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## Utilization the Aquatic Plants as a Filtration Media in A Closed Bio-Aquatic System to Improve Water Quality and Common carp Growth Performance

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### ABSTRACT

The experiment was conducted in the animal production field, College of Agriculture, Tikrit University, for the period from 14/6/2022 to 14/8/2022 inside a semi-exposed building with three treatments. Each treatment included three replicates, and each replicate included (6) fish in a closed bio-aquatic system, by weight  $13.75 \pm 1.09$ , a filtration tank representing the mechanical and biological filter in an intensive aquatic system with a capacity of 100 liters each, two aquatic plants were grown Water thyme (*Hydrilla Verticillata*), Torpedo grass (*Panicum Repens*), except for the control treatment, which was similar to all treatments except for the presence of aquatic plants. The water was treated in a mechanical filter unit through sponge layer, then directing the water to the biological treatment by means of crushed gravel, in addition to the aquatic plants to filter the dissolved organic pollutants. The results showed a significant effect of aquatic plants at ( $p \leq 0.05$ ) on pH, Cloredait (Cl) nitrate ( $\text{NO}_3$ ), nitrite ( $\text{NO}_2$ ), and ammonia. Water properties reflect to the biological system of organisms. There was a significant improvement in gain weight (G.W), specific growth rate (SGR), and feed conversion efficiency (FCR) for treatments T2 and T3 compared with control treatment. While, the result of the correlation or interactions between water characteristics with growth parameters for Common carp fish showed significant effects at ( $P \leq 0.05$ ) and ( $P \leq 0.01$ ) level.

### KEY WORDS:

Water thyme, Torpedo Grass, Aquatic plants, biological filtration, water quality

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## استخدام النباتات المائية كوسائط فلترية في النظام الأحيومائي المغلق لتحسين صفات الماء وأداء نمو سمكة الكارب الشائع

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### الخلاصة

أجريت التجربة في حقل الإنتاج الحيواني، كلية الزراعة، جامعة تكريت، للمدة من 2022/6/14 إلى 2022/8/14 داخل مبنى شبه مكشوف (جملون) بثلاث معاملات. تضمنت كل معاملة ثلاث مكررات، وتضمن كل مكرر (6) أسماك في نظام أحيومائي مغلق، بمعدل وزن  $1.09 \pm 13.75$ ، يمثل خزان الفلترية المرشح الميكانيكي والحيوي في نظام تربية مكثف بسعة 100 لتر، تم استزراع نباتين مائيين هما الكطل (*Hydrilla Verticillata*) و السلهو (*Panicum Repens*) باستثناء معاملة التحكم والتي كانت مماثلة لجميع المعاملات باستثناء وجود النباتات المائية. تمت معالجة المياه في وحدة تصفية ميكانيكية من خلال طبقة إسفنجية، ثم توجيه المياه إلى المعالجة الحيوية عن طريق الحصى المكسر، بالإضافة إلى النباتات المائية لتصفية الملوثات العضوية الذائبة. أظهرت النتائج تأثيراً معنوياً للنباتات المائية عند ( $p \leq 0.05$ ) على الأس الهيدروجيني، الكلوريدات، نترات النترت، والأمونيا. تؤثر خصائص الماء على النظام الحيوي للكائنات الحية. كان هناك تحسن كبير في زيادة الوزن، ومعدل النمو النوعي، وكفاءة تحويل الغذاء، للمعاملات T2 و T3 مقارنة بمعاملة السيطرة. أظهرت نتيجة الارتباط بين خصائص المياه مع معايير النمو لأسماك الكارب الشائع تأثيرات معنوية عند مستوى ( $P \leq 0.05$ ) و ( $P \leq 0.01$ ).

**الكلمات المفتاحية:** نبات الكطل، عشبة السلهو، نباتات مائية، ترشيح حيوي، جودة المياه.

## INTRODUCTION

Aquatic plants are sensitive to the environmental conditions they live in because they are affected by a number of environmental factors, so the flowering of particular plant growth in a special environment and conditions is considered as evidence of the presence of those conditions (AlSaadi and AlMiah, 1983). The importance of aquatic plants comes as one of the effective ways to purify water, as they work to remove pollutants with their plant composition (Korsbrekke., et al, 2001).

