# A Study of Phosphorus and Calcium Dynamics in an Integrated Rainbow Trout and Spinach (*Nores* variety) Aquaponic System with Different Crop Densities

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**Abstract**

The goal of this study is to quantify both calcium and phosphorus budgets for an integrated rainbow trout – spinach (*Nores* variety) aquaponic system, where three crops densities were used (BH1–59 crops/$m^2$, BH2–48 crops/$m^2$ and BH3–39 crops/$m^2$ and a control variant). Fish were fed with two types of feed (41% and 50% protein), using 3 different feeding regimes. Total calcium and total phosphorus retention rates for each of the three tested spinach biomass densities were individually determined by water chemical and plant biochemical analysis. Also, the concentration of those two macroelements was determined from fish meat and fish faeces. Significant differences ($p<0.05$) were recorded between fish faeces total phosphorus content and between total calcium and total phosphorus retention rates for each of the three variants of tested crops densities (significant higher at BH1 compared to BH3, $p<0.05$). It is recommended that lower densities to be used for a better crop absorption of both calcium and phosphorus or a lower hydraulic flow regime and a better light intensity to be applied in case of the used integrated aquaponic system.

**Keywords**: aquaponic system, calcium, faeces, phosphorus, rainbow trout, spinach

## 1. Introduction

Most of the recirculating aquaculture systems replace 5% to 10% of system water daily to prevent the buildup of toxic levels of ammonia and other fish by-products and provide makeup water for evaporation and for backwashing filters [1]. Related to this, crops can be cultured hydroponically in recirculating systems to produce a valuable by-product, while improving the water quality is highly desirable [2]. The main attraction for the implementation of those integrated aquaponic systems has financial considerations because nutrient recovery from aquaculture effluents reduces hydroponic chemical costs [3], but also ecological reason heavily weighs. High quality water is repeatedly used to support the growth of both fish and vegetables, further reducing costs [3]. The system involves no control of root pathogens, as these are controlled biologically by the broad spectrum of antagonistic micro-organisms that develop in the natural environment [4, 5]. Plants extract nutrients from wastewater and convert the metabolic products which could be toxic for fish, fact that makes some authors [6, 7] to characterize aquaponics as a friendly method in relation with the environment, due to the reuse of wastes and nutrients in the resulting effluents from the fish farming activity. The process of by-products (wastes) revalorization from one species in a second crop generated by the co-cultured species enhances the profitability

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due to lower refreshment rate and water consumption or to the crop itself which represent another source of revenue for the farmer [8, 9]. After nitrogen, phosphorus is the second most frequently limiting macronutrient for plant growth, making up about 0.2% of a plant's dry weight [10]. Plants deficient in phosphorus are stunted in growth and often have an abnormal dark-green color [11]. Also, phosphorus is an important mineral in nucleic acids and cellular membranes, the main representative of the structural components of the skeletal tissues, and it is directly involved in energy processes [12]. Fish can absorb this mineral from the water, but due to the low waterborne P concentration, dietary supplementation is necessary [13]. Phosphorus retention is also directly affected by fish growth rate and higher values were obtained when growth performances were good [14]. Nowadays, the evaluation of micronutrients and essential trace elements levels of fruits and vegetables is a growing trend in nutritional studies throughout the world [15].

Therefore, the present study aims to quantify both calcium and phosphorus budgets for a floating rafts integrated rainbow trout–spinach (*Nores* variety) aquaponic system, where three crops densities were used.

## 2. Materials and methods

*Integrated aquaponic system description*

The present experiment took place between February 20$^{th}$ and April 4$^{th}$, 2013 at the recirculating system pilot station of Aquaculture, Environmental Science and Cadastral Measurements Department of Food Science and Engineering Faculty–"*Dunarea de Jos*'' University of Galati. Figure 1 describes for the first time, the new emplacement and configuration of this second recirculating pilot station as follows: 12 rectangular shape rearing units with a volume of 0.15 $m^3$/unit–No. 1; 2 rectangular sump units with a volume of 0.29$m^3$/unit–No. 2; 1 mechanical-quartz sand water conditioning unit with backwash–No. 4; 1 biological trickling filtration unit–No. 5; one sterilization UV filter (TETRA POND, Type UV-C 35000 and 36 Watt)–No.7; 3 recirculating pumps–No. 3, oxygenation unit (compressor Resun Air- Pump, Model: ACO-018 A with a flow of 260 l/min) and also water quality control sensors–No. 6.

The aquaponic modules (No. 8) consist in 4 rectangular glass made units (900x600x200mm), placed above the recirculating system, on a metal support (Figure1). A lighting system made of 3 fluorescent lamps, with reddish wavelength and a luminous power of 1080 lm was placed above the hydroponic units (Figure 1–No. 9).

