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Yield and Nutrients Leaf Content of Butterhead Lettuce (*Lactuca Sativa*) in Response to Fish Nutrient Solution in a Small Scale of Aquaponic Systems

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ABSTRACT

This study was conducted over 52 days to evaluate the potential of using the nutrient solution produced from different fish stocking densities on the yield and nutrients leaf content of lettuce cultivated in decoupled recirculation aquaponic systems (DRAPS). In this study, three stocking densities of tilapia were used 8, 10 and 12 kg $m^{-3,}$ respectively. The result showed the highest total yield 448 g m^{-2} was obtained at a low stocking density. Also, it has been showing that with an increased fish stocking density, the leaf nutrient content of butterhead lettuce for potassium, calcium, magnesium, sodium, iron and copper was increased. While for the nitrogen, phosphorus and manganese, the higher leaf content was obtained at lower stocking density. Considering the lettuce yield, leaf nutrient contents and nitrogen dynamics, the stocking density of 8 kg m^{-3} could be suggested as the ideal stocking density for DRAPS.

Keywords: Butterhead lettuce, decoupled recirculation aquaponic systems, stocking density, tilapia.

INTRODUCTION

Aquaponics system (APS) plays an influential and significant role in improving sustainable food production in the future (Goddek *et al*., 2016) 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. Aquaponics combines RAS with soilless culture systems through the nitrification process to produce a non-toxic form of nitrate (NO_3-N) as the final product of nitrogen (N) (Rakocy *et al*., 2006; Al Tawaha *et al*., 2021). This system was one of the fastest-growing agricultural food production systems (Rakocy *et al*., 2006; Graber and Junge 2009; Rakocy 2012; Somerville *et al*., 2014).

Stocking density (SD) is a crucial factor in DRAPS with a high planting density. It affects the productivity of macronutrients through the biological process such as NO_3-N, P and K, which is required for plant growth and development (Al-Tawaha *et al*., 2021). Furthermore, a high stocking density affects the water quality parameters of a small-scale APS by reducing the DO and increasing the NH_3-N, NO_3-N, total suspended solids, salinity, and EC (Al-Tawaha *et al*., 2021). Hence, if the stocking density of a DRAPS is high, the conditions such as the surface area of biofilter materials, size ratio of the rearing tank to biological tank, and the efficiency of the mechanical filter need to be optimized to have a high amount of nitrifying bacteria in the system. In other words, high stocking density means more N is produced and movement to the lettuce root zone in the second circulation from the first circulation. Al-Hafedh *et al*. (2008) examined the lettuce yield under different planting densities, ranging from 25 to 42 plants m^{-2} and integrated with tilapia in the deepwater culture system. The study showed that low planting density at 25 to 30 plants m^{-2} was ideal for high lettuce yield. The possible reason could be that the stocking density was not optimal for high planting density at 42 plants m^{-2} and hence, the sufficient of NO_3-N for the plants.

Lettuce is one of the most economical crops (Sala and Costa, 2012) traditional APS (Effendi *et al*., 2015; Wahyuningsih *et al*., 2015; Anderson *et al*., 2017) and decoupled aquaponics (Monsees *et al*., 2019; Al Tawaha *et al*., 2021) because of its low nutrients requirement (Diver, 2006; Rakocy, 2012). In addition, lettuce is a hardy plant with a fast growth rate ranging from four to five weeks of vegetative growth phases to harvest and has high global consumption. Similarly, tilapia is one of the most important worm fish species in APS (Rakocy *et al*., 2006; Love *et al*., 2015). It has high availability and an easily cultivable nature, is fast-growing, and possesses high tolerance water conditions such as pH, water temperature, dissolved oxygen (DO) (El-Sayed, 2006). As a result, tilapia culture has been practiced widely to reduce the rising worldwide demands for protein sources and its high economic value (Diver, 2006). Therefore, the success of the symbiotic integration of tilapia and lettuce in APS has a significant role in sustaining the agriculture production system (Tyson *et al*., 2011; Effendi *et al*., 2017; Al Tawaha *et al*., 2021). In APS, SD is a vital factor in maintaining water quality for growth and yield for fish and plants. There were limited studies on the response of lettuce to a nutrient solution produced at different SD under DRAPS. Therefore, the objective of this study was to determine the effect of nutrient solution of different stocking densities of tilapia (*Oreochromis niloticus*) on the yield and nutrients leaf content of lettuce (*Lactuca sativa*) grown under DRAPS without the addition of inorganic fertilizers.

