CHAPTER 23

Use of Enzymes in DDGS Diets for Poultry and Swine

Introduction

THE DEVELOPMENT AND USE OF FEED ENZYMES for use in animal feeds has been one of the most significant nutritional advances in the past 50 years (Khattak et al., 2006). The global feed enzyme market represents more than $550 million U.S. dollars and it has been estimated in saving the feed industry $3 to 5 billion annually (Adeola and Cowieson, 2011). The feed enzyme market is generally comprised of phytases (60 percent) and nonphytase (carbohydrases and proteases; 40 percent), and the use of these exogenous feed enzymes in swine and poultry diets has been one of the most researched nutritional topics for many years (Adeola and Cowieson, 2011). Phytases have been widely used as an economical and effective replacement of supplemental inorganic phosphorus sources in swine and poultry diets, and are classified as 3- or 6-phytases which is based on which phosphate they initiate catalysis on the myo-inositol nucleus of phytic acid (Adeola and Cowieson, 2011). Furthermore, about 80 percent of the global carbohydrase market is comprised of xylanases and glucanases, with lesser amounts of α-amylase, β-mannanase, α-galactosidase, and pectinases (Adeola and Cowieson, 2011). Almost all of these carbohydrases hydrolyze carbohydrate polymers, resulting in reduced molecular weight oligo- or polysaccharides but no free sugars (Adeola and Cowieson, 2011).

Supplementing exogenous enzymes in swine and poultry diets have numerous potential benefits including: reduction of digesta viscosity to enhance lipid and protein digestion; increase the metabolizable energy content of the diet; increase feed intake, growth rate and feed conversion; decreased size and alter the microbial population of the gastrointestinal tract; reduce water consumption and water content of excreta in poultry; reduce the amount of excreta as well as ammonia, nitrogen and phosphorus content (Khattak et al., 2006). However, several factors determine whether these beneficial effects are realized including: matching the specific enzyme with the appropriate target substrates in the diet; concentrations of antinutritional factors in grain-based diets; spectrum and concentrations of enzymes used; animal species, age and stage of production; characteristics of the microflora in the gastrointestinal tract; and the physiological status of the pig or bird (Khattak et al., 2006). In general, poultry tend to be more responsive to dietary enzyme supplementation than pigs, and young animals are more responsive than older animals (Khattak et al., 2006). Super-dosing (greater than 2,500 FTU/kg feed) has also become a major research topic because several studies have shown additional growth performance benefits compared with conventional does of 500 to 1,000 FTU/kg of feed for both poultry and pigs (Adeola and Cowieson, 2011). The mechanisms of these benefits may be due to greater phosphorus release to restore suboptimal calcium:digestible P; less residual phytate in the diet to serve as an antinutritional factor; and/or the generation of myo-inositol which has vitamin-like properties and lipotropic effects (Adeola and Cowieson, 2011).

Although there is a tremendous wealth of research information on various animal responses and modes of action from feeding various commercial enzymes, it has also created a tremendous challenge for nutritionists to determine the appropriate enzymes to use, optimal conditions for using enzymes and modifications in diet formulation strategies to achieve their potential benefits in swine and poultry diets. Several excellent reviews have been published to summarize the benefits and challenges of using various types of enzymes in swine (Adeola and Cowieson, 2011; de Vries et al., 2012; Kerr and Shurson, 2013; Jha and Berrocoso, 2015; Swiatkiewicz et al., 2015) and poultry (Khattak et al., 2006; Adeola and Cowieson, 2011; Slominski, 2011; de Vries et al., 2012; Ravindran, 2013; Swiatkiewicz et al., 2015; Dida, 2016). However, most of these reviews focused on enzyme responses in swine and poultry diets containing a wide variety of ingredients, and not specifically responses to diets containing DDGS. Therefore, the purpose of this review is to summarize numerous swine and poultry studies that have evaluated the repsonses of various exogenous enzymes in DDGS diets.

Fiber Characteristics of DDGS

Dietary fiber is perhaps the most poorly understood constituent of swine and poultry diets, and is generally described as a complex and highly variable component of plant-based feedstuffs (**Figure 1**, NRC, 2007). It is important to note that the analytical methods used to characterize the fiber component of animal feeds often overlap or exclude fractions of other distinctly different carbohydrate fractions in a feedstuff. As shown in **Figure 1**, common analytical methods used to measure complex carbohydrates in high fiber feed ingredients and feeds include: crude fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF), soluble and insoluble fractions of total dietary fiber (TDF), and non-starch polysaccharides (NSP). Each of these fiber methods measures several fractions of complex carbohydrates, but

they do not adequately relate to the energy value of feeds for swine. Consequently, our ability to adequately relate analytical measures of fiber to its physiological functions have been problematic. Some fiber types are more digestible than others, and although they cannot be degraded by mammalian enzymes, they can be fermented by bacteria in the hindgut (Grieshop et al., 2001). The fermentable fiber types are often called nonstarch polysaccharides (NSP). Up to 90 percent of the cell walls of plants are made up of NSPs; of which, cellulose, hemicellulose, and pectins are most abundant (Selvendran and Robertson, 1990). Other less abundant NSPs include fructans, glucomannans, galactomannans, mucilages, β-glucans, and gums. Cellulose is found in tightly bound aggregates in plants, while hemicellulose and pectins have sugar side chains that allow them to be more readily degraded during the digestive and lower gut fermetation processes. Lignin is not a polysaccharide, but is a high molecular weight polymer that is not considered a functional dietary constituent because it is indigestible by swine (Grieshop et al., 2001).

