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# Water-soluble fractions obtained by enzymatic treatment of wheat grains promote short chain fatty acids production by broiler cecal microbiota

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ABSTRACT

The use of exogenous cell wall degrading enzymes to improve the nutritional values of cereal grains has increased during the last decades. In this study, products from enzymatic hydrolysis of wheat grain were isolated and their ability to induce short-chain fatty acids was investigated using *in vitro* fermentation with cecal microbiota. Water-soluble fractions were obtained following incubation without and with a multicomponent enzyme preparation (MEP) (2 mL/kg) containing essentially endoxylanase and endoglucanase activities. These were further fractionated by size exclusion chromatography into high molecular weight (HMw) and low molecular weight (LMw) sub-fractions. The MEP treatment increased the content in water-soluble arabinoxylan **(AX)** and decreased the degree of polymerization (DP) of the xylan-backbone. *In vitro* fermentation assays using broiler cecal content as inoculum demonstrated that low substituted AX with reduced molecular weight isolated after MEP treatment yielded higher concentrations of acetate and butyrate. This result indicates that cecal fermentation of enzymatic degradation products into short-chain fatty acids, particularly butyrate, is one of the important mechanisms of MEP action on wheat-based diets that can contribute to the improvement of intestinal health and animal performance.



*Abbreviations:* AGp, arabinogalactan-proteins; AX, arabinoxylans; AXOS, arabino-*xylo*-oligosaccharides; BWG, body weight gain; DP, degree of polymerization; EIF, ethanol-insoluble fraction; FCR, feed conversion ratio; FOS, fructo-oligosaccharides; GLC, gas liquid chromatography; HBSS, Hank's buffered salt solution; HCl, hydrogen chloride; HMw, high molecular weight; HPLC, high-performance liquid chromatography; HPSEC, high performance size-exclusion chromatography; LMw, low molecular weight; MEP, multicomponent enzyme preparation; $NaBH_4$, sodium borohydrure; NaClO, sodium hypochlorite; NSP, non-starch polysaccharides; PBS, phosphate-buffered saline; PITC, phenyl isothiocyanate; SCFA, short chain fatty acids; TEA, triethylamine; WSF, water-soluble fraction; XOS, *xylo*-oligosaccharides.

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**Table 1**
Characterization of Rovabio Excel[a].

| Designation | Activity[b] |
|---|---|
| *endo*-1,4-β-xylanase | 811 U/g |
| *endo*-1,3(4)-β-glucanase | 1215 U/g |

[a] Major enzyme activities of Batch Rovabio Excel LC 13.M.002152.
[b] Activities as measured by DNS colorimetric method. For *endo*-1,3(4)-β-glucanase, one unit corresponds to the amount of enzyme which produces 1 μmole of glucose per minute from barley β-glucan at pH 5.0 and 50 °C. For xylanase, one unit corresponds to the amount of enzyme which produces 1 μmole of xylose per minute from birchwood xylan at pH 4.0 and 50 °C.

## 1. Introduction

Wheat is the most commonly used cereal in poultry diets in Europe. However differences in metabolisable energy are observed amongst wheat based diets, partly due to the presence of non-starch polysaccharides (NSP)(Gutierrez del Alamo et al., 2008). In wheat, the NSP content ranges from 12 to 16% of dry matter. Starchy endosperm NSP consist mainly of arabinoxylans (AX), that represent 70% of the cell walls (Saulnier et al., 2012). Arabinoxylans are composed of a linear backbone of β-(1,4)-linked D-xylose residues that is partially substituted with individual α-L-arabinose residues attached through O-2 and/or O-3 (Izydorczyk and Biliaderis, 1995). The second most important wheat NSP are mixed-linked β-glucans, which are linear homoglucans arranged as blocks of consecutive (1,4)-linked β-D-glucose residues separated by single (1,3)-linkages (Li et al., 2006). It is well demonstrated that these two major components of cereal grain endosperm exert anti-nutritional effects through the viscosity-inducing property of their water-soluble fraction. In the case of wheat, the water-soluble NSP are almost exclusively composed of AX.