The term is Aquaponics an expression of the system of plant and fish farming, which is the cultivation of plants and aquatic life in a recycled environment, therefore it is a combination of fish farming and plant cultivation without soil. The water is filtered to remove extra nutrients, metabolic waste, and particulates (Hassan et al., 2022). Water treatment methods that guarantee an improvement in quality also guarantee a high energy and financial investment and operational cost, the requirement to search for a novel aquaponics system by using a biological filtration unit, then test the best aquatic filtration plants (Zaidan et al., 2023). Due to environmental factors impacting fishes physical condition, growth performance, and yield, the management of water quality conditions is important, especially if the fish are to be produced in aquaculture systems (Hassan et al., 2021a).

However, rapid media filters were examined with much less frequency in the literature on production systems, highlighting a research gap (Mori and Smith, 2019). There is no research concerning the closed bio-aquatic system to comparing between Water thyme (*Hydrilla Verticillata*), Torpedo Grass (*Panicum Repens*), effect on water quality, Common carp growth performance, biochemistry, kidney and liver plasma enzymes.

## MATERIALS AND METHODS

### Experiment design

The current study was conducted in the Animal Production Department, College of Agriculture, Tikrit University, Salah Al-deen, Iraq. There was a total of three treatments, each three replicate 100L tanks in an intensive system, as shown in Fig. 1. No other aquatic biological filtration media were used in the control treatment other than 50 kg of gravel fine mesh since the biological filtration were linked to the treatment tanks in such a way that the biological treated the water before it returned to the grow-out tanks. Each treatment contains a tank that represents the mechanical and biological filter to maintain water quality to examine the effect of Water thyme, Torpedo grass on water quality and Common carp growth performance. The water was treated first through spongy layer, then the water was directed to biological treatment by crushed gravel, in addition to the presence of aquatic plants to filter organic dissolved pollutants. There were three treatments, each treatment contained three replicate of tanks with a capacity of 100 liter in a bio-composite system, and then pumping water from the biomechanical filtration tanks through a pump to circulate the water 15 time a day. The system is designed to allow water to flow into the grow-out tanks with a capacity of per treatment to ensure that each treatment is filtered in a separate tank to avoid mixing and maintain the effects of different aquatic plants for each treatment. In addition, each treatment contained Water thyme, Torpedo grass as a biological filter in the filtration tank above the gravel, which helps to the water filtration. The water was treatment before returning to the grow-out tanks, a partial water change 10% was carried out every week to maintain water quality and wash the mechanical filters. The water used for all transactions was supplied from one source (Figs. 2).

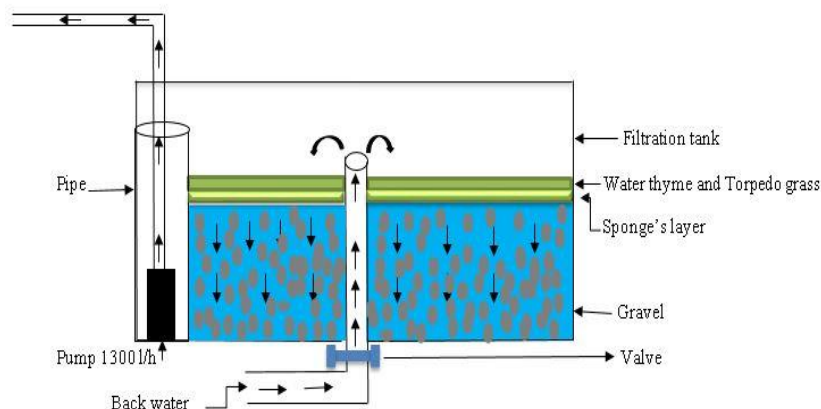


Figure 1: Longitudinal of the mechanical, biological filtration tanks of a closed bio-aquatic system.

