



# Potential of corn distiller's dried grains with solubles (DDGS) in the diet of European catfish (*Silurus glanis*)

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## ABSTRACT

Two experiments were conducted to evaluate the suitability of corn DDGS as a protein source for European catfish (*Silurus glanis*). The first experiment consisted of an *in vivo* digestibility assessment to determine apparent digestibility coefficients for protein, lipid, phosphorus and amino acids available in DDGS. One hundred twenty juveniles (average weight,  $154.29 \pm 2.73$  g) were distributed in recirculation water system equipped with six  $1\text{m}^3$  fiberglass tanks (20 fish per tank), which were allotted to two experimental group in triplicates. Juveniles were fed either of the two diets, with or without DDGS (DDGS diet or reference diet) *ad libitum* till saturation 3 times per day for two weeks. Apparent digestibility coefficient of dry matter, crude protein, crude fat and phosphorus of DDGS ingredient for European catfish were found 49.42 %, 73.39 %, 77.38 % and 87.98 % respectively. The second experiment was conducted for eight weeks to evaluate the effect of corn DDGS on growth, nutrient utilization and metabolism of European catfish. Two hundred forty juveniles of European catfish (average weight,  $272.7 \pm 37.8$  g) were stocked in 1000 L glass fibre tanks in a recirculation system in triplicates. Juveniles were fed with either of the four iso-nitrogenous and iso-caloric (37 % crude protein and 6 % crude fat) experimental diets formulated with the inclusion of 0, 10, 20 and 30 % corn DDGS with partial replacement of soybean and wheat. No significant differences were found between the experimental groups regarding growth performance and plasma biochemical parameters. The liver histopathological observations showed that 20 and 30 % DDGS groups had less vacuolized hepatocytes than the other groups. Both experiments conclude that apparent digestibility of corn DDGS is auspicious for European catfish and 30 % DDGS can be included in the diet of European catfish without compromising the growth performance and nutrient utilization.

## 1. Introduction

Corn distiller's dried grains with soluble (DDGS) is left over dry by-product obtained after fermentation of corn by enzymes and yeast which produces bioethanol as major product. Starch available in corn converted to ethanol by fermentation however, other components, like fibre, protein and fat get concentrated in the remaining material i.e DDGS. Therefore, available of relatively high level of energy, protein, amino acid, non-phytate phosphorus and yeast make DDGS a suitable ingredient for fish feed. Nevertheless, its composition varies between different processing plants (Liu, 2008) and among the grains used for processing (Randall and Drew, 2010). Number of variables in the raw

materials and processing factors that have been listed by Olentine (1986) contribute to variation in nutrient composition of distiller's by-products viz. soil conditions, applied fertilizers, weather, production and harvesting methods and different processing factors like grind procedures, cooking, conversion, dilution of converted grains, fermentation, etc. Between 2006 and 2015 remarkable peer-reviewed publications have been published, and the composition data are reviewed and summarised by Zeng et al. (2017). DDGS became a very attractive ingredient for partial replacement for some of the more expensive traditional energy (corn), protein (soybean meal) and phosphorus (mono- or dicalcium phosphate) ingredients used in animal feeds. Inclusion of DDGS in animal feeds that reported excellent animal

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performance, health and food product quality.

The growth of the fuel ethanol and biodiesel industries increased the quantities of the corn products and opened new possibilities for feed industry, including aquafeed production. The global biofuels industry produces about 52 million tons of by-products for use in animal feed, and about 85 percent of these by-products are produced by the ethanol industry (Popp et al., 2016). The United States ethanol industry is the largest producer of corn co-products, with annual production of about 38 million tons. This amount of corn co-product production is comparable to the amount of soybean meal produced in the U.S. annually, and is being used in large quantities in animal feeds all around the world. (USGC-DDGS Handbook, 2018). At present, less than 2% of total DDGS produced is used in aquaculture feeds (Shurson, 2012). However, expansion of aquaculture followed by rise in global aquafeed consumption potentiates corn DDGS in fish feed.

Major challenges for the successful use of corn DDGS in aquaculture feeds are having limited knowledge of amino acid composition and its digestibility. Despite of relatively high crude protein content (30–32 %), lysine, methionine, threonine and tryptophan concentrations are relatively low in corn DDGS ((USGC-DDGS Handbook, 2018). Furthermore, amino acid content and its digestibility are variable due to different period of heating during the DDGS production processes. Consequently, fish diets with high protein levels require synthetic amino acids supplement when significant amounts of DDGS are included. Digestibility of protein, lipid, dry matter and energy have been determined for several fish species like common carp (Revesz et al., 2020), rainbow trout (Overland, 2013), channel catfish (*Ictalurus punctatus*) (Lim et al., 2009; Li et al., 2013), hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) (Thompson, 2008) and Nile tilapia (*Oreochromis niloticus*) (Haidar et al., 2016). However, very few studies have been conducted to determine the amino acid digestibility of corn DDGS in fish such as European seabass (*Dicentrarchus labrax*) and meagre (*Argyrosomus regius*) (Magalhães et al., 2015), pompano (*Trachinotus carolinus*) (Lech and Reigh, 2012) and rainbow trout (*Ornitorchus mykiss*) (Cheng and Hardy, 2004). Apparent digestibility of amino acids in DDGS were found relatively high (90 % for all essential amino acids except threonine) in rainbow trout (*Ornitorchus mykiss*) (Cheng and Hardy, 2004).

There are several recommendations for inclusion level of DDGS in the diet of fish. In most of the cases, replacement of fish meal in diet can only be possible with supplementation of synthetic amino acids. For Nile tilapia up to 30 % DDGS could be included without synthetic lysine and methionine supplementation and 8% fish meal in the diet (Li et al., 2011a). In rainbow trout, only 15 % replacement is advised without synthetic lysine and methionine in order to replace 50 % fishmeal or 22.5 % in case of 75 % replacement of fishmeal (Cheng and Hardy, 2004). Recently, Overland et al. (2013a, 2013b) demonstrated that 50 % DDGS inclusion could be advised in the diet with equal amounts of lysine and methionine supplementation. Channel catfish can tolerate higher DDGS in the diet (Li et al., 2010, 2011b) with 40 % inclusion in place of soybean with minimum 10 % fish meal level in the diet. Moreover, 70 % of DDGS could be utilized without inclusion of any fish meal, but lysine supplementation must be required. The plant ingredients such as soybean or corn other plants, could be replaced much easily in the diets of carnivorous species with DDGS. A maximum dietary DDGS inclusion level of 33 % was established for hybrid striped bass, *Morone chrysops* × *M. saxatilis* (Trushenski and Gause, 2013), less (10–20 %), for rainbow trout (Welker et al., 2014), for olive flounder, *Paralichthys olivaceus* (Rahman et al., 2015), and for turbot (Diógenes et al., 2018).

