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Hydrolysed wheat gluten as part of a diet based on animal and plant proteins supports good growth performance of Asian seabass (*Lates calcarifer*), without impairing intestinal morphology or microbiota

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ABSTRACT

A trial was conducted to determine the effect of 6% hydrolysed wheat gluten (HWG) inclusion in a low-fishmeal diet on growth performance and intestinal microbiota and morphology of Asian seabass (*Lates calcarifer*). Fish (initial average weight of 36.3 g) were allocated into floating cages (eight replicates per diet, 30 fish per cage). They were fed either a fishmeal-based diet (positive, diet code: POS; 36% fishmeal), or a diet based on a mix of animal and plant proteins (negative, diet code: NEG). The fishmeal inclusion rate in this diet was reduced to 6%, with the major protein sources being soybean meal and animal by-products. The third diet (diet code: HWG) was similar to the NEG diet with the exception that 6% HWG was included in replacement of the other proteins. At the end of the 48 day trial, fish were sampled for intestinal microbiology and histology. Growth parameters were also assessed. Final body weight, SGR and daily feed intake were not significantly different across diets. However, the feeding rate was significantly affected, with the lowest rate observed in the POS treatment and the highest with the NEG treatment. The FCR was significantly lower for both POS and HWG fed fish than for the NEG fed fish. High throughput sequencing revealed that the majority of reads derived from the mucosa samples belonged to members of Proteobacteria (70.3% of the reads), Cyanobacteria (10.0%) and Firmicutes (7.6%). In the digesta reads were mainly assigned to Proteobacteria (34.5%), Fusobacteria (34.5%), and Firmicutes (22.6%). The alpha diversity did not differ among dietary treatments. Some differences in OTU relative abundances were obtained between diets, however, the overall community was not modified to a large extent by HWG. Histological appraisal revealed that the HWG fed fish exhibited significantly higher posterior intestinal perimeter ratio than that of the POS treatment. Overall, including HWG in a low fishmeal diet positively affects feed efficiency. Concomitantly the absorptive surface area of the posterior intestine was improved while the intestinal microbiota, described comprehensively here for the first time in Asian seabass, was similar to the microbiota of other healthy carnivorous marine fish species. The mechanisms involved in these changes may be related to the high glutamine content and to the high protein digestibility of HWG.

Statement of relevance

Fishmeal used to be the major source of proteins, especially for marine fish and salmonids. Nevertheless, its inclusion in diets has been reduced. Some commercial fish feeds may contain less than 10% fishmeal, largely replaced by a mix of animal and plant proteins. Our manuscript demonstrates that hydrolysed wheat gluten may be used to formulate high quality low fishmeal diets.

1. Introduction

Intensive production of farmed fish, fed with compound feeds, has expanded considerably during the past two decades (Olsen and Hasan, 2012). In such feeds, fishmeal (FM) used to be the major source of proteins, especially for marine fish and salmonids (Tacon et al., 2011). Nevertheless, because of the limited availability of FM in the market and

Abbreviations: HWG, hydrolysed wheat gluten; FM, fishmeal; POS, positive diet; NEG, negative diet; LP, lamina propria; E, epithelium layer; GC, goblet cells; CP, crude protein.

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its increasing price, its inclusion in diets has been progressively reduced. Today, some commercial fish feeds may contain less than 10% FM, largely replaced by a mix of animal and plant protein sources. However, such a diet may be challenging for carnivorous fish such as the Asian seabass (*Lates calcarifer*). High-quality proteins have to be used in order to maintain high growth rate and the intestinal health of fish.

Hydrolysed wheat gluten (HWG) is a protein source obtained by enzymatic hydrolysis of vital wheat gluten, a promising plant protein for fish feed (Apper-Bossard et al., 2013). A recent study performed in rainbow trout (*Oncorhynchus mykiss*) reported that replacing up to 50% dietary crude protein, from high-quality FM to HWG, supports similar growth performance without impairing intestinal microbiota and morphology (Apper et al., 2014). Furthermore, replacing 1 to 5% of soy protein concentrate with HWG in a diet based on animal and plant proteins increased growth performance and digestive enzyme activities, and positively modulated intestinal morphology, immunity and antioxidative systems of juvenile hybrid sturgeon (Qiyou et al., 2011; Zhu et al., 2011). Such a result suggests that HWG may be a suitable protein source to decrease dietary FM inclusion whilst maintaining a high quality diet, high level of growth performance, whilst preserving intestinal health.

The Asian seabass is a carnivorous fish fed with extruded diets which requires high level of dietary proteins. To our knowledge, the effect of including different protein sources in a low FM-diet on microbiota and intestinal morphology is not described in this species. The present study aimed to investigate the effect of 6% HWG inclusion in a low-FM diet, based on a mix of animal and plant proteins, on the growth performance and intestinal microbiota and morphology of Asian seabass.

