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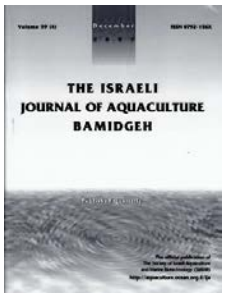

The Effect of Distiller's Dried Grains with Solubles on Carcass Composition, Fatty Acid Composition, Skin and Fillet Coloration of Rainbow Trout (*Oncorhynchus mykiss*)

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Keywords: DDGS; fish meal; alternative feedstuff; sustainable diets; flesh quality; colour

Abstract

The aim of the study was to evaluate the effects of distiller's dried grains with solubles (DDGS) as an alternative protein source on carcass composition, fatty acid composition, skin, and fillet coloration of rainbow trout (*Oncorhynchus mykiss*). Four isoproteic (45.5% crude protein) and isocaloric (17.42 MJ/kg) diets were formulated using DDGS (0, 10, 20 and 30%) for a feeding trial of 84 days. Fish (mean initial weight of 19.88 g) were distributed into triplicate treatments at a rate of 25 fish per 200 L tanks and fed to satiation with the experimental diets three times a day. The results of the present study show that the coloration of experimental fish was not modified negatively by the use of DDGS protein sources in diets. Carcass composition values were also not affected by increased DDGS, although some fatty acids were affected. Total saturated fatty acids, total monounsaturated fatty acids, total n-3 polyunsaturated fatty acids (∑n-3 PUFA) and ∑n-3 PUFA/total n-6 polyunsaturated fatty acids (∑n-6 PUFA) values of the fish in the experimental groups were found to be statistically similar. On the other hand, palmiteloic acid, docosahexaenoic acid and ∑n-3 PUFA decreased, while linoleic acid, ∑n-6 PUFA and ∑PUFA increased significantly. In conclusion, the fatty acid composition of fish muscle was affected by the use of DDGS up to a rate of 30% in rainbow trout diet, while no negative effect on the skin and fillet coloration could be found.

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Introduction

Fish meal (FM) has been widely used in carnivorous fish feed as a high-quality feed ingredient. FM production cannot meet the increasing demand as fish stocks worldwide are diminishing, and costs are high relative to other protein sources. In an attempt to solve the impending crisis in the expanding aquaculture sector, research on cheaper and plentiful vegetal or animal protein source alternatives to replace FM has increased. Distiller's dried grains with solubles (DDGS), a major by-product of the dry mill ethanol industry, is a vegetal protein source which is readily available, has high feeding values, and is competitively priced relative to other alternative protein sources (Magalhães et al., 2015). Studies regarding replacement of lowered FM with DDGS as an alternative feed protein source have been carried out on cultured fish species like rainbow trout *Oncorhynchus mykiss* (Barnes et al., 2012), European seabass *Dicentrarchus labrax*, meagre *Argyrosomus regius* (Magalhães et al., 2015), and the Nile tilapia *Oreochromis niloticus* (Schaeffer et al., 2010).

Research into complete or partial replacement of FM with vegetal alternative protein sources has had both positive and negative changes on fish skin, fillet coloration, and quality (Kalinowski et al., 2005; James et. al., 2009; García-Romero et al., 2014; Aydın et al., 2015; Yi et al., 2015; Grassi et al., 2016; Karadal et al., 2016). Some studies have shown negative effects of vegetal protein source replacement for FM on the chemical composition, a lowering of n-3 and polyunsaturated fatty acid (PUFA) values as compared to the fish fed with FM (Francesco et al., 2004; Gümüş and Aydın, 2013; Aydın et al., 2015). Others have shown that partial substitution does not affect body composition of tilapias (Yigit and Demir, 2016). Changes in color and quality of the fish directly affect price and preference (Kalinowski et al., 2011). Therefore, some research has been focused on the effects of the feed content on skin and fillet color (Francesco et al., 2004; Kalinowski et al., 2005; García-Romero et al., 2014; Yi et al., 2015; Karadal et al., 2016). Although several studies have examined the effects of DDGS on coloration on poultry and livestock (Cortes-Cuevas et al., 2015), no reports on the effects of DDGS supplementation to fish feed are available.