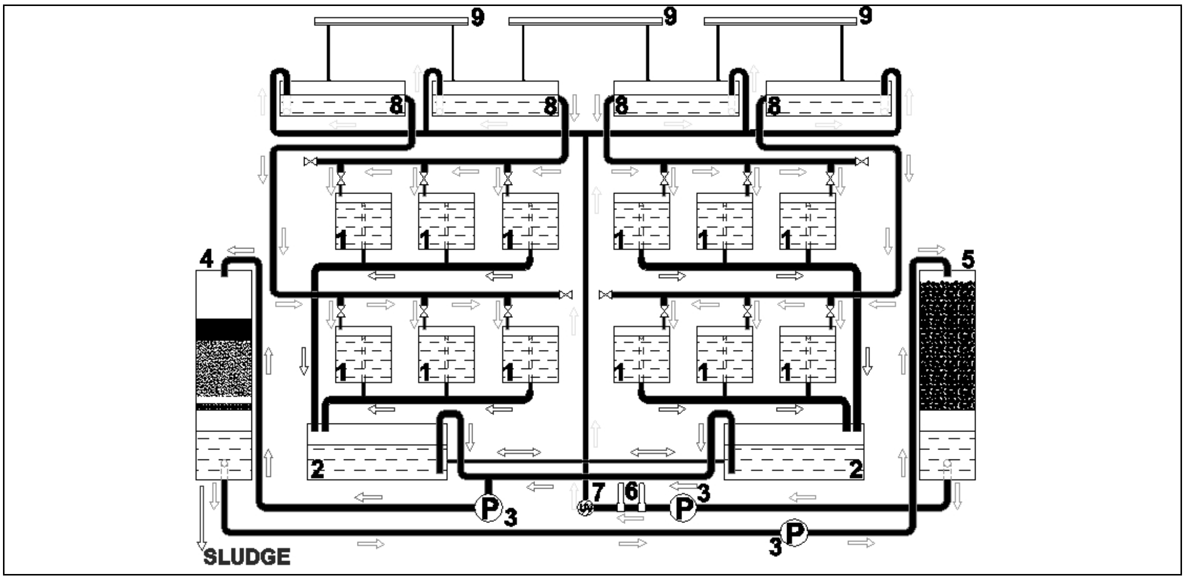


**Figure 1.** The configuration and emplacement of second pilot recirculating system station

Regarding the water cycle inside the integrated system, it can be seen from the direction indicated by the arrows in figure 1, that water flows out from the rearing units, pass through the mechanical filter first and after that, by using a recirculating pump, it goes through the biological filtration unit and then to the aquaponic modules, that output the treated water back to rearing units, as described in previous studies [16, 17]. The total volume of water from the integrated system is around the value of 2.5-2.7 $m^3$. An equal water flow of 6 L/minute was set for the inlet of all 4 hydroponic units. The support media of spinach consisted of polystyrene plates with holes for plastic special supports (Figure 2).

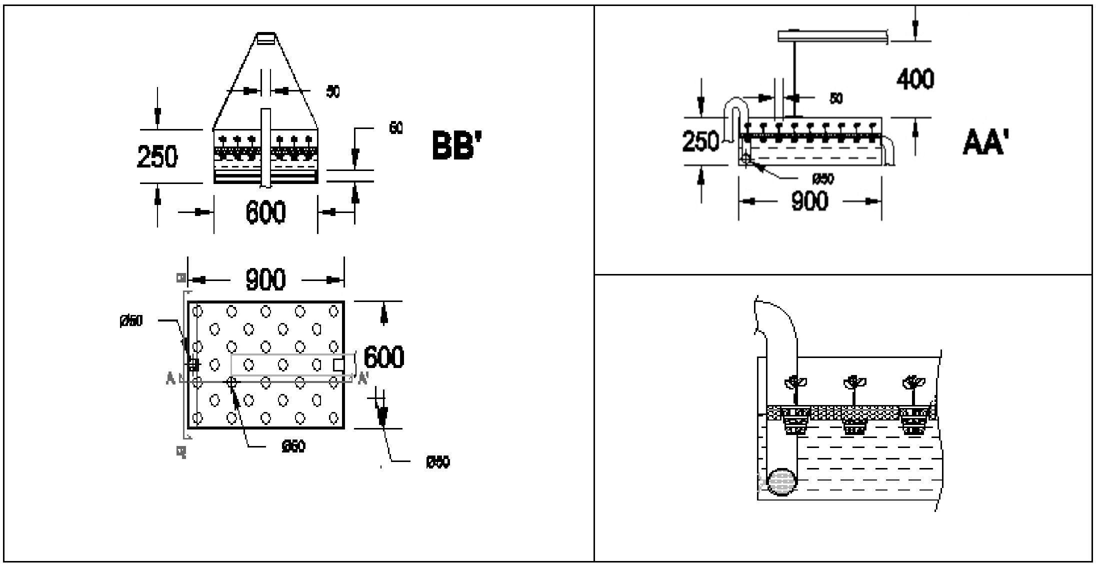


**Figure 2.** Aquaponic modules: **AA'.** Longitudinal section view; **BB'.** Crossing section view

Plants were placed in plastic supports and then, these were filled with a few hydroton balls to ensure their stability–figure 2 [16, 17]. The distance between plants was 15cm length and widthwise and the maximum capacity of an aquaponic unit was 32 plants (Figure 2) [16, 17].

