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Microbiological analysis of lettuce (*Lactuca sativa* L.) grown in an aquaponic and hydroponic system

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ABSTRACT

Aquaponics is the integration of aquaculture and hydroponics and it generally corresponds to a recirculating aquaculture system where the waste produced by aquatic organisms becomes nutrients through bacterial action for plant growth. Water consumption as well as the environmental impact are lower in this system compared to hydroponic system and traditional aquaculture system. The recent study evaluated the microbiological quality of lettuce (*Lactuca sativa* L.) farmed in two production systems: aquaponics and hydroponics. At the same time, we evaluated fresh mass gain and feed conversion ratio (FCR) of rainbow trout (*Oncorhynchus mykiss*). The lettuce was farmed in an aquaponic system with waste from the fish, and in a hydroponic system with nutrient solution (Hoagland II-modified) for 21 days. At the end of this term, large lettuce was gotten from 8 to 12 cm. The lettuces that grew in both systems did not showesignificant differences in the microbiological quality. The aquaponic system started with rainbow trout, which have an average mass of 27.1 ± 0.8 g, and duringthe experiment rainbow trout gained 13.6 g, getting a FCR of 0.74 after the experiment. These results indicate that the aquaponic system used is a sustainable alternative for the production of high quality lettuce, allowing at the same time the farming simultaneous of fish with a good feed conversion ratio.

Keywords: Aquaponic, hydroponic, microbiological quality, feed conversion rate, rainbow trout.

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INTRODUCTION

Worldwide lettuce (*Lactuca sativa* L.) is the most economically important farming among leafy vegetables, due to the possibility of annual farming, under different production systems and for the diversity of botanical varieties and crops (Suslow et al., 2003). This vegetable is grown mostly in soils irrigated mainly by furrows (Flaño, 2013), leading to a large consumption of water and the risk for its safety in case the water is polluted by pathogenic bacteria (e.g. *Escherichia* coli 0157:H7, Salmonella or Listeria monocytogenes) (Sirsat and Neal, 2013).

Hydroponics is a method of farming without the use of soil. Instead of that, we can use inert solid substrates or liquid means without substrate. In these systems, all the necessary nutrients for the growth of plants are gotten from synthetic fertilizers (Tonet et al., 2011; FAO, 2015). Besides that, hydroponics is a more controlled system than direct farmingon the soil, which allows more efficient use of water and fertilizers with less risk of plagues and diseases. However, complete dependence on manufactured fertilizers make their implementation very expensive for small farmers (FAO, 2015; Stefanelli et al., 2011; Hashida et al., 2014). In this sense, integration with other complementary crops, such as fish production, can be a strategy to facethe necessityfor fertilizers by plants.

Aquaculture is the production of aquatic organisms in captivity as fish, shellfish, etc. The main categories of production systems including open systems (cages),

ponds, recirculating aquaculture systems (RAS), etc. (Woynarovich et al., 2011). Aquaculture is an increasingly important activity in global fish production, but it shows sustainability problems due to the treatment of nutrient-rich wastewater, whisch is an aquaculture sub-product (FAO, 2015). This partially happens because alowamount of dietary nutrients is retained by the fish. Most of these nutrients are excreted by the fish as solid and dissolved fractions, which are gathered in systems with low water exchange and then, when this nutrientsare separated, the wáter quality changes (FAO, 2015; Endut et al., 2010).

A suggestion of solution to this kind of problem is the use of aquaponic systems integrating aquaculture and hydroponics (Figure 1). The goal of this system is to produce fish and vegetables in a closed circuit, where the use of synthetic fertilizers and the removal of waste is practically zero (Rakocy et al., 2003; Guzmán and Moreno, 2012; Nelson and Pade, 2008; Rakocy et al., 2006). In this typeof system fish waste is become to nutrients for plants by the action of nitrifying bacteria. These bacterias oxidize ammonia to nitrite and this to nitrate, prevaling the group *Nitrosomonas* spp. And *Nitrobacter* spp. respectively for each transformation (Hollyer et al., 2009). This is why this food production system has been described as very efficient and ecological (Sirsat and Neal, 2013).