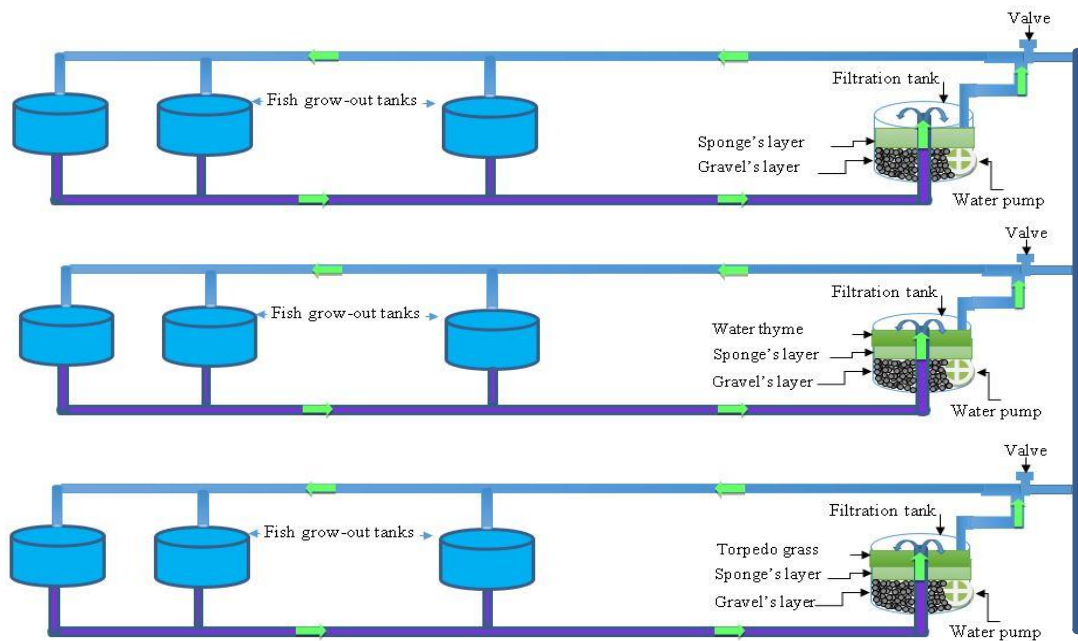


Figure 2: The grow-out, biological filtration plants treatment tanks of a closed bio-aquatic system of the experiment.

### ***Plant's Source and Experimental Preparation***

Water thyme, Torpedo grass aquatic plants were collected from the Tigris River and fingerlings of Common carp fish (*Cyprinus carpio*) weighing an average of 13.75 g/fish were obtained from one of the private hatchery, the fish were transferred to Tikrit University, Faculty of agriculture fields in 7/6/2023 by an aquarium car equipped with a pump to circulate water, reduce stress on fish and provide oxygen, then the fish were placed in a 500 liter for the purpose of regionalization to the new environment to avoids the thermal shock and remained until the start of the experiment (Donaldson et al, 2008). Nonetheless, three treatments were created and applied randomly. A commercial pellet 4mm (30% protein, 3% lipid (Arbel fed) was fed to the fish twice daily (09:30 and 15:00) 4% of body weight. Daily feed intake was monitored, and water quality meters were used to measure the temperature, dissolved oxygen (DO), pH, Ammonia-N (mg l<sup>-1</sup>), Nitrate NO<sub>3</sub>, Nitret NO<sub>2</sub>, total dissolved solids (TDS) and Chloride (CL) (ppm) on a weekly basis from each replicate.

The fish in each tank were euthanized with around 100ppm of clove oil at the end of the experiment, and their ultimate weight and length were recorded (Hassan *et. al.*, 2021b). Using the following formulae, the specific growth was calculated:

SGR for length = specific growth rates (% day<sup>-1</sup>) =  $[(\ln L_1 - \ln L_0) / T] \times 100$ ; where L<sub>1</sub> = final length, L<sub>0</sub> = initial length and T = time in days

SGR for weight = specific growth rates (% day<sup>-1</sup>) =  $[(\ln W_1 - \ln W_0) / T] \times 100$ ; where W<sub>1</sub> = final weight, W<sub>0</sub> = initial weight and T = time in days

The feed conversion ratio (FCR) was determined using the following equation:

FCR = total dry weight of diet fed (g) / wet weight gain (g)

In order to calculate the hepatosomatic index (HSI) and viscerasomatic index (VSI), respectively, from the six fish, the viscera were separated, removed, and weighed, followed by the liver.

VSI = [viscera weight (g) / fish body weight (g)]. × 100

HSI = [liver weight (g) / fish body weight (g)]. × 100

The condition factor is expressed using the equation,  $K = 100W/Lb$ .

The survival rate is calculated using the following equation.

$$SR (\%) = (N_2/N_1) \times 100$$

where SR is the survival rate,  $N_2$  is the final total number of fish alive, and  $N_1$  is the initial total number of fish.

