



UNIVERSITI PUTRA MALAYSIA

***INCREASED BIOMASS PRODUCTION OF LETTUCE (*Lactuca sativa* L.)
THROUGH NUTRIENT MANAGEMENT IN A RED HYBRID TILAPIA
(*Oreochromis spp.*) BASED ON DECOUPLED RECIRCULATION
AQUAPONICS SYSTEM***

ABDEL RAZZAQ MOHAMMAD SAEED ALTAWAHA

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By

ABDEL RAZZAQ MOHAMMAD SAEED ALTAWAHA

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

May 2021

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DEDICATION

To my father's soul, and to my aunt Khadija. To my mother, who waited for me all the time through my doctorate studies and she counted each minute to return to her. To my wife and children, to my brothers, with special notice to Abdullah and Abdel Rahman, and to my sisters and my father and mother in law.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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May 2021

Chairman : Puteri Edaroyati bt Megat Wahab, PhD
Faculty : Agriculture

Improved aquaponics systems (APS) by adopting the decoupled recirculating aquaponics system (DRAPS) that has two independent circulations where can be controlled the physical and chemical characteristics of the water for the fish and plant independently and ability to supply macronutrients and micronutrients at an optimum concentration for plants is one of the most effective solutions to address food security issues. Thus, this study was conducted, and it consisted of four experiments. The first experiment was conducted based on a randomized complete block design (RCBD) to determine the ideal stocking density among 8 kg m^{-3} , 10 kg m^{-3} , and 12 kg m^{-3} and its effects on water quality, weight gain of tilapia and butterhead lettuce in the DRAPS. The results of the first experiment showed that the highest stocking density produced the highest concentrations of ammonia-nitrogen ($\text{NH}_3\text{-N}$), ammonium (NH_4), nitrate-nitrogen ($\text{NO}_3\text{-N}$), and potassium (K). It was reported that DRAPS that relied solely on fish waste would have an insufficient concentration of nitrogen (N), phosphorus (P), K, and iron (Fe). However, the results also showed that while the lowest stocking density (8 kg m^{-3}) produced the highest yield of butterhead lettuce, they were lower than the marketable yield of 150 g plant^{-1} .

The lowest stocking density of 8 kg m^{-3} was subsequently used in the second experiment to evaluate the effects of three ratios of nitrate to urea±nickel (Ni) at (60:40–Ni, 60:40+Ni, and 100:0 as control) and two different seedling ages (14 days and 21 days) on the growth, yield, and the leaf nutrient content of the butterhead lettuce in DRAPS. The experimental design was based on an RCBD split-plot design with nitrate: urea ratio as the main plot, seedling age as the sub-plot, and three replicates for each treatment. The results showed that the shoot fresh weight of the butterhead lettuce transplanted at the seedling age of 21 days and supplemented with nitrate urea ratios of 60:40–Ni and 60:40+Ni decreased by 40.8% and 43.5%, respectively as compared to that

supplemented with nitrate urea at the ratio of 100:0. When the lettuce was supplemented with fertilizer at the ratio of 100:0, the total shoot weight of the lettuce transplanted at the seedling age of 14 days decreased by 20.8% as compared to that of the 21-day. It was observed that the lettuce that was supplemented with Ni produced higher leaf N, K, sodium (Na), and Fe contents than those without Ni. Controlling the pH and introducing inorganic fertilizer in the second loop of DRAPS had significantly increased the total fresh yield per unit area by 91% compared to that as reported in Chapter 3. The nitrate urea ratio of 100:0 and seedling age of 21 days were determined as the ideal treatments and they were used in the third experiment. It was observed from experiments 1 and 2 that Fe is one of the most limited micronutrients in APS. Therefore, the third experiment was conducted to evaluate the effects of Fe sources and nutrient solution on the growth, yield, and leaf nutrient content of the salad in DRAPS. The experiment was set up as a factorial with two factors (first factor: Fe-sources [Fe-DTPA and Fe-EDTA]; second factor: Nutrient solution [inorganic fertilizer and recycled nutrient solution]) based on an RCBD with three replicates for each treatment. The results showed that the highest total fresh weight of the salad was detected in the DRAPS treated with Fe-DTPA and complemented nutrient solution which was 6.2% higher than those in the DRAPS treated with Fe-EDTA from the same season.

