EVALUATING THE EFFICACY OF XYLANASE ON BROILER PERFORMANCE AND NUTRIENT DIGESTIBILITY IN CORN-SOYBEAN MEAL DIETS

A Dissertation

by

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ABSTRACT

The objective of this research was to evaluate the influence of ingredient and diet nutrient differences on xylanase efficacy in broilers. Experiment one consisted of reduced nutrient diets with supplemental fat at two inclusion rates and the supplementation of xylanase at two inclusion rates, as well as a positive control as an industry reference diet. Dietary nutrient reductions led to increases in FCR at 49 d. Fat level had an effect on starter and grower FCR, but had no effect on growth performance beyond 28 d. Xylanase inclusion increased BW at 13 and 28 d and reduced FCR at 49 d in reduced energy diets regardless of supplemental fat level. These results demonstrate that variable supplemental fat inclusion does not affect xylanase efficacy or final broiler growth performance.

Experiment two consisted of diets with corn from three distinct geographical locations within the US and the supplementation of xylanase at two inclusion rates. The variation in nutrient profile between corn sources influenced BW, energy digestibility, and short-chain fatty acid profile. Xylanase inclusion reduced FCR during the finisher phase and from 1 to 42 d. These results highlight the importance of understanding accurate ingredient nutrient profiles when formulating diets, as nutrient profile variability can effect broiler performance and digestibility, and demonstrate the consistency of xylanase inclusion on broiler feed efficiency improvement.

Experiment three consisted of diets with three dietary AME levels and the supplementation of xylanase at two inclusion rates. Increasing AME level increased

early BW and led to increases in energy and crude protein digestibility at 18 d, 33 d, and 42 d. Xylanase inclusion reduced FCR from 1 to 33 d and 1 to 42 d of age and increased energy and crude protein digestibility at 33 d and 42 d. These results demonstrate the positive effect of increased levels of dietary energy on performance and nutrient digestibility, as well as the ability of xylanase to improve digestibility resulting in increased efficacy. This research program indicates the importance of adequately understanding ingredient and diet nutrient differences and outlines the benefit of exogenous xylanase inclusion as a nutritional strategy to improve diet value or reduce production costs.

DEDICATION

I would like to dedicate this dissertation work to my family and friends. Thank you for all of the years of unwavering love, support, and advice that has motivated me to be successful in all that I do. I love you all.

To my parents Kevin and Gena, and brother Matt, I cannot thank you enough for all of the love and support that you have provided not only throughout my education, but also throughout my entire life. You have always been there for me. No matter how challenging or stressful times may have got, you encouraged me to stay focused and chase my goals. Words cannot express how much I appreciate everything you have done to help me get to where I am today.

To family and friends no longer with me, thank you for the guidance and inspiration to push through every challenge I have ever faced. Knowing that you are always with me in spirit motivates me to work hard and constantly better myself.

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Contributors

This work was supervised by a dissertation committee consisting of Professor Jason Lee [advisor], Professor Tri Duong [co-advisor], and Professor Gregory Archer of the Department of Poultry Science and Professor Tryon Wickersham of the Department of Animal Science.

All work conducted for the dissertation was completed by the student independently.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Poultry Diets: Formulation and Ingredients

Over the past 30 years, increased demand for poultry products, in both domestic and international markets, has led to a rise in US broiler meat production of approximately 35% (Davis et al., 2013). The expansion of this poultry market can be attributed to several factors including advances in broiler genetics, improved feeding programs and rearing facilities, improved efficiency of broiler production and processing, changes in consumer demands, and increased broiler meat usage in food service (Larbier and Leclercq, 1994; Davis et al., 2013). As production efficiency and producer goals have changed over time, research has allowed for improvements in the estimation of broiler nutrient requirements (NRC, 1994). Feed represents approximately 60-70% of the total input costs of poultry production (Leeson and Summers, 2009), which presents the need to continuously evaluate current and potential alternative ingredients to ensure diets are manufactured with the appropriate nutrient quality and lowest cost . Multiple feedstuffs can be utilized in poultry diet manufacturing, consisting of cereal grains, cereal-by products, plant and animal protein sources, supplemental fats and oils, vitamin and mineral premixes, synthetic amino acids, and feed additives. The combination of these feedstuffs and water provide the birds with the essential nutrients necessary for growth, reproduction, and overall health (NRC, 1994). Energy and protein sources constitute the largest component of dietary formulations at approximately 90-95% of the diet, while vitamin and mineral premixes account for 3-4%, and feed

additives account for the remaining 1-2% (Ravindran, 2013b). Globally, corn is the most predominately used cereal grain as a source of energy, and soybean meal is the most commonly used plant protein source. However, other energy sources such as wheat and sorghum, and other plant protein sources such as canola and sunflower meal are used outside of the US.

Feed Formulation

Feed formulation is the process of quantifying the total amount of ingredients needed to create a single diet that meets the nutritional requirements of the animal. To do so, it is important to understand the nutrient requirements of the specific animal, composition of feed ingredients and any related nutritional or processing constraints, and the cost and availability of ingredients. The precision feeding style used in poultry is referred to as least cost formulation, which matches the nutrient contents of available ingredients to animal nutrient requirements in the most economic manner possible. Nutritionists must also consider any ingredient restrictions that can have non-nutritional effects, such as decreases in feed mill throughput or reductions in feed form related quality.

Cereal Grains

Cereal grains are the primary ingredient used to satisfy the energy requirement of poultry. Available energy can be stored in the starch, cellulose, and oil components of the cereal. However, majority of the energy is derived from the starch component, making starch digestibility and utilization a crucial component in poultry feeding (Classen, 1996). Starch is an omnipresent polysaccharide stored in cells found within the

endosperm of cereals. The complete endosperm of these grains consists of two different tissues, the starchy endosperm and the aleurone layer. The starchy endosperm, which is sometime mistakenly referred to by the general term “endosperm”, is the largest morphological component of the cereal and provides the greatest contribution to animal nutrition. The aleurone layer is comprised of block-like cells that create layers of proteins, lipids, vitamins, and minerals that surround the starchy endosperm (Evers and Millar, 2002). Together, the starchy endosperm and aleurone layer provide nutrients for the embryo during plant germination. The embryo is the plant component that is capable of developing into a plant and consists of lipids and lipid-soluble vitamins (Evers and Millar, 2002). Although multiple cereal grains are used to satisfy energy requirements across different countries and regions, corn is the dominant cereal grain within the US.

Cereal by-products

Recently, the diversion of grains from the feed market to energy production entities has presented grain supply issues, pushing nutritionists to consider alternative ingredients, such as cereal by-products, with a greater availability and potential cost savings. Distillers dried grains with solubles (DDGS) is a commonly used by-product created through the fermentation process of cereal grains during ethanol manufacturing (Stein, 2006; Świątkiewicz and Koreleski, 2008). During this process, the starch component of cereal grains is converted to ethyl alcohol and CO_2 that is utilized in the biofuel industry (Rosentrater, 2006). The non-fermentable substrates remaining are rich in protein, lipids, fiber, vitamins, and minerals at a concentration that is more than double that of the original cereal grain (Świątkiewicz and Koreleski, 2008; Salim et al.,

2010). Corn DDGS have been used in poultry diet formulations since originally becoming available, and usage has steadily increased with the expansion of ethanol production. It has been recommended that DDGS inclusion should not exceed 6% in broiler starter diets and be included at no more than 12% in subsequent feeding phases. Multiple studies have been conducted to evaluate the effect of DDGS inclusion on broiler growth performance. Although it has been reported that DDGS could potentially be used at levels up to 25% when dietary energy is maintained (Waldroup et al., 1981), research has shown that there are no adverse effects on growth performance or carcass quality when DDGS are included at a maximum of 12 to 15% in reduced energy diets (Lumpkins et al., 2004; Wang et al., 2007).

Protein Sources

Protein-yielding feed ingredients are the second largest component of monogastric animal diets behind those that provide energy. Oilseed meals are a protein-rich residue that is the product left over from the oil extraction process of oil-bearing seeds like soybeans, cottonseed, rapeseed, and peanuts. Soybean meal (SBM) is the preferred plant-based protein source for animal diets within the US, and supplies the majority of the dietary protein requirements of the animal (Ravindran, 2013a). Not only does SBM provide crude protein levels ranging from 40 to 48% with a balanced profile of highly digestible amino acids, but it also provides greater metabolizable energy and a lower crude fiber content compared to other oilseed meals (Willis, 2003; Frias et al., 2008). Soybean meal provides an excellent ratio of most essential and non-essential amino acids sought after in feed formulation; however, is limited in the amount of the

sulfur containing amino acids methionine and cysteine (Dozier et al., 2011; Mukherjee et al., 2016). The inclusion of SBM generally ranges from 25 to 40% in commercial poultry diets, but when included at larger levels can present anti-nutritional factors such as NSP, trypsin inhibitors, lectins, and saponins (Yasothai, 2016).

Plant-based protein sources, other than SBM, are generally imbalanced in amino acid profiles. Because of this, there is a need to supplement either synthetic amino acids or an animal-based protein product to meet nutritional requirements for growth and production (Ravindran, 2013a). Animal-based protein sources are a dry-rendered product derived from the bone, tendons, ligaments, muscle, and organs of condemned animals (Ravindran and Blair, 1993). Porcine meat and bone meal (MBM) is a common animal protein source used in the US, and contains relatively high levels of protein, calcium, and phosphorus. Products defined as MBM have a crude protein level up to 55% and calcium and phosphorus levels of approximately 8% and 4%, respectively. The inclusion of MBM in commercial poultry diets rarely exceeds 5 to 7% due to concerns with protein quality and phosphorus availability (Sell, 1996; Leeson and Summers, 2009).

Supplemental Fats and Oils

Feed-grade fats and oils are commonly added to poultry diets for multiple reasons to include supplying energy, improving the absorption of fat-soluble vitamins, improving pellet durability, increasing diet palatability, and reducing rate of passage in the gastrointestinal tract improving absorption of other dietary nutrients (Moav, 1995; Balevi and Coskun, 2000; Palmquist, 2002; Baião and Lara, 2005). These products can be available in many different forms to include restaurant greases, the rendering of

animal carcasses, or refuse from the vegetable oil industry, and are normally selected based on cost and product availability (NRC, 1994). It is recommended that supplemental fat or oil should be included in dietary formulations at levels less than 5% to meet demands for pellet durability. In diets manufactured with cereal grains other than corn, supplemental fat or oil inclusion should be at least 1% of the diet to ensure the essential requirement for linoleic acid is met (Leeson and Summers, 2009).

Anti-Nutritional Factors

Non-starch Polysaccharides

Polysaccharides are polymers of monosaccharides linked together by glycosidic bonds and represent the most important group of nutrients found in plant-based feedstuffs (Sethy et al., 2015). The term NSP covers multiple polysaccharide molecules, excluding α-glucans (starch) and free sugars. Variation in the amount and structure of NSP can differ across different feedstuffs and is classified by the monosaccharides present, monosaccharide ring forms (pyranose or furanose), position of the glycosidic linkages, configuration of the glycosidic linkages (α or β), sequence of monosaccharide residues, and those non-carbohydrate substituents present or not (Choct, 1997). Dietary fiber is defined as plant material that is not normally hydrolyzed by endogenous intestinal enzymes but could be potentially digested by microorganisms in the gut. This fiber can be divided into two types defined by their water solubility. Insoluble dietary fiber includes celluloses, some hemicelluloses, and lignin, while soluble dietary fiber includes β-glucans, pectins, gums, mucilages, and some hemicelluloses (Căpriță et al., 2010) (Table 1). All components grouped within insoluble and soluble dietary fiber, with

the exception of lignin, are now generally classified as NSP (Leeson and Summers, 2001).

Non-starch polysaccharides that are of most concern to poultry, are found in corn and soybean meal, and consist of arabinoxylans, β-glucans, pentosans, arabinogalactans, mannans, galactomannans, xylans, and oligosaccharides, which can be grouped into the three main larger classifications of cellulose, hemicellulose, and pectins (Bacic et al., 1988; Choct, 2006; Căpriţă et al., 2010; Slominski, 2011). Cellulose is the most important cell wall polysaccharide described as a linear homopolymer consisting of several hundred to thousands of D-glucose units linked via consecutive β- 1, 4 linkages (Figure 1). The combination of these D-glucose units creates a crystalline microfibril that is insoluble and resistant to enzymatic degradation. Hemicelluloses are heteropolymers with side chains of different sugar units that can include the five-carbon sugars xylose and arabinose, six-carbon sugars mannose and galactose, and acidified forms of these sugars, such as glucuronic acid. Unlike cellulose, hemicelluloses have random, amorphous structure with shorter chain lengths of sugar units, promoting easy hydrolysis with enzymes.

Cellulose is present in the cell wall of both mono- and dicotyledonous plants, whereas the other cell wall polysaccharides differ between flowering plants. Mixed linkage β-glucan and arabinoxylan are the main cell wall polysaccharides observed in cereal grains, and xyloglucans, glucomannans, galactomannans, and pectins are the main cell wall polysaccharides of protein-rich feedstuffs (Bach Knudsen, 2014). Corn, which is the primary cereal grain fed within the US, contains minimal amounts of soluble NSP

and approximately 8% insoluble NSP, majority of which is arabinoxylans (Choct, 2006; Slominski, 2011; Bach Knudsen, 2014). Arabinoxylans are classified as hemicelluloses with linear xylose units combined in β- 1, 4 linkages with side chains of arabinose. Beta-glucans consist of linear D-glucose units combined together with β- 1, 3 glycosidic bonds (Choct, 1997). Soybean meal, which is the predominately used plant-based protein in the US, contains approximately 3% soluble NSP and 16% insoluble NSP, majority of which are mannans (Irish and Balnave, 1993; Bach Knudsen, 1997; Slominski, 2011). Mannans are also classified as hemicelluloses with mannose units combined in β- 1, 4 linkages with side chains of glucose or galactose. These NSP have different physical and chemical characteristics that have shown to have various effects on the intestinal physiology of poultry, as well as having detrimental effects on broiler growth and performance.

Encapsulation Effect

In plant-based feedstuffs, the cytoplasmic matrix consists of proteins, lipids, and starches that provide nutritional value to poultry. These nutrients are surrounded by the cell wall which is a rigid, semi-permeable layer comprised primarily of polysaccharides that provides the cell with structure and protection. Previous research has shown that the physical entrapment of these intracellular nutrients by the cell wall inhibits the access of digestive enzymes and reduces the overall nutrient availability (Annison, 1993; Grundy et al., 2016; Rovalino-Córdova et al., 2019). In monogastrics, such as poultry, it is estimated that approximately one-fourth of a standard diet goes undigested because the animal lacks the endogenous enzyme production necessary to degrade certain anti-

nutritional factors (Ojha et al., 2019). Reduced nutrient availability and digestibility has been observed with water-soluble NSP, and can lead to decreases in dietary AME and negative effects on broiler performance (Annison, 1993). Proper feeding strategies must be applied to target the cell wall fraction of plant-based feedstuffs in order to achieve maximum growth performance.

Table 1. Chemical classifications of dietary fiber**.**

Fiber[1]	Main Chain	Side Chain	Description
Cellulose	Glucose	None	Main structural component of plant cell wall. Insoluble in concentrated alkali and soluble in concentrated acid.
Hemicellulose	Xylose Mannose Galactose Glucose	Arabinose Galactose Glucuronic acid	Cell wall polysaccharides containing backbone of 1-4 linked pyranoside sugars. Vary in degree of branching and uronic acid content. Soluble in dilute alkali.
Pectic substances	Galacturonic acid	Rhamnose Arabinose Xylose Fucose	Components of primary cell wall and middle lamella. Vary in methyl ester content. Generally, water-soluble and gel forming.
Lignin	Sinapyl alcohol Coniferyl alcohol p-Coumaryl alcohol	3-D structure	Non-carbohydrate cell wall component. Complex cross-linked phenyl propane polymer. Insoluble in 72% sulfuric acid. Resists microbial degradation.

[1]Data modified from McPherson (1985) and (Căpriță et al., 2010).

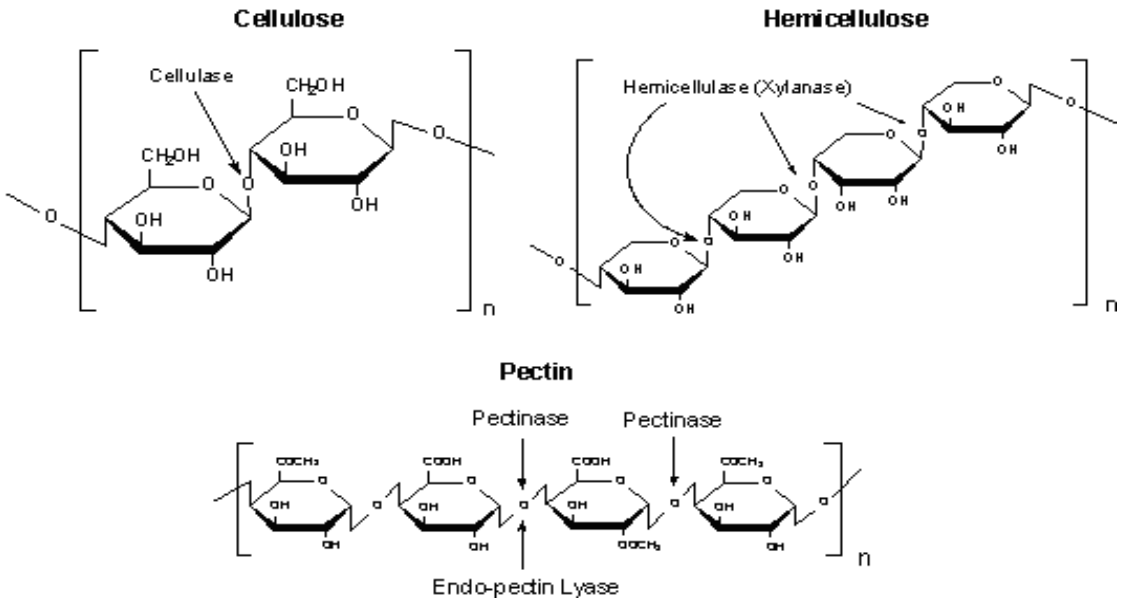

Figure 1. Structural models of cellulose, hemicellulose, and pectin with proposed cites of hydrolization. Adapted from [Chapter 2. The cell wall. Pages 52–108 in Biochemistry & Molecular Biology of Plants. (Carpita and McCann, 2000; Buchanan et al., 2015)]

Viscosity

Viscosity is defined as the measure of internal fluid resistance to deformation at a specific rate, and in the simplest terms, is regarded as the "thickness" of a substance. The viscosity is a physiochemical property of NSP and is associated with the hydration properties, cation exchange, and absorptive properties of that substance (Bach Knudsen, 2011). The presence of dietary polysaccharides, specifically the water-soluble portion of NSP, have the ability to increase intestinal digesta viscosity. However, the severity of viscous consistency can be affected by the degree of polymerization of the sugar units and the overall concentration of those water-soluble polysaccharides (Izydorczyk and Biliaderis, 1992; Nilsson et al., 2000; Cowieson and Adeola, 2005). Poultry lack the digestive capacity to breakdown NSP observed in ruminant animals (Bedford, 1995; Choct and Cadogan, 2001; Meng et al., 2005), and when feeding viscous grains, such as wheat, barley, and rye, the viscosity-inducing NSP can have several effects reducing overall growth performance (Ward, 1996; Adeola and Bedford, 2004). Changes in viscosity can reduce or prolong passage time in the broiler's small intestine depending on the digesta consistency (Ward, 1996; Bedford and Schulze, 1998; Choct et al., 2010). Overall, the anti-nutritive effects of increased intestinal digesta viscosity limits the availability of nutrients to the bird by reducing the exposure of nutrients to enzymes or decreasing digesta contact with intestinal enterocytes (Bedford, 1995; Dänicke et al., 1999a; Teirlynck et al., 2009). Improvements in nutrient absorption and digestibility have been observed with reductions in intestinal digesta viscosity due to increases in facilitated diffusion of substrates, digestive enzymes, and digestive products;

improvements in the connection of digesta by intestinal contractions; and improvements in the contact of available nutrients with enterocyte surfaces (Simon, 1998).

