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Benefits in the Tilapia Growth, by Vetiver Grass in an Aquaponics System

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

Article Information

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ABSTRACT

Tilapia aquaculture has growing vertiginously in the world, reaching 7.02 million tons in 2020. In Mexico, the same year reached 72.6 thousand tons. 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 the benefits in tilapia aquaculture, by Vetiver grass. 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 O₂, pH etc., and also tilapia weights were recorded along experiment. Results indicated that, ammonia decreased, nitrate increased and tilapia 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; it was 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.

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1. 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 [3]. 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. Currently 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. arow toaether. Unfortunately, it is the only place in the Country where this system is practiced, Fig. 1.



Fig. 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 wastes and other substances produced by aquatic animals, can supplies nutrients for the plants growing, which in turn purify the water

Based on this system, but in a more simplified way, the aim of this work was to demonstrate the benefits of an aquaponics system make up by tilapia (*Oreochromis aureus*) and Vetiver grass (*Chrysopogon zizanioides*) growing together into small ponds.

1.1 Theoretic Concepts and Backgrounds

Several authors have reported in past years that Vetiver grass can to improve the soil and water quality, reducing some toxic compounds produced by plants and aquatic animals, such as ammonia and some pollutants to aquatic ecosystems [4,5,6]. The ammonia is a nitrogen waste derivate from protein metabolisms of aquatic animals and then released to the water; the fishes release it through the gill. This happens in any aquatic systems such as seas, lakes, rivers and also in aquaculture ponds.

Ammonia (NH₃) is the most toxic form of the nitrogen species in the water; it is much more toxic than NO⁻₂ and ionized ammonia (NH₄⁺). In addition, ammonia toxicity increases as temperature rises and as pH decrease [7]. The chemical equation that drives the relationship between ammonia (NH₃) 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 % [7].

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 [7].

2. MATERIALS 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 miniponds 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 fish. In addition, this adaptation period, served for the roots of Vetiver grass developed enough, in order to it might be used in the aquaponics system experiment. Once the adaptation period finished, the experiment was initiated. Basically, the experiment consisted in comparing the growth of tilapia and the water quality in the P2, P3 and P4 mini-ponds with tilapias and Vetiver grass, i.e., in an aquaponics system, Vs. the growth of tilapias alone in the mini-pond P1. The Vetiver grass was planted in 5 plastic containers with the Vetiver roots introduced in river sand, intensively washed. The containers of mini-ponds P2, P3 and P4 were partially submerged into the water; in this way, the nutrients required by Vetiver grass, only could be taken in by the roots (Fig.2).

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 minipond 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 NO₃⁻ concentrations were quantified by the methods of salicylate, proposed by [8] and Morris & Riley, modify by [9] respectively. The filters were transferred to centrifuge tubes, and 5 ml of 90% acetone was added to each tube and left in dark 24 h. at -2°C, for the subsequent quantification of Chlorophyll "a", following the method proposed in [9].



Fig. 2. Mini-ponds with tilapia and Vetiver grass in aquaponics system. As can see the miniponds 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

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 Figs. 3 and 4.

In the above equation: the intercepts 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 mini-ponds water, from Absorbance values.

Similarly, in the above equation: the intercepts 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 of mini-ponds, from Absorbance values.

Once obtained the correlation equations for (NH^3, NH^4+) and NO^3 , the ammonia and nitrate concentrations in the water samples of miniponds, were calculated using the respective equations.

Regarding Chlorophyll "a", the concentration of the water samples in the mini-ponds was calculated by measuring the absorbance of acetone extracts at 665, 645 and 630 nm wavelength, using the same spectrophotometer indicated above. and the equation proposed by [8]. Chlorophyll "a" = C/V (μ g/l) where C=11.6xAbs-665-1.31xAbs645-0.14xAbs-630, and V= volume in liter of mini-ponds 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 Hunan®, China (range -20 to 110 ° C), a pH meter Orion Star model A121 Thermo Scientific® Massachusetts, USA, a portable TDS (range 0.00 to 1000 ppt.) Water Quality® and a portable dissolved oxygen meter Hanna Instruments® model HI 98192, Milan, Italy, respectively. Also, for quantified the tilapias growth of each mini-pond, they were weighted weekly, using a Mettler PM-100® Greifensee, Switzerland semi-analytical balance.

2.1 Data Analysis

All experimental data were analyzed by ANOVA one via, using the Statistica 7.0 software, Vince Stat Software® 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.

