# Supplemental Daily light integral by LED light to improve the growth of leafy vegetables in aquaponics system

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# Abstract

Aquaponics is a highly efficient production system that relies on the fish food as the only energy system's input for growing both fishes and vegetables, without chemicals. In Mediterranean climates, during winter, due to the combined effects of low solar radiation and low-temperature levels, plant metabolic activity, growth rate, and nutrient uptake decrease, which may, in turn, cause harmful accumulation of nitrogen compounds for fishes in the water. Growers renew the water periodically by discharging nutrients enriched water with severe environmental and economic costs. In winter, aquaponics cultivation cycles can be integrated with supplemental light to guarantee a constant Daily Light Integral (DLI) that could promote plant growth and nutrient uptake. The aim of the study was to evaluate the morpho-physiological response 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 (Oreochromis niloticus L.) in a Recirculating Aquaponics System (RAS). Plant were grown under natural light (NL) or natural light integrated with 16 hours of supplemental white LED lighting (IL, PPFD: 173 µmol m<sup>-2</sup> s<sup>-1</sup>, DLI, 10 mol m<sup>-2</sup> d<sup>-1</sup>). The results showed species-specific morpho-physiological responses, with higher productivity in the endives than lettuce, under both light regimes. Compared to NL, plant biomass and leaf area were promoted under IL, accounting for higher plant photosynthetic rates. IL boosted endive growth, while lettuce leaf expansion and biomass allocation into the canopy were increased. Both lettuce and endives performed well in the adopted RAS system. It appears recommendable to provide a fixed DLI during the winter months to shorten the crop cycle by improving plant growth and nutrient uptake. However, further studies are needed to optimize the lighting application protocols and reduce energy costs too.

Keywords: white spectrum, photochemistry, water use efficiency, root growth

# **INTRODUCTION**

Urban agriculture stands as a valuable tool to reduce agri-food production environmental impacts and shorten the food supply chain (Ruff-Salís et al., 2020). Furthermore, a necessity for the cities is to provide high-quality proteins and vegetables at local level. In this scenario, aquaponics can be considered a sustainable solution to create sustainable food systems. Aquaponics, the merge of aquaculture and hydroponics, allows to convert fish faeces rich in ammonia, thanks to microbial activity, into nitrates for plants with mutual benefit by reducing the need to discharge water by aquaculture plans and the need for chemical fertilizers to grow vegetables (Greenfeld et al., 2019). In coupled aquaponics, fish production in recirculating aquaculture systems (RAS) and plants in hydroponics are combined in a single loop, entailing systemic compromises on the optimal 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* 

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L.)(Yep and Zheng, 2019), that nowadays, it 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 greenhouse, vertical farms, and other controlled environment agricultural (CEA) plans (Oliver et al., 2018). In Mediterranean climates, during winter, due to the combined effects of low solar radiation and low-temperature levels, plant metabolic activity, growth rate, and nutrient uptake decrease, which may, in turn, cause harmful accumulation of nitrogen compounds for fishes in the water (Anderson et al., 2017). In the winter months, the use of supplemental lighting could promote plant growth and mitigate nutrient accumulation in the water.

Therefore, the study aimed to evaluate the morpho-physiological response 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<sup>-2</sup> s<sup>-1</sup>, DLI, 10 mol m<sup>-2</sup> d<sup>-1</sup>) 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) inside a cold 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 December 2020 to February 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 sterilization unit. Ambient air insufflation was set at 0.05 v v<sup>-1</sup> min <sup>-1</sup>. 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 density.

# Plant material and culture

Two weeks old seedlings of lettuce ((L), *Lactuca sativa* L. *cv*. Meraviglia d'inverno (L'ortolano), escarole endive (EE) (*Chicorium endivia var latifolia cv*. Bionda a cuore pieno (Esasem), 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 unit of the RAS system at a plant density of 25 plant m<sup>2</sup>. Plants were harvested after 52 days. Water temperature was set to 24°C, pH and electrical conductivity were monitored daily over the entire period and were on average 8 and 1042  $\mu$ S cm<sup>-1</sup>, respectively.

