



# Nutritional and economic benefits of using DDGS (distiller' dried grains soluble) as feed ingredient in common carp semi-intensive pond culture

Zsuzsanna J. Sándor<sup>a,\*</sup>, Norbert Révész<sup>a</sup>, Dániel Varga<sup>b</sup>, Flórián Tóth<sup>a</sup>, László Ardó<sup>a</sup>,  
Gergő Gyalog<sup>a</sup>

<sup>a</sup> Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety, Research Centre for Aquaculture and Fisheries, H-5540, Szarvas, Anna-Liget Str. 35, Hungary

<sup>b</sup> Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety, Department of Applied Fish Biology, Kaposvár, Guba Sándor Str. 40, Hungary

## ARTICLE INFO

### Keywords:

Common carp  
Semi intensive carp monoculture  
DDGS  
Meat quality  
Economics

## ABSTRACT

A survey was conducted to evaluate the suitability and profitability of corn DDGS (distiller' dried grains soluble) as a protein source in feed for common carp (*Cyprinus carpio* L.) in semi-intensive pond production. Six ponds of 0.17 ha were stocked with 70 pc 2+ and 1050 pc 1+ old carp with average weight  $362 \pm 10$  g and  $45 \pm 1$  g, respectively, and fed with two types of feed in triplicates. Fish were kept on natural pond food supplemented periodically with wheat grains in the first part of feeding season in order to minimize nutrition costs. When the sampling harvest result indicated that growth rates were slowing down wheat was replaced with formulated feeds (control and experimental diets) as external food source till end of experiment. The experimental feed contained 40 % DDGS substituting the plant ingredients of the control feed. Growth, nutrient utilization, health, flesh quality of the fish was compared at the end of rearing season and finally the economic performance of the diets was investigated. Significantly better performance of experimental group was found in most of the production parameters (final weight, weight gain, specific growth rate) at juvenile age and in feed conversion rate (1.56 vs 1.78 g/g), protein efficiency ratio (2.32 vs 2.08 g/g), gross yield (3520 vs 3020 kg/ha) of the ponds. Economic advantage of high DDGS inclusion in carp feeds was demonstrated by significantly improved per-hectare profit and benefit-cost ratio. These are attributed to higher yields, better feed conversion and lower cost of novel feed formulation. Health and flesh quality of the fish were not affected by the diet composition. It was concluded that combining the use DDGS-based compound feeds with maximal exploitation of pond food results in better production, nutrient utilisation and economic performance than traditional cereal-based semi-intensive carp farming.

## 1. Introduction

Common carp (*Cyprinus carpio*) is one of the most important freshwater finfish species cultured worldwide. In 2018, global common carp production reached 4.2 million tones, ranked 4th in global fish production (FAO, 2020). In Europe, carps are reared predominantly in earthen ponds in extensive production systems, and the conventional nutrition technology is based on natural food supplemented with cereal grains, such as wheat, maize and barley, depending on price and local availability (Szűcs et al., 2007). Recently, extruded and pelleted feeds have become relatively widely used in Central and Eastern European countries as they provide a higher weight gain to fish (Ciric et al., 2015).

Using complex feeds instead of cereals, shorten the production cycle and last but not least amends the fish flesh quality (Mráz et al., 2012; Trbovic et al., 2013; Dickson et al., 2016; Marković et al., 2016; Stoycheska and Stamenkovska, 2017). Recently, different terrestrial plants, such as soybean, wheat, corn and marine origin sources were utilized as ingredients in carp feed manufacturing (Hlavak et al., 2016; Mazurkiewicz, 2009). Increased Hungarian production of protein-rich plants such as feed peas, lupine, horse beans and apparent availability of agricultural and industrial by-products serves as a ground for economically sustainable feed ingredients. Taking into consideration the promising results in the field of nutrient digestibility of some of these, there is a scope to increase environmental sustainability of future carp diets

\* Corresponding author.

E-mail address: [jakabne.sandor.zsuzsanna@uni-mate.hu](mailto:jakabne.sandor.zsuzsanna@uni-mate.hu) (Z.J. Sándor).

<https://doi.org/10.1016/j.aqrep.2021.100819>

Received 26 April 2021; Received in revised form 14 July 2021; Accepted 3 August 2021

Available online 13 August 2021

2352-5134/© 2021 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

with the inclusion of alternative protein ingredients (Roy et al., 2019).

Distiller's Dried Grain with Solubles (DDGS) is a by-product from the bioethanol industry, which is a suitable alternative feed ingredient to replace unsustainable feed components that either have limited supply or are applicable to direct human consumption (DDGS Handbook, 2018). It was successfully applied in the diet of different animals and fish species as well (Abouel et al., 2021; Allam et al., 2020; Diógenes et al., 2018; Overland et al., 2013; Lammer et al., 2015 etc.) and was concluded that DDGS could replace the fish meal or soybean meal in the diet in some extent depending on the fish feeding behavior. The advantage of DDGS compared to other plant-based feed ingredients is that it does not contain antinutritive substances and it has highly utilizable phosphorus content. On the other hand, it contains a low proportion of the essential amino acids (Liu, 2011). Inclusion of DDGS into aquafeed formulations decreases feed ingredient costs for farming of omnivorous species such as tilapia (*Oreochromis niloticus*), striped catfish (*Pangasianodon hypophthalmus*) and Pacu (*Piaractus mesopotamicus*); however, feed conversion and growth rates are often compromised above certain inclusion levels. (Coyle et al., 2004; Allam et al., 2020; Oliveira et al., 2020). Therefore, economic benefits can be exploited if reduction in feed formulation costs compensates the economic loss associated with higher feed use per unit of production.

In our previous research we tested different inclusion levels of DDGS in carp feeds and it was demonstrated that the plant components could be replaced with DDGS up to a proportion of 40 % without imposing any negative effect on growth and feed utilization, health and other metabolic processes. We found that carps digest DDGS as well as corn and wheat (Révész et al., 2019, 2020), moreover the highly digestible phosphorus available in the DDGS may decrease the eutrophication of the fish ponds.

In continuation of our earlier studies, in order to step forward toward commercialization, we formulated a pond carp feed using the optimal DDGS inclusion level identified in the previous experiment and tested its performance under semi-intensive farming conditions in ponds stocked with mixed fish age population. The objectives of the experiment were to investigate the impact of the novel diet on growth, feed conversion, quality of fish meat and economic performance in comparison with a commercially available carp feed used as a control feed.

## 2. Materials and methods

The trials were conducted in line with the European Union Directive (2010/63/EU) on the protection of animals for scientific purposes. All animal experiments have been approved by the Ethical Committee of HAKI, which was established according to Hungarian State law (10/1999. (I. 27.)) and operated according to different Hungarian State laws concerning animal experiments, transportation of animal, welfare etc. (40/2013. (II.14)).

### 2.1. Experimental design and feeding protocol

Two carp feeds (conventional formula vs. DDGS-based experimental formula), were tested with 3 replicates per treatment. Six earthen ponds with an average surface of 1808 ( $\pm 53$ ) m<sup>2</sup> and a depth of 1.5 m were used for the experiment which lasted for a production season. On 2nd May 2018 each of the ponds was stocked with 70 two-years-old and 1050 one-year-old scaly carp individuals with a mean weight ( $\pm$  STD) of 362  $\pm$  10 g and 45  $\pm$  1 g, respectively. The ponds were harvested on 3<sup>rd</sup> October 2018. Growth and health status of the fish were checked by sampling harvest every 3 weeks. Before these harvests, the water level was reduced by 50 %, and after that, it was filled up to operational level immediately.

The feeding technology during the experiment was set in line with the principles of carp nutrition under semi-intensive technology as described by Ruttkay (2016). This protocol suggests that due to the availability of pond food (zooplankton and zoobenthos) providing

enough protein and other elementary nutrients for carp, only cereal grains are recommended to use in order to supplement pond food as energy sources in the first part of the season (Horvath et al., 2002). By feeding the carp moderately with cereals during May-June zooplankton peak and switching to compound feeds in July when biomass of pond food start to shrink good feed conversion rate can be achieved, moreover the fat content of the fish can also be kept down at acceptable level (Ruttkay, 2016). Following this protocol in the present experiment, cow manure (4 t.ha<sup>-1</sup>) was added to the ponds in two installments (once before the experiment and once at 8<sup>th</sup> July) in order to keep the plankton production at a high level. Fish were kept on natural pond food supplemented periodically with wheat grains as long as sampling harvest results shown acceptable growth. On 12<sup>th</sup> July, the sampling harvest result indicated that growth rates were slowing down. Therefore, from the subsequent day wheat was replaced with formulated feeds (control and experimental diets) as external food source till harvest. The feeding was managed manually, twice per day with 2–3 % of fish biomass (depending on the water temperature). Dissolved oxygen and water temperature were determined twice per week, while the water quality parameters of the ponds (total inorganic nitrogen, total nitrogen, orthophosphate phosphorus, total phosphorus, and total ammonium nitrogen) were assessed bi-weekly. The methods and obtained results are presented by Tóth et al., 2021.

### 2.2. Experimental diets

The control group was fed with a commercially available feed (crude protein: 35 %; crude fat: 7 %), while the experimental group was fed with a pellet containing 40 % DDGS produced by the same feed manufacturer. The feeds were iso-nitrogenic, iso-lipidic and iso-caloric. The experimental feed was formulated such that the soybean and other plant ingredients of the control feed were replaced by DDGS, while the proportion of the animal origin ingredients remained unchanged. The feeds were in form of semi-floating pellets with the diameter of 4.5 mm. The formulation of the experimental is presented in Table 1. Proximate composition, amino acid and fatty acid profile of both diets are summarized in Table 2. Formulation of the control diet is not presented here due to intellectual property issues; nevertheless, the ingredients are in descending order as follows: wheat, soybean meal (C.P. 46), corn gluten (C.P. 60 %), poultry meal (C.P. 62 %), extruded soy meal, blood meal, yeast, fish meal (C.P. 60 %), premix.

