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Soybean meal induces enteritis in turbot *Scophthalmus maximus* at high supplementation levels

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ABSTRACT

The goal of the present study was to find whether higher soybean meal (SBM) levels might trigger soybean meal-induced enteritis (SBMIE) in turbot. If so, caution must be taken when mixing ingredients containing saponins and other antinutrients to avoid SBMIE like symptoms. In a 8 week feeding trial conduced on turbot, three isonitrogenous and isolipidic diets were formulated to include 26%, 40% and 54% SBM to progressively replace 30%, 45% and 60% fish meal (FM) in a FM based diet, respectively. The results showed that SBM caused dose-dependent decreases in growth performance and nutrient utilization. Enteritis developed in the distal intestine in the inclusion range of 26–54%. Dose-dependent increases in severity of the inflammation, with concomitant alterations in brush border membrane enzymes and inflammatory marker genes expression were seen. Our results confirm the hypothesis that high inclusion level of SBM may cause similar inflammatory changes as observed in several other fish species. Thus, caution must be taken when formulating turbot diets based on ingredients that may contain saponins and other antinutrients. Moreover, turbot is also a candidate species for the study of causes and mechanism of diet induced inflammation in the intestine of fish.

Statement of relevance: The present work first describes the soybean meal induced enteritis in turbot and provides the information that caution must be taken when formulating turbot diets based on ingredients that may contain saponins of other antinutrients.

1. Introduction

Turbot *Scophthalmus maximus* has become the most important cultured flatfish in Europe and Asia because of its high quality flesh and rapid growth, with a global production of around 70,000 t per year (FAO FishStatJ, 2013). Global aquaculture has continued to grow while the production of fish meal is stable, at best. Thus, research on alternatives to fish meal has been an international priority for more than two decades (Hardy and Kissil, 1997). Among the ingredients investigated as alternatives to fish meal, soy products are some of the most promising because of the security of supply, price and good reasonable amino acid profile (Storebakken et al., 2000). However, soybean meal (SBM) of standard quality is used in carnivorous fish diets only at relatively low levels due to its negative effects on gut health in several fish species (Krogdahl et al., 2010; Merrifield et al., 2011). Specifically, soybean meal has been observed to cause proliferative or inflammatory conditions in the distal intestinal mucosa of cultured fish species such as Atlantic salmon, rainbow trout, common carp and zebra fish (van den Ingh et al., 1991; Rumsey et al., 1994; Baeverfjord and Krogdahl, 1996; Yamamoto et al., 2008; Urán et al., 2008; Hedrera et al., 2013). The histopathological changes, commonly referred to as soybean meal-induced enteritis (SBMIE), have been extensively studied, and are characterized by a shortening of the mucosal folds, a swelling of the lamina propria and subepithelial mucosa, a strong infiltration of various inflammatory cells, and decreased numbers of absorptive vacuoles in the enterocytes, a situation that decrease the capacity of the distal intestine to digest and absorb nutrients (van den Ingh et al., 1991; Baeverfjord and Krogdahl, 1996; Bakke-McKellep et al., 2000; Bakke-McKellep et al., 2007). In Atlantic salmon, the effects of SBM were proved to be dose-dependent; the worst symptoms were observed at the highest inclusion level (30%), but even the lowest evaluated amount of SBM (10%) generated adverse effects in salmon (Krogdahl et al., 2003). The key antinutrient responsible for the enteritis was recently confirmed to be saponins (Krogdahl et al., 2015). Based on research conducted by Bonaldo et al. (2011) on turbot juveniles, turbot appear insensitive to SBM, at least up to 22% in the diet, a level which was found not to affect the digestibility and intestinal histology. As saponins may be supplied also by other plant feed ingredients and in total reach high levels, there is a need to investigate whether SBM might induce enteritis in turbot when included at levels higher than 22%.

