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Xylanase and protease increase solubilization of non-starch polysaccharides and nutrient release of corn- and wheat distillers dried grains with solubles

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ABSTRACT

The use of distiller dried grains with solubles (DDGS) as alternative to conventional animal feed for non-ruminants is challenged by the high content of non-starch polysaccharides and varying protein quality. In this study the enzymatic degradation of corn- and wheat DDGS was evaluated, in vitro, by use of four xylanases from two different glycoside hydrolase families, GH10 and GH11, along with protease and phytase. Wheat DDGS showed the highest degree of enzymatic degradation due to a lower degree of cell wall complexity compared with that of corn DDGS. For corn DDGS,the combination of xylanase and protease yielded the highest degree of enzymatic degradation, indicating close association of arabinoxylan and protein within the cell wall matrix. Collectively, the GH10xylanase degraded DDGS more efficiently than the GH11 xylanases, due to the complexity of the DDGS substrate and the substrate affinity of the GH10xylanase. The current in vitro results indicate a high potential of xylanase in combination with protease to efficiently degrade DDGS and promote nutrient release in diets for non-ruminant animals.

1. Introduction

During the past decade attention on implementing alternatives to conventional feed stuffs (e.g., corn, wheat, and soybean meal) in animal production has become more and more prevalent. The main reason is the increased demand for and consequently increased costs of conventional raw materials together with an increased availability of new low-cost raw materials, many of these being co-products from the grain processing industries. One of these co-products is distillers dried grains with solubles (DDGS), a dried co-product from the production of fuel ethanol [1,2].

As a feed ingredient, DDGS has high potential with a digestible- and metabolizable energy content similar to that of corn, along with a high content of digestible phosphorous [3,4]. In general, DDGS has greater concentrations of protein, fat, vitamins and minerals compared to the parent grain [5,6]. However, disadvantages of using DDGS in the feeding of non-ruminant animals are the 3–3.5 fold higher content of non-starch polysaccharides (NSP), compared to the parent grain [3,7]. Since non-ruminant animals have a limited capacity to utilize NSP, high inclusion levels will inevitably limit feed utilization [8,9,10]. Varying protein quality and the risk of mycotoxin contamination further challenge a high inclusion rate of DDGS in animal diets.

The NSP in DDGS originate from cell walls in the botanical grain fractions; aleurone layer, pericarp, endosperm, germ and tip cap (corn), with arabinoxylan and cellulose as the major components of the NSP fraction [7]. Arabinoxylan consists of D-xylose units joined by β-linkages and substituted with arabinose residues along the chain [11,12], including other substitutes like D-glucuronic acid and acetyl groups [13]. These substitutes together with feruloylated arabinose residues contribute to arabinoxylan cross-linking to form strong intermolecular complexes, affecting the enzymatic degradation and encapsulation of nutrients [14,15]. Xylanases belong to two main families based on their primary sequence and structure

Abbreviations: ADF, acid detergent fiber; AH, acid hydrolysis; A:X, arabinose:xylose ratio; BSA, bovine serum albumin; cDDGS, corn DDGS; DDGS, distillers dried grains with solubles; DF, dietary fiber; DM, dry matter; EE, ether extract; GH, glucoside hydrolase family; NCP, non-cellulosic polysaccharides; NDF, neutral detergent fiber; NIRS, near infrared reflectance spectroscopy; NSP, non-starch polysaccharides; PLS, partial least square; TAXI, Triticumaestivumxylanase inhibitors; UA:X, uronicacid:xylose ratio; wDDGS, wheat DDGS; XIP, xylanase-inhibiting proteins.

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[16], i.e., glycoside hydrolase family 10 and 11 (GH10, GH11). GH10 xylanases have been shown to exhibit greater catalytic versatility or broader substrate specificity than xylanases from the GH11 family. GH11 cleaves primarily unsubstituted regions of the arabinoxylan backbone, whereas GH10 is capable of cleaving in more substituted regions, hence being less hindered by the presence of substitutions [17,18].

Several studies have been conducted to investigate the effects of NSP degrading enzymes on nutrient digestibility of DDGS containing diets. However, the in vivo results are inconclusive [11,19,20], providing evidence for further investigation of the effect of NSP degrading enzymes and other enzymes on DDGS hydrolysis and nutrient release.

The current study was undertaken to investigate the enzymatic degradation of corn DDGS (cDDGS) and wheat DDGS (wDDGS), in vitro, using four different xylanases, alone and in combination with protease and phytase. It is hypothesized that the xylanases comprise different affinities toward c- and wDDGS, thus, affecting DDGS degradation differently. In addition, it is hypothesized that addition of protease and phytase may contribute to an increased degradation by disrupting the feed matrix interactions through hydrolysis of protein and phytate. The effect of enzyme treatment was evaluated by NSP degradation defined as pentosan solubilization, and nutrient release defined as protein solubilization.

2. Materials and methods

2.1. Chemicals

Phloroglucinol (1,3,5-trihydroxybenzene) and D-Xylose ($C_5H_{10}O_5$) were from Merck, and BCA Protein Assay Kit was from Thermo Fischer Scientific. All other chemicals used were of analytical grade.

2.2. Materials

The wDDGS was supplied from a European bioethanol plant and cDDGS from a North American bioethanol plant. These two samples were previously analyzed and characterized together with 136 DDGS samples by Pedersen et al. [7].

