Nutrient accumulation, growth and quality of leafy vegetables in aquaponics system are modulated by supplemental LED lighting

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Abstract

Aquaponics is a highly efficient production system that relies on the food introduced for fish as the only nutrients input for growing both fishes and vegetables. However, plant ability to absorb nutrients in an aquaponics system may be reduced in the winter months characterised by low evaporative demand (low temperature and radiation) which in turn could cause accumulation of nitrate, nitrite, and ammonia in the water. These by-products are harmful for fishes, forcing growers to renew the water more often. The aim of the study was to evaluate growth, physiological response, nutrient accumulation, and quality of lettuce and curly endive, grown in floating raft Recirculating Aquaponic System (RAS) combined with tilapia, under natural light (NL) or NL integrated with 16 hours of supplemental white LED lighting (IL, PPFD: 173 μmol m^{-2} s^{-1}, daily light integral [DLI], 10 mol m^{-2} d^{-1}). Results show a species-specific response to the lighting regimes. Compared to NL, IL promoted plant growth and nutrient accumulation in both species. Particularly, endive it increased leaf area and induced new leaf formation. Supplemental lighting increased whole plant assimilation capacity with no effect on pigments content and photochemical efficiency. However, supplemental lighting decreased the maximal photochemical efficiency (F_v/F_m) in lettuce. The different lighting regimes affected nutrient accumulation and translocation in both leaves and roots. To summarize, curly endive performs better than lettuce in aquaponics. Supplemental lighting can guarantee a stable filtration capacity during the winter season, improving overall system performances and plant qualitative attributes of the tested crops.

Keywords: pigments, mineral content, daily light integral, net photosynthesis

INTRODUCTION

Urban agriculture stands as a valuable tool to reduce agri-food production environmental impacts, economic costs and shorten the food supply chain (Stoknes et al., 2019; Rufí-Salís et al., 2020). Moreover, a common necessity for the current and future cities is to provide high-quality and safe proteins and fiber sources at local level. In this scenario, aquaponics can be considered a valuable solution to create sustainable food systems. Aquaponics like other integrated production techniques will keep spreading in the coming years, to reduce the high demand for plant fertilizers and increase the overall sustainability (Armanda et al., 2019; Specht et al., 2019).

Aquaponics, combining aquaculture and hydroponics, allows to convert fish faeces, thanks to microbial activity, into available nitrogen form for the plants with mutual benefit by reducing the need to discharge water by aquaculture plans and chemical fertilizers reliance to grow hydroponic vegetables (Greenfeld et al., 2019).

Among the different system typologies, the most common is the coupled aquaponics fish or 1-loop systems, fish production in recirculating aquaculture systems (RAS) and plants in hydroponics are combined in a single loop, entailing systemic compromises on the optimal

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production parameters for both fishes and plants (e.g., pH). Coupled RAS systems are well suitable to grow leafy vegetables, especially lettuce, in combination with tilapia (*Oreochromis niloticus* L.)(Yep and Zheng, 2019), which nowadays, is the most grown fish species worldwide (Wang and Lu, 2016). These systems can have several advantages if implemented into an urban context, such as rooftop greenhouses, vertical farms, and other controlled environment agricultural (CEA) systems (Wortman, 2015; Oliver et al., 2018; Armanda et al., 2019). Plant's metabolism directly depends on the surrounding environment, in fact, variation in day length, light intensity and temperature, affects plant photosynthetic process, growth and may reduce nutrients absorption capacity, which may, in turn, cause harmful accumulation of nitrate, nitrite, and ammonia for fishes in the water (Anderson et al., 2017). In the winter months or when the solar radiation is low, supplemental lighting could promote plant growth and mitigate nutrient accumulation in the water.

Therefore, the study aimed to evaluate morpho-physiological response and mineral and pigment content of lettuce (*Lactuca sativa* L.), escarole endive (*Cichorium endivia var. latifolia*), and curly endive (*Cichorium endivia var. crispum*) grown in a floating raft in combination with tilapia, under natural light (NL) or natural light integrated with 16 hours of supplemental white LED lighting (IL, PPFD: 173 μmol m-^{2} s-1, DLI, 10 mol m-^{2} d-1) in a coupled RAS.

