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# Combined *endo*-β-1,4-xylanase and α-L-arabinofuranosidase increases butyrate concentration during broiler cecal fermentation of maize glucurono-arabinoxylan

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ABSTRACT

Solubilisation of prebiotic arabinoxylooligosaccharides from complex arabinoxylans in *e.g.* maize by xylanases may be increased by addition of auxiliary debranching enzymes. In this study, the hydrolysis and fermentation of maize fibre was investigated *in vitro* using a xylanase and an α-L-arabinofuranosidase. Combining the enzymes induced a higher ($P < .001$) xylose solubilisation and higher ($P < .05$) butyrate production during *in vitro* fermentation of maize fibre with cecal broiler inoculum compared to applying enzymes separately after 48 h. Subsequently, fibre degradation and fermentation was investigated in ROSS 308 broiler chickens supplemented with the enzyme combination to test the effects on gut morphology and microbiota composition along with performance. However, to address the relevance of combining the enzymes *in vivo*, further full factorial studies using individual enzymes at lower dosages are needed. Birds were fed a maize/soy based diet with 100 g/kg maize DDGS and 50 g/kg rapeseed meal. Enzymes supplementation increased ($P < .001$) body weight (+5.4%) and improved ($P < .001$) feed conversion ratio (−5.8%) after 29 days compared to control birds. Non-starch polysaccharide analysis and confocal microscopy of jejunum digesta visualised and confirmed solubilisation of the insoluble maize (glucurono)arabinoxylan. Birds receiving enzyme supplementation had increased ($P < .001$) duodenum villi length (+120 μm) and reduced ($P < .002$) CD3 T-cell infiltration (−22.1%) after 29 days. Cecal butyrate levels were increased ($P < .05$) compared to controls. Although the microbiota composition was not significantly altered, numerical increases in cecal *Ruminococcaceae* and *Lachnospiraceae* genera were observed in birds supplemented with enzymes.

## 1. Introduction

Degradation and solubilisation of non-starch polysaccharides (NSP) by exogenous enzymes increases available substrates for microbial fermentation in the cecum (Cadogan and Choct, 2015) and thereby the total production of short chain fatty acids (SCFA). SCFA may be used as a direct energy source by broilers (Choct et al., 1996; Steenfeldt et al., 1998) and therefore the NSP fraction

*Abbreviations:* araF, arabinofuranosidase; AX, arabinoxylan; AXOS, arabinoxylo-oligosaccharide; CD3, cluster of differentiation 3; CLSM, confocal laser scanning microscopy; DDGS, dried distiller's grains with soluble; EP, enzyme protein; GAX, (glucurono)arabinoxylan; GH, glycoside hydrolase; NSP, non-starch polysaccharide; RI, refractive index; SCFA, short chain fatty acids; xyl, xylanase

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represents another potential energy reservoir to increase performance of broilers if rendered fermentable.

The NSP content in maize is around 90 g/kg of the dry matter (DM) on average (Knudsen, 2014). Approximately 64% of the NSP in maize kernels is (glucurono)arabinoxylan (GAX). Only 2% of this GAX is water-soluble (Choct, 1997). Xylanases are well-known for their ability to degrade arabinoxylan (AX) from wheat (Courtin and Delcour, 2001). Maize GAX however, has a higher degree of substitution compared to wheat AX (Knudsen, 1997). The insolubility and complexity of maize GAX structures affects susceptibility to microbial fermentation or hydrolysis by xylanases (Malunga and Beta, 2016). Maize GAX indeed is a heterogenous and highly branched structure heavily decorated with arabinose and several other substituents such as galactose, ferulic acid (Bunzel, 2010) and glucuronic acid. In addition, some of these substituents are often acylated or methylated (Agger et al., 2010). The branched structure sterically impedes xylanases from *e.g.* family 11 and 10 glycoside hydrolases (GH) to bind and cleave the β-1,4-linked xylose backbone (Akin, 2008; Ravn et al., 2016). In general, GH10 xylanases have a higher preference to degrade soluble GAX than GH11 xylanases (Pell et al., 2004; Ravn et al., 2016). Addition of auxiliary de-branching enzymes may increase the solubilisation capacity of xylanases by removing substituents present on the xylan chain, *e.g.* removal of arabinose by arabinofuranosidases (Bachmann and McCarthy, 1991), acetyl groups by acetyl xylan esterases (Selig et al., 2009) or ferulic acid by ferulic acid esterases (Topakas et al., 2005).