To understand the opportunities to improve energy content and nutrient digestion when using enzymes in DDGS diets, we first need to know the NSP composition of the fiber fraction in DDGS. Pedersen et al. (2014) determined the non-starch polysaccharide (NSP) profile of 47 corn and 11 wheat DDGS samples and showed that NSP's represent about 25 to 34 percent of the composition of corn DDGS samples (**Table 1**), and most of it is insoluble. This suggests that the fiber fraction in corn DDGS has limited digestibility in the small intestine, and limited fermentability in the lower gastrointestinal tracts of swine, poultry and fish. Cellulose represents about 5 to 9 percent of corn DDGS content, and the predominant non-cellulosic polysaccharides are xylose (7.7 percent) and arabinoxylose (12.3 to 17.2 percent), which are also mainly insoluble. The mannose content in corn DDGS (1.7 percent) is substantially greater than found in corn grain, and is likely due to the mannan content in residual yeast cell walls that are present in DDGS. Corn DDGS has greater arabinose (6.2 percent) and uronic acid (1.6 percent) content than wheat DDGS (5.7 and 0.8 percent, respectively), which results in relatively high arabinose to xylose and uronic acid to xylose ratios. This indicates that the fiber (heteroxylan) structure is more complex and variable in corn DDGS compared to wheat DDGS, and therefore, is more difficult to degrade with exogenous enzymes. However, the Klason lignin content, which is indigestible, in wheat DDGS was greater than in corn DDGS samples. Klason lignin is not well defined as a chemical constituent, and may contain protein (Maillard products), residual fat and waxes, and cutin in addition to true lignin. These results suggest that the concentrations of substituted xylan and souluble NSP's are altered during DDGS production from their original structure in corn grain.

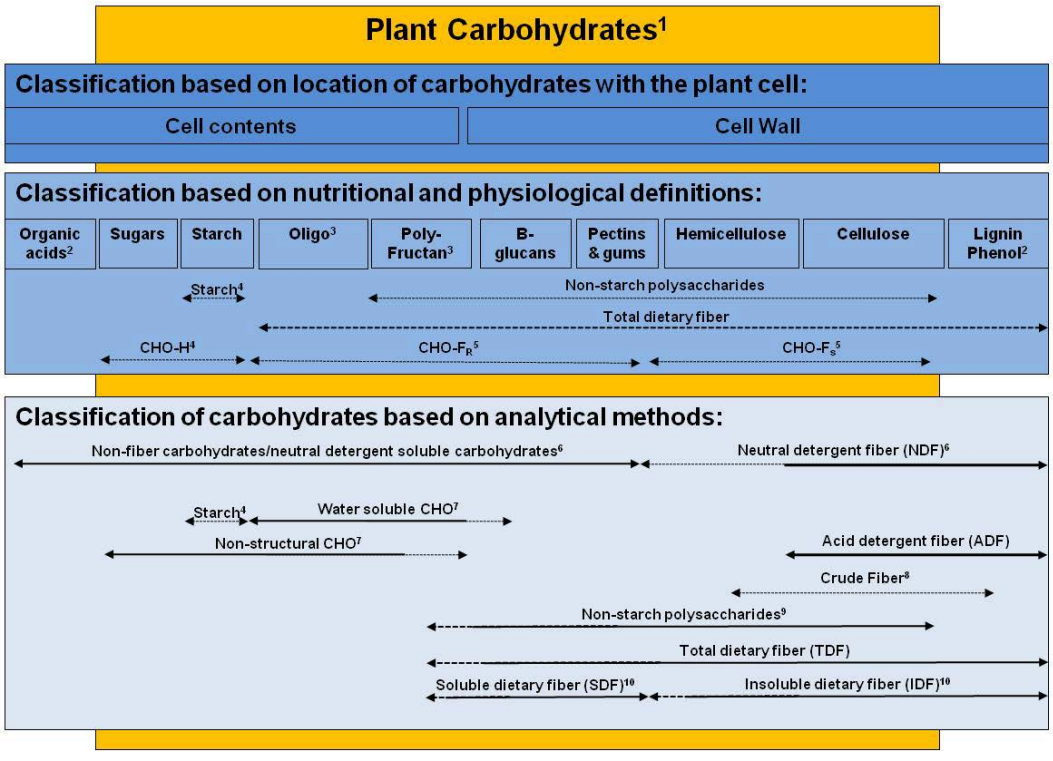

Figure 1. Nutritional and analytical classifications used to characterize plant carbohydrates (adapted from NRC, 2007).

