



Evaluation of plant-fish ratio in an aquaponic system with different stocking densities of *Cyprinus carpio* and *Carassius auratus*

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Abstract. Aquaponic systems (AS) produce clean food, generate higher yields, consume few resources and are friendly to the environment. In aquaponics, it is important to define the relationship between the stocking density of fish and plants to maintain an appropriate balance among food supply, nutrient production and nutrient assimilation. Therefore, this study aimed to evaluate plant-fish ratio in an aquaponic system with different stocking densities of *Cyprinus carpio* and *Carassius auratus*. Three stocking densities were evaluated: Exp 1 with 1.23 ± 0.027 kg, Exp 2 with 2.79 ± 0.022 kg and Exp 3 with 5.23 ± 0.025 kg, each treatment with two repetitions. Each treatment had a planting density of 25 plants m^{-2} . During the experiments, water quality parameters were registered, total ammonia, nitrogen, nitrites, nitrates, calcium, phosphate, iron, general hardness, carbonate hardness, dissolved oxygen, pH and temperature. Plant and fish growth were recorded monthly and productive parameters were estimated. Survival was good in the three experiments, obtaining values higher than 90% for *Origanum vulgare* and 95% for fish. The growth, yield and harvest time of *O. vulgare* improved remarkably by increasing the fish biomass due to an increase and availability of nutrients. These results demonstrate that AS with the highest stocking densities increased the performance of *O. vulgare*.

Key Words: aromatic plants, eco-friendly product, nutrient cycle, ornamental fish, water quality.

Introduction. *Origanum vulgare* (Linnaeus, 1753) is a species that has a wide composition of chemical substances used in medicine as phytoconstituents, improving the efficacy of certain drugs (Pezzani et al 2017). In gastronomy, its organoleptic characteristics make it possible to enhance the flavor of food, and in some cases, it is used as a natural preservative (Granados-Sánchez et al 2013). Worldwide, by 2019 the main exporter of *O. vulgare* was India with 381.77 tons, followed by China with 295.07 tons, Germany with 211.51 tons, United States with 128.26 tons and Egypt with 117.17 tons, whilst the main importers were the United States, Germany, Japan, South Korea and France with values between 389.82 and 101.52 tons (<https://www.tridge.com>). However, its cultivation is mainly conventional, generating negative impacts on the environment (Cogollo-Ospina & Durán Palacio 2021). This problem makes necessary the implementations of alternative crops, such as aquaponics.

Aquaponic systems (AS) are the combination of a recirculating aquaculture system (RAS) for production of aquatic organisms (usually fish) and a hydroponic system for production of plants. In the latter, organic waste is produced by fish, by the excretion of nitrogenous products and ions and the food not consumed, added to the nitrification processes carried out by bacteria. These generate nutrients in the system that serve as a source of nutrients for the growth and production of plants, which act in turn as a biological filter (Goddek et al 2019). This association has the following advantages: clean products, better control and management of diseases, decrease in the use of fertilizers,

ease in local food production, higher density in the production of plants and fish, efficient use of space and water, among others (Hammond Wagner et al 2020).

However, there are not many studies about the optimal fish-plant ratio, which can lead to problems in the maintenance of organisms. For example, an excess in fish biomass may not be maintained by the biofiltration capacity, generating an accumulation of total ammonia nitrogen (TAN) or release of nutrients that can pollute the environment. In contrast, if there is a higher density of plants in relation to the stocking density of fish, an appropriate amount of nutrients will not be produced, presenting deficiencies in the plants. In AS with *Oreochromis niloticus* and *Lactuca sativa*, it has been reported that 60-100 g of food per day are necessary to supplement the nutritional requirements of 25 plants per meter (Rakocy 2012). In an AS with *Clarias gariepinus* and *L. sativa*, 15-42 g of fish feed per m² of plant growing area are needed (Endut et al 2010). According to Somerville et al (2014), 40-50 g of food day⁻¹ for foliage plants and 50-80 g of food day⁻¹ for fruit plants are needed.

In the present work, a polyculture of oregano (*O. vulgare*) was established, with two species: goldfish (*Carassius auratus*) and carp (*Cyprinus carpio*) in AS, obtaining a multipurpose system with ornamental fish for sale and fish for consumption, which would improve the local economy and food security by implementing this technology. For this reason, the objective of this study was to evaluate plant-fish ratio in an aquaponic system with different stocking density of *C. carpio* and *C. auratus*.

