Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)
http://dx.doi.org/10.1590/1983-21252023v36n103rc

Nutrients in lettuce production in aquaponics with tilapia fish compared to that with hydroponics

Nutrientes na produção de alface em aquaponia com peixes tilápia em comparação com hidroponia

Eglerson Duarte[1]*, Enilson de B. Silva[1], Fernanda da C. Moreira[1], Dermeval Braga[1], Shara G. dos Santos[1]

[1]Department of Agronomy, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, MG, Brazil.

ABSTRACT - In aquaponic systems, the residual water from the cultivation of fish is not enough to meet the nutritional demands of plants. The main objective of this study was to investigate how to adjust a nutritive solution for the cultivation of lettuce *(Lactuca sativa L.)* in aquaponics, based on hydroponics. The treatments included six separate crops of lettuce in an aquaponic system, while the hydroponic system served as the control. In each treatment, three blocks were used to quantify the parameters of 'head' diameter, number and dry weight of leaves, shoot dry weight, root dry weight, total dry weight, and the concentration and accumulation of nutrients in the total dry weight of the lettuce plants. The lower nutrient supply led to the occurrence of the lowest total dry weight in the C2 leaf crops when compared to that of the other crops of aquaponics, which had adequate time for system maturation. The system maturation and the use of a balanced solution in the C5 and C6 crops allowed the head diameter of the aquaponics plants to be equal to those of the hydroponics plants. In the aquaponic system, N and Fe were the most limiting macronutrient and micronutrient, respectively. To produce lettuce in an aquaponic system, it is necessary to 'ripen' the crop water for at least 30 days, and supplement micronutrients in the form of mineral fertiliser.

Keywords: *Lactuca sativa* L. Plant nutrition. *Oreochromis niloticus*. Intensive system.

RESUMO - No sistema de aquaponia, a água residual do cultivo dos peixes não é suficiente para suprir toda a demanda nutricional das plantas. O objetivo principal deste estudo foi investigar como ajustar uma solução nutritiva para o cultivo de alface *(Lactuca sativa L.)* em aquaponia, tendo como base à hidroponia. Os tratamentos foram seis culturas distintas de alface em sistemas de aquoponia e tendo como controle o sistema de hidroponia. Foram utilizados três blocos em cada tratamento para quantificar os parâmetros: diâmetro da "cabeça", número de folhas e massa seca das folhas, massa seca da parte aérea, massa seca da raiz e massa seca total e a concentração e acúmulo de nutrientes na massa seca total de plantas de alface. O menor aporte de nutrientes, fez com que o peso seco total das folhas do cultivo C2 fossem menor quando comparadas às demais safras da aquaponia, que possuíam um adequado tempo de maturação do sistema. A maturação do sistema e o uso da solução balanceada nos cultivos C5 e C6 permitiram que o diâmetro da cabeça das plantas da aquaponia fosse igual ao da hidroponia. No sistema de aquaponia, o N foi o macronutriente mais exigido e o Fe dentre os micronutrientes. Para a produção de alface em sistema aquopônico, é necessário a maturação da água das culturas no mínimo 30 dias, juntamente com a suplementação de micronutrientes na forma de fertilizante mineral.

Palavras-chave: *Lactuca sativa L*. Nutrição de plantas. *Oreochromis niloticus*. Sistema intensivo.

Conflict of interest: Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.

Received for publication in: August 16, 2021.
Accepted in: June 22, 2022.

***Corresponding author:**
<eglerson.duarte@ufvjm.edu.br>

INTRODUCTION

Recently, consumer interest in foods produced with more environmentally responsible and low-cost methods has increased (RIBEIRO; JAIME; VENTURA, 2017; ALI et al., 2020). This study focused on developing a combined fish and lettuce production method in an aquaponic system, which differs from the focus of studies published in the United States, Europe (HAO et al., 2020), and even Brazil (CARNEIRO et al., 2015).

Aquaponics, a system that integrates both fish farming and hydroponics, meets the demand for sustainability by minimising the loss of the water and nutrients required for agricultural production (OBIRIKORANG et al., 2021). The supply of feed to fish is the main form of nutrient input in the system (CARNEIRO et al., 2015; BAKHSHANDEH et al., 2020; NAVARRO, et al., 2021). Aquaponics is a closed system of water recirculation where the water containing fish waste and the accumulation of organic matter are used as nutrient sources. In this system, there are interactions among fish, plants, and the nitrifying bacteria *Nitrosomonas* and *Nitrobacter* (SOMERVILLE et al., 2014). Nitrifying bacteria transform the ammonia produced by fish (DUARTE et al., 2013) into nutrients that can be assimilated by plants (ZOU et al., 2016), thus improving the quality of the water returned to the fish (DELAIDE et al., 2017).

Aquaponics allows production in places that are not conducive to agriculture, such as urban centres, thereby providing diversity in production, and the quality animal and vegetable protein (PANTANELLA et al., 2012; CARNEIRO et al., 2015; SOMERVILLE et al., 2014). A requirement for aquaponics cultivation is a fish species that can tolerate intensive management systems. Furthermore, it is important to use small vegetables adapted to soil-less cultivation. Lettuce (*Lactuca sativa* L.) stands out among the plant species with this potential.

Lettuce is among the most important vegetables produced worldwide, which leads to its production in Brazil. It can be cultivated throughout the year, with a variety that yields good leaf mass and rapid growth. Depending on the region of cultivation, it can be harvested within 30 days after transplantation. It carries great social importance in family farming and human consumption, with production concentrated close to consumer centres due to its high perish ability.

Commercial rations are formulated to meet the nutritional demand of fish, and not the requirements of plants grown in fish-plant systems. Nutrients such as P, Ca, K, and Fe are generally not available in ideal quantities for the proper development of vegetables; thus, mineral fertilisers are used as supplements (CARNEIRO et al., 2015; SOMERVILLE et al., 2014). Supplementation may differ according to factors such as the fish cultivation phase, feed composition, temperature, water quality parameters, and water flow rate (CARNEIRO et al., 2015). An adequate supply of nutrients allows for adequate growth and development of crops (ZOU et al., 2016; ENDUTA et al., 2011), but this procedure needs to be performed carefully to avoid generating extra costs or causing the collapse of the productive system.

In this study, we hypothesised that identifying the nutritional requirements of lettuce grown in aquaponics would allow for the formulation of a nutrient solution adjusted to optimise the productivity of lettuce grown with aquaponics. The main objective of this study was to develop a nutritive solution for cultivating lettuce plants in aquaponics, based on hydroponics.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse (UFVJM) in Diamantina-MG (Lat. 18°12'S, Long. 43°34'W; altitude 1250 m above sea level) from February to October 2018, and from April to September 2019.

The experimental design used randomised complete blocks to mitigate the difference in light intensity in the greenhouse. In each treatment, three blocks, each containing seven plants per block, were used to quantify the following parameters: ‘head’ diameter, number and dry weight of leaves, shoot dry weight, root dry weight, and total dry weight. To quantify the concentration and accumulation of nutrients in the total dry weight of the lettuce plants, four plants were randomly collected from each block, totalling 12 plants from each treatment.

The treatments comprised six distinct crops of lettuce (C1, C2, C3, C4, C5, and C6) produced at different times in an aquaponic system, using six crops of lettuce grown in a hydroponic system as a control. For each treatment, the lettuce plants were cultivated for 30 days.

The lettuce plants from C1 to C6 were cultivated from February to October 2018 and from April to September 2019. These were tested and adjusted when necessary using the aquaponics nutrient solution, thereby optimising lettuce production. The cultivation of lettuce plants was conducted in three phases.

