

Preliminary results of the combined production of duckweed *Spirodela polyrhiza* and common carp (*Cyprinus carpio*) in an aquaponic system

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SUMMARY

As a result of population growth, increasing amount of food is needed, and agriculture is under an increasing burden to meet these needs. Traditional agriculture is often attacked due to its environmental impact. We must find alternative, environmentally friendly ways to produce more food. Aquaponics is a closed system in which we can produce both fish and plants at the same time. Duckweed species are small, aquatic, floating plants belonging to angiosperms. It can potentially be an alternative protein source, due to its high protein content, good amino acid supply, and rapid growth. Under suitable conditions, it doubles its weight in 2–4 days, and can reach a yield of 30 t ha⁻¹ year⁻¹ in dry matter. It forms a carpet on top of the water and can be found in slow-moving or still waters. Since they are resistant to a wide range of nutrient concentrations, they are also suitable for cleaning wastewater (such as eutrophicated lakes, sewage reservoirs, liquid manure storage). Fish feed is the primary nutrient source for aquaponic systems, which usually contains fishmeal. If duckweed can be used as an alternative for fishmeal in the feed, it could improve the sustainability of the aquaponic and aquaculture systems. In this study, the aim was to develop an optimal harvesting protocol for duckweed *Spirodela polyrhiza* under aquaponic conditions. In a four weeks experiments, four harvesting protocols were set up, a control where only biomass measurements were made, a 25% group where 25% of the biomass at the time of measurement was harvested, and a 50% and a 75% group where at the time of measurement 50% and 75% of the biomass was harvested. Three replicates were used per treatment. We weighed the biomass every week and removed the amount corresponding to the group. Based on the preliminary results, it can be said that more biomass was obtained in the groups with the 25% harvesting protocol and the control group.

Keywords: aquaponics; duckweed; alternative protein source

INTRODUCTION

Traditional agriculture is often attacked due to its impact on the environment. We must find alternative, environmentally friendly ways to produce food (Breitenstein and Hicks, 2022). Aquaponics is a closed system (Yep and Zheng, 2019) in which we can produce both fish and plants at the same time. It takes on many different sizes and shapes, as producers often start aquaponic production as a hobby, so it can range from a very small, table-top size to several cubic meters of water and hundreds of square meters of plant growing area. The wastewater from the fish unit (aquaculture) is transferred to the biofilter where the bacteria breaks down the ammonia to nitrite and then nitrate, then the water goes to the plant production (hydroponics) unit, where the plants absorb inorganic substances from the water (e.g., ammonia, nitrite, nitrate, phosphate), and then this filtered water is returned to the fish. An aquaponic system can also be upgraded from existing aquaculture systems by attaching a hydroponic system. Many fish species can be reared in an aquaponic system, like *Oreochromis aureus*, *Oreochromis niloticus*, *Cyprinus carpio*, *Tinca tinca*, *Clarias gariepinus*, *Silurus glanis* (Calone et al., 2019; Birolo et al., 2020).

While there cannot be an aquaponic system without fish, plants also play an important role in the system by maintaining water quality. They remove dissolved nutrients from the system which they use for their growth, allowing the reuse of nutrients, at the same time significantly reducing the wastewater effluents and minimizing the water turnover rate which means a

minor environmental impact. Plants like *brassica campestris l. subsp. chinensis*, *ipomoea aquatica*, *lactuca sativa*, *mentha arvensis*, *ocimum asilicum*, *beta vulgaris var. bengalensis* can be grown in aquaponic systems. *capsicum annum*, *solanum lycopersicum*, *fragaria vesca*, *citrullus lanatus* (Li et al., 2018; Fernandez-Cabanás et al., 2022) can also be cultivated. Aquaponics is also suitable for saltwater or brackish water production, using halophytic plants (Spradlin & Saha, 2022). Some plants can even yield more in an aquaponic system compared to that grown traditionally. The causes of multiple yields are the continuous balanced water and the nutrient uptake of plants. (Csorvási et al., 2014). The continuous nutrient intake can be utilized by plants that are capable of rapid, continuous growth.

