Original Research Article

Benefits in Tilapia Growth, by Vetiver Grass in Aquaponics System, Improving The Water Quality and Obtaining an Aggregate Value

Abstract

Tilapia aquaculture has growing vertiginously in the world, reaching 7.02 million tons in 2020. In Mexico reached 72.6 thousand tons, same year; this production values have increased environmental impact and production costs. In Mexico there is a pre-Hispanic aquaponics system, where people culture corn, bean fishes and other organisms. Taken this system as basis, the aim this work was demonstrate benefits of Vetiver grass in tilapia aquaculture. Three mini-ponds make up by tilapia juveniles and Vetiver in aquaponics system, and one mini-pond without Vetiver, were cultured during ten weeks. The ammonia, nitrates, dissolved O_2, pH etc., also tilapia weight were recorded along experiment. Results indicated that, ammonia decreased, nitrate increased and tilapias weight increased significantly in mini-ponds with Vetiver than without Vetiver. A von Bertalanffy simplified model was used to calculate time required for tilapias reached 500 g (commercial size) with Vetiver and without Vetiver, obtaining 48.6 and 54.4 weeks respectively. An extrapolation to commercial tilapia aquaculture, will decreased production cost, due to lower amount of feed and water in tilapia hatcheries; also, a lower environmental impact by wastes discharged to coastal ecosystems, e.g., the amount of ammonia produced in mini-ponds with Vetiver was 4.56 times less than mini-pond without Vitier at week ten. Moreover, tilapia culture with Vetiver, have an aggregated value, because Vetiver is used in perfumes production, reaching 45.2 billons US dollars in 2020.

Keywords : Tilapia growth, Vetiver grass, Aquaponics, Water quality, Aggregated value.

Introduction

During the last decades the tilapia culture has been developing intensively, since the growth of tilapia is a relative easy culture, and because the demand of cheap foods rich in protein, particularly in the developing countries, has been increasing during last decades, very fast. However, the great majority of aquaculture systems, such in Mexico as in other countries, are based in monoculture systems with an increasing water demand and feed supply, which increase the production costs. The world aquaculture tilapia production in 2019 was 6.8 million of metric tons, whereas for 2020 was estimated in 7.02 [1]. Although, this increase was lower than the average growth rate during 2010 to 2019, which was 7.7 percent/year; the price during the last ten years decreased from 4.5 in 2010 to 3.8 in 2020 US dollars/kg; even so, the cultivation of tilapia is very attractive [1]. In Mexico, the aquaculture production of tilapia in 2020 was 72.6 thousand of metric tons, with a value of 2066.43 million pesos; about 3.4 millions of US dollars [2]. From an ecological point of view, the tilapia culture in ponds in their different modalities (extensive, intensive and super intensive) becomes to be the principal problem, due that in natural ecosystems, the tilapia growths together and interacting with many other animals and plants species, which make the system in equilibrium.

In Asian countries, there are some examples of tilapia and channel fish, growing in the rice fields, which is an ancient way of farming rice and fishes. In Mexico there is also a pre-Hispanic system where corn, bean, flowers, shrimp and freshwater fishes, and other species grow together. This system is known as Chinampas. Formay it is practiced in the Xochimilco lake, at south of Mexico City. The Chinampas are small floating islands, where the plants and aquatic animals above referred, grow together, Fig. 1. Unfortunately, it is the only place in the Country where this system is practiced.

Figure 1. The Chinanapa systems in the Xochimilco lake, at South of Mexico City. As can be observed the system are floating islands where are growing together corn, bean, flowers, shrimp and freshwater fishes, also, other species like Ahuehuetes trees. (*Taxodium huegelii* C. Lawson1851). In fact, it is an aquaponics system; i.e., a system in which the waste produced by aquatic animals supplies nutrients for plants growing hydroponically, which in turn purify the water.

Based in this system, but in a much more simplified way, the aim of this work was to assay an aquaponics system make up by tilapia (*Oreochromis aureus*) and Vetiver grass (*Chrysopogon zizanioides*) growing together into small ponds; since several authors have reported in past years, that the Vetiver grass can to improve the soil and water quality, reducing some toxic compounds produced by plants and aquatic animals, such as ammonia in aquatic ecosystems, [3,4,5]. The ammonia is a nitrogen waste derivate from protein metabolisms, and then released to the water by aquatic animals; the fishes release it through the gill. This happens in any aquatic systems such as seas, lakes, rivers and also in aquaculture ponds.

