Animal Feed Science and Technology 171 (2012) 181–191

Contents lists available at SciVerse ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

# Effects of exogenous enzymes on apparent nutrient digestibility in rainbow trout (*Oncorhynchus mykiss*) fed diets with high inclusion of plant-based protein

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ARTICLE INFO

*Article history:*
Received 17 May 2011
Received in revised form 12 October 2011
Accepted 12 October 2011

*Keywords:*
Rainbow trout
Digestibility
β-Glucanase
Protease
Xylanase
Plant protein

ABSTRACT

Plant-based protein ingredients are increasingly used in feed for carnivorous fish as replacement for fish meal. This study investigated if supplementing diets with high inclusion levels of plant-based protein with three different enzymes could improve the apparent nutrient digestibility in juvenile rainbow trout (*Oncorhynchus mykiss*). Three diets with high inclusion levels of either de-hulled, solvent extracted soybean meal (344 g/kg), sunflower meal (246 g/kg), or rapeseed meal (264 g/kg) were produced and feed batches were coated post-extrusion with the different enzymes: β-glucanase at 67 mg/kg, xylanase at 208 mg/kg, protease at 228 mg/kg, or a combination of the three enzymes at the same doses. Three consecutive digestibility trials were carried out using a flow-through, modified Guelph System. Each trial was designed to include five dietary treatment groups: a non-supplemented control diet and 4 diets supplemented with either β-glucanase, xylanase, protease or a combination of the three enzymes. Diets were fed to triplicate tanks and two faecal sampling periods for digestibility measurements for each tank were scheduled. Each experiment comprised a 10-day digestibility trial succeeded by a water sampling period for measuring the dissolved nitrogen (N) output. Each experiment lasted 17–19 days in total.

Apparent digestibility coefficients (ADCs) of protein, fat, ash, phosphorus and dry matter (DM) were derived from the three digestibility trials, along with calculations of the specific growth rate (SGR, %/d) and feed conversion ratio (FCR). Nitrogen mass-balance and energy retention were evaluated for each dietary treatment group to elucidate on the utilization of digested nutrients and energy.

Enzyme supplementation had only moderate effect on apparent nutrient digestibility in the sunflower and rapeseed experiments, while β-glucanase and protease improved the apparent digestibility of all dietary nutrients in the soybean experiment ($P<0.05$). The effect was more pronounced for lipid than for other nutrients. β-Glucanase had a positive effect on energy retention in the soybean experiment ($P<0.05$), while there were no effects on nitrogen retention or fish performance in any of the three experiments ($P>0.05$) during the short feeding periods. The study thus provides preliminary results on the potential of β-glucanase and protease to increase apparent nutrient digestibility of soybean meal in fish feed.



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doi:10.1016/j.anifeedsci.2011.10.005

## 1. Introduction

Current research in carnivorous fish nutrition focuses on the replacement of fish meal with plant-based protein ingredients in order to support the globally expanding aquaculture industry and to ensure that aquaculture remains sustainable (FAO Fisheries Department, 2006; Gatlin et al., 2007; Hardy, 2010). The digestive system of carnivorous fish has not evolved to deal with the wide variety of anti-nutritional factors (ANFs) that are present in most plant-based feedstuffs, and which may interfere with fish performance and health due to impaired nutrient utilization (reviewed by Francis et al., 2001; Gatlin et al., 2007; Hardy, 2010; Krogdahl et al., 2010). High levels of ANFs in carnivorous fish diets may consequently depress growth and have a negative impact on the environment due to elevated nutrient discharge. The influence of ANFs on nutrient utilization must therefore be reduced before carnivorous fish diets can be formulated to include more plant-based feedstuffs.

The use of exogenous enzymes could be one way to reduce the impact of ANFs in carnivorous fish diets containing high levels of plant-based feedstuffs. They are already widely applied in feed for terrestrial animals such as pigs and poultry as a way to reduce the anti-nutritional effects of primarily non-starch polysaccharides (NSPs) and phytic acid, and increase the utilization of carbohydrates and phosphorus, respectively (reviewed by Campbell and Bedford, 1992; Bedford and Schulze, 1998; Bedford, 2000; Choct, 2006; Cowieson et al., 2006).