Ingredient and Nutrient Variability

Energy

According to the NRC (1994), energy is not considered a nutrient, but a property of energy-yielding dietary components that is created after oxidation of starches, proteins, and lipids, and can be expressed in multiple different ways. In poultry, the primary unit for energy measurements is apparent metabolizable energy (AME). Apparent metabolizable energy is defined as the difference between gross energy of feed consumed and the gross energy contained in the feces, urine, and gaseous products, which represents the energy available for growth, reproduction, and metabolic processes. Multiple reports show that dietary energy reductions will negatively affect broiler BW gain, FCR, and nutrient digestibility (Cowieson, 2010; Coppedge et al., 2012; Masey O'Neill et al., 2012; Singh et al., 2012). Anti-nutritional factors may also impede energy utilization; therefore, poultry nutritionists are presented with the constant need to evaluate nutritional strategies to ensure dietary energy is provided to the animal in the most effective way.

Corn

Corn is the primary cereal grain utilized in US poultry diet manufacturing due to its high energy availability and relatively low NSP content and can contribute up to 65% of the metabolizable energy and 20% of the protein in a standard broiler starter diet (Cowieson, 2005). Although corn has advantages over other cereal grains, the nutritive

value of any cereal can still vary between, and within, each specific grain species. Those factors that contribute to variability in corn can include plant variety, geographical location and climate, post-harvest processing and storage, lipid/protein/starch matrices, fiber content, and heat treatment (Socorro et al., 1989; Herrera-Saldana et al., 1990; Leeson et al., 1993; Brown, 1996; Cromwell et al., 1999; Collins et al., 2001; Gehring et al., 2012).

Apparent metabolizable energy of cereal grains can be greatly affected by starch digestibility (Rogel et al., 1987). In corn-based poultry feeds, approximately 60% of the AME is derived from the starch content, and small variation in the digestibility can have a significant effect on AME of the diet (Weurding et al., 2001). There are two endosperm types found within corn that have different characteristics, and the proportion of these can vary between corn cultivars which greatly affects the nutritional value and functionality of the grain (Yu et al., 2015) (Figure 2). The floury endosperm has a soft, loose structure with spherical starch granules and a large amount of air space, while the vitreous endosperm has a hard, compact structure with polygonal starch granules and very little air space (Rooney and Pflugfelder, 1986; Cowieson, 2005; Miao et al., 2014). Increased vitreousness is associated with reductions in starch digestibility due to starch-protein interactions and physical entrapment of the nutrients within the endosperm (Weurding et al., 2001; Yu et al., 2015). This suggests that encapsulated nutrients could reduce the nutritional value of the corn and have an overall effect on dietary AME. Previous research evaluating more than 50 different corn samples resulted in starch

content ranges from 628 to 720 g/kg and presented potential AME variation of approximately 440 kcal/kg (Cowieson, 2005).

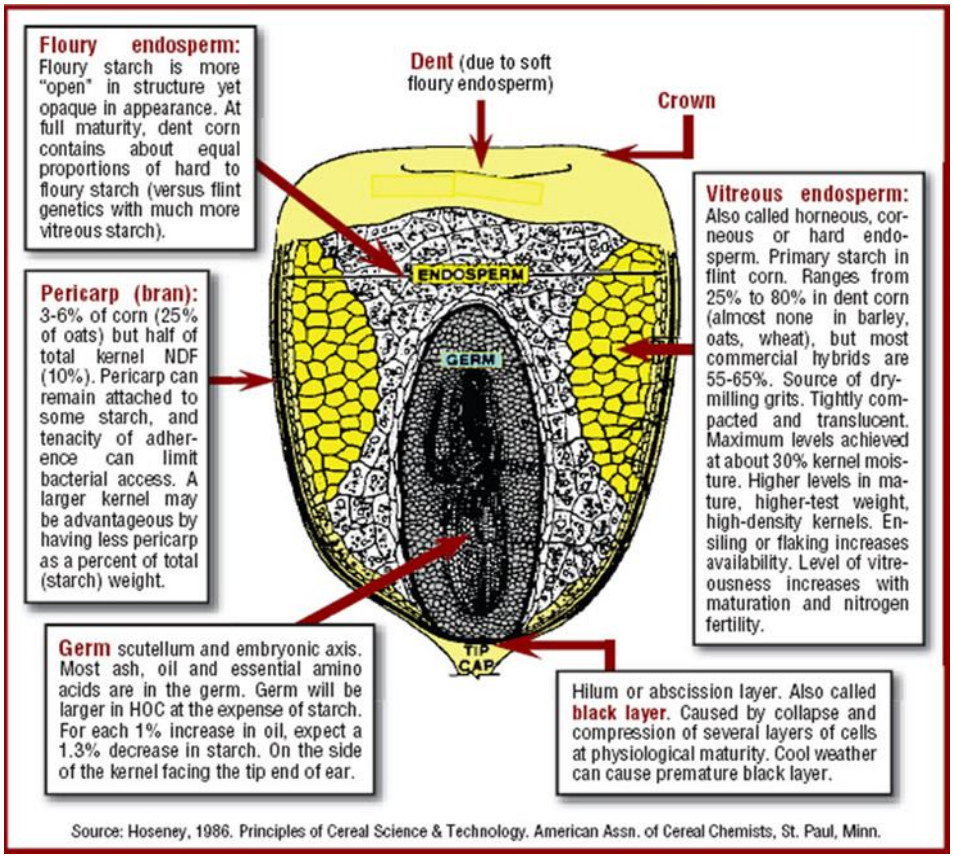

Figure 2. Structure and physical properties of corn. Adapted from [Principles of Cereal Science & Technology (Hoseney, 1986).]

Alternative Ingredients

Although corn DDGS can be included in dietary formulations as a rich source of protein, amino acids, minerals, and energy, there is large variation associated with the nutrient concentration and quality across different sources of DDGS (Świątkiewicz and Koreleski, 2008). Separate studies analyzing 9 (Cromwell et al., 1993) or 10 (Spiehs et al., 2002) sources of DDGS from different production systems resulted in varying content of crude protein, fat, neutral detergent fiber, acid detergent fiber, ash, and multiple amino acids which did not correspond with nutrient reference data (NRC, 1994). Distillers dried grains with solubles also contains a large amount of soluble NSP (approximately 35%) that provide a potential source of dietary energy (Świątkiewicz and Koreleski, 2008; Youssef et al., 2008). Variation observed among different sources of DDGS, as well as the elevated NSP content in the ingredient are both important factors that could negatively affect broiler performance and nutrient utilization.

Supplemental Fats and Oils

Fats and oils are considered a raw feedstuff commonly included into poultry diets as an energy-yielding ingredient. A wide variety of fats and oils are available for use in poultry feed manufacturing, all of which are compositionally different. Although these products can be supplemented in poultry diets for multiple beneficial reasons, it is also important to understand product complexity can present a lack of uniformity in available energy content. The evaluation of approximately 10 different sources at multiple inclusion levels resulted in variation of AME ranging from 5,230kcal/kg to 11,106 kcal/kg (Ravindran et al., 2016). Two methodologies are generally accepted to determine

the AME of fats and oils in poultry diets (Mateos and Sell, 1981; Irandoust et al., 2012). Both of these methods assume that diet composition has no effect on lipid digestion, and supplemental fats and oils do not have any effect on the utilization of other dietary nutrients (Ravindran et al., 2016). However, significant interactions have been observed between dietary NSP and fat digestibility, and true metabolizable energy of fats can vary depending on cereal grain type (Sibbald, 1978; Ward and Marquardt, 1983).

There are also non-diet related factors that can affect the utilization of fats and oils, with the most important being bird age. Younger birds, do not have the ability to digest and absorb dietary fat efficiently (Ravindran et al., 2016), due to limited bile and lipase secretion. As bird age increases, so does the ability to replenish bile salts lost by excretion. It is estimated that duodenal bile secretion can double between the ages of 4 to 7 and 7 to 10 days, however it has still not progressed to the point of sufficiently supporting the absorption and digestive processes (Krogdahl, 1985; Noy and Sklan, 1995). With the potential of such variation between feed ingredients, as well as non-diet related factors, it is important to consistently analyze all feed ingredients prior to diet manufacturing and consider outside factors that affect nutrient utilization.

Exogenous Enzymes

The supplementation of exogenous NSP-degrading enzymes into dietary formulations has been considered common practice for most commercial poultry integrators within the US. These enzymes are selected for use based on the target substrate, as well as the overall objective to be achieved. Having a thorough understanding of feed ingredient composition and the chemistry of targeted substrates

allows for the most effective inclusion of these enzymes, and promotes the greatest advantage to broiler performance and cost savings.

Two primary reasons for choosing to utilize exogenous enzymes in feed formulation is to increase the feeding value of raw ingredients or to reduce the variation in nutrient quality of feedstuffs (Bedford, 2000), which can lead to cost savings and improvements in broiler growth performance. In feed ingredients, carbohydrates, proteins, and lipids exist as complexes and the limited endogenous enzyme activity in poultry restricts access to these nutrients. Ileal digestibility of essential nutrients can vary from approximately 70% to greater than 95% depending on diet composition, and the inclusion of NSP-degrading enzymes can allow for up to 25% of the undigested nutrients to become available to the animal (Cowieson, 2010).

Previously, two modes of action have been presented for which NSP-degrading improve digestibility (Cowieson, 2010). In non-viscous grain diets, the use of these enzymes allows for the degradation of cell walls and increases the availability of carbohydrates, proteins, and lipids previously entrapped in the plant (Annison, 1993; Bedford, 2018). In viscous grain diets, enzyme inclusion partially degrades soluble NSP, which can reduce the water holding capacity of the NSP and decrease digesta moisture leading to improvements in digestibility (Almirall et al., 1995; Ward, 1996; Bedford, 2018). More recently, a third mode of action has been proposed that NSP-degrading enzymes can present a prebiotic effect within the intestine. The hypothesis suggests that inclusion of these enzymes produce fermentable substrates that can provide energy for intestinal microorganisms leading to increased energy recovery volatile fatty acid

production and increased gizzard efficiency (González-Ortiz et al., 2016; Bedford, 2018).

Xylanase Inclusion

Xylanases, officially known as endo-1, 4-β-xylanase, are hydrolytic enzymes that catalyze the endo-hydrolysis of the backbone of xylans, and create smaller carbohydrate fragments that can be better digested by the bird (Collins et al., 2005) (Figure 3). Xylanase supplementation has been observed to improve FCR with improvements of 15 and 9 points at 21 d and 49 d, respectively (Gao et al., 2008); 4 and 6 points at 35 d and 42 d, respectively (Masey O'Neill et al., 2012); and 6 and 3 points at 15 d and 23 d, respectively (Williams et al., 2014). Increases in BW, as well as improvements in FCR have been reported in multiple studies (Esmaeilipour et al., 2011; Coppedge et al., 2012). The improvements observed with xylanase inclusion could be attributed to increases in nutrient retention and digestibility. In both corn and wheat-based diets, xylanase inclusion increased nitrogen retention and improved energy digestibility and crude protein digestibility (Hew et al., 1998; Dänicke et al., 1999a; Stefanello et al., 2016; Amerah et al., 2017). It has been suggested that xylanase can be credited an energy value, because degradation of NSP could contribute to increases in dietary energy level (Slominski, 2011; Masey O'Neill et al., 2012). Increased energy utilization with xylanase supplementation has been confirmed where performance was able to overcome dietary reductions ranging from 50 kcal/kg to 132 kcal/kg (Coppedge et al., 2012; Singh et al., 2012; Williams et al., 2014).

The inclusion of xylanase has led to improvements in growth performance and nutrient utilization of broilers fed corn-based diets (Aftab, 2012; Masey O'Neill et al., 2012; Stefanello et al., 2016), broilers fed wheat-based diets (Engberg et al., 2004; Cowieson and Masey O'neill, 2013), and turkeys fed low viscosity wheat-based diets (Persia et al., 2002), even with variation in the amount of NSP substrate. This supports the idea that xylanase can be effective in diets that are compositionally different. However, there is a different mechanism in which the enzymes work, which depends on diet makeup and substrate availability.

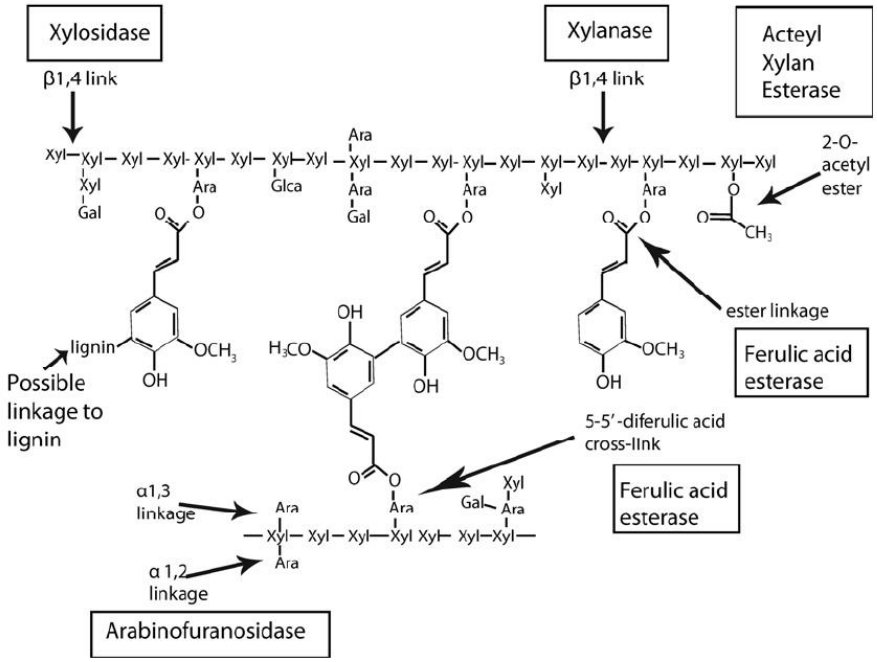

Figure 3. Structural features of arabinoxylan with enzymatic methods of cleavage for various linkages in the arabinoxylan molecule. Adapted from [Cereal Chemistry (Kiszonas et al., 2013)].

β-Glucanase Inclusion

Beta-glucanases, officially known as endo-1, 3(4)-β-glucanase, are hydrolytic enzymes that catalyze the endo-hydrolysis of the backbone of cellulose, lichenin, and cereal β-D-glucans, and create smaller carbohydrate fragments that can be better digested by the bird (Bedford and Partridge, 2010) (Figure 4). Beta-glucanases are primarily included in viscous grain-based diets due to the increased soluble NSP content; however, previous research also indicates benefits of β-glucanase supplementation in corn-based diets. The inclusion of β-glucanase in oat and barley-based diets led to increases in BW, improvements in FCR, and reductions in viscosity in broilers through 35 d (Józefiak et al., 2006). In wheat and barley-based diets, the inclusion of β-glucanase increased BW, improved FCR, and reduced small intestine weight compared to non-supplemented diets broilers fed through 40 d (Mathlouthi et al., 2011). The beneficial effect of β-glucanase in corn-based diets has been observed with increases in energy digestibility of broilers through 21 d (Leslie et al., 2007). Furthermore, increases in BW at 21 d, and improvements in FCR at 21 d and 42 d have also been observed in corn-based diets; however, an additive effect was observed with the addition of xylanase leading to greater improvements in performance (Cowieson et al., 2010). Overall, research indicates β-glucanase supplementation as a viable nutritional strategy to mitigate the negative effects associated with NSP, specifically β-glucan.

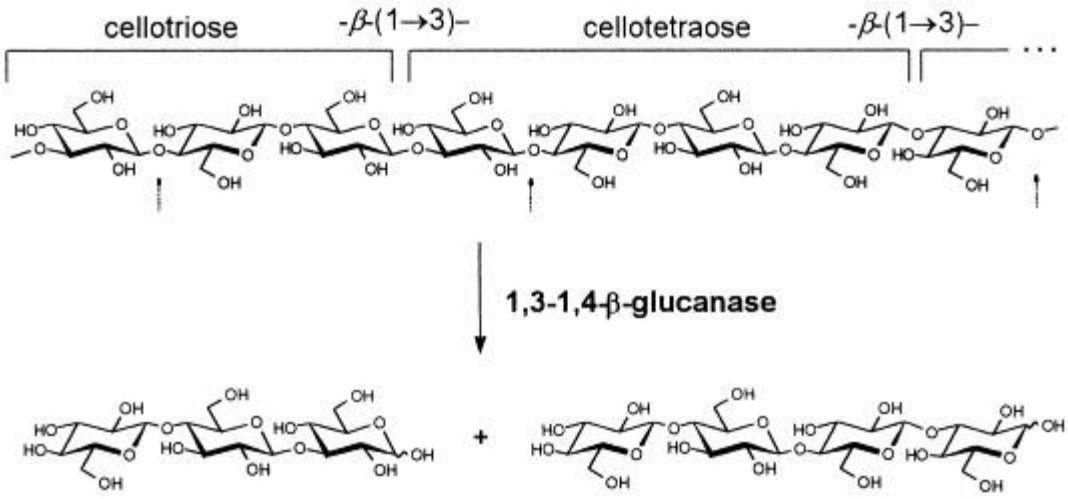

Figure 4. Structural features of β-glucans with enzymatic methods of cleavage for the molecule. Adapted from [Bacterial 1,3-1,4-β-glucanases: structure, function and protein engineering (Planas, 2000)].