Duckweed (*Lemnaceae*) species are small, aquatic, floating plants belonging to angiosperms. They can potentially be an alternative protein source suitable for human consumption as well as animal feed, due to its high protein content, good amino acid supply, and rapid growth (Zhao et al., 2012; Bog et al., 2019). Under suitable conditions, it doubles its weight in 2–4 days, and can reach a yield of 10–30 t ha⁻¹ year⁻¹ (Stejskal et al., 2022; Leng et al., 1995) harvested two to three times a week, is about 55 t ha⁻¹, with a protein content of 30%. Duckweed biomass production can reach up to 106 t/ha/year dry weight according to Sun et al. (2015). It forms a carpet on top of the water, it can be found in slow-flowing or stagnant waters. Since they are resistant to a wide range of nutrient concentrations, they are also suitable for cleaning wastewater (such as

eutrophicated lakes, sewage reservoirs, liquid manure storage) (Utami et al., 2018).

Fish feed is the primary nutrient source for aquaponic systems, which usually contains fishmeal. Fishmeal made from pelagic fish used to be the major dietary protein source in compounded feed for many important farmed species, but it is available in a limited amount (Olsen and Hasan, 2012). Fishmeal is made from captured fish the fishmeal content of fish feed influences the sustainability of aquaculture production.

If we can use duckweed as an alternative for fishmeal in the fish feeds, it could improve the sustainability of the aquaponic and aquaculture systems. Duckweed can also be used as an energy plant, due to its fast growth, and because of certain treatments (such as the removal of sulfur), the starch fraction can be enriched in it, so it can be used to produce biofuel (Chen et al., 2022). Fish fed with feed containing higher levels of duckweed had higher carcass moisture and lower lipid content compared to the control diet (Noor et al., 2000). It has been scientifically demonstrated that the use of duckweed in moderate amounts or as a partial replacement of other protein feed materials, including soybean meal, has a beneficial effect on the productivity, fattening, and slaughter performance of livestock and poultry as well as on the quality of their meat and eggs (Hu et al., 2022).

Fasakin (et al., 2001) used solar dried duckweed as a dietary protein component for tilapia (*Oreochromis niloticus* L.) and found that there were no significant differences in growth performance and nutrient utilization of fish fed with diets containing up to 20% duckweed inclusion compared to the control. However, increases in dietary duckweed inclusion resulted in progressively reduced growth performance and nutrient utilization of fish. Diet without fish meal (100% duckweed) gave the poorest result. The most cost-effective diet in terms of cost per unit gain in weight of fish was obtained with 30% duckweed dietary inclusion. The result showed that solar dried up to 30% duckweed dietary inclusion as a replacement for fishmeal in practical diets supported fish growth and was cost-effective. Contrary to this Irabor et al. (2022) examined the effect of duckweed (*Lemna minor*) meal inclusion levels (0%, 20%, 40%, 60% and 80%) on the growth, blood, and serum profiles of African catfish (*C. gariepinus*) juvenile cultured for 56 days under well managed condition. Proximate analysis on the test ingredient revealed a significantly ($P \leq 0.05$) high crude protein content in the test ingredient. At the end of the experiment, data evaluated revealed that at 40% duckweed meal inclusion level significant ($P \leq 0.05$) increase in growth was observed in *C. gariepinus* juvenile, while as inclusion level increased (60% and above), decline in the growth rate was observed. The test diets had no negative impact on the blood and serum profile of the sampled fish because all parameters observed were within the normal range. The water quality parameters examined revealed no adverse effect of the test diets on the water quality. Conclusively, 40% inclusion level of duckweed meal in

the diet of *C. gariepinus* juvenile is best for optimum growth performance without any adverse effect.

According to Mustofa et al. (2022) fishmeal can be replaced with up to 35% fermented duckweed meal without adversely affecting the growth, survival, or physiological parameters of the juvenile barramundi. Decreased plasma ALP observed in the fish fed the fermented duckweed meal diets have been attributed to the healthier state of the plasma membranes of these fish compared to those in the control group. Therefore, duckweed besides acting as an efficient biological filter in IRAS can be recycled to reduce dependency on fishmeal-based diets. Duckweed meal can replace fishmeal up to 50% ~ without affecting growth and nutrient utilization significantly in the feed of grass carp (*Ctenopharyngodon Idella*), however beyond this growth and stored protein reduces as the level of inclusion increases (Srirangam, 2016).

