Effects of diets containing dried distiller's grain with solubles (DDGS) on the water quality of the carp rearing ponds

[1]Flórián Tóth, [1,2]Norbert Révész, [1]Ferenc Demény, [3]Attila Uhljar, [1]László Berzi-Nagy, [1]Zsuzsanna Molnár, [1]Janka Bíró, [1]Dénes Gál, [1]Éva Kerepeczki, [1]Zsuzsanna J. Sándor

[1] Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety, Research Centre for Aquaculture and Fisheries, Hungary; [2] DSM Nutritional Products, Hungary; [3] Hungarian University of Agriculture and Life Sciences, Hungary. Corresponding author: F. Tóth, toth.florian@uni-mate.hu

Abstract. With the intensification of aquaculture, the water quality of the receiving water bodies is highly modified. Thus, it is essential to investigate that aspect when testing an alternative feed ingredient. In this paper, we assess how corn dried distiller's grain with solubles (DDGS) affects the water chemistry parameters and the growth performance of carps (*Cyprinus carpio*). The trial was carried out in an outdoor experimental fishpond system with six earthen ponds, in three concurrent repetitions with one control feed. Each pond was stocked with two-years old and one-year old *C. carpio* individuals. Total nitrogen, ammonium nitrogen, total inorganic nitrogen, total phosphorus, orthophosphate phosphorus, chlorophyll-a concentrations and production parameters were compared. It was shown that in the ponds receiving DDGS feed, the growth rate and final body weight of the fish were higher, while the daily specific growth rate was significantly higher compared to the control group. No significant difference was found between the treatments for the water chemistry parameters, except for the total nitrogen. The higher total nitrogen concentration observed with the DDGS use did not exceed the limit prescribed by the current environmental regulation. In conclusion, the DDGS is a promising feed ingredient for *C. carpio* nutrition, used as supplementary or complete diet in pond culture.
Key Words: alternative feed ingredient, corn DDGS, carp, intensification of aquaculture, water quality.

Introduction. The rapid growth of the human population challenges agriculture as well as aquaculture which respond with continuous improvements. At present, aquaculture is one of the fastest growing livestock sectors (FAO 2018). In parallel, human exploitation of natural resources continues, habitats are changing, and pathogens of domesticated and exotic animals and plants are spreading. As a result, ecosystems are losing their genetic and phylogenetic, taxonomic and functional diversity at a rapid rate and on a large scale (Naeem et al 2012).

As aquaculture production increases, it results that the amount of feed intake is also increasing, and side by side its environmental load shows also an elevating trend of discharged organic matter, nutrients and suspended solids amounts (Edwards 2015), implying a potentially negative environmental impact of aquaculture (Naylor et al 2000) through the effluent water. Organic matter, phosphorus and nitrogen from feed residuals and metabolites are the most widespread concern in water pollution, causing eutrophication and oxygen depletion (Gál et al 2016). As feed ingredients have a significant effect, not only on growth performance, but also on the state of receiving water bodies, studies on water quality are of utmost importance (Wahab et al 2002; Ćirić et al 2015; Davidson et al 2016; Nagy et al 2017).

Common carp (*Cyprinus carpio*) contributes to around 7.7% of the total aquaculture production globally (FAO 2020). The most widely used *C. carpio* production technology in Europe is the polyculture in earthen ponds (Szűcs et al 2007). Extensive and semi-intensive *C. carpio* farming is based on natural food resources and supplementary grain feeding. However, *C. carpio* adapts well to intensive pond fish production conditions and this technology has a lower environmental impact compared to other intensive systems (Roy et al 2019; Kestemont 1995). *C. carpio* production dominates in table fish production in Hungary. In 2018, the production volume reached 18,300 tons, corresponding to 81.6% of all pond production. The average yield in 2018 per hectare was 595 kg (Kiss 2019).

Traditionally, extensive systems are forced to be intensified due to the market expectations. Replacing the traditional grain feeding with compound feeds makes production more profitable and has beneficial effects on the fish meat quality (Dickson et al 2016; Marković et al 2016; Stoycheska et al 2017). However, the inclusion of new, alternative plant raw materials in production has its own challenges, both in terms of product performance and impact on the water quality (Hardy 2010). The availability and cost of different feed ingredients should also be considered when formulating a diet. From a sustainable and energy-efficient perspective, local by-products from the food and fermentation industries can be particularly important as they are typically cheaper and have a lower environmental impact. For these considerations, the Dried Distiller's Grain with Solubles (DDGS), a by-product of bioethanol production, can contribute to the sustainable development of aquaculture. With its medium energy content, medium protein, digestible fibre and accessible phosphorus quantity it makes possible to produce high nutritional feed (Lim et al 2011). An additional advantage over other plant ingredients is the lack of antinutrient factors (Makkar 2012).