**Table 1**  
Formulation of the experimental diet.

Ingredients	%
DDGS <sup>1</sup>	40.0
Wheat <sup>2</sup>	20.5
Soybean meal (C.P.46) <sup>3</sup>	8.0
Corn gluten (C.P. 60 %) <sup>4</sup>	8.0
Poultry meal (C.P. 62 %) <sup>5</sup>	5.0
Extruded soy meal <sup>6</sup>	5.0
Blood meal <sup>7</sup>	4.0
Yeast <sup>8</sup>	4.0
Fish meal (C.P.60 %) <sup>9</sup>	4.0
Premix <sup>10</sup>	1.5

<sup>1</sup>PANNONIA BIO Ltd; <sup>2</sup>GEOMARK Ltd; <sup>3</sup>AGRO-TRIÓ Ltd. (distributor); <sup>4</sup>HUNGRANA Ltd; <sup>5</sup>KATECH Ltd.; <sup>6</sup>HAGE Ltd; <sup>7</sup>KATECH Ltd; <sup>8</sup>EUROPROTEIN Ltd. (distributor) <sup>10</sup>CARGILL Ltd.

<sup>10</sup>Composition/kg: Vitamin A (E672) 1003400 NE; D3 (E671) 80650 NE; vitamin E (3a700) 5000 mg; vitamin E equivalent antioxidant 0 mg; vitamin K3 337 mg; Ca 12.2 %; P 7.8 %; Na 0.1 %; Lys 6.9 %; Met 18.8 %; Fe (E1) 670 mg; Zn(E6) 1070 mg; Mn(E5) 160 mg; Cu (CuSO<sub>4</sub>\*5 H<sub>2</sub>O) 200 mg; Se(E8) 20 mg.

**Table 2**  
Feed composition (as fed).

Proximate composition (%)	Experimental diet	Control diet
Dry matter	92.56 ± 0.11	92.26 ± 0.04
Crude protein	35.04 ± 0.51	34.47 ± 0.01
Crude fat	7.80 ± 0.45	6.60 ± 0.38
Crude fibre	4.46 ± 0.05	2.98 ± 0.04
Crude ash	5.93 ± 0.55	6.24 ± 0.03
NFE (calculated)	46.78	49.71
Gross energy (MJ kg <sup>-1</sup> ) (calculated)	19.46	19.37
Protein/Energy (mg KJ <sup>-1</sup> )	18.00	17.79
<b>Essential amino acids and micro elements (calculated) (%)</b>		
Lysine	1.34	1.92
Methionine	0.69	0.87
Methionine + Cystine	1.04	1.39
Threonine	0.95	1.36
Tryptophan	0.26	0.38
Arginine	0.69	0.21
Isoleucine	0.07	0.15
Leucine	1.57	0.36
Valine	0.66	0.19
Ca	0.64	0.98
P	0.52	0.83
Mg	0.005	0.005
Na	0.04	0.05
<b>Fatty acid profile (w%)</b>		
16:0	15.46 ± 0.03	14.83 ± 0.08
18:0	3.93 ± 0.02	4.68 ± 0.08
18:1n-9	26.88 ± 0.10	25.18 ± 0.02
18:2n-6	44.60 ± 0.03	44.10 ± 0.01
18:3n-3	2.66 ± 0.05	3.86 ± 0.04
20:4n-6	0.23 ± 0.01	0.28 ± 0.01
20:5n-3	0.27 ± 0.02	0.41 ± 0.01
22:6n-3	0.85 ± 0.02	1.03 ± 0.02
Total n-3	3.89 ± 0.03	5.40 ± 0.06
EPA+DHA	1.13 ± 0.00	1.43 ± 0.01

NFE: Nitrogen free extract; Gross energy: = (23.9 × CP (g kg<sup>-1</sup>) + 39.8 × CL (g kg<sup>-1</sup>) + 17.6 × NFE (g kg<sup>-1</sup>)) 10<sup>-3</sup>.

Total n-3: 18:3n-3 + 18:4n-3 + 20:3n-3 + 20:4n-3 + 20:5n-3 + 22:3n-3 + 22:4n-3 + 22:5n-3 + 22:6n-3 + 24:5n-3 + 24:6n-3.

### 2.3. Sampling

During stocking and at the harvest all fish were measured in stock of 50 pc from which the biometric indices were determined. Although studying the link between quantity of pond food and fish growth was not the subject of this study, zooplankton biomass was assessed four times in order to know whether there are differences in nutrients sources above feed between ponds or treatments. Each time 100 L of surface water were taken and filtered using 50 µm mesh plankton net, then concentrated to 100 mL. The zooplankton samples were preserved in a centrifuge tube with added formaldehyde (4%) and the biomass volume was estimated.

At end of experiment after 154 day feeding fish were harvested and production parameters determined. Five individuals from each pond were dissected to measure biometrical indices. Before processing, the following biometric traits were registered: body length, standard length, body height and body width. After the processing, separated parts of the body (hepatopancreas, viscera, head, gonad) were measured, and the dressing yield, filleting yield, viscerosomatic index, hepatosomatic index, gonadosomatic index were calculated. Dressing yield was calculated as edible part of the fish without head, fins and viscera. The sex of each individual was determined. Carps were processed after percussive stunning in accordance with the rules of the Carp Performance Testing Codex (OMMI, 2001).

Three whole fish samples for body composition were taken as well as fillet samples for meat quality studies. One ml of blood was taken from the caudal vein of three adult and three juvenile fish per pond, using heparinised needles and syringes. Blood samples were put into heparinised microcentrifuge tubes and centrifuged at 1,400 g for 20 min at 4 °C. After centrifugation, blood plasma was collected and stored at -20

°C for further analysis. Liver samples were taken from three fish per pond for gene expression analysis (only from the juvenile fish). 100 mg aliquot of samples were collected and put into 1 ml RNA later for one day at 4 °C and after that kept at -20 °C still analysis.

### 2.4. Analytical measurements and data evaluation

The proximate composition of the filet has been analyzed by standard methods of the AOAC (1998) (Table 2). The experimental diet's total carbohydrate (TC) and gross energy (GE) values were calculated as TC = 100 - (crude protein + crude fat + crude fibre + ash), with GE = values of carbohydrates, proteins and lipids of 17.2, 23.6 and 39.5 KJ g<sup>-1</sup>, respectively (Halver and Hardy, 2002). Lipids were extracted from the samples with a 2:1 mixture of chloroform and methanol. The extracts were purified according to the method by Folch et al. (1957). Aliquots of total lipid samples were trans-esterified using a methanolic solution of HCl (Stoffel et al., 1959). Fatty acid methyl esters (FAME) were separated on fused silica capillary columns (DB-225) in an AGILENT (HP) gas chromatograph system (type "6890N") equipped with a Flame Ionisation Detector (FID) and a mass spectrometer (MS) detector (MSD, type "5973N"). FAME were identified using authentic primary (SUPELCO, Bellefonte, NJ) or secondary standards (e.g., linseed oil, cod liver oil) and by means of the relationship between the logarithms of relative retention times and the carbon number (Cn) of fatty acids. The amino acid composition is given following the calculation made with the known data of the ingredients.

### 2.5. Blood biochemistry

Blood plasma chemistry was done with a Samsung PT10 V, semi-automatic clinical chemistry analyser using Comprehensive Samsung Kit. Plasma enzymes and metabolites, such as creatine (CREA), Glutamine (GLU), phosphate (PHOS), total protein (TP), globulin (GLOB), alkaline phosphatase (AP), cholesterol (CHOL), triglyceride (TRIG) and amylase (AMY), were measured according to IFCC (International Federation of Clinical Chemistry).

### 2.6. Gene expression

Expression levels of genes involved in growth (IGF-1) and stress response (HSP70) were measured in liver samples, respectively, by real-time quantitative PCR (qPCR), using β-actin as an internal reference gene. Description of the methodology and the reference genes are presented in Supplementary data S.1.

### 2.7. Fillet flesh quality investigation

Assessment of the quality parameters of the fillet was done using method presented by Varga et al. (2013a,b). According to the protocol for determination of the physical characteristics of the flesh, the freshly collected fillet were kept in vacuum safe plastic bags at 4 °C during transport to laboratory. Fillet pH was measured at 45 min and 24 h post mortem by a Testo 205 precision pH meter (Testo AG, Lenzkirch, Germany). The colour (CIE Lab, L – lightness, a\* – redness, b\* – yellowness) of the fresh fillet was determined by a Minolta ChromaMeter 300 apparatus (Minolta, Osaka, Japan). Dripping loss was determined by the method of Honikel (1998). To determine the cooking loss, fillet samples (100 g) were closed into sealed bags and were cooked at 75 °C for 20 min. The exudate weight, as expressed in the percentage of the initial sample weight, was referred to as cooking loss. The thawing loss was determined by the same manner, i.e. samples (25 g) were frozen (-20 °C) and thawed to room temperature after 2 days.

### 2.8. Calculations and statistical analysis

Growth performance of fish such as specific growth rate (SGR),

apparent feed conversion ratio (AFCR), apparent protein efficiency ratio (APER) and apparent protein production value (PPV) and gross yield (GY) was calculated based on the following standard formulae:

Weight gain (%) = {(Final weight – Initial weight) / Initial weight} × 100;

Specific growth rate (SGR) = (Ln final body mass – Ln initial body mass) × 100 / days;

Apparent feed conversion ratio (AFCR) = Total dry feed offered (g) / wet weight gain (g);

Apparent protein efficiency ratio (PER) = Net weight gain (wet weight) / Protein offered;

Apparent protein productivity value (%) (PPV) = 100 × (total final biomass (g) × final whole-body crude protein – total initial biomass (g) × initial whole-body crude protein) / (feed protein × total feed offered);

Gross yield (%): Final weight (kg) / pond area (ha).