European catfish (*Silurus glanis*) is an important carnivorous freshwater species in Central and Eastern European aquaculture due to their fast growth, robustness, stress tolerant capability and high market value. Recently the protein content of its commercial feeds was based mostly on high quantity of fish meal (ca. 60 %). However, increasing demand, reduced availability and increased price of marine ingredients made it necessary to replace with other alternative sources of protein. Fish meal was successfully replaced with soybean and rendered animal protein by

Havasi et al. (2015) and Kumar et al. (2017), but replacing the soybean is getting more attention in European feed production. Utilization of agriculture and food industry by products became a current emergent topic. The objective of the present study was to determine the apparent digestibility of nutrients in DDGS for European catfish and to evaluate the effect of partial replacement of soybean and wheat by DDGS in the feeds.

## 2. Materials and methods

Two experiments were conducted to evaluate the suitability of corn DDGS as a protein source for European catfish. The first experiment consisted of an *in vivo* digestibility assessment to determine apparent digestibility coefficients for protein, lipid, phosphorus and amino acids available in DDGS. The second experiment was dedicated to eight weeks long nutritional trial with growth performance, nutrient utilization, feed conversion and diet digestibility. The experiments were conducted according to 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. *In vivo* ingredient digestibility determination

#### 2.1.1. Experimental diets

A reference diet was formulated (Table 1) to contain 43 % protein and 9 % fat according to the requirement of European catfish and was supplemented with 0.1 % yttrium oxide as indigestible marker. The test diet was prepared by mixing 70 % of the reference diet and 30 % of the test ingredient- corn DDGS (Cho et al., 1982; Bureau et al., 1999). The DDGS was supplied by Pannonia Bio, Hungary. Both diets were produced by extrusion in the pilot plant for animal feed production of the Feed-to-Food Research Centre at the Institute of Food Technology, University of Novi Sad, Serbia. Dry ingredients were ground by a hammer mill (ABC Inženjering, Pančevo, Serbia) equipped with the 0.8 mm sieve and then mixed in Muiyang SLHSJ0.2A double-shaft pedal mixer (Muiyang, Yangzhou, China). Fish oil was added directly into mixer during mixing of dry ingredients through nozzles that were positioned in the upper part of the mixer, above the mixing pedals. The material was preconditioned by direct addition of steam and water in aforementioned double-shaft mixer until final temperature of the material reached 95 °C. Targeted final moisture content of the material after preconditioning was 25 %. Preconditioned material was extruded using co-rotating twin-screw extruder (Bühler BTSK-30, 7 sections, length/diameter ratio = 28:1, Bühler, Uzwil, Switzerland) with 4 mm die opening (die open area of 12.56 mm<sup>2</sup>). The pellets were collected when temperature at the die was in range of 105–110 °C. The pellets were then dried in fluidized bed vibro dryer (FB 500 × 200, Amandus Kahl, Hamburg, Germany) at 80 °C until product reached final moisture content of approximately 10 %. The final pellets were 4.5 mm in diameter and semi-floating.

The digestibility trial was set up in the recirculation system of the institute with European catfish juveniles having average weight of 154.29 ± 2.73 g. The experimental unit consisted of six 1 m<sup>3</sup> glass fibre tanks. During the trial water flow was established at average 4.5 L/min per tank, dissolved oxygen level was kept above 80 % of saturation, ammonia-N was below 0.1 mL/L and pH varied between 8.6–8.8. Water temperature was set to 24 ± 1 °C. A total of 120 catfish juveniles were stocked in tanks with 20 fish/tank stocking density and acclimatized for two weeks. Digestibility trial was carried out in triplicate. The fish were fed *ad libitum* till saturation 3 times per day during the trial.

#### 2.1.2. Faeces collection

After two weeks of feeding, all the fish stock were satisfied in order to

**Table 1**

Formulation (%), proximate composition (% wet weight), amino acid profile of the reference and experimental diets used in the digestibility experiment.

Ingredients	Reference diet	DDGS diet
Wheat flour T850 <sup>1</sup>	28.50	20.00
Corn DDGS <sup>2</sup>	–	30.00
Fish meal <sup>3</sup>	40.00	28.00
Soybean flour (defatted, 50 % Pr) <sup>4</sup>	20.00	14.00
Fish oil <sup>5</sup>	5.00	3.50
Blood meal <sup>6</sup>	2.50	1.75
Mono-Ca-Phosphate <sup>7</sup>	1.80	1.26
Vitamin premix <sup>7</sup>	1.00	0.70
CaCO <sub>3</sub> <sup>7</sup>	0.60	0.42
NaCl <sup>7</sup>	0.50	0.35
Yttrium-oxide <sup>8</sup>	0.10	0.07
<b>Proximate Composition</b>		
Crude Protein	43.25 ± 0.29	39.70 ± 0.60
Crude Fat	5.99 ± 0.20	7.68 ± 0.68
Crude Fibre	1.37 ± 0.02	3.95 ± 0.05
Crude Ash	10.07 ± 0.12	8.68 ± 0.05
Phosphorus	1.37 ± 0.05	1.24 ± 0.02
Gross energy (KJ g <sup>-1</sup> )	17.76	17.82
<b>Amino Acid Profile</b>		
<b>Essential Amino Acid (EAA)</b>		
Arginine (ARG)	2.30	1.61
Cysteine (CYS)	0.38	0.31
Histidine (HIS)	1.38	1.23
Isoleucine (ILE)	1.90	1.71
Leucine (LEU)	3.04	3.21
Lysine (LYS)	2.65	2.31
Methionine (MET)	1.17	1.25
Phenylalanine (PHE)	1.84	1.64
Threonine (THR)	1.75	1.81
Valine (VAL)	2.15	2.01
<b>ΣEAA</b>	<b>18.56</b>	<b>17.19</b>
<b>Non-Essential Amino Acid</b>		
Alanine (ALA)	2.16	2.19
Aspartic acid (ASP)	3.76	3.45
Glutamic acid (GLU)	2.25	1.99
Glycine (GLY)	2.25	1.99
Proline (PRO)	2.11	2.05
Tyrosine (TYR)	1.50	1.43
Serine (SER)	2.42	2.22

<sup>1</sup> Union SP Commerce mill, Temerin, Serbia.<sup>2</sup> Pannonia Gold, Dunaföldvár, Hungary.<sup>3</sup> 999 Fish meal LT, Triple Nine Fish Protein A/S, Esbjerg, Denmark.<sup>4</sup> SOPRO-TB200, Soja protein, Bečej, Serbia.<sup>5</sup> Sardina DOO, Postira, Brač, Croatia.<sup>6</sup> ATEV Fehérjefeldolgozó Zrt., Hungary.<sup>7</sup> Supplied by DTD Ribarstvo, Bački Jarak, Serbia.<sup>8</sup> Alfa Aesar, Thermo Fisher (Kandel) GmbH, Karlsruhe, Germany.

collect faeces from intestine (Austreng, 1978). Before harvesting fish were anesthetized with Norcaicum/Tonogen based anaesthesia (Matuk and Gulyás, 1987). Faecal samples from a given tank were pooled and stored at –20 °C till analysis.