Révész et al (2019, 2020) have demonstrated that the DDGS at high inclusion level is also suitable for *C. carpio* production without any negative effects on the growth and health. Moreover, benefits in fat metabolism and high digestibility of phosphorus have been observed. However, to be able to use DDGS as a sustainable ingredient in the fish feed of the future, examination of its environmental impact is needed as well. The aim of this study was to compare the effects of an experimental DDGS-containing diet and a commercially-available conventional *C. carpio* feed on the main water quality parameters. The effects on nitrogen and phosphorus forms together with the chlorophyll-a concentrations were examined. Our hypothesis was that the experimental DDGS containing diet has not more adverse effects on the water quality than a commercially available feed.

Material and Method

Description of the study sites. The trial was accomplished at the experimental site of Hungarian University of Agriculture and Life Sciences – Institute of Aquaculture and Environmental Safety - Research Centre for Aquaculture and Fisheries (MATE AKI HAKI, Szarvas, Hungary). The experiment was carried out in six earthen ponds, in three concurrent repetitions (Figure 1). The ponds had an average surface of 1,808 ± 53 m^2 and an average depth of 1.5 m. The ponds were filled from the oxbow lake of River Körös, located next to the institute. Firstly, the water was pumped into a water reservoir, then, connected by canals to the experimental ponds, the water flew under control, by gravity. The six ponds were located parallel and their effluent water was collected in a common dedicated drainage channel. Paddle-wheel aeration devices were placed at the surface of the ponds to keep the dissolved oxygen concentration at a favourable level.

Two different age groups of *C. carpio* (1+ and 2+ years old) were stocked into the ponds on the 3rd of May in 2018. Each pond was stocked with 70 two-years old and 1,050 one-year old *C. carpio* individuals, with average weights of 360 and 50 g, respectively. In order to ensure the proper quantity of natural plankton production, we added cow manure (2 t ha^{-1}) to the ponds before launching the experiment, repeating the treatment in the middle of the season. In the nutritional design of the traditional semi-intensive technology, cereals (wheat) and natural resources (zooplankton and zoobenthos) are the food sources of *C. carpio*. The semi-intensive feeding with compound feeds started on the 23rd of July. In half of the ponds, fish were fed with 40% DDGS-based experimental compound feed, that had 35.04% crude protein content and 7.8% crude fat content. In the other three ponds, the fish were fed with a commercial feed as control, with similar nutritional characteristics (34.47% crude protein, 6.60% crude fat produced by Haltáp Kft., Szarvas, Hungary).

Figure 1. The experimental pond system of Hungarian University of Agriculture and Life Sciences – Institute of Aquaculture and Environmental Safety - Research Centre for Aquaculture and Fisheries. Orange squares indicate the ponds fed with DDGS, while blue squares indicate control ones. (Source: Hungarian University of Agriculture and Life Sciences - Institute of Environmental Sciences - Research Center of Irrigation and Water Management).

The experimental feed has been formulated to replace the plant ingredients of the commercial feed with DDGS, leaving unchanged the animal origin ingredient level. The feeding trial lasted for 174 days, during the rearing season. Feeding was carried out by hand, twice per day, with a quantity equal to 2-3% of the fish biomass (depending on the water temperature). In total, the fish consumed 2,370 kg of feed in the control ponds 2,484 kg of feed in the experimental DDGS treatment ponds. Every third week, the growth and health status of the stock was checked by test harvesting and the feeding rate was modified according to the estimated biomass of the ponds. At the end of the trial, the whole stock was measured to evaluate the growth performance. During the trial, only four fish died, which proves that there were no external harmful effects.

The studies were conducted according to the European Union Directive (2010) on the protection of animals for scientific purposes. All animal experiments have been approved by the Ethical Committee of MATE AKI HAKI, which was established according to the Hungarian State law (1999) and operated according to different Hungarian State laws (2013) concerning animal experiments, transportation of animal, welfare etc.