Recent studies in Atlantic salmon have described the SBMIE at the transcriptional level, which is characterized by induction of acute inflammatory-related cytokines and chemokines, NF-κB and TNF-a

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related genes and regulators of B and T lymphocytes function (Skugor et al., 2011; Sahlmann et al., 2013; Grammes et al., 2013; De Santis et al., 2015). To our knowledge, no studies have been reported to investigate the molecular mechanisms on SBMIE in turbot. The purpose of this study was to investigate whether the high inclusion level of SBM in the range of 26–54% may affect nutrient utilization, digestibility and induce enteritis in turbot. To further elucidate mechanisms leading to the development of SBMIE, the present study examined the expression of genes related to inflammatory responses (qPCR).

2. Materials and methods

2.1. Feed ingredients and diet formulation

Based on Yun et al. (2012), a basal diet (named as FM) were formulated to contain 48% crude protein and 12% crude lipid with fish meal as the primary protein source, fish oil and soybean oil as lipid source and wheat flour as the carbohydrate source. This diet was used as control. Based on the FM diet (control diet), another three isonitrogenous and isolipidic diets were formulated to contain 260 g kg^{-1}, 400 g kg^{-1} and 540 g kg^{-1} soybean meal as replacement of 30%, 45% and 60% fish meal in the basal diet, named as SBM26, SBM40 and SBM54, respectively (Table 1). As list in Table 2, all four diets could meet the essential amino acid (EAA) requirements of juvenile turbot based on the whole body amino acid profile (Kaushik, 1998; Peres and Oliva-Teles, 2008), no crystalline amino acids were supplemented. The diet preparation and storage were as described by Yun et al. (2012).

2.2. Experimental procedure

Disease-free juvenile turbot were obtained from a commercial farm in Haiyang, China and transferred to an indoor flow-through water system in the Haiyang Yellow Sea Aquatic Product Co., Ltd. Fish were acclimated to the system and fed with the FM diet for 2 weeks. After that, turbot with initial body weight of about 8.5 g were randomly distributed into 12 tanks, 30 fish per tank (filled with 300 l seawater). The seawater was pumped from coast adjacent, filtered through a sand filter and distributed to each tank at approximate 2.0 l/min. Each diet was fed to fish in three tanks. Fish were fed with the experimental diets to apparent satiation twice daily at 07:00 and 18:00. During the 8-week feeding trial, water temperature was 12–16 °C, pH 7.8–8.2 and salinity 28–30.

Table 1
Ingredients and compositions of experimental diets (dry-matter basis).

	Experimental diet[a]			
	FM	SBM26	SBM40	SBM54
Ingredients (g kg^{-1})				
Fish meal[b]	600	420	330	240
Soybean meal	0	260	400	540
Wheat meal	280	210	150	80
Fish oil	20	38	48	58
Soybean oil	20	13	9	6
Soybean lecithin	20	20	20	20
Vitamin and mineral premix[c]	25	25	25	25
Monocalcium phosphate	0	5	5	5
Choline chloride	5	5	5	5
Yttrium premix	1	1	1	1
Calcium propionic acid	1	1	1	1
Ethoxyquin	0.5	0.5	0.5	0.5
Cellulose	27.5	1.5	5.5	18.5
Proximate composition (%)				
Dry matter	94.1	94.0	94.8	94.6
Crude protein	48.8	48.7	48.7	48.8
Crude lipid	12.5	12.3	12.0	12.3
Ash	12.5	11.6	11.2	11.0
Gross energy (KJ/g)	20.2	20.4	20.6	20.6