Biometric indices and post-harvest indices determined were:

Viscerosomatic index (VSI) = total wet viscera (g) / body weight (g);

Hepatosomatic index (HSI) = 100 × wet hepatopancreas weight (g) / wet body weight (g);

Dressing yield (%) = weight of eviscerated edible fish part / body weight;

Filleting yield (%) = 100 × fillet weight / body weight;

Gonadosomatic index (%) = 100 × weight of gonad / body weight.

All values were checked for normality of data distribution and homogeneity of variance using the Kolmogorov – Smirnov and Levene tests, respectively. Differences between treatments were analysed using one-way ANOVA, with statistical significance set at 0.05. For the biochemical parameters the effect was analysed by using two-way analysis of variance (ANOVA) with age (juveniles and adult group) and dietary treatment (control diet and DDGS experimental diet) as two fixed factors. Where significant interactions were found between main effects, one-way ANOVA was used to compare simple effects. These tests were performed by IBM SPSS 22. software package. To compare the biomass of the zooplankton communities of the treatments we used T-test in R software environment (R Development Core Team, 2013) with vegan package (Oksanen et al., 2012). Shapiro-Wilk test was used to test normal distribution and the analysis of variances was checked by F-test.

In order to address the effect of diet on the slopes of growth curves a linear regression analysis was conducted using data on average individual weights measured at three-weekly sampling harvest in the period when formulated diets were fed. Two equations were parameterized where average individual juvenile weight and adult weight were used as response variables, while time (as covariate), diet (as a fixed factor) and their interaction term were used as explanatory variables. 12 July, the last day before using formulated feed, was set as time zero. If the interaction effect between diet and time was significant ( $P < 0.05$ ), the slopes of the linear growth curves differed between diets. Similarly, if coefficient of diet factor was significant, the individual weight on 12 July would differ between two treatments, meaning that differences in yield and other production indicators calculated for the whole season would not only be attributed to difference in diets but to other non-intended factors occurring in the first half of the season. For these calculations the “lm” function of R software environment was used.

## 2.9. Economic evaluation

Per-hectare profit ( $\pi$ ) was used in the study as an indicator of economic performance. This was defined as *income above feed, seed and labour costs* and it was calculated as total revenue (TR) minus cost of stocking material ( $C_S$ ), feeding costs ( $C_F$ ) and labour costs ( $C_L$ ). The units

of  $\pi$ , TR,  $C_S$ ,  $C_F$  and  $C_L$  were €.  $\text{ha}^{-1}$ , whereas calculation of these items are described by Eqs. 1–5, respectively.

$$\pi = \text{TR} - C_S - C_F - C_L \quad (1)$$

$$\text{TR} = P_{Cc2} \times W_{hCc2} + P_{Cc3} \times W_{hCc3} \quad (2)$$

$$C_S = \text{Stock}_{Cc1} \times P_{Cc1} + \text{Stock}_{Cc2} \times P_{Cc2} \quad (3)$$

$$C_F = \text{Wheat} \times P_{Wh} + CF \times P_{CF}; \text{ for the control group} \quad (4a)$$

$$C_F = \text{Wheat} \times P_{Wh} + DF \times P_{DF}; \text{ for the experimental group} \quad (4b)$$

$$C_L = \text{wage} \times L \quad (5)$$

where

$W_{hCc2}$  and  $W_{hCc3}$  are the harvest weight of 2+ and 3+ years old C. carp biomass, respectively ( $\text{kg. ha}^{-1}$ );

$\text{Stock}_{Cc1}$  and  $\text{Stock}_{Cc2}$  ( $\text{kg. ha}^{-1}$ ) are the stocked biomass of 1+ and 2+ years old C. carp, respectively ( $\text{kg. ha}^{-1}$ );

$P_{Cc1}$ ,  $P_{Cc2}$  and  $P_{Cc3}$  are the unit prices of corresponding age classes (€.  $\text{kg}^{-1}$ );

*Wheat*, *CF* and *DF* are the amount of wheat, control feed and experimental feed fed during the trial, respectively ( $\text{kg. ha}^{-1}$ );

$P_{Wheat}$  and  $P_{CF}$  are the market price of wheat and control feed, respectively (€.  $\text{kg}^{-1}$ );

*wage* is the cost of a Full-time equivalent (FTE) for a year period (€.  $\text{FTE}^{-1} \cdot \text{year}^{-1}$ );

*L* represents the estimated labour requirement for carp production ( $\text{FTE. ha}^{-1}$ );

$P_{DF}$  is the projected sale price of experimental feed (€.  $\text{kg}^{-1}$ ).

Values for per-unit prices of carp, feed, stocking material and labour are determined based on prevailing market prices and listed in Table S.1. In order to arrive at economic conclusions more relevant at industrial level, per-hectare labour input (*L*) was derived from findings of a farm-level survey. This is detailed in Supplementary data S.1.

Projection of sale price of experimental feed was based on the sum of ingredient prices as of 2018 and additional costs covering cost of operating the production line, cost of drying loss and packaging cost (Table S.2.). The sum of these additional costs was identical to that is used when pricing the commercialized conventional feed.

## 3. Results

### 3.1. Growth performance and nutrient utilization

Growth performance and feed efficiencies are presented in Table 3. During the 155 days of the experiment, the one-year-old group increased their body weight nearly tenfold in both groups. The SGR and weight gain of juveniles were significantly higher in the experimental group. Although statistical significances in these indicators were not detected in the adult group, the final body weight differed significantly here, as well. The mortality rates over the season were between 8.5–17.1 % for the older age class, and 1.8–8.9% for the juvenile's stock. Feed as well as protein utilization efficiency also differed significantly in favour of experimental feed (AFCR: 1.56 vs 1.78; APER: 2.32 vs 2.08). The protein production value (APPV) reached 36.3 % in the experimental group compared to 31.8 % in the control group. Considering both age classes, per hectare gross was significantly higher under experimental diet than in the control group (3520 vs 3020  $\text{kg. ha}^{-1}$ ). The measured biomass of zooplankton and pond water temperature is presented in the Supplementary material in Figure S1 and S2. Significant differences between the ponds were not detected in pond food supply.

Parameter values of the linear growth model are presented in the Supplementary text (Table S.3). Among the juveniles, the coefficients of *time* (3.25) and *interaction effect between feed and age* (0.76) were significant ( $P < 0.05$ ), indicating that the slopes of the linear growth curves differed between diets in the second part of the season. These values



**Table 3**  
Growth performances and feed utilization parameters.

	Experimental group	Control group	p-value
<b>Juveniles</b>			
Initial weight juveniles (g)	45.4 ± 0.6	46.0 ± 0.5	0.252
Final weight juveniles (g)	497.9 ± 25.7	433.5 ± 18.0	<b>0.024</b>
Stocked biomass (kg ha <sup>-1</sup> )	280.3 ± 3.85	284.1 ± 3.11	NR
Harvested biomass (kg ha <sup>-1</sup> )	2973 ± 169.5	2542 ± 185.8	<b>0.041</b>
Weight gain (%) (juveniles)	996.9 ± 48.23	842.2 ± 28.9	<b>0.009</b>
Mortalities (%) (juveniles)	3.33 ± 0.91	5.14 ± 3.60	0.445
SGR juveniles (% day <sup>-1</sup> )	1.56 ± 0.03	1.46 ± 0.02	<b>0.010</b>
<b>Adults</b>			
Initial weight adult (g)	363.3 ± 8.3	358.6 ± 12.5	0.611
Final weight adult (g)	1533.9 ± 194.4	1323.4 ± 37.1	0.053
Stocked biomass (kg ha <sup>-1</sup> )	149.6 ± 3.43	147.6 ± 5.13	NR
Harvested biomass (kg ha <sup>-1</sup> )	546.8 ± 65.2	475.0 ± 28.5	0.156
Weight gain (%) (adult)	322.4 ± 54.4	269.6 ± 23.6	0.199
Mortalities (%) (adult)	13.33 ± 4.12	12.86 ± 3.78	0.891
SGR adult (% day <sup>-1</sup> )	0.91 ± 0.04	0.86 ± 0.04	0.210
total wheat offered (kg ha <sup>-1</sup> )	1441 ± 73	1419 ± 58	NR
total pellets offered (kg ha <sup>-1</sup> )	3430 ± 219	3228 ± 173	NR
AFCR (g g <sup>-1</sup> )	1.56 ± 0.02	1.78 ± 0.04	<b>0.002</b>
APER (g g <sup>-1</sup> )	2.32 ± 0.03	2.08 ± 0.04	<b>0.002</b>
APPV (%)	36.27 ± 2.05	31.77 ± 2.26	0.063
Gross yield (kg ha <sup>-1</sup> )	3520 ± 230	3020 ± 180	<b>0.041</b>
Biomass weight gain ratio	7.79 ± 0.53	6.57 ± 0.35	<b>0.029</b>

Weight gain (%):  $100 \times (\text{final average body weight} - \text{initial average body weight}) / \text{initial average body weight}$ .

Mortalities (%):  $100 \times \text{total number of individuals initial} / \text{total number of individuals final}$ .

AFCR: apparent feed conversion ratio = total feed offered / (total biomass final - total biomass initial).

SGR: Specific growth rate =  $100 \times (\ln \text{initial average weight} - \ln \text{final average weight}) / \text{feeding time}$ .

APER: apparent protein efficiency ratio = net weight gain (wet weight) / protein offered.

APPV: apparent protein production value =  $100 \times (\text{total biomass final} \times \text{final whole-body crude protein} - \text{total biomass initial} \times \text{initial whole-body crude protein}) / (\text{feed protein} \times \text{total feed offered})$ .

Gross yield = total biomass final (kg)/ha.

Biomass weight gain ratio = total biomass final / total biomass initial.

For statistical analysis Independent samples test were used, at 0.05 significance level.

