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Effect of stocking density on growth performance of monosex tilapia (*Oreochromis niloticus*) with Indian spinach (*Basella alba*) in a recirculating aquaponic system

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Abstract— *An experiment was conducted to compare effect of stocking density on growth performance of monosex tilapia (Oreochromis niloticus) with Indian spinach (Basella alba) in a recirculating aquaponic system. The experiment was set-up for 8 weeks under 4 treatments with three replications, where stocking density of tilapia were 30, 50, 70 and 90 fish/tank (300 litre) in treatments T1, T2, T3 and T4, respectively. Water from the tank was recirculated through a vegetable growing tray. Each of the tray was 0.15* m^3*in size, which was planted with 12 plants (Indian spinach). The fish of all the treatments was fed two times a day. During the experimental period,the range of water temperature was 27.1 to 31.50 C, pH 7.48 to 8.28, ammonia 0.2 to 2.0 mg/l and dissolve oxygen 5.11 to 6.58 mg/l. At the end of the experiment, average weight gain, final length, specific growth rate (%/day), survival rate was significantly higher in* T_1 *(30 fish/tank) treatment while the net yield of fish and plant biomass was higher in* T_2 *(50 fish/tank) treatment.Therefore, the study suggests that stocking density of 50 fish/tank for tilapia, i.e. 167 fish/*m^3*, is suitable for production of both plant and fish in a recirculating aquaponic system.*

Keywords*— *Aquaponic, Stocking density, Tilapia, Indian spinach, Growth.

I. INTRODUCTION

The demand of aquaculture products is increasing to the consumers, together with the costs related with land and water and also increasing environmental constraints, have determined producers to advance their technological facilities or to implement new engineering solutions to assure the culture of high stocking densities, thus gaining enough fish supplies to cover the production costs and equally, to meet the marketed and s[1].To overcome such situation the aquaponics, an environmental friendly and sustainable food production system may be appeared as a weapon to fight against water scarcity, soil degradation, climate change and the increased population[2].

Aquaponics is a novel alternative method of fish and crop production system by combining aquaculture and hydroponics, a way of growing plants without using soil substrate. The elements which are essential for an aquaponic systemare fish rearing tank, a suspended solid removal component, a bio filter, a hydroponic component and a sump[3].In this method, plants filter waste product means ammonia which is harmful to the fish from the system and utilized them as a nutrient source[4].Very simply, the principle of aquaponic system is fish excrete contains potentially toxic nitrogen compounds, including ammonia which processed into nitrite and then nitrate by nitrifying bacteria which provided in the system. Released ammonia by the fish is not only transformed to nitrate but also removed by the plants from the water [5].The plants utilize this biologically available nutrient from the water for growth, on the other hand, fishes get suitable water quality for their health that also decrease the need to replace water for the fish tanks[3], [6].In an aquaponics system, waste input in the fish tank is reduced through a closed looped system. This symbiotic relationship between fish and plants facilitates to produce multiple crops at a time that results in increased yields while reducing costs and maintenance[7]. In the aquaponic system water, energy and fish feed are the three main physical inputs although vary in size and type of production system[8]. There also need to createa balance of the macro- and micro-nutrient amount that fish can release in the water for a given feed in the aquaponic system; this highly depends on fish species, fish density, temperature, and type of plants[9].Now, it is clear that the supplied feed and stocking density is directly related to maintain the metabolites flow into the aquaponic system

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on the other hand, in aquaculture, 'stocking density' is considered to be one of the important factors that affect fish growth, feed utilization, gross fish yield and economic returns[10], [11].

Tilapia has become the third most significant fish in aquaculture after carps and salmonids [12]. Nile tilapia is commonly cultured tilapia species all over the world that produced over 70% of the cultured tilapia[13]which is also a common choice in aquaponic system[4]. On the contrary, *Basella alba* or Indian spinach is a popular tropical leafy-green vegetable, commonly grown as backyard herb in the home gardens which is a rich source of Vitamin A and C[14].Therefore, the present investigation is carried out to identify the production of mono sex Nile tilapia and Indian spinach with varying stocking density in a recirculating aquaponic system.