MATERIALS AND METHODS

Set-up of DRAPS

This experiment was conducted at the Faculty of Agriculture, UPM Serdang, Selangor, Malaysia and the total area for this experiment 85 m^2. The APS used in this experiment was composed of two loops. The first loop of APS formed of the complete recirculation aquaculture system to produce nutrient-rich water through biological processes. The first loop composed of a single tank (350 liter) for a fish tank connected to a mechanical tank (45 liter). At the end of the mechanical filtering step, the filtered water flowed into a biofilter tank (45 liter), and then the water was then connected to a sump tank (300 liter) to complete the first circulation. The first loop had a total capacity of 740 liter, and its flow rate of the water was 6.4 L min^{-1} (Endut *et al*., 2010). While the second loop of DRAPS composed of the second sump tank (300 liter) with an HP component (NFT), and the water flow rate in the second loop was between 1-2 liter

Figure 1. Schematic illustrated the original DRAPS was used in this experiment

per minute. The first circulation's nutritional solution was supplied to the second circulation via a one-way valve. The DRAPS's total water volume was kept at 1040 liter. No water was discharged during the trial period except for loss via evaporation, transpiration, and sludge clearance, which was less than 5% under tropical circumstances.

Plant and fish materials

Before transplantation, the butterhead lettuce (*Lactuca sativa*) seeds from a company called (Green World Genetics) were planted and cultivated in a seed tray for 14 days to guarantee that the seedlings were of the same size at the time of transplanting. Then, the seedlings of 14 days old were moved to the HP units. The planting density 32 plants m^{-2}, with a spacing of 15 cm between each plant. The red tilapia (*Oreochromis niloticus*) utilized in this study were purchased from an aquaculture farm at Universiti Putra Malaysia in Puchong, where they were raised in a standard aquaculture system. All red tilapia were first stocked at an average weight of 125 g ± 20 g in each of the nine DRAPS. The daily feeding rate was 2% of the tilapia's body weight. As indicated in Table 1, the feed was a commercial floating pellet from Dindings Company. The diameter of pellet was 3.2 mm Company. The fish were hand-fed twice daily for 30 minutes at 9.00 am. and 5.00 pm.

Experimental design set up and treatments

The study was conducted between April 6, 2019 and May 16, 2019. The experimental design was a randomized complete block design (RCBD) in its construction. Stocking densities were established using the lowest density required to provide sufficient nitrogen for butterhead lettuce development in a small-scale APS. In this investigation, stocking densities of (8, 10 and 12 kg m^{-3}) were employed as T1, T2, and T3, respectively. Thus, stock densities were (2.8, 3.5 and 4.2 kg $tank^{-1}$), respectively, per 350 liter fish tank. Each stocking density was replicated three times, with each replicate linked to an HP unit. Thus, the total number of HP was nine units.

Table 1. The proximate nutrient composition of the fish feed

Proximate nutrient composition	Percentage (%)
Protein	32
Fat	5
Ash	10
Fiber	5
Moisture Content	10

Chemical water quality measurement

Each week, the concentrations of N compounds (i.e. NH_3-N, NH_4-N, and NO_2-N) in the fish tanks were monitored. HI 83200, HANNA instruments, Woonsocket, RI, USA, was used for the measurement.

Yield and relative chlorophyll content of lettuce

At the harvest stage, The total fresh yields of the lettuce were measured. The lettuce samples were divided into shoot and root. These samples weighted using a ME analytical Weighing Balance (Mettler Toledo Inc.). The relative chlorophyll content was measured on the three youngest fully expanded leaves using a portable chlorophyll meter (Konica Minolta SPAD-502 Plus) at 14 and 21 days after transplanting (DAT) lettuce.