Material and Method

Description of the study sites. This work was carried out in the Animal Physiology Laboratory and in the Ichthyology Greenhouse at the Campus of the Nueva Granada Military University, Cajica, Colombia (04°56'33.18" N; 74°00'46.28" W). Experiments 1 and 2 lasted 90 days, while experiment 3 lasted 60 days. The location of the experiments was at 2558 m above sea level, with an average temperature of 14°C (<https://en.climate-data.org/>).

Design of aquaponic systems. Two AS per experiment were installed, consisting of a fish tank with a 1000 L capacity (polyethylene; Colempaques), a submersible water pump of 6000 L h⁻¹ (King 6F: Resun®), a clarifier of 200 L (polyethylene; Distrienvases) to remove suspended solids with a size between 100 µm - 1.5 mm (Timmons & Ebeling 2010), a 40 L mechanical filter (polyethylene; Colempaques) with a polyethylene mesh of 2 mm, a 1000 L capacity submerged biofilter (polyethylene; Colempaques), with 200 L of inert gravel substrate for the adhesion of nitrifying bacteria. The hydroponic system was a raft bed type for a total crop area of 3 m² and a volume of 1200 L (polyethylene; Bestway), where floating polystyrene boards were placed to support the plants (Figure 1). In addition, the aeration in the two systems was supplied by a blower (GF-370; Resun®). Each system had 14 aeration lines with air stones (25x50 mm; Air Stone Cylinder; Pawfly), distributed as follows: six lines for the hydroponic system, four for the fish tank and four for the biofilter (Figure 1).

Biological material. The acquisition of *C. auratus* and *C. carpio* fingerlings was carried out from authorized distribution centers. The initial weight of *C. auratus* and *C. carpio* are presented in Table 3. The different initial weight values are due to the fact that the ornamental and consumer fish markets commercialize different sizes. Therefore, it was necessary to implement 40 L (polyethylene) cylindrical floating cages to avoid predation. When *C. carpio* reached approximately 4.5 cm in length, they were stocked into the fish tank. For the evaluation of the different stocking densities, three experiments (Exp), each with two repetitions were conducted. In each AS, the same number of fish was maintained: 330 fingerlings of *C. auratus* and 500 of *C. carpio*, with variations only in the initial size of the organisms and total biomass. The stocking density values assessed were the following: Exp 1: 1.23±0.02 kg m⁻², Exp 2: 2.79±0.02 kg m⁻² and Exp 3: 5.23±0.02 kg m⁻². The seedlings of *O. vulgare* propagated by seed were acquired from the Bio-systems Center of the Jorge Tadeo Lozano University. When plants presented more than

four true leaves, they were transplanted into the AS, at a density of 25 plants m⁻², for a total of 75 plants per AS.

