Effects of replacement of fishmeal with other alternative protein sources in the feed on hydrochemical and technological parameters in African catfish (*Clarias gariepinus*)

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Abstract. The African catfish (*Clarias gariepinus*) is a perspective fish species for Bulgaria, because it has a rapid growth rate, it can be grown at high stocking densities, it is not pretentious about the quality of water and it has relatively high market price. The sustainable aquaculture practices aimed to replace the fish meal in fish feed with the alternative protein sources. The results from studies connected with the replacement of fish meal in the feeding of African catfish with soya meal, worm meal, etc. were very contradictory. The aim of the present study was to investigate the effect of feed where fishmeal has been replaced by algal meal (Alltech Neogreen®) on the hydrochemical and technological parameters in African catfish cultivated in a recirculation aquaculture system. The trial was conducted in closed recirculation system. The weight of fish from both experimental variants - C (fish were fed feed containing fish meal) and D variant (catfish were fed with feed containing algae meal instead of fish meal) were measured at the start and at the end of trial. Feed conversion ratio was also calculated. The hydrochemical parameters (oxygen, pH, conductivity, ammonium, nitrite, nitrate and phosphate) were measured during the 60 days experimental trial. The use of feed in which fishmeal was replaced by microalgae (Alltech Neogreen®) did not affect the hydrochemical parameters and survival of experimental fish, but improved their growth and feed conversion ratio.
Key Words: African catfish, alternative protein source, hydrochemical parameters, growth.

Introduction. The African catfish (*Clarias gariepinus*) is a thermophilic species which does not tolerate temperatures below 12°C (Zaykov & Staykov 2013). In the changing climate, the trend for Bulgaria is an increase in the average annual temperatures, which will allow the cultivation of more thermophilic species (Slavcheva et al 2005a; Slavcheva et al 2005b; Sirakov & Slavcheva-Sirakova 2015; Slavcheva-Sirakova 2015).

Currently, the African catfish is acclimatized in China, Vietnam, Thailand, Brazil, Indonesia, the Netherlands, Hungary and others. It is grown in countries with temperate climates in closed recirculation systems. The advantage of this species is that it is unpretentious to the concentration of oxygen in the water because it has a special apparatus for breathing of atmospheric oxygen (Alves et al 1999; Eding & Kamstra 2001; Wachirachaikarn et al 2009).

The African catfish has a rapid growth rate and can be grown at high stocking densities (Haylor 1991; Hengsawat et al 1997; Hossain et al 1998; van de Nieuwegiessen et al 2009) and is not pretentious about the quality of water (Schram et al 2010; Roques et al 2015). This makes this species a promising candidate for cultivation in closed recirculation systems. Recirculation systems are environmentally friendly technology for growing hydrobionts and have recently been the subject of strong development in Bulgaria, especially because of their ability to combine the fish cultivation with the cultivation of plant species (Sirakov et al 2017; Sirakov et al 2018; Sirakov & Velichkova 2018; Sirakov et al 2019; Velichkova & Sirakov 2019; Velichkova et al 2020a, b).

The average price of the African catfish on the Bulgarian market varies from 4 to 6 EUR kg^{-1} live weight and from 7.5 to 10 EUR kg^{-1} for smoked fish meat. The average cost of growing of this catfish in a recirculation system is about 1.50 EUR kg^{-1}, which makes it economically viable for cultivation in the above mentioned systems (Akvafermer.bghttp://bulaquatechnics.com).

In the recent years, with the increasing of interest towards the production of fish species in intensive farms, the main requirement for hydrobionts is intensive growth with minimal impact on the environment. The basis for the cultivation of predatory fish species is fishmeal in extruded feed (Hardy 1999). The production of fishmeal in recent years is about 5 million tons according to FAO (2020). For the production of 1 ton of this product it is necessary to process and use approximately 5-6 tons of raw materials mainly fish and their products. Fishmeal in the diet of different aquaculture species reduces the sustainability of aquaculture practices. Therefore its replacement with other products (plant or of other origin) in the diet of aquatic organisms has been the subject of research in this sector in recent years (Marinho et al 2013; Stadtlander et al 2013; Basri et al 2015).