# **Lighting treatment**

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 9 h 13 min and 10 h 40 min from December 2020 to February 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, the light spectral composition is reported in Figure 1. IL was applied at an average photosynthetic photon flux density (PPFD) of 173.5 ± 6.2  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> guaranteeing a

minimum daily light integral (DLI) of 10.0  $\pm$  0.4 mol m<sup>-2</sup> d<sup>-1</sup>. IL spectra composition was analyzed with a spectral light meter (MSC15, Gigahertz-Optik GmbH, Türkenfeld, Germany).



Figure 1. Spectral irradiance distribution (W m<sup>-2</sup> nm<sup>-1</sup>) of the white LED (Hortimol TLed 40W Full Spectrum FSG, The Netherlands). The spectra was obtained with a portable spectral light meter (MSC15, Gigahertz-Optik GmbH, Türkenfeld, Germany)

# Gas exchanges measurements

Gas exchanges were measured at 36 days after planting (DAP) on one fully expanded leaves of 12 plants x *species x lighting treatment* using a photosynthesis yield analyzer (LCi T, ADC Bioscientific Ltd, UK); measurements were carried out at noon at ambient  $CO_2$  (457± 1.55 ppm) at a mean temperature of 28.5°C, humidity of 43.15%, and Photosynthetic Photon Flux Density (PPFD) of 689.5 µmol m-<sup>2</sup> s<sup>-1</sup>.

### Chl a fluorescence emission and SPAD measurements

On the same leaves used for gas exchanges measurements, Chl *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  $\Phi_{PSII}$  meter by applying a saturating pulse of 4286 µmol m<sup>-2</sup> s<sup>-1</sup> for 1.1 s, to obtain the maximum light-adapted fluorescence (F<sub>m</sub>') and steady-state fluorescence (F<sub>s</sub>). For measurements in the dark, leaves were dark-adapted for 30 min with a dark leaf clip, then using an F<sub>v</sub>/F<sub>m</sub> meter a 1.0 s saturating pulse light (3429 µmol m<sup>-2</sup> s<sup>-1</sup>) was given to obtain the F<sub>m</sub> and F<sub>o</sub> 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  $(\Phi_{PSII})$  was calculated as  $\Phi_{PSII} = (F_m'-F_s)/F_m'$ ; The non-photochemical quenching (NPQ) was calculated as  $(F_m/F_m')$  -1.

### Stomatal traits

One leaflet of fully expanded leaf was sampled from 6 plants per *species x lighting treatment* and immediately fixed in FAA solution (40 % formaldehyde, 10% glacial acetic acid, 50 % ethanol). Stomatal traits were determined by peeling the abaxial epidermis in each leaflet's median region, avoiding the midrib and the margin. Three epidermal strips per leaflet were peeled and mounted with water on microscope slides. Afterwards, each epidermal strips were observed under a transmitted light microscope (BX51, Olympus, Hamburg, Germany) at a magnification of 20x, and the images were collected using a digital camera (EP50, Olympus); finally, each image was processed using the ImageJ software 1.50i version (Wayne Rasband

National Institute of Health, USA). Five stomata per epidermal strips were randomly selected to estimate their density (number of stomata per surface unit, mm<sup>2</sup>).

### **Plant growth**

At harvest on 12 plants per *species x lighting treatment*, leaf number was recorded. The total leaf area was obtained by digitally analyzing pictures with ImageJ software 1.50i version (Wayne Rasband National Institute of Health, USA). Shoot and fresh root biomass were recorded with an electronic balance, and dry weights were obtained after drying samples at 70°C for 48 hours.