Leaf tissue analysis of lettuce

The lettuce leaf tissues were analyzed for the concentration of total N, P, K, Calcium (Ca), Magnesium (Mg), Iron (Fe), Copper (Cu), Manganese (Mn) and Sodium (Na). The leaves were prepared for analysis by the following method. First, the fresh lettuce leaves obtained at the harvest time were dried to constant weight in an oven. Approximately 0.25 g of the powdered sample was added into a digestion tube. Exactly 5 ml of concentrated sulphuric acid (H_2SO_4) was then added to the tube, and the tube was rotated repeatedly until the sample was well mixed. The tube was left to stand for at least two hours or overnight. After that, inside a fume cupboard, 2 ml of 50% hydrogen peroxide (H_2O_2) was added slowly down the sides of the tilted tube, and the tube was gently shaken. The tube was then placed in a digestion block after the reaction with H_2O_2 had subsided. The lettuce sample was digested for 45 minutes at 285 °C and allowed to cool. After that, H_2O_2 was again added slowly in drops until the digested lettuce sample turned to a clear or colorless solution. The clear solution was then cooled and diluted with distilled water and filtered. The filtrate was topped up with distilled water until 100 ml mark and was analyzed using the Perkin Elmer Analyst 400 Spectrophotometer Flame method (USEPA, 1983) at the AAS Laboratory, which was located in the Department of Land Management, Faculty of Agriculture, UPM.

Statistical analysis

The experimental design was a randomized complete block design (RCBD) with three replicates. Using the Statistical Analysis System (SAS), version 9.4, the data were examined using an ANOVA (SAS Institute Inc., Cary, NC, USA). The means were compared by using the LSD test with a significance level of 0.05.

RESULTS

Water quality parameters

The dynamic trends of NH_3-N, NH_4^+, total ammonia nitrogen (TAN), and NO_2-N are presented in Figure 2. Effendi *et al*. (2020) reported that NH_3 in APs was generally measured as TAN, which composed of NH_3-N in Figure 2a and NH_4^+ in Figure 2b. The variation of TAN of all treatments is presented in Figure 2c. The fluctuation of nitrite level during the experiment is presented in Figure 1d. In this study, the concentrations of NH_3-N, NH_4^+, and NO_2-N in Figure 2d in all the treatments increased from day zero to day seven due to the accumulation of excess feeds and fish feces, and began to decrease from the eighth day of the study until the end. The decreased in levels of NH_3-N, NH_4^+, NO_2-N, and NO_2 in this study could be an indication of a well-developed microbial community in different stocking densities. The NO_3-N and PO_4-P, K, Ca and Fe as mentioned in (Al Tawaha *et al*., 2021).

Total fresh weight of lettuce

Figure 3 and 4 illustrates the possibility of utilizing the water from the different fish stocking densities to support lettuce growth. The results showed that there were significant differences ($p<0.05$) in the parameters like total fresh weight (g m^{-2}) and plant height. The highest total fresh weight of 448 g m^{-2} was observed in the lowest stocking density (T3) (Fig. 3). At the same time, the lowest total fresh weight of 302.82 g m^{-2} was observed in T2. Moreover, the highest lettuce height was found in T1 and the lowest in T2 (Fig. 4).

Relative chlorophyll content

The difference in the relative chlorophyll content of the lettuce was insignificant ($P>0.05$) among the treatments during the vegetative growth. The relative chlorophyll content values ranged from 15.49 to 18.47 after two weeks of