NR - not relevant.

suggest a linear model where individual weight gain of carp juveniles is 3.25 g.day<sup>-1</sup> in the control group and 4.01 g.day<sup>-1</sup> in the experimental group. As coefficient of age factor was not significant, average individual weight of juveniles did not differ between treatments on 12 July, the last day before the start of feeding with formulated diets. Although the coefficient of the interaction effect (2.87) was not statistically significant in the adult group, modelled daily weight gain was 42 % higher in the experimental group than in the control group (9.63 vs 6.76 g.day<sup>-1</sup>). For better visualization of model results and predicted difference between treatments in weight development over time, measured individual weights and fitted regression lines are depicted in Fig. 1.

### 3.2. Composition and quality parameters of the marketable fish

Composition, slaughtering indices and some physical quality parameters of the market size fish flesh were determined at the end of season from adult fish population. There were no significant differences observed in the nutrient content of the fish fillets between the groups, as the difference between the individuals was very high (Table 4). The crude fat content of the meat was measured in a wide range, between 3.14 and 10.96 % (mean: 6.16 %) in the experimental group, and between 2.51 % and 8.92 % (mean: 6.43 %) in the control group. However, negative correlation was found between the water content and fat content of the fillet. The amount of crude protein differed only slightly between individuals and groups.

A similar result was obtained for conventional meat quality parameters as dripping loss, cooking loss, thawing loss, pH and colours. No statistically significant difference could be detected between the control and the experimental group for these parameters. The water holding capacity of the fish in the experimental group proved to be better than the control, although statistically not significantly. Both spontaneous and induced water loss were lower in this group. (Table 5)

For the fatty acid profile, minor difference was found in the total polyene fatty acids (TOTAL PUFA), TOTAL omega-6 content, but substantial individual differences suppressed the feed effect and statistically significant difference was not observed in most parameters. Level of the essential long chain poly-unsaturated fatty acids, EPA, DHA, and ARA, was relatively low, the amount varied between 0.19 and 0.65 mg/g, the highest amount determined for ARA. Significant differences were recorded for linoleic acid, 18:2n-6, in favour to the experimental group due to the inclusion of DDGS. Oleic acid (18:1n-9) level is almost higher in the control group (18.9 mg/g) compared to DDGS containing group (17.4 mg/g). We did not find significant differences (except for the hepatosomatic index) in the dressing indices at the end-of-season between the groups, although the higher filleting yield, hepatosomatic index and viscera index indicate a better weight gain of the experimental group (Table 5). In contrast, a small increase in oleic acid was observed in the control group, which demonstrates the appearance of fat depositions in the body.

### 3.3. Serum biochemical parameters and gene expression

As shown in Table 6, all the plasma biochemical indices analysed were not influenced significantly by the diet, except the phosphate, when higher values were measured for experimental group in both age classes. Significant differences between the age classes were found only in the amylase activity, but interaction within both factors were not observed. The amylase activity in the juveniles' fish was determined. The four experimental groups (feed\*age) had similar levels in creatine (0.18–0.24 mg/dl), glucose (60–77 mg/dl), Ca (9.3–10.4 mg/dl) total protein (3.0–3.15 g/dl), globulin (1.6–1.9 g/dl), alkaline phosphatase (66–144 U/L), cholesterol (143–154 mg/dl) and triglyceride (229–292 mg/dl).

Similarly, expression of the two examined genes did not differ significantly between the experimental and the control group. Fold differences were  $1.062 \pm 2.061$  for IGF-1 and  $0.89 \pm 1.829$  for HSP70 in the experimental group, relative to the control group (Figure S3.).

### 3.4. Economic calculations

Economic simulations indicate that ( $p < 0.01$ ) calculated profit, which is defined as 'income above feed, seed and labour costs', is higher for experimental groups than for control groups (9264 vs. 7938 €. ha<sup>-1</sup>). This is mainly attributed to increased revenue resulting from higher carp yields under DDGS-based diets. Feed costs between diets did not differ significantly, because savings associated with reduced feed cost (0.56 vs. 0.60 €.kg feed<sup>-1</sup> for experimental and control feed, respectively) was eliminated by increased use of feed due to better growth and higher standing biomass in experimental groups in the second part of the season.

Calculated benefit-cost ratio is also significantly higher for the experimental group than for the control group (Table 7.). Sensitivity analysis shows that even a 200 % increase in DDGS prices, raising the price of the experimental feed from 0.56 to 0.74 €.kg feed<sup>-1</sup>, would still result in a significantly better economic performance of the experimental group.

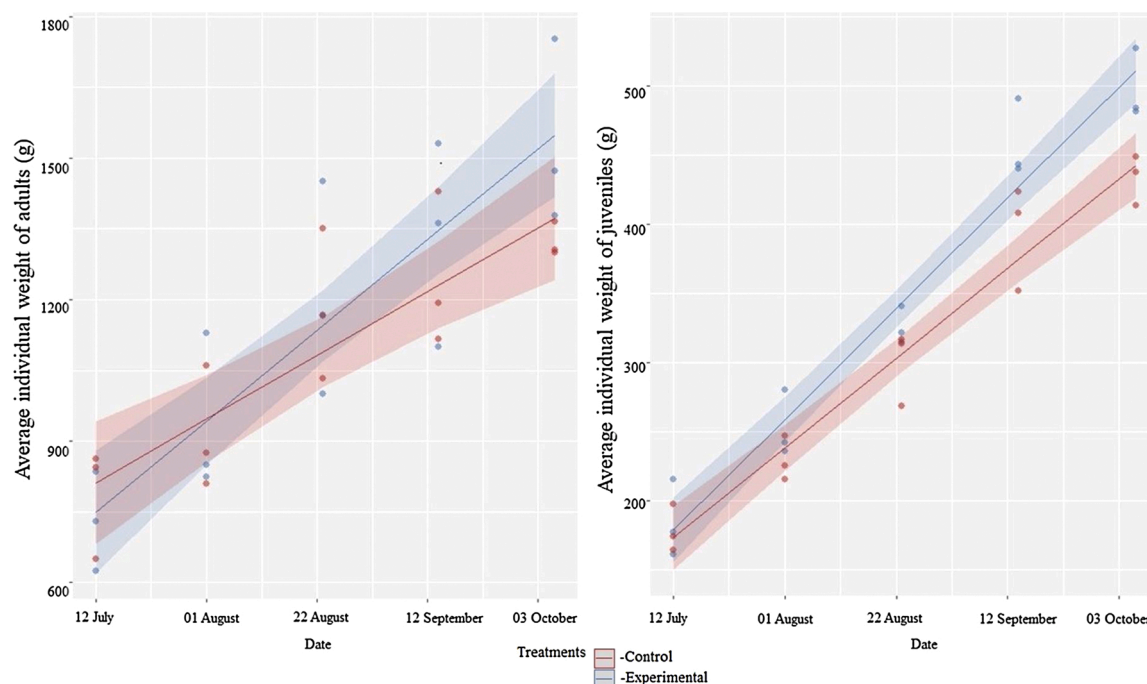


Fig. 1. Average individual weight development per treatment over the second part of the season. Dots represent result of tri-weekly sampling harvest in each pond, lines are fitted linear regressions based on model parameters presented in Table S.3.

Table 4

Composition of the market size (adult) fish flesh.

Filet composition (n = 9) (w.w.)	Experimental group	Control group	p value
Water (%)	74.40 ± 1.80	74.66 ± 1.90	0.852
Crude protein (%)	16.79 ± 0.31	16.81 ± 0.46	0.901
Crude fat (%)	6.16 ± 2.11	5.92 ± 1.94	0.801
Crude ash (%)	1.29 ± 0.12	1.19 ± 0.12	0.459
<b>Fatty acid composition (mg g<sup>-1</sup>)</b>			
16:0	7.78 ± 1.54	7.56 ± 1.24	0.784
16:1n-9	0.24 ± 0.05	0.27 ± 0.04	0.388
16:1n-7	2.46 ± 0.58	2.50 ± 0.29	0.878
18:0	1.94 ± 1.02	2.36 ± 0.51	0.392
18:1n-9	17.41 ± 4.02	18.94 ± 3.95	0.520
18:2n-6	8.34 ± 1.31	6.26 ± 1.68	<b>0.038</b>
18:3n-3	0.51 ± 0.06	0.54 ± 0.13	0.710
20:4n-6 (ARA)	0.65 ± 0.13	0.62 ± 0.06	0.710
20:5n-3 (EPA)	0.19 ± 0.04	0.22 ± 0.05	0.209
22:6n-3 (DHA)	0.38 ± 0.04	0.42 ± 0.04	0.617
TOTAL SFA	10.51 ± 2.07	10.58 ± 1.85	0.785
TOTAL MUFA	22.57 ± 5.00	24.46 ± 4.72	0.516
TOTAL PUFA	11.21 ± 1.55	9.04 ± 2.04	0.064
TOTAL n-3	1.23 ± 0.09	1.33 ± 0.18	0.241
TOTAL n-6	9.98 ± 1.48	7.70 ± 1.87	<b>0.041</b>
Total lipid	47.21 ± 8.68	47.16 ± 8.73	0.993

Total SAT: 12:0 + 14:0 + 15:0 + 16:0 + 16:0 + 17:0 + 17:0 + 18:0 + 19:0 + 20:0 + 21:0 + 22:0 + 23:0.

Total MUFA: 14:1n-5 + 15:1 + 16:1n-9 + 16:1n-7 + 16:1n-5 + 17:1n-8 + 18:1n-9 + 18:1n-7 + 18:1n-5 + 19:1n-10 + 20:1n-11 + 20:1n-9 + 20:1n-7 + 22:1n-11 + 22:1n-9 + 22:1n-7 + 24:1n-11 + 24:1n-9 + 24:1n-7.

Total n-3: 18:3n-3 + 18:4n-3 + 20:3n-3 + 20:4n-3 + 20:5n-3 + 22:3n-3 + 22:4n-3 + 22:5n-3 + 22:6n-3 + 24:5n-3 + 24:6n-3.

Total n-6: 18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 + 20:4n-6 + 22:2n-6 + 22:4n-6 + 22:5n-6.

Total PUFA: Total n-3 + Total n-6.

