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Effect of feed protein level on water chemical and technological parameters of a recirculating aquaponics system for carp *(Cyprinus carpio* L.*)* and lettuce (*Lactuca sativa* L.) farming

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Abstract

Aquaponics is a system integrating hydrobionts farming with cultivation of plant species (vegetables, spice herbs, flowers etc.). The aim of the present study was to establish the effect of feeds with different protein content (24% and 28%) on water chemical and technological parameters of a recirculating aquaponic system for carp (*Cyprinus carpio* L.) and lettuce (*Lactuca sativa* L.) farming. The experiment was conducted in 2 variants, each of 30-day duration, comprising feeding carps with pelleted feed either with 24% crude protein or 28% crude protein, with pellet size of 3.5 mm. Each of variants comprised 12 fish with uniform initial weight (563±0.1 and 0.566±0.3 g respectively), without significant difference (P≥0.05) and 17 plants in 2nd germination week. In fish growth performance traits at the end of experiments showed higher body weight in carps in the variant with 28% dietary protein (by 4.4%) and total body length (by 1.4%) as compared to those with 24% dietary protein. Fish fed feed with higher dietary protein level exhibited lower feeding coefficients vs those fed 24% protein (by 13.04%). The lettuce biomass and the length of their root system by the end of the trial were higher in the experimental variant with 28% crude protein.

Introduction

By definition, aquaponics is the combined farming of fish and plant species in a recirculating system (Timmons, Ebeling, Wheaton, Summerfelt, & Vinci, 2002; Diver, 2006), including plants growing without soil. Aquaponics allows to grow plants substituting the use of large areas of land with waste waters from aquaculture systems, which provides a twice larger area for cultivation (Graber & Junge, 2009). Aquaponic units are modern biotechnological systems with huge potential producing organic cultures. This method of food cultivation is the only one excluding totally the use of chemicals (Salam, Asadujjaman, & Rahman, 2013.).

Various fish species could be farmed in aquaponic units – tilapia, barramundi, silver perch, catfish, crabs, shrimps, and from vegetables – green vegetables (lettuce, Chinese cabbage etc.), beans, green peas, leaf cabbage, radishes, strawberries, melon, onion, turnip, herbs. The advantages of such combined farming come from lower initial investment costs, operation and infrastructure costs compared to the separate organization of both production systems, namely aquaculture water purification by plants that reduces water consumption, and environmental pollution by wastewater. The integrated cultivation in the so-called aquaponic system allows to obtain increased profit from two types of produce: fish and vegetables with the same production capacities (Diver & Rinehart, 2010; Rakocy, 1999; Timmons, et al., Ebeling, Wheaton, Summerfelt, & Vinci 2002). The aquaponics is very close to the definition for sustainable aquaculture as it combines production of fish and plants and integrates the flow of nutrients through a natural biological cycle as nitrification allowing for most efficient utilization of non-renewable resources (Gold, 1999).

The successful farming of fish in aquaponic systems is possible only if complete rations are fed. Feeding compliant with the biological needs of the farmed species, its current conditions and the environment is essential (Zaykov & Staykov 2013). So far, the research on aquaponics has investigated the effects of frequency of feeding, various ratios of fish feed to plant area, various water flow rates in the aquaponics units (Al-Hafedh, Alam, & Beltagi, 2008; Liang & Chien, 2013). One of the main parameters characterizing feed is its protein content.

The analysis of available literature has shown that there are no studies investigating the effect of dietary protein level on fish production performance in recirculating aquaponic systems.

The aim of the present study was to establish the effect of feeds with different protein content (24% and 28%) on water chemical and technological parameters of a recirculating aquaponic system for carp (*Cyprinus carpio* L.) and lettuce (*Lactuca sativa* L.) farming.