II. MATERIALS AND METHODS

Site and design of the experiment

The experiment was conducted for 8 weeks in a recirculating aquaponic system in the wet-field laboratory of Faculty of Fisheries, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh from 6 May to 6 July 2015. The experiment was carried out in completely randomized design having four stocking density, (30, 50,70 and 90 fish/tank for T1, T2, T3 and T4, respectively) with triplicate.

Setting up fish rearing tank and vegetable growing bed

In this experiment, fish were reared in 12 circular plastic tanks having 300-liter capacity and vegetable were grown in rectangular trays prepared with steel sheet of (125×80×15) cm^3 or 0.15 m^3 size.All the vegetable growing trays were filled with small pieces of bricks (works as bio filter). Twelve water pumps (each of 12 watts) were used for elevating water from tank to tray. Fish rearing tanks were arranged arbitrary among the treatments. All the settings were kept under a transparent polythene shed surrounded by bamboo fencing. In this systemfish ate food and release metabolites into the water derived from the food. The metabolites containing water was pumped in the vegetable bed. These metabolites were further metabolized by bacteria (living in small pieces of bricks), and the products of this metabolism were used by plants for nourishment.

Rearing of fish, transplanting of vegetable and feeding

The tilapia juvenileswere collected from a commercial hatchery, Mymensingh, Bangladesh. After acclimatizing and nursing up to desired sized (Table 5), the fingerlings were stocked in the rearing tank. On the other hand, Indian spinach seed were collected from the local market and were grown in the earthen bed up to 15-20 cm size of the seedlings (three weeks) having at least 2–3 true leaves. After three days of stocking fish in the rearing tank, 12 vegetable seedlings/ tray were transplanted in the vegetable bed. Commercially made floating pellets for tilapia from Mega feed limited, Bangladesh were fed twice a day up to satiation. Proximate compositions of floating pellets according to manufacturer and laboratory analysis are shown in Table 1.

Table.1: Composition of experimental pellets as labeled by the manufacturer and by the laboratory analysis

Composition	**Manufacturer value**	**Laboratory analyzed value** (As fed-basis)
Moisture (%)	11	13
Crude Protein (%)	30	28
Crude lipid (%)	3	4
Ash (%)	10	9

Monitoring water quality

The water quality parameters from fish growing tanks were monitored fortnightly during the study period. Water temperature (^{0}C) was recorded using a Celsius thermometer (digi-thermo WT-2) at the experimental site. Dissolved oxygen (DO) and pH were measured using digital oxygen meter (HQ40d multi) and pH meter (sensIONTM + NH_3), respectively. Ammonia level was measured fortnightly using hach kits (Hach Co., Loveland, Colorado).

Fish sampling and growth performance

At the end of 8 weeks of rearing period, all fish from each tank were counted, measured length and weight to observe survival and growth performances. Ten fish carcasses from each tank were pooled, washed with distilled water and stored at -20^{0}C for whole body chemical composition analysis. The following formulas were used to observe the growth performances. *Weight gain (g) = Mean final weight (g) - Mean initial weight (g)*

$$\text{Specific Growth Rate (\% body weight day}^{-1}) = \frac{\ln W_2 - \ln W_1}{T} \times 100$$

Where,

W_1 = Average initial live body weight (g)

W_2 = Average final live body weight (g)

T = Number of days (Culture period)

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Net yield = No. of fish harvested × Mean weight gainof fish

Survival rate was calculated by the following equation.

$$\text{Survival Rate (\%)} = \frac{\text{Total number of fish at harvest}}{\text{Total number of fish at stocking}} \times 100$$

The utilization of feed was observed through Feed conversion ratio (FCR)by following equation.

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Live weight gain (g)}}$$

Proximate analysis of the feed and fish carcass

The proximate composition of feed and fish was determined according to standard method given by the Association of Official Analytical Chemists (AOAC, 1995).

Moisture content (%) was determined by a thermostat oven at 105^{0}C for 24 hours until a constant weight obtained. Crude protein was determined indirectly by measuring total nitrogen content by standard Micro-kjeldahl method by using digestor, Model DK20 and automatic distillation by Model¬ UDK152. Crude lipid content was determined by extracting in diethyl ether in Soxhlet apparatus. Ash content was determined by igniting the sample in muffle furnace at 550°C for 6 hours. The following equations were used.

% Nitrogen =

Error!