Table 1. Average concentration (percent) and variation in the nutrient and non-starch polysaccharide (NSP) composition of 47 corn and 11 wheat DDGS samples (dry matter basis; adapted from Pedersen et al., 2014)

	Corn DDGS				Wheat DDGS			
	Mean	**Range**	**SD**	**CV %**	**Mean**	**Range**	**SD**	**CV %**
Moisture	8.7	6.5 – 12.4	0.8	10	7.6	6.8 – 8.7	2.0	2
Crude protein	31.4	27.1 – 36.4	2.1	7	33.4	30.3 – 37.9	2.8	9
Ether extract	9.1	6.5 – 11.8	1.5	17	5.2	4.4 – 6.5	0.8	16
Acid hydrolyzed ether extract	11.1	8.4 – 13.5	1.4	13	7.3	6.5 – 8.8	0.8	11
NDF	35.1	30.2 – 39.7	2.4	7	30.6	27.3 – 34.2	2.6	8
ADF	10.1	8.9 – 11.9	0.6	6	10.5	9.5 – 12.2	0.8	7
Crude fiber	7.7	6.4 – 9.5	0.6	7	6.7	5.5 – 8.8	0.9	14
Starch	6.0	2.9 – 13.9	2.7	45	4.0	<1.0 – 8.8	4.2	103
Total sugars	9.0	5.4 -12.6	1.7	19	9.8	4.6 – 12.4	2.2	23
Ash	7.1	5.4 – 9.0	0.7	9	9.1	8.1 – 10.0	0.4	5
Total NSP	28.3	25.0 - 33.7	2.0	9	26.2	24.2 – 29.1	0.9	4
Soluble NSP	3.1	1.6 - 6.5	0.8	47	6.7	5.3 – 8.0	0.1	2
Cellulose	6.7	5.2 - 9.1	0.8	16	5.0	3.5 – 6.7	1.6	32
Non-cellulosic polysaccharides								
Total xylose	7.7	6.7 - 10.0	0.7	10	8.6	7.0 – 9.3	0.7	8
Soluble xylose	0.6	0.1 - 1.6	0.3	62	2.3	1.5 – 3.2	0.5	22
Total arabinose	6.2	5.6 - 7.2	0.4	7	5.7	5.1 – 6.2	0.0	0
Soluble arabinose	0.7	0.2 - 1.5	0.3	45	1.7	1.2 – 2.2	0.3	15
Total glucose	2.8	2.1 - 4.4	0.4	13	3.3	2.7 – 3.7	0.1	5
Soluble glucose	0.3	0.0 - 1.6	0.4	190	1.1	0.1 – 2.1	1.0	89
Total mannose	1.7	1.2 - 2.0	0.2	12	1.6	1.3 – 1.8	0.2	13
Soluble mannose	0.6	0.4 - 0.9	0.1	19	0.7	0.4 – 0.8	0.1	18
Total galactose	1.5	1.3 - 2.1	0.2	11	1.1	1.0 – 1.2	0.1	11
Soluble galactose	0.3	0.2 - 0.5	0.1	29	0.6	0.4 – 0.7	0.1	18
Total uronic acids	1.6	1.4 - 2.0	0.1	8	0.8	0.7 – 0.9	0.1	12
Soluble uronic acids	0.5	0.3 - 0.6	0.1	11	0.3	0.2 – 0.4	0.0	15
Klason lignin	2.5	1.5 - 4.7	0.7	26	6.6	4.4 – 9.3	2.1	32
Arabinose:Xylose	0.80	0.71 - 0.85	0.0	5	0.66	0.62 – 0.70	0.01	9
Uronic acid:Xylose	0.20	0.16 - 0.23	0.0	8	0.09	0.08 – 0.11	0.0	21

Effects of Adding Exogenous Enzymes to DDGS Diets for Swine

The starch content of DDGS ranges from 3.8 to 11.4 percent, but it is unknown if it is resistant starch or if is digestable and contributes to metabolizable energy content (**Table 2**). Although most of the dietary fiber in DDGS is insoluble, the apparent total tract digestibility of total dietary fiber varies from 23 to 55 percent. As a result, some of the fiber in DDGS is digested and fermented to contribute significant amounts of energy when feeding DDGS to swine. This likely explains why a measure of fiber (e.g. NDF, TDF) is a significant predictor in recent equations developed to estimate the ME content of reduced-oil DDGS sources for swine (Urriola et al., 2014).

A recent review conducted by Swiatkiewicz et al. (2015) summarized the various responses from adding various enzymes to corn DDGS diets for swine and are summarized in **Table 3**. In general, the majority of these studies showed improvement in nutrient digestibility when enzymes were added to corn DDGS diets, but this benefit was not usually observed in an improvement in growth performance. Furthermore, several studies summarized in this review evaluated only phytase responses, and not combinations of phytases with carbohydrases and proteases. Several studies summarized in the Swiatkiewicz et al. (2015) review were excluded from **Table 3** because they were comparisons with wheat- or wheat-corn blends of DDGS which are not representative of responses to adding enzymes to corn DDGS diets due to different fiber and nutrient characteristics. Furthermore, several studies evaluating enzyme addition to corn DDGS diets for swine have been published since the review by Swiatkiewicz et al. (2015).

To provide a more comprehensive and detailed evaluation of the effects of adding various exogenous enzymes to corn DDGS diets for swine, a meta-analysis was conducted to summarize the overall effects of studies evaluating pig growth performance, with or without phytase, in corn-soybean meal diets (**Table 4**). Comparisons of growth performance responses from enzyme supplementation in corn-soybean meal and DDGS diets are shown in **Table 5**, apparent total tract digestibility of nutrients in DDGS diets supplemented with various enzymes are shown in **Table 6**, and apparent total tract digestibility of fiber components in DDGS diets with various enzymes is shown in **Table 7**. Data from numerous studies that evaluated the effects of adding various carbohydrases, carbohydrases + proteases, mannases, xylansases, and phytases to corn-soybean meal diets containing DDGS for swine were used in this analysis (Agyekum et al., 2016; Agyekum et al., 2012; Asmus et al., 2012; Barnes et al., 2011; de Vries et al., 2014; de Vries et al., 2013; Graham et al., 2012; Jacela et al., 2010; Jakobsen et al., 2015; Jang et al., 2017; Jones et al., 2010; Kerr et al., 2013; Kiarie et al., 2016; Kiarie et al., 2012; Koo et al., 2017; Li et al., 2012; Moran et al., 2016; Ndou et al., 2015; Passos et al., 2015; Pedersen et al., 2014; Sandberg et al., 2016; Shrestha, 2012; Swiatkiewicz et al., 2013a; Tsai et al., 2017; Widyaratne et al., 2009; Woyengo et al., 2015; Yanez et al., 2011; Yoon et al., 2010).