The aim of this study was to investigate the effects of partial replacement of FM with DDGS on carcass composition, muscle fatty acid composition, skin, and fillet coloration of rainbow trout (*Oncorhynchus mykiss*).

Materials and Methods

Feed formulation and preparation. The fish meal (FM) used as the protein source in experimental feeds was obtained from Skretting Feed Production Co. Inc. (İzmir, Turkey), and the distillers dried grains with solubles (DDGS) made of corn was obtained from Agricultural Chemistry Technologies Ind. and Co. Inc. (Bursa, Turkey).

Four isoproteic (45.47% crude protein) and isocaloric (17.42 MJ/kg digestible energy) experimental diets were formulated using DDGS (0 [Control], 10 [DDGS10], 20 [DDGS20], and 30% [DDGS30]) to meet NRC (2011) requirements for rainbow trout (*Oncorhynchus mykiss*) (Table 1). The ground feed content was weighed using digital balances with 0.5 g (KERN DS 65K) and 0.001 g precision (Scaltec SPB 42), homogenized in a food mixer (Jinan Eagle Machine DP-40, Shandong, China), then pelleted using a twin-screw extruder (DP65-II Twin Screw Inflating Food Machine, Jinan Eagle Machine Co., Ltd., Shandong, China) into 3 mm pellets. After decreasing the moisture content below 11.5% using a ventilated oven (DP-DKX-II Multi-Layer Auto Oven, Jinan Dapeng Machine Co., Ltd., Shandong, China) the food contents were left to cool down at room temperature and stored in sealed plastic bags at below -20°C for future use.

Table 1. Feed ingredients and proximate composition of the experimental diets

Ingredients (%)	*Experimental diets*[1]			
	DDGS0 (Control)	*DDGS10*	*DDGS20*	*DDGS30*
Fish meal[2]	65.00	61.38	57.77	54.16
DDGS[3]	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[4]	1.00	1.00	1.00	1.00
Mineral premix[5]	1.00	1.00	1.00	1.00
CMC[6]	1.00	1.00	1.00	1.00
Cr_2O_3[7]	0.50	0.50	0.50	0.50
Proximate composition[8]				
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 (%)[9]	22.69	22.25	20.67	19.12
DE (MJ/kg)[10]	17.42	17.43	17.45	17.47

[1] Experimental diet code indicating DDGS in diet (0%, 10%, 20%, and 30%, respectively);
[2] Anchovy *Engraulis encrasicolus*, Black sea. Crude protein 69.8%, crude lipid 10.4% (wet weight)
[3] Distiller's dried grains with solubles. Crude protein 25.5%, crude lipid 12.2% (wet weight)
[4] Per kg vitamin mix (Rovimix 107): 16 000 000 IU vitamin A, 1 600 000 IU vitamin D3, 170 000 mg vitamin E, 10 800 mg vitamin K3, 16 000 mg vitamin B1, 25 500 mg vitamin B2, 17 000 mg vitamin B6, 43 vitamin B12, 170 000 mg vitamin C, 170 000 mg niacin, 5 100 mg folic acid, 42 500 mg calcium D-pantothenate, 425 mg D-biotin, 255 000 mg inositol.
[5] Per kg mineral mix (Remineral): 42 500 mg manganese, 42 500 mg iron, 42 500 mg zinc, 8 500 mg copper, 128 mg cobalt, 680 mg iodine, 128 mg selenium
[6] Carboxymethyl cellulose
[7] Chromium oxide
[8] Proximate composition values are mean of triplicate analyses (%, wet weight basis)
[9] Nitrogen-free extract = 100 - (%crude protein + %crude lipid + %crude ash + %crude fiber + %moisture)
[10] Digestible energy of experimental diets was calculated according to values 20.5 kJ/g protein, 37.7 kJ/g fat, and 14.6 kJ/g carbohydrate (Aydın and Gümüş, 2013)