Ammonia (NH_3) is the most toxic form of the nitrogen species in the water; it is much more toxic than NO^{-}_{2} and ionized ammonia (NH_4^+). In addition, ammonia toxicity increases as temperature rises and as pH decrease. The chemical equation that drives the relationship between ammonia (NH_3) and ion ammonium NH_4^+ is:

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$$

When the pH is low, the reaction is driven to the right, and when the pH is high, the reaction is driven to the left. The aqueous concentration of ammonia is much lower at low temperatures than at high. This means that at low temperatures and low pH the activity as NH_3 is even lower, and NH_4^+ is even higher. On the other hand, as temperature increase also increase the volatility of NH_3, since it a gaseous compound; for example, at pH around 9.5 the volatility of ammonia is around of 60%, and that of ion ammonia (NH_4^+) is 40 %.

Another important biological processes which can change the ammonia concentration in the water, are the photosynthesis and respiration. Chemically the photosynthesis is the fixation of CO_2 dissolved in the water for the synthesis of carbohydrates, and the respiration is the inverse process, but both are coupled. In the aquaculture ponds, carbon dioxide is released during respiration and consumed by photosynthesis. As a result, pH in the pond varies throughout the day because the light intensity changes along the day.

Material and Methods

Groups of 9 tilapia juveniles, between 8.4 to 10.4 g weight. were distributed in 4 plastic vessels (mini-ponds) of 100 liters' volume. The mini-ponds were nominated as P1, P2, P3 and P4. The mini-ponds P2, P3 and P4 were the experimental group; whereas P1, was the control group. All the mini-ponds were filled with 70 liters of filtered freshwater, passing tap water through a filter of 20 µm of pore size. Also, mini-ponds were supplied with air bubbling, using air pumps. The mini-ponds were placed outside, but under a shaded area;

however, they received sunlight for a couple of hours during morning (8 to 10) and in the afternoon (17 to 19) hours, before sunset. Before to start the experiment, the tilapias were leave 4 weeks in the mini-ponds, to observe diseases, mortality or any other alteration signs in the fishes. In addition, this adaptation period served for the Vetiver grass developed enough roots, in order to it might be used in the experiment as an aquaponics system. Once the adaptation period finished, the experiment was started. Basically, the experiment consisted in comparing the growth of tilapia and the water quality in the P2, P3 and P4 mini-ponds make up by fishes and Vetiver grass, i.e., in an aquaponics system, Vs. the growth of tilapias alone in the mini-pond P1, as control group. The Vetiver grass was planted in 5 plastic containers with her Vetiver roots into river sand, intensively washed. The containers were introduced into the water of mini-ponds P2, P3 and P4; in this way, the nutrients required by Vetiver grass, only could be take in from the water (Fig.2).

