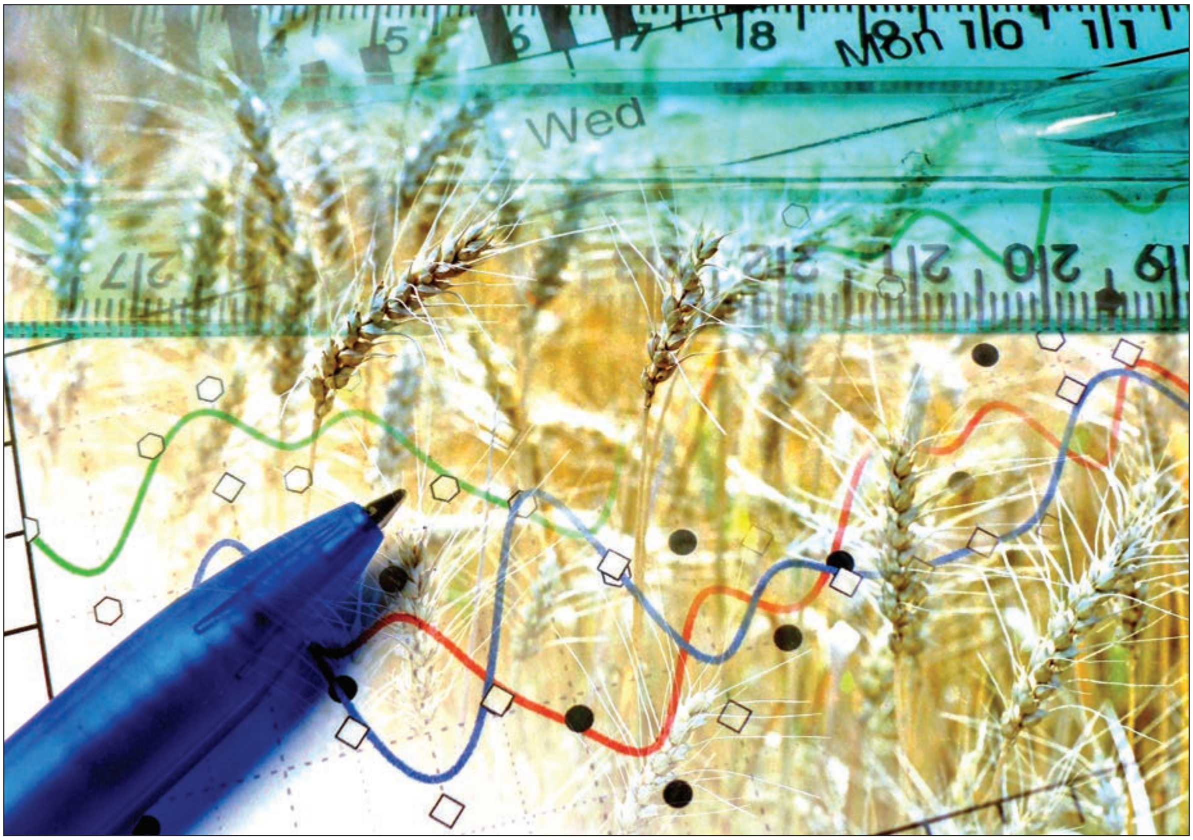

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Nitrate Content on Summer Lettuce Production Using Fish Culture Water*

[1]E. Rico-García, [2]V.E. Casanova-Villareal, [1]A. Mercado-Luna, [1]G.M. Soto-Zarazúa, [1]R.G. Guevara-González, [1]G. Herrera-Ruiz, [1]I. Torres-Pacheco and [1]R.V. Velázquez-Ocampo
[1]División de Estudios de Posgrado, Facultad de Ingeniería, Universidad Autónoma de Querétaro, Cerro de las Campanas, Colonia las Campanas, C.P. 76010, Querátaro, Qro. México
[2]Instituto Tecnológico de la Zona Maya, Kilometro 21.5 Carretera Chetumal-Escarcega Municipio Othon, P. Blanco, Ejido Juan Sarabia, C.P. 77060, Quintana Roo, México