Fishmeal is a major protein component but is often scarce and expensive in many countries and its use in African catfish feed is 40 to 60% (van Weerd 1995). Many efforts have been made by experts in recent years to replace fishmeal in the diet of African catfish (Crețu et al 2016). For fast-growing and sustainable aquaculture, the desired results for replacing fishmeal have not yet been achieved. Algae is an excellent dietary source of protein, vitamins and carbohydrates (Kumar et al 2008). Protein is a key ingredient in fish food as it provides amino acids for the synthesis of body protein and provides some energy for the body.

Tacon (1987) recommends a crude protein level of 35 to 42% for omnivorous fish species. Soy flour and the addition of methionine allow 50% substitution of fish meal (Viola et al 1982). A 12-week experiment for feeding African catfish was conducted and in its ration 75% of the fish meal was replaced with the flour from poultry production and other domestic animals; the obtained results were satisfactory with the shown growth of fish in the experimental group (Goda et al 2007). Replacing fishmeal in the diet of African catfish up to 25% with worm meal can take place without affecting its growth parameters (Dedeke et al 2013). The results from experiments aimed to replace the fishmeal in feeding of African catfish with the alternative protein sources are very contradictory.

The aim of the present study was to investigate the effect of feed where fishmeal has been replaced by algal meal (Alltech Neogreen®) on the hydrochemical and technological parameters in African catfish cultivated in a recirculation aquaculture system.

Material and Method

Recirculation system. The experiment with African catfish (*Clarias gariepinus*) was conducted in 2019 between October and December (for 60 days) at the Experimental Aquaculture Base at the Department of Biology and Aquaculture, Agriculture Faculty, Trakia University (Stara Zagora Bulgaria). For the purposes of the experiment, a closed recirculation system was used (Figure 1).

Tank Control group C	Tank Experimental group D	Tank	Tank	Tank	Tank	Tank	Tank
Tank Experimental group D1	Tank Control group C1	Tank	Tank	Mechanical filter	Mechanical filter	Biofilter	Pump section

Figure 1. Recirculation aquaculture system used in current trial.

The number of tanks in the used recirculation system was 12 and 4 of them were used for the current experiment. The useful volume of fish tanks was 0.8 m^3. The flow rate in RAS was maintained at 25 L min^{-1}. The bottoms of fish tanks as well as the bottoms of filters were cleaned by syphoning every day. The cleaning section of RAS consisted from 2 settling tanks (mechanical filters) and 1 moving bed biofilter. To compensate the water's loss from the systems, as a result of evaporation, 10-15% of fresh water was added daily from the total volume of the recirculation system. The water in the system was heated throughout the experimental period by a heating system to ensure suitable temperature conditions. During the experiments, additional aeration of the water was provided by an aerator system.

Feeding of the experimental fish. The fish were fed 3 times per day, manually at 8:00, 12:00 and 16:00. In the first experimental variant, the fish were fed extruded pellets containing fishmeal as a protein source (C) (Table 1). In those of the second group, the used feed in which the fish meal was replaced with an alternative protein source - microalgae (D) (Table 2) (Alltech Neogreen®). The daily ration for experimental and control fish was 2% of their biomass.

Table 1

Nutrition value of control feed (Alltech Ultra®)

Nutritional content	*Values*
Protein (%)	44
Fat (%)	25
Ash (%)	5.2
Crude fibre (%)	1.6
Total P (%)	0.84
Energy	
Gross (MJ kg^{-1})	23.4
Digestible (MJ kg^{-1})	21.3
Vitamins added	
Vitamin A (IE kg^{-1})	10.000
Vitamin D (IE kg^{-1})	2,950
Vitamin E (mg kg^{-1})	200
Vitamin C (stable) (mg kg^{-1})	250

Table 2

Nutrition value of experimental feed (Alltech Neogreen®)

Nutritional content	*Values*
Protein (%)	43
Fat (%)	28
Ash (%)	7.4
Crude fibre (%)	1.7
Total P (%)	1.20
Energy	
Gross (MJ kg^{-1})	23.2
Digestible (MJ kg^{-1})	21.2
Vitamins added	
Vitamin A (IE kg^{-1})	10.000
Vitamin D (IE kg^{-1})	1,330
Vitamin E (mg kg^{-1})	200
Vitamin C (stable) (mg kg^{-1})	250

Hydrochemical parameters. Methods adapted for fish farming were used for retracing the hydrochemical parameters in the recirculation system during the trial:
- dissolved oxygen, mg L-1 (HQ40D Hach Lange with oxygen probe);
- pH (HQ40D Hach Lange with pH probe);
- water temperature, oC (HQ40D Hach Lange);
- electrical conductivity, µS cm-1 (HQ40D Hach Lange with conductivity probe).