*Experimental design*

Before starting the experiment, the activation of the biological trickling filtration unit was made as described in previous studies [8]. Daily ammonia, nitrite and nitrate levels were monitored to determine the degree of ammonia oxidation to nitrate and therefore to observe when a stable state of bacterial biomass is obtained [16, 17]. For the 44 days experiment, a total number of 228 rainbow trout (*Oncorhynchus mykiss*), with an average initial weight of 111.77 grams, was used in parallel with spinach (*Spinacia oleracea*), *Nores* variety, at an age of 25 days, as described in previous studies [16, 17]. Total fish biomass from the recirculating aquaculture system, at the beginning of the experiment, was 25.51 kg [16, 17]. Fish were divided in six groups, in duplicate, as described in previous studies [16, 17]. Three of them (G1) were fed with Clasic Extra 1 P–41% brute protein; 0.9% phosphorus and the other three (G2) with Nutra PRO-MP-T–50% brute protein; 1.3% phosphorus; 1% calcium, as in the protocol described in previous studies [18]. A total amount of 12 363.32 g of Clasic Extra 1 P feed and 11 579.54 g Nutra PRO-MP-T was administrated during all 44 experimental days [16, 17]. *Nores* variety spinach was placed in the hydroponic units with the following stocking densities: (BH1–59crops/$m^2$, BH2–48crops/$m^2$ and BH3–39crops/$m^2$). The seedlings were obtained at the Natural Sciences Museum Complex Galaţi. A daily percentage of 10% water exchange was applied. The technological water was analyzed in terms of phosphorus and calcium concentration by using Spectroquant Nova 400 spectrophotometer, with Merck compatible kits. Samples of water were taken from the outlet of biological filter (inlet of hydroponic units) and outlet of each hydroponic unit. The luminous intensity was measured with TESTO 545 light meter. Both hydraulic loading rate (HLR)=flow rate (Q)/total surface area of hydroponic module (m/day) and hydraulic retention time (HRT)=surface area x water depth x porosity of hydroponic module/flow rate (min), were calculate according to previous studies [19]. The values obtained were 16m/day for hydraulic loading rate and 0.48 min for hydraulic retention time. The phosphorus and calcium removal rates in hydroponic units were calculated with the following formula [8]: phosphorus/calcium retained $(g/m^2/day)=((Q/V*(C_{in}-C_{out})–dC_{out}/dt)*d$, where, Q=the flow rate $(m^3/day)$, V=system volume $(m^3)$, C=concentration of TAN $(g/m^3)$, d=depth (m), t=time (d). The obtained results were then expressed in $m^2/day$. The fish faeces collection was made with a special EHEIM water vacuum cleaner provided with a mesh compartment for solids retention. Chemical analyses concerning both phosphorus and calcium levels were carried out over a number of 5 samples. For determining the phosphorus pentoxide, SR ISO 2294:2009 reference method was used. Also, for determining the total calcium content, the organic substance is oxidized by oxygen through calcination at a constant temperature of $450^oC±25^oC$ and the obtained extract is solubilized with 0.5N HCl, neutralized with NaOH -1N until a 12-13 pH range. Finally, the complexometric method is used, with murexide as indicator.

*Statistical methods*
Statistical analysis was performed using the IBM SPSS Statistics 20 for Windows. Statistical differences between treatments were tested using T test (α=0.05) after a normality test (Kolmogorov-Smirnov). Comparisons between variants were assessed using post-hoc Duncan test for multiple comparisons (ANOVA). Also, bivariate correlations were made for obtaining Pearson coefficient.

## 3. Results and discussion

*Phosphorus and calcium in fish meat*
A total quantity of 12 363.33 g of Clasic Extra 1 P and 11579.54g of Nutra PRO-MP-T were distributed in the integrated system throughout the 44 experimental days. The fodder biochemical analysis confirmed a content of 0.9% phosphorus for Clasic Extra 1 P and 1.3% phosphorus for Nutra PRO-MP-T. The total phosphorus input into the recirculating integrated system, through administrated feed quantity, is presented in figure 3. Also, Nutra PRO-MP-T feed contains 1% calcium, fact that makes a total calcium input of around 116 g throughout the experimental period.