Sampling and water chemistry analysis methods. During the trial, water samples were collected every second week from the outlet of the ponds, by a column sampler. The water samples were sent to the accredited Laboratory of Environmental Analytics of Hungarian University of Agriculture and Life Sciences - Institute of Environmental Sciences - Research Center for Irrigation and Water Management (MATE KÖTI ÖVKI) right after sampling, without preservation. Total nitrogen (ISO 1997), ammonium nitrogen (ISO 2005), total inorganic nitrogen (ISO 1996, 2005), total phosphorus (ISO 2004), orthophosphate phosphorus (ISO 2004) and chlorophyll-a (ISO 1992) were analysed according to the mentioned standards of the Hungarian Standards Institution. The vast majority of the water samples were immediately processed in the laboratory and the remaining ones were cooled down to a temperature of +4°C for a maximum storage time of 6 hours. The dissolved reactive phosphorous and nitrogen forms were analysed by spectrophotometer from the filtered water samples. The absorbances were measured by a SPEKOL-11 type spectrophotometer in cuvettes of 1 cm.

***Calculations and statistical analysis**.* Growth performance of *C. carpio*, such as specific growth rate (SGR), feed conversion ratio (FCR) and gross yield (GY) were calculated based on the following standard formula (Ruttkay 2016):

$$SGR = 100\left(\ln w_f - ln\ w_i\right) * t^{-1}$$

Where:
w_i and w_f - the initial and final average weight (g);
t - the time (day).

$$FCR = TF * \left(w_f - w_i\right)^{-1}$$

Where:
TF - the total feed offered (g);
w_i and w_f - the initial and final average weight (g).

$$GY = \left(w_f - w_i\right) * A$$

Where:
w_i and w_f - the initial and final average weight (t);
A - the area (ha).

For statistical analyses we used Microsoft Excel and „dplyr" (Shimko & Andersen 2014) and „ggpubr" (Kassambara 2018) packages of „R" (R Development Core Team 2013), an open source statistic software. Shapiro-Wilk test was used to test the normality of the distribution of the measured water chemistry parameters and the F test was used for the analysis of variance. In the case of normal distributions and corresponding variances, a two-sample t-test was used to examine the means for each treatment to be compared, otherwise, the Mann-Whitney non-parametric test was used.

Results

Production parameters. By the end of the feeding experiment, the performances of the fish receiving DDGS-containing feed and of the *C. carpio* fed with conventional commercial feed were comparable. The specific growth rate for control juvenile fish (1+) was significantly lower (1.46 g day^{-1}) than in DDGS group (1.56 g day^{-1}). A similar favourable growth was observed for the 2-year old stage fish, with an SGR of 0.91 g day^{-1}, compared to 0.86 g day^{-1} in the control group. Moreover, in the case of the feed conversion ratio (FCR) and gross yield, differences were also statistically significant (in the FCR 1.56 g g^{-1} vs 1.78 g g^{-1} and in the gross yield 3.32 t ha^{-1} vs 2.85 t ha^{-1}, for the DDGS and control treatments, respectively).

Water chemistry - Total ammonium nitrogen. Ammonium nitrogen concentrations measured in the water samples ranged between 0.186 and 0.283 mg L^{-1} for DDGS-fed ponds, with an outlier of 0.549 mg L^{-1} (Figure 2a). This concentration was similar for the control ponds, ranging between 0.162 and 0.258 mg L^{-1}, with an outlier value (0.536 mg L^{-1}), at the same sampling time as for the experimental diet fed group. Due to the outliers, we used the Shapiro-Wilk test and none of the data sets had a normal distribution, which would be a prerequisite for the t-test. Thus, this value was omitted for statistical analysis, which resulted in normal distribution. Thereafter, the non-parametric Mann-

Whitney test showed no significant differences between the effects of the two treatments on the water ammonium-nitrogen concentration.

The average concentration of ammonium-nitrogen in DDGS fed fish ponds was 0.279±0.123 mg L^{-1}. The average concentration of ammonium-nitrogen in control ponds was 0.263±0.126 mg L^{-1}. The effect of DDGS feed was not statistically different from the commercially available feed, for this water chemistry parameter. The ammonium-nitrogen concentration did not reach the toxic value of 1 mg L^{-1} in either treatment.

Water chemistry - Total inorganic nitrogen. Concentrations ranged between 0.328-0.690 mg L^{-1} in the "DDGS" ponds and 0.311-0.517 mg L^{-1} in the control ponds and outlier values were 0.919 and 0.917 mg L^{-1} (Figure 2b). For the control ponds there were outlier values at the same sampling time as in the case of ammonium-nitrogen, thus the data sets did not have a normal distribution. Therefore, in the case of total inorganic nitrogen, we analysed the data similarly to the ammonium-nitrogen sets.

