EVALUATION OF NUTRITIONAL STRATEGIES FOCUSED ON EXOGENOUS ENZYMES AIMED AT MAXIMIZING BROILER PERFORMANCE AND NUTRIENT UTILIZATION

A Dissertation

by

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ABSTRACT

The objective of this research was to quantify improvements in nutrient digestibility and performance using multiple exogenous enzymes which target different substrates in diets varying in nutrient density. Experiment one consisted of three control diets varying in dietary energy with the supplementation of xylanase at two inclusion rates. Reducing the dietary energy level negatively influenced feed conversion ratio (FCR) and decreased the fat pad weight of broilers in the reduced energy diet compared to the positive control. The inclusion of xylanase reduced FCR throughout the trial compared to the control. These results demonstrate the effectiveness of xylanase inclusion in reduced energy diets to improve FCR of broilers.

Experiment two evaluated the inclusion of a cocktail NSPase and β-mannanase, separately or intermittently, on broiler growth performance and processing. Broiler performance was improved with the inclusion of a cocktail NSPase and β-mannanase throughout the experiment. When evaluating weight gain from d 22 to 47, the intermittent application of cocktail NSPase and β-mannanase improved weight gain compared to the positive control diet. The results of this experiment confirm the ability of exogenous enzymes to improve the nutritive worth of feed ingredients by enzymatic degradation.

Experiment three determined the impact of corn source on broiler performance and nutrient digestibility with or without the inclusion of xylanase. The variability of nutrient profile between corn sources influenced body weight, FCR, and nutrient

digestibility throughout the trial. Xylanase inclusion improved FCR in the finisher phase suggesting that the length of time feeding enzymes may be impactful to the final outcome of performance. The results of this experiment indicate the importance of rapid and accurate evaluation of corn nutrient content to maximize observed growth performance.

Experiment four evaluated the effects of calcium (Ca) and phosphorus (P) level in a diet containing super-dose (> 3X dose) levels of phytase on male broiler performance and breast meat yield. Calcium and P level influenced broiler performance, tibia ash percent, fecal mineral content, and litter mineral content. These data illustrate the importance of utilizing the correct Ca and P matrix value in diets containing phytase. This research program outlines the importance of adequate understanding of nutritional value of raw ingredients for accurate formulation when utilizing exogenous enzymes.

DEDICATION

I dedicate my dissertation work to my family. A special feeling of gratitude for my husband, Jason, who has always supported my decision to pursue a PhD. I will always appreciate your words of encouragement and pushing me to excel at everything I do. You have always put me first and I cannot express how grateful I am for everything you have done.

To my daughter, Pyper Jo, I started this journey when you were eighteen months old. Some days were tough but I would not trade a single moment. You have always been my biggest fan and my inspiration to pursue my goals.

To my parents, Wes and Brenda, thank you for the continued love and support throughout the years. You have always taught me the importance of education and for that I am forever grateful.

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Contributors

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

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NOMENCLATURE

ADF	Acid Detergent Fiber
AME	Apparent Metabolizable Energy
AOAC	Association of Agriculture Chemists
BW	Body Weight
BXU	Birchwood Xylanase Unit
DDGS	Distillers Dried Grains with Solubles
FC	Feed Consumption (gram/bird/day)
FCR	Feed Conversion Ratio
FTU	Phytase Unit
IACUC	Institutional Animal Car and Use Committee
IDE	Ileal Digestible Energy
IEDC	Ileal Energy Digestibility Coefficient
INDC	Ileal Nitrogen Digestibility Coefficiant
ME	Metabolizable Energy
NC	Negative Control
NSP	Non-Starch Polysaccharides
PC	Positive Control
VFA	Volatile Fatty Acids
WOG	Without Giblets

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CHAPTER I

INTRODUCTION

Poultry feed is an important factor controlling profitability and production cost of the poultry industry. Feed accounts for up to 70 percent of total production cost per bird. With energy and protein requirements representing approximately 95 percent of total feed cost, energy sources make up the largest component of poultry diets, followed by plant and animal protein sources. Corn and soybean meal are the most abundant ingredients used in U.S. poultry diets to supply the birds energy and protein requirements. Corn has a high available energy content and is low soluble non-starch polysaccharides (NSP), which are anti-nutritive factors present in many plant-based feedstuffs (Bedford, 1995; Smits and Annison, 1996; Bach Knudsen, 1997). Soybean meal is the by-product of the oil extraction process that has a high crude protein content (44 to 50 percent) and a balanced amino acid composition which compliments corn for feed formulation. Although nutrient needs vary by species, age, and production phase; digestible amino acids, metabolizable energy, and available phosphorus are considered to be the most costly nutrients that make up the composition of the diet. Nutritionist are challenged to formulate diets using ingredients with the highest quality, consistency, and nutritive value.

An adequate understanding of the nutritional value of raw ingredients is vital information a nutritionist must have for the accurate formulation of a feed to meet desired nutrient specifications. Variation between ingredients can impact digestibility and utilization of the feed which can ultimately affect bird performance. For example,

the nutritional value of corn is variable and dependent on the plant variety, growing climate conditions, and post-harvest processing and storage conditions. All of these factors can affect starch structure and lipid/protein/starch matrices (Socorro et al., 1989; Herrera-Saldana et al., 1990; Leeson et al., 1993; Leigh, 1994; Brown, 1996; Collins et al., 1998; Cromwell et al., 1999; Collins and Moran, 2001). It has been reported that variability in corn samples can yield differences in (nitrogen corrected apparent metabolizable energy) AME_n of more than 400 kcal/kg (Cowieson, 2005). Indigestible components present in feed ingredients are also considered limiting factors during feed formulation. Dietary fibers and NSP constitute a significant part of all plant feedstuffs. Some of the common NSP of concern in corn and soybean meal-based diets include arabinoxylans, β-glucans, pentosans, arabinogalactans, mannans, galactomannans, xylans, oligosaccharides, cellulose, hemicellulose, and pectins (Bacic et al., 1988; Choct, 2006; Caprita et al., 2010; Slominski, 2011). Phytate is the principle storage form of phosphorus in plants, and is considered to be an anti-nutrient because it can form insoluble complexes with minerals and proteins. Monogastric animals lack effective endogenous enzymes for the hydrolysis of NSP and phytate. Monogastric animals typically lack the endogenous enzymes necessary to effectively breakdown NSP and phytate in the gastrointestinal tract. As a result, these feed ingredient constituents are poorly digested in poultry.

The inclusion of exogenous enzymes in poultry diets to improve the digestibility and efficiency of nutrient utilization has become a common practice in the poultry industry in recent years. Exogenous enzyme supplementation increases the range of feed

ingredients that can be used and reduces the variability in the ultimate nutritive value that may exist among different batches of ingredients. Inclusion of exogenous enzymes also improves the degree of precision in diet formulation. To achieve maximum benefits from an enzyme product, it is important to ensure that the enzymes are chosen based on the substrates in the ingredients that will be used in feed formulations.

The nutritive and economic value of corn, soybean meal, and other ingredients can be improved by the addition of appropriate preparations of enzyme activities targeting substrates within the diet. The purpose of the research described herein was to investigate the improvements in nutrient digestibility and bird performance that can be achieved using multiple exogenous enzymes in diets with varying nutrient density.

CHAPTER II

LITERATURE REVIEW

Feed Ingredients in Poultry Diets

Poultry meat and eggs have been recognized worldwide as affordable sources of high-quality protein for human nutrition. The rapid growth of the poultry sector has been attributed to several factors such as development and transfer of feed, short production cycle, advances in breeding, and improved processing technologies (Larbier and Leclercq, 1994). Because feed represents the largest input of poultry production, constituting up to 70 percent of the total cost of production, it is recognized as an extremely important factor influencing profitability and product quality (Larbier and Leclercq, 1994). Poultry rations are formulated from a mixture of feedstuffs, including cereal grains, cereal by-products, fat, plant protein sources, animal by-product meals, vitamin and mineral premixes, crystalline amino acids and feed additives. Energy and protein requirements make up approximately 95 percent of total feed cost. The remaining costs are composed of approximately 3 to 4 percent for major minerals including calcium and phosphorus, trace mineral, and vitamin requirements, and 1 to 2 percent for various feed additives. Ingredients that supply mainly energy comprise the largest component of poultry diets, followed by plant protein sources and animal protein sources. Cereals, such as corn, produce edible starchy grains which are used in commercial poultry diets. In the U.S., corn is the most common ingredient used in commercial poultry diets to supply dietary energy, while soybean meal (SBM) is used to meet protein requirements.

Corn is efficient at converting large amounts of solar energy into stable forms of chemical energy stored as starch, cellulose, and oil. The endosperm of yellow dent corn is composed mostly of starch, which serves as the energy storage. The pericarp is the outer covering that protects the kernel and preserves the nutrients. The germ is the living organism that contains genetic information, enzymes, vitamins, and minerals. Approximately 25 %of the germ is oil. Yellow dent corn is primarily composed of starch (62%), protein and fiber (19%), water (15%), and oil (4%) (C. B. Clifford, 2018). The starch in yellow dent corn is a polymer of D-glucose units composed of two different polymeric molecules: amylose and amylopectin. Amylopectin is 50 % of the whole kernel (80% of the starch) and amylose is 12 % of the kernel (20% of the starch). The endosperm cell wall is composed of branched arabinoxylans with small amounts of mixed-linked β-glucan and cellulose. Xylans and cellulose are abundant in the cell walls of hull fractions.

Soybean meal is the most important and preferred source of high quality vegetable protein for the manufacture of animal feed. Soybean meal is the by-product of the soy oil extraction process. It has a high crude protein content of 44 to 50 % and a balanced amino acid composition that compliments corn well for feed formulation. Amino acids in soybean meal are the key elements for proper growth and development of animals. An inclusion level of 25 to 40 % is used in diets for monogastric animals such as poultry. Soybean meal contains approximately 30 % carbohydrates, including 20 % non-starch polysaccharides (NSP) and 10 %oligosaccharides (Hollung et al., 2006).

Animal nutrient requirements vary by species, age and production phase of the animal. Although nutrient needs vary, digestible amino acids, metabolizable energy, and available phosphorus are considered to be three of the most costly nutrients in the the diet composition. Nutritionist are challenged to formulate diets using ingredients with the highest nutritive composition, consistency, and nutritive value.

Nutrient Variability in Dietary Ingredients

It is important to precisely know the nutrient specification for each feed ingredient for accurate formulation of the nutrient content of the diet. Variation between ingredients, and within the same ingredient, can potentially affect bird performance due to differences in digestibility and nutrient utilization. The impact of the indigestible components present in feed ingredients become limiting factors during feed formulation that can influence feed utilization.

Corn. Energy-contributing ingredients constitute approximately 65 % of the dietary cost for broiler chickens (Donohue and Cunningham, 2009). Corn contributes approximately 65 % of the metabolizable energy and 20 % of the protein in a broiler starter diet and is by far the most commonly used cereal grain in the U.S. (Coweison, 2004). The composition of corn has been shown to have variability in terms of starch, protein, fiber, oil, and amino acid content (D' Alfonso, 2002; Song et al., 2004). Samples of corn obtained from various locations, seasons or years indicate various metabolizable energy (ME) values and varied nutrient composition (Leeson and Summers, 1976; Maier, 1995; Collins et al., 1998). Starch contributes approximately 60 % of the apparent

metabolizable energy (AME) content of corn-based poultry feeds (Weurding et al., 2001), and relatively small differences in starch digestibility can impact dietary AME content. Cowieson (2005), reported that the AME_n of corn for broilers may vary by more than 400 kcal/kg. Although corn is considered to be highly digestible by poultry, there is evidence to suggest that some nutrients in corn are not completely digested in the small intestine and resistant starches and protein escape digestion and undergo hindgut fermentation, limiting the energy value (Brown, 1996; Weurding et al., 2001a; Slominksi, 2001; Noy and Sklan, 1995).

Starch. Starch is a polymer of D-glucose units linked by α-(1,4) and α-(1,6) glycosidic bonds (Carre, 2004). The two molecules found in starch are amylose and amylopectin (Figure 2-1). The size of the starch granule is an important factor in determining the energy value of starch. Smaller granules have a larger surface area, and therefore have a greater potential for hydrolysis by endogenous amylase (Carre, 2004).

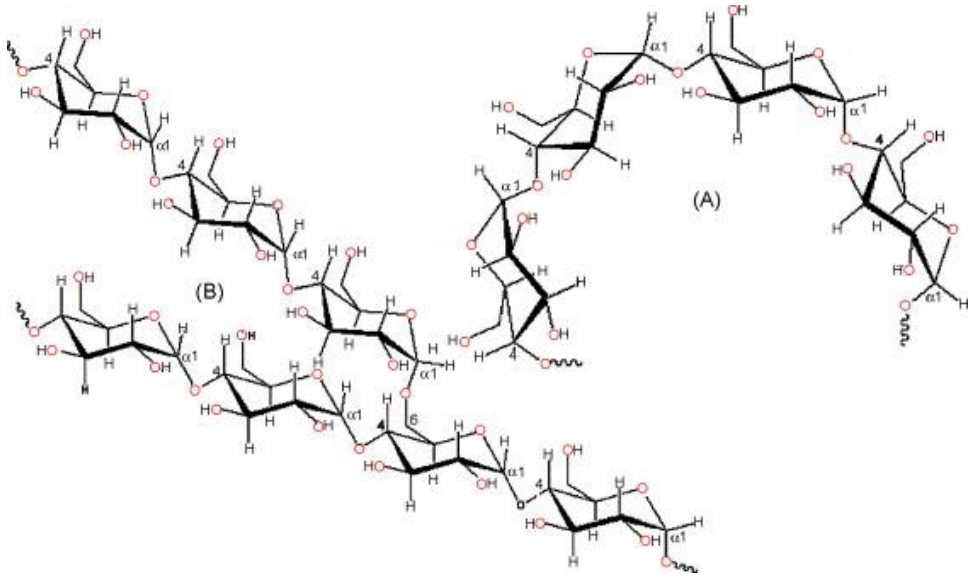

Figure 2-1. Representation of the basic structure of amylose (A) and amylopectin (B) Reprint from [Factors that affect the nutritional value of maize for broilers. (Coweison, 2005)]

Indigestible Components of Dietary Ingredients

Non-starch Polysaccharides. Poultry diets consist of feed ingredients that provide nutrients for efficient digestion and utilization. As meat production increases so do challenges in maintaining efficiency while sustaining production. Dietary NSP constitute a significant part of all plant feedstuffs. Corn and soybean meal are common ingredients used to formulate poultry diets in the U.S. and contain various amounts of NSP. Some of the common NSP of concern in corn and SBM include arabinoxylans, β-glucans, pentosans, arabinogalactans, mannans, galactomannans, xylans, oligosaccharides, cellulose, hemicellulose, and pectins (Bacic et al., 1988; Choct, 2006; Caprita et al., 2010; Slominski, 2011) (Figure 2-2).

