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The effect of dissolved oxygen on common carp (*Cyprinus carpio*) and basil (*Ocimum basilicum*) in the aquaponics system

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SUMMARY

*Aquaponics is an integrated system that combines fish farming (aquaculture) and hydroponic plant production. The objective of this study was to examine how the level of dissolved oxygen with or without an air pump affects water quality, fish output and plant growth parameters for common carp (*Cyprinus carpio*) and basil (*Ocimum basilicum*).*

Ebb – and flood aquaponics systems (with automatic syphon) was used. Two treatments were set in this experiment, one of which was the aquaponics system without air pump (unit I), where water of the plant bed was pumped two directions, one falling back to the fish tank oxygenating the water the other was pumped to the hydroponics unit. The other system (unit II) was designed with an air pump.

In the course of the study, water quality parameters, such as oxygen saturation, dissolved oxygen (DO), electrical conductivity (EC) and nitrite were significantly different ($p<0.05$). Total basil biomass was higher in unit II. (5367.41 g). The final biomass of common carp were 2829.45 g ± 79.24 and 2980.6 g ± 64.13 g in unit I and unit II respectively. Weight gain (WG) and specific growth rate (SGR) showed no significant differences ($p>0.05$) between the treatments.

Keywords: *aquaponics; common carp; basil; flood- and- drain system; air pump*

INTRODUCTION

Aquaponics is an integrated system that combines recirculating aquaculture and hydroponic plant production (Diver, 2006). A typical aquaponics consists of a fish tank (aquaculture), a biofilter (for nitrification) and a plant grow bed (hydroponics) (Love et al., 2015). The latter is practically a biological filter because the nitrogen forms are excellent nutrients for plants. Fish wastewater in an aquaponics system, is delivered to hydroponics unit where the plants use the nitrogen forms provided by nitrification. Nitrification is the main process that transforms NH_4^+ to NO_3^- in the presence of oxygen (Hu et al., 2015). This process is carried out by three groups of bacteria, ammonia oxidizing bacteria (*Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus, Nitrosovibrio sp*), ammonia oxidizing archaea and the nitrite oxidizing bacteria (*Nitrobacter, Nitrococcus, Nitrospira, Nitrospina sp*) (Ebeling et al., 2006; Panuvatvanich et al., 2009). Somerville et al. (2014) believed that ammonia (NH_4^+) concentration should not reach levels above 1 mg L^{-1}. The nitrite (NO_2^-) is usually within the range s 0.4 to 1.1 mg L^{-1} in a well-operated aquaponics system. The nitrate (NO_3^-) is not toxic up to 150-300 mg L^{-1} (Graber and Junge, 2009; Hu et al., 2014). The concentration of nitrogen forms are the result of conversion by nitrifying bacteria which are oxygen dependent.

The oxygen is most important determinant factor of plant and fish-rearing. It is an essential element in aquaponics too, necessary for nitrification and plays critically important role in fish biomass in aquaponics production. Rakocy (2007) believed if DO level is insufficient root rot symptoms may occur therefore it is important also from a plant health point of view.

Dissolved oxygen level is influenced by many factors. The environmental factors (air temperature, light intensity, and air humidity) and water quality (pH, water temperature, bacteria flora) are the main parameters influencing the fish water. (Kasozi et al., 2019). Dissolved oxygen (DO) optimal for the aquaponics is in the range of 4–8 mg L^{-1}. (Tyson et al., 2008). The demands of fish species will determine this value. Baßmann et al (2017) in study found that, the African Catfish (*Clarias gariepinus*) showed good growth parameters in aquaponics at 8 mg L^{-1} oxygen (DO)level. Tilapia (*Oreochromis niloticus*) do not require a high oxygen concentration because it can survive at 0.5–1.0 mg L^{-1} (El-Sayed, 2006). Another study showed that the oxygen contents of fish tanks were 6 mg L^{-1}. The Nile tilapia (*Oreochromis niloticus*) showed also good specific growth rate (SGR) (Abentin et al., 2018). Similar parameters were reported by Hussain et al. (2015) when the effect of water flow rate on the production potential of koi carp (*Cyprinus carpio* var. *koi*) under aquaponics system was examined with specific growth rate (SGR) 1.22 (% day^{-1}) and the dissolved oxygen 6.89 (mg L^{-1}). The carp (*Cyprinus carpio*) is known to be tolerant to higher oxygen fluctuations in ponds between 0.5–20 mg L^{-1} (Lukowicz, 1982).