The Mann-Whitney test showed no difference between the two feeds, in terms of total inorganic nitrogen in the water. The average concentration of total inorganic nitrogen in the experimental feed ponds was 0.541±0.205 mg L^{-1}. The average concentration of total inorganic nitrogen in the control ponds was 0.483±0.207 mg L^{-1}. For this water chemistry parameter, the effects of DDGS and commercially available feeds were not different.

Water chemistry - Total nitrogen. The range of total nitrogen concentration in the DDGS ponds was between 1.507-2.197 mg L^{-1}, while in the control ponds it ranged between 1.003-1.790 mg L^{-1} (Figure 2c). The average total nitrogen concentration in the DDGS ponds was 1.841±0.264 mg L^{-1}, while in the control ponds it was 1.384±0.245 mg L^{-1}. Based on the distributions and variances, it was possible to use t-test for the full data set, which showed that the DDGS feed caused a significantly higher total nitrogen level.

Water chemistry - Orthophosphate phosphorus. The average concentration of orthophosphate phosphorus was 0.060±0.026 mg L^{-1} in the DDGS ponds and 0.053±0.021 mg L^{-1} in the control ponds (Figure 2d). As the data set had a normal distribution and the variances were the same, we used a two-sample t-test. In the case of the orthophosphate phosphorus concentration in the water, there was no difference between the two feed types. The effects of DDGS and commercially available feeds were not different for this water chemistry parameter.

Water chemistry - Total phosphorus. Similarly to the orthophosphate phosphorus, the t-test showed no significant difference between the two feed types, in terms of total phosphorus concentration in water. The average concentration of the total phosphorus was 0.204±0.033 mg L^{-1} in the DDGS ponds and 0.190±0.030 mg L^{-1} in the control ponds (Figure 2e).

Water chemistry - Chlorophyll-a. The average value was 66.002±41.780 μg L^{-1} in the DDGS ponds and 50.396±21.664 μg L^{-1} in the control ponds (Figure 2f). The Mann-Whitney test showed no significant difference between the two treatments, in terms of chlorophyll-a concentrations. There was no chlorophyll-a content difference between the effects of the two feeds.