Abstract: The aim of this study was to investigate the nitrate concentration on lettuce (*Lactuca sativa* cv. Px06516006) grown in fish culture water. This study was carried out in Queretaro State, Mexico. Lettuce cultivars were grown in a floating system inside a plastic greenhouse. The cultivation of lettuce was divided into 10 beds; four beds were used as a control group, with a standard nutrient solution and the other six beds were used as treatment group, fish culture water with the addition of missing nutrients. A density of 30 plants m^{-2} was used. Two trial periods were tested in the summer of 2008, 29 days from April 11 to May 9 for the first experiment and 37 days from May 22 to June 27 for the second experiment. The system was assessed on basis of leaves nitrate content, total fresh weight and total dry weight. In both trials the final nitrate content of leaves was less than 2400 mg kg^{-1}. No significant differences ($p<0.05$) were found for fresh and dry weights among treatments. Based on these results it is shown that fish culture water is suitable for low nitrate content lettuce production with no detriment to plant quality neither yields during summer.

Key words: Floating, greenhouse, lettuce, nitrates, tilapia culture water

INTRODUCTION

Nitrate and ammonium are the major sources of nitrogen absorbed by plants. Most of the ammonium has to be incorporated into organic compounds in the roots, whereas nitrate is readily mobile in the xylem and can also be stored in the vacuoles of roots, shoots and storage organs. Nitrate accumulation in vacuoles can be of considerable importance for cation-anion balance, osmoregulation, particularly in so-called nitrophilic species, and in regards to the quality of vegetables and forage plants (Marschner, 1997). Sources which provide humans with nitrate can be of exogenous and endogenous origins. One of the main exogenous sources is the consumption of vegetables which represents as much as 60 to 90% of daily nitrate intakes. The main endogenous source is the L-arginine-NO pathway, which is always active throughout the body and produces NO from the amino acid L- arginine and oxygen (De Graaf, 2006).

Nowadays still there is a controversy whether nitrate has harmful or beneficial effects on human health (L'hirondel *et al*., 2006; Ward *et al*., 2006). Harmful effects of nitrate arise when nitrate is reduced to nitrite by bacteria in the gastrointestinal tract. Nitrate and nitrite to smaller extend are

Corresponding Author: Enrique Rico-García, Facultad de Ingeniería, Universidad Autónoma de Querétaro, Centro Universitario Cerro de las Campanas, S/N, Colonia Las Campanas, Querétaro, Qro, Código Postal 76010, México
Tel: +52 01 442 192 12 00/ 6016 Fax: +52 01 442 192 12 00/6006

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involved in metabolisms that can result in formation of N-nitrosamines, which are carcinogenic and can caused gastric cancer and urinary bladder cancer. Another harmful effect is methamoglobinaemia caused by reduction of nitrate to nitrite and nitric oxide that oxidizes hemoglobin in red blood cells to abnormal form known as methaemoglobin. This compound can not transport oxygen causing lack of oxygen in body tissues. This condition generally affects infants up to 12 months old. Beneficial effects consist of relations between nitrate and the killing of pathogens. It may also help to kill ingested pathogens in the stomach and improve gastric mucosal blood flow and mucus secretion. Dental caries, skin infections, urinary tract infections may be inhibited by growth-inhibition or self-destruction of harmful bacteria that are exposed to acidified nitrite (De Graaf, 2006).

The leaves of lettuce plants can accumulate nitrates of up to 6000 mg kg^{-1} when grown in hydroponics as was done in northern Europe in winter (Gent, 2006). Ioslovich and Seginer (2002) also proposed a control policy to reduced nitrate in lettuce cultivars for fixed spacing systems that consisted to start with the highest permissible temperature and with abundant nitrate supply and then to switch down to the feasible temperature and the lowest permissible nitrate supply, this reduced the final nitrate concentration on lettuce plants. Due to concerns about harmful effects of nitrate on human health, the European Union has tried to minimize accumulation of these chemicals in the enviroument and food. In lettuce and other leafy vegetables nitrate content has become a quality mark (Abubaker *et al*., 2007; D'Antuono *et al*., 2007; Prasad and Chetty, 2007), for greenhouse lettuce cultivations maximum allowable nitrate concentration for lettuce harvested in the winter from October 1 to March 31 is 4500 mg kg^{-1} and in summer from April 1 to September 30 it is 3500 mg kg^{-1} (De Graaf, 2006).