### **Statistical analysis**

The experiment was carried out on 27 plants per *species x lighting treatment* with a complete randomized distribution between the species. Except for stomatal traits, the sampling, measurements, and ANOVA were carried out on the average of 4 plants x 3 repetition x species x light treatment 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

### **Plant growth**

Compared to L and EE, EC plants produced more leaves, a higher total leaf area, and fresh and dry leaves and root weight (Table 1) and showed the highest dry matter content in both leaves and roots, while L the lowest (Table 1). Compared to NL, IL significantly increased the total leaf area in all the species differently. In fact, in EE and EC, IL promoted new leaf formations increasing the leaf area, whereas, in L plants, IL promoted leaf expansion accounting to a higher leaf area. Compared to NL plants, plants grown under IL developed a significantly longer root apparatus in all the species (Table 1). In addition, IL increased fresh and dry weight and decreased the dry matter content in both leaves and roots (Table 1). Under NL, L plants allocated more biomass to the canopy being the canopy: root ratio significantly higher in L plants compared to EE and EC (Table 1). IL increased in all species fresh canopy and root biomass (Table 1), with the strongest responses observed in EC (+78% and +79% for canopy and root, respectively). Compared to NL, IL did not increase canopy dry biomass content (on average 4.36%), and it increased the root dry biomass only in EE (+12%). In contrast, the canopy:root ratio decreased only in L (Table 1).

# **Eco-physiological responses**

Among the three species, leaf net photosynthesis (*Pn*) was higher in EE plants than in L plants, while stomata conductance (*gs*)did not differ between the species. In contrast, leaf transpiration (*E*), the maximal photochemical efficiency of PSII ( $F_v/F_m$ ) and stomatal density were higher in EE and EC compared to L (Table 2). The non-photochemical quenching (NPQ) and the yield of PSII ( $\Phi_{PSII}$ ) were similar between the species. Compared to NL, *Pn* decreased under supplemental lighting, whereas *gs*, *E* and stomatal density increased (Table 2). In specific, under NL, gas exchanges in terms of leaf *Pn* was on average +27% higher in EE than the others (Table 2).

Instead, *gs* and *E* did not vary between the species, and it was on average 0.11 mol m<sup>-2</sup> s<sup>-1</sup> and 3.48 mol H<sub>2</sub>0 m<sup>-2</sup> s<sup>-1</sup>, respectively (Table 2). IL light significantly interacted with the species; in detail, *Pn* increased by 59 % only in EE while it did not vary in the others (Table 2).

Table 1. Plant growth measurements at 52 DAT: leaf number, total leaf area (TLA), root length (RL), leaves fresh weight (LFW), leaf dry weight (LDW), leaf dry matter (LDM); root fresh weight (RFW), root dry weight (RDW), root dry matter (RDM), canopy:root ratio (C:R) in plants of lettuce (L), escarole endive (EE) and curly endive (EC) 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). Non-significant or significant differences at P  $\leq 0.05$ , 0.01, or 0.001 are indicated as ns, \*\*, and \*\*\*, respectively.

Treatment	Leaf	TLA	LFW	LDW	LDM	RL	RFW	RDW	RDM	C·R
	(n)	(cm² plant-1)	(g plant-1)	(g plant <sup>-1</sup> )	(%)	(mm)	(g plant <sup>-1</sup> )	(g plant <sup>-1</sup> )	(%)	0.13
Species (S)										
L	31.5 b	1680 b	86.1 b	2.8 c	3.6 c	29.7	8.3 b	0.4 b	5.3 c	10.6
EE	34.0 b	1880 ab	79.5 b	4.1 b	5.3 b	22.8	7.3 b	0.6 b	7.2 b	8.2
EC	67.7 a	2120 a	125.2 a	6.0 a	4.8 a	23.3	12.8 a	0.8 a	6.0 a	8.3
Lighting treatment (LT)										
NL	33.0 b	1220 b	45.3 b	2.0 b	4.6 a	15.7 b	3.6 b	0.2 b	6.1	11.0 a
IL	55.8 a	2570 a	148.6 a	6.6 a	4.4b	34.9 a	15.3 a	0.9 a	6.3	7.2 b
Species × Lighting treatment										
L×NL	29.8 cd	1271 c	53.5 c	1.9 c	3.5 c	17.8 bc	2.7 с	0.1 c	5.6 cd	15.1 a
L×IL	32.2 c	2096 b	118.7 b	3.8 c	3.2 c	41.7 a	13.9 b	0.7 b	5.0 d	6.2 b
EE×NL	22.6 c	1180 c	38.2 c	2.1 c	5.5 a	13.9 c	3.7 c	0.3 c	6.8 b	9 b
EE×IL	45.5 b	2580 c	120.8 b	6.2 b	5.1 ab	31.6 ab	10.9 b	0.8 b	7.7 a	7.5 b
EC×NL	46.7 b	1205 c	44.2 c	2.1 c	4.8 b	15.2 bc	4.4 c	0.3 c	5.9 c	8.9 b
EC×IL	88.7 a	3031 a	206.3 a	9.8 a	4.8 b	31.4 ab	21.1 a	1.3 a	6.2 bc	7.9 b
Significance										
S	**	*	***	**	**	ns	**	**	**	ns
LT	**	**	**	**	**	**	**	**	ns	**
S×LT	**	**	***	**	ns	**	**	***	***	**