The content of ammonium ions, nitrates, nitrites and phosphates in the water of the experimental and control tanks was determined by the following methods:
- ammonium ions, mg L-1 (BDS-ISO 3587);
- nitrates, mg L^{-1} (BDS-ISO 3758);
- nitrites, mg L^{-1} (BDS-ISO 3762);
- phosphates, mg L^{-1} (BDS-ISO 6878).

Experimental fish. After transportation from fish farm Aquafish Pazardzhik OOD, the fish were adapted for 7-day acclimatization period in tanks of experimental recirculation system. At the start of experiment all fish were weighed individually on the technical balance (Elicom S300PM), with an accuracy of 0.1 g. The initial weight of fish from two experimental groups were as follows: control group (C) - 12.45±0.46 g; experimental variant (D) - 13.6±0.43 g.

The total number of catfish for each of the experimental groups was 100 individuals and stocking density was 25 ind m^{-3}. Mortality was monitored during the experimental period and the survival (%) of each of the experimental variant was finally determined. At the end of the experiment, all fish were individually weighed and the final weight was determined. The feed conversion ratio (FCR) was also calculated according to the following formula:

$$FCR = \frac{\text{Total feed (g)}}{\text{Weight gain (g)}}$$

Statistical analysis. All data were statistically analyzed by Anova single factor (Microsoft Office 2010). All differences between replicate groups were considered significant at $p < 0.05$.

Results

Hydrochemical parameters. According to Suleiman & Solomon (2017), the optimal temperature range for African catfish is 24-28°C. During the experiment, the water temperature was monitored, and it was optimal for the cultivation of African catfish, as the average water temperature in the tanks of variant C was 25.73±0.11°C and in those of variant D - 25.72±0.11°C, a statistically significant difference in the values of this indicator of the two experimental variants was not proved ($p > 0.05$) (Figure 2).

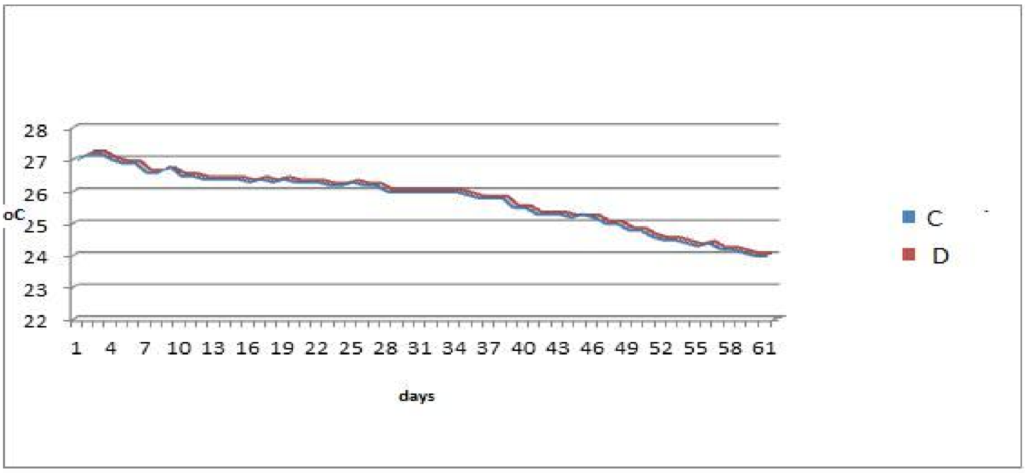

Figure 2. Temperature in experimental and control tanks during the trial.