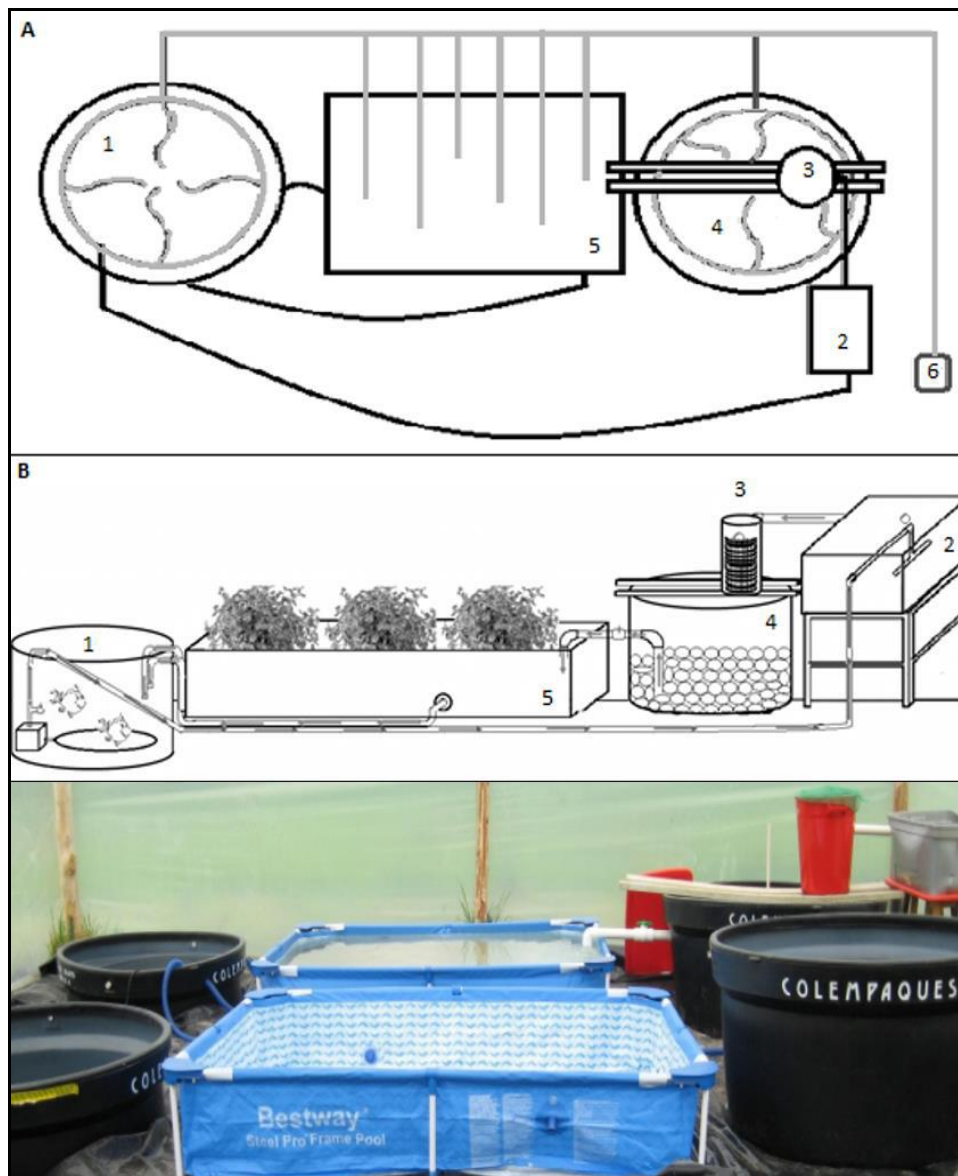


Figure 1. Design of aquaponic systems (AS). A: top view of the AS with the aeration system. B - general diagram of the AS, with 1 - fish tank, 2 - clarifier, 3 - mechanical filter, 4 - biofilter, 5 - plant bed, 6 - air turbine. C - photo of the systems.

Water and nutrient quality parameters. Chemical maturation was used in Exp 1 and 2. In this modified technique, 40 mg L⁻¹ of solid sea salt, 40 mg L⁻¹ ammonium nitrate, 40 mg L⁻¹ di-basic sodium phosphate and 250 mg L⁻¹ of calcium carbonate were added (Neissi et al 2021).

The water quality parameters were measured twice a week: total ammonia nitrogen (TAN), nitrite (NO₂⁻), nitrate (NO₃⁻), iron (Fe²⁺), phosphate (PO₄²⁻) and calcium (Ca²⁺) were determined with a photometer Spectroquant Multy® (Merk®) and High Sensitivity Kits (Merk®). The pH and dissolved oxygen (DO) were determined with Exttech DO700 multi-probe equipment. Temperature was determined with a maximum and minimum thermometer (Alla France, Reference 75000- 001-bl), and general hardness (GH) and carbonate hardness (CH) with Tetra® kits. Once the water quality parameters were in the appropriate ranges: pH 6.5-7, TAN<1.0 mg L⁻¹, NO₂⁻<0.3 mg L⁻¹

and $\text{NO}_3^- > 10 \text{ mg L}^{-1}$, fingerlings of *C. auratus* and *C. carpio* were stocked (Chen et al 1990; Fivelstad 1988; Merino et al 2007).

Water quality corrections. After monitoring the possible plant nutritional deficiencies and the data obtained, weekly amendments of iron chelate (DTPA-Fe), potassium nitrate and calcium nitrate were carried out. When the pH was between 7.5-8, 25% sulfuric acid or 37% nitric acid was used, and when it was between 5-6.5, potassium hydroxide, magnesium carbonate or calcium carbonate were added.