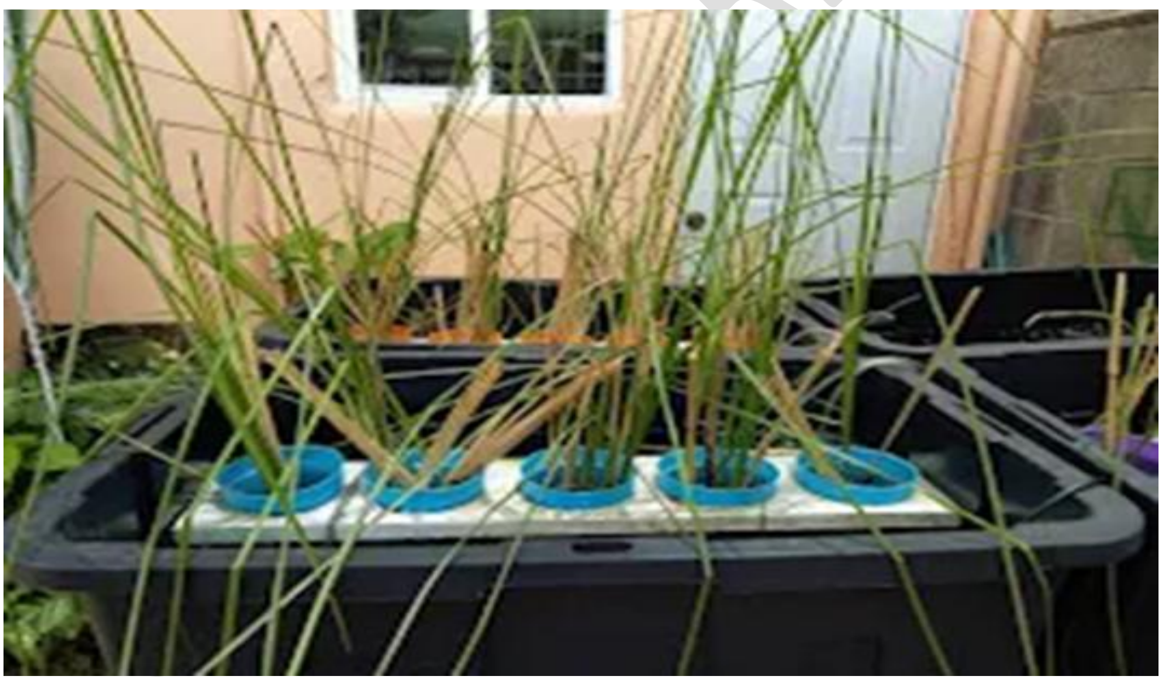

Figure 2. Mini-ponds with tilapia and Vetiver grass in aquaponics system. As can see the mini-ponds P2, P3 and P4 was made with 5 plastic containers with the roots of Vetiver into river sand; in this way, nutrients required by the grass, only could be take in from the water. Also the control mini-pond P1 can be seen in the left corner without Vetiver grass.

During experimental time, the tilapias were feed daily at rate of 3-4 % their total biomass, using a 3 mm pellets feed composed by soybeans meal, fish meal, corn and wheat meal, calcium, phosphorus, vitamins, folic acid, etc., supplied by Lomas®. Each week the mini-ponds water, was changed around 75 % total volume. Previous to water change, 20-30 ml of water from each mini-pond was taken, and then filtered through, cellulose esters filters of 1,2 µm pore size x 1.9 mm diameter, MF-Millipore supplied by MercK®. After, total ammonia (NH_3, NH_4^+) and nitrates NO_3^- concentrations were quantified by the methods of salicylate, proposed by [6] and Morris & Riley, modify by [7] respectively. The filters were transferred to centrifuge tubes, 5 ml of 90% acetone was added to each tube and left in dark 24 h. at -2 ^oC, for the subsequent quantification of Chlorophyll "a", following the method proposed in [7].

To know the (NH_3, NH_4^+) and NO_3^- concentrations in the water samples, standards reference solutions of (NH_3, NH_4^+) and NO_3^-, were prepared, and the absorbance measured using an Thermo Scientific Evolution 600® UV-Vis Spectrophotometer; then with the values of standard concentration and absorbance, linear correlations were made Fig. 3 and 4

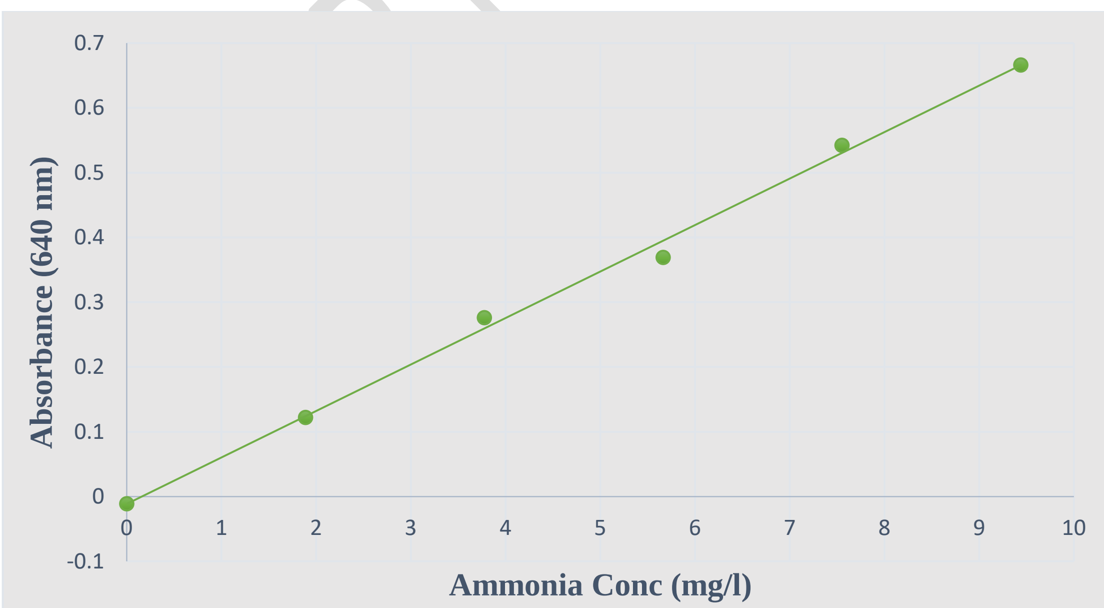

Figure 3. Standard curve of (NH_3, NH_4^+) and correlation equation, where Y is Absorbance, and X is Ammonia concentration (mg/l).