The objective of this study was to examine how the level of dissolved oxygen changes with or without an air pump and what effect it has the water quality, fish

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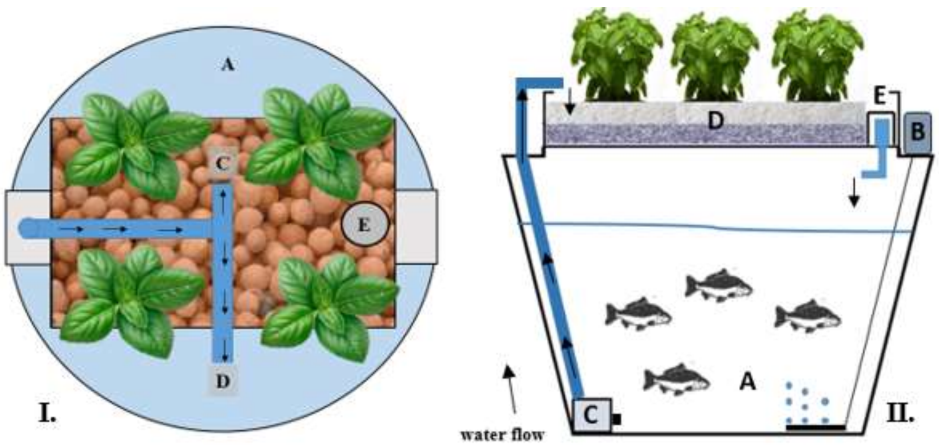
output and plant growth parameters for common carp (*Cyprinus carpio*) and basil (*Ocimum basilicum*).

MATERIALS AND METHODS

Aquaponic systems and experimental design

The experiment was conducted in the Fish Biology Laboratory of University of Debrecen Faculty of Agricultural and Food Sciences and Environmental Management. Two ebb and flood aquaponics systems with auto siphon and gravel bed hydroponic were connected. The aquaponics systems (*Figure 1/II.*) were operated in double sheet plastic tunnel greenhouse under shade cloth. The capacity of fish tank and hydroponics unit (plant boxes) were 225 liters and 32 liters, respectively. These fish rearing tanks contained 150 L freshwater. Crushed stones were used as substrate in the plant boxes with the dimensions: 25 cm in height and surface area of 43 cm (width) x 75 cm (length). The total surface area of the hydroponics boxes of the three replicates were approx. 1 m^2. The maximum flood status was 15 cm water level at the plant boxes. A submersible pump (capacity 1650 L h^{-1}) were used to drive the water to the hydroponics units from the fish tanks There were two treatments in this experiment, one without aeration (unit I), and the other (unit II) with air pump (Air pump 400, Eheim, Germany). Water of the plant bed were split into two parts in unit I, one falling back to the fish tank providing oxygenation, and the other was pumped into the hydroponics unit (*Figure 1/I.*). There were three replicates for each treatment (*Figure 2).* The environmental factors inside the plastic greenhouse were measured daily during experiment. Light intensity was measured with a digital luxmeter (PKT-5065 Luxmeter, spectral, range: 0.1–100.000 Lux; 0.1 Lux; ±4%). The lux values were ranging between 7305 Lux and 9039 Lux e depending on the weather conditions. The air humidity (PCE-THB 40 Thermo Hygrometer Barometer) were 65.8% on average and air temperature 30.6 °C on average. There was no water exchange, only the evaporated water was refilled once a week. The experiment was carried out in 58 days.

Figure 1. **Aquaponics system**



I. = The water division of the plant bed in unit I (A = fish tank, C = water falling into the fish tank, D = In the plant bed running water, E = bell siphon. II. = The structure of unit II (A = fish tank; B = air pump (There was not in other aquaponics system); C = pump; D = plant box; E = bell siphon).

Fish and plant species

Mixed sex common carp (*Cyprinus carpio*) was stocked in all fish rearing tanks (n=15). They were acclimated for 1 week before the experiment. The mean mass (or average body weight) was 140.90g in unit I and 140.57 g in unit II (8.43 kg m^{-3}). The fish were fed two times daily at 8.00 am and 4.00 pm (10 g/fish tank/ day, 1.43% of total fish biomass) with sinking carp feed (BioMar- EFICO Alpha 756), containing 40% crude protein, 23% crude fat, and 3.9% crude fiber. The fish were weighed weekly.