The objective of this research was to determine whether fish culture water is suitable for lettuce (*Lactuca sativa* L.) productions in floating bed system using nitrate plant content as an assessing variable. It is well known that fish can supply plants with nitrogen and phosphorous (Hayashi *et al*., 2008; Lennard and Leonard, 2006). In this study the phosphorous fish water content was neglected. So, fish culture water was considered as a partial source of nitrogen.

MATERIALS AND METHODS

Experimental Site

The lettuce was grown inside a plastic greenhouse, 9 m wide, 12 m long with the gutter at 4.2 m and the ridge at 5.9 m high. The lateral ventilation area was 48 m^2 and the roof ventilation area was 7.2 m^2, 44 and 6.6% of the total covered area, respectively. The fish production tank was inside a plastic greenhouse with almost no ventilation, to keep the water temperature above 25°C. These experimental greenhouses are located in Queretaro State University, campus Amazcala, Mexico at a longitude of 100° 16'W; latitude, 20° 42'N; altitude, 1920 m.

Lettuce Crop System

Lettuce seeds (*Lactuca sativa* cv. Px06516006) were germinated (22 days) using a commercial substrate at about 25°C and 70% of relative humidity. Then they were transplanted into the floating system for two trial periods of 29 days for the first experiment from April 11 to May 9 and 37 days for the second experiment from May 22 to June 27 of 2008.

Lettuce cultivars were grown in a floating bed system divided into 10 beds, 1.2 m wide, 2.5 m long and 0.12 m deep with a total cultivation area of 30 m^2 (Fig. 1). Each bed had a plant density of 30 plants m^{-2}, 90 plants per bed. Four beds were used as a control; with a standard nutrient solution, and the other six beds were used as treatment; with fish culture water with the addition of missing nutrients. It was assumed that fish ouly provide a part of nitrogen to water so nitrogen was complemented and all other nutrients were added content. The nutrient solution was proposed based on that of Rodríguez-Delfin *et al*. (2001) all quantities were reduced by 0.75 factor in order to induce

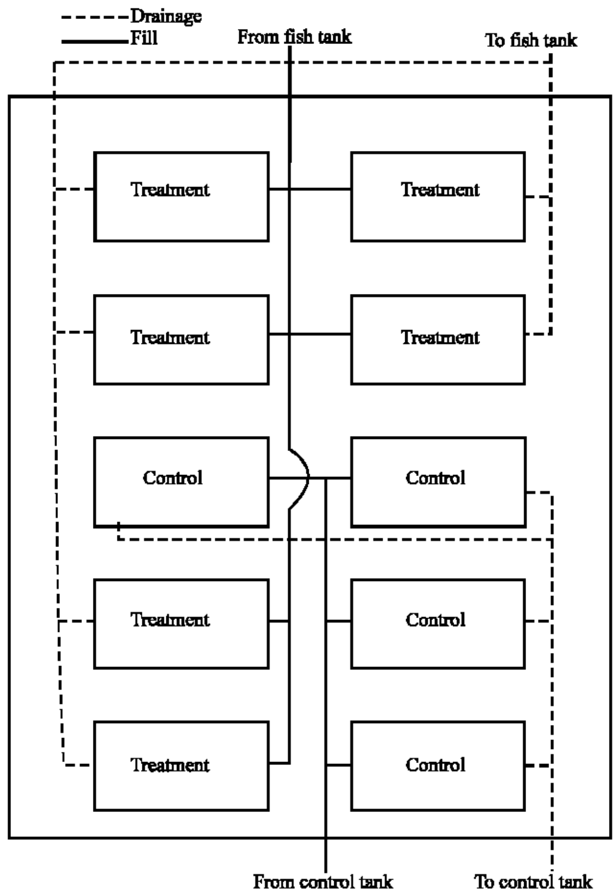

Fig. 1: Schematic distribution of beds

Table 1: Nutrient solutions 75% (ppm) and chemical compounds employed for the nutrient solutions

