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# Dietary replacement of fishmeal by corn distillers dried grains with solubles (DDGS) in diets for turbot (*Scophthalmus maximus*, Linneaus, 1758) Juveniles



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#### ABSTRACT

The aim of this study was to determine the potential of corn distillers dried grain with solubles (DDGS) to partially replace fishmeal (FM) in practical diets for turbot. For that purpose, a control diet was formulated to include 40% FM and a mixture of plant protein ingredients (soybean meal, corn gluten, and wheat gluten). Three other diets were formulated based on the control but with 10, 17.5, or 25% of DDGS replacing FM. Diets were tested in triplicate, in an 84-days growth trial with juveniles of 29 g initial body weight. Feed intake was not affected by diet composition, but growth and feed efficiency linearly decreased with the increase of dietary DDGS level. Whole-body dry matter and protein contents were not affected by diet composition, but lipid and energy content were higher in fish fed the control diet than the 17.5DDGS and 25DDGS diets and the 25DDGS diet, respectively. The apparent digestibility coefficients (ADCs) of protein and amino acids were similar among diets, while the ADCs of energy decreased with the increase of dietary DDGS level. Digestive amylase and lipase activities in posterior intestine were lower in fish fed the 17.5DDGS and 25DDGS diets than the control diet, while proteases activity was not affected by diet. No differences among dietary treatments were observed on plasma glucose, but plasma total protein, albumin, triglycerides, and cholesterol were lower in fish fed the DDGS diets. Activity of key enzymes of glycolysis, gluconeogenesis, and lipogenesis was not affected by diet composition, but activity of alanine aminotransferase increased with the increase of dietary DDGS. Moreover, oxidative status of liver and intestine was not affected by dietary treatments, but susceptibility to oxidative stress was higher in the intestine than in the liver. Overall, it is concluded that replacing FM by DDGS in practical diets for turbot juvenile reduced growth performance and impaired overall nitrogen and energy metabolism.

### 1. Introduction

Fishmeal (FM) is becoming a scarce commodity in the world market, due to availability fluctuations and increasing global demand (Gatlin et al., 2007; Tacon and Metian, 2015). Given this context, various attempts have been made to reduce aquafeeds reliance on FM using alternative protein sources of good nutritional value in fish diets (Gatlin et al., 2007; Oliva-Teles et al., 2015). This is particularly critical for high trophic level species that are less flexible in terms of feed ingredient, and so more dependent upon the dietary use of FM and protein-rich plant ingredients than low-trophic level species (Tacon and Metian, 2015).

Turbot (Scophthalmus maximus) is an important aquaculture flatfish

species in Europe and East Asia due to its high market acceptability, fast growth rate, and tolerance to intensive production conditions. Being a high trophic level species (4.4; FishBase), turbot has high dietary protein requirements, ranging from 50% to 65% (Andersen and Alsted, 1993; Danielssen and Hjertnes, 1993; Lee et al., 2003; Cho et al., 2005). Although FM is traditionally used as the main protein source in turbot diets, previous studies demonstrated that it can be replaced up to 20% by corn gluten meal (Regost et al., 1999) or by 25% of soy protein concentrate (Day and González, 2000) without adverse effects on growth performance and feed efficiency. Bonaldo et al. (2015) also reported similar growth performances of turbot when dietary FM was reduced from 50% to 35% by the incorporation of plant proteins mixture (wheat gluten meal, soybean meal, and soy protein concentrate).

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Though plant protein concentrates proved suitable feedstuffs to partially replace FM in turbot diets, they are expensive commodities, and search for alternative feed ingredients is required. Within these alternative ingredients are distillers dried grains with solubles (DDGS), which are by-products of cereal distillation for ethanol production, that are increasingly available at relatively low cost in the market (Pahm et al., 2008; Cozannet et al., 2011). DDGS is the most important byproduct of the bioethanol manufacture industry, representing 0.3 t per ton of processed cereal (Alagón et al., 2016). DDGS protein content ranges from 26 to 33%, fat from 9 to 14%, and neutral detergent fibres from 33 to 44% (Liu, 2012; Nyachoti et al., 2005; Kluth and Rodehutscord, 2010; Abdel-Raheem et al., 2011). Except for its fiber content. DDGS does not contain other anti-nutritional factors as those found in other plant protein sources such as soybean meal, rapeseed meal or cottonseed meal, and that usually interfere with fish performance and health (Gatlin et al., 2007; Kluth and Rodehutscord, 2010; Oliva-Teles et al., 2015). Moreover, DDGS is locally available, including in Europe, increasing its potential as an alternative to imported traditional plant ingredients.