### 2.1.3. Analytical methods

The chemical compositions of feed and faeces were analysed by standard methods of the AOAC (1998) (Table 1). 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). The fatty acid compositions of feed samples were analysed by capillary gas chromatography (AGILENT 6890 N) according to the method by Folch et al. (1957). Amino acid contents of the diets and faeces samples were analysed by the accredited laboratory of the Hungarian Food Chain Safety Office ([www.nebih.gov.hu](http://www.nebih.gov.hu)) following the ISO 13903:2005. Yttrium and

phosphorus content were analysed by ICP method. The digestion of samples was made with mixtures of acids, including nitric acid (R.G. 65 %) and hydrogen peroxide (R.G. 30 %). The extraction was realized by using microwave digestion technique under high pressure. The type of microwave apparatus was Milestone Ethos Plus. The concentrations of elements were measured by Thermo Scientific 6500 ICP-OES equipment.

### 2.1.4. Digestibility equations

Apparent digestibility coefficients (ADCs) of dry matter, protein and phosphorus of the diets were determined by the following formula (Cho et al., 1982; Bureau et al., 1999):

$$ADC_{\text{diet}} = [1 - (\{Y_{\text{diet}} / Y_{\text{faeces}}\} \times \{D_{\text{faeces}} / D_{\text{diet}}\})] \times 100$$

Where,  $Y_{\text{diet}}$  is the dietary yttrium level,  $Y_{\text{faeces}}$  is the faeces yttrium level,  $D_{\text{diet}}$  is the dietary nutrient level and  $D_{\text{faeces}}$  is the faeces nutrient level.

The apparent digestibility coefficients of the test ingredient (DDGS)

were calculated according to Bureau et al. (1999) as follows:

$$ADC_{DDGS \text{ ingredient}} = ADC_{DDGS \text{ diet}} + [(ADC_{DDGS \text{ diet}} - ADC_{\text{reference diet}}) \times (0.7 \times D_{\text{ref}} / 0.3 D_{DDGS \text{ ingredient}})]$$

Where,  $D_{\text{ref}}$  is the % nutrient (or  $\text{kJ g}^{-1}$ ) of reference diet (dry matter basis) and  $D_{DDGS}$  is the % nutrient (or  $\text{kJ g}^{-1}$ ) of DDGS ingredient (dry matter basis).

## 2.2. Nutritional trial

### 2.2.1. Experimental diets

A control diet meeting the nutritional requirement of European catfish juveniles was formulated to include fish meal and poultry meal in 45 %, soybean defatted product and wheat another 45 % (Table 2.). The experimental diets were formulated with inclusion of DDGS in different ratio (10 %, 20 %, 30 %) in order to replace the fish meal in the diet. Yttrium oxide was incorporated to the diets as inert digestibility marker. The feeds were also produced by twin-screw extruder in the pilot plant for animal feed production of the Feed-to-Food Research Centre at the Institute of Food Technology, University of Novi Sad, Serbia. The diets were produced using same equipment and production parameters as the two diets used in digestibility trial. The feeds were in form of semi-floating pellets with the diameter of 4.5 mm. Amino acid composition and fatty acid profile of the diets are summarised in Table 4.

### 2.2.2. Experimental design and sampling

20 individuals of catfish juveniles with  $272.7 \pm 37.8$  g initial weight was stocked in 1000 L glass fibre tanks in a recirculation system in triplicates after 3 weeks of acclimatization. The water temperature was set to 24 °C, with minimum 80 % oxygen saturation. The fish were fed manually, 3 times per day up to 2.5 % of body weight. The fish were measured every two weeks to follow the growth performance and to adjust the daily feed amount. After 8 weeks of feeding at the end of the

experiment, fish were dissected to measure biometrical indices (condition factor, hepatosomatic index, viscerosomatic index) and to take different type of samples. Whole fish and filet samples were taken for proximate composition, liver samples for histology, gene expression and fatty acid profile analysis, and blood plasma for blood chemistry measurements. One ml of blood was taken from the caudal vein of two fish, using heparinised needles and syringes. Blood samples were put into heparinised micro centrifuge tubes and centrifuged at 1400 g for 20 min at 4 °C. After centrifugation, blood plasma was collected and stored at -20 °C for further analysis. Finally, faeces samples were collected from the posterior intestine after sacrificing the fish in order to determine the apparent digestibility coefficients of the diets.

### 2.2.3. Chemical analysis

Proximate analysis, amino acid and fatty acid profile of diets, fish and faeces were determined by standard methods of the AOAC (1998); Folch et al. (1957) and ISO 13903 (2005) (Tables 2 and 3.). Blood plasma chemistry was done with a Samsung PT10 V, semi-automatic clinical chemistry analyser. Plasma enzymes and metabolites, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma-glutamyl transferase (GGT), lipase, amylase, total cholesterol (TC) and triglyceride (TG) were measured according to IFCC (International Federation of Clinical Chemistry).

### 2.2.4. Histology

For histological analysis liver samples ( $n = 6$  per treatment) were immediately immersed in Bouin's solution for 16 h and then transferred to 70 % ethanol (Culling, 1974). Subsequently, the samples were embedded in paraffin and thin sections (5  $\mu\text{m}$ ) were obtained and stained with Mayer's hematoxylin and eosin. The morphological structures of these tissues were observed using an imaging microscope (ECLIPSE 80i, Nikon, Japan).

**Table 2**

Formulation and chemical composition of the diets with different inclusion level of DDGS.

Ingredients	DDGS 0	DDGS 10	DDGS 20	DDGS 30
Poultry meal <sup>1</sup>	25.00	20.50	16.00	12.00
Soybean flour 50 % Pr <sup>2</sup>	21.02	22.00	23.00	23.50
Wheat <sup>3</sup>	25.00	18.94	12.80	6.72
Fish meal 60 % <sup>4</sup>	20.00	20.00	20.00	20.00
DDGS <sup>5</sup>	0.00	10.00	20.00	30.00
Yeast <sup>6</sup>	5.00	5.00	5.00	5.00
Fish oil <sup>7</sup>	1.50	1.50	1.50	1.50
Soybean oil <sup>3</sup>	1.80	1.20	0.60	0.00
Lysin 78 % <sup>3</sup>	0.06	0.15	0.22	0.28
Methionine DL 99 % <sup>3</sup>	0.04	0.06	0.08	0.10
Premix <sup>3</sup>	0.50	0.50	0.50	0.50
NaCl <sup>3</sup>	0.08	0.15	0.30	0.40
Y <sub>2</sub> O <sub>3</sub> <sup>8</sup>	0.10	0.10	0.10	0.10
<b>Proximate Composition % wet weight basis</b>				
Crude Protein	39.39 $\pm$ 0.32	38.40 $\pm$ 0.19	37.85 $\pm$ 0.16	37.68 $\pm$ 0.32
Crude Fat	7.58 $\pm$ 0.01	6.92 $\pm$ 0.01	6.66 $\pm$ 0.00	6.09 $\pm$ 0.00
Crude Fibre	3.32 $\pm$ 0.16	3.69 $\pm$ 0.20	4.23 $\pm$ 0.05	4.46 $\pm$ 0.03
Crude Ash	8.66 $\pm$ 0.08	8.14 $\pm$ 0.06	7.86 $\pm$ 0.02	7.92 $\pm$ 0.03
Gross Energy (KJ g <sup>-1</sup> )	19.43	19.26	19.18	18.94
Phosphorus	1.23	1.13	1.08	1.06

<sup>1</sup> BRO-MK Processed animal protein, Brovis DOO, Visoko, Bosnia & Herzegovina.