Fish growth measurements. Weight and standard length (SL) were measured for 10% of the AS fish population monthly, with a weight scale (Vibra, Model DJ 820E, sensitivity of 0.01 g) and a calliper. From the data in each experiment, the productive parameters were obtained: weight gain (WG) (Alatorre-Jacome et al 2012), specific growth rate (SGR) (Alatorre-Jacome et al 2012) and survival (S) (Aguilar et al 2010). In addition, an adjustment of the feeding rate was made: the fish were fed three times a day at 8:00, 12:00 and 17:00 h with a commercial feed with 45% crude protein (CP) (Ruales & Vásquez-Torres 2014; Bijoy et al 2018).

Plant growth measurements. *O. vulgare* were planted into the hydroponics when NO_3^- concentration reached 25 mg L^{-1} . At the beginning and end of the culture, fresh and dry weight data were taken from the aerial part using a scale (Vibra, Model DJ 820E, sensitivity of 0.01 g). The difference between fresh weight and dry weight was used to calculate the water content of the plants. The length of the stems of 20 randomly selected plants was recorded monthly. For harvest, it was taken into account that the percentage of export-type stems (stems > 8 cm) was greater than 70% (Pedraza Luengas et al 2016). From these data, the productive parameters were estimated: relative growth rate (RGR), absolute growth rate (AGR) and survival (S) (Pal et al 2018; Debnath et al 2022). The amount of nitrogen (N) and the proportion of NH_4^+ , -N and $\text{NH}_3\text{-N}$ were established in relation to the data of NO_3^- , TAN, temperature and pH (Colt & Armstrong 1981; Fivelstad 1988; Merino et al 2007).

Results. During the development of the experiments, the levels of nitrogenous compounds (TAN, NO_2^- and NO_3^-) were appropriate and between the tolerance levels for *C. carpio* and *C. auratus*. Also, TAN and NO_2^- behaved similarly in all three experiments. With respect to NO_3^- , Exp 1 and 2 started with levels of 27.5 and 74.5 mg L^{-1} , respectively, compared to Exp 3 with 247.50 mg L^{-1} . The water quality parameters are presented in Table 1.

Table 1

Water quality variables in the aquaponics system

Parameter	Exp 1		Exp 2		Exp 3		Tolerance
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	
TAN ¹ (mg L ⁻¹)	0	1.1	0.01	1.48	0	1.86	<2
Nitrite ¹ (mg L ⁻¹)	0	0.8	0.04	0.68	0.01	0.71	<1
Nitrate ¹ (mg L ⁻¹)	10	180	40	160	18.5	250	0-400
Calcium ^{1,2} (mg L ⁻¹)	4	91	4	93	7	120	4-400
Phosphate ¹ (mg L ⁻¹)	0	50	0	125	2	125	0.02-180
Iron ^{1,2} (mg L ⁻¹)	0	0.36	0	0.2	0	0.36	0.1-5
GH ¹ (mg L ⁻¹)	35	262.5	35	297.5	52.5	297.5	>100
KH ¹ (mg L ⁻¹)	0	105	0	52.5	0	52.5	50-300
pH ¹	6.65	7.4	6.01	7.7	6.34	7.91	6.5-7.2

Note: TAN - total ammonia nitrogen; CH - carbonate hardness; GH - general hardness; 1 - Timmons & Ebeling (2010); 2 - Nelson (2008).

An increase in the availability of nutrients was observed as the fish biomass grew. This was evidenced in the amount of nitrogen available in the form of TAN and NO_3^- for each of the experiments, with values between 0.23-0.4 $\text{mg day}^{-1} \text{ plant}^{-1}$. The growth of *O.*

vulgare was higher in Exp 3, where a biomass of $5.23 \pm 0.025 \text{ kg m}^{-2}$ was maintained (Table 2).

Table 2

Yield of *Origanum vulgare*

Exp	Initial weight (g ind ⁻¹)	N mg L ⁻¹	N plant day ⁻¹ (mg)	Stem length (cm)	Final Average Weight (g ind ⁻¹)	Yield (kg m ⁻²)
1	1.83±1.07	17	0.23	6-8.5	4.07±3.29	0.1
2	1.82±0.65	22	0.28	6.92-9.53	22.58±13.29	0.56
3	5.5±1.35	30	0.40	7.76-9.8	37.58±29.27	0.93

Note: Exp - experiment; Ind - individual; N - nitrogen calculated from TAN + NO₃⁻.