Basil (*Ocimum basilicum*, Genovese F1) plants were chosen in the hydroponics units, because they are fast growing and have favorable nutrient uptake (Kurd et al., 2017; Mostafavi et al., 2019), also it is widely used in soilless cultures (Roosta, 2014; Mangmang et al., 2016). The basil seeds were planted in universal potting soil (Premi Vit deluxe, containing N, 1% P_2O_5, 0.1% K_2O, 0.3% pH, 6.4 (±0.5). After this plant lets were transferred to the grow beds (initial plant biomass: 4.64g ±1.7). The basil planting density was 12 seedlings per 3 m^{-2}. No additional fertilizers were provided during the experiment and the plants were harvested only once at the end of experiment.

Physical and chemical analysis

Physical water parameters were measured each day in the fish tanks. Dissolved oxygen (DO, mg L^{-1}) oxygen (%), temperature (ºC) (Hach-Lange HQ40D, Germany) electrical conductivity (EC, µs cm^{-1}, Adwa

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AD332, Romania), pH (HANNA combo HI 98120, Romania), redox potential (ORP, mv, HM digital ORP-200). Chemical water parameters were analyzed weekly with a spectrophotometer (Hach Lange DR 3900, Germany) for NH_3-N (mg L^{-1}), NO_2-N (mg L^{-1}), NO_3-N (mg L^{-1}). Plant growth intensity (with a hand ruler) (n=6) and chlorophyll level of the leafs chosen randomly was weighed with a SPAD-502 Plus Chlorophyll Meter (Konica Minolta, Japan) once a week. The leaves were selected from the top, bottom and center of the plant (n=3).

Figure 2. **The experimental aquaponics units**

Mathematical and Statistical analyses

Fish growth parameters were calculated at the end of the experiment. Specific growth rate SGR (% d^{-1}) = (ln W_t - ln W_0) x t^{-1} × 100, where W_t = final biomass, W_0 = initial biomass, t = time in days (Pucher et al., 2015), Feed conversion ratio FCR (g g^{-1}) = fish feed quantity (g) × weight gain $(g)^{-1}$, Protein efficiency ratios PER (g g^{-1}) = wet weight gain (g)/total protein intake (g), Weight gain WG (%) =100 × (final body weight−initial body weight)/initial body weight. The data were analyzed with Microsoft Excel (2016) and statistical software package SPSS version 22. Variations in fish and plant growth as well as water quality parameters were determined with independent t-test (P<0.05).

RESULTS

Growth parameters of common carp (*Table 1*), final weight (unit I = 188.63 g ± 79.24, unit II = 198.71 g ± 64.13), final biomass (unit I=2829.45g ± 79.24, unit II = 2980.6 g ± 64.13 g), feed conversion ratio FCR (unit I = 1.76 ± 22.52, unit II = 1.44 ± 0.36), protein efficiency ratios PER (unit I = 1.03 g g^{-1} ± 0.85, unit II = 1.25 g g^{-1} ± 0.27), weight gain WG (unit I = 33.9% ± 28.02, unit II = 41.3% ± 8.78) and specific growth rate (SGR) showed no significant differences (P>0.05) between the treatments. The SGR (unit I = 0.50% d^{-1} ± 0.39, unit II = 0.60% d^{-1}± 0.11) was better in unit II. No mortality was observed during the experiment.

Basil total biomass was higher in the unit II. (5367.41 g) than in unit I. (4254.9 g). *Chlorophyll* content of leaves showed significant difference (P<0.05) at young leaves. Plants of unit II. had more *chlorophyll* 35.59 (± 5.04). Plant height (59.68 ± 3.56, 61.32 ± 3.48), SPAD total (32.20 ± 8.39, 36.07 ± 7.53), SPAD of bottom leaves (30.12 ± 9.47, 38.14 ± 6.60), and SPAD of center leaves was not significantly different (P>0.05) (*Table 2*). Equation of plant height (*Figure 3*) showed better values in the unit. II (y=8.1471x + 16.104, R^2 = 0.9321) than in the unit. I (y=7.5674x + 19.86, R^2 = 0.9183).

