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The Usage of Aquatic Floating Macrophytes (*Lemna And Wolffia*) as Biofilter in Recirculation Aquaculture System (RAS)

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Abstract

Conventional water treatment in recirculation aquaculture systems (RAS) is a limited technology to answer the challenges of so called “sustainable aquaculture”. This is why new and innovative technologies need to be invented and introduced in RAS. The aim of the conducted study was to determine the possible advantages of using two macrophytic plants – *Lemna minor* and *Wolffia arrhiza* and their quality as biological filter in a RAS for the cultivation of fingerlings from common carp. The temperature, dissolved oxygen, pH and conductivity were measured daily with a portable combined meter and with a probe appropriate for the parameters in the newly constructed control and experimental RAS (with floating macrophytes as a biological filter). Ammonium, nitrite, nitrate, total nitrogen and phosphorus were measured spectrophotometrically. At the end of the trial the fish were weighed and individual weight gain, specific growth rate and FCR (feed conversion ratio) were calculated. The utilization of two macrophytes (*Lemna* and *Wolffia*) in their quality as a biofilter in RAS increased dissolved oxygen in the water, significantly decreased the quantity of total dissolved solids, ammonia, nitrite, orthophosphate as well as total phosphorus in water, and significantly increased the growth of the cultivated carp’s fingerlings.

Keywords: Lemna, Wolffia, biofilter, RAS, water quality, carp’s fingerlings.

Introduction

One of the main problems in recirculation aquaculture systems (RAS) which are still object of investigation from aquaculture scientists is the removal of nitrogenous (Burrows, 1964; Leitritz and Lewis, 1976; Crab *et al*., 2007) and phosphorous metabolite compounds. The nitrogenous waste products are being created and excreted from fish through gill diffusion, gill cation exchange, as well as urine and feces excretion; in addition to this some nitrogenous wastes are accumulated from the organic debris of dead and dying organisms, uneaten feed, and from nitrogen gas in the atmosphere (Timmons *et al*., 2002). Phosphorous waste compounds, on the other hand are supplied together with feed, particularly compound feeds.

Piedrahita (2003), Gutierrez-Wing and Malone (2006) reported that approximately 75% of nitrogenous and phosphorous compounds in food for hydrobionts remain unused and are accumulated in water like waste products.

Biological filtration is the method used for the destruction of organic and inorganic waste compounds in RAS (Boyd, 1990).

In the past few decades there has been considerable interest towards using floating macrophytes for the removal of nitrogenous and phosphorous compounds in the water (Steward, 1970). Macrophyte plants act as biological filters which accumulate nutritional compounds and inorganic waste products. Duckweed is a floating aquatic macrophyte which consists of four genera: *Lemna*, *Spirodela*, *Wolffia* and *Wolffiella*. Duckweed in wastewater treatment was found to be very effective in the removal of nutrients, soluble salts, organic matter, heavy metals and in the eliminating suspended solids, algal abundance and total and fecal coliform densities (El-Kheir *et al*., 2007). Duckweed has a high mineral absorption capacity and can tolerate high organic loading as well as high concentrations of micronutrients (Hasan and Chakrabarti, 2009).

Fresh duckweed is well suited to intensive fish farming systems with relatively rapid water exchange rates for waste removal (Gaigher et al., 1984), and duckweed is converted efficiently to live weight by certain fish, which include carp and tilapia (van Dyke and Sutton, 1977; Hepher and Pruginin, 1979;

Robinette et al., 1980; Hassan and Edwards, 1992). The research conducted with duckweed as a biological filter in the field of aquaculture is connected with the filtering of effluent water from fish farms (Sipaúba-Tavares *et al*., 2002) or improving water quality in fish ponds (Ferdoushi *et al*., 2008). The research pertaining to questions connected to the usage of duckweed as a biofilter in RAS are limited (Jo *et al*., 2002).

The aim of the conducted study was to determine the possible advantages of using two macrophytic plants – *Lemna minor* and *Wolffia arrhiza* and their quality as biological filter in RAS for the cultivating of fingerlings from common carp.