Chemical compounds				
Name	Chemical formula	Elements	Control	Fish
Potassium nitrate	KNO_3	N	120	105
Mono potassium phosphate	KH_2PO_4	P	27	27
Potassium sulfate	K_2SO_4	K	195	195
Calcium nitrate decahydrate	$Ca(NO_3)_2 \cdot 10H_2O$	Ca	113	95
Magnesium sulfate heptahydrate	$MgSO_4+7H_2O$	Mg	34	34
Sulfuric acid	H_2SO_4	S	176	176
Iron chelate 13.12% (LO)	Fe	Fe	0.75	0.75
Manganese chelate 13%	Mn	Mn	0.37	037
Boric acid 18%	H_3BO_3	B	0.37	0.37
Zinc chelate 14%	Zn	Zn	0.11	0.11
Copper chelate 14%	Cu	Cu	0.07	0.07

This table was modified from Rodríguez-Delfín *et al.* (2001)

a reduction on the final nitrate content on lettuce plants (Ioslovich and Seginer, 2002; Petropoulos *et al.*, 2008; Andersen and Nielsen, 1992). The elements quantities and the chemical compounds used in the nutrient solution are shown in Table 1. The initial nitrogen content was determined as nitrates using an NO_3 meter (Horiba Ltd. Kyoto, Japan). In both, the control and treatment, all water was recirculated twice a day to provide aeration for the lettuce root system at 9:00 and 18:00 h.

Aquaculture System

The fish used in the aquaponic system were tilapia (*Oreochromis niloticus*). At the beginning of first trial period, the tank was stocked with 920 Tilapias of an average weight of 15 g at approximately five weeks old. Fish feeding was calculated as function of fish age and body weight. Fed times and food rations were determined using the growth table for tilapia (*O. niloticus*) production under intensive conditions presented by Morales-Díaz (2003). Commercial floating food pellets of 1.5 mm which contains 35% protein, 3% fat and 5% fiber were used to feed the fish.

The tank was equipped with an aeration system built with stone diffusers, hose and a blower of 2.5 Hp. The aeration system was controlled with an ON-OFF control. Ten minutes each hour from 19:00 to 8:00 h and five min from 9:00 to 18:00 h.

Assessed Variables

Leaves nitrate content; total fresh weight and total dry weight were the variables, taken nitrate content as a principal variable. At the beginning of each trial a sample of 10 small plants was taken to determine the variables initial values. During the experiments a sample of nine plants, chosen randomly was harvested from each bed, 90 plants in total every week. To determine nitrate content 30 plants were used (12 for the control and 18 for the treatment) and 60 plants were used to determined total fresh weight and total dry weight (24 for the control and 36 for the treatment). All plants were harvested between 8:30 and 9:00 h and then placed in a dark cold container to minimize plant physiological activity in order to avoid changes in plant nitrate content (Chandra *et al*., 2008; Prasad and Chetty, 2007; Enninghorst and Lippert, 2003).

Nitrate content was determined using an NO_3 meter (Horiba Ltd. Kyoto, Japan). The time used to measure nitrate content in plants was determine taking into account that in Mexico lettuce plants are usually harvested in the morning time to avoid quality detriment due to solar heat. On the other hand, it was determined that at morning the content of nitrate in lettuce plants is higher than in afternoon (Fig. 2). To obtain plant sap complete plants were smashed until a homogenous paste was obtained from which the sap samples were taken.

To measure fresh and dry weights a balance (Adventurer, Ohaus Corp. Pine Brook. NJ. Max. Cap. 210 g, Readability 0.0001 g) was used. All plants were dried at 75°C, using a stove (RIOSSA Model HSF-41) until the constant weight was achieved.