In the first phase, cultivation C1was used to identify, through visual diagnosis, the symptoms of nutritional deficiency in lettuce that appeared during this phase. Deficiencies of Fe and B that were still present in the C1 culture were identified. Ca was added preventively before the onset of deficiency symptoms. Measures of 180 mg L^{-1}, 1,955µg L^{-1}, and 216 µg L^{-1} of Ca, Fe, and B, respectively, were supplemented. This was done using the concentrations and reagents used in the hydroponic system proposed by Martinez (1999), while keeping the proportions adjusted for the volume of water used in the aquaponic system.

In the second phase, C2, C3, and C4 crops, served to calibrate the solution supplemented with Fe, B, and Ca (C1 crop), using the concentrations and reagents proposed by Martinez (1999) for hydroponics. The third phase had the ideally treated crops, C5 and C6. It served to validate the solution calibration, using the results of water nutrient concentration from previous crops. Thereafter, aquaponic supplementation was performed. It was not necessary to supplement macronutrients, as only micronutrients (B, Cu, Fe, Mn, and Zn) were in short supply using the reagents of the hydroponic nutrient solution at the concentrations proposed by Martinez (1999), adjusted for the aquaponics water volume. The dynamics of the aquaponics crops are shown in Figure 1.

Before the beginning of the experiments (C1, C2, and C5), the biofilter media of the aquaponic system were colonised with bacteria (matured media). Because it naturally contains bacteria, the biological filter of the C1 crops was matured for 30 days using water reused from fish farming, which was located 100 m away from the experimental area. However, because of the risk of the pathogenic contamination of fish, this maturation method was not used for the other crops. The biological filters of the C2 and C5 crops were matured according to the methodology described by Duarte et al. (2013), which provides adequate conditions in the biological filter for the growth of bacteria, while avoiding the risk of the pathogenic contamination of fish.

The water used in the aquaponic system undergoes a maturation process, which consists of the fish inhabiting the system for 30 days, so that there is an increase in the supply of nutrients in the water before the plants are added to the system. For maturation of the C1 crop system, reused fish water was used, which did not require the 30 days waiting period for the plants to enter the system because it already had a suitable supply of nutrients. In C2 crops, only the biological filter was matured, and there was no maturation of the aquaponics water. The plants were added to the system together with the fish, without a supply of nutrients, to evaluate the effect of system maturation on plant growth. For C3 crops, the water used was already mature because it was used for the C2 crops. In the C4 crops, the mature water from the C3 crops was used. For the C5 crops, the biological filter was matured according to the methodology described by Duarte et al. (2013), and the aquaponics water was matured with the fish inhabiting the system for 60 days to simulate the supply of nutrients from the C4 crops. In the C6 crops, matured water from the C5 crops was used (Figure 1).

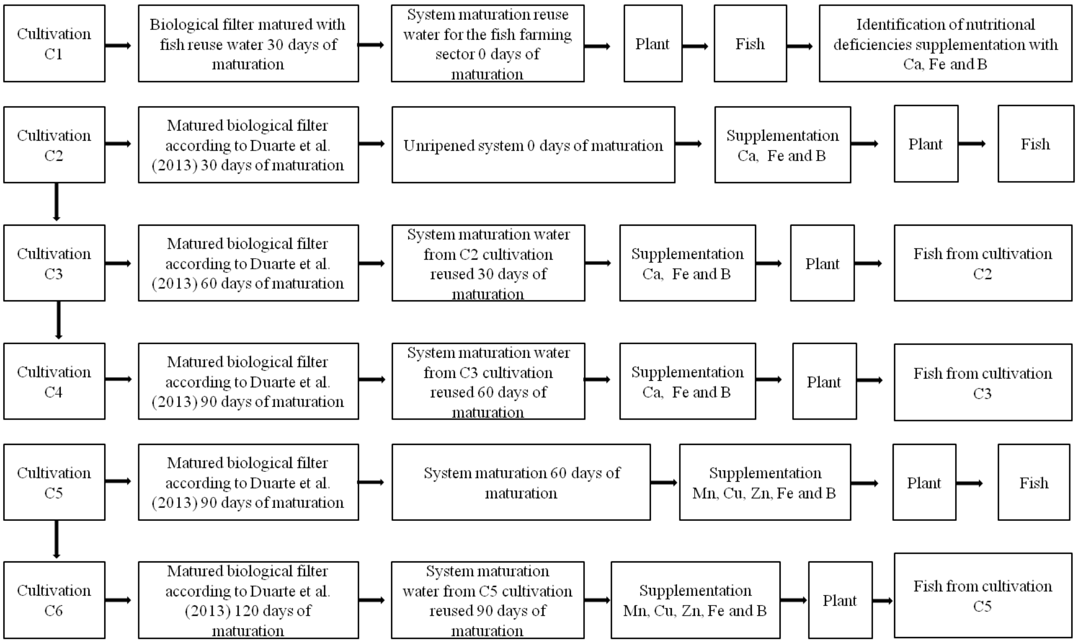

Figure 1. Flowchart of the six lettuce crops in an aquaponic system containing tilapia fingerlings.

To produce the hydroponic solution, the nutrient solution in the hydroponic system used water from an artesian well and macronutrients, which was composed of (mg L^{-1}): 168 N (KNO_3, $NH_4H_2PO_4$, Ca $(NO_3)_2$), 31 P ($NH_4H_2PO_4$), 234 K (KCl), 180 Ca (Ca $(NO_3)_2$), 48 Mg ($MgSO_4$), and 112 S (K_2SO_4); and micronutrients (µg L^{-1}), which comprised: 497 B (H_3BO_3), 19 Cu ($CuSO_4$), 2.513 Fe ($FeCl_3$-EDTA), 950 Mn ($MnSO_4$), 48 Mo ($Na_2MoO_4.2H_2O$), and 98 Zn ($ZnSO_4$) from pure reagents, with an electrical conductivity of 2.3 dS m^{-1} (MARTINEZ, 1999).

The curly lettuce of the cultivar Monica SF31Feltrin® was used for all six crops. The seeds were sown manually in polyethylene trays of 128 cells filled with a commercial substrate from Bioplant® that is based on pin bark and sawdust. After germination, the seeds were placed in a nursery where 25% of the complete nutrient solution was applied in the first week, 50% was added in the second week, and 75% in the third week. After the plants had obtained four to five leaves (0.05 m high), the roots of the plants were washed to remove the adhered substrate, and were transferred to aquaponic and hydroponic systems, where they remained until harvest.

The floating type aquaponic and hydroponic systems were used, where a cultivation table with a 0.02 m-thick Styrofoam plate, with 0.05-mdiameter holes given 0.25 × 0.25 m of spacing between plants, floats in a reservoir (0.30 × 0.50 × 0.30 m) containing a 0.20 m blade. This allows the roots to be submerged with constant oxygenation from a radial compressor with a maximum flow rate of 2.02 m^3 min^{-1} of air flow. Both systems, with the aid of an analogue timer, recirculated the solution every 30 min, driven by a submerged Salobetter® pump with a flow rate of 2 m^3 h^{-1}. The aquaponic system used 100% of this flow, whereas the hydroponic system used 50% of the flow.

The hydroponic system was composed of a 0.2 m^3 reservoir for nutrient solutions (MARTINEZ, 1999). The aquaponic system comprised a circular tank for growing fish (1 m^3), a solid waste filter containing a Perlon acrylic mat (0.2 m^3), and a biological filter (0.2 m^3) filled with biomedia (17 holes, 20 × 15 mm, area of 314 m^2 m^{-3}) that had already been cleaned and matured.

Tilapia fingerlings (*Oreochromis niloticus*) were fed 66 g (in all crops) of Guabi® commercial feed composed of 32 g $kg^{-1}$and 42 g kg^{-1}crude protein (CP), three times a day (7:00 am, 1:00 pm, and 5:00 pm), which is the required amount for the cultivation of 1.32 m^2 of leafy vegetables (SOMERVILLE et al., 2014).

