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Use of corn distiller's dried grains with solubles as a feed ingredient for rainbow trout (*Oncorhynchus mykiss*) feeds: Growth, digestibility, liver and intestine histology

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Abstract

A feeding trial was performed to investigate the effects of the partial replacement of fish meal with distiller's dried grains with solubles (DDGS) on growth, feed assessment, economic assessment, digestibility of diets, as well as liver and intestinal histology of rainbow trout (*Oncorhynchus mykiss*). Four isonitrogenous and isocaloric diets were prepared using DDGS in rates of 0%, 10%, 20% and 30% (Control, DDGS10, DDGS20, and DDGS30, respectively) instead of reducing the fish meal content. Triplicate groups of 25 rainbow trout (mean initial weight 19.88±0.02 g) were fed with the diets for 84 days. The results revealed that dietary DDGS30 had the highest final weight and specific growth rate. Feed conversion and protein efficiency rates were similar among the dietary treatments. Economic conversion ratio was reduced depending on DDGS increase in diet that resulted in an increased economic profit index. The apparent digestibility of the diets did not differ significantly among treatments. There were no significant differences in the diameter of hepatocyte nuclei but there was a detected amount of vacuolization in the hepatocyte cytoplasm of fish fed the dietary DDGS20 and DDGS30 diets. Distal intestine histomorphometric parameters were unaffected by dietary treatments, however, increased lamina propria width was observed as the rate of DDGS increased in diets. These results indicate that DDGS can be added to rainbow trout diets by up to 30% without negatively affecting growth performance, diet digestibility, as well as the histology of the liver and intestine.

Keywords: Alternative protein sources, DDGS, Fish meal replacement, Growth metrics, Feed utilization, Histomorphology

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Introduction

In order to provide sustainable aquaculture production, it is necessary to seek alternative feed ingredients that may be used partially or completely instead of fish meal, and study their applicability (Aydın *et al.*, 2015; Gümüş *et al.*, 2016; Ye *et al.*, 2016; Acar *et al.*, 2019). Some plant-based ingredients such as soybean meal (SBM) have been used to replace dietary fish meal owing to their stable supply, and low price. However, it has been demonstrated that SBM and other plant-based feed ingredients contain a variety of anti-nutritional substances like protease inhibitors, phytic acid, tannins, lectins, saponins, alkaloids, gossypols, oligosaccharides and non-starch polysaccharides, which can negatively affect the health status of fish including levels of bile acids, and the histology of the liver and intestine (Iwashita *et al*., 2008; Ferrara *et al.*, 2015). Some studies showed that inclusion levels of SBM and other plant-based ingredients induce histomorphological changes in the fish tissues such as cytoplasmic vacuolization and displacement of hepatocyte nuclei, reduction in the supranuclear vacuolization of the enterocytes, shortening of the mucosal folds, and increase in the width and cellular infiltration of the submucosa and lamina propria in the distal intestine (Krogdahl *et al*., 2003; Matsunari *et al*., 2010; Martínez-Llorens *et al*., 2012; Venold *et al.*, 2012; Ferrara *et al.*, 2015; Monge-Ortiz *et al*., 2016; Ye *et al*., 2016). Thus, they were used in limited amounts in feeds of carnivorous fish such as rainbow trout.

When plant-based ingredients are included in aquafeeds, either the ingredients that contain less anti-nutritional substances are used, or several enzymes are added to the feed, or various technologies are used before the use of plant-based ingredients to reduce the negative effects of anti-nutritional substances (Güroy *et al.*, 2013; Diógenes *et al*., 2018a). Several studies report that the distiller's dried grains with solubles (DDGS) are obtained as a result of the separation of carbon dioxide and ethanol after the fermentation of sugar in grains with yeast in the ethanol industry that may be used as alternative feed ingredients in fish feed (Welker *et al.*, 2014). The nutrient composition of DDGS, except starch, is approximately three-folds higher than those in the original grain due to the fermentation process (Magalhães *et al.*, 2015; Aydın and Gümüş, 2016). Although DDGS contain a high content of non-starch polysaccharides, they lack other common anti-nutritional factors namely trypsin inhibitor, phytate, gossypol, glucosinolates, erucic acid that are usually found in other plant feedstuff (Magalhães *et al.*, 2015), and their lower costs is also an edge when compared to the other plant-based ingredients (Cheng and Hardy, 2004a; Welker *et al.*, 2014). The determination of the applicability of DDGS instead of fish meal is vital because of their

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advantage in quality and cost. Studies have been conducted to determine the effects of DDGS as a feed ingredient for rainbow trout (Cheng *et al.,* 2003; Cheng and Hardy, 2004a; Barnes *et al.,* 2012a; Welker *et al.,* 2014) and other carnivorous fish species (Bae and Lee 2015; Magalhães *et al.,* 2015; Diógenes *et al*., 2018b). However, there is limited information to determine the effects of DDGS on liver and intestine histology, and economic efficiency of DDGS-based diets for rainbow trout *Oncorhynchus mykiss*. Therefore, the present study was conducted to investigate the effect of a partial replacement of dietary fish meal with different levels of DDGS in rainbow trout diets on growth performance, feed utilization, nutrient digestibility, economic efficiency, and histology of the liver and intestine.