<sup>2</sup> SOPRO-TB200, Sojaprotein, Bečej, Serbia.

<sup>3</sup> Supplied by DTD Ribarstvo, Bački Jarak, Serbia.

<sup>4</sup> Sardina DOO, Postira, Brač, Croatia.

<sup>5</sup> Pannonia Gold, Dunaföldvár, Hungary.

<sup>6</sup> Biofood, Tambou, Russia.

<sup>7</sup> Sardina DOO, Postira, Brač, Croatia.

<sup>8</sup> Alfa Aesar, Thermo Fisher (Kandel) GmbH, Karlsruhe, Germany.

**Table 3**

Amino acid and fatty acid composition of the diets (% wet weight).

Essential Amino Acid	DDGS 0	DDGS 10	DDGS 20	DDGS 30
Arginine (ARG)	2.68	2.51	2.40	2.41
Cysteine (CYS)	0.56	0.52	0.57	0.52
Histidine (HIS)	1.40	1.48	1.42	1.42
Isoleucine (ILE)	1.84	1.84	1.82	1.77
Leucine (LEU)	2.96	3.03	3.29	3.35
Lysine (LYS)	2.50	2.39	2.48	2.54
Methionine (MET)	0.62	0.57	0.59	0.49
Phenylalanine (PHE)	1.77	1.73	1.94	1.82
Threonine (THR)	1.35	1.37	1.46	1.46
Valine (VAL)	2.26	2.66	2.40	2.57
<b>ΣEAA</b>				
<b>Non-Essential Amino Acid</b>				
Alanine (ALA)	2.28	2.14	2.30	2.30
Aspartic acid (ASP)	4.19	4.21	4.50	4.00
Glutamic acid (GLU)	7.49	6.54	6.67	6.94
Glycine (GLY)	3.11	2.65	2.67	2.32
Proline (PRO)	2.32	2.06	2.69	2.39
Serine (SER)	1.93	2.07	2.06	2.12
Tyrosine (TYR)	1.35	1.37	1.46	1.46
<b>Fatty Acids w%</b>				
14:0	1.63 ± 0.14	1.59 ± 0.04	1.75 ± 0.02	1.87 ± 0.02
16:0	16.49 ± 0.64	16.14 ± 0.06	15.80 ± 0.06	15.47 ± 0.21
16:1n-9	0.49 ± 0.27	0.28 ± 0.00	0.26 ± 0.02	0.37 ± 0.14
16:1n-7	3.01 ± 0.07	2.75 ± 0.06	2.57 ± 0.04	2.63 ± 0.05
18:0	4.32 ± 0.08	4.29 ± 0.02	3.97 ± 0.04	3.57 ± 0.06
18:1n-9	23.49 ± 0.71	24.19 ± 0.28	23.24 ± 0.11	22.65 ± 0.38
18:1n-7	2.32 ± 0.58	1.90 ± 0.06	1.81 ± 0.01	2.01 ± 0.36
18:2n-6	28.38 ± 0.49	30.08 ± 0.19	30.31 ± 0.16	29.88 ± 0.33
18:3n-6	0.26 ± 0.20	0.15 ± 0.01	0.12 ± 0.01	0.25 ± 0.17
18:3n-3	3.17 ± 0.11	3.10 ± 0.00	2.81 ± 0.02	2.36 ± 0.08
20:0	0.26 ± 0.02	0.27 ± 0.00	0.29 ± 0.00	0.31 ± 0.02
20:4n-6	0.43 ± 0.01	0.40 ± 0.03	0.39 ± 0.01	0.39 ± 0.06
20:5n-3	1.87 ± 0.05	1.97 ± 0.00	2.24 ± 0.01	2.34 ± 0.03
22:6n-3	4.77 ± 0.29	4.82 ± 0.02	5.52 ± 0.16	5.69 ± 0.23
Total SFA	23.42 ± 0.99	22.97 ± 0.09	25.57 ± 0.06	22.02 ± 0.46
Total MUFA	33.87 ± 0.19	33.87 ± 0.27	33.19 ± 0.09	33.33 ± 0.12
Total n-6	29.66 ± 0.43	31.09 ± 0.25	31.35 ± 0.08	31.19 ± 0.09
Total n-3	10.43 ± 0.17	10.48 ± 0.03	11.22 ± 0.18	11.14 ± 0.18
Total PUFA	40.08 ± 0.26	41.57 ± 0.29	42.57 ± 0.10	42.33 ± 0.27
Total lipid mg FA/ g	71.78 ± 6.02	65.54 ± 0.11	66.44 ± 3.46	59.22 ± 4.29

SFA- saturated fatty acids, MUFA-monounsaturated fatty acids, PUFA-polyunsaturated fatty acids.

### 2.2.5. Calculations and statistical analysis

Growth performance of fish such as percentage weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and protein production value (PPV) was calculated based on the following standard formulae: Weight gain (%) = [(Final total biomass weight – Initial total biomass) / Initial total biomass] × 100; Specific growth rate (g/day) (SGR) = (Ln final average weight – Ln initial average weight) × 100 / days; Feed conversion ratio (g/g) (FCR) = Total dry feed offered (g) / Weight gain (g); Protein efficiency ratio (g/g) (PER) = Weight gain / Protein offered. 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 (g)); Condition factor (g/cm<sup>3</sup>) (CF) = body weight (g) × 100 / body length<sup>3</sup>; viscerosomatic index (%) (VSI) = total viscera (g) / body weight (g); hepatosomatic index (%) (HSI) = hepatopancreas weight (g) / body weight (g) × 100; Visceral fat index (%) (VFI) = visceral fat (g) / body weight (g) × 100.

The statistical analyses were performed by IBM SPSS 22. software package. All data were tested with one-way analysis of variance (ANOVA) with Tukey's Post Hoc test. The statistical IDs marked with different letters translate into a deviation on a significance level of  $p < 0.05$ .

## 3. Results

### 3.1. In vivo digestibility

The determined apparent digestibility coefficients (ADC) for dry matter, crude protein, crude fat, phosphorous and for essential amino acids are illustrated in Table 4. Digestibility coefficients of most of the nutrients, except phosphorus, were lower in test diet compared to the reference diet, however significant differences ( $p < 0.05$ ) were revealed only for crude fat and dry matter. In respect of essential amino acids, the ADC of cystine, lysine and arginine in test diet were found significantly lower ( $p < 0.05$ ) compared to reference diet. The ADC of the DDGS, as ingredient, had relatively high value for crude protein and crude fat compared to other fish species, 73.4 % and 77.4 %, respectively. Meanwhile high phosphorus digestibility was demonstrated with value of almost 88 %. Regarding the ADC (%) of amino acid such as lysine, cystine, arginine and histidine, presented significantly reduced values (Table 4)

### 3.2. Nutritional trial

After the 8 weeks of feeding trial, statistical differences in terms of growth performance and nutrient protein utilization (Table 5) were not



**Table 4**

Apparent Digestibility Coefficients (ADC) of the feeds and the test ingredient (DDGS).