The harvest time of *O. vulgare* was considerably reduced in Exp 3, 60 days, while in the other two crops the harvest criteria (70% of the stems with export quality) were reached in 90 days (Figure 2).

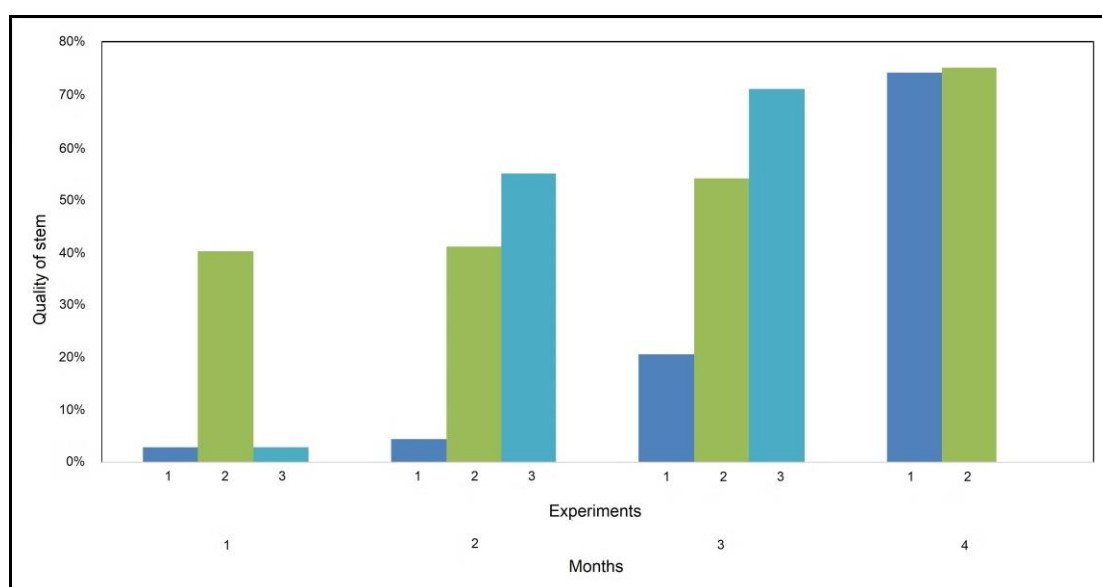


Figure 2. Yield quality of *Origanum vulgare*; data represents the stems with length higher than 8 cm (export quality).

The WG in terms of biomass for *C. carpio* was: Exp 1 - 0.83 kg; Exp 2 - 1.52 kg; Exp 3 - 3.19 kg. The RGR showed a decreasing trend in Exp 1, 2 and 3, with values between 3.89-1.44 g day⁻¹ (Table 3). WG of *C. auratus* varied between the experiments, with a higher value of 1.65 kg in Exp 1 and the lowest value in Exp 2, with 0.73 kg. In the 3 experiments, the two species presented survival percentages higher than 95% (Table 3).

Table 3

Yield parameters of *Cyprinus carpio* and *Carassius auratus*

Species	Exp	Initial SL (cm)	Final SL (cm)	WG (g ind ⁻¹)	RGR (g day ⁻¹)	SGR (%)	S (%)
<i>Cyprinus carpio</i>	1	2.44±0.22	3.79±1.02	1.71	3.89	1.76	98±1.73
	2	3.42±0.47	5.13±1.23	3.09	2.22	1.30	99.2±0.32
	3	4.85±1.20	6.35±1.28	6.64	1.44	1.49	97.8±1.56
<i>Carassius auratus</i>	1	5.34±1.26	6.14±0.98	5.52	0.95	0.74	95.4±2.54
	2	5.16±1.36	5.74±1.23	2.36	0.37	0.35	98.4±1.02
	3	5.8±1.21	6.02±1.49	4.72	0.53	0.71	97.2±1.15

Note: each value represents the mean±SD; SL - standard length; WG - weight gain; RGR - relative growth rate; SGR - specific growth rate; S - survival rate; ind - individual.

Regarding the yield parameters (WG, RGR and AGR) of *O. vulgare*, it was also observed that when animal biomass increases it improves the performance of these growth variables in plants. Regarding survival, high values above 90% were obtained for the three experiments (Table 4).