Materials and Methods

The experiment was conducted in two newly built recirculation aquaculture systems situated in the experimental aquaculture base at the Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria.

The Recirculation Aquaculture Systems (RAS)

For the purpose of our study two independent recirculation aquaculture systems were constructed and built. Each of them consisted of four tanks (50 litres each) and a module where the process of filtering the water was realized. The cleaning block of the experimental RAS consisted of a mechanical and biological filter (Figure 1). The biological filter consisted of two macrophytic plants – *Lemna minor* and *Wolffia arrhiza* freely floating on the surface of the water. The control RAS filtering block only consisted of a mechanical filter (Figure 1).

Experimental Fish

From a fish farm situated on the Jrebchevo dam, fingerlings from common carp were selected and transported to the experimental aquaculture base of the Faculty of Agriculture. The average fish weight at the start of the trial was:

- Experimental group fish – 8.13 g;
- Control group fish – 8.18 g;

Between the average weight of the carp from the two experimental variants a significant difference could not be found ($P \geq 0.05$). The stocking density was at 333 pcs/m^3. The feeding level which we used in our trial was 2% of the biomass of the fish. The fish were fed manually three times per day. The content of granulated feed which we used in our experiment can be seen in Table 1.

At the end of the trial the fish were weighed and individual weight gain, specific growth rate and FCR (feed conversion ratio) were calculated.

The specific growth rate was calculated using the following formula:

$$SGR = [(Bf \ln - \ln Bi)/T]*100$$

Bf - final biomass; Bi - initial biomass; T - time interval (days). The food conversion ratio was calculated using the following formula:

$$FCR = F/(Bf-Bi)$$

F amount of food administered; (Bf-Bi) growth gain; Bf, Bi - final and initial biomass;

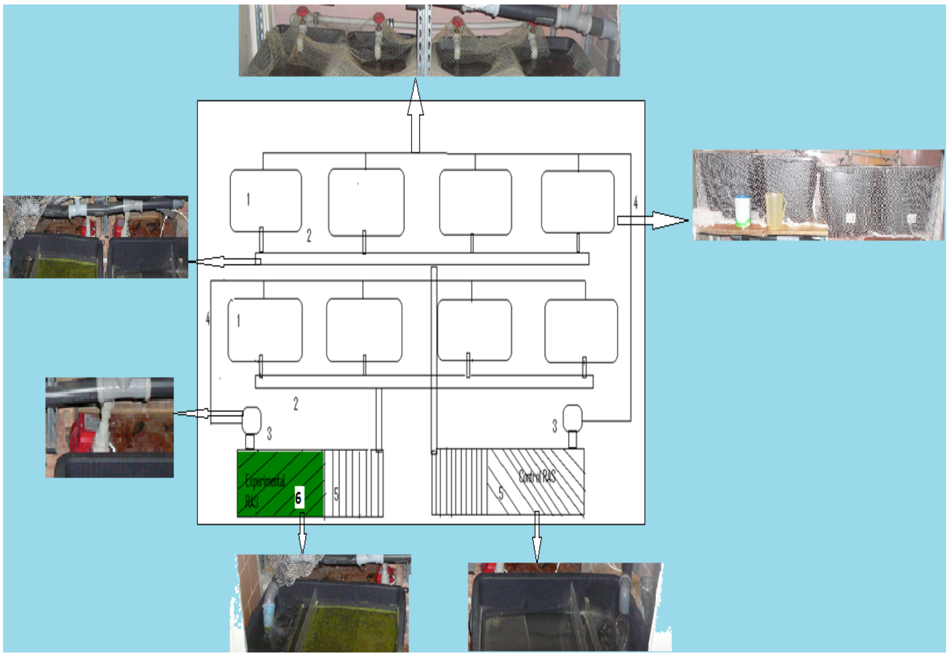

Figure 1. Scheme of experimental and control RAS. 1: raising tank; 2: outlet water; 3: pump; 4: water inlet; 5: mechanical filter; 6.biological filter.

