IN VITRO EVALUATION OF THE INTERACTION BETWEEN EXOGENOUS CARBOHYDRASES PRODUCED BY SOLID-STATE FERMENTATION OF BREWERS' SPENT GRAIN AND DIGESTIVE ENZYMES

Helena Fernandes[1,5]; Francisco J. Moyano[2]; Nelson Fernandes[1]; José Salgado[3]; Tiago Aires[4]; Isabel Belo[3]; Aires Oliva-Teles*[1,5]; Helena Peres[1,5]; Carolina Castro[5]

[1]*Department of Biology, Faculty of Sciences, University of Porto, Portugal
[2]Dpto. Biología y Geología, CEIMAR, University of Almería, Almería, Spain
[3]CEB – Centre of Biological Engineering of University of Minho, Campus de Gualtar 4710-057 Braga, Portugal
[4]Sorgal, Sociedade de Óleos e Rações, SA, Ovar, Portugal
[5]CIMAR/CIIMAR-Centro Interdisciplinar Marinha e Ambiental,, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos 4450-208 Matosinhos, Portugal
e-mail: aoteles@fc.up.pt

Introduction

The presence of non-starch polysaccharides (NSP) in most plant feedstuffs (PF) has been associated with adverse effects on carnivorous fish nutrient digestibility and, ultimately, on growth and health. The lack of enzymatic machinery and well-developed microbiota in the digestive tract of carnivorous fish for processing NSP are possible causes for such effects. A promising nutritional strategy to improve nutrient digestibility of plant-based diets is the use of exogenous enzymes (e.g. carbohydrases, proteases) as feed additives. Solid-state fermentation (SSF) is an eco-friendly and cost-effective biotechnology process that allows converting inexpensive agro-industrial by-products into added-value products such as carbohydrases. As a low-cost and lignocellulosic by-product of the brewing industry, brewery spent grain (BSG) is an attractive substrate for microbial enzyme production by SSF. The present study was designed to assess the potential of a carbohydrase enzyme extract obtained by SSF to release total amino acids (AA) and monosaccharides (pentoses) from plant-based diets in an *in vitro* gastrointestinal model with enzyme extracts of European sea bass (*Dicentrarchus labrax).*

Material and methods

Three isoproteic (48% crude protein) and isolipidic (16% crude lipids) diets were formulated with 15% fishery products (fish meal and fish protein concentrate), 5% hemoglobin, and 61% plant feedstuffs (wheat gluten, soybean, wheat, rice bran, sunflowe , rapeseed). In all diets fish oil was the main lipid source. Diets were similar in starch (10%), hemicellulose (2.4%), cellulose (1.6-%) and lignin (2.3%) contents. The diets were unsupplemented (control) or supplemented with an enzyme extract obtained by SSF at 0.1 or 0.4%. The enzyme extract was obtained from SSF of BSG using *Aspergillus ibericus* MUM 03.49 as the inducer for enzyme production. The enzyme extract was a combination of cellulase (1343U g^{-1} crude extract) and xylanase (15885U g^{-1} crude extract).

Table I. Total amino acids (AA, mg g^{-1}) and pentoses ($\mu g\ g^{-1}$) released by gastric (G) and intestinal (I) digestion of the experimental diets with active (A) or inactive (W) fish enzyme extracts[1]

Diet	Control				0.1% BSG				0.4% BSG			
Digestion phase	G		I		G		I		G		I	
Incubation mixture	A	W	A	W	A	W	A	W	A	W	A	W
AA	18.7 ±0.1	14.9 ±0.1	27.3 ±0.5	24.3 ±0.2	19.2 ±0.3	18.3 ±0.4	27.7 ±0.5	11.4 ±0.0	18.9±0.0	17.3 ±0.3	29.4 ±0.1	23.9 ±0.6
Pentoses	179.1 ±26.8^{a}	197.2 ±10.9^{a}	232.1 ±32.0^{a}	267.0 ±27.5^{a}	198.3 ±40.5^{b}	217.1 ±7.5^{b}	282.3 ±65.3^{b}	355.2 ±10.1^{b}	261.5 ±48.4^{c}	291.2 ±11.3^{c}	328.3 ±26.2^{c}	394.3 ±13.2^{c}

3-way ANOVA (P-value)

Factor	diet	digestion phase	incubation mixture	diet x digestion phase	diet x reaction mixture	digestion phase x reaction mixture	diet x digestion phase x reaction mixture
AA	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Pentose	≤0.001	≤0.001	≤0.001	0.179	0.663	0.103	0.774

[1]Value are mean ± SD (n = 3). When significant interaction between factors was found, one-way ANOVA was performed for each factor. Different superscript letters stand for statistical differences across experimental diets as determined by the Tukey test ($P < 0.05$).

(Continued on next page)

The *in vitro* methodology involved two-step hydrolysis designed to simulate stomach and intestinal digestion of European sea bass, as described in Morales and Moyano (2010). Briefl , the digestion was simulated using the following conditions: samples of each diet (between 20 and 80 mg mL^{-1}) were incubated in triplicate in a 10 mL closed reactor at 25ºC simulating gastric and intestinal digestions. The gastric digestion was done at pH of 5.0 with 9846 U mg^{-1} acid proteases and the intestinal digestion was done at pH of 8.5 with 507 U mg^{-1} alkaline proteases from sea bass stomach and intestine, respectively. A negative control assay including inactivated fish enzyme extracts was also carried out. The release of hydrolysis products, AA and pentoses, were monitored along the course of each digestion phase (at 0, 0.5, 1, 2h for gastric digestion; at 3, 4, 5, and 6h for intestinal digestion). Results are presented as total amount of product released during the different digestion phases.

Results and Discussion

After the pelleting process, 0.1 and 0.4 % BSG supplemented diets presented 1.76 and 5.76 U g^{-1} cellulase activities and 5.11 and 8.80 U g^{-1} xylanase activities, respectively. The simulated gastric and intestinal digestion demonstrated that AA release remained unaffected by dietary treatments (Table I). In contrast, a significant increment of pentoses release in the presence of increased incorporation of extracts in the diets was observed throughout gastric and intestinal incubations. Independently of dietary treatment, AA and pentoses release were higher at intestinal than at gastric digestion. In all dietary treatments, inactivation of fish enzyme extracts (W), promoted higher pentoses release than active fish enzyme extracts(A). Contrarily, inactivation of fish enzyme extracts did not affect AA release from experimental diets, except in the 0.1% BSG diet.

In conclusion, dietary supplementation of plant-based diets with 0.1 or 0.4 % enzyme extracts obtained by SSF of BSG seems to contribute to enhancing monosaccharide availability. Results also suggest that fish enzyme extracts interact with carbohydrases extract reducing their ability to hydrolyze NSP. Thus, coating the enzyme extract should be considered to maximize their potential. Studies *in vivo* still need to be performed to confirm the results of the present *in vitro* results.

Acknowledgments: supported by POCI-01-0145-FEDER-030377 and MAR-02.01.01-FEAMP-0111. HF and CC supported by grants SFRH/BD/131219/2017; SFRH/BPD/114942/2016, respectively.

References: Morales GA, Moyano FJ (2010). Aquaculture 306: 244–251.