A protein content ranging from 15 to 45% dry weight can be achieved with duckweed depending on specific species and strain within species and on growing conditions (Cheng and Stomp, 2009). The Crude fiber content ranges between 8.8–29.7% (dry matter basis) (Xu et al., 2022)

The choice of fish species in an aquaponic system depends on many factors like, value and environmental tolerance. The common carp (*Cyprinus carpio*) belongs to the order Cypriniformes and the family Cyprinidae, which is considered the largest family of freshwater fish. The common carp is an important fish species satisfying ornamental, food and recreational fisheries' needs worldwide, but in common with other cyprinid fishes, it is particularly renowned for its environmental tolerance. Its hardiness, fast growth, easy propagation, omnivorous feeding habit, ability to readily accept supplementary feed, resistance to disease and tolerance of a wide range of climatic conditions have made common carp a popular species. It generally inhabits freshwater environments, especially ponds, lakes, and rivers, and also rarely inhabits brackish-water environments. Common carp originated in Eastern Europe and Central Asia. Farming of common carp started in European countries and China many centuries ago and has been eventually introduced to other parts of the world. The majority of the EU's common carp production takes place in eastern Europe. (Satoh, 2017; Rahman, 2014; Jeney and Jian, 2009; Williams et al., 2008).

The aim of recent study was to examine the effect of different harvesting protocols on the growth of duckweed (*Spirodela polyrhiza*) in an aquaponic setting with the use of common carp. The impact of the harvesting protocols on the fish in the aquaponic system (*Cyprinus carpio*) was investigated. At the end of the experiment, the protein content of the duckweed biomass was determined.

MATERIALS AND METHODS

12 small-scale aquaponic systems were used with 200 l of water each (150 l fish tank, 50 l plant tank), in a greenhouse at the Aquaculture Laboratory of the

University of Debrecen during the beginning of autumn. A system had two units: one tank for the fish, and another for duckweed, the systems included a pump (800 l h⁻¹ a heater (200 W in each system), a filter sponge (20x20x5) and air stones connected to an air pump the greenhouse used only natural light. Six Common carp individuals were stocked in each system, each system received 527±2 grams of fish biomass (average weight: 88 grams individually) and 60 grams of duckweed. The oxygen concentration (7.98 mg l⁻¹ ±0.2 mg l⁻¹), and water temperature (21.79 °C±0.4 °C) was measured each day at the same time with Hach HQ30d portable meter, weekly we measured the nitrogen forms of the system (ammonia, nitrite, nitrate) with HACH Lange DR/3900 spectrophotometer.

The harvesting and measuring took place every week at the same time, we used fishnets to collect the duckweed, let the water run down for 2 minutes, and weighed it on a scale. Four harvesting protocols were used as treatments, a control where only biomass measurements were made, a 25% group where 25% of the biomass at the time of measurement was harvested, and a 50% and a 75% group where at the time of measurement 50 and 75% of the biomass was harvested. Three replicates were used per treatment. The duration of the experiment was 3 weeks (21 days).

The fish received 5% of their bodyweight of commercially available dry feed (30% protein, 7% fat – Aller Aqua, Danish Kingdom, Christiansfeld) each day. The fish received their daily feed in two parts, so we could ensure they consumed all the feed. To determine the nutritional value of duckweed, samples were sent for proximate composition analysis to the Agricultural Laboratory Center of the University of Debrecen. The following methods were used for the examinations: preparation MSZ EN ISO 6498:2012, drying, measuring MSZ ISO 6496:2001, crude protein: (Kjeldahl method) MSZ EN ISO 5983-2:2009, crude fat: MSZ EN ISO 11085:2015, crude fiber: MSZ EN ISO 6865:2001, ash: MSZ ISO 5984:1992.