ARA - arachidonic acid, EPA - eicosapentaenoic acid, DHA - docosahexaenoic acid.

Table 5

Slaughtering indices and some physical quality parameters of the market size fish.

Slaughtering indices (n = 9)	Experimental group	Control group	p value
Filleting yield (%)	36.6 ± 2.4	35.2 ± 2.5	0.366
Dressing yield (%)	70.0 ± 2.1	70.2 ± 1.4	0.538
Hepatosomatic index (%)	3.9 ± 0.4	3.5 ± 0.4	<b>0.040</b>
Visceral index (%)	15.6 ± 1.9	14.9 ± 2.5	0.530
Gonadosomatic index (%)	5.8 ± 2.4	6.1 ± 2.7	0.786
<b>Physical quality parameters</b>			
Dripping loss (%)	2.81 ± 0.73	2.97 ± 1.27	0.732
Cooking loss (%)	21.32 ± 3.95	22.89 ± 2.67	0.345
Thawing loss (%)	5.69 ± 1.54	5.7 ± 1.82	0.991
pH	6.39 ± 0.04	6.39 ± 0.07	0.991
Lightness (L)	44.34 ± 3.08	44.34 ± 0.99	0.993
Redness (a)	1.45 ± 0.15	1.28 ± 0.94	0.775
Yellowness (b)	1.6 ± 0.77	1.76 ± 0.59	0.621

## 4. Discussion

### 4.1. Growth performance and nutrient utilization

One of the aims of the Central European aquafeed industry is to reduce the cost of formulations by substituting imported protein-rich ingredients with locally available with cheaper ones such that growth performance of target species is not compromised. Therefore, DDGS has a great potential in carp feeds since it is cheaper than soybean meal and performed well in growth experiments when common carp was reared on both soybean meal and DDGS-based diets (Révész et al., 2019, 2020). An additional challenge of feed producers is to make formulated feeds economically viable for Central European carp farmers the majority of which follow a semi-intensive farming technology and use only cereal grains to complement pond food resources in order to minimize nutrition costs (Gyalog et al., 2011; Marković et al., 2016). Given this background, in our experiment use of compound feeds was combined with a grain-fed period in order to utilise pond food efficiently and optimize requirement for external nutrient inputs. Although pond food plays an important role in carp biomass gain under semi-intensive

**Table 6**

Biochemical parameters of blood plasma (n = 9) at the end of trial.

FEED/AGE	CREA mg. dl <sup>-1</sup>	GLU mg. dl <sup>-1</sup>	PHOS mg. dl <sup>-1</sup>	Ca mg. dl <sup>-1</sup>	TP g. dl <sup>-1</sup>	GLOB g. dl <sup>-1</sup>	ALP U. L <sup>-1</sup>	CHOL mg. dl <sup>-1</sup>	TRIG mg. dl <sup>-1</sup>	AMY U. L <sup>-1</sup>
<b>Exp. juveniles</b>	0.24 ± 0.17	74.00 ± 26.07	9.97 ± 2.27	9.62 ± 1.09	3.15 ± 0.43	1.92 ± 0.23	95.42 ± 31.20	150.57 ± 22.99	233.85 ± 29.79	207.57 ± 47.21
<b>Contr. juveniles</b>	0.19 ± 0.09	60.77 ± 12.48	6.73 ± 1.50	9.43 ± 0.51	3.15 ± 0.31	1.84 ± 0.28	102.11 ± 58.18	154.22 ± 17.67	254.88 ± 81.65	192.88 ± 55.43
<b>Exp. adult</b>	0.18 ± 0.09	77.12 ± 25.60	8.66 ± 3.99	10.45 ± 2.50	3.11 ± 0.43	1.83 ± 0.28	144.25 ± 95.43	143.87 ± 31.11	229.50 ± 69.53	144.37 ± 34.67
<b>Contr. adult</b>	0.26 ± 0.11	66.37 ± 18.49	6.16 ± 2.63	9.32 ± 1.13	3.00 ± 0.56	1.66 ± 0.44	66.00 ± 24.35	146.50 ± 17.15	292.75 ± 131.75	160.87 ± 45.88
<b>FEED</b>	0.809	0.118	<b>0.006</b>	0.223	0.720	0.271	0.104	0.701	0.185	0.957
<b>AGE</b>	0.834	0.563	0.341	0.506	0.531	0.246	0.768	0.380	0.594	<b>0.008</b>
<b>FEED * AGE</b>	0.146	0.869	0.707	0.387	0.728	0.696	0.056	0.950	0.502	0.355

Exp. Juveniles: Experimental feed, age: juveniles; Contr. Juveniles: Control feed, age: juveniles; Exp. Adult: experimental feed, age: adult; Contr. Adult: control feed, age: adult.

CREA - Creatin, GLU - Glucose, PHOS - Phosphate, Ca - Calcium, TP - Total Protein, GLOB - Globulin, ALP - Alkaline Phosphatase, CHOL - Cholesterol, TRIG - Triglyceride, AMY - Amylase.

**Table 7**

Economic indicators of the treatments.

Parameters	Units	Experimental group	Control group	p value
Cost of feed <sup>1</sup>	€, kg <sup>-1</sup>	0.56	0.60	NR <sup>2</sup>
Feed costs	€, ha <sup>-1</sup>	2158 ± 110	2142 ± 91	NR
Seed costs	€, ha <sup>-1</sup>	1225 ± 7	1231 ± 9	NR
Labour cost	€, ha <sup>-1</sup>	1374 ± 52	1235 ± 41	0.041
Feed, seed and labour costs	€, ha <sup>-1</sup>	4756 ± 168	4607 ± 132	0.380
Gross income	€, ha <sup>-1</sup>	9264 ± 485	7938 ± 398	0.040
Income above feed, seed and labour costs	€, ha <sup>-1</sup>	4509 ± 318	3331 ± 268	0.016
Benefit-Cost Ratio (BCR)	€, € <sup>-1</sup>	1.95 ± 0.03	1.72 ± 0.04	0.003

<sup>1</sup> Cost of feed: market price of commercial feed in control group and projected sale price of DDGS-based experimental feed in experimental group.

<sup>2</sup> NR-not relevant.

conditions, in our experiment zooplankton availability did not differ between the ponds implying that difference in fish growth cannot be attributed to pond food.

In Central Europe gross yields in carp farming range from 500 to 1500 kg ha<sup>-1</sup> under semi-intensive cereal-based technologies, while production intensity may be increased up to 3000 kg ha<sup>-1</sup> with the use of compound feeds (Gyalog et al., 2017; Marković et al., 2016; Roy et al., 2020). The average yield (3420 kg ha<sup>-1</sup>) of experimental groups in our study was high in comparison with these values, which is important in an era when climate change and societal drivers stress the need of efficient use of land and water resources (Gyalog et al., 2021). Gross yield of experimental treatment was also significantly higher than that of control treatment.

The weight gain ratio calculated for the combined age class was significantly higher under DDGS-based diet (7.8) than with commercial feed (6.6). Within the experimental group the weight gain ration of the juvenile (1year+) biomass was 10.6, while for the adult (2year+) biomass this metric was 3.7. These values are superior to those calculated for cereal-based semi-intensive technology. Under Hungarian conditions Horvath et al. (2002) calculates that weight gain ratio of carp juveniles is around six at a stocking rate of 300 kg ha<sup>-1</sup>. Weight gain ratio of adult (2 year+) Common carp stocked at a density of 300–400 kg ha<sup>-1</sup> is generally around 3–4 assuming a 6–7 months-long production season (Horvath et al., 2002; Varga et al., 2020). Mráz et al. (2012) reported a similar ration for biomass gain under experimental conditions in the Czech Republic. However, industry average is somewhat lower in Hungary due to higher mortalities, as reported by Gyalog et al. (2017).

APPV (sometimes also referred as protein retention efficiency and can directly be translated to nitrogen efficiency) and APER (a measure of biological weight increases per weight unit of protein fed) values are often used to evaluate the nutrient efficiency of production technologies.

In our experiment, APER differed significantly between treatments having much better values for DDGS experimental group, even though the essential amino acid supply was more balanced in the control group. Similar tendency is observed for PPV without statistical significance. The protein utilization efficiency calculated (APPV = 36.3 %) for the experimental treatment is comparable to the upper side of the range of nitrogen utilisation efficiencies modelled for cereal based Common carp farming in the Czech Republic (Roy et al., 2020), and higher than that is calculated for Hungarian semi-extensive carp farms (Gál et al., 2016). Calculated feed conversion rates (1.56 in experimental group and 1.78 in control group) should be interpreted in the context of pond culture conditions. In China, the biggest carp producer a median value of 1.5 for the feed conversion rate was reported for carps (Chiu et al., 2013), while the global average is 1.8 (Tacon and Metian, 2008). Taking into account that compound feed accounted for only 70 percent of total feed intake in our experiment, the DDGS-based formulation proved to be nutritionally efficient.

Several studies demonstrated that the DDGS content feed positively impacted the growth of fish and also the production parameters (Li et al., 2011; Overland et al., 2013; Wu et al., 1996, 1997; Sándor et al., 2021). According to these studies the DDGS inclusion level varying between 15–60 % depending on the feeding behaviour of the targeted species. In our trial in the pilot farm condition 40 % DDGS level in the carp feed demonstrated a positive impact to the production parameters. Similarly, Révész et al. (2019, 2020) presented worthwhile results for common carp in closed rearing system. This investigation confirms that corn DDGS is highly utilizable by common carp juveniles, furthermore, advantages in production and nutrient utilization of adult aged class carp could be observed.