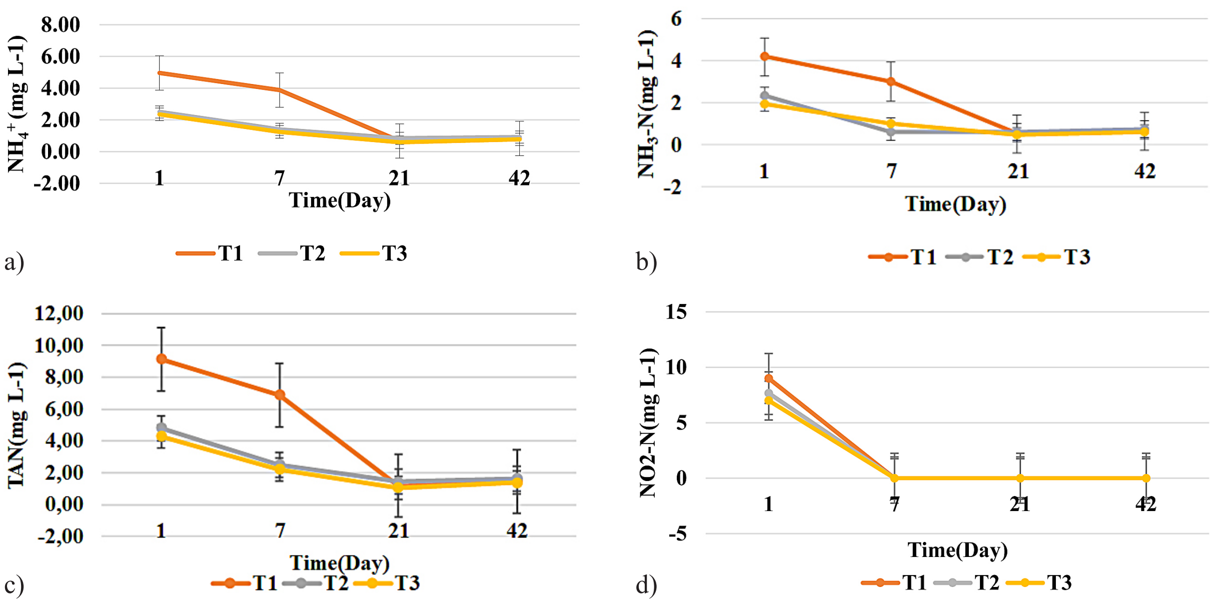

Figure 2. Dynamic of water quality parameters from tilapia rearing tank in different stocking densities in DRAPS. (a) Unionized ammonia nitrogen (NH_3-N), (b) ammonium (NH_4^+) (c) total ammonia nitrogen (TAN) and (d) nitrite-nitrogen (NO_2-N)

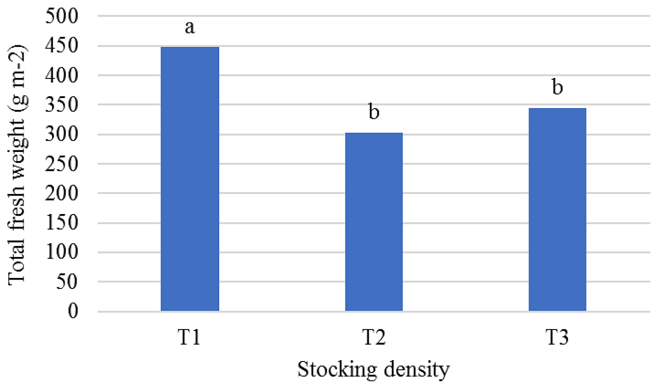

Figure 3. Total fresh weight of lettuce at different stocking densities under DRAPS conditions. Means for each treatment followed with the same letter are not significantly different at p-value 0.05 using LSD

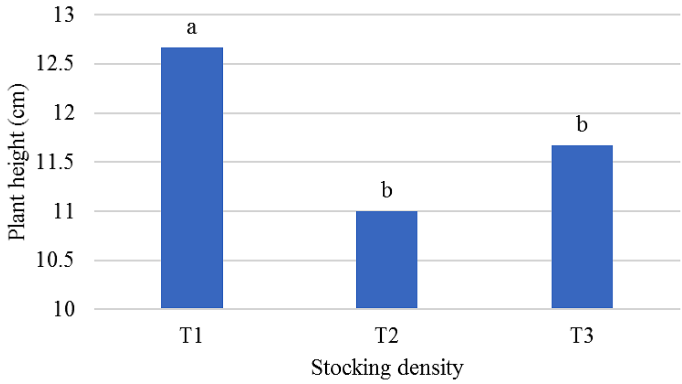

Figure 4. Plant height of lettuce at different stocking densities under DRAPS conditions. Means for each treatment followed with the same letter are not significantly different at p-value 0.05 using LSD

Figure 5. Lettuce yield in NFT under DRAPS. T1: Stocking density (8 kg m^{-3}), T2: stocking density (10 kg m^{-3}) and T3: stocking density (12 kg m^{-3})

transplanting, whereas the content values after three weeks ranged from 9.20 to 10.22.