Hydrochemical Parameters

The temperature, dissolved oxygen, pH and conductivity were measured daily with a portable combined meter and with a probe appropriate for the parameters.

Samples for other analysis were taken once every ten days by the order showed below:

B1 – 10th day after start of the trial
B2 – 20th day after start of the trial
B3 – 30th day after start of the trial
B4 – 40th day after start of the trial

Table 1. Content of used feed in trial

Content	%
Crude Protein	42
Crude Fat	22
Ash	5.5
Crude fibre	4.6
Total phosphorus	0.9
Ca	1.9
Na	0.2
Vitamine	-
E300	100 mg kg^{-1}
E307	175 mg kg^{-1}
Antioxidants	-
E324	19 mg kg^{-1}
E321	2 mg kg^{-1}
Cu	9.4
Mn	30
Zn	130
Fe	140

They were measured with a spectrophotometer DR 2800 (Hach Lange). The methods and range of tests which were used during the experiment are shown it Table 2.

The macrophytes were determined by Flora Reipublicae Popularis Bulgaricae, vol II (Jordanov *et al.*, 1963). Data analysis were conducted by using ANOVA (MS Office, 2010).

Results

During the experimental period, the values of water temperature were similar in both RAS's (Figure 2) and fluctuated between 19.7°C and 22.9°C (Table 3). Average values of temperature for the control RAS were 21.82°C and 21.97°C, but without statistically proven differences (Table 3).

The values of dissolved oxygen during the experimental period were higher in the experimental RAS in comparison to these of the controlled RAS and fluctuation in the values in the first system were much more supple than these showed in the second system (Figure 3). Average values of dissolved oxygen were higher with 5.14% in experimental RAS compared with the measured values in the control RAS ($P \leq 0.01$) (Table 3).

During our trial the measured pH was weakly alkaline in both recirculation systems too (Figure 4) and without statistically proven differences between control and experimental RAS (Table 3).

The values of conductivity in the controlled RAS were higher compared to those showed in the

Table 2. Methods and range of tests used for monitoring the water quality parameters during experiment

Quality parameters	Determination method	Measuring range (mg L^{-1})
Ammonium-nitrogen	Indophenol blue	0.015-2
Nitrite – nitrogen	Diazotization	0.015-0.6
Nitrate - nitrogen	2.6 dimethylphenol	5-35
Total nitrogen	Koroleff digestion +2.6 dimethylphenol	5-40
Phosporus (ortho + total)	Phosphormolybdenum blue	0.05-1.5 mg L^{-1} PO_4-P 0.15-4.5 mg L^{-1} PO_4

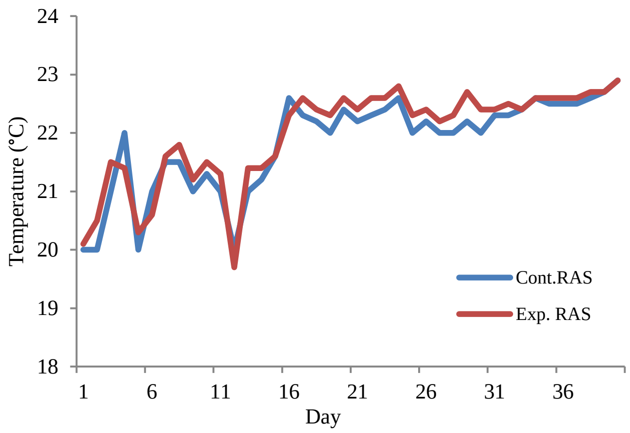

Figure 2. Temperature in control and experimental RAS during experiment.