Materials and methods

Experimental diets

Four experimental isonitrogenous (43.47% crude protein) and isocaloric (17.44 MJ/kg digestible energy) diets were formulated to meet (NRC, 1993) the requirements of rainbow trout. The control diet was the same as used previously (Kaushik *et al.,* 1995), where fish meal (65% in diet) was used as the sole protein source. The remaining three diets were prepared by using distiller's dried grains with solubles (DDGS) in rates of 10%, 20% and 30% (DDGS10, DDGS20 and DDGS30, respectively) instead of reducing the fish meal content. The formulation and proximate analysis of the experimental diets are shown in Table 1.

Table 1: Feed ingredients (g 100g^{-1} diet, as is basis) and proximate composition of the experimental diets.

Ingredients	Experimental diets			
	Control	DDGS10	DDGS20	DDGS30
Fish meal[a]	65.00	61.38	57.77	54.16
DDGS[b]	0.00	10.00	20.00	30.00
Corn starch	25.50	19.57	13.63	7.67
Fish oil	6.00	5.55	5.10	4.67
Vitamin premix[c]	1.00	1.00	1.00	1.00
Mineral premix[d]	1.00	1.00	1.00	1.00
CMC[e]	1.00	1.00	1.00	1.00
Cr_2O_3[f]	0.50	0.50	0.50	0.50
Proximate composition[g]				
Dry matter (%)	89.24	89.14	88.84	88.54
Crude protein (%)	43.79	43.32	43.44	43.87
Crude lipid (%)	12.18	12.48	12.96	13.24
Crude ash (%)	9.34	9.32	9.41	9.34
Crude fibre (%)	1.24	1.78	2.36	2.98
NFE (%)[h]	22.69	22.25	20.67	19.12
DE (MJ/kg)[i]	17.42	17.43	17.45	17.47

[a] Crude protein 69.8%, crude lipid 10.4% (wet weight).
[b] Distiller's dried grains with solubles. Crude protein 25.5%, crude lipid 12.2% (wet weight).
[c, d] Vitamin premix and mineral premix as described in Aydın *et al.* (2017)
[e] Carboxymethyl cellulose
[f] Chromium oxide
[g] Proximate composition values are mean of triplicate analyses (%, wet weight).
[h] Nitrogen-free extract = 100 - (crude protein% + crude lipid% + crude ash% + crude fiber% + moisture%).
[i] Digestible energy of experimental diets was calculated according to values 20.50 kJ/g protein, 37.70 kJ/g fat, and 14.60 kJ/g carbohydrate (NRC, 1993).

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Experimental diets were prepared at the facilities of Art-Aqua Yem ve Katkı Maddeleri Tur. San. Tic. Ltd. (Izmir, Turkey). The fish meal (Anchovy, Black Sea) used as the protein source in diets was obtained from Skretting Feed Production Co. Inc. (Muğla, Turkey) and DDGS was made of corn from Agricultural Chemistry Technologies Ind. and Co. Inc. (Bursa, Turkey). Fish oil (Anchovy, Black Sea; Skretting Feed Production Co. Inc., Muğla, Turkey) and corn starch (Sunar Mısır, Adana, Turkey) were added as lipid and carbohydrate sources, respectively to balance the nutrient composition of the diets. Carboxymethyl cellulose (Sigma-Aldrich) as a binder and chromic oxide (Sigma-Aldrich) as an inert marker were used at 1% and 0.5% inclusion level in diets, respectively.

All the dry ingredients were thoroughly mixed and extruded in a twin screw extruder (DP65-II Double Screw Inflating Food Machine, Jinan Eagle Food Machinery Co., Ltd., Shandong, China) with a 3 mm die after adding fish oil and water (30%, v/w). The barrel temperatures could be adjusted from 36°C to 80°C. After decreasing the moisture content of the pellets below 11.5% using a ventilated dry oven (DP-DKX-II Multi-Layer Auto Oven, Jinan Dapeng Machine Co., Ltd., Shandong, China), sealed in plastic bags and stored at -20 °C until used.

Rearing conditions of fish and feeding

The feeding trial was carried out on a commercial trout farm (Toklu Fish Farm Co., Ltd., Antalya, Turkey). Juvenile rainbow trout were obtained from another fish farm (Aydemir Trout Co., Ltd., Isparta, Turkey) and were hand-fed three times a day until apparent satiation using commercial feed (45% crude protein, 18% crude lipid and 17 MJ/kg digestible energy; Blueaq, Abalıoğlu Feed-Soy and Textile Ind. Inc., Denizli, Turkey). After 15 days of acclimation period, a total of 300 fish (initial mean body weight 19.88±0.02 g) were randomly divided among 12 fiberglass circular tanks (200 L) with 25 fish/per tank. Four experimental diets were randomly assigned to tanks with three replicates/treatment. During the experiment, the fish were fed by hand three times a day (at 09:00, 13:00 and 17:00 h) with the diets to apparent satiation for 84 days. The amount of feed distributed per tank was recorded and uneaten feed, if any, was collected from the bottom of the tank using a siphon 30 min after the onset of feeding, dried in an oven at 70 °C and weighed to determine the feed intake. The fish were subjected to a natural photoperiod of approximately 11 h light/13 h darkness. Each tank was supplied with 10 L/min flow-through freshwater with temperature varied between 9.3 and 15.2 ºC, averaging 14.3 ºC due to seasonal climate variation that affects all tanks equally.

