2019

# Susceptibility of fibre to exogenous carbohydrases and impact on performance in swine

John F. Patience
*Iowa State University*, jfp@iastate.edu

A. L. Petry
*Iowa State University*

# Susceptibility of fibre to exogenous carbohydrases and impact on performance in swine


## Abstract

Feed represents a very large portion of the cost of raising a pig to market; indeed, the cost of meeting the energy specifications of a diet is the largest single item in the cost of production budget. Within this context, fibre plays a significant role as it represents a substantial but poorly utilized portion of typical commercial diets. It is therefore not surprising that enzymes attract a great deal of attention as a vehicle by which fibre can be used more effectively. Interestingly the mode of action of enzymes within the diet is poorly understood. Indeed, enzymes are providing unexpected health benefits, including but not limited to reduced mortality in the grow-finish phase. In any event, enzymes improve energy and nutrient digestibility – not always translated into faster or more efficient gain – and also impact the microbiome, gut barrier function and possibly oxidative stress. Suggestions are provided for future research topics and applications.



## Keywords

pig, xylanase, β-mannanase, cellulase, β-glucanase


## Disciplines

Agriculture | Animal Sciences | Biochemical Phenomena, Metabolism, and Nutrition | Microbial Physiology

## Comments


This is a manuscript of a chapter published as Patience, J.F. and A.L. Petry. 2019. Susceptibility of fibre to exogenous carbohydrases and impact on performance in swine. Page 99-115 In: G. Gonzalez-Ortiz, M.R. Bedford, K. E. Bach Knudsen, C. Courtin and H.L. Classen, editors, The Value of Fibre. Engaging the Second Brain for Animal Nutrition. Wageningen Academic Press, Wageningen, The Netherlands. doi:10.3920/978-90-8686-893-3_5. Posted with permission

**Susceptibility of fibre to exogenous carbohydrases and impact on performance in swine**

J.F. Patience* and A.L. Petry

Department of Animal Science, Iowa State University, 201B Kildee Hall, Ames, IA 50011-1178, USA;

jfp@iastate.edu

Running title: Fibre, carbohydrases and performance in swine

## Abstract

Feed represents a very large portion of the cost of raising a pig to market; indeed, the cost of meeting the energy specifications of a diet is the largest single item in the cost of production budget. Within this context, fibre plays a significant role as it represents a substantial but poorly utilized portion of typical commercial diets. It is therefore not surprising that enzymes attract a great deal of attention as a vehicle by which fibre can be used more effectively. Interestingly the mode of action of enzymes within the diet is poorly understood. Indeed, enzymes are providing unexpected health benefits, including but not limited to reduced mortality in the grow-finish phase. In any event, enzymes improve energy and nutrient digestibility – not always translated into faster or more efficient gain – and also impact the microbiome, gut barrier function and possibly oxidative stress. Suggestions are provided for future research topics and applications.

## 5.1 Introduction

Feed represents between 55% and 75% of the total cost of pork production, depending on the capital intensity of the individual farm. Meeting the energy specification of the diet represents about 85% of the total cost of a diet formulation. It can therefore be argued that dietary energy is the single greatest expense in pork production. Furthermore, energy impacts many aspects of pig performance, including feed intake, growth rate, feed efficiency, carcass composition and even meat quality (Beaulieu *et al.,* 2009; Patience, 2017).

The information in Table 5.1 illustrates that whether one is using the digestible energy (DE), metabolisable energy (ME) or net energy (NE) system, or feeding a lower fibre or higher (primarily insoluble) fibre diet, the contribution of fibre to the pig's daily supply of energy is very, very small – between 1 and 4 percent. This could be higher –- perhaps 8% - if the diets were based on more fermentable fibre sources (Bach Knudsen and Hansen, 1991); in Table 5.1, the fibre will be relatively poorly fermented, reflecting the type of diets and ingredients utilized in North America. Nonetheless, using these diets as examples, on a weight basis, fibre represents 11 to 18% of the total diet, but at most, 4% of total energy supply; thus, the yield of energy is very poor. Clearly, there is a huge reward if the pig industry can find ways to increase the extraction of energy from the fibre component of the diet.

**Table 5.1.** Profile of energy supplied by dietary components in lower and higher fibre diets[1]

| | Nutrient Composition, % | | % of DE Contribution | | DE Contribution (kcal) | | △ |
|---|---|---|---|---|---|---|---|
| **Item, %** | Low Fibre | High Fibre | Low Fibre | High Fibre | Low Fibre | High Fibre | |
| Protein | 14 | 17 | 21 | 26 | 718 | 910 | +27% |
| Fat | 5 | 10 | 11 | 21 | 391 | 742 | +90% |
| Fibre | 9 | 18 | 2 | 4 | 62 | 135 | +118% |
| CHO | 59 | 43 | 66 | 50 | 2304 | 1764 | -23% |
| Other | 13 | 12 | - | - | - | - | |
| Total | 100 | 100 | 100 | 100 | 3473 | 3551 | |

| | Nutrient Composition, % | | % of ME Contribution | | ME Contribution (kcal) | | △ |
|---|---|---|---|---|---|---|---|
| **Item, %** | Low Fibre | High Fibre | Low Fibre | High Fibre | Low Fibre | High Fiber | |
| Protein | 14 | 17 | 19 | 23 | 640 | 792 | +24% |
| Fat | 5 | 10 | 12 | 22 | 396 | 738 | +86% |
| Fibre | 9 | 18 | 2 | 4 | 63 | 135 | +114% |
| CHO | 59 | 43 | 67 | 51 | 2277 | 1765 | -22% |
| Other | 13 | 12 | - | - | - | - | |
| Total | 100 | 100 | 100 | 100 | 3375 | 3429 | |

| | Nutrient Composition, % | | % of NE Contribution | | NE Contribution (kcal) | | △ |
|---|---|---|---|---|---|---|---|
| **Item, %** | Low Fibre | High Fibre | Low Fibre | High Fibre | Low Fibre | High Fibre | |
| Protein | 14 | 17 | 14 | 18 | 375 | 463 | +23% |
| Fat | 5 | 10 | 14 | 26 | 359 | 667 | +86% |
| Fibre | 9 | 18 | 1 | 2 | 25 | 58 | +132% |
| CHO | 59 | 43 | 71 | 54 | 1834 | 1401 | -24% |
| Other | 13 | 12 | - | - | - | - | |
| Total | 100 | 100 | 100 | 100 | 2593 | 2588 | |

[1]Low and high fibre diets were formulated to be identical in NE (2.59 Mcal/kg) as well as standardized ileal digestible (SID) lysine, methionine, threonine, and tryptophan. The low fibre diet was composed of 79% corn, 16% soybean meal and 2% fat, resulting in a diet with 50% starch, 9% neutral detergent fibre (NDF), 5% acid hydrolysed ether extract (aEE), and 3% acid detergent fibre (ADF). The high fibre diet was composed of 41% corn, 20% corn distillers dried grains with solubles (DDGS), 10% wheat middlings, 10% corn bran with solubles, 11% soybean meal and 5% added fat resulting in a diet with 33% starch, 18% NDF, 10% aEE, and 6% ADF. CHO represents starch and other carbohydrates not considered in NDF. Other = moisture + ash. Adapted from National Research Council, 2012 and Noblet *et al*., 2004).