Rearing conditions of fish and feeding. This study was conducted at the Toklu Fish Farm Co., Ltd. (Antalya, Turkey), between January 1 and March 29 of 2015. The rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) were obtained from Aydemir Trout Co., Ltd. (Isparta, Turkey). During the acclimation period of 15 days the fish were fed 3 mm diameter commercial trout feed composed of 45% crude protein, 18% crude lipid and 16.7 MJ/kg energy (Blueaq, Abalıoğlu Feed-Soy and Textile Ind. Inc., Denizli, Turkey). At the beginning of the experiment, each of 12 cylindrical fiberglass tank (200 L water/tank) was randomly stocked with 25 fish (initial mean weight of 19.88±0.02 g) with three replicate tanks for each dietary treatment (4 treatments x 3 replicates). Each tank was supplied with 10 L/min flow-through freshwater at a temperature, 14.3±1.2ºC; dissolved oxygen, 8.4±0.4 mg/L; and pH 7.2±0.1. Fish were fed by hand to apparent satiation with experimental diets three times a day (09:00, 13:00 and 17:00 h) for 84 days. Photoperiod was set at natural conditions. Fish weight and amount of feed consumed was measured biweekly. Any uneaten feed was collected 1 h after each feeding, dried to constant weight at 70°C and weighed.

Chemical analysis. At the end of the trial, five fish per tank were selected and anesthetized with 0.75 ml/L clove oil solution (1:10 ratio; clove oil: 95% ethanol). Fish samples were stored at -20°C until they were analysed for proximate carcass and muscle fatty acids composition. Chemical composition of experimental diets and fish carcass

were performed according to standard AOAC (1995). Moisture was determined by drying to constant weight at 105°C in laboratory oven (Elekro-mag M 5040 p), crude protein (N × 6.25) was determined by the Kjeldahl method after an acid digestion (Gerhardt Analytical Systems; Königswinter, Germany), crude lipid was determined by the ether-extraction method using the Soxtec System HT (Behr Soxtec System KV5M; Düsseldorf, Germany), and ash content was determined by a muffle furnace (Elekro-mag M 1813) at 550°C for 5 h. Total lipids were extracted by the method of Folch et al. (1957). Fatty acids were methylated according to the procedure of Metcalfe and Schmitz (1961) and fatty acid methyl esters (FAME) were analyzed as described by Czesny and Dabrowski (1998). Fatty acids were determined by Agilent 6890 N Network Gas Chromatography (Agilent Technologies; Santa Clara, CA, United States).

Color measurements. At the end of the feeding experiment, five fish from each tank were randomly selected and anesthetized with 0.75 ml/L clove oil solution for color measurements. Color measurements were taken at the end of the procedure (Average weight of these fish: 104.1 ± 11.0 g) using a Minolta CR400 Chroma Meter (Minolta Camera Co. Ltd., Asaka, Japan). Triplicate measurements were taken at each site, to obtain a mean value for each area. The color measurements were performed on the left body surface area of each fish. Skin color measurements were performed at three sites (Kalinowski et al. 2005): opercular site above the lateral line 1 cm posterior to the operculum, dorsal site 1 cm below the dorsal fin, and the caudal site just above the lateral line in line with anal fin (Fig. 1). Fillet color measurements were performed at two different sites (the dorsal and caudal sites) after removal of the fillets. Color measurements of FM, and DDGS were performed in petri dishes. Data for each sample was expressed in terms of International Commission on Illumination CIE (1976) values for L* (lightness), a* (redness) and b* (yellowness). Angle of hue (H*) and chroma (C*) which defines the saturation of color were calculated by the equation, $H^* = \arctan(b^*/a^*)$ and $C^* = (a^{*2}+b^{*2})^{1/2}$, respectively (Hunt, 1977). Referential A standard white tile with reflectance values of L*= 95.23, a= -0.31 and b= +3.01 was used as the reference.