Materials and Methods

Experimental Fish

Two experiments were conducted in the experimental training base of the Faculty of Agriculture – Trakia University. Carps in good health with uniform average body weight (0.563±0.1 kg and 0.566±0.3 kg) were provided by Nomikom – FISH Ltd fish farm, without statistically significant differences in weight. In each of the experiments, fish were put in concrete tanks (8 fish per tank) with dimensions 2.0x1.0x1.0 m, The fish tank's utilizable volume was1.6 m^3. The stocking densities in both variants were 4.504 кg/m^3 and 4.528 кg/m^3. Fish were allowed to adapt to the environment for a week. During that period they were fed pelleted feed with appropriate composition and pellet size of 3.5 mm.

Two experiments of 30- day duration were performed, with 8 fish with uniform body weight (0.563±0.1 and 0.566±0.3 kg).

The growth performance of experimental carp was monitored by control catches by the 15th day and live weight measurement (kg).

Experimental Feed

Carp were fed pelleted feed with 24% and 28% crude protein content, respectively.

The daily ration of carp amounted to 1% of their live weight and was corrected according to the weight by the 15th experimental day. The feed was offered manually, three times a day (at 8.30, 12.30 and 16.30 h).

Feed composition and nutritional values are presented in Table 1.

Experimental Plants

Before the experiment, 17 lettuce (*Lactuca sativa* L.) seedlings were purchased from a nursery garden (Plovdiv) at the 2nd week of germination (second leaf formation). The plants were put into a specialized substrate for aquaponic culturing. Trace elements for lettuces were added by pulverization of B-essentials onto leaves (leaf fertilization). To avoid anaerobic areas around the root systems, constant aeration was provided in the growing tanks. Adequate lighting was ensured by the installation of two Fluora type lamps. The water entering the cultivation system was with a very low flow rate – 0.5 Lmin. It was regulated by water taps maintaining a specified flow rate of the water entering the aquaponic unit. Once a week, the bottoms

Table 1. Composition and nutritional values of experimental carp' feeds

Ingredients	Feed with 24% CP	Feed with 28% CP
Corn, %	20,00	15,00
Wheat, %	28,50	23,50
Soybean meal (49% CP), %	25,50	36,00
Sunflower meal (34% CP), %	19,00	19,00
Sunflower oil, %	2,30	2,30
Salt, %	0,50	0,50
Limestone, %	0,60	0,30
Dicalcium phosphate, %	3,00	3,00
Vitamin mineral premix, %	0,40	0,40
Lysine, %	0,20	-
Total:	100,00	100,00
The feed provides:		
Metabolisable energy, kcal/kg	3010,00	3012,00
Crude protein, %	23,85	28,03
Crude fat, %	4,48	4,32
Crude fibre,%	5,89	6,22
Calcium, %	1,12	1,12
Phosphorus,%	1,11	1,16
Sodium, %	0,25	0,26

of the experimental tank and the sedimentation tank were cleaned with fresh water which was added at 10% of the volume to compensate evaporation losses.

Experimental Recirculating Aquaponics System

The experimental design (Raft system) was placed in one of the tanks of the recirculating aquaponic system (Figure 1). The tank for lettuce growing had an area of 0.6 m^2, and comprised floating Styrofoam sheets and hydroponic pots for plants growing.

In both variants, at the end of experimental periods, the biomass of plants (g) was determined by means of analytical balance and the root length (cm) – with a linear measurement device.

Water Chemical Parameters

Throughout the experiment, the efficiency of wastewater purification in the system was monitored at a daily basis through measurement of dissolved oxygen, water pH and electrical conductivity with portable HQ30D multimeter(Hach Lange). The changes in dynamics of nitrogen and phosphorus compounds in the system were determined spectrophotometrically (DR 2800)(Hach Lange).

Technological Traits

At the end of the experiment, the average daily weight gain of carps, feeding coefficient and survival rate in the variants with different dietary crude protein levels were calculated as followed:

$$\text{Daily weight gain (g/day)} = \frac{[W2-W1]}{D}$$

where:

W1 – the weight of fish at the end of the experiment;

W2 – the weight of fish at the beginning of the experiment;

D – days of the period.

$$\text{Feeding coefficient: } K= [\frac{F}{Wf-Wi}]$$

where:

K- feeding coefficient;

F – the amount of consumed feed (g);

Wf – total biomass of fish at the end of the experiment (g);

Wi – total biomass of fish at the beginning of the experiment (g).

$$\text{Survival (\%)}=\frac{\text{number of surviving fish}}{\text{number of fish at the beginning of the experiment}}\text{x100}$$

The data were submitted to statistical analysis by means of ANOVA (Ms Office, 2010).