Knowing the microbiological quality of lettuce is important, especially in aquaponic systems since fish waste is used as nutrients that can contaminate the water and vegetables (FAO, 2015).

Finally, aquaponic system is a good alternative for vegetable production using waste fish and optimizing water use. However, there is a limited information about the microbiological quality of vegetables, which was produced thanks to the aquaponic system, because of that, the objective of this study was to test the functioning of an aquaponic system and then evaluate the microbiological quality of farming lettuce (*Lactuca sativa* L.) in this system compared to others produced in a conventional hydroponic system. The gained fresh mass of rainbow trout (*Oncorhynchus mykiss*) produced in the aquaponic system was a study too.

MATERIALS AND METHODS

Trial location

This recent research was done in the greenhouse and the laboratories of Centro de Estudios Postcosecha (CEPOC), Facultad de Ciencias Agronomicas, Universidad de Chile. This place is located at 33°57′ south latitude and 70°60′ west longitude and 627 meters avobe sea level, Comuna de La Pintana, Provincia de Santiago, Region Metropolitana, Chile (Figure 2).

Experimental design

The design was completely randomized with 2 treatments and 3 repetitions per treatment, total 6 experimental pieces. The treatments belong to an aquaponic system and a hydroponic system, both with a system of lettuce farming by the floating root technique with a density of 70 plants/m^2.

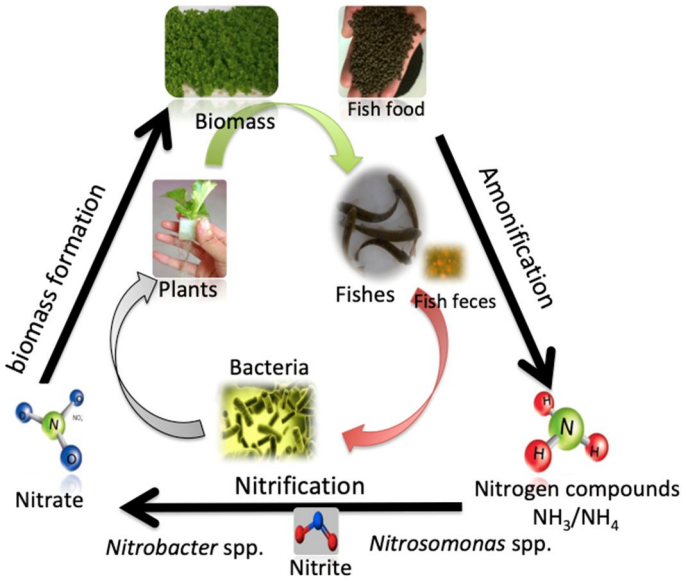

Figure 1. Symbiotic aquaponic cycle.

Crop management

Lettuce of the botanical variety Acephala, cultivar levistro was used. The seeds were planted in 200 pieces alveolate trays, with a mixture of granulated rock-wool substrate (Agrolan® Compañía Industrial El Volcán SA, Chile) and expanded-perlite A6 (Harbolite Chile Ltda., Chile) pre-hydrated in 1: 1 volumetric ratio respectively. Seeds were planted one by alveolus at 1 cm depth. Subsequently, the trays were placed in a stove (LabTech Co. Ltda., Korea), at 25°C until the emission of the radicle and then placed on a seedbed inside the greenhouse. The irrigation was carried out with potable water until the seedlings reached the development state of expanded cotyledons. Then they were irrigated with nutritive solution Hoagland II-modified (Hoagland and Arnon, 1950), diluted at 50% maintaining a pH between 5.5 and 6.5.Each seedling was transplanted when it reached 3rd state or 4th true leaf to the hydroponics components of each system.

