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Modifications of intestinal nutrient absorption in response to dietary fish meal replacement by plant protein sources in sea bream (*Sparus aurata*) and rainbow trout (*Onchorynchus mykiss*)

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ABSTRACT

The effects of partial (75%; diet PP75) and total (100%; diet PP100) replacement of fish meal by plant protein sources on the intestinal nutrient absorption of gilthead sea bream and rainbow trout were examined over a 12-week growth trial. The diets comprised a mixture of plant ingredients (corn gluten meal, wheat gluten, extruded peas and rapeseed meal) and met the amino acid requirements of the fish. A third group of fish on a fish meal diet was used as a control for comparative purposes. Diets were tested in triplicate and fish were fed twice a day until visual satiation.

At the end of the trial, we measured amino acid (L-leucine, L-lysine, L-phenylalanine, L-alanine and L-proline) and D-glucose absorption at short-term (6 h) and long-term (36 h and 48 h in rainbow trout and sea bream respectively) post-feeding by means of brush border membrane vesicles obtained from pyloric caeca, proximal intestine and distal intestine. The absorption pattern at 6 h post-feeding was modified in both species in response to fish meal replacement. In PP75-fed trout absorption was delayed from pyloric caeca to proximal and/or distal intestinal segments, thus total absorption capacity was maintained in this group. On the contrary, total uptake was significantly decreased in trout fed the PP100 diet and in sea bream on both PP75 and PP100 diets. Glucose transport capacity was increased in both experimental sea bream groups and in PP75 trout. Long-term transport capacity was up-regulated for both species.

Our results show that intestinal nutrient absorption is modified in response to the use of high levels of plant protein sources and that these changes are species-specific.

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1. Introduction

Fish meal (FM) has traditionally been the main source of protein in feed for the culture of carnivorous fish; however, as a result of the increase in aquaculture activity in recent years, the availability of FM is now limited (SOFIA, 2008). Thus, to allow the sustainable development of this industry, while at the same time ensuring optimal growth rates and a final product of high quality, other protein sources are required to replace FM in finfish carnivorous diets. In this regard, high percentages of FM can be replaced by plant protein (PP) meals without compromising fish growth (Albrektsen et al., 2006; de Francesco et al., 2007) as long as diets are balanced to match amino acid requirements of the different fish species. However, PP sources are also possibly rich in antinutritional factors (Francis et al., 2001; Gatlin et al., 2007; Kaushik, 1990) that can compromise fish health (Olsen et al., 2007) and impair fish performance when not treated (Knudsen et al., 2006) or when PP is used at high levels (Hansen et al., 2007). In this regard, PP-based diets diminish intestinal alkaline protease activity in carnivorous fish (Lilleeng et al., 2007; Santigosa et al., 2008). These results suggest that nutrient luminal availability could be modified as a consequence of the depletion of intestinal enzymatic activity. Moreover, previous studies have shown that fish on PP diets show histological modifications in the intestine (Krogdahl et al., 2003; Santigosa et al., 2008), which might modify the capacity of transporters immersed in the lipid bilayer.

Extensive information concerning absorption mechanisms in fish is available (Boge et al., 2002; Collie and Ferraris, 1995; Sala-Rabanal et al., 2004). In vertebrates, absorption occurs by diffusion, facilitated transport, or active transport (Mailliard et al., 1995). Facilitated and active transport mechanisms are used for the uptake of amino acids, oligopeptides and glucose. They follow asymptotic kinetics and depend on the presence of protein transporters immersed in the lipid bilayer (Palacin et al., 1998). In fish, nutrient uptake is mediated by transport proteins similar to those of mammals, although substrate specificity between fish and mammals differs (Collie and Ferraris,

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1995). Moreover, in contrast to mammals, amino acid transporters in fish are present along the entire intestine (Buddington et al., 1997).