The 32 g kg^{-1} CP diet had macronutrient concentrations (g kg^{-1}) of 6 N, 24 P, 54 K, 47 Ca, 25 Mg, and 33 S; as well as micronutrient concentrations (mg kg^{-1}) of 77 B, 11.8 Cu, 306.4 Fe, 31.7 Mn, and 141.0 Zn. The 42 g kg^{-1} CP had a macronutrient content (g kg^{-1}) of 8 N, 20.5 P, 52 K, 41 Ca, 26 Mg, and 54 S; and a micronutrient content (mg kg^{-1}) of 3.0 B, 24.6 Cu, 507.8 Fe, 125.1 Mn, and 277.2 Zn. The sample used for bromatological analysis was wet-digested in a microwave oven, and its concentration was quantified using an argon-induced plasma spectrophotometer in a method adapted from Silva (2009).

The control of algae in the water of aquaponic systems was performed with the aid of an Atman® 36 W ultraviolet steriliser. The temperature of the hydroponic nutrient solution was kept the same as that of aquaponics (Table 1), using Atman® 300 W thermostats in both systems. The water lost due to evaporation was replaced daily. The pH was monitored every 3 days, and adjusted as necessary using NaOH and HCl of 100 mmol L^{-1}. In the hydroponic system, the pH was maintained at 6.2. Monitoring the replacement of hydroponic nutrient solution was done based on electrical conductivity (EC), and the nutrient solution was replaced whenever it reached 30% of the initial EC (MARTINEZ, 1999).

The physicochemical parameters of nitrate and ammonium water concentrations in the aquaponic and hydroponic systems were quantified according to the method of SILVA (2009). pH, EC, and alkalinity were measured according to the method of Macêdo (2005); and the nutrient concentrations were quantified using an argon-induced plasma spectrophotometer.

The plant material collected at the end of each cycle was cleaned as follows: chlorinated water was cleaned using diluted detergent, chlorinated water, 100 mmolL^{-1} HCl solution, and then distilled water to remove any residue.

After collection, all plants were packed in paper bags and dried in an oven with a circulating air temperature of 65 °C until a constant mass was obtained. Subsequently, the vegetable material was weighed on an analytical balance to obtain the dry weights of the leaves and roots. The vegetable material was then crushed and subjected to wet nutrient analysis in a microwave oven, and its concentration was quantified using an argon-induced plasma spectrophotometer (SILVA, 2009). The accumulation of nutrients per plant was obtained from the product of the dry weight and the nutrient concentration of each evaluated part.

The experimental design comprised randomised blocks, with three blocks in each treatment. Four lettuce plants per block were randomly selected from each block, totalling 12 repetitions for each treatment (aquaponics and hydroponics). The blocking was intended to mitigate the difference in light intensity in the greenhouse, and the different cultivation conditions within each reservoir of the floating aquaponic and hydroponic cultivation system for the lettuce. The data were subjected to analysis of variance using Sisvar® software 5.6 (FERREIRA, 2019). Mean aquaponic and hydroponic treatments were compared using the F test at a 5% significance level within each lettuce crop.

RESULTS AND DISCUSSION

The complete nutritional solution, balanced with an EC of 2.3 $dSm^{-1}$and a pH of 6.2, was maintained among the six cultivations in the hydroponic system. The concentration or formulation of the solution did not change from crop to crop.

In aquaponics, the nutrient solution is dynamic and depends on factors such as the maturation time of the ration provided, feeding rate, and fish density. This dynamism directly reflects the nutrient concentration, and makes parameters such as the pH and EC variable (Table 1). This influences the availability and accumulation of nutrients by the plant, making it different from the EC of 2.3 dSm^{-1} and pH of 6.2 used in the hydroponic system.

Table 1. Average values of temperature from the nutrient solution and the environment, pH, electrical conductivity, and nutrient contents at 0 and 30 days in the aquaponic system, in six 30 days old lettuce crops grown with tilapia.

Crops	C1		C2		C3		C4		C5		C6	
Days	0	30	0	30	0	30	0	30	0	30	0	30
Temp. H_20 (C°)	23±2.8		24±1.8		26±1.3		26±1.9		24±2.5		25±4.0	
Temp. Env. (C°)	25±6.5		22.4±6.7		21±5.0		21±5		15±5.0		14±.4.2	
pH	7.0	7.3	7.7	7.7	7.7	7.7	7.6	7.3	7.0	7.3	7.2	7.3
	.... dS m^{-1} to 25°C											
Conductivity	0.50	0.68	0.78	0.70	0.75	0.70	0.73	0.70	0.50	0.47	0.52	0.39
	.... Macronutrients (mg L^{-1})											
Ammonium	2.92	2.92	2.92	2.92	2.92	2.19	2.19	1.46	2.92	2.92	2.92	2.19
Nitrate	13.84	10.20	3.65	3.65	3.65	6.57	6.57	13.15	13.84	10.23	10.20	5.84
P	40.00	38.50	23.00	18.00	18.00	30.20	30.20	40.20	49.09	20.19	20.19	14.81
K	12.30	15.30	13.06	13.06	13.06	13.06	13.06	15.30	14.14	12.59	12.59	13.54
Ca	21.24	169.97	157.22	135.97	135.97	123.22	123.22	108.35	51.44	46.62	46.62	35.44
Mg	4.87	5.63	4.88	6.00	6.00	7.13	7.13	7.13	3.13	8.15	8.15	8.32
S	1.32	4.94	28.86	26.70	26.70	16.50	16.50	6.88	3.28	8.58	8.58	8.71
	.... Micronutrients (µg L^{-1})											
B	0.23	62.00	216.00	60.00	210.00	59.00	210.00	63.00	210.00	58.80	214.00	64.20
Cu	0.10	0.10	0.10	0.01	0.01	0.01	0.01	0.01	31.00	9.30	30.00	9.00
Fe	0.01	580.00	1955.00	574.00	1950,00	578.00	1952.00	585.6	1953.00	527.31	1950.00	526.50
Mn	0.01	0.01	0.76	0.01	0.01	0.01	0.01	0.01	275.00	82.50	272.00	81.60
Zn	0.01	0.25	0.19	0.03	0.03	0.16	0.16	0.06	46.00	13.80	47.20	14.16

± Confidence interval

Because three very different organisms (fish, plants, and bacteria), with different pH requirements for their survival, occupy the same body of water in aquaponics, the pH must be maintained between 6.5 and 7.0 to satisfy all the biological components present in the system (SOMERVILLE et al., 2014; CARNEIRO et al., 2015). In the six aquaponics crops, the pH ranged from 7.0 to 7.7, with an average of 7.4 (Table 1). pH variations of 7.2 to 7.8, with an average of 7.5, have been reported in the literature (LENZI et al., 2017); however, among the crops, the pH fluctuation was small when compared with that of the fish-plant crops grown for up to 20 days (ZOU et al., 2016).

Alkalinity below 100 mg L^{-1}in the aquaponic system (Figure 2C) provided less pH fluctuation (Table 1) between 0 and 30 days in each cultivation. This small oscillation is related to the buffering power of alkalinity (Figure 2). Some impurities present in the water, and the salts added to the system, can react with acids and can neutralise a certain amount of these reagents, thereby conferring the characteristic water of alkalinity, which allows the acid-basic balance (buffering power) of the culture system to be below 100 mg L^{-1} (SOMERVILLE et al., 2014). Alkalinity below 100 mg L^{-1}in the aquaponic system (Figure 2C) favoured the acid-base balance of the water, causing less fluctuation in the pH system (Table 1). Cultivation C2 (Table 1) did not show a change in the pH between 0 and 30 days, even with an alkalinity level above 100 mg L^{-1}, owing to the use of the protocol established in the Materials and Methods.

