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Original Paper

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Farming largemouth bass (*Micropterus salmoides*) with lettuce (*Lactuca sativa*) and radicchio (*Cichorium intybus*) in aquaponics: effects of stocking density on fish growth and quality, and vegetable production

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The present study assessed the effects of two initial stocking densities (low – LD, 4.23 kg m⁻³, moderate – MD, 8.05 kg m⁻³) on growth, health and fillet quality of largemouth bass (*Micropterus salmoides*), and on yield of lettuce (*Lactuca sativa*) and radicchio (*Cichorium intybus* group Rubifolium) produced in a low-tech recirculating aquaponic system. A total of 104 largemouth bass (initial body weight: 236 ± 38 g) were randomly stocked in eight 500 L tanks (four per stocking density) and monitored during a 70-day period. Vegetables yield was similar in LD and MD groups. Lettuce yield (6.33 kg m⁻²) was in line with typical values, whereas radicchio showed a negligible yield (1.34 kg m⁻²). Likewise, fish final weight (263 g, on average), specific growth rate (0.17% d⁻¹), feed conversion ratio (2.72), and mortality (4.8%) did not differ between treatments. Fish morphometric indices, slaughter results and fillet quality were not affected by stocking density. In conclusion, the production of lettuce was successful in the tested system, whereas the production of radicchio did not achieve satisfactory results. Growth performances of the largemouth bass were poor and further investigations are required to optimize the rearing of this fish species in low-tech aquaponic systems.

Keywords: largemouth bass, lettuce, radicchio, water quality, flesh quality

1 Introduction

The 2030 Agenda for a Sustainable Development indicates food production as one of the world's greatest challenges (FAO, 2017). Climate changes and environmental pollution, coupled with limited water and arable land, impose to identify new alternatives for producing nutritious and economically viable food, concurrently with the reduction of waste, carbon and ecological footprints (Goddek et al., 2019).

Aquaponics, an innovative technology that combines recirculating aquaculture systems and soil-less culture (Lennard and Goddek, 2019), might represent a solution to 2030 Agenda challenges both for fish and vegetable production, limiting nutrient losses, water consumption and land use (Goddek et al., 2019). In aquaponics, the stocking density of fish can largely affect water quality (Maucieri et al., 2019), altering the levels of dissolved solids, nutrient concentrations and fish waste by-products (i.e.

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nitrogen compounds), and thus potentially altering the balance of the aquaponic system set-up. Moreover, stocking density is critical for the productivity and profitability of a fish farm (Wang et al., 2019). On the other hand, high stocking densities can impair fish growth, health and welfare (Yildiz et al., 2017).

Species diversification is crucial for a sustainable fish production (Chaves-Pozo et al., 2019). In aquaponics, the main cultured species are Nile tilapia (*Oreochromis niloticus*) (Diem et al., 2017), African catfish (*Clarias gariepinus*) (Baßmann et al., 2017) and koi carp (*Cyprinus carpio* var. koi) (Nuwansi et al., 2016) because of their high adaptability and tolerance to sub-optimal environmental conditions (Vitule et al., 2006; Guyon et al., 2012; Rahman, 2015). To date, studies assessing the suitability of other fish for aquaponics production are still limited. Largemouth bass (LMB; *Micropterus salmoides*, Lacépède, 1802) has been gradually introduced worldwide, both as game and aquaculture fish (Hussein et al., 2020). Global aquaculture production of LMB amounted to 458,000 tons in 2017, 99.8% from China (FAO, 2020). LMB farming showed a sharp increment (+88.1%) from 2012 to 2017 (Hussein et al., 2020), and it continues to expand. LMB is mainly farmed outdoor in raceway systems (Yuan et al., 2019) and high-density ponds (Sun et al., 2020), requiring 2 growing seasons (from 18 to 24 months) to reach the commercial size (approximately 0.5 kg live weight) (Watts et al., 2016). Due to its high growth rates, excellent flesh quality, and high tolerance to handling and water conditions (Hussein et al., 2020), LMB is a potential candidate for aquaponic systems. Nevertheless, to the best of our knowledge, no information to this regard is currently available.