Sampling procedures

At the end of the 84-day feeding trial, all the fish in each tank were caught and

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individually weighed on an analytical balance (Precisa BJ 410C, Dietikon/Switzerland) to determine growth performance, feed utilization and economic efficiency parameters. Prior to weighing, the fish were anaesthetised with eugenol (EMD Millipore Corperation, Billerica, Massachusetts, USA). Since eugenol is poorly soluble in water, it was first dissolved in ethanol at a ratio of 1:10 (eugenol:ethanol 95%) and used at a concentration of 75 μL L^{-1} (Taheri Mirghaed *et al*., 2018). Five fish, from each tank were randomly sampled and starved for 24 h prior to sampling for organosomatic indices and histological examinations. From these five fish, the liver, intestine and viscera were individually weighed to calculate organosomatic indices. Meanwhile, three of these five fish per tank with a total of nine fish for each dietary treatment were also sampled for histological analyses. Liver and approximately 1-cm distal intestine section samples were taken as described by Bullerwell *et al.* (2016). The samples were fixed in 10% phosphate-buffered formalin (pH 7.2) for 24 h, then transferred to 70% ethanol and stored at 4 °C until further processing.

Digestibility trial

After sampling at the end of the feeding trial, the rest of the fish (15 fish per tank) were used for the digestibility trial. The fish were fed by hand twice a day (at 09:00 and 17:00 h) with the experimental diets to apparent satiation for 21 days. Uneaten feed and fecal residue were removed from the tank using a siphon 30 min after feeding. Feces were siphoned from each of the 12 tanks through a fine mesh netting (80 μm) before the next feeding. Sufficient amount of feces were collected from each tank, centrifuged at 2500 rpm for 15 min, separated from supernatant, and stored in sealed plastic bags at -20°C until analysis. The fecal samples from each tank were dried in an oven at 70 °C and ground to a powdery consistency and analyzed separately to determine their chemical composition and chromic oxide concentrations. Apparent digestibility coefficients (ADC) of dry matter, protein, lipids, and energy of the experimental diets were calculated using the following equations (Cho *et al.*,1982):

ADC of nutrients (%) = 1 - [100 x (dietary Cr_2O_3 / fecal Cr_2O_3) x (fecal nutrient / dietary nutrient)]

ADC of energy (%) = 1 - [100 x (dietary Cr_2O_3 / fecal Cr_2O_3) x (fecal energy / dietary energy)]

Economic analysis

The feed costs ($/kg) calculated were the cost of diet required to produce 1 kg of biomass. They were calculated from using the price of feed ingredients (October 2014: Fish meal 1.7 $/kg, DDGS 0.25 $/kg). The sale price of rainbow trout was considered at 3.13 $/kg in Turkey during the period of the experiment (Anonymous, 2015).

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Economic indexes were calculated according to standard formulae (Martínez-Llorens *et al*., 2007) at the end of the feeding trial:

Economic conversion ratio (ECR) ($/kg) = Amount of feed consumption (kg) x feed cost ($/kg) / weight gain (kg)

Economic profit index (EPI) ($/fish) = [final weight (kg/fish) x fish sale price ($/kg)] - [ECR ($/kg) x weight increase (kg)]

Chemical analysis

Chemical compositions of the experimental diets and feces were determined using the official methods by AOAC (1995). Chromic oxide concentrations of the experimental diets and feces were determined according to Furukawa and Tsukahara (1966).

Histological analysis

Histological analysis were carried out at the Histology Laboratory, Faculty of Fisheries, Akdeniz University, Antalya, Turkey. Fixed liver and distal intestine samples were dehydrated in a graded ethanol series, equilibrated in xylene, embedded in paraffin according to the standard histological analysis procedures. Sections of 5 μm were cut using a rotary microtome (RM2135 RT, Leica Instruments, Nussloch, Germany) and stained according to the hematoxylen and eosin (H&E) staining technique (Khojasteh *et al*., 2009; Jalili *et al*., 2019). Light microscopy evaluation was performed using a microscope (Olympus CX41, Olympus Corporation, Tokyo, Japan), and images were taken using a camera (The Imaging Source DFK 72AUC02, Bremen, Germany) which was connected to the microscope. Liver sections were evaluated based on the general abnormalities such as vacuole formation within the hepatocytes, nuclear location and nuclear diameter (Caballero *et al.,* 2004; Martínez-Llorens *et al.,* 2012). The intestinal morphology was evaluated according to Bullerwell *et al.* (2016) and Ye *et al.* (2016). Measurements of villi height, villi width, villi area, lamina propria, submucosa, stratum compactum and muscular layer were performed (Figs. 1 and 2). The number of goblet cells was also assessed according to Baeza-Ariño *et al*. (2016) and Ye *et al* (2016). The images were processed and analysed using NIS-Elements D software (Nikon Instruments Europe B.V., Amsterdam, Netherlands).