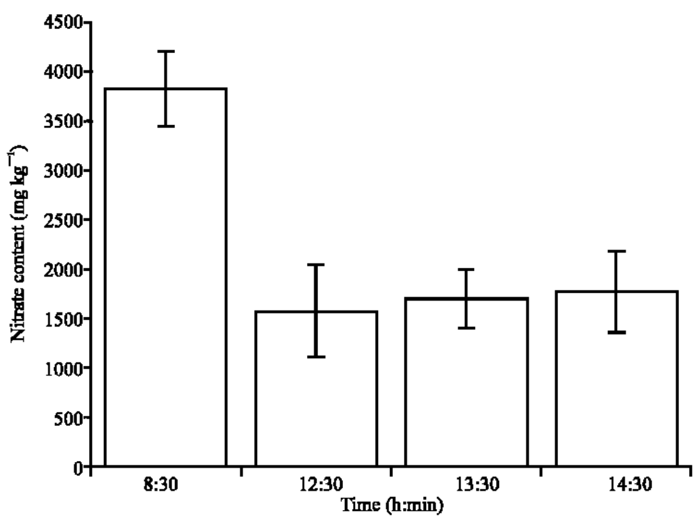

Fig. 2: Nitrate content in lettuce plant according time of the day

Extra Analysis

At the end of each trial of lettuce production a bacteriological analysis was done checking for *Escherichia coli* and *Salmonella* sp. (NOM-112-SSA1-1994 and NOM-114-SSA1-1994). None of these bacteria were found. Also, at the end of both lettuce production cycles a fish tissue analysis was done in order to looking for any excessive metal mineral concentration taking a sample of 50 g for the control and treatment. These concentrations were determined by atomic absorption spectrometry (NOM-117-SSA1-1994).

Statistical Analysis

The experiment design adopted was a completely randomized design. The statistical analysis was done using the SAS, v.9 system software with one way ANOVA with a significance level of $p<0.05$.

RESULTS AND DISCUSSION

Nitrate Concentration on Leaves

Figure 3 shows nitrate behavior along the second trial period. The content of nitrates was higher in the beginning than at the end in both the control and treatment samples. It can be explained if we consider that plants need large quantities of nitrogen at the beginning.

In both trial periods the final nitrate content of the leaves was less than 2400 mg kg^{-1}. According to EU standards for lettuce harvested between April 1 and September 30 the maximum nitrate content is 3500 and 2500 mg kg^{-1} for production in greenhouse and on open field, respectively. In Fig. 3 it can be seen how the nitrate content of lettuce leaves remained high during most of the time when it had