The dynamics of the oxygen concentrationin in the tanks of variants C and D is shown in Figure 3. The average content of dissolved oxygen in the experimental tanks from variant C was 6.13±0.049 mg L^{-1}, and in those of experimental variant D - 6.13±0.50 L^{-1} with no significant statistical differences ($p > 0.05$) (Figure 3).

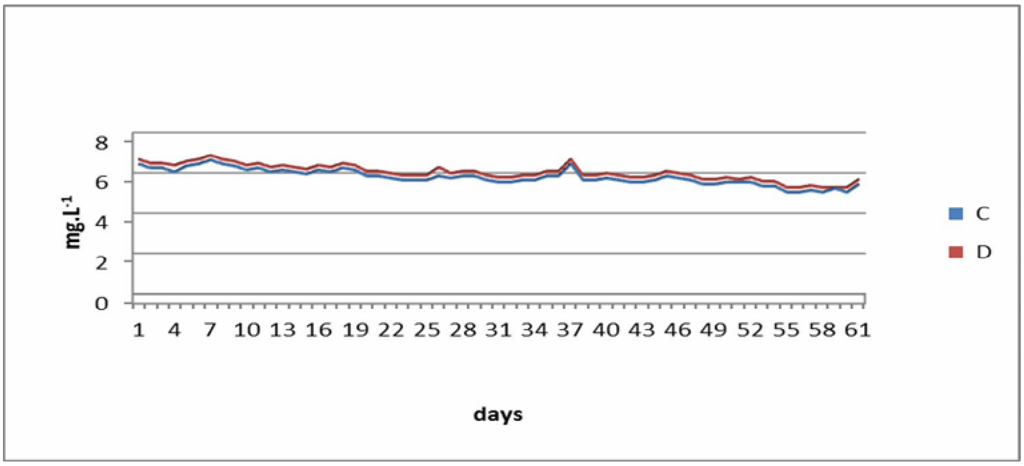

Figure 3. Dissolved oxygen in experimental and control tanks during the trial.

During the experiment, the pH values of the water were optimal for the cultivated species. The mean value of this indicator from the experimental replicates was 7.14±0.01 in the tanks of variant C and 7.13±0.01 for D, with no statistically significant difference ($p > 0.05$) (Figure 4). According to Zaykov & Staykov (2013) African catfish is not oversensitive to the water pH and could survive even in acidic water.

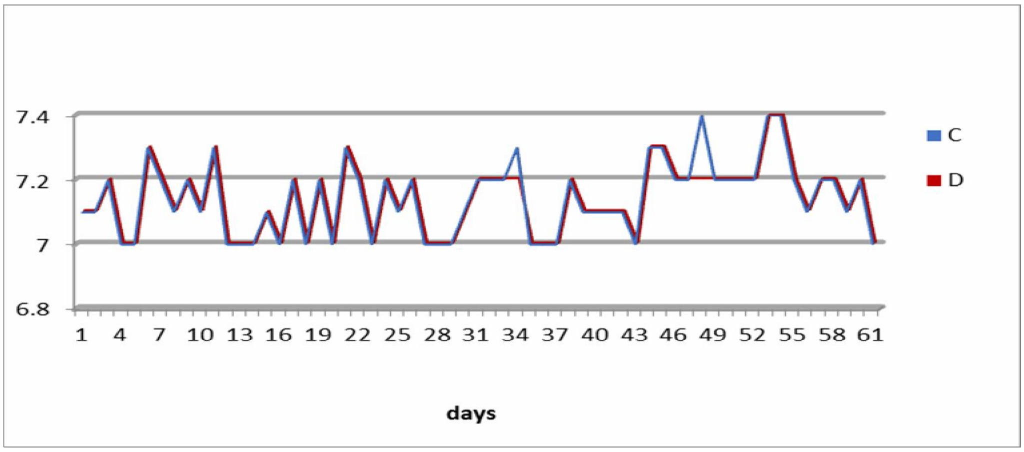

Figure 4. pH in experimental and control tanks during the trial.

The electrical conductivity was also monitored during the experiment and the average value of electrical conductivity for tanks C was 284.5±1.01 μS cm^{-1} and for tanks D was 284.6±1.01 μS cm^{-1}, but there were no statistically significant differences ($p > 0.05$) (Figure 5).