% Crudeprotein = 6.25 × % Nitrogen

$$\text{Total lipid (\%)} = \frac{\text{Weight of lipid (g)}}{\text{Weight of sample (g)}} \times 100$$

$$\text{Ash content (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

Harvesting ofvegetable

The first harvesting was done at 26 days after planting (DAP). Then vegetable was harvested after 10 days interval. The plants were cut manually at a length of 6 inch from the bed level by a scissor. The crop was allowed to grow, and the subsequent three harvests were done at 36 DAP, 46 DAP and 56 DAP. After harvesting weight (g) of the vegetable was recorded.

Statistical Analysis

The obtained data from the experiment were analyzed statistically by one-way ANOVA using statistical software Statistix 10. This analysis was then followed by Tukey test where significant differences in means were observed. Significance level was determined at the 5% level.

III. RESULTS

Water Quality Parameters

In aquaponics system, water quality is one of the most important factor to determinesuitable stocking density. Different physico-chemical parameters such as temperature (°C), dissolved oxygen (mg/l), hydrogen ion concentration (pH), nitrate-nitrogen (mg/l), and ammonia-nitrogen (mg/l) were recorded. Average fortnightly variations of water temperature in different treatments during the rearing period are shown in Table 2. The range of average water temperature of the treatments during the experimental period was 27.1 to 31.50C. The highest temperature was recorded on the 8th week (31.5±1.10C) in the treatment T3 where stocking density was 70 fish/tank and the lowest temperature was recorded on the 6th week (27.1±2.60C) in the treatment T1 where stocking density was 30 fish/tank. However, there was no significant difference (P> 0.05) among the treatments.

Table.2: Fortnightly variation of water temperature (°C) during the experimental period in different treatments

Treatment	2nd week	4th week	6th week	8th week
T_1	28.5±2.1	30.6±1.4	27.1±2 .6	31.3±3.2
T_2	28.4±2.3	30.4±3.5	27.3±3.1	31.4±2.4
T_3	28.2±1.5	30.2±2.6	27.4±2.2	31.5±1.1
T_4	28.3±2.2	30.1±3.2	27.5±1.4	31.2±2.3

Note: Values are given as mean ± standard deviation.

Average fortnightly variations of dissolved oxygen in different treatments during the rearing period are shown in Table 3. The range of dissolved oxygen (DO) concentration was found between 5.11 to 6.58 mg/l. The highest dissolved oxygen concentration was recorded in the 8th week (6.58±0.56 mg/l) in the treatment T_1, where stocking density was 30 fish/ tank, and the lowest dissolved oxygen concentration was recorded in the 8th week (5.11±0.93 mg/l) in the treatment T_4, where stocking density was 90 fish/tank. There was no significant difference (P>0.05) of mean values of dissolved oxygen concentration among different treatments.

Table.3: Fortnightly dissolved oxygen level (mg/l) during the experimental period in different treatments

Treatment	2nd week	4th week	6th week	8th week
T_1	6.40±0.98	6.33±0.67	6.12±0.78	6.58±0.56
T_2	6.30±0.66	6.23±0.87	6.10±0.59	6.20±0.75
T_3	5.80±0.12	5.44±0.64	5.30±0.32	5.12±0.33
T_4	5.20±0.34	5.12±0.67	5.20±0.86	5.11±0.93

Note: Values are given as mean ± standard deviation.

Hydrogen ion concentration (pH) in water body generally controls considerably the water chemistry. Any sudden fluctuation of pH causes the death of many aquatic species. Average fortnightly variations of pH in different treatments during the rearing period are shown in Table 4. The range of average values of pH were 7.48 to 8.28. The highest pH value was recorded in the 2nd week (8.28±1.11) in the treatment T_2, where stocking density was 50 fish/ tank, and the lowest dissolved oxygen concentration was recorded in the 8th week (7.48±1.34) in the treatment T_2, where stocking density was 50 fish/tank. There was no significant difference (P> 0.05) among the different treatments.

Table.4: Fortnightly water pH measured during the experiment period in different treatments

Treatment	2nd week	4th week	6th week	8th week
T_1	8.26±1.12	7.58±1.23	7.78±1.04	7.72±1.32
T_2	8.28±1.11	7.58±1.53	7.68±1.09	7.48±1.34
T_3	8.23±1.44	7.52±1.13	7.74±1.45	7.59±1.33
T_4	8.23±1.08	7.55±1.53	7.75±1.00	7.63±1.45

Unionized ammonia (NH_3) is highly toxic to fish, but ammonium ion (NH_4^{+}) is relatively nontoxic. In culture condition, the lower the value of total ammonia, the better water quality for fish. Average fortnightly variations of ammonia in tanks under different treatments are shown in Figure 1.