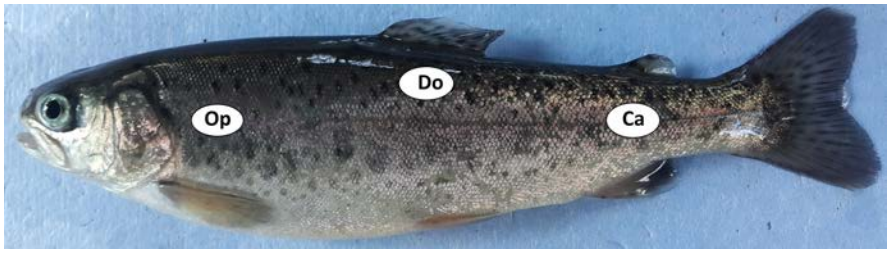

Fig. 1. Location of skin and fillet color measurement sites on a rainbow trout. Op: Opercular site, Do: Dorsal site, Ca: Caudal site (Original photo).

Statistical analysis. All data were subjected to one-way analysis of variance (ANOVA) using the software of the SPSS 22.0 (SPSS INC., Chicago, IL, USA). Differences among the means were compared by Duncan's multiple range test at a 5% probability level. All data were presented as mean ± standard deviation (mean ± SD) throughout the text.

Results

Proximate composition. The proximate carcass composition values of the rainbow trout after 84 days of feeding with the experimental feeds containing DDGS as protein source replacement for FM for are given in Table 2. According to the statistical analyses made, no significant differences among the experimental groups regarding the carcass moisture, crude protein, crude lipid and crude ash (P>0.05).

Table 2. Proximate carcass composition of rainbow trout fed the experimental diets

	Experimental diets[1]			
Parameters[2]	*Control*	*DDGS10*	*DDGS20*	DDGS30
Moisture (%)	68.04±0.87	69.08±0.62	68.13±0.76	67.92±0.40
Crude protein (%)	18.33±0.33	18.23±0.39	18.47±0.65	18.95±0.47
Crude lipid (%)	10.57±0.42	10.38±0.55	10.71±0.73	10.82±0.16
Crude ash (%)	1.68±0.03	1.67±0.09	1.72±0.05	1.73±0.04

[1] Experimental diet code indicating DDGS in diet (0%, 10%, 20%, and 30%, respectively) [2] No significant difference among means according to one-way variance analysis (P>0.05). Values are mean of triplicate analysis. Crude protein, crude lipid and crude ash values are given over wet weight

Fatty acid composition. At the end of the experiment, fatty acid composition in the fish muscles of the treatment groups is given in Table 3. No statistically significant effect of partial replacement of FM with DDGS on saturated fatty acids (SFA) could be observed ($P>0.05$), as can be seen in ∑SFA (Table 3). Despite a statistically significant decrease in palmiteloic acid (C16:1) in DDGS20 and DDGS30 group, the DDGS ratio supplementation in the feed did not significantly influence the other monounsaturated fatty acids (MUFA) and ∑MUFA values. Among the n-3 series polyunsaturated acids (PUFA) of the DDGS20 group eicosapentaenoic acid (C20:5n-3; EPA) was significantly lower compared to the control group ($P<0.05$), no significant difference between the DDGS30 group and the control could be seen ($P>0.05$). Also no significant difference was found among groups in α-linolenic acid (C18:3n-3), eicosatrienoic acid (C20:3n-3), docosahexaenoic acid (C22:6n-3; DHA) and ∑n-3 PUFA values ($P>0.05$). Among the n-6 series fatty acids, linoleic acid (C18:2n-6) value increased along with the DDGS increase in feed, as did the ∑n-6 PUFA value ($P<0.05$). ∑PUFA and the ∑n-3/∑n-6 ratio were also found to be similar among the groups ($P>0.05$).