The main difference was detected between the water quality parameters of fish tanks (*Table 3*). Significant differences (P<0.05) were observed in dissolved oxygen (DO) levels 5.55 mg L^{-1} (± 1.6); 6.30 mg L^{-1} (± 1.0) (*Figure 4*), oxygen saturation 65.92% (± 18.2); 76.29% (± 11.1), and electric conductivity (EC) 729.20 μs cm^{-1} (± 101.96); 839.76 μs cm^{-1} (± 204.05). The higher values were measured in the fish rearing tanks of unit II. No significant differences (P<0.05) were observed in temperature 23.66 ºC (± 2.16); 24.20 ºC (± 2.11), redox potential (ORP) 124.5mv; 110.4mv and pH (8.4 (± 0.34); 8.4 (± 0.31) of water. Levels of nitrite (NO_2-N) were lower in fish tanks of unit II while ammonia (NH_3-N) 1.01 mg L^{-1} (± 0.76); 0.90 mg L^{-1} (± 0.76) and nitrate (NO_3-N) 3.98 mg L^{-1} (± 2.40); 3.68 mg L^{-1} (± 1.70) were not different (P>0.05).

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Figure 3. **Mean weekly height of Basil (*Ocimum basilicum*) in aquaponics systems**

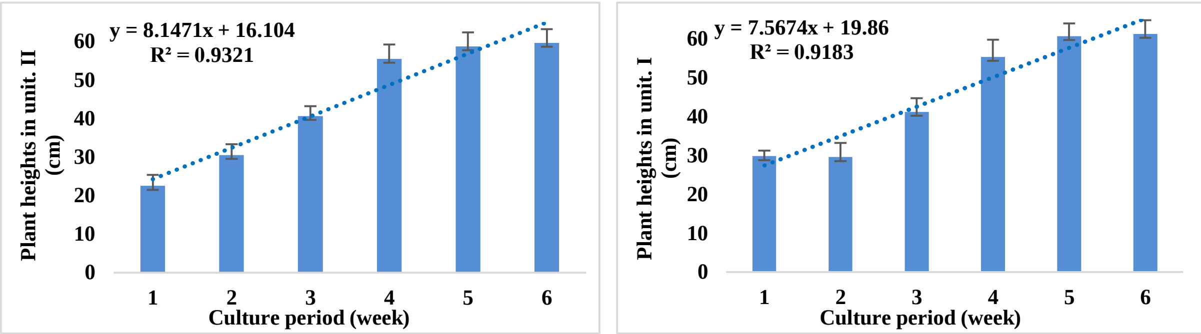

Table 1. **Growth performance of the *C. carpio* in the study**

Parameters	Unit I (aquaponics without air plump)	Unit II (aquaponics)
Initial weight (g)	$140.91^a \pm 69.73$	$140.57^a \pm 30.23$
Final weight (g)	$188.63^a \pm 79.24$	$198.71^a \pm 64.13$
Initial biomass (g)	$2113.6^a \pm 69.73$	$2108.55^a \pm 30.23$
Final biomass (g)	$2829.45^a \pm 79.24$	$2980.6^a \pm 64.13$
SGR (% d^{-1})	$0.50^a \pm 0.39$	$0.60^a \pm 0.11$
FCR (g g^{-1})	$1.76^a \pm 22.52$	$1.44^a \pm 0.36$
PER (g g^{-1})	$1.03^a \pm 0.85$	$1.25^a \pm 0.27$
WG (%)	$33.9^a \pm 28.02$	$41.3^a \pm 8.78$

Note: Mean values ± SEM of fish parameters, significance (P<0.05).

Table 2. **Basil (*Ocimum basilicum*) parameters**

Parameters	Unit I (aquaponics without air plump)	Unit II (aquaponics)
Initial plant height (cm)	$22.53^a \pm 2.84$	$21.98^a \pm 1.40$
Final plant height (cm)	$59.68^a \pm 3.56$	$61.32^a \pm 3.48$
Total biomass (kg m^{-2})	4.256	5.367
Plant wet weight gain (kg m^{-2})	$1.77^a \pm 0.20$	$1.40^a \pm 0.38$
$SPAD_{(total)}$	$32.20^a \pm 8.39$	$36.07^a \pm 7.53$
$SPAD_{(top\ leafs)}$	$33.97^a \pm 8.86$	$35.59^b \pm 5.04$
$SPAD_{(center\ leafs)}$	$32.51^a \pm 6.56$	$34.47^a \pm 10.01$
$SPAD_{(bottom\ leafs)}$	$30.12^a \pm 9.47$	$38.14^a \pm 6.60$

Note: Mean values ± SEM of plant parameters, significance (P<0.05).