Non-starch polysaccharides degrading enzymes (NSP-enzymes) constitute a category of safe additives that improve body weight gain (BWG) and feed conversion ratio (FCR) in broilers (Mathlouthi et al., 2002a; Choct et al., 2004; Wang et al., 2005; Walk et al., 2011). The NSP-enzymes like *endo*-1,4-β-xylanases and *endo*-1,3(4)-β-glucanases are widely used separately, in combination or in a more complex mixture to decrease the viscosity of the intestinal content and enhance the nutritional value of cereal diets (Choct et al., 2004; Mathlouthi et al., 2002b). However, the improvement of nutritional performance of wheat-based diets by NSP-enzymes is not only linked to viscosity effect. NSP-enzymes also increase short-chain fatty acids concentration (SCFA) in the ceca (Choct et al., 1999) and alter the microbiota (Van der Wielen et al., 2000).

However, it is not fully explained in the literature what substrates are released by the NSP-enzymes which contribute favorably to the SCFA production in the caeca. The aim of this paper is to contribute to the understanding of the beneficial effects of NSP-enzymes supplementation to wheat-based diets for broilers. Therefore, water-soluble NSP were isolated from wheat grain treated or not with the multicomponent enzyme preparation (MEP) Rovabio® Excel. Water-soluble NSP were further separated by size exclusion chromatography into high molecular weight (HMw) and low molecular weight (LMw) sub-fractions. Fractions were characterised for their chemical composition and physico-chemical features, and their SCFA production pattern was determined *in vitro* using broiler cecal microbiota.

## 2. Materials and methods

### 2.1. Materials

The multicomponent enzyme preparation(MEP) Rovabio® Excel was provided in liquid form by Adisseo SAS (Commentry, France) (Table 1). The MEP contains glycosyl hydrolases, mainly *endo*-1,3(4)-β-glucanase (1200 U/g) and *endo*-1,4-β-xylanase (800 U/g), produced from the fermentation of *Talaromyces versatilis* sp. nov. (previously named *Penicillium funiculosum)*.

The wheat cultivar Barok, harvested in 2012, was purchased from Euronutrition (St Symphorien, France). Xylo-oligosaccharides (XOS), XOS95P, were purchased from Shandong Longlive Bio-technology CO.LTD, Yucheng, China. Fructo-oligosaccharides (FOS), Fibrulose® F97, were purchased from Cosucra, Warcoing, Belgium.

### 2.2. Isolation of the wheat fractions

The flowchart of the isolation of the wheat fractions is described in Fig. 1.

Wheat grains were decontaminated with successive washing in water, 70% ethanol, sodium hypochlorite (NaClO) and water. After drying in a stove at 40 °C for 24 h, grains were ground using a 0.5 mm grid (Retsch, Haan, Germany). Ground grains (300 g) were suspended in Milli-Q water (1/3, w/w) leading to pH around 6.0. The incubation lasted 6 h at 40 °C under gentle agitation in a water-bath orbital shaker at 60 rpm, with or without MEP addition (2 mL/kg). After 6 h, the water-soluble fraction (WSF) was recovered by centrifugation at 11800*g* for 20 min in a Sigma 6K10 centrifuge (Osterode am Harz, Germany). The WSF was placed for 10 min in a boiling water bath to deactivate enzymes, and then centrifuged (11800*g*, 20 min). The supernatant was then precipitated at 4 °C overnight with 5 vols of 96% ethanol to get a final concentration of 80% ethanol. Ethanol-insoluble fraction **(EIF)** was recovered by centrifugation and successively washed with 80% ethanol, 96% ethanol, acetone and finally dried overnight in a stove at 40 °C. An EIF sample (3 g) was dissolved in Milli-Q water (150 mL)