The use of phytase is developing quite fast in aquaculture. However, apart from phytase in fish feed (*e.g.*, Lanari et al., 1998; Cheng et al., 2004; Vielma et al., 2004; Cao et al., 2007; Denstadli et al., 2007; Dalsgaard et al., 2009), there are relatively few studies on the effects of exogenous enzymes on salmonid performance and nutrient utilization (Carter et al., 1992, 1994; Drew et al., 2005; Ogunkoya et al., 2006; Farhangi and Carter, 2007). Drew et al. (2005) thus showed an increase in the apparent nutrient digestibility and an improvement in the feed efficiency when supplementing a commercial protease to a rainbow trout (*Oncorhynchus mykiss*) diet containing a mixture of rapeseed and pea. Ogunkoya et al. (2006) added graded levels of a commercial enzyme cocktail with variously not clearly defined enzyme activities to rainbow trout diets containing up to 200 g/kg of soybean meal (SBM). Apparent nutrient digestibility was improved in relation to SBM, but no effects were observed on growth and feed efficiency. Farhangi and Carter (2007) supplemented protease and carbohydrases alone or in combination to de-hulled, lupin-based, juvenile rainbow trout diets. No effects on performance were observed, but the mixed enzyme significantly improved the protein efficiency ratio, and the apparent digestibility of dry matter, protein and gross energy was improved in fish fed a carbohydrase supplemented diet. Carter et al. (1994) reported a positive effect on Atlantic salmon smolt (*Salmo salar* L) performance and feed efficiency when supplementing a combination of proteolytic enzymes and carbohydrases to a diet containing 340 g/kg SBM. However, they could not correlate this with an increase in the apparent digestibility of nitrogen and carbon.

The purpose of the present study was to evaluate the effects on the apparent nutrient digestibility in juvenile rainbow trout (*O. mykiss*) of supplementing three enzyme products to three diets with high inclusion levels of different plant-based protein ingredients. The three evaluated enzyme products consisted of β-glucanase, xylanase, and protease, while the plant-based protein ingredients consisted of soybean meal, sunflower meal and rapeseed meal.

## 2. Materials and methods

### 2.1. Culture conditions and fish

Three consecutive experiments were conducted with juvenile rainbow trout obtained from local, Danish trout farms [Troelstrup fish farm (experiments 1 and 2) and Fousing fish farm (experiment 3)] and transferred to DTU Aqua's experimental facilities located in Hirtshals, Denmark. Fish in similar size range (±10 g) were sorted out prior to each trial, pooled and subsequently distributed randomly among 18, 189-L, flow-through, cylindrical-conical thermoplastic tanks mounted in a modified Guelph setup for faeces sedimentation and collection (Dalsgaard and Pedersen, 2011). The initial biomass (mean ± SD) in the tanks was 6.4 ± 0.2, 5.1 ± 0.2, and 5.4 ± 0.1 kg/m$^3$ with fish having an initial body weight of 110 ± 12, 106 ± 11, and 73 ± 6 g in experiments 1, 2, and 3, respectively. Each tank was supplied with tap water at a flow rate of 40 L/h, and an average temperature of 12 °C was maintained throughout the experiments along with a 15 h light:9 h dark diurnal photoperiod.

### 2.2. Experimental diets

Four iso-nitrogenous, iso-lipidic, and iso-caloric diets (Table 1) were formulated and prepared by BioMar A/S (Brande, Denmark). The amino acid composition of the diets was not optimised. The four diets comprised of a fish-meal based control diet and three diets in which fish meal was partly replaced by either soybean meal (de-hulled, solvent extracted soybean cake Hi-Pro; SOY, 344 g/kg, experiment 1), sunflower meal (de-oiled sunflower cake; SUN, 246 g/kg, experiment 2), or rapeseed meal (de-oiled rapeseed cake; RAP, 264 g/kg, experiment 3). The diets were prepared as 3 mm pellets using a Clextral BC-45 extruder (Clextral S.A., Firminy, France). Each of the three plant-based diets were subsequently divided into five batches, four of which were coated post-extrusion with either: (i) a fungal, multi-component enzyme comprising endo-1,3(4)-β-glucanase as the main activity (GLU, RONOZYME® VP (L), DSM Nutritional Products, Saint-Louis Cedex, France) at 67 mg/kg; (ii) a fungal mono-component enzyme as endo-1,4-β-xylanase (XYL, RONOZYME® WX (L), DSM Nutritional Products, Saint-Louis Cedex, France) at 208 mg/kg; (iii) a bacterial mono-component enzyme as serine protease (PRO, experimental enzyme,