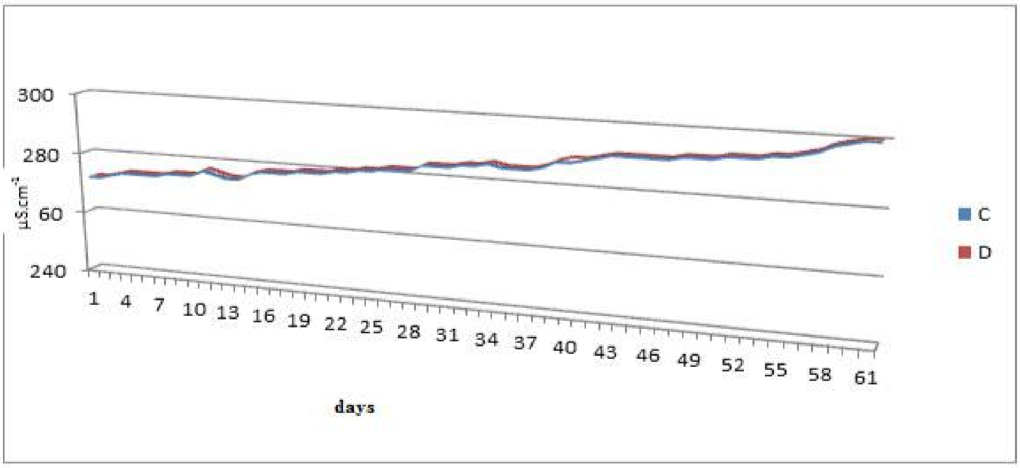

Figure 5. Conductivity in experimental and control tanks during the trial.

The concentration of ammonium ions during the experiment is given in Table 3. The difference between the average values of ammonium ions in experimental variants C and D was 46.5%, but the differences were not statistically significant ($p > 0.05$). The difference in the mean values of nitrates measured in the tanks of variants C and D was 63.14%, but the difference was not statistically proven ($p > 0.05$) (Table 3). The difference in the mean values of the nitrates measured in the tanks of the two variants C and D was 39. 31% but the difference was not statistically proven ($p > 0.05$). The difference between the mean phosphate values in the tanks of variant C and D was 28.86%, but the difference was not statistically proven ($p > 0.05$).

Table 3

Concentration of some N and P-compounds during the trial

Experimental variant	*Ammonium ion (mg L^{-1})*	*Nitrate (mg L^{-1})*	*Nitrite (mg L^{-1})*	*Phosphate (mg L^{-1})*
C	0.062±0.51	0.073±0.65	19.24±5.6	1.154±0.3
D	0.116±0.12	0.198±0.7	31.70±7.1	1.622±0.6

Technological parameters. The survival of the African catfish during the experiment is given in Table 4. It was 100% in both variants - control and in the experimental. The mean initial weight of the fish from variant C was 12.45±0.46 g and in the group fed with an alternative source of protein - algae 13.6±0.43 g. The minimal difference in this indicator in the fish from the two experimental variants was not statistically significant ($p > 0.05$) (Table 4). The final weight of the fish from group C was 158±7.26 g and in the D variant it was 189.7±8.94 g. The difference in final weight between the two experimental variants was 17.7% and it was statistically significant (Table 4) ($p \leq 0.05$). The FCR was lower in fish from D variant with 16.2% compared with this calculated for fish from C variant and the difference was statistically significant ($p \leq 0.05$).

Table 4

Technological parameters in African catfish (*Clarias gariepinus*)

Experimental variant	*Survival (%)*	*Initial weight (g)*	*Final weight (g)*	*FCR*
C	100	12.45±0.46	158±7.26	1.23±0.01
D	100	13.6±0.43	189.7±8.94	1.03±0.01

Discussion. At the time of the experiment, the hydrochemical parameters were within the acceptable technological parameters for the cultivation of African catfish. The temperature during the present experiment was in the range of 24 to 27°C, which temperatures correspond to the optimal vales for the cultivation of African catfish in a recirculation system. The water temperature in the present study were similar to the water temperature in the recirculation systems used for African catfish cultivation in experiments conducted from Akinwole & Faturoti (2007).