Brush border membrane vesicles (BBMVs) have been used to study intestinal nutrient transport in several fish species (Ahearn and Storelli, 1994; Drai et al., 1990; Sala-Rabanal et al., 2004). This technique allows the study of uptake rates without substrate metabolization. However, other approaches, such as the everted sleeve technique or *in vivo* apparent absorption, can be used to determine uptake capacity (Bakke-McKellep et al., 2000; Nordrum et al., 2000; Refstie et al., 2006).

On the basis of these premises, here we studied the effects of partial (75%) and total replacement of FM by PP on the nutrient absorption capacity along the intestine of gilthead sea bream (*Sparus aurata*) and rainbow trout (*Oncorhynchus mykiss*).

2. Materials and methods

2.1. Diets

Three isoproteic and isolipidic diets for each species (Table 1) were formulated at the *Institute National de la Recherche Agrobiologique* (I.N.R.A., St-Pée-sur-Nivelle, France). The FM diet contained fish meal as the major source of protein. Fish meal was replaced by a blend of protein sources of plant origin (corn gluten, wheat gluten, extruded peas, rapeseed meal and sweet white lupin) at 75% and 100% in diets PP75 and PP100, respectively. Diets were balanced with free crystalline amino acids to meet the essential amino acid requirements of fish (NRC, 1993). For a more detailed description of the diets (including proximate composition) see Gómez-Requeni et al. (2004, 2005).

2.2. Animals and sampling

At the *Instituto de Acuicultura de Torre de la Sal* (C.S.I.C., Spain), acclimated sea bream (16.5 ± 0.7 g) were randomly distributed into nine 0.5-m^3 circular fiberglass tanks (90 fish per tank). Triplicate groups of fish were fed one of the diets twice a day until visual satiety for 12 weeks (April to July). Trial was conducted under natural photoperiod and a natural water temperature that ranged from 17 to 25 °C. Rainbow trout (19.0 ± 0.2 g) were randomly distributed in nine 1-m^3 circular fiberglass tanks (75 fish per tank) at the INRA experimental fish farm (Donzacq, Landes, France). During 12 weeks (April to July), triplicate groups of trout were fed one of the experimental diets twice a day until visual satiety. Trial was conducted under natural photoperiod and constant water temperature (17 ± 1 °C).

Data on growth performance and nutrient retention for sea bream and trout are detailed in Gómez-Requeni et al. (2004) and Gómez-Requeni et al. (2005), respectively.

At the end of each growth trial, 20 fish per experimental group were starved for 24 h, lightly anaesthetized with MS-222 (0.1 g L^{-1}) and force-fed the diet corresponding to their group at 1% of their body weight by the stomach intubation method (Schuhmacher et al., 1997) using a polyethylene catheter (0.3 mm, i.d., 10 cm length) and a 5 cm^3 syringe. In order to produce an acceptable food paste, diets were ground and mixed with 2 parts of water. At two postprandial times (6 and 48 h for sea bream; 6 and 36 h for trout) 10 fish per condition and time were anaesthetized, weighed, and then killed by severing their spinal cord. Digestive tracts were isolated on ice and adherent tissue was removed. Intestines were divided into pyloric caeca (PC), proximal intestine (PI) and distal intestine (DI) on the basis of morphological appearance. Segments were opened lengthwise, washed in isosmotic saline containing protease inhibitor phenyl-sulphonyl-methyl-fluoride (PMSF 0.1 M) and rapidly frozen.

Table 1
Composition of the experimental diets for sea bream (sb) and rainbow trout (rt).