Results and Discussion

During the experimental period, water chemical parameters in the aquaponic system for farming carps and lettuce in both experimental variants (24% and 28% dietary crude protein level) are presented on Figures 2 - 4. All monitored parameters ranged within the allowances for integrated species.

The average values of dissolved oxygen and water pH (Figure 2C; A) in the variant with 28% crude protein in feed did not differ considerably throughout the experiment. Nevertheless, the highest dissolved oxygen concentration was determined in tanks where carp were offered feed with 28% protein. The differences between both variants were not statistically significant as dissolved oxygen and water pH were concerned ($P \geq 0.05$). The recommendations for pH in aquaculture systems range between 6.5 and 8.5. It was found out that water pH increased when ammonia levels decreased (Timmons, et al., 2002).

The water electric conductivity in both variants (Figure 2B) showed lower values in tanks where the feed with 28% crude protein was offered but the difference as compared to the other variant was not significant ($P \geq 0.05$).

Ammonium ion concentrations before and after aquaponics (Figure 2E) were higher in the variant where the carp were fed with the higher dietary crude protein levels 28% but the difference between variants was not statistically significant ($P \geq 0.05$).

Water nitrate content in experimental tanks before and after the aquaponics (Figure 2F) was higher in the tanks of the 28% CP variant before the aquaponics, but the opposite was found after the aquaponics, i.e. higher

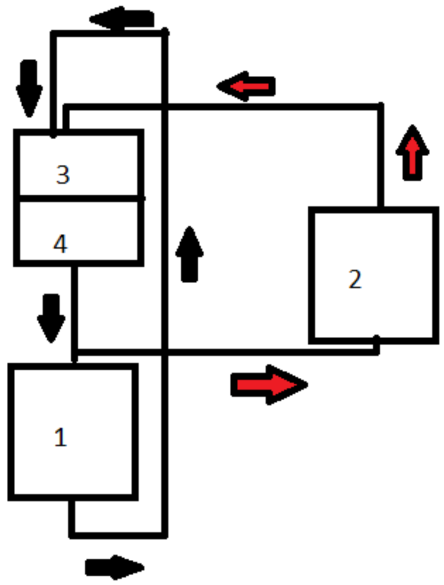

Figure 1. Experimental recirculating aquaponic system: 1-fish; 2-plants; 3- mechanical filter; 4- biological filter; ➡ water flow direction in the recirculating system; ➡ water flow direction in the aquaponic system.

levels in the 24% CP variant. There was no statistically significant difference between the two crude protein dietary levels (P≥0.05).

Total phosphorus content in water (Figure 2D) increased in the 24% CP experimental variant. After the aquaponics, it began to decrease as it passed through the plants due to the gradual increasing of plant biomass. With regard to this parameter, more beneficial conditions were detected in the tanks where carps were fed 28% CP, and again, the differences were insignificant (P≥0.05).

The carps selected P≥0.05). During the middle of the studied period, the live weight of carp fed feed with 28% crude protein was 1.68% higher as compared to carpfed 24% CP. This tendency was observed until the end of the trial for both variants. As shown in Figure 3, the weight gain of carp fed feed with 28% CP was by 2.09 g higher and they attained higher live body weight compared to fish reared in 24% CP tanks. The live weight of fish fed 28% and 24% CP ranged between 628.6±209.4 and – 601.8±155.8 g, respectively and was by 4.4% higher for the former variant but without statistically significant differences (P≥0.05). In aquaponic systems, good water conditions are needed for the growth and health of the fish. Water quality parameters to be analyzed are dissolved oxygen ammonia, nitrates, pH and other characteristics. Thefish feed can also affect water quality, requiring constant water quality monitoring (Diver & Rinehart, 2010).