ADC %	Reference feed	DDGS feed	p-value	DDGS ingr.
Dry Matter	67.44 ± 0.61	61.97 ± 1.21	0.002	49.42 ± 3.98
Crude Protein	82.89 ± 0.41	80.82 ± 0.65	0.010	73.39 ± 2.98
Crude Fat	97.35 ± 0.05	90.06 ± 0.32	0.000	77.38 ± 0.86
Phosphorus	60.14 ± 1.59	65.41 ± 1.09	0.009	87.98 ± 5.95
Essential Amino Acids (EAA)				
Arginine	93.01 ± 0.13	89.41 ± 0.34	0.000	72.64 ± 1.90
Cystine	62.34 ± 0.71	46.97 ± 1.69	0.000	24.49 ± 4.15
Histamine	66.95 ± 0.62	64.74 ± 1.12	0.041	58.00 ± 4.55
Isoleucine	86.94 ± 0.25	86.19 ± 0.44	0.061	83.43 ± 2.05
Leucine	85.28 ± 0.28	86.11 ± 0.44	0.051	87.85 ± 1.37
Lysine	85.56 ± 0.27	82.96 ± 0.54	0.002	66.30 ± 4.02
Methionine	86.06 ± 0.26	86.15 ± 0.44	0.765	86.39 ± 1.56
Proline	85.56 ± 0.27	86.12 ± 0.44	0.137	87.19 ± 1.29
Threonine	82.81 ± 0.32	82.97 ± 0.54	0.682	83.48 ± 2.27
Valine	83.51 ± 0.31	83.36 ± 0.53	0.684	82.82 ± 2.37

observed for European catfish juveniles. FCR values varied between 1.29–1.36 g/g, SGR 1.43–1.50 g/day, PER and PPV 1.78–1.94, and 27.7–30.2 % respectively. No mortality was observed during the trial. The examined fish did not showed differences in biometric indices, irrespective of the dietary composition (Table 5). The plasma biochemical parameters such as Glucose, Phosphatase, Ca, Total protein, Globulin, Alanine aminotransferase, Alkaline phosphatase, Cholesterol, Triglyceride and Amylase (Table 6) were not differing significantly. The triglyceride level of the serum presented appreciable differences between DDGS 0 (393 mg/dl) and DDGS 30 (270.8 mg/dl) groups with high standard deviations. Cholesterol level varied between 129–136 mg/dl and glucose 60–64 mg/dl, having the lower value in DDGS 30 group.

Proximate composition was determined in the whole body and filet on dry weight basis. In the filet, crude protein level was found between 79.0–80.5 %, the crude fat between 11.5–12.1 % and crude ash had approximately 5.4 %. Significant differences were not detected between treatments (Table 7.). Crude fat content of the whole fish body differs significantly at  $p = 0.070$ , the highest level in the DDGS 30 treatment was observed. The crude protein content was similar in all of the treatments.

Structure, shape and consistency of hepatocytes, shape and localization of cell nucleus of liver were studied. The liver histopathological observations (Fig. 1) showed that 20 % and 30 % DDGS fed groups had less vacuolized hepatocytes than the other groups. There were no differences were observed between fishes of different experimental groups

**Table 5**

Growth performance, nutrient utilization parameters (n=20) and biometric indices (n=6).

Parameters	DDGS 0	DDGS 10	DDGS 20	DDGS 30	p-value
Initial Body Weight (g)	271.60 ± 35.60	273.60 ± 33.80	273.20 ± 40.20	272.30 ± 41.80	0.964
Final Body Weight (g)	629.90 ± 24.04	630.80 ± 110.80	609.30 ± 151.20	629.00 ± 130.50	0.717
Yield (g)	358.30 ± 21.20	357.10 ± 17.20	336.10 ± 23.50	356.70 ± 29.00	0.611
FCR (g/g)	1.29 ± 0.06	1.30 ± 0.04	1.36 ± 0.07	1.29 ± 0.08	0.608
SGR (g/day)	1.50 ± 0.05	1.49 ± 0.04	1.43 ± 0.07	1.49 ± 0.06	0.498
PER (g/g)	1.78 ± 0.12	1.94 ± 0.04	1.93 ± 0.07	1.89 ± 0.08	0.163
PPV (%)	27.68 ± 1.70	29.18 ± 0.50	30.21 ± 1.01	28.87 ± 1.13	0.144
CF (%)	0.60 ± 0.05	0.66 ± 0.08	0.61 ± 0.02	0.60 ± 0.02	0.181
VSI (%)	7.45 ± 0.76	7.66 ± 0.70	7.55 ± 0.25	7.30 ± 0.64	0.777
HSI (%)	1.87 ± 0.34	2.08 ± 0.25	1.84 ± 0.24	1.84 ± 0.15	0.330
VFI (%)	0.78 ± 0.41	0.83 ± 0.40	0.56 ± 0.20	0.53 ± 0.29	0.333
GI (%)	2.89 ± 0.25	2.80 ± 0.25	3.01 ± 0.34	2.79 ± 0.20	0.585
Filleting Yield (%)	43.53 ± 2.32	45.07 ± 3.42	43.47 ± 3.40	43.96 ± 3.90	0.824

FCR - feed conversion rate, SGR - specific growth rate, PER - protein efficiency ratio, PPV - protein production value; CF - condition factor; VSI - viscerasomatic index; HSI - hepatosomatic index; VFI - visceral fat index; GI - gut index. Values are means of six replicates.

on gut morphology in respect of length of epithelial cells and number and size of goblet cells. (Fig. 2).

Fatty acid composition of the liver samples presented differences in some of the fatty acids (Table 8.). 16:0 palmitic acid differs significantly in DDGS 20 and 30 to control represent in almost 21 % of the fatty acids. Mono-unsaturated fatty acids, as 16:1n-7 and 18:1n-9 decreased significantly with increase of DDGS level in the diet. Consequently, total saturated and total unsaturated fatty acids follow similar trend with individual fatty acids, namely total SFA increased with DDGS inclusion, while total MUFA decreased. Enrichment with total PUFA of the liver is also observed, but this difference is not significantly demonstrated at  $p < 0.05$ . Total lipid content in liver tissue varied between 8.46 and 17.31 mg FA/g, less amount in DDGS 30 treatment was determined.

The apparent digestibility coefficients of the diets determined for different nutrients are presented in Table 9. ADC for dry matter and crude protein were not significantly ( $p < 0.05$ ) differing with DDGS inclusion, but for crude fat was highly digestible in all of the diets (ranging between 96–98 %) except DDGS 20 treatment where an outlier values were found (88 %). Phosphorus digestibility was determined for these diets and significant differences found between the treatments. The lowest digestible diet was the control DDGS 0 diet with ADC for P around 29 %, compared to DDGS 10 with 54 %, DDGS 20 with 44 % and DDGS 30 with 47 % phosphorus ADC values.