Table 3. The fatty acid composition of rainbow trout fed the experimental diets

Fatty acids[2] (%, on total fatty acids)	Experimental diets[1]			
	Control	DDGS10	DDGS20	DDGS30
C14:0	4.16±0.18	4.28±0.26	3.88±0.24	4.01±0.41
C15:0	0.78±0.16	0.83±0.23	1.19±0.48	0.94±0.57
C16:0	23.16±2.03	23.13±1.51	25.56±1.85	23.82±4.12
C17:0	1.29±0.45	1.31±0.52	1.63±0.50	0.98±0.06
C18:0	5.75±0.53	5.69±0.62	5.98±0.34	5.56±0.14
C20:0	0.57±0.18	0.52±0.23	0.54±0.21	0.48±0.02
C21:0	0.55±0.07	0.73±0.09	0.85±0.08	0.82±0.28
C23:0	0.49±0.07	0.45±0.02	0.42±0.12	0.41±0.05
∑SFA[3]	36.76±3.50	36.92±3.03	40.05±2.89	37.02±5.26
C16:1	5.53±0.11^{a}	5.71±0.94^{a}	4.44±0.10^{b}	4.47±0.11^{b}
C18:1n-9	25.81±1.50	25.26±2.49	24.83±1.30	26.38±1.13
C20:1n-9	1.49±0.19^{a}	1.44±0.12^{a}	1.05±0.09^{b}	1.39±0.07^{a}
C22:1n-9	0.69±0.13	0.82±0.13	0.80±0.23	0.66±0.04
∑MUFA[4]	33.52±1.85	33.23±3.21	31.12±1.38	32.89±1.21
C18:3n-3	1.28±0.03	1.19±0.05	1.18±0.24	1.17±0.05
C20:3n-3	0.14±0.06	0.14±0.04	0.12±0.02	0.11±0.02
C20:5n-3 (EPA)	2.49±1.24^{a}	2.25±1.52^{ab}	1.74±0.53^{b}	2.16±0.04^{ab}
C22:6n-3 (DHA)	12.00±1.16	11.69±4.29	9.49±0.62	11.05±1.44
∑n-3 PUFA[5]	15.92±1.25	15.26±4.73	12.52±0.90	14.49±1.45
C18:2n-6	10.01±2.07^{c}	9.32±0.90^{c}	12.75±0.52^{b}	15.49±1.06^{a}
C20:2n-6	0.72±0.16	0.86±0.42	0.87±0.12	0.88±0.10
C20:3n-6	0.22±0.04	0.24±0.07	0.34±0.13	0.33±0.11
C20:4n-6	0.17±0.02	0.19±0.02	0.21±0.04	0.21±0.04
∑n-6 PUFA[6]	11.24±2.09^{c}	10.70±1.17^{c}	14.17±0.75^{b}	17.02±1.30^{a}
∑PUFA[7]	27.16±3.31^{ab}	25.96±3.73^{b}	26.70±1.62^{ab}	31.51±0.53^{a}
∑n-3/∑n-6	1.44±0.18	1.47±0.58	0.88±0.02	0.86±0.15

[1] Experimental diet code indicating DDGS in diet (0%, 10%, 20%, and 30%, respectively)
[2] Fatty acids composition values are mean of two analysis. Values in the same row with different superscripts are significantly different ($P<0.05$)
[3] Total saturated fatty acids included C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C:21, and C:23
[4] Total monounsaturated fatty acids included C16:1, C18:1n-9, C20:1n-9, and C22:1n-9
[5] Total n-3 polyunsaturated fatty acids included C18:3n-3, C20:3n-3, C20:5n-3, and C22:6n-3
[6] Total n-6 polyunsaturated fatty acids included C18:2n-6, C20:2n-6, C20:3n-6, and C20:4n-6
[7] Total polyunsaturated fatty acids included C18:2n-6, C18:3n-3, C20:2n-6, C20:3n-3, C20:3n-6, C20:5n-3, C20:4n-6, and C22:6n-3

Color parameters. Coloration data for the diet protein sources (FM and DDGS samples) and the experimental diets are presented in Table 4. DDGS L* (lightness), a* (redness), b* (yellowness), C* (brightness) and H* (hue) were found to be higher than that of FM, meaning that DDGS is lighter, with more yellow and red in comparison. Statistical analyses showed that L* and H* values were similar among experimental feeds (P>0.05), while a*, b* and C* were significantly different (P<0.05), although this difference is between the control and experimental groups (P<0.05), each being otherwise similar. The higher b* value of the protein source DDGS, is reflected by the higher b* values of the DDGS containing experimental feeds.