Table 3. Temperature and hydrochemical parameters in control and experimental RAS

Parameters	Control RAS (x±Sx)	Experimental RAS (x±Sx)
Temperature (°C)	21.82±0.12	21.97±0.13
Dissolved oxygen (mg L^{-1})	7.37±0.11	7.77±0.06**
Conductivity (µS cm^{-3})	613.05±8.39	549.45±12.12***
pH	8.38±0.02	8.33±0.02
Ammonium (mg L^{-1})	0.19±0.00	0.15±0.00*
Nitrate (mg L^{-1})	12.65±1.8	16.07±8.8
Nitrite (mg L^{-1})	0.13±9.09	0.08±0.0**
Total nitrogen (mg L^{-1})	18.3±20	13.9±15
Total phosphorus (mg L^{-1})	0.6±0.05	0.48±0.04*
Ortho-Phosphate (mg L^{-1})	0.4±0.05	0.3±0.05*

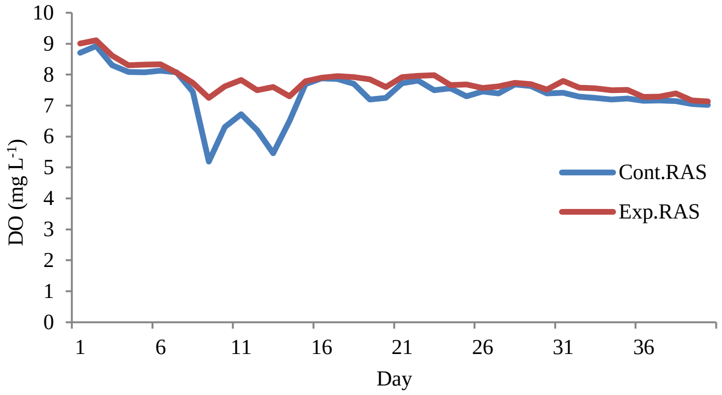

Figure 3. Temperature in control and experimental RAS during experiment

experimental RAS (Figure 5). The average value of conductivity in experimental RAS were lower by 10.37% contrasted to those of the control variant (Table 3).

The minimal measured value of ammonia was 0.14 mg L^{-1} in the experimental RAS and the maximum value - 0.216 mg L^{-1} in the control system (Figure 6). The concentration of ammonium in experimental RAS were lower in every taken samples during the trial compared with the results which were received for control RAS (Figure 6).

The same tendency was found, even much more better expressed, for nitrite (Figure 7), the higher values were measured in control RAS, compared with these which we received from our measurement in the experimental RAS and differences were statistically proven at a high level ($P \leq 0.01$) (Table 3). The minimal measured values of nitrite during our experiment were 0.053 mg L^{-1} and it was measured in the experimental RAS. The maximum value was 0.144 mg L^{-1} and it was measured in the control recirculation system (Figure 7).

During the experiment all measured quantities of nitrates were higher in the experimental RAS than those which we found for the control recirculation system (Figure 8), but differences between the control and the experimental variants were not statistically proven (Table 3). The maximum measured value for this parameter was 20.3 mg L^{-1} and the minimal quantity of nitrate was found to be 11.1 mg L^{-1} in the control system. Total nitrogen was higher in the control RAS compared to recirculation system using macrophytes as a biofilter every day when we took samples (Figure 9).

With respect to phosphorus compound we found a higher quantity of orthophosphate and total phosphorus in the control RAS compared to the experimental RAS in all conducted measurements (Figure 10 and 11).

Average values of the final weight, individual weight gain and specific growth rate of carp cultivated in the recirculation system using two macrophytes from genera *Lemna* and *Wolfia* as biofilter were by 10.8%, 28.1% and 25.6% respectively higher than those which we find for the carp's fingerlings cultivated in the control RAS (Table 4). The average value of the feed conversion ratio for the fish from the experimental RAS was by 27.8% lower than those showed by fish cultivated in the control RAS (Table 4). The survival of the fish was not affected by the type of filter which we used in our trial.