The corresponding equation is: Y=-0.0112+ (0.0717) X; where Intercept in Y axis= -0.0112, Slope=0.0717 and Correlation coefficient =0.998314282.

Therefore, X=(Abs.+0.0112)/0.0717, which permit to calculate the ammonia concentration in water, from Absorbance values.

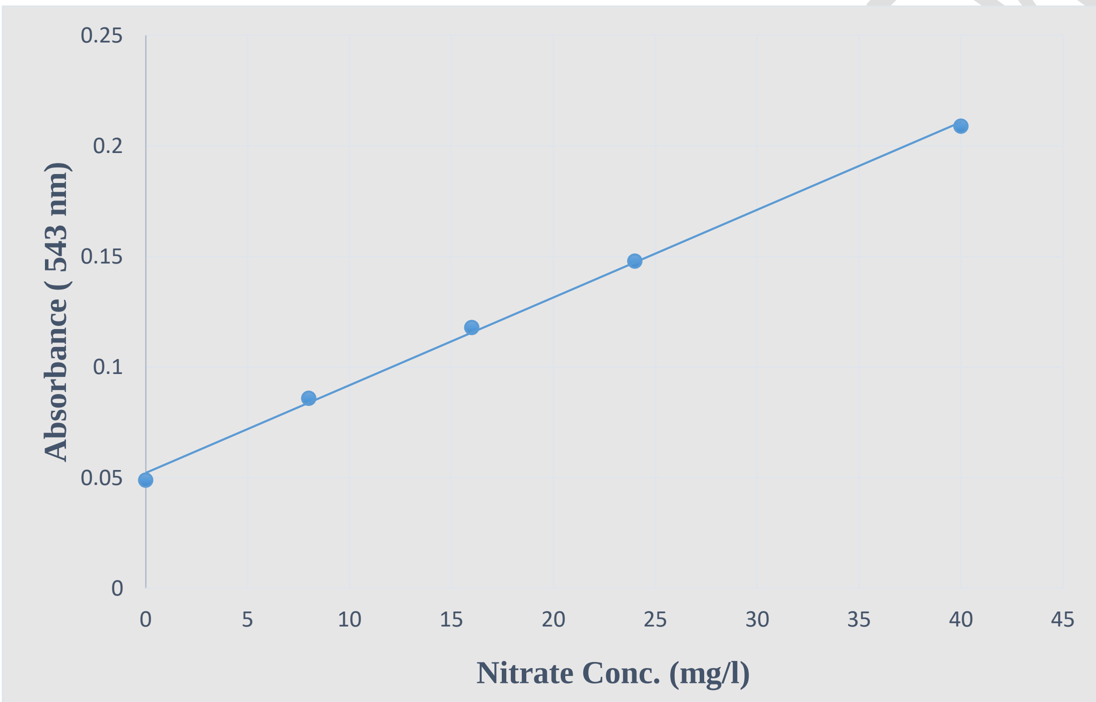

Figure 4. Standard curve of NO_3^-, and correlation equation, where Y is Absorbance, and X is Nitrate concentration (mg/l).

Similarly, the equation is: Y=-0.05228 +(0.00396) X; where Intercept in Y axis=0.05228, Slope=0.00396 and Correlation coefficient =0.998403179

Therefore, X=(Abs.-0.05228)/0.000396, which permit to calculate the nitrate concentration in water, from Absorbance values.

Once the correlation equations for (NH_3, NH_4^+) and NO^-_3 have been obtained, the ammonia and nitrate concentrations in the water samples in mini-ponds, were calculated using the respective equations.

Regarding to Chlorophyll "a", the concentration in water samples, were calculate measuring the absorbance at 665, 645 and 630 nm of wavelength, using the same spectrophotometer above indicated, and the equation proposed by [7]. Chlorophyll "a"= C/V (µg/l) where C=11.6xAbs-665-1.31xAbs645-0.14xAbs-630, and V= volume in liters of filtered water.

At same time that the water samples were taken, the temperature, pH, total dissolved solids (TDP) and dissolved oxygen were measured "in situ", using a mercury thermometer (range -20 to 110 ° C) an Orion Star model A121 pH meter, a Hanna model HI 98192 portable TDS (range 0.00 to 1000 ppt.) and a portable dissolved oxygen meter Hanna model HI 98193, respectively. Also in order to quantified the tilapias growth increase, weekly the fishes of each mini-pond, were weighted, using a Mettler PM-100 semi-analytical balance.

Results

The tilapias growth (increase in weight) during the experimental time is shown in Fig.5. As can be see, the growth in the mini-ponds with Vetiver (P2, P3 and P4) was significantly higher than in control mini-pond P1 (P<0.05). Consequently, the aquaponics system has a benefic effect on fish growth with Vetiver at same stock density, than in control mini-pond.