#### 4.2. Composition and quality parameters of the marketable fish

There was no adverse effect of ingredients source on the proximate composition of the common carp fillets determined from the market size individuals. The fat content, which is the most dominant indicator of the flesh quality, varied as usual in the pond systems did with high individual variability. The fat content of the carp fillet produced in traditional carp farming using grain supplementation is usually much higher (10–15 %) compared to the carps fed with composed, high protein content diet (Geri et al., 1995; Trbovic, 2014; Sándor et al., 2020). High energy content feeds increased the growth and in same time the fat content of the flesh, while the protein remain unchanged (Kaushik, 1995). It seems that body weight positively affected the visceral and hepatosomatic indices, but slightly contributed to the increase in the filleting and dressing yield. These findings are in accordance with the results of Bauer and Schlott (2009). It is generally accepted, filleting yield is affected by many factors, such as age, weight, sex, body shape reproduction (Souza et al., 2015). For example, a significant decrease of fillet yield can be observed on female carps prior to spawning (Dubost et al., 2007). Following earlier findings (Bauer and Schlott, 2009; Dubost et al., 2007) our results are in accordance with attainable 70 % dressing yield and 36 % filleting yield for common carp. Interestingly, Varga et al. (2013a) described the fillet mean yield 44 % investigated four Hungarian common carp strains. Prchal et al. (2020) found that the filleting yield of Amur mirror carp was 50 %, which is higher than usual values in common carp. These differences are most likely due to the different filleting methods (commercial vs experimental).

It is well known that the fatty acid composition of the fish is markedly influenced by the lipid pattern of the given feed. In our case this presumption is reflected in linoleic acid level (18:2n-6) and in total n-6 and total PUFA level which is caused by fatty acid profile of DDGS. The rest of the fatty acids were not differing significantly between treatments. The fatty acid composition of the feeds differing mainly in LC n-3 FAs which are in higher level in the control feed. Since this tendency is not visible in the fillet, it is assumed that n-3 FAs were strongly utilized in oxidation process in control group compared to DDGS group. However, this observation should be smoothed out by the possible zooplankton intake during the season. Unfortunately, reduced level of long chain polyunsaturated fatty acids (LcPUFA) is observed in both groups, notwithstanding pond fish can take long chain n-3 from the natural food feeding (Mráz and Pickova, 2011; Steffens, 2016). For this reason, feeding a final diet over the last period of fish rearing and slaughtering is advised when the tissue n-3 level could be enriched (Mráz et al., 2012).

The measured data on the fillet physical quality are similar to previous results on the meat quality of carp under domestic conditions (Varga et al., 2013a). Considering the physical quality parameters (water holding capacity, pH, colour) all fillet samples were identical. All type of moisture loss (induced: cooking and thawing loss, and spontaneous: dripping loss) were lower in experimental group compared to the control, without significant difference. The muscle intracellular water loss is introduced by tetanic contraction of the muscle fibers (spontaneous dripping loss), developing the *rigor mortis*. In this process the protein components of the third filament are degraded. Regarding the provoked thawing loss, freezing disrupts cellular membranes, the Z-line, and the filamentous structure as well (Takahashi et al., 1993). The degradation process revealed after the thawing is associated with the increase of the  $\text{Ca}^{2+}$  concentration around the myofibrils, thus enabling further contraction and fluid loss through the damaged membrane structure. In addition, the extent of thawing loss is primarily bound to the proteins (Varga et al., 2013a).

After the death of fish, the physical and chemical degradation processes of tissues were started. Lactate as an end-product of anaerobic metabolism is accumulated in muscles. It results a pH fall of the fish flesh in the first 24 h *post mortem*. Our results on carp fillet pH are similar to previous findings (Fauconneau et al., 1995; Varga et al., 2013a). The

colour of carp flesh is mainly affected by the feed. Cereal feed, independently of the different environment results an identical meat colour (Varga et al., 2013a). Using unusual feeds in carp feeding, such as walnut, only slight changes can be detected in flesh lightness (L value) (Varga et al., 2013b).

In our study no difference was found between the control and experimental group regarding the colour. Herath et al. (2016) showed that the dietary treatments did not affect the lightness, redness, yellowness of the flesh of Nile tilapia fed DDGS. Our results suggest that dietary inclusion of DDGS (40 %) does not negatively affect fillet water holding capacity, pH and color.

#### 4.3. Serum biochemical parameters and gene expression

Biochemical parameters of the blood plasma are dependent on fish size, species, nutritional status and environmental circumstances (Chen et al., 2003; Tan et al., 2013; Velisek et al., 2009; Peres et al., 2014). In our experiment, the level of phosphate significantly increased in both age classes fed with the experimental feed, and amylase activity was significantly higher in the older age classes. Other biochemical parameters did not differ significantly between the treatments or age classes. The high phosphate level is attributed to the available digestible phosphor present in DDGS compared to other plant ingredients (Révész et al., 2020).

IGF-1 mediates many of the growth-promoting effects of growth hormone (GH) and it is synthesized in various tissues, mainly in the liver of fish (Duan, 1998). Nutritional status has a profound effect on hepatic IGF-1 mRNA level in fish. Starvation caused a significant decrease of IGF-1 mRNA level in the liver of coho salmon (*Oncorhynchus kisutch*), grouper (*Epinephelus coioides*) and channel catfish (*Ictalurus punctatus*) (Duan and Plisetskaya, 1993; Pedroso et al., 2006; Peterson and Waldbieser, 2009). Growth-promoting effect of diets correlated with elevated expression of hepatic IGF-1 in gibel carp (*Carassius auratus gibelio*) and Nile tilapia (*Oreochromis niloticus*) (Tu et al., 2015; Hassaan et al., 2019). Our results are consistent with these previous findings, as dietary inclusion of DDGS did not positively or negatively affect growth of adult fish and IGF-1 expression in their livers. Heat shock proteins are the main regulators of stress response in fish (Basu et al., 2002; Shatiyaa and Vijayan, 2003). Elevated expression of HSP70 in fish liver can be a sign of malnutrition, as it was demonstrated in gilthead sea bream (*Sparus aurata*) (Kokou et al., 2016). In our experiment, 40 % inclusion of DDGS into the fish feed did not change the expression level of HSP70 in the liver of common carp, which means that this level of DDGS is tolerable for this species, and it is in concern with the result of our previous studies (Révész et al., 2019).

#### 4.4. Economics

Economic benefits of applying DDGS in aquafeeds may arise due to cheaper feed formulations or improved production efficiency, which either can be reflected in increased yield per unit of infrastructure (measured in  $\text{m}^3$  or ha) or improved feed conversion. For some species trade-offs occur between production efficiency and cost of formulations when expensive ingredients with high nutritional value (i.e. fish meal) are substituted with DDGS. Coyle et al. (2004) and Allam et al. (2020) reported reduced costs of feed formulations but worse growth performance and feed conversion for tilapia and striped catfish, respectively. In these trade-off situations, economic benefit arises only if reduction in unit cost of feed compensates the economic loss associated with lower yields and higher feed conversion. However, in case of some omnivorous species for which commercial feeds already formulated dominantly with plant proteins, DDGS can replace vegetable ingredients such that cost of formulation is decreased and growth performance is increased at the same time. From economic point this means that savings made on cost of feed is topped with further economic gains stemming from better growth of fish (Oliveira et al., 2020). In our study, we demonstrated a marked



economic advantage of applying DDGS in carp feed in comparison with a commercial formula. Although the cost of feed formulation containing 40 % DDGS is only slightly lower than that of commercial formula, significant benefit arises because of increased per-hectare revenues associated with increased growth rates.

Benchmarking our results against the economic performance of cereal-based semi-intensive carp farming is a hard task, because empirically derived financial data is not available in a regional context. However, using the Climepond software, which performs both biological and economic simulations for Hungarian carp farms assuming a feeding technology based on cereal grains, it can be calculated that best performing pond management scenarios come with an 'income above feed, seed and labour costs' in the range of 1200–1300 € ha<sup>-1</sup> and with benefit cost ratios around 1.5–1.6 (Gyalog et al., 2021). These numbers suggest that combining the use of DDGS-based compound feed with periods of grain feeding (our experimental treatment) is a viable carp farming technology.

## 5. Conclusions

Based on a carp feeding experiment conducted throughout the season, it can be said that the feed containing 40 % DDGS performed well in terms of production and feed utilization parameters and could be a promising component of carp feed to be placed on the market in the future. Based on the results of the economic assessment, the use of experimental feed formula in carp culture generates much higher net income per hectare compared to the use of conventional feeds. At the same time, quality of the fish produced with DDGS-based diet is favorable and they lead to similar fillet quality values as commercial feeds, which has been used in production for a long time.

## Author statement

**Zsuzsanna J. Sándor:** Conceptualization, Methodology, Writing, Original draft preparation, Visualization, Reviewing.

**Norbert Révész:** Conceptualization, Methodology, Data curation.

**Dániel Varga:** Investigation, Writing.

**Flórián Tóth:** Formal analysis, Investigation.

**László Ardó:** Investigation, Writing.

**Gergő Gyalog:** Methodology, Writing, Validation, Reviewing.

## Declaration of Competing Interest

The authors report no declarations of interest.

## Acknowledgment

The work was supported by the European Regional and Development Fund and the Government of Hungary within the projects GINOP-2.3.2-15-2016-00025 and 2020-4.1.1-TKP2020 funding scheme.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.aqrep.2021.100819>.