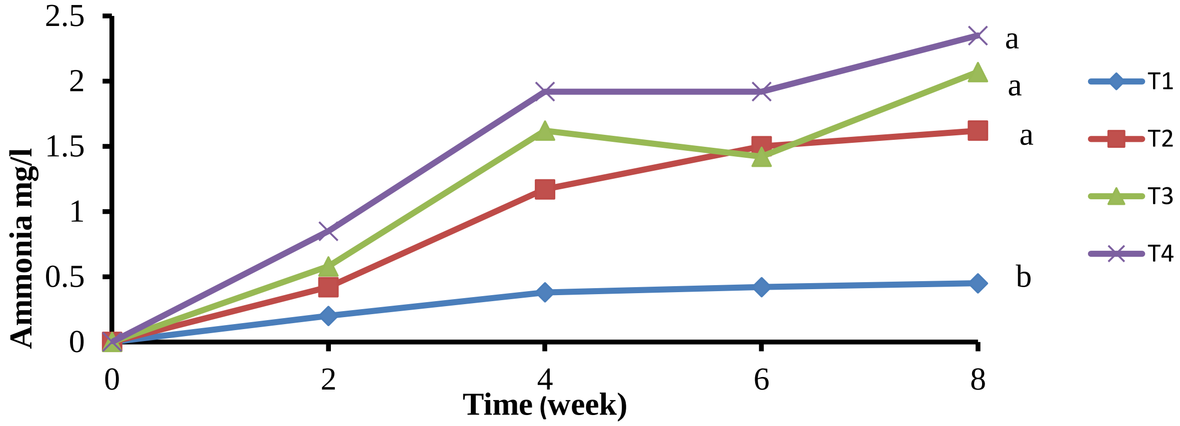

Fig.1: Fortnightly observed ammonia level (mg/l) during the experiment period in different treatments.

The maximum and minimum ammonia concentration was found in the 8th week (2 mg/l) in treatment T_4 where stocking density was 90 fish/tank and 2nd week (0.2 mg/l) in the treatment T_1 where stocking density was 30 fish/tank respectively. In all treatments ammonia level was increased with the increase in culture time. However, it was ammonia was lower in treatment T_1 compared to other treatments throughout the experimental period.

Growth performance of fish

After 8 weeks of rearing, significant difference(P<0.05) was found in growth performances of fish (Table 5). The highest average weight gain and final length of Tilapia were observed 44.29±5.35g and 15.34±0.64 cm, respectively in the treatment T_1 where stocking density was 30 fish/tank. The lowest average weight gain and final length 19.77±0.47 g and 13.14±0.06 cm, respectively that was observed in the treatment T_4, where stocking density was 90 fish/tank. The specific growth rate (SGR, % day-1) of fish in different treatments was 3.68±0.17, 3.44±0.22, 2.86±0.11 and 2.56±0.04 in the treatment T_1, T_2, T_3 and T_4, respectively. There was significant difference in SGR (P<0.05) among different treatments. The highest SGR value and the lowest SGR value were found in treatment T_1 and treatment T_4, respectively. SGR value in treatment T_2, where stocking density was 50 fish/tank was statistically similar to that of treatment T_1.

Table.5: Growth performance of O. niloticus fed with floating pellets in different stocking density

Growth parameters	**T_1**	**T_2**	**T_3**	**T_4**	**Level of significance**
Initial weight (g)	5.23±0.04	5.25±0.02	5.24±0.00	5.25±0.20	NS
Weight gain (g)	44.29±5.35^{a}	37.72±5.64^{ab}	24.66±2.02^{bc}	19.77±0.47^{c}	*
Initial length (cm)	5.25±0.35	5.50±0.00	5.60±0.28	5.70±±0.14	NS
Final Length (cm)	15.34±0.64^{a}	14.60±0.18^{ab}	13.51±0.29^{b}	13.14±0.06^{b}	*
Survival Rate (%)	100±00^{a}	99±1.41^{ab}	95.00±3.02^{ab}	92.78±0.78^{b}	*
FCR	1.34±0.09	1.12±0.10	1.38±0.05	1.34±0.06	NS
SGR (%/day)	3.68±0.17^{a}	3.44±0.22^{ab}	2.86±0.11^{bc}	2.56±0.04^{c}	*
Net Yield (kg/m^3)	4.43±0.36	6.23±1.10	5.47±0.62	5.50±0.18	NS

Note: Values are given as mean ± standard deviation. Value in the same column bearing different letters are significantly different at 5%.