Cocktail Enzyme Combinations

Commercially available products that are labeled as xylanases are often composed of a blend of multiple different exogenous enzymes and credited with xylansae as the main enzymatic activity (Aftab, 2012). There are also enzyme mixtures available that include NSP-degrading enzymes, as well as other enzymes like proteases, amylases, and phytases. The inclusion of proper exogenous enzymes, to target their corresponding substrates, partially degrades soluble NSP and other nutrient complexes (proteins and phytate) into smaller fragments increasing the availability of the nutrients and can reduce unwanted complexes and viscous-like gels in the intestine (Ward, 1996). Nutritionists can utilize these blended enzyme preparations as a way to target varying substrates across multiple feed ingredients and improve performance and nutrient utilization.

Previous research indicates that, in some cases, cocktail enzyme preparations may not positively influence growth performance or nutrient utilization. The inclusion of a xylanase, amylase, and protease combination in corn-based mash diets, with or without corn DDGS, resulted in no performance or energy digestibility differences compared to the control diets (Olukosi et al., 2007; Olukosi et al., 2010). However, there are also instances in which enzyme combinations have provided benefits to broilers. A cocktail preparation of xylanase, glucanase, and pectinase resulted in improvements in FCR of broilers though 18 d (Meng et al., 2005), while a preparation of xylanase, glucanase, mannanase, cellulase, and pectinase resulted in increases in BW and dietary AME of broiler through 18 d (Meng and Slominski, 2005). Improvements in BW gain,

FCR, and increases in dietary AME and starch digestibility were also observed in broilers fed corn-based diets supplemented with xylanase and amylase through 25 d (Stefanello et al., 2015).

Significant amounts of research have reported the positive effects of NSPase inclusion to include increases in nutrient digestibility and increasing feed efficiency however, other reports have indicated inconsistent responses. Thus, the objectives of this research program is to evaluate the influence of ingredient and diet nutrient differences on xylanase efficacy with the specific aims:

1. Determine the influence of dietary supplemental fat inclusion on nutrient digestibility and broiler growth response with the inclusion of NSPase
2. Identify if corn source effects xylanase efficacy as measured by broiler performance and nutrient digestibility
3. Investigate the effect of varying dietary AME level on the ability of xylanase to improve broiler growth and nutrient digestibility.

CHAPTER II

EVALUATION OF THE EFFECT OF DIETARY FAT INCLUSION ON XYLANASE EFFICACY IN BROILER DIETS

Introduction

Since the early 2000's, the production of ethanol and other biofuels has presented an increase in the demand of corn supplies for energy applications and has led to a rise in cereal grain prices for the animal feed industry. With feed accounting for up to 70 percent of the total production costs per animal, increases observed in feed ingredient prices has encouraged nutritionists to look at alternative ingredients and feed additives to help improve nutrient utilization and feed efficiency as a strategy to reduce costs. Corn and soybean meal are the two most predominantly used ingredients in US poultry feed manufacturing. Approximately 60% of a standard nutrient density diet is comprised of corn, with another 25% of that diet made up of soybean meal (Leeson and Summers, 2009). Other cereal grains such as wheat, barley, and sorghum can be used as alternative energy sources, whereas canola and peanut meals may be used as alternative protein sources. The variety of alternative ingredients that can be utilized during feed manufacturing, not only presents variation in the nutrient profiles but also potential increases in anti-nutritional factors which can have an effect on both feed value and usage (Bedford and Schulze, 1998).

Non-starch polysaccharides (NSP) are anti-nutritional factors found in plant-based feedstuffs, that may impede digestion or negatively affect the digestive tract in poultry due to the lack of specific endogenous enzymes (Khattak et al., 2006). The

primary types of NSP present in cereal grains and cereal by-products consist of arabinoxylans, β-glucans, and cellulose. In protein feedstuffs, numerous NSPs exist including arabinans, arabinogalactans, galactans, galactomannans, mannans, and pectins (Choct, 1997; Slominski, 2011). Multiple studies have been conducted to evaluate the effect of exogenous enzyme supplementation on the anti-nutritional effects of dietary NSP, in which improvements in FCR, increases in nutrient digestibility, and reductions of intestinal viscosity have been observed (Bedford and Classen, 1992; Bedford and Morgan, 1996; Meng et al., 2005; Choct, 2006).

In addition to cereal grains, broiler diets are generally formulated to contain a plant oil or animal fat for multiple reasons to include supplying energy, improving the absorption of fat-soluble vitamins, improving pellet durability, increasing diet palatability, and reducing rate of passage in the gastrointestinal tract improving absorption of other dietary nutrients (Moav, 1995; Balevi and Coskun, 2000; Palmquist, 2002; Baião and Lara, 2005). Supplemental fats and oils constitute a great source of metabolizable energy and supply the most concentrated caloric value of all feed ingredients, which can be more than twice that of carbohydrates or proteins (NRC, 1994). Despite being broadly accepted by nutritionists, supplemental fats and oils are very complex and present a lack of uniformity in available energy content (Ravindran et al., 2016). Differences in the digestibility of these lipid sources depends on the physical and chemical properties of each individual fatty acid. Higher degrees of saturation and longer fatty acid chain length leads to poorer digestibility (Ward and Marquardt, 1983; Wiseman et al., 1991).

Although there have been numerous studies and review articles evaluating the effect of diet-related factors on fat digestibility and interactions between fat type and enzyme inclusion, there is a lack of literature evaluating any interactions between dietary fat level and enzyme inclusion. Therefore, the objective of the current study was to determine the effects of dietary fat inclusion on xylanase efficacy on performance and nutrient digestibility of the male Cobb 500 broiler.

Materials and Methods

Experimental Animals and Husbandry

Male broiler chicks (Cobb) were obtained from a commercial hatchery on day of hatch, weighed, wingbanded, and assigned randomly to pens to ensure statistically similar starting pen weights at a stocking density of 0.065 m^2 per bird. Experimental animals were raised in floor pens, provided age appropriate heat and ventilation, and given access to water and experimental diets for ad libitum consumption for the duration of the study. Each 3.35 m^2 pen contained a tube feeder, a nipple drinker line, and fresh pine shavings as litter. Temperature was monitored, recorded daily, and adjusted to maximize bird comfort. The lighting program was as follows: 1 to 3 d, 24 h of light at 21.53 lux; 4 to 8 d, 23 h of light at 21.53 lux; 9 to 18 d, 16 h of light at 8.07 lux; 19 to 32 d, 18 h of light at 1.08 lux; and 33 d to termination, 20 h of light at 0.54 lux. All experimental procedures were performed as approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC).

Experimental Design and Diets

The effects of dietary fat inclusion on the efficacy of xylanase in improving

growth performance and nutrient digestibility of male Cobb 500 broilers was evaluated. Broiler chicks (n=2,385) were allocated to five experimental treatment groups arranged as a randomized complete block design with 9 replicate pens of 53 birds per treatment and fed diets with varying levels of supplemental fat inclusion with or without added xylanase. The five experimental treatment groups were as follows: positive control (**PC**), negative control 1 (**NC1**), negative control 2 (**NC2**), negative control 1 with xylanase (**NC1 + X**), and negative control 2 with xylanase (**NC2 + X**). The PC diet was formulated as a standard industry corn-SBM diet. The NC1 and NC2 diets were formulated with a 110 kcal/kg reduction in AME and a 1-2% reduction in digestible AA (**dAA**) compared to the PC diet, and with supplemental fat inclusion rates for NC1 and NC2 of a maximum of 0.5% and minimum of 1.5%, respectively. The remaining diets consisted of the NC1 and NC2, each supplemented with 1,500 EPU/kg of xylanase (Hostazym®X; Huvepharma, Peachtree City, GA). An endo-pentosanase unit (EPU) is defined as the amount of enzyme that releases low-molecular fragments from dyed xylan in amount equal to the amount of such fragments liberated from 1 unit enzyme standard at pH 4.7 and 50°C.

Diets were formulated on a least-cost basis and composed primarily of corn, soybean meal, corn distillers' dried grains with solubles, and porcine meat and bone meal (Tables 2 and 3). Distillers' dried grains with solubles were included in the diet throughout all feeding phases at levels ranging between 5 and 10%. Meat and bone meal was included in the diet throughout all feeding phases at levels ranging between 2 and

4%, and titanium dioxide was included in all dietary phases as an indigestible marker to determine ileal digestible energy (IDE), ileal digestible energy coefficient (IDEC), and ileal digestible nitrogen coefficient (IDNC).

Three basal diets were mixed consisting of the PC, NC1, and NC2 (Table 2 and 3). The NC1 and NC2 were divided into two equal amounts and one-half received the addition of the enzyme premix and the other received cornstarch. Experimental diets were fed for the duration of the study using a 4-phase feeding plan: starter (0-13 d, crumble), grower (14-28 d, pellet), finisher (29-40 d, pellet), and withdrawal (41-49 d, pellet). Diets were manufactured for each phase using a 2-ton double-ribbon, horizontal Scott mixer and a 1 ton/h California Pellet Mill equipped with a conditioner and a 4.4 mm diameter die; and, when appropriate, crumbled using a roller mill. Pelleting temperatures were maintained between 74° and 76°C to ensure the maximum level of enzyme recovery was achieved as directed by the manufacturer.

Triplicate feed samples were collected from each dietary treatment for proximate analysis of composite samples. Crude protein was determined using combustion (AOAC 990.03), crude fat was determined using petroleum ether extraction (AOAC 945.16), ADF was determined gravimetrically (AOAC 973.18), and total phosphorous was determined by wet ash inductively coupled with plasma spectroscopy (AOAC 985.01M). Enzyme activity in the finished feed was verified by the manufacturer to ensure that assayed levels recovered in the allocated diet were within acceptable ranges.

Table 2. Ingredient composition and nutrient content of basal starter and grower diets varying in supplemental fat levels and nutrient profiles

	Starter			Grower		
Item (%)	PC	NC1	NC2	PC	NC1	NC2
Ingredient						
Corn	59.94	62.26	58.05	63.23	66.00	61.47
Soybean meal (48%)	26.87	27.68	26.11	21.37	19.23	19.57
DDGS[1]	5.00	5.00	5.00	7.5	8.92	9.69
Meat and bone meal	4.00	2.07	4.18	3.23	3.14	3.14
A/V fat	1.89	---	1.50	2.19	---	1.50
DL-Met (98%)	0.24	0.22	0.24	0.23	0.22	0.22
L-Lys HCL	0.19	0.18	0.19	0.25	0.28	0.28
L-Thr (98%)	0.04	0.03	0.04	0.06	0.07	0.07
Limestone	0.64	1.05	0.61	0.72	0.75	0.75
Monocalcium PO_4	0.02	0.32	---	---	---	---
Sodium Chloride	0.39	0.41	0.34	0.25	0.21	0.21
Sodium Bicarbonate	0.02	0.02	0.06	0.21	0.26	0.25
Trace Minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05
Vitamins[3]	0.25	0.25	0.25	0.25	0.25	0.25
Phytase[4]	0.01	0.01	0.01	0.01	0.01	0.01
Coccidiostat[5]	0.05	0.05	0.05	0.05	0.05	0.05
Titanium Dioxide	0.40	0.40	0.40	0.40	0.40	0.40
Sand	---	---	2.92	---	0.16	2.09
Calculated Nutrients						
CP	22.00	21.56	21.56	20.00	19.60	19.60
ME (kcal/kg)	3058	2948	2948	3102	2992	2992
Ca	0.90	0.92	0.90	0.84	0.84	0.84
P	0.52	0.53	0.51	0.49	0.49	0.49
Available P	0.45	0.45	0.45	0.42	0.42	0.42
dig Met	0.55	0.52	0.54	0.51	0.50	0.50
dig Lys	1.10	1.08	1.08	1.00	0.98	0.98
dig Thr	0.72	0.70	0.70	0.66	0.65	0.65
Crude fat	5.12	3.12	4.67	5.52	3.49	4.85
Na	0.20	0.20	0.19	0.20	0.20	0.20
Analyzed Nutrients						
CP	21.70	21.60	20.80	19.90	19.40	20.00
Crude fat	4.92	3.89	4.92	5.94	3.76	5.38
ADF	5.20	4.20	5.40	3.30	4.50	6.60

[1]Distiller's dried grains with solubles

[2]Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3]Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D3, 46 IU vitamin E, 0.0165 mg B12, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[4]OptiPhos 2000 PF. This product is a source of phytase which increases the digestibility of phytin-bound phosphorus in poultry and swine rations.

[5]Active drug ingredient Salinomycin at a use level of 40-60 g/ton. For the prevention of coccidiosis caused by *Eimeria tenella, Eimeria necatrix, Eimeria acervulina, Eimeria maxima, Eimeria brunette,* and *Eimeria mivati.*

Table 3. Ingredient composition and nutrient content of finisher and withdrawal diets varying in supplemental fat levels and nutrient profiles.

	Finisher			Withdrawal		
Item (%)	PC	NC1	NC2	PC	NC1	NC2
Ingredients						
Corn	68.00	71.38	67.80	69.75	72.94	69.88
Soybean meal (48%)	19.56	18.58	18.81	17.65	16.98	17.10
DDGS[1]	5.00	5.00	5.26	5.00	5.00	5.00
Meat and bone meal	2.36	2.34	2.38	2.44	2.11	2.47
A/V fat	2.60	0.23	1.50	2.71	0.42	1.50
DL-Met (98%)	0.20	0.19	0.20	0.18	0.16	0.17
L-Lys HCL	0.21	0.21	0.21	0.21	0.21	0.20
L-Thr (98%)	0.06	0.05	0.06	0.05	0.04	0.05
Limestone	0.74	0.75	0.74	0.73	0.80	0.73
Monocalcium PO_4	---	---	---	---	0.05	---
Sodium Chloride	0.19	0.17	0.17	0.14	0.13	0.13
Sodium Bicarbonate	0.32	0.34	0.35	0.38	0.40	0.40
Trace Minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05
Vitamins[3]	0.25	0.25	0.25	00.25	0.25	0.25
Phytase[4]	0.01	0.01	0.01	0.01	0.01	0.01
Coccidistat[5]	0.05	0.05	0.05	0.05	0.05	0.05
Titanium Dioxide	0.40	0.40	0.40	0.40	0.40	0.4
Sand	---	---	1.76	---	---	1.61
Calculated Nutrients						
CP	18.30	18.09	18.00	17.53	17.30	17.29
ME (kcal/kg)	3168	3058	3058	3190	3080	3080
Ca	0.76	0.76	0.76	0.76	0.76	0.76
P	0.44	0.44	0.43	0.43	0.44	0.43
Available P	0.38	0.38	0.38	0.38	0.38	0.38
dig Met	0.47	0.45	0.46	0.43	0.42	0.42
dig Lys	0.90	0.88	0.88	0.85	0.83	0.83
dig Thr	0.61	0.60	0.60	0.58	0.57	0.57
Crude fat	5.87	3.63	4.78	6.04	3.84	4.84
Na	0.20	0.20	0.20	0.20	0.20	0.20
Analyzed Nutrients						
CP	17.80	18.10	17.20	17.40	17.20	16.40
Crude fat	5.90	3.89	5.08	5.98	3.61	5.00
ADF	4.80	4.60	4.00	3.30	2.80	4.80

[1]Distiller's dried grains with solubles

[2]Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3]Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D3, 46 IU vitamin E, 0.0165 mg B12, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[4]OptiPhos 2000 PF. This product is a source of phytase which increases the digestibility of phytin-bound phosphorus in poultry and swine rations.

[5]Active drug ingredient Salinomycin at a use level of 40-60 g/ton. For the prevention of coccidiosis caused by *Eimeria tenella, Eimeria necatrix, Eimeria acervulina, Eimeria maxima, Eimeria brunette,* and *Eimeria mivati*.

Performance Measurements

Experimental animals and residual feed were weighed by pen at 0, 13, 28, 40, and 49 d post-hatch for determination of BW and feed consumption. Mortalities and post-mortem weight were recorded daily for the calculation of percent mortality, BWG, ADFI, and mortality adjusted FCR.

Digestibility Measurements

Ileal digestible energy (IDE) and crude protein (CP) digestibility were determined at 13 d (6 birds per replicate pen), 28 d (5 birds per replicate pen), 40 d (4 birds per replicate pen), and 49 d (3 birds per replicate pen). Ileal samples were pooled and homogenized by pen and then divided into 2 aliquots with one used for the determination of IDE and the other used for the determination of CP digestibility. For IDE determination, samples were dried at 100° C for 24 h and gross energy of feed and ileal digesta was determined using bomb calorimetry (Parr Instrument Company, Moline, IL). Titanium concentration was determined via protocol outlined by Short et al. (1996). This procedure consisted of a half gram of each dried sample being weighed and ashed. Following ashing, each sample was titrated with 10 mL of sulfuric acid (7.4 M) and then boiled at 200°C for 2 h until dissolved. Samples were then titrated with 20 mL of 30% hydrogen peroxide, and brought to 100 mL total volume using distilled water. Samples were then analyzed for absorption using a Thermo Fisher Scientific Genesys 10S UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) at 410 nm. IDE was calculated using the following equation (Scott et al., 1982):

$$GrossEf\ -ExcretaEi\ where\ ExcretaEi\ =\ GEx\ (Tif/Tii)$$

where Gross Ef is gross energy present in the feed, GE is gross energy in the ileal contents, Tif/Tii is the ratio of titanium presence in the feed and ileal contents. Samples used for CP digestibility determination were freeze-dried using a FreeZone 12 L Console Freeze Dry System (Labconco Corporation, Kansas City, MO) prior to analysis. Nitrogen concentration of each dried sample was determined via combustion method, using an Elementar Rapid N Cube Analyzer (Elementar Americas, Inc., Mt. Laurel, NJ). Ileal digestible energy coefficients (IDEC) and ileal digestible nitrogen coefficients (IDNC) were calculated using the following equation (Scott et al., 1982):

$$[(NT/Ti)_d - (NT/Ti)_i] / [(NT/Ti)_d]$$

Where NT represents kcal in the sample, Ti represents the percentage of titanium, the subscript "i" represents the ileal contents and subscripts "d" represents the diet.

Statistical Analysis

All data were analyzed by ANOVA using the General Linear Model (GLM) procedure (SPSS Statistics V26.0, SPSS Inc., Chicago, IL) with statistically different means ($p<0.05$) separated using Duncan's Multiple Range Test post-hoc. Additionally, a factorial ANOVA was conducted using a 2 (diet) x 2 (enzyme) factorial design, with the fixed factors of diet (max of 0.5% fat vs. min of 1.5% fat) and enzyme (control vs.

xylanase), and block as a random factor. For each parameter, pen was used as the experimental unit.