## References

- Abouel Azm, F.R., Kong, F., Tan, Q., Zhu, Y., Yu, H., Yao, J., Luo, Z., 2021. Effects of replacement of dietary rapeseed meal by distiller's dried grains with solubles (DDGS) on growth performance, muscle texture, health and expression of muscle-related genes in grass carp (*Ctenopharyngodon idellus*). *Aquaculture* 533, 736169. <https://doi.org/10.1016/j.aquaculture.2020.736169>.
- Allam, B.W., Khalil, H.S., Mansour, A.T., Srour, T.M., Omar, E.A., Nour, A.A.M., 2020. Impact of substitution of fish meal by high protein distillers dried grains on growth performance, plasma protein and economic benefit of striped catfish (*Pangasianodon hypophthalmus*). *Aquaculture* 517. <https://doi.org/10.1016/j.aquaculture.2019.734792>.
- Basu, N., Todgham, A.E., Ackerman, P.A., Bibeau, M.R., Nakano, K., Schulte, P.M., Iwama, G.K., 2002. Heat shock protein genes and their functional significance in fish. *Gene* 295, 173–183.
- Bauer, C., Schlott, G., 2009. Fillet yield and fat content in common carp (*Cyprinus carpio*) produced in three Austrian carp farms with different culture methodologies. *J. Appl. Ichthyol.* 25, 591–594. <https://doi.org/10.1111/j.1439-0426.2009.01282.x>.
- Chen, C.Y., Wooster, G.A., Getchell, R.G., Bowser, P.R., Timmons, M.B., 2003. Blood chemistry of healthy, nephrocalcinosis-affected and ozone-treated tilapia in a recirculation system, with application of discriminant analysis. *Aquaculture* 218 (1–4), 89–102.
- Chiu, A., Li, L., Guo, S., Bai, J., Fedor, C., Naylor, R.L., 2013. Feed and fishmeal use in the production of carp and tilapia in China. *Aquaculture* 414–415, 127–134. <https://doi.org/10.1016/j.aquaculture.2013.07.049>.
- Ciric, M., Subakov-Simic, G., Dulic, Z., Bjelanovic, K., Cicovacki, S., Markovic, Z., 2015. Effect of supplemental feed type on water quality, plankton and benthos availability and carp (*Cyprinus carpio* L.) growth in semi-intensive mono - culture ponds. *J. Aquac. Res. Dev.* 46, 777–788. <https://doi.org/10.1111/are.12230>.
- Coyle, S.D., Mengel, G.J., Tidwell, J., Webster, C.D., 2004. Evaluation of growth, feed utilization, and economics of hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus*, fed diets containing different protein sources in combination with distillers dried grains with solubles. *J. Aquac. Res. Dev.* 35 (4), 365. <https://doi.org/10.1111/j.1365-2109.2004.01023.x>.
- DDGS, 2018. 4<sup>th</sup> Revised Edition of the U.S. Grains Council DDGS User Handbook – Precision DDGS Nutrition.
- Dickson, M., Nasr-Allah, A., Kenawy, D., Kruijsen, F., 2016. Increasing fish farm profitability through aquaculture best management practice training in Egypt. *Aquaculture* 465, 172–178. <https://doi.org/10.1016/j.aquaculture.2016.09.015>.
- Diógenes, A.F., Castro, C., Miranda, A.C., Oliva-Teles, A., Peres, H., 2018. Dietary replacement of fishmeal by corn distillers dried grains with solubles (DDGS) in diets for turbot (*Scophthalmus maximus*, Linnaeus, 1758) Juveniles. *Aquaculture* 492, 113–122. <https://doi.org/10.1016/j.aquaculture.2018.04.005>.
- Duan, C., 1998. Nutritional and developmental regulation of insulin-like growth factors in fish. *J. Nutr.* 128, 306S–314S.
- Duan, C., Plisetskaya, E.M., 1993. Nutritional regulation of insulin-like growth factor I mRNA expression in salmon tissues. *J. Endocrinol.* 139, 243–252.
- Dubost, N., Masson, G., Moreteau, J.C., 2007. Gonad development and filleting yield of common carp *Cyprinus carpio* L. Reared in ponds in Eastern France. *J. Appl. Ichth.* 13 (1), 15–20. <https://doi.org/10.1111/j.1439-0426.1997.tb00092.x>.
- FAO, 2020. Fishery and aquaculture statistics. Global Aquaculture Production 1950–2018 (FishstatJ). FAO Fisheries and Aquaculture Department, Rome [online] Updated 2020. [www.fao.org/fishery/statistics/software/fishstatj/en](http://www.fao.org/fishery/statistics/software/fishstatj/en).
- Fauconneau, B., Alami-Durante, H., Laroche, M., Marcel, M., Vallot, D., 1995. Growth and meat quality relations in carp. *Aquaculture* 129, 265–297. [https://doi.org/10.1016/0044-8486\(94\)00309-C](https://doi.org/10.1016/0044-8486(94)00309-C).
- Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226, 497–509. <https://doi.org/10.1007/s10858-011-9570-9>.
- Gál, D., Pekár, F., Kerepeczki, É., 2016. A survey on the environmental impact of pond aquaculture in Hungary. *Aquac. Int.* 24, 1543–1554. <https://doi.org/10.1007/s10499-016-0034-9>.
- Geri, G., Poli, B.M., Gualtieri, M., Lupi, P., Parisi, G., 1995. Body traits and chemical composition of muscle in the common carp (*Cyprinus carpio* L.) as influenced by age and rearing environment. *Aquaculture* 129, 329–333. [https://doi.org/10.1016/0044-8486\(94\)00300-D](https://doi.org/10.1016/0044-8486(94)00300-D).
- Gyalog, G., Váradi, L., Gál, D., 2011. Is intensification a viable way for pond culture in Central and Eastern Europe? *AAEL Bioflux* 4, 584–589.
- Gyalog, G., Oláh, J., Békefi, E., Lukácsik, M., Popp, J., 2017. Constraining factors in Hungarian carp farming: an econometric perspective. *Sustainability* 9, 2111. <https://doi.org/10.3390/sul1236726>.
- Gyalog, G., Sturm, A., Wätzold, F., Berzi-Nagy, L., Csukás, B., Varga, M., 2021. ClimePond: A software-based decision support system integrating biophysical and economic modelling for pond aquaculture production under climate change in Hungary. *Aquaculture in review*.
- Halver, J.E., Hardy, R.W., 2002. Fish Nutrition, 3<sup>rd</sup> ed. Academic Press, pp. 259–308. <https://doi.org/10.1016/b978-012319652-1/50006-50009>.
- Hassan, M.S., El-Sayed, A.I.M., Soltan, M.A., Iraqi, M.M., Goda, A.M., Davies, S.J., El-Haroun, E.R., Ramadan, H.A., 2019. Partial dietary fish meal replacement with cotton seed meal and supplementation with exogenous protease alters growth, feed performance, hematological indices and associated gene expression markers (GH, IGF-1) for Nile tilapia, *Oreochromis niloticus*. *Aquaculture* 503, 282–292. <https://doi.org/10.1016/j.aquaculture.2019.01.009>.
- Herath, S.S., Haga, Y., Satoh, S., 2016. Effects of long-term feeding of corn co-product-based diets on growth, fillet color, and fatty acid and amino acid composition of Nile tilapia, *Oreochromis niloticus*. *Aquaculture* 464, 205–212. <https://doi.org/10.1016/j.aquaculture.2016.06.032>.
- Hlavak, D., Másilko, J., Anton-Pardo, M., Hartman, P., Regenda, J., Vejsada, P., 2016. Compound feeds and cereals as potential tools for improved carp *Cyprinus carpio* production. *Aquacult. Environ. Interact.* 8, 647–657. <https://doi.org/10.3354/aei00206>.
- Honikel, K.O., 1998. Reference methods for the assessment of physical characteristics of meat. *Meat Sci.* 49 (4), 447–457. [https://doi.org/10.1016/S0309-1740\(98\)00034-5](https://doi.org/10.1016/S0309-1740(98)00034-5).
- Horvath, L., Tamas, G., Seagrave, C., 2002. Carp and Pond Fish Culture, 2 ed. Blackwell Science, Oxford, UK. <https://doi.org/10.1002/9780470995662>.
- Kaushik, S.J., 1995. Nutrient requirements, supply and utilization in the context of carp culture. *Aquaculture* 129, 225–241. [https://doi.org/10.1016/0044-8486\(94\)00274-R](https://doi.org/10.1016/0044-8486(94)00274-R).