The pH is a critical factor in aquaponics as it influences the availability of nutrients in the culture solution (SOMERVILLE et al., 2014). Most plants support pH variations between 5.5 and 6.5 (BUGBEE, 2004); therefore, a pH above 7.0, which is considered ideal for the development of fish and bacteria, would directly interfere with the solubility and availability of nutrients in the growing water. Moreover, nutrients such as Fe, Mn, B, Zn, and Cu decrease dramatically in availability at pH levels above 7.0 (SOUZA et al., 2010). However, the pH levels of the C5 and C6 crops were close to 7.2, which allowed for better availability and absorption of nutrients by the plants in an aquaponic system (CEROZI; FITZSIMMONS, 2016; ASHKIANI et al., 2020).

The number of C5 leaves in the aquaponic system was lower than that in the hydroponic system, where as the number of leaves in the C6 crops of both systems were equal (Figure 3B). The lower supply of nutrients from C2 crops, and the supplementation of Fe and B in C1 crops, which was carried out only after visual diagnosis, influenced the number of leaves and the shoot dry weight (Figures 3B and 3D). The lower supply of nutrients, due to the lack of water maturation (Table 1), caused the dry weight of leaves and total dry weight in C2 crops (Figures 3C and F) to be lower than those of the other aquaponics crops, which had adequate system maturation times.

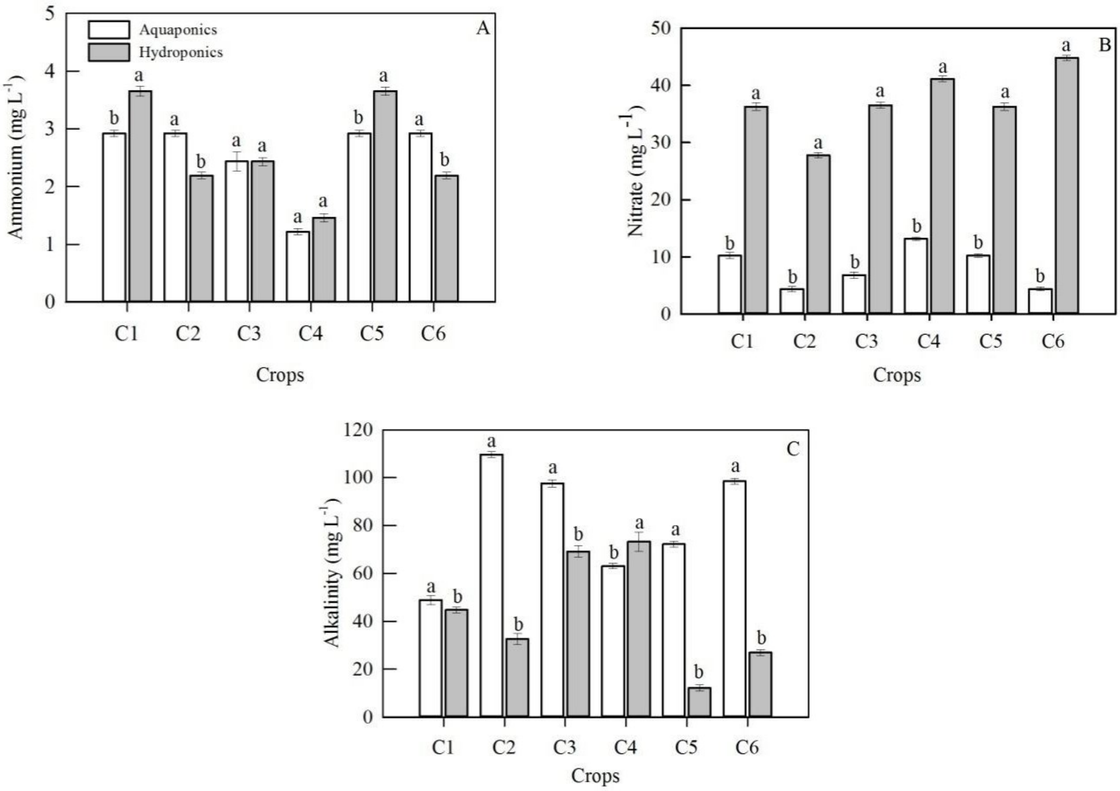

Figure 2. Ammonium (A), nitrate (B), and alkalinity (C) concentrations in the water bearing six lettuce crops grown for 30 days in an aquaponic and hydroponic system. The bars at each crop with the same letter are not significantly different according to the F test at a 5% level. Data are means ± confidence intervals (n = 12).

Except for the C1 and C2 crops, the shoot dry weight of the other aquaponics crops was similar to that of plants cultivated in hydroponics (Figure 3D). The shoot dry weight (Figure 3D) was not negatively influenced by the alkaline pH of the aquaponic system. A greater supply of nutrients favoured the increase in the root dry weight, making it possible for the root dry weight of the C1 aquaponics plants to surpass that of the hydroponic plants (Figure 3E). Moreover, in the C2 crops, the root dry weight was lower than that in aquaponics, whereas in the other crops (C3, C4, C5, and C6) it was equal (Figure 3E).

The adjustment made in the C2, C3, and C4 crops, with the addition of Fe and B to the aquaponics water, was enough to prevent symptoms of nutritional deficiency as evaluated using visual diagnosis, but not yet adequate so that the diameter of the 'head' of the plants being equal to that in the hydroponic system suggests that it was not enough for improved growth (Figure 3A). Despite Fe and B supplementation, factors such as the pH of the solution, and the competition of macronutrients for the same active site of the enzymes, can reduce these elements in the mineral solution of the aquaponic system of C5 and C6. Only having a matured system and using a balanced solution, allowed the C5 cultivation in aquaponics to have a different 'head' diameter than that of the hydroponics plants and that of C6 (Figure 3A).

In the aquaponic system in all cultivations, the level of ammonium was below 4 mg L^{-1} and that of nitrate was below 20 mg L^{-1} (Figures 2A and B), which demonstrates the efficiency of the conversion of the excreted ammonia into nitrate using the experiment's biological filtration (CARNEIRO et al., 2015). In contrast, in hydroponics, the highest levels of nitrate (Figure 2B) came from the use of reagents, based on calcium nitrate and potassium nitrate, used in the formulation of the nutrient solution. This resulted in N levels that were higher than the 'residual' waters of the fish culture. These reagents are associated with a pH of 6.2 in the system, which is ideal for the plant (SOMERVILLE et al., 2014), and enhanced the absorption of N by hydroponic lettuce.

The maturation of the aquaponic system for at least 30 days, as done in the C3 cultivation, is necessary to increase the supply of nutrients. The addition of plants together with fish in the C2 crops demonstrated that there was insufficient time for the maturation of the system. This influenced the low availability of nutrients in the cultivation water (Table 1), with a lower concentration than that of the hydroponics solution, thereby compromising the growth of lettuce, which was reflected in the shoot dry weight (Figure 3D). The greater supply of nutrients in the water has significant effects on the aquaponic system, increasing the plant biomass produced (ZOU et al., 2016). Although the total P content was higher in relation to the other cultivations, the non-differentiation in P values between 0 and 30 days (Table 1) confirmed that P was in an unusable form in the aquaponic solution. This caused an increase in growth (Figure 3E), manifested in an increase in the nutrient absorption area (ALMEIDA et al., 2011), to explore the solution in search of available P at the expense of shoot growth (Figure 3D).

