Debranching enzymes enhance corn/soybean meal diets

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The cell wall is a highly interconnected matrix of various polysaccharides, proteins and lignin and, as such, requires a collaborative effort of different enzymes. The propensity to bond and interact with smaller molecular units allows non-starch polysaccharides (NSPs) to polymerize into highly branched and complex structures. Chemical identities and important degradation sites become disguised and blurred.

A unique group of enzymes — the debranching enzymes — targets attachments on primary NSPs in corn and other feed ingredients (Banerjee et al., 2010). Removing these attachments or bridged structures is a crucial first step in the process of effectively dismantling main-chain NSPs and releasing the entrapped nutrients (Santiago et al., 2007).

**Biomass recalcitrance**

NSPs in corn/soybean meal-based diets are notoriously recalcitrant, that is, highly resistant to degradation. The insoluble and tightly packed nature of the cell wall NSPs severely limits access by the primary NSP endo-acting xylanases, glucanases, pectinases and others during gastrointestinal passage (Chesson et al., 1997). Biomass recalcitrance refers to the limited degradability of plant cell walls. Cultivar, plant organ and stage of development influence NSP composition and complexity. Plant cell wall properties dictate a number of factors that ultimately affect degradability.

**Corn AX structure**

Arabinoxylan (AX) is the most prevalent NSP in all cereal grains, comprising 5-10% of ingredient dry weight. In corn, AX is a backbone of beta-1,4-linked xylose residues with attached arabinose units. Glucuronic acid, xylose and galactose add to the structural attachments (Rogowski et al., 2015). Hydroxycinnamic acid residues and acetyl groups attach to both the arabinose branches and the xylose backbone to make corn AX one of the most complex of all cereals (Rogowski et al., 2015; Agger et al., 2010). The heterogeneous nature of these attachments confers an insoluble and recalcitrant character to corn NSP.

**Enzymatic degradation**

The heterogeneous nature of corn AX requires two groups of enzymes (Laegert et al., 2013; Wong, 2008): (1) de-branching or exo-acting enzymes to remove sidechains and crosslinks with other plant components, and (2) endoxylanases and beta-xylanosidases to break the xylan backbone.

**Role of debranching enzymes**

Debranching enzymes consist of a heterogeneous group of exo-acting enzymes that provide greater enzyme access to substrates. Sometimes called “helper enzymes,” they primarily include glycoside

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The sidechain-degrading glycoside hydrolases attack xylans and include several different alpha-L-arabinofuranosidases. Many esterases are also known, each targeting different substrates. Acetyl xylan esterases release acetyl groups linked to the xylan backbone. On the other hand, ferulic acid esterases (FAE) — among others — detach critical acids from sidechains of xylans and deconstruct ferulic acid cross-links (Kuhnel et al., 2011).

Xylan-degrading enzymes

Xylanases are the primary xylan-degrading enzymes for cereal grains. These enzymes are categorized according to their structural similarity into glycoside hydrolase (GH) families 5, 10 and 11 and 30 of the Cazy database (van Gool et al., 2012; www.cazy.org). Common feed xylanases belong to GH families 10 and 11 and cleave glycosidic bonds that unite xylose units in the xylan chain.

GH family 10 xylanases need at least two consecutive unsubstituted xylose constituents, whereas GH family 11 xylanases require three (Goesaert et al., 2011; Biely, 1997). Yet, most studies find that eight to nine of every 10 xylose units in corn AX are occupied, which is insufficient for most xylanases. Figure 1 illustrates the highly branched nature of the main xylan chain that shields the xylan.

Sidechain substituents impede xylanase attack of AX in corn (Agger et al., 2011). Wheat AX contains fewer sidechains and was far easier to degrade than corn when several different xylanases were tested (Rose et al., 2011). As noted earlier, xylanase efficacy increases with sidechain removal.

Xylanase, debranching synergism

Debranching enzymes create sites for enzymatic assault by xylanases in corn. Particularly with corn-based diets found in North America, debranching enzymes can improve carbohydrase efficacy.

Debranching enzymes facilitated the release of arabinose (by arabinofuranosidase) from corn AX prior to the xylose liberation by xylanase (Figure 2; Husiman et al., 2000). The removal of the arabinose provides space for xylanase function and also helps improve substrate solubility. Hence, AX degradation is a process that relies heavily on the removal of attachments to precede xylan degradation.

In corn bran, debranching enzymes nearly doubled the release of xylose — a direct indication of the importance of the debranching function (Agger et al., 2010). In a more remarkable example, xylanase alone yielded less than 2% xylose and arabinose from wet-milled corn (Nghiem et al., 2011). With FAE, the yield of xylose and arabinose rose 44% and 53%, respectively, which established FAE as one of the important debranching enzymes for corn AX.

DSM has developed an enzyme composite product — Victus — in which debranching enzymes (FAE, arabinofuranosidases and acetyl xylan esterases) target corn AX, along with a similar group for soybean meal pectin. Figure 3 shows how the composite product first released arabinose from corn AX, followed by xylose, which is evidence of the synergism expected. Again, once the side attachments are removed, the degradation of the cell walls occurs more completely to release the internalized “caged” nutrients and other anti-nutritional aspects of NSPs.

Square one

An estimated 25-30% of the energy in most corn/soybean meal-based diets for poultry goes unutilized (National Research Council, 1994). This is partly attributed to the NSP entrapment of proteins, lipids and starches in cereal grains.

Corn AX is especially difficult to degrade because of the complex branching that confers recalcitrance. Debranching enzymes remove these attachments and prepare polysaccharides for the primary endo-acting carbohydrases.

References

The list of references can be obtained by emailing Nelson-E.Ward@DSM.com.