The highest survival rate was found in the treatment T_1 where stoking density was 30 fish/tank and the lowest was in T_4 treatment where stoking density was 90 fish/tank. Survival rate of Tilapiain treatment T_2, was statistically similar to that of treatment $T_{1.}$ In case of FCR and net yield (kg/m^3) were found in case of T_2 though there was no significant difference among the treatments.

Proximate composition of whole body carcasses of fish

The average moisture (%) of *O. niloticus* was 72.67±3.32, 71.36±0.64, 72.75 ±3.18 and 71.15±1.20 % in the treatments T1, T2, T3 and T4, respectively. The average protein (%) of *O. niloticus* was 20.82±0.26, 20.33±0.29, 20.07±2.01 and 20.01±1.97 in the treatments T1, T2, T3 and T4, respectively. The average lipid % of *O. niloticus* was 6.72±0.24, 6.52±0.12, 6.40±0.20 and 6.22±0.11 in the treatments T1, T2, T3 and T4, respectively. The average ash percentage was1.75, 1.69, 1.73 and1.65 % in the treatments T1, T2, T3 and T4, respectively. The average protein, lipid, moisture and ash (%) of in different treatment did not differ significantly (P>0.05) (Table 6), which indicated that stocking density did not affect the proximate composition of *O. niloticus.*

Table.6: Proximate composition of whole body carcasses of O. niloticus fed with floating pellets in different stocking density

Treatments	Moisture	Protein	Lipid	Ash
T_1	72.67±3.32	20.82±0.26	6.72±0.24	1.75±0.12
T_2	71.36±0.64	20.33±0.29	6.52±0.12	1.69±0.23
T_3	72.75±3.18	20.07±2.01	6.40±0.20	1.73±0.34
T_4	71.15±1.20	20.01±1.97	6.22±0.11	1.64±0.41
Level of significance	NS	NS	NS	NS

Note: Values are given as mean ± standard deviation. Value in the same column bearing different letters are significantly different at 5%.

Yield of Indian spinach

The yield of Indian spinach per treatment varied significantly due to different stocking density of tilapia at 26, 36, 46 and 56 DAP (Table 7). At 26 DAP, the highest (3.21±0.18 kg/m^2) yield was recorded in treatment T_2 where the stocking density of tilapia was 50 fish/tank, while the lowest (1.38±0.08 kg/m^2) yield was found in treatment T_1 where the stocking density of tilapia was 30 fish/tank. The highest yield (3.21±0.18, 2.75±0.44, 2.38±0.35 and 1.31±0.04 kg/m^2) was recorded in treatment T_2 where the stocking density of tilapia was 50 fish/tank, at 26, 36, 46 and 56 DAP, respectively while the lowest yield (1.38±0.08, 0.82±0.24, 0.53±0.34 and 0.48±0.07 kg/m^2) was recorded in treatment T_1 where the stocking density of tilapia was 30 fish/tank.at 26, 36, 46 and 56 DAP, respectively.

Table.7: Yield (kg/m^2) of Indian spinach in different treatments

Treatment	26 DAP	36 DAP	46 DAP	56 DAP	Total Production
T_1	1.38±0.08^{c}	0.82±0.24^{c}	0.53±0.34^{c}	0.48±0.07^{b}	3.21±0.76^{d}
T_2	3.21±0.18^{a}	2.75±0.44^{a}	2.38±0.35^{a}	1.31±0.04^{a}	9.65±0.45^{a}
T_3	2.75±0.04^{b}	2.15±0.25^{a}	1.65±0.16^{b}	1.28±0.09^{a}	7.83±0.23^{b}
T_4	2.12±0.14^{b}	1.73±0.41^{b}	1.90±0.64^{b}	0.85±0.05^{b}	6.60±0.34^{c}

Note: Values are given as mean ± standard deviation. Value in the same column bearing different letters are significantly different at 5%.