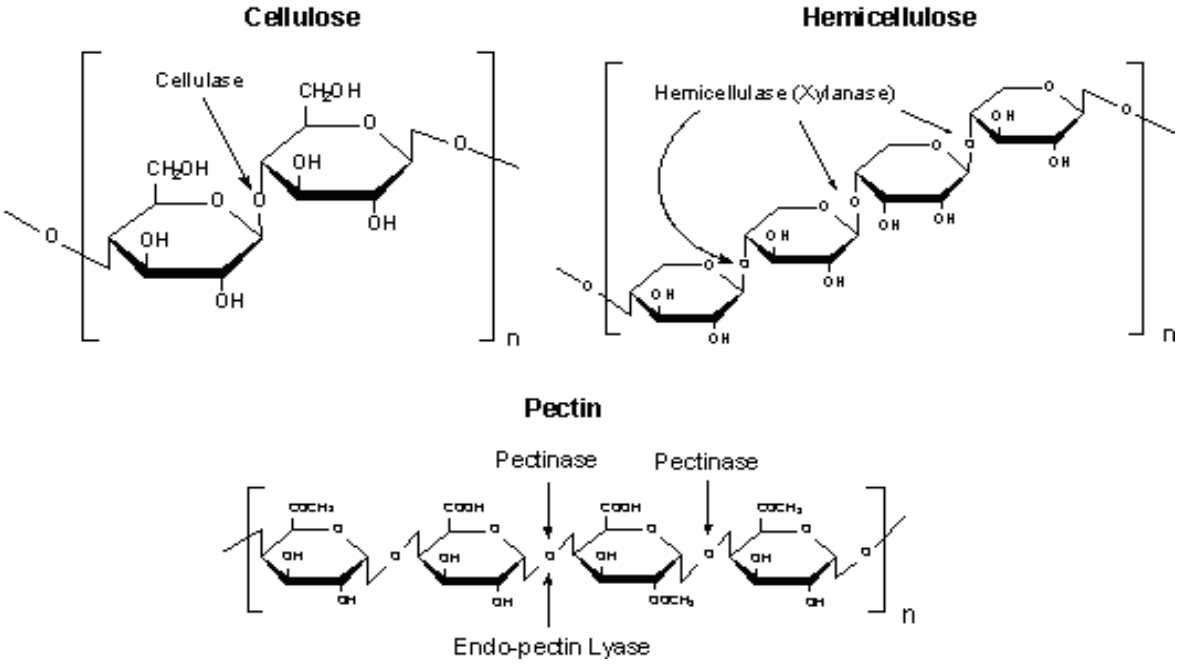

Figure 2-2. Structural models of cellulose, hemicellulose, and pectin with proposed cites of hydrolization. Reprint from [The cell wall. Pages 52–108 in Biochemistry & Molecular Biology of Plants. (Carpita and McCann, 2000)]

Corn contains negligible amounts of soluble NSP and approximately 8 % of insoluble NSP, predominately arabinoxylans and β-glucans (Choct, 2006; Slominski, 2011). Soybean meal contains approximately 3 % of soluble NSP and 16 % of insoluble NSP (Irish and Balnave, 1993), consisting mainly of arabinans, arabinogalactans, galactans, galactomannans, mannans, and pectins (Slominski, 2011).

The variation in the amount and structure of NSP is different between plant materials. Non-starch polysaccharides consist of a series of soluble and insoluble polysaccharides predominantly present in primary or secondary plant cell walls

(Selvendran, 1984; Carpita and Gibeaut, 1993; McDougall et al., 1996; Vincken et al., 2003). Polysaccharides in the plant cell wall consist of pentoses including arabinose and xylose; hexoses including glucose, galactose, and mannose; 6-deoxyhexoses; and uronic acids glucuronic and galacturonic. Polysaccharides are built from monosaccharides that can exist in 2-ring (pyranse and furanose) forms. These residues can be linked through glycosidic bonds at any one of their available hydroxyl groups in α or β orientations (Bach Knudsen, 2014). Therefore, polysaccharides can adopt a large number of 3-dimensional shapes and offer a wide range of functional surfaces. Additionally, charged groups on polysaccharides can affect the ionic properties and be esterified at different degrees.

The main constituent of plant cell wall polysaccharide is cellulose, which is present in all cell walls of both mono- and dicotyledonous plants. The main cell wall polysaccharide of cereals include mixed linked β-glucan and arabinoxylan, whereas xyloglucans, gluco- and galactomannans, and pectic polysaccharides are the main cell wall polysaccharides of protein-rich seeds and grains. Cellulose is an insoluble substance composed of linear polymers of D-glucose residues linked via consecutive β-1,4 linkages. Arabinoxylan is a hemicellulose consisting of a linear backbone of β- 1, 4 xylose residues with arabinose substitution. Beta-glucans are composed of a linear backbone of β-D-glucose residues linked via β- 1, 3 glycosidic bonds. Beta-mannan or galactomannan is a polysaccharide with repeating units of mannose residues linked via β- 1, 4 linkages, with glucose or galactose often found attached to the β-mannan backbone (Figure 2-3).

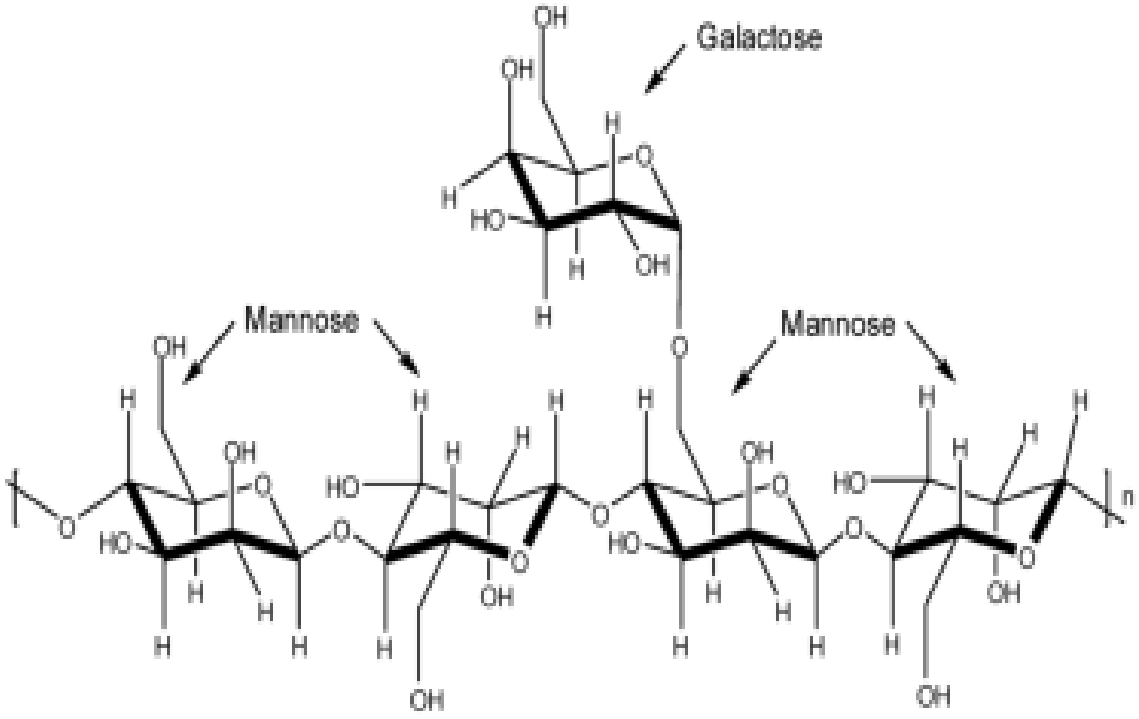

Figure 2-3. Segment of galactomannan showing mannose backbone with a branching galactose unit. Reprint from [Dept. of Chem. Federal University of Vicosa (Teixeira et al., 2014)].

Phytate. Phosphorus (P) in an essential nutrient for growing animals, and therefore a sufficient supply of P in animal feed is required (O'Dell and Sunde, 1997). Phosphorus is necessary for skeletal mineralization and is involved in the regulation of key enzymes in metabolism and in several physiological processes. Considerable amount of P are present in plant-sourced feed ingredients but is bound as phytate. Phytate-P is unavailable to monogastric animals as they lack effective endogenous enzymes for the hydrolysis of phytate. Phytate, the mixed salt of phytic acid (myo-inositol

hexaphosphate; IP_6) exists predominately in feed ingredients as IP_6 (Kasim and Edwards, 1998). Furthermore, IP_6 is a potent anti-nutrient that can impair the digestibility of carbohydrates, amino acids, proteins and minerals and can increase the secretion of endogenous compounds (Ravindran et al., 1999; Selle et al., 2000; Coweison et al., 2004). Phytic acid is unstable in the free acid form and occurs as a complex with metal cations such as calcium (Ca), iron (Fe), zinc (Zn), magnesium (Mg), potassium (K), and manganese (Mn). These salts are called phytates (Cheryan, 1980; Morris, 1986). Phytate carries a maximum of 12 negative charges (Cosgrove, 1966);therefore, it has a great potential for chelating positively charged multivalent cations, especially Fe, Zn, Mg, and Ca (Cosgrove and Irving, 1980) (Figure 2-4).

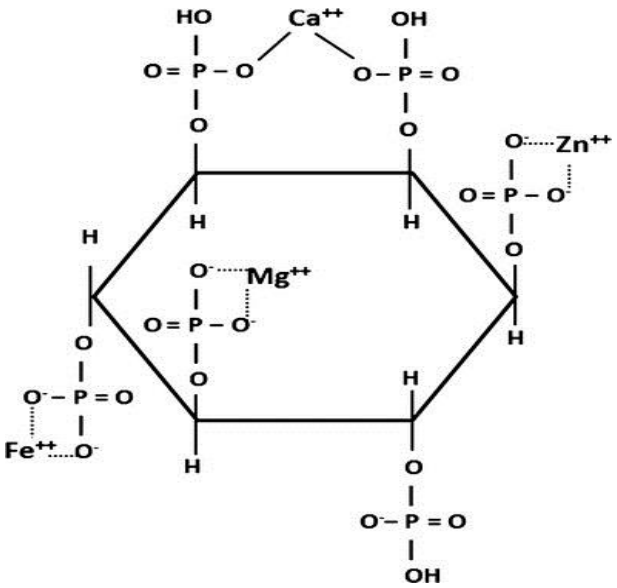

Figure 2-4. Phytic acid-chelate at neutral pH. Reprint from [Oilseed phytates – nutritional implications (Erdman, 1979)]

Even though Ca has the lowest affinity for phytate, it has the greatest impact on P utilization because it is the mineral present in the diet at the highest concentration. Insoluble Ca-phytate complexes can be formed. Phytate limits the availability of Ca and P due to this complex formation. Insoluble Ca-phytate complexes are resistant to enzymatic hydrolysis by phytases (Taylor, 1965). As a result, Ca is a limiting factor on phytate degradation.

The pH is an important factor influencing phytate solubility, with increased solubility at lower pH values than at higher pH values. Complexes between phytic acid and minerals are soluble under the acidic conditions of the gastric digesta. The increasing pH during the passage from the stomach to the small intestine, leads to poor absorption of minerals and trace elements due to insoluble mineral-phytate complexes (Schlemmer et al., 2009; Selle et al., 2009). The inhibitory effect of phytate on mineral bioavailability is governed by several factors: pH, content of minerals and phytate, solubility of phytates and concentration of enhancers or inhibitors.

Several authors report that phyate depresses protein and amino acid utilization due to the formation of complexes. Phytate-protein complexes alter protein structure and in turn decrease protein solubility, enzymatic activity and proteolytic digestibility (Lillford and Wright, 1981; Deshpande and Damodaran, 1989; Urbano et al., 2000). Phytate has the ability to form complexes with proteins at high and low pH values, as phytic acid can react directly with charged group of proteins or indirectly with negatively charged group of proteins mediated by a mineral cation. Phytate-protein

complexes are less likely to be digested by proteolytic enzymes therefore other pancreatic digestive enzymes like lipase and amylase may be inhibited by phytate (Macholz, 1986; Caldwell, 1992).

Exogenous Enzymes in Poultry Diets

The nutritional and economic value of corn and SBM can be improved by the addition of appropriate preparations of carbohydrases, phytases, and other enzyme activities. Increasing the value of diets with exogenous enzymes can be achieved by elimination of the nutrient encapsulating effect of the cell walls, solubilization of cell wall NSP, and the release of P from phytate hydrolysis.

Enzymes are highly effective biological catalyst capable of accelerating chemical reactions. Chemically, they are proteins with a complex three-dimensional molecular structure. Enzymes can be denatured during high-temperature feed manufacture and transit through the low pH conditions of the stomach. Each enzyme hydrolyzes specific substrates at specific reaction sites. To achieve maximum benefits from the enzyme inclusion, it is important to ensure that the enzymes are chosen on the basis of substrates in the ingredients used in dietary formulations.

In feed ingredients, the substrates exist as complexes, limiting the accessibility to enzymes. Despite advances in technology, the chemistry and structure of most target substrates are not well defined. The chemistry of NSP varies widely in different ingredients. Although the type of sugars composing the NSP is known for cereal grains, corresponding information for other ingredients is not understood as well.

Understanding the chemistry of targeted substrates can greatly improve the efficacy of enzymes. Enzyme affinity also needs to be considered when evaluating the enzyme effectiveness. For an enzyme to be effective, an adequate enzyme to substrate ratio must be present in the diet.

The largest user of feed grade exogenous enzymes in animal agriculture is the poultry industry. The highly integrated structure of the poultry sector has enabled the adoption of new technologies and the inclusion of exogenous enzymes has now become the standard to improve digestibility and efficiency of nutrient utilization. The ultimate goal of adding enzymes to poultry feeds is to improve bird performance and profitability through enhanced digestion of dietary components in ingredients. The use of enzymes increase the range of feedstuffs that can be used and increases the flexibility in feed formulations. Supplementation with exogenous enzymes reduces the variability in the nutritive value between batches of ingredients, thus improving the degree of precision diet formulation. The benefits of using exogenous enzymes go beyond simply improving nutrient digestion and have implications for global poultry production in terms of environmental impact, gut health, bird welfare, and sustainability.

Non-starch Polysaccharide Degrading Enzymes (NSPase). A number of potential mechanisms by which exogenous enzymes improve the digestibility of corn and SBM-based diets are possible. Exogenous enzymes work to hydrolyze polysaccharides that are involved in encapsulation of starch, protein, and minerals. Exogenous enzymes allow compounds to be available for digestion that were previously not accessible for

endogenous enzymatic digestion (Bedford, 1996). The majority of the available energy in cereal grains comes from starch. Starch is stored intracellularly, and is partly inaccessible to monogastric animals in the absence of exogenous enzymes to degrade plant cell wall material. The use of effective combinations of NSP-degrading enzymes could reduce the nutrient encapsulating effect of cell walls which could result in an increase in protein, starch and energy utilization.