Results

Growth Performance

A significant treatment effect on BW was observed at 13 d (p=0.049) (Table 4). Body weight of broilers fed the NC1 + X diet was greater than that of those fed the PC, whereas the BW of broilers fed the remaining treatments were not significantly different from the PC. Although a main effect of diet was not observed on BW at 13 d (p=0.053), broilers fed the NC1 diet tended to be heavier than those fed the NC2 diet. However, a significant main effect of enzyme was observed on BW at 28 d (p=0.027), where broilers fed xylanase supplemented diets were approximately 30 g heavier than those that were not. . Following 28 d, significant treatment effects were not observed on BW. Additionally, no differences were observed in ADFI or mortality throughout the duration of the study (data not shown).

A significant main effect of diet (p=0.008) was observed during the starter phase where reduced fat levels in the NC1 diets improved FCR by 2.1 points compared to NC2 diets (table 5). The opposite was observed during the grower phase where increased fat levels in the NC2 diets improved phase FCR by 2.1 points compared to NC1 diets (p=0.033). A treatment effect (p=0.004) was also observed during the grower phase where reductions in dAA and energy led to increases in FCR compared to the PC regardless of fat level. The inclusion of xylanase into the NC2 diet led to improvements in grower FCR to levels similar of the PC. Finisher FCR in the NC1 and NC1 + X were

similar to the PC; however, the NC2 and N2 + X had elevated ($p<0.05$) FCR compared to the PC. A significant treatment effect was observed in cumulative FCR 1-28 d ($p=0.023$), 1-40 d ($p=0.015$), and 1-49 d ($p=0.005$). From 1-28 d, an increase in FCR was observed in the NC1 and NC2 fed broilers compared to the PC fed broilers. Inclusion of xylanase into both NC treatments improved cumulative FCR to levels similar of the PC. Similar results were observed from 1-40 d with increases in FCR of NC1 and NC2 treatments compared to the PC; however, during this period only inclusion of xylanase into the NC1 improved FCR to levels comparable to the PC. At the conclusion of the study, increases were observed in cumulative FCR (1-49 d) with all dietary treatments compared to the PC regardless of dietary fat level or enzyme inclusion; however, the inclusion of xylanase reduced cumulative weight corrected FCR (1-49 d) to levels similar of the PC regardless of dietary fat level.

Table 4. BW of male broilers fed diets varying in supplemental fat levels and nutrient profiles, with or without xylanase inclusion.

Treatment		13 d (g)	28 d (kg)	40 d (kg)	49 d (kg)
Positive Control		362.9[b]	1.510	2.817	3.746
NC1[1]		369.4[ab]	1.490	2.753	3.641
NC2[2]		363.1[b]	1.489	2.736	3.622
NC1 + X[3]		375.2[a]	1.522	2.812	3.705
NC2 + X		369.0[ab]	1.519	2.771	3.665
ANOVA					
Pooled SEM		1.4	0.007	0.014	0.020
Pooled CV		2.8	3.5	3.8	4.2
P-value		0.049	0.259	0.157	0.317
Main Effects					
Diet					
NC1	(n=18)	372.3	1.506	2.782	3.673
NC2	(n=18)	366.1	1.504	2.753	3.643
Enzyme					
Control	(n=18)	366.2	1.489[b]	2.744	3.632
Xylanase	(n=18)	372.1	1.520[a]	2.791	3.685
P-value					
Diet		0.053	0.896	0.318	0.534
Enzyme		0.065	0.027	0.114	0.269
Diet × Enzyme		0.993	0.956	0.691	0.828

[a,b,c] Means within columns with different superscripts are significantly different ($P \leq 0.05$)

[1]Energy content reduced by 110 kcal/kg, 1-2% reduction in dAA, and maximum of 0.5% fat compared to the PC.

[2]Energy content reduced by 110 kcal/kg, 1-2% reduction in dAA, and minimum of 1.5% fat compared to the PC.

[3]Inclusion of 1,500 EPU/kg of xylanase (Hostazym® X; Huvepharma, Peachtree City, GA).

Table 5. Mortality corrected FCR (feed:gain) of male broilers fed diets varying in supplemental fat levels and nutrient profiles, with or without xylanase inclusion.

		FCR (Feed:Gain)							bwcFCR[4]
Item		1 - 13 d	14 - 28 d	29 - 40 d	41 - 49 d	1 - 28 d	1 - 40 d	1 - 49 d	1 - 49 d
Treatment									
Positive Control		1.224	1.455^{c}	1.631^{b}	2.053	1.397^{b}	1.477^{b}	1.600^{b}	1.564^{b}
NC1[1]		1.219	1.512^{a}	1.665^{ab}	2.105	1.436^{a}	1.522^{a}	1.639^{a}	1.642^{a}
NC2[2]		1.238	1.489^{ab}	1.699^{a}	2.116	1.425^{a}	1.527^{a}	1.648^{a}	1.659^{a}
NC1 + X[3]		1.208	1.494^{ab}	1.657^{ab}	2.103	1.420^{ab}	1.503^{ab}	1.628^{a}	1.608^{ab}
NC2 + X		1.231	1.475^{bc}	1.686^{a}	2.102	1.413^{ab}	1.513^{a}	1.633^{a}	1.627^{ab}
ANOVA									
Pooled SEM		0.003	0.004	0.006	0.012	0.003	0.004	0.003	0.009
Pooled CV		1.94	2.2	3.1	5.3	1.8	2.2	1.8	4.7
P-value		0.059	0.006	0.047	0.751	0.023	0.015	0.005	0.052
Main Effects									
Diet									
NC1	(n=18)	1.214^{b}	1.503^{a}	1.661	2.100	1.428	1.513	1.633	1.625
NC2	(n=18)	1.235^{a}	1.482^{b}	1.692	2.109	1.419	1.520	1.641	1.643
Enzyme									
Control	(n=18)	1.229	1.500	1.682	2.110	1.431	1.524	1.644	1.650
Xylanase	(n=18)	1.219	1.484	1.672	2.102	1.417	1.508	1.630	1.618
P-value									
Diet		0.008	0.033	0.077	0.898	0.242	0.463	0.366	0.450
Enzyme		0.216	0.105	0.559	0.828	0.066	0.095	0.109	0.182
Diet × Enzyme		0.745	0.839	0.876	0.875	0.805	0.818	0.730	0.967

[a,b,c] Means within columns with different superscripts are significantly different ($P \leq 0.05$)

[1]Energy content reduced by 110 kcal/kg, 1-2% reduction in dAA, and maximum of 0.5% fat compared to the PC.

[2]Energy content reduced by 110 kcal/kg, 1-2% reduction in dAA, and minimum of 1.5% fat compared to the PC.

[3]Inclusion of 1,500 EPU/kg of xylanase (Hostazym® X; Huvepharma, Peachtree City, GA).

[4]Weight corrected FCR: FCR was adjusted to 3.65 kg using 1 point of FCR (0.01) equivalent to 27 g of BW.

Nutrient Digestibility

No significant main effects were observed with diet or enzyme inclusion on IDE or IDEC at any sample period throughout the current study although a significant interaction was observed between diet and enzyme on day 49 measurements (Table 6). A treatment effect was observed on IDE at 14 d (p=0.008), 40 d (p=0.014), and 49 d (p=0.021). A similar trend was observed on IDE at 14 d and 40 d, where all dietary treatments had a significantly lower IDE than the PC regardless of fat level or enzyme inclusion. Nutrient reductions and a maximum of 0.5% supplemental fat in the NC1 led to a significantly lower IDE at 49 d; however, the inclusion of xylanase into the NC1 treatment increased IDE to levels comparable of the PC. Although no differences were observed on IDEC through 40 d, a treatment effect was observed at 49 d (p=0.050). Enzyme supplementation in the NC1+ X treatment increased IDEC compared to the NC1. Although this increase was observed, no negative control treatments were statistically different from the PC regardless of fat level or enzyme inclusion. A significant diet x enzyme interaction was observed on IDE (p=0.010) and IDEC (p=0.010) at 49 d.

A significant main effect of diet was observed on IDNC at 14 d (p=0.018) and 49 d (p=0.025), as well as an enzyme effect at 49 d (p=0.023) (Table 7). Elevated levels of supplemental fat increased IDNC at 14 d in the NC2 treatments compared to the NC1 treatments. The opposite was observed at 49 d where lower levels of supplemental fat in the NC1 treatments increased IDNC compared to the NC2 treatments. The only effect of enzyme supplementation was observed at 49 d with xylanase inclusion increasing IDNC

compared to non-enzyme supplemented treatments. A significant treatment effect was observed on IDNC at 14 d ($p=0.047$), 40 d ($p=0.013$), and 49 d ($p=0.004$). Decreased nutrients and lower fat levels in the NC1 led to reductions in IDNC compared to the PC, regardless of enzyme inclusion. All negative control treatments produced similar IDNC at 40 d, regardless of the fat level or enzyme inclusion. Additionally, IDNC of all negative control treatments was significantly lower than that of the PC at 40 d. At 49 d, the inclusion of xylanase into the NC1 increased IDNC compared to the NC1 treatment. A significant diet x enzyme interaction was observed on IDNC at 49 d, where the NC1 + X yielded the greatest value compared to all dietary treatments, including the PC.

Table 6. Ileal digestible energy (IDE) and ileal digestible energy coefficients (IDEC) of male broilers fed diets varying in supplemental fat levels and nutrient profiles, with or without xylanase inclusion.

	IDE				IDEC			
Item	13 d	28 d	40 d	49 d	13 d	28 d	40 d	49 d
Treatment								
Positive Control	3542[a]	3433	3534[a]	3297[a]	0.787	0.768	0.787	0.742[ab]
NC1[1]	3387[b]	3307	3278[b]	3109[b]	0.767	0.762	0.753	0.724[b]
NC2[2]	3301[b]	3248	3334[b]	3222[ab]	0.747	0.747	0.759	0.741[b]
NC1 + X[3]	3304[b]	3318	3347[b]	3268[a]	0.748	0.764	0.769	0.761[a]
NC2 + X	3342[b]	3351	3385[b]	3170[ab]	0.756	0.770	0.771	0.729[b]
ANOVA								
Pooled SEM	28	25	30	23	0.006	0.006	0.006	0.005
Pooled CV (%)	5.1	4.7	5.1	4.1	4.8	4.5	4.5	3.9
P-value	0.008	0.146	0.014	0.021	0.087	0.658	0.280	0.050
Main Effects								
Diet								
NC1 (n=18)	3349	3312	3313	3189	0.758	0.763	0.761	0.742
NC2 (n=18)	3322	3303	3359	3195	0.752	0.759	0.765	0.729
Enzyme								
Control (n=18)	3344	3280	3306	3163	0.757	0.755	0.756	0.732
Xylanase (n=18)	3325	3336	3366	3219	0.753	0.767	0.770	0.745
P-value								
Diet	0.713	0.914	0.366	0.770	0.671	0.791	0.694	0.476
Enzyme	0.625	0.317	0.247	0.210	0.625	0.318	0.246	0.205
Diet × Enzyme	0.213	0.278	0.856	0.010	0.213	0.279	0.852	0.010

[a,b,c] Means within columns with different superscripts are significantly different ($P \leq 0.05$)

[1]Energy content reduced by 110 kcal/kg, 1-2% reduction in dAA, and maximum of 0.5% fat compared to the PC.

[2]Energy content reduced by 110 kcal/kg, 1-2% reduction in dAA, and minimum of 1.5% fat compared to the PC.

[3]Inclusion of 1,500 EPU/kg of xylanase (Hostazym® X; Huvepharma, Peachtree City, GA).

Table 7. Ileal digestible nitrogen coefficients (IDNC) of male broilers fed diets varying in supplemental fat levels and nutrient profiles, with or without xylanase inclusion.

Treatment		13 d	28 d	40 d	49 d
Positive Control		0.822[a]	0.732	0.800[a]	0.759[b]
NC1[1]		0.779[b]	0.749	0.762[b]	0.753[b]
NC2[2]		0.811[ab]	0.731	0.757[b]	0.765[b]
NC1 + X[3]		0.777[b]	0.728	0.774[b]	0.801[a]
NC2 + X		0.816[ab]	0.750	0.772[b]	0.754[b]
ANOVA					
Pooled SEM		0.007	0.011	0.005	0.006
Pooled CV		5.0	9.0	3.6	4.7
P-value		0.047	0.900	0.013	0.004
Main Effects					
Diet					
NC1	(n=18)	0.777[b]	0.740	0.768	0.780
NC2	(n=18)	0.814[a]	0.740	0.765	0.759
Enzyme					
Control	(n=18)	0.794	0.740	0.760	0.758
Xylanase	(n=18)	0.798	0.740	0.773	0.780
P-value					
Diet		0.018	0.947	0.742	0.025
Enzyme		0.996	0.844	0.134	0.023
Diet × Enzyme		0.868	0.343	0.917	0.010

[a,b,c] Means within columns with different superscripts are significantly different ($P \leq 0.05$)

[1]Energy content reduced by 110 kcal/kg, 1-2% reduction in dAA, and maximum of 0.5% fat compared to the PC.

[2]Energy content reduced by 110 kcal/kg, 1-2% reduction in dAA, and minimum of 1.5% fat compared to the PC.

[3]Inclusion of 1,500 EPU/kg of xylanase (Hostazym® X; Huvepharma, Peachtree City, GA).

Discussion

The supplementation of NSP degrading enzymes as a strategy to improve the nutritive value of feed ingredients has become common practice within today's industry. Arabinose and xylose are two common soluble NSPs that when combined produce arabinoxylan (Choct, 1997). Arabinoxylan is the primary polymer found in the cell wall of most cereal grains, including rye, triticale, wheat, sorghum, and corn (Bach Knudsen, 1997). Elevated levels of these soluble NSPs in poultry diets has shown to reduce nutrient digestibility and absorption, and negatively affect overall bird performance (Antoniou et al., 1981; Choct and Annison, 1990). There is an abundance of literature showing benefits of xylanase in wheat-based diets (Choct et al., 1999; Wang et al., 2005; Gao et al., 2008) instead of corn-based diets, which may be related to a better understanding of the modes of action of these NSPs in viscous cereals (Bedford, 2000). Generally speaking, NSP degrading enzymes provide benefits by either decreasing intestinal digesta viscosity or by softening the encapsulation effect and increasing the availability of nutrients to broilers (Simon, 1998). These mechanisms are rather complex and not completely understood in non-viscous cereals. However, improvements in growth performance and nutrient digestibility have still been observed with xylanase inclusion in corn-based diets (Williams et al., 2014; Amerah et al., 2017).

Besides being the most concentrated source of energy in poultry diets, supplemental fats and oils can improve absorption of vitamins, increase palatability, and reduce the rate of food passage (Baião and Lara, 2005; Firman et al., 2008). Supplemental fat source and level has been observed to have a significant effect on

broiler growth performance, nutrient digestibility, and can potentially change the composition and quality of the carcass (Dänicke et al., 1999b; Baião and Lara, 2005).

In our study, a factorial analysis was conducted to observe the main effects of supplemental fat level and xylanase inclusion in which no significant interactions were present with broiler growth performance. There were no differences observed in BW between the PC and NC1 or NC2 treatments at any point during this study. However, the inclusion of xylanase into the NC1 increased BW beyond that of the PC at 13 d. Multiple studies have reported no differences in BW with energy reductions and xylanase inclusion in both corn and wheat based diets (Singh et al., 2012; Cowieson and Masey O'neill, 2013; Amerah et al., 2017). The only increases in BW were observed in non-energy reduced diets supplemented with xylanase. In our study, the only enzyme main effect on growth performance was observed on BW at 28 d. The inclusion of xylanase increased BW by 31 g compared to the non-enzyme supplemented treatments regardless of supplemental fat level. Increased BW is observed more commonly with the addition of xylanase in wheat-based diets, rather than corn-based diets (Esmaeilipour et al., 2011; Zhang et al., 2014). This limited effect of xylanase in corn-based diets is likely related to the modes of action of xylanase being different in viscous and non-viscous grains. There were also no differences observed in ADFI regardless of the 110 kcal/kg AME reduction or fat level. Energy reductions of 100 kcal/kg were reported not to effect ADFI in a previous study evaluating broiler diets with or without supplemental fat (Masey O'Neill et al., 2012).

In the current study, energy reductions of 110 kcal/kg in the NC1 and NC2 treatments led to increases in FCR during the grower phase and cumulatively 1-28 d, 1-40 d, and 1-49 d. Previous research has shown nutrient reductions to negatively affect broiler performance, specifically FCR, when dietary energy is reduced by 66, 97, and 132 kcal/kg (Williams et al., 2014; Latham et al., 2016b). During the grower phase, we observed that addition of xylanase improved FCR in both NC diets, however, xylanase supplementation was only able to bring performance back to PC levels in the NC2 diet.

Performance benefits in later growth phases can be attributed to xylanase promoting a beneficial bacteria population and increasing the fermentative capacity of the ceca (Masey O'Neill et al., 2012). This may not be the case in the current study, as the differences in FCR became less apparent from 1-28 d to 1-49 d, with all NC treatments having a higher FCR than the PC regardless of fat level or enzyme inclusion. During the starter phase, increased level of fat in the NC2 led to increases in FCR. The opposite was observed during the grower phase with increases of supplemental fat in the NC2 improving FCR. The effect of fat level on FCR from the starter to grower of the current study may be related to an age effect on the digestibility of lipids. Previous research shows physiological limitations of younger birds to effectively digest and utilize fats, regardless of source, which can lead to potential effects on performance (Tancharoenrat et al., 2010; Tancharoenrat et al., 2013).

Research conducted evaluating fat type (10% soybean oil or 10% beef tallow), enzyme supplementation (with or without xylanase), and NSP level (7.7 g/kg to 17.6 g/kg soluble pentosans) determined energy utilization and crude protein digestibility

decreased with increasing levels of dietary NSP but could be improved with the addition of xylanase (Dänicke et al., 1999b). The effect of xylanase was more pronounced in the tallow diets as compared to the soybean oil diets. In the current study, supplemental fat level was evaluated instead of source; however, the inclusion of xylanase at 49 d still presented improvements in nutrient digestibility by increasing IDE in the NC1 diet and IDNC regardless of diet. The only diet × enzyme interactions observed in the current trial were on IDE, IDEC, and IDNC at 49 d. The inclusion of xylanase into the NC1 improved energy and nitrogen digestibility to levels similar or beyond that of the PC.

