Aquaculture 306 (2010) 233–237

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Influence of partial substitution of dietary fish meal on the activity of digestive enzymes in the intestinal brush border membrane of gilthead sea bream, *Sparus aurata* and goldfish, *Carassius auratus*

Flávia C.P. Silva [a], Jacques R. Nicoli [a], José L. Zambonino-Infante [b], Marie-Madeleine Le Gall [b], Sadasivam Kaushik [c], François-Joël Gatesoupe [b,c,*]

[a] Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
[b] Ifremer, Nutrition Aquaculture et Génomique UMR1067, F-29280 Plouzané, France
[c] INRA, Nutrition Aquaculture et Génomique UMR1067, F-64310 St Pée Sur Nivelle, France

ARTICLE INFO

Article history:
Received 14 March 2010
Received in revised form 15 May 2010
Accepted 19 May 2010

Keywords:
Sparus aurata
Carassius auratus
Fish meal replacement
Brush border membrane
Digestive enzymes
Alkaline phosphatase
Aminopeptidase N
Maltase
Glutamyl transpeptidase

ABSTRACT

The sustainable composition of diets of high nutritional value is of the utmost importance for intensive aquaculture. Digestion and absorption of nutrients depend on the activity of the digestive enzymes, in particular those located in the brush border membrane of enterocytes, which are responsible for the final stages of breaking down and absorption of nutrients. In the present study, the substitution of fish meal by lupin or rapeseed meal in the diet was evaluated on gilthead sea bream (*Sparus aurata*) and goldfish (*Carassius auratus*). The objectives were to compare the activities of intestinal brush border enzymes in both species fed the control and experimental diets. When gilthead sea bream were fed the vegetable diets, significantly lower activities compared with the control group were observed for alkaline phosphatase and γ-glutamyl transpeptidase, but these differences were not significant in goldfish. Maltase activity was found decreased in the group fed lupin meal, both in sea bream and in goldfish. However, in spite of these differences in enzyme activities, growth characteristics of the fishes were similar with the three diets. It seemed that both fish were able to adapt to partial substitution of fish meal, but it remains to investigate the mechanism for compensating the decrease in specific enzymatic activity in the enterocytes of carnivorous gilthead sea bream.

1. Introduction

Marine fish aquaculture is a worldwide expanding industry, in which production has been concentrated in Europe on species such as gilthead sea bream, *Sparus aurata* L., and sea bass, *Dicentrarchus labrax* (L.) (Chabrillón et al., 2005). Limited supplies and the high cost of fish meal have forced fish nutritionists to search for alternative protein sources. Plant-derived protein sources, such as soybeans and lupin, are considered as interesting alternatives for fish meal, because they are widely available and do not conflict with human food security interests (Leenhouwers et al., 2006). Such sustainable composition of diets of high nutritional value is extremely important for intensive aquaculture. The formulation of diets is usually based on the digestibility data of the dietary nutrients used and this information is of great importance for assessing the nutritional value and quality of these nutrients. However, the digestion and absorption of the nutrients directly depend on the activity of the digestive enzymes, in particular those located in the brush border section of the intestine, which are responsible for the final stages of breaking down and assimilation of food (Fountoulaki et al., 2005). Fish meal has been a major ingredient in compound feeds for *S. aurata*, but alternative sources of protein are being used increasingly as fish meal availability decreases and prices increase. The vast majority of these alternative ingredients are plant-based, notably soybean products. The use of such ingredients introduces components which are known to affect digestive physiology along the digestive tract and which reduce the digestibility and utilization of the nutrients (Deguara et al., 2003).