Discussion

Two RAS's were not disposed with heating elements, which is the reason, the temperature inside

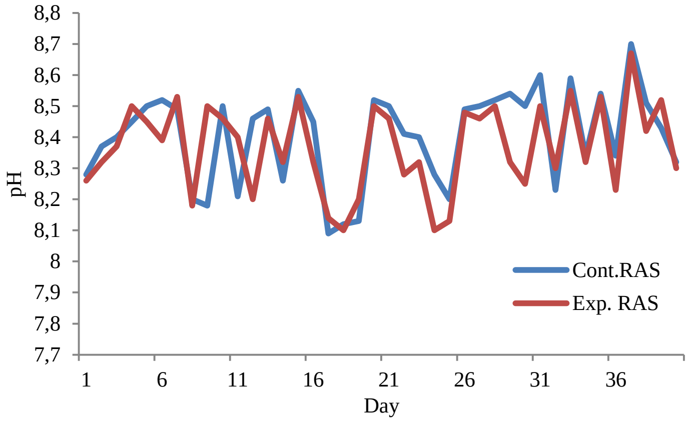

Figure 4. pH in control and experimental RAS during the experiment.

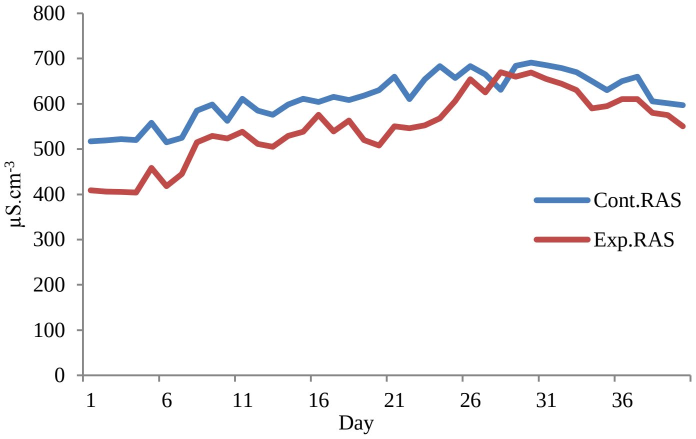

Figure 5. Conductivity in control and experimental RAS during the experiment.

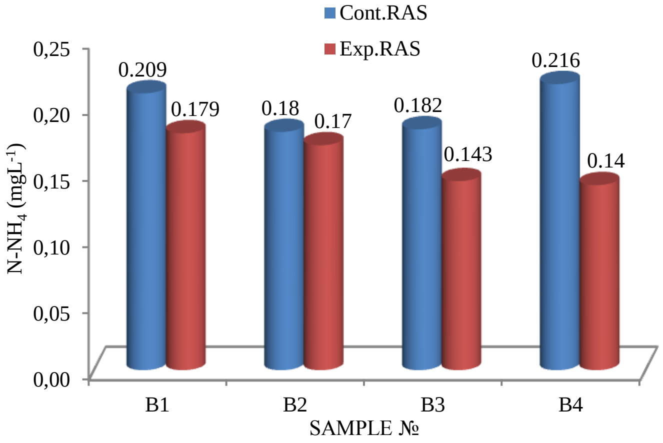

Figure 6. Dynamic evolution of ammonium (N-NH_4^+).

them are dependent from air temperature. Nevertheless the temperature during the experiment was optimal for the growth of experimental carp, because according to Huet (1970) a range of 20-28°C is the optimum temperature for the growth of common carp under laboratory conditions.

Our received results with respect to oxygen concentration are confirmed from the research conducted by Ferdoushi *et al.* (2008), which investigated the impact of macrophytic plants (*Lemna*)

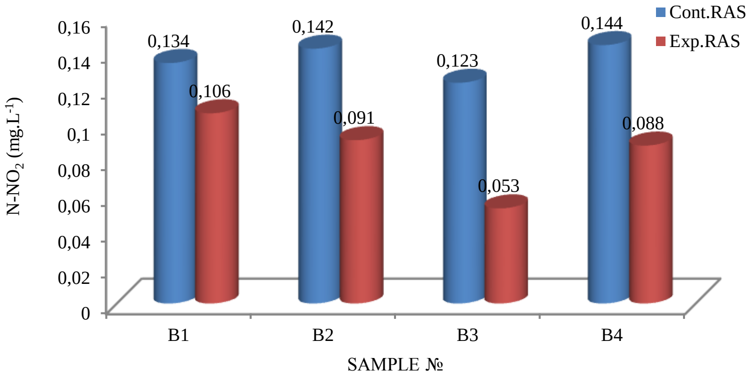

Figure 7. Dynamic evolution of nitrite ($N\text{-}NO_2^-$).