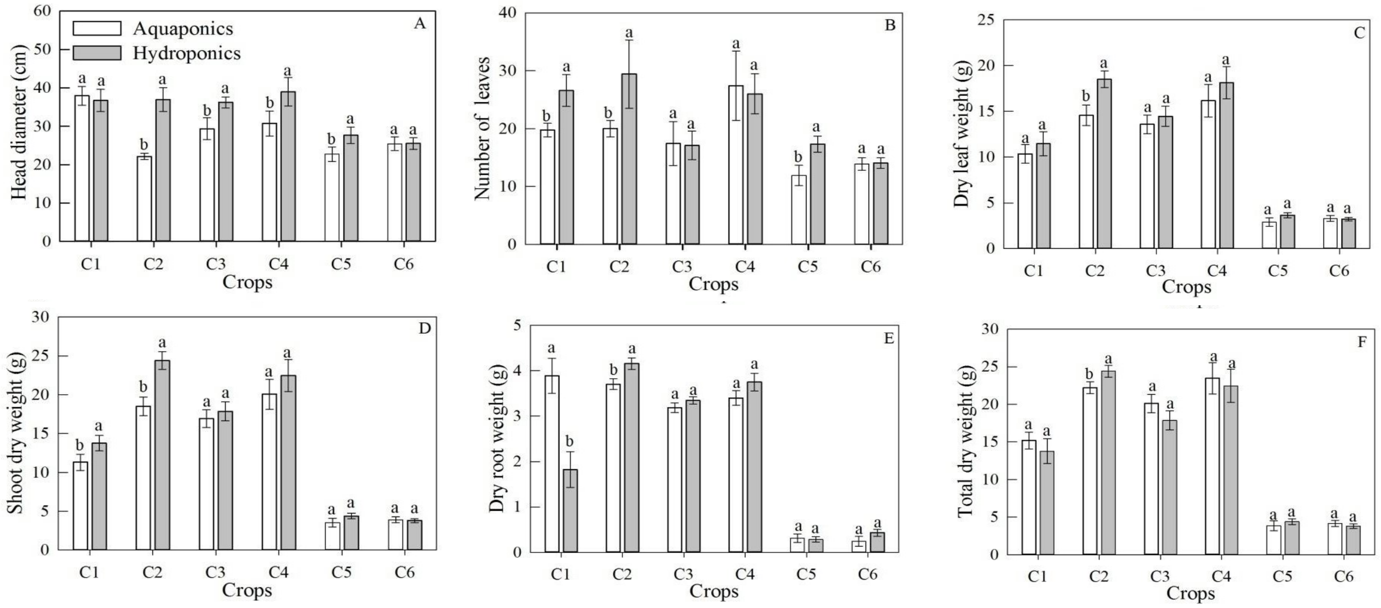

Figure 3. 'Head' diameter (A), number of leaves (B), dry leaf weight (C), shoot dry weight (D), Root dry weight (E), and total dry weight (F) of six 30 days old lettuce crops grown in an aquaponic and hydroponic system. The bars at each crop with the same letter are not significantly different according to the F test at a 5% level. Data are means ± confidence intervals (n = 12).

In the C1 crops, owing to the absence of mineral supplementation, the nutrient concentration (Table 1) was below that demanded by the plant, causing the appearance of visual symptoms of a nutritional deficiency in Fe and B. In the leaves of the aquaponics system's C5 and C6, the concentrations of all macronutrients (Figures 4A, B, C, D, E, and F), and the micronutrients Fe and Zn (Figures 4I and K) were adequately within the critical cultivation range (MARTINEZ, 1999); meanwhile, Mn was adequate in C5 crops (Figure 4J). In C5 and C6 crops, for B and Cu, the concentration was below the critical range (Figures 4G and H). In C6 crops, Mn was below the critical level (Figure 4J). In general, the C5 and C6 crops in the aquaponic system had an adequate supply of macronutrients and micronutrients (Figure 4L).

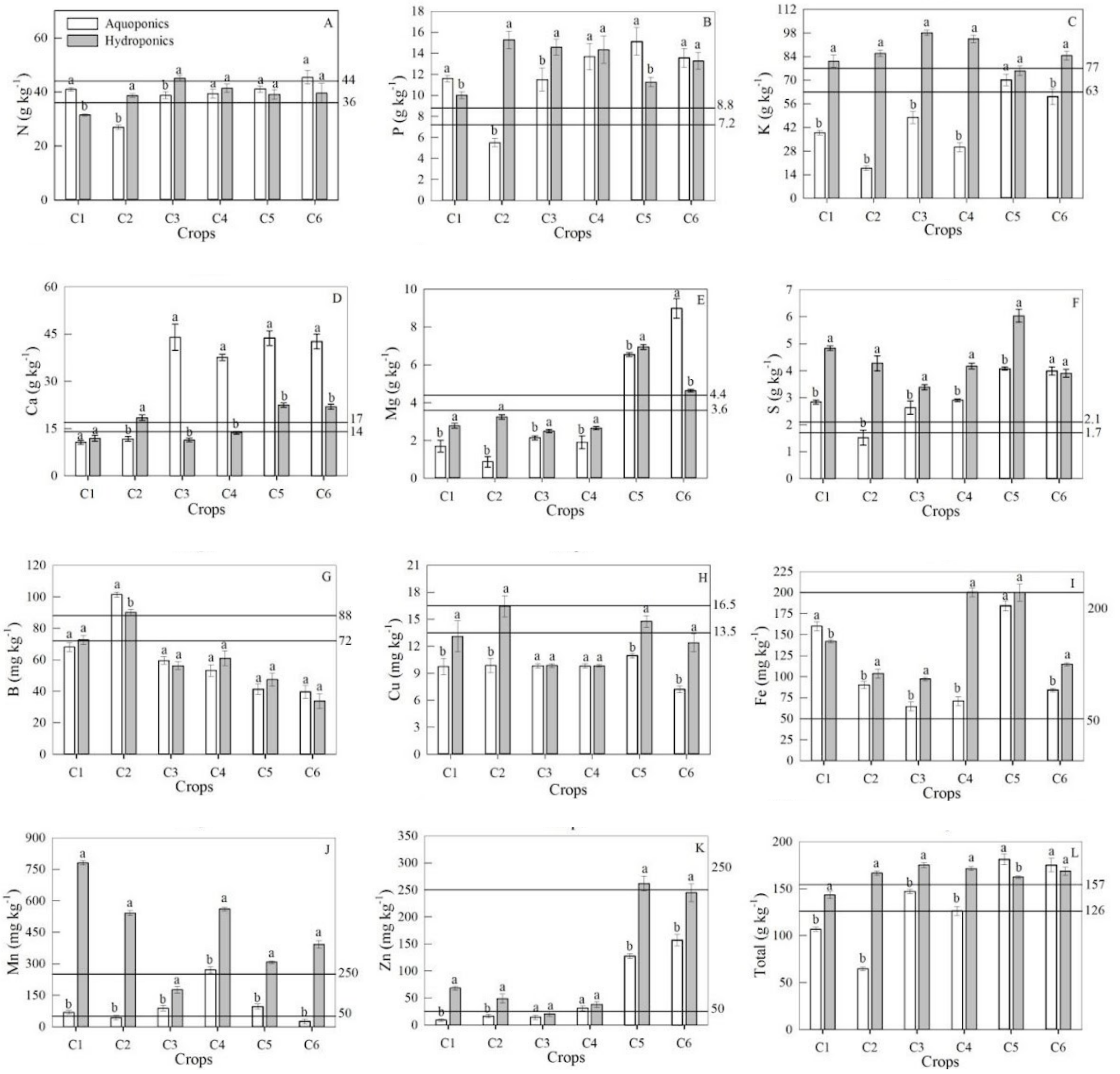

Figure 4. Nutrient concentration in lettuce leaves in six30days old crops, in aquaponic and hydroponic systems. The critical range (MARTINEZ, 1999) of crop nutrients is represented by perpendicular lines. The bars at each crop with the same letter are not significantly different according to the F test at a 5% level. Data are means ± confidence intervals (n = 12).

In the C1 crops in aquaponics, despite the greater supply of nutrients from the fish culture reuse water used in the maturation of the system and the biological filter (Table 1), the nutrient concentrations of K, Ca, Mg, Cu, B, and Zn in the leaves of the lettuce plant (Figures 4C, D, E, G, K, H, and L) were below the critical range for the crops. This prevented the plant from being able to express its maximum production level.