Thus, our study aimed at evaluating the effect of rearing LMB at 2 stocking densities on fish growth performance, slaughter results, flesh quality traits, and lettuce (*Lactuca sativa*, L.) and radicchio (*Cichorium intybus*, L., group Rubifolium) yields in a low-tech recirculating aquaponic system.

2 Material and methods

2.1 Ethics statement

The study was approved by the Ethical Committee for Animal Experimentation (Organismo Preposto al Benessere degli Animali - OPBA) of the University of Padova (project no. 6/2017; prot. n. 15132). All animals were handled according to the principles stated by the EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

2.2 System and experimental conditions

The experimental system was located at the experimental farm of the University of Padova, North-East Italy (45°20'N, 11°57'E, 6 m a.s.l.), inside a plastic greenhouse. It consisted of 8 independent units (Figure 1), 4 of which containing fish at low initial stocking density (LD, 4.23 kg m⁻³) and 4 with fish at initial moderate stocking density (MD, 8.05 kg m⁻³).



Figure 1 Rendering of the aquaponic unit. A, main tank for fish (500 L); B, settling vessel (100 L); C, tanks for vegetables/biofilters (275 L); D, storage tank for water collection (50 L) and pumping to A tank

Each unit consisted of: A) a main tank (volume 500 L, height 0.80 m) in which the fish were kept; B) a settling vessel (100 L) to remove main suspended solids; C) 2 tanks for vegetables (volume 275 L each, height 0.35 m), filled with 225 L of expanded clay (LECA Laterlite, Solignano, Italy), which received the water from the main tank and acted both as hydroponic substrate for vegetable growth and biofilter; D) a water storage tank (volume 50 L, height 0.45 m) in which water was collected from

the tanks with vegetables before being pumped back into the tank with fish (Figure 1). Water flow was guaranteed by overflow (from the main tank to the tanks with vegetables, and from the latter ones to the storage tank), as the 3 parts presented water at different heights. A single pump (Newa Jet 1700, NEWA TecnoIndustria Srl, Loreggia, Italy) located in the storage tank returned the water to the main tank. The flow rate was 300 L h^{-1} corresponding to a complete recirculation of water every 2 h. Fish tanks were covered by a net to prevent fish from jumping and were provided with 2 porous stones connected to 2 aerators (Scubla D100, Scubla Srl, Remanzacco, Italy).

2.3 In vivo trial

The experiment lasted 70 days from September to November under natural photoperiod. Twenty days before the beginning of the trial, all experimental units were filled with water enriched with the same nutritive solution (141 mg L^{-1} of KH_2PO_4 , 517 mg L^{-1} of K_2SO_4 , 405 mg L^{-1} of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 31 mg L^{-1} of Fe-EDTA, and 11 mg L^{-1} of micronutrients). On the first day of trial, the main tanks were stocked with a total of 104 LMB of about 11 months of age. Fish were provided by a commercial farm (Azienda Agricola Vincenzi Marco, Modena, Italy) at average live weight of $236 \pm 38 \text{ g}$ and underwent to 2 initial stocking densities, 4.23 kg m^{-3} for LD (9 fish per tank) and 8.05 kg m^{-3} for MD (17 fish per tank). The LD value was chosen based on the minimum density capable of providing sufficient N for the growth of plants (Somerville et al., 2014), and the MD value was chosen in view of a maximum final biomass of fish (estimated to 1.5–2.0 times the initial weight) consistent with recommendations for organic aquaculture (Commission Regulation 889/2008). The fish were fed manually, initially twice a day (9 am and 4 pm) and then (after 21 days of trial) once a day (3 pm), with a commercial diet as floating extruded pellets (Aller Acqua, Pordenone, Italy; composition: 37% crude protein, 12% crude fat, 2.8% crude fibre, 7.0% ash, 0.1% sodium, 1.0% calcium, and 1.2% phosphorus, as-fed basis). Initially, the feeding rate was 1.0% of fish biomass. After one week, due to the scarce feeding activity, feed was distributed till apparent visual satiation. Considering the whole cycle, the feeding rate averaged 0.4% of the total biomass, ranging from 0.3% to 1.0%, according to fish feeding activity.