The optimum planting density (PD) should be determined according to agronomic assessment, which takes growth performance, water use efficiency (WUE), and recycled nutrient solution use efficiency into consideration. In this context, the effects of two PD (16 plants m⁻² and 32 plants m⁻²) and two nutrient solutions on the salad in DRAPS were studied in the fourth experiment. The ideal stocking density (Experiment 1), nitrate urea ratio, seedling age (Experiment 2), and Fe source (Experiment 3) were used in this experiment. The highest shoot fresh weight of the butterhead lettuce was recorded in the interaction of PD of 16 plant m⁻² and complemented nutrient solution, while the highest total fresh weight was recorded in the PD of 32 plants m⁻² and Complemented nutrient solution. The results collected from all four experiments demonstrated the potential of DRAPS in optimizing nutrient availability and increasing the butterhead lettuce biomass production. In conclusion, this study had successfully used DRAPS to produce butterhead lettuce and tilapia, which has proven the sustainability of DRAPS with two independent loops as an agricultural production system capable of increasing WUE and minimizing the inorganic nutrient supplements by integrating the production of fish and plant.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENINGKATAN HASIL BIOJISIM SALAD (*Lactuca sativa* L.) MELALUI
PENGURUSAN NUTRIEN DALAM TILAPIA MERAH HIBRID (*Oreochromis
spp.*) BERASASKAN SISTEM AKUAPONIK EDARAN SEMULA
NYAHGANDING**

Oleh

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Sistem akuaponik (APS) yang ditambah baik dengan menggunakan sistem akuaponik kitar semula yang terpisah (DRAPS) dengan dua kitaran tersendiri yang dapat mengawal ciri fizikal dan kimia air untuk ikan dan tumbuhan secara berasingan dan kemampuan untuk membekalkan makronutrien dan mikronutrien pada kepekatan optimum untuk tanaman adalah salah satu penyelesaian yang paling berkesan untuk mengatasi masalah keselamatan makanan. Oleh itu, kajian ini dijalankan dan ia terdiri daripada empat eksperimen. Eksperimen pertama dilaksanakan berdasarkan reka bentuk blok rawak lengkap (RCBD) untuk menentukan ketumpatan stok optimum antara 8 kg m^{-3} , 10 kg m^{-3} , dan 12 kg m^{-3} dan kesannya pada tilapia dalam DRAPS. Hasil eksperimen pertama menunjukkan bahawa ketumpatan stok tertinggi menghasilkan kepekatan ammonia-nitrogen ($\text{NH}_3\text{-N}$), amonium (NH_4), nitrat-nitrogen ($\text{NO}_3\text{-N}$), dan kalium (K) yang tertinggi. Dilaporkan bahawa DRAPS yang hanya bergantung pada sisa ikan akan mempunyai kepekatan nitrogen (N), fosforus (P), K, dan besi (Fe) yang tidak mencukupi. Meskipun demikian, hasil eksperimen juga menunjukkan bahawa walaupun hasil salad butterhead adalah tertinggi pada ketumpatan stok terendah (8 kg m^{-3}), hasil tersebut adalah lebih rendah daripada hasil yang dapat dipasarkan, iaitu $150 \text{ g tumbuhan}^{-1}$. Ketumpatan stok terendah, iaitu 8 kg m^{-3} digunakan dalam eksperimen kedua untuk menilai kesan-kesan tiga tahap nitrat (urea±nikel (Ni) pada 60:40–Ni, 60:40+Ni dengan 100:0 sebagai kawalan) dan dua umur anak benih yang berbeza (14 hari dan 21 hari) pada pertumbuhan, hasil, dan kandungan nutrien daun salad butterhead dalam DRAPS. Reka bentuk eksperimen didasarkan pada rancangan petak terpisah RCBD dengan nisbah nitrat:urea sebagai petak utama, umur anak benih sebagai anak petak, dan tiga ulangan untuk setiap olahan. Hasil eksperimen menunjukkan bahawa berat segar tunas salad butterhead yang ditanam dengan menggunakan anak benih berumur 21 hari dan dibaja dengan baja bernisbah 60:40–Ni dan 60:40+Ni masing-masing menurun sebanyak 40.8% dan 43.5% berbanding dengan salad butterhead yang dibaja dengan baja bernisbah 100:0. Apabila salad butterhead dibaja dengan baja bernisbah 100:0, jumlah berat tunas salad butterhead yang ditanam dengan menggunakan anak benih berumur 14