Generalised chlorosis with green ribs on new leaves was one of the first symptoms of nutritional deficiency to appear in the C1 crops in aquaponics because of the lack of Fe. This is easily identified because Fe is a relatively immobile element in the phloem with a low translocation rate (TAIZ et al., 2017). Fe deficiency decreased the number of leaves and the dry mass of the aerial part of the C1 crops (Figures 3B and D). It also caused changes in the physiological processes of the plant, such as unbalanced redox reactions, reduction in respiratory rates, and alteration in photosynthesis (LI; WANG; YANG, 2015).

Although the maturation period was adequate for C3 and C4 crops, the imbalance in the nutrient solution did not compromise the number of leaves in the C3 and C4 crops (Figure 3B); however, it did compromise the 'head' diameter (Figure 3A). Only having the adequate availability of nutrients, as in theC6 crops, were the lettuce from aquaponics able to express their full productive potential. For this, adequate time was necessary for the maturation of the system, along with the incorporation of micronutrients in the water from tilapia cultivation, thereby creating a balance. Thus, these lettuce plants matched those in hydroponics in relation to the 'head' diameter, as well as in the number of leaves, dry weight of leaves, shoot dry weight, root dry weight, and total dry weight (Figures 3A, B, C, D, E and F). In C5 and C6, the diameter of the head (Figure 3) was smaller than that in other aquaponics cultivations, and was associated with the variation in environmental temperature (Table 1), which was lower in C5 and C6. The Monica SF31Feltrin® variety is better adapted to higher temperatures.

An equal number of leaves was observed in the C6 lettuce plants grown in aquaponic and hydroponics (Figure 3B).This showed that the plants of the systems had the same photosynthetic conditions in which to produce carbohydrates. The photosynthetic process that occurs directly in leaves to form carbohydrates depends on the interception of light energy and its conversion into chemical energy (TAIZ et al., 2017).

N was the nutrient with the highest total dry weight in C5 and C6 crops in aquaponics (Figure 5A). The C5 and C6 crops grown in the aquaponic system showed a total accumulation of macronutrients that was similar to that found in hydroponics (Figures 5A, B, and C). Exceptions to this were Ca (Figure 5D), which surpassed that seen in hydroponics, and Mg, which was lower than that in hydroponics (Figure 5E). Nutrient accumulation is related to the efficiency of root cell absorption in relation to the transport and use of nutrients by the plant (TAIZ et al., 2017).

The concentration of N compounds (NH_3, NO_2^-, and NO_3^-) are determined by the action of bacteria of the genera *Nitrosomonas* and *Nitrobacter* (ZOPPAS; BERNARDES; MENEGUZZI, 2016) that are present in the bio filter. In this experiment, they increased the nitrification rates due to the more alkaline pH of the water (Table 1), as has already been demonstrated in the literature (LENZI et al., 2017). Commercial feeds used in fish farming have a high proportion of protein, which is a source of nitrogen compounds in water. Organic nitrogen present in feed residues and ammonia excreted by fish are converted by bacteria to ammonium (NH_4^+), nitrite (NO_2^-), and finally to nitrate (NO_3^-) (DUARTE et al., 2013). Nitrates are the main form of assimilation, which are directly absorbed by the roots where they are metabolised or transported to the shoot of the plant (ZOU et al., 2016; HU et al., 2015; WONGKIEW et al., 2017).

Fe was the micronutrient that was most in demand in aquaponics (Figure 5I), with symptoms of visual impairment appearing in C1 crops when not supplemented. Among the lettuce plants grown in the aquaponic system, C5 and C6, showed similar values for total accumulation of B, Fe, and Mn to that in hydroponics (Figures 5G, I, and J). The Zn (Figure 5K) in C5 and C6 crops, along with the Cu in C5 crops, were lower than those in hydroponics (Figure 5H).

In C5 and C6 crops, the decreasing order of the sum of the accumulated macronutrients in aquaponics and hydroponics was N>K>Ca>P>Mg>S. The order of micronutrients in aquaponics were Fe>Zn>Mn>B>Cu, and Mn>Fe>Zn>B>Cu in hydroponics.

The N in the aquaponic system in C5 and C6 crops was the macronutrient with the highest accumulation in plants (Figure 5A). The availability method and volume of N supplied in aquaponics facilitated its accumulation in lettuce plants. However, the increase in availability caused non-competitive inhibition with Mg and B (RAMOS et al., 2011; WAHYUNINGSIH; EFFENDI; WARDIATNO, 2015), thereby considerably decreasing the absorption of these nutrients, with symptoms of B nutritional deficiency appearing in the absence of mineral fertiliser supplementation (C1crops). However, both the addition of mineral fertiliser to the growing water and the maturation of the system were enough to minimise the effect of B inhibition.

At pH 3.0 to 5.5 there is a greater availability of reactive soluble P (orthophosphate) in the culture solution (CEROZI; FITZSIMMONS, 2016). Between the concentrations of 24 g kg^{-1} and 20.5 g kg^{-1} of P present in the diet of 32 g kg^{-1}and 42 g kg^{-1} of CP supplied, P was sufficient in the C5 and C6 crops to maintain the nutrient content (Figure 4B) and accumulate to a level that is suitable (Figure 5B) for the plant, even with the most alkaline pH. In this way, it allowed the dry root weight and shoot dry weight to match those of the plants in hydroponics, demonstrating that there is no need for P supplementation.

Lettuce has a high demand for P, especially in the final phase of its cycle, which in cases of the absence of P, lack of P, or pH action, there is a reduction in the fresh weight of the shoot and roots; a marked reduction in plant diameter; poor head formation; and a notable reduction in the accumulated P levels in the leaves (LANA et al., 2004).

The residual water from the fish farming system is not able to meet all the nutritional demands of lettuce (CORTEZ et al., 2009). In C1 crops, the K concentration was below the critical range (Figure 4C), and absorption was lower than that in hydroponics (Figure 5C), which caused a decrease in the number of lettuce leaves (Figure 3B). Potassium is one of the most extracted macronutrients in lettuce plants. It performs several physiological functions in the plant, and is directly associated with product quality (ALMEIDA et al., 2011). Its deficiency causes reductions in shoot growth and malformations of the shoot (PETRAZZINI et al., 2014). The use of potassium chloride (KCl) in the hydroponics solution favoured its accumulation. Moreover, the Cl^- ion acts as a companion, facilitating the entry of K into the root epidermis (ALBUQUERQUE et al., 2012), thereby enhancing the absorption of K by hydroponic lettuce.

