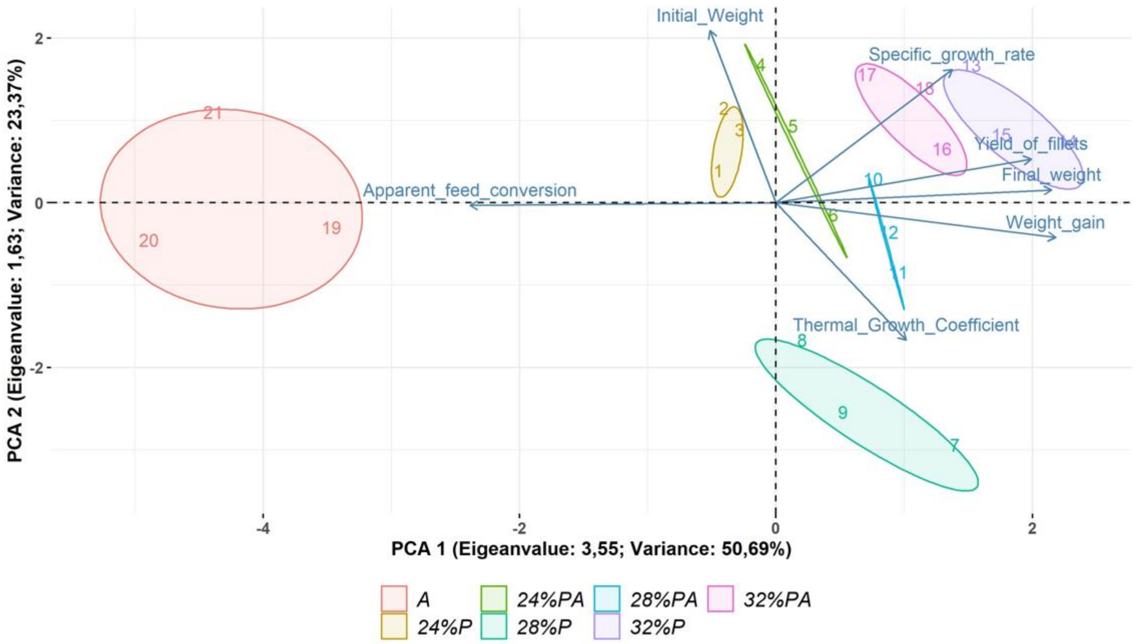


**Figure 2.** Principal component analysis (PCA). Weight gain and final weight have a high positive correlation and both have a high negative correlation with apparent feed conversion.

## Discussion

### *Treating effluents*

The results obtained in this study show that the use of *A. pinnata* for purifying the effluent of the aquaculture is a low-cost, eco-friendly, technology very efficient and a way to adopt sustainable practices in aquaculture. Studies show that the impacts of aquaculture can be minimized through management and planning based on practices that provide the maintenance of biodiversity and become the activity sustainable and profitable, and also improve the use of the natural resource (Bohnes and Laurent, 2020; Valenti *et al*., 2018). In this way, the use of technologies accessible to small producers to treat effluents makes them more competitive and adds value to the final product (Boyd *et al*., 2020; Mohd Nizam *et al*., 2020; Vasconcelos *et al*., 2020).

The use of the *A. pinnata* in the present study was efficient due to the significant amounts of the retention of phosphorus and nitrogen observed after 7 days. In general, *Azolla* is highly efficient in reducing aquatic contamination through the bioaccumulation of contaminants (Ansari *et al*., 2020). Upadhyay and Tripathi (2007), reported that *A. pinatta* is efficient in phytoremediate and hyperaccumulate metals, metalloids and other contaminants. Bennicelli *et al*. (2004), showed the use of *A. caroliniana* has the potential to purify the waters polluted by mercury and chromium. *A. caroliniana* also is contributed to reducing nitrate and phosphate concentration of fish farming effluent (Toledo and Penha, 2011). *A. pinnata* has proved effective in domestic wastewater phytoremediation studies reducing the total phosphorus and total nitrogen (Rai, 2008).

The results of bioaccumulation obtained in the present study was similar in previous studies (Arora and Singh, 2003; Muvea *et al*., 2019). In particular, the results showed greater removal capacity of nitrogen in the water on the 14th day. *A. filiculoides* showed the same retention on the 14th day of cultivation (Arora and Singh, 2003). According to Forni *et al*. (2001), the use of phytotreatment systems with *A. filiculoides* also was efficient in the removal of nitrogen and phosphorus during the period of two weeks.

Nitrogen and phosphorus are essential for plant metabolism and growth (Bi *et al*., 2019). Besides that, macrophytes take up nutrients to build up their biomass over time (Muvea *et al*., 2019). In this way, it was expected the retention the phosphorus and nitrogen over the study period. Despite that, *A. pinnata* expected effects of water purification were not achieved to phosphorus for a time above 7 days. The reduction of phosphorus on the 14th day may be a result of the growth of the macrophyte, as well as the sedimentation mechanism (Arora and Singh, 2003; Forni *et al*., 2001; Redding *et al*., 1997). Redding *et al*. (1997), report the risks of *A. filiculoides* remaining for a longer period in the treatment system and its decomposition brings a return to the previous levels of nutrients. Thus, our results suggests that *A. pinnata* should be removed on the 7th day and the and the brownish-green coloring of the plant indicates the time to do this environmental management.