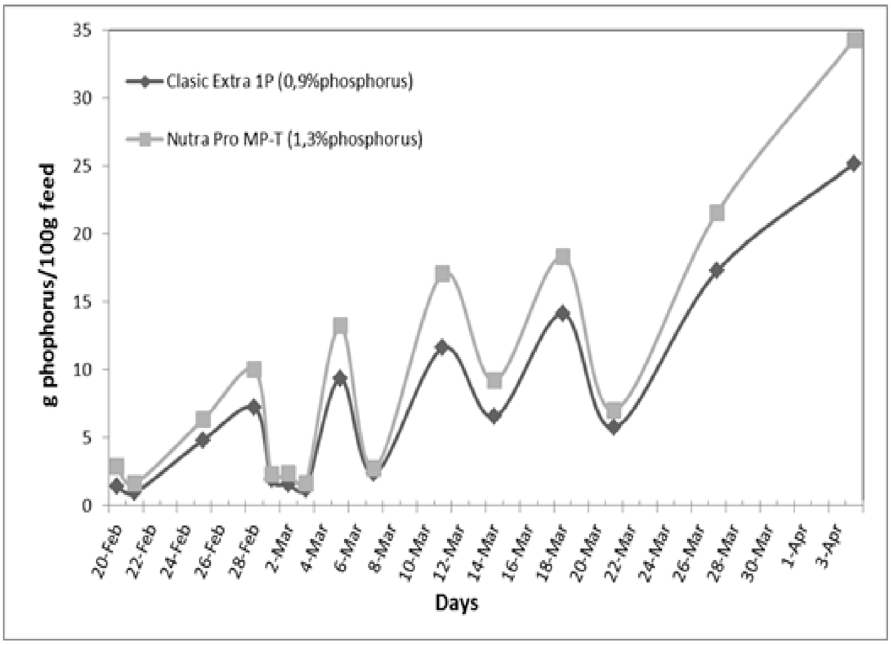


**Figure 3.** The dynamics of phosphorus input into the recirculating integrated system

Regarding rainbow trout meat phosphorus and calcium content, before the start of the experiment, a mean value of 252.66±7.25 mg $P_2O_5$/100 g phosphorus and 31.11±0.96 mg/100g total calcium were registered. At the end of the experiment, the following mean values were obtained: 248.7±12.5 mg $P_2O_5$/100g and 31.97±2.11 mg/100g total calcium at G1 fish group; 249.9±13.62 mg $P_2O_5$/100g and 37.84±1.96 mg/100g total calcium at G2 fish group. The results were lower compare to previous studies [20], where two types of feed were administrated, one with 1.8% phosphorus and the other with 0.99% and that registered a rainbow trout meat phosphorus content of 370 mg $P_2O_5$/100 g, respectively 430 mg $P_2O_5$/100 g, given the fact that at the beginning of the experiment, the rainbow trout mean phosphorus content was 500 mg $P_2O_5$/100g. The maximum phosphorus absorption in rainbow trout is 520 mg $P_2O_5$/100g and higher dietary levels only increase the amount of excreted phosphorus [21]. It has been mentioned that rainbow trout fed fish meal based-diets showed higher phosphorus retention than fish fed soy protein concentrate [22]. Also, diets with high lipid levels and lower phosphorus content improved phosphorus retention [23]. Values between 42-58 mg/100g total calcium in trout meat, higher than our current values, were registered in previous studies [24] and also it was mentioned that an increase of 10 g in body weight is accompanied by a storing of 0.04 g of total calcium. According to other studies [25], the accumulation of dietary calcium in the body of rainbow trout was higher in a diet containing no phosphorus than in a diet whose Ca/P ratio was l.

Regarding the data series distribution of final total calcium meat content (Figure 4), by analyzing skewness and kurtosis, it can be said that the mesokurtic distribution has a little platikurtic tendency, a bit flatter than a normal distribution, with the tendency of values scattering over a longer interval around the mean, a little tilted to the right, with more extreme values to the left, fact that goes also for G1 variant–meat phosphorus content. The values indicated by the median were close to average values. By using two multiple comparisons test (Tukey and Duncan–ANOVA),

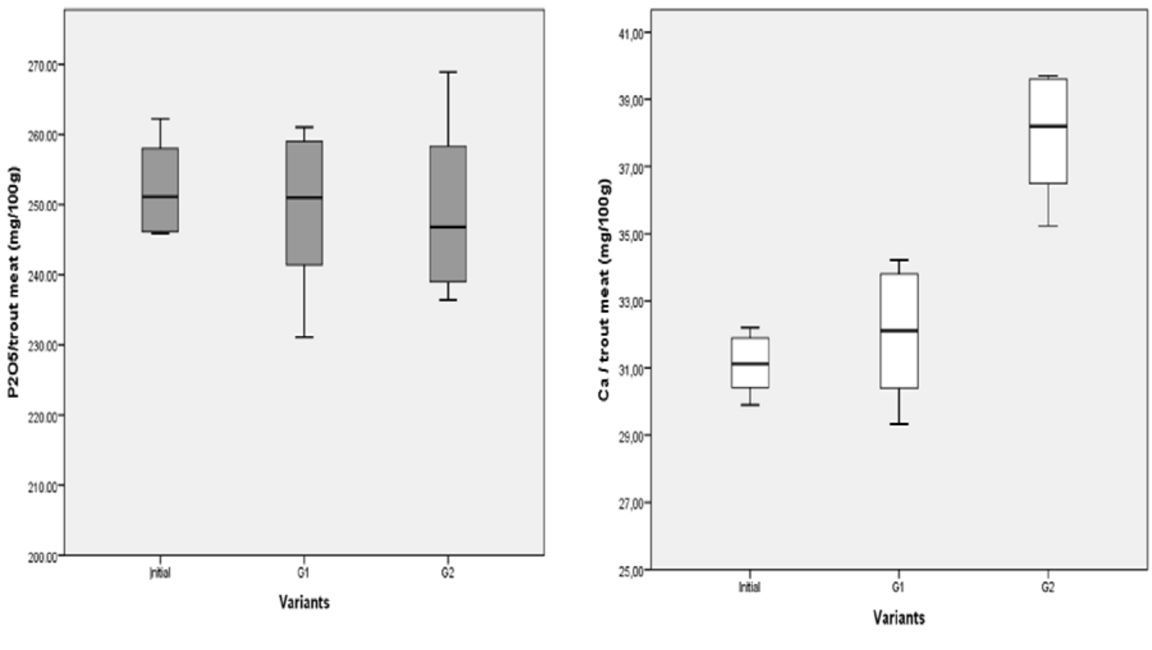


**Figure 4:** Phosphorus ($P_2O_5$) and total calcium content in rainbow trout meat

it was observed that differences between the initial meat phosphorus values and final G1 and G2 values are not significant ($p>0.05$)–1 data subset: Initial+G1+G2. Also, the differences between the initial meat calcium values and final G1 values are significant, comparing with final G2 values ($p<0.05$)–2 data subset: Initial+G1; G2. For measuring the correlation intensity between phosphorus and calcium meat accumulation, Pearson correlation was used. The value of Pearson correlation coefficient was-0.329, stating that there is not an indirect strong correlation.