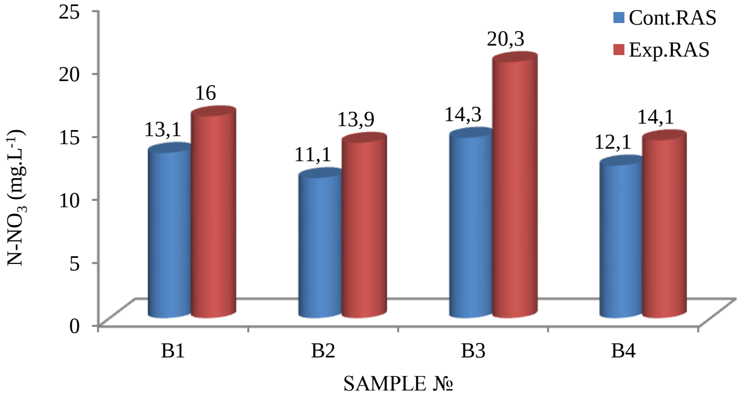

Figure 8. Dynamics evolution of nitrate ($N\text{-}NO_3^-$).

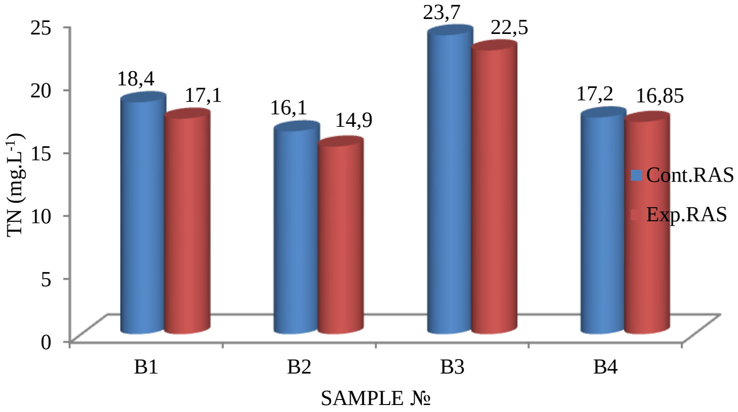

Figure 9. Dynamics evolution of total nitrogen (TN).

as biofilters in fish ponds. They found out, that the quantity of dissolved oxygen was higher in the pond treated with *Lemna*. According Ondok *et al*. (1984) macrophytes oxygenate the water very effectively.

Sengupta *et al*. (2010) explore the impact of duckweed growth on water quality in sub-tropical pondsand found out that pH values were mostly alkaline in the ponds studied and varied between 6.9 and 9.1. pH values of experimental ponds with macrophytes were found to be slightly alkaline and mean values of pH were 7.61±0.39 (Ferdoushi *et al*., 2008).

