USE OF ENZYMES IN PIG DIETS

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Introduction

Corn and soybean meal are important ingredients to the feed industry in the United States. In total, 122 million metric tons of corn and 27 million metric tons of soybean meal are consumed by the feed industry per year (AFIA, 2012). The swine industry consumes 14% of the total feed production needing 23 million tons of feed to sustain pork production (IFIF, 2012). Traditionally, between 65 and 70% of the swine production cost are related to feed cost and efforts to improve nutrient digestibility can have significant effects on pork industry profitability (National Pork Board, 2012). Corn is the main ingredient in typical swine diets, and it is also consumed for ethanol production. Consequently, its price fluctuates dramatically increasing from $3/bushel in 2008 to $7/bushel in 2013 (USDA, 2013a). Soybean meal is the main protein source in swine diets, and its price fluctuates from $10/bushel in 2008 to $14/bushel 2013 (USDA, 2013b). Price fluctuations of feed ingredients significantly affect the economy of swine production.

Yellow dent corn (IFN 4-02-861, AAFCO, 1992) is an important source of energy in swine diets, as it has 3,395 kcal/kg of metabolizable energy (ME), 62.6% of it is starch (NRC, 2012). Regarding the indigestible components corn contains 9.7% of non-starch polysaccharides (NSP) (Knudsen, 1997) and 0.21% of phytate P (NRC, 2012). The arabinoxylans are the main NSP accounting for 4.3% of the corn composition (Ward et al., 2008). Soybean meal (IFN 5-04-612, AAFCO, 1992) has 47.7% of crude protein (CP), and the apparent ileal digestibility (AID) of CP is 82% (NRC, 2012). Therefore it is an important source of protein in swine diets. Regarding the indigestible components, soybean meal contains 21.7% NSP (Knudsen, 1997) and 0.38% phytate P (NRC, 2012). Soybean meal also contains 3.8% raffinose and 7.3% stachyose (NRC, 2012), both considered as flatulence-producing factors (Liener et al.,
Feed enzymes aiming to degrade the indigestible components of swine diets have been studied (Pettey et al., 2002; Kim et al., 2003; Kim et al. 2006; Ji et al., 2008; Li et al., 2010; Wang et al., 2011a; Wang et al., 2011b; Jo et al., 2012, Almeida and Stein, 2012) in order to provide economic benefits to the swine industry.

Pigs do not produce digestive enzymes to degrade NSP (Hartman et al., 1961; Lindemann et al., 1986; Huguet et al., 2006). The presence of NSP can limit the digestibility of nutrients (Moeser et al., 2002; Urriola and Stein, 2010). Even though NSP can be fermented to generate volatile fatty acids as a source of energy in the large intestine the contribution of volatile fatty acids as a source of energy is limited to 18% of the digestible energy (Anguita et al., 2006; Gutierrez et al., 2013). Therefore, feed enzymes targeting polymeric carbohydrates are being studied in swine nutrition (Cozanet et al., 2012; Nortey et al., 2007; Woyengo et al., 2008). It is estimated that the total non-phytase enzymes market accounts for to 40% of the total enzyme market (Adeola and Cowieson, 2011). There is growing interest in using supplemental enzymes to degrade NSP in order to mitigate their negative effect on nutrient digestibility (Choct and Annison, 1992, Choct et al., 2010). Arabinoyxylans are the main NSP in corn (Ward et al., 2008; Knudsen, 1997) and a typical corn-soybean meal based diets has 3% of arabinoyxylans. The negative effect of arabinoyxylans on nutrient digestibility was previously described (Choct and Annison, 1992). Soybean meal contains 3.8% raffinose and 7.3% stachyose (NRC, 2012) and their anti-nutritional effect on swine nutrition was described (Kim and Baker, 2003; van Kempen et al. 2006; Choct et al., 2010).

Phytate P degradation by microorganisms in the large intestine (Schlemmer et al., 2001) does not enable pigs to utilize P from phytate. It is estimated that a typical swine diet contains 0.24% of phytate P which represents by 67% of the total amount of phosphorus provided by a typical corn-soybean meal based diet respectively. Considering that pigs need between 0.18 and 0.36% of ATTD P (NRC 2012), the intestinal absorption of P from phytate P can contribute as a significant nutritional source of P to pigs. Therefore, there are several studies about phytase improving P digestibility in pigs (Almeida and Stein,
Recent reports observed that protease supplementation could improve protein and amino acid digestibility (Guggenbuhl et al., 2012; Mc Alpine et al., 2012b). These data indicated that there is opportunity to augment digestion of protein by supplementing exogenous protease.