The total yield of Indian spinach per meter square was significantly different due to different stocking density of tilapia (Table 7). The highest (9.65±0.45 kg/m^2) yield was recorded in treatment T_2 where the stocking density of tilapia was 50 fish/tank, while the lowest (3.21±0.76 kg/m^2) yield was found in treatment T_1 where the stocking density of tilapia was 30 fish/tank.

IV. DISCUSSION

In this aquaponic system, four different stocking densities were trialed to determine optimum stocking density of *O. niloticus*with Indian spinach. During the experiment the temperature was within suitable range for the grow out of the fish as well as for the aquaponic system. [15]exhibited that the range of water temperature of 26.06 to 31.97°C is preferable for fish culture. In aquaponics, tilapia is usually reared between 22.2 and 23.3 °C in order that the requirements of the fish, the nitrifying bacteria and the aquaponic plants are met, as plants grows better at slightly lower temperatures[16]. At a time, low concentration of dissolved oxygen can decrease water uptake by the roots and thereby decrease leaf growth of lettuce[17].In the present investigation, it is observed a decreasing trend of DO with increasing stocking density. However, the DO range 5.11±0.93 to 6.58±0.56 mg/l exhibited a suitable condition for fish culture and the system as well. pH is one of the key features in aquaponics and should be kept around 7 for smooth nitrification; converting ammonia and providing nitrate for the plants[9], [18].

It is identified that disruption may occur in the nitrification process while pH<6.5 with eventual risk of ammonia and nitrite toxicity. However, the pH was in suitable range for the system. In this present trial it was found that the level of ammonia was increased with the increasing stocking density. Accumulation of urine of fish might have caused the higher ammonia content in higher stocking density. The unionized form of ammonia (NH_3) is highly noxious to fish and other aquatic life, while the ammonium ion ($NH4^+$) is much less toxic. In the aquaponic system at pH of 7, the majority of ammonia nitrogen is in the ammonium ion form. Therefore, the ammonia level was within suitable for the system.

In terms of growth performance, lower the stocking density better the weight gains, SGR (%/day) and final length. This indicated an inverse relationship with increasing stocking density. Some researchers observed similar results, where stocking density was related to average weight gain, SGR (%/day) and length in tilapia[11]. In this trial 50 fish/tank showed the best result. The cause might be the suitable environment for this stocking density. FCR is one of the crucial parameter for the economic consideration. In this trial, the lowest FCR, 1.12±0.10 also observed in case of 50 fish/ tank, though there were no significant differences among the treatments. On the other hand, higher the stocking density lower the survival rate was observed. The cause might be the crowed condition as well as the competition though the fed was supplied up to satiation. In case of net yield highest figure 6.23±1.10 kg/m^3 was observed in T_2 where 50fish/tank were stocked. The lower production in the highest stocking density might be attributed to the fact that the growth and survival rate of fish in treatment T_3 and T_4 was the lowest and the increase in biomass was limited by available space and greater competition. The present study demonstrated that 50 fish/tank was the best stocking density in terms of production for tilapia cultured in the aquaponic system.

In aquaponic system, vegetable production is also an important factor to identify the optimum stocking density. In the present study, the highest (9.65±0.45 kg/m^2) yield was recorded in treatment T_2 where the stocking density of tilapia was 50 fish/tank. The possible reason might be theplant of treatment T_2 got suitable amount of nitrogen for their growth on the other hand in treatment T_3 and T_4 got excessive amount of nitrogen that hampered growth of plant and in treatment T_1 plant did not get enough nitrogen for their growth.

V. CONCLUSION

The effects of stocking density were determined for the *O. niloticus* growth and Indian spinach biomass in one-loop system. Though, the average weight gain, specific growth rate was higher in the stocking density of 30 fish/tank, considering the net yield of *O. niloticus* as well as biomass of Indian spinach in50 fish/tank stocking density is preferable. In aquaponic system, water quality parameters are very much important to run the system smoothly. More precisely, pH and nitrogenous substances arecrucial for the growth of the plant in the system. This nitrogen flow mostly comes from the fish. So, the optimum stocking density should be considered in aquaponic system.

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