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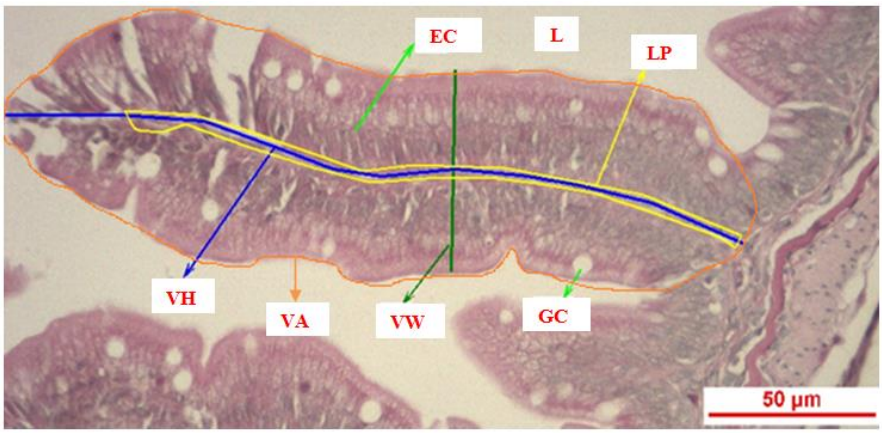


Figure 1: Detail of distal intestine villus with measures of VH (Blue line): Villi height, VW (Green line): Villi width, LP (yellow line): Lamina propria width, VA (Orange line): Villi area. GC: Goblet cell, L: Lumen, EC: Enterosit cell (Original) (H&E, 200x).



Figure 2: Detail of distal intestine wall with measures of mm (Green line): muskularis layer, sc (red line): stratum compactum, sm (yellow line): submucosa (Original) (H&E, 400x).

Statistical analysis

Data were submitted to Kolmogorov-Smirnov and Levene's tests, to verify normal data distribution and homogeneity of variances, respectively. All data analysis was performed using one-way analysis of variance (ANOVA). Differences among the means were compared using Duncan's multiple range tests. All statistical tests were performed using the SPSS software (IBM SPSS Statistics Base v23, IBM Corporation, New York, USA). Regression analysis of the relationship between the feed intake, ECR, EPI, lamina propria width and the replacement levels of dietary DDGS were calculated using Microsoft Excel. Limits for all critical ranges were set at $p<0.05$. Results were expressed as means ± SD throughout the text.

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Results

Growth performance, biometrical parameters and survival

The growth performance and survival rate of rainbow trout fed with the four different levels of DDGS as a replacement for fish meal are presented in Table 2. At the end of the 84-day feeding trial, final weight and specific growth rate were significantly different among the fish fed with the Control, DDGS10, DDGS20, and DDGS30 diets, with the highest specific growth rate in the treatment group fed the DDGS30 diet and lowest growth performance was recorded in trout in the Control group. No significant differences were detected for growth performance in the DDGS10 and DDGS20 groups. No significant differences were observed in condition factor, viscerosomatic indices, hepatosomatic indices and intestine somatic indices among the dietary treatments. The fish survival rates ranged from 96.0% to 98.7%, but no significant difference was found among treatments.

Table 2 Growth performance, biometrical parameters and survival of rainbow trout fed the different experimental diets.

Parameters	Experimental diets			
	Control	DDGS10	DDGS20	DDGS30
Initial weight (g/fish)	19.87±0.49	19.88±0.55	19.87±0.59	19.89±0.36
Final weight (g/fish)	100.21±3.32^{b}	102.79±1.79^{b}	104.18±1.95^{b}	109.70±3.91^{a}
SGR (%/day)	1.93±0.04^{b}	1.95±0.03^{ab}	1.97±0.02^{ab}	2.03±0.06^{a}
CF	1.20±0.01	1.21±0.01	1.21±0.01	1.22±0.01
VSI (%)	11.56±0.68	11.41±0.50	11.18±1.08	11.14±0.89
HSI (%)	1.42±0.11	1.40±0.07	1.32±0.11	1.36±0.15
ISI (%)	1.01±0.06	1.05±0.09	1.06±0.10	1.07±0.11
SR (%)	97.3±2.3	96.0±4.0	98.7±2.3	98.7±2.3

$^{a-b}$ Values in the same row with different superscripts are significantly different from each other (p<0.05).

SGR: Specific growth rate = [Ln final weight (g) - Ln initial weight (g)] / days x 100; CF: Condition factor = [final weight (g) / total length^{3}(cm)] x 100; VSI: Viscerosomatic indices = [visceral weight (g) / final weight (g)] x 100; HIS: Hepatosomatic indices = [liver weight (g) / final weight (g)] x 100; ISI: Intestine somatic indices = [intestine weight (g) / final weight (g)] x 100; SR: Survival rate = (final number of fish / initial number of fish) x 100

Feed utilization and digestibility

The feed utilization and digestibility of experimental groups are shown in Table 3. Feed intake was significantly greater for fish fed the DDGS30 diet compared to those fed the Control diet. However, feed intake of fish fed the DDGS10 and DDGS20 diets was similar to that of the Control diet. There was a significant cubic regression between DDGS content in diets and feed intake (R^2=0.7002; P=0.017). Feed conversion ratio (ranging from 0.94 to 0.97) and protein efficiency ratio (ranging from 2.25 to 2.31) were not significantly different among the dietary treatments.

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The apparent digestibility coefficient (ADC) of dry matter, protein, lipid and energy ranged from 76.27-77.25%, 91.64-92.53%, 94.09-94.56% and 89.10-89.72%, respectively, and were similar among all diets.