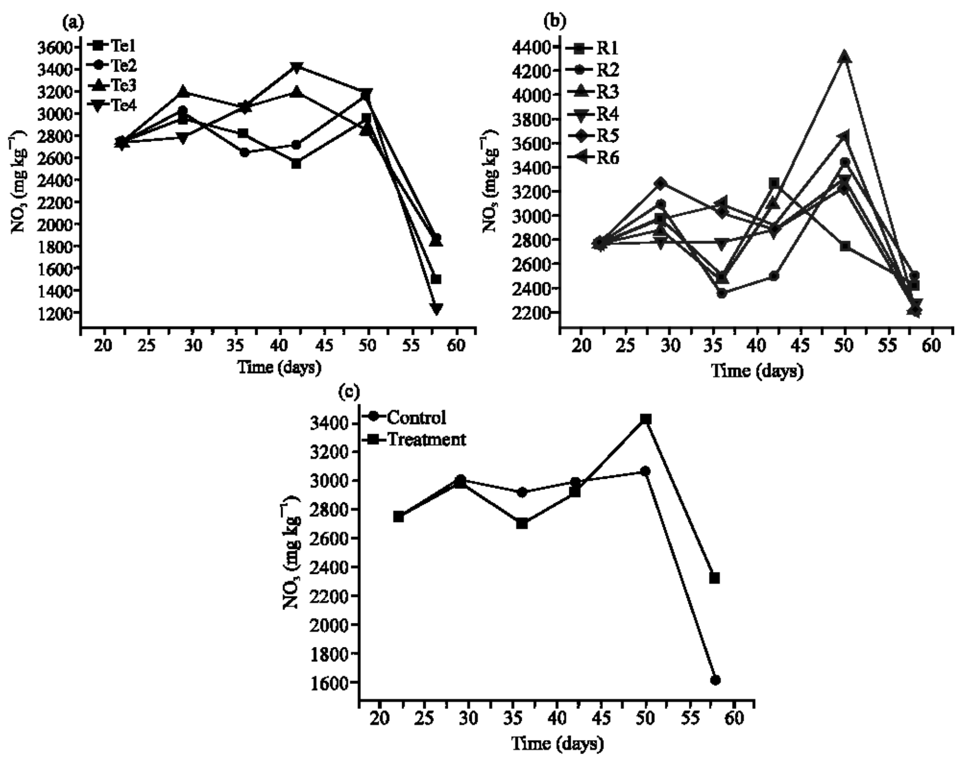

Fig. 3: Nitrate content development during the second trial period (a) control (b) treatment and (c) average values

been expected to decrease from the third week onward. In fact, in some of the treatment samples nitrate content was higher, in some cases, more than that of the EU standard. This behavior was observed to be due to the lack of sun light caused by cloudy days. According to De Graaf (2006), the nitrate content on lettuce leaves increases when the photosynthesis plant rate is less than the conversion plant rate and vice versa, Behr and Wiebe (1992) also found that there exist a close negative correlation between nitrate content and photosynthesis. In this case the cloudy days caused low sun radiation and low temperatures so the plant photosynthesis plant rates decreased causing the nitrate content of the leaves to rise. This explains why the length of the second trial period was longer than that of the first. As these investigators were waiting for sunny days to confirm the decrease of nitrate content in lettuce plants.

Fresh and Dry Matters

In Fig. 4, it can be seen the behavior of average values for fresh and dry matter during the second trial period. Figure 5 depicts the behavior for fresh to dry weights ratio. This ratio decreases rapidly in the first days decreasing from 0.102, in both control and treatment samples, and reaching values from 0.049 and 0.060 respectively. Similar values for fresh weight as those found in study have been reported by Leunard and Leonard (2006). Dry matter was always less than 10% of fresh weight. In fact this relation tends to decrease from 10 to 5%. Seawright *et al*. (1998) carried out an experiment in aquaponic systems with a complete nutrient solution with different fish biomass. All treatments showed not statistically differences for plant biomass. However, nitrate content in plants were not reported.

Fish Analysis

At the end of the first and second trials, a sample of 30 fish were captured and weighed. The average fish weight was 29.5 and 49.5 g, respectively. The fish growth corresponds with that on the table by Morales-Díaz (2003) in which was based the feeding management. The fish tissue analysis for the control and the treatment were similar showing no abnormal mineral concentration. Mohammad and Hossam (2007) found that heavy metals accumulate in fish organs in different concentrations which trend was liver, gills and muscle. In this study ouly muscle samples were taken and this can explained way not differences were found. From these results it can be said that the fish may grow in a nutrient solution for lettuce hydroponic production without any adverse effect on fish growth.

Statistical Analysis

For the first trial in the plant nitrate content there were no significant differences between the control and the treatment, however in the second trial period there were significant differences. This