For all growth performance measures, the overall percentage of improvement in ADG, ADFI, and gain:feed from enzyme supplementation without or with phytase was minimal, which indicates that there are minimal benefits of adding these commercially available enzymes to corn-soybean meal diets (**Table 4**) and corn-soybean meal diets containing DDGS (**Table 5**) to justify their cost in commercial swine diets. In fact, the overall ADG and gain:feed responses, when corn-soybean meal (15 comparisons) and DDGS (12 comparisons) diets were supplemented with carbohydrases, were negative (**Table 5**). However, although the incremental improvements were small, the addition of phytase in

Table 2. Concentration of carbohydrates and apparent total tract digestibility (ATTD) of dietary fiber in corn distillers dried grains with solubles in pigs (adapted from Urriola et al., 2010)

Item	Average	Lowest value	Highest value	SD
Starch, total %	7.3	3.8	11.4	1.4
Starch, soluble %	2.6	0.5	5.0	1.2
Starch, insoluble %	4.7	2.0	7.6	1.5
ADF %	9.9	7.2	17.3	1.2
NDF %	25.3	20.1	32.9	4.8
Insoluble total dietary fiber %	35.3	26.4	38.8	4.0
Soluble total dietary fiber %	6.0	2.36	8.54	2.1
Total dietary fiber %	42.1	31.2	46.3	4.9
ATTD, total dietary fiber %	43.7	23.4	55.0	10.2

Table 3. Summary of responses to adding dietary enzymes to corn DDGS diets for swine (adapted from Swiatkiewicz et al., 2015)

Production Stage	DDGS %	Enzymes	Enzyme Responses	Reference
Nursery	20	Phytase	Increased phosphorus digestibility and reduced phosphorus excretion in manure	Xu et al., 2006a
Growing-finishing	20	Phytase	Increased phosphorus digestibility and reduced phosphorus excretion in manure	Xu et al., 2006b
Sows, late gestation and lactation	15	Phytase	Decreased fecal phytate phosphorus excretion; No effect on sow and litter performance	Hill et al., 2008
Growing-finishing	20	Phytase	Improved dry matter, gross energy and nitrogen digestibility	Lindemann et al., 2009
Growing-finishing	15 to 60	Xylanase, β-glucanase, mannase, cellulose, and protease	No effect on growth performance	Jacela et al., 2010
Nursery	30	Xylanase, β-glucanase, mannanase	No effect on growth performance	Jones et al., 2010
Growing-finishing	10 or 15	Mannanase	Improved growth performance and protein digestibility	Yoon et al., 2010
Growing	50	Phytase	Less improvement in phosphorus digestibility than in corn grain	Almeida and Stein, 2012
Finisher	35 to 50	Xylanase	No effect on nutrient digestibility in 35 percent DDGS, and decreased nutrient digestibility in high DDGS diets	Asmus et al., 2012
Growing-finishing	10 or 15	Xylanase, β-glucanase, phytase, protease, cellulose, and amylase	Tended to improve growth performance	Li et al., 2012
Growing-finishing	7.5 or 10	Xylanase, β-glucanase	Improved nutrient digestibility and growth performance in gilts up to 55 kg in body weight, but not in barrows	Kiarie et al., 2012
Nursery and growing-finishing	30	Multiple commercial products including xylanases, β-glucanases, proteases, and phytases	Minimal and inconsistent improvements in nutrient digestibility of some enzymes and no effect on growth performance in nursery or growing-finishing pigs	Kerr et al., 2013
Growing-finishing	15 or 20	Xylanase and β-glucanase	Tended to improve growth performance, reduced carcass backfat and increased carcass primal cut weights	Swiatkiewicz et al., 2013a
Growing-finishing	30	Xylanase and protease	Reduced odor emission in manure in pigs fed xylanase; protease improved gross energy digestibility	O'Shea et al., 2014
Growing	20	Phytase, xylanase, protease	Increased phytate degradation and improved energy and nitrogen digestibility	Passos and Kim, 2014

combination with carbohydrases, carbohydrases + proteases and xylanases appeared to slightly improve ADG and gain:feed compared with no phytase supplementation in corn-soybean meal diets (**Table 4**). These responses are in agreement with several published studies indicating that the combination of phytase and carbohydrases generally result in greater growth performance and digestibility responses than either one alone. However, the impact of dietary phytase supplementation on the digestibility of energy has not been consistent. Some studies (Adeola et al., 2004, 2006; Liao et al., 2005; Jendza et al., 2006; Beaulieu et al, 2007) have observed no impact of phytase on energy digestibility, while other studies (Brady et al., 2002; Sheltonet al., 2003; Jendza et al., 2005; Veum et al., 2006) have reported positive effects. Results from Kerr et al. (2010) suggested that if there is an effect of phytase on energy digestibility, it is relatively small in magnitude and highly variable. These disappointing growth performance improvements, or lack thereof, from feed enzyme supplementation are a result of minimal positive effects on dry matter and gross energy digestibility, and negative effects on nitrogen (crude protein) and ether extract (crude fat) digestibility (**Table 6**). These responses are further confirmed by the relatively minimal overall improvements in apparent total tract digestibility of various fiber fractions in DDGS diets (**Table 7**).