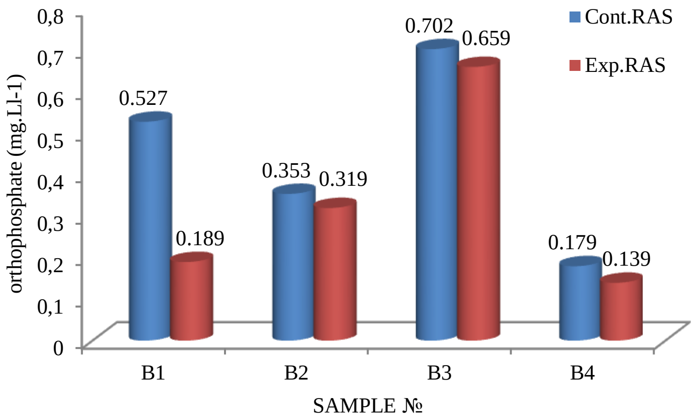

Figure 10. Dynamics evolution of orthophosphate

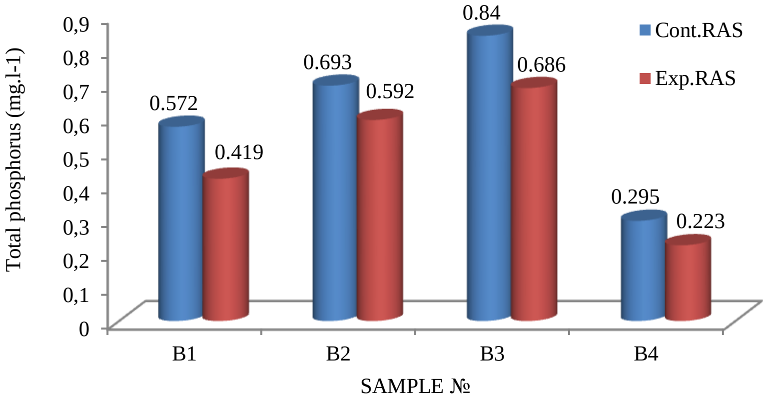

Figure 11. Dynamics evolution of total phosphorus.

Table 4. Technological indicators of common carp's fingerlings growth

Experimental variant	Control RAS	Experimental RAS	P
Fish (recalculated pcs/m^3)	333	333	
Initial fish weight (g/fish)	8.13	8.18	ns
Final fish weight (g/fish)	11.63	13.04	***
Individual weight gain (g)	3.49	4.86	***
SGR (Specific growth rate) (%/day)	0.90	1.21	***
FCR Feed conversion ratio (g/g)	2.33	1.68	***
Feeding level (% biomass)	2	2	
Survival (%)	100	100	
Days of growth (days)	40	40	

The electrical conductivity (or specific conductance) of water depends on the concentration and charge of the dissolved ions. Because of this relationship, conductivity often is used as an indicator of the total dissolved solids (TDS) in the water. The slightly lower values of conductivity in the experimental RAS compared to those which we received in the control recirculation system are by our opinion the result from the decrease of total dissolved solids (TDS) in the water from the biofilter consisting of two macrophytes. Azeez and Sabbar (2012) stated that duckweed used in phytoremediation of the pollutants in wastewater from oil refinery reduced 48.9% from TDS in the water.

Ammonia toxicity is thought to occur from osmoregulatory imbalance causing renal failure and gill epithelial damage resulting in suffocation, decreased excretion of endogenous ammonia and general neurological and cytological failure (Meade, 1985). The total ammonia in the water is the sum of the un-ionized ammonia and the ionized form (ammonium). The main factor which determines the direction of the reaction from which the ammonia/ammonium ratio in water depends is the pH of the water. In general, at a temperature around room temperature and pH of around 8.0, the ammonia's quantity is equal to 10% from total ammonia in the water. The calculations made have shown that at the highest ammonium value which was received in our trial (in control RAS-0.216 mg L^{-1}) the value of ammonia is 0.024mg.l^{-1}. For salmonids, long term exposure to concentrations between 0.05 to 0.2 mg L^{-1} of ammonia can significantly reduce their growth rate, fecundity and disease resistance and increased gill ventilation, metabolic rate, erratic and quick movements and also can cause mortality (Isla Molleda, 2008). The average value of ammonium was 19.6% lower in the RAS with a biofilter consisting of two macrophytes compared to its concentration in the control RAS. Pernial *et al.* (1998) also found out that *Lemna minor* monoculture consistently removed the largest amount of ammonia and phosphorus from storm water within 8 weeks.

Problems connected to nitrite are typically more likely in closed aquaculture systems due to an insufficient and inefficient process of removing the waste product ammonia from the biofilter by means of nitrification process (Kroupova *et al*., 2006). Nitrite accumulates in plasma, gills, liver, brain and muscles (Bath and Eddy, 1980; Margiocco *et al*., 1983; Gisbert *et al*., 2004). The toxic effect of nitrite on fish is the result of haemoglobin oxidizing to methaemoglobin, which is unable to transport oxygen (Cameron, 1971). Voslářová *et al*. (2008) stated that the rate of nitrites is determined at the amount of $\leq$0.9 mg L^{-1} (for Cyprinidae) and this concentration is suitable for life and reproduction. In our trial the concentration of nitrite are lower than 0.9 mg L^{-1} in all measurements made.