Conflicting information has been published regarding the ability of single and cocktail type enzyme products to positively influence growth performance. A study conducted by Olukosi et al. (2010) reported no effect on broiler performance with the inclusion of a xylanase, amylase, and protease in corn and SBM- based diets or with diets including distillers' dried grains with solubles (DDGS). On the contrary, Meng et al. (2005) fed a multi-carbohydrase cocktail containing 1,000 U of xylanase, 400 U of glucanase, 1,000 U of pectinase, and trace amounts of cellulose, mannanase, and galactanase and reported improvements in feed conversion ratio (FCR) in broilers from 5 to 18 d of age. Meng and Slominski (2005) fed varying enzyme preparations containing xylanase, glucanase, cellulase, pectinase, and mannanase to broiler chicks grown in batteries from 5 to 18 d of age and reported increased body weight (BW) and dietary AME values with the inclusion of enzymes in diets containing SBM, canola meal and wheat. A study conducted by Coppedge et al. (2012) yielded improvements in BW and FCR in corn and SBM- based diets supplemented with an NSPase. Cowieson and Ravindran (2008) reported the inclusion of a xylanase, amylase, and protease cocktail in a corn and SBM diet increased AME, dry matter retention, and apparent ileal

digestibility coefficients of dry matter, nitrogen, and energy. The goal of multi-enzyme preparations is to target multiple substrates to improve digestibility of dietary fiber in feed ingredients.

Xylanase. Commercial enzymes described as xylanases are often blends or cocktails with xylanase as a main activity (Choct 1999; Aftab, 2012). Xylanase is often used independently or in combination with other enzymes as part of an enzyme cocktail. Xylanases degrade the backbone of xylans, such as arabinoxylans, by hydrolyzing the carbohydrates into more digestible components (Figure 2-5).

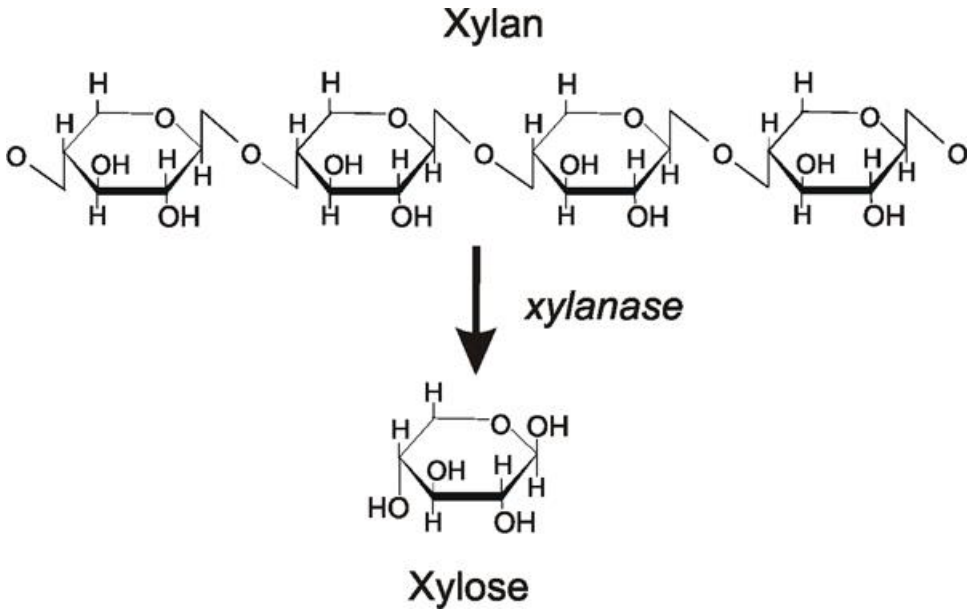

Figure 2-5. Xylanase structural model. Reprint from [A Biofactory of Novel Enzymes, Actinobacteria - Basics and Biotechnological Applications (Vaijayanthi et al., 2016)].

Additionally, xylanase may also be used to improve phosphorus utilization by increasing cell wall permeability or liberating phytate which was previously bound (Esmaeilipour et

al., 2011). A study conducted by Esmaeilipour et al. (2011) reported that supplementation with xylanase improved cumulative FCR through d 15 and d 23 between 3 and 6 points. Several other authors have also reported improvements in FCR with the inclusion of xylanase (Choct et al., 1999; Gao et al., 2007, Masey O'Neill et al., 2012). These improvements in FCR and average daily gain (ADG) have been observed in wheat and barley based diets (Choct et al., 1999; Gao et al., 2007; Esmaeilipour et al., 2011; Kalmendal and Tauson, 2012), as well as corn and SBM- based diets (Masey O'Neill; Coppedge et al., 2012).

Improvements in FCR with the inclusion of xylanase may be attributed to increases in nutrient retention. Esmaeilipour et al. (2011) reported an increase in retention of crude protein, dry matter, and energy with xylanase supplementation. Kalmendal and Tauson (2012) also observed improvements in energy retention, with an increase in AME_n with supplementation of xylanase. Xylanase inclusion has been reported to overcome energy reductions by several reporting authors. Coppedge et al. (2012) reported that xylanase inclusion could improve performance with a 132 kcal/kg reduction in AME. It can be concluded that xylanase supplementation has a positive effect on nutrient utilization and broiler performance.

B-Mannanase. Soybean meal contains approximately 3 percent soluble NSP and 16 percent insoluble NSP (Irish and Balnave, 1993), consisting mainly of arabinans, arabinogalactans, galactans, galactomannans, mannans, and pectins (Slominski, 2011). Among NSP, mannans occur in the forms of glucomannans, galactomannans,

glucogalactomannans, and glucuronomannans in plant cell walls (Aman and Graham, 1990). The exogenous enzyme β-mannanase fragments the high molecular weight and soluble β-galactomannan into mannooligosaccharides through enzymatic degradation.

Beta-mannan has been reported to inhibit animal performance by compromising weight gain and FCR (Anderson and Warnick, 1964), as well as glucose and water absorption (Rainbird et al., 1984). The beneficial effect of enzymatic degradation of β-mannan with the inclusion of a β-mannanase has been reported in broilers fed diets containing SBM (Lee et al., 2003; Jackson et al., 2004; Daskiran et al., 2004). The inclusion of β-mannanase has been shown to have immunological benefits by reducing lesion development in broilers subjected to a necrotic enteritis model through a combined *Eimera* species and *Clostridium perfringens* challenge (Jackson et al., 2003). Jackson et al. (2004) demonstrated that β-mannan can stimulate the innate immune response, potentially leading to unnecessary energy expenditure (Hsiao et al., 2006). A study conducted by Jackson et al. (2004) reported that reducing the amount of β-mannan content within the intestine decreased the energy expenditure through an active innate immune response, leading to a more efficient nutrient utilization and energy expenditure. Supplementation with β-mannanase can be used by integrators as another strategy to reduce dietary energy levels and reduce diet cost.

Phytase. The enzyme phytase (myo-inositol hexaphosphate phosphohydrolase) catalyzes the stepwise hydrolysis of IP_6 to inorganic phosphate and myo-inositol. Phytases have been categorized by two criteria: 1) by the position of hydrolysis onset

and 2) by the preferred pH conditions (Kumar et al., 2010). The two internationally classified phytases, 3-phytases and 6-phytases, are named after the site where the hydrolysis of the phytate molecule is initiated (Selle and Ravindran, 2007). Exogenous microbial phytases are isolated from bacteria, yeast and fungi (Harland and Morris, 1995). Hydrolysis of phytate by exogenous microbial phytase was first investigated by Nelson et al. (1971) who reported improvements in P utilization by broilers fed corn and SBM- based diets. Until the late 1980s, there was little environmental pressure to reduce P excretion, and adding the extrinsic enzyme was very costly. Since then, advances in biotechnology have led to techniques for the genetic modification of fungi. Improved fermentation technology led to the development of commercial phytase products that could be used in swine and poultry diets (Kornegay, 2001). The first feed grade phytases were produced mostly by fungi, however development in the production of enzymes by bacteria and yeasts has resulted in new exogenous phytase products. Studies suggest that increased liberation of phytate bound P in diets supplemented with bacterial phytases may be due to a higher resistance against pepsin activity associated with *E. coli* phytases compared to fungal phytases (Rodriguez et al., 1999; Igbasan et al., 2000).

Several factors can influence the efficacy of exogenous phytase supplementation on P digestibility. Factors include variations in the basal diet (particularly Ca and total P concentration), the origin and concentration of phytate P, Ca-phytate complexes (Nelson et al., 1984; Angel et al., 2002), source of inorganic P and the level of phytase inclusion (Selle and Ravindran, 2008). It has been suggested that Ca

may inhibit phytase activity due to the insoluble Ca-phytate complexes (Applegate et al., 2003). Other studies report that the Ca:P ratio of the diet may impact the response to phytase supplementation (Qian et al., 1996; Lui et al., 2000). Peer reviewed journal articles have evaluated the negative effects of high dietary Ca levels on broiler performance. Akter et al. (2016) conducted a study to determine the effects of different Ca and available P (aP) levels on phytase activity and the impact on broiler performance. In that study, three Ca levels and two levels of aP were evaluated with or without microbial phytase. Akter reported that increasing Ca level reduced feed intake and body weight gain in phytase supplemented diets. Similar results were reported by Lei et al. (1994), concluding that higher Ca levels and larger Ca:P ratios depressed exogenous phytase efficacy. Relatively low Ca levels and smaller Ca:P ratios may be advantageous to corn and SBM- based poultry diets. The reduction in broiler performance is likely due to the insoluble Ca-phytate complexes, limiting the availability of Ca and P, thus hindering phytase activity (Angel et al., 2002). Authors have also suggested that Ca may inhibit phytase activity by competitive inhibition of the phytase competing for active sites with Ca (Qian et al., 1996). Including exogenous phytase at elevated levels to broiler diets presents the question as to the appropriate adjustments in dietary Ca and P levels and Ca:P ratios. The ability of phytate to chelate Ca needs to be established so that adequate adjustments to dietary Ca levels can be made in diets supplemented with elevated levels of exogenous phytase. To maximize phytase activity, dietary Ca levels should be kept to a minimum in phytase-supplemented poultry diets but without compromising skeletal integrity and growth performance.

CHAPTER III

EVALUATION OF XYLANASE IN LOW ENERGY BROILER DIETS*

Introduction

Feed cost accounts for up to 70 percent of total production cost per bird. In 2008 and 2009, the cost of broiler feed in the United States more than doubled from $120 to $250 per ton (Donahue and Cunningham, 2009), increasing the overall cost of broiler production. The increase in feed cost during this time can be directly attributed to the increased use of corn for ethanol production, thus increasing the demand and price of this commodity. The increase in dietary ingredient cost has encouraged nutritionist to maximize nutrient utilization and improve feed efficiency. Improved utilization of dietary nutrients has both economic and environmental aspects, because less feed is required to produce a certain amount of meat and fewer excess of nutrients are excreted by the birds. In efforts to maximize nutrient utilization, the addition of exogenous enzymes in broiler diets has become a common practice in the poultry industry.

The major component of dietary fiber in ingredients commonly used in poultry diets is comprised of cellulose and non-cellulosic NSP. In cereal grains such as corn, the non-cellulosic polysaccharides consist of arabinoxylans and β-glucans, whereas in soybean and canola meals NSP such as arabinans, arabinogalactans, galactans,

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* Reprinted with permission from "Evaluation of Xylanase in Low Energy Broiler Diets" by M. P. Williams, J. T. Klein, C. L. Wyatt, T. W. York, and J. T. Lee. 2014. Journal of Applied Poultry Research, 23, 188-195.

galactomannans, mannans, and pectic polysaccharides predominate (Slominski, 2011). Chickens lack the digestive ability to hydrolyze dietary NSP except for small amounts in the lower intestine. Non-starch polysaccharides are essentially indigestible by monogastric animals, resulting in a reduction in nutrient utilization. Efforts to ameliorate the negative effects of the anti-nutritional components of dietary NSP by supplementation with exogenous enzymes have been recently investigated. Multiple studies have shown the effects of NSP-degrading enzymes on hydrolyzing NSP, reducing intestinal viscosity, and improving nutrient utilization and bird performance. Such results are evident in diets containing high levels of soluble fiber (Choct and Annison, 1992; Ravindran et al., 1999; Wang et al., 2005; Boguhn and Rodehtscord, 2010; Coppedge et al., 2012).

Various dietary considerations are made when supplementing exogenous enzymes in broiler diets. Energy can be reduced in the diet by substituting fat with corn or through dilution with a high fiber ingredient. Non-starch polysaccharide degrading enzymes, such as xylanase, can be used to improve energy utilization in the diet, resulting in reduced dietary cost. Previous studies have shown that supplementation with xylanase in reduced energy diets for broilers resulted in an improvement in FCR (Coppedge et al., 2012; Masey O'Neill et al., 2012). The objective of the current study was to evaluate the impact of xylanase inclusion in reduced energy corn and SBM-based diets on broiler growth performance and processing yield.

Materials and Methods

Experimental Design

The effect of xylanase inclusion on broiler growth performance and processing yields in diets varying in nutrient densities was evaluated in a completely randomized experimental design with 7 dietary treatments during a 45 d grow-out. The experimental design consisted of a positive control diet (PC) and two negative control diets with the AME reduced by 66 kcal per kg (NC1) and 132 kcal per kg (NC2). The negative control diets were used to evaluate varying reductions in energy with the addition of xylanase inclusion at two different inclusion rates.

Experimental Diets

Diets were corn and SBM based with varying energy profiles and increasing levels of DDGS (5 to 10%) as broiler age increased. The PC diet was formulated to total amino acid and energy levels of that found in a typical industry diet. The NC2 diet was the energy value of the PC diet reduced by 132 kcal/kg (Table 3-1). The NC1 diet was obtained by blending equal portions of the PC and NC2 diets, resulting in an energy reduction of 66 kcal/kg. Xylanase was added to the NC1 and NC2 diets prior to pelleting. Two levels of a commercially available xylanase[1] were used in this experiment with the first being included at a rate of 60 g/metric ton for all dietary phases (X1) and the second inclusion level being included at a rate of 60 g/metric ton in the starter and grower diets and 100 g/metric ton in the finisher and withdrawal diets (X2).

[1] Econase®XT 25 - AB Vista Feed Ingredients, Chesterfield, MO

The xylanase had an activity of 160,000 BXU/g (one BXU is the amount of enzyme that produces 1 nMol of reducing sugars from birch xylan as xylose in 1 sec at pH 5.3 and 50 C). All diets contained 750 FTU/kg of phytase[2] (one FTU is the amount of enzyme that catalyzes the release of 1 µMol of inorganic phosphate per minute from 5.1 mM sodium phytate in pH 4.5 buffer at 37 C). A starter diet was fed from d 1 to d 15, grower 1 diet was fed from d 16 to d 23, grower 2 was fed from d 24 to d 31, finisher diet was fed from d 32 to 38 d, and a withdrawal diet was fed from d 39 to d 45 of age (termination of the trial). All diets were pelleted with exception of the starter diet which was pelleted and then crumbled. The conditioning and pelleting temperature did not exceed 70°C to preserve enzyme activity. Samples were collected in duplicate during feed manufacturing for nutrient analysis and enzyme activity. Samples were sent to the manufacturer for xylanase and phytase activity. The activity level for xylananse inclusion at 60 g/metric ton (X1) was above 11,800 BXU/kg. The activity level for xylanase inclusion at 100 g/metric ton (X2) was above 19,700 BXU/kg. The activity level for phytase inclusion was above 798 FTU/kg. Crude protein was determined using AOAC by combustion (AOAC 990.03), total phosphorus determined by wet ash ICP (AOAC 985.01M), acid detergent fiber determined using an ANKOM[3] digestion unit (AOAC 973.18), and an ether extraction to determine crude fat (AOAC 920.39).