DDGS has been mainly used in farm animal feeds (US Grains Council, 2012). Studies in fish evaluating FM replacement by DDGS are limited to a few fish species, such as in rainbow trout, *Oncorhynchus mykiss* (Cheng and Hardy, 2004), channel catfish, *Ictalurus punctatus* (Tidwell et al., 1990; Webster et al., 1991, 1992; Robinson and Li, 2008; Lim et al., 2009; Li et al., 2011), sunshine bass, *Morone chrysops* × *Morone saxatilis* (Thompson et al., 2008), hybrid tilapia, *Oreochromis niloticus* Q × *Oreochromis aureus* of (Welker et al., 2014a), meagre, *Argyrosomus regius*, and European seabass, *Dicentrarchus labrax* (Magalhães et al., 2015).

Thus, the aim of the present study was to evaluate the effect of partial replacement of FM by DDGS in diets for turbot juveniles on growth performance, diet digestibility, and some key actors involved in nutrient metabolism and oxidative defence mechanisms.

# 2. Materials and methods

This experiment consisted of a growth trial and a digestibility trial performed with turbot (*Scophthalmus maximus*) juveniles provided by a commercial fish farm. The trials were conducted at the Marine Zoological Station, University of Porto, Portugal, by certified scientists (following the Federation of European Laboratory Animal Science Associations —FELASA category C recommendations) and the experiment was performed according to the European Economic Community animal experimentation guidelines directive of 24 November 1986 (86/609/EEC).

## 2.1. Experimental diets

A control diet was formulated to contain 40% FM (corresponding to 55% of dietary protein) and a mixture of plant ingredients (soybean meal, corn gluten, wheat gluten) as protein sources, and fish oil as lipid source (Table 1). Three other diets were formulated similar to the control but with FM partially replaced by 10%, 17.5%, or 25% of DDGS (corresponding to a FM protein replacement of circa 8, 14, and 20%, respectively). All dietary ingredients were finely ground, mixed, and dry pelleted using a laboratory pellet mill (CPM: California Pellet Mill, Crawfordsville, IN, USA) through a 3 mm dye, dried at 50 °C for 24 h, and then kept in a freezer until used.

# 2.2. Growth trial

The growth trial was performed in a thermo-regulated recirculating water system, equipped with a battery of 12 fiberglass cylindrical tanks (100 L water capacity each), supplied with a continuous flow of filtered seawater (6 L min  $^{-1}$ ). During the trial, water temperature and salinity averaged 18  $\pm$  1  $^{\circ}$ C and 35.5  $\pm$  0.8‰, respectively. Oxygen levels

Table 1
Composition and proximate analysis (% dry matter) of the experimental diets.

Diet	Control	10DDGS	17.5DDGS	25DDGS
Ingredients (% DM)				
Fish meal <sup>a</sup>	40.0	36.9	34.5	32.2
DDGS <sup>b</sup>	-	10	17.5	25
Wheat gluten <sup>c</sup>	5	5	5	5
Corn gluten <sup>d</sup>	15	15	15	15
Soybean meal <sup>e</sup>	10	10	10	10
Wheat meal <sup>6</sup>	16.8	10.3	5.5	0.7
Fish oil	9.7	9.3	9.0	8.6
Vitamin premix <sup>f</sup>	1.0	1.0	1.0	1.0
Choline chloride (50%)	0.5	0.5	0.5	0.5
Mineral premix <sup>g</sup>	1.0	1.0	1.0	1.0
Binder <sup>h</sup>	1.0	1.0	1.0	1.0
Proximate composition				
Dry matter (%)	88.5	86.5	85.8	86.3
Crude protein	53.5	54.4	54.4	53.7
Crude lipid	15.5	15.9	14.7	14.8
Ash	10.1	9.7	9.1	9.4
Gross energy (kJ g <sup>-1</sup> )	23.1	21.8	23.0	22.28
Essential amino acids conten	ıt			
Lysine	3.52	3.46	3.31	3.28
Arginine	3.81	3.79	3.71	3.65
Histidine	1.86	1.88	1.87	1.89
Isoleucine	2.55	2.49	2.57	2.55
Leucine	4.91	5.19	5.51	5.86
Valine	2.94	2.83	2.88	2.91
Methionine	1.41	1.43	1.39	1.36
Phenylalanine	2.62	2.68	2.81	2.84
Threonine	2.1	2.16	2.33	2.38
Non-essential amino acids co	ontent			
Tyrosine	2.10	2.22	2.38	2.27
Aspartic acid	4.20	4.25	3.88	3.86
Glutamic acid	9.18	9.16	9.05	8.74
Serine	2.54	2.63	2.37	2.27
Glycine	2.63	2.53	2.53	2.48
Alanine	3.34	3.43	3.56	3.52
Proline	3.77	3.81	3.79	3.31