Table 4. Color parameters of feed ingredients and experimental diets

			Experimental diets[1]			
Parameters	*FM*	*DDGS*	*Control*	*DDGS10*	*DDGS20*	*DDGS30*
L*	40.90±2.76	45.06±2.85	28.61±0.55	28.76±0.58	29.10±0.41	29.40±0.25
a*	-0.13±0.14	6.43±0.92	-3.29±0.11[a]	-3.71±0.12[b]	-3.64±0.14[b]	-3.63±0.14[b]
b*	20.47±0.80	36.36±0.87	14.82±0.39[b]	16.38±0.50[a]	16.57±0.92[a]	17.14±0.66[a]
C*	20.47±0.65	36.94±0.73	15.19±0.36[b]	16.79±0.51[a]	16.97±0.87[a]	17.52±0.62[a]
h*	-1.56±0.01	1.40±0.02	-1.35±0.01	-1.35±0.01	-1.35±0.02	-1.36±0.02

[1] Experimental diet code indicating DDGS in diet (0%, 10%, 20%, and 30%, respectively)
[2] Values in the same row with different superscripts are significantly different (P<0.05, n = 5)
L*, lightness; a*, redness; b*, yellowness; C, brightness; H, hue

The results of the skin and fillet color measurements at the end of the 84 day experiment are given in Tables 5 and 6. According to the opercular site skin color measurements, as well as in the fillet color measurements, all L*, a*, b*, C*, and H* values among groups were not statistically significant (P>0.05), while only the L* value of the skin color measurements at the dorsal and caudal sites were statistically different compared to the experimental groups DDGS10 and the control (P<0.05).

Table 5. Color parameters of rainbow trout skin fed the experimental diets

	Experimental diets[1]			
Parameters	*Control*	*DDGS10*	*DDGS20*	*DDGS30*
Opercular				
L*	51.74±4.69	44.57±3.88	51.45±5.71	48.91±0.11
a*	3.41±1.13	3.46±1.30	3.25±0.66	3.65±0.67
b*	5.44±2.46	4.53±2.28	6.60±0.75	6.04±0.62
C*	6.66±1.66	6.04±1.00	7.36±0.92	7.08±0.65
h*	0.96±0.33	0.87±0.43	1.12±0.06	1.03±0.09
Dorsal site				
L*	35.67±2.45[a]	29.75±1.90[b]	33.92±2.05[ab]	32.85±2.56[ab]
a*	-0.03±1.26	-0.09±0.42	-0.85±0.45	-0.14±0.88
b*	5.27±1.22	4.62±0.54	5.45±0.37	4.78±0.95
C*	5.38±1.14	4.63±0.54	5.52±0.42	4.83±0.95
h*	0.55±1.55	0.54±1.73	-1.42±0.07	-0.48±1.63
Caudal site				
L*	55.79±1.80[a]	47.15±4.40[b]	52.80±3.64[ab]	51.14±1.67[ab]
a*	3.20±1.01	3.28±0.51	1.93±1.00	3.53±0.59
b*	5.53±1.79	6.13±1.65	6.72±0.61	6.33±0.86
C*	6.45±1.76	7.02±1.21	7.06±0.34	7.27±0.74
h*	1.03±0.16	1.06±0.18	1.29±0.16	1.06±0.10

[1] Experimental diet code indicating DDGS in diet (0%, 10%, 20%, and 30%, respectively)
[2] Values in the same row with different superscripts are significantly different (P<0.05). Data represent the mean of three replicates per dietary treatments, n = 15 for each treatment. L*, intensity of lightness; a*, redness; b*, yellowness; C, chroma; H, hue