2. Materials and methods

2.1. Experimental diets

Three diets were formulated according to recommended guidelines and commercial nutritional specifications for Asian seabass (Table 1; NRC, 2011). These three diets differed by their protein sources. The first diet (positive control, POS) was a FM-based diet and contained 36.2% FM. The second diet was based on a mix of animal and plant proteins and was considered as the negative control (NEG). The FM inclusion rate in this diet was reduced to 6%, with the major protein sources being soybean meal, haemoglobin powder, and poultry meal. The third diet was similar of the NEG diet with the exception that 6% hydrolysed wheat gluten (HWG, Solpro® 508, Tereos Syral, France) was included in order to partially replace the other protein sources (Table 1). The diets were produced by In Vivo NSA (Binh Duong, Vietnam). Feed was extruded floating pellets.

Table 1
Composition of experimental diets (% as fed basis) for Asian seabass (feed producer: In Vivo NSA). Diet codes — POS: fish-meal-based diet; NEG: plant and animal protein-based diet; HWG: diet with hydrolysed wheat gluten.

	Diets		
	POS	NEG	HWG
Ingredients			
Broken rice	10.0	9.5	8.8
Wheat flour	13.0	13.0	12.0
Hydrolysed wheat gluten 80% CP[a]	–	–	6.0
Soybean meal 47% CP, Brasil	19.3	25.9	24.4
Animal proteins (Poultry by-products meal 66% CP and Haemoglobin 93.9% CP)	12.2	33.9	31.8
Fish meal (FM 60% CP and 65% Premium)	36.2	6.0	5.6
Salmon oil	8.2	8.3	7.8
Premix[b]	1.1	3.5	3.7
Composition			
Protein	46.0	45.8	47.0
Fish meal, % CP	53.0	9.0	8.0
Animal proteins, % CP	21.0	57.0	51.0
Plant proteins, % CP	26.0	34.0	30.0
HWG, % CP	–	–	11.0
Starch	16.5	16.1	15.4
Lipid	12.0	12.4	12.3
Fibre	0.9	0.8	0.7
Ash	11.2	8.3	8.2
Ca	2.7	1.8	1.5
P	1.3	1.4	1.3

[a] Solpro®, Tereos Syral, Marckolsheim, France.
[b] Premix vitamins, minerals and amino-acids.

2.2. Fish and experimental design

The feeding trial was conducted at the In Vivo NSA Experimental facilities, Nha Be, Vietnam and lasted 48 days.

Seven hundred and twenty fish were randomly distributed into 3 m^3 ($1 \times 2 \times 1.5$ m) floating cages with eight replicate cages per diet, and 30 fish per cage (average weight = 36.3 ± 0.0 g). Fish were fed experimental diets to apparent satiation by hand twice daily. Feed waste was collected some minutes after feeding. Wet uneaten feed was dried to convert wet weight into as fed weight to calculate real feed intake and feed conversion ratio (FCR).

Water temperature and dissolved oxygen were recorded daily in the morning and in the afternoon. Every two days, turbidity and ammonia were recorded. These parameters remained within a normal range for this species: the average temperature was 31.1 ± 1.1 °C in the morning (7:30 am) and 32.6 ± 1.2 °C in the afternoon (3:00 pm), while average dissolved oxygen was 3.6 ± 0.6 mg/L in the morning (7:30 am) and 6.1 ± 1.0 mg/L in the afternoon (3:00 pm) during the course of the trial. The fish were subjected to natural photoperiod.

2.3. Zootechnical parameters

Daily distribution of feed and initial and final individual body weight were measured in order to assess growth performance by net weight gain, FCR, protein efficiency ratio (PER), and specific growth rate (SGR). The feed conversion ratio was calculated as FCR = g dietary intake × (g weight gain)$^{-1}$. The protein conversion ratio was calculated as PER = g dietary crude protein intake × (g weight gain)$^{-1}$. Specific growth rate was calculated as SGR (%body weight gain/day) = [(Log_n Final fish weight − Log_n Initial fish weight) / Time Interval] * 100.

2.4. Intestinal microbiology

At the end of the trial eighteen fish (n = 6 per treatment) were sampled to investigate the intestinal microbiota. The gastrointestinal tract was aseptically removed according to Merrifield et al. (2009). The posterior intestinal digesta and mucosa were isolated separately and were kept in 70% molecular-grade ethanol and stored at −20 °C until further analysis.

2.4.1. DNA extraction and PCR

Prior to DNA extraction samples were centrifuged and the ethanol was removed. DNA was extracted from 90 mg of sample material after lysozyme incubation (50 mg mL^{-1} in TE buffer) for 60 min at 37 °C, using the QIAamp® Stool Mini Kit (Qiagen, Crawley, UK) according to manufacturer's instructions. Some modifications were added in the clean-up and precipitation steps as described by Falcinelli et al. (2015). Briefly, after protein removal, equal volumes of sample and ice-cold Tris-buffered phenol solution were mixed, allowed to stand on ice for 15 min, then washed twice with chloroform and centrifuged for 5 min at 3000 *g*. The upper aqueous layer was retained, mixed with ice-cold isopropanol, left for 15 min at −20 °C to precipitate the DNA, before cold centrifugation at 14,000 *g* for 15 min. The resultant pellets were then washed twice with 70% ethanol, air dried for 5 min and resuspended overnight at 4 °C in 30 μL sterile water. DNA concentrations were analysed using a Nanodrop™ 1000 (Thermo Scientific Ltd., DE, USA). All reagents used were molecular grade and were