Aquaponic system

Aquaculture

Samples of rainbow trout (*Oncorhynchus mykiss*) were cultivated in a rectangular pond with a flat bottom (0.7 × 0.4 × 0.5 m), of 120 L of capacity with dechlorinated drinking water (Figures 3 and 4A). The rainbow trout was acquired in the fish farming Rio Blanco located in Los Andes, Quinta Region. It worked with 40 specimens per repetition, and at the beginning, each fish sample had an average fresh mass of 24.4 ± 0.8 g. They were fed twice a day with pelletized commercial feed (Ewos® transfer, Chile, containing 48% protein) at 1.44% of their fresh body mass.

Biofilter

The pond and the section of the biofilter (Figures 3 and 4B) were

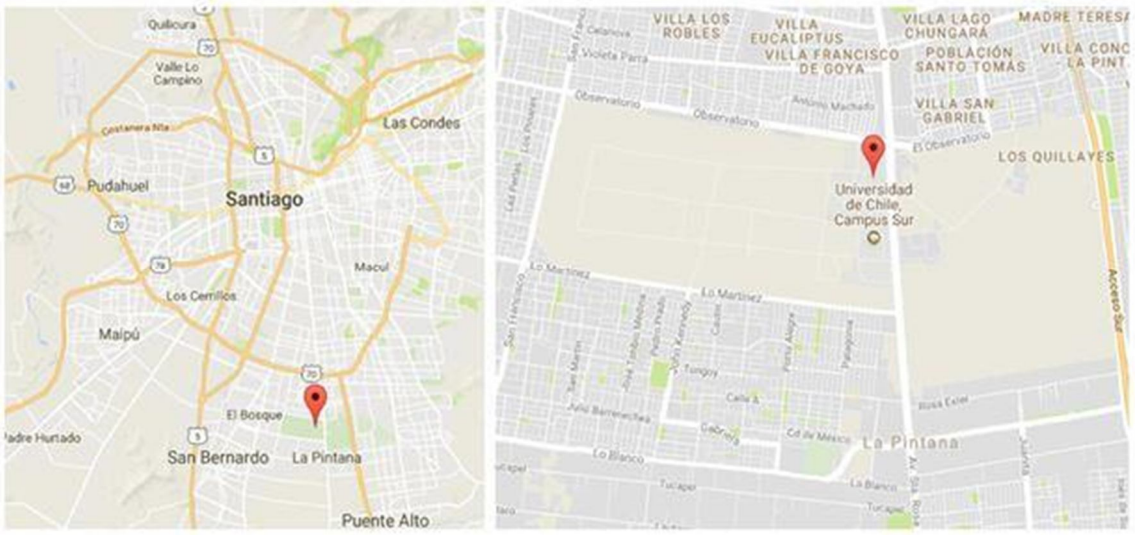

Figure 2. Geographic location, Universidad Campo Sur, Chile.

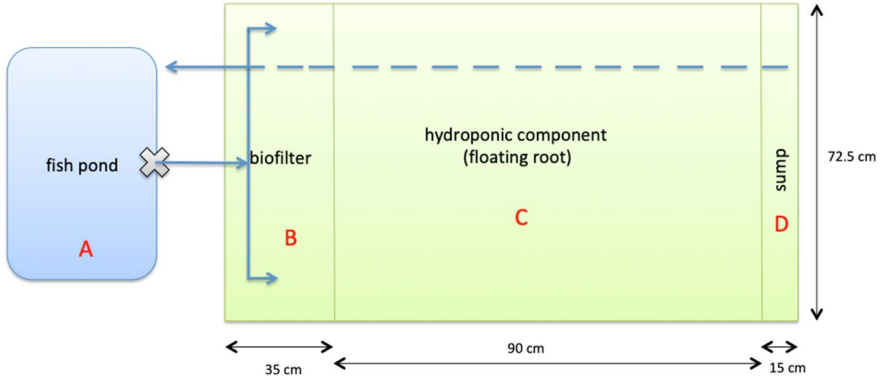

Figure 3. Chart of the modified aquaponic system.