[2] Quantum®TR - AB Vista Feed Ingredients, Chesterfield, MO
[3] Ankom Technology – Macedon, NY

Table 3-1. Calculated and analyzed nutrient content and ingredient profiles of the two basal diets positive control (PC) and negative control 2 (NC 2) for the starter (d 1-15), grower 1 (d 16-23), grower 2 (d 24-31), finisher (d 32-38), and withdrawal (d 39-45) dietary phases.

Ingredient Profile	Starter (%)		Grower 1 (%)		Grower 2 (%)		Finisher (%)		Withdrawal (%)	
	PC	NC 2	PC	NC 2	PC	NC 2	PC	NC 2	PC	NC 2
Corn	54.56	57.65	60.75	60.03	58.20	62.09	64.33	65.56	65.75	67.83
Soybean Meal (48%)	33.19	33.07	25.61	28.98	24.44	23.81	19.33	20.53	18.53	19.22
Distillers dried grains with solubles	5.00	5.00	7.00	7.00	10.00	10.00	10.00	10.00	10.00	10.00
Meat and bone meal	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	--	--
A/V Fat	3.57	0.50	2.93	0.50	3.96	0.69	3.15	0.50	3.37	0.50
Limestone	1.25	1.26	1.29	1.27	1.26	1.27	1.23	1.27	1.36	1.41
Sodium chloride	0.28	0.28	0.27	0.27	0.25	0.25	0.25	0.49	0.27	0.32
Mono-calcium PO_4	0.49	0.59	0.35	0.32	0.21	0.20	0.04	0.03	0.16	0.14
L-Lysine HCL	0.11	0.11	0.22	0.10	0.18	0.19	0.21	0.17	0.14	0.11
DL-Methionine	0.18	0.17	0.21	0.17	0.14	0.14	0.15	0.13	0.12	0.16
Vitamins[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coban 90[3]	0.05	0.05	0.05	0.05	0.05	0.05	--	--	--	--
Phytase[4]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Calculated Nutrient Content										
Protein	23.59	23.80	20.95	22.44	20.79	20.83	18.81	19.47	17.95	18.47
Metabolizable energy (kcal/kg)	3058	2926	3080	2948	3102	2970	3124	2992	3146	3014
Calcium	0.90	0.92	0.86	0.86	0.82	0.82	0.76	0.78	0.72	0.74
Phosphorus	0.56	0.59	0.52	0.53	0.50	0.50	0.44	0.45	0.41	0.41
Available phosphorus	0.45	0.47	0.42	0.42	0.4	0.4	0.36	0.34	0.33	0.33
Methionine total	0.54	0.54	0.543	0.52	0.47	0.47	0.45	0.44	0.42	0.47
Lysine total	1.34	1.34	1.22	1.23	1.18	1.18	1.06	1.07	0.96	0.96
Threonine total	0.86	0.87	0.76	0.82	0.76	0.76	0.68	0.71	0.66	0.67
Crude fat	5.71	2.86	5.36	3.05	6.51	3.49	5.86	3.37	6.01	3.34
Sodium	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.24	0.14	0.16
Analyzed Nutrient Content										
Crude protein	24.30	24.90	21.20	23.70	21.30	21.30	19.10	19.90	17.10	18.10
Crude fat	6.56	3.64	6.63	3.62	7.97	5.01	6.92	4.29	5.54	4.43
Acid detergent fiber	3.34	3.75	4.02	3.35	3.40	3.63	2.20	3.17	2.66	3.23

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 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.

[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] Active drug ingredient monensin sodium, 90 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necarix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*.

[4] Quantum®TR - AB Vista Feed Ingredients, Chesterfield, MO

Animals and Management Practices

On d of hatch, 2,352 Cobb 500 straight-run broiler chicks were randomly allotted to floor pens and dietary treatments based on initial body weight. Chicks were provided age appropriate supplemental heat and given access to feed and water *ad libitum.* Chicks were placed in 1.83m x 1.83m rearing pens equipped with tube feeders and nipple drinkers with fresh pine shavings as bedding material. Animal care was provided in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC). All broilers and feed were weighed on the d of dietary changes (d 15, 23, 31, 38 and 45) for determination of average BW and feed consumption for the calculation of mortality corrected feed conversion ratio (FCR). Upon completion of the trial (d 45), 4 male and 4 female broilers/ replicate were individually weighed and processed to obtain processing yield data.

Termination of Trial

All broilers were bulk weighed on the evening of d 45 prior to an 8 hour feed withdrawal period for processing on d 46. Four male and 4 female broilers from each replicate pen (64 broilers/treatment) were removed and individually weighed before processing. Carcass weight without giblets (WOG) and fat pad weights were determined and yields were calculated following processing prior to emerging chilling.

Statistical Analysis

All data were subjected to a one way ANOVA with statistically different means ($p \leq 0.05$) separated using Duncan's Multiple Range Test. Additionally, a factorial ANOVA was conducted using either a 2 (Diet) x 2 (Enzyme) (d 1-31) or 2 (Diet) x 3

(Enzyme) (After d 31) factorial design depending on age with the factor of diet (NC1 v NC2) and enzyme (Control, X1, and X2). If necessary, main effect means were separated using a protected Duncan's Multiple Range Test.

Results

A difference ($p<0.05$) was observed in BW through 15 d of age between the PC and NC2 diet, with the positive control diet yielding the highest observed weight (Table 3-2). The inclusion of xylanase (X1) in the NC2 diet increased ($p < 0.05$) d 15 BW when compared to the NC2 fed broilers. On d 23, no difference was observed in BW with the reduction in energy in the NC1 or NC2 diets relative to the PC.

Table 3-2. Average body weight and mortality of broilers fed diets with three varying energy levels with inclusion of xylanase at two inclusion rates.

Diet	Xylanase	Day 15 (g)	Day 23 (kg)	Day 31 (kg)	Day 38 (kg)	Day 45 (kg)	Mortality (%)
PC	--	508.7[a]	1.07[ab]	1.82	2.36	2.95	5.9
NC1[1]	--	506.0[ab]	1.08[ab]	1.84	2.37	2.93	3.3
NC2[2]	--	493.1[b]	1.05[b]	1.82	2.37	2.94	4.2
NC1	X1[3]	508.7[a]	1.08[ab]	1.82	2.37	2.94	2.9
NC2	X1	510.9[a]	1.09[a]	1.86	2.41	2.96	4.5
NC1	X2[4]	---	---	---	2.39	2.96	5.1
NC2	X2	---	---	---	2.41	2.98	4.5
SEM		4.6	0.02	0.02	0.03	0.03	0.01

[a,b] Means within columns with different superscripts differ significantly at $p \leq 0.05$.
[1] Energy content reduced by 66 kcal/kg compared to the PC
[2] Energy content reduced by 132 kcal/kg compared to the PC
[3] Inclusion of 60 g/metric ton of xylanase (Econase®XT 25 - AB Vista Feed Ingredients)
[4] Inclusion of 100 g/metric ton of xylanase (Econase®XT 25 - AB Vista Feed Ingredients)

However, xylanase (X1) inclusion in the NC2 diet increased ($p<0.05$) BW as compared to the NC2 fed broilers. Following d 23, no differences were observed in BW. Additionally, no differences were observed in mortality throughout the trial.

An increase was observed in FCR through the starter phase and cumulative FCR through d 23 as dietary energy levels were reduced (Table 3-3). The inclusion of xylanase (X1) in the NC1 diet yielded the lowest observed FCR during these phases. With regards to FCR during the withdrawal phase, the reductions in energy (NC1 and NC2) increased ($p<0.05$) FCR when compared to the positive control diet. The inclusion of xylanase (X1 and X2) reduced FCR during the withdrawal phase to levels that were comparable to the positive control. A difference was also observed in cumulative FCR through 45 d of age between the PC and NC2 ($p<0.05$), which resulted in the highest observed FCR. The inclusion of xylanase at both inclusion rates (X1 and X2) in the NC2 diet reduced ($p < 0.05$) cumulative FCR to levels that were comparable to the PC.

Table 3- 3. Dietary phase and cumulative mortality corrected feed conversion ratio (feed:gain) of broilers fed diets with three varying energy levels with inclusion of xylanase at two inclusion rates.

Diet	Xylanase	Starter	Grower 1	Grower 2	Finisher	Withdrawal	Day 1-23	Day 1-31	Day 1-38	Day 1-45
PC	--	1.33[ab]	1.42	1.66	2.35	2.29[b]	1.38[ab]	1.51	1.69	1.81[c]
NC1[1]	--	1.37[ab]	1.42	1.69	2.34	2.44[a]	1.40[ab]	1.52	1.70	1.84[ab]
NC2[2]	--	1.40[a]	1.42	1.71	2.36	2.45[a]	1.41[a]	1.54	1.72	1.86[a]
NC1	X1[3]	1.32[b]	1.41	1.72	2.32	2.41[ab]	1.36[b]	1.51	1.68	1.82[bc]
NC2	X1	1.34[ab]	1.41	1.67	2.38	2.42[ab]	1.38[ab]	1.51	1.70	1.83[bc]
NC1	X2[4]	---	---	---	2.25	2.36[ab]	---	---	1.69	1.82[bc]
NC2	X2	---	---	---	2.33	2.33[ab]	---	---	1.69	1.81[bc]
SEM		0.02	0.02	0.03	0.07	0.05	0.02	0.01	0.01	0.01

[a-c] Means within columns with different superscripts differ significantly at $p \leq 0.05$.

[1] Energy content reduced by 66 kcal/kg compared to the PC

[2] Energy content reduced by 132 kcal/kg compared to the PC

[3] Inclusion of 60 g/metric ton of xylanase (Econase®XT 25 - AB Vista Feed Ingredients)

[4] Inclusion of 100 g/metric ton of xylanase (Econase®XT 25 - AB Vista Feed Ingredients)

Energy level did not affect carcass weight or yield although the elevated inclusion of xylanase (X2) in the NC1 diet yielded the highest observed percentage, resulting in an increase ($p<0.05$) when compared to the lower inclusion of xylanase (X1) (Table 3-4). Energy level of the diet did impact fat pad weight and fat pad yield. A difference was observed in fat pad weight and yield between the PC and NC2 diets ($p<0.05$) with the PC yielding the highest fat pad weight. A linear decrease was observed in fat pad weight and yield as dietary energy levels were reduced. The NC2 diet yielded the lowest observed fat pad weight and yield. The inclusion of xylanase at both inclusion rates (X1 and X2) in the NC2 diet reduced ($p<0.05$) fat pat yield when compared to the PC diet. With regards to males and females, males yielded higher ($p<0.05$) live weight and WOG weight, however females had an increased ($p<0.05$) fat pad weight and yield when compared to males.

Table 3-4. Processing parameters including live weight, carcass weight without giblets (WOG) weight, fat pad weight, WOG yield, fat pad yield of broilers fed diets with three varying energy levels with inclusion of xylanase at two inclusion rates.

TRT	Sex	Live Wt.	WOG Wt.	Fat Pad Wt. (g)	WOG Yield (%)	Fat Pad Yield (%)
PC	Male	3214.1	2417.3	34.3	75.25	1.41
	Female	2695.2	2049.9	38.4	75.98	1.84
NC1[1]	Male	3210.3	2442.1	31.9	76.06	1.31
	Female	2692.7	2047.8	32.1	76.02	1.55
NC2[2]	Male	3291.3	2501.7	27.4	75.99	1.02
	Female	2693.4	2042.2	29.5	75.78	1.46
NC1+X1[3]	Male	3248.0	2459.6	30.0	75.71	1.22
	Female	2649.2	2013.2	36.7	76.00	1.83
NC2+X1	Male	3227.4	2465.1	24.6	76.35	1.00
	Female	2708.3	2071.1	32.8	76.45	1.58
NC1+X2[4]	Male	3256.1	2495.8	30.2	76.66	1.21
	Female	2703.3	2076.2	33.8	76.78	1.67
NC2+X2	Male	3208.3	2438.3	27.2	75.95	1.12
	Female	2688.3	2044.2	26.6	76.01	1.30
Main Effects						
TRT						
PC		2954.6	2233.6	36.3^{a}	75.62^{bc}	1.63^{a}
NC1		2951.5	2244.9	32.0^{ab}	76.04^{abc}	1.43^{abc}
NC2		2987.6	2268.3	28.5^{b}	75.89^{bc}	1.25^{cd}
NC1+X1		2963.3	2247.4	33.2^{ab}	75.85^{bc}	1.51^{ab}
NC2+X1		2967.8	2268.1	28.7^{b}	76.40^{ab}	1.29^{bcd}
NC1+X2		2979.7	2286.0	32.0^{ab}	76.72^{a}	1.44^{abc}
NC2+X2		2948.3	2241.2	26.9^{b}	75.98^{abc}	1.21^{d}
Sex						
Male		3238.9^{a}	2461.2^{a}	29.2^{b}	75.98	1.21^{b}
Female		2687.3^{b}	2043.8^{b}	32.4^{a}	76.02	1.60^{a}
Pooled SEM		15.1	11.9	0.6	0.09	0.03

[a-d] Means within columns with different superscripts differ significantly at $p \leq 0.05$.

[1] Energy content reduced by 66 kcal/kg compared to the PC

[2] Energy content reduced by 132 kcal/kg compared to the PC

[3] Inclusion of 60 g/metric ton of xylanase (Econase®XT 25 - AB Vista Feed Ingredients)

[4] Inclusion of 100 g/metric ton of xylanase (Econase®XT 25 - AB Vista Feed Ingredients)

Factorial analysis was conducted on the two NC diets evaluating enzyme inclusion. The factorial analysis indicated the inclusion of xylanase reduced ($p<0.05$) cumulative FCR throughout the experiment on d 15, 23, 31, 38, and 45. Confirming that the inclusion of xylanase reduced FCR in all diets (Table 3-5). Increasing xylanase inclusion (X2) in the finisher and withdrawal phases reduced ($p<0.05$) FCR when compared to the control diets, yielding the lowest observed FCR through d 32 to 45.

Table 3-5. Main effect means of diet (NC1 and NC2) and enzyme (X1 and X2) on dietary phase mortality corrected feed conversion ratio (feed:gain).