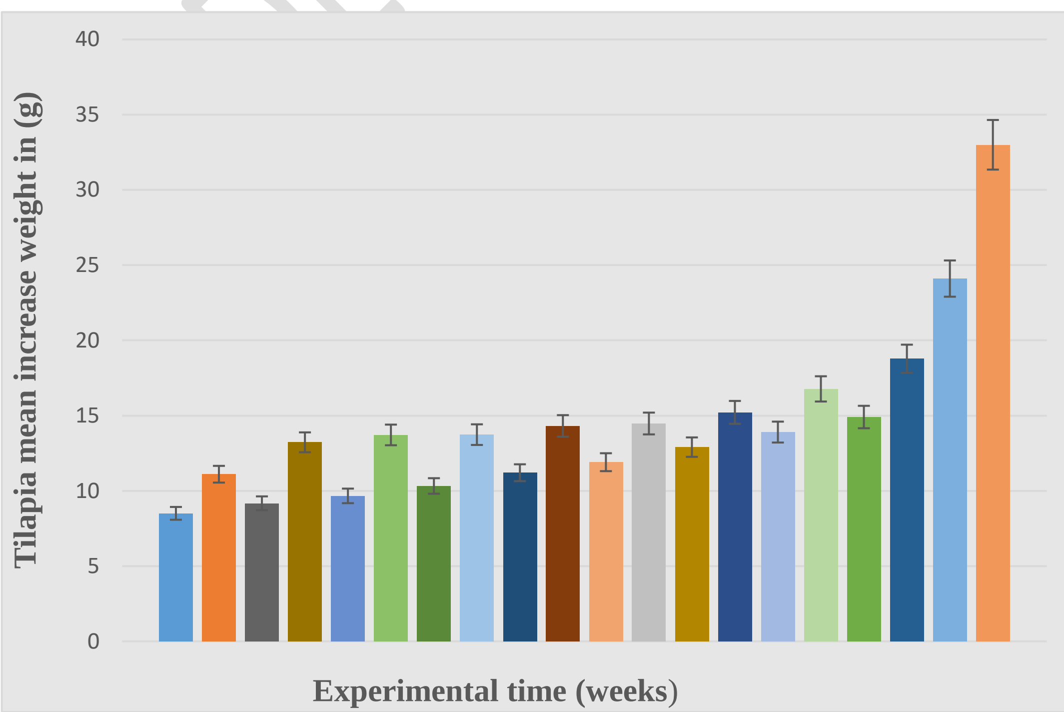

Figure 5. Tilapia weigh increase in grams per week. The first one of each two bars groups, correspond to control mini-pond (P1), whereas the second bars from each group, are the values of experimental mini-ponds (P2, P3 and P4). Also the values of experimental mini-ponds were significant higher than control (P<0.05).

Regarding to ammonia concentration, it was decreasing as the experimental time ran. As can be observed in Fig. 6, the ammonia concentration was significant higher (P<0.05) in the control mini-pond P1, than the mean values in the experimental mini-ponds (P2, P3 and P4) Therefore, it is possible said that Vetiver grass consumed an important amount of ammonia excreted by the tilapias; consequently, the aquaponics system is working.