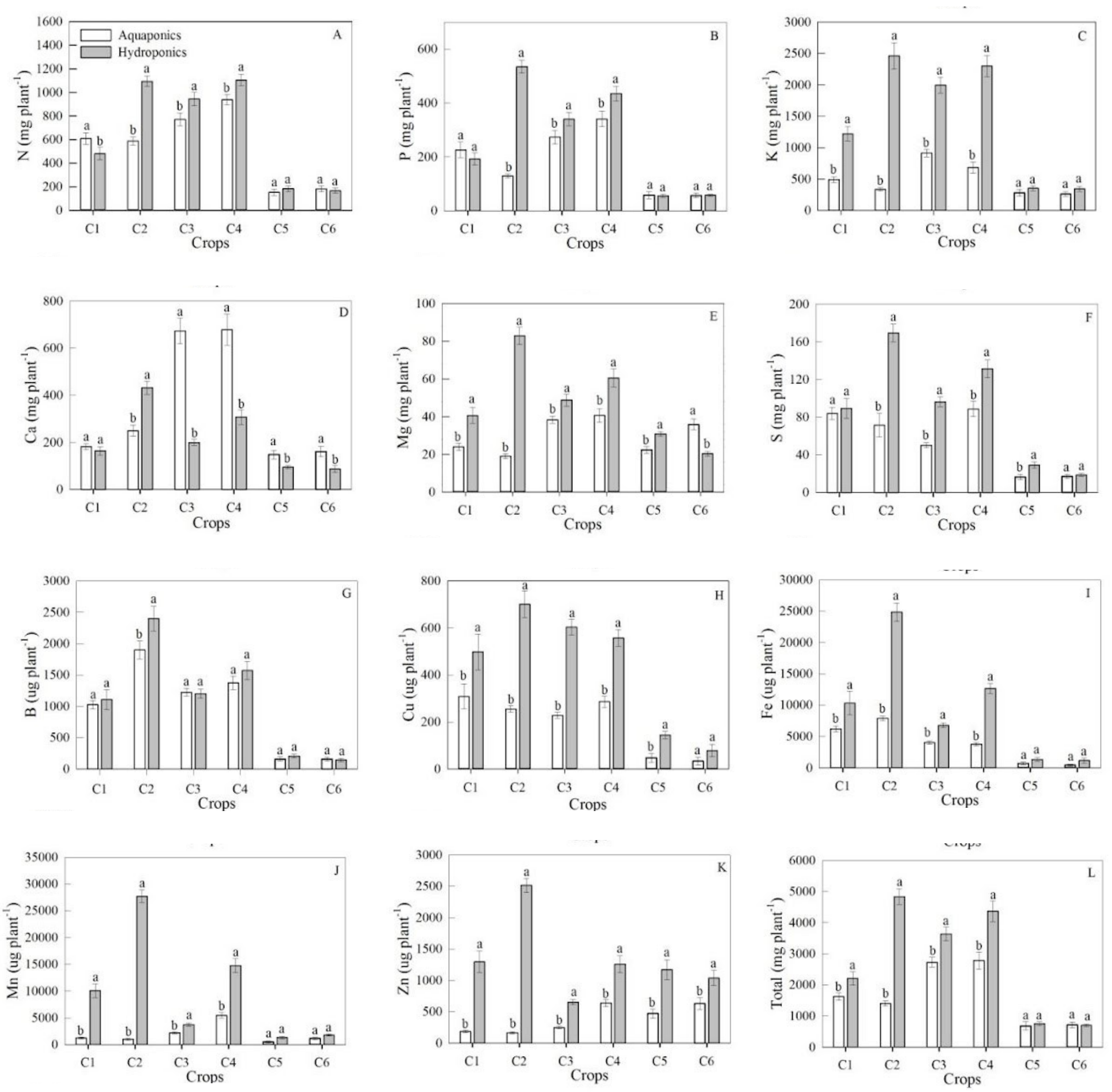

Figure 5. Nutrient accumulation in the total dry weight of six 30days old lettuce crops in aquaponic and hydroponic systems. The bars at each crop with the same letter are not significantly different according to the F test at a 5% level. Data are means ± confidence intervals (n = 12).

In solutions with alkaline pH, there is an increase in Ca concentration and high levels of Ca in the aquaponics water (Table 1), which contributes to a decrease in the absorption of Mg and Mn due to competitive inhibition (MOREIRA et al., 2000). It can also affect the plant's absorption of P because of the decrease in solubility and availability of phosphate ions in the solution. This is because Ca reacts with phosphate-forming complexes such as $CaHPO_4$ and CaH_2PO_4 (NOLLA; ANGHINONI, 2006).

Calcium is very important in tissue formation, epidermal stability, and root development (PETRAZZINI et al., 2014). The quantities present in the fish ration allowed the aquaponics plants to accumulate a greater quantity than the hydroponics plants (Figure 5 D). The C6 crops contributed to avoiding a reduction in the head diameter, number of leaves, dry leaf weight, shoot dry weight, root dry weight, and total dry weight (Figures 3 A, B, C, D, E, and F).

The absorption of S in the C6 aquaponics crops was equal to that of the hydroponics crops (Figure 5 F), while staying above the critical level of the crops, which is between 1.7 g kg^{-1}and 2.1 g kg^{-1}(Figure 4 F). In C2 crops, the concentration may have been below 1.7 g kg^{-1} (Figure 4 F), which may have contributed to the reduction in the number of leaves (Figure 3B). It is important to maintain adequate levels of S in aquaponic systems, as this nutrient is directly linked to the number of leaves and plant height (ALMEIDA et al., 2011; HU et al., 2015), which are important commercial parameters.

Fe was the most accumulated micronutrient in the lettuce grown in aquaponic, and the accumulation was equal to that of the C5 and C6 hydroponics crops (Figure 5 I). In the C5 and C6 crops, the Fe concentration in the leaves was adequately within the critical range (Figure 4 I). This may be explained by the process of chelating Fe^{2+} with EDTA, which neutralises positive Fe charges and results in a chelate with negative residual charge. This increases the availability of Fe, thereby preventing precipitation because of the pH (MORUZZI; REALI, 2012). However, the chelate may have suffered decomposition due to the action of bacteria present in the system, thereby releasing Fe^{2+} into the culture solution, which precipitates with $H_2PO_4^-$ when oxidised to Fe^{3+}. The chemical behaviour of Fe, including its solubility, depends on the intensity of oxidation or reduction that occurs in the system, and is influenced by physicochemical factors, such as pH and microorganisms (TAIZ et al., 2017). In solutions with higher alkalinity and pH levels above 6.5, such as those of our crops in aquaponics, Fe salts have a constant low stability (SOMERVILLE et al., 2014), and easily precipitate in aqueous solutions (LENZI et al., 2017). The carbonates present in the solution interact with Fe^{2+} to form $Fe(OH)_2$ or $FeCO_3$, and Fe^{3+} can form $Fe(OH)_3$, which is unavailable to plants (MORUZZI; REALI, 2012).

In the C5 cultivation, the accumulation of Cu and Zn was less than that in hydroponics (Figures 5 H and K). Furthermore, the Cu concentration was below the critical range in the C5 and C6 aquaponics crops (Figure 4 H), and Mn was below the critical level in the C6 crops. The Cu and Zn accumulation (Figures 5 H and K) in the C5 and C6 aquaponic crops was lower than that in hydroponics, and the leaf copper content was below the critical level in both crops (Figure 4 H). The C6 crops also had Mn concentrations below the critical level for lettuce crops (Figure 4 J). These nutrient concentrations did not influence plant growth, and there were no visual symptoms of deficiency. This is because the high demand for these nutrients did not occur in the vegetative development phase, but rather in the reproductive phase of lettuce, where it accumulates to more than 50% during flowering (KANO; CARDOSO; VILAS BÔAS, 2011).

Even at a more alkaline pH, the EC was lower than that of the hydroponic solution. The fact that the commercial fish feed does not meet all the nutritional demands of the lettuce plants shows that with the adjustment of the nutrient content in the water and proper management techniques, it is possible to achieve production equal to that of hydroponics in the aquaponic systems. However, for this to occur, it is necessary to add mineral fertiliser to the water before the beginning of the cultivation to increase the nutrient supply with the maturation of the system. It is also important to use adequate feeding rates, and to respect the density of fish and plants per cultivation area. Thus, as long as the fish reach an adequate size for the market, the producer can generate income from successive lettuce crops, thereby limiting fish production costs.

CONCLUSION

To produce lettuce in an aquaponic system, it is necessary to allow the crop water to mature for at least 30 days, as well as to supplement micronutrients in the form of mineral fertiliser.

The correct recommendation of mineral supplementation of micronutrients, crude protein content of the ration, and fish density must be considered because of the interaction among them in the aquaponic system.

ACKNOWLEDGMENTS

The Research Supporting Foundation for the State of Minas Gerais (Fapemig) funded this project. The Federal University of the Jequitinhonha and Mucuri Valley (UFVJM) provided the infrastructure required to conduct this study.