MATERIALS AND METHODS

Aquaponics system design and fish feed rate

The experiment was carried out in a Recirculating aquaponics system (RAS) prototype inside a unheated greenhouse (40°48'57.9"N 14°21'01.6"E) at the Department of Agricultural Sciences of the University of Naples Federico II (Portici, Italy) from the 27th of April the 21st of May 2021. The RAS unit consisted of 4 tilapia fish rearing tanks, each of 2800 L. The system was equipped with an 800 L Superbead system for mechanical and biological filtration, 400 L trickling filter, 40 W UV sterilisation unit. Ambient air insufflation was set at 0.05 v v^{-1} min $^{-1}$. A preformulated feed containing 35% of the protein was adopted as fish feed. The daily fish feed target was adjusted based on fish age and stocking.

Plant material and experimental conditions

Two weeks old seedlings of lettuce ((L), *Lactuca sativa* L. *cv*. Meraviglia d´Inverno (L´ortolano), and curly endive (EC) (*Cichorium endivia var crispum cv*. De Louvriers (Seedsselect) grown on polystyrene sowing tray were used as plant material. Roots were gently washed with tap water to remove the peat cube and planted into a floating raft system of the RAS unit at a plant density of 20 plant m^2.Plants were harvested after 23 days, when they reached their commercial maturity. Water temperature was set to 23°C, pH and electrical conductivity (EC) were monitored daily over the entire period and were on average 6.9 and 855.7 μS cm $^{-1}$, respectively.

Lighting treatments consisted of 1) natural sunlight control (NL) with a photoperiod (or daylength, which was calculated for the site locations as the time interval between sunrise and sunset) ranging between 13 h 20 min and 14 h 29 min from April 2021 to May 2021 and 2) natural sunlight integrated with 16 hours (6:00-22:00) of supplemental lighting (IL) provided by white LED (Hortimol TLed 40W Full Spectrum FSG, The Netherlands), B: R ratio of 0.44, at an average photosynthetic photon flux density (PPFD) of 173.5 ± 6.2 μmol m^{-2} s^{-1} guaranteeing a minimum daily light integral (DLI) of 10.0 ± 0.4 mol m^{-2} d^{-1}.

Plant growth

At harvest on 18 plants per *species x lighting treatment*, leaf number was recorded. The total leaf area was obtained by analysing digital images with ImageJ software 1.50i version (Wayne Rasband National Institute of Health, USA). Fresh weights of canopy and roots were recorded with an electronic balance, and dry weights were obtained after drying samples at 70°C for 48 hours. Specific leaf area (SLA) was calculated as the ratio between fully expanded leaf area and its dry weight.

Leaf gas exchanges and Chl *a* fluorescence emission measurements

Gas exchanges measurements were performed at 23 days after trasnplanting (DAT) on one fully expanded leaves of 3 plants × 3 replicates × *species* × *lighting treatment* using a photosynthesis yield analyser (LCi T, ADC Bioscientific Ltd, UK); measurements were carried out at noon at ambient CO_2 (434 ppm) at a mean temperature of 31.1°C, humidity of 45%, and Photosynthetic Photon Flux Density (PPFD) of 1251.8 µmol m^{-2} s^{-1}.

On the same leaves used for gas exchanges measurements, Chlorophyll *a* fluorescence emission, was determined using a portable fluorimeter kit (Plant stress Kit, Opti-Sciences, Hudson, USA). Measures in the light were carried out with a ΦPSII meter by applying a saturating pulse of 4286 µmol m^{-2} s^{-1} for 1.1 s, to obtain the maximum light-adapted fluorescence (F_m') and steady-state fluorescence (F_s). For measurements in the dark, leaves were dark-adapted for 30 min with a dark leaf clip (Opti-Sciences Inc., Hudson, USA), then using an F_v/F_m meter (Opti-Sciences Inc., Hudson, USA) a 1.0 s saturating pulse light (3429 µmol m^{-2} s^{-1}) was given to obtain the F_m and F_o values. The PSII maximum photochemical efficiency (F_v/F_m) was calculated as $F_v/F_m = (F_m - F_0)/F_m$. The quantum yield of PSII electron transport (Φ_{PSII}) was calculated as $\Phi_{PSII} = (F_m' - F_s)/F_m'$ following Genty et al. (1989).

Leaf chlorophyll and carotenoids content determination

At harvest (23 DAT), leaf photosynthetic pigments content was determined on 1 fully expanded leaf per 3 plants × 3 replicates × *species* × *lighting treatment.* Leaf samples were immediately frozen at -20°C till analysis. An aliquot of 0.5 g of leaf tissue was grinded together with 5 mL of acetone (80%) into 15 mL tube flask. The solution was incubated in the dark, at room temperature for 15 min, followed by a 5 min centrifugation at 3000 *g* pigments content was determined by their light absorbance at 662, 645 and 470 nm for chlorophyll a, b and total carotenoids, using a Hach DR 2000 spectrophotometer (Hach Company, Loveland, CO, USA). Total Chlorophylls was calculated as the sum of chlorophyll *a* and *b* according to. Lichtenthaler and Burkart (1999).