During the entire trial, lettuce and radicchio were cultivated simultaneously. At the beginning of the trial, 16 plants per experimental unit (8 plants of lettuce in one tank and 8 plants of radicchio in the other one; plant density of about 9 plants m^{-2}) were transplanted at the third true leaf stage. Neither pesticides nor antibiotics in water or feed were used during the trial.

2.4 Water quality

Throughout the trial, water lost by evapotranspiration from each unit was manually and daily refilled with tap water. Twice a week, water was monitored in the outflow of fish tanks for temperature, dissolved oxygen, pH, and electric conductivity using a portable multi-parameter apparatus (HQ40d Portable Multi-Parameter Meter, Hach Lange GmbH, Germany). The chlorophyll content was measured with a fluorescence detector (HHLDFluorescence-Chlorophyll, Turner Designs, USA). The water contents of NO_2^- , NO_3^- , PO_4^{3-} and NH_4^+ were determined by ion chromatography (Nicoletto et al., 2014).

2.5 In vivo recordings on fish

Fish health and feed intake were daily monitored. Fish were individually weighed at the beginning of the trial (0 days) and at the end of the rearing period (70 days). To this purpose, fish were harvested with a handling net, placed in a separate one and slightly anaesthetized with 10 mg L^{-1} of clove oil containing 87% eugenol. At initial weighing, fish were fasted for 48 h before and 24 h after the procedure. Before slaughtering, fish were fasted for 24 h. Daily growth rate, specific growth rate and feed conversion ratio were calculated according to Lugert et al. (2016).

2.6 Recordings at slaughtering of fish and harvest of vegetables

At slaughter, fish were anaesthetized as described above and then shot down by a percussion applied to the head of fish restrained manually using a plastic knob. All fish were weighed, individually tagged and stored in polystyrene boxes with ice in a cold room ($0\text{-}2^\circ\text{C}$). One day after slaughter, the fish were dissected, the viscera separated, the carcasses weighed and the viscerosomatic index calculated according to Ighwela et al. (2014). Moreover, a selection of 64 fish (8 per tank; 32 per experimental treatment), representative of their reference groups in terms of average body weight and variability, were measured for total length, standard length, head length and maximum height. The morphological indices (condition factor, cranial index, relative profile) were calculated according to Luxinger et al. (2018). The fish were also dissected to measure the hepatosomatic index (Ighwela et al., 2014) as well as the carcass and fillet yields. On the same fish, the muscle pH was recorded at three points on

the dorsal side of the right fillet with a pH meter (Basic 20; Crison Instruments SA, Carpi, Italy) equipped with a specific electrode (cat. # 5232; Crison Instruments SA). Thereafter, the skin was removed and L*, a* and b* colour indices of the flesh were measured at 3 points on the dorsal side of each fillet with a Minolta CM-508 C spectrophotometer (Minolta Corp., Ramsey, NJ, USA).

Regarding vegetables, at harvesting (63 days - end of the lettuce cycle; 80 days - end of the radicchio cycle), all plants were divided into aboveground and belowground parts, and the fresh weights of leaves and roots were immediately recorded to determine the marketable yield and the root yield.

2.7 Statistical analysis

The data of water characteristics, growth performance, slaughter results and flesh quality of fish, and vegetable yields were analysed through *t*-test statistics with the stocking density of fish as the main effect. The PROC TTEST of SAS software (SAS, 2013) was used for all analyses. Differences between means with *P* <0.05 were assumed to be statistically significant.