Table 6. Color parameters of rainbow trout fillet fed the experimental diets

	Experimental diets[1]			
Parameters[2]	*Control*	*DDGS10*	*DDGS20*	*DDGS30*
Opercular				
L*	62.50±1.66	66.11±1.95	63.26±1.37	64.31±2.31
a*	3.16±1.03	2.78±0.16	3.00±0.72	3.83±0.25
b*	5.89±0.48	5.26±0.23	5.31±0.31	5.79±0.21
C*	6.71±0.91	5.95±0.20	6.12±0.51	6.94±0.27
h*	1.09±0.10	1.08±0.03	1.06±0.10	0.99±0.03
Caudal site				
L*	56.36±0.95	58.66±0.84	55.33±2.63	57.67±1.77
a*	3.64±1.49	3.17±1.31	3.52±0.75	3.25±0.08
b*	4.67±0.55	4.14±0.53	4.30±1.31	5.73±0.56
C*	5.98±1.22	5.27±1.13	5.59±1.29	6.59±0.49
h*	0.93±0.17	0.94±0.16	0.87±0.14	1.06±0.04

[1] Experimental diet code indicating DDGS in diet (0%, 10%, 20%, and 30%, respectively)

[2] No significant difference among means according to one-way variance analysis ($P>0.05$). Data represent the mean of three replicates per dietary treatments, n = 15 for each treatments. L*, lightness; a*, redness; b*, yellowness; C, chroma; H, hue

Discussion

According to the results of our study, no negative effects of DDGS use as a partial replacement for FM on the rainbow trout whole body composition values (moisture, crude protein, crude lipid and crude ash) were found (Table 2). These results are in agreement with the findings of Barnes et al. (2012), and Aydın and Gümüş (2013).

Fish fatty acid composition is generally accepted to be affected by the fatty acid composition of the feed (Francesco et al., 2004; Borquez et al., 2011). This view is supported by the results of the present study (Table 3). Use of ethanol production by-product DDGS as a protein source in place of decreasing amounts of FM, is shown to be effective on some rainbow trout fatty acid composition parameters, while apparently not effective on some fatty acids (Table 3). Lipid content of DDGS is about 10%, linoleic acid (C18:2n-6) being the dominant fatty acid species (55.7% of total fatty acids), followed by the moderate levels of oleic acid (C18:1n-9) at 25.0%, palmitic acid (C16:0) at 14.9% and linolenic acid (C18:3n-3) at 7.8% (Aydın and Gümüş, 2016). Fatty acids of FM, the most frequently used raw material in fish feeds, is largely composed of palmitic acid (22%), oleic acid (C18:1n-9) (11%), docosahexaenoic acid (DHA; C22:6n-3, 18%) and eicosapentaenoic acid (EPA; C20:5n-3, 12%) (Gümüş and Aydın, 2013). Considering the fatty acid compositions of the protein sources DDGS and FM, this study shows that replacement of FM with increasing amounts of DDGS led to a significant increase in linoleic acid content, largely due to the high linoleic acid content of DDGS (Hanson et al., 2012). Similarly, the increase in the carcass linoleic acid content with the addition of alternative protein source replacement of FM was also reported by Francesco et al. (2004), Gümüş and Aydın (2013), and Aydın et al. (2015), although no change was reported by Borquez et al. (2011). SFA and ∑SFA values in this study were not affected by the relative decrease of FM and the increase of DDGS. Increased carcass palmitic acid and palmiteloic acid (C16:1) values were reported in Atlantic salmon (*Salmo salar*) after treatment with a plant protein source mix (Corn gluten and soy concentrate) as a partial replacement for FM; these changes were attributed to lowered feed consumption in addition to the relative increase in the plant protein sources in feed (Pratoomyot et al. 2010). In the present study, feed consumption did not decrease in the DDGS groups. The significant decrease in n-3 series polyunsaturated acid eicosapentaenoic acid is in agreement with the findings of Cabral et al. (2013), Hu et al. (2013), and Aydın et al. (2015). Despite a slight decrease in eicosapentaenoic acid, no significant change corresponding to the relative increase of DDGS rate was observed in docosahexaenoic acid. However, no significant difference in eicosapentaenoic acid values between the