Table 3: Feed utilization of rainbow trout and apparent digestibility coefficients (ADC) of the experimental diets.

Parameters	Experimental diets			
	Control	DDGS10	DDGS20	DDGS30
FI (g/fish 84 days)	78.14±1.85[b]	80.88±1.56[b]	81.27±2.22[ab]	84.86±1.98[a]
FCR	0.97±0.02	0.97±0.01	0.96±0.01	0.94±0.03
PER	2.26±0.04	2.25±0.01	2.28±0.03	2.31±0.06
Dry matter (%)	77.25±0.29	77.14±0.38	76.32±0.40	76.27±0.95
Crude protein (%)	91.99±0.32	91.64±0.32	92.06±0.53	92.53±0.66
Crude lipid (%)	94.09±0.22	94.52±0.10	94.56±0.46	94.33±0.29
Energy (%)	89.10±0.19	89.49±0.21	89.56±0.28	89.72±0.50

Values are mean (±SD) of triplicate analysis. [a-b]Values on the same line and different superscripts are significantly different (p<0.05).
FI: Feed intake = [amount of feed supplied (g) - amount of unconsumed feed (g)] / number of fish in tank; FCR: Feed conversion ratio = feed intake (g) / [final weight (g) - initial weight (g)]; PER: Protein efficiency ratio = [final weight (g) - initial weight (g)] / protein intake (g).

Economical parameters

The feed cost ($/kg) decreased from 1.93 in the Control diet to 1.84, 1.76 and 1.67 in diets DDGS10, DDGS20 and DDGS30, respectively (Table 4). However, significant differences were observed in ECR and EPI among treatments. The highest ECR (1.98 $/kg) was found in the fish fed the Control diet, while the lowest ECR (1.77 $/kg) was found in the fish fed the DDGS30 diet. A strong positive cubic relationship was determined between dietary DDGS inclusion and the ECR (R^2= 0.9089; P= 0.000). Likewise, there was a strong positive cubic relationship between dietary DDGS inclusion and the EPI (R^2= 0.9089; p=0.000). EPI was the lowest in fish fed the Control diet (0.15 $/fish) and the highest in fish fed the DDGS30 diet (0.18 $/fish).

Table 4: Economical parameters of rainbow trout fed the different experimental diets.

Parameters	Experimental diets			
	Control	DDGS10	DDGS20	DDGS30
Feed cost ($/kg)	1.93	1.84	1.76	1.67
ECR ($/kg)	1.980±0.04[a]	1.883±0.01[b]	1.820±0.02[c]	1.767±0.04[c]
EPI ($/fish)	0.153±0.01[c]	0.163±0.01[b]	0.174±0.01[a]	0.180±0.01[a]

Values are mean (±SD) of triplicate analysis. [a-c]Values in the same row with different superscripts are significantly different from each other (p<0.05).
ECR: Economic conversion ratio; EPI: economic profit index.

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Liver histology

The liver from fish fed the Control and DDGS10 diets showed uniform, normal-shaped hepatocytes and centrally located nuclei (Fig. 3). The liver samples of fish fed the DDGS20 and DDGS30 diets demonstrated variable levels of cytoplasmic vacuolization and the displacement of nuclei toward the periphery of the hepatocyte. The diameter of hepatocytes nucleus in the Control group (5.94±0.13 μm) was bigger than those in the DDGS10 (5.77±0.12 μm), DDGS20 (5.81±0.18 μm) and DDGS30 (5.70±0.06 μm) treatments, but there were no significant differences in the diameter of hepatocytes nucleus among the treatments (Table 5). The hepatocytes of fish fed the Control diet and DDGS-based diets had round or elliptic nuclei stained clearly with hematoxylin.

Table 5: Effect of the DDGS dietary inclusion on liver and distal intestine histomorphometric parameters.

Parameters	Experimental diets			
	Control	DDGS10	DDGS20	DDGS30
Liver				
Nucleus (μm)[a]	5.94±0.68	5.77±0.12	5.81±0.18	5.70±0.06
Distal intestine				
Villi width (mm)	0.12±0.02	0.13±0.03	0.13±0.02	0.13±0.02
Villi height (mm)	0.71±0.23	0.70±0.20	0.73±0.25	0.75±0.29
Villi area (mm^2)	0.08±0.03	0.08±0.03	0.09±0.02	0.09±0.03
Lamina propria (μm)	14.71±3.16[a]	15.33±4.80[ab]	17.41±5.19[c]	16.65±5.80[bc]
Submucosa (μm)	14.19±3.45	14.91±5.60	15.25±4.71	14.82±5.76
Stratum compactum (μm)	9.92±2.62	10.05±3.01	10.92±3.77	10.65±4.32
Muscular layer (μm)	77.92±17.35	78.70±17.07	78.52±13.01	78.64±17.76
Goblet cells[b]	9.13±2.88	9.67±3.94	10.13±3.77	9.97±3.60

[a-c]Values in the same row with different superscripts are significantly different from each other (p<0.05).

Values are the means ± SD; n=90 (10 measurements x 3 fish x 3 tanks).