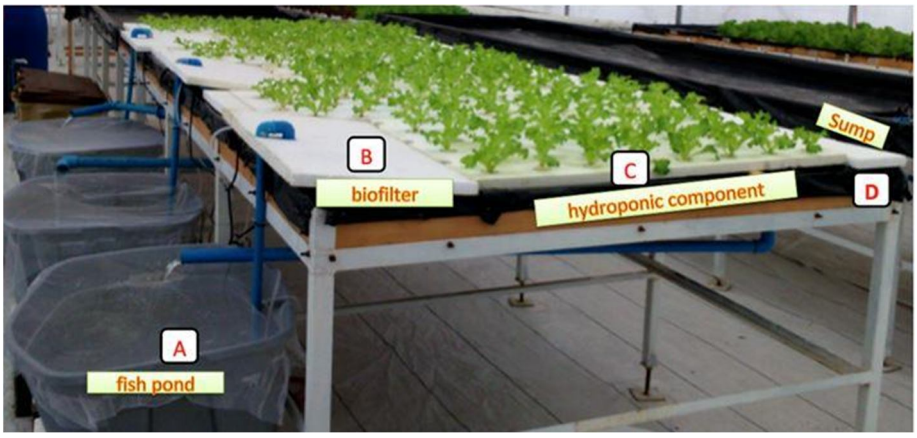

Figure 4. Components of the modified aquaponic system.

connected through a hydraulic PVC pipe of 2.54 cm in diameter. A submersible pump (Sicce IDRA, Italy) located in the pond boosted water (10 L/min) from the fish pond to the biofilter section. The biofilter was connected to the hydroponic component (Figure 3 and

4C) formed by a floating root table through 8 pipes of 1.6 cm diameter. These two sections were at the same height, 0.7 m above the pond with fish. The biofilter was constituted by a section of 0.35 × 0.725 m, where 400 bio-balls were placed (Bio-ball Sunsun, China) with a total surface area of 20 m^2 (Rakocy et al., 2006). Water passes by gravity from the biofilter section hydroponically component.

Hydroponic component

The tables had dimensions of 1.05 × 0.725 m, and as support for the plants were used plates of expanded polystyrene of medium density (20 kg/m^3) and 2.54 cm thick. The water passes by gravity from the hydroponic section to the sink (Figures 3 and 4D), coming back to the fish pond through a hydraulic PVC pipe of 2.54 cm diameter, thus closing the circuit (Figures 3 and 4) (Rakocy et al., 2006).

Hydroponic system

The tables had dimensions of 1.5 × 0.6 m and, as a support for the plants were used expanded polystyrene sheets of medium density (20 kg/m^3) and 2.54 cm, drilled with a hole of 5 cm diameter. A plant was placed in each hole and 70 plants in a square meters following a Zigzag design. The roots of the plants were in direct contact with the liquid mean (water and nutrient solution). With the help of an air pump (Tetratec APS 300, China) oxygen was introduced into each table to oxygenate the roots of the plants. The concentration of nitrate in the nutrient solution at the start of the experiment was 150 mg/L.

35 lettuce plants were harvested from each repetition of both systems when the leaves reached 8-12 cm in length since they were transplanted 21 days ago. The harvested leaves were placed in 18 bags (3 bags per repetition, 50 g) of low density polyethylene with which the microbiological analyzes were carried out.

Determinations

Microbial count

The analysis was done by the time of harvest. Three samples (per replication) of 10 g of whole lettuce per bag was taken and mixed with 90 ml of 0.1% sterile peptonated water (Merck, Germany) in a sterile bag and homogenized in a mixer (IUL, Masticator Classic, Spain) for 1 min. Serial dilutions (1:10) were made in 9 ml of 0.1% peptonated water.

- ✓ **Mesophilic aerobic count (AMC):** A depth seeding (1 ml) of the appropriate dilution was carried out on the plate counting agar mean (Merck, Germany) and incubated at 37°C for 48 h.
- ✓ **Enterobacteriaceae:** A deep farming was carried out in the red glucose violet bile agar mean (VRBD) (Merck, Germany) and incubated at 37°C for 48 h.
- ✓ **Psychrophilic bacteria:** Deep farming was done on the agar plate counting agar (Merck, Germany) and incubated at 5°C for 10 days.

Total counts were expressed as the logarithm of colony forming units per gram (log cfu/g).