Leaf mineral content analysis

According to Pannico et al. (2019) protocol, a 250 mg aliquot of ground-milled (model MF10.1, IKA-Werke GmbH & Co. KG, Staufen, Germany) dry leaf sample was used for the determination of leaf mineral (nitrate, P, K, Ca and Mg) composition. Mineral analysis was then carried out after 0.45 µm filtering using an ion chromatographer (model ICS-3000, Dionex, Sunnyvale, CA, USA), quantified using an electrical conductivity detector equipped with an IonPac CS12A and IonPac AS11-HC analytical columns for the analysis of cationic and anionic contents, respectively (Dionex, Sunnyvale, CA, USA). All the minerals were expressed as g kg^{-1} on dry weight (DW) basis. Considering the dry matter content, nitrates concentration was as mg kg^{-1} on fresh weight (FW) basis. The nutrient accumulation was calculated by multiplying the canopy dry weight of each plant by the concentration of a given nutrient.

Statistical analysis

The experiment was carried out on 18 plants per species x lighting treatment with a complete block randomised distribution between the species. the sampling, measurements, and analysis of variance were carried out on the average of 3 plants × 3 replicates × *lighting treatment* per each species using the SPSS software package v27 (www.ibm.com/software/analytics/spss). Means were compared by Tukey HSD post-hoc test (P<0.05).

RESULTS

Compared to natural light, in curly endive, supplemental lighting promoted new leaves formation (+19.7%), increased total leaf area (+37%) and canopy and root fresh and dry biomass, +99.2%, +92.1% and +169.1 % and 11.4%. for fresh and dry biomass in the canopy and in roots respectively. In lettuce, supplemental lighting promoted canopy and root fresh and dry biomass, +50.9%, +55.2% and +66.4% and + 22.0% respectively, without influencing the leaf number and the total leaf area that were on average 39.25 and 2803.05 cm^2, respectively (Table 1).

Table 1. Plant growth measurements at 23 DAT: leaf number, total leaf area (TLA), canopy fresh weight (CFW), canopy dry weight (LDW); root dry weight (RDW) in plants of curly endive and lettuce, and grown in a floating raft in a coupled RAS system. Mean values (n=3), followed by different letters within each parameter, are significantly different based on Tukey HSD post-hoc ($P<0.05$).

Species	Light treatment	Leaf number (no. plant^{-1})	Total leaf area (cm^2 plant)	Canopy FW (g plant^{-1})	Canopy DW (g plant^{-1})	Root DW (g plant^{-1})
Curly Endive	NL	43.2b	2585.6b	121.2b	4.2b	1.3b
	IL	51.8a	3550.2a	241.4a	11.4a	2.8a
	mean	*47.5*	*3067.9*	*181.3*	*7.8*	*2.0*
Significance		0.039*	0.011**	0.001***	0.001***	0.002**
Lettuce	NL	35.3	3183.9	199.2b	3.9b	1.1b
	IL	43.2	2803.1	300.6a	6.5a	1.3a
	mean	*39.2*	*2993.5*	*249.9*	*5.2*	*1.2*
Significance		0.071^{ns}	0.052^{ns}	0.007**	0.028*	0.05*

Non-significant or significant differences at $P \leq 0.05$, 0.01, or 0.001 are indicated as ns, **, and ***, respectively

Supplemental lighting did not affect, at the time of measurement, gas exchanges in both species (Table 2) compared to control. In endive the mean leaf net photosynthetic rate averaged around 7.46 µmol of CO$_2$ m^{-2} s^{-1}, g_s was on average 0.27 while in lettuce leaf net photosynthesis was on average 5.95 µmol of CO$_2$ m^{-2} s^{-1}, g_s 0.27.

Table 2. Plant eco-physiological traits at 23 DAT: leaf net photosynthesis (P_n), stomatal conductance (g_s), maximal photochemical efficiency (F_v/F_m), quantum yield of PSII (Φ_{PSII}); specific leaf area (SLA) in plants of curly endive and lettuce, grown in floating rafts in a coupled RAS system. Mean values (n=3), followed by different letters within each parameter, are significantly different based on Tukey HSD post-hoc ($P<0.05$).