***Azolla meal***

In general, the proximate composition of the *Azolla* meal were similar to those found in previous studies (Basak *et al.*, 2002; Cherryl *et al.*, 2014; Magouz *et al*., 2020). Crude fiber content of *Azolla* meal varies from 12.7% to 15.71%, and the lipids and ash vary from 3.4% to 3.7% and 15.6% to 24.2%, respectively (Alalade and Iyayi, 2006; Basak *et al*., 2002; Cherryl *et al*., 2014; Kumar *et al*., 2019). The values obtained in the present study to neutral detergent fibre was higher compared with that report by Alalade and Iyayi (2006), however the value of the acid detergent fibre was lower.

The value of the crude protein of *Azolla* meal range between 21.4% to 25.7% (Alalade and Iyayi, 2006; Basak *et al.*, 2002; Cherryl *et al.*, 2014; Kumar *et al*., 2019). Additionally, the protein content showed in the present study was similar to the *A. pinnata* plant and higher than plant *A. filiculoides* (Souza *et al*., 2008). The variation of the proximate composition of *Azolla* meal could be attributed to environmental conditions that can affect the growth morphology and composition of plant as such soil nutrient, light intensity and temperature (Alalade and Iyayi, 2006). Despite that, our results of proximal composition suggests the use of the *Azolla* meal as a potential natural protein source.

The *Azolla* meal presented a rich content of the minerals such as calcium, magnesium, phosphorus and potassium. The values obtained of minerals and amino acid in this study were similar to report by Alalade and Iyayi (2006). Pillai *et al.* (2002), reported that the *A. pinnata* plant is naturally rich in minerals and could contain some probiotics. Additionally, the meal showed a good content of essential amino acids and the glutamic acid presented the highest value. Roy *et al.* (2016), also reported high content of the glutamic acid in *Azolla* plant (12.6%), near to *Azolla* meal. Glutamic acid is important to metabolism of plant and intervenes in the assimilation of nitrogen (Cao *et al*., 2010). In this way, *Azolla* meal showed good nutrient and minerals contents and contains almost all essential amino acids found in others ingredients used to feed animals.

***Zootechnical performance***

The use of vegetable biomass produced in fish farming effluent treatment systems is an alternative to promote sustainability (Vasconcelos *et al*., 2020; Zhao *et al*., 2019). In this context, our results suggested that *Azolla* meal offered with the commercial feed with 28% of the crude protein is a positive effect on feed conversion ratio in tilapia. In this way, the biomass of *A. pinnata* can be reused and can be used as a complementary diet for tilapia raised in ponds.

*Azolla pinnata* has been used as feed or an alternative protein source due their high biomass and good nutritional composition (Magouz *et al*., 2020; Mosha, 2018). Azolla meal incorporation partially or fully as a component in the tilapia feed has been related in previous studies (Alalade *et al*., 2007; Mounes *et al*., 2020; Magouz *et al*., 2020; Mosha, 2018; Souza *et al*., 2008). The studies reported the improvement in the growth performance of the fish with the dietary inclusion of Azolla meal (Mounes *et al*., 2020; Magouz *et al*., 2020; Mosha, 2018; Souza *et al*., 2008). Fiogbe *et al*. (2004), showed that *O. niloticus* juveniles feed with diet containing 45% incorporated *Azolla* meal exhibited weight gain and growth. Juvenile grass carp (*Ctenopharyngodon idella*) was feed with a diete of the five parts of feed to one part of *A. filiculoides* showed a high specific growth rate and daily growth gain (Souza *et al*., 2008). Magouz *et al*. (2020), reported increase significantly in feed conversion ratio in Nile tilapia fed with 30% *Azolla* meal.

Despite that the *Azolla* meal did not incorporate as a component in feed-in the present study, their use combined with commercial feed showed the highest feed conversion as observed in treatment 28% PA and 32% PA. However, the results in the treatment with pure *Azolla* showed final weight and apparent feed conversion with values less than others treatments. Thus, although the *Azolla* meal showed a higher crude protein content and essential amino acid composition than most other aquatic macrophytes (Mosha, 2018), their use as pure *Azolla* is not recommended.

For better extraction of information and a more complete assessment of the data, the PCA was applied, based on the correlation of variables, and the variables with the greatest effect on treatment discrimination were observed. Among the variables analyzed, apparent feed conversion was the most representative. Mengistu *et al*. (2020), using PCA, also reported that the best performers in tilapia farms are affected by differences in growth rate and feed conversion ratio. In the present study, it is observed in PC1, which explained 50.69% of the data variance, that the variable's final weight and weight gain are directly correlated with the highest levels of apparent feed conversion.

Thus, the treatments with the highest values of weight gain and final weight exhibited the lowest values of apparent feed conversion. The treatments of the 28% PA and 32% PA showed the best zootechnical performance.

## Conclusions

Treat effluent of aquaculture with aquatic plants and use reusing the plant to develop a new product is a way to promote the circular economy in aquaculture. *A. pinnata* showed potential as eco-friendly technology to the retention of phosphorus and nitrogen after seven days and the *Azolla* meal offered with commercial feed with 28% crude protein showed the best efficiency rates in tilapia. The use of the *Azolla* is a new marketing strategy for the small producer that you can diversify your products and become more competitive. Therefore, the development of the new product of the reuse of biomass of plant may permit reduction of production costs and improvements in the yield of the fish produced. Thus, the use of the *A. pinnata* meal in combination with commercial feed can be used to development of fish farming and is a viable alternative in the search for sustainable products to promote a bioeconomy in small fish farms.

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