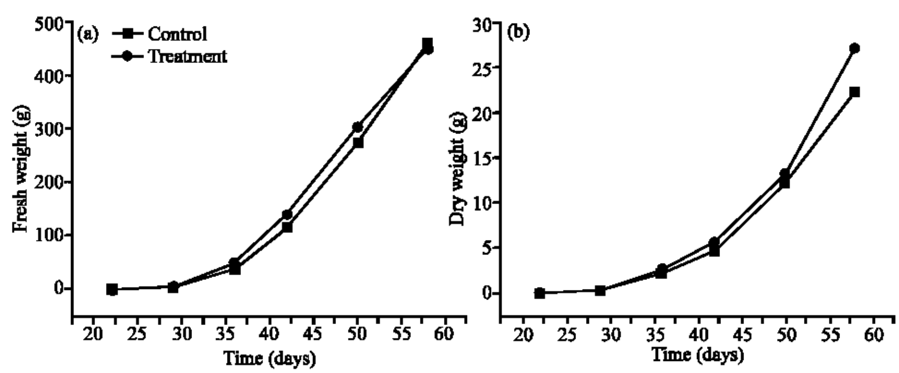

Fig. 4: Development of average values for (a) fresh weight and (b) dry weight during the second trial period

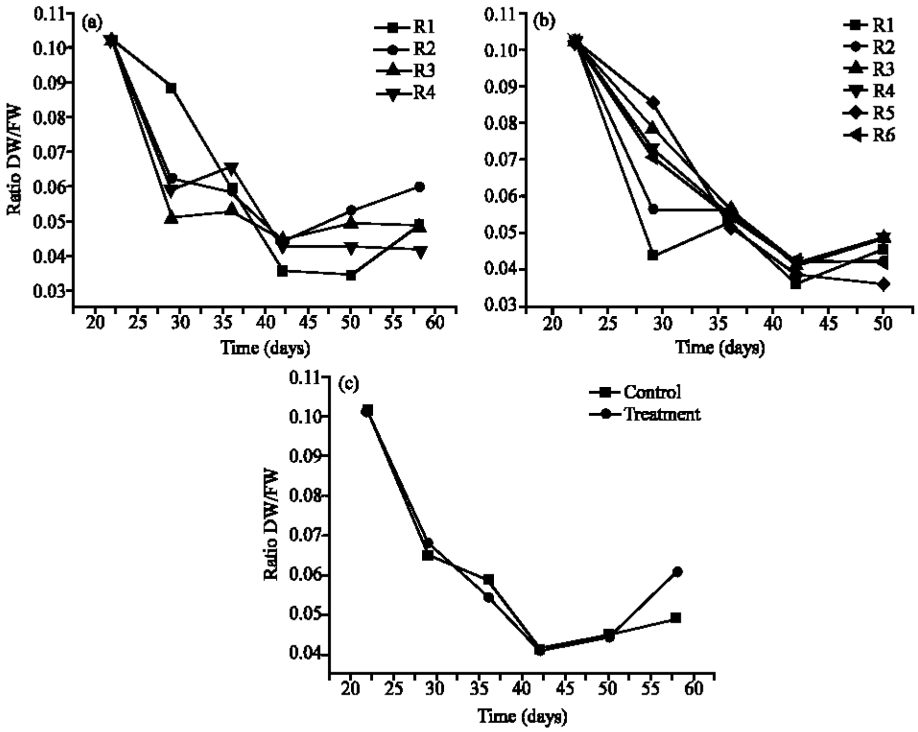

Fig. 5: Dry to fresh weight ratio development during the second trial period (a) control (b) treatment and (c) average values. DW: Dry weight; FW: Fresh weight

Table 2: Final values for the two trial periods

Variable	NO_3(mg kg^{-1})	TFW(g)	TDW(g)
First trial period			
Control	2083a	239a	15a
Treatment	2382a	227a	13a
Second trial period			
Control	1644b	433a	22a
Treatment	2394a	449a	27a

NO_3: Nitrate content; TFW: Total fresh weight; TDW: Total dry weight. Different letter mean significant differences ($p<0.05$). Length of the first trial period 29 days. Length of the second trial period 37 days trial

is attributed to the lack of sun light that took place during the second experiment. In general the plant nitrate content was higher in the treatment than in the control. In both trial periods no significant differences were observed between the control samples and treatment samples for fresh matter and dry matter (Table 2).

According to the results found in this research it could be concluded that fish culture water can provide nitrogen to the plants and it is suitable for production of lettuce plants with low levels of nitrate on leaves and no detriment on plant quality and yield during summer. It also contributes to the reuse of fish culture water.

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