3 Results and discussion

Water losses due to evaporation, transpiration and fish splashing during the trial were not affected by fish stocking density (on average, 370 L). The amount of water daily added to refill the experimental units never overcome 2% of the total volume of the system. On average, 5.8 L of water per day, equal to 0.9% of the total volume of the system, were added (data not reported in tables). Water temperature changed with external environmental conditions, ranging from 16.1°C to 25.4°C (Figure 2a). Dissolved oxygen ranged from 7.5 ppm to 11.0 ppm (Figure 2b), and was significantly lower (*P* <0.001) in MD than in LD tanks from the 31st day onwards, likely due to a higher oxygen consumption related to the higher fish biomass (Maucieri et al., 2019). The water chlorophyll content remained stable during the experiment with no significant differences between treatments (Figure 2c). Water pH was never found below 8 and was higher in LD compared to MD tanks at 57 and 63 days (Figure 2d); this might be linked to the nitrification processes that have been accentuated with increasing stocking density and that resulted in a greater production of ammonia in the fish tanks (Yildiz et al., 2017). Water electrical conductivity ranged from 1,123 to 1,315 $\mu\text{S cm}^{-1}$ during the trial, without differences between LD and MD treatments.

The contents of NO_3^- (+18.6 mg L^{-1}), Cl^- (+2.4 mg L^{-1}) and PO_4^{3-} (+5.5 mg L^{-1}) were higher in MD than in LD tanks (*P* <0.05), whereas no differences were found for NH_4^+ (0.44 mg L^{-1} , on average) (Table 1). The ammonium contents, far below the critical threshold for LMB (Tidwell et al., 2000), demonstrate the efficiency of the biofilter activity in both LD and MD treatments (Tyson et al., 2004).

Table 1 Contents of ions measured in the water of fish tanks during the trial (70 days)

Item	Stocking density		<i>P</i> -value
	LD	MD	
Tanks (n)	4	4	
NO_3^- (mg L^{-1})	42.3 ± 4.4	60.9 ± 6.5	0.022
Cl^- (mg L^{-1})	19.8 ± 0.5	22.2 ± 0.4	0.001
PO_4^{3-} (mg L^{-1})	11.4 ± 0.3	16.9 ± 0.8	<0.001
NH_4^+ (mg L^{-1})	0.52 ± 0.15	0.36 ± 0.05	0.361

LD – low fish stocking density; MD - moderate fish stocking density. Data are expressed as means ± standard error.

Notwithstanding water characteristics remained within acceptable limits for LMB growth, health and welfare (Brown et al., 2009), throughout the trial, fish daily growth rate (0.41 g d^{-1} , on average), specific growth rate (0.17% d^{-1} , on average) and feed conversion ratio (2.73, on average) were rather unsatisfactory (Table 2). In studies that dealt with recirculating aquaculture system tanks, LMB juveniles (15 g initial weight) reached average specific growth rate and feed conversion ratio of 3.14% per d and 1.25, respectively (Li et al., 2018; Guo et al., 2019). According to Yuan et al. (2019), LMB (8 g initial weight) reared in ponds achieved a final weight of 539 g in 240 days, showing daily growth rate, specific growth rate and feed conversion ratio of 2.21 g d^{-1} , 1.75% per d and 0.95, respectively. The poor fish performance of the present study might be explained by the difficulties LMB experienced in adapting to the environmental conditions of the tested aquaponic system.

On the other hand, even if the diet used in the current study was the same offered (without problems) in the commercial farm where fish were purchased, it was little appetized and could have played a role in reducing fish growth performance (Junjie and Shengjie, 2018; Li et al., 2018; Guo et al., 2019).

During the trial, only 5 fish died (4 from MD group and 1 from LD group) without previous symptoms of disease. The stocking density did not affect fish mortality (4.8% on average), growth performance or feed efficiency. Thus, the fish biomass remained higher in MD treatment compared to LD one from the beginning to the end of the trial (Table 2).