[a] FM, a basal diet; SBM26, 26% of the soybean meal inclusion level to replace 30% fish meal in basal diet; SBM40, 40% of the soybean meal inclusion level to replace 45% fish meal in basal diet; SBM54, 54% of the soybean meal inclusion level to replace 60% fish meal in basal diet.
[b] Fish meal: steam dried fish meal, (COPENCA Group, Lima, Peru).
[c] Vitamin premix supplied the diet with (mg kg^{-1} diet) the following compounds: retinyl acetate, 32; vitamin D_3, 5; DL-α-tocopherol acetate, 240; vitamin K_3, 10; thiamin, 25; riboflavin (80%), 45; pyridoxine hydrochloride, 20; vitaminB_{12} (1%), 10; Lascorbyl-2-monophosphate-Na (35%), 2000; calcium pantothenate, 60; nicotinic acid, 200; inositol, 800; biotin (2%), 60; folic acid, 20; cellulose, 11,473. Mineral premix consisted of (mg kg^{-1} diet) the following ingredients: $FeSO_4 \cdot H_2O$, 80; $ZnSO_4 \cdot H_2O$, 50; $CuSO_4 \cdot 5H_2O$, 10; $MnSO_4 \cdot H_2O$, 45; KI, 60; $CoCl_2 \cdot 6H_2O$ (1%), 50; Na_2SeO_3 (1%), 20; $MgSO_4 \cdot 7H_2O$, 1200; calcium propionate, 1000; zeolite, 17,485.

Table 2
Essential amino acid profile of the diets and requirements of turbot (g 16 g^{-1} N).

	Experiment diet				EAA requirements	
Amino acid	FM	SBM26	SBM40	SBM54	1[c]	2[d]
Threonine	3.8	3.6	3.6	3.6	2.9	2.4
Phenylalanine	4.0	4.1	4.1	4.1	5.3[a]	2.5
Lysine	6.2	5.9	5.7	5.6	5.0	5.0
Valine	2.9	3.3	3.6	3.8	2.9	2.7
Leucine	6.9	6.9	6.9	6.9	4.6	4.5
Isoleucine	3.2	3.2	3.6	3.5	2.6	2.6
Methionine	2.8	2.2	2.2	2.0	2.7[b]	1.7
Arginine	5.6	5.6	5.8	5.8	4.8	4.2
Histidine	1.8	1.9	2.0	2.0	1.5	1.3

[a] Phenylalanine + tryptophan.
[b] Methionine + cysteine.
[c] From Kaushik (1998).
[d] From Peres and Oliva-Teles (2008).

2.3. Sampling

The fish were weighed at start and at end of the experiment. After 8 weeks of feeding, six fish per tank were randomly selected and their body weight and length were recorded. Then, the fish were killed by a sharp blow to the head and the ventral belly surface was opened to expose the abdominal cavity. Only fish with digesta throughout the intestinal tract were sampled to ensure intestinal exposure to the diets. The intestine and liver were removed, cleared of any mesenteric, adipose tissue, rinsed with ice-cold distilled water to remove the eventual remaining gut contents and weighed. Sample was taken from the following gastrointestinal (GI) sections for brush border membrane enzyme activity analysis: central part of the proximal intestine (PI), midintestine (MI, 1/2 the distance between the pyloric caeca and the intestinal constriction), distal intestine (DI, from the constriction to the anus). All digestive organ samples were placed in tubes and immediately frozen in liquid nitrogen and stored at −80 °C until enzyme assay. For the histological evaluation, DI samples were taken from four of the six sampled fish per tank, placed in 4% phosphate-buffered formaldehyde solution for 24 h, and subsequently stored in 70% ethanol until further processing. For mRNA extraction, DI samples from the four fish selected for histological evaluation were placed in RNA later (Ambion) at 4 °C for 24 h and then stored at −20 °C until use.

2.4. Chemical analysis

Standard methods (AOAC, 1995) were used for analyzing experimental diets and carcasses samples. Moisture and ash content were determined gravimetrically to constant weight in an oven at 105 °C and 550 °C, respectively. Crude lipid was determined gravimetrically after extraction with ethyl ether (Extraction System B-811, BUCHI, Switzerland). Crude protein was determined by Kjeldahl method with a FOSS Kjeltec System (2300, Sweden) using boric acid to trap released ammonia. Gross energy was determined by calorimetric bomb (Parr, Moline, IL, USA). Amino acids in feed ingredients and diets (Table 2) were determined by amino acid analyser (Biochrom 30, GE Health care Co. Ltd., Cambridge, UK).