This literature review will focus on the undigested substrates in feedstuffs and primary enzymes available for feed supplementation. The objective is to analyze the available information regarding mode of action and nutrient digestibility.

**Non-starch polysaccharides and phytate**

**Xylans**

The NSP increases intestinal endogenous losses of nitrogen (Grala et al., 1998) and affect intestinal morphology (Montagne et al., 2005; Willamil et al., 2012). It also increases viscosity of digesta (Choct and Annison, 1992) which can be related to low nutrient digestibility (Choct et al., 1999).

NSPs of corn are mainly composed of D-xylopyranose (xylose) and corn contains 9.7% of NSPs (Knudsen, 1997). Xylan structure (Figure 1) is composed of 1,4-β-linked D-xylopyranose and corn contains 3.0% of xylose (Knudsen, 1997). Arabinoxylans are composed of a xylan back bone with L-arabinose attached to xylose units (Figure 1) and corn contains 4.3% of arabinoxylans (Ward et al., 2008). Arabinoxylans are present in the endosperm and pericarp tissues of the grain (Ebringerova and Heinze, 2000). The arabinoxylan of corn is characterized to be branched with L-arabinose, glucoronic acid (Huisman et al., 2000), and ferulates (Grabber et al., 1998). Under low pH, similar to that in the stomach, L-arabinose can be partially released from arabinoxylans (Zhang et al., 2003; Craeyveld et al., 2009). Soybean meal contains xylose as xyloglucans and soybean contain xylose as xylans in its hulls (Karr-Lilienthal et al., 2005). Consequently, corn is the main source of xylans in a corn-soybean meal based diet. The non-ruminant animals do not produce enzymes to degrade the arabinoxylan, therefore
degradation of the arabinoxylans in the cell wall would enable digestive enzymes to digest the nutrients inside the cell wall (Tervila-Wilo et al., 1996; Masey O’Neil et al., 2014).

Arabinoxylans increases viscosity of digesta (Choct and Annison, 1992) which can be related to low nutrient digestibility (Choct et al., 1999). However, comparing different ingredients, corn and soybean meal yield less viscous solutions than rye, barley, oats, and wheat (Mathlouthi et al., 2002). The differences on viscosity are related to amount of water extractable arabinoxylan and other NSPs (Mathlouthi et al., 2002). The effect of viscosity on nutrient digestibility of pigs is controversial. Owusu-Asiedu et al. (2006) indicate that by adding 7% of guar gum or cellulose to the diet yielded greater viscosity of ileal digesta, slowed the passage rate of digesta through the small intestine, and decreased apparent total tract digestibility (ATTD) of protein and digestible energy. However, Hooda et al. (2011) observed that high viscous and low fermentable fiber can reduce passage rate and increase AID of dry matter, energy, and protein. Studying different sources of DDGS (wheat DDGS, corn DDGS, and wheat corn DDGS) to the swine diets Yang et al. (2010) observed that wheat corn DDGS yielded the highest digesta viscosity and also the highest AID of amino acids compared to the other sources. The nutrient digestibility of pigs seems to be determined by other factors such as digesta passage rate and intestine bacterial activity (Bartelt et al., 2002). Thus the mechanism of feed enzymes that involves viscosity problems might not be always important in pigs. Consequently, the main benefits of degradation of arabinoxylan in the cell wall is related to access of digestive enzymes to nutrients inside the cell wall (Tervila-Wilo et al., 1996; Masey O’Neil et al., 2014).

**Galactosides and galactomannans**

Soybean meal contains 4.1% of galactose (Knudsen, 1997), 3.8% of raffinose, 7.3% of stachyose (NRC, 2012). It also contains mannose as 1% of β-mannans (Hsiao et al., 2006). Corn contains little amount of galactose (0.5%), raffinose (0.2%), stachyose (0.1%), and mannose (0.3%) compared to soybean meal (Knudsen, 1997). Due to lack of digestive enzymes to degrade α-1,6-galactosyl and β-1,4-mannosyl bonds, pigs cannot digest those components in the small intestine (Hartman et al., 1961;
Lindemann et al., 1986; Kim et al., 2003; Huguet et al., 2006). The indigestible galactosides from soybean meal are known as flatulence-producing factors, the microorganism in the large intestine will degrade them and produce gases such as carbon dioxide and methane (Liener et al., 1994). Reviewing the antinutritional factors of galactosides, Martinez-Villaluenga et al. (2008) mentioned about the osmotic changes that lead to diarrhea, microbial imbalance, abdominal pain, reduction of ME, and lower amino acids digestibility. Kim et al. (2003) and van Kempen et al. (2006) observed that the stachyose composition has a negative correlation with AID of dry matter and energy. Growth of pigs was also affected by galactosides and galactomannans (Kim et al., 2006). It was also reported that mannans form viscous solutions and reduced the intestinal absorption of glucose (Rainbird et al., 1984; Nunes and Malmlof, 1992) and water (Rainbird et al., 1984).