The oxygen concentration in the two experimental variants varied from 6.10 to 6.13 mg L^{-1}. According to Zaykov & Staykov (2013), the oxygen in the cultivation of this species should not fall below 3 mg L^{-1}.

The usage of an algae meal supplement in the feed did not affect the pH. According to Marimuthu et al (2019), the optimal pH of the cultivated species should be around 7.00, but it can also survive in slightly alkaline or slightly acidic environments. According to Zaykov & Staykov (2013), the African catfish is tolerant to the active reaction of water and can tolerate low and high values of this indicator.

The electrical conductivity in both variants was 289.5-289.6 µS cm^{-1}. During the trial, the experimental and control tanks were cleaned by siphoning their bottoms in order to prevent the accumulation of organic matter. There is a tendency to increase the values of this indicator due to the accumulation of certain metabolites in the water of the recirculation system over time. Electrical conductivity shows the ability of a solution, in this case water, to conduct an electric charge. For this reason, conductivity can be used as an indicator of the concentration of dissolved ions in water and thus indirectly showed its contamination.

The large accumulation of organic matter in recirculation system could be the reason for low values of the pH and high electrical conductivity which can negatively affect the health status of fish, something that is confirmed by the study of Martins et al (2009). In our study, the differences between the values of the indicators dissolved oxygen, pH and electrical conductivity in the water of the experimental tanks of C and D variants were minimal and without statistical significance. The results obtained for these indicators are in accordance with those obtained by Sirakov et al (2012) in which the use of meal from *Spirulina* as an additive did not show a significant effect on the hydrochemical parameters. Studies determining the effect of feed using algae meal as a protein source in fish on hydrochemical parameters are rare in the literature.

The content of ammonium ions in the water of the tanks in which the African catfish was fed Alltech Neogreen® feed did not differ significantly from that in the water of the tanks in which the fish were fed with the control feed. A biofilter was used to regulate ammonium ions in the recirculation system. According to Wicaksana et al (2015), the usage of a biofilter to produce African catfish is important to keep ammonium ion levels within acceptable limits.

The nitrate concentration was acceptable for the production of cultivated species and varied between 19.24 and 31.70 mg L^{-1}. According to Monsees et al (2017) the high concentration of nitrates above 500 mg L^{-1} can affect the growth of fish. The values of nitrites were within the allowable limits for the production of the species 0.073-0.198 mg L^{-1}.

The concentration of orthophosphates in both variants was in the range of 1.154-1.622 mg L^{-1}. According to Strauch et al (2019) acceptable values of orthophosphates for the cultivated species are up to 80 mg L^{-1}.

The replacement of fishmeal with microalgae did not have a significant impact on African catfish survival and it was 100% in both the experimental and control variants, which corresponds to the results obtained in the study of Osibona & Ayoola (2018) on the same species.

At the beginning of the experiment, no statistically significant differences were found for the initial weight of the control fish and those of the experimental variant. At the end of the scientific study, the fish from the experimental variant had a higher live weight (by 16.71%) than that of the fish from the group fed with feed containing fishmeal, and the difference was statistically proven ($p \leq 0.05$). Our results are in line with the results obtained by Sirakov et al (2012), which also reported an increased final weight in rainbow trout (*Oncorhynchus mykiss*) fed with the addition of *Spirulina* meal. Our results are confirmed by the experiment of Abdel-Warith et al (2016) which reported higher growth rates in African catfish when adding algae meal as a source of protein. Different studies (Nyina-Wamwiza et al 2010; Abdel-Warith et al 2020) showed that African catfish is able to digest and assimilate the diets containing significant quantity of plant compounds. This was confirmed in current study and better FCR was received for fish from D variant where catfish were fed with the feed with alternative protein source (Alltech Neogreen®).

Conclusions. The use of feed in which fishmeal was replaced by microalgae (Alltech Neogreen®) did not affect the hydrochemical parameters and survival of experimental fish, but improved their growth and feed conversion ratio. According to the results we obtained in this study the final weight in the catfish fed with Alltech Neogreen® was with 17.7% higher than that found for the fish from the control group. The usage of algae in fish feeding increases the sustainability in aquaculture, allowing for a decrease in fish meal quantity used for fish feeding purposes.

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