*Phosphorus and calcium in spinach variants*

Regarding spinach final phosphorus and calcium content, for each one of the three experimental variants and for market spinach, the following mean values were obtained: 21.68±0.39 mg $P_2O_5$/100 g fresh weight (FW) and 17.88±1.39 mg total Ca/100g FW at BH1; 26.88±0.63 mg $P_2O_5$/100 g FW and 24.17±1.62 mg total Ca/100g FW at BH2; 32.23±1.32 mg $P_2O_5$/100 g FW and 30.87±0.97 mg total Ca/100g FW at BH3; 31.83±1.79 mg $P_2O_5$/100g FW and 65.64±1.37 mg total Ca/100g FW for market spinach. The current values were near the ones from previous studies [26], where a phosphorus content between 29-37 mg $P_2O_5$/100 g FW in spinach leaves was reported. It must be pointed out that although other studies [27] regarding phosphorus level of green lettuce, cultured in an integrated aquaponic floating rafts system together with pikeperch, found higher phosphorus levels of aquaponic green lettuce (34.81–37.33 mg $P_2O_5$/100g Fw), comparing with normal cultured one (20.13mg $P_2O_5$/100 g FW) on soil, in case of the current experiment, only the BH3 crops phosphorus level registered this tendency (32.23 mg $P_2O_5$/100g FW, comparing with 31.83 mg $P_2O_5$/100 g FW at market spinach). Regarding total calcium content, significant lower levels were registered for spinach grown in aquaponic conditions, comparing with market spinach, cultured in soil. From figure 5 it can be concluded that the data series distribution of final phosphorus spinach content, by analyzing skewness and kurtosis, have a mesokurtic distribution with a little platikurtic tendency, a bit flatter than a normal distribution, with the tendency of values scattering over a longer interval around the mean, a little tilted to the right, with more extreme values to the left and the values indicated by the median close to average values. Also, regarding total calcium spinach content, the distribution is almost the same, except that here the histogram is a little tilted to the left, with more extreme values to the right (Figure 5) with the values indicated by the median close to average values. By using two multiple comparisons test (Tukey and Duncan–ANOVA), it was observed that differences between the experimental variants (BH1, BH2, BH3) in term of phosphorus spinach content, are significant ($p<0.05$) and the ones between BH3 and market spinach in term of phosphorus content are not significant ($p>0.05$)-3 data subset: BH1; BH2; BH3+Market spinach.

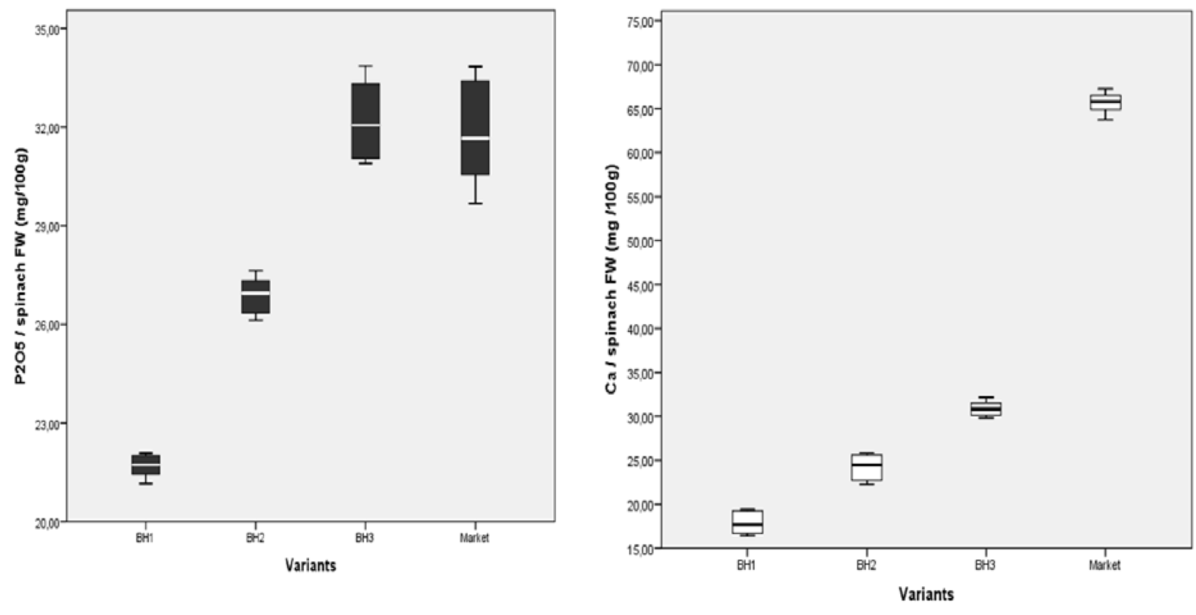


**Figure 5.** Phosphorus ($P_2O_5$) and total calcium spinach leaves content

Also, the differences between the experimental variants (BH1, BH2, BH3, market spinach) in term of total calcium spinach content, are significant ($p<0.05$)-4 data subset: BH1; BH2; BH3; Market spinach. For measuring the correlation intensity between phosphorus and calcium spinach leaves content, Pearson correlation was used. The value of Pearson correlation coefficient was 0.675, stating that there is direct strong correlation between them.