Table 2. Eco-physiological responses in terms of leaf net photosynthesis (*Pn*), Stomatal conductance (*gs*), net leaf transpiration rate (*E*), Maximal photochemical efficiency of PSII ( $F_v/F_m$ ), the effective quantum yield of PSII ( $\Phi_{PSII}$ ), non-photochemical quenching (NPQ), SPAD and stomatal density in plants of Lettuce (L), Escarole Endive (EE) and Curly Endive (EC) grown in a floating raft in a coupled RAS system. Mean values (n=3), followed by different letters within each parameter indicated significant differenced based on Tukey HSD Post-hoc (P<0.05). Non-significance or significant differences at P ≤ 0.05, 0.01, or 0.001 are indicated as ns, \*\*, and \*\*\*, respectively.

Treatment	Pn (µmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	g₅ (mol m⁻² s⁻¹)	E (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	F <sub>v</sub> /F <sub>m</sub>	Φρςιι	NPQ	SPAD Index (SPAD unit)	Stomatal Density (%)
Species (S)								
L	3.46 b	0.12	3.46 b	0.61 b	0.25 b	1.45	26.07 a	37.26 c
EE	4.54 a	0.14	4.30 a	0.71 a	0.37 a	1.33	24.15 a	58.46 b
EC	4.10 ab	0.13	4.02 a	0.70 a	0.34 a	1.60	21.0 b	48.53 a
Lighting treatment (LT)								
ŇL	4.56 a	0.11 b	3.48 b	0.68	0.35 a	1.40	23.6	38.82 b
IL	3.51 b	0.15 a	4.38 a	0.67	0.28 b	1.55	24.3	57.34 a
Species × Lighting treatment								
L×NL	3.58 b	0.12 c	3.23 c	0.60 b	0.28 ab	1.44	15.9 abc	37.0 b
L×IL	3.35 b	0.13 bc	3.69 c	0.63 ab	0.21 b	1.55	18.1 ab	37.5 b
EE×NL	3.51 b	0.12 c	3.80 bc	0.74 a	0.39 a	1.27	13.4 bc	43.7 b
EE×IL	5.58 a	0.17 a	4.80 a	0.68 ab	0.36 ab	1.38	19.7 a	73.2 a
EC×NL	4.52 ab	0.10 c	3.41 c	0.71 ab	0.41 a	1.49	11.9 c	35.7 b
EC×IL	3.68 b	0.16 ab	4.64 ab	0.70 ab	0.27 ab	1.71	17.3 ab	61.4 a
Significance								
(S)	*	ns	**	**	*	ns	**	**
(ÌLŤ)	**	**	**	ns	**	ns	ns	**
Ś×LT	*	**	**	ns	ns	ns	**	**

Furthermore, in plants grown under IL, *gs* and *E* increased significantly only in EE and EC (+21%, +26%, and +32%, +39% respectively) while it did not vary in L (Table 2). SPAD index was lower in EC compared to EE and L (Table 2), in addition, IL significatively interacted with the species increasing SPAD values by +32% only EE (Figure 2).  $F_v/F_m$  under NL was higher in EE than L, 0.74 and 0.60, respectively (Table 2). All values except for L were close to 0.8.  $\Phi_{PSII}$  did not differ between the species under NL, and it was on average 0.359 (Table 2). Under NL conditions, NPQ was 17% higher in L compared to EE and EC (Table 2). IL did not decrease the ability to intercept light. However, the lower  $\Phi_{PSII}$  values were observed in L (0.21). The stomata density, under NL, was similar between the species (Table 2). while it increased in both the endives grown under IL compared to NL (+ 42 % and + 40 % for EC and EE, respectively), while L showed no significant variation (Table 2).