The utilisation in pig diets of ingredients rich in fibre has wide geographical origins. Europe has been feeding fibre-rich ingredients in larger quantities and for a longer period of time than other major pork producing regions of the world. It therefore comes as no surprise that Europe has traditionally led the world in its understanding of the chemistry and physiology of dietary fibre. While western Canada has a fairly long history of feeding fibrous ingredients to pigs, the corn belt of the United States is a relative newcomer. The rapid rise in the price of corn a decade ago to more than ~USD300 per tonne, from a more typical USD120 to USD160, precipitated growing interest in alternative ingredients; the coincident expansion of the ethanol industry producing large quantities of distillers dried grains (DDGS) provided such a feedstuff.

Initially, fibre in pig diets was viewed as an antinutritional factor that impaired feed intake and lowered the digestibility of energy and nutrients in the diet (Agyekum and Nyachoti, 2017; Beaulieu *et al.,* 2009; Gutierrez *et al.,* 2016;). Indeed, Gutierrez *et al.* (2014) reported that the xylose concentration could explain about 70% of the variation in ME content among 9 different corn co-products – ingredients as diverse as dehulled, degermed corn, corn bran and corn germ meal. Across the 9 ingredients, the total non-starch polysaccharide (NSP) content ranged from 1.1% to 44.4%. Concurrently, research in human nutrition was identifying favourable health and nutrition outcomes through the use of various forms and sources of fibre (Holscher, 2017; Reynolds et al., 2019). Gradually, the view of fibre in the diet of pigs evolved as well; fibre certainly could negatively impact digestibility and certain performance outcomes, but it could also provide health and welfare advantages as well (Jha *et al.,* 2019).

In order to overcome the adverse effects of dietary fibre, enzymology emerged as a logical technology to provide benefit in terms of improved energy and nutrient utilisation and more favourable growth performance outcomes in swine (Torres-Pitarch *et al.,* 2019). Since the target of exogenous enzymes is fibre, various types of carbohydrases have been evaluated and are now widely used in commercial practice. However, some of the benefits of carbohydrase use, especially those related to animal health, were unexpected but provided meaningful financial benefit to the industry.

## 5.2 Definition of dietary fibre

The definition of dietary fibre has evolved over the past 70 years. In 1953, the Australian Hipsley defined it as cellulose plus hemicellulose plus lignin. In a letter to the editor of Lancet, Trowell broadened the definition of dietary fibre to one which has more or less survived to current time with only minor modifications: “dietary fibre is composed of the remnants of plant cells resistant to hydrolysis by …. alimentary enzymes and that it includes all indigestible polysaccharides (celluloses, hemicelluloses, oligosaccharides, pectins, gums) plus waxes and lignin” (Trowell *et al.,* 1976). The Codex Alimentarius Committee (2010) defined fibre as those carbohydrate polymers with ten or more monomeric units which are not hydrolysed by the endogenous enzymes nor absorbed in the small intestine.” The Committee actually allows polymers with as few as three sugar residues to be included, but suggests individual nations define the limit between three and ten sugars.

The above definitions could be reasonably considered “physiological” in nature, but their application in practical animal nutrition is problematic due to analytical challenges. Therefore, dietary fibre can also be defined in chemical terms, such as ‘the sum of NSP and lignin’ as proposed by Theander *et al.* (1994). However, this definition ignores resistant starch and non-digestible oligosaccharides, making it also less than ideal for practical pig diet formulation (Bach Knudsen, 2001). Each definition has its place in the overall discussion of dietary fibre as it relates to swine nutrition, but all of them are less than comprehensive. This perhaps explains further efforts to define fibre by the American Association of Cereal Chemists – International, the Food and Nutrition Board, the European Commission and the Food Standards Australia and New Zealand.

Overall, the physicochemical properties of fibre include fermentability, solubility and viscosity (Holscher, 2017); because fibres differ widely in their physicochemical characteristics, it should come as no surprise that they also differ in their physiological and nutritional impact on the pig. Thus, fibres can be classified as soluble versus insoluble, fermentable versus poorly fermented, and viscous versus non-viscous (Dikeman and Fahey, 2006). It is sometimes assumed that insolubility is predictive of fermentability; this is not always the case. The fibre in corn fits this "rule," as it is both predominantly insoluble and poorly fermented; however, soybean hulls are an important exception, because they are insoluble but also fermentable.

In swine diets, dietary fibre comes primarily from plant cell walls of cereal grains and protein sources of vegetable origin plus co-products of both. Typical co-products include soybean meal, canola meal, corn DDGS, wheat middlings, wheat bran, corn bran, corn germ meal, soybean hulls and rice hulls. Thus, the majority of fibre consists of NSP and lignin. The main NSP would include cellulose, arabinoxylans, β-glucans, xyloglucans, rhamnogalacturonans and arabinogalactans (Bach Knudsen, 2001); depending on the ingredients, diets could also contain resistant starch, fructans, and pectins. The carbohydrate polymers may contain pentoses (arabinose, xylose), hexoses (glucose, galactose, mannose), uronic acids (galacturonic acid, glucuronic acid) and 6-deoxyhexose (rhamnose, fucose) residues (BeMiller, 2010).

A detailed discussion on the chemistry and physiology of fibre is beyond the scope of this chapter. For additional information, readers are encouraged to read Chapters 1, 4 and 19 of this book as well as other reviews such as Bach Knudsen (2001), Cummings and Stephen (2007) and Lunn and Buttriss (2007). A description of the fibre components of common feed ingredients in swine is presented in Table 2.