Boyd and Queiroz (1997) verified that aquatic plants in biofilter systems were capable of removing 94% of nitrite. The average value of nitrite in the recirculation system using macrophytes as biofilter in our experiment was just 37.7% lower in comparison to those which were found in the control RAS (Table 3).

Our results concerning nitrate's concentration contrasted with the findings from Leslie *et al*. (1983) who detected a significant increase in nitrate-nitrogen concentration in two Florida lakes following macrophytes removed by grass carp. A higher nitrate concentration in RAS using macrophytes as biofilter is probably the result of fact that duckweeds prefer ammonia nitrogen (NH_4-N) as a source of nitrogen and will remove ammonia preferentially, even in the presence of relatively high nitrate concentrations. Our opinion is confirmed by Hasan and Chakrabarti (2009), who reported that duckweed plants utilize all available ammonium before beginning to assimilate nitrate and appear to grow more quickly in the presence of ammonium than with nitrate. The higher oxygen level in the recirculation system using macrophytic plants as biofilter could stimulate the growth of bacteria from genus Nitrobacter, which in their turn are involved in the nitrification process. This possibly leads to an accumulation of significant quantity of nitrate in the water, that could be another possible reason of the higher concentration of nitrate in the experimental recirculation system, compared with the nitrate concentration in the control RAS.

Contrary to ammonia and nitrite, nitrate is relatively non-toxic to aquatic organisms. However, the research with octopus (Hyrayama, 1966), trout (Berka *et al*., 1981), shrimp (Muir *et al*., 1991) and eel (Kamstra *et al*., 1998) showed that a high concentration of nitrate can affect the growth of commercially grown hydrobionts.

The quantity of nitrate in our trial was higher in both RAS's than recommended for the cultivation of common carp - 2 mg L^{-1} (Zajkov, 2006). The general reason for that was the lack of a denitrification process in both systems. Average values of total phosphorus and orthophosphate were respectively by 20% and 25.8% lower in experimental RAS compared with values of phosphorus compound measured in control RAS ($P\leq0.05$) (Table 3).

Our results are in confirmation to those received from Boyd and Queiroz (1997) who stated that aquatic plants in biofilter systems are able to remove 97% of the phosphorus compound in the water. The lower purification effect of a biofilter consisting of the two above cited macrophytic plants, concerning phosphorus and some of the other waste products voided in water from fish, could be results from the lower temperature during the experiment, which reduced the assimilation of soluble compounds from the plants. For example Landolt, (1986) stated that the optimal temperature for *Lemna minor* is 26°C.

Ferdoushi *et al*. (2008) who conducted an experiment with macrophytes as biofilter in a fish pond throughout the study period found out that phosphate phosphorus (PO_4-P) were 3.5 mg L^{-1} higher in the treatment without macrophytes. Landolt and Kandeler (1987) reported that *Lemna* sp. requires high phosphorus concentrations to grow in water.

The better water quality in the experimental RAS compared with those in the control recirculation system are logically expressed in better growth rate and better food assimilation in carps fingerlings from recirculation system which used floating macrophytes like biological filter. Timmons *et al*. (2002) stated that deterioration of the water in RAS caused negative effects on fish growth, increased fish stress and caused health problems in the fish.

Conclusion

The utilization of two macrophytes (*Lemna* and *Wolffia*) in their quality as a biofilter in RAS increased dissolved oxygen in water and decreased significantly the quantity of total dissolved solids, ammonia, nitrite, orthophosphate and total phosphorus in water.

Better water quality in a RAS using macrophytes (*Lemna* and *Wolffia*) as biofilter result in better growth and better assimilation of food in carp's fingerlings compared to growth parameters and FCR of fingerlings from the control RAS whose cleaning section consists just from a mechanical filter.

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