hari menurun sebanyak 20.8% berbanding dengan salad butterhead yang ditanam menggunakan anak benih berumur 21 hari. Salad butterhead yang dibaja dengan Ni diperhatikan menghasilkan kandungan N, K, sodium (Na), dan Fe daun yang lebih tinggi daripada yang tanpa Ni. Pengoptimuman pH dan penambahan baja sintetik pada lingkaran kedua DRAPS telah meningkatkan jumlah hasil segar bagi setiap unit kawasan sebanyak 91% berbanding dengan yang dilaporkan dalam Bab 3. Nisbah baja 100:0 dan umur anak benih 21 hari ditentukan sebagai olahan optimum dan digunakan dalam eksperimen ketiga. Dari eksperimen 1 dan 2, dapat diperhatikan bahawa Fe ialah salah satu mikronutrien yang paling terhad dalam APS. Oleh itu, eksperimen ketiga dijalankan untuk menilai kesan-kesan sumber Fe dan musim tanam pada pertumbuhan, hasil, dan kandungan nutrien daun salad butterhead dalam DRAPS. Eksperimen ini menggunakan reka bentuk faktorial dengan dua faktor (faktor pertama: sumber Fe [Fe-DTPA dan Fe-EDTA]; faktor kedua: musim tumbuh [baja sintetik dan larutan nutrien kitar semula]) berdasarkan RCBD dengan tiga ulangan untuk setiap olahan. Hasil eksperimen menunjukkan bahawa jumlah berat segar salad butterhead yang paling tinggi didapati daripada DRAPS yang diolah dengan Fe-DTPA pada musim tanam pertama dan ia adalah 6.2% lebih tinggi daripada jumlah berat segar salad butterhead dalam DRAPS yang diolah dengan Fe-EDTA pada musim yang sama. Fe-DTPA diperhatikan sedikit meningkatkan kepekatan N, K, kalsium (Ca), dan magnesium (Mg) dalam daun, sedangkan Fe-EDTA meningkatkan kepekatan Fe, kuprum (Cu), dan mangan (Mn) dalam daun. Ketumpatan penanaman (PD) optimum harus ditentukan dengan penilaian agronomi yang mempertimbangkan prestasi pertumbuhan, kecekapan penggunaan air (WUE), dan kecekapan penggunaan larutan nutrien kitar semula. Dalam konteks ini, kesan-kesan dua PD (16 tumbuhan m^{-2} dan 32 tumbuhan m^{-2}) dan dua musim tanam pada salad butterhead dalam DRAPS dikaji dalam eksperimen keempat. Ketumpatan stok (Eksperimen 1), nisbah baja, umur anak benih (Eksperimen 2), dan sumber Fe (Eksperimen 3) yang optimum digunakan dalam eksperimen ini. Berat segar tunas salad butterhead tertinggi dicatat pada musim tanam pertama dengan PD 16 tumbuhan m^{-2} , sementara jumlah berat segar tertinggi dicatat pada musim tanam pertama dengan PD 32 tumbuhan m^{-2} . Hasil yang dikumpulkan daripada keempat-empat eksperimen ini memperagakan potensi DRAPS dalam mengoptimumkan ketersediaan nutrien dan meningkatkan pengeluaran biomas salad butterhead. Kesimpulannya, kajian ini telah berjaya menggunakan DRAPS untuk pengeluaran salad butterhead dan tilapia. Hal ini telah membuktikan kelestarian DRAPS yang mempunyai dua lingkaran bebas sebagai sistem pengeluaran pertanian yang mampu meningkatkan WUE dan meminimumkan suplemen nutrien sintetik dengan mengintegrasikan pengeluaran ikan dan tumbuhan.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