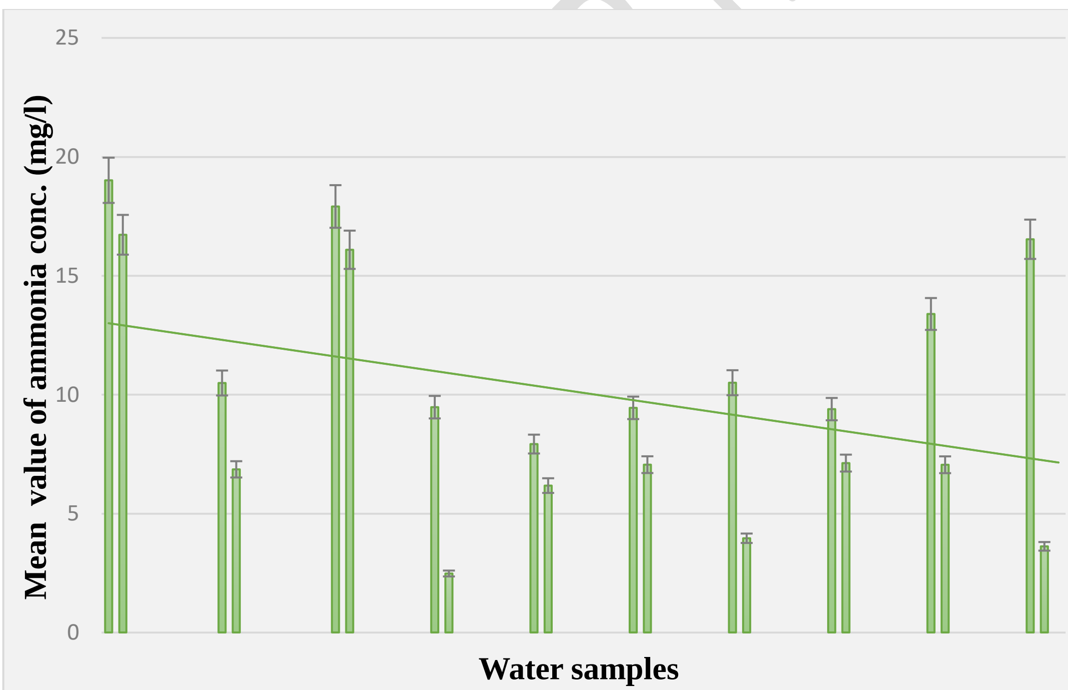

Figure 6. Water ammonia concentration of control and experimental mini-ponds P1, and (P2, P3 and P4) respectively. The values are the mean concentration in (mg/l). The Control correspond to the first of each couple of columns; whereas the second bars of each coupe,

correspond to experimental mini-ponds. The concentrations are significant higher in control than experimental mini-ponds ($P<0.05$).

The fact that ammonia increase along experiment, also can be due to nitrification processes by bacteria, which contribute to reduce ammonia concentration in water., e.g. the amount of ammonia produced in mini-ponds with Vetiver was 4.56 times less than mini-pond without Vitier at week ten.

Concerning to nitrate concentration, it was increasing along the experimental time Fig.7. The nitrate concentration was significant higher ($P<0.05$) in experimental mini-ponds (P2, P3 and P4) than in control mini-pond P1. As can be see, the tendency was inverse to observed in the ammonia concentration; i.e., whereas the ammonia decreased as the experiment ran, the nitrate increased; which may due, to an increase in the population of nitrifying bacteria during the experimental time.

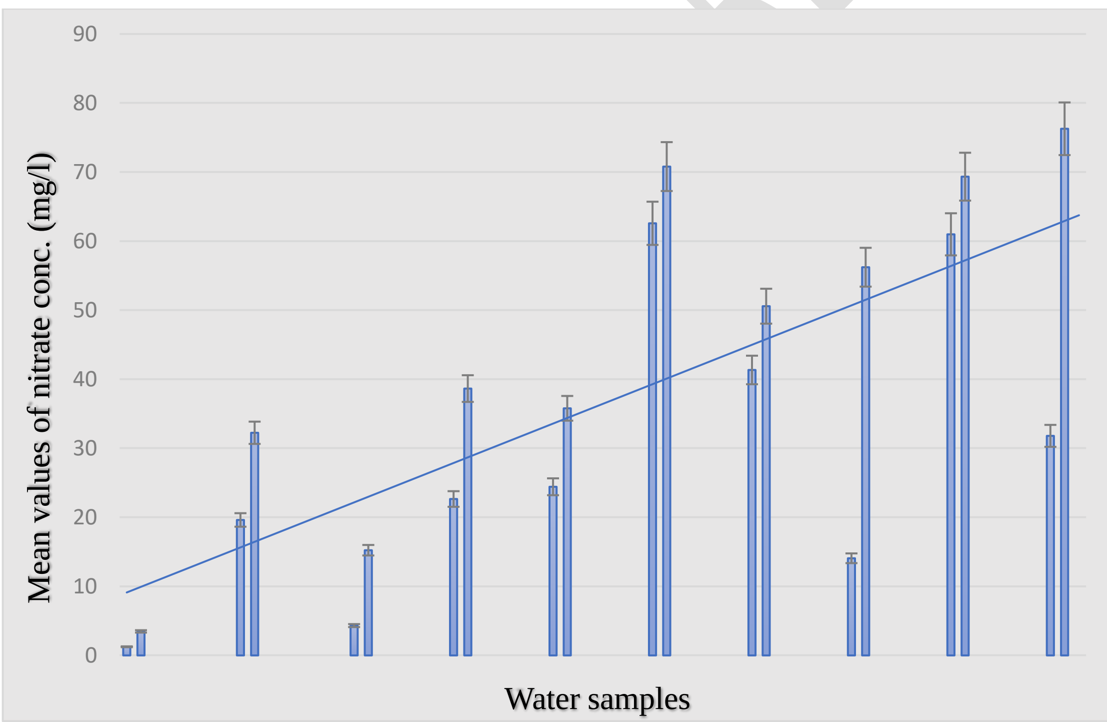

Figure 7. Water nitrate concentration of control P1and experimental mini-ponds (P2, P3 and P4). The values are the mean concentration in (mg/l). The control corresponds to the second bars of each couple of bars; whereas the first bar of each coupe, correspond to

experimental mini-ponds. The concentrations are significant higher in control than experimental mini-ponds ($P<0.05$).