Table 3. **Water quality parameters**

Parameters	Unit I (aquaponics without air plump)	Unit II (aquaponics)
Dissolved oxygen (mg L^{-1})	$5.55^a \pm 1.6$	$6.30^b \pm 1.0$
Oxygen saturation (%)	$65.92^a \pm 18.2$	$76.29^b \pm 11.1$
Electric conductivity (µs cm^{-1})	$729.20^a \pm 101.96$	$839.76^b \pm 204.05$
Temperature (°C)	$23.66^a \pm 2.16$	$24.20^a \pm 2.11$
Redox potential (mv)	124.5^a	110.4^a
pH	$8.4^a \pm 0.34$	$8.4^a \pm 0.31$
NH_3-N (mg L^{-1})	$1.01^a \pm 0.76$	$0.90^a \pm 0.76$
NO_2-N (mg L^{-1})	$1.82^a \pm 3.28$	$0.55^b \pm 1.17$
NO_3-N (mg L^{-1})	$3.98^a \pm 2.40$	$3.68^a \pm 1.70$

Note: Mean values ± SEM of water quality parameters, significance (P<0.05).

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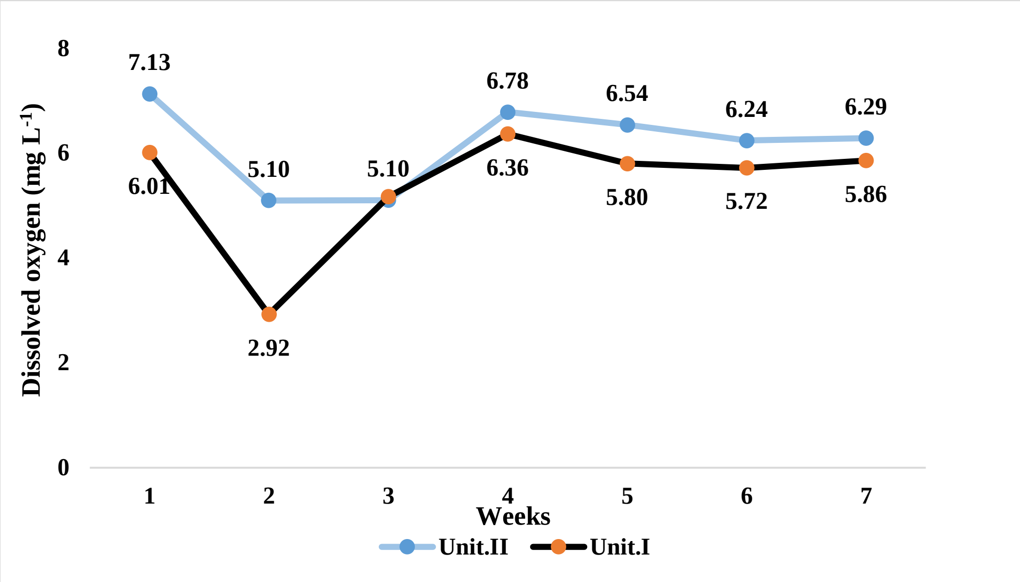
Figure 4. **Changes in oxygen levels during the experiment**



DISCUSSION

The present study describes effect of the low oxygen level on water parameters, carp and basil growth. The presence and proportion of nitrogen forms is determined by the amount of oxygen (Hu et al., 2015). The EC (electrical conductivity) determine the quantity of nitrogen ions dissolved in water. This nutrient source basically determines plant growth.

No fish mortality was recorded during the experimental period. The water quality was adequate for carp growth in both systems. The carp is well adapted to lower oxygen levels (Somogyi et al., 2019). Sometimes there were extremely low dissolved oxygen levels during the experiment, but it did not lead to fish death. The feed conversion ratios (FCR) of common carp were 1.76 g g^{-1} and 1.44 g g^{-1} in the study. Paudel, (2020) reported similar FCR values of 1.73–1.75 g g^{-1} at *C. carpio* after 125 days. Shete et al. (2016) published better feed conversion ratio of 1.06 g g^{-1} with initial and final weight 0.30–8.07 g at 6.5 mg L^{-1} dissolved oxygen after 2 months. Majeed and Najim, (2019) reported higher 2.01 g g^{-1} feed conversion ratio in their experiment with 41.3–48.42 g initial and final weight at 5.36 mg L^{-1} oxygen after 60 days. Specific growth rate (SGR) for unit II (0.60% d^{-1}) was better in the present experiment. Majeed and Najim, (2019) published lower SGR values. The experiment was conducted with *C. carpio* in aquaponics system with 0.26% d^{-1} SGR as a result. The dissolved oxygen level was 5.36 mg L^{-1} in the fish tank, during the experiment (60 days). The relationship between oxygen and SGR values in the literature is shown in *Table 4* as a function of stocking density and duration.