[a] Diameter of hepatocyte nucleus

[b] Goblet cells per villi

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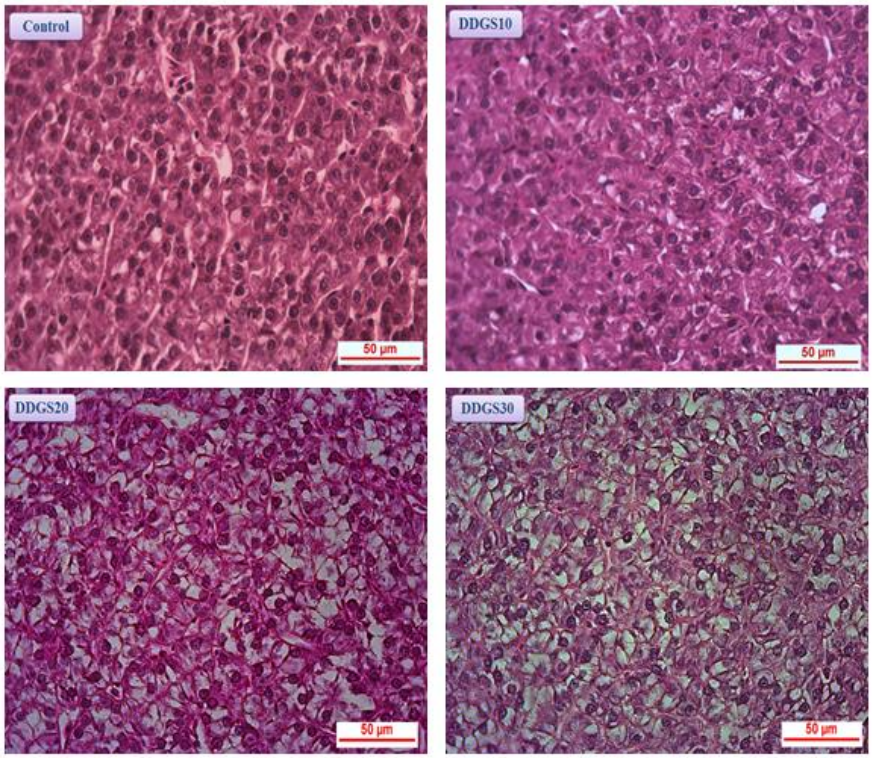


Figure 3: Histological sections of hepatocytes of the liver of rainbow trout fed the experimental diets (H & E, 400x). Livers from fish fed with the Control and DDGS10 diets showed uniform, normal-shaped hepatocytes and centrally located nuclei. The livers of fish fed with DDGS20 and DDGS30 diets demonstrated variable levels of cytoplasmic vacuolization and the displacement of nuclei toward the periphery of the hepatocyte.

Intestine histology

The results of the measurements of the evaluated histomorphometric parameters in the distal intestine of rainbow trout are presented in Table 5. Generally, there were no significant histomorphometric changes in the distal intestine of fish fed the different experimental diets. However, lamina propria width of fish fed the DDGS20 and DDGS30 diets increased significantly compared to those in the Control group. Dietary inclusion of DDGS had no significant effect on villi height, villi width and villus area (Table 5; Fig. 4). The submucosa, stratum compactum and muscular layer did not present any significant differences, whereas lamina propria width showed significant differences among dietary treatments (Table 5; Fig. 5). There was a significant cubic relationship between lamina propria width and dietary inclusion of DDGS (R^2=0.7926). The number of goblet cells per villi ranged from 9.13±2.88 to 10.13±3.77 although no significant differences were found among dietary treatments. However, a trend toward increasing numbers of goblet cells with increasing inclusion levels of DDGS was observed.

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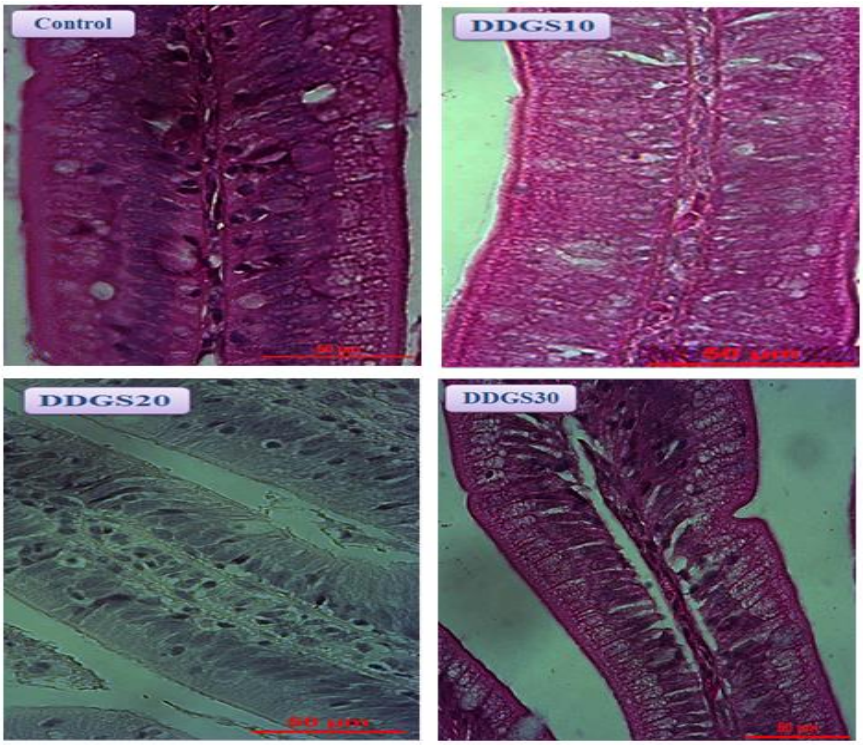


Figure 4: Histological sections of mucosal folds of the distal intestine of rainbow trout fed the experimental diets (H & E, 400x). The figure demonstrated changes in lamina propria witdhin the fish fed the DDGS10, DDGS20 and DDGS30 diets compared to the Control.