## 5.3 Carbohydrases and their dietary targets

The utilisation of exogenous enzymes in animal nutrition has been studied for many decades, and in recent years, has advanced in sophistication in terms of production and application. Overall, their role is to enhance utilisation of otherwise poorly digested components of the diet, notably but not exclusively NSP and phytate. Research is actively pursuing such topics as heat stability to survive the pelleting process, specificity of activity to achieve more predictive outcomes, assay methods to improve quality assurance and new enzyme development to expand market function and penetration (Li *et al.,* 2012)

At the present time, close to 4,000 enzymes have been identified, and of these, about 200 of microbial origin are used commercially. It should be noted, however, that only about 20 enzymes are produced on an industrial scale and are therefore available for commercial application. There are 12 major enzyme producers in the world, and around 400 minor producers (Habte-Tsion *et al.,* 2018). In 2011, almost 90% of total world production was controlled by only 3 companies (Li *et al.,* 2012).

.

**Table 5.2.** Carbohydrate composition of common grain sources and fibrous ingredients used in young pig diets (DM basis, g/kg)

| Item[1] | Corn | Sorghum | Rye | Wheat | Wheat bran | Wheat middlings | Hulled Barley | Hulless Barley | Hulled Oat | Hulless Oat | Corn DDGS | Sugar beet pulp |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Starch | 620 | 690 | 613 | 618 | 169 | 168 | 587 | 645 | 468 | 557 | 60 | - |
| Cellulose | 17 | 15 | 15 | 13 | 64 | 67 | 39 | 10 | 82 | 14 | 67 | 203 |
| NDF | 118 | 137 | 118 | 101 | 359 | 389 | 203 | 140 | 281 | 123 | 351 | 499 |
| ADF | 54 | 51 | 40 | 32 | 122 | 67 | 64 | 24 | 152 | 41 | 101 | 261 |
| NCP | | | | | | | | | | | | |
| Soluble | 25 | 4 | 42 | 19 | 38 | 12 | 56 | 50 | 40 | 54 | 30 | 290 |
| Insoluble | 38 | 47 | 94 | 62 | 243 | 227 | 88 | 64 | 110 | 49 | 185 | 207 |
| Arabinose | 17 | 17 | 36 | 23 | 77 | 72 | 28 | 20 | 18 | 13 | 62 | 201 |
| Xylose | 21 | 13 | 61 | 36 | 144 | 116 | 56 | 28 | 80 | 23 | 77 | 14 |
| Mannose | 2 | 1 | 5 | 2 | 5 | 3 | 4 | 4 | 3 | 3 | 17 | 11 |
| Galactose | 8 | 3 | 5 | 3 | 8 | 7 | 3 | 3 | 7 | 4 | 15 | 55 |
| Glucose | 6 | 10 | 26 | 11 | 34 | 25 | 47 | 58 | 33 | 56 | 28 | 14 |
| Uronic acids | 8 | 4 | 4 | 4 | 15 | 15 | 6 | 2 | 10 | 5 | 16 | 188 |
| Total NSP | 81 | 66 | 152 | 95 | 345 | 307 | 186 | 124 | 232 | 116 | 283 | 700 |
| Lignin | 8 | 16 | 21 | 18 | 69 | 73 | 35 | 9 | 66 | 32 | 25 | 37 |
| Dietary fibre | 89 | 83 | 174 | 112 | 414 | 381 | 221 | 133 | 298 | 148 | 308 | 737 |

[1]NDF = neutral detergent fibre; ADF = acid detergent fibre; NCP = non-cellulosic polysaccharides; NSP = non-starch polysaccharides; total NSP = cellulose + NCP; dietary fibre = total NSP + lignin.
Source: Li (2018)

There are many carbohydrases currently used in animal nutrition. The most common are xylanase, β-glucanase, mannanase and cellulase. Amylase is frequently used in poultry diets to enhance starch digestion but is infrequently added to swine diets. There are many other carbohydrases available, but they are not routinely used in swine diets; they include maltase, pectinase, galactosidase and lactase.

### 5.3.1 Xylanase

The primary target for xylanase (EC 3.2.1.8), also known as endo-1,4-β-xylanase, is arabinoxylan, a key component of hemicellulose. However, arabinoxylan is a complex structure with numerous forms. Success in breaking it down would require an array of enzymes possessing different targets *in vivo* (Coral *et al.,* 2006). Another form of xylanase, referred to as β-1,4-xylosidase (EC 3.2.1.37), also attacks the primary main chain structure. Other enzymes, including α-L-arabinofuranosidases and α-D-glucuronidases, target the side chains of arabinoxylan.

Xylanase is frequently used in diets based on corn, wheat and rye, and their related co-products. The arabinoxylan content of common feed ingredients varies by a factor greater than 5, ranging from a high of 221 g/kg in corn bran to a low of about 45 g/kg in corn (Huntley, 2018). Both barley (84 g/kg) and wheat (73 g/kg) contain about a third more arabinoxylans than corn.

Xylanases can be produced from numerous organisms, including *Aspergillus oryzae, Aspergillus aculeatus, Humicola insolens, Trichoderma longibrachiatum, Bacillus subtilis, Penicillium funiculosum, Mycothermus thermophiloides* and *Thermomyces lanuginosus* (Li *et al.,* 2012). Because they differ in their origin, xylanases may also vary in their substrate specificity. For example, Choct *et al.* (2004) compared xylanases from three different sources - *Thermomyces lanuginosus*, *Humicola insolens* and *Aspergillus aculeatus* - and reported quite different functionality, including their affinity for soluble versus insoluble arabinoxylans. Interestingly, these functional differences did not result in differences in the birds' growth responses.

### 5.3.2 Beta-glucanase

The primary target for β-glucanase (EC 3.2.1.6), also known as endo-1-3(4)-β-glucanase, is β-glucans, found in greater quantities in oats and barley than in wheat. They are also abundant cell wall constituents of sugar cane and hulless barley. These complex structures consist of glucosyl residues linked by β-1,3 or β-1,4 glycosidic bonds, in a typical ratio of 1:2 (Keitel *et al.,* 1994; Qiao *et al.,* 2009). Beta-glucanases exist in at least 4 types; in addition to the previously mentioned and most common β-1,3(4)-glucanase (EC 3.2.1.6), there is also β-1,3-1,4-glucanase (lichenase, EC 3.2.1.73), β-1,4-glucanase (cellulase, EC 3.2.1.4) and β-1,3-glucanase (laminarinase, EC 3.2.1.39).

Like xylanase, β-glucanase can be produced from multiple organisms, including *Aspergillus niger, Bacillus subtilis, Bacillus brevis, Bacillus licheniformis, Bacillus circulans, Trichoderma longibrachiatum, Mycothermus thermophiloides* and *Penicillium funiculosum* (Furtado *et al.,* 2011; Li et al., 2012).

