



# Evaluation of enzyme- and *Rhizopus oligosporus*-treated high oil residue camelina meal on rainbow trout growth performance and distal intestine histology and inflammatory biomarker gene expression

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## ABSTRACT

The effect of high oil residue camelina meal (HORM) and treated HORM on rainbow trout growth performance and related parameters was determined. Two isocaloric, isonitrogenous diets containing fish meal at a level of 100 g/kg and fish oil at a level of 50 g/kg were formulated. One was a control diet, and the other contained HORM at an 80 g/kg inclusion level. In an additional five test diets, HORM was replaced with treated HORM (water-soaked HORM, pectinase HORM, Superzyme HORM, High-Pectinase Superzyme HORM (HP-SZ HORM) or *Rhizopus* HORM/wheat (1:3 wheat:HORM fermented with *R. oligosporus*; R-HORM). Test ingredients in treated HORM diets were mixed with water and/or their respective enzymes, then incubated for 24 h at 40 °C or fermented by *R. oligosporus* fungus for 72 h. Diets were fed to rainbow trout (average weight: 33.7 g; 30 fish/126 L tank; 3 tanks/treatment) in a freshwater (11.8 ± 0.7 °C), flow-through system for 112 days. Treatment of HORM with water or enzyme provided intermediate growth between the control and R-HORM diets and the initial four weeks on test. On day 28, fish fed the control diet had a higher specific growth rate (SGR) than fish fed HORM and R-HORM and a higher thermal growth coefficient than fish fed R-HORM ( $P < 0.05$ ). On day 56, control-fed fish had a significantly higher weight gain and fork length than water-soaked HORM- and R-HORM-fed fish. On day 112, fish fed the control diet had a higher SGR than fish fed R-HORM and fish fed all diets except water-soaked HORM had a longer fork length ( $P < 0.05$ ) than fish fed R-HORM ( $P < 0.05$ ). There were no significant differences in the transcript levels of eight inflammatory biomarker genes (GILT $\alpha$ , GILT $\beta$ , PAR2 $\alpha$ , PAR2 $\beta$ , IL1 $\beta$ , MyD88, TGF $\beta$ 1 $\alpha$  and TGF $\beta$ 1 $\beta$ ) in the distal intestines of fish fed the HORM, HP-SZ HORM or R-HORM diets, compared with fish fed the control diet. An inclusion level of 80 g/kg HORM and water- and enzyme-treated HORM (all three treatments) are acceptable in juvenile rainbow trout diets. Future studies may involve similar treatments at higher dietary inclusion levels and HORM fermented with *R. oligosporus* using a substrate other than wheat.

## 1. Introduction

Camelina (*Camelina sativa*), an oilseed crop commonly called false flax or gold of pleasure (Zubr, 1997), is being developed as a potential alternative feed ingredient for fish. High oil residue camelina meal (HORM) is a by-product generated when oil produced by the camelina seed is extracted using a mechanical expeller process. It contains approximately 34% crude protein and 10 to 13% residual oil (NRC, 2011). Camelina oil contains high levels of unsaturated fatty acids, especially

$\alpha$ -linolenic (18:3 n-3) which accounts for 37.8% of the total fatty acids (Zubr, 2003).

Despite their nutritional value, a potential limitation of the use of plant-based aquafeed ingredients such as camelina is that they contain antinutritional factors (ANFs) that affect the taste and digestibility of the feed (Matthäus and Zubr, 2000). These ANFs include glucosinolates, mucilage, phytic acid, sinapine and tannins (Matthäus and Zubr, 2000; Russo and Reggiani, 2012; Schuster and Friedt, 1998). Glucosinolates, as well as their degraded products, are some of the primary ANFs of