Indeed, previous studies reported that largemouth bass can be farmed at largely different stocking densities and under a wide range of conditions, i.e. from 7 kg m⁻³ in indoor tanks (Guo et al., 2019) to 35 kg m⁻³ in in-pond raceway systems (Wang et al., 2019). Nevertheless, previous studies showed no effect of the stocking density on LMB performance (initial weight 36 g) farmed in in-pond raceway systems at two stocking densities (initial stocking density 2.45 vs. 4.10 kg m⁻³) (Wang et al., 2019) or in LMB (initial weight 112 g) reared in a recirculating aquaculture system and stocked at 2.24, 6.72 or 13.4 kg m⁻³ (Watts et al., 2016).

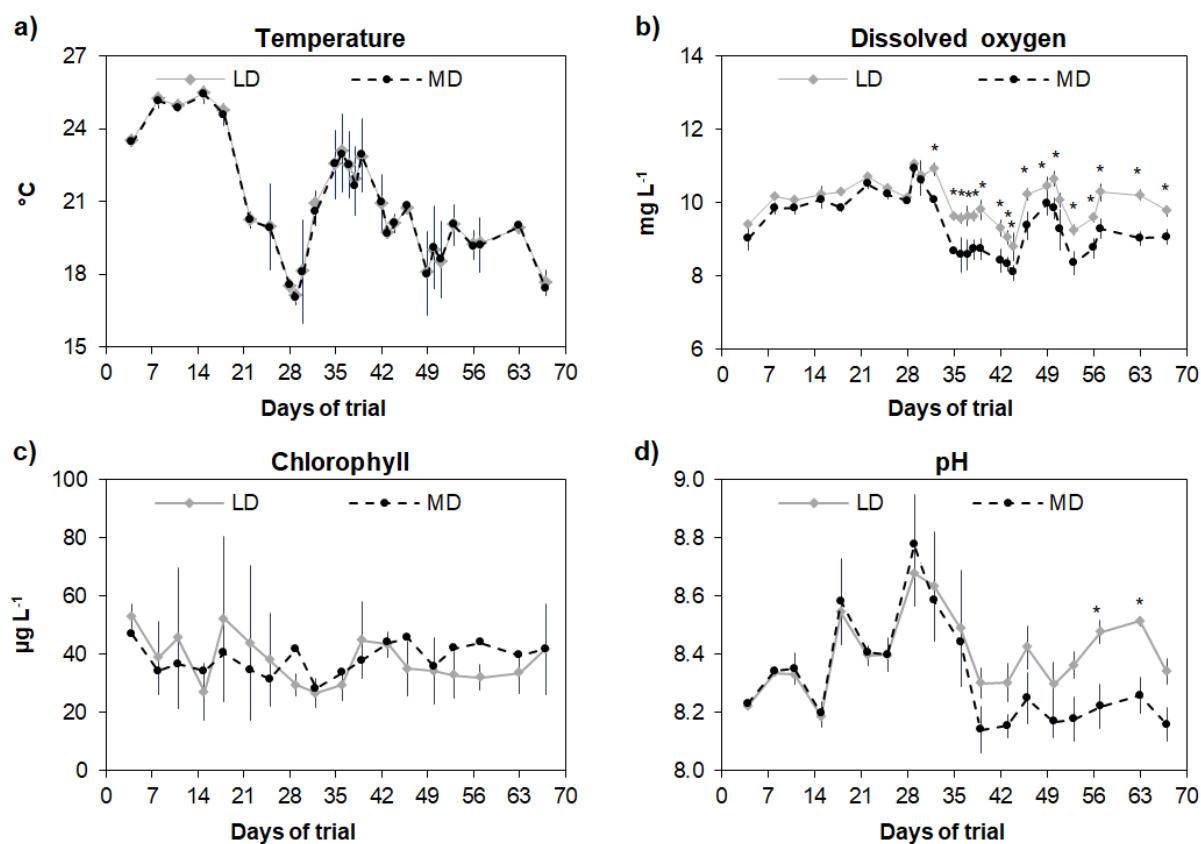


Figure 2 Temperature (a), dissolved oxygen (b), chlorophyll (c) and pH (d) values (means ± standard deviation) measured in the water of fish tanks during the trial. LD - low fish stocking density; MD - moderate fish stocking density. The asterisk indicates significant differences between means ($P < 0.05$)