*Phosphorus in rainbow trout faeces*

Fish faeces tend to be highly variable in their chemical content, which is also the case with other animal faeces [28- 30]. According to other studies [28], it was found difficult to compare faeces phosphorus ($P_2O_5$) values with those from other studies because of the differences in conditions under which the solids were produced, separated, stored, and collected. The mean faeces phosphorus ($P_2O_5$) values, registered in the current research were 2.95±0.48% $P_2O_5$/faeces DW for G1 fish group, where Clasic Extra 1P–0.9% phosphorus feed was administrated and 3.63±0.47% $P_2O_5$/faeces DW where Nutra Pro MP-T–1.3% phosphorus feed was used. The differences between G1 and G2 phosphorus faeces content were statistical significant ($p<0.05$). In Figure 6 it can be observed that the faeces phosphorus content has a decreasing trend throughout the experimental period, most probably because of a progressive positive tendency manifested toward fish phosphorus retention.

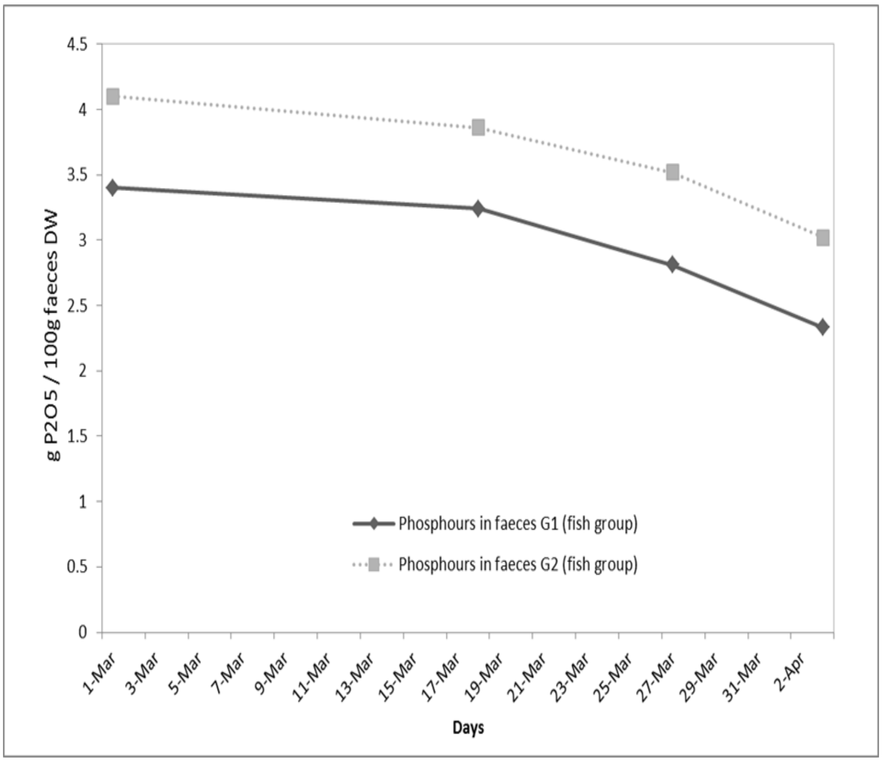


**Figure 6**. The evolution of faeces phosphorus content

In previous studies [28], a phosphorus level for rainbow trout faeces of 2.54% $P_2O_5$ was registered. Also, similar values were reported in other studies [31]-2.22% $P_2O_5$ and [30] 3.51% $P_2O_5$. Lower values were reported in [29]-0.35% $P_2O_5$-1.85% $P_2O_5$; [32]-1.79% $P_2O_5$, 1.49% $P_2O_5$ and [33] 0.94% $P_2O_5$, all for rainbow trout faeces. Also, other studies [34], registered different concentration of phosphorus in rainbow trout faeces, by administrating different feed types: 2.51±0.13% $P_2O_5$ in faeces for 1.12±0.07% phosphorus feed type; 3.86±0.13% $P_2O_5$ for 1.2±0.10% phosphorus in feed; 2.25±0.07% $P_2O_5$ for 0.90±0.02% phosphorus in feed and 2.87±0.86% $P_2O_5$ for 1.08±0.16 phosphorus in feed. According to other studies [20], it has been concluded that if fish are fed available phosphorus above their requirement, an increment of the nonfecal phosphorus excretion at some level can be expected in large-sized fish due to a reduction of the retention efficiency. Also, it is known that while the efficiency of phosphorus retention into the carcass is decreased, more is discharged as soluble (presumably urine) and insoluble (presumably faeces) waste [35].

*Phosphorus and calcium in technological water*

The mean water phosphorus and calcium concentrations at the inlet of aquaponic modules and also at the outlet of each one of them, registered the following values: 9.95±3.47 mg $P_2O_5$/L and 94.48±4.12 mg $Ca^{2+}$/L at the aquaponic modules inlet and 8.11±3.18 mg $P_2O_5$/L and 92.6±4.76 mg $Ca^{2+}$/L at BH1 outlet; 8.49±3.21 mg $P_2O_5$/L and 93.18±4.71 mg $Ca^{2+}$/L at BH2 outlet; 8.9±3.2mg $P_2O_5$/L and 93.54±4.65 mg $Ca^{2+}$/L at BH3 outlet; 9.91±3.46 mg $P_2O_5$/L and 94.4±4.14 mg $Ca^{2+}$/L at control variant outlet.