In this study, we investigated the interaction of supplemental fat incorporation and xylanase inclusion, and evaluated broiler performance and nutrient digestibility. We observed that reductions in dietary energy effected broiler performance, specifically through differences in FCR, IDE, and IDNC regardless of supplemental fat level. The inclusion of xylanase helped mitigate these effects by improving FCR during the finisher phase and 1-40 d, as well as increasing IDE at 49 d. The significance of dietary fat level was most apparent on starter and grower FCR, as well as IDNC at 14 d; however, minimal interactions were observed between fat level and xylanase throughout the duration of the study. These data support the idea that there is no significant effect of varying supplemental fat inclusion on xylanase efficacy when evaluating broiler growth performance and nutrient digestibility. However, further research is needed to determine if supplemental fat levels of other fat sources may have an effect on xylanase efficacy.

CHAPTER III

EVALUATION OF XYLANASE ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY OF BROILERS FED DIETS WITH CORN FROM DIFFERENT GEOGRAPHICAL LOCATIONS

Introduction

Cereal grains are the primary source of energy in poultry feed manufacturing and can represent up to 70 percent of the total diet composition (Black et al., 2005). The top two grains cultivated in the world are corn and wheat, making up approximately 43 and 28 percent of total grain production, respectively (USDA-NASS-FAS, 2019). Corn has been used as the primary cereal grain within the United States due to its high available energy and relatively low non-starch polysaccharide (**NSP**) content. Approximately 65 percent of the ME and 20 percent of the protein in a standard broiler starter ration can be attributed to corn (Cowieson, 2005). Despite some advantages of corn over other grains, the nutrient content of any cereal can still vary between, and within, each specific grain species. Factors contributing to variability in corn include plant variety, geographical location and climate, post-harvest processing and storage, lipid/protein/starch matrices, fiber content, and heat treatment, in which all can greatly affect the performance and profitability of poultry production (Socorro et al., 1989; Herrera-Saldana et al., 1990; Leeson et al., 1993; Brown, 1996; Cromwell et al., 1999; Collins et al., 2001; Gehring et al., 2012; Williams et al., 2018). Understanding these factors is critical for maximizing the nutritive value you can get out of corn, which is dependent on the total content of starch, oil, protein, and anti-nutritive components (Cowieson, 2005).

The AME of cereal grains is largely affected by starch digestibility (Rogel et al., 1987), where even small differences in digestibility can have a large effect on total AME of the diet. Chemical analysis of more than 50 different corn samples presented starch content ranging from 628 to 720 g/kg and leads to a potential AME variation of more than 400 kcal/kg (Cowieson, 2005). Starch is contained in a starch-protein matrix of the corn endosperm, and surrounded by the aleurone layer. The aleurone layer is essentially a barrier that prevents endogenously produced enzymes from fully accessing the starch and protein component, limiting nutrient digestibility (Theander et al., 1989). Starch digestibility is affected by the starch structure and starch-protein associations within the feedstuff (Weurding et al., 2001), suggesting that restricted access to these nutrients in corn may lead to a reduction in digestion limiting the overall AME value of the cereal.

Non-starch polysaccharides are anti-nutritive factors that consist of multiple soluble and insoluble polysaccharides found within the cell wall of plant-based feedstuffs. The presence of NSP, such as arabinoxylan and β-glucan, are crucial in determining the utilization of starch in broilers. Multiple mechanisms have been proposed for the anti-nutritive activity of NSP; however, two are consistently recognized. The first mechanism is that, following ingestion of plant-based feedstuffs, the proportion of NSP in the cell wall solubilizes and results in increased intestinal digesta viscosity. The second mechanism is known as the “cage effect”, where insoluble NSP in the cell wall encapsulate essential nutrients reducing the availability to monogastric animals. Furthermore, these NSP are secured together by inter- and intramolecular hydrogen bonding that renders the carbohydrate portion resistant to

enzymatic hydrolysis (Classen, 1996). This, combined with poultry's limited endogenous enzymatic capacity, allows nutrients to bypass digestion completely or not be broken down until bacterial fermentation occurs in the lower gut.

One way to minimize the effect of ingredient variability on dietary nutrient content is to supplement exogenous enzymes and alleviate the negative effects of anti-nutritive factors. Non-starch polysaccharide degrading enzymes have shown to be highly effective in wheat-based diets by hydrolyzing water-soluble NSP, reducing the intestinal digesta viscosity, and improving nutrient digestibility (Bedford and Schulze, 1998; Choct et al., 1999; Adeola and Bedford, 2004; Gao et al., 2008). Benefits of these enzymes in corn-based diets has been much more variable (Bedford and Classen, 1992; Meng et al., 2005), and is likely related to the lower NSP level compared to wheat-based diets (Choct, 1997). Enzyme inclusion, specifically with xylanase, has been demonstrated to have an effect on intestinal microbial populations within poultry. The hydrolysis of structural fiber produces short-chain xylo-oligomers that can be partially fermented in the hindgut, changing the profile of volatile fatty acids and improving broiler performance (Cowieson and Masey O'neill, 2013). It is crucial to understand all factors that can affect the nutritive value of feed ingredients as well as the efficacy of exogenous enzymes. In this study, we evaluated the efficacy of xylanase in diets with corns from different geographical locations on the growth performance, nutrient digestibility, and ileal short-chain fatty acid profile of male Cobb 500 broilers.

Materials and Methods

Experimental Animals and Husbandry

Male broiler chicks (Cobb) were obtained from a commercial hatchery on day of hatch, weighed, wingbanded, and assigned randomly to pens to ensure statistically similar starting pen weights at a stocking density of 0.093 m^2 per bird. Experimental animals were raised in floor pens, provided age appropriate heat and ventilation, and given access to potable water and experimental rations for ad libitum consumption for the duration of the study. Each 1.67 m^2 contained a tube feeder, a nipple drinker line, and fresh pine shavings as litter. Temperature was monitored, recorded daily, and adjusted to maximize bird comfort. The lighting program was as follows: 1 to 3 d, 24 h of light at 21.53 lux; 4 to 8 d, 23 h of light at 21.53 lux; 9 to 18 d, 16 h of light at 8.07 lux; 19 to 32 d, 18 h of light at 1.08 lux; and 33 d to termination, 20 h of light at 0.54 lux. All procedures were performed as approved by the Texas A&M University Institutional Animal Care and Use Committee.

Experimental Design and Diets

The effect of different corn samples on the efficacy of xylanase in improving growth performance and nutrient digestibility, and volatile fatty acid profile of male Cobb 500 broilers was evaluated. Broiler chicks (n=1,080) were allocated to six experimental treatment groups arranged as a randomized complete block design with 10 replicate pens of 18 birds per treatment. Broilers were fed one of six dietary treatments in a 3 × 2 factorial arrangement with three corn sources and two xylanase levels.

Experimental rations were fed for the duration of the study using a 3-phase feeding plan: starter (0-18 d, crumble), grower (19-33 d, pellet), and finisher (34-42 d, pellet).

Experimental Rations

Corn and soybean meal based diets were formulated on a least-cost basis to be iso-nitrogenous and iso-caloric using the NRC (1994) value for corn within each feeding phase (Table 8). During feed manufacturing, one large premix was created containing all ingredients with the exception of corn to eliminate nutrient variability among the experimental diets. An equal amount of premix was then combined with the same amount of each corn source allowing all differences in the diet to be attributable to the corn. The final diets manufactured for each corn source were then divided into two equal parts receiving supplementation of xylanase (Econase XT, AB Vista, Marlborough, Wiltshire, UK) at 0 or 16,000 BXU/kg. The xylanase preparation contained 160,000 units of endo-1,4- β -xylanase activity (EC 3.2.1.8) per gram. One birch xylan unit (BXU) is defined as the amount of enzyme that liberates 1 nmol reducing carbohydrates from birchwood xylan, measured as xylose equivalents, under the conditions of the assay (AB Enzymes, Germany). Titanium dioxide was included in the finisher phase diets as an indigestible marker to determine ileal digestible energy (IDE).

Diets were manufactured for each phase using a 2-ton double-ribbon, horizontal Scott mixer and a 1 ton/h California Pellet Mill equipped with a conditioner and a 4.4 mm diameter die; and, when appropriate, crumbled using a roller mill. Pelleting temperatures were maintained between 74° and 76°C to ensure the maximum level of enzyme recovery was achieved.

Triplicate feed samples were collected from each dietary treatment, and proximate analysis of composite samples was performed by a third-party laboratory (Midwest Laboratories, Omaha, NE). Crude protein was determined using combustion (AOAC 990.03), crude fat was determined using petroleum ether extraction (AOAC 945.16), ADF was determined gravimetrically (AOAC 973.18), and total phosphorous was determined by wet ash inductively coupled with plasma spectroscopy (AOAC 985.01M). Finished feeds were analyzed for xylanase activity using an ELISA method (Envirologix method AP019, Enzyme Services & Consultancy, Cordova, TN) and reported (Table 10).

Table 8. Ingredient composition and nutrient content of diets fed to male Cobb 500 broilers.

Item (%)	Starter	Grower	Finisher
Ingredients			
Corn	64.30	69.85	73.45
Soybean meal (48%)	32.44	27.10	23.62
DL-Met (98%)	0.27	0.24	0.21
L-Lysine HCL	0.18	0.18	0.17
L-Threonine (98%)	0.05	0.05	0.06
Soy oil	0.20	0.20	0.41
Limestone	1.19	1.11	0.98
Monocalcium PO_4	0.75	0.66	0.52
Sodium Chloride	0.27	0.26	0.23
Sodium Bicarbonate	0.05	0.05	0.05
Trace Minerals[1]	0.05	0.05	0.05
Vitamins[2]	0.25	0.25	0.25
Phytase[3]	0.01	0.01	0.01
Titanium Dioxide	----	----	0.40
Calculated Nutrients			
CP	21.57	19.42	18.00
ME (kcal/kg)	2950	3000	3050
Ca	0.90	0.84	0.76
P	0.74	0.69	0.64
Available P	0.45	0.42	0.38
dig Met	0.56	0.51	0.46
dig Lys	1.18	1.05	0.95
dig Thr	0.77	0.69	0.65
Crude fat	3.06	3.17	3.45
Na	0.17	0.16	0.15

[1]Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[2]Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D3, 46 IU vitamin E, 0.0165 mg B12, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[3]Quantum Blue 5G (AB Vista, Marlborough, Wiltshire, UK). This product is a source of phytase which increases the digestibility of phytin-bound phosphorus in poultry and swine rations, included at 500 FTU/kg.

Corn Source

Corn used in this study was obtained from from three different geographical locations in the United States (Texas (**A**), Ohio (**B**), Iowa (**C**)) to represent the variability in corn available to feed manufacturers. Prior to initiation of the study, proximate and physiochemical analyses (Table 9) were conducted using Near Infrared Reflectance (NIR) spectroscopy which was carried out using a Foss 6500 NIR spectrophotometer (FOSS NIR Systems, Inc., Laurel, MD). The analyses were conducted at Aunir (a division of AB Agri, Towcester, UK) and the calibrations were based on wet chemistry analyses of more than 1,000 corn samples, as described by Piotrowski et al. (2011).

Performance Measurements

Experimental animals and residual feed were weighed by pen at 0, 18, 33, and 42 d post-hatch for determination of BW and feed consumption. Mortalities and post-mortem weight were recorded daily for the calculation of percent mortality, BWG, ADFI, and mortality adjusted FCR.

Digestibility Measurements

Ileal digestible energy (**IDE**) and crude protein (**CP**) digestibility were determined at 42 d (3 birds per replicate pen). Ileal samples were pooled and homogenized and then used for the determination of IDE. For IDE determination, samples were dried at 100° C for 24 h and gross energy of feed and ileal digesta was determined using a Parr 6400 bomb calorimeter (Parr Instrument Company, Moline, IL). Titanium concentration was determined via protocol outlined by Short et al. (1996). This procedure consisted of a half gram of each dried sample being weighed and ashed.

Following ashing, each sample was titrated with 10 mL of sulfuric acid (7.4 M) and then boiled at 200°C for 2 h until dissolved. Samples were then titrated with 20 mL of 30% hydrogen peroxide, and brought to 100 mL total volume using distilled water. Samples were then analyzed for absorption using a Thermo Fisher Scientific Genesys 10S UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) at 410 nm. IDE was calculated using the following equation (Scott et al., 1982):

$$GrossEf\ -ExcretaEi\ where\ ExcretaEi\ =\ GEx\ (Tif/Tii)$$

where Gross Ef is gross energy present in the feed, GE is gross energy in the ileal contents, Tif/Tii is the ratio of titanium presence in the feed and ileal contents. Samples used for CP digestibility determination were freeze-dried using a FreeZone 12 L Console Freeze Dry System (Labconco Corporation, Kansas City, MO) prior to analysis. Nitrogen concentration of each dried sample was determined via combustion method, using an Elementar Rapid N Cube Analyzer (Elementar Americas, Inc., Mt. Laurel, NJ). Ileal digestible nitrogen coefficients (IDNC) were calculated using the following equation (Scott et al., 1982):

$$[(NT/Ti)_d - (NT/Ti)_i] / [(NT/Ti)_d]$$

Where NT represents kcal in the sample, Ti represents the percentage of titanium, the subscript "i" represents the ileal contents and subscripts "d" represents the diet.

Short-Chain Fatty Acid Determination

Short-chain fatty acid (**SCFA**) concentration was determined at 42 d (3 birds per replicate). Ileal SCFA were analyzed as described previously (Williams et al., 2018). Briefly, ileal contents collected from each sampled bird were pooled by pen, homogenized, acidified using 1 ml 25% (w/v) metaphosphoric acid, brought to a total volume of 5 mL using diH_2O, and frozen for storage (-20^oC). Frozen samples were thawed, vortexed, and centrifuged (20,000 × g, 20 min), and supernatants were analyzed for SCFA using gas chromatography with flame ionization detection. The polyethylene glycol GC column was operated at 100 to 150^oC with carrier gas flow (N_2) at 1.8 mL min^{-1}.

Statistical Analysis

All data were analyzed as a 3 (corn source) x 2 (xylanase) factorial ANOVA using the General Linear Model (GLM) procedure (SPSS Statistics V26.0, SPSS Inc., Chicago, IL), with the fixed factors of corn source (A, B, C) and xylanase (0 or 16,000 BXU/kg), and block as a random factor. In the event that a significant interaction was detected, data were analyzed using a one-way ANOVA. Main effect and treatment means were deemed significant at $P \leq 0.05$ and separated using Duncan's Multiple Range Test post-hoc. The experiment unit used for each parameter was pen.

Results

Corn Analysis

Pre-study analysis of corn from different geographical locations provided the intended nutrient variation between each corn source (Table 9). Differences across the three sources included 1% moisture, 0.8% CP, 0.1% fiber, 1.6% starch, 2.2% protein solubility index (PSI), and 0.2% oil. Proximate analysis values were included in a prediction equation to determine estimated AME of each corn source. A 40 kcal/kg reduction was observed from Corn A to Corn B, and a further reduction of 39 kcal/kg was observed from Corn B to Corn C to achieve a total difference in AME of 79 kcal/kg between Corn A and Corn C. Finished diet analysis determined differences among corn sources during each feeding phase (Table 10).

Table 9. Analyzed nutrient profiles[1] of corn sourced from different geographical locations.

Source[2]	Moisture (%)	CP (%)	Fiber (%)	Starch (%)	PSI[3] (%)	Oil (%)	AME[4] (kcal/kg)
Corn A	14.6	8.3	2.5	77.9	33.7	3.7	3,232
Corn B	15.6	7.7	2.4	78.5	32.4	3.9	3,192
Corn C	15.5	8.5	2.6	76.9	31.5	3.9	3,153

[1] Results are expressed per 100% of dry matter.
[2] Corn sources: A, Texas; B, Ohio; C, Iowa
[3] Protein Solubility Index.
[4] Predicted AME value on an as-is basis using a prediction equation based on NIR values.

Table 10. Analyzed nutrient content of basal diets and enzyme recovery from finished diets with various corn sources[1] fed to male Cobb 500 broilers.

	Starter			Grower			Finisher		
Item (%)	Corn A	Corn B	Corn C	Corn A	Corn B	Corn C	Corn A	Corn B	Corn C
Basal Diets									
CP	18.7	20.0	19.4	16.9	18.7	19.2	17.9	16.5	17.0
Crude Fat	3.57	2.93	3.02	3.56	3.08	2.60	2.73	2.66	2.58
ADF	2.1	2.5	3.1	2.7	2.8	3.1	3.0	3.5	3.0
Total Ca	0.75	0.79	0.72	0.79	0.79	0.68	0.83	0.77	0.71
Total Na	0.12	0.13	0.13	0.11	0.12	0.12	0.12	0.11	0.12
Total P	0.54	0.55	0.55	0.48	0.53	0.51	0.50	0.48	0.46
Ash	3.97	4.16	4.16	3.88	4.09	3.94	4.17	4.02	4.08
ME (kcal/kg)	3042	2932	2954	3042	2976	2932	2976	2954	2976
Enzyme Recovery from Finished Diets (BXU/kg)									
Control	<2,000	<2,000	~3,500	<2,000	<2,000	~3,500	~3,500	~3,500	~2,500
Xylanase	12,100	14,200	12,900	14,800	14,900	15,100	15,500	15,300	14,900

[1] Corn sources: A, Texas; B, Ohio; C, Iowa

Growth Performance

A significant main effect of corn source was observed at 33 d ($p = 0.013$), where BW of broilers fed Corn C were 60 g heavier that those fed Corn B (Table 11). No other differences were observed in BW throughout the duration of the study.

A significant corn source × xylanase interaction was observed on ADFI during the grower phase ($p = 0.005$), as well as cumulatively 1 to 33 d ($p = 0.008$) and 1 to 42 d ($p = 0.049$) (Table 11). During the grower phase, the inclusion of xylanase into the Corn C diet increased ADFI compared to broilers fed Corn C without xylanase, while xylanase supplementation into the Corn B diet reduced ADFI ($p = 0.001$) compared to broilers fed Corn B without xylanase. During the finisher phase, a significant main effect of xylanase was observed, where xylanase inclusion reduced ADFI by approximately 5.5 g/bird/d compared to non-xylanase supplemented treatments. From 1 to 33 d, the inclusion of xylanase had variable effects on ADFI depending on the corn source. The inclusion of xylanase into Corn B diets led to the lowest cumulative ADFI, while supplementation of xylanase into Corn C diets led to the highest cumulative ADFI. Similar treatment effects were observed on ADFI from 1 to 42 d as inclusion of xylanase in Corn C led to the highest ADFI observed which was significant higher than broilers fed diets with xylanase and fed Corn A and B.