Table 4. **SGR values in common and Koi carp reared in aquaponics systems**

Fish species	DO (mg L^{-1})	SGR (% d^{-1})	Stocking density (kg m^{-3})	Time of experiment (day)	Reference
common carp	6.0–7.0	0.2–0.3	10	52	Zou et al. (2016)
common carp	6.3	0.39	12.2	70	Knaus and Palm (2017)
common carp	5.7–6.6	5.4–5.5	0.18	60	Shete et al. (2016)
common carp	6.8–6.9	0.8–1.0	4.6	30	Sirakov et al. (2018)
common carp	6.4–6.6	1.4–2.0	5	40	Sirakov and Velichkova (2018)
Cyprinus carpio var. Koi	5.6–6.8	0.3–0.8	2.8	60	Hussain et al. (2015)
Cyprinus carpio var. Koi	5.4–6.7	0.7–0.9	1.4	60	Nuwansi et al. (2019)

Plant biomass of basil was 4 kg m^{-2} (5.5 mg L^{-1}, DO; 65,9 O_2%; 0.73 mS cm^{-1}, EC; 1 mg L^{-1}, NH_3-N; 1.8 mg L^{-1}, NO_2-N; 3.98 mg L^{-1}, NO_3-N) and 5 kg m^{-2} (6.3 mg L^{-1}, DO; 76.2%, 0.84 mS cm^{-1}, EC; 0.9 mg L^{-1}, NH_3-N; 0.55 mg L^{-1}, NO_2-N; 3.68 mg L^{-1}, NO_3-N) at the end of the experiment. Basil height grew from 22.5 cm to 59.6 cm (in Unit I) and from 21.9 cm to 61.3 cm (in Unit II) in 42 days. Rakocy et al. (2004) reported the basil yield over 2 kg m^{-2} per harvest (28 days) beside 4–5 mg L^{-1} (DO), 0.8 mS cm^{-1} (EC). The nitrogen forms

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were 2 mg L^{-1}, TAN; 1 mg L^{-1} >, NO_2-N; 42 mg L^{-1}, NO_3-N in the aquaponics system. Other aquaponics studies using basil reported yields of 0.531 kg m^{-2} biomass production (over 30 days), where water parameters were 4–6 mg L^{-1} (dissolved oxygen), 0.6–0.9 mS cm^{-1} (electrical conductivity) and 0.1–0.4 mg L^{-1}, NH_4; 0.3 mg L^{-1}, NO_2; 10–12 mg L^{-1}, NO_3 (Espinosa et al., 2016). Dissolved oxygen 6.5–7.5 mg L^{-1}, oxygen saturation 80–90% and 00.8–04 mg L^{-1}, NH_4; 0.05–0.2 mg L^{-1}, NO_2; 11–106 mg L^{-1}, NO_3 were in the aquaponics system where basil production was 5 kg m^{-3} at final harvest, and length of plants changed from 27 cm to 131 cm in 5 weeks during the experiment (75 days) of Selek et al. (2015). Lower basil lengths were measured by Saha et al. (2016). The plants mean height was 90 cm in the aquaponics system.

The chlorophyll content in the present study were higher in unit II. Saha et al., (2016) analyzed SPAD index of basil leaf in aquaponics. Different results were observed in the SPAD values. Mean of basil leaves chlorophyll content was not reaching 30 SPAD reading. As well lower SPAD values were measured by Rakocy and Hargeaves (1993).