A number of researchers investigated the effect of feed protein level in different fish species confirmed that higher dietary protein level resulted in higher growth performance (Hoffman, Prinsloo, & Rukan, 1997; Erfanullah & Jafri, 1998; Giri, Sahoo, Sahu, & Meher, 2003; Salhi, Bessonart, Chediak, Bellagamba, & Camevia, 2004;). The higher feed protein level results in better growth performance in fish is a well known fact (Kim & Lee, 2005). There are studies investigating the ratio between fish and plant section are existed (Shete, Verma, Chadha, Prakash, & Chandrakant, 2015; Rakocy,

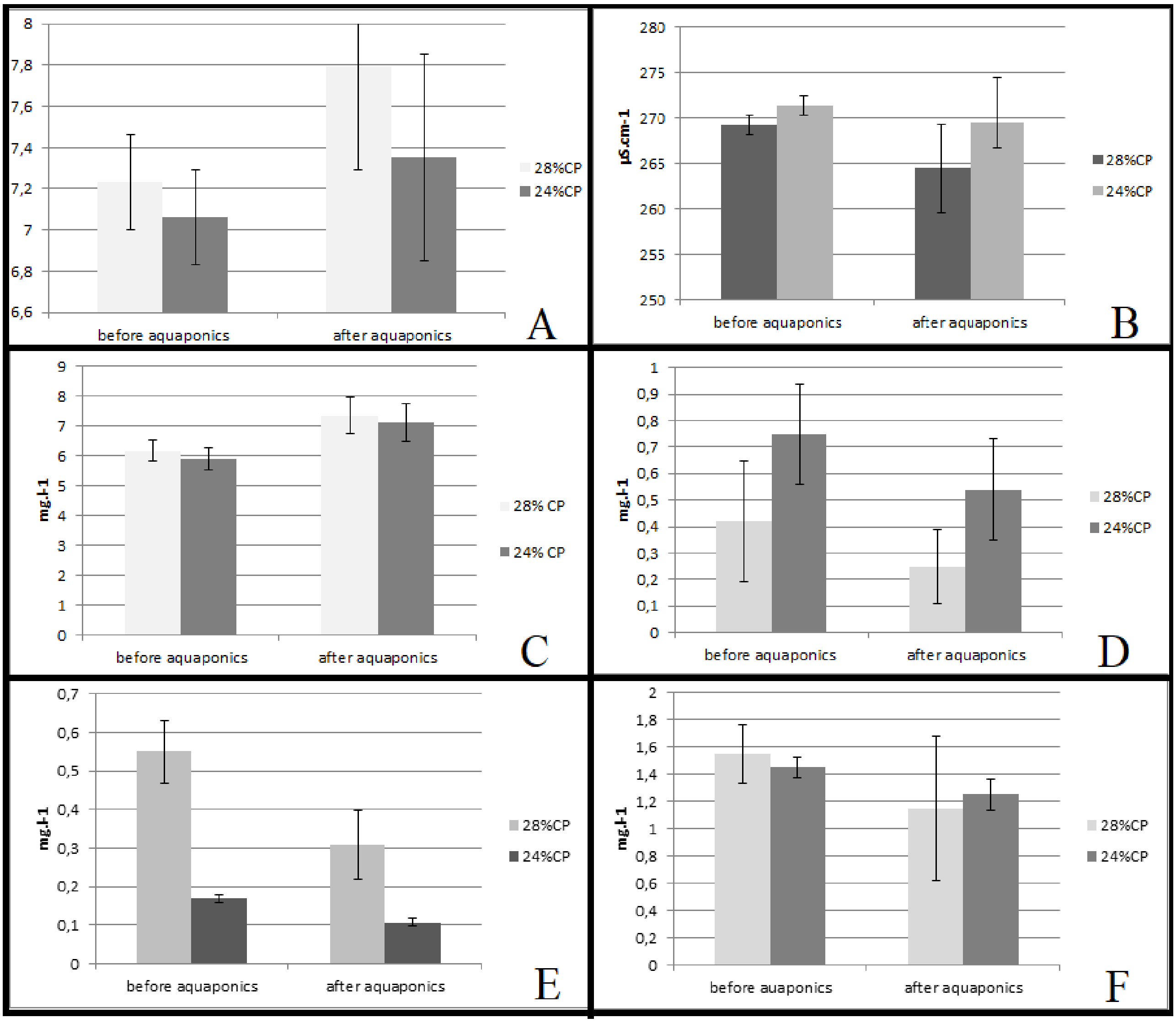

Figure 2. Hydrochemical parameters during the trial: A)pH; B)conductivity; C)dissolved oxygen; D)phosphorus; E)ammonium ions (NH_4^+); F)nitrates.