REFERENCES

ALI, L. G. et al. Enhancement of germination and early seedling growth of rice (*Oryza sativa*) var. FARO44 by seed priming under normal and drought stressed conditions. **Journal of Plant Nutrition**, 43: 1579-1593, 2020.

ALBUQUERQUE, F. S. et al. Nutrientes minerais em pimentão fertirrigado sob lâminas de irrigação e doses de potássio. **Horticultura Brasileira**, 30: 681-687, 2012.

ALMEIDA, T. B. F. et al. Avaliação nutricional da alface cultivada em soluções nutritivas suprimidas de macronutrientes. **Biotemas**, 24: 27-36, 2011.

ASHKIANI, A. et al. Effects of foliar zinc application on yield and oil quality of rape seed genotype sunder drought stress. **Journal of Plant Nutrition**, 43: 1594-1603, 2020.

BAKHSHANDEH, E. et al. Effects of plant growth promoting microorganisms inoculumson mineral nutrition, growth and productivity of rice (*Oryza sativa* L.). **Journal of Plant Nutrition**, 43: 1643–1660, 2020.

BUGBEE, B. Nutrient management in recirculating hydroponic culture. **Acta Horticultura**, 648: 99-112, 2004.

CARNEIRO, P. C. F. et al. **Aquaponia**: produçãosustentável de peixes e vegetais.1. ed. Aracaju, SE: Embrapa Tabuleiros Costeiros, 2015. 30 p.

CEROZI, B. S.; FITZSIMMONS, K. The effect of pH on phosphorus availability and speciation in an aquaponics nutrient solution. **Bioresource Technology**, 219: 778-781, 2016.

CORTEZ, G. E. P. et al. Qualidade química da água residual da criação de peixes para cultivo de alface em hidroponia. **Revista Brasileira de Engenharia Agrícola e Ambiental**, 13: 494-498, 2009.

DELAIDE, B. et al. Plant and fish production performance, nutrient mass balances, energy and water use of the PAFF Box, a small-scale aquaponic system. **Aquacultural Engineering**, 78: 130-139, 2017.

DUARTE, E. et al. Post-larval culture of Nile tilapia with different ratios of calcareous hell/gravel substrate in biofilters. **Revista Brasileira de Ciência Veterinária**, 20: 43-48, 2013.

FERREIRA, D. F. SISVAR: A computer analysis system to fixed effects split plot type designs. **Revista Brasileira de Biometria**, 37: 529-535, 2019.

ENDUTA, A. et al. Nutrient removal from aquaculture waste water by vegetable production in aquaponics recirculation system. **Desalination and Water Treatment**, 32: 422-430, 2011.

HAO, Y. et al. Review States, Trends, and Future of Aquaponics Research. **Sustainalibity**, 12: 77-83, 2020.

HU, Z. et al. Effect of plants species on nitrogen recovery in aquaponics. **Bioresource Technology**, 188: 92-98, 2015.

KANO, C.; CARDOSO, A. I. I.; VILLAS BÔAS, R. L. Acúmulo de nutrientes pela alface destinada à produção de sementes. **Horticultura Brasileira**, 29:70-77, 2011.

LANA, R. M. Q. et al. Produção da alface em função do uso de diferentes fontes de fósforo em solo de Cerrado. **Horticultura Brasileira**, 22: 525-528, 2004.

LENZI, G. L. et al. Produção de alface (*Lactuca sativa*) em efluentes de um cultivo de tilapias mantidas em sistema BFT em baixas salinidade. **Boletim Instituto da Pesca**, 43: 614-630, 2017.

LI, H.; WANG, L.; YANG, Z. M. Co-expression analysis reveals a group of genes potentially involved in regulation of plant response to iron-deficiency. **Gene**, 554: 16-24, 2015.

MACÊDO, J. A. B. **Métodos laboratoriais de análises físico -químicas e microbiológicas**. 3. ed. Belo Horizonte, MG: Conselho Regional de Química, 2005. 602 p.

MARTINEZ, H. E. P. Hidroponia. In: RIBEIRO, A. C.; GUIMARÃES, P. T. G.; ALVAREZ, V. V. H. (Eds.). **Recomendações para o uso de corretivos e fertilizantes em Minas Gerais**: **5ª aproximação**. Viçosa, MG: CFSEMG, 1999, v. 1, cap. 16, p. 131-166.

MOREIRA, A. et al. Efeito da relação cálcio e magnésio do corretivos sobre micronutrientes na alfafa. **Pesquisa Agropecuaria Brasileira**, 35: 2051-2056, 2000.

MORUZZI, R. B.; REALI, M. A. P. Oxidação e remoção de ferro e manganês em águas para fins de abastecimento públicoou industrial – uma abordagem geral. **Revista de Engenharia e Tecnologia**, 4: 29-43, 2012.

NAVARRO, R. D. et al. Growth of fingerlings in different stocking densities in tropical aquaponic system of basil production. **Ciência e Natura**, 43: 1-35, 2021.

NOLLA, A.; ANGHINONI, I. Atividade e especiação química na solução afetadas pela adição de fósforo em latossolo sob plantio direto em diferentes condições de acidez. **Revista Brasileira de Ciência do Solo**, 30: 955-963, 2006.

OBIRIKORANG, K. A. et al. Aquaponics for Improved Food Security in Africa: A Review. **Frontiers in Sustainable Food Systems**, 5: 1-10, 2021.

PANTANELLA, E. et al. Aquaponics vs. Hydroponics: production and quality of lettuce crops. **Acta Horticulture**, 927: 887-894, 2012.

PETRAZZINI, L. L et al. Deficiências de nutrientes em alface americana cultivada em hidroponia. **Horticultura Brasileira**, 32: 310-313, 2014.

RAMOS, M. J. M. et al. Deficiência de macronutrientes e de boro em abacaxizeiro 'Imperial': Composição mineral. **Revista Brasilelira de Fruticultura**, 33: 261-271, 2011.

RIBEIRO, H.; JAIME, P. C.; VENTURA, D. Alimentação e sustentabilidade. **Estudos Avançados**, 31:185-198, 2017.

SILVA, F. C. **Manual de análises químicas de solos, plantas e fertilizantes**. 2. ed. Brasília, DF: Embrapa Informações Tecnológicas, 2009. 623 p.

SOMERVILLE, C. et al. **Small-scale aquaponic food production. Integrated fish and plant farming**. 1. ed. Roma: FAO Fisheries and Aquaculture Technical, 2014. 262 p.

SOUZA, L. H. et al. Efeito do pH do solo rizosférico e não rizosférico de plantas de soja inoculadas com *Bradyrhizobium japonicum* na absorção de boro, cobre, ferro, manganês e zinco. **Revista Brasileira de Ciência do Solo**, 34: 641-1652, 2010.

TAIZ, L. et al. **Fisiologia e Desenvolvimento Vegetal**. 6. ed. Porto Alegre, RS: Artmed, 2017. 888 p.

ZOU, Y. et al. Effects of pH on nitrogen transformations in media-based aquaponics. **Bioresource Technology**, 210: 81-87, 2016.

ZOPPAS, F. M.; BERNARDES, A. M.; MENEGUZZI, A. Parâmetros operacionais na remoção biológica de nitrogênio de águas por nitrificação e desnitrificação simultânea. **Engenharia Sanitaria Ambiental**, 21: 29-42, 2016.

WAHYUNINGSIH, S.; EFFENDI, H.; WARDIATNO, Y.

Nitrogen removal of aquaculture wastewater in aquaponic recirculation system. **AACLBioflux**, 8: 491-499, 2015.

WONGKIEW, S. et al. Nitrogen transformations in aquaponic systems: A review. **Aquacultural Engineering**, 76: 9-19, 2017.