<sup>&</sup>lt;sup>a</sup> Pesquera Centinela, Steam Dried LT, Chile (CP: 74.2%; CL 10.1%). Sorgal, S.A. Ovar, Portugal.

were kept above  $7.0\,\mathrm{mg\,L}^{-1}$  and a 12L:12D photoperiod was adopted. For the growth trial, 12 homogenous groups of 18 fish each (initial body weight 30 g) were constituted and each diet was randomly assigned to triplicate of these groups. The trial lasted 84 days and fish were fed by hand, twice a day, six days a week, to apparent visual satiety. Utmost care was taken to avoid feed waste and to assure that all feed supplied was consumed. Fish were bulk weighted at the beginning, after two weeks, and at the end of the trial, following 1 day of feed deprivation. Ten fish from the initial stock population and 6 from each experimental tank at the end of the trial were sampled, pooled, and frozen at  $-20\,^{\circ}\mathrm{C}$  for whole-body composition analysis. Whole fish, viscera and liver weights of these fish were recorded for determination

<sup>&</sup>lt;sup>b</sup> DDGS (CP: 32.8%; CL:9.0%; ash: 5.1%; crude fiber 7.5; acid detergent fiber: 14.4; neutral detergent fiber: 42.3; nitrogen free extract: 45.5; Pannonia Gold®).

<sup>&</sup>lt;sup>c</sup> Wheat gluten (CP: 84.3%; CL: 3.9%), Sorgal, S.A. Ovar, Portugal.

<sup>&</sup>lt;sup>d</sup> Corn gluten (CP: 68.3%; CL: 2.9%), Sorgal, S.A. Ovar, Portugal.

<sup>&</sup>lt;sup>e</sup> Soybean meal (CP: 53.7%; CL:2.1%), Sorgal, S.A. Ovar, Portugal.

<sup>&</sup>lt;sup>6</sup> Wheat meal (CP: 14.6%; CL: 2.2%), Sorgal, S.A. Ovar, Portugal.

 $<sup>^{\</sup>rm f}$  Vitamins (mg kg $^{-1}$  diet): retinol, 18,000 (IU kg $^{-1}$  diet); calciferol, 2000 (IU kg $^{-1}$  diet); alpha tocopherol, 35; menadion sodium bis., 10; thiamin, 15; riboflavin, 25; Ca pantothenate, 50; nicotinic acid, 200; pyridoxine, 5; folic acid, 10; cyanocobalamin, 0.02; biotin, 1.5; ascorbyl monophosphate, 50; inositol, 400.

 $<sup>^{\</sup>rm g}$  Minerals (mg kg $^{-1}$  diet): cobalt sulphate, 1.91; copper sulphate, 19.6; iron sulphate, 200; sodium fluoride, 2.21; potassium iodide, 0.78; magnesium oxide, 830; manganese oxide, 26; sodium selenite, 0.66; zinc oxide, 37.5; dicalcium phosphate, 8.02 (g kg $^{-1}$  diet); potassium chloride, 1.15 (g kg $^{-1}$  diet); sodium chloride, 0.4 (g kg $^{-1}$  diet).

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