**Glucans and cellulose**

The cereal glucan is a mixed linkage 1-3, 1-4 \( \beta \)-D-glucan. The 1-3 linkage makes the structure soluble. Corn and soybeans contain negligible amount of glucans (Knudsen et al., 1997; Ko and Lin, 2004). Cellulose is a water insoluble \( \beta \)-glucan consisting of linear molecule of D-anhydroglucopyranose linked by \( \beta \)-(1-4) bond. Corn contains 2.2% of cellulose and soybean meal contain 6.2% (Knudsen et al., 1997). Oats and barley have greater content of cellulose and \( \beta \)-glucans than corn and soybean meal (Knudsen et al., 1997). Therefore, research with enzymes targeting those substrates were done mainly with barley and oats (Graham et al., 1989; Li et al., 1996; O'Shea et al., 2010; O'Shea et al., 2011; Kong and Adeola, 2012;).

**Phytate**

Phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakis phosphate) is the storage form of P in cereal grains and oil seeds (Cheryan, 1980). The corn grain will store P as phytin (phytic acid bound to Ca and Mg) mainly in the germ, but there is also phytin in the endosperm and in the hull (O’Dell et al., 1972). The soybean meal will have phytin stored together with protein (Erdman, 1979). Corn and soybean meal will
have 0.21% and 0.36% of phytate P, respectively (NRC, 2012), thus limiting the phosphorus utilization of these feedstuffs by the pigs (Schlemmer et al., 2001).

Upon dissociation, the phytic acid will leave negative charges that can bind to cations such as Ca, Zn, Cu, Fe, Mn, Mg (Maenz et al., 1999). Phytate can also bind to protein (Rajendran and Prakash, 1993; Kies et al., 2006), and a high phytate diet decreases the absorption of amino acids (Liao et al., 2005). There is evidence that phytate interacts with fats, forming complexes of Ca, lipids, and phytate (Cosgrove, 1966), which have a negative effect on AID of energy (Liao et al., 2005).

**Feed enzymes**

**Xylanase**

The enzyme endo-1,4-β-xylanase (xylanase) carries the enzyme commission identifier 3.2.1.8 and catalyzes the endohydrolysis of (1→4)-β-D-xylosidic linkages in xylans (International Union of Biochemistry and Molecular Biology, 1992). Xylanases are classified into the glycoside hydrolase families based on the catalytic domains, structure, and molecular mechanism (Collins et al., 2005). Xylanases utilized by the feed industry belong to the glycosidic hydrolase families 10 and 11, both have glutamate in the catalytic site (Paloheimo et al., 2011). The family 11 works exclusively on substrates containing D-xylose and family 10 can be active in other substrates such as cellulose (Collins et al., 2005). Most of xylanases are active between pH 4-6 and there are thermostable xylanases available for feed application (Paloheimo et al., 2011).
Pigs do not produce enzymes to degrade arabinoxylan, therefore one mode of action proposed for xylanases involves degradation of the arabinoxylans in the cell wall enabling endogenous enzymes to digest the nutrients inside the cell wall (Tervila-Wilo et al., 1996; Masey O’Neil et al., 2014). Choct and Annison (1992) reported that dietary arabinoxylans increased digesta viscosity and dietary supplementation of xylanase reduced the effect of arabinoxylans on viscosity. The benefit of xylanase supplementation is related to NDF degradation. Passos and Kim (2014) observed that xylanase supplementation from 0 to 1,400 LXU/kg enhanced ileal NDF digestibility of a corn-soybean meal based diet fed to growing pigs. Zanotto et al. (2010) reported that combination of xylanase and amylase improved the digestible energy and metabolizable energy of a corn by 2.8 and 2.9 % respectively, whereas supplementation of amylase was not different than corn not supplemented with enzyme. The supplementation of xylanase also improved digestibility of a wheat-based diet fed to pigs (Woyengo et
Therefore, there is evidence that xylanase can improve digestibility of feedstuffs by degrading arabinoxylans of the cell wall.

