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The Influence of Aquaponically Grown Duckweed (*Lemna minuta* Kunth) Used for Composition of Sustainable Diets on Hydrochemical and Technological Parameters in Carp (*Cyprinus carpio* L.)

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Abstract

The aim of the current study was to investigate the influence of aquaponically grown duckweed (*Lemna minuta*) used as part of a biofilter in recirculation aquaculture systems, when its included in the composition (10 and 30% content of daily feed ratio) of sustainable diets on hydrochemical and technological parameters in common carp (*Cyprinus carpio* L) fingerlings cultivated in recirculation aquaponic systems.The inclusion of *L. minuta* in diets for carp fingerlings influenced the hydrochemical parameters and decreased the quantity of nitrogen and phosphorus compounds in water of tanks where carps were fed with feed containing duckweed, but difference was statistically significant only for ammonium (0.087±0.008)($P \leq 0.05$). The carps fed with a diet containing 30% (L30) duckweed of their daily feed ratio showed better survival but similar growth and FCR than fish fed with L0 and differences were not significant ($P \geq 0.05$). The lower growth and higher FCR were measured in carps fed with L10 compared to the values of these parameters in carp's fingerlings fed with L0 diet and the differences were significant ($P \leq 0.05$). The duckweed presents cheap and easy accessible ingredients for feeding of carp. Furthermore it could be used for treatment of wastewater in recirculating aquaculture systems this way increasing their sustainability.

Keywords: Duckweed, Common Carp, Feed Substitution, Aquaponics, Growth

Introduction

The search for alternative components instead expensive and deficit ones for the composition of feed for hydrobionts is a problem with a primary importance for the aquaculture sector. During the last few decades significant attention from researchers was paid on the usage of different plants with high nutritional value-soy (Kaushik et al., 1995; Deng et al., 2006), beans (Azaza et al., 2009; Mahmoud, Kilany, & Dessouki, 2014) and different vegetables (Hossain, Focken, & Becker, 2001; Mundheim, Aksnes, & Hope, 2004).

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The plants which are still subject of studies from researchers and probably could be the answer on the posed challenges from the aquaculture sector are the species from genera *Lemna*.

Another possible application of plants from genus *Lemna* is their utilization in the waste water treatment process. Ferdoushi, Haque, Khan, and Haque (2008) studied water treatment capacity of plants from genus *Lemna* when they are used as biological filter. They stated that the duckweed could remove a significant quantity of nitrogen compounds. The duckweed was used in treatment of water from dairy livestock (Rashid & West, 2007), municipality waste water (Dalu & Ndamba, 2003; El-Kheir, Ismail, El-Nour, Tawfik, & Hammad, 2007) and aquaculture (Velichkova & Sirakov, 2013). The plants from genus *Lemna* by the process of bioaccumulation could remove up to 99% of nutrient compounds in the water during the waste water treatment process (Skillicorn, Spira & Journey, 1993).

One possible technology that could establish the link between the application of plants from genus *Lemna* in wastewater treatment process and their use for animal feeding purposes is aquaponics. The aquaponics technology is integrating aquaculture with the production of different plants (Graber & Junge, 2009; Diver & Rinehart, 2010). In most cases the received plant production in this type of system consists of vegetables and spices for human consumption (Rakocy, Losordo, & Masser, 2006; Karimanzira, Keesman, Kloas, Baganz, & Rauschenbach, 2016). There is a lack of studies which consider other possible plant applications of this sustainable aquaculture technology.

One species from genera *Lemna*-*L. minuta* was recently found in Bulgaria (Kirjakov & Velichkova, 2016). The numerous researches connected with the investigation of usage of duckweed as a feed component for fish feeding used *L. minor* (Yılmaz, Akyurt, & Günal 2004; El-Shafai, El-Gohary, Verreth, Schrama, & Gijzen, 2004; Talukdar, Shahjahan, & Rahman, 2012). The studies connected with the test of other species from *Lemna* as a feed substitution in fish feed were practically missing or were very rare (Bairagi, Ghosh, Sen, & Ray, 2002).