### ***Plasma biochemistry***

Two fish from each replication tank had their caudal veins' blood drawn using 2.5 ml syringes. The blood sample was centrifuged at 360 g for 15 minutes, and the plasma was removed and stored at -20 °C pending determination of the total protein, mg/ dl, creatinine, albumin, and urea concentrations as well as the AST and GPT levels.

### ***Statistical analysis***

The data were analyzed using one-way ANOVA at a significance level of (P) 0.05, and Duncan's multiple range test was employed to identify significant differences between the treatments. SAS Version 9 was used for analysis. The data were presented as means with standard deviations (SD) (n=3).

## **RESULT AND DISCUSSION**

### ***Water Properties***

The pH values of the four coefficients in a row were all basal in the current study, the treatment of Water thyme, Torpedo grass, respectively, outperformed the control treatment were 8.7, 8.8, 8.7 at a significant deference as shown in Table 1. Also, a significant difference was observed between the T1 and T2 treatment. Hamdani (2009) found that the pH tends to the base in his study removed pollutants from some sewage in the city of Mosul. The current study agreed with Shiblawi (2021) that the pH value is fixed for the length of the experiment within the limits of (8-9). Heydamjad (2012) confirmed that common carp fish have the ability to survive and grow at pH levels ranging from 6-9. The results showed that the PH values ranged from 7.3-7.6 (Shet et al, 2015). Hassan et al. (2022) found that the pH of the water used for fish farming in the closed system is 7.45-7.51, which is not consistent with the current study, and the reason may be due to a difference in the susceptibility of aquatic plants to filtering between the media growing on beneficial bacteria. Al-Rubaie (2008) stated that the pH value is affected by the presence of organic matter in the soil. Kazim (2005) noted that basicity is affected by high temperature increased decomposition of organic substances, increased CO<sub>2</sub> concentration.

The oxygen values in the current study were between (4.5-4.6), where there was no significant superiority of the Torpedo grass with the control treatment. Fernandes (2016) noted an inverse relationship between temperature and oxygen. Hamdani (2009) showed that oxygen values increase over a longer period of time from the use of water lentil plant in the removal of pollutants.

Oxygen diffuses in water in two ways, the most important of which is diffusion from atmospheric air and then metabolism, where the product of the process of extracting plants for carbon dioxide is the production of O<sub>2</sub>. The results of Irhayyim et al (2020) showed that the O<sub>2</sub> values in all coefficients were (8.16, 8.19, 8.24, 8.34) at (0%-25%-33%-50%) respectively. Ahmed and AlShakrchy (2013) stated that the percentage of dissolved oxygen inside the closed water system between 8.5-10.1 mg/L due to the low temperature, the oxygen portability higher during the experiment period, which is an appropriate ratio for growth and breeding of Common carp fish. The results of the current study showed ammonia values (0.27,0.32,0.11) for T1, T2 and T3 respectively. The current study also agreed with Shiblawi (2021) that the ammonia concentration is low due to the ability of rice to consume ammonia, which was confirmed by (Li et al., 2018). Shete et al (2015) the reason for the high non-elimination of ammonia values in a control treatment (0.38) mg/L in his study evaluating the different aquaculture of mints with Common carp in an aquaculture system. Tavaras et al, (2010) found that the aquatic plant has the ability to absorb organic substances in wastewater at a pH of eight obtained by removing nitrates and nitrites. The results of Shete et al. (2015) and Shete et al. (2017) showed a decrease in the values of both nitrates and nitrites. That the nitrite(NO<sub>2</sub>) values were 0.26,0.20,0.15 for treatment T1, T2, T3, respectively, where a significant superiority was observed between the Torpedo grass and control treatment. The results of the current study agreed with Hamdani (2009) and Knode (2017) regarding the ability of the water lentil plant to absorb pollutants in wastewater. Irhayyim et al (2020) stated that the water quality coefficients in aquariums were similar in all coefficients including the nitrite averages fluctuated over time. Water quality is determined by many indicators, including nitrite, which affects the growth and health of fish (Sun et al, 2020).

Table 1: The mean (±SD) of various water quality factors over two months of rearing common carp.