Table 4

Yield parameters of *Origanum vulgare* from the aerial part

Exp	Days	Final average biomass (kg)	WG (kg AS ⁻¹)	RGR (g g ⁻¹ day ⁻¹)	AGR (g day ⁻¹)	S (%)
1	90	0.28±0.003	0.14	0.009	0.02	93.33±4.62
2	90	1.53±0.018	1.39	0.028	0.23	90.67±3.12
3	60	2.7±0.025	2.29	0.032	0.53	96±2.48

Note: each value represents the mean±SD of the repetitions; Exp - experiment; WG - weight gain; RGR - relative growth rate; AGR - absolute growth rate; S - survival rate; ind - individual; AS - aquaponic system.

Discussion. The yield of *O. vulgare* in Exp 3 had good values, with 0.93 kg m⁻², higher than in the other two treatments. However, it was surpassed by the results of Ramírez-Sánchez (2016) in AS, with 1.23 kg m⁻². In Exp 1, the production was 0.1 kg m⁻², higher than that obtained by Kosakowska et al (2019) in open field conditions with a yield of 0.03 kg m⁻² and in tunnel with 0.06 kg m⁻². However, the density was less than 6.4 plants m⁻², lower than in the present study. Additionally, *O. vulgare* used were one year old and may have lost plant vigor (Kosakowska et al 2019). For Exp 2, the production was 0.56 kg m⁻², greater than the 0.25 kg m⁻² obtained by Ramírez Sánchez et al (2016), who worked in AS with densities greater than 32 plants m⁻². Despite the higher density used by Ramírez Sánchez et al (2016), Exp 2 exhibited better performance, which can be attributed to the substrate used in the floating bed and the type of propagation used (cuttings). Ramírez Sánchez et al (2016) mention that the polystyrene prevented the adequate growth of the cuttings, generating fewer adventitious stems. Exp 2 presented higher values than those obtained by Murillo-Amador et al (2013), who had a density of 4.2 plants m⁻² in an open field crop with a yield of 0.11 kg m⁻², and in shade of 0.37 kg m⁻². The work of Ramírez-Sánchez (201) in AS showed a yield of 1.23 kg m⁻². They used plants propagated by seeds and a mesh as a floating bed, which increased the number and favored the development of adventitious stems. This work highlights the higher yields and densities that can be achieved in AS compared to traditional crops.

The growth of *O. vulgare* increased considerably with a higher fish biomass. Therefore, in Exp 3, the productivity was higher than in Exp 1 and 2, resulting in a harvest time reduced to 30 days for Exp 3, obtaining more than 70% of the stems with a height greater than 8 cm. The RGR in Exp 3 was similar to that obtained in AS by Ramírez Sánchez et al (2016), while the AGR in Exp 3 was higher. This behavior could be attributed to the low fish biomass used (5 g m⁻³ to 26 g m⁻²). Survival was high in all experiments, being better in Exp 3 and exceeding the values obtained by Ramírez Sánchez et al (2016) in AS, with 69.23%±10.5.

The growth in terms of fresh weight was affected by the variation in the water content of the plants, being 50% in Exp 1, 80% in Exp 2, and 90% in Exp 3, indicating a lower turgor, possibly caused by a potassium deficiency (K⁺) (Smolenski et al 1967). Savidov (2004) reported that Ca²⁺ is an antagonist of K⁺, so that its excess can induce a deficiency of the latter if it is not presented in a 2:1 ratio (Ca²⁺: K⁺). In Exp 1, there was a higher concentration of Ca²⁺ with respect to K⁺ (6:1), while for Exp 2 and 3, the concentration of these compounds remained in equilibrium (1.7:1) due to the addition of K⁺ periodically, allowing the adequate intake of these two cations. Not maintaining an adequate ratio of these two macronutrients causes a reduction in the stem elongation (Smolenski et al 1967). This could be evidenced in the production of export-type stems, since the time needed by plants in Exp 1 and 2 was 90 days, 30 days higher than that in Exp 3.

The pH and DO remained similar in the experiments, with values ranging between 6.01-8.1 and 5-6.4 mg L⁻¹, respectively. In AS, it is recommended to maintain the pH between 6.5-7, since the requirements of the three organisms are different (Rakocy

2012). Similarly, it has been reported that the adequate range for DO is 4-7 mg L⁻¹ (Bernstein 2011; Eck et al 2019).