A significant main effect of corn source was observed on starter FCR ($p = 0.002$), in which broilers fed diets containing Corn A exhibited a lower FCR as compared to broilers fed either Corn B or Corn C diets with a 2.1 and 1.2 point advantage, respectively (Table 12). A significant interaction between corn source and

xylanase was observed for grower FCR as the addition of xylanase resulted in different outcomes depending on the dietary corn source. The addition of xylanase to diets containing Corn C increased observed FCR as compared to the control fed Corn C broilers while inclusion of xylanase to Corn A and B fed broilers had no significant effect on observed grower FCR. This observation in the grower phase resulted in a similar response for cumulative FCR from 1 to 33 day of age with broilers fed Corn C with xylanase exhibiting a higher FCR than Corn C fed broilers without xylanase. A significant main effect of xylanase was observed during the finisher phase ($p = 0.001$) on FCR with a 5.6 point reduction in FCR in broilers fed xylanase as compared to non-xylanase fed broilers. At the conclusion of the trial, significant main effect differences were observed for corn source ($p = 0.035$) and xylanase ($p = 0.040$) on FCR from 1 to 42 d. Broilers fed diets with Corn B yielded a cumulative FCR of 1.3 points higher than those fed diets with Corn A, and the inclusion of xylanase reduced FCR 0.9 points from 1 to 42 d.

Table 11. BW and ADFI of male broilers fed diets containing different corn sources with varying levels of xylanase to 42 d post-hatch.

Treatments			BW			ADFI (g/bird-day)				
Corn[1]	Xylanase[2]		18 d (g)	33 d (kg)	42 d (kg)	Starter	Grower	Finisher	1 to 33 d	1 to 42 d
A	Control		668.4	2.052	3.005	46.08	152.19^{ab}	204.51	94.32^{ab}	117.93^{ab}
A	Xylanase[1]		677.2	2.048	2.984	45.87	150.34^{b}	197.54	92.84^{bc}	115.27^{bc}
B	Control		673.0	2.045	2.983	46.95	150.13^{b}	204.90	93.85^{ab}	117.64^{ab}
B	Xylanase		669.8	1.988	2.921	46.95	145.48^{c}	196.90	91.39^{c}	114.00^{c}
C	Control		676.1	2.086	3.002	46.55	150.47^{b}	203.81	93.79^{ab}	117.36^{ab}
C	Xylanase		677.7	2.068	3.017	46.91	155.21^{a}	202.31	95.78^{a}	118.61^{a}
ANOVA										
P-value							0.001		0.003	0.019
Main Effects										
Corn Source										
A		(n=20)	672.8	2.050^{ab}	2.995	45.98	151.32	201.02	93.58	116.60
B		(n=20)	671.4	2.017^{b}	2.952	46.95	147.92	200.90	92.62	115.82
C		(n=20)	676.9	2.077^{a}	3.010	46.73	152.72	203.06	94.79	117.99
Xylanase										
Control		(n=30)	672.5	2.061	2.997	46.53	150.93	204.40^{a}	93.98	117.64
Xylanase		(n=30)	674.9	2.035	2.974	46.58	150.34	198.92^{b}	93.34	115.96
P-values										
Corn Source			0.640	0.013	0.100	0.112	0.003	0.766	0.015	0.110
Xylanase			0.630	0.106	0.312	0.899	0.570	0.048	0.274	0.049
Corn × Xylanase			0.610	0.374	0.373	0.835	0.005	0.577	0.008	0.049
Pooled SEM			3.0	0.012	0.021	0.23	0.98	2.09	0.49	0.75

[a,b,c] Means within columns with different superscripts are significantly different ($P \leq 0.05$)

[1] Corn Source: A, Texas; B, Ohio; C, Iowa

[2] Xylanase added at 16,000 BXU/kg feed

Table 12. Mortality corrected FCR (feed:gain) and nutrient digestibility of male broilers fed diets containing different corn sources with varying levels of xylanase to 42 d post-hatch.

Treatments		FCR					Digestibility[3]	
Corn[1]	Xylanase[2]	Starter	Grower	Finisher	1 to 33 d	1 to 42 d	IDE[3]	IDNC[4]
A	Control	1.346	1.658[a]	1.934[b]	1.559[ab]	1.678	3352	0.814
A	Xylanase[1]	1.330	1.646[a]	1.931[b]	1.544[bc]	1.662	3413	0.815
B	Control	1.359	1.650[a]	1.989[a]	1.556[ab]	1.690	3130	0.810
B	Xylanase	1.360	1.668[a]	1.917[b]	1.566[a]	1.677	3163	0.833
C	Control	1.351	1.606[b]	2.014[a]	1.525[c]	1.673	3381	0.810
C	Xylanase	1.348	1.667[a]	1.917[b]	1.565[ab]	1.677	3413	0.826
ANOVA								
P-value			<0.001	0.002	0.001			
Main Effects								
Corn Source								
A	(n=20)	1.338[b]	1.652	1.933	1.551	1.670[b]	3380[a]	0.815
B	(n=20)	1.359[a]	1.659	1.953	1.561	1.683[a]	3147[b]	0.821
C	(n=20)	1.350[a]	1.636	1.967	1.545	1.675[ab]	3394[a]	0.818
Xylanase								
Control	(n=30)	1.352	1.638	1.979	1.547	1.681[a]	3288	0.811
Xylanase	(n=30)	1.346	1.660	1.923	1.558	1.672[b]	3317	0.825
P-values								
Corn Source		0.002	0.073	0.212	0.077	0.035	0.024	0.886
Xylanase		0.204	0.007	0.001	0.044	0.040	0.681	0.227
Corn × Xylanase		0.322	0.002	0.054	0.001	0.122	0.920	0.708
Pooled SEM		0.003	0.006	0.010	0.004	0.002	49	0.006

[a,b,c] Means within columns with different superscripts are significantly different ($P \leq 0.05$)

[1] Corn Source: A, Texas; B, Ohio; C, Iowa

[2] Xylanase added at 16,000 BXU/kg feed

[3] Digestibility measures: IDE, Ileal digestible energy; IDNC. Ileal digestible nitrogen coefficient

Nutrient Digestibility

A significant main effect of corn source was observed on IDE at 42 d ($p = 0.024$). Ileal digestible energy of Corn B was approximately 240 kcal lower than that of Corn A and Corn C (Table 12). No differences in nitrogen digestibility were observed regarding corn source. Addition of xylanase did not significantly increase ileal digestibility of energy or nitrogen although increases of 29 kcal/kg and 1.4% were observed, respectively.

Short-Chain Fatty Acid Determination

A significant main effect of corn source was observed on ileal isobutyric acid at 42 d ($p = 0.049$) (Table 13). Broilers fed Corn B diets exhibited lower isobutyric levels than those fed Corn C, while those fed Corn A diets were intermediate. Additionally a significant main of xylanase was observed on ileal lactic acid ($p = 0.036$) and total SCFA ($p=0.033$), where xylanase supplementation reduced lactic acid and SCFA levels by 44 and 34.4 percent respectively, compared to non-xylanase supplemented treatments. No other differences were observed in ileal SCFA content, regardless of corn source or xylanase inclusion.

Table 13. Short-chain fatty acid profile (mM) of ileal contents in 42 d male broilers fed diets containing different corn sources with varying levels of xylanase to 42 d post-hatch.

Corn[1]	Xylanase	Acetic	Propionic	Butyric	Valeric	Isobutyric	2-me-butyric	Isovaleric	Lactic	BCFA[3]	VFA[4]	SCFA[5]
A	Control	10.64	1.753	0.598	4.808	0.132	1.056	0.630	72.91	1.818	19.88	92.80
A	Xylanase[1]	9.46	1.865	0.508	4.944	0.076	0.711	0.379	32.93	1.166	17.55	52.26
B	Control	10.58	1.642	0.483	5.046	0.026	0.796	0.482	52.29	1.303	19.05	71.34
B	Xylanase	10.33	1.529	0.506	4.727	0.020	0.706	0.419	50.67	1.144	17.79	68.47
C	Control	10.85	1.694	0.521	5.206	0.153	0.799	0.412	78.00	1.364	20.08	98.08
C	Xylanase	10.49	1.853	0.798	4.856	0.197	0.866	0.651	30.21	1.803	19.40	51.64
Main Effects												
Corn Source												
A	(n=20)	10.08	1.809	0.553	4.876	0.104[ab]	0.883	0.505	52.92	1.492	18.78	72.53
B	(n=20)	10.46	1.585	0.495	4.887	0.023[b]	0.751	0.450	51.48	1.224	18.42	69.90
C	(n=20)	10.68	1.773	0.666	5.041	0.174[a]	0.833	0.531	54.10	1.583	19.75	74.86
Xylanase												
Control	(n=30)	10.69	1.696	0.534	5.027	0.104	0.884	0.508	67.73[a]	1.495	19.67	87.40[a]
Xylanase	(n=30)	10.09	1.745	0.604	4.838	0.094	0.761	0.483	37.93[b]	1.371	18.23	57.46[b]
P-values												
Corn Source		0.696	0.453	0.555	0.930	0.049	0.607	0.798	0.988	0.413	0.553	0.957
Xylanase		0.311	0.747	0.440	0.489	0.865	0.263	0.803	0.036	0.587	0.231	0.033
Corn × Xylanase		0.828	0.781	0.293	0.853	0.724	0.305	0.142	0.353	0.158	0.813	0.374
Pooled SEM		0.31	0.092	0.051	0.177	0.021	0.066	0.057	8.45	0.125	0.59	8.39

[a,b] Means within columns with different superscripts are significantly different ($P \leq 0.05$)

[1] Corn Source: A, Texas; B, Ohio; C, Iowa

[2] Xylanase added at 16,000 BXU/kg feed

[3] BCFA, total branched-chain fatty acids; includes Isobutyric, 2-me-butyric, and Isovaleric

[4] VFA, total volatile fatty acids; includes Acetic, Propionic, Butyric, Valeric, and BCFA

[5] SCFA, total short-chain fatty acids; includes VFA and Lactic

Discussion

Corn can contribute up to 65 percent of the metabolizable energy requirement in broilers (Cowieson, 2005). The physical composition and nutritional value of corn can fluctuate with plant variety, geographical location and climate, and post-harvest processing and storage (Socorro et al., 1989; Herrera-Saldana et al., 1990; Leeson et al., 1993; Brown, 1996; Cromwell et al., 1999; Collins et al., 2001; Gehring et al., 2012). Previous research evaluating corn crops across 16 different states showed differences in crude protein ranging from 7.31 to 9.06 percent and lysine content differences between 0.25 to 0.30 percent (Cromwell et al., 1999). Evaluation of corn crops across 15 different countries presented total starch content ranging from 64.5 to 69.2 percent (D'Alfonso and McCracken, 2002). These observed differences propose that variation in corn quality and nutrients may have a negative effect on broiler growth performance.

In the current study, three sources of corn were obtained from different regions within the United States to represent variation of corn that could be available to the feed industry. Proximate analysis of these corn sources determined differences of 1% moisture, 0.8% CP, 0.1% fiber, 1.6% starch, 2.2% PSI, and 0.2% oil. Analyzed values were included in a prediction equation that estimated an AME difference of 79 kcal/kg between corns; a 40 kcal/kg reduction was observed from Corn A to Corn B, and a further reduction of 39 kcal/kg was observed from Corn B to Corn C. Exogenous enzymes are commonly included in dietary formulations as a strategy to alleviate some of the negative effects associated with nutrient variation in feed ingredients. The efficacy of these enzymes are dependent on the diet composition and total anti-nutritive activity,

and the inclusion of xylanase has led to improvements in performance and nutrient digestibility in broilers fed corn-soybean meal based diets (Williams et al., 2014; Amerah et al., 2017).

Corn source had an effect on BW at 33 d, with observed differences of approximately 60 g. There were no other significant differences observed on BW regardless of corn source or xylanase inclusion; however, the 60 g difference was still present at 42 d. Previous research shows differences in BW at 49 d while feeding two different corn sources; however these sources had similar nutrient profiles (CP, amino acids, fat, fiber, ash, AME) but different grain size and hardness (Collins et al., 2001), suggesting composition characteristics may have a significant effect on performance independent of nutrient profile. Greater differences in corn nutrient profile of the current study may be associated with BW differences being observed at a younger age.

During enzyme supplementation, ADFI can vary due to the hydrolysis of NSP releasing encapsulated protein and starch, making some nutrients more readily available to broilers (Bedford, 1996). In the current study, finisher ADFI was reduced with xylanase supplementation regardless of corn source. During the grower phase, and cumulatively from 1 to 33 d and 1 to 42 d, an interaction was observed between corn source and xylanase. The effect of xylanase was inconsistent across corn sources, with enzyme inclusion reducing ADFI in corn B, increasing ADFI in Corn C, and having no effect on ADFI in Corn A. Inconsistent results of enzyme supplementation has been well-documented, with different enzyme combinations either increasing or having no effect on ADFI (Yegani and Korver, 2013; Latham et al., 2016a). Nutrient quality has

been reported to be the greatest determining factor on enzyme responses (Cowieson, 2010), and variation of corn nutrient profile with equal addition of each source in the current study, may be the primary factor determining the effect of xylanase inclusion on ADFI.

An interaction was observed between corn source and xylanase on grower and cumulative FCR from 1 to 33 d. Similar to ADFI, the enzyme response on FCR was inconsistent across the different corn sources during these periods. Xylanase supplementation to broilers fed Corn C led to increases in grower FCR but reductions to finisher FCR, for overall increases in FCR from 1 to 33 d. The inclusion of xylanase in Corn A led to reductions in FCR from 1 to 33 d, whereas inclusion in Corn B led to no differences from 1 to 33 d. Corn source had an effect on starter FCR and cumulative FCR from 1 to 42 d, with differences of 2.1 and 1.3 points, respectively. Xylanase has been demonstrated to be successful in improving FCR in multiple published reports, which is similar to data observed in this experiment (Gao et al., 2008; Esmaeilipour et al., 2011; Masey O'Neill et al., 2012). Variable data on FCR demonstrates the negative effect that differences in corn nutrient profile can have on performance; however, finisher FCR in the current study shows the ability of xylanase to mitigate the differences of corn nutrient profile. Xylanase inclusion led to differences in finisher FCR of 0.3, 7.2, and 9.7 points in Corn A, B, and C, respectively, to yield similar FCR across all xylanase-supplemented treatments.

The mode of action of enzymes in corn-soybean meal based diets has been linked to improved access to cell wall contents by a reduction of cell wall integrity, improved

starch digestibility or increased digestion of resistant starches, modification of intestinal microbe populations, and an overall reduction in the adverse effects of corn and soy-derived anti-nutritive factors (Cowieson and Ravindran, 2008; Tang et al., 2014). In our study, corn source had a significant effect on IDE; however, no other differences were observed on IDE or IDNC, regardless of corn source or xylanase inclusion. Others have reported inconsistent results of enzyme inclusion on IDE and CP digestibility and determined that corn source, enzyme product, dietary phase, or an interaction of multiple factors may play a role in enzyme response (Mahagna et al., 1995; Yegani and Korver, 2013). Similar differences were observed on IDE in previous research evaluating four different corn sources (Tang et al., 2014), and is consistent with data reporting that the AME of corn can vary by more than 400 kcal/kg in different samples, making general energy matrix values for corn less accurate (Cowieson, 2005). Increases observed in IDE during the current study were inversely related to the total starch content of each corn source. Although IDE improvements have been linked to starch digestibility, total starch content of corn sources may not be a practical indicator in estimating this, suggesting grain composition and nutrient profile as a whole can play a significant role in the effect on broiler performance and nutrient digestibility.

At 42 d, corn source had an effect on ileal isobutyric levels, with Corn B having levels 86.8 percent lower than Corn C. Xylanase had an effect on ileal lactic acid and total SCFA levels, with enzyme supplementation reducing lactic levels by 44 percent and SCFA by 34.3 percent. No other differences were observed on ileal short-chain fatty acid profile, regardless of corn source or xylanase inclusion. Previous research

shows that increased amounts of arabinoxylan affects the microbial populations in the crop, gizzard, and ileum. Enzyme supplementation into these diets led to reductions in the lactic acid concentration of the ileum (Jozefiak et al., 2007), which is consistent with the xylanase response observed in the current study on reductions of lactic acid and SCFA. It is indicated that the fermentation of low molecular weight carbohydrates yield a larger percentage of VFA than large molecular carbohydrates (Marounek et al., 1999; Wang et al., 2005). With differences in corn nutrient profile, the nutrient profile and available substrate may be associated with the reduction of isobutyric acid in Corn B, compared to Corn C.

In our study, a factorial analysis was conducted to observe the main effects of corn source and xylanase on broiler growth performance, nutrient digestibility, and the ileal volatile fatty acid profile of male Cobb 500 broilers. Differences in analyzed nutrients and estimated AME of corn sources, combined with differences observed in growth performance and nutrient digestibility, indicate the importance of accurately analyzing corn nutrient profile and formulating diets on individual ingredient analysis rather than standard nutrient values. Enzyme responses were generally not significant, but in cases of significance lacked consistency or had a negative effect on performance, such as, increasing FCR during the grower phase and reducing FCR during the finisher phase. The expected benefit of xylanase inclusion may have been limited to the varying nutrient content related to equal corn inclusion across treatments, or possible variation in the amount of substrate available for xylanase to act on. Lack of enzyme response to performance variables has been reported in other studies supplementing xylanase or a

xylanase, amylase, and protease combination in corn-soybean meal based diets through 21 d of age (Kocher et al., 2003; Olukosi et al., 2007). Furthermore, there were no observed enzyme responses on BW or FCR in a series of three experiments feeding an enzyme combination of xylanase and β-glucanase to broilers with diets varying in AME or CP levels through 49 d (West et al., 2007). Overall, the results of this study demonstrate the effect that corn nutrient profile can have on broiler growth performance and nutrient digestibility, with and without xylanase supplementation, and present the importance of formulating diets on individual ingredient values rather than general matrix values.

CHAPTER IV

EVALUATION OF XYLANASE ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY OF BROILERS FED DIETS WITH VARYING AME LEVELS

Introduction

To meet the energy requirement of broilers, cereal grains are included into dietary formulations at levels up to 60% (Leeson and Summers, 2009). However, as the inclusion level and type of grain changes, this presents varying levels of non-starch polysaccharides (**NSP**). These NSP are poorly digested fibers that have shown to negatively affect broiler growth and performance due to anti-nutritional properties, which can reduce nutrient availability and digestibility (Bedford and Classen, 1992; Bedford and Morgan, 1996; Meng et al., 2005). The detrimental effect that NSP have on an animal is generally related to one of two mechanisms, with the first being that the cell wall of non-viscous cereals (corn) create a physical barrier that can encapsulate essential nutrients, rendering them less available due to the limited endogenous enzyme production in poultry. The second mechanism is that the viscous properties of soluble NSP in viscous grains (wheat, barley, and oat) cause it to dissolve in the intestine and increases the resistance for nutrient transport through the epithelial surface, depresses feed passage rate, and interferes with nutrient digestibility (Bedford and Morgan, 1996; Bach Knudsen, 2014; Sethy et al., 2015).