APS	Aquaponic systems
AOA	Ammonia-Oxidizing Archaea
AOB	Ammonia-Oxidizing Bacteria
CNS	Complemented nutrient solution
DRAPS	Decoupled recirculation aquaponics system
DWC	Deepwater culture
DTPA	Diethylenetriaminepentaacetic acid
DO	Dissolved oxygen
EC	Electric conductivity
EDTA	Ethylenediaminetetraacetic acid
FCR	Feed conversion ratio
L	Liter
LAR	Leaf area ratio
LWR	Leaf weight ratio
NOB	Nitrite-oxidizing bacteria
WUE	Water use efficiency
NUE	Nitrogen use efficiency
NFT	Nutrient film technique
PWU	Product water use
RZT	Root zone temperature
SC	Substrate culture
RGR	Relative growth rate
RNS	Recycled nutrient solution

SRAPS	Single recirculation aquaponics system
SLA	Specific leaf area
SR	Survival rate
TDS	Total dissolved solids
T	Water temperature
WG	Weight gain



CHAPTER 1

INTRODUCTION

1.1 Background to the study

In the last three decades, the single recirculation aquaponics system (SRAPS) achieved extraordinary attention due to an influential and significant role in improving sustainable food production (Goddek et al., 2015) by providing sufficient plant and fish products (Rakocy et al., 2006) for human consumption, which has a direct impact on the future of global food security (Maucieri et al., 2019; Rakocy et al., 2006). However, managing SRAPS was anticipated to be a complicated process to achieve optimal conditions towards the sustainable production of three organisms: fish, bacteria, and plants, in terms of pH, nutrients, and water quality. Interestingly, an innovation was proposed by Kloas et al. (2015), who named this approach as decoupled recirculation aquaponics system (DRAPS) to address the issue.

DRAPS is an alternative approach (2-loop systems) and has been gaining a significant amount of interest among the community as a sustainable agricultural production since 2015. Moreover, this system received tremendous attention due to its many advantages over SRAPS (Suhl et al., 2016; Kloas et al., 2015). One of the most critical features is the separate and highly controlled conditions for fish, microbes, and plants, as reported by Suhl et al. (2016) and Kloas et al. (2015), where the imbalances can be managed accurately for various parameters such as pH, electrical conductivity (EC), dissolved oxygen (DO), root zone temperature (RZT), and N compounds (NH₃-N, NH₄-N, NO₂-N, NO₃-N). In addition, the significance of DRAPS is the ability to supply macronutrients and micronutrients at an optimum concentration as independent factors for maximizing the growth and yield of plants.

The first study for DRAPS was conducted by integrating tomato and tilapia under nutrient film technique (NFT) by Kloas et al. (2015). Then, Suhl et al. (2016) and Kloas et al. (2015) identified a research gap that required further improvement: optimising and adjusting the production of the nutrient solution generated from the fish culture to plant ratio for different species. Thus, the optimal red hybrid tilapia (*Oreochromis spp.*) stocking density to lettuce (*Lactuca sativa*) ratios were not comprehended because the majority of the past studies prioritized the evaluation of the efficiency of DRAPS for producing tomato and tilapia (Monsees et al., 2017; Suhl et al., 2016; Kloas et al., 2015). Optimizing fish stocking density is critical for producing optimum N level by converting ammonia to nitrate through nitrifying bacteria, with nitrate as the basic requirement for plant functions such as photosynthesis and regulating plant morphology, physiology, and mineral content.