Leaf mineral concentrations

The statistical analysis showed that there were significant differences ($p<0.05$) in the leaf content of N, Ca, Mg, Na, Fe, Cu and Mn among the treatments excluding P and K. The N leaf content was significantly reduced at T1 and T2 by 13% and 9% than T3 (Fig. 7). In contrast, higher P leaf content was obtained from T1 (Fig. 7). The leaf content of K ranged from 69.36 to 73.76 mg g^{-1} (Fig. 7). The Ca leaf content was reduced by 27.9% and 19.9% at T3 and T2 than T1 (Fig. 7), while Mg content was significantly reduced by 30.9% and 18.3% at T3

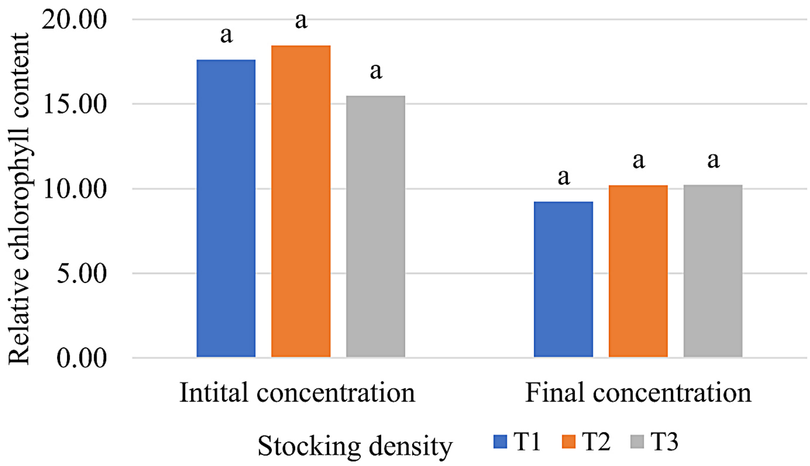

Figure 6. The initial and final relative chlorophyll content in lettuce leaf at different stocking densities under DRAPS Conditions. Means followed with the same letter are not significantly different at p-value 0.05 using LSD

and T2 than T1 (Fig. 7). The micronutrients such as Na content were significantly reduced by 48.6% and 47.7% at T3 and T2 than T1. However, the Fe content was reduced by 41.9% and 29.2% at T3 and T2 than T1. The Cu leaf content was significantly reduced by 37.5% and 68% at T3 and T2 than T1. The Mn leaf content was significantly reduced by 16.5% and 27.9% at T1 and T2 than T3 (Fig. 8).

DISCUSSION

Total fresh yields were less than reported in Licamele (2009), Schmautz *et al*. (2017), and Madar *et al*. (2019). Nevertheless, the total fresh yield of lettuce in this study at low stocking density was higher than the yield obtained in the study by Johnson *et al*. (2017), which was 0.418 $kg \cdot m^{-2}$. Under the experimental conditions, it was observed that the lettuce seedling grew well for the first two weeks in the NFT trough. However, the stunted growth of lettuce plants was observed at the beginning of week three, which may have caused by insufficient nutrients produced by the nitrification process in DRAPS, especially nitrate. The function of nitrate in the performance of DRAPS and the optimum development of leafy plants such as lettuce is critical. The low nitrate and iron levels in all the treatments and the high plant density of 32 plants per square meter have restricted the lettuce's development. The deficiency symptoms of chlorosis and growth suppression began to appear on the young leaves of lettuce. The insufficient level of phosphorus, potassium, magnesium, and calcium produced by the nitrification process stunted the growth of lettuce. This consists of Rakocy et al. (2007) stating that low yield is associated with inadequate levels of phosphorus, potassium, iron and manganese in the nutrient solution. In addition, to high water temperature for a nutrient solution was not in the optimal range, which is decreasing the absorption of the nutrients.

The chlorophyll content in leafy plants such as lettuce is one of the most important physiological indicators of the level of N and Fe in the plants. Nitrogen plays a significant role in forming chlorophyll pigments, whereas Fe is important in leaf photosynthesis by increasing the number of photosynthetic units per area (Spiller and Terry 1980). The initial and final SPAD values for the lettuce plants in this study were lower than the SPAD values reported by Pantanella *et al*. (2010), Maucieri *et al*. (2019), and Nozzi *et al*. (2018), which were 34.1, 24.1, and 22.0, respectively. Low concentrations of N and Fe in the nutrient solutions of the NFT units may be the possible explanation for the low SPAD values. However, the SPAD value of the lettuce in this study for all the treatments, which was 9.6 at average, was higher than that reported by Maucieri *et al*. (2019). However, the SPAD value of the lettuce in this study for all the treatments, which was 9.6 at average, was higher than that reported by Maucieri *et al*. (2019).