Purified endo-1,4-β-xylanases (EC 3.2.1.8); XylA (GH10), XylB (GH11), XylC (GH11) and XylD (GH11) from different microbial origins, protease (EC 3.4.21.62) of *Bacillus* origin (*syn.* Multifect P-3000, Danisco Animal Nutrition, Marlborough, UK), and purified microbial phytase (EC 3.1.3.26), were supplied by DuPont Industrial Biosciences, Denmark. The purity of the enzyme solution was confirmed using gel electrophoresis (NU-PAGE Bis/Tris precast gel, Invitrogen, Life Technologies Corp.). Thermostable α-amylase (E-BLAAM 53.7 U/mg) and amyloglucosidase (E-AMGDF 36 U/mg) were obtained from Megazyme International.

2.3. Chemical analysis and calculations

2.3.1. Near infrared reflectance spectroscopy

Ground DDGS (Retsch ZM 200 centrifugal mill fitted with a 0.5 mm sieve) was scanned from 1100 to 2498 nm using near infrared reflectance spectroscopy (NIRS) on a FOSS NIRSystems 5000 (Foss). The spectral data were predicted by Aunir (AB Agri Ltd., UK) for the composition of moisture, fat (ether extract), fat (acid hydrolysis), protein, crude fiber, ash, starch, total sugars, neutral detergent fiber (NDF) and acid detergent fiber (ADF), using the calibration available for DDGS as previously described [7].

2.3.2. Compositional analysis of NSP

Total- and soluble NSP along with their constituent sugars were determined by gas–liquid chromatography for neutral sugars and by a colorimetric method for uronic acids, basically as described earlier [21], except that 2 M of sulfuric acid for 1 h was used for the hydrolysis of the non-cellulosic polysaccharides (NCP) rather than 1 M of sulfuric acid for 2 h. Klason lignin was measured gravimetrically as the residue resistant to hydrolysis by 2 M sulfuric acid after swelling with 12 M sulfuric acid [21].

Percentage solubilization of total pentosan as

$$\frac{0.88 \times \text{measured pentose}(\mu\,\text{g/ml})}{\text{Total content of anhydrous xylose and arabinose}(\mu\,\text{g DM/ml})} \times 100$$

Percentage solubilization of total protein as

$$\frac{\text{measured protein}(\mu\,\text{g/ml})}{\text{Total content of protein}(\mu\,\text{g DM/ml})} \times 100$$

2.4. Enzyme purification and quantification

The protein content of the purified enzyme solutions were quantified using a NanoDrop 1000 spectrophotometer (Thermo Fischer Scientific Inc.) based on UV absorbance at 280 nm according to Lambert Beer's Law with extension coefficient calculated from sequence. The preparation of protease was performed just prior to incubations, by diluting the stock solution in ice-cooled MQ-water and mixed while kept on ice. One protease unit was defined as the release of 1.0 μg of phenolic compound, expressed as tyrosine equivalents, from a casein substrate per minute.

2.5. Substrate preparation

DDGS was ground and sieved (<212 μm) before mixing with 25 mM citrate buffer pH 6.0–10% w/v suspension followed by final pH-adjustment. Under constant stirring, 175 μl/well of this suspension was dispensed into 96-well plates using Biomek NX (Beckman Coulter, Inc.). Prepared substrate plates were stored at −20 °C, and thawed just prior to incubations.

For the preparation of insoluble substrate (hereon referred to as insoluble DDGS), removal of soluble NSP was performed basically as previously described [21]. Ground DDGS (<212 μm) in sodium acetate/$CaCl_2$-buffer (0.1 M/20 mM, pH 5.0) was mixed with thermostable α-amylase (E-BLAAM 53.7 U/mg) and incubated at 100 °C for 1 h with frequent mixing. Complete degradation of starch was done by incubation with amyloglucosidase (E-AMGDF 36 U/mg) for 2 h at 60 °C. After removal of starch, the soluble NSP was extracted in sodium phosphate buffer (0.2 M, pH 7.0) at 100 °C for 1 h, followed by centrifugation at 3000 rpm. The pellet was then thoroughly washed sequentially with the phosphate buffer, ethanol (85% v/v), and acetone, with centrifugation and discard of supernatant in between these washes. The sample was placed at room temperature until completely dried.

2.6. Enzymatic hydrolysis of DDGS by xylanase, protease and phytase

For each of the four xylanase treatments (0.2 g xylanase/kg feed) a full factorial 3^2 experiment was setup in duplicates with 2 factors in 3 levels; protease (0 U/kg feed, 4.3×10^5 U/kg feed, 8.6×10^5 U/kg feed) and phytase (0 μg/well, 0.1 g/kg feed, 0.2 g/kg feed). A volume of 25 μl of mixed enzyme solution was transferred to the thawed 96-well substrate plates and mixed. Then the substrate plates were sealed and placed in iEMS incubators (Thermo Scientific) at 39 °C, 1100 rpm for 4 h. After incubation, the solution was transferred to a 96-well filter plate (0.22 μm) and centrifuged at 3600 rpm, 5 °C until the retentate was completely dried. Finally, the filtrate was mixed, proper diluted and aliquots taken out for protein- and pentosan quantification.