Arabinoxylooligosaccharides (AXOS) with a degree of polymerization < 10 are recognized as prebiotic compounds, which pass undigested through the small intestine (Broekaert et al., 2011). They are fermented in the large intestine and promote growth of beneficial bacteria (Gibson and Roberfroid, 1995), such as butyrate-producing bacteria (De Maesschalck et al., 2015; Ravn et al., 2017). AXOS generated from wheat bran AX by *endo*-1,4-β-xylanases improve performance in broilers (Courtin et al., 2008). Soluble AXOS are fermented in the hindgut of broilers into SCFA, *e.g.* acetate, propionate and butyrate (den Besten et al., 2013). Butyrate is the preferred energy source of intestinal colonocytes (Hamer et al., 2008) and the beneficial effects on intestinal gut health and morphology are well described across species (Place et al., 2005; Onrust et al., 2015). To achieve a positive intestinal biological effect of solubilised AXOS, it is necessary that the xylanases are *endo*-acting, cleaving the polymer mid-chain rather than an *exo*-acting enzyme whose action may result in high amounts of monomeric xylose which, at high level, is detrimental to broiler performance (Schutte, 1990). It is therefore necessary to apply enzymes with a suitable substrate activity, efficacy, thermo- and pH tolerance and gastric stability in order to solubilise prebiotic AXOS in the gut. One possible route to increase soluble AXOS from cereal plant cell walls is the addition of xylanases (Courtin et al., 2008; Ravn et al., 2017) along with debranching enzymes. In GAX the most common substituent is arabinose. Removing these arabinoses with arabinofuranosidases may increase accessibility for cleavage by xylanases (Biely, 2012a, 2012b).

The purpose of the present study was to investigate the effect of a combined endoxylanase and arabinofuranosidase enzyme mixture on maize glucurono-arabinoxylan breakdown and cecal microbial fermentation. The hypothesis was that the enzymes could increase butyrate production during cecal fermentation.

The enzyme combination with highest hydrolytic activity and butyrate production was then tested *in vivo* using a fibre-rich diet to support the *in vitro* data.

## 2. Materials and methods

### 2.1. Materials

Maize fibre containing 364 g/kg crude fibre, 25.5 g/kg starch, 375 g/kg protein, 98 g/kg fat and 46 g/kg ash was obtained by double de-starching and de-proteinization. A total of 100 kg milled maize grits (Semolina 011, de-germed and milled) were supplied from a local feed mill (Institute of Technology Sdr. Stenderup, Denmark) was incubated in 250 kg tap water heated to 95 °C, pH 6.2 (achieved by addition of 1 M NaOH) with 1 kg Termamyl 120 L (Novozymes, Denmark) for 4 h followed by centrifugation (5300 rpm) and particle separation by decanting. Another de-starching step was performed under same conditions and subsequently, the fibre residue was re-dissolved in 150 kg tap water, heated to 55 °C, pH 8.1 and incubated with 80 g 2.4 L FG Alcalase (Protease, Novozymes, Denmark) for 4 h and then centrifuged (5300 rpm), decanted and freeze dried yielding 2.4 kg of a fibre-rich product. The maize fibre contained 110.5 g/kg xylose. Purified mono component *endo*-β-1,4- xylanase (EC 3.2.1.8): xyl (GH11) was obtained from *B. stearothermophilus* and expressed in *B. subtilis.* Purified mono component α-L-arabinofuranosidase (EC.3.2.1.55): araF (GH62) was obtained from *Penicillium oxalicum* and expressed in *A. Oryzae*. The *endo*-β-1,4- xylanase and the α-L-arabinofuranosidase will henceforth be termed 'xyl' and 'araF'. Both enzymes were supplied from Novozymes, Denmark and are stable under gastrointestinal conditions in the stomach and small intestine (data not shown).

### 2.2. Activity assay

Xyl activity was tested in a xylan solubilisation assay used to select the best combination of enzymes to test *in vivo*. Maize fibre (400 mg) substrate (n = 4) was incubated with xyl (10 mg EP/kg) with and without araF (5 mg EP/kg). EP = enzyme protein as previously described by Ravn et al. (2016) for 3 h at 40 °C with stirring (500 rpm) in 4 mL sodium acetate (NaOAc) buffer. The xyl dosage can approximately be compared to 10 x commercials dosage and 5 x for the araF. Solubilised AX oligomers and polymers released into the supernatant were hydrolysed to monomers using 1.63 M HCl at 99 °C for 1 h and subsequently cooled and neutralized with 1.3 M NaOH. Total xylose was quantified using a kit from Megazyme (Bray, Ireland), per the manufacturer's instructions.