Table 4. Comparison of the percent change (relative differences) in ADG, ADFI and gain:feed from feed enzyme supplementation in corn-soybean meal diets compared with unsupplemented diets of pigs

Dietary treatment	No. comparisons	ADG % change	ADFI % change	Gain:Feed % change
Without phytase	43	+0.74	+0.43	+0.22
+ carbohydrases	15	-1.10	+0.52	-1.21
+ carbohydrases and proteases	11	+2.03	+1.07	+1.00
+ mannanase	10	+2.35	-0.37	+2.74
+ xylanase	7	+0.33	+0.33	-1.56
With phytase	30	+1.83	+0.38	+1.82
+ carbohydrases	9	-0.14	+2.92	-1.70
+ carbohydrases and proteases	6	+2.22	+1.06	+0.81
+ mannanase	1	+0.47	+0.71	-0.61
+ xylanase	14	+3.03	-1.57	+4.70

Table 5. Comparison of the percent change in ADG, ADFI and gain:feed from feed enzyme supplementation in corn-soybean meal and corn DDGS diets compared with unsupplemented control diets for pigs

Dietary treatment	No. comparisons	ADG % change	ADFI % change	Gain:Feed % change
Corn-soybean meal	43	+0.74	+0.43	+0.22
+ carbohydrases	15	-1.10	+0.52	-1.21
+ carbohydrases and proteases	11	+2.03	+1.07	+1.00
+ mannanase	10	+2.35	-0.37	+2.74
+ xylanase	7	+0.33	+0.33	-1.56
DDGS	30	+1.39	+1.10	+0.58
+ carbohydrases	12	-0.74	+1.42	-1.40
+ carbohydrases and proteases	7	+2.49	+1.92	+1.06
+ xylanase	11	+2.82	+0.24	+2.44

Table 6. Comparison of the absolute differences (percent) change of feed enzyme supplementation in corn DDGS diets compared with unsupplemented control diets on apparent total tract digestibility of nutrients

Nutritional component	No. comparisons	% change
Dry matter	15	+ 0.75
Gross energy	34	+ 0.53
Nitrogen	26	- 0.25
Ether extract	20	- 0.88
Phosphorus	24	+2.15

Table 7. Comparison of the absolute differences (percent) change of feed enzyme supplementation in corn DDGS diets compared with unsupplemented control diets on apparent total tract digestibility (ATTD) of fiber

Fiber component	No. comparisons	% change
ADF	19	- 0.77
NDF	24	+ 0.54
Total arabinoxylose	5	+1.84
Total NSP	5	+4.66
Insoluble NSP	5	+4.84

To improve the effectiveness of enzymes, and other methods of degrading the fiber structure to improve energy utilization in DDGS, a better understanding of the physical structure of fiber is needed. The primary cell wall structure in cereal grains is comprised of a skeletonof cellulosic microfibrils embedded in a matrix of hemicelluloses and smaller amounts of pectins, glycoproteins and hydroxycinnamates. Subsequently as the secondary cell wall continues to develop, p-coumaryl, coniferyl and sinapyl alcohols are co-polymerized to form mixed lignins. (Santiago et al., 2013). The addition of these mixed lignins to the cell wall structure add significant strength to fiber and resistance to degradation.

In corn, the most abundant hemicelluloses are arabinoxylans, which are comprised of a β (1→4)-d-xylan backbone with substitutions of arabinose, glucuronic acid and acetic acid. The hemicellulose is tangled with cellulose microfibrils by hydrogen bonds (**Figure 1**). These hydrogen bonds give the cell wall greater inaccessibility to degradation (Somerville et al., 2004), but also implies that the removal of arabinoxylans from the surface region of fiber by the addition of xylanases can result in exposure of cellulose microfibrils (crystalline structure), which is highly resistant to acids and enzymatic hydrolysis (Hall et al., 2010). In fact, the apparent ileal digestibility of cellulose (11.9 percent) is less than in other fiber components (37 percent), and the apparent total tract digestibility of cellulose (29.0 percent) is also less than other components of fiber (43.8 percent) in pigs fed wheat DDGS (Pedersen et al., 2015). Therefore, it is possible that the more stable cellulosic microfibrils embed or trap arabinoxylans, resulting in decreased apparent total tract digestibility of fiber and prevent xylanase from accessing its substrates.