### 5.3.3 Beta-mannanase

The target of endo-1,4-β-mannanase (EC 3.2.1.78) is β-mannans, which consist of repeating β-1,4-linked mannose residues; by cleaving these bonds, β-mannanases produce β-1,4-manno-oligosaccharides. Beta-mannans are linear in structure but also possess β-1,6 linkages with galactose or glucose.

Galactomannans are found most frequently in legumes such as soybeans. However, the richest sources of β-mannans are palm kernel meal (367 g/kg) and copra meal (250 g/kg); this compares to only 13 g/kg in dehulled soybean meal or 21 g/kg in non-dehulled soybean meal. Wheat (0.9 g/kg) and corn (0.8 g/kg) contain very small quantities of β-mannans (Huntley, 2018). In any event, mannans represent the second most abundant hemicellulosic polysaccharide.

One of the justifications for using β-mannanase in animal feed is the β-mannans which are believed to possess a molecular pattern which is similar to immuno-stimulatory carbohydrate forms on the surface of pathogenic bacteria. It was suggested that the β-mannans would initiate an immune response, sometimes referred to as a Feed Induced Immune Response (FIIR; Huntley *et al.,* 2018). Cleaving these bonds was believed to reduce these false immune signals, thus reducing energy and other nutrients directed to an unnecessary and unproductive immune response. Given the high energetic cost of a stimulated immune system, it was believed that a favourable outcome in terms of reduced maintenance energy could be achieved. This theory has not been supported by recent experimental outcomes in pigs. Using indirect calorimetry to quantify fasting heat production and maintenance energy requirements, Huntley *et al.* (2018) showed no effect of β-mannanase supplementation in pigs fed diets based on corn and soybean meal with 10% soybean hull and containing 1.33% β-mannan. A subsequent growth trial using nursery-aged pigs also failed to show a benefit from feeding β-mannanase (Huntley, 2018). It is entirely possible that the hypothesis of a FIIR is correct, as it has been supported in poultry, but perhaps has not yet been applied correctly in swine.

Beta-mannanase is typically produced from *Bacillus lentus* (Li *et al*., 2012) and *Mycothermus thermophiloides*.

### 5.3.4 Cellulase

Cellulase (EC 3.2.1.4), more appropriately called endoglucanase, is one of three enzymes required to function synergistically to depolymerize cellulose; the other two are exoglucanase (EC 3.2.1.176; EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21; Juturu and Wu, 2014). Cellulose is a water-insoluble polymer consisting of repeated β-D-glucopyranose joined by β-1,4-glycosidic linkages to form microfibrils which in turn combine to form macrofibrils. Cellulases cleave these β-1,4-glycosidic linkages to produce glucose, cellobiose and cello-oligosaccharides (Juturu and Wu, 2014).

## 5.4 Possible mode of action of carbohydrases

It is not clear exactly how carbohydrases exert their influence in the gastrointestinal tract (GIT) of the pig to elicit desired phenotypic outcomes. Improvements in nutrient and energy digestibility logically evolves from the degradation of fibre in the diet. However, more recent observations that enzymes alter gut structure and function, modify the microbiome and possibly impact oxidative stress are more difficult to explain. Below is a list and brief discussion of a number of proposed modes of action of exogenous enzymes. The actual effect of carbohydrases will be discussed in more detail later in this chapter.

1. Eliminates nutrient encapsulation effect, thus increasing energy and nutrient availability (deLange *et al.,* 2010).

   A considerable portion of the energy and nutrients supplied by grains and plant protein sources are encapsulated within a fibre matrix. It is believed that one of the potential modes of action of carbohydrases is to degrade the fibre structure, thus releasing these otherwise unavailable nutrients and energy.

2. Reduces loss of nutrients to fermentation

   Fermentation is an inefficient process. If carbohydrases are able to hydrolyse sugars for absorption into the bloodstream, thus avoiding fermentation, a net increase in energy available to the pig will occur. By the same token, if amino acids can be absorbed as such rather than being fermented, there will be substantial benefit to the pig.

3. Improves utilisation by releasing monosaccharides from polysaccharide chains

   There is controversy as to the extent to which polysaccharide chains can be hydrolysed to the level of individual sugars in the small intestine. However, if this does occur, it is known that pentoses and obviously hexoses can be utilised by the pig, although the latter with much greater efficiency than the former (Huntley and Patience, 2018).

4. Produces beneficial polysaccharide hydrolysis products

   A mode of action more commonly studied in poultry, but plausible in swine, is the modulation of the intestinal microbiome by arabinoxylan-oligosaccharides (AXOS) or xylo-oligosaccharides (XOS), which are produced from xylanase's hydrolysis of NSPs (Morgan *et al.,* 2017; Bedford, 2018). These oligosaccharides may increase microbial diversity and shift microbial communities to the benefit of fibre-degrading species, thereby improving the ability of the large intestine to ferment fibre (Craig *et al.,* 2018; Zhang *et al.,* 2018).

5. Improves pig health by enhancing gut barrier function

   This action of carbohydrases has been demonstrated in a number of papers so far, but the exact mechanism by which it occurs has been elusive. Of particular interest is the fact that such improvements in gut barrier integrity have been associated with improvements in growth rate, an example of growth performance enhancement reflecting positive changes at the physiological level (Li *et al.,* 2018, 2019).

6. Improves gut health generally

   Whether it is changes to the gut microbiome, the physical structure of the villus/mucus interface or viscosity of the digesta, there are many ways in which carbohydrases could be affecting gut health. For example, one possibility is the suppression of the pathogenic component of the microbiome. There is a great deal of research on-going in this area, so the prospects for breakthroughs on the topic are encouraging.

7. Reduces endogenous losses associated with sloughing of epithelial cells and erosion of mucin
   One of the factors that reduces energy digestibility is losses of endogenous secretions into the faeces. Adeola and Cowieson (2011) suggest that enzymes may reverse this effect, thus improving the efficiency of digestion in the pig.
8. Reduces the impact of antinutritional factors (ANFs)
   Another possible mode of action could be the destruction of ANFs, or the rendering of them inactive in the gut (deLange *et al.,* 2010).