**Galactosidase and mannanase**

The enzyme α-1,6-galactosidase (galactosidase) carries the enzyme commission number, 3.2.1.22 and catalyzes the hydrolysis of terminal, non-reducing α-D-galactose residues in α-D-galactosides (International Union of Biochemistry and Molecular Biology, 1992). The enzyme endo-1,4-β-mannanase (mannanase) carries the enzyme commission number 3.2.1.78 and catalyzes the random hydrolysis of (1→4)-β-D-mannosidic linkages in mannans (International Union of Biochemistry and Molecular Biology, 1992).

The feed supplementation of enzymes targeting α-1,6-galactosides and β-galactomannans was reported in pigs. Kim et al. (2003 and 2006) studied galactosidase and mannanase in corn-soybean meal based diets fed to nursery, grower, and finishing pigs. It was reported improvements on AID of GE, lysine, threonine, and tryptophan due to enzyme supplementation. Moreover, the pigs fed diet supplemented with galactosidase and mannanase had a greater G:F ratio, and raffinose and stachyose concentration in the small intestine was reduced. Pettey et al. (2002) reported greater G:F ratio in nursery pigs fed a corn-soybean meal based diet supplemented with β-1,4-mannanase. Studying β-1,4-mannanase in corn-soybean meal-DDGS based diet fed to finisher pigs Yoon et al. (2010) observed greater ADG, ATTD of dry matter, GE, and protein.

**Glucanase**

The enzyme endo -1,4- β-glucanase (cellulase) carries the enzyme commission number 3.2.1.4 and catalyzes the endohydrolysis of (1→4)-β-D-glucosidic linkages in cellulose, and cereal β-D-glucans. The enzyme 1,3(4)- β-glucanase carries the enzyme commission number 3.2.1.6 and catalyzes the reaction endohydrolysis of (1→3)- or (1→4)-linkages in β-D-glucans. (International Union of Biochemistry and Molecular Biology, 1992). Corn and soybean meal has lower content of glucans and cellulose than barley and oats (Knudsen, 1997). Therefore most of the research with glucanase has been reported on barley and
oats (Graham et al., 1989; Li et al., 1996; O'Shea et al., 2010; O'Shea et al., 2011; Kong and Adeola, 2012;). Li et al (1996) observed that glucanase in a barley-based diet can increased digestible energy by 3% and ATTD of CP by 6%. Kong and Adeola (2012) fed a barley-corn-soybean meal based diet to growing pigs supplemented with glucanase. There was no effect of glucanase on ATTD and AID of DM, GE, and N. However, it was reported that supplementation of glucanase increase AID of starch by 1.8% in a diet formulated to contain 87% barley (Graham et al., 1989).

**Amylase**

The enzyme α-amylase (amylase) catalyzes the endohydrolysis of (1→4)-α-D-glucosidic linkages in polysaccharides containing three or more (1→4)-α-linked D-glucose units (International Union of Biochemistry and Molecular Biology, 1992). The interest in amylase supplementation relies on the digestion of the resistant starch that is not digestible by the animals (Isaksen et al., 2011). It was observed that amylase from *Bacillus amyloliquefaciens* hydrolyses amylopectin faster than pancreatic amylase (Bijttebier et al., 2010). Supplementation of amylase in corn-soybean meal based diet improved weight gain by 9% and feed conversion by 4% in poultry (Gracia et al., 2003). However, no improvement was observed on growth performance and ATTD of nutrients in growing pigs due to amylase supplementation to a corn-soybean meal based diet (Jo et al., 2012).

**Phytase**

The enzymes myo-inositol (1,2,3,4,5,6) hexakiphosphate phosphohydrolases (phytases) carry the enzyme commission identifiers 3.1.3.8 (3-phytase), 3.1.3.2 (4-phytase), 3.1.3.72 (5-phytase), or 3.1.3.26 (6-phytase). These enzymes catalyze the reaction: myo-inositol hexakiphosphate phosphohydrolases + H₂O = myo-inositol pentakiphosphate + phosphate (International Union of Biochemistry and Molecular Biology, 1992). The phytases can be classified based on the optimum pH (acid or alkaline), based on the carbon in the myo-inositol ring of phytate where the phosphorylation starts (3- phytases, 5 phytases, or 6 phytases), or based on the catalytic mechanism (histidine phytase, cysteine phytase, purple acid phytase, or β-propeler phytase). The phytases utilized by the feed industry belong to the histidine acid phytase
Their utilization in animal nutrition depends on their proteolitic resistance (Wyss et al., 1999), pH of optimum activity (Boyce and Walsh, 2006), and thermal tolerance (Garret et al., 2004).