For the total yield we used one-way ANOVA and for the weekly measurements we used two-way ANOVA where the two factors were time and treatment. For the statistical analysis we used one- and two-way analysis of variances with IBM SPSS Statistics and Microsoft Excel programs. For the measurement of duckweed yield (that were taken out of the tanks) we used one-way ANOVA. To measure the fresh duckweed that was left in the systems every week after the measurements, we used two-way ANOVA. Regarding the carp we measured the Survival rate, Weight Gain (WG), Specific Growth Rate (SGR) and the Feed Conversion Ratio (FCR) of the fish were calculated with the following formulas:

$$\text{Survival rate (\%)} = 100 \times (\text{nf}/\text{ni}) \quad (1)$$

$$\text{WG} = \text{Wf} - \text{Wi} \quad (2)$$

$$\text{SGR (\%/day)} = 100 \times (\text{lnWf} - \text{lnWi})/\text{days of experiment} \quad (3)$$

$$\text{FCR} = \text{WF (g)}/\text{WG (g)} \quad (4)$$

nf: Final number of individuals

ni: Initial number of individuals

WF: Weight of Feed

WG: Weight of Growth

lnWf: logarithmic value of the final weight

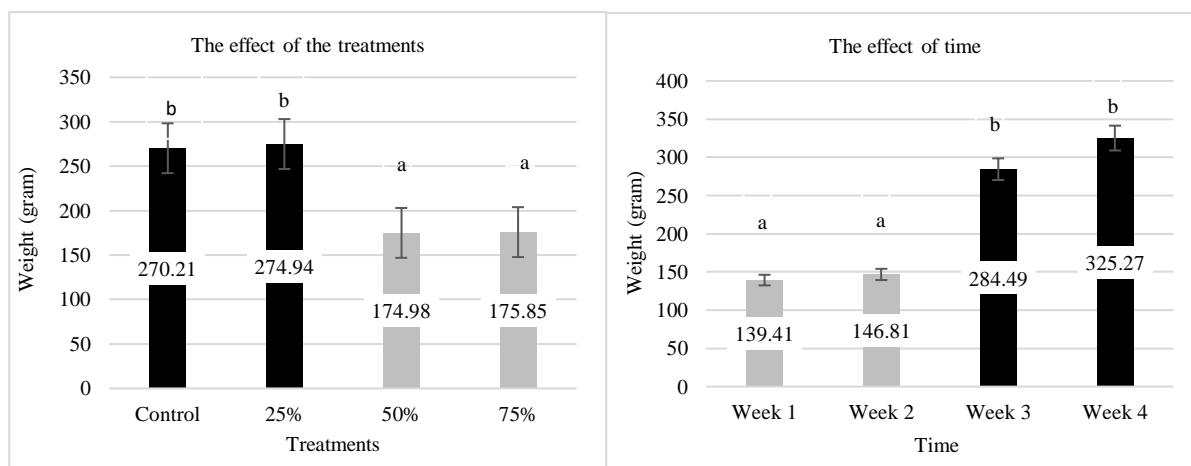
lnWi: logarithmic value of the initial weight

RESULTS AND DISCUSSION

The two factors were time and treatments (harvesting protocol). The two-way ANOVA showed (Figure 1), that the treatments had a significant effect on duckweed's biomass production. Between the control and 25% harvest groups no significant difference was found, but these groups produced more biomass at the end of the experiment, than the 50% and 75% groups. The 50% and 75% groups had no difference between them and fell behind in the biomass production. Time also has a significant effect on growth because duckweed can double its mass in 2-4 days' time (Stejskal et al., 2022). The 2nd weeks results fell behind expectations, as the 75% harvesting group removed a significant amount of biomass from the systems. The effect of the treatments can be explained, with the number of duckweed individuals that remained in the systems. By harvesting less biomass, more individuals are left in each system these can replenish their numbers faster. More individuals can make more offspring.