Digestion and absorption of nutrients depend on the activity of the digestive enzymes, in particular those located in the brush border section of the intestine, which are responsible for the final stages of breaking down and assimilation of the food (Klein et al., 1998). Much research has been conducted in the last two decades to study the digestive ability and specific nutritional requirements of fish larvae and juveniles (Cahu and Zambonino Infante, 2001). Intestinal epithelium is considered to be the structure most likely associated with the terminal digestion of luminal peptides in vertebrates. Intestinal peptide hydrolases are found in two main sub-cellular locations, the cytosol and the brush border membrane of enterocytes (Zambonino Infante and Cahu, 2007). When enterocyte maturation occurs in fish larvae, the activity of the cytosolic enzymes decrease concurrently with the development of several enzymes like alkaline phosphatase, maltase, γ-glutamyl-transpeptidase

* Corresponding author. Ifremer, Nutrition Aquaculture et Génomique UMR1067, F-29280 Plouzané, France. Tel.: + 33 2 98 22 43 89; fax: + 33 2 98 22 46 53.
E-mail address: joel.gatesoupe@ifremer.fr (F.-J. Gatesoupe).

0044-8486/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2010.05.018

and aminopeptidase N located in the brush border membrane (Cahu and Zambonino Infante, 1995; Ma et al., 2005). Alkaline phosphatase is a dominant enzyme of the intestinal brush border, and is often used as a marker of intestinal integrity (Wahnon et al., 1992). Its activity is increased in a few hours in presence of its substrates. The functional significance of this enzyme is far to be fully understood, however, it hydrolyzes phosphoester bounds in various organic compounds like proteins, lipids, and carbohydrates (Nikawa et al., 1998). Maltase is a disaccharidase whose activity is rapidly (in less than 24 h) altered by the presence of disaccharides in the intestinal lumen (Santos et al., 1992). γ-Glutamyl-transpeptidase and aminopeptidase N are among the major enzymes of the intestinal microvilli, and play an essential role in the final hydrolysis and assimilation of dietary proteins (Douglas et al., 1999). The activity of these two enzymes is greatly enhanced by intraluminal peptide nutrients (Sonoyama et al., 1994).

The adaptive responses of these enzyme activities occur within hours and are particularly well recommended for assessing the impact on the intestinal function and integrity of non-conventional protein sources.

The present study evaluated the effect of partial substitution of fish meal by lupin or rapeseed meals in the diet to evaluate growth response and activities of intestinal brush border enzymes in two different model species having different feeding behaviors and trophic levels: (1) a marine temperate fish, gilthead sea bream (*S. aurata*), with a short digestive tract, and (2) a fresh warmwater species, goldfish (*Carassius auratus*), having a long relative intestine length.

2. Materials and methods

2.1. Animals and general rearing conditions

Gilthead sea bream (*S. aurata*) were provided by Ferme Marine de Douhet (FMD, La Brée les Bains, France), and the experiment was conducted at the laboratory 'Adaptation, Reproduction et Nutrition des poissons' (ARN, Ifremer, Centre de Brest, France). The fish were distributed into two tanks (1000 l). They were supplied with running seawater at 18–20 °C, and 35 ppt salinity. Photoperiod was maintained at 12–12 light/dark. After an adaptation period, the fish were distributed into 12 conical fiberglass tanks (60 l capacity; 40 individuals per tank). Until the start of the experiment, they were fed a commercial diet. The goldfish (*C. auratus*) were reared at the laboratory 'Nutrition, Métabolisme et Aquaculture' (INRA, Saint Pée Sur Nivelle, France). Goldfish juveniles were distributed into nine conical fiberglass tanks (60 l capacity; 30 individuals per tank). They were supplied with running freshwater at 23–25 °C. Photoperiod was maintained at 12–12 light/dark.