The utilization of urea [CO(NH₂)₂] as a supplementary N source has become possible due to the two-loop design of DRAPS. Therefore, finding the optimal ratio of urea with inorganic nitrate will reduce fertilizer costs to produce leafy crops. Urea is well known for its potential as a reliable N source due to its high N content (46%). However, modern agricultural systems such as hydroponics and coupled APS seldom use urea as the source of N due to the concern in the build-up of NH₄-N. This is because urea will be broken down to NH₄-N, and if the concentration becomes 3 mg L⁻¹ or higher, it can be toxic to the fish. Conversely, this would not be a concern in DRAPS due to the advantages of having two loops. In DRAPS, the fish culture is located in the first loop, which will not be affected by the application of urea or its conversion to NH₄-N. However, the utilization of urea is necessary for APS, especially DRAPS, because NO₃-N is the main product of the nitrification process and is the main source of N for most crops such as lettuce. Previous studies reported that urea supplied together with NO₃-N has reduced nitrate accumulation in leafy vegetables (Zhu et al., 1997). Gunes et al. (1994) also reported that the fresh or dry yield of lettuce under NFT was not affected by the replacement of 20% NO₃-N by urea in the nutrient solution.

Nickel (Ni) is one of the most effective micronutrients of urea in the nutrient solution as it is a component of the urease enzyme (Dixon et al., 1980) and other enzymes that are responsible for nitrate reduction. The urease enzyme was essentially used in urea assimilation by plant tissues to hydrolyze urea (Witte, 2011). Thus, Ni is needed to increase urease activity and avoid any urea toxicity symptoms in crop tissues (Marschner, 1995; Benchemsi-Bekkari and Pizelle, 1992). Moreover, Ni also plays a crucial role in N metabolism (Brown et al., 1990) in higher plants (Hansch and Mendel, 2009; Barker and Pilbeam, 2007). However, limited studies were available on urea optimization through the supplementation of micronutrients such as Ni in the nutrient solution for producing lettuce (Khoshgoftarmanesh et al., 2011).

Seedling age is an important agronomic factor and is a vital strategy to increase the plants' growth, development, and yield in APS. Numerous studies have reported on the production of lettuce under APS. However, the seedling age varied from ten to 21 days (Monsees et al., 2019; Pérez-Urrestarazu et al., 2019; Velichkova et al., 2019; Jordan et al., 2018b; Delaide et al., 2016). Therefore, the standardization of seedling age of lettuce with an ideal number of leaves, leaf area and root canopy in an APS plays a vital role in growth and yield in improving the uptake of nutrients, enhancing nutrient use efficiency, and preventing stunted growth of the plants. Furthermore, the seedling age at the time of transplantation is expected to have significant impacts on plant growth and yield. To date, the effect of seedling age at the time of transplantation on subsequent plant performance in DRAPS were not studied.

Iron (Fe) is one of the most limited micronutrients in APS (Kasozi et al., 2019) and is the third most limiting nutrient for plant growth and metabolism (Rout and Sahoo, 2015; Samaranyake et al., 2012; Zuo and Zhang, 2011). Fe is not released in an adequate concentration by the nitrification process in APS (Roosta and Hamidpour, 2011; Rakocy et al., 2006). Therefore, the Fe levels will be too low to sustain hydroponic vegetable production in APS that solely depends on fish metabolism to provide nutrients for plants