No significant effect of stocking density was observed for LMB morphological indices or flesh quality (Table 3). Fillet quality traits of LMB reared in our aquaponic system were similar to those reported in other studies on LMB cultured in ponds (Li et al., 2018; Chen et al., 2020) and were not affected by stocking density. To our knowledge, no information on the effect of stocking density on the flesh quality of fish reared in aquaponic systems is currently available. On the other hand, findings of the present study agree with results on LMB cultured in ponds (Tidwell et al., 2007; Wang et al., 2019), whereas studies performed in other fish species cultured in aquaculture showed that carcass yield, viscerosomatic index and hepatosomatic index can be significantly influenced by stocking density (Ni et al., 2014; Liu et al., 2016). Indeed, high stocking density is the major chronic stressor in farmed fish (Hoyle et al., 2007), thus being a possible cause of alterations in the end-product quality such as reduced lightness of skin, and decreased muscle pH and water holding capacity (Suárez et al., 2014).

In the present study, the low and moderate initial stocking densities adopted, and the poor growth performance of fish may explain the absence of significant effects on the flesh quality of fish.

Table 2 Growth performance of largemouth bass

Item	Stocking density		P-value
	LD	MD	
Fish (n)	34	65	
Tanks (n)	4	4	
Fish weight (g)			
0 days	234 ± 6	236 ± 5	0.784
70 days	266 ± 6	261 ± 6	0.561
Biomass (kg m ⁻³)			
0 days	4.10 ± 0.16	7.56 ± 0.06	<0.001
70 days	4.66 ± 0.15	8.35 ± 0.03	<0.001
Daily growth rate (g d ⁻¹)	0.46 ± 0.03	0.36 ± 0.02	0.096
Specific growth rate (% d ⁻¹)	0.19 ± 0.01	0.14 ± 0.01	0.114
Feed conversion ratio	2.30 ± 0.25	3.15 ± 0.33	0.087
Biomass growth (kg m ⁻³)	0.56 ± 0.05	0.78 ± 0.07	0.037

LD – low fish stocking density; MD - moderate fish stocking density. Data are expressed as means ± standard error.

Table 3 Morphometric indices, slaughter results and fillet quality traits of largemouth bass

Item	Stocking density		P-value
	LD	MD	
Fish (n)	32	32	
Tanks (n)	4	4	
Condition factor	1.50 ± 0.02	1.50 ± 0.02	0.493
Relative profile	0.273 ± 0.003	0.280 ± 0.003	0.310
Cranial index	0.280 ± 0.002	0.280 ± 0.002	0.984
Viscerosomatic index	9.40 ± 0.23	8.9 ± 0.19	0.155
Hepatosomatic index	3.30 ± 0.10	3.00 ± 0.10	0.061
Carcass weight ¹ (g)	243 ± 6	240 ± 5	0.708
Carcass yield (%)	90.6 ± 0.2	91.1 ± 0.2	0.155
Fillet weight ¹ (g)	100 ± 2	100 ± 3	0.943
Fillet yield (%)	41.1 ± 0.5	41.7 ± 0.4	0.391
Fillet pH	6.39 ± 0.02	6.40 ± 0.02	0.853
Flesh colour			
L*	44.2 ± 0.3	43.5 ± 0.4	0.120
a*	-2.50 ± 0.10	-2.30 ± 0.10	0.109
b*	5.10 ± 0.30	5.50 ± 0.40	0.430

¹Carcass weight and fillet weight were measured on all slaughtered fish (n=99). LD - low fish stocking density; MD - moderate fish stocking density. Data are expressed as means ± standard error.