![Diagram of dephosphorylation of myo-inositol hexakiphosphate by phytases.](adapted_from_Greiner_and_Konietzny_2011)

The dephosphorylation of myo-inositol hexakiphosphate (phytate) by phytases (Figure 1) involves sequential removal of phosphate groups (Greiner et al., 2002). The removal of P from phytate and the further P absorption in the small intestine (Jones et al., 2010; Guggenbuhl et al., 2012; Rojas and Stein, 2012) is the main reason for phytase supplementation in swine diets.

As previously mentioned, it was indicated that phytate can bind to protein (Rajendran and Prakash, 1993; Kies et al., 2006) and decrease absorption of protein and amino acids (Liao et al., 2005). But the literature regarding the benefits of phytase on amino acid digestibility is not consistent. Some studies indicated benefits due to phytase supplementation (Kemme et al., 1999; Zeng et al., 2014), while others
did not (Traylor et al., 2001; Liao et al., 2005). The inconsistent results in the literature (Adeola and Sands, 2003) could be related to the source of protein provided in the diet (Pomar et al., 2008).

As mentioned before, there is evidence that phytate interact with fats forming complexes of Ca, lipids, and phytate (Cosgrove, 1966), which have a negative impact on the AID of energy (Liao et al., 2005). Johnston et al. (2004) observed greater AID of energy and DM due to phytase supplementation to a corn-soybean meal based diet formulated with low Ca and P levels. Shelton et al. (2003) studied the effect of phytase on growth performance of pigs and observed an increase in the back fat deposition, but not on the ADG, ADFI and G:F. Selle and Ravidran, 2007 speculated that phytase can improve fat digestibility and evidence in poultry supported this hypothesis (Liu et al., 2010). However other studies concluded that dietary phytase supplementation had no effect on AID of energy (Liao et al., 2005; O’Quinn et al., 1997).

**Protease**

Protease is the general term for enzymes that degrade proteins. The majority of the proteases are classified as serine proteases because the amino acid serine is in the active site (Hedstrom, 2006). Serine proteases catalyze the hydrolysis of peptide bonds and carry the enzyme commission number 3.4.21 (International Union of Biochemistry and Molecular Biology, 1992). Effective utilization of supplemental protease in diets depends upon resistance to low pH and the ability to degrade soybean meal as it is the major protein ingredient in swine diets (Glitsø et al., 2012). Pedersen et al. (2012) studied different commercial proteases and observed that they are active between pH 5.5 and 7.0. The first use of protease in pig nutrition was reported by Cunningham and Brisson (1957) where they predigested feed ingredients with the enzymes, but no improvement of growth performance was observed. Recent studies reported supplementation of protease. Supplementation of protease to corn-soybean meal based diet improved AID of nitrogen in nursery (Guggenbuhl et al. 2012), growing (Wang et al. 2011b) and finishing pigs (Mc Alpine, 2012b). There is evidence that protease hydrolyze glycinin and β-conglycinin of soybean and improve growth performance of nursery pigs (Wang et al., 2011a).
**Conclusion and implications**

Arabinoxylans and galactosides are the main indigested components of corn and soybean meal, respectively. Therefore, supplementation of xylanase and galactosidase can potentially improve nutrient digestibility of corn-soybean meal based diets. There is enough evidence to support phytase supplementation to improve phosphorus digestibility from corn-soybean meal based diets. However, more research is necessary to clarify the phytase effect on protein and energy digestibilities. The few reports about protease supplementation indicate the potential benefits on protein digestibility. However, more research is necessary to study the amino acid digestibility in order to adjust the diet formulation according the nutritional requirement of pigs. Furthermore, little is known about combinations of enzymes and how protease could interact with other enzymes. The adequate supplementation of feed enzymes depends on the estimation of energy and protein digestibilities. Most of the studies demonstrated benefits of feed enzymes on complex diets and more information is necessary about supplementation of feed enzymes to specific feedstuffs.

**Literature cited**