Increase in fresh mass of rainbow trout

A portable scale was used to measure the fresh mass of the fish. Weekly samplings were made, taking 15 samples of trout (3 groups of 5) at random from each pond. The feed conversion ratio was calculated (FCR = [kg of food consumed / (final fresh mass - initial fresh mass)]). Thus, the percentage increase in fresh mass was calculated using the following formula [(final fresh mass * 100) / initial fresh mass]] of the rainbow trout.

Statistical analysis

The results obtained were subjected to an analysis of variance (ANOVA) with significance level of 5%, and in the case of significant differences between treatments, Tukey's multiple rank comparisons test was applied using the statistical program InfoStat (2014p, Argentina).

RESULTS AND DISCUSSION

Microbiological counts

In both farming systems dit not show significant differences in the microbiological counts by the time of harvest. The average counts were 3.2 ± 0.1 log CFU/g for aerobic mesophiles 1.0 ± 0.5 log CFU/g for enterobacteria and 2.3 ± 0.3 log CFU/g for psychrophilic bacteria in lettuce leaves farmed by the aquaponic (AS) and hydroponic (HS) systems (Figure 5).

Mesophilic

The count of mesophilic aerobic bacteria (AMC) in leaves was similar to the value reported by Sirsat and Neal (2013), who found 3.2 log CFU/g for Romaine lettuce, which was farmed byaquaponics system in a greenhouse. While Selma et al. (2012) and Scuderi et al. (2011) reported counts of 4.0 and 6.0 log CFU/g for lettuce, which was cultivated by hydroponic system NGS and floating root, respectively.

Enterobacteriaceae

The enterobacteria count was less than 2.3 log CFU/g as reported by Scuderi et al. (2011) in a similar study.

Psychrophiles

The count of psychrophilic bacteria in leaves was lower than the value reported by Orellana (2011), who found 4.9 log CFU/g reported for arugula leaves, which were farmed by hydroponics system in the greenhouse. It is important to have low counts of psychrophilic bacteria, since when westored the vegetable in refrigeration, they could multiply quickly and affect their quality (Selma et al., 2012).

The low microbiological counts on the leaves of lettuce at the time of harvest could be due to the fact that drinking water was used in the farming systems as a

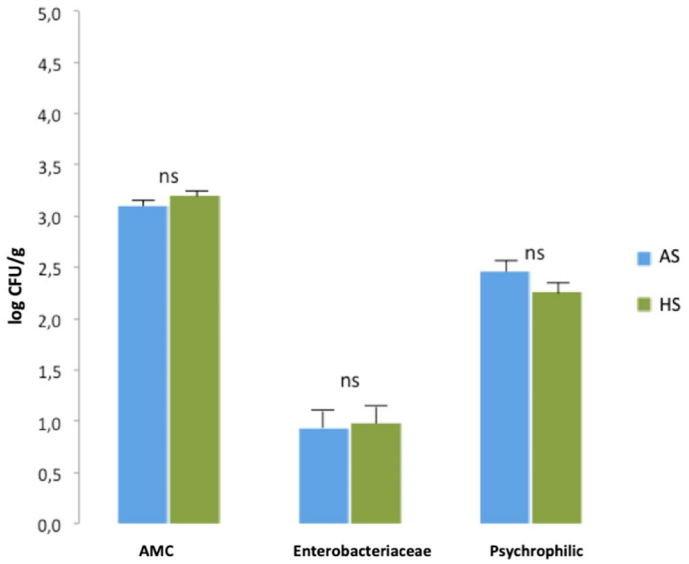

Figure 5. Mesophilic aerobic count (AMC), enterobacteriaceae and psychrophilic bacteria (log CFU/g) at the time of harvest in lettuce leaves farmed in both systems. The bars represent the arithmetic average (n = 3) ± ES. Ns, not significant (P > 0.05).

source of water for the start-up and recharge of the system (Hollyer et al., 2009). In addition, the leaves of the lettuces were never in contact with the water of fish in the aquaponic system nor with the nutritive solution in the hydroponic system (Erickson, 2012).