Water quality	Treatments		
	Control(T <sub>1</sub> )	T <sub>2</sub>	T <sub>3</sub>
Temperature (°C)	27±0.577a	28 ±1 a	28±1a
PH	8.7±0.07d	8.8±0.01c	8.7±0.047d
DO(mg L)	4.5±0.1b	4.6±0.10ab	4.6±0.06b
TDS	369±1.5b	364±20.2b	373±0.57b
Nitrate NO <sub>3</sub>	7.24±0.22c	5.73±0.16d	3.16±0.20e
Nitrit NO <sub>2</sub>	0.26±0.04ab	0.205±0.09bc	0.152±0.03c
Ammonia NH <sub>3</sub>	0.27±0.07ab	0.32±0.08a	0.11±0.15b
Cloredait CL	37±1.15d	42 ±2c	35 ±3.06 d

Means are± SD and various superscripted letters in each row reference significant differences (p < 0.05).

### Survival and growth performance

It was noted from the table 2, of growth parameters of common carp that the survival rate of fish is 100% for all transactions, it was not affected by the use of aquatic plants. As for the specific growth rate(SGR), there were significant differences between T2 and control treatment. The current study showed that there are significant differences between the Torpedo grass (T3) compared to the control treatment in the feed conversion ratio. The results showed that there were significant differences in the gain weight between the Torpedo grass and T1, where the best treatment was the Torpedo grass treatment, which amounted to 62 g followed by the Water thyme 51g, the control 48.67g. The use of sponge or sawdust led to a significant improvement in the rate of food conversion and thus improved fish productivity (Hassan et al, 2022).

### ***Liver and kidney plasma biochemistry Characteristic***

The lowest urea ratio was in the Water thyme(T2) treatment, which amounted to 3.26, and the highest urea ratio was in the control treatment(T1), which amounted to 4.45, and there was Torpedo grass treatment, table 3. Hematological and biochemical qualities are vital indicators for diagnosing the health and physiological condition of fish and are of great importance in farming a clear picture and an indicator of some positive and negative cases of environmental and nutritional factors (Gabrielois et al, 2015). Albumin performs a wide vital functions in fish and has a major role in transporting various compounds such as drugs and stimulating the production of proteins by the liver and leads to an increase in the immune response (Tan et al., 2017), whose values ranged between 1.1, 0.9, 0.8, 0.9 respectively for the four coefficients and that the total protein values ranged 1.3, 1.1, 1.6, 1.3. Proteins play an important role in the process of osmotic regulation, so the presence of fish in an unbalanced concentration of osmosis leads to the continuation of protein depletion until it stabilizes as a result of the fish adapting to the new situation (Wijayasinghe et al, 2017). Fish respond to organs and this response is accompanied by changes in the level of proteins Logan (Bukley, 2015). Exposure of fish to pollutants and stress at high levels leads to a decrease in protein in the blood plasma (Al-Khshali and Al- Hilalli, 2017). In the current study, the ALT values ranged 7.4, 3.13, 2.3 for T1, T2 and T3 respectively, where the highest value was in the control treatment followed by the treatment of Water thyme, then Torpedo grass. AST, ALT values indicate the presence of stress on the liver, as the alt enzyme is present in large quantities in the liver and increases in plasma when destruction or deterioration of the organ occurs, He et al , (2006), A high index of these enzymes is considered an indicator of the presence of vital damage in the liver, kidneys and blood (Adrich, 2003). However, changes in liver and kidney function may occur when AST and ALT values rise above normal limits (Al-Shakrchy, 2020). As for the biochemical blood parameters when they Sirkov et al, (2020) were not significantly affected by probiotic supplements. High levels of these enzymes in the blood of fish indicate the occurrence of stress, illness or damage caused by environmental pollutants, including poor water quality or improper breeding conditions (Ghafarifarsani et al, 2021). Increasing the feeding rate in Common carp, stress can affect appetite and feeding habits, reduce digestibility and absorption of nutrients, including impaired liver function, reduced growth rate and immunity, and fish may require a different nutritional strategy to maintain a balance of nutrients and ensure water quality (Abdel Gany et al, 2023).

### **Chemical composition**

The results of the chemical synthesis showed that there were no significant differences in the dry matter quality between all the parameter compared with the control treatment, table 4. As for the protein, there was a significant difference level ( $p \leq 0.05$ ) between the T2, T3 with the control treatment. Sirkov et al (2020) Showed that the fat content in Carp fed on a feed containing probiotics was higher at 2.34 and the percentage of fat in fish is from 1-24% (Ali, 2006). Al Taie (1986) also explained the percentage of fat in common carp ranged from 0.2-2.5% of wet weight. The chemical composition of fish meat in terms of moisture, protein, fat and carbohydrates varies depending on the variation of environmental conditions (Al-Jubouri, 2017).