- Kokou, F., Adamidou, S., Karacostas, I., Sarropoulou, E., 2016. Sample size matters in dietary gene expression studies – a case study in the gilthead sea bream (*Sparus aurata* L.). Aquac. Rep. 3, 82–87. <https://doi.org/10.1016/j.aqrep.2015.12.004>.
- Lammers, P.J., Kerr, B.J., Honeyman, M.S., 2015. Biofuel co-products as swine feed ingredients: combining corn distillers dried grains with solubles (DDGS) and crude glycerin. Anim. Feed Sci. Technol. 201, 110–114. <https://doi.org/10.1016/j.anifeeds.2014.12.013>.
- Li, M.H., Oberle, D.F., Lucas, P.M., 2011. Evaluation of corn distiller's dried grains with solubles and brewer's yeast in diets for channel catfish *Ictalurus punctatus* (Rafinesque). Aquac. Res. 42, 1424–1430. <https://doi.org/10.1111/j.1365-2109.2010.02734.x>.
- Liu, K., 2011. Chemical composition of distillers grains, a review. J. Agric. Food Chem. 59, 1508–1526. <https://doi.org/10.1021/jf103512z>.
- Marković, Z., Stanković, M., Rašković, B., Dulić, Z., Živić, I., Poleksić, V., 2016. Comparative analysis of using cereal grains and compound feed in semi-intensive common carp pond production. Aquac. Int. 24, 1699–1723. <https://doi.org/10.1007/s10499-016-0076-z>.
- Mazurkiewicz, J., 2009. Utilization of domestic plant components in diets for common carp *Cyprinus carpio* L. Arch. Polish Fish. 17, 5–39. <https://doi.org/10.2478/v10086-009-0001-4>.
- Mráz, J., Picková, J., 2011. Factors influencing fatty acid composition of common carp (*Cyprinus carpio*) muscle. Neuroendocrinol. Lett. 32 (Suppl. 2), 3–8. NEL320811R01.
- Mráz, J., Máčková, J., Kozák, P., Picková, J., 2012. Lipid content and composition in common carp optimization of n-3 fatty acids in different pond production systems. J. Appl. Ichthyol. 28, 238–244. <https://doi.org/10.1111/j.1439-0426.2011.01904.x>.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Wagner, H., 2012. Vegan: Community Ecology Package. R Package Version 1.17-2 <https://cran.r-project.org/http://vegan.r-forge.r-project.org/>. Accessed on 18 Marc 2020.
- Oliveira, K., Segura, J.G., Oliveira, B., Medeiros, A.C.L., Zimba, R.D., Viegas, E.E.M., 2020. Distillers' dried grains with soluble in diets for Pacu, *Piaractus mesopotamicus* juveniles: growth performance, feed utilization, economic viability, and phosphorus release. Anim. Feed Sci. Technol. 262, 114393 <https://doi.org/10.1016/j.anifeeds.2020.114393>.
- OMMI, 2001. Állattenyésztés évkönyve. In: Ferenc, Flink (Ed.), Országos Mezőgazdasági Intézet. OMMI, Budapest, Hungary, p. 200. (In Hungarian). <http://www.odrportal.hu/web/guest/record/-/record/MOKKAI0008709403>.
- Overland, M., Krogdahl, Å., Shurson, G., Skrede, A., Denstadli, V., 2013. Evaluation of distiller's dried grains with solubles (DDGS) and high protein distiller's dried grains (HPDDG) in diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 416–417, 201–208. <https://doi.org/10.1016/j.aquaculture.2013.09.016>.
- Pedroso, F.L., de Jesus-Ayson, E.G.T., Cortado, H.H., Hyodo, S., Ayson, F.G., 2006. Gen. Comp. Endocrinol. 145, 237–246. <https://doi.org/10.1016/j.ygcen.2005.09.001>.
- Peres, H., Santos, S., Oliva-Teles, A., 2014. Blood chemistry profile as indicator of nutritional status in European seabass (*Dicentrarchus labrax*). Fish Phys. and Biochem. 40 (5), 1339–1347. <https://doi.org/10.1007/s10695-014-9928-5>.
- Peterson, B.C., Waldbieser, B.C., 2009. Effects of fasting on IGF-1, IGF-II and IGF-binding protein mRNA concentrations in channel catfish (*Ictalurus punctatus*). Dom. Anim. Endocrin. 37, 74–83. <https://doi.org/10.1016/j.domaniend.2009.03.004>.
- Prchal, M., Kocour, M., Vandeputte, M., Kause, A., Vergnet, A., Zhao, J., Gela, D., Kašpar, V., Genestout, L., Bestin, A., Haffray, P., Bugeon, J., 2020. Morphological predictors of slaughter yields using 3D digitizer and their use in a common carp breeding program. Aquaculture 734993. <https://doi.org/10.1016/j.aquaculture.2020.734993>.
- R Development Core Team, 2013. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Accessed on 18 Marc 2020. <http://www.R-project.org/>.
- Révész, N., Havasi, M., Lefler, K.K., Hegyi, Á., Ardó, L., Sándor, Zs., 2019. Protein replacement with Dried Distiller's Grain with Solubles (DDGS) in practical diet of common carp (*Cyprinus carpio*). AACL Bioflux 12 (4), 1174–1188.
- Révész, N., Kumar, B., Bogevis, A.S., Fazekas, Gy., Jeney, Zs., Hegyi, Á., Sándor, Zs.J., 2020. Effect of temperature on digestibility, growth performance and nutrient utilization of corn distiller's dried grains with soluble (DDGS) in Common carp juveniles. Aquac. Res. 51, 828–835. <https://doi.org/10.1111/are.14432>.
- Roy, K., Vrba, J., Kaushik, S.J., Mraz, J., 2019. Feed-based common carp farming and eutrophication: is there a reason for concern? Rev. in Aquac. 2016, 1–23. <https://doi.org/10.1111/raq.12407>.
- Roy, K., Vrba, J., Kaushik, S.J., Mraz, J., 2020. Nutrient footprint and ecosystem services of carp production in European fishponds in contrast to EU crop and livestock sectors. J. of Cleaner Prod. 270, 122268 <https://doi.org/10.1016/j.jclepro.2020.122268>.
- Ruttay, A., 2016. In: Szarvas, Péter A. (Ed.), Az édesvízi akvakultúra alapjai és a magyar haltenyésztés sajátosságai. NAIK Halászati Kutatóintézet (In Hungarian).
- Sándor, Z.J., Révész, N., Bíró, J.N., Rónyai, A., 2020. Comparison of carp filet quality produced in semi-intensive pond system using different type of feeds. AACL Bioflux 13 (5), 2970–2981.
- Sándor, Z.J., Révész, N., Lefler, K.K., Čolović, R., Banjac, V., Kumar, S., 2021. Potential of corn distiller's dried grains with solubles (DDGS) in the diet of European catfish (*Silurus glanis*). Aquac. Rep. 20, 100653 <https://doi.org/10.1016/j.aqrep.2021.100653>.
- Shatya, R., Vijayan, M.M., 2003. Autoregulation of glucocorticoid receptor by cortisol in rainbow trout hepatocytes. Am. J. Physiol., Cell Physiol. 284, C1508–C1515. <https://doi.org/10.1152/ajpcell.00448.2002>.
- Souza, M.L.R., Macedo-Viegas, E.M., Zuanon, J.A.S., Carvalho, M.R.B., Reis-Goes, E.S., 2015. Processing yield and chemical composition of rainbow trout (*Oncorhynchus mykiss*) with regard to body weight Acta Scientiarum. Animal Sciences 37, 103–108. <https://doi.org/10.4025/actascianimsci.v37i2.24165>.
- Steffens, W., 2016. Aquaculture produces wholesome food: cultured fish as a valuable source of n-3 fatty acids. Aquac. Int. 24, 787–802. <https://doi.org/10.1007/s10499-015-9885-8>.
- Stoffel, Wilhelm, Chu, Florence, Ahrens, E.H., et al., 1959. Analysis of Long-Chain Fatty Acids by Gas-Liquid Chromatography. Anal. Chem. 31 (2), 307–308. <https://doi.org/10.1021/ac60146a047>.
- Stoycheska, A.M., Stamenkovska, I.J., 2017. Profitability of carp production on Macedonia and Serbia. Biotechnol. Anim. Husb. 33, 103–113. <https://doi.org/10.2298/BAH1701103M>.
- Szűcs, I., Stündl, L., Váradi, L., 2007. Carp farming in Central and Eastern Europe and a case study in multifunctional aquaculture. In: Leung, P.S., Lee, C.S., O'Bryan, P.J. (Eds.), Species and System Selection for Sustainable Aquaculture. Blackwell Publishing, Ames, pp. 389–413.
- Tacon, A.G.J., Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. Aquaculture 285 (1), 146–158. <https://doi.org/10.1016/j.aquaculture.2008.08.015>.
- Takahashi, K., Inoue, N., Shinano, H., 1993. Effect of storage temperature on freeze denaturation of carp myofibrils with KCl and NaCl. Nippon. Suisan Gakkaishi 59, 519–527. <https://doi.org/10.2331/suisan.59.519>.
- Tan, Q., Liu, Q., Chen, X., Wang, M., Wu, Z., 2013. Growth performance, biochemical indices and hepatopancreatic function of grass carp, *Ctenopharyngodon idella*, would be impaired by dietary rapeseed meal. Aquaculture 415, 119–126. <https://doi.org/10.1016/j.aquaculture.2013.07.036>.
- Tóth, F., Révész, N., Demény, F., Uhljár, A., Molnár, Z., Bíró, J., Sándor, Zs.J., 2021. Effects of diets containing dried distiller's grain with solubles (DDGS) on the water quality of the carp rearing ponds. AACL Bioflux 14 (2), 1057–1067.
- Trbovic, D., 2014. The Influence of Diet on Lipid Content and Fatty Acid Composition of Carp Meat (*Cyprinus Carpio* L., 1758) in the Semi-intensive Rearing System. Dissertation [In Serbian]. University of Belgrade.
- Trbovic, D., Markovic, Z., Milojkovic-Opsenica, D., Petronijevic, R., Spiric, D., Djinic-Stojanovic, J., Spiric, A., 2013. Influence of diet on proximate composition and fatty acid profile in common carp (*Cyprinus carpio*). J. Food Anal. 31, 75–81.
- Tu, J., Xie, S., Han, D., Yang, Y., Jin, J., Liu, H., Zhu, X., 2015. Growth performance, digestive enzyme, transaminase and GH-IGF-1 axis gene responsiveness to different dietary protein levels in broodstock allogynogenetic gibel carp (*Carassius auratus gibelio*) CAS III. Aquaculture 446, 290–297. <https://doi.org/10.1016/j.aquaculture.2015.05.003>.
- Varga, D., Romvári, R., Horn, P., Hancz, Cs., Molnár, T., Szabó, A., 2013a. Environmental factors influencing the slaughter value and the flesh quality of common carp in four typical fish farms in Hungary. Acta Aliment. 42, 495–503.
- Varga, D., ifj. Horváth, Z., Horváth, Z., Andrásyné Baka, G., Szabó, A., 2013b. Dió törtszem etetésének hatása ponty (*Cyprinus carpio* L.) filé húsmínőségére, zsírsav összetételére és fogyasztói megítélésére. Acta Agraria Kaposvariensis 17, 41–49. In Hungarian.
- Varga, M., Berzi-Nagy, L., Csukas, B., Gyalog, G., 2020. Long-term dynamic simulation of environmental impacts on ecosystem-based pond aquaculture. Environ. Model. Softw. 134, 104755 <https://doi.org/10.1016/j.envsoft.2020.104755>.
- Velisek, J., Svobodova, Z., Machova, J., 2009. Effects of bifenthrin on some haematological, biochemical and histopathological parameters of common carp (*Cyprinus carpio* L.). Fish Phys. and Biochem. 35 (4), 583–590.
- Wu, Y.V., Rosati, R.R., Brown, P.B., 1996. Effects of diets containing various levels of protein and ethanol coproducts from corn on growth of tilapia fry. J. Agric. Food Chem. 44, 1491–1493.
- Wu, Y.V., Rosati, R.R., Brown, P.B., 1997. Use of corn-derived ethanol products and synthetic lysine and tryptophan for growth of tilapia (*Oreochromis niloticus*) fry. J. Agric. Food Chem. 45, 2174–2177.