## 4. Discussion

### 4.1. Digestibility of DDGS

Dry matter ADC provides an estimate for overall digestibility of the test ingredient, and a low value usually indicates that a high quantity of indigestible material is present in the feedstuff. In the present study, ADC of dry matter determined in DDGS (49.4 %) is comparable with data obtained for channel catfish (50.8 %) (Li et al., 2013). The low ADC is due to high level of nitrogen free extract in DDGS, which is mainly composed of non-soluble protein (NSP) and not digestible by carnivorous fish. Recently for protein ADC 86.9 % was reported for channel catfish as long as for European seabream and meagre values were ranging between 92–96 % (Magalhães et al., 2015). The ADC for protein in corn DDGS is clearly lower in European catfish (73.4 %) compared to common carp (86.1 %) (Révész et al., 2020) and rainbow trout (80.8 %) (Øverland et al., 2013a). The difference in ADC of protein may be due to different feeding habit and metabolism differences of the species.

The digestibility and nutritional value of the protein correlates with amino acid profile of the protein. The digestibility of essential amino acid in the present study is above 80 % for most of the essential amino acid, except the cystine, lysine, arginine and histamine. ADCs in channel

**Table 6**  
Biochemical parameters of blood plasma (n = 6) at the end of trial.

	GLU	PHOS	Ca	TP	GLOB	ALT	ALP	CHOL	TRIG	AMY
	mg/dl	mg/dl	mg/dl	g/dl	g/dl	U/L	U/L	mg/dl	mg/dl	U/L
DDGS 0	64.33 ± 0.45	7.06 ± 0.45	7.06 ± 0.45	9.92 ± 0.31	2.71 ± 0.40	1.93 ± 0.19	14.00 ± 2.82	88.00 ± 7.21	136.60 ± 18.64	393.20 ± 45.02
DDGS 10	62.00 ± 0.32	7.45 ± 0.32	7.45 ± 0.32	9.90 ± 0.37	2.75 ± 0.20	1.86 ± 0.27	12.50 ± 3.14	88.16 ± 7.73	130.80 ± 11.26	310.20 ± 61.74
DDGS 20	62.83 ± 0.76	7.34 ± 0.76	7.34 ± 0.76	10.22 ± 0.39	2.93 ± 0.21	2.00 ± 0.14	14.50 ± 1.87	90.83 ± 11.85	140.60 ± 21.32	364.50 ± 116.70
DDGS 30	60.33 ± 0.28	7.25 ± 0.28	7.25 ± 0.28	9.81 ± 0.33	2.70 ± 0.17	1.76 ± 0.12	14.66 ± 2.87	90.33 ± 4.76	129.30 ± 10.28	270.80 ± 58.40
p-value	0.292	0.406	0.406	0.242	0.535	0.181	0.531	0.512	0.483	0.245
										0.880

GLU – Glucose, PHOS – Phosphate, Ca – Calcium, TP – Total Protein, GLOB – Globulin, ALT – Alanine aminotransferase, ALP – Alkaline Phosphatase, CHOL – Cholesterol, TRIG – Triglyceride, AMY – Amylase.

catfish determined by Li et al. (2013) are slightly higher compared to our data. Meanwhile the less digestible AA in European catfish is the lysine (72.4 %). Similarly, ADC for the lysine was the lowest (50.7 %) in corn for stripped catfish, but 94.2 % in the soybean meal (Da et al., 2013).

Digestibility of crude fat in corn DDGS is higher (93.8 %) in channel catfish compared to European catfish determined in current study (77.4 %) and in sunshine bass (68.7 %)(Thompson et al., 2008). The differences in ADCs of DDGS among different studies may have resulted due to differences in processing conditions, nutrient compositions of diet, faecal collection methods, digestive physiology of different fish species.

It is well known that the bioavailability of phosphorus is higher for fish from DDGS (USGC-DDGS Handbook, 2018) compared to other feed ingredients of animal and plant origin. Similar result was observed in the present trial for European catfish also. The ADC for phosphorus is dependent on the ingredient like 25–29 % in soy protein concentrate and 34 % in herring meal (Kim et al., 1998), 80–82 % in corn DDGS (Révész et al., 2020). However, in the present study, for European catfish, ADC of phosphorus in corn DDGS was registered 88 %.Prabhu et al. (2019) obtained 78.1 % ADC of phosphorus in wheat dried distillers for common carp, which represented the highest value within other feed stuffs investigated (whole peas, sunflower meal, hydrolysed feather meal). Higher digestibility of corn DDGS is due to hydrolyzation of corn during the fermentation process which reduces the phytate level. Replacement of fish meal with plant sources in aquafeeds highlighted the role of phytate present in plants seeds, because it has negative impact on growth, nutrient, energy utilization and mineral uptake. From 50–80% of total P in plants is stored in phytate (Ravindran et al., 1995), and this form is not bioavailable for fish due to the lack of intestinal digestive enzymes, phytase. Moreover, phytate can reduce digestibility of other nutrients through connection as cation to the amino acids, lipids, protein (Kumar et al., 2012). As a conclusion we can assume that DDGS is good source for available phosphorus and satisfy the requirement for several fish species ranging between 0.3–1.0 % (National Research Council (NRC), 2011). Other benefit of using DDGS in aquaculture should be the low phosphorus excretion into the environment due to its relatively high digestible phosphorus content.

#### 4.2. Nutritional aspects of DDGS

DDGS is considered as an acceptable ingredient in diets for some of the fish species investigated (USGC-DDGS Handbook, 2018). Very few studies have been conducted on the replacement of fish meal with plant ingredients in the diet of European catfish (Bekcan et al., 2006). In the present study different experimental feeds were formulated replacing some ingredients (poultry meal, wheat, soy oil) with increasing amount of DDGS, as long as level of fish meal and fish oil was kept constantly low (20 % and 1.5 %). The diets were isonitrogenous and were enriched with synthetic lysine and methionine in order to balance the potential essential amino acid deficit occurring with DDGS utilization (Kim et al., 2006). The variability of EAA compositions of DDGS may be due to several factors that include differences in non-protein-nitrogen substances, temperature and duration of drying, and the contribution of yeast AA to total AA in DDGS (Liu, 2011). The findings of the present result showed that inclusion of 0, 10, 20 and 30 % DDGS did not affect the growth parameters, nutrient utilization and biometric indices of European catfish. Similar study was conducted by Webster et al. (1993), when cage reared juvenile catfish were fed diets containing 0, 10, 20, or 30 % DDGS which partially replaced corn and soybean meal in the diets. There were no differences in individual fish weights, survival, feed conversion, carcass composition, carcass waste (head, skin, viscera), and organoleptic properties of the filets among dietary treatments. Results from Robinson and Li (2008) study also suggest that adding up to 30 % DDGS to channel catfish diets supports satisfactory growth performance when the diet is supplemented with synthetic lysine. Similarly, Zhou et al. (2010) replaced soybean meal and maize meals with DDGS in juvenile hybrid catfish (channel catfish × blue catfish *I. Furcatus*) diets and