**5.5 Carbohydrase effects on growth performance**

There are dozens if not hundreds of published studies on the use of carbohydrases in general, and xylanase specifically, in pig diets. The following will discuss a few recent and representative papers. The impact of xylanase will, of course, depend on the available substrate in the diet. For example, in diets based on corn or corn DDGS, rate and efficiency were improved by xylanase in some studies (Lan *et al.,* 2017), only rate of gain in others (Tsai *et al.,* 2017) and no effect in still others (Jones *et al.,* 2010; Jones *et al.,* 2015). Similar observations have been reported using wheat as the basal ingredient. Owusu-Asiedu *et al*. (2010) found that a combination of xylanase and β-glucanase improved efficiency, but not rate of gain; the individual enzymes fed alone had no effect. Zijlstra *et al.* (2004) fed nursery pigs diets based on wheat and canola meal with graded levels of a blend of xylanase and β-glucanase; they reported an improvement in feed intake and feed efficiency, but only a numerical increase in average daily gain. Interestingly, the response in some cases was curvilinear, suggesting that excessive supplemental enzymes may be contraindicated. Also using diets based on wheat, Omogbenigun *et al.* (2004) compared three different enzyme preparations. All contained xylanase, β-glucanase, amylase, protease, invertase and phytase; individual treatments also included 1) cellulase, galactanase and mannanase, 2) cellulase and pectinase, or 3) cellulase, galactanase, mannanase and pectinase. This study was unusual in the number of enzymes included in each treatment, in contrast to most experiments in which one or two enzymes are compared. In any event, the approach was successful, as all three resulted in improved rate and efficiency of gain.

The above experiments all demonstrated that substrate, enzyme or enzyme combination and enzyme dose impact results. Other factors such as age of the pig and the length of the experiment could be included as well. Improvements in energy digestibility appear to occur more frequently than improvements in growth performance; it is puzzling to try to explain why improvements in digestibility are not more frequently associated with more rapid or efficient gain. The fact of the matter remains that improvements in digestibility without concomitant improvement in growth performance is of limited value to the industry (Aftab and Bedford, 2018). Thus, there is still a need to develop a more thorough understanding of carbohydrases in order to achieve more predictable and consistent outcomes, especially as it relates to growth performance.

Adeola and Cowieson (2011) completed a survey of published studies on the effect of exogenous NSP enzymes on growth performance. The most commonly used enzyme in these reports was xylanase. The authors concluded that there was no consistent effect on growth performance in swine.

Zeng *et al.* (2018) undertook a meta-analysis representing 101 studies and reported that the addition of a carbohydrase or protease to diets improved average daily gain by 2.1% in corn-based diets, 3.0% in wheat-based diets and 1.5% in barley-based diets. Feed efficiency was improved by 1.6%, 2.8% and 2.7% in diets based on corn, wheat and barley, respectively. The authors reported that in further evaluations, performance was not increased more in higher fibre co-products than in diets based on cereal grains.

Even more recently, Torres-Pitarch *et al.* (2019) reported the results of their meta-analysis of carbohydrases on growth performance in growing pigs. They identified 302 potential publications dealing with growing pigs, of which only 67 met all of their selection criteria; these papers reported a total of 139 comparisons. The most common reason for exclusion was failure to report the results of assays of the diets for enzyme activity. Of the total reports, 32% reported a positive response to enzyme use in terms of feed efficiency, 65% reported no change, and 3% reported a negative outcome. Average daily gain was improved by xylanase in corn diets and in co-product diets; mannanase improved ADG in corn diets only. Enzyme complexes appeared to be most effective, as they improved ADG in corn, wheat, barley or co-product diets. With respect to feed efficiency, mannanase in corn diets, and multi-enzyme complexes in corn, wheat, barley and co-product diets were effective. The authors concluded from their study that the response to enzyme was influenced by both the enzyme product used and the composition of the basal diet, Notably, they did not consider the effect of length of time on the enzyme nor initial body weight.

### 5.6 Carbohydrase effects on energy and nutrient digestibility

Carbohydrases and their application have improved over time such that biological responses to their use, measured in terms of improved digestibility of the diet, is increasingly frequent, if not routinely expected. For example, in a large meta-analysis conducted in 2017, Torres-Pitarch *et al.* (2019) reported that overall total tract digestibility of dry matter, crude protein and gross energy was improved by xylanase, blends of xylanase and β-glucanase and mannanase. However, one of the great mysteries of enzyme utilisation is the fact that far too frequently, xylanase improves digestibility but does not translate into either faster or more efficient growth. The reasons for this are unclear at this time.

Part of the challenge is the fact that the mode of action of carbohydrases is not yet fully elucidated, in part because there has been very little research conducted *in vivo*; the vast majority of mechanistic research on this subject has been conducted *in vitro*. The inconsistency of response to carbohydrases may be due to numerous factors. These include the nature of the dietary substrate, such as the level and structure of the NSP, the level of dietary energy and amino acids present in the diet, the source and nature of the enzyme, the age of the pig and the duration of the study (Li, 2018). As an example, Zhang *et al.* (2018) reported that xylanase C, produced from *B. subtilis*, was more effective in diets based on wheat, while xylanase A, produced from *F. verticillioides*, was more effective in corn-based diet.

Perhaps one of the greatest difficulties in quantifying the impact of carbohydrases on energy and nutrient digestibility is the very methodology used to conduct such studies; this may also explain why improvements in energy or dry matter digestibility often do not translate into faster or more efficient gain. There are a number of flaws in our methodology. The typical experiment measures the quantity of energy and nutrients ingested into the GIT and then quantifies the amount exiting in the faeces and urine. Thus, the measurement is quite crude, and can easily be confounded by 1) changes in endogenous losses which are rarely if ever measured effectively in their totality, 2) losses due to gaseous emissions which are even less frequently measured, 3) accurately collecting 100% of all faecal excretion if using the total collection method or accurately assaying the marker in the marker method, and 4) in the case of energy, accounting for the differences in utilisation of energy from difference sources. There is also the distinction between digestion and absorption; sometimes in conversation, one is considered equal to the other and this is clearly not the case. Based on the potential modes of action of enzymes described previously, digestibility studies that differentiate effects in the upper and lower gut would be warranted. For example, if energy substrates are absorbed as such in the small intestine, they will be used with greater caloric efficiency than if they are released and absorbed as the products of fermentation in the lower gut. Finally, there is the issue of experimental precision. Enzyme effects on digestibility are often in the range of 1 to 5 percent; even a 3 to 5 percent change in digestibility can sometimes be difficult to detect statistically when measured at the terminal ileum. Conversely, over the total tract, differences in the range of only 1 to 2 percent can often be detected, due to the smaller standard errors (Newman, 2014).

By the same token, positive responses to enzyme use may be observed but are artefacts of the methodology. The solution may not be easy, but slope ratio measurements of availability may be necessary, as opposed to direct measurement of digestibility. The use of indirect calorimetry could be another useful option although precision is once again a challenge in identifying small treatment effects.