Diet (kcal/kg reduction)	Enzyme (g/metric ton)	Day 1-15	Day 1-23	Day 1-31	Day 32-45	Day 1-38	Day 1-45
NC 1[1]	--	1.37	1.40	1.52	2.37	1.70	1.84
NC 2[2]	--	1.40	1.41	1.54	2.40	1.72	1.86
NC 1	X1[3]	1.32	1.37	1.51	2.35	1.68	1.82
NC 2	X1	1.34	1.39	1.50	2.39	1.70	1.83
NC 1	X2[4]				2.30	1.69	1.82
NC 2	X2				2.30	1.69	1.81
Marginal Means							
NC 1		1.34	1.38	1.51	2.34	1.69	1.82
NC 2		1.36	1.40	1.51	2.36	1.70	1.83
	--	1.39[a]	1.41[a]	1.53[a]	2.39[a]	1.71[a]	1.85[a]
	X1	1.33[b]	1.38[b]	1.51[b]	2.37[a]	1.69[b]	1.82[b]
	X2				2.30[b]	1.69[b]	1.81[b]
Pooled SEM		0.01	0.01	0.01	0.01	0.01	0.00

[a,b] Main effect means within columns with different superscripts differ significantly at $p \leq 0.05$.
[1] Energy content reduced by 66 kcal/kg compared to the PC
[2] Energy content reduced by 132 kcal/kg compared to the PC
[3] Inclusion of 60 g/metric ton of xylanase (Econase®XT 25 - AB Vista Feed Ingredients)
[4] Inclusion of 100 g/metric ton of xylanase (Econase®XT 25 - AB Vista Feed Ingredients)

Discussion

The results of this study indicate that reducing dietary energy (-66 and -132 kcal/kg) negatively impacted broiler performance throughout the experiment. A difference was observed in BW through d 15 between the PC and NC2 diets with the positive control yielding the highest observed BW. With regards to FCR, the reductions in energy increased FCR through the starter phase and cumulative FCR through d 23 of age. During the withdrawal phase, the birds fed reduced energy diets (NC1 and NC2) had an increased FCR when compared to the PC. Differences were also observed at the conclusion of the trial with increased cumulative FCR in broilers fed reduced energy diets; with the NC2 yielding the highest observed FCR. Similar results were reported by Masey O'Neill et al. (2012) with increased cumulative FCR through d 35 and 42 in birds fed energy deficient diets when compared to the positive control.

The inclusion of xylanase in reduced energy diets resulted in improved broiler performance. The inclusion of xylanase in the NC2 diet (-132 kcal/kg) increased d 15 BW when compared to its negative control. On d 23, the inclusion of xylanase in the NC2 diet increased BW compared to the NC2 diet without enzyme inclusion. Improved performance of broilers was also observed by Esmaeilipour et al. (2011) when xylanase was supplemented in the diet. With regards to FCR, xylanase inclusion improved cumulative FCR through d 15 and 23 between 3 and 6 points. During the withdrawal phase, the inclusion of xylanase at both inclusion rates (X1 and X2) improved FCR to levels that were comparable to the positive control. Many studies have shown the effects

of xylanase and other NSP-degrading enzymes on hydrolyzing NSP, reducing viscosity, and improving nutrient utilization, ultimately improving the performance of broilers (Choct and Annison, 1992; Ravindran et al., 1999; Wang et al., 2005; Boguhn and Rodehtscord, 2012; Masey O'Neill et al., 2012). The results of the current study support the previous findings such that the negative impact of reducing dietary energy can be improved through with xylanase supplementation (Masey O'Neill et al., 2012). The benefit on FCR within individual dietary phases was observed in the starter and withdrawal phases of production. The early improvements indicate a positive benefit in young broilers which yet have a fully functional gastrointestinal tract and/or a mature microflora. The benefits observed later during grow out with xylanase inclusion have been related to establishment of a more beneficial bacteria in lower gastrointestinal tract of the broiler through the production of xylo-oligomers (Masey O'Neill et al., 2012).

Exogenous enzymes have been reported to impact processing parameters. A study conducted by Coppedge et al. (2012) reported an improvement in breast meat yield with the supplementation of an NSP-degrading enzyme. In the current study, the elevated inclusion of xylanase (X2) in the NC1 diet yielded the highest observed carcass yield, resulting in an increase when compared to the lower inclusion of xylanase (X1). This also suggests that increasing the xylanase inclusion may positively influence carcass yield. A linear decrease was observed in fat pad weight and yield as dietary energy was reduced. This is similar to results observed by Arafa et al. (1983) where fat pad weights of the broilers were decreased as the daily energy intake was decreased. The inclusion of xylanase did not increase fat pad weight in either of the NC diets.

A factorial analysis was conducted to observe the main effects of dietary energy and xylanase inclusion on growth performance as no significant interactions were present. Xylanase inclusion improved cumulative FCR throughout the trial regardless of energy level. During the finisher and withdrawal phase, the increased level of xylanase (X2) improved FCR when compared to the control diets and low level of enzyme, yielding the lowest observed FCR through d 32 to 45.

In conclusion, reducing dietary energy negatively impacts broiler performance specifically through an observed increase in FCR. However, the inclusion of xylanase eliminated the negative impact of energy reduction by improving cumulative FCR overall. Increasing xylanase inclusion during the finisher and withdrawal phases (X2) improved cumulative FCR. A linear reduction in fat pad weight was observed as dietary energy was decreased. The inclusion of a xylanase had no impact on fat pad weight regardless of energy reduction or when it was applied. These data support the use of xylanase in reduced energy diets for broilers. The ability to reduce in corn and SBM based diets without negatively impacting performance provides nutritionists an additional cost saving measure in diet formulation.

CHAPTER IV

EVALUATION OF BETA-MANNANASE AND NSPASE INCLUSION SEPARATELY OR INTERMITTENTLY IN REDUCED ENERGY DIETS FED TO MALE BROILERS ON PERFORMANCE PARAMETERS AND CARCASS YIELD[*]

Introduction

Corn and soybean meal (SBM), 2 of the major ingredients used in poultry diets, contain various amounts of fibrous material classified as NSP. Some of the common NSP of concern in corn and SBM include β-glucans, pentosans, arabinogalactans, galactomannans, xylans, and pectins. The major components of hemicelluloses in plant cell walls include mannans and xylans (Bacic et al., 1988). Corn, the main source of energy in many poultry diets, contains negligible amounts of soluble NSP and approximately 8 percent insoluble NSP, predominately in the form of arabinoxylans and β-glucans (Choct, 2006; Slominski, 2011). Soybean meal, the most widely used source of vegetable protein in poultry diets, contains approximately 3 percent soluble NSP and 16 percent insoluble NSP (Irish and Balnave, 1993). Consisting mainly of arabinans, arabinogalactans, galactans, galactomannans, mannans, and pectins (Slomski, 2011). Aside from representing a potential source of energy, the presence of dietary NSP can

[*] Reprinted with permission from "Evaluation of Beta-Mannanase and NSPase Inclusion Separately or Intermittently in Reduced Energy Diets Fed to Male Broilers on Performance Parameters and Carcass Yield" by M. P. Williams, B. Brown, S. Rao, and J. T. Lee. 2014. Journal of Applied Poultry Research, 23, 1-9. Copyright [2014] by Poultry Science Association Inc.

result in increased intestinal viscosity, reduced nutrient digestibility, FCR, and ultimately decreased bird performance (Bedford and Classen, 1992; Bedford and Morgan, 1996; Lazaro et al., 2003; Meng et al., 2005). In recent years, nutritionist have made a concerted effort to limit the negative effect of dietary NSP in poultry diets and improve the nutritive worth of feedstuffs through the use of exogenous enzymes.

Supplementation of exogenous enzymes in poultry diets in efforts to ameliorate the negative effects of NSP has been shown to be effective in high fiber wheat-based diets (Bedford and Classen, 1992; Meng et al., 2005; Choct et al., 1999; Gao et al., 2007). The success of enzyme inclusion in corn and SBM based diets, which are the primary ingredients used in the U.S., have varied (Bedford and Classen, 1992; Gracia et al., 2003; Meng and Slominski, 2005). When the NSP in corn and SBM are analyzed it becomes clear that the sole supplementation with xylanase and β-glucanase is a likely explanation of this variation in results (Slominski, 2011). Rather, supplementation with a complex blend of cocktail carbohydrases (NSPase), including cellulases, pectinases, xylanases, glucanases, mannanases, and galactanases, may be the most effective strategy in the degradation of NSP.

Exogenous enzymes function to hydrolyze indigestible bonds in the cell wall of the plant tissues into smaller fragments, thus allowing for improved digestibility by monogastric animals. Water soluble β-glucans and arabinoxylans are the NSP of major concern when feeding poultry diets with high cereal grain content (Carpita et al., 2010). Among NSP, mannans occur in the forms of glucomannans, galactomannans, glucogalactomannans, and glucuronomannans in plant cell walls (Aman and Graham,

1990). Soluble β-galactomannan is a polysaccharide composed of D-mannose units attached by β-1,4 linkages, with galactose or glucose often found attached to the β-mannan backbone (Carpita and McCann, 2000). Beta-mannan is commonly found in a wide variety of vegetable feed ingredients, including SBM. The exogenous enzyme, β-mannanase, fragments the high molecular weight and soluble β-galactomannan into mannooligosaccharides through enzymatic degradation.

The objective of the current study was to determine if an intermittent application of β-mannanase and a cocktail NSPase is advantageous as opposed to individual supplementation. This is based on the fact that β-mannanase targets β-mannans in SBM which is in higher concentrations in starter and grower diets and the cocktail NSPase targets arabinoxylans in corn which increases in concentration in finisher and withdrawal diets.

Materials and Methods

Experimental Design

The effect of β-mannanase[4] and cocktail NSPase[5] inclusion, separately and intermittently, on broiler growth performance and processing yields in reduced energy diets was evaluated. The trial consisted of a completely randomized experimental design with 5 dietary treatments during a 47 d grow out. The dietary treatments included a 1) positive control (PC), 2) negative control (NC) with a reduction of 88 kcal/kg ME

[4]Hemicell – HT, Elanco Animal Health, Greenfield, IN
[5] Enspira®_– Enzyvia LLC, Sheridan, IN

through the starter and grower 1 phase and 132 kcal/kg reduction in the grower 2, finisher, and withdrawal phases compared to the PC, and the NC supplemented with 3) β-mannanase, 4) cocktail NSPase (cocktail carbohydrase – xylanase, β-glucanase, α-galactosidase, and cellulase), and 5) intermittent application of β-mannanase/cocktail NSPase. The intermittent treatment included β-mannanase from d 1-21 (starter and grower 1 phases) and cocktail NSPase from d 22-47 (grower 2, finisher, and withdrawal phases). Each treatment included nine replicate pens with 35 male broilers placed per replicate (1,575 total chicks placed).

Experimental Diets

Diets were corn and SBM based with the PC diet formulated to amino acid and energy levels of that found in an industry type broiler diet (Table 4-1). The NC diet contained 88 kcal/kg less AME in the starter and grower 1 phase and contained 132 kcal/kg less AME in the grower 2, finisher, and withdrawal phases. During feed manufacturing, all reduced energy treatments (trt 2 to 5) were mixed as one large basal diet and divided into four equal batches prior to enzyme inclusion. All diets contained 250 FTU/kg of phytase[6]. Premixes including ground corn and enzymes (400 g/ton) were added to all NC treatments prior to pelleting. Beta-mannanase and cocktail NSPase were included at a rate of 363.2 g/ton (63,800 U/kg of finished feed) and 113.5 g/ton (330 U/kg xylanase of finished feed) respectively, for all dietary phases. One unit of β-mannanase activity is defined as the amount of enzyme which generates 0.72 microgram

[6] Optiphos PF – Enzyvia, LLC Sheridan, IN

of reducing sugars per minute from a mannose-containing substrate at pH 6.6 and temperature of 40°C. One unit of xylanase activity is defined as micromoles of total reducing sugars released per minute at 40°C and pH 4.5. The dietary treatments consisted of the sole inclusion of β-mannanase and cocktail NSPase, and an intermittent treatment with the inclusion of β-mannanase during the starter and grower 1 phase (d 1-21) and cocktail NSPase inclusion during the grower 2, finisher, and withdrawal phases (d 22-47). The dietary program consisted of 5 dietary phases with the starter diet being fed from d 1 - 10, grower 1 from d 11 - 21, grower 2 from d 22 - 32, finisher from d 33 - 40, and withdrawal from d 41 - 47 of age (termination of the trial). All diets were pelleted with exception of the starter diet which was pelleted and then crumbled. The conditioning and pelleting temperature did not exceed 70°C to preserve enzyme activity. Samples were collected in duplicate during feed manufacturing for nutrient analysis. Crude protein was determined using AOAC by combustion (AOAC 990.03), total phosphorus determined by wet ash ICP (AOAC 985.01M), acid detergent fiber determined using an ANKOM digestion unit (AOAC 973.18), and an ether extraction to determine crude fat (AOAC 920.39). Enzyme activity was verified by each manufacturer to ensure that assayed levels were within acceptable range.

Table 4-1: Dietary formulations and calculated and analyzed nutrient content of the Positive Control (PC) and Negative Control (NC) diets fed to male market broilers.

	Starter (%)		Grower 1 (%)		Grower 2 (%)		Finisher (%)		Withdrawal (%)	
Ingredient	PC	NC	PC	NC	PC	NC	PC	NC	PC	NC
Corn	53.72	55.45	58.13	60.35	61.32	64.43	66.96	70.30	68.20	71.15
Dehulled soybean meal (48%)	39.30	39.00	34.67	34.27	31.91	31.51	26.86	26.26	26.02	25.48
DL-Methionine (99%)	0.29	0.29	0.29	0.29	0.23	0.23	0.22	0.22	0.18	0.18
L-Threonine	0.04	0.04	0.05	0.05	0.03	0.03	0.04	0.04	0.01	0.01
L-Lysine HCL	0.30	0.12	0.13	0.14	0.09	0.10	0.13	0.14	0.08	0.09
Fat, A/V Blend	3.11	1.45	3.47	1.64	3.32	0.61	2.91	0.16	3.11	0.50
Limestone	1.67	1.85	1.62	1.62	1.53	1.54	1.52	1.53	1.62	1.76
Mono calcium phosphate	0.93	0.93	0.82	0.82	0.74	0.73	0.54	0.53	--	--
Sodium Chloride	0.46	0.51	0.46	0.46	0.46	0.46	0.28	0.27	0.27	0.32
Sodium bicarbonate	--	--	--	--	--	--	0.19	0.19	0.19	0.20
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coban 90[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	--	--
Phytase [4]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Calculated Nutrient Content (%)										
Protein	23.94	23.94	22.09	22.09	20.93	21.01	19.00	19.00	18.60	18.60
dig-Lysine	1.26	1.26	1.16	1.16	1.06	1.06	0.96	0.96	0.90	0.90
dig-Methionine	0.62	0.62	0.59	0.59	0.52	0.52	0.49	0.49	0.44	0.44
dig-TSAA	0.93	0.93	0.88	0.88	0.81	0.81	0.75	0.75	0.70	0.70
dig-Threonine	0.82	0.82	0.77	0.77	0.71	0.71	0.65	0.65	0.61	0.61
Calcium	0.90	0.97	0.85	0.85	0.80	0.80	0.75	0.75	0.70	0.70
Available phosphorus	0.45	0.45	0.42	0.42	0.40	0.40	0.35	0.35	0.32	0.32
Total phosphorus	0.59	0.59	0.55	0.55	0.53	0.53	0.47	0.47	0.43	0.43
Sodium	0.20	0.22	0.20	0.20	0.20	0.20	0.18	0.18	0.18	0.18
AME (Kcal/kg)	3036	2948	3102	3014	3124	2992	3157	3025	3190	3058
Analyzed Nutrient Content (%)										
Crude protein	24.00	23.10	21.10	21.00	21.10	19.40	17.90	17.50	18.00	16.00
Crude fat	4.62	4.27	5.89	4.57	5.25	2.98	5.40	2.96	4.69	3.43
Total phosphorous	0.62	0.61	0.56	0.59	0.57	0.54	0.45	0.45	0.33	0.33
Acid detergent fiber	1.30	2.10	2.10	2.10	2.60	1.50	3.00	2.60	2.80	4.00

[1] Vitamin premix added at this rate yields per kg diet 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 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. The carrier is ground rice hulls.