The butterhead lettuce quality, in terms of leaf mineral content in response to nutrients solution composition and concentration, resulted in different fish stocking densities in DRAPS under non-controlled environmental conditions (Fig. 7 and 8). The general view of the statistical analysis showed there was a significant

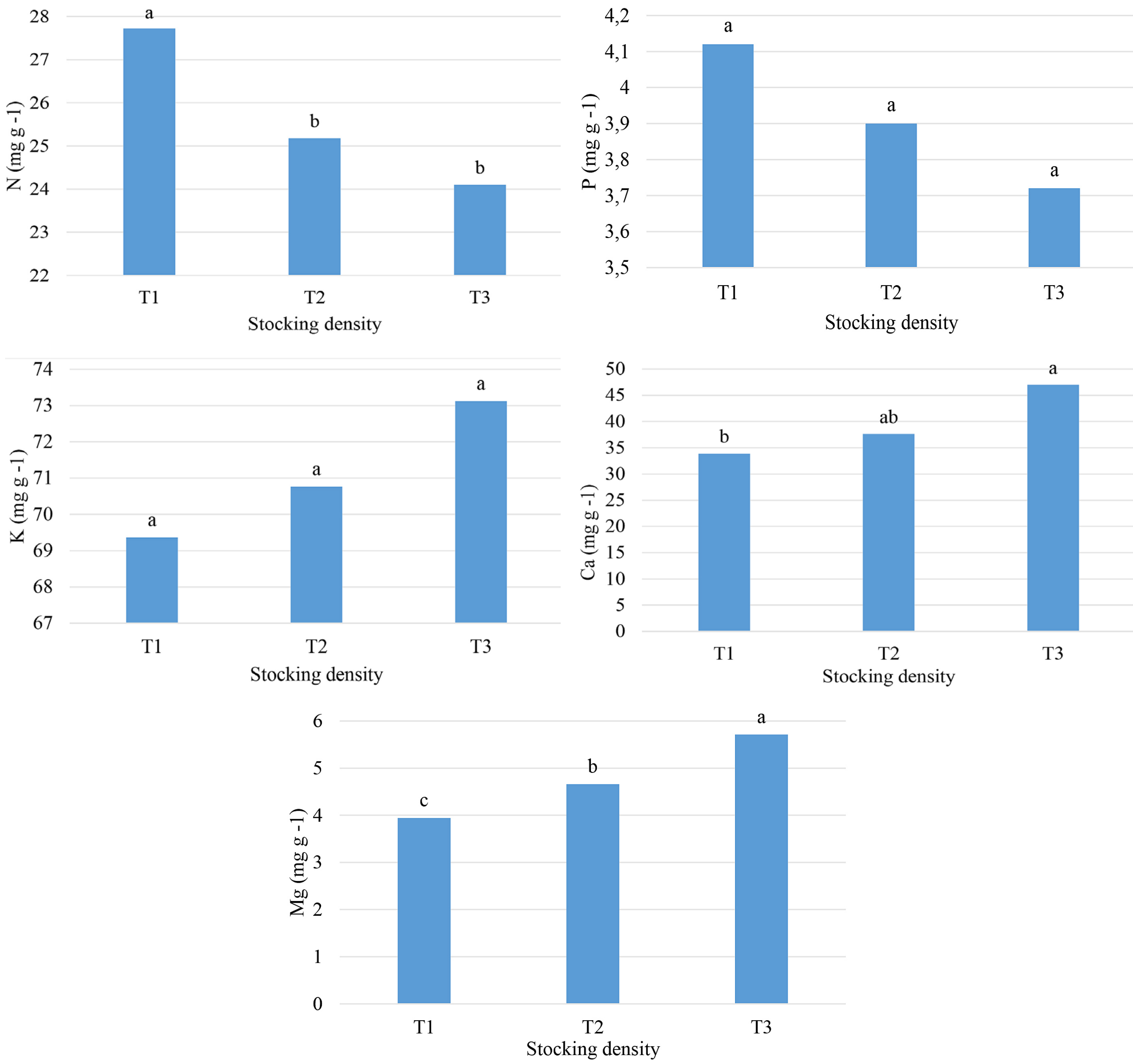

Figure 7. Macronutrients leaf content of lettuce in response to nutrient solution of different stocking density under DRAPS conditions. Means for each treatment followed with the same letter are not significantly different at p-value 0.05 using LSD