Shultz, Bailey, & Thoman, 2003), but so far, data on the relationships between the protein level in feed, water quality, and plant biomass are not available.

According to Ballestrazzi, Lanari, D'agaro, & Mion (1994) the quantity of excreted ammonia and phosphate in wastewater increased proportionally to the protein level in fish feed. This statement agrees with our results. The higher protein level in fish feed is connected with higher quantity of excreted ammonium ion and phosphorus (Al-Hafedh, et al., 2008). The preferable form of nitrogen for vegetables is ammonia the higher ammonium concentration in 28% CP in the current study resulted in higher biomass in plants from this experimental variant.

The total body length of carp fed different dietary protein levels is presented in Figure 3B. Throughout the experiment, there were no statistically significant differences with respect to this trait. At the end of the trial, carps fed feed with 28% CP exhibited higher total length by 1.4% than those fed lower dietary protein level (24%).

Li and Robinson (1998) and Li and Lovell (1992a, 1992b) investigated the effects of different dietary protein levels and their results are in line with ours as growth performance of fish was associated with dietary crude protein content. These results are also in agreement with experimental data in salmonids (Arzel, Metailler, Kerleguer, Delliou, & Guillaume, 1995) and American eel (Tibbetts, Lall, & Anderson, 2000).

The average daily weight gain at the end of the experiment was 39% lower in carp fed feed with 24% crude protein than that of carp offered feed with 28% CP, but the difference was not significant (P≥0.05) (Figure 3C). Feeds with higher protein content in the aquaponics system showed better growth performance. These results are probably due to the higher content of valuable nutrients in the variant with 28% CP. Other studies (Bekcan, Dogankaya, & Cakirogullari, 2006; Soon & Hashim, 2001) reported an increase in body weight of bagrid and European catfish parallel to dietary crude protein level.

Fish fed higher dietary protein level (28%) demonstrated lower feed coefficient – by 13.04%, but the difference was not significant (P≥0.05) (Figure 3D).

According to the main principle of aquaponics, ammonia is formed from metabolism of fish but it could be harmful to fish and plants at excessive concentrations. It could be however converted into nitrates with the help of bacteria, and nitrate-rich water benefits the development of plants. According to Blidariu and Grozea (2011) only about 10% of water in traditional agricultural systems, is absorbed by plants, whereas about 90% is lost to runoff or evaporation. In recirculating aquaponic systems, there is an efficient use of water, with typical losses of less than 10% of water volume per day (Blidariu & Grozea, 2011). Goddek et al. (2015) established that recirculating aquaponics systems were efficient as they reuse 95%-99% of water. Aquaponic systems have to be sized in a way that the plant production area should be large enough to re-use this excess water through plant uptake (Tyson, Danyluk, Simonne, & Treadwell, 2012). Evapotranspiration increases system sustainability by reducing wastewater effluent to the environment while supplying nitrogen and other nutrients for the plants' growth. The aquaponic systems could help reduce the waste,