[2] Trace mineral premix added at this rate yields per kg of diet 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. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3] Active drug ingredient monensin sodium, 90 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necarix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*.

[4] Optiphos – Enzyvia LLC, Sheridan, IN

Animals and Management Practices

On d of hatch, 1,575 Cobb 500 male broiler chicks were randomly allotted to floor pens and dietary treatments based on initial BW. Chicks were provided age appropriate supplemental heat and given *ad libitum* access to feed and water. Chicks were placed in 1.83m x 1.83m rearing pens equipped with tube feeders and nipple drinkers with fresh pine shavings as bedding material. Animal care was provided in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC). All broilers and feed were weighed on the d of dietary changes (d 10, 21, 32, 40 and 47) for determination of average BW and feed consumption for the calculation of mortality corrected FCR.

Termination of Trial

All broilers were bulk weighed on the evening of d 47 prior to an 8 hour feed withdrawal period for processing on d 48. Six broilers from each replicate pen (54 broilers/treatment) were removed and individually weighed before processing. Carcass weight without giblets (WOG) and fat pad weights were determined and yields were calculated following processing prior to emersion chilling.

Statistical Analysis

All data were subjected to a one way ANOVA with statistically different means ($P \leq 0.05$) separated using Duncan's Multiple Range Test. Percentage data (mortality and yield) were subjected to an arc sin transformation prior to statistical analysis.

Results

For the duration of the trial, the reduction in energy in the NC diet reduced (P<0.05) male broiler BW compared to the PC diet (Table 4-2). The inclusion of β-mannanase and cocktail NSPase, separately and intermittently, increased BW compared to the NC diet throughout the trial. Specifically, during the starter and grower 1 phase, the inclusion of β-mannanase and cocktail NSPase in the reduced energy diets increased (P<0.05) BW to levels that were comparable to that of the PC diet. During the grower 2, finisher, and withdrawal phases, the inclusion of β-mannanase, cocktail NSPase, and intermittent application of β-mannanase/cocktail NSPase increased (P<0.05) BW to levels that were similar to the PC diet with the intermittent application yielding the highest observed BW. With regards to weight gain during the grower 2, finisher, and withdrawal phases (d 22 to 47), the inclusion of β-mannanase and cocktail NSPase increased (P<0.05) weight gain compared to the NC to levels that were similar to that of the PC diet, however the intermittent application of β-mannanase/cocktail NSPase further increased (P<0.05) weight gain compared to the PC diet.

Table 4-2. Body weight (BW), weight gain, and mortality of male broilers fed low energy diets with the inclusion of β-mannanase[1], NSPase[2], and intermittent application of β-mannanase/NSPase.

TRT d 1-21	TRT d 22-47	BW (g) d 10	BW (g) d 21	BW (kg) d 32	BW (kg) d 40	BW (kg) d 47	Wt Gain (kg) (d 1-21)	Wt Gain (kg) (d 22-47)	Total Mortality (%)
Positive Control	Positive Control	234.3[a]	890.1[a]	1.788[a]	2.474[a]	2.938[a]	0.848	2.048[b]	4.1[b]
Negative Control	Negative Control	163.6[b]	591.3[b]	1.297[b]	1.946[b]	2.409[b]	0.549	1.818[c]	15.2[a]
β-mannanase	β-mannanase	226.0[a]	863.8[a]	1.758[a]	2.446[a]	2.923[a]	0.820	2.064[ab]	4.7[b]
β-mannanase	NSPase			1.796[a]	2.528[a]	3.054[a]		2.190[a]	5.4[b]
NSPase	NSPase	220.3[a]	848.2[a]	1.776[a]	2.508[a]	2.959[a]	0.806	2.110[ab]	2.2[b]
SEM		0.004	0.018	0.033	0.036	0.041	0.018	0.028	0.010
P-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a,b] Treatment means within a column with different superscripts differ significantly at p≤0.05
[1]Hemicell – HT, Elanco Animal Health, Greenfield, IN (363.2 g/ton; 63,800 U/kg of finished feed)
[2]Enspira – Enzyvia LLC, Sheridan, IN (113.5 g/ton; 330 U/kg xylanase of finished feed)

The reduction in energy in the NC diet increased (P<0.05) mortality corrected FCR compared to the PC diet (Table 4-3). During the starter and grower 1 phase, the inclusion of β-mannanase and cocktail NSPase improved (P<0.05) cumulative FCR compared to the NC diet to levels that were similar to that of the PC diet through d 21. The inclusion of β-mannanase, cocktail NSPase, and intermittent application of β-mannanase/cocktail NSPase improved (P<0.05) cumulative FCR compared to the NC diet to levels that were similar to that of the PC diet for the duration of the trial (d 22-47).

Table 4-3. Dietary phase and cumulative mortality corrected feed conversion ratio (FCR) of male broilers fed low energy diets with the inclusion of β-mannanase[1], NSPase[2], and intermittent application of β-mannanase/NSPase.

TRT d 1-21	TRT d 22-47	Starter d 0-10	Grower 1 d 10-21	Grower 2 d 21-32	Finisher d 33-40	Withdrawal d 41-47	d 1-21	d 1-32	d 1-40	d 1-47
Positive Control	Positive Control	1.076[b]	1.421	1.644	2.063	2.884	1.322[b]	1.487[b]	1.644[b]	1.814[b]
Negative Control	Negative Control	1.218[a]	1.566	1.704	2.062	2.696	1.458[a]	1.587[a]	1.722[a]	1.878[a]
β-mannanase	β-mannanase	1.118[b]	1.407	1.668	2.068	2.769	1.328[b]	1.499[b]	1.653[b]	1.824[b]
β-mannanase	NSPase			1.645	2.004	2.662		1.492[b]	1.639[b]	1.798[b]
NSPase	NSPase	1.105[b]	1.393	1.620	1.996	2.913	1.312[b]	1.479[b]	1.626[b]	1.816[b]
SEM		0.012	0.015	0.010	0.018	0.063	0.012	0.008	0.007	0.008
P-value		0.001	0.001	0.277	0.650	0.582	<0.001	<0.001	<0.001	0.031

[a,b] Treatment means within a column with different superscripts differ significantly at p≤0.05
[1]Hemicell – HT, Elanco Animal Health, Greenfield, IN (363.2 g/ton; 63,800 U/kg of finished feed)
[2]Enspira – Enzyvia LLC, Sheridan, IN (113.5 g/ton; 330 U/kg xylanase of finished feed)

During the starter phase, the reductions in energy in the NC diet increased (P<0.05) FCR compared to the PC diet. The inclusion of β-mannanase and cocktail NSPase in the reduced energy diets improved (P<0.05) FCR in the starter phase to levels comparable to the PC diet.

Energy reductions in the NC diet decreased (P<0.05) all processing parameters evaluated including, live weight, WOG weight and yield, and fat pad weight and yield, when compared to the PC diet (Table 4-4). Individual enzyme inclusion increased (P<0.05) individual bird live weight to levels that were comparable to that of the PC diet. The intermittent application of β-mannanase/cocktail NSPase resulted in a further increase (P<0.05) in live weight when compared to the PC diet. Inclusion of β-mannanase, cocktail NSPase, and intermittent application of β-mannanase/cocktail NSPase increased (P<0.05) WOG weight and yield compared to the NC diet to levels that were comparable to the PC diet. Inclusion of β-mannanase and cocktail NSPase,

separately and intermittently, increased fat pad weight compared to the NC diet with the intermittent application yielding similar results to the PC diet. The separate inclusion of β-mannanase and cocktail NSPase did not influence fat pad yield compared to the NC, however the intermittent application of β-mannanase/cocktail NSPase resulted in an increase (P<0.05) in fat pad yield compared to the NC diet.

Table 4-4. Processing parameters including live weight, WOG weight and yield, and fat pad weight and yield, of male broilers fed low energy diets with the inclusion of β-mannanase[1], NSPase[2], and intermittent application of β-mannanase/NSPase.

TRT d 1-21	TRT d 22-47	Live Weight (g)	WOG Weight (g)	WOG Yield (%)	Fat Pad Weight (g)	Fat Pad Yield (%)
Positive Control	Positive Control	3063^{b}	2369^{a}	77.34^{a}	37.1^{a}	1.57^{a}
Negative Control	Negative Control	2710^{c}	2017^{b}	74.39^{b}	24.4^{c}	1.20^{c}
β-mannanase	β-mannanase	3101^{ab}	2382^{a}	76.82^{a}	30.1^{b}	1.26^{bc}
β-mannanase	NSPase	3166^{a}	2439^{a}	77.05^{a}	33.9^{ab}	1.39^{b}
NSPase	NSPase	3130^{ab}	2416^{a}	77.17^{a}	30.9^{b}	1.27^{bc}
SEM		16	14	0.13	0.6	0.02
P-value		<0.001	<0.001	<0.001	<0.001	<0.001

[a-c] Treatment means within a row with different superscripts differ significantly at p≤0.05
[1]Hemicell – HT, Elanco Animal Health, Greenfield, IN (363.2 g/ton; 63,800 U/kg of finished feed)
[2]Enspira – Enzyvia LLC, Sheridan, IN (113.5 g/ton; 330 U/kg xylanase of finished feed)

Discussion

Supplementation with exogenous enzymes in broiler diets containing corn and SBM is a widely used strategy to improve the nutritive worth of feedstuffs. Enzyme supplementation allows for energy reductions in the diet by substituting fat with corn or through dilution with a high fiber ingredient. In the current study, the reduction in energy level (-132 kcal/kg) in the negative control (NC) diet reduced BW and increased cumulative FCR in broilers for the duration of the trial. Similar results were observed by

Coppedge et al. (2012) with a decrease in BW through d 26 when the energy levels were reduced by 133 kcal/kg compared to the PC. Masey O'Neill et al. (2012) also observed negative impacts when reducing dietary energy resulting in an increase in FCR through d 42. In efforts to eliminate these negative effects and to recover this caloric value, exogenous enzymes are supplemented in corn and SBM based broiler diets.

The efficacy of exogenous enzymes is significantly influenced by substrate variability and interactive factors. Inclusion of a cocktail carbohydrase containing several activities has the ability to target different components of feedstuffs, possibly having a greater effect than individual supplementation targeting one substrate. In the current study, an improvement in BW and FCR was observed throughout the trial with the inclusion of the cocktail NSPase in the reduced energy diets as compared to the NC. Meng et al. (2005) observed reductions in FCR in broilers fed reduced energy corn and SBM based diets with the inclusion of a cocktail NSPase containing similar enzymes to the NSPase fed in the current study. The strategy of supplementing with multiple enzymes as opposed to individual inclusion was confirmed by Cowieson et al. (2010) where simultaneous inclusion of xylanase and glucanase resulted in a subadditive effect on growth performance compared to individual inclusion. Similarly, Coppedge et al. (2012) reported an increase in BW and FCR with the inclusion of a cocktail NSPase in reduced energy diets, whereas the sole inclusion of xylanase did not increase BW. Meng and Slominski (2005) reported improved FCR in broilers fed a reduced energy diet consisting of 69 percent corn with the inclusion of a multi-carbohydrase containing xylanase, glucanase, pectinase, cellulase, mannanase, and galactanase activity. It is

evident that the use of a multi-carbohydrase to target various NSP content in corn and SBM may provide a potential for further improvements in the nutritive value of these macro-ingredients, ultimately leading to improved performance of birds.

The identification of factors inhibiting nutrient utilization is necessary for selecting the appropriate inclusion of enzymes or enzyme complexes to improve the nutritive value of feedstuffs. Beta-mannan, also referred to β-galactomannan, is commonly found SBM which is one of the primary ingredients used in poultry diets. Beta-mannan has been reported to inhibit animal performance, compromising weight gain and FCR (Anderson and Warnick, 1964), as well as glucose and water absorption (Rainbird et al., 1984). The beneficial effect of enzymatic degradation of β-mannan with the inclusion of a β-mannanase has been reported in broilers fed diets containing soybean meal (Lee et al., 2003; Jackson et al., 2004; Daskiran et al., 2004). In the current study, the inclusion of β-mannanase in reduced energy diets improved BW and FCR in broilers throughout the trial compared to the NC diet. Similar results were observed by Zangia and Torki (2010) with the addition of β-mannanase resulting in improved BW gain and FCR in broilers throughout the experimental period. Zou et al. (2006) also observed an increase in BW in broilers fed corn and SBM based diets supplemented with β-mannanase as compared to the control. Improvements in FCR of broilers fed diets supplemented with β-mannanase have been documented as well (Lee et al., 2003; Jackson et al., 2004; Daskiran et al., 2004).

The objective of the current study was to determine if an intermittent application of β-mannanase and a cocktail NSPase will have an increased effect opposed to individual inclusion. Beta-mannanase targets substrates in SBM which is in higher concentrations in starter and grower diets and the cocktail NSPase targets substrates in corn which increases in concentration during the finisher and withdrawal phases. The intermittent application of β-mannanase and cocktail NSPase improved BW and cumulative FCR throughout the experiment compared to the NC diet. No differences were observed in BW or FCR with the intermittent application compared to individual inclusion of β-mannanase and cocktail NSPase. Interestingly, the intermittent application of β-mannanase/cocktail NSPase increased weight gain from d 22 to d 47 and individual bird live weight on d 48 compared to the PC, where as individual supplementation yielded similar results to that of the PC diet. The inclusion of β-mannanase and cocktail NSPase, separately and intermittently, increased carcass weight and yield compared to the NC diet to levels that were similar to that of the PC. Similar results were reported with improvements in processing parameters with the inclusion of an exogenous enzyme (Coppedge et al., 2012; Tahir et al., 2005). The inclusion of β-mannanase and cocktail NSPase, separately and intermittently, increased fat pad weight compared to the NC diet with the intermittent application yielding similar results to the PC diet. The individual inclusion of β-mannanase and cocktail NSPase did not influence fat pad yield compared to the NC; however the intermittent application of β-mannanase/cocktail NSPase increased fat pad yield compared to the NC diet.