**Table 7**

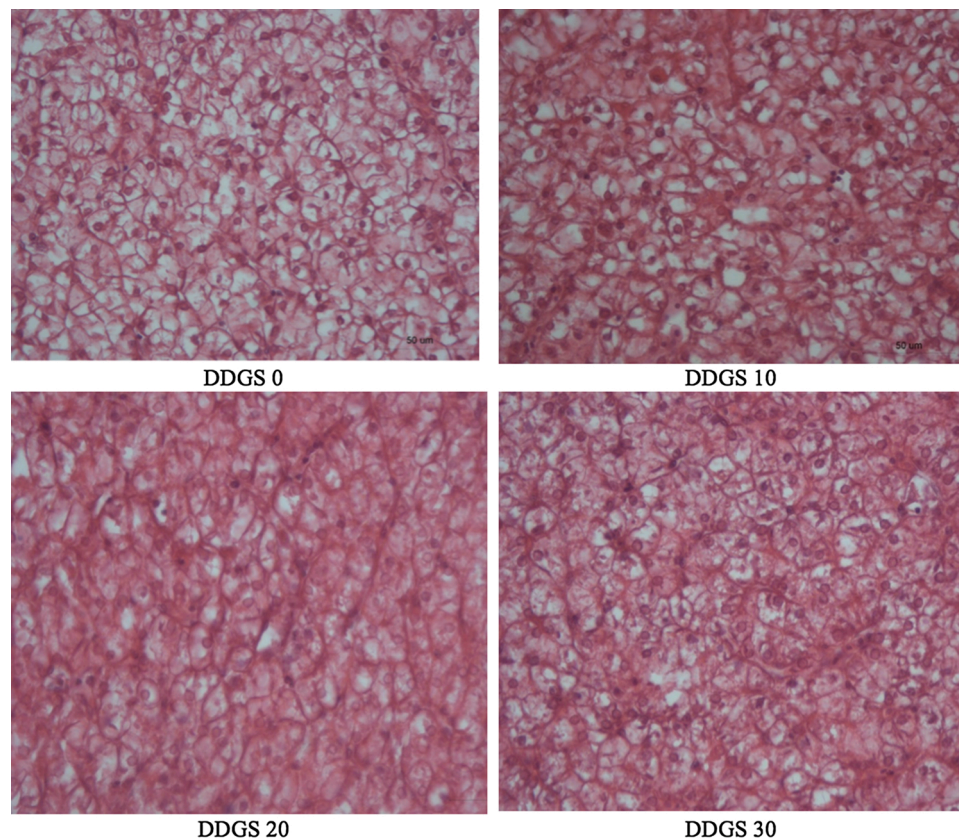
Proximate composition of the whole body and filet (% dry weight basis) (n = 6).

Filet	DDGS 0	DDGS 10	DDGS 20	DDGS 30	p-value
Crude Protein	78.96 ± 2.07	80.46 ± 1.55	80.08 ± 1.06	79.0 ± 0.96	0.366
Crude Fat	11.58 ± 2.02	11.54 ± 1.39	11.78 ± 2.59	12.07 ± 1.18	0.873
Crude Ash	5.28 ± 0.33	5.36 ± 0.38	5.47 ± 0.27	5.49 ± 0.16	0.573
<b>Whole body</b>					
Crude Protein	61.32 ± 1.04	61.23 ± 0.88	60.24 ± 1.09	59.39 ± 2.23	0.313
Crude Fat	23.87 ± 1.60	23.76 ± 2.29	26.95 ± 1.21	27.45 ± 2.00	0.070
Crude Ash	10.27 ± 1.03	9.70 ± 0.13	8.44 ± 1.12	8.56 ± 0.64	0.069

observed that feeds containing 30 % DDGS provided good growth, feed conversion, and protein retention. Results from these studies indicate that up to 30 % DDGS can be added to channel catfish diets without adversely affecting survival, growth or feed conversion and flavour qualities of the filets. Furthermore, when fish meal was replaced in the diet of striped catfish (Allam et al., 2019), with High Protein Dried Distilled Grain (HP-DDGS) in different ratio negative tendency was reported in growth and nutrient utilization due to the amino acid imbalance.

Whole body carcass composition and filet composition of European catfish was not affected by DDGS replacement in the present trial. There were no statistical differences in the plasma biochemical parameters, however, DDGS 20 and DDGS 30 groups had less vacuolated hepatocytes in liver. For other species, it has been studied that high dietary DDGS inclusion levels reduced wholebody lipids and energy content (Webster et al., 1993; Robinson and Li, 2008; Diógenes et al., 2018) that was attributed to a reduction of digestible energy intake. Diógenes et al. (2018) observed that fish meal replacement by DDGS reduced both plasma triglycerides and cholesterol levels in turbot, and the authors

attributed this reduction with lower feed intake of fish fed with DDGS diets. However, when soybean meal was replaced with DDGS, the same parameters increased with inclusion level of DDGS (Peres et al., 2014). In present study, both fish meal and soybean meal level were unchanged in the diet, however, lower values for the particular parameters in DDGS 30 group was observed. The reason for decrease in plasma triglycerides and cholesterol level may be due to lower dietary fat in the DDGS treatment groups. It is well known that dietary replacement of fish oil by vegetable oils affects plasma cholesterol and triglycerides levels of gilthead seabream (Caballero et al., 2006; Castro et al., 2016). Moreover, it was hypothesized that the presence of yeast cells in DDGS (Ingledew, 1999) may affect plasma lipid profile. Kumar et al. (2013) and Øverland et al. (2013b) found a decrease in plasma cholesterol, while, Mohebbi et al. (2013) reported increase in plasma cholesterol and decreased triglycerides level when yeast or yeast derived products were utilized. Allam et al. (2019) found a decrease in total protein, albumin, globulin and glucose level in the serum of striped catfish fed on HP-DDG with increasing the inclusion level in the diet, but all of the parameters are higher compared to our data obtained in the experiment. Similarly

**Fig. 1.** Histological sections of liver.



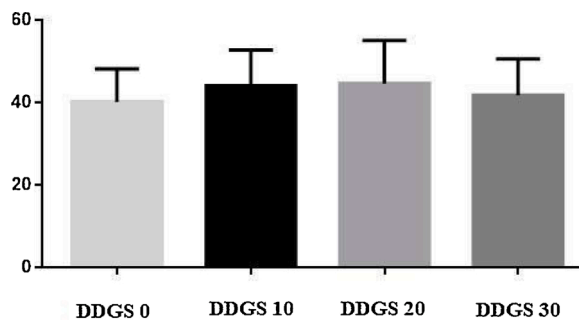


Fig. 2. Length of epithelial cells (μm).

no impact have been reported for gilthead seabream (*Sparus aurata*) juveniles by Diógenes et al. (2019) in total protein, globulin and albumin level with dietary inclusion of DDGS as long as triglyceride level and cholesterol were changing significantly compared to the control.