Regarding vegetable production, fish stocking density did not affect plant growth (Figure 3a). On average, lettuce showed a marketable yield of 6.33 kg m⁻² and root yield of 0.38 kg m⁻², whereas radicchio reached a final marketable yield of 1.34 kg m⁻² and root yield of 0.08 kg m⁻² (Figure 3b). Similar results were obtained for the same crops in an aquaponic system with pangasius fish and nutrient film technique soilless system (Maucieri et al., 2017). In other studies, significant differences were reported for the vegetables yield as a consequence of different fish stocking density. Maucieri et al. (2019) obtained higher vegetable productions (+49%, on average) in system with European carp (*Cyprinus carpio*) farmed at low density, compared to systems at high density (2.34 kg m⁻³ vs. 3.88 kg

m⁻³). Similar results were obtained by Pantanella et al. (2012) in aquaponic systems farming Nile tilapia (*Oreochromis niloticus*) at 2 stocking densities (5 kg m⁻³ vs. 8 kg m⁻³). Overall, the marketable yields of lettuce obtained in the present trial were similar to those reported in other studies (Pantanella et al., 2012; Maucieri et al., 2019).

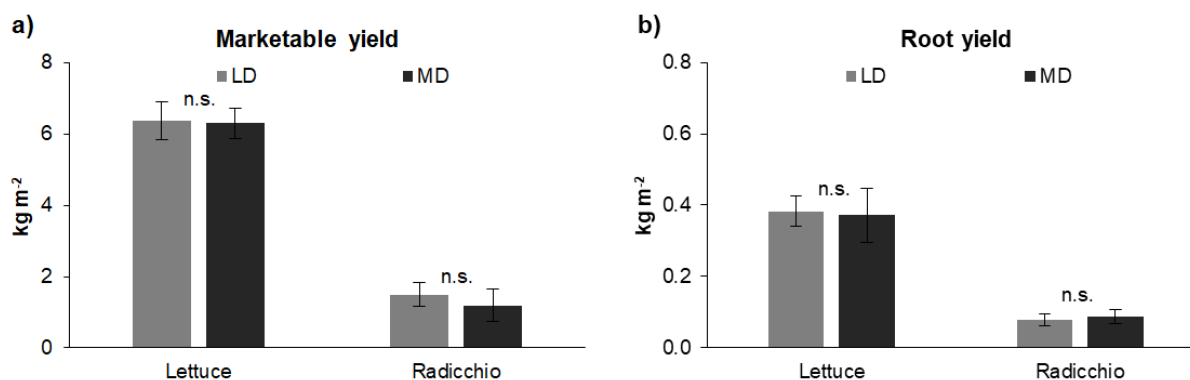


Figure 3 Marketable yield (a) and root yield (b) of lettuce and radicchio measured at the end of their crop cycle (63 days and 80 days, respectively). LD - low fish stocking density; MD - moderate fish stocking density

On the contrary, the production of radicchio was lower (~30%) when compared with the productions obtained in field cultivation (Filippini et al., 2011). This result may be related to an insufficient nutrient flow in the system, a reduced nutrient uptake by plants and a lower suitability of chicory adult plants to soilless conditions as reported by Maucieri et al. (2017). Indeed, even if the pH range maintained during the cycle was consistent with the requirements for bacteria growth (7.0 < pH < 9.0; Goddek et al., 2015), the values registered were not optimal for an aquaponic system, since basicity limits the absorption of nutrients by plants (Rakocy, 2012).

4 Conclusions

The present study demonstrated that aquaponics is a promising and potentially sustainable production system, especially in view of the saving of water resources and the production of lettuce, while radicchio resulted less suitable than lettuce for such systems. Further investigations are necessary to clarify the correct methods of farming LMB in aquaponic systems, focusing on its behaviour, feeding technique, and stocking density. In fact, despite LMB is a species of growing interest for the aquaculture market, available information related to its environmental and nutritional requirements is still very limited both under conventional and, especially, under alternative farming systems.

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