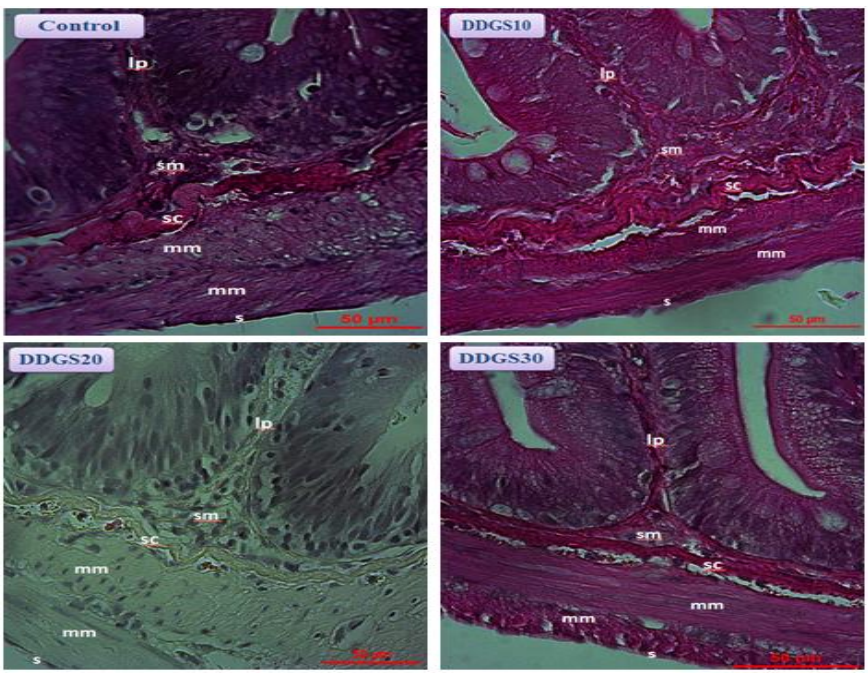


Figure 5: Histological sections of the distal intestine wall of rainbow trout fed the experimental diets. mm: muskularis layer, sc: stratum compactum, sm: submucosa (H & E, 400x). No significant differences were observed in submucosa, stratum compactum and muscular layer between experimental groups.

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Discussion

Results showed that after using dietary DDGS instead of fish meal did not have any significant negative effect on the growth performance of rainbow trout. This result indicated that DDGS used in this feeding trial could be a potential candidate as an alternative feed ingredient for rainbow trout. Cheng and Hardy (2004b) and Barnes *et al.* (2012b) reported that DDGS could be used in the diet at a rate of 15% and 20%, respectively without observing decrease in the growth of the fish. These result were similar to the findings of the present study. In contrast to the present study, Bae and Lee (2015) reported that final weight and specific growth rate decreased with the decrease in fish meal and with an increase in DDGS in juvenile rockfish *Sebastes schlegeli* diets. They reported that the fermentation of DDGS during production, and depending on this, the amount of anti-nutritional substances decreased and yeast *Saccharomyces cerevisiae* (3.9% of DDGS, 5.3% of the total DDGS protein), which is beneficial for fish growth, might have a role in this (Yamamoto *et al.*, 2010; Gümüş *et al*., 2016). Researchers underlined that since the nutrient amount in DDGS might show variations due to many factors, there might be different results in growth parameters reported by different studies (Barnes *et al.*, 2012a; Aydın and Gümüş, 2016). The feed utilization parameter values in this study were similar to those reported by Cheng and Hardy (2004b), and Bullerwell *et al.* (2016). Unlike the results of this study, FCR values deteriorated depending on the increase of the DDGS in rainbow trout (Barnes *et al.* 2012c) and in rockfish diet (Bae and Lee, 2015).

According to the previously conducted study by Barnes *et al.* (2012b) feed costs might be reduced by using DDGS in diet. The feed cost, which is one of the economic assessment parameters of this study, decreased with the increase of DDGS and the reduction of fish meal used in the feed (Table 5). The most important factor in the decrease in feed costs is the low value of DDGS when compared to fish meal. Martínez-Llorens *et al.* (2007) and Sánchez Lozano *et al.* (2007) showed that using soybean and sunflower meal instead of fish meal improved feed costs, ECR and EPI values, which is in agreement with the results of this study. In the study on rainbow trout by Barnes *et al.* (2012b), the cost of one kilogram of fish meal was computed as $ 0.56 when the fish were fed with Control diet containing fish meal; and this amount decreased to $ 0.44 when 20% DDGS was used in the diet. The results of the present study are similar to the findings of Barnes *et al.* (2012b), who reported that important economic profits may be acquired by increasing the DDGS rate and decreasing the fish meal rate.