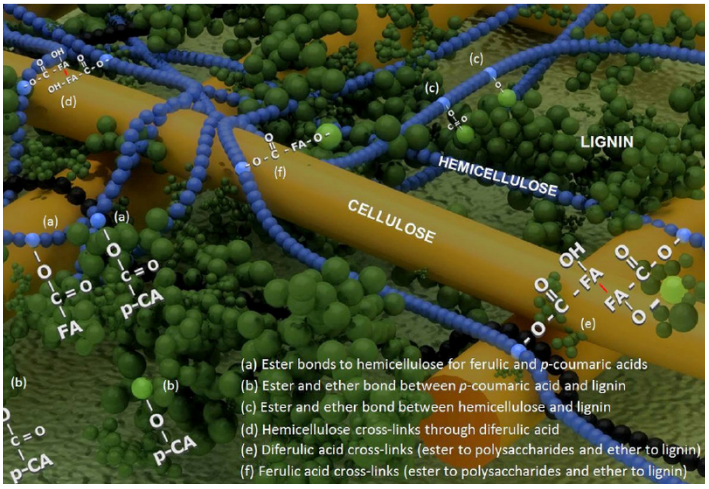

Figure 1. Representation of the secondary cell wall structure in corn (adapted from Santiago et al., 2013)

Furthrmore, understanding the changes in morphology of fiber before and after the degradation processes may be useful in identifying approaches to improve the utilization of fiber in DDGS for pigs. Results from several studies have shown that crystalline celluloses are much more resistant to enzymatic hydrolysis compared to those with low crystallinity (Fan et al., 1980; Zhang and Lynd, 2004; Hall et al., 2010). In addition, the crystallinity and crystal size of natural fiber sources have been shown to increase during thermal processing (Poletto et al., 2014). It is well known that the production of DDGS involves drying temperatures greater than 100 °C as it exits the dryer (Rosentrater et al., 2012). This indicates that the most readily degradable fiber may have already been partially degraded during DDGS production and thus, limits the effectiveness of feed enzyme or other processing technologies in high DDGS diets. In fact, Urriola et al. (2010) showed that variability of fiber digestibility varies among DDGS sources, which may be due to ethanol plants using various processing conditions.

Processing methods can be used to modify plant cell wall structure and improve NSP degradability, but use of common feed processing methods such as grinding and pelleting are inadequate for degrading NSP structures (de Vries et al., 2012). Although hydrothermal pretreatments using acid catalysts have been shown to be effective in degrading lignocellulosic material (Sun and Cheng, 2002), they can cause protein damage and increase acid or mineral content (van den Borne et al., 2012). In contrast, the use of mild acid hydro thermal treatments with maleic acid has been shown to increase solubilization of NSP in DDGS (de Vries et al., 2013). However, although acid extrusion aided in more rapid degradation of NSP, and shifted fermentation to more proximal locations in the gastrointestinal tract, more than 35 percent of NSP in DDGS were not degraded (de Vries et al., 2014). As a result, these authors suggested that enzymes and/or process technologies may be more effective if ester-linked acetyl, feroyl, or coumarol groups of the fiber structure are targeted. Cereal grains, ferulic acid, *p*-coumaric acid and sinapic acid are involved in coupling of arabinoxylans, cell wall trapped protein and lignin like polymers (Ralph et al., 1995; Bunzel et al., 2004; Piber and Koehler, 2005). Ferulic acid and derivatives are the most important cross-links in grain cell walls and are bound to arabinoxylans and pectins (Bunzel, 2010). Dimers, trimmers and oligomers of ferulic acid cross-link with two or more polysaccharide chains to strengthen the cell wall, but impair enzymatic degradation (Grabber et al., 1998a,b) leading to reduced fiber digestibility in DDGS. In fact, Pedersen et al. (2015) reported that the concentrations of ferulic acid dimers and trimmers were five to six times greater in corn DDGS than in wheat or grain blends of DDGS, indicating that the ferulic acid cross-links in the corn cell wall do not appear to be modified during fermentation and production of DDGS.

Ammonia fiber expansion (AFEX) is one alkaline pre-treatment technology that disrupts the crystalline structure of cellulose and significantly enhances enzymatic digestibility from fiber rich biomass (Mosier et al., 2005; Gao et al., 2010). In ruminants, AFEX treated forages were reported to have improved NDF digestibility when evaluated *in vitro* with rumen inoculum (Bals et al., 2010). This research group also attempted to optimize AFEX pre-treatment conditions in corn DDGS and reported that almost all cellulose in DDGS was degraded after 72 hours of enzymatic hydrolysis, and released 190 g of glucose dry biomass (Bals et al., 2006). Corn DDGS contains 5.8 percent cellulose and accounts for about 23.3 percent of total NSP (Jaworski et al., 2015). If cellulose of DDGS were hydrolyzed before entering the lower gastrointestinal tract of pigs, it may contribute about 242 kcal/kg DE (Noblet and van Milgen, 2004) to the energy value of DDGS. More importantly, the proportion of arabinoxylans imbedded in cellulose may be exposed and more accessible to degradation from exogenous enzymes, bacteria, organic acids and their combination.

Effects of Adding Exogenous Enzymes to DDGS Diets for Poultry

The addition of feed enzymes to poultry diets have many potential benefits including reduction in digesta viscosity, enhanced digestion and absorption of nutrients, increased AME content, increased feed intake, body weight gain and feed conversion, reduced beak impaction and vent plugging, decreased size of the gastrointestinal tract, alter the microbial population in the gastrointestinal tract, reduce water intake and water content of excreta, reduced excreta output and N and phosphorus excretion, and reduced ammonia emissions (Khattak et al., 2006). In general, the supplementation of carbohydrases in poultry diets containing corn DDGS have been more effective than in pig diets containing DDGS.

A recent review conducted by Swiatkiewicz et al. (2015) summarized the various responses from adding different feed enzymes to corn DDGS diets for poultry, and these results are summarized in **Table 8.** While the majority of these studies showed some benefit of enzyme supplementation in DDGS diets for broilers and layers for at least a few of the response criteria measured, results were inconsistent.