Table 2: Mean (SD±M) Common carp growth performance Parameters in the different filtration aquatic plants over two months.

Parameters	Treatments		
	Control(T <sub>1</sub> )	T <sub>2</sub>	T <sub>3</sub>
Initial weight (g)	14.16±1.22 a	13.5±1.803a	13.6±0.26a
Final weight (g)	23.7±2.29a	23.2±2.25a	24.0±0.5a
Initial length (cm)	9.41±0.06 a	9.3 ± 0.47a	9.4±0.12a
Final length (cm)	11.16±0.59a	12.3±1.37a	11.9±1.21a
Initial body depth (cm)	2.8±0.19a	2.53±0.23a	2.55±0.1a
Final body depth	3.66±0.27ab	3.32±0.17b	3.35±0.05b
SGR weight	0.159±0.035a	0.16±0.013b	0.17±0.004a
SGR Length	0.029±0.009a	0.05±0.017a	0.04±0.019a
Condition factor	1.70±0.174cb	1.28±0.306a	1.49±0.47ab
Hepatosomatic index	1.46±0.744a	1.73±0.42a	1.83 ±0.16a
Viscerasomatic index	3.88±7.275b	9.93±1.34ab	10.9±1.68ab
Feed intake	481±1.15b	476±0.58b	499±2.52ab
FCR	3.425±0.762a	3.44±1.46a	2.67±0.10c
Gain weight	48.67±5.13c	51±1c	62±1.53ab
PER	2.6±0.1a	2.6±0.45a	2.7± 0.1a
Survival rate%	100% a	100% a	100% a

Means are± SD and various superscripted letters in each row reference significant differences ( $p < 0.05$ ).

Table 3: Mean (SD±M) kidney plasma biochemistry Parameters in different filtration aquatic plants over two months of rearing Common carp.

Parameters	Treatments		
	Control (T <sub>1</sub> )	T <sub>2</sub>	T <sub>3</sub>
Urea mg/dl	4.45 ±0.97 <sub>abc</sub>	3.26±1.06 <sub>c</sub>	4.31 ±0.90 <sub>abc</sub>
Creatinine mg/dl	1.7±0.1 <sub>a</sub>	0.066±0.06 <sub>a</sub>	0.13±0.06 <sub>a</sub>
Total protein mg/dl	1.33±0.58 <sub>a</sub>	1.66± 0.6 <sub>a</sub>	1.66± 0.58 <sub>a</sub>
Albumin g/dl	0.996±0.13 <sub>abc</sub>	1.25±0.13 <sub>a</sub>	1.19±0.06 <sub>ab</sub>
AST/GOT IU/MI	139±72.96 <sub>a</sub>	193.8± 27.66 <sub>a</sub>	121.23±28.27 <sub>a</sub>
ALT/GPT IU/MI	7.4±0.95 <sub>a</sub>	3.13±0.709 <sub>b</sub>	2.3±0.5 <sub>b</sub>

Means are± SD and various superscripted letters in each row reference significant differences ( $p < 0.05$ ).

Table 4: Mean (SD±M) proximate analysis Parameters in different filtration aquatic plants over two months of rearing Common carp.

Parameter	Treatments		
	Control (T <sub>1</sub> )	T <sub>2</sub>	T <sub>3</sub>
Moisture	78±2.54 a	73±8.021 a	75±10.02a
protein	16.24±0.51 b	15.5±0.56 a	18.4±0.40 a
Fat	4.35±1.74 a	6.89±0.49 a	2.89±1.28 a
Ash	1.62±0.09 a	3.4±0.62a	2.4±0.87 a

Means are± SD and various superscripted letters in each row reference significant differences ( $p < 0.05$ ).

## CONCLUSION

The use of aquatic plants Water thyme (*Hydrilla Verticillata*) and Torpedo grass (*Panicum Repens*) led to an improvement in the water properties (ammonia, nitrates, nitrites and chlorides). Improved the growth performance criteria is an indicator that the closed bio-aquatic system was improved. The aquatic plants has positive effect on the plasma biochemical characteristics such as Urea mg/dl, AST/GOT IU/MI and ALT/GPT IU/MI.



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