difference ($P<0.05$) leaf content for N, Calcium (Ca), Magnesium (Mg), Sodium(Na), iron (Fe) and Copper (Cu) and Manganese (Mn) except for P and K among the stocking density treatments. In DRAPS, the first loop provided the second loop by the nutrient solution in HP units. This nutrient solution composed of complex nutrients from nitrifying bacteria. However, during the experiment period, the pH, EC and water temperature were not controlled for the HP units.

In this study, the highest N concentration (27.72 mg g^{-1}) in leaves recorded under low stocking density and its lower than the levels considered as optimal by other authors such as Windsor and Adams (1987), report the optimal values from 39.0 to 50.0 mg g^{-1} N. However, the N content in Fallovo *et al*. (2009) study was ranged 46.2 48.0 mg g^{-1} of lettuce cultivated in soilless cultures. The previous study by De Kreij *et al*. (1990) reported that the optimum N content in lettuce cultivated in a greenhouse is 56.0 mg g^{-1} while Albornoz and Lieth (2015) reported that the lettuce was grown with different concentrations of nutrients, the N content ranged from 51.9 to 71.1 mg g^{-1}. In this study, the higher leaf P content was 4.12 mg g^{-1} at the lower stocking density, in contrast to the previous studies, it was higher than the concentration found out by Madar *et al*. (2019) and lower than Fallovo *et al*. (2009) study of P content in lettuce cultivated in the soilless substrate was 5.5–5.9 mg·g^{-1}. However, Delaide *et al*. (2016) reported that the concentration of P leaf content varied