The protein digestibility values (91.6-92.5%) in this study were found to be similar between the treatments (Table 3). Protein and amino acid digestibility rates of DDGS are high in rainbow trout, which was also reported by

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Cheng and Hardy (2004a), Barnes *et al.* (2012a) and Barnes *et al.* (2012b). In the study conducted by Barnes *et al.* (2012a), the protein digestibility values (93.4-94.1%) of the rainbow trout diet with DDGS was higher when compared to that in the control (91.4%). The lipid digestibility values of this study (94.1-94.6%) were found to be higher than those reported by Cheng and Hardy (2004a) (79-89%), and lower than those determined by Magalhães *et al.* (2015) (95.8%). Refstie *et al*. (2005) displayed that the fermentation process in plant-based protein sources is influential in increasing the digestibility rates of the nutrients.

Changes in diet content have been reported to have significant effects on the histological structure of tissues. Thus, histological analysis is used as a valuable tool for fish species to provide more information about tissue changes caused by diet ingredients. The histomorphometric parameters of liver and distal intestine are significantly affected by factors such as the variety and quantity of prebiotics, fatty acids, antinutrients, enzymes, and bile salt (Caballero *et al*., 2004; Yamamoto *et al*., 2010; Ye *et al*., 2016; Fronte *et al*., 2019). In this study, it was determined that the increase of DDGS in the diets does not affect the diameter of hepatocyte nucleus of the fish; however, there was a decrease, which was similar to the results reported by Matsunari *et al.* (2010). Martínez-Llorens *et al.* (2012) and Baeza-Ariño *et al.* (2016) demonstrated that using plant-based ingredients in sea bream diet caused an increase in the vacuoles in the liver cell cytoplasm of the fish and led to replacements in some cell nuclei, which were similar to the findings reported in our study about the livers of the fish fed with the DDGS20 and DDGS30 diets. Previous studies showed that replacement of fish meal with increasing amounts of DDGS led to a significant increase in linoleic acid content of rainbow trout muscle, largely due to the high linoleic acid content of DDGS in the diet (Aydın and Gümüş, 2016; Aydın *et al.*, 2017). In the present study, the increase in the vacuoles in the liver cell cytoplasm and migration of some cell nuclei in fish in the DDGS20 and DDGS30 groups may be due to the fatty acid composition of the diets. These results were in agreement with the findings of Caballero *et al.* (2004), who claimed that the rate of fish oil decreased and that of vegetable oil increased in diets.

This study demonstrated that using DDGS in feeds instead of fish meal did not cause any inflammations in the intestinal structure of the fish. The results obtained by Ye *et al.* (2016) showed similarities with our findings. Bansemer *et al.* (2015) used up to 20% solvent-extracted soybean meal and up to 40% soy protein concentrate in yellowtail kingfish diets, and Ye *et al.* (2016) used up to 20% solvent-extracted camelina meal instead of fish meal in Atlantic salmon *Salmo salar* diets and found no significant effect on the villi height, villi width and villi

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area, which were similar to the results of the present study. It was observed that only the width of villus lamina propria significantly increased as the DDGS rate increased in diets (Table 5). The results were similar to the findings of Martínez-Llorens *et al.* (2012), Ferrara *et al.* (2015), Baeza-Ariño *et al.* (2016), Monge-Ortiz *et al.* (2016) and Ye *et al.* (2016), who reported that partly using plant-based ingredients in diets instead of fish meal greatly increased the villus lamina propria width. Bansemer *et al.* (2015), on the other hand, indicated that using plant-based ingredients in the diet at varying amounts instead of fish meal did not have any negative effects in the lamina propria width when compared with the control group. Venold *et al.* (2012) noted that the duration of the feeding was important in terms of histopathological effects occurring in the distal intestines, and as the feeding duration increased, fish adapted to the diet and tolerated the anti-nutritional substance contents in the diet at certain rates. Similar to the results of this study, Martínez-Llorens *et al.* (2012) used carob meal, Monge-Ortiz *et al.* (2016) used plant protein mixtures and Ye *et al.* (2016) used solvent-extracted camelina meal in their studies, and these applications did not have any significant effects on the submucosa and muscular layer values of the fish. In this study, it was determined that the goblet cell count per villus was between 9.1 and 10.1; no significant differences were recorded in the goblet cell count between the dietary treatments; however, there was a certain increase with the increase of DDGS in the diet. Similar to the our findings, Martínez-Llorens *et al.* (2012) and Ye *et al.* (2016) reported that when up to 34% and up to 20% plant-based ingredients were used in the diets of sea bream, and Atlantic salmon, respectively, there were no significant differences in the goblet cell count. However, Baeza-Ariño *et al.* (2016) reported that when a mixture of vegetable protein concentrates were used in sea bream diets instead of fish meal, there were statistically significant increases in the goblet cell count per villus, which is similar to the increasing trend in the results of the present study.

In conclusion, it was determined that up to 30% of DDGS can be incorporated instead of decreasing fish meal without significantly affecting growth performance, feed utilization, and apparent nutrient digestibility of rainbow trout. The results also indicated that using DDGS in the diets in place of fish meal did not negatively affect distal intestine histomorphometric parameters except for lamina propria width, and liver histology. In addition, it was seen that using DDGS as a feed ingredient in rainbow trout diets would bring economic benefits.

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