In the present study, supplementing reduced energy corn and SBM diets with β-mannanase and/or cocktail NSPase improved performance and processing parameters of male broilers. The results of this study confirm the ability of exogenous enzymes to improve the nutritive worth of feed ingredients by enzymatic degradation targeting specific substrates in corn and SBM. Observations conclude that intermittent application of enzymes targeting specific substrates determined by dietary ingredient profile could be beneficial.

CHAPTER V

EFFECTS OF NUTRIENT VARIABILITY IN CORN AND XYLANASE INCLUSION ON BROILER PERFORMANCE, NUTRIENT UTILIZATION, AND VOLATILE FATTY ACIDS[*]

Introduction

Corn has been utilized as a major feed ingredient in poultry diets due to its high available energy content and low soluble NSP. Non-starch polysaccharides are anti-nutritive factors present in many plant-based feedstuffs (Bedford, 1995; Smits and Annison, 1996; Bach Knudsen, 1997). Despite these advantages, the nutritional value of corn is variable and dependent on the variety, climate conditions, post-harvest processing and storage, starch structure, and lipid/protein/starch matrices (Socorro et al., 1989; Herrera-Saldana et al., 1990; Leeson et al., 1993; Leigh, 1994; Brown, 1996; Collins et al., 1998; Cromwell et al., 1999; Collins and Moran, 2001). The nutritional value for corn is a function of the content of starch, oil, protein and anti-nutrients, including phytate, enzyme inhibitors and resistant starches. Samples of corn obtained from various locations, seasons or years indicate various ME as well as varied composition (Leeson and Summers, 1976; Maier, 1995; Collins et al., 1998).

Starch contributes around 60 % of the AME content of corn-based poultry feeds (Weurding et al., 2001). Relatively small differences in starch digestibility can have a

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substantial impact on dietary AME content. Cowieson, (2005) reported that the AME_n of corn for broilers may vary by more than 400 kcal/kg. Branched arabinoxylans predominate in the endosperm cell walls; however small amounts of mixed-linked β-glucan and cellulose are present. Xylans and cellulose are abundant in the cell walls of hull fractions. Although corn is considered to be highly digestible by poultry, there is evidence to suggest that some nutrients in corn are not completely digested in the small intestine and resistant starches and protein escape digestion and undergo hindgut fermentation, limiting the energy value (Brown, 1996; Weurding et al., 2001a; Slominksi, 2001; Noy and Sklan, 1995). Noy and Sklan (1995) reported that the ileal digestibility of corn starch rarely exceed 85 percent in broilers. The physical barrier created by the aleurone layer inhibit the animals' endogenous enzymes in accessing and fully digesting the starch and protein components enclosed within the cells (Theander et al., 1989; Slominski et al., 1993; Bedford, 2002). The undigested nutrients limit the energy value of corn, presenting an opportunity for the use of exogenous enzymes in corn-based diets.

Supplementation of exogenous enzymes in poultry diets has been shown to be effective in high soluble NSP wheat-based diets (Bedford and Classen, 1992; Meng et al., 2005; Choct et al., 1999; Gao et al., 2007). The success of enzyme inclusion in corn and SBM based diets is variable (Bedford and Classen, 1992; Gracia et al., 2003; Meng and Slominski, 2005). The main mechanism of action of exogenous enzymes in wheat-based diets is the hydrolysis of water soluble NSP, reducing the viscosity of the intestinal contents and improving the nutritive value of the diet (Bedford and Schultz,

1998; Adeola and Bedford, 2004). This is unlikely to be the primary mechanism in corn-based diets as the soluble NSP concentration is less than 1g/kg (Choct, 1997). A so-called "ileal brake effect" has been suggested, mediated by changes in microflora and volatile fatty acids (VFA) in the large intestine (Cowieson and Masey O'Neill, 2013). Changes in VFA have been reported in response to enzyme, which correspond with improved performance (Masey O'Neill et al., 2014). Response to enzyme supplementation in corn and SBM based diets is likely to be influenced by corn quality and the presence of resistant starch in the ingredient affecting overall ME value. Therefore, it is vital to have adequate understanding of nutritional value of raw ingredients and the likely response to exogenous enzyme supplementation.

The objective of the current study was to determine the effects of corn nutrient variation with different corn sources and xylanase inclusion on broiler growth performance, dietary AME, ileal digestible energy, VFA profiles, and the interaction of these two factors. The working hypothesis is that nutrient variation in corn will impact broiler performance, dietary AME, ileal digestible energy (IDE) and VFA profiles, and xylanase inclusion will improve associated parameters.

Materials and Methods

Experimental Design

The effect of corn source and xylanase inclusion on broiler performance, dietary AME, IDE, VFA profiles, and the interaction of these two factors, was evaluated in a 6 x 2 factorial design yielding a total of 12 treatments for a 41 d trial. The 12 treatments were derived using corn source as the variable, with each corn diet being fed either with

or without xylanase. The diets were fed to 2,160 male broilers with 10 replicates, each containing 18 birds per replicate. On d of hatch, chicks were randomly allotted to floor pens and dietary treatments based on initial average BW. Chicks were provided age appropriate supplemental heat and given access to feed and water *ad libitum.* Chicks were placed in 1.67 m^2 rearing pens equipped with tube feeders and nipple drinkers with fresh pine shavings as bedding material. Animal care was provided in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC).

Experimental Diets

Six corn sources were obtained from 6 different regions (Iowa, Minnesota, Nebraska, North Dakota, South Dakota, Texas – harvested in 2012) in the United States to represent the variability in corn available to feed manufactures. Corn and BM- based diets were formulated using the NRC (1994) value for corn and fed in three dietary phases (Table 5-1): starter (d 0 - 18), grower (d 19 - 31) and finisher (d 32 - 41). During feed manufacturing, one large premix containing all ingredients with the exception of corn was mixed to eliminate nutrient variability among the experimental diets. An equal amount of premix was then mixed with the same amount of each corn source. Therefore, all differences in the diet should have been attributable to the corn. The finished diet for each corn source was then divided into 2 equal parts and xylanase was added at 100 g/metric ton to one part prior to pelleting. All diets were pelleted at 70°C with a conditioning time of 10 s; the starter diet was fed as a crumble while the grower and finisher diets were fed as a pellet. Dietary samples of all treatments were collected and analyzed to confirm xylanase recovery (Table 5-1).

Table 5-1. Dietary formulation, calculated and analyzed nutrient content of diets, based on percentages, and xylanase recovery associated with each corn source, formulated for male broilers fed diets based on corn from various geographical locations, with and without xylanase inclusion.

Ingredient	Starter (%)	Grower (%)	Finisher (%)
Corn	61.56	64.95	70.98
Dehulled soybean meal (48%)	32.55	29.70	23.96
DL-Methionine (99%)	0.26	0.23	0.23
Lysine	0.17	0.14	0.21
L-Threonine	0.02	--	0.03
Fat, animal/vegetable blend	1.50	1.30	1.10
Limestone	1.58	1.46	1.36
Mono calcium phosphate	1.57	1.40	1.29
Sodium chloride	0.46	0.40	0.18
Sodium bicarbonate	--	0.09	0.31
Vitamin premix[1]	0.25	0.25	0.25
Trace minerals[2]	0.05	0.05	0.05
Coban 90[3]	0.05	0.05	0.05
Calculated Nutrient Content			
Protein	21.3	20.2	18.0
Dig-Lysine	1.13	1.04	0.95
Dig-Methionine	0.55	0.52	0.49
Dig-TSAA	0.84	0.79	0.74
Dig-Threonine	0.71	0.65	0.60
Calcium	0.95	0.87	0.80
Available phosphorus	0.45	0.41	0.38
Total phosphorus	0.70	0.66	0.62
Sodium	0.20	0.20	0.20
Metabolizable energy (Kcal/kg)	3000	3025	3075
Xylanase Recovery (BXU)			
A	19,354	21,922	24,045
B	18,845	23,261	22,593
C	19,057	22,491	20,519
D	21,440	21,555	21,672
E	21,772	18,275	15,523
F	21,942	19,669	19,092

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 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.

[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] Active drug ingredient monensin sodium, 90 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necarix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*.The xylanase used in the current study was supplied by AB Vista (Marlborough, UK).

The xylanase preparation (Econase XT) contained 160,000 units of endo-1,4- β - xylanase activity (EC 3.2.1.8) per gram. One unit of xylanase (BXU) is defined as the amount of enzyme that liberates 1 nmol reducing sugars from birchwood xylan, measured as xylose equivalents, under the conditions of the assay (AB Enzymes, Germany). All broilers and feed were weighed on the d of dietary changes (d 18, 31 and 41) for determination of average BW and feed consumption for the calculation of mortality corrected FCR. Starter and finisher diets contained 0.5 percent titanium dioxide as an indigestible marker for the determination of nutrient utilization.

Corn Source

Prior to initiation of the study, proximate and physiochemical analyses were conducted using Near Infrared Reflectance (NIR) spectroscopy which was carried out using a Foss 6500 NIR spectrophotometer[7] (Table 5-2). The analyses were conducted at Aunir (Towcester, UK) and the calibrations were based on wet chemistry analyses of 1,000 corn samples, as described by Piotrowski et al. (2011).

Nutrient Digestibility

On d 18 and 41, ileal and excreta contents were collected and pooled per replicate pen for the determination of AME and IDE. On d 18 (5 birds per replicate) and d 41 (3 birds per replicate), birds were euthanized via carbon dioxide asphyxiation, and ileal contents were collected from 4 cm posterior to Meckel's diverticulum to 4 cm anterior the ileal-cecal junction.

[7] FOSS NIR Systems, Inc., Laurel, MD

Table 5-2. Nutrient profiles[1] taken from three samples of each corn source associated with different geographical locations

Corn Source	Starch (%)	Crude Protein (%)	Oil (%)	Crude Fiber (%)	Moisture (%)	PSI[2] (%)	Vitreousness (%)	AME[3] (kcal/kg)
A	76.5	8.61	3.61	2.48	12.4	41.3	58.7	3296
B	75.8	8.46	3.70	2.59	11.2	38.5	59.5	3295
C	77.2	8.38	3.66	2.47	13.7	32.1	58.8	3212
D	74.7	9.55	3.70	2.75	12.9	41.1	62.4	3241
E	76.0	8.47	3.74	2.61	14.5	35.5	60.9	3163
F	77.6	8.15	3.72	2.47	14.9	33.6	59.1	3183

[1]Results are expressed per 100% of dry matter

[2]Protein Solubility Index

[3]Predicted AME value on an as-is basis using a prediction equation based on NIR values.

Excreta contents were collected by placing stainless steel pans in each pen for the collection of clean fresh excreta material. A minimum of 8 defecations were collected per replicate pen. Ileal and excreta collections were homogenized and stored at -20°C prior to sample analysis. A sub-sample was dried at 100°C for 24 h for moisture determination. Samples were then ground for gross energy and titanium concentration determination.

Titanium concentration was determined using a modified protocol outline by Short et al. (1996). For this procedure, 0.5 g of each dried sample was 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 filled to 100mL using distilled water. Samples were then analyzed by absorption using a Thermo Fisher Scientific Genesys 10S UV-Vis

(Model 10S UV-Vis) Spectrophotometer[8] at 410 nm. Gross energy of feed, ileal and excreta samples were determined using a Parr 6400 bomb calorimeter[9]. Nitrogen concentration of each dried sample was determined via combustion method, using an Elementar Vario Max Analyzer[10]. Ileal digestible energy (IDE) was calculated using the following equation (Scott, Neshiem, and Young, 1982):

$$\text{Gross } E_f - \text{Ileal } E_i \text{ where Ileal } E_i = GE \times (Ti_f/Ti_i)$$

Ileal energy digestibility coefficients (**IEDC**) and ileal nitrogen digestibility coefficients (**INDC**) were calculated using the following equation (Scott, Neshiem, and Young, 1982):

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

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

Volatile Fatty Acid Determination

Volatile fatty acid (VFA) concentration was determined on d 18 (5 birds per replicate) and d 41 (3 birds per replicate). Ileal, large intestine and cecal contents were collected and pooled per replicate pen. Contents were then homogenized and 2 g of each sample was added to a tube with one mL of 25% wt/vol metaphosphoric acid and distilled water was added to achieve a constant volume of 5 mL. The tube was vortexed and frozen at -20°C. Samples were thawed, vortexed, and centrifuged at 20,000 × *g* for 20 min, and using gas-liquid chromatography (GLC) as described by Vanzant and

[8]Thermo Fisher Scientific, Waltham, MA
[9] Parr Instrument Company, Moline, IL
[10] Elementar Inc., Hanau, Germany

Cochran (1994). The GC was equipped with a flame ionization detector (FID) and polyethylene glycol column. The column was operated at 100 to 150°C with a highly purified N_2, at 1.8 mL/min, as the gas carrier.

Statistical Analysis

Data were analyzed as a 6 x 2 factorial Analysis of Variance (ANOVA) using the General Linear Model (GLM) procedure (SPSS V 18.0). In cases of the presence of significant interactions, 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. The experiment unit used for each parameter was pen.

Results

Broiler Performance

Early BW differences (P=0.001) were observed between corn source through d 18 of age. The difference in BW resulted in a range of 40.1 g between corn sources (Table 5-3). Broilers fed corn E yielded the highest observed BW, with corn B and D yielding comparable results. Body weights of broilers fed corn A and F yielded similar results to corn B and D, but did not reach the BW of broilers fed corn F. Broilers fed corn C yielded a lower (p=0.001) BW compared to all other corn sources. No main effect differences were observed between xylanase inclusion and the control-fed broilers. On d 31, corn source did impact BW, resulting in a range of 91 g (P=0.001); however an interaction was observed between corn source and enzyme, with xylanase inclusion negatively impacting broiler BW when fed corn source A (P=0.006). At the conclusion

of the trial (d 41), no main effect differences were observed regarding corn source or xylanase inclusion.

Table 5-3. Body weight, dietary phase FCR (f:g) and cumulative FCR of male broilers fed corn of varying nutrient profiles, with or without xylanase[1] inclusion[2].