Table 8. Summary of responses to adding dietary enzymes to corn DDGS diets for poultry (adapted from Swiatkiewicz et al., 2015)

Production Stage	DDGS %	Enzymes	Enzyme Responses	Reference
Broilers, 8–21 days of age	30 or 40	Phytase	Improved phosphorus bioavailability but no consistent effects on ME content and amino acid digestibility	Martinez-Amezcua et al., 2006
Broilers, 1–21 days of age	10	Carbohydrases, protease, phytase	Phytase improved dry matter and N digestibility but enzymes had no effect on growth performance	Olukosi et al., 2010
Broilers, 1–42 days of age	10 or 20	Xylanase	Improved feed intake, body weight gain, and dry matter, protein, and hemicellulose digestibility	Liu et al., 2011
Broilers, 18–23 days of age	30	Multi-enzyme containing xylanase, β-glucanase, mannanase, and phytase	No effect on growth performance or nutrient digestibility	Min et al., 2011
Broilers, 12–21 or 7–21 days of age	7 or 10	Multi-enzyme 1 (xylanase, amylase) Multi-enzyme 2 (xylanase, amylase, protease)	Both multi-enzymes improved ME, and Multi-enzyme 2 improved amino acid digestibility except methionine	Romero et al., 2013
Broilers, 1–42 days of age	12 (starter) or 18 (finisher)	Xylanase, phytase	Combination of enzymes improved growth performance, dry matter and organic matter digestibility, retention of Ca and P, and bone characteristics	Swiatkiewicz et al., 2014b
Layers, 26–68 weeks of age	20	Xylanase and β-glucanase	Reduced some of the negative effects of feeding DDGS on egg production during the second phase of the laying cycle	Swiatkiewicz and Korelski, 2006
Layers, 30–40 weeks of age	5, 10, 15 or 20	Multi-enzyme containing xylanase, β-glucanase, amylase, and protease	Improved egg production and lipid digestibility in 15 and 20 percent DDGS diets	Shalash et al., 2010
Layers, 40–56 weeks of age	7, 15 or 23	Multi-enzyme complex containing xylanase, β-glucanase, amylase, and protease	Improved egg production and feed conversion in 7 and 15 percent DDGS diets, but no effect on egg quality, nutrient digestibility, or hematological and biochemical blood charactersitics	Ghazalah et al., 2011
Layers, 40–52 weeks of age	15	Phytase	No effect on egg production or egg quality	Koksal et al., 2012
Layers, 28–36 weeks of age	5, 10, 15 or 20	Multi-enzyme containing protease, pentosanase, pectinase, cellulose, β-glucanase, amylase, and phytase	Reduced N and phosphorus concentrations in excreta but no effect on egg production or egg quality	Deniz et al., 2013a

Table 8. Summary of responses to adding dietary enzymes to corn DDGS diets for poultry (adapted from Swiatkiewicz et al., 2015)

Production Stage	DDGS %	Enzymes	Enzyme Responses	Reference
Layers, 64–72 weeks of age	10	Phytase	Improved feed intake and feed conversion, decreased phosphorus concentration in excreta, but no effect on egg production or egg quality	Deniz et al., 2013b
Layers, 20–44 weeks of age	20	Xylanase, phytase	Combination of enzymes improved egg production	Swiatkiewicz et al., 2013b
Layers, 20–44 weeks of age	10 or 15	Xylanase	Improved egg production and egg mass	Bobeck et al., 2014
Layers, 26–55 weeks of age	20	Xylanase, phyase	No effect on femur and tibia bone measurements	Swiatkiewicz et al., 2014a

To provide a more comprehensive and detailed evaluation of the effects of adding various feed enzymes to corn DDGS diets for broilers and layers, a meta-analysis was conducted. The comparison of growth performance responses from adding carbohydrases, carbohydrases and proteases, proteases and xylanases to corn-soybean meal and DDGS diets in broilers is shown in **Table 9**. Similar to responses for swine, adding carbohydrases to corn-soybean meal and corn-soybean meal-DDGS slightly reduced growth performance of broilers, but adding proteases or xylanases appear to be more effective in improving growth performance of broilers when fed DDGS diets compared with responses from feeding corn-soybean meal diets. Depending on the cost of enzymes and diet cost, these responses suggest that adding xylanases to broilers diets may result in growth performance responses great enough to justify the cost of their use. Although the magnitude of protease responses are also relatively high, limited studies have been published that have shown these effects, and caution should be used when considering the likelihood of achieving similar responses when adding commercial proteases to broiler diets. Furthermore, improvements in AME content and dry matter digestibility from adding xylanases to broiler diets containing DDGS were greater than from only supplementing carbohydrases or proteases, but data are limited (**Table 10**). Two studies showed an average of 4.5 percent improvement in protein digestibility by adding proteases to DDGS diets for broilers, and the combination of carbohydrases and proteases improved AME and dry matter digestibility in DDGS diets for broilers (**Table 10**).

Although adding the combination of carbohydrases and proteases, or xylanases, to layer diets generally result in improvements in body weight gain, feed intake and gain:feed in layers, the magnitude of improvement is less than in broiler diets but greater than in corn-soybean meal diets for layers (**Table 11**). Adding enzymes to layer diets appears to slightly improve egg production, egg weight and egg yolk color, but may negatively effect Haugh units of eggs (**Table 12**). Based on the results from this meta-analysis, nutritionists can use this summary of responses to determine if the magnitude of improvement in economically important production responses is great enough to justify the cost of adding combinations of carbohydrases and proteases, or xylanases to layer diets.