### 5.7 Other effects of carbohydrases

#### 5.7.1 Reduction in digesta viscosity

There is no question that one of the important benefits of using carbohydrases in poultry diets is the reduction in digesta viscosity (Adeola and Bedford, 2004; Raza *et al.,* 2019). How important this is in swine is a topic of debate. The physiological conditions of the upper intestinal tract of the pig differs substantially from that in the bird. For example, Duarte *et al.* (2019) reported that xylanase reduced digesta viscosity in newly weaned pigs fed diets based on corn, corn DDGS and soybean meal, but provided no benefit in terms of growth performance or ileal digestibility of energy, crude protein or dry matter.

#### 5.7.2 Alterations in gut health, immune function, physiology, microbiota and antioxidant status

One of the evolving areas of investigation of exogenous enzymes relates to their overall impact on animal health, with a specific focus on both the upper and lower intestinal tract. Various studies have suggested that enzymes improve immune function, overall physiology of the gut, the nature and composition of the microbiota, the balance between pathogens and commensal bacteria and antioxidant status. Both the quantity and quality of the information is expanding at a rapid rate (Adeola and Cowieson, 2011; Kiarie *et al.,* 2013); encouraging results will no doubt spur an even greater level of activity. What is really missing in the literature at the present time is the results of studies conducted on a larger scale under commercial-like conditions to see if the more basic studies lead to practices that are successful on the farm.

One of these outcomes is reduced grow-finish mortality, first reported by Zier-Rush *et al.* (2016); xylanase added at 0.15% of the diet reduced mortality from 3.99% to 1.90% during the growth phase from 12 to 138 kg body weight. Studies able to distinguish mortality of this magnitude are rare, but numerous subsequent field comparisons, though less rigorous scientifically, have supported this conclusion. There are a number of possible explanations for this favourable impact on livability. Li *et al.* (2018) reported that an enzyme blend (cellulase, β-glucanase, xylanase) improved rate of gain and gut barrier integrity. In a subsequent experiment, the same research group studied pigs exposed to a defined F18 *Escherichia coli* challenge and again reported that a carbohydrase, in combination with a highly fermentable fibre source, improved growth performance and gut barrier integrity (Li *et al.,* 2019). These results were confirmed by Tiwari *et al.* (2018). It is therefore possible that the reduction in mortality reported above is due to improved gut barrier integrity protecting the pig from the translocation, and thus systemic exposure, to luminal pathogens or antigens.

Carbohydrases may also improve gut health through a reduction in ammonia production, enhancing volatile fatty acid (VFA) concentrations or increasing the production of lactic acid, or simply supporting the proliferation of commensal bacteria (Adeola and Cowieson, 2011). It is also possible that carbohydrases are releasing monosaccharides such as xylose, which have been shown to stimulate the growth of bifidobacteria (He *et al.,* 2010).

Another possibility is the reduction in the degree of oxidative stress to which the pig is exposed. However, data supporting this effect, while encouraging, are limited at this time.

### 5.7.3 Release of mono-sugars

Due to a lack of adequate *in vivo* data, it is not currently known to what extent carbohydrases release individual monosaccharides. The current belief is that if it does exist, it is quantitatively very limited. Most of this is based on deductive reasoning, which may or may not bear out under experimental scrutiny. Furthermore, the metabolism of individual sugars, other than glucose, is not well understood. Recently, however, some light has been shed on the metabolism of xylose. Xylose, a pentose as opposed to a hexose, has been shown to be able to supply energy to the pig albeit with an efficiency of 40 to 60%, substantially lower than that of glucose (Huntley and Patience, 2018).

## 5.8 Going forward

There is a very strong motivation to maximize the effectiveness and thus utilisation of exogenous carbohydrases in swine diets. Fibre is poorly utilised by the pig, yet represents a considerable portion of the diet. The level of fibre in the diet is likely to grow as the industry increasingly depends on co-product ingredients to manage feed costs. Any enhancement in fibre digestion will therefore contribute to the financial success and long-term viability of the pig industry.

Nonetheless, the focus of research on enzymes should go beyond the traditional digestibility model since health and survivability benefits appear to be real and reproducible – and financially rewarding. The economic impact of reducing mortality by even 1 percentage point represents an improvement in net income equal to 2 to 3 points in feed conversion.

A much better understanding of the mode of action of carbohydrases will logically lead to more effective and focused utilisation by the pig industry. Included in such studies should be the enunciation of the conditions under which enzymes are most effective and benefits are most reproducible. One particularly important question is the time required for a pig to be on a given enzyme in order to elicit a consistent response. There are data to support the conclusion that a longer exposure is necessary to achieve maximum effect, but the literature fails to define the exact length of time required – and why.

There is also a need in studies on enzymes to provide more complete information on research conditions, including the composition of the basal diet, the health and genetic background of the pigs, the design and operation of animal housing and the outcome of diet assays – especially as they relate to the enzymes being evaluated (Patience, 2016). Failure to provide such detail impairs the ability of readers to understand experimental conditions, and thus more effectively interpret outcomes in the context of the total body of knowledge as presented in the literature. In the same vein, the scope and scale of comprehensive meta-analyses are often diminished by the necessity of excluding large numbers of studies due to a lack of complete information on experimental conditions (Schweer et al., 2017; Torres-Pitarch *et al*., 2019).

With respect to achieving more predictable and reproducible outcomes, the literature seems to suggest that increasing focus should be placed on the use of enzyme blends. Their effectiveness in the literature is striking. The early days of enzyme research tended to involve enzyme blends, but that was often motivated by an inability to produce single enzymes free of contamination at a cost acceptable to the industry. This situation has obviously changed, and the current generation of feed enzymes is much more consistent in both concentration and composition. Consequently, research has followed the technology, and the majority – but not all – employed single enzyme preparations. Perhaps the time has come to expand studies to intentionally using enzyme blends to most effectively meet the needs of the pig and feed industries.

## References

Adeola, O. and Bedford, M.R., 2004. Exogenous dietary xylanase ameliorates viscosity-induced anti-nutritional effects in wheat-based diets for White Pekin ducks (*Anas platyrhynchos domesticus*). British Journal of Nutrition 92:87-94.

Adeola, O. and Cowieson, A.J., 2011. Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. Journal of Animal Science 89:3189-3218.

Aftab, U. and Bedford, M.R., 2018. The use of NSP enzymes in poultry nutrition: myths and realities. World's Poultry Science 74:277-286.

Agyekum, A.K. and Nyachoti, C.M., 2017. Nutritional and metabolic consequences of feeding high-fiber diets to swine: A review. Engineering 3:716-725.

Bach Knudsen, K.E., 2001. The nutritional significance of "dietary fibre" analysis. Animal Feed Science and Technology 90:3-20.