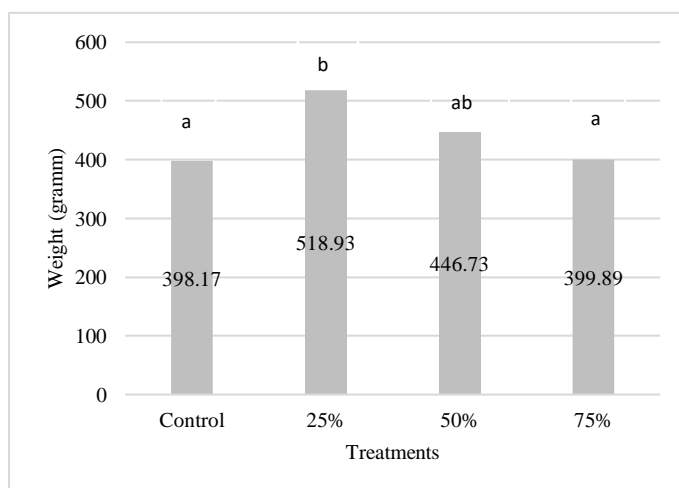
The overall biomass was calculated by adding the last weeks measurements and the previous weeks' harvests together. Evaluating the overall biomass production (Figure 2), the 25% treatment showed to be the most productive (with a biomass production of 518.93 g), the control (398.17 g) and 75% (399.89 g) treatments showed to be the least productive, the 50% (446.73 g) treatment was between them. Xu and Shen (2011) showed that harvesting less duckweed biomass at a shorter time interval resulted in a higher biomass density. So, after reaching complete coverage duckweed cannot produce as much biomass as the harvested groups. The difference between the groups is the harvested amount during the tests. The 75% group could not recover properly during the entirety of the study, hence the difference. The weekly harvesting regime produced more biomass in those groups which harvested less biomass percentage at a time. The control group and the 75% group can be similar in production, because the surface area – or production area, where duckweed can grow – could define the production capacity regarding duckweed.

Figure 1: The effect of treatments and time on duckweed biomass production



p<0.05

Figure 2: The overall biomass production of duckweed



p<0.05

Table 1 shows the data of the duckweed harvests. The ‘Left in’ column represents what was left in the tanks after harvests. The ‘harvest’ column represents what was taken out each week, and the ‘sum’ column is the sum of the previous two. In Week 1 the experiment started, there were no harvests so the

amount that was put in is equal to the sum. The ‘sum’ is equal of the three repetitions of each treatment. On Week 2 the growth and the harvests can be seen and the total biomass growth. On Week 3 the same can be said, except for the 75% group which wasn’t harvested.

Table 1: Data of the duckweed harvests (data shown in grams)

Treatments	Week 1 (Start)			Week 2			Week 3			Week 4 (End)
	Left in	harvest	sum	Left in	harvest	sum	Left in	harvest	sum	sum
control	180	0	180	376	0	376	1138.4	0	1138.4	1194.5
25%	180	0	180	281.3	93.9	375.2	970.2	323.5	1293.7	1139.4
50%	180	0	180	236	236	472	396.5	397.2	793.7	706.9
75%	180	0	180	112.32	337.28	449.6	533.2	0	533.2	862.4

The nutritional value of the duckweed (Table 2) based on dry matter were the following: crude protein content is 34.47%, crude fat 0.17%, crude fiber 13.83%, ash 22.13%. This protein content is high, but

it can be much higher as Hu et al. (2022) under artificial conditions determined protein content of a high protein strain duckweed in 50.89% of dry matter.



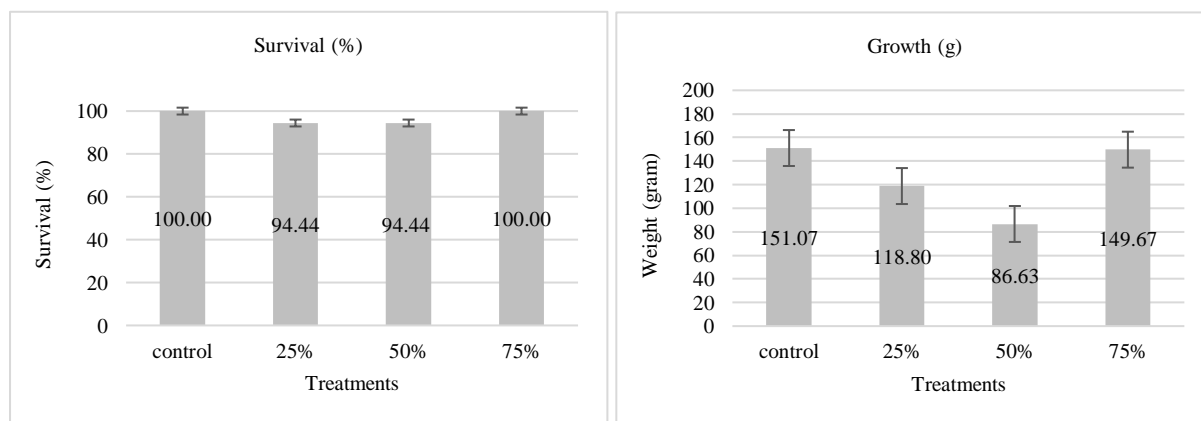
Table 2: The nutritional value of duckweed