Species	Light treatment	P_n (µmol CO$_2$ m^{-2} s^{-1})	g_s (mol H$_2$O m^{-2} s^{-1})	F_v/F_m	Φ_{PSII}	SLA (cm^2/g)
Curly Endive	NL	7.99	0.28	0.78	0.35	462.39
	IL	6.93	0.26	0.75	0.34	440.96
	mean	*7.46*	*0.27*	*0.76*	*0.34*	*451.67*
Significance		0.541^{ns}	0.639^{ns}	0.067^{ns}	0.926^{ns}	0.848^{ns}
Lettuce	NL	7.09	0.29	0.74	0.35a	447.77
	IL	4.81	0.25	0.75	0.23b	408.05
	mean	*5.95*	*0.27*	*0.75*	*0.29*	*427.91*
Significance		0.133^{ns}	0.281^{ns}	0.65^{ns}	0.004**	0.589^{ns}

Non-significant or significant differences at $P \leq 0.05$, 0.01, or 0.001 are indicated as ns, **, and ***, respectively

Supplemental lighting had no effect in endive on the maximal photochemical efficiency of PSII and the quantum yield of PSII that were on average 0.76 and 0.34 respectively, while in lettuce and quantum yield of PSII decreased by 34.2%. In both species, leaf traits like specific leaf area were not affectedby supplemental lighting.

Photosynthetic pigments in terms of total chlorophylls, carotenoids and chlorophyll a/b ratio were not affected by lighting regime in both species (Table 3). The endive and lettuce plants developed a total chlorophyll and carotenoids concentration of 1.14 mg g^{-1} and 0.26 mg g^{-1} of fresh weight respectively.

The mineral composition was significantly affected by lighting treatments in both species (Table 4). In endive, nitrates, phosphate, potassium and calcium accumulation increased under supplemental lighting by 28.8%, 140.6%, 247.6%, 172.9% and 60.8% respectively, while no difference were observed regarding magnesium accumulation.

Table 3. Plant photosynthetic pigments content at 23 DAT: Total chlorophyll (a+b) (mg g^{-1}) content; Carotenoids (mg g^{-1}) content and Chlorophyll a/b ratio in plants of curly endive (EC) and lettuce (L), and grown in a floating raft in a coupled RAS system. Mean values (n=3), followed by different letters within each parameter, are significantly different based on Tukey HSD post-hoc (P<0.05).

Species	Light treatment	Total chlorophyll (mg g^{-1})	Carotenoids (mg g^{-1})	Chl (a/b)
Curly Endive	NL	1.07	0.25	2.01
	IL	1.21	0.27	1.75
	mean	*1.14*	*0.26*	*1.88*
Significance		0.586 ^{ns}	0.399 ^{ns}	0.289 ^{ns}
Lettuce	NL	1.15	0.26	1.81
	IL	1.1	0.27	1.65
	mean	*1.13*	*0.26*	*1.73*
Significance		0.68 ^{ns}	0.694 ^{ns}	0.539 ^{ns}

Non-significant or significant differences at P ≤ 0.05, 0.01, or 0.001 are indicated as ns, **, and ***, respectively

In lettuce supplemental lighting promoted nitrate concentration, nitrogen, phosphate and potassium accumulation by 35.1%, 110.6%, 90.9% and 180.6% respectively, while calcium and magnesium accumulation were not affected by lighting regime and they were on average of the light treatment 51.5 and 20.4 respectively.

Table 4. Plant nitrates concentration (mg kg FW^{-1}) and nutrient accumulation (g plant DW^{-1}) at 23 DAT: nitrate, phosphate, potassium, calcium and magnesium in plants of curly endive and lettuce, and grown in a floating raft in a coupled RAS system.

Species	Light treatment	N-NO_3 (mg kg plant FW^{-1})	N-NO_3 (g plant DW^{-1})	PO_4 (g plant DW^{-1})	K (g plant DW^{-1})	Ca (g plant DW^{-1})	Mg (g plant DW^{-1})
Curly Endive	NL	2584.9	313.7b	70.1b	388.1b	30.9b	13.5a
	IL	3330.6	754.9a	243.7a	1059.5a	49.6a	16.7a
	mean	*2957.8*	*534.3*	*156.9*	*723.8*	*40.2*	*15.1*
Significance		0.023*	0.001***	0.001***	0.000**	0.007**	0.321 ^{ns}
Lettuce	NL	1456.5	285.7b	60.4b	237.0b	41.1a	15.2a
	IL	1968.9	601.9a	115.3a	665.1a	61.9a	25.7a
	mean	*1712.7*	*443.8*	*87.9*	*451.1*	*51.5*	*20.4*
Significance		0.044*	0.031**	0.008**	0.007**	0.147 ^{ns}	0.074 ^{ns}