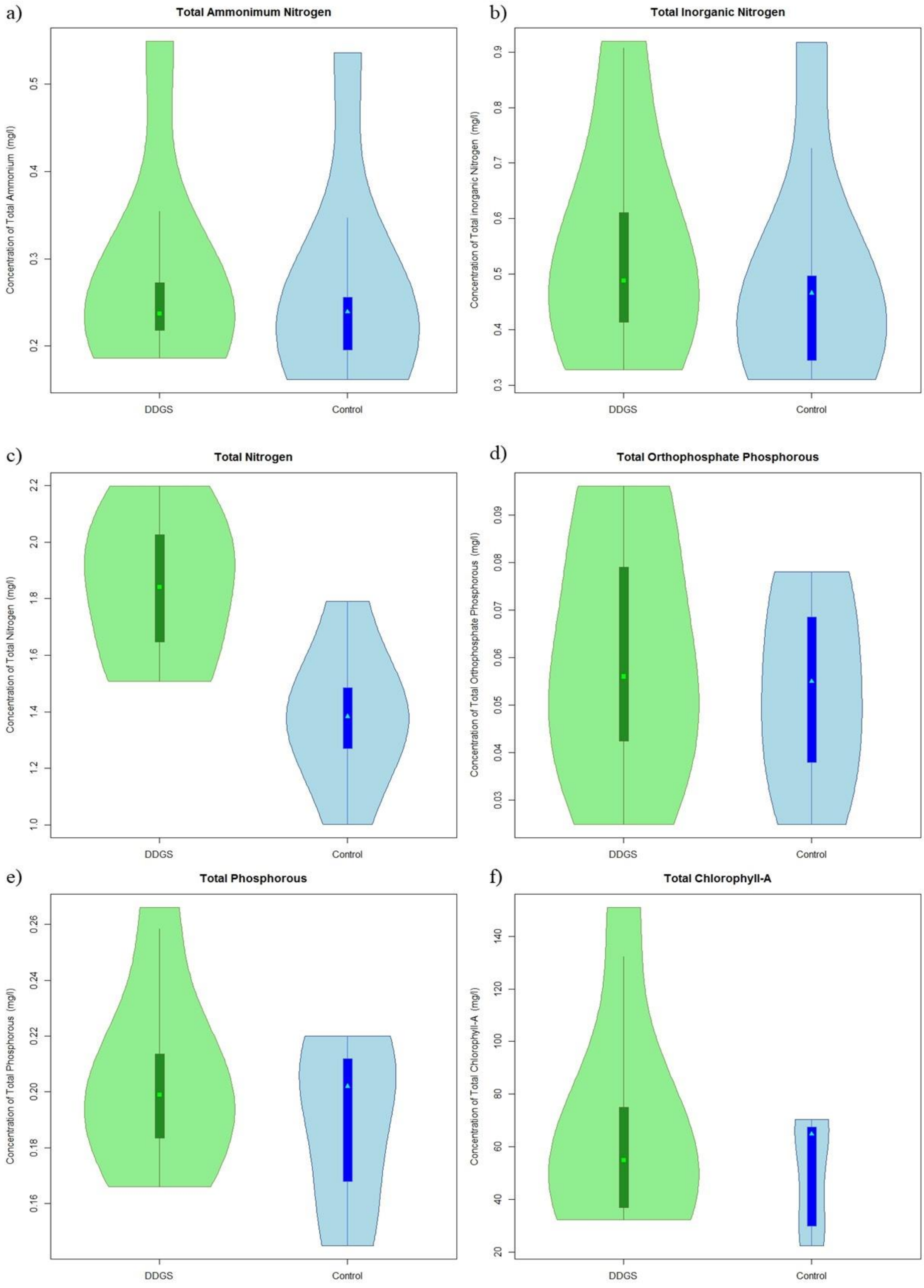

Figure 2. Violin plots of water chemistry parameters: a) Total ammonium-nitrogen, b) Total inorganic nitrogen, c) Total nitrogen, d) Total orthophosphate phosphorus, e) Total phosphorus, f) Total chlorophyll-A.

Discussion. The replacement of soybean and corn meal by corn-DDGS seemed to be successful in the diet of *C. carpio*. According to the fish growth and production parameters during the rearing season, it was shown that in the ponds which received DDGS feed, the growth rate and final body weight of the fish were also higher. The one-year old population gained nearly tenfold their body weight, in both the treatment and control groups, during the 155 days of the feeding experiment, but the daily specific growth rate was significantly higher in the experimental group. The DDGS-based feed had a positive effect on the growth of *C. carpio* and feed utilization under semi-intensive pond conditions. Révész et al (2019) also found better growth parameters for *C. carpio* fingerlings fed with a 40% DDGS diet.

The effect of the corn-DDGS based and a commercially available fish feed on the water quality was examined in terms of nitrogen and phosphorous forms as well as chlorophyll-a concentrations. Confirming our hypothesis, the results demonstrated that the DDGS-based feed had not a higher impact on the water quality than the conventional control fish feed. With regard to the nitrogen forms, we detected significantly higher concentrations of total nitrogen in the DDGS-treated ponds, but it should be noted that the concentration did not exceed the limit prescribed by the current environmental regulation in Hungary (Emission limit value for total nitrogen discharged directly into the receiver is 35 mg L^{-1})(Hungarian State 2004). The permissible limits of 0.4 mg L^{-1} for orthophosphate phosphorous and 1 mg L^{-1} for ammonium-nitrogen have been set for the *C. carpio* culture waters (EU directive 2006). As no statistical difference was found for the inorganic nitrogen forms between the treatments, the high concentrations may be due to the amount of the organic nitrogen forms. The lower essential amino acid levels in DDGS, compared to those available in soybeans, can explain that they decreased the protein efficiency and increased non-utilized nitrogen ratio in fish excreta. According to the absence of significant differences in the case of the other measured chemical or biological parameters, we can conclude that the DDGS-based experimental *C. carpio* feed does not cause a more threatening environmental load than the conventional fish feed.

The DDGS User Handbook of U.S. Grains Council (2018) presented an overview of the evaluation of the DDGS in environmental sustainability terms and suggested that it generates moderate metabolic by-product amounts, compared to other feed ingredients. Although the eliminated phosphorus and nitrogen in various farmed animals are included, the DDGS metabolic by-product amounts in aquaculture feed are not yet discussed. Henriksson (2017) summarized the environmental impact of feed ingredients in aquatic feeds in Indonesia and classified them based on categories of impact such as: global warming, acidification, eutrophication, land use and freshwater consumption, through life cycle assessments. The environmental effects of DDGS were generally moderate, among the listed feed ingredients. A study on the effect of a plant-based aquafeed (containing soybean meal and linseed oil) found that, under semi-intensive fish pond conditions similar to our results, there were no significant differences in water chemistry parameters, compared to the commercial feed or supplementary grain feeding (Berzi-Nagy et al 2017). In the zooplankton communities, as bioindicator organisms for the same experimental setup, no overall difference was detected between plant-based and fish-meal / fish oil-based diets (Tóth et al 2020).