The water source used in aquaponic systems has a significant impact on the quality of the final products, as fish or plants (Chalmers, 2004). One of the most studied bacteria is *Escherichia coli*, which is found in the intestines of warm-blooded animals such as pigs, birds, warm-blooded animals, etc. To be present this bacterium in aquaponic system, it should come from birds or from the same operators, in case that in the farm doesn't practice good farming practices (Fox et al., 2012). It is considered that the average temperatura of the poikilotherm animals (whose body temperature varies according to the temperature of the environment) as fish, which is low to make a favor the optimal proliferation of most enteric bacteria that can infect humans (Sugita et al., 1996). The worries about the microbiological quality is each time more important, because this vegetable is consumed raw, without any lethal treatment for microorganisms and can affect consumer health (Franz et al., 2008). An increased risk of pollution and a subsequent increase of microbial populations occurs when the surfaces of the lettuce are contacted directly with the soil (Fallovo et al., 2009), unlike the aquaponic and hydroponic systems cultivated by the floating root or NFT techniques, where only the roots of the plants are in contact with the nutritive solution and not the comestible aerial part (FAO, 2015). For these reasons in this study, the lettuce grown in both systems had microbiological counts lower than that established by the Food Sanitary Regulation for fruits and other pre-prepared vegetables ready for consumption (Ministerio de Salud Pública de Chile, 2014). This regulation establishes a maximum limit for AMC and enterobacteriaceae of 6.69 and 4.69 log CFU/g, respectively.

Increase in fresh mass of rainbow trout

Table 1 shows the increase in fresh mass and the feed conversion factor (FCR) of rainbow trout cultivated in the aquaponic system. The average fresh mass at the beginning of the experiment was 27.1 ± 0.83 g per fish. After 3 weeks the fish increased their fresh mass by 13.6 ± 1.5 g with a feeding rate of 1.44% of their fresh body mass per day. The feed conversion factor of the fish was 0.74.

For any aquaculture system, the survival of the fish and the growth parametersparametros are really important (Lennard and Leonard, 2006). Waynarovich et al. (2011) indicate a normal mortality of 5% (in a period of 4 to 6.5 months of farming) for rainbow trout with a fresh mass greater than 25 g within a traditional aquaculture system. In the recent study, there was no rainbow trout mortality in any of the replicates for the 21-day trial. In terms of feed conversion efficiency, the FCR value got in the present study (Table 1) are within the range described by Merino and Von-Brand (2015). This author indicates an adequate FCR <1 for rainbow trout with a fresh mass less than 100 g and fed with 42% protein pellets grown in a commercial aquaponic system. In another research, Lennard and Leonard (2006) and Palm et al. (2014) got an ACF of 0.85 and 0.93 for codfish Murray (*Maccullochellapeelii peelii*) and Tilapia de Nilo which are kinds of fish (*Oreochromis niloticus*) respectively, both associated with lettuce farming. The growth and FCR of the fish depends directly on the quality and quantity of the food and the water quality during the crop (Palm et al., 2014). The food that was used in the present study contains 48% protein and the different water quality parameters were within the recommended range for rainbow trout (Endut et al., 2010).

CONCLUSION

In view of the results and considering other investigations, we can affirm the microbiological quality of the vegetables and a synergic effect among the two sub productive systems (hydroponic and aquaculture), obtaining good efficiency in vegetable and fish. It should be taken into account that this study lasted 21 days, with the main purpose of designing and testing an aquaponic system that reaches the balance between the sub systems and that ensures the production of fresh and innocuous lettuces together with a live fish.

Table 1. Increase in fresh dough and feed conversion factor (FCR) and weekly fresh mass increase of 40 fish from the aquaponics system. The values of fresh biomass (number of fish x fresh mass (g) of fish) are the arithmetic average (n = 3) ± ES.

AS	Farming weeks				Consumed food	FCR
	0	1	2	3		
Accumulated biomass (g)	1085 ± 33	1294 ± 53	1453 ± 52	1614 ± 78	393 ± 4	0.74
Increased fresh weight (%)	-	19	12	11		

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