Bach Knudsen, K.E., and Hansen, I., 1991. Gastrointestinal implications in pigs of wheat and oat fractions 1. Digestibility and bulking properties of polysaccharides and other major constituents. British Journal of Nutrition 65:217-232.

Beaulieu, A.D., Williams, N.H. and Patience, J.F. 2009. Response to dietary digestible energy concentration in growing pigs fed cereal-grain based diets. Journal of Animal Science 87:965-976.

Bedford, M.R., 2018. The evolution and application of enzymes in the animal feed industry: the role of data interpretation. British Poultry Science 59:486-493.

BeMiller, J., 2010. Carbohydrate analysis. In: S. Nielsen (ed.) Food analysis. Springer US. p. 147-177.

Choct, M., Kocher, A., Waters, D.L.E., Pettersson, D. and Ross, G., 2004. A comparison of three xylanases on the nutritive value of two wheats for broiler chickens. British Journal of Nutrition 92:53-61.

Codex Alimentarius Committee of the Food and Agriculture Organization of the United Nations (FAO). 2010. Guidelines on nutrition labelling CAC/GL 2-1985 as last amended 2010. Available at http://www.fao.org/fao-who-codexalimentarius/thematic-areas/nutrition-labelling/en/

Corral, O.L. and Vallasenor-Ortega, F., 2006. Xylanases. In: Guevara-Gonzalez, R.G., Torres-Pacheco, I., (eds.) Advances in Agricultural and Food Biotechnology: Research Signpost. Trivandrum, India, p. 305–22.

Craig, A.D., Bedford, M.R., Hastie, P., Khattak, F. and Olukosi, O.A., 2018. The effect of carbohydrases or prebiotic oligosaccharides on growth performance, nutrient utilisation and development of small intestine and immune organs in broilers fed nutrient-adequate diets based on either wheat or barley. Journal of the Science of Food and Agriculture.

Cummings, J.H. and Stephen, A.M., 2007. Carbohydrate terminology and classification. European Journal of Clinical Nutrition 61(Suppl 1):S5-S18.

De Lange, C.F.M., Pluske, J., Gong, J. and Nyachoti, C.M., 2010. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livestock Science 134:124-132.

Dikeman, C.L. and Fahey, G.C., 2006. Viscosity as related to dietary fiber: a review. Critical Reviews in Food Science and Nutrition 46:649-663.

Duarte, M.E., Zhou, F.X., Dutra, W.M. and Kim, S.W., 2019. Dietary supplementation of xylanase and protease on growth performance, digesta viscosity, nutrient digestibility, immune and oxidative stress status, and gut health of newly weaned pigs. Animal Nutrition (In press).

Furtado, G.P., Ribeiro, L.F., Santos, C.R., Tonoli, C.C., de Souza, A.R., Oliveira, R.R., Murakami, M.T. and Ward, R.J., 2011. Biochemical and structural characterization of a -1,3–1,4-glucanase from *Bacillus subtilis* 168. Process Biochemistry 46:1202-1206.

Gutierrez, N.A., Serao, N.V.L., Kerr, B.J., Zijlstra, R.T. and Patience, J.F., 2014. Relationships among dietary fiber components and the digestibility of energy, dietary fiber, and amino acids, and energy content of 9 corn co-products fed to growing pigs. Journal of Animal Science 92:4505-4517.

Gutierrez, N.A., Serao, N.V.L. and Patience, J.F., 2016. Effects of distillers' dried grains with solubles and soybean oil on dietary lipid, fiber, and amino acid digestibility in corn-based diets fed to growing pigs. Journal of Animal Science 94:1508-1519.

Habte-Tsion, H-M., Kumar, V. and Rossi, W., 2018. Perspective of non-starch polysaccharide enzymes in nutrition. In: Nunes, C. and Kumar, V. (eds) Enzymes in human and animal nutrition. Academic Press. Cambridge, MA, p. 239-254.

He, J., Yin, J., Wang, L., Yu, B. and Chen, D., 2010. Functional characterisation of a recombinant xylanase from *Pichia pastoris* and effect of the enzyme on nutrient digestibility in weaned pigs. British Journal of Nutrition 103:1507-1513.

Hipsley, E.H., 1953. Dietary "fibre" and pregnancy toxemia. British Medical Journal, 2:420-422.

Holscher, H.D., 2017. Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes 8:172-184.

Huntley, N.F., 2018. Swine carbohydrate metabolism in relation to dietary energy partitioning. Ph.D. Dissertation, Iowa State University, Ames, IA.

Huntley, N.F., and Patience, J.F., 2018. Xylose metabolism in the pig. PLoS ONE 13:e0205913.

Huntley, N.F., Nyachoti, C.M. and Patience, J.F., 2018. Lipopolysaccharide immune stimulation but not β-mannanase supplementation affects maintenance energy requirements in young weaned pigs. Journal of Animal Science and Biotechnology 9:901-916.

Jha, R., Fouhse, J.M., Tiwari, U.P., Li, L. and Willing, B.P., 2019. Dietary fiber and intestinal health of monogastric animals. Frontiers in Veterinary Science.

Jones, C.K., Bergstrom, J.R., Tokach, M.D., DeRouchey, J.M., Goodband, R.D., Nelssen, J.L. and Dritz, S.S., 2010. Efficacy of commercial enzymes in diets containing various concentrations and sources of dried distillers grains with solubles for nursery pigs. Journal of Animal Science 88:2084-2091.

Jones, C.K., Frantz, E.L., Bingham, A.C., Bergstrom, J.R., DeRouchey, J.M. and Patience, J.F., 2015. Effects of drought-affected corn and nonstarch polysaccharide enzyme inclusion on nursery growth performance. Journal of Animal Science 93:1703-1709.

Juturu, V. and Wu, J.C., 2014. Microbial cellulases: Engineering, production and applications. Renewable and Sustainable Energy Reviews 33:188-203.

Keitel, T., Meldgaard, M. and Heinemann, U., 1994. Cation binding to a Bacillus (1,3-1,4)-β-glucanase. Geometry, affinity and effect on protein stability. European Journal of Biochemistry 222:203-214.

Kiarie, E., Romero, L.F. and Nyachoti, C.M., 2013. The role of added feed enzymes in promoting gut health in swine and poultry. Nutrition Research Reviews 26:71-88.

Lan, R., Li, T. and Kim, I., 2017. Effects of xylanase supplementation on growth performance, nutrient digestibility, blood parameters, fecal microbiota, fecal score and fecal noxious gas emission of weanling pigs fed corn-soybean meal-based diets. Animal Science Journal 88:1398-1405.