Ingredient (g kg^{-1})	FMsb	PP75sb	PP100sb	FMrt	PP75rt	PP100rt
Fish meal	703.7	176	0	637.9	159.5	0
Maize gluten	0	180	250	0	177.1	232.4
Wheat gluten	0	180	250	0	150	200
Extruded peas	0	90	120	0	120	163.3
Rapeseed meal	0	67.2	27.3	0	75	100
Sweet white lupin	0	0	6.9	0	0	0
Extruded wheat	142	30.6	15.5	203.4	42.5	0
Fish oil	124.3	149.8	158	128.6	151.1	158.7
Ligand	10	10	10	10	10	10
Mineral mix	10	10	10	10	10	10
Vitamin mix	10	10	10	10	10	10
$CaHPO_4 \cdot 2H_20$	0	30.7	51.1	0	37.8	40
L-Arginine	0	11.3	15.9	0	9.5	12.5
L-Histidine	0	3.4	4.8	0	2.9	3.9
L-Lysine	0	23.6	32.5	0	20.8	27.6
DL-Meteonine	0	3.6	5.4	0	3.06	4.1
L-Triptophane	0	2.2	2.9	0	2.1	2.7
L-Treonine	0	7.4	10.4	0	6.2	8.3
L-Isoleucine	0	6.2	8.4	0	5.3	7.1
L-Valine	0	8	11.1	0	7.0	9.4

For a more detailed description of the diets (including proximate composition) see Gómez-Requeni et al. (2004, 2005).

2.3. Nutrient uptake studies

BBMVs of the different intestinal segments were obtained following the technique described by Sala-Rabanal et al. (2004). Briefly, tissue was homogenized (in mM: 100 mannitol, 2 HEPES for sea bream; 60 mannitol, 2 HEPES for trout; and pH 7.4) and basolateral membranes were precipitated by addition of $MgCl_2$ and then centrifuged. Subsequent selective centrifugations allowed the purification and concentration of apical enterocyte membranes, which were vesiculated (in mM: 300 mannitol, 20 HEPES, 0.1 $MgSO_4 \cdot 7H_20$, 4.08 LiN_3 for sea bream; 285 mannitol, 20 HEPES, 0.1 $MgSO_4 \cdot 7H_20$, 4.08 LiN_3 for trout; and pH 7.4) using an insulin syringe.

2.3.1. Validation of the BBMV suspensions

To ensure the enrichment in BBMVs, as well as depletion in other cellular fractions, the activity of membrane enzymes considered as suitable cellular markers (Scalera et al., 1980) was measured (20 °C) in the initial homogenate and in the final BBMV preparations. Thus, alkaline phosphatase activity was measured following Weiser (1973) to assure an increase in brush border membrane during vesiculation. To rule out basolateral membrane contamination, Na^+/K^+–ATPase activity was determined using the method described by Colas and Maroux (1980), as modified by Sala-Rabanal et al. (2004). Similarly, depletion in citrate synthase activity (Srere, 1969) discarded the presence of mitochondrial debris.

The orientation of BBMVs was studied using a modification of the approach described by Del Castillo and Robinson (1982). Thus vesicle preparations were incubated in the presence or absence of a buffer containing 2 mM sodium deoxicolate plus 15 mM EDTA, which disrupts cellular membranes. The total sucrase activity of the solution was then measured by adding 100 μM of saccharose to the preparations. An increase in sucrase activity in disrupted BBMVs versus the non-disrupted vesicles indicates the presence of vesicles in the non-physiological orientation.

The lineal uptake zone in the BBMVs obtained was determined for 3 essential amino acids (L-leucine, L-lysine and L-phenylalanine), 2 non-essential amino acids (L-alanine and L-proline), and D-glucose, as described by Sala-Rabanal et al. (2004). For the analysis, 10 μL of BBMVs was mixed with 40 μL of incubation buffer (in mM: 250 NaSCN, 100 mannitol, 40 HEPES, 0.1 $MgSO_4 \cdot 7H_2O$, 8.16 LiN_3, 0.15 unlabeled nutrient, 0.01 ^{3}H-nutrient. Osm 320; pH 7.4 for sea bream; 250 NaSCN, 70 mannitol, 40 HEPES, 0.1 $MgSO_4 \cdot 7H_2O$, 8.16 LiN_3, 0.15 unlabeled nutrient, 0.01 ^{3}H-nutrient. Osm 305; and pH 7.4 for trout).

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