Water content	96%
Composition	% in Dry Matter
Crude protein	34.47
Crude fat	0.17
Crude fiber	13.83
Crude ash	22.13

Regarding the production parameters of fish (Figure 3), the survival rate and growth, showed no significant differences between the treatments. This means that it is possible to harvest any amount of duckweed biomass, the removal does not affect the fish production in any way.

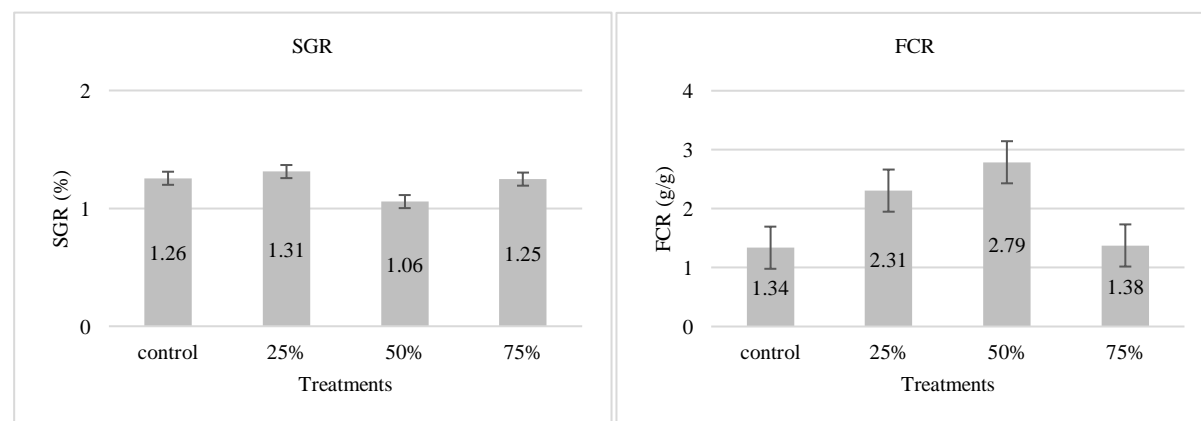
The FCR and SGR (Figure 4) did not show any difference between the different groups. The harvesting protocols weren't enough environmental change, to affect the fish production.

Figure 3: The survival rate and growth parameters of *Cyprinus carpio*



p<0.05

Figure 4: The SGR and FCR parameters of *Cyprinus carpio*



p<0.05

CONCLUSIONS

This study aimed to determine the best harvesting protocol for the duckweed species *Spirodela polyrhiza*. In terms of weekly biomass growth, the control and 25% treatments proved to be the best which means that while the duckweed is growing, we can harvest 25% of the overall biomass weekly and because of the regular harvests we can produce more overall biomass. By harvesting less biomass, more individuals are left in each system these can replenish their numbers faster. More individuals can reproduce at a faster rate.

The control group and the 75% group can be similar in production, because the surface area – or production area, where duckweed can grow – defines the production capacity regarding duckweed. So, after reaching complete coverage duckweed cannot produce as much biomass as the harvested groups. The difference between the groups is the harvested amount during the tests. The 75% group could not recover properly during the entirety of the study, hence the difference and that is the reason why we didn't harvest it during week 3.

The Common carp individuals were not affected by the harvesting protocol, this means that regardless



which protocols we use on-site, it will not have any effect on the fish growing parameters. By harvesting 25% of the biomass, we can produce more overall duckweed in the long-term. This study provides a way to further increase the production of those, who benefit from the use of duckweed.

In this study the protein content of duckweed was measured 34.47 % in dry matter. Though according to Hu et al. (2022) under artificial conditions it can reach 50.89% of dry weight. The measurements of the nitrogen forms didn't show any difference between the treatments, so this couldn't have any effect on the growth of the fish.

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