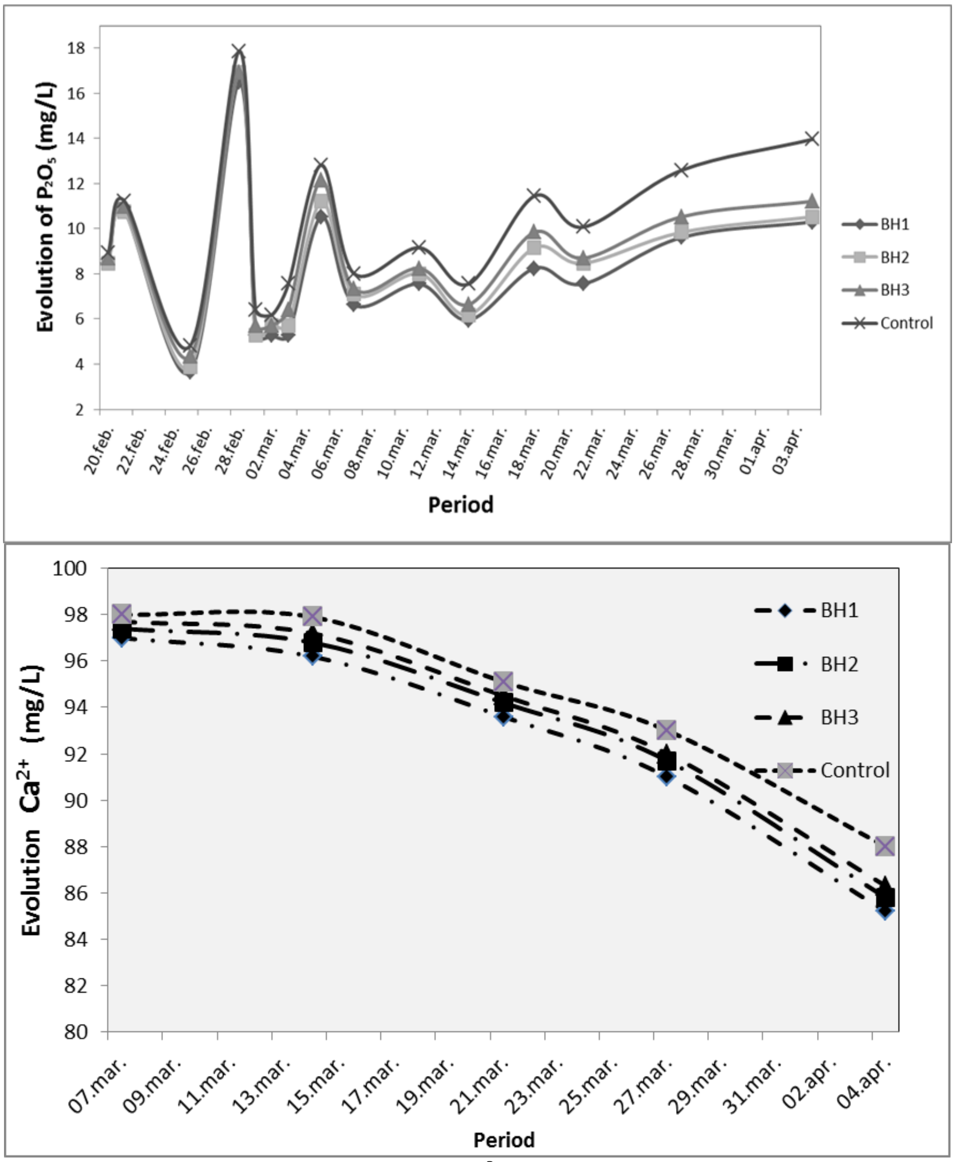


**Figure 7.** The evolution of $P_2O_5$ and $Ca^{2+}$ concentration in technological water

The evolution of $P_2O_5$ and $Ca^{2+}$ concentration in technological water is presented in figure above. It can be observed that $Ca^{2+}$ concentrations have a downward trend, while $P_2O_5$ concentrations had some fluctuations at the beginning of the experimental period, but immediately after, had a constantly evolving tendency (Figure 7).

*Water treatment capacity*

Regarding phosphorus and calcium removal rates, the following mean values were obtained: 3.83±1.15 mg/day $P_2O_5$ and 3.9±1.39 mg/day $Ca^{2+}$ at BH1; 3.04±0.91 mg/day $P_2O_5$ and 2.69±1.27 mg/day $Ca^{2+}$ at BH2; 2.18±0.54 mg/day $P_2O_5$ and 1.95±1.12 mg/day $Ca^{2+}$ at BH3. The evolution of phosphorus and calcium removal rates is presented in Figure 8. It can be observed that phosphorus removal rate has some fluctuations given by the variations of the crops nutritional demand for phosphorus, but the general impressions one of upward tendency. The evolution of calcium removal rate, comparing with the one of phosphorus, presents a less abrupt upward tendency, revealing a constant increase of crops calcium absorption rate.