Plant protein meals may have lower digestibility and therefore they can cause elevated nutrient levels in the water column, mainly due to antinutritional factors, like fibres and non-starch polysaccharides (Francis et al 2001; Kokou & Fountoulaki 2018). However, these indigestible compounds are characterized rather for soybean, pea or rapeseed meals, since corn DDGS contains a very low concentration of antinutrient factors (Makkar 2012; US Council 2018). The value of 86% for the apparent protein digestibility of the DDGS feedstuff, reported by Révész et al (2020), is one of the highest value observed for the *C. carpio* (Roy et al 2019). The phosphorus digestibility is also high in the DDGS, compared to other plant ingredients. This is particularly important, due to the biological limitation of the stomachless species' ability to digest phosphorous (Hua & Bureau 2010).

Roy et al (2019) have examined the role of several feed ingredients in the *C. carpio* pond farming as a source for nitrogen and phosphorous saturation of the pond ecosystem. They found that eutrophication caused by an increased level of nitrogen and phosphorus could be mitigated by an appropriate pond management and by using highly digestible feeds, including brewery yeast and corn DDGS. Waste nutrients can be absorbed and eliminated by abundant planktonic and benthic microbial communities in earthen ponds, and partly recycled into fish biomass. But for this purpose, the key approach is to optimize the size of fish stock in order to allow the zooplankton population to propagate properly (Sommer et al 2012).

Conclusions. In conclusion, DDGS, a by-product of bioethanol production, is a potential feed ingredient for carp nutrition as a supplementary diet in pond culture. Based on the results obtained for the performance of *C. carpio* production and for the ponds' water quality parameters, it can be inferred that the DDGS utilization in semi-intensive cultivation technology is promising. Thus, in the future, DDGS may be able to gain a prominent role as ingredient for fish feed, replacing the soybean or corn meals.

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Conflict of interest. The authors declare that they have no conflicts of interest.

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Authors:
Flórián Tóth, Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety, Research Centre for Aquaculture and Fisheries, Anna-liget str 35, H-5540 Szarvas, Hungary, e-mail: toth.florian@uni-mate.hu
Norbert Révész, Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety, Research Centre for Aquaculture and Fisheries, Anna-liget str 35, H-5540 Szarvas, Hungary, e-mail: revesznorbert89@gmail.com
Ferenc Demény, Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety, Research Centre for Aquaculture and Fisheries, Anna-liget str 35, H-5540 Szarvas, Hungary, e-mail: demeny.f@gmail.com
Attila Uhljar, Hungarian University of Agriculture and Life Sciences, Szabadság út 1-3, H-5540 Szarvas, Hungary, e-mail: a.uhljar97@gmail.com
László Berzi-Nagy, Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety, Research Centre for Aquaculture and Fisheries, Anna-liget str 35, H-5540 Szarvas, Hungary, e-mail: laszlo.berzi-nagy@agroloop.eu
Zsuzsanna Molnár, Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety, Research Centre for Aquaculture and Fisheries, Anna-liget str 35, H-5540 Szarvas, Hungary, e-mail: brlas-molnar.zsuzsanna@uni-mate.hu
Janka Biró, Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety - Research Centre for Aquaculture and Fisheries, Anna-liget str 35, H-5540 Szarvas, Hungary, e-mail: nagyne.biro.janka@uni-mate.hu
Dénes Gál, Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety, Research Centre for Aquaculture and Fisheries, Anna-liget str 35, H-5540 Szarvas, Hungary, e-mail: gal.denes.73@gmail.com
Éva Kerepeczki, Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety, Research Centre for Aquaculture and Fisheries, Anna-liget str 35, H-5540 Szarvas, Hungary, e-mail: kerepeczki.eva@uni-mate.hu
Zsuzsanna Jakabné Sándor, Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety, Research Centre for Aquaculture and Fisheries, Anna-liget str 35, H-5540 Szarvas, Hungary, e-mail: jakabne.sandor.zsuzsanna@uni-mate.hu

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