3. RESULTS

The tilapia 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, than without Vetiver.



Fig. 3. Standard curve of (NH₃, NH₄⁺) and correlation equation Y=-0.0112+ (0.0717) X; where Y is Absorbance, and X is Ammonia concentration (mg/l). This equation permits to calculate ammonia concentrations in the mini-pond during experimental time







Fig. 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)

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





Data	Sample	Total Dissolved Solids	Chlorophyla	Temperature	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

Table 1. Other physicochemical parameters recorded during the experimental time; the values of Temperature, pH and O₂ dissolved, had small changes, whereas Total Dissolved Solids and Chlorophyll "a" decreased along the experimental time

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Data	Sample	Total Dissolved Solids	Chlorophyla	Temperature	Hidrogen	Dissolved
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						
i	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						
t	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



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

The fact that ammonia decreased along experiment, 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 Vitiver 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.

In this case, the nitrate increasing, is benefit to Vetiver, because it is more easy to assimilate from the water of the mini-ponds.

The other physicochemical parameter recorded during the experiment, such as the Total dissolved solids (TDS), Chlorophyll "a", (pH), Temperature, etc., are shown in Table 1. As can be observed, the temperature and dissolved oxygen presented low variation along the experiment. The pH, and Chlorophyll, were higher in control mini-pond (P1) than in mini-ponds with Vetiver. Also the TDS decreasing as experiment ran; this could be a consequence of the reduction in phytoplankton which is directly related to Chlorophyll amount; therefore, it contributes to increase the water quality in the mini-ponds with Vetiver grass (P2, P3 and P4).

4. 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 alone. 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 (NO₂⁻) by Nitrosomonas bacteria, and then Nitrobacter bacteria can oxidase nitrites to nitrates (NO_3) which are taken in by Plants [10] 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 water quality is improved and there is not any increase in production cost, because the tilapias growth more in same time and with same food amount; which reduce the aquatic pollution due to decreasing the organic matter, derived from the unconsumed feed and by tilapia feces. Also this system can to reduce the water required; i.e., the tilapia production in Kg/ ha., will be higher by the aquaponics system than traditional aquaculture. Therefore, it is possible to conclude, that tilapia aquaculture using aquaponics system with Vetiver grass, have many benefits that traditional methods.

On the other hand, the Vetiver grass have many uses and benefits in perfumery; due to stabilizing and preserving properties, its uses in perfumes production, reached 45.2 billions of US dollars in 2020 [11]. 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 [12]. Therefore, the tilapia aquaculture in aquaponics system with Vetiver grass can give an important aggregated 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 [4,5,6,13]. 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 in some cases, the pollutants and wastewater are treated before to be discharged [14]. Other authors have reported that some plants such as O. basilicum, Menta piperita and M. spicata integrated to the production of tilapia O. niloticus in aquaponics, can reduce the ammonia and nitrite concentration in the water; however, input of oxygen to water must be increased, due to elevate O_2 demand by these species [15]. Therefore, this system is more expensive that the presented in this work. In other paper, the authors report that a combination of aquaponics

system with greenhouses, modern technology and information technology, can reduce the water consumption and the amount of fertilizer in fish aquaculture. The equipment needed for planting and breeding will gradually transition away from manual control, to automation, intelligence and high efficiency, [16]. It is obvious that the automation and intelligent systems will be used in future, but these systems will be more expensive and increased the unemployed, which is not recommended to emergent countries; therefore, the system proposed in this work, is better.

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 [17]. The von Bertalanffy growth model, has been chosen as an optimal model, between a lot of fish growth models [18]. 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, there is a direct relationship between length and weight; then the growth can be expressed as increase of fish weight. Based on this, the follow equation was used, which is a simplified equation of von Bertalanffy model

 $W_t = W_{\infty}$ (I-e^-K(t-t_0); then dW_t / W_{∞} = - K(dt/t_0); therefore, Ln W_t = -- K(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 data, expressed in (g/week).

 $\tilde{t_o}$ 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 [19]. 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 was very simplified, the results obtained are in concordance with works more sophisticated [20,21,22], because the authors report similar times to get tilapias of same weight, and also, they applied the von Bertalanffy model; therefore, can be conclude that results obtained in this work, can be useful in commercial tilapia aquaculture, with Vetiver grass, using aquaponics system.

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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COMPETING INTERESTS

Author has declared that no competing interests exist.

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