The proportion of polysaccharides within the NSP fraction of cereal grains can vary significantly depending on the individual grain species, as well as within each specific species, and can lead to variation in overall nutritive value (Bach Knudsen,

2014). Corn and wheat are the most predominately used cereals in the poultry industry, both of which are rich in the pentosan arabinoxylan but have differences in total substrate compositions (Theander et al., 1989; Bach Knudsen, 1997). Total NSP content for corn and wheat is 9.0% and 11.3%, respectively. On a percentage of dry matter, corn grain has 4.7% arabinoxylan in a 0.74 arabinose: xylose ratio, 0.1% β-glucan, 1.1% lignin, and 10.1% fiber. Wheat grain has 7.3% arabinoxylan in a 0.62 arabinose: xylose ratio, 1.0% β-glucan, 1.8% lignin, and 13.1% fiber (Bach Knudsen, 1997).

It is important to consider that the NSP content, along with lignin, represent the fiber component of the ingredient and has shown to have the largest negative effect on nutrient digestibility in broilers (Bach Knudsen, 2014). The nutritive value of 13 wheat cultivars from different regions was assessed using a classical AME assay and provided differences of approximately 550 kcal/kg and a significant negative correlation ($r=-0.91$, $p<0.001$) between AME values and total NSP content (Annison, 1991). Previous research has shown that elevated levels of dietary pentosans resulted in depressed AME, reduced starch digestion and nitrogen retention, and negative effects on overall broiler growth performance (Choct and Annison, 1990). Regardless of which grain is used in diet manufacturing, it is crucial that nutrient specifications match with ingredient nutrient analysis to ensure the nutritional requirements of the animal are met.

One way to alleviate some of the negative effects of dietary NSP is with the supplementation of xylanase and other NSP degrading enzymes (**NSPase**). Inclusion of these enzymes allows for the hydrolysis of indigestible bonds within the cell wall of plant-based ingredients into smaller fragments, increasing availability and improving

digestibility. Utilization of NSPase has proved to be effective at increasing the amount of AME that is available from cereal grains (Choct et al., 1994; Zhou et al., 2009; Coppedge et al., 2012). The use of these enzymes in wheat-based diets has proven to be effective in reducing intestinal digesta viscosity and improving feed efficiency (Bedford and Classen, 1992; Meng et al., 2005). More recently, a newer mechanism has been proposed that NSP degrading enzymes produce fermentable prebiotics that provide energy for intestinal bacteria. As a result, there is increased energy recovery from volatile fatty acid production, and elevated butyrate levels increase the efficiency of the gizzard, which leads to more effective digestion (Bedford, 2018). It is believed that these enzymes work in corn-based diets by reducing the encapsulation effect of the cell wall, which increases nutrient availability, or by potentially providing a prebiotic effect allowing increases in energy recovery; however, the success of these enzymes in corn based diets vary in past research (Bedford, 2000; Meng et al., 2005; Cowieson and Masey O'neill, 2013). Therefore, the objective of the current study was to evaluate xylanase efficacy on growth performance and nutrient digestibility in broilers fed diets with vary AME levels in corn-soybean meal diets.

Materials and Methods

Experimental Animals and Husbandry

Male broiler chicks (Cobb) were obtained from a commercial hatchery on day of hatch, weighed, wingbanded, and assigned to pens to ensure statistically similar starting pen weights at a stocking density of 0.056 m^2 per bird. Experimental animals were raised in floor pens, provided age appropriate heat and ventilation, and given access to potable water and experimental rations for ad libitum consumption for the duration of each study. Each 1.67 m^2 replicate pen contained a tube feeder, a nipple drinker line, and fresh pine shavings as litter. Temperature was monitored, recorded daily, and adjusted to maximize bird comfort. The lighting program was as follows: 1 to 3 d, 24 h of light at 21.53 lux; 4 to 8 d, 23 h of light at 21.53 lux; 9 to 18 d, 16 h of light at 8.07 lux; 19 to 32 d, 18 h of light at 1.08 lux; and 33 d to termination, 20 h of light at 0.54 lux. All experimental procedures were performed as approved by the Texas A&M University Institutional Animal Care and Use Committee.

Experimental Design

The effect of different AME levels on the efficacy of xylanase in improving growth performance and nutrient digestibility of male Cobb 500 broilers was evaluated. Broiler chicks (n=1,800) were allocated to six experimental treatment groups arranged as a randomized complete block design with 10 replicate pens of 30 birds per treatment. Broilers were fed one of six dietary treatments in a 3 × 2 factorial arrangement with three AME levels and two xylanase levels. Experimental rations were fed for the

duration of the study using a 3-phase feeding plan: starter (0-18 d, crumble), grower (19-33 d, pellet), and finisher (34-42 d, pellet).

Experimental Rations

Corn and soybean meal based diets were formulated on a least-cost basis to be iso-nitrogenous using the breeder nutrient recommendations for all values with the exception of AME (Table 14). During feed manufacturing, three basal diets were created for each growth phase with three AME levels. A medium AME (**Med**) diet was formulated to be similar to breeder recommendations for AME, along with low AME (**Low**) diet that had a 75 kcal/kg reduction and high AME (**High**) diet that had a 75 kcal/kg increase compared to the medium AME diet. The final diets manufactured for each AME level were then divided into two equal parts receiving supplementation of xylanase (Econase XT, AB Vista, Marlborough, Wiltshire, UK) at 0 (**control**) or 16,000 BXU/kg (**xylanase**). The xylanase preparation contained 160,000 units of endo-1,4- β - xylanase activity (EC 3.2.1.8) per gram. One birch xylan unit (BXU) is defined as the amount of enzyme that liberates 1 nmol reducing carbohydrates from birchwood xylan, measured as xylose equivalents, under the conditions of the assay (AB Enzymes, Germany). Titanium dioxide was included in the diets of all growth phases as an indigestible marker to determine ileal digestible energy (IDE).

Diets were manufactured for each phase using a 2-ton double-ribbon, horizontal Scott mixer and a 1 ton/h California Pellet Mill equipped with a conditioner and a 4.4 mm diameter die; and, when appropriate, crumbled using a roller mill. Pelleting

temperatures were maintained between 74° and 76°C to ensure the maximum level of enzyme recovery was achieved.

Triplicate feed samples were collected from each dietary treatment, and proximate analysis of composite samples was performed. Crude protein was determined using combustion (AOAC 990.03), crude fat was determined using petroleum ether extraction (AOAC 945.16), ADF was determined gravimetrically (AOAC 973.18), and total phosphorous was determined by wet ash inductively coupled with plasma spectroscopy (AOAC 985.01M). Finished feeds were analyzed for xylanase activity using an ELISA method (Envirologix method AP019, Enzyme Services & Consultancy, Cordova, TN) and reported (Table 14).

Table 14. Ingredient profile, nutrient content, and xylanase recovery of the three basal diets representing the different AME levels for the starter, grower, and finisher dietary phases.

	Starter			Grower			Finisher		
Item (%)	Low	Med	High	Low	Med	High	Low	Med	High
Ingredients									
Corn	63.12	61.52	59.93	70.30	68.85	68.36	74.84	73.38	71.92
Soybean meal	33.54	33.82	34.11	26.68	26.84	26.89	22.34	22.49	22.65
DL-Met	0.24	0.24	0.24	0.20	0.20	0.20	0.19	0.19	0.20
L-Lys HCL	0.22	0.21	0.21	0.26	0.26	0.26	0.27	0.27	0.27
L-Thr	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03
Soy oil	0.34	1.65	2.97	0.14	1.44	1.87	0.23	1.52	2.82
Limestone	1.18	1.18	1.18	1.11	1.11	1.11	0.99	0.98	0.98
Monocalcium PO_4	0.74	0.74	0.74	0.66	0.66	0.66	0.52	0.53	0.53
NaCl	0.25	0.25	0.25	0.26	0.26	0.26	0.27	0.27	0.27
$NaHCO_3$	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Trace Minerals[1]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamins[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Phytase[3]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
TiO_2	0.40	0.40	0.40	0.40	.40	0.40	0.40	0.40	0.40
Calculated nutrient content									
CP	22.0	22.0	22.0	19.3	19.3	19.2	17.6	17.5	17.5
AME (kcal/kg)	2,950	3,025	3,100	3,000	3,075	3,150	3,050	3,125	3,200
Ca	0.90	0.90	0.90	0.84	0.84	0.84	0.76	0.76	0.76
P	0.74	0.74	0.74	0.69	0.69	0.69	0.64	0.64	0.64
Available P	0.45	0.45	0.45	0.42	0.42	0.42	0.38	0.38	0.38
dig Met	0.53	0.54	0.54	0.47	0.47	0.47	0.44	0.44	0.44
dig Lys	1.24	1.24	1.24	1.10	1.10	1.10	1.00	1.00	1.00
dig Thr	0.75	0.75	0.75	0.66	0.66	0.66	0.60	0.60	0.60
Crude fat	3.17	4.42	5.66	3.12	4.35	4.76	3.30	4.53	5.75
Na	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15
Analyzed nutrient content									
CP	22.1	19.4	21.0	18.9	16.5	16.4	15.4	16.0	14.5
Crude fat	3.5	4.3	5.2	3.0	4.3	4.2	3.6	4.2	5.7
ADF	3.0	3.6	1.3	2.7	2.2	2.6	1.9	2.3	2.0
Total Ca	0.69	0.76	0.72	0.64	0.65	0.73	0.69	0.64	0.68
Total Na	0.13	0.10	0.11	0.12	0.09	0.10	0.11	0.12	0.12
Total P	0.53	0.52	0.48	0.46	0.43	0.43	0.40	0.44	0.47
Ash	4.79	5.24	7.27	4.72	5.71	9.47	7.44	6.28	4.87
Enzyme Recovery from Finished Diets (BXU/kg)									
Control	<2,000	<2,000	~3,500	<2,000	<2,000	~3,500	~3,500	~3,500	~2,500
Xylanase	12,100	14,200	12,900	14,800	14,900	15,100	15,500	15,300	14,900

[1]Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[2]Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D3, 46 IU vitamin E, 0.0165 mg B12, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[3]Quantum Blue 5G (AB Vista, Marlborough, Wiltshire, UK). This product is a source of phytase which increases the digestibility of phytin-bound phosphorus in poultry and swine rations, included at 500 FTU/kg.

Performance Measurements

Experimental animals and residual feed were weighed by pen at 0, 18, 33, and 42 d post-hatch for determination of BW and feed consumption. Mortalities and post-mortem weight were recorded daily for the calculation of percent mortality, BWG, ADFI, and mortality adjusted FCR.

Digestibility Measurements

Ileal digestible energy (**IDE**) and crude protein (**CP**) digestibility were determined at 18 d (5 birds per replicate pen), 33 d (5 birds per replicate pen), and 42 d (5 birds per replicate pen). Ileal samples were pooled and homogenized and then divided into 2 aliquots with one used for the determination of IDE and the other used for the determination of CP digestibility in triplicate. For IDE determination, samples were dried at 100° C for 24 h and gross energy of feed and ileal digesta was determined using a Parr 6400 bomb calorimeter (Parr Instrument Company, Moline, IL). Titanium concentration was determined via protocol outlined by Short et al. (1996). This procedure consisted of a half gram of each dried sample being weighed and ashed. Following ashing, each sample was titrated with 10 mL of sulfuric acid (7.4 M) and then boiled at 200°C for 2 h until dissolved. Samples were then titrated with 20 mL of 30% hydrogen peroxide, and brought to 100 mL total volume using distilled water. Samples were then analyzed for absorption using a Thermo Fisher Scientific Genesys 10S UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) at 410 nm. IDE was calculated using the following equation (Scott et al., 1982):

$$GrossEf\ -ExcretaEi\ where\ ExcretaEi\ =\ GEx\ (Tif/Tii)$$

where Gross Ef is gross energy present in the feed, GE is gross energy in the ileal contents, Tif/Tii is the ratio of titanium presence in the feed and ileal contents. Samples used for CP digestibility determination were freeze-dried using a FreeZone 12 L Console Freeze Dry System (Labconco Corporation, Kansas City, MO) prior to analysis. Nitrogen concentration of each dried sample was determined via combustion method, using an Elementar Rapid N Cube Analyzer (Elementar Americas, Inc., Mt. Laurel, NJ). Ileal digestible energy coefficients (**IDEC**) and ileal digestible nitrogen coefficients (**IDNC**) were calculated using the following equation (Scott et al., 1982):

$$[(NT/Ti)_d - (NT/Ti)_i] / [(NT/Ti)_d]$$

Where NT represents kcal (IDEC) or nitrogen content (IDNC) in the sample, Ti represents the percentage of titanium, the subscript "i" represents the ileal contents, and subscripts "d" represents the diet.

Statistical Analysis

All data were analyzed using 3 (AME level) x 2 (xylanase) factorial ANOVA using the General Linear Model (GLM) procedure (SPSS V 18.0), with the fixed factors of AME (Low, Med, High) and xylanase (control or xylanase), and block as a random factor. In the event that a significant interaction was detected, data were analyzed using a one-way ANOVA. Main effect and treatment means were deemed significant at P ≤

0.05 and separated using Duncan's Multiple Range Test. The experiment unit used for each parameter was pen.

Results

Growth Performance

A significant main effect of AME (P=0.007) was observed on BW at 18 d but not at 33 or 42 d (Table 15). Body weight of broilers fed the high AME diet was greater than those fed low or med AME diets. A significant main effect of AME was observed on ADFI over the starter (P=0.003) and grower phases (P=0.040), but not over the remaining periods (Table 15). Average daily feed intake of broilers fed the low energy diet consumed more feed than the med and high energy diets during the starter phase, whereas broilers fed the med AME diet consumed less feed than the low or high AME diets during the grower phase. Xylanase inclusion was not observed to have a significant main effect on BW or ADFI.

A significant main effect of AME was observed on FCR during the starter phase (p=<0.001) and cumulatively over 1 to 33 d (p=<0.001) and 1 to 42 d (p=<0.001) (Table 16). Over the starter phase, stepwise decreases in FCR were observed as AME increased with FCR being reduced 3.1 and 5.2 points when broilers were fed the med and high AME diets, respectively, as compared with those fed the low AME diet. Over 1 to 33 d, a 2.9 point reduction in FCR was observed in broilers fed the med AME diet as compared with the low AME diet with no further reduction observed with the high AME diet. Over 1 to 42 d, decreases in FCR were observed as AME increased with a 1.4 point

reduction between the low and med AME diets and a 1.7 point further reduction between the med and high AME diets.

A significant main effect of xylanase was observed on cumulative FCR from 1 to 33 d ($p=0.012$) and 1 to 42 d ($p=0.006$) in which xylanase inclusion improved cumulative FCR over the two periods by 1.6 and 1.8 points, respectively. Additionally, a significant AME × xylanase interaction ($p=0.024$) was observed on FCR during the grower phase. Grower FCR was greatest when broilers were fed the low AME diet without xylanase when compared with the remaining treatments ($p=0.017$).). The effect of xylanase on FCR during the grower phase of production varied depending on AME level of the diet with a 4.6 point reduction in the low AME diet, a slight numerical increase in the med AME diet, and a slight numerical reduction in the high AME diet. No differences were observed in mortality throughout the duration of the study (not shown).

Table 15. BW and ADFI of male broilers fed diets varying in AME levels with or without the inclusion of xylanase.

Treatments		BW			ADFI (g/bird-day)				
AME[1]	Xylanase[2]	18 d (g)	33 d (kg)	42 d (kg)	Starter	Grower	Finisher	1 to 33 d	1 to 42 d
Low	Control	668.2	2.005	2.746	44.63	144.05	172.86	95.95	118.83
Low	Xylanase	670.4	2.046	2.814	44.26	144.38	178.88	96.90	121.42
Med	Control	672.4	2.031	2.770	43.91	141.88	172.59	94.76	117.66
Med	Xylanase	675.4	2.005	2.756	43.49	139.55	173.64	93.65	117.11
High	Control	683.3	2.047	2.790	43.19	144.31	171.50	96.81	120.68
High	Xylanase	682.1	2.065	2.830	43.60	144.12	173.67	95.75	118.84
Main Effects									
AME									
Low	(n=20)	669.2[b]	2.024	2.778	44.46[a]	144.21[a]	175.72	96.40	120.06
Med	(n=20)	673.9[b]	2.018	2.763	43.70[b]	140.71[b]	173.11	94.21	117.39
High	(n=20)	682.7[a]	2.056	2.810	43.39[b]	144.22[a]	172.58	96.28	119.76
Xylanase									
Control	(n=30)	674.6	2.027	2.768	43.91	143.41	172.32	95.84	119.06
Xylanase	(n=30)	676.1	2.038	2.800	43.77	142.63	175.29	95.39	119.05
P-values									
AME		0.007	0.150	0.262	0.003	0.040	0.642	0.070	0.245
Xylanase		0.736	0.623	0.252	0.554	0.510	0.284	0.636	0.968
AME × Xylanase		0.875	0.371	0.469	0.266	0.691	0.807	0.552	0.444
Pooled SEM		0.002	0.010	0.015	0.153	0.718	1.515	0.478	0.769

[a,b] Means within columns with different superscripts are significantly different ($P \leq 0.05$)

[1] Calculated AME density (kcal/kg): Starter - Low 2,950, Med 3,025, High 3,100; Grower - Low 3,000, Med 3,075, High 3,150; Finisher - Low 3,050, Med 3,125, High 3,200

[2] Xylanase added at 16,000 BXU/kg feed

Table 16. Mortality corrected FCR of male broilers fed diets varying in AME levels with or without the inclusion of xylanase.