(Roosta and Mohsenian, 2012; Seawright et al., 1998). Nonetheless, Fe is an essential nutrient for crop production in APS and must be optimized to an adequate level (2-5 mg L⁻¹) in the hydroponic production unit (Kasozi et al., 2019; Roosta and Mohsenian, 2012; Tyson et al., 2011; Rakocy et al., 2006). The deficiency in Fe will result in poor yield and low nutritional quality (Rout and Sahoo, 2015), inhibit photosynthesis, cause lower stomatal conductance, and decrease the transpiration rate of plants (Larbi et al., 2006). Fe can be supplemented through different methods, such as chelated to water or as a foliar application. Different sources of chelated Fe can be used in APS, such as Fe-EDTA and Fe-DTPA. Consequently, Randall et al. (1999) reported the safe level of Fe concentration for aquatic culture as 1.69 mg L⁻¹. In a subsequent study, Phippen et al. (2008) endorsed a total Fe concentration at an optimum level of 0.35 mg L⁻¹ to 1.0 mg L⁻¹ for aquatic culture.

Using recycled nutrient solution (RNS) in aquaponics and hydroponic systems has become increasingly popular in ecosystems sustainability (Bugbee, 2004). Moreover, Paulus et al. (2012) reported the feasibility of using nutrient solutions prepared with low-quality water or RNS for producing lettuce in hydroponic systems, which possessed multiple benefits (Askari-Khorasgania and Pessaraki, 2020). Hence, the benefits of RNS as compared to conventional and hydroponic cultivation are promised by APS and DRAPS (Askari-Khorasgania and Pessaraki, 2020), which thereby increases the energy, water, and nutrient uptake efficiency, financial gains, and sustainability of plants and ecosystems (Askari-Khorasgania and Pessaraki, 2020). Previous studies by Zekki et al. (1996) reported that the RNS does not restrict crop yields. Bugbee. (2004) had also reported that there is a limited understanding of nutrient management in RNS. Thus, the management of RNS alongside fish nutrient solution is highly needed.

Planting density (PD) is one of the most crucial agronomic factors for optimizing the quality and quantity of lettuce yield (Maboko, 2013) in APS and hydroponics (Makhadmeh et al., 2017). PD plays a vital role in promoting the efficient use of sunlight for photosynthesis, water, and nutrients. PD is an effective crop management factor that affects productivity by regulating growth, development, and yield. Moreover, high PD will result in intense competition between plants for radiation needed for leaf photosynthetic capacity (Ma et al., 2014), water, and nutrients (Bugbee, 2004). Thus, the optimization of PD is a vital step towards improving lettuce performance.

1.2 Statement of the Problem

Managing a single recirculation aquaponics system was anticipated to be a complicated process; it is a complex system due to needing to optimize the physical and chemical properties such as pH, EC, DO, and temperature of the water for three types of organisms, which are fish, nitrifying bacteria, and plants in a single loop. In addition, it is challenging to apply a mineral salt without adversely impacting the fish and nitrifying bacteria. Therefore, the decoupled recirculation aquaponics system with a two-circulation design is the key advantage that optimizes water's physical and chemical parameters such as pH, EC, DO, root-zone temperature, and N compounds to maximize the yield of plants and fish. Also, its capability to supply the plants with the optimal

concentrations of inorganic macronutrients and micronutrients in producing the highest quantity and quality of crops without toxifying the fish culture and causing adverse effects on the nitrifying bacteria.

1.3 Objectives of the study

The general aim of this study was to optimize the DRAPS as a sustainable food production system through the integration of tilapia (*Oreochromis spp*) and lettuce (*Lactuca sativa* L.) in small-scale production. The specific objectives of this study to improve and optimize DRAPS were as follow.

1. To determine the ideal stocking density of tilapia (*Oreochromis spp*) for enhanced water quality, the growth performance of both fish and lettuce (*Lactuca sativa*) yield without the addition of fertilizers in DRAPS.
2. To evaluate the potential of different nitrogen form/ratios with and without Nickel and seedling ages on biomass production, relative growth rate and leaf nutrient contents of lettuce in DRAPS.
3. To evaluate the effect of different chelated iron and planting densities with recycled nutrient solutions on the growth, yield, water use efficiency and leaf nutrients contents of lettuce in DRAPS

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