Changes in the structure of hepatocytes are commonly caused by toxicity, but it can induce by nutritional influences. The status of vacuolation may indicate energy stores in form of glycogen or lipid (Wolf and Wheeler, 2018). Bilen and Bilen (2013) reported that fish in captivity due to less physical activity store much more carbohydrate in the liver than their needs. This symptom has been reported by several authors as it is one of the most common non-infectious nutritional diseases (Shefat and Karim, 2018). Besides increasing of DDGS incorporation in feed, present result showed decreasing tendency of liver vacuolation and hypertrophy. However, the experimental diets have been set to isonitrogenous and isoenergetic, the DDGS30 group had the lowest crude fat content. In this case this small difference and better fatty acid composition due to DDGS resulted less vacuolation, better

nutrient utilization and healthier liver tissue. Some ingredients can destroy the health and the integrity of the intestinal epithelia (Atalah et al., 2007), but no such observations were found in the present study.

Differences in saturated and mono unsaturated fatty acids level in the liver between different treatments suggest that DDGS inclusion positively affected the fatty acid metabolism of European catfish. Decrease of monoenoic fatty acids in the liver is occurred to the decrease in poultry meal level in the diet, which refers to the usefulness of high amount of rendered animal protein in the diet. High level of MUFA in the DDGS 0 group indicate liver fattening (Torstensen et al., 2011), which is confirmed by higher total lipid (mg FA/g) determined. Similar effect was observed in common carp, when the highest fat deposition in DDGS 0 group have been found (Révész et al., 2019). Nonetheless, based on histological study of the liver and on the ALT and ALP activities in the plasma, damage of the liver tissue was not observed in any of the fish groups. The increase of saturated fatty acids in liver with inclusion level of DDGS in diet could not be explained by the minor differences in saturated fatty acid, crude fat or total lipid content of the diets. Such tendency was not observed in the case of common carp (Révész et al., 2019).

The apparent digestibility coefficients (ADC) of different experimental diets for dry matter and crude protein were similar, this fact pointed out that 30 % inclusion of DDGS in the diet containing 20 % fishmeal is preferable and utilizable by European catfish. Moreover, the available phosphorus increases with inclusion of DDGS in the diet, which is a benefit for phosphorus intake and waste discharge.

In conclusion, results from the present study revealed that ADCs of crude fat and phosphorus of corn DDGS were high and reflected in the DDGS as a suitable ingredient for the use in European catfish diet up to 30 %.

Table 8

Fatty acid composition of the liver (w%) (n = 6) (as is).

Fatty acids w%	DDGS 0	DDGS 10	DDGS 20	DDGS 30	p-value
14:0	0.83 ± 0.09	0.92 ± 0.22	0.75 ± 0.09	1.02 ± 0.34	0.170
16:0	15.47 ± 1.94 <sup>a</sup>	17.19 ± 1.12 <sup>ab</sup>	18.42 ± 0.98 <sup>b</sup>	21.10 ± 0.80 <sup>c</sup>	0.000
16:1n-9	1.04 ± 0.18	0.97 ± 0.10	1.03 ± 0.15	1.02 ± 0.13	0.843
16:1n-7	5.81 ± 2.45 <sup>a</sup>	4.85 ± 1.62 <sup>ab</sup>	3.39 ± 0.51 <sup>ab</sup>	2.69 ± 1.14 <sup>b</sup>	0.013
18:0	7.05 ± 1.14 <sup>a</sup>	7.72 ± 0.40 <sup>a</sup>	9.28 ± 0.69 <sup>b</sup>	10.19 ± 0.55 <sup>b</sup>	0.000
18:1n-9	27.93 ± 7.94 <sup>a</sup>	24.62 ± 4.70 <sup>ac</sup>	18.87 ± 2.05 <sup>bc</sup>	15.90 ± 3.27 <sup>b</sup>	0.002
18:1n-7	4.49 ± 0.93	4.53 ± 0.94	4.04 ± 0.37	3.56 ± 0.92	0.176
18:2n-6	5.40 ± 0.65	4.89 ± 0.70	5.07 ± 0.28	5.11 ± 0.27	0.404
18:3n-6	0.31 ± 0.17	0.38 ± 0.40	0.42 ± 0.47	0.24 ± 0.03	0.578
18:3n-3	0.25 ± 0.09	0.21 ± 0.60	0.28 ± 0.15	0.15 ± 0.09	0.191
20:0	0.09 ± 0.00	0.09 ± 0.01	0.29 ± 0.00	0.12 ± 0.04	0.135
20:4n-6	6.10 ± 1.91	6.83 ± 1.34	8.35 ± 0.55	8.72 ± 2.52	0.052
20:5n-3	0.66 ± 0.20	0.73 ± 0.13	0.79 ± 0.08	0.67 ± 0.18	0.468
22:6n-3	11.69 ± 4.40	13.02 ± 2.65	14.76 ± 0.75	14.86 ± 0.65	0.143
Total SFA	23.74 ± 3.15 <sup>a</sup>	26.20 ± 1.40 <sup>ad</sup>	28.92 ± 1.31 <sup>bd</sup>	32.92 ± 1.21 <sup>c</sup>	0.000
Total MUFA	43.20 ± 11.42 <sup>a</sup>	38.57 ± 7.05 <sup>ac</sup>	30.51 ± 3.24 <sup>bc</sup>	26.00 ± 5.06 <sup>b</sup>	0.003
Total n-6	17.89 ± 3.79	18.55 ± 3.27	21.70 ± 1.53	22.51 ± 4.40	0.075
Total n-3	13.13 ± 4.83	14.57 ± 2.89	16.48 ± 0.92	16.43 ± 0.75	0.164
Total PUFA	31.03 ± 8.60	33.12 ± 6.07	38.17 ± 2.30	38.94 ± 0.27	0.084
Total lipid mg FA/g	17.31 ± 4.59 <sup>a</sup>	14.42 ± 2.55 <sup>ac</sup>	10.30 ± 2.25 <sup>bc</sup>	8.46 ± 4.81 <sup>b</sup>	0.000

SFA- saturated fatty acids, MUFA-monounsaturated fatty acids, PUFA-polyunsaturated fatty acids Values are means of six replicates; values within the same row with different letters are significantly different ( $p < 0.05$ ).

Table 9

Apparent digestibility coefficients of the diets with different inclusion level of DDGS.

ADC <sub>feed</sub> (%)	DDGS 0	DDGS 10	DDGS 20	DDGS 30	p-value
Dry Matter	56.62 ± 1.76	54.65 ± 0.23	55.86 ± 0.23	55.85 ± 0.23	0.140
Crude Protein	77.09 ± 1.33	78.60 ± 1.16	74.49 ± 2.32	76.95 ± 0.53	0.054
Crude Fat	98.01 ± 0.16 <sup>a</sup>	96.80 ± 0.11 <sup>a</sup>	88.03 ± 1.50 <sup>b</sup>	97.12 ± 0.07 <sup>a</sup>	0.000
Phosphorous	29.59 ± 2.86 <sup>a</sup>	54.66 ± 0.23 <sup>b</sup>	44.69 ± 0.30 <sup>c</sup>	47.21 ± 0.29 <sup>c</sup>	0.000

Values are means of three replicates; values within the same row with different letters are significantly different ( $p < 0.05$ ).

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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