The aim of the current study was to investigate the influence of aquaponically grown duckweed (*L. minuta*) used as a part of biofilter in recirculation aquaculture systems, when its included in the composition (10 and 30% content of daily feed ratio) in sustainable diets on hydrochemical and technological parameters in common carp (*Cyprinus carpio* L) fingerlings cultivated in recirculation aquaponics systems.

Materials and Methods

Experimental Fish

One hundred and eight pieces from fish species common carp in good health and without visible injuries were chosen from fish farm "Tundja-73" and transported to the aquaculture base at Agriculture faculty (Trakia University). The average weight of the experimental fish was 12±0.1 g., without significant differences in weight (P≥0.05) between different individuals. The carps were acclimatized to the experimental aquaponics recirculation system for one week. The stocking density of the carps in the system was 5 kg.m^{-3}. During the acclimatization period the fish were fed with feed of appropriate size and content.

The trial continued for 40 days. The carps were fed with 3 experimental diets – L0 (diet without substitution of duckweed (*L. minuta)* meal), L10 (diet substituted with 10% duckweed (*L. minuta*) meal) and L30 (diet substituted

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with 30% duckweed (*L. minuta*) meal). The trial was conducted in 3 replications. The fish were fed manually tree times per day with daily feed ratio of 3% from fish body weight.

Experimental Aquaponic System

The experimental aquaponic system consisted of fish tanks (50 l), a sedimentation tank (100l) and biological filter (50 l), which consists of zeolite compartment, and the aquaponic section contained floating duckweed as well. Two months prior the start of feeding trial the same aquaponic recirculation system was used for cultivation of duckweed. Oxygen was added to every single fish tank and biological filter by aerator. The flow rate was maintained to 1 $l.min^{-1}$. The bottom of the fish tanks and the sedimentation tank were cleaned every three days by siphoning.

Experimental Diets

The duckweed was collected from Banya – Plovdiv region (42°32'226''N 24°50'213''E) and determined by Flora of North America (Landolt, 1993). The species *L. minuta* Kunth were chosen and used for aquaponics cultivation. . The received from biological filter duckweed was dried. The analysis of protein content and moisture was made prior to the diet formulation (Table 1) AOAC (1995). Three isocaloric and isonitrogenous diets were formulated (Table 2 and Table 3).

The different components of each diet were mixed thoroughly and by addition of warm water they were mixed until a homogeneous dough was obtained. The received dough was then passed through a mincer to produce the pellets of 0.5 mm in diameter. The received feed was dried at +40°C and afterwards was kept in a refrigerator (+4°C) until use (Yilmaz et al., 2004).

Hydrochemical Parameters

The temperature, oxygen quantity and pH were measured daily with a portable meter HQ40D (Hach Lange) with appropriate for the aim probes. The concentration of ammonium ($mg.l^{-1}$), nitrite ($mg.l^{-1}$), nitrate ($mg.l^{-1}$) and total phosphate ($mg.l^{-1}$) were measured spectrophotometrically (DR2800) with cuvette tests (Hach Lange). The methods used for spectrophotometrical measurement of hydrochemical parameters were shown in Table 4.

Technological Parameters

The survival (%) of fish, specific growth rate (% per day) and feed conversion ratio (FCR) were calculated by following equitations:

$$\boldsymbol{Survival}\ (\%) = \frac{\boldsymbol{Number\ of\ survival\ fish}}{\boldsymbol{Number\ of\ fish\ in\ the\ start\ of\ experiment}}$$

$$\boldsymbol{FCR} = \frac{\boldsymbol{Amount\ of\ feed\ fed\ (kg)}}{\boldsymbol{Weight\ gained\ (kg)}}$$

$$\boldsymbol{Specific\ growth\ rate}\ (\%\ \boldsymbol{per\ day})\ \frac{\boldsymbol{ln\ (Final\ weight - Initial\ weight)}}{\boldsymbol{experimental\ period}}\boldsymbol{x100}$$

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Statistical Analysis

The received data were analyzed by ANOVA single factor at a significance level of $P<0.05$. The analyses were made by using the SPSS program.

Results

Hydrochemical Parameters

The temperature was optimal for the raised fish and varied between 24.1 and 25.5°C without statistical differences in different experimental variants (Fig.1). The content of oxygen in tanks where carps were fed with the diet containing the highest substitution of duckweed (30%) was higher accordingly with 1.9% and 3.8% compared to the values in the tanks where fish were fed with L0 and L10 diets, but the differences were not significant ($P\geq0.05$). The average value of pH was closer to neutral in tanks where carps were fed with the L30 diet and its value was higher than 8 in tanks where the experimental diets L0 and L10 were given to fish (Fig.1), but the differences were not statistically proven ($P\geq0.05$).

The quantity of nitrogen compounds and phosphate was lower in the water of tanks where carps were fed with the diet substituted with 30% duckweed, compared with the values of these parameters measured in the water of tanks where fish were fed with diets L0 and L10 (Fig.2). The amounts of ammonium, nitrite, nitrate and phosphate in the water of tanks where carps were fed with the L30 diet were lower accordingly with 49.7%, 34.1%, 20.8% and 11.9% compared to their values in the tank's water where fish were fed with the control diets L0, but the differences showed statistical significance only for ammonium (0.087±0.008) ($P\leq0.05$) (Fig.2). The amounts of ammonium, nitrite, nitrate and phosphate in the water of tanks where carps were fed with the L10 diet were lower accordingly with 36.9%, 12.9%, 0.63% and 6.71% compared to the values in the tank's water where fish were fed with control diets L0, but the differences were not statistically proven ($P\geq0.05$) (Fig.2).

Technological Parameters

The survival of carp during the trial was higher in the experimental group fed with L30 (100%), followed by the survival found for carp fed with the L10 diet (93%) and a lower survival was calculated for fish fed with the control diet (L0) (85%). The highest final weight (27.6 g±0.7) was found in carp fed with the L0 diet and it was accordingly with 21.01% and 30.2% higher than the weight calculated for carps fed with L10 (21.8 g±1.9) and L30 (25.5 g±2.1) diets. The highest specific growth rate (2.08%/day±0.2) was found in carp fed with the control diet (L0) and it was higher accordingly with 7.6% and 7.92%, compared to these calculated for carps fed with the L10 (1.45%/day±0.2) and L30 (1.86%/day±0.3) diets. The best FCR (1.28±0.1) was found in carp fed with the L0 diet, followed by the FCR found in carp fed with the L30 diet (1.49±0.4) and a higher FCR was found in fish fed with the experimental diet L10 (2.08±0.3).

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Discussion

Hydrochemical Parameters

The protein content of *L. minuta* in the current study was lower compared with the protein content found for duckweed cultivated under ideal conditions (Leng, Stambolie, & Bell, 1995). The reason for the lower protein content in aquaponically cultivated *duckweed* in our experiment could be the result from the partial removal of nutrients from the zeolite compartment and a decreased availability of nitrogen and phosphorus compounds for cultivated duckweed in the biological compartment. This assumption was in confirmation with the conclusion made from Goopy and Murray (2003) which stated that the levels of available nutrients can strongly influence both the quantity and quality of the received yield from duckweed.

The hydrochemical parameters were most favorable for the fish from the experimental variant fed with L30 (the diet with the highest substitution ofduckweed). The content of nitrogen and phosphorus compounds was the lowest in tanks where carp was fed with the diet containing the highest level of *L. minuta*. We observed a tendency of decreasing quantity of nitrogen and phosphorus compounds along with a raising percentage of duckweed content in the carp' diet and a decrease of other plant constitutes (wheat, corn meal and soybean meal) in experimental diets (L10 ad L30). There are no studies which dealt with the excretion of nitrogen and phosphorus in fish fed with the diets containing *L. minuta*. The received results are in confirmation with Lazzari and Baldisserotto (2008) which indicated that the inclusion of plant protein sources in fish diets could have different effects on fish growth and nitrogen wastes. This feed which has a poorer amino acids balance, reducing N retention, and consequently, increasing N excretion (Lazzari & Baldisserotto, 2008). The protein of duckweed has good balanced amino acid content (Gwaze & Mwale, 2015) and compared with most other plants, duckweed' leaves contain a small quantity of fiber and no indigestible material (Chaturvedi, Langote, & Asolekar, 2003). The proportionally decreased quantity of wheat, corn and soybean meal in the experimental diets containing 10% and 30% duckweed and a higher value of nitrogen excretion in carp from the control diet L0 compared with the other experimental group could be explained with better digestibility and a balanced amino acid profile of the meal made from *L. minuta*.

Jahan et al., 2003 stated that the reduction of phosphorus discharge from aquaculture is possible if the level of bioavailable phosphorus in the diets is higher. It could be concluded on basis of the received results in the current study that phosphorous bioavailability of duckweed in experimental diets was higher than phosphorous bioavailability for wheat and corn meal in the L0 diet. This can be confirmed in the study conducted by Schneider, Amirkolaie, Vera-Cartas, Eding, Schrama, & Verreth (2004) where the fish fed with a duckweed containing diet showed the lowest faecal losses of phosphorus (329 $g.kg^{-1}P$) compared with the content of P in five alternative diets - wheat gluten diet; soybean extract diet; soybean meal diet; single-cell protein diet and fishmeal diet where the contents of phosphorus in fish faeces were higher (350-401 $g.kg^{-1}P$).

Technological Parameters

Duckweed was successfully used as a diet for tilapia and carp (Hepher & Pruginin, 1979; Dyke & Sutton, 1977; Hassan & Edwards, 1992). The results from the current study showed that the survival rate of common carp fed with feed

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containing *L minuta* increased along with an increased percentage of duckweed substitution. The received results of the current study are controversial with those received from Yilmaz et al. (2004) for common carp's fry where the highest survival rate was found for the group fed with feed containing 5% duckweed and the value of this parameter decreased along with an increased percentage in duckweed substitution in the feed. The difference concerning this parameter between the current study and the one conducted by Yilmaz et al. (2004) is probably the result from highest requirements of fry to the protein quality in the received feed.

The final weight and specific growth rate was higher for carp fed with the L0 diet, but the difference were not statistically significant ($P \geq 0.05$) compared with the value of these parameters for carp fed with the L30 diet. This result is in line with the research conducted by Yilmaz et al. (2004) who stated that a diet containing up to 20% duckweed didn't significantly affect the growth of the experimental carp's fry. In the current research the plant ingredients (wheat, corn and soybean meal) from the L0 diet were replaced with duckweed which didn't significantly affect the assimilation of nutrients and gave the opportunity for the higher percentage of duckweed to substitute without significantly affecting the fish growth.

Anti-nutritonal factors are substances which either by themselves or through their metabolic products, have influence on the feed utilization and affect animal health or which pose an effect to reduce nutrient intake, digestion, absorption and utilization and may have other adverse effects (Akande, Doma, Agu, & Adamu, 2010). The better growth in carp fed with L30 compared with the growth found for carp fed with L10 could be result of the presence of a higher quantity of antinutrional factors in the diet containing a higher soybean level. Anti-nutritional factors of soybean are in most cases urease and trypsin (Nwanna, Ajani & Bamidele, 2006). Ismail (1998) stated that duckweed contains low content of anti-nutritional factors and Yilmaz et al. (2004) stated that the inclusion of duckweed doesn't need processing to destroy any antinutrients.

The received results for FCR for the experimental fish fed with a diet containing *L. minuta* were in line with other studies where the FCR values of carp cultivated under condition of polyculture and fed with substitution of duckweed, wheat and rice bran were found to vary between 1.5 and 3 (Devaraj, Krishna Rao, & Keshavappa, 1981).

The chosen experimental period was comparatively short, but some tendencies were established in current study. More experiments with long trial period were needed in the future to confirm the received results.

In conclusion the inclusion of up to 30% duckweed (*L. minuta*) in feed for common carp fingerlings decreases the quantity of ammonium, nitrite, nitrate and total phosphorus accordingly with 49.7%, 34.1%, 20.8 and 11.9% compared with the quantities of these parameters found for experimental groups fed with feed without inclusion of *L. minuta*. The carps fed with L30 showed better survival and similar growth of fish compared with fish fed with a diet without substitution of duckweed.

The duckweed (*L. minuta*) presents cheap and easy accessible ingredients for feeding common carp. Furthermore it could be used for wastewater treatment in recirculating aquaculture systems, this way increasing their sustainability.

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Table 1. The protein and moisture content in duckweed received after two months of operation in aquaponics recirculation system

Parameters	Value (%)
Crude protein	16.04
Moisture	5.51

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Table 2. Composition (g.kg^{-1} dry weight) of the diets for feeding trial (40 days) in common carp

Ingredients, %	Experimental diets		
	L0*	L10**	L30***
Wheat meal	21.190	7.190	-
Corn meal	15.000	15.000	-
Wheat bran	10.000	10.000	10.000
Sunflower meal	10.000	10.000	6.000
Soybean meal, 44% (crude protein)	27.000	26.000	19.770
Fish meal, 62%	10.000	10.000	12.000
Sunflower oil	-	5.000	15.000
Lemna minuta	-	10	30
Di-calcium phosphate	4.5	4.7	4.95
Chalk	1.4	1.2	0.7
Salt	0.5	0.5	0.5
L-lysine	-	-	0.170
DL- methionine	-	-	0.500
Vanilla	0.010	0.010	0.010
Vitamine premix	0.400	0.400	0.400

*L0-diet without substitution of duckweed (*L. minuta)* meal); **L10 (diet substituted with 10% duckweed (*L. minuta*) meal); *** L30 (diet substituted with 30% duckweed (*L. minuta*) meal);

Table 3. Nutritional value of composed diets for feeding trial (40 days) in common carp

Ingredients, %	Experimental diets		
	L0*	L10**	L30***
Digestible energy, kcal/kg	2625	2590	2550
Crude protein, %	26.07	26.11	25.40
Crude fiber	5.16	5.21	4.48
Calcium, %	2.30	2.30	2.30
Phosphorus, %	1.60	1.60	1.58
Linoleic acid , %	0.91	4.44	11.36
Lysine, %	1.80	1.84	1.71

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Methionine+cystine, %	1.42	1.40	1.24
Threonine, %	1.42	1.46	1.05
Tryptophan, %	0.39	0.37	0.23
Vitamin A, IU/kg	8000	8000	8000
Vitamin D_3, IU/kg	1500	1500	1500
Vitamin E, mg/kg	150	150	150
Vitamin B_1, mg/kg	5	5	5
Vitamin B_2, mg/kg	15	15	15
Vitamin B_6, mg/kg	8	8	8
Vitamin B_{12}, mcg/kg	20	20	20
Nicotinic acid, mg/kg	80	80	80
Pantothenic acid, mg/kg	40	40	40
Folic acid, mg/kg	5	5	5
Biotin, mg/kg	0.60	0.60	0.60
Iron, mg/kg	20	20	20
Manganese, mg/kg	25	25	25
Copper, mg/kg	2.5	2.5	2.5
Zinc, mg/kg	18	18	18
Iodine, mg/kg	1	1	1
Cobalt, mg/kg	0.48	0.48	0.48
Chlorine, %	0.58	0.58	0.58
Sodium, %	0.37	0.37	0.37

*L0-diet without substitution of duckweed (*L. minuta)* meal); **L10 (diet substituted with 10% duckweed (*L. minuta*) meal); *** L30 (diet substituted with 30% duckweed (*L. minuta*) meal);

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Table 4. Spectrophotometrically measured hydrochemical parameters during the feeding trial (40 days) in common carp

Hydrochemical parameters (mg.l^{-1})	Methods	Test №
Ammonium	Indophenol Blue	LCK304
Nitrite	Diazotisation	LCK341
Nitrate	2.6-Dimethylphenol	LCK339

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OrthoPhosphate	Phosphormolybdenum Blue	LCK349

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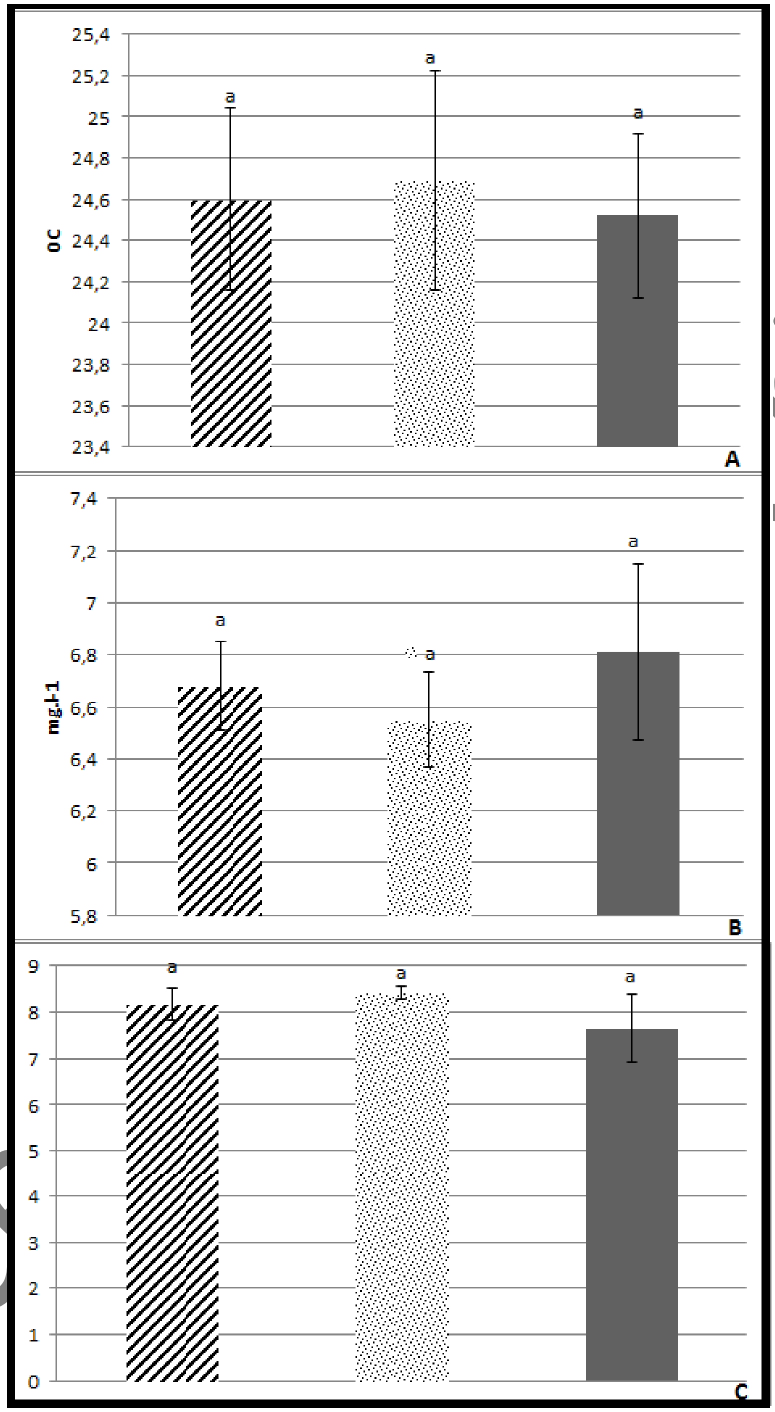

Figure 1. Average values of hydrochemical parameters during the feeding trial (40 days) with different experimental diets in common carp : A) temperature B) oxygen; C) pH; Each bar represents mean ± S.D. Bars with different letters are significantly different (P<0.05);Experimental diets: L0 (▨), L10(░), L30 (■);

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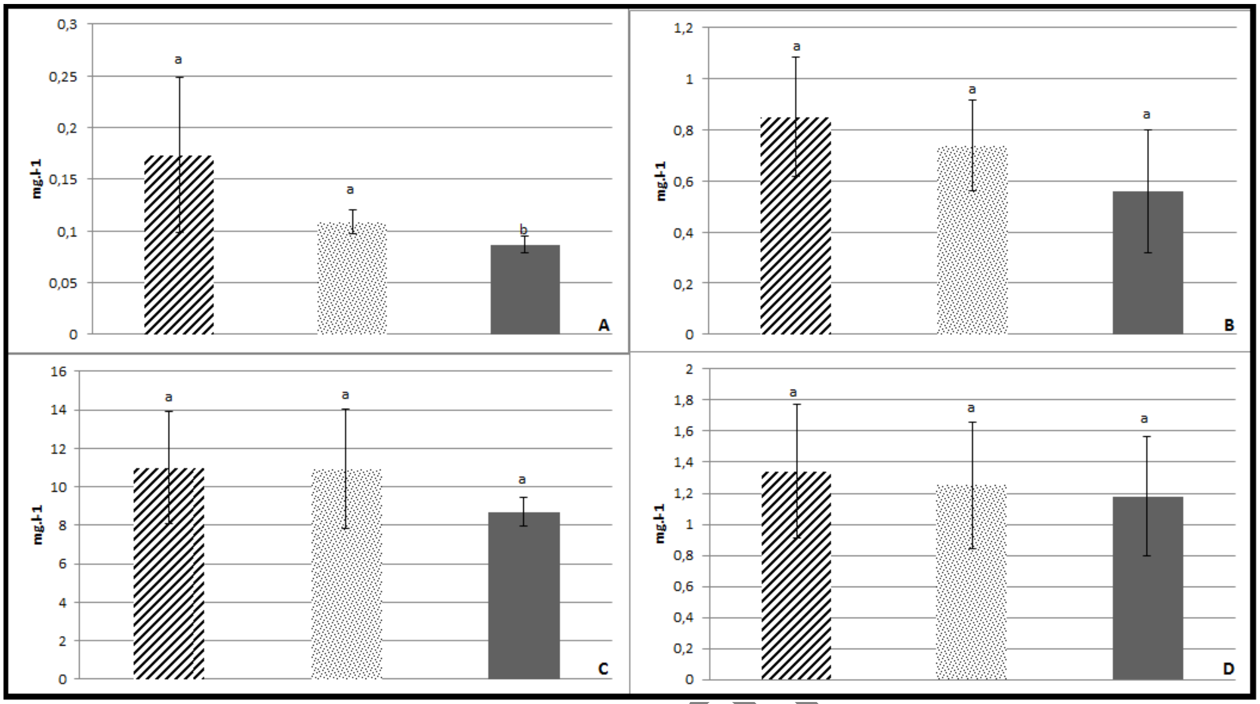