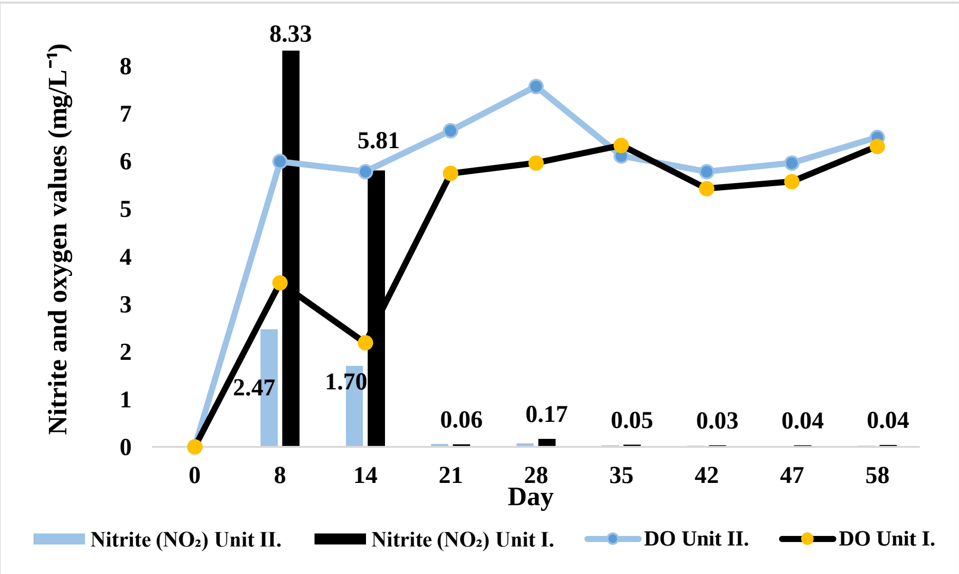
The water parameters were favorable for carp in both units. Dissolved oxygen level of unit I was decreasing during the one-week acclimatization period for the fish (2.92 mg L^{-1}). Then 30 percent of water change was applied and faster water flow was provided in the unit I. From the first week of the experiment oxygen values were over 5 mg L^{-1} and it did not go lower during the whole duration. Knaus and Palm, (2017) reported similar oxygen distribution. The dissolved oxygen level decreased until day 10 of the experiment (phase I), then increased in the middle phase from day 12. Zou et al. (2016) and Stoyanova et al. (2020) reported similar mean DO values too. Oxygen content in the fish tank was measured in aquaponics system in summer the mean was 6 mg L^{-1} during the experiment. The optimum oxygen level was between 5-6 mg L^{-1} by Bernstein, (2011).

The electrical conductivity (EC) was lower in unit I (729.20 µs cm^{-1}), it refers to better mineral use by plants. Knaus and Palm (2017) published different results when Carp was reared in aquaponics. The EC was 588.8 µs cm^{-1}.

The redox potential (ORP) affects biological and chemical processes of the water. (Ge et al., 2015). Wang et al. (2015) demonstrated that the decrease of ORP is related to the decrease of dissolved oxygen (DO). Therefore, the ORP affects low NH4 + contents through oxygen. The redox potential of fish tanks was lower in the unit II equipped with air pump (124.5 mv) where dissolved oxygen level was higher (6.30 ± 1.0) that in the unit I (5.55 ± 1.6). Similar results were published with Nile tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*) by Knaus and Palm (2019) at 5.8 mg L^{-1} oxygen the ORP 123.6 mv (*O. niloticus*) in aquaponics. At other treatment ORP was 121.4 mv and DO 6.3 mg L^{-1} in fish tanks.

The nitrite levels were different. The mean lower oxygen level (5.55 mg L^{-1}) affected higher mean NO_2-N (1.82 mg L^{-1}) in unit I. Extreme high values were detected in both units during the beginning of the experiment (*Figure 5*). Results of Paudel (2020) where the Nitrogen transformation was examined in aquaponics, higher nitrite levels were observed during the first of the three parts of the experiment. Wongkiew et al. (2017) examined the effect of the oxygen levels on the nitrification and demonstrated that the nitrite oxidation was affected by low dissolved oxygen conditions. This is also supported by the changes in nitrite concentrations.

Figure 5. **Change of nitrite values (mg L^{-1}) as a function of oxygen**



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CONCLUSIONS

The dissolved oxygen quantity is very important in the aquaponics systems. It is essential not only for fish but also for the optimum status of other water quality parameters. Results in the present study pointed out that oxygen level has a direct impact on nitrification, fish production parameters, nutrient uptake by plants and, as a result leads to increase in plant and fish biomass. The results show that basil (*Ocimum basilicum*) production can be combined with common carp (*Cyprinus carpio*). It proved to be environmentally sustainable at lower dissolved oxygen levels. Furthermore, there is an alternative to bypass the air pump, which can provide more cost effective operation in aquaponics.

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