		Body Weight			Dietary Phase FCR			Cummulative FCR	
Corn Source	**Xylanase**	**Day 18 (g)**	**Day 31 (kg)**	**Day 41 (kg)**	**Starter**	**Grower**	**Finisher**	**Day 1-31**	**Day 1-41**
A	Enzyme	735	1.856^{c}	2.696	1.377	1.675	1.859	1.539	1.629
A	Control	754	1.993^{ab}	2.835	1.373	1.611	1.928	1.506	1.621
B	Enzyme	746	1.953^{ab}	2.732	1.362	1.622	2.021	1.506	1.634
B	Control	755	1.969^{ab}	2.783	1.359	1.675	1.957	1.533	1.646
C	Enzyme	721	1.914^{bc}	2.729	1.407	1.635	1.939	1.536	1.648
C	Control	726	1.943^{ab}	2.749	1.377	1.654	1.998	1.534	1.660
D	Enzyme	754	2.030^{a}	2.855	1.385	1.635	1.920	1.528	1.632
D	Control	744	2.003^{a}	2.814	1.379	1.658	1.996	1.538	1.658
E	Enzyme	768	1.991^{ab}	2.781	1.354	1.622	1.994	1.503	1.631
E	Control	759	1.969^{ab}	2.720	1.356	1.634	2.016	1.511	1.639
F	Enzyme	750	1.988^{ab}	2.803	1.385	1.644	1.925	1.533	1.640
F	Control	729	1.948^{ab}	2.744	1.377	1.608	2.055	1.508	1.654
Main Effect Means									
Corn Source									
A		745^{b}	1.925	2.766	1.375	1.643	1.893^{b}	1.522	1.625
B		751^{ab}	1.961	2.757	1.360	1.648	1.989^{a}	1.519	1.640
C		724^{c}	1.928	2.739	1.392	1.644	1.968^{a}	1.535	1.654
D		749^{ab}	2.016	2.834	1.382	1.647	1.958^{ab}	1.533	1.645
E		764^{a}	1.980	2.750	1.355	1.628	2.005^{a}	1.507	1.635
F		740^{b}	1.968	2.774	1.381	1.626	1.990^{a}	1.521	1.647
Enzyme Inclusion									
-	Control	745	1.971	2.774	1.370	1.640	1.992^{a}	1.522	1.646
-	Enzyme	746	1.956	2.766	1.378	1.639	1.943^{b}	1.524	1.636
	p-value								
Corn Source	-	0.001	0.001	0.105	0.199	0.658	0.017	0.159	0.186
Enzyme	-	0.842	0.493	0.777	0.314	0.960	0.026	0.560	0.131
Corn x Enzyme		0.092	0.006	0.118	0.900	0.021	0.078	0.080	0.706
SEM		0.002	0.008	0.012	0.004	0.005	0.010	0.003	0.003

[a-c] Main effect and treatment means differ significantly ($P \leq 0.05$)
[1] Econase XT, AB Vista, Marlborough, UK
[2] n = 10 replicate pens

Regarding starter and grower dietary phase FCR, no main effect differences were observed with corn source or xylanase inclusion. During the finisher phase, main effect differences were observed for corn source (P=0.017). Broilers fed corn source A yielded

the lowest observed FCR, which was significantly reduced from birds fed corn B, C, E and F. Intermediate results were observed with birds fed corn source D. Enzyme inclusion reduced FCR (P=0.026). The variability associated with the 6 sources of corn resulted in a range of 11 points in FCR during the finisher phase. No main effect differences were observed in cumulative FCR for corn source and xylanase inclusion.

Nutrient Digestibility

On d 18, corn source influenced nitrogen and energy digestibility on all evaluated parameters, including IDE (P=0.005), ileal nitrogen digestibility coefficient (INDC) (P=0.015), ileal energy digestibility coefficient (IEDC) (P=0.011), and apparent metabolizable energy (AME) (P=0.001) (Table 5-4). Nutrient variability resulted in a range of 152 kcal/kg for IDE between corn sources, with birds fed corn source A yielding the lowest value. An increase (P=0.005) in IDE was observed in birds fed corn sources C, D, E and F compared to corn A and B fed broilers. Variability in corn source resulted in a range of 4.4 percent for INDC between sources, with corn B yielding the lowest at 79.7 percent and corn D yielding the highest at 84.1 percent. A decrease (P=0.015) in INDC was observed in birds fed corn source A and B compared to broilers fed corn source D. Intermediate results were observed in all other corn sources, including C, E and F. Nutrient variability influenced IEDC, yielding a 3.1 percent range between the various sources of corn. Birds fed corn source C, D and E yielded the highest values of IEDC with a decrease (P=0.011) in birds fed corn source A and B. Corn F fed broilers yielded intermediate results. The range of AME for the 6 corn

Table 5-4. Nutrient utilization (kcal/kg) including ileal digestible energy (IDE), ileal energy digestibility coefficient (IEDC), ileal nitrogen digestibility coefficient (INDC) and apparent metabolizable energy (AME) of male broilers fed sources of corn varying in nutrient profiles, with and without xylanase[1] inclusion, on 18 and 41 days of age[2].

Main effect Means		Day 18				Day 41			
Corn Source		IDE	INDC	IEDC	AME	IDE	INDC	IEDC	AME
A		3433^{b}	0.811^{bc}	0.786^{bc}	3533^{b}	3316^{b}	0.742^{c}	0.759^{c}	3493^{ab}
B		3435^{b}	0.797^{c}	0.782^{c}	3515^{b}	3508^{a}	0.803^{a}	0.800^{a}	3582^{a}
C		3540^{a}	0.825^{ab}	0.813^{a}	3617^{a}	3295^{b}	0.769^{abc}	0.757^{c}	3472^{ab}
D		3585^{a}	0.841^{a}	0.810^{a}	3655^{a}	3392^{ab}	0.784^{ab}	0.771^{bc}	3519^{ab}
E		3543^{a}	0.833^{ab}	0.808^{a}	3691^{a}	3453^{a}	0.797^{a}	0.792^{ab}	3436^{b}
F		3540^{a}	0.826^{ab}	0.805^{ab}	3686^{a}	3323^{b}	0.759^{bc}	0.760^{c}	3388^{b}
	Enzyme Inclusion								
	Control	3490	0.816	0.795	3610	3394	0.777	0.776	3466
	Enzyme	3536	0.829	0.806	3621	3368	0.775	0.771	3493
	p-value								
Corn Source		0.005	0.015	0.011	<0.001	0.001	0.002	0.003	0.041
Enzyme		0.126	0.097	0.092	0.632	0.439	0.829	0.441	0.394
Corn X Enzyme		0.800	0.515	0.800	0.288	0.923	0.807	0.923	0.173
SEM		14.2	0.004	0.003	13.3	33.2	0.008	0.008	19.9

[a-c] Main effect and treatment means differ significantly ($P \leq 0.05$)
[1]Econase XT, AB Vista, Marlborough, UK
[2] n = 10 replicate pens

sources was 176 kcal/kg, with a decrease (P<0.001) observed in birds fed corn source A and B compared to C, D, E and F fed broilers. The digestibility data observed on d 18 corresponded with differences in observed BW as corn source D and E yielded broilers with 2 of the highest observed body weights. Increases in IEDC and INDC with xylanase inclusion were observed, although neither reached the level of statistical significance (P=0.097 and 0.092, respectively).

On d 41, nutrient variability between corn sources resulted in a main effect difference in energy and nitrogen digestibility on all evaluated parameters, including IDE (P=0.001), INDC (P=0.002), IEDC (0.003), and AME (P=0.041). Nutrient variability associated with corn source resulted in a range of 213 kcal/kg for IDE, with birds fed corn source C yielding the lowest value. Broilers fed corn source B and E increased (P=0.001) IDE compared to A, C and F corn sources, with corn source D yielding intermediate results. Nitrogen digestibility was influenced by nutrient variability, resulting in a range of 6.1 percent for INDC. Similarly, broilers fed corn source B and E yielded the highest INDC value, resulting in an increase (P=0.002) compared to birds fed corn source A and F; intermediate results were observed in birds fed C and D corn. An increase (P=0.003) in IEDC was observed in birds fed corn source B compared to A, C, D and F fed broilers, with corn source E yielding intermediate results. The nutrient variability resulted in a range of 4.3 percent for IEDC between corn sources. The range of AME for the 6 corn sources was 194 kcal/kg, with corn source B fed broilers yielding the highest value. Birds fed corn source B resulted in an increase (P=0.041) in AME compared to E and F corn fed broilers, with all other sources of corn

yielding intermediate results. No main effect differences were observed with xylanase inclusion, and an interaction was not observed between corn source and xylanase inclusion.

Volatile Fatty Acid Determination

At d 18 the inclusion of xylanase increased the concentration of butyrate in the ceca (Table 5, P=0.031). The inclusion of xylanase increased total VFA concentration in the ceca; however not at a significant level (P=0.062). On d 41, an interaction between corn source and xylanase inclusion was observed in isovalerate percentage in the cecal contents (Table 5, p=0.038). Differences were not observed in the other sections of the intestine that were sampled (data not shown).

Table 5-5. VFA compound concentration (mM/g) and percent (%) of fresh cecal contents of male broilers fed sources of corn varying in nutrient profiles, with and without xylanase[1] inclusion, on 18 and 41 days of age[2].

	Corn							Enzyme				
Compound	**A**	**B**	**C**	**D**	**E**	**F**	**p-value**	**Control**	**Xylanase**	**p-value**	**corn x xylanase**	**SEM**
Concentration	**Day 18**											
Acetate	11.664	12.456	12.759	11.444	10.564	11.726	0.430	11.234	12.293	0.103	0.873	0.323
Proprionate	1.319	1.221	1.322	1.168	1.017	1.112	0.195	1.149	1.234	0.271	0.957	0.040
Isobutyrate	0.106	0.110	0.108	0.113	0.890	0.100	0.558	0.103	0.105	0.787	0.697	0.004
Butyrate	4.141	4.336	3.912	3.750	3.634	3.652	0.552	3.612[b]	4.184[a]	0.031	0.930	0.133
Isovalerate	0.128	0.150	0.135	0.149	0.127	0.121	0.426	0.132	0.138	0.596	0.706	0.005
Valerate	0.300	0.323	0.315	0.327	0.277	0.298	0.805	0.298	0.314	0.492	0.826	0.011
Total VFA	17.646	18.580	18.538	16.938	15.694	16.995	0.445	16.516	18.255	0.062	0.888	0.465
Percent (%)												
Acetate	66.42	68.00	68.55	67.16	67.30	70.05	0.452	68.59	67.30	0.230	0.975	0.005
Proprionate	7.74	6.50	7.20	6.95	6.40	6.35	0.142	6.81	6.88	0.804	0.724	0.002
Isobutyrate	0.74	0.50	0.65	0.63	0.45	0.65	0.449	0.67	0.53	0.130	0.120	0.001
Butyrate	22.89	22.20	21.30	22.42	23.00	20.60	0.644	21.29	22.80	0.110	0.950	0.005
Isovalerate	0.95	1.00	0.90	0.95	1.00	0.90	0.958	0.97	0.93	0.695	0.367	<0.001
Valerate	1.68	1.70	1.80	1.84	1.70	1.70	0.959	1.76	1.72	0.696	0.944	0.001
Concentration	**Day 41**											
Acetate	9.896	10.415	11.277	10.877	10.340	11.380	0.321	10.440	10.943	0.244	0.174	0.224
Proprionate	3.017	3.058	3.880	3.525	3.339	3.758	0.118	3.440	3.417	0.937	0.536	0.109
Isobutyrate	0.248	0.233	0.262	0.255	0.251	0.254	0.870	0.253	0.249	0.754	0.301	0.006
Butyrate	3.110	3.253	3.501	3.480	3.586	3.431	0.602	3.357	3.428	0.679	0.353	0.084
Isovalerate	0.299	0.295	0.341	0.328	0.301	0.318	0.714	0.318	0.308	0.599	0.095	0.010
Valerate	0.550	0.524	0.603	0.579	0.550	0.505	0.834	0.556	0.546	0.823	0.973	0.022
Total VFA	17.105	17.766	19.855	19.030	18.353	19.633	0.188	18.351	18.878	0.447	0.195	0.365
Percent (%)												
Acetate	57.60	57.95	56.74	57.42	56.65	58.30	0.843	56.95	57.93	0.247	0.966	0.004
Proprionate	17.60	17.84	19.53	18.16	17.95	18.90	0.751	18.53	18.12	0.650	0.855	0.004
Isobutyrate	1.50	1.26	1.21	1.32	1.40	1.15	0.228	1.33	1.29	0.639	0.731	0.001
Butyrate	18.20	17.95	17.63	18.32	19.40	17.40	0.235	18.24	18.07	0.685	0.720	0.002
Isovalerate	1.75	1.84	1.95	1.58	1.75	1.55	0.359	1.79	1.68	0.400	0.038	0.001
Valerate	3.25	3.00	3.05	3.05	3.00	2.55	0.578	3.09	2.88	0.336	0.731	0.001

[a,b] Main effect and treatment means differ significantly ($P \leq 0.05$)
[1]Econase XT, AB Vista, Marlborough, UK
[2] n = 10 replicate pens

Discussion

In the current study, various sources of corn were obtained from 6 different regions in the US to represent the variability of corn available to feed manufactures. Corn source impacted early BW on d 18. Similar results were observed in a trial conducted by Collins and Moran (2001) in which corn source impacted feed consumption, BW gain and FCR. The differences in BW validates that the nutrient profile varies between corn sources. On d 31, an interaction was observed between corn

source and xylanase inclusion, suggesting that xylanase benefit may be related to corn nutrient value. Yegani and Korver (2013) conducted a study feeding three different sources of corn with the inclusion of xylanase, a xylanase/amylase/protease blend, or a xylanase/ β-glucanase blend. Similar to the current results, they observed that the inclusion of xylanase in only one corn source having negative impacts on performance variables. The same experiment observed bird responses to enzyme supplementation mainly in the grower phase, which correlates to the interaction between corn source and xylanase inclusion on d 31 BW in the present study. At the conclusion of the trial, FCR was influenced by corn source with values ranging from 1.893 to 2.005. Xylanase inclusion reduced FCR during the finisher phase compared to the diets without xylanase inclusion. Improvements in FCR with the inclusion of xylanase has been previously reported by several authors (Gao et al., 2007; Esmailipour et al., 2011; Kalmendal and Tauson, 2012; Masey O'Neill et al., 2012). The ability of xylanase to improve performance at the end of the trial is supportive of the concept that the length of time feeding enzymes may be impactful on the final outcome (Rosen, 2002).

One of the challenges associated with exogenous enzyme supplementation is that enzyme inclusion may not always lead to enhanced growth performance or nutrient digestibility (Cowieson et al., 2006). This inconsistency was observed in the current study in which supplementation with xylanase improved FCR; however this improvement was not reflected with nutrient digestibility. Nutrient digestibility improvements were observed in a study conducted by Cowieson et al. (2006), but these improvements were dependent on diet, resulting in a diet x enzyme interaction. Similar

results were observed by Yegani and Korver (2013) in which enzyme inclusion increased IDE in one of the corn sources, but there were no differences in the other two sources of corn, indicating that enzyme effectiveness was dependent on corn source. Cowieson (2010) reports that the nutritional quality of the diet is probably the most important factor that influences responses to enzyme inclusion. In the current study, no xylanase x corn source interactions were observed regarding nutrient digestibility. On d 18, differences in corn source were observed in all evaluated parameters including IDE, INDC, IEDC and AME. Similar results were previously reported in which differences were observed between corn sources regarding IDE and AMEn (Gehring et al., 2012). A main effect difference was observed between corn source regarding d 41 IDE, INDC and IEDC. Gehring et al. (2012) reported ileal N digestