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Use of *Lemna minuta* Kunth. for composition of sustainable diets and influence on hydrochemical, technological and blood biochemical parameters in common carp (*Cyprinus carpio* L.) cultivated in aquaponics

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Abstract

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The purpose of this study was to investigate the influence of aquaponically grown *L. minuta* used as a part of biofilter in recirculation aquaculture systems, with its subsequent inclusion in the composition (50 and 100% content of daily feed ratio) in sustainable diets on hydrochemical, biochemical blood and technological parameters in common carp (*Cyprinus carpio* L.) fingerlings cultivated in aquaponics recirculation systems.At the end of the experiment were calculated average final weight, specific growth rates, feed conversion ratio, meat quality and blood biochemical parameters. The inclusion of up to 100% duckweed (*L. minuta*) in feed for common carp fingerlings decreases the quantity of ammonium, nitrite, nitrate and phosphate accordingly with 66%, 71.4%, 38.8% and 44.1%compared with the quantities of these parameters found for experimental groups fed with feed without inclusion of *L. minuta*. The carps fed with inclusion of up to 50% duckweed showed better growth of fish, SGR and FCR compared with fish fed with a diet without substitution of duckweed.

Keywords: aquaponic recirculation system; blood parameters; carp; growth; *Lemna minuta*

Introduction

The usage of expensive and deficit components for the composition of feed for hydrobionts imposes the search of alternative components. This problem in the last years is of primary importance for the aquaculture sector. The rapid development of aquaculture in the world leads to the bigger needs of fish meal and its price rising (Hardy & Tacon, 2002). This has a detrimental effect on the populations of open-water fish from which fishmeal is made. Increasingly sought appropriate and cheap raw materials to replace the fish meal, but with good nutritional content.Many researchers investigated the usage of different plants with high nutritional value like beans (Azaza et al., 2009), soy (Deng et al., 2006), sunflower (Rinchard et al., 2002), cereals (Stoyanova & Delchev, 2014; Stoyanova & Kuneva, 2018), vegetables (Mundheim et al., 2004).

The plants from genera Lemna probably could be the answer to the posed challenges from the aquaculture sector because they contain more than 50% protein (Sirakov & Velichkova, 2018). Duckweed is the smallest flowering plant in the world witch fast growing and easily adapt to various aquatic condition. Also, they used for water treatment and bioremediation (Velichkova & Sirakov, 2019). Duckweed was 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 in wastewater treatment (El-Kheir et al., 2007, Velichkova et al., 2018). These plants were used in the treatment of water from municipality waste water (Dalu & Ndamba, 2003), dairy livestock (Rashid & West, 2007), swine farm (Pena et al., 2017). Plants from genus *Lemna* are used as a biological filter in the recirculation system in aquaculture and could remove up to 99% of nutrient compounds in the water during the wastewater treatment process (Ferdoushi et al., 2008, Velichkova & Sirakov, 2013).

One good technique is a possibility for the application of plants from the genus *Lemna* in the wastewater treatment process and their subsequent use for animal feeding purposes in aquaponics systems. Aquaponics is a recirculation system, where the cultivation of fish and plants. Received plant production in this type of system consists of vegetables and spices for human consumption. There are no studies for other possible plant applications of this sustainable aquaculture technology. A large number of studies have been conducted with *L. minor* as a feed component for fish feeding (Yilmaz et al., 2004, Talukdar et al., 2012). The studies related to other species from *Lemna* as a feed substitution in fish feed were practically missing or were very rare (Bairagi et al., 2002). In Bulgaria one species from genera *Lemna* – *L. minuta* was recently found by Kirjakov & Velichkova (2016).The purpose of this study was to investigate the influence of aquaponically grown *L. minuta* used as a part of biofilter in recirculation aquaculture systems, with its subsequent inclusion in the composition (50 and 100% content of daily feed ratio) in sustainable diets on hydrochemical, biochemical blood and technological parameters in common carp (*Cyprinus carpio* L) fingerlings cultivated in aquaponics recirculation systems.

Material and Methods

Aquaponic recirculation system

The aquaponic recirculation system consisted of 10 cultivation tanks for fish and 4 plant tanks. Next to the fish tanks is situatedthe cleaning block of the system which consisted of one mechanical filter (settling tank) and moving bed biofilter. Two heaters and pump assuring recirculation of water were located in the next tank. The wastewaterfrom the fish tanks was directed to hydroponic system consisted of 4 tanks for plant cultivation. Three plant growth-promoting lamps FLUORA T8 (18 w, Osram Fluorescent Fluora Tubular Linear Lamp) were used. The lamps were turned on at 6.30 am and stopped at 6.00 pm. Plant tanks were with dimensions 1/0.50/0.25 m. The useful volume of tanks for fish cultivation was 0.3 m^3. The flow rate in the recirculation system was maintained at 50 $l.min^{-1}$. Every day the bottoms of fish tanks as well as the bottoms of filter's units were cleaned by opening the valve located at the bottoms of tanks. The water loses connected with cleaning process and evaporation were 10% of total volume of the recirculation system. This quantity of water was refilled daily with clean water.

Experimental fish and feeding

Common carp's in a good health and without visible injuries were chosen from a fish farm – Tundja-73 and transported to the aquaculture base at Agriculture faculty (Trakia University). Fifteen carps with average weight of the 41.83±9.88 g (control, L0), 41.26±12.61 g (experimental fish L50) and 41.46±11.03 g (experimental fish L100), without significant differences in weight ($P \geq 0.05$) between different individuals in good health condition were placed in each tank. During the acclimatization period the fish were fed with feed of appropriate size and content. The trial continued for 60 days. The carps were fed with 3 experimental diets – L0 (diet without substitution of duckweed (*L. minuta)* meal), L50 (diet substituted with 50% duckweed (*L. minuta*) meal) and L100 (diet substituted with 100% duckweed (*L. minuta*) meal). The trial was conducted in 2 replications. The fish were fed manually tree times per day with daily feed ratio of 3% from fish body weight.

The used duckweed for a trial was collected from Banya – Plovdiv region (42°32′226″N 24°50′213″E) and determined by Flora of North America (Landolt, 1993). *L. minuta* were reared for one month in the aquaponic system. This produced sufficient biomass and at the same time aided the purification of water. The collected biomass was dried. The analysis of protein content was 26.42%, moisture was 5.72% and made prior to the diet formulation AOAC (1995). Three isocaloric and isonitrogenous diets were formulated (Table 1).

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

Hydrochemical parameters

The oxygen content ($mg.l^{-1}$), pH, water temperature (°C) and electrical conductivity ($\mu S.cm^{-1}$) were measured daily with a portable meter (HQ30D), accordingly with LDO, pH (liquid) and conductivity electrodes. 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 appropriate cuvette tests (Hach Lange) weekly.

Table 1. Composition (g.kg^{-1} dry weight) and nutritional value of composed diets for feeding trial (60 days) in common carp

Ingredients, %	Diets		
	L0	L50	L100
Wheat meal	21.19	24.59	19.09
Corn meal	15.00	15.00	15.00
Wheat bran	10.00	–	–
Sunflower meal	10.00	10.00	10.00
Soybean meal	27.00	33.00	38.50
Fish meal	10.00	5.00	–
Lemnaminuta	–	5	10
Di-calcium phosphate	4.5	5	5
Chalk	1.4	1.5	1.5
Salt	0.5	0.5	0.5
Vanilla	0.01	001	0.01
Vitamine premix	0.40	0.40	0.40
Nutritional value of composed diets:			
Digestible energy, kcal/kg	2625	2544	2360
Crude protein, %	26.07	26.02	26.06
Crude fiber	5.16	5.10	5.74
Calcium, %	2.30	2.22	1.99
Phosphorus, %	1.62	1.50	1.36
absorb phosphorus, %	1.05	1.10	1.09
Linoleic acid , %	0.91	0.80	0.78
Lysine, %	1.80	1.74	1.68
Methionine + cystine, %	1.42	1.38	1.34
Threonine, %	1.42	1.41	1.40
Tryptophan, %	0.39	0.39	0.39
Methionine	0.74	0.69	0.64
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	10	10	10
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	0.01	0.01	0.01
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, %	0.29	0.29	0.29
Sodium, %	0. 71	0.43	0.43

Fish growth performance

The average individual weight (g) of the fish was calculated at the start, middle and end of experiment. At the end of the trial the weight gain (g), survival rate (%) and the feed conversion ratio in the fish were determined.

The biometrical calculations were carried out according tothe following formulas:

Specific growth rate (*SGR*) (Zhou et al., 2006):

$$SGR = \frac{(LnWf - LnWi)}{n} \times 100,$$

where *SGR* – specific growth rate, %; *Wi* – initial weight, g; *Wf* – final weight, g; *n* – number of days

Feed conversion ratio (FCR):

$$FCR = \frac{Feed\ given}{Fish\ weight\ gain},$$

where *FCR* – feed conversion ratio; feed given, g; fish weight gain, g.

Chemical analyses of meat samples

The musculature samples of common carp were determined on atomic absorption spectrometer (AAS) "A Analyst 800" – PerkinElmer. Crude protein content (%) was calculated by converting the nitrogen content, quantified by Kjeldahl's method, using an automatic Kjeldahl system (Kjeltec 8400, FOSS, Sweden). Lipid content (%) was determined by the method of Soxhlet, using an automatic system (Soxtec 2050, FOSS, Sweden). Ash content (%) was investigated by incineration in a muffle furnace (MLW,Germany) at 550°C for 8 h. Crucibles were brought about the room temperature and weighed (mg).

Biochemical blood analyses

Blood was taken from the examined fish directly from the heart with disposable sterile plastic syringes (3 ml) with a needle. As an anticoagulant Heparine sodium (1%) was used. The blood samples were instantly transmitted and analyzed in a hematological laboratory (NCPTC – Trakia University) and reported in Mindray BC – 120 hematology analyzer. Follow biochemical blood parameters were investigated: glucose (mmol.l^{-1}), urea (mmol.l^{-1}), creatinine (µmol.l^{-1}), total protein (g.l^{-1}), albumin (g.l^{-1}), ASAT (U.l^{-1}), ALAT (U.l^{-1}), Ca (mmol.l^{-1}), P (mmol.l^{-1}), Mg (mmol.l^{-1}), *triglycerides* (mmol.l^{-1}) and *cholesterol* (mmol.l^{-1}).

Statistical analysis

The data were analyzed statistically with ANOVA single factor STATISTICA 6.0 software (StatSoft Inc., 2002).

Results and Discussion

Hydrochemical parameters

An important indicator for the optimal development of the cultivated species is water temperature. In our trial it was optimal for the raised fish and varied between 21.3 and 22.1°C (Table 2). The average value of pH was closer to neutral in tanks where carps were fed with the L100 and L50 diets and its value was higher than 8 in tanks where the L0 diet were given to fish (Table 2), and the differences were statistically proven ($P < 0.001$). The content of oxygen in tanks where carps were fed with the diet containing thehighest substitution of duckweed (100%) was higher accordingly with 7.5% and 2.2% compared to the values in the tanks where fish were fed with L0 and L50 diets, and the differences were significant ($P < 0.001$). The values of this parameter during the trial period were higher with 5.4% in the experimental tanks where fish were fed with L50 comparison to these of L0 ($P < 0.001$). Electric conductivity of water varied from 296.7 $\mu S.cm^{-1}$ to 307.4 $\mu S.cm^{-1}$ (Table 2). The conductivity values of experimental variant L100 and L50 were with 3.5% and 2.8% higher compared to these one respectively of L0 diets ($P < 0.001$). The analysis of hydrochemical data showed that duringthe experiment, they were optimum for the farmed species. Tanks were cleaned three times per day, with addition of fresh water in amount of 10% from the total recirculation system volume. To maintain the optimum water chemical parameters during the experiment, the mechanical filter and the biofilter in particular was of major significance. This led to good results with respect to survival, weight gainand feed conversion ratio in experimental fish.

The quantity of nitrogen compounds and phosphate was lower in the water of tanks where carps were fed with the diet substituted with 100% duckweed, compared with the values of these parameters measured in the water of tanks where fish were fed with diets L0 and L50 (Table 2). The amounts of ammonium, nitrite, nitrate and phosphate in the water of tanks where carps were fed with the L100 diet were lower accordingly with 66%, 71.4%, 38.8% and 44.1% compared to their values in the tank's water where fish were fed with the control diets L0, and the differences showed statistical significance ($P < 0.05$, $P < 0.001$) (Table 2). The amounts of ammonium, nitrite, nitrate and phosphate in the water of tanks where carps were fed with the L50 diet were lower accordingly with 45.1%, 38.1%, 24.1% and 18.6% compared to the values in the tank's water where fish were fed with control diets L0, but the differences were statistically proven only for ammonium and nitrate ($P < 0.001$) (Table 2).

Growth performance and feed utilization efficiency

Survival rate during the experiment showed 100% in fish in the two experimental and control group (Table 3).The highest final weight (92.5 g±11.2) was found in carp fed with the L50 diet and it was accordingly with 5.7% ($P < 0.05$) and 3.24% higher than the weight calculated for carps fed with L0 (87.2 g±9.2) and L100 (89.5 g±9.7) diets.The highest specific growth rate (1.35%/day±0.1) was found in carp fed with the L50 diet and it was higher accordingly with 9.6% ($P < 0.05$) and 5.2%, compared to these calculated for carps fed with the L0 (1.22%/day±0.01) and L100 (1.28%/day±0.05) diets. The best FCR (1.28±0.07) was found in carp fed with the L50 diet, followed by the FCR found in carp fed with the L100 diet (1.37±0.09) and a higher FCR was found in fish fed with the control diet L0 (1.45±0.01).

Biochemical blood parameters

The blood glucose level depends by season and water temperature (Coz-Rakovac et al. 2005). In this study, the glucose level was 28.9% and 14.6% lower in carps from L50 group compare to L0 and L100, but the differences was not statistically proven ($P > 0.05$) (Table 4). The urea level was 42.5% and 27.9% lower for L50 diet compare to these ones from the L0 and L100 variant, but with not statistically significant differences ($P > 0.05$). The creatinine level was

Table 2. Average values of hydrochemical parameters during the feeding trial (60 days) with different experimental diets in common carp

Parameters	n	L0	L50	L100
T°C	60	22.16±0.17	21.64±0.14	21.31±0.33
pH	60	8.10±0.18***	7.96±0.14***	7.89±0.16***
O_2 mg.l^{-1}	60	7.69±0.19***	8.13±0.08***	8.31±0.11***
conductivity $\mu S.cm^{-1}$	60	296.7±4.70***	304.9±1.98***	307.4±2.39***
NH_4+ mg.l^{-1}	8	0.206±0.04***	0.113±0.03***	0.07±0.01***
NO_2- mg.l^{-1}	8	0.21±0.12*	0.13±0.11	0.06±0.01*
NO_3- mg.l^{-1}	8	5.51±0.35***	4.18±0.91***	3.37±0.95***
PO_4- mg.l^{-1}	8	0.59±0.16*	0.48±0.17	0.33±0.07*

$P < 0.05$*, $P < 0.001$***

Table 3. Growth performance of common carpduring the feeding trial (60 days) with different experimental diets

Parameters	n	L0	L50	L100
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Initial body weight, g	15	41.83±9.88	41.26±12.61	41.46±11.03
Final body weight, g	15	87.2±9.2*	92.5±11.2*	89.5±9.7
Survival rate, %		100	100	100
SGR % per day		1.22±0.01*	1.35±0.1*	1.28±0.05
Average individual weight gain, g	15	45.37±0.4*	51.3±2.8*	48±3.2
FCR		1.45	1.28	1.37

P < 0.05*

Table 4. Biochemical blood parameters of common carpduring the feeding trial (60 days) with different experimental diets