In this case, the nitrate increasing, is benefit to Vetiver, because it is more easy to assimilate.

The other physicochemical parameter recorded during the experiment, such as the Total dissolved solids (TDS), Chlorophyll “a”, Hydrogen potential (pH), etc., are shown in Table I. As can be observed the values of Temperature, pH and Dissolved oxygen has low variation during experimental time; whereas the TDS and Chlorophyll decreased along experiment.

Table I. Other physicochemical parameters recorded during the experimental time; the values of Temperature, pH and O_2 dissolved, had small changes, whereas Total Dissolved Solids and Chlorophyll “a” decreased along the experimental time.

Data	Sample	Total Dissolved Soli	Chlorophy	Temperatur	Hidrogen	Dissolved
April 30 2021	Aquarium	TDS (mg/l)	(µg/l)	°C	potencial (pH)	Oxigen (mg/l)
	P1	1940	31.153	28.7	8.4	7.4
	P2	1828	9.9115	29.1	8.04	7.5
	P3	1724	7.8745	28.3	8.06	7.6
	P4	1788	11.0645	28.5	8.08	7.7
May 06 2021						
	P1	1910	5.358	28.7	7.85	7.52
	P2	1586	3.3935	29.2	7.48	7.44
	P3	1502	4.909	29.1	7.28	7.57
	P4	1570	2.182	28.4	7.26	7.65
May 15 2021						
	P1	1868	17.538	28.4	7.82	7.23
	P2	1370	14.5865	29.5	7.3	7.34
	P3	1358	16.0085	28.4	7.31	7.51
	P4	1288	12.5285	28.3	7.32	7.68
May 31 2021						
	P1	585	9.8815	28.5	7.87	7.41
	P2	393	7.169	29.7	7.19	7.24
	P3	330	7.498	28.4	7.29	7.31
	P4	384	8.36	28.5	7.42	7.35
Jun 03 2021						
	P1	485	2.136	27.8	7.85	6.4
	P2	293	1.063	28.8	7.16	6.5
	P3	230	1.015	27.9	7.27	6.6
	P4	284	0.768	27.6	7.12	6.7
Jun 19 2021						
	P1	154	7.9966	27.8	7.84	6.5
	P2	120	1.6138	28.8	6.87	6.2
	P3	116	1.5031	27.9	7.05	6.1
	P4	117	1.0251	27.8	7.05	6.2
July 2th 2021						
	P1	125	4.06	27.2	7.85	5.9
	P2	69	1.068	28.6	6.98	5.7
	P3	44	3.1463	27.5	7.14	5.55
	P4	31	0.174	27.7	6.79	5.76
July 8th 2021						
	P1	207	1.111	27	7.87	5.8
	P2	88	0.338	27	7.28	5.4
	P3	87	0.5846	28	7.16	5.6
	P4	96	0.338	28	7.07	5.6
July 15th 2021						
	P1	120	9.432	27.3	7.94	5.6
	P2	83	1.0875	27.5	6.84	5.27
	P3	97	0.5075	27.4	7.01	5.34
	P4	129	0.5075	28.1	7.14	5.22
July31th 2021						
	P1	135	9.939	29.2	7.92	5.9
	P2	81	1.588	29.7	6.77	5.47
	P3	89	4.053	29.7	6.99	5.74
	P4	102	0.749	29.8	6.91	5.62

Data Analysis

All experimental data were analyzed by ANOVA one via, using the Statistica 7.0 software, VinceStatSoftware® for obtaining the mean values, standard deviations, and significant value. Data which did not meet normality requirements, were analyzed non-parametrically by Kruskal-Wallis ANOVA and median test.