2.2. Experimental design

The experiment lasted 30 days, during which the fish were fed one of the three diets, tested in quadruplicate for gilthead sea bream and in triplicate for goldfish. The experiments were intended to compare the effect of the partial substitution of fish meal with either rapeseed or lupin meal in isoproteic diets (42% on a dry matter basis, Table 1). The level of substitution with the single plant protein source was limited to 20% of the diet based on earlier studies, particularly with relevance to limit the levels of possible antinutritional factors in ingredients such as lupin (Burel et al., 1998; Glencross et al., 2003) or rapeseed meal (Burel and Kaushik, 2008). The diet with lupin meal was balanced with lysine, methionine and leucine. After 10 days of acclimation, the tanks were randomly allotted to the three experimental groups F, L and R which were fed the fish meal diet, the lupin diet, and the rapeseed diet, respectively. The fish were fed twice a day, at 2% of daily ration on a body-weight basis. No mortality was observed during the experiment. The fish were weighed under anesthesia with benzocaine at start, and by the end of the 30 days of experiment. During the two experiments, the gilthead sea bream grew from 18.8 ± 2.1 g to 33.8 ± 5.5 g, and the goldfish grew from 21.5 ± 0.3 g to 29.2 ± 7.3 g. No significant difference was observed between the mean weights of the experimental groups (Table 2).

Table 1
Ingredients and chemical composition of the experimental diets.

Diets	F	L	R
Ingredients *(g kg^{-1}, on a dry matter basis)*			
Fish meal [a]	570	451	478
Rapeseed [b]	0	0	200
Lupin [c]	0	200	0
Starch	276	193	168
Fish oil	124	124	124
Carob gum E410	5	5	5
Xanthan gum E415	5	5	5
Mineral mix [d]	10	10	10
Vitamin mix [d]	10	10	10
Amino acid mix[e]	0	2	0
Analyzed composition			
Dry matter (DM, %)	97.8	94.8	97.6
Crude protein (% DM)	45.7	45.4	45.5
Crude fat (% DM)	19.0	20.5	18.6
Aminoacid composition (% crude protein)[f]			
Arginine	5.4	6.7	5.8
Histidine	2.0	2.2	2.3
Isoleucine	4.7	4.6	4.6
Leucine	7.5	7.0	7.5
Lysine	7.2	6.8	6.8
Methionine + Cystine	4.1	3.6	4.4
Phenylalanine + Tyrosine	6.8	6.9	6.9
Threonine	4.4	4.2	4.5
Tryptophane	1.1	1.1	1.1
Valine	4.8	4.6	5.0

[a] Norse-LT 94 supplied by La Lorientaise, Lorient, France. The amounts were adjusted to obtain isoproteic diets (42 %, dry matter basis).
[b] SAIPOL, Grand Couronne, France.
[c] Lup'Ingredients, Martigne Ferchaux, France.
[d] According to Cahu et al. (1999).
[e] Leucine 36.8%; lysine 31.6%, methionine 31.6%.
[f] Computed after data from raw feedstuffs.

2.3. Sampling and dissection of gilthead sea bream and goldfish

The fish were sampled in each tank by the end of the experiments, after 4 weeks of experimental feeding. The fish were euthanized with 2-phenoxythanol, the corporal surface was disinfected with ethanol (70%), and the abdominal cavity was opened. The mucosa of the digestive tract was collected by scrapping the anterior intestine for further purification of brush border membranes (see below) for enzymatic determinations.

2.4. Enzymatic determinations

The intestinal mucosa was homogenized to purify brush border membranes (BBM) according to a method described by Crane et al. (1979). The intestinal mucosa was homogenized with a homogenizer (Polytron, PT-MR 2100) at maximum speed for 30 s. Then, a 1-ml

Table 2
Initial and final mean weights of gilthead sea bream and goldfish (in g, ± standard deviation). At the end of the experiments, the differences were not significant.

Diets	F	L	R
Gilthead sea bream			
Initial weight (g)	18.80 ± 2.10	18.80 ± 2.10	18.80 ± 2.10
Final fish weight (g)	31.90 ± 4.11	34.20 ± 5.65	35.20 ± 6.63
Goldfish			
Initial weight (g)	21.50 ± 0.30	21.50 ± 0.30	21.50 ± 0.30
Final fish weight (g)	27.72 ± 6.44	26.53 ± 5.51	33.44 ± 10.09