Non-significant or significant differences at P ≤ 0.05, 0.01, or 0.001 are indicated as ns, **, and ***, respectively

DISCUSSION

In the framework of an increased interest on and diffusion of urban agriculture (UA) due to its potential to increase access to healthy and nutritious food, strengthen local economies and promote a sense of community, research efforts on developing and evaluating food production systems that can be at the same time fully sustainable and potentially integrable in compact cities (Zhang et al., 2022) Aquaponics is an efficient production technique, suitable to be embedded into different urban food production contexts, from rooftop gardens to vertical farming systems, so fat aquaponics has been proved, thanks to the nitrates rich water, to be an ideal technique to grow several leafy vegetables like lettuce (Delaide et al., 2016), basil (Ferrarezi and Bailey, 2019), whereas no study seems to evaluate endives performance in similar conditions. Our study provides useful information for both costumers and urban growers on aquaponics products in terms on crop growth, physiological behavior and in particular quality. In terms of quality, usually nitrate accumulation in leaf tissue is a serious threat for human health, causing different potential disorders (Buscaroli et al., 2021), as regards maximum levels for nitrates in the European commission regulation (EU) No 1258/2011 sets the maximum level of nitrates in lettuce between the 1st of April till the 30th of September to 4000 mg NO_3 kg $^{-1}$ production FW basis, accordingly in our experimental condition nitrate concentration in both species and light regimes was below this level and supplemental lighting increased its content. Usually, shorter daylength and lower solar radiation contribute to nitrate accumulation into leaf tissue, in addition, reduced solar radiation reduce plant metabolism, growth and overall reduced nutrient accumulation and hence system filtration capacity in an aquaponics system. Low nutrients accumulation in aquaponics system can cause the increase in the system of harmful concentrations for fishes, imposing the release of nutrient enriched water into the environment, with eutrophication risks (Lam et al., 2015; Yang and Kim, 2019). Light influences several aspect in plant life, from circadian rhythm regulation to photosynthesis and hence grow (Pattison et al., 2018). Different studies shows the benefits of supplemental lighting to guarantee a constant minimum day light integral, required to match production targets, especially in controlled environment soilless cultivation systems (Paucek et al., 2020).

In our experimental growing condition, in both crops supplemental lighting promoted growth and nutrients accumulation, with little or no influence on pigment content and photochemistry. Since only few studies are available in literature on the soilless cultivation of endives, especially under aquaponics condition or different lighting conditions, our findings contribute to highlight that curly endive is suitable to be grown in aquaponics and that supplemental DLI can increase plant production and can be easily applied into any aquaponics systems. In fact, endive plant growth was promoted by increasing both leaf number and total leaf area, and hence carbon assimilation who contributed to a higher plant fresh and dry biomass accumulation. The higher root biomass development induced by supplemental lighting contributed to a higher nutrients accumulation.

From an eco-physiological perspective, supplemental lighting did not increase the specific leaf area and it did not affect leaf ability to absorb light as revealed by the absence of difference in the leaf photosynthetic pigment concentration. This result was in line with the maximal photochemical efficiency of PSII in both species. However, the actual fraction of light effectively absorbed by the PSII decreased in lettuce when grown under supplemental lighting, according to literature. Indeed, in lettuce different studies demonstrated that Φ_{PSII} decreases under increasing light intensity (van Iersel et al., 2016; Weaver and van Iersel, 2019). On the contrary, the absence of differences in Φ_{PSII} observed in endive suggests higher saturating light requirements compared to lettuce. However, at the time of measurements (midday) any significant difference in photosynthetic rate under the two lighting regimes was observed. It may be ascribed to the fact that supplemental lighting accounts less in the overall radiation at the time of measurements, whereas it could be more effective in the morning or in the afternoon, when the solar radiation is low.

CONCLUSIONS

Aquaponics is a suitable technique to grow leaf vegetables especially in combination with tilapia. The two species well suit to be grown in aquaponics systems. Supplemental lighting promotes growth and nutrients accumulation without detrimental effect on plant photochemical performances. Our results suggest that providing a fixed DLI to promote plant growth could shorten the growing cycle, especially in the winter growing season, when the solar radiation and daylength is reduced. In addition, supplemental lighting can increase the overall system performance, reducing the risk of nutrients and water depletion. However, the environmental impact of supplemental lighting strategies in aquaponics in relation with the effectively gained yield in the different seasons should be considered to improve a sustainable use of artificial lighting.

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