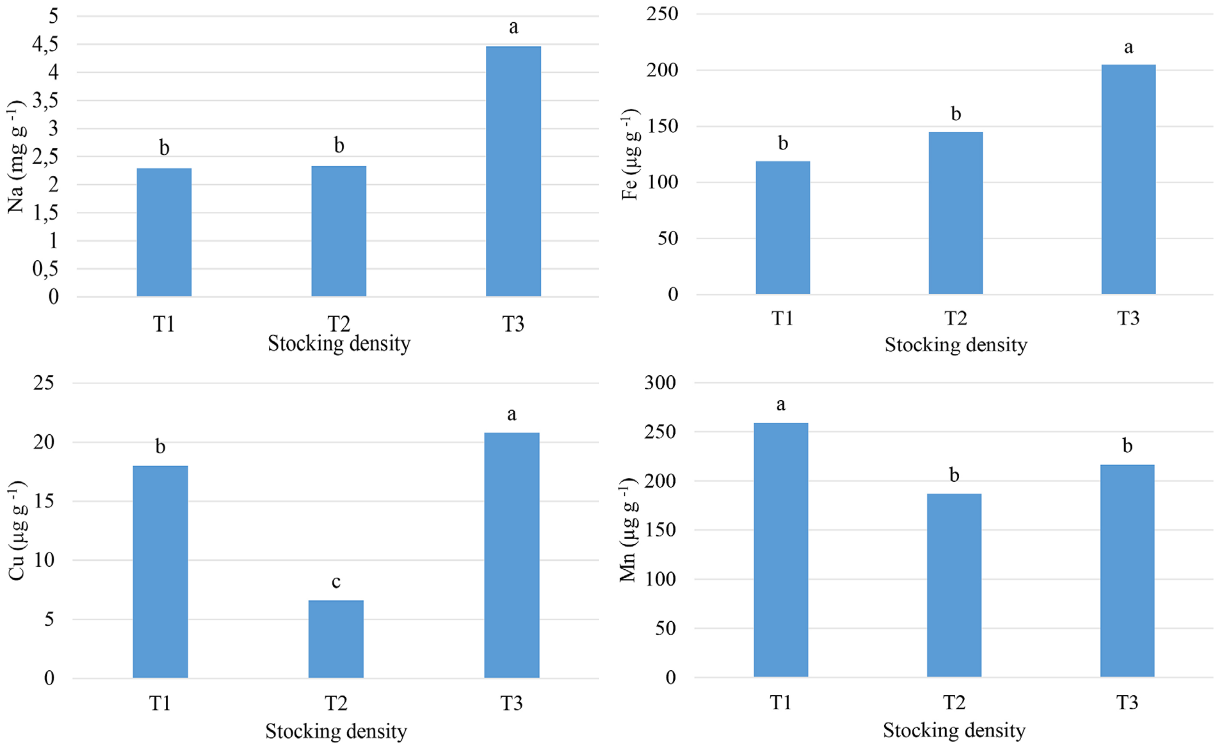

Figure 8. Micronutrients leaf content of lettuce in response to nutrient solution of different stocking density under DRAPS conditions. Means for each treatment followed with the same letter are not significantly different at p-value 0.05 using LSD

from 5.47 mg g^{-1} under aquaponic solution and 9.25 mg·g^{-1} under complemented aquaponic solution under DRAPS. Leaf K content in our result ranged from (69.36-73.12 mg g^{-1}) and was higher than content that obtained in Delaide *et al*. (2016) study for K leaf content; it was ranged from 24.6 mg g^{-1} under aquaponic solution and 29.8 mg g^{-1} under complemented aquaponic solution under DRAPS. While under soilless cultivation, Fallovo *et al*. (2009) found that lettuce's K leaf content ranged from 58.6–71.1 mg g^{-1}. The study of De kreij *et al*. (1990) found out that the optimum potassium content in lettuce ranges from 78.2 to 136.8 mg g^{-1}. From the point of view, it was shown that with an increased stocking density of fish, the leaf nutrient content of butterhead lettuce for K was raised and for the N, P decreased.

Delaide *et al*. (2016) reported that the Ca leaf content varied from 6.36 mg g^{-1} under aquaponic solution and 11.3 mg g^{-1} under complemented aquaponic solution under DRAPS, lower the Ca leaf content in our result. The higher content of Ca was 46.98 mg g^{-1} at a higher stocking density. Consequently, the Mg leaf content in this study was 5.71 mg g^{-1}, which higher than that obtained in the Delaide *et al*. (2016) study. In this study, the highest Na content in the leaves of butterhead lettuce was obtained at a higher fish stocking density. Consequently, the Na leaf content was higher than that concentration reported by Delaide *et al*. (2016) study. It was ranged from 3.70 mg g^{-1} under aquaponic solution and 2.60 mg g^{-1} under complemented aquaponic solution under DRAPS. In general view, it was shown that with increased the stocking density of fish, the leaf nutrient content of butterhead lettuce for Ca, Mg, Na was increased.

As a result of Fe content, it was stated that the highest leaf content (204.80 µg g^{-1}) was achieved at high stocking density and the lowest Fe leaf content was obtained at low stocking density. The concentration of our result was higher than the Fe content in Madar *et al*. (2019) and lower than the Fe leaf content in Delaide *et al*. (2016) study, and they founded the Fe ranged from 739 µg g^{-1} under aquaponic solution and 935 µg g^{-1} under complemented aquaponic solution under DRAPS. Regarding Cu leaf content, the highest leaf content was 20.80 µg g^{-1} was obtained at the higher stocking density. The Cu leaf content in the current study, regardless of the main factors, was lower than the leaf content reported by Delaide *et al*. (2016). For Mn leaf content, the highest contents (259 µg g^{-1}) was recorded at low stocking density. In contrast to Delaide *et al*.

(2016) findings, the leaf content of our study was lower than Cu leaf content obtained at complemented aquaponic solution under DRAPS. Finally, it was documented that with increased the stocking density of fish, the leaf nutrient content of butterhead lettuce for Fe and Cu was increased and Mn leaf content was decreased.

CONCLUSION

It can be concluded that the lowest stocking density of 8 kg m^{-3} gave the highest yield of lettuce. The low level of macronutrients was the major reason for the low growth and yield of lettuce. It has been showing that with an increased stocking density of fish, the leaf nutrient content of butterhead lettuce for K, Calcium (Ca), Magnesium (Mg), Sodium (Na), iron (Fe) and Copper (Cu) was increased. The higher leaf content for the N, P and Manganese (Mn) was obtained at lower stocking density.

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