In summary, the type of carbohydrase, protease or xylanase should be considered in this evaluation to determine the likelihood of achieving these responses under commercial conditions for both broilers and layers. Nutritionists are encouraged to obtain the published references cited in these tables to learn more about the diet formulation and experimental conditions used to achieve these responses before deciding if enzyme supplementation is a wise decision in DDGS diets for broilers and layers.

Conclusions

The supplementation of various feed enzymes in swine and poultry diets containing plant-based feed ingredients for swine and poultry has been studied for many years. Growth performance responses in swine and broilers, and egg production responses in layers, have been inconsistent and are a result of properly matching the target substrates (i.e. non-starch polysaccharides, proteins and phytate) with the appropriate enzymes to degrade them and improve digestibility. In general, consistent improvements in phosphorus and nutrient digestibility have been shown from adding phytase to corn-soybean meal diets fed to swine and poultry, but not for carbohydrases, proteases and xylanases. Corn DDGS has unique chemical characteristics that prevent significant degradation from feed enzymes, but imrovements in energy, protein and fiber digestibility are generally greater for broilers than for swine and layers. However, measurable improvements in energy and nutrient digestibility from feed enzyme supplementation do not necessarily improve growth performance and egg

production. Based on the results from this meta-analysis, nutritionists can use this summary of responses to determine if the magnitude of improvement in economically important production responses is great enough to justify the cost of adding various types of feed enzymes to swine, broiler or layer diets. The type of carbohydrase, protease or xylanase should be considered in this evaluation to determine the likelihood of achieving these responses under commercial conditions. Nutritionists are encouraged to obtain the published references cited in this chapter to learn more about the diet formulation and experimental conditions used to achieve these responses before deciding if enzyme supplementation is a wise decision in DDGS diets for swine, broilers, and layers.

Table 9. Comparison of the percent change in ADG, ADFI, and Gain:Feed from feed enzyme supplementation in corn-soybean meal and corn DDGS diets compared with unsupplemented control diets for broilers

Dietary treatment	No. comparisons	Weight gain % change	Feed intake % change	Gain:Feed % change
Corn-soybean meal	16	-1.07	-0.77	0.66
+ carbohydrases	4	-6.13	-4.82	1.41
+ carbohydrases and proteases	3	0.75	-0.64	-1.31
+ protease	1	4.34	8.14	3.64
+ xylanase	8	0.11	0.10	0.65
DDGS	33	2.73	1.18	-1.97
+ carbohydrases	7	-0.70	-1.12	-0.33
+ carbohydrases and proteases	7	1.02	-1.23	-2.16
+protease	2	5.95	1.79	-3.89
+ xylanase	17	4.47	3.04	-2.33

References: Olukosi et al., 2010; Liu et al., 2011; Min et al., 2011; Barekatain et al., 2013a,b,c; Waititu et al., 2014; Campasino et al., 2015

Table 10. Comparison of the absolute differences (percent) of feed enzyme supplementation in broiler diets containing corn DDGS on apparent metabolizable energy and apparent total tract digestibility of nutrients

Enzymes	AME[1]		Dry Matter		Crude Protein		Phosphorus	
	n	%	n	%	n	%	n	%
Carbohydrases	8	+0.07	0	ND	3	-0.29	0	ND
Carbohydrases and proteases	7	+4.21	4	+2.75	5	+1.20	5	-1.82
Proteases	0	ND	2	+1.00	2	+4.50	0	ND
Xylanases	6	+5.83	8	+3.63	8	+2.50	0	ND
Overall	21	+3.09	14	+3.00	18	+1.90	5	-1.82

[1]AME = apparent metabolizable energy

[2]ND = not determined

References: Min et al., 2009; Olukosi et al., 2010; Liu et al., 2011; Min et al., 2011; Barekatain et al., 2013a; Romero et al., 2013; Waititu et al., 2014

Table 11. Comparison of the percent change in body weight (BW), ADFI, and feed conversion ratio (FCR) from feed enzyme supplementation in corn-soybean meal and corn DDGS diets compared with unsupplemented control diets for layers

Dietary treatment	No. comparisons	BW % change	ADFI % change	FCR % change
Corn-soybean meal	4	+0.07	+0.48	+0.51
+ carbohydrases and proteases	4	+0.07	+0.48	+0.51
DDGS	16	+3.52	+0.13	-1.86
+ carbohydrases	2	+1.70	-0.87	-2.64
+ carbohydrases and proteases	11	+4.34	+0.30	-1.61
+ xylanase	3	+1.66	+0.19	-2.26

References: Swiatkewicz et al., 2006; Shalash et al., 2010; Ghazalah et al., 2011; Bobeck et al., 2014

Table 12. Comparison of the absolute differences (percent) of feed enzyme supplementation in corn DDGS diets compared with unsupplemented control diets on egg production and quality

Item	No. comparisons	percent change
Egg production	26	+1.26
Egg weight	26	+ 0.33
Egg yolk color	19	+4.94
Haugh units	12	- 0.12

References: Swiatkewicz et al., 2006; Shalash et al., 2010; Ghazalah et al., 2011; Koksal et al., 2012; Deniz et al., 2013a,b; Swiatkewicz et al., 2013; Bobeck et al., 2014

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