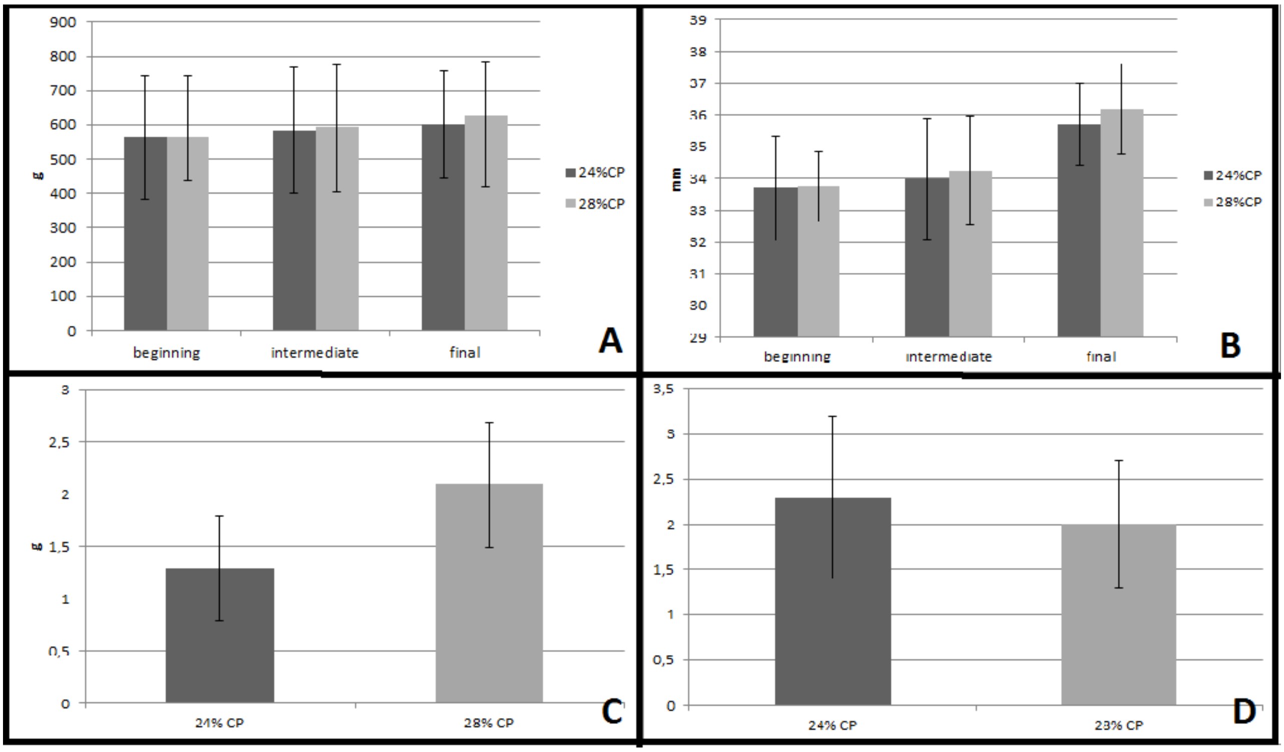

Figure 3. Growth parameters in carp during the trial: A)live weight; B)total body length; C)daily weight gain; D)FCR.