Treatments		FCR (feed:gain)				
AME[1]	Xylanase[2]	Starter	Grower	Finisher	1 to 33 d	1 to 42 d
Low	Control	1.285	1.731[a]	1.904	1.571	1.640
Low	Xylanase	1.282	1.685[b]	1.883	1.540	1.613
Med	Control	1.261	1.681[b]	1.961	1.528	1.617
Med	Xylanase	1.245	1.699[b]	1.919	1.527	1.609
High	Control	1.233	1.697[b]	1.928	1.526	1.606
High	Xylanase	1.232	1.673[b]	1.875	1.514	1.589
ANOVA						
P-value			0.017			
Main Effects						
AME						
Low	(n=20)	1.284[a]	1.685	1.893	1.556[a]	1.627[a]
Med	(n=20)	1.253[b]	1.689	1.940	1.527[b]	1.613[b]
High	(n=20)	1.232[c]	1.706	1.901	1.520[b]	1.597[c]
Xylanase						
Control	(n=30)	1.259	1.700	1.931	1.542[a]	1.621[a]
Xylanase	(n=30)	1.252	1.685	1.892	1.526[b]	1.603[b]
P-values						
AME		<0.001	0.070	0.232	<0.001	<0.001
Xylanase		0.099	0.071	0.109	0.012	0.006
AME × Xylanase		0.098	0.024	0.851	0.140	0.493
Pooled SEM		0.003	0.037	0.013	0.004	0.003

[a,b,c] Means within columns with different superscripts are significantly different ($P \leq 0.05$)

[1] Calculated AME density (kcal/kg): Starter - Low 2,950, Med 3,025, High 3,100; Grower - Low 3,000, Med 3,075, High 3,150; Finisher - Low 3,050, Med 3,125, High 3,200

[2] Xylanase added at 16,000 BXU/kg feed

Nutrient Digestibility

No AME level × Xylanase interactions were observed on nutrient digestibility throughout the duration of the study. However, multiple differences were observed in energy and protein digestibility based on the varying level of AME in the diet and the inclusion of xylanase (Table 17). A significant main effect of AME level was observed on IDE on all evaluated days and on crude protein digestibility on 18 d and 33 d. Increasing dietary AME level led to a stepwise increase in IDE at 18 d ($p<0.001$) and 33 d ($p<0.001$), where feeding diets containing med AME vs. low AME and high AME vs. low AME led to increases of 81 kcal/kg and 164 kcal/kg, respectively at 18 d, and increases of 68 kcal/kg and 150 kcal/kg, respectively at 33d. At 42 d, there were no differences observed in IDE between broilers fed the low and med AME diets; however, there was an increase ($p<0.001$) of 184 kcal/kg with broilers fed high AME diets compared to those fed med AME diets. Increases were observed in IDEC at 33 d ($p=0.003$) and 42 d ($p<0.001$) in broilers fed high AME diets compared to those fed low or med AME diets. At 18 d, increases in IDNC ($p=0.014$) were observed in broilers fed low and high AME diets compared to those fed med AME diets. Broilers fed high AME diets at 33 d had a greater IDNC ($p=0.049$) than those fed low AME diet, while broilers fed med AME diets were intermediate. A significant main effect of xylanase was observed on IDE at 33 d ($p<0.001$), IDEC at 33 d ($p<0.001$), and IDNC at 33 d ($p=0.033$) and 42 d($p=0.048$). The inclusion of xylanase increased IDE, IDEC, and IDNC at 33 d by 89 kcal/kg, 2.1%, and 1.5%, respectively. Crude protein digestibility was increased at 42 d compared to non-xylanase supplemented diets by 1.8%.

Table 17. Nutrient digestibility[1] of male broilers fed diets varying in AME levels with or without the inclusion of xylanase.

Treatments		18 d			33 d			42 d		
AME[2]	Xylanase[3]	IDE[3]	IDEC	IDNC	IDE	IDEC	IDNC	IDE	IDEC	IDNC
Low	Control	2956	0.699	0.753	2966	0.693	0.735	3028	0.713	0.734
Low	Xylanase	3033	0.718	0.764	3102	0.725	0.757	3072	0.723	0.743
Med	Control	3053	0.696	0.732	3084	0.714	0.750	3082	0.712	0.716
Med	Xylanase	3101	0.707	0.745	3121	0.723	0.752	3047	0.704	0.748
High	Control	3133	0.705	0.763	3138	0.721	0.756	3233	0.737	0.743
High	Xylanase	3184	0.716	0.773	3230	0.742	0.777	3264	0.744	0.756
Main Effects										
AME										
Low	(n=20)	2994[c]	0.709	0.759[a]	3034[c]	0.709[b]	0.746[b]	3049[b]	0.717[b]	0.738
Med	(n=20)	3075[b]	0.701	0.738[b]	3102[b]	0.719[b]	0.751[ab]	3065[b]	0.708[b]	0.731
High	(n=20)	3158[a]	0.710	0.768[a]	3184[a]	0.732[a]	0.767[a]	3249[a]	0.741[a]	0.749
Xylanase										
Control	(n=30)	3047	0.700	0.749	3062[b]	0.709[b]	0.747[b]	3114	0.721	0.731[b]
Xylanase	(n=30)	3106	0.714	0.762	3151[a]	0.730[a]	0.762[a]	3132	0.724	0.749[a]
P-values										
AME		<0.001	0.676	0.014	<0.001	0.003	0.049	<0.001	<0.001	0.240
Xylanase		0.064	0.062	0.115	<0.001	<0.001	0.033	0.590	0.585	0.048
AME × Xylanase		0.936	0.919	0.958	0.214	0.208	0.419	0.288	0.286	0.550
Pooled SEM		17.934	0.004	0.004	14.435	0.003	0.004	15.854	0.003	0.005

[a,b,c] Means within columns with different superscripts are significantly different ($P \leq 0.05$)

[1] IDE, ileal digestible energy (kcal/kg); IDEC, ileal digestible energy coefficient; IDNC, ileal digestible nitrogen coefficient

[2] Calculated AME density (kcal/kg): Starter - Low 2,950, Med 3,025, High 3,100; Grower - Low 3,000, Med 3,075, High 3,150; Finisher - Low 3,050, Med 3,125, High 3,200

[3] Xylanase added at 16,000 BXU/kg feed

Discussion

Corn and wheat are the most predominately used cereal grains within the poultry industry and can contribute over 60% of the metabolizable energy requirement in broilers (Cowieson, 2005; Leeson and Summers, 2009). Physical composition and nutritional value of these grains varies between grain species, as well as within each species as inclusion level fluctuates. Utilizing these plant-based feedstuffs in diet manufacturing presents NSP that have anti-nutritional properties and are a potential energy source that is not digestible with endogenous enzyme production. These NSP have shown to increase intestinal digesta viscosity, reduce nutrient availability and digestibility, and negatively affect broiler growth performance (Bedford and Classen, 1992; Bedford and Morgan, 1996; Meng and Slominski, 2005). It is common practice to include NSP-degrading enzymes in dietary formulations as a strategy to alleviate the detrimental effects of anti-nutritional factors in feed ingredients and improve overall energy utilization of the diet. When supplementing NSP-degrading enzymes, such as xylanase, dietary energy can be reduced by substituting supplemental fat with corn, leading to potential cost savings for integrators. While the efficacy of exogenous enzymes can differ depending on diet composition and anti-nutritional substrates, xylanase has led to improvements in broiler growth performance and nutrient digestibility in both corn and wheat diets (Meng et al., 2005; Williams et al., 2014; Amerah et al., 2017).

Dietary AME level had an effect on early BW, with an observed differenceof 13.5 g between the low and high AME diet. However, these differences were lost

beyond 18 d. Previous research has shown dietary energy increases to promote increases in BW (Leeson et al., 1996), but there has also been reports of energy reductions up to 133 kcal/kg leading to no significant differences in BW (Coppedge et al., 2012). In certain cases, birds fed diets with different AME levels had similar body weights but as AME was reduced ADFI increased, suggesting that those fed reduced energy diets utilized feed less efficiently over a 49 d period (Leeson et al., 1996).

During the starter phase, energy reductions in the low AME diets led to a significantly increased ADFI compared to the med and high AME diets. As expected, similar results were observed between broilers fed the low and med AME diets during the grower phase. These observed differences between the low and med AME diets can be attributed to 75 kcal/kg increase between the diets, agreeing with the idea that dietary energy can regulate feed consumption (Ahiwe et al., 2018). No differences were observed on BW or ADFI with xylanase inclusion in our study. There are multiple reports of different exogenous enzymes having no effect on growth performance parameters of poultry fed corn-soybean meal diets (Kocher et al., 2003; Olukosi et al., 2007; West et al., 2007). In the current study, FCR was the only growth parameter observed to be affected by xylanase inclusion, with reductions observed from 1 to 33 and 1 to 42 d. The lack of significant enzyme response on other parameters could potentially be related to the dietary AME differences preventing detectable differences in BW and ADFI (Troche et al., 2007; West et al., 2007).

The main effect of AME level was apparent on FCR during the current study, specifically during the starter phase and cumulatively from 1 to 42 d. Overall, the

advantage of increasing AME density was more than 5.0 points in the starter phase and 3.0 points from 1 to 42 d. In previous research, reductions in dietary energy proved to be effective in increasing FCR and was attributed to observed increases in feed intake similar to starter ADFI in the current study (Masey O'Neill et al., 2012).

A main effect of xylanase on FCR was not observed during the individual growth phases. However, the inclusion of 16,000 BXU/kg improved cumulative FCR from 1 to 33d and 1 to 42 d. In wheat-based diets, effective xylanase activity is related to a reduction in intestinal digesta viscosity. In corn-based diets, it is proposed that improved access to cell wall contents by a reduction of cell wall integrity, improved starch digestibility, or a prebiotic effect that modifies the intestinal microbe population and allows exogenous enzymes to alleviate the negative effects associated with anti-nutritive factors (Cowieson and Ravindran, 2008; Tang et al., 2014). Early improvements in FCR can sometimes be related to the additional enzymatic activity that is provided, since broilers have limited endogenous enzyme activity that limits digestive efficiency during early stages of life (Olukosi et al., 2007). Improvements during later stages of growth have been attributed to a prebiotic effect of xylanase establishing greater numbers of beneficial bacteria in the lower intestine with the provision of xylo-oligomers following the degradation of corn fiber (Masey O'Neill et al., 2012). The mechanism responsible in the current study is unclear; benefits of xylanase inclusion on FCR were not observed during specific stages of growth, rather on a cumulative basis from 1 to 33 d and 1 to 42 d.

In the current study, dietary AME level and xylanase inclusion had a significant effect on nutrient digestibility, with increases in AME level leading to stepwise increases in energy digestibility, energy digestibility coefficients, and nitrogen digestibility coefficients. The inclusion of xylanase at 16,000 BXU/kg led to increases in energy digestibility, energy digestibility coefficients, and nitrogen digestibility coefficients, as well. Previous research has shown inconsistent results with exogenous enzyme inclusion on nutrient digestibility and proposed that multiple factors, such as the availability of substrate, enzyme type, growth phase, and starch digestion play a role in enzyme response (Mahagna et al., 1995; Meng and Slominski, 2005; Yegani and Korver, 2013). It is also suggested that NSP-degrading enzymes can hydrolyze or solubilize protein-carbohydrate complexes, facilitating the breakdown of these structures and increasing ileal crude protein digestibility (Marsman et al., 1997; Meng et al., 2005). Considering there are multiple factors that can affect enzyme efficacy on nutrient digestibility, results observed in the current study indicate favorable conditions were achieved to increase energy and nitrogen digestibility.

In our study, a factorial analysis was conducted to observe the main effects of dietary AME level and xylanase inclusion on growth performance and nutrient digestibility of male Cobb 500 broilers. Reductions in dietary AME level negatively affected broiler performance, specifically through increases in FCR and reduced nutrient digestibility. Responses with the inclusion of xylanase were focused on improved efficiency with reductions in cumulative FCR from 1 to 33 d and 1 to 42 d which were facilitated by increases in both energy and crude protein digestibility. No AME level ×

Xylanase interactions were observed throughout the duration of the current study, with the exception of grower FCR, where xylanase inclusion at 16,000 BXU/kg improved FCR of broilers fed low AME diets but elicited no significant response on FCR of broilers fed med and high AME diets. Lack of interactions with xylanase has been previously reported in research evaluating xylanase inclusion in reduced energy diets (Masey O'Neill et al., 2012), and xylanase inclusion in diets with varying supplemental fat and cereal grain levels (Dänicke et al., 1999a). The lack of an interaction in the current study as well as other published reports is of significance as it confirms xylanase efficacy regardless of dietary AME level, which is important for practicing nutritionists and how they determine to utilize xylanase in their nutritional programs. Overall, the results of this study demonstrate that the efficacy of xylanase with regard to increasing nutrient digestibility and feed efficacy is not influenced by the dietary AME value of the diet.

CHAPTER V

CONCLUSIONS

Increases in the price of current feed ingredients has pushed nutritionists to look at alternative ingredients and changes in dietary nutrient content as a way to reduce broiler diet costs. The inclusion of exogenous enzymes in these diets is common practice as a nutritional strategy to enhance nutrient utilization, which can lead to further cost savings and improvements in broiler growth performance. The current set of experiments provides a better understanding of the effects ingredient and dietary nutrient variation can have on broiler performance and nutrient digestibility and demonstrates the ability of xylanase to consistently improve nutrient utilization and feed efficiency.

In chapter II, we evaluated the influence of dietary supplemental fat inclusion on nutrient digestibility and broiler growth response with or without the inclusion of xylanase. Early BW tended to be heavier in birds fed the low fat NC1 diets compared to those fed NC2 diets with higher fat levels. At 28 d, broilers fed diets with the inclusion of xylanase were approximately 30 g heavier than those fed diets without xylanase. Reductions in supplemental fat improved starter FCR in broilers fed NC1 diets by 2.1 points compared to those fed NC2 diets. The opposite was observed during the grower phase where increased fat levels in the NC2 diets improved phase FCR by 2.1 points compared to NC1 diets. Reductions in dAA and energy in the NC1 and NC2 diets increased grower FCR compared to the PC regardless of fat level. However, the inclusion of xylanase in the NC2 diet reduced FCR to levels similar of the PC. During the finisher phase, broilers fed NC2 diets had an increased FCR compared to the PC

regardless of xylanase inclusion. From 1-28 d, an increase in FCR was observed in the NC1 and NC2 fed broilers compared to the PC fed broilers. Inclusion of xylanase into both NC treatments improved cumulative FCR to levels similar of the PC. Similar results were observed from 1-40 d; however, during this period only inclusion of xylanase into the NC1 improved FCR to levels comparable to the PC. At the conclusion of the study, increases were observed in cumulative FCR (1-49 d) with all dietary treatments compared to the PC regardless of dietary fat level or enzyme inclusion which could be related to dAA and energy reductions. However, the inclusion of xylanase reduced cumulative weight corrected FCR (1-49 d) to levels similar of the PC regardless of dietary fat level.

Results observed on IDE were similar at 14 and 40 d, where all dietary treatments had a significantly lower IDE than the PC regardless of fat level or enzyme inclusion. Nutrient reductions and a maximum of 0.5% supplemental fat in the NC1 led to a significantly lower IDE at 49 d; however, the inclusion of xylanase into the NC1 treatment increased IDE to levels comparable of the PC. Elevated levels of supplemental fat increased IDNC at 14 d in the NC2 treatments compared to the NC1 treatments. The opposite was observed at 49 d where lower levels of supplemental fat in the NC1 treatments increased IDNC compared to the NC2 treatments. The inclusion of xylanase increased IDNC at 49 d compared to non-xylanase supplemented treatments. Decreased nutrients and lower fat levels in the NC1 led to reductions in IDNC compared to the PC at 14 d, regardless of enzyme inclusion. All negative control treatments produced similar IDNC at 40 d, regardless of the fat level or enzyme inclusion. Additionally, IDNC of all

negative control treatments was significantly lower than that of the PC at 40 d. At 49 d, broilers fed NC1 + X diets had increased IDNC compared to those fed the NC1 diet that reached levels beyond that of the PC.

In chapter III, we identified if corns sourced from three different geographical locations effected xylanase efficacy as measured by broiler performance, nutrient digestibility, and ileal volatile fatty acid content. Differences in analyzed nutrients and estimated AME of corn sources are consistent with differences observed in growth performance and nutrient digestibility. Corn source had an effect on BW at 33 d, where broilers fed Corn C were 60 g heavier than those fed Corn B. An interaction between corn source and xylanase was observed on ADFI during the grower phase, as well as cumulatively 1 to 33 and 1 to 42 d. During the grower phase, as well as cumulatively from 1 to 33 and 1 to 42 d the inclusion of xylanase led to variable effects on ADFI depending on corn source. However, during the finisher phase xylanase inclusion reduced ADFI by approximately 5.5 g/bird/d compared to non-xylanase supplemented treatments. An effect of corn source was observed on starter FCR, in which broilers fed diets containing Corn A exhibited a lower FCR as compared to broilers fed either Corn B or Corn C diets with a 2.1 and 1.2 point advantage, respectively. FCR response to xylanase during the grower phase and cumulatively from 1 to 33 d varied depending on the dietary corn source. The effect of xylanase on FCR was most apparent during the finisher phase with a 5.6 point reduction in FCR of broilers fed xylanase as compared to those fed diets not containing xylanase. At the conclusion of the trial, both corn source and xylanase inclusion had an effect on FCR where broilers fed diets with Corn B

yielded a cumulative FCR of 1.3 points higher than those fed diets with Corn A, and the inclusion of xylanase reduced FCR 0.9 points compared to those fed non-xylanase supplemented diets.

Corn source had an effect on nutrient digestibility, where ileal digestible energy of Corn B was approximately 240 kcal lower than that of Corn A and Corn C. Additionally, corn source and xylanase inclusion had an effect s content where broilers fed Corn B diets exhibited lower isobutyric levels than those fed Corn C diets. Xylanase supplementation reduced lactic acid and SCFA levels by 44 and 34.4 percent respectively, when compared to non-xylanase supplemented treatments. Overall, the results of this study demonstrate the effect that corn nutrient profile can have on broiler growth performance and nutrient digestibility, with and without xylanase supplementation, and present the importance of formulating diets on individual ingredient values rather than general matrix values. Differences present in analyzed nutrient content and estimated AME are consistent with differences observed in broiler growth performance.

In chapter IV, we evaluated the effect of varying dietary AME level on the ability of xylanase to improve broiler growth performance and nutrient digestibility. Increasing dietary AME level led to differences in early BW, where broilers fed high AME diets were approximately 13.5 g heavier than those low AME diets at 18 d. During the starter phase, energy reductions in the low AME diets led to a significantly increased ADFI compared to broilers fed med or high AME diets. The effect of AME level was apparent on FCR, specifically during the starter phase and cumulatively from 1 to 42 d. The

observed advantage of increasing dietary energy from the low to high AME diets led to more than a 5.0 point improvement in the starter and 3.0 point improvement from 1 to 42 d. The inclusion of xylanase at 16,000 BXU/kg reduced cumulative FCR by 1.6 points from 1 to 33 d and by 1.8 points from 1 to 42 d.

Dietary AME level and xylanase inclusion had a significant effect on nutrient digestibility, with increases in AME level leading to stepwise increases in energy digestibility, energy digestibility coefficients, and nitrogen digestibility coefficients. The inclusion of xylanase at 16,000 BXU/kg led to increases in energy digestibility, energy digestibility coefficients, and nitrogen digestibility coefficients, as well. Overall, the results of this study demonstrate that reductions in dietary AME level can negatively affect broiler growth performance, but the efficacy of xylanase with regard to increasing nutrient digestibility and feed efficiency is not influenced by the dietary AME value of the diet.

Ultimately, results of the current group of experiments quantified improvements in broiler growth performance and nutrient digestibility with xylanase supplementation without the efficacy of these enzymes being affected by ingredient and dietary nutrient variation.

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