Blood parameters	n	L0 $\bar{x}$ ± SD	L50 $\bar{x}$ ± SD	L100 $\bar{x}$ ± SD
Glu mmol.l^{-1}	6	6.56±4.22	4.66±1.08	5.46±1.33
Urea mmol.l^{-1}	6	9.95±7.94	5.72±2.88	7.94±2.19
Crea µmol.l^{-1}	6	71.50±7.7	28.60±1.9	54.60±8.9
TP g.l^{-1}	6	151.68±24.19**	136.56±45.97	187.80±7.09**
Alb g.l^{-1}	6	61.4±9.22	51.62±9.00	61.14±1.36
ASAT U.l^{-1}	6	20.50±17.40	75.0±33.90	47.60±22.80
ALAT U.l^{-1}	6	33.66±13.30	8.00±5.14	38.10±28.60
GGT	6	28.33±13.7	19±9.18	13.33±7.6
ALP U.l^{-1}	6	16.50±10.40	5.60±7.70	8.00±4.30
Ca mmol.l^{-1}	6	6.98±4.4	6.90±6.03	5.70±0.83
P mmol.l^{-1}	6	6.6±3.79	7.06±4.12	9.33±1.00
Mg mmol.l^{-1}	6	2.9±0.67	2.86±1.10	3.19±0.44
TG mmol.l^{-1}	6	11.79±4.25	7.59±3.55	10.78±1.44
CHOL mmol.l^{-1}	6	8.95±2.16	6.71±2.57	8.90±0.87

P < 0.01**

60% and 47.6% lower for L50 diet compare to L0 and L100 group (P > 0.05). The level of the total protein was higher by 27.2% and 19.2% (P < 0.01) for L100 diet compared to L50 and L0. The amount of albumin in the blood is lower of experimental fish of L50 (51.62±9.00). ALAT values are lower with 76.23% and 79% respectively in blood of carps from L50 compare to L0 and L100. The triglyceride concentrations in serum of carps fed with L50 were lower with 35.6% and 29.5% compared than in L0 and L100 (Table 4). The measured cholesterol are higher with 25% in L0 variant compare to L50 group, but with not statistically significant differences (P > 0.05). The levels of ALP were lower with 66% and 30% in blood fish L50 compare to L0 and L100. The blood phosphorus and magnesium of carps in L100 diet is higher than the L0 and L50 variant, but it is not statistically proven (P > 0.05).

Chemical analyses of meat samples

The analysis of chemical composition of the meat of the common carp, cultivated in aquaponic recirculation system showed that the lower dry matter and ash were found in the fillets of L50 diet and the differences was statistically significant (p < 0.05) for ash (Table 5). The crude protein concentrations in fillets of carps fed with L0 were higher with 0.5% and 1% compared to L50 and L100 but only with statistically significant differences (p < 0.05) for L100. In this study was received with 0.9% and 7.7% lower fat in the fillets from experimental fish fed with L50 compared to L0 and L100 variant but the differences was not statistically significant (p > 0.05).

Table 5. Chemical composition of the fillets of the common carpduring the feeding trial (60 days) with different experimental diets (%)

Indicator	n	L0	L50	L100
Moisture	6	77.49±0.05	77.65±0.27	77.51±0.09
Dry matter	6	22.5±0.05	22.35±0.28	22.49±0.1
Crude protein	6	19±0.10*	18.9±0.3	18.8±0.13*
Fat	6	2.15±0.06	2.13±0.08	2.31±0.14
Ash	6	1.35±0.01*	1.34±0.01	1.4±0.02*

P < 0.05*

Conclusion

The inclusion of up to 100% *L. minuta* in feed for common carp fingerlings decreases the quantity of ammonium, nitrite, nitrate and phosphate accordingly with 66%, 71.4%, 38.8% and 44.1% compared with the quantities of these parameters found for experimental groups fed with feed without inclusion of *L. minuta*. The carps fed with L50 showed better growth of fish, SGR and FCR compared with fish fed with a diet without substitution of duckweed. Most of the blood parameters are better in fish from L50 diet compared to L0 and L100. With 0.9% and 7.7% lower fat in the fillets from experimental fish fed with L50 compare to L0 and L100 variant were established.

These results show that the *L. minuta* can be used as a substitute for fishmeal on the one hand and on the other for wastewater treatment in an aquaponic recirculation system.

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