Discussion and Conclusion

From results obtained, it is possible to say that the aquaponics system formed by tilapias and Vetiver grass, work better than tilapias alones. In other words, the ammonia produced as a metabolic waste by tilapias, supplies nutrients for growth of Vetiver plants, which in turn purify the water; this can be corroborate in the Fig. 6 since the ammonia concentration was decreasing along the experimental time; e.g., the ammonia in control mini-pond was 4.56 times less, than experimental mini-ponds in week ten. On the other hand, the nitrates concentration was increased as the experiment ran Fig. 7. That's mean ammonium was oxidized to nitrite (NO_2^-) by Nitrosomonas bacteria, and then Nitrobacter bacteria can oxidase nitrites to nitrates (NO_3^-) which are taken in by Plants [8] like Vetiver. This aquaponics system, can be applied easily to tilapias aquaculture production, such in Mexico as in other countries. Moreover, this system has an ecological and economical, benefit since there is not any increase in production cost, because the tilapias growth more in same time period and with same food amount, which reduce the aquatic pollution because decrease the amount of organic matter, derived from the feed supplied, and by tilapia feces; just as can be observed in the results obtained. Also this system may be reducing the water required by tilapia aquaculture; i.e., the tilapia production in Kg/ ha., will be higher than in traditional cultures. Therefore, it is possible to say, that the tilapia aquaculture using aquaponics system with Vetiver grass, have many benefits that traditional methods.

Concerning the others physicochemical parameters recorded in this work, they presented low variation along the experiment (Table I); however, the pH, and Chlorophyll, were slightly higher in control aquarium (P1) than mini-ponds with Vetiver. Also the TDS were higher in the first eight weeks of experimental time than following weeks. This could be a consequence of the reduction in phytoplankton which is directly related to Chlorophyll amount; which also contributed to increase the water quality in the mini-ponds with Vetiver grass (P2, P3 and P4).

On the other hand, the Vetiver grass have many uses and benefits. Due to its stabilizing or preserving properties, it is widely used in perfumes production, which in 2020 reached 45.2 billons of US dollars [9]. The oil and extracts of Vetiver, are contained in approximately 36% of Western perfumes (Guerlain, Givenchy, Chanel No 5, Christian Dior, etc.), which demand is around 250 tons per year [10]. Therefore, the aquaculture tilapia in aquaponics system with Vetiver grass can give an important aggregate value, for the producers.

Moreover, since ancient times, several plants such as Lotus spp., Lemna minor or duckweed, Vetiver grass, etc. have been used in bioremediation, to recover soils and water from contamination [3,4,5,11]. This becomes relevant since the contamination levels in soil and water has drastically increased due to population growth, industrialization, etc., because a lot of chemicals wastes has been discharged to soils and water bodies, by several industries, municipals drainages, hospital and agriculture fields; despite that pollutants substances and wastewater are treated before to be discharged [12].

On the other hand, although the objective of this work was not evaluate the tilapias rate growth, in the Fig. 5 can be observed that the increase in weight has an exponential tendency (first part of biological growth curve, or (sigmoid curve). Diverse mathematical models have been used to estimate the fish growth, and so provide reliable information for aquaculture systems [13]. The von Bertalanffy growth model, has been chosen as an optimal model, between a lot of fish growth models [14,15]. So the von Bertalanffy model, can be applied to the tendency observed in Fig. 5. Although, the original model was developed for determine the fish growth or age, expressed as length of fish; but since there is a direct relationship between length and weight, the growth can be expressed as increase of fish weight. Based in this, the follow equation was used, which is a simplified equation of von Bertalanffy model

$W_t = W_\infty (l\text{-}e^{-K(t-t}{}_0\ ); \text{ then } dW_t / W_\infty = - K(dt/t_0); \text{ therefore, } \mathrm{Ln}\ W_t = -K(\mathrm{Ln}\ t).$

Where W_∞ is the mean weight in (g) of the fish at infinitum (in practice at a time t)
K is the growth coefficient, which can be calculated from experimental datum's, expressed in (g/week)
t_o is the "age" that fish would have at time zero (in fact is zero).
and W_t is the weight of fish at time t (expressed in weeks).

This equation corresponds to a review of the von Bertalanffy model, proposed by [16]. Therefore, applying this equation for calculate the time required by tilapias to reach a commercial weight of (500 g.), in the mini-ponds with Vetiver grass, was 54.64 weeks;

whereas, the time for reach same weight by tilapias in the mini-pond without Vetiver, was 60.42 weeks; i.e.,10.57% more time. The times above referred do not consider that the age of tilapias in the beginning of experiment was around 5.8-6.2 weeks; therefore, the real time will be 48.6 and 54.4 weeks respectively.

Although the estimated tilapia growth in this work is very simplified, the results obtained are in concordance with works more sophisticated [17,18], since the authors report similar times to get tilapias of same weight, and they also applied the von Bertalanffy model; therefore, can be conclude that the results can be useful for tilapia aquaculture, combined with Vetiver grass in aquaponics systems.

Ethical Approval

The author declare that tilapias do not were damage in any of its parts So once finished the experiment, the fishes were returned to place where they were taken: The Education Center for Environmental and Agriculture Sustainable.

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