Li, S., Yanga, X., Yang, S., Zhu, M. and Wang, X., 2012. Technology prospecting on enzymes: application, marketing and engineering. Computational and Structural Biotechnology Journal 2: e201209017.

Li, Q.Y., and Patience, J.F., 2017. Factors involved in the regulation of feed and energy intake of pigs. Animal Feed Science and Technology 233:22-33.

Li, Q.Y., 2018. Impact of dietary fiber and exogenous carbohydrases in weaned pigs. Ph.D. Dissertation, Iowa State University, Ames, IA.

Li. Q.Y., Gabler, N.K., Loving, C.L., Gould S.A. and Patience, J.F., 2018. A dietary carbohydrase blend improved intestinal barrier function and growth rate in nursery pigs fed higher fiber diets. Journal of Animal Science 96:5233-5243.

Li. Q.Y., Burrough, E.R., Gabler, N.K., Loving, C.L., Sahin, O., Gould S.A. and Patience, J.F., 2019. A soluble and highly fermentable dietary fiber with carbohydrases improved gut barrier integrity markers and growth performance in ETEC challenged pigs. Journal of Animal Science 97:2139-2153.

Lunn, J. and Buttriss, J.L., 2007. Carbohydrates and dietary fibre. Nutrition Bulletin 32:21-64.

Morgan, N.K., Wallace, A., Bedford, M.R. and Choct, M., 2017. Efficiency of xylanases from families 10 and 11 in production of xylo-oligosaccharides from wheat arabinoxylans. Carbohydrate polymers 167:290-296.

National Research Council, 2012. Nutrient requirements of swine. 11th edition. National Academies Press. Washington D.C., USA.

Newman, M., 2014. Defining the energy and nutrient content of corn grown in drought-stressed conditions and determining the relationship between energy content of corn and the response of growing pigs to xylanase supplementation. M.S. Thesis. Iowa State University, Ames, IA.

Noblet, J., Seve, B., and Jondreville, C., 2004. Nutritional values for pigs. In: Sauvant, D., Perez, J., and Tran, G. (eds.) Tables of composition and nutritional value of feed materials: pigs, poultry, cattle, sheep, goats, rabbits, horses and fish. Wageningen Academic Publishers, Wageningen, The Netherlands. p.28.

Omogbenigun, F.O., Nyachoti, C.M. and Slominski, B.A., 2004. Dietary supplementation with multienzyme preparations improves nutrient utilization and growth performance in weaned pigs. Journal of Animal Science 82:1053-1061.

Patience, J.F., 2016. Designing, conducting and reporting swine and poultry nutrition research. In: Bedford, M.R., Choct, M. and Masey O'Neill, H. (eds) Nutrition Experiments in Pigs and Poultry: A Practical Guide. CABI, Wallingford, Oxfordshire, UK. p. 99-120.

Patience, J.F., 2017. Meeting energy requirements in pig nutrition. In: Wiseman, J. (eds.) Achieving Sustainable Production of Pig Meat. Volume 2: Animal Breeding, and Nutrition. Burleigh Dodds Science Publishing., Cambridge, UK. p.127-143.

Qiao, J., Dong, B., Li, Y., Zhang, B. and Cao, Y., 2009. Cloning of a β-1,3-1,4-Glucanase gene from *Bacillus subtilis* MA139 and its functional expression in *Escherichia coli*. Applied Biochemistry and Biotechnology 152:334-342.

Raza, A., Bashir, S. and Tabassum, R., 2019. An update on carbohydrases: growth performance and intestinal health in poultry. Heliyon 5:e01437.

Reynolds, A., Mann, J., Cummings, J., Winter, N., Mete, E. and Morenga, L.T., 2019. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. Lancet 393:434-445.

Schweer, W.P., Patience, J.F., Schwartz, K., Linhares, D., Rademacher, C., Allen, H., Loving, C., Ramirez, A. and Gabler, N.K., 2017. A review and evaluation of antibiotic alternatives in the literature. Journal of Animal Science 95 (Suppl 2):148.

Theander, O., Aman, P., Westerlund, E. and Graham, H., 1994. Enzymatic/chemical analysis of dietary fiber. Journal of AOAC International 99:703-709.

Tiwari, U.P., Chen, H., Kim, S.W. and Jha, R., 2018. Supplemental effect of xylanase and mannanase on nutrient digestibility and gut health of nursery pigs studied using both in vivo and in vitro models. Animal Feed Science and Technology 245:77-90.

Torres-Pitarch, A., Manzanillaac, E.G., Gardiner, G.E., O'Doherty, J.V. and Lawlor, P.G., 2019. Systematic review and meta-analysis of the effect of feed enzymes on growth and nutrient digestibility in grow-finisher pigs: Effect of enzyme type and cereal source. Animal Feed Science and Technology 251:153-165.

Trowell, H. C., Southgate, D. A. T., Wolever, T. M. S., Leeds, A.R., Gassull, M. A., and Jenkins, D. J. A., 1976. Dietary fiber redefined. Lancet 307:967.

Tsai, T., Dove, C.C., Cline, P.M., Owusu-Asiedu, A., Walsh, M.C. and Azain, M., 2017. The effect of adding xylanase or β-glucanase to diets with corn distillers dried grains with solubles (CDDGS) on growth performance and nutrient digestibility in nursery pigs. Livestock Science 197:46-52.

Zeng, Z.K., Trudeau, M., Li, Q.Y., Wang, D.J., Jang, C., Shurson, G.C., and Urriola, P.E., 2018. Effects of exogenous proteinases and carbohydrases on growth performance in pigs fed diets with corn distillers dried grains with solubles: A meta-analysis. Journal of Animal Science 97(Suppl 3):307-308.

Zhang, Z., Tun, H.M., Li, R., Gonzalez, B.J.M., Keenes, H.C., Nyachoti, C.M., Kiarie, E. and Khafipour, E., 2018. Impact of xylanases on gut microbiota of growing pigs fed corn- or wheat-based diets. Animal Nutrition 4:339-350.

Zier-Rush, C.E., Groom, C., Tillman, M., Remus, J. and Boyd, R.D., 2016. The feed enzyme xylanase improves finish pig viability and carcass feed efficiency. Journal of Animal Science 94(Suppl 2):115.

Zijlstra, R.T., Li, S., Owusu-Asiedu, A., Simmins, P.H. and Patience, J.F., 2004. Effect of carbohydrase supplementation of wheat- and canola-meal-based diets on growth performance and nutrient digestibility in group-housed weaned pigs. Canadian Journal of Animal Science 84:689-695.