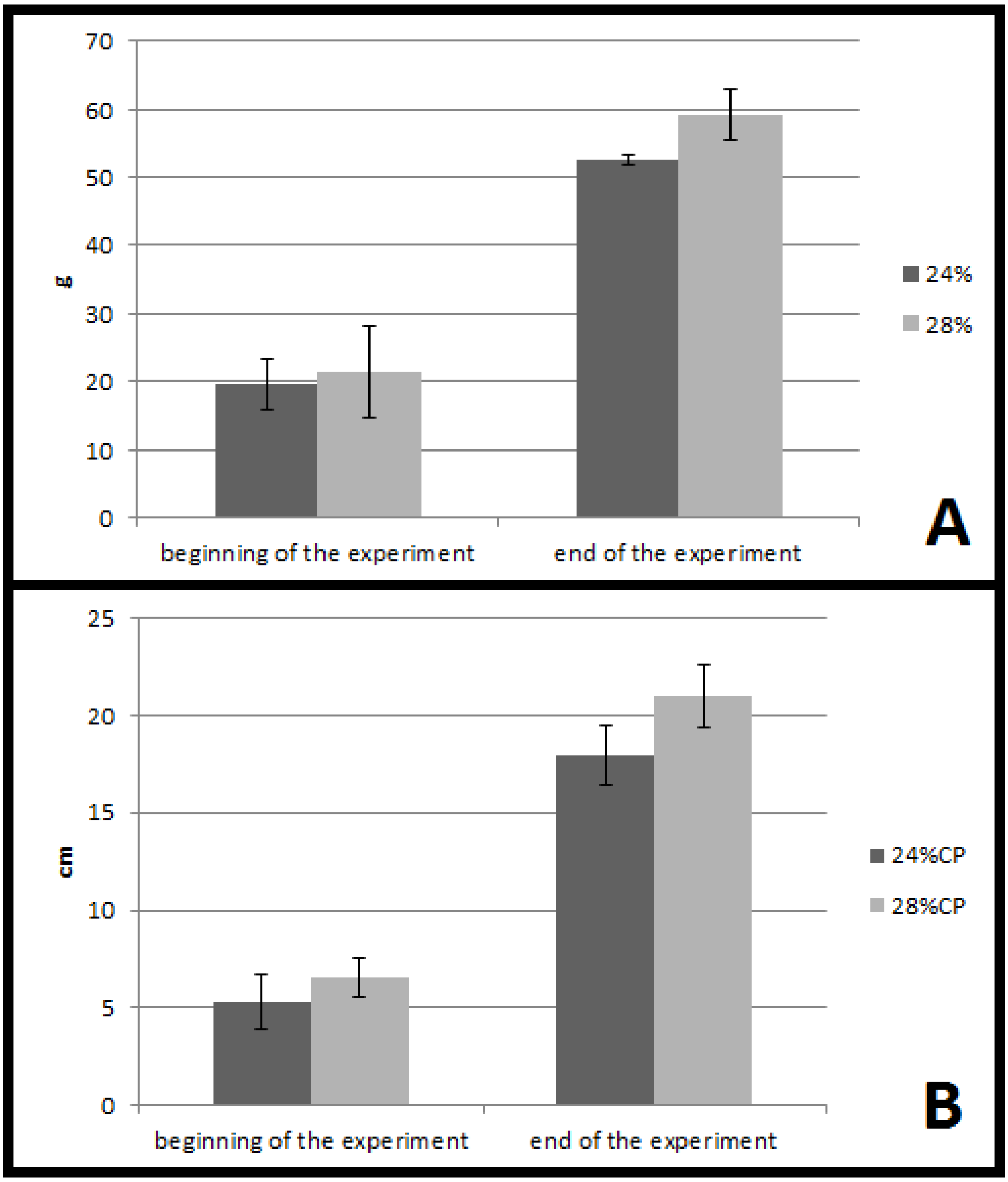

Figure 4. Growth parameters in lettuce during the trial: A) average weight of head in lettuce; B) average root length in lettuce.

pollution of traditional agriculture and reduce the amount of ecological degradation from excessive use of fertilizers and pesticides. Apart from the ecological aspect, aquaponic systems also show very good yields of the cultivated crops. In this experiment, Figure 4 presents the data for average lettuce biomass in both experimental variants in the beginning and the end of the trial. Higher values were determined for the variant with 28% CP, where the biomass was by a 12% higher. This research shows that high-protein feed correlates with the biomass of lettuce. Figure 4 presents the average length of the root of lettuce. At the end of the experiment, the lettuce root in the second experimental variant (28% CP) was 14% longer compared to that of the other variant, but the differences were not statistically significant ($P \geq 0.05$). Higher crude protein content in fish feed led to better developed roots in cultivated plants.

The survival of fish in the aquaponics system was 100% in both studied variants.

Conclusions

In the aquaponics system, fish fed feed with 28% crude protein exhibited better growth performance than those fed 24% CP: higher final body weight by 4.4%, higher total body length by 1.4%. Daily weight gain of carps fed 24% CP was 39% lower than that of fish fed 28% CP.

The feed coefficient in fish fed feed containing 28% CP was 13.04% lower than that of fish fed feed with 24% CP.

The lettuce biomass and root length were superior by 12% and 14% respectively in the experimental variants in which carp received the feed with higher crude protein content (28%).

Water chemical parameters allowed affirming that aquaponics was highly efficient for purification of water in aquaculture systems and could be used in designing fish farming recirculating systems.

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