control and DDGS30 could be detected in response to the decrease in FM and fish oil ratio, along with the increase of protein source DDGS. Docosahexaenoic acid like groups, ∑n-3 PUFA and ∑n-3 PUFA/∑n-6 PUFA, were also not affected by supplementation of DDGS. In addition, use of lupin meal as a replacement of FM in rainbow trout feed had no negative effects on muscle eicosapentaenoic acid, docosahexaenoic acid, ∑n-3 PUFA and ∑n-3 PUFA/∑n-6 PUFA values (Borquez et al., 2011). As seen in Table 3, DDGS increase led to a slight decrease in ∑n-3 PUFA/∑n-6 PUFA values, and caused an increase in the ∑n-6 PUFA value. The absence of a significant drop in ∑n-3 PUFA in DDGS including experimental feeds may be linked to higher amounts of FM (minimum 54.2% in diet) or lower amount of DDGS (max. 30.0%). Increase in the muscle ∑n-6 PUFA value along with the increase of DDGS amount should be linked with proportional increase of linoleic acid found in great amounts in DDGS. Similarly, an increase in ∑n-6 PUFA of rainbow trout carcass with the increase in vegetal protein source was reported by Francesco et al. (2004). In another study, although there was a reduction in ∑n-3 PUFA/∑n-6 PUFA ratio with the rise of linoleic acid and ∑n-6 PUFA values of rainbow trout liver correlated to the increase of the canola meal which replaced and lowered FM content. In addition, no negative impact of canola meal on ∑n-3 PUFA was found (Shafaeipour et al. 2008).

The b* value of DDGS used as the alternative protein source in this study, was significantly higher than that of FM (Table 4), but this was not reflected on the skin (Table 5) and fillet (Table 6) coloration parameters (L*, a*, b*, C*, and H*) of the fish at the end of the trial period. However, according to the skin color measurements of the fish at operculum and caudal sites, there was a slight increase in the b* of the experimental groups as compared to the control. The addition of sea urchin as the feed supplement had no significant effect on the skin color of the red porgy (*Pagrus pagrus*), according to the study of García-Romero et al. (2014). Full replacement of FM in the diet of the experimental group (63.8% of the diet) with a mixture of plant protein sources (corn gluten, wheat gluten, extruded peas, and rapeseed) resulted in a significant increase in b* and H* values in the fillets of the rainbow trout fed the experimental feed as compared to the control, while a* value decreased, and on the other hand no change for L* and C* was reported (Francesco et al. 2004). On the contrary, an increase in a*, b*, C*, and H* values in the fillets due to the oleoresin paprika use in rainbow trout feed, with no significant change in the L* value was reported (Yesilayer and Erdem 2011). There were increased fillet a* values, while no significant change in L* and b* values as a response to bacterial pigment use in Nile tilapia feed (Grassi et al. 2016). Increased skin color L*, a*, b*, C*, and H* values of red porgy, fed for 120 days trial with feed which included shrimp shell meal at a rate of 16% as compared to the control, were found (Kalinowski et al. 2007). Spirulina as an additive was reported to be effective in improving the skin color of gold fish (*Carassius auratus*) and kenyi cichlids *Maylandia lombardoi* (James et al., 2009; Karadal et al., 2016). Fish skin and fillet color are affected by the types of raw materials used as additives, relative ratios applied, and the fish species used (Kalinowski et al. 2007; Yi et al. 2015; Karadal et al. 2016). According to the results of the present study, substitution of protein source by DDGS at a maximum of 30% resulted in an increase in skin color b* value only at operculum and caudal sites, while the dorsal site and fillet color values were not affected.

In conclusion, the coloration of rainbow trout skin and fillets were not modified negatively by the use of DDGS protein sources in diets. Despite a slight decrease in fish muscle fatty acid ∑n-3 PUFA/∑n-6 PUFA value, it has been concluded that DDGS can be used in rainbow trout feed at rates up to 30% with no decrease in docosahexaenoic acid, ∑n-3 PUFA, ∑n-6 PUFA and ∑PUFA values.

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