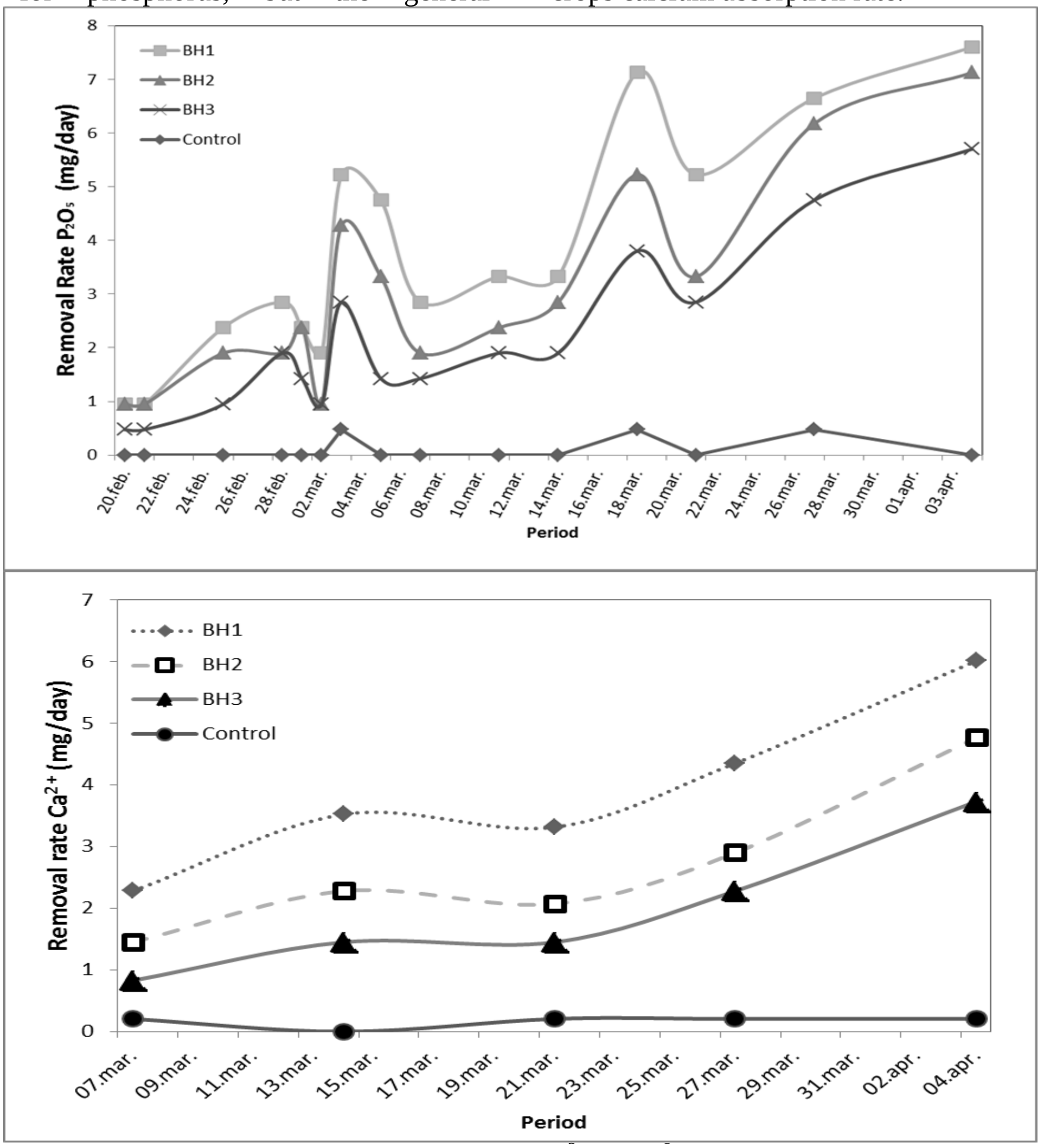


**Figure 8.** The evolution of P-$PO_4^{3-}$ and $Ca^{2+}$ removal rates

By using two multiple comparisons test (Tukey and Duncan–ANOVA), it was observed that differences between the experimental variants and the control variant in terms of phosphorus retention rate are significant ($p<0.05$). Also significant differences are revealed between BH1 and BH3 experimental variants ($p<0.05$)-3 data subset: BH1+BH2; BH2+BH3; Control. The differences between the experimental variants and the control variant in terms of calcium retention rate are significant ($p<0.05$). Also significant differences are revealed between BH1, BH2 and 3 experimental variants ($p<0.05$)-4 data subset: BH1; BH2; BH3; Control. Pearson correlation was used. The value of Pearson correlation coefficient was 0.901, stating that there is a direct strong correlation between phosphorus and calcium removal rates.

## 4. Conclusions

As a main conclusion to this study it can be stated that plant density applied in BH1 case is the best of all three tested densities in terms of water chemical treatment, given the highest values of phosphorus ($P_2O_5$) and calcium ($Ca^{2+}$) removal rates. By analyzing the phosphorus ($P_2O_5$) and calcium ($Ca^{2+}$) removal rates, it must be pointed out that plants have different evolution periods in their lifetime and therefore different nutrient absorption rates, more constant in case of calcium. Also, the balance between plants absorption rates and administrated feed quantity in terms of calcium and phosphorus budgets is found to be important. Feed phosphorus and calcium concentration was found to be less important in relation with rainbow trout meat phosphorus ($P_2O_5$) and total calcium content. From the faeces phosphorus ($P_2O_5$) content evolution, we can conclude that fish has the upward tendency to retain phosphorus, manifested especially after the first 20 days of the experimental period. Regarding the spinach phosphorus ($P_2O_5$) concentration, in BH3 experimental variant there have been registered values similar to market spinach, in comparison with the other two experimental variants where phosphorus deficiency was observed. Also, total calcium deficiencies were observed in spinach from all three experimental variants, compared with marketable spinach. Visual signs of these deficiencies were manifested by necrotic leaf margins on young leaves or curling of the leaves, especially in case of BH1 and BH2 experimental variants. It is recommended that others feeding regimes or other feed types to be used, by respecting the plants stocking density applied in case of BH3 variant, to achieve an equilibrium between plants calcium absorption rates and $Ca^{2+}$ concentration in water.

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