# DISCUSSION

Thanks to the nitrate-rich water, leafy vegetables are easy to grow crop in aquaponics systems. However, crop production and performance may vary among species, variety, and even seasons. In our growing condition, the three species showed morpho-physiological differences, with similar traits observed in the EE and EC compared to L that accounted, under NL, despite the similar yield in all the three species to a higher dry matter content in the endives. Chl *a* fluorescence measurements indicate plants in a healthy status (Baker, 2004). It is well known that aquaponics systems are deficient in essential nutrients like Fe, essential in the photosynthetic process (Kasozi et al., 2019). In our experiment, all plants showed a SPAD index lower than values retrieved in other studies in L and other leafy vegetables (Yang and Kim, 2019). However, this did not inhibit plant gas exchanges from being similar within the three species. It is well known that beside the direct influence on plant growth through the photosynthetic process (Pattison et al., 2018), light shapes and regulates several morphoanatomical modifications. Furthermore, most lighting strategies adopted in the cultivation systems aim to reach market goals by achieving species-specific DLI targets. Generally, in controlled environment cultivation of lettuce , a DLI of 17 mol m<sup>-2</sup> d<sup>-1</sup> is suggested and a minimum DLI of 10 mol  $m^{-2} d^{-1}$  is recommended in the winter period (Pennisi et al., 2020) There are no literature information on endives DLI requirements currently. In our study, we supplied plants with a LED light DLI of 10 mol m<sup>-2</sup> d<sup>-1</sup> under a 16 hours fixed photoperiod. As a result, plant growth was enhanced under IL treatment in all the species with different adaptation mechanisms. Indeed, IL promoted plant growth in the endives, by increasing leaf number, while it increased total leaf area in L. In both cases fresh and dry biomass increased compared to NL plants, likely for the higher plant light interception. It is also well known that when light intensity increases the  $\Phi_{PSII}$  may lower and, in turn, the ability to absorb light too (van Iersel et al., 2016). However, in our growing condition, despite  $\Phi_{PSII}$ decreased significantly in L, no effects were detected on the thermal dissipation, as revealed by the NPQ values, compared to the other species. These results correlate with the lower F<sub>v</sub>/F<sub>m</sub> values and gas exchange measurements, registered in L. On the other hand, under IL, gs and, in turn *Pn* and *E* rate increased in EE, whereas IL promoted only *gs* and *E* in EC and had no effect on L. It is worth noting that instantaneous gas exchange measurements are single photography over time that does not consider the previous plant's daily light history and temporary shadings due to greenhouse structures. It could explain the absence of difference in EC *Pn* values under both light regimes. In addition, at the time of measurement, IL plants already received the previous 6 hours, approximately 3.74 mol m<sup>-2</sup> d<sup>-1</sup> of light; therefore, IL light can be better used when solar radiation accounts less for the total light intensity. These findings positively correlate with the increase in stomatal density observed only in the endives, where this morpho-anatomical adaptation accounted for higher light use efficiency by increasing  $CO_2$  assimilation and water transpiration fluxes at the plant level, contributing to an increase in plant growth (Amitrano et al., 2021), Our results agree with Formisano et al (2021) who reported cultivar depended response of stomatal index in lettuce to light levels in greenhouse.

# CONCLUSIONS

To conclude, coupled aquaponics floating raft system can be a valuable means to produce leafy vegetables in urban areas and at a commercial scale, all the three species well performed. IL lighting was more effective on endives, and its application does not decrease plant photochemical performances. Our results suggest providing a fixed DLI to promote plant growth and shorten the growth cycle, especially in the winter period where NL is reduced. However, morpho-anatomical variations are species and/or cultivar dependent; this highlights the need to evaluate the species-variety pheno-plasticity to different lighting regimes to select a variety with traits able to adapt to different lighting environments improving crop performance and production.

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