The concentrations of the nitrogen compounds did not affect the adequate growth of the fish, since they remained within the appropriate levels (Rakocy 2012). This shows that the nitrification process was efficient, with low concentrations of TAN and NO₂⁻ and an increase in NO₃⁻ (Harris 1993; Timmons & Ebeling 2010). Even so, it was difficult to meet the requirements of *O. vulgare*. Nitrogen levels (N) of 90 mg L⁻¹ are recommended for soil crops (redtractor.org.uk). However, the total N in the form of TAN, NO₂⁻ and NO₃⁻ available in the experiments was lower, with values of 17, 22 and 30 mg L⁻¹. This can be explained by the fish biomass, with increased biomass increasing the amount of N available to plants. In AS, the phosphorus in fish food contains a large amount of phytates. These are excreted by the fish, making them available to the plants. Plant roots cannot take up these compounds, which can lead to P deficiencies for plants (Hien et al 2015; da Silva Cerozi & Fitzsimmons 2016).

The low N and P (PO₄²⁻) availability led *O. vulgare* to show symptoms of deficiency. Therefore, young leaves presented dark green coloration with the presence of anthocyanins and thin stems, while chlorosis in old leaves is specifically related to N deficiency (Patel et al 2020). Exp 3 reached 70% of export-type stems in a much shorter time (60 days), coinciding with a lower deficiency of N. In these AS, 87-164 g of food was used per day to produce *C. carpio*, *C. auratus*, *O. vulgare*. The noticeable difference in food supply is possibly because in terms of N, *O. vulgare* is more demanding than *L. sativa*, which is 37.6 mg L⁻¹ by than *L. sativa* (Ako & Baker 2009). Another important consideration is the CP level, which is generally low in AS. The works carried out by Rafiee & Saad (2005) used 24% CP, Endut et al (2010) used 32% PC, Sikawa & Yakupitiyage (2010) used 34% CP. In these studies, fish of 100-200 g were used, which require lower protein levels (Vásquez-Torres et al 2011). However, in this study, a 45% CP food was used for two reasons. The first is that fish were smaller in size than those commonly used in AS, so it was expected that they would have a higher protein requirement to meet their metabolic needs (Vásquez-Torres et al 2011). Riaño-Castillo (2015) demonstrated this fact when evaluating *C. auratus* of different sizes, since 2.7 g fish with a high CP level (45%) produced a greater amount of TAN, compared to 9 g fish under similar conditions. The second reason is that, as the protein level was higher, an increase in TAN excretion via protein catabolism was expected. This is also demonstrated by Riaño-Castillo (2015) when determining the effect of the CP level in the diet (30, 40 and 45%) on the excretion of TAN.

In Exp 3, a WG of 6.64 g was obtained with a density of 5.23 g L⁻¹, similar to that of Riaño-Castillo (2015) of 4.05-6.14 g with a density of 2.5 g L⁻¹. Regarding the RGR and SGR for *C. carpio*, it was found that small fish had higher growth rates compared to larger fish (Bijoy et al 2018).

For *C. auratus*, the WG per individual for Exp 1 was 5.52 g higher than that obtained by Martínez Moreno et al (2016) in three treatments (0.86 g, 1.2 g, 0.87 g), despite handling a lower density (0.38 g L⁻¹, 0.65 g L⁻¹, 1 g L⁻¹).

The fish survival was high for *C. carpio* with values between 97.8-99.2%, while for *C. auratus* it was between 95.4-98.4%. The values are better than those obtained in other works, 90% for *C. carpio* obtained by Ramírez-Sánchez (2016), from 69.23 to 92.31% obtained by Ramírez Sánchez et al (2016) and 93.5% obtained by Carrascal et al (2016). Martínez Moreno et al (2016) obtained a survival rate for *C. auratus* from 90 to 92.8%. This could be due to the fact that the water quality parameters remained within the tolerance ranges reported for these species.

Conclusions. For Exp 1 and 2, the relationship between the stocking density of fish and the growth of *O. vulgare* was not adequate, since the plants presented several deficiencies and the growth was lower compared to Exp 3, where the stocking biomass of the fish was higher, increasing the availability of nutrients for the plants, which performed better.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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