Figure 2. Average values of hydrochemical parameters during the feeding trial (40 days) with different experimental diets in common carp : A) ammonium; B) nitrite; C) nitrate; D) total phosphorus; Each bar represents mean ± S.D. Bars with different letters are significantly different (P<0.05);

Experimental diets: L0 ( ), L10 ( ), L30 ( )

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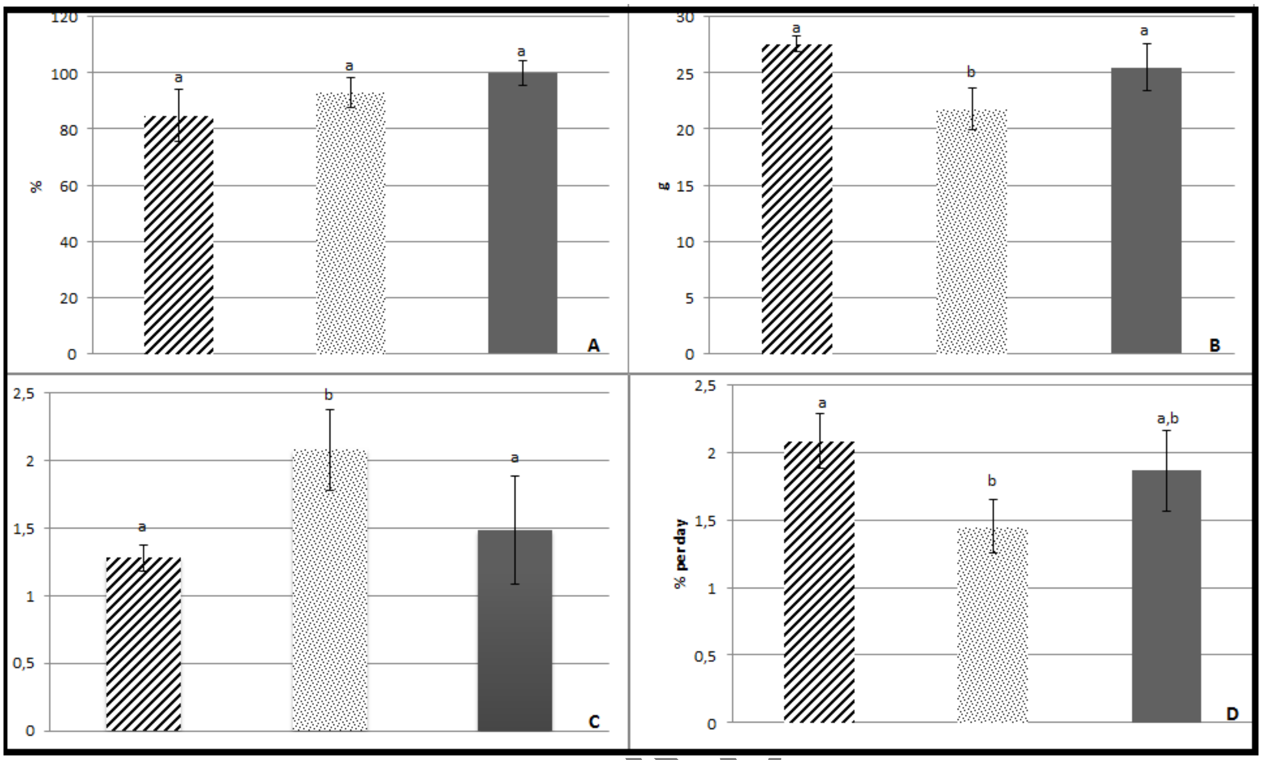

Figure 3. Average values of technical parameters during the feeding trial (40 days) with different experimental diets in common carp : A) survival carp (%); B) final weight (g); C) feed conversion ratio; D) specific growth rate (% per day); Each bar represents mean ± S.D. Bars with different letters are significantly different (P<0.05);

Experimental diets: L0 ( ), L10 ( ), L30 ( )

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