Abbreviations: HORM, High oil residue camelina meal; FCR, Feed conversion ratio

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concern in camelina meal (Schuster and Friedt, 1998). Mucilage is a gel-forming fiber, which in camelina, consists of acidic and neutral polysaccharides (Zubr, 2010). The crude fiber content of camelina seed ranges from 7.6–13%, with a mucilage content of 6.7% (Zubr, 1997; Zubr, 2010). Phytic acid is found in many legumes and oilseeds and reduces the solubility and biological availability of phosphorus and other mineral elements such as calcium, magnesium, zinc and iron, by producing complexes that cannot be digested or absorbed by the animal (Russo and Reggiani, 2012; Francis et al., 2001; Hossain and Jauncy, 1993). Tannins can precipitate proteins, thereby inhibiting the activity of digestive enzymes. Tannins also hinder the use of vitamins and minerals in the digestive tract (Russo and Reggiani, 2012). Matthäus and Zubr (2000) reported a range of 21.9–30.1 mg/g total inositol phosphate (phytic acid) in 15 samples of camelina oilseed cake. In these same 15 samples, sinapine (a bitter component of Brassica oilseeds) levels ranged from 1.7–4.2 mg/g and condensed tannin levels were between 1.0 and 2.4 mg/g.

Previous work by Hixson et al. (2014) has shown that the substitution of fish meal with 10% solvent-extracted camelina meal inhibited the growth of Atlantic salmon (*Salmo salar*). As camelina meal contains ANFs, this inhibited growth may have been a direct result of ANFs in the feed. Therefore, investigating methods to reduce the ANFs present in camelina meal and related products is paramount to increase the value of this n-3 fatty-acid-containing, plant-based protein source for use in aquafeeds.

The effects of some ANFs can be tempered based on the level that is included in the diet; whereas others can be inactivated or eliminated through ingredient processing. Processing methods such as water-soaking and dietary enzymes (Egounlety and Aworh, 2003) and fermentation with the *Rhizopus oligosporus* fungus may help eliminate or alleviate the negative influence of some ANFs in camelina and other plant meals. For example, water-soaking can reduce about 90% of the glucosinolates in Brassica vegetables (Song and Thornalley, 2007). Research involving the application of carbohydrase enzymes, such as xylanases, cellulases and pectinases, to plant-based feed ingredients included in aquafeeds is also increasing in effort to elucidate methods of improving the nutrient availability and utilization of these ingredients (Castillo and Gatlin, 2015).

*Rhizopus*, a fungus with a low pathogenicity (level 1 biosecurity), is used to make tempeh, a pre-fermented dish from soybeans. *Rhizopus* grows at a maximum temperature of 48 °C, enabling inactivation at higher temperatures (Hartanti et al., 2015). *Rhizopus* treatment (*R. oligosporus*) of lupins effectively reduced alkaloids and increased crude protein composition during the fermentation process (Ortega-David and Rodriguez-Stouvenel, 2014). *R. oligosporus* may also have potential detoxifying applications in reducing glucosinolate levels in Brassica meals and, as a fungus that digests cellulose fibers, it may also reduce the negative impact of mucilage.

The objective of this project was to determine the effect of water, enzyme and *R. oligosporus* treatment on the ANF content of HORM, as well to determine the suitability of these treated HORM products as feed ingredients for rainbow trout.

## 2. Materials and methods

### 2.1. Experimental ingredients

Camelina (Calena cultivar) was grown and harvested in Canning, Nova Scotia (Canada) under the supervision of Dalhousie University, Faculty of Agriculture (Truro, NS, Canada) staff. Atlantic Oilseed Processing, Ltd. (Kinkora, Prince Edward Island, Canada) extracted the oil from the seeds using a KEK P-0500 expeller-press (EGON KELLER GMBH and CO. KG, Remscheid, Germany). The remaining high-oil presscake was hammer-milled (screen size 8 mm) to yield a high oil residue camelina meal (HORM). Ethoxyquin (60% ethoxyquin, 40% silica) was added to the HORM at an inclusion rate of 0.2%

of the predicted oil content of the HORM, in order to prevent oxidation.

Six ingredients (Table 1) were tested in this experiment: untreated HORM, water-soaked HORM, pectinase-treated HORM (pectinase HORM), multi-carbohydrase (Supzyme™-OM Concentrate)-treated HORM (Supzyme HORM), High-Pectinase Supzyme™-OM Concentrate-treated HORM (HP-SZ HORM), and *R. oligosporus*-treated HORM and wheat (R-HORM). The water-soaked and enzyme-treated HORM products were prepared using the following ingredient ratios: Water-soaked HORM: 1000 g HORM + 7000 mL water; pectinase HORM: 1000 g HORM + 7000 mL water + 0.015 g pectinase, Supzyme HORM: 1000 g HORM + 7000 mL water + 0.06 g Supzyme™-OM Concentrate; HP-SZ HORM: 1000 g HORM + 7000 mL water + 0.015 g High Pectinase Supzyme™-OM Concentrate. Once all ingredients were combined to make a treated HORM product, the mash was mixed for 10 min, then incubated for 24 h at 40 °C in a forced-air oven. After incubation, the mash was spread thinly over parchment paper laid out on large metal sheets and dried in a forced-air oven for 48 h at 45 °C. The Supzyme™-OM Concentrate contained (unit/g): 14,000 cellulase; 13,675 amylase; 9800 xylanase; 5000 protease; 4025 glucanase; 2800 invertase; 2075 mannanase, 1350 pectinase. The Pectinase was in liquid form and contained 2500 units of pectinase activity/g. The High-Pectinase Supzyme™-OM Concentrate contained (units/g): 9225 cellulase; 8975 amylase; 6450 xylanase; 3300 protease; 2750 glucanase; 1850 invertase; 1375 mannanase, 1350 pectinase. All enzymes were provided by Canadian Biosystems Inc. (Calgary, AB, Canada) and were administered based on the instructions of the manufacturer.

To make the R-HORM test ingredient via surface-activated bio-fermentation (SAB), a combination of HORM and whole wheat kernels was subjected to hot water pasteurization to prepare the HORM/wheat material for inoculation with *R. oligosporus* fungus (ATCC 22959). To pasteurize the HORM/wheat in preparation for inoculation, HORM, wheat and water were combined at a ratio of 1.33 kg camelina: 0.67 kg wheat: 2.56 kg water and held at 70 °C for 2 h. The post-pasteurized HORM/wheat was inoculated with ATCC-approved *R. oligosporus* at a rate of 10<sup>6</sup> conidia per mL. The inoculated product was then spread onto shallow trays in a grow-out pilot system developed at the Atlantic BioVenture Centre (previously Nova Scotia Agricultural College, currently Dalhousie University, Faculty of Agriculture, Truro, NS, Canada) and incubated for five days within a controlled access sterilized warm room environment with filtered air and heat provided and CO<sub>2</sub> removal. Upon completion of the SAB process, the HORM/wheat material was dried in a forced-air oven at 45 °C for 72 h in order to deactivate the *R. oligosporus* fungus and to attain a product with a moisture level < 8%. The stabilized product was ground using a hammer mill with a 0.50 mm mesh size, resulting in the R-HORM test ingredient.

### 2.2. Diet preparation

Seven experimental diets were fed in this trial. Two isocaloric, isonitrogenous diets were formulated: 1) one control diet containing 80 g/kg herring meal and 50 g/kg herring oil and 2) one diet that contained the same ingredients as the control diet, as well as HORM at an 80 g/kg inclusion level (Table 2). All other feed ingredients were typical of feed production used in rainbow trout diets and each diet was balanced to be 40% available crude protein and 4400 kcal digestible energy (DE)/kg to meet requirements for rainbow trout (NRC, 2011). To make the remaining experimental diets, the HORM in the second diet was replaced with one of the other five test ingredients listed in Section 2.1 (water-soaked HORM, pectinase HORM, Supzyme HORM, HP-SZ HORM, R-HORM; Table 3).

All feeds were mixed and steam-pelleted at the Chute Nutrition Centre, Dalhousie University, Faculty of Agriculture (Truro, NS, Canada). A Hobart mixer (Hobart Corporation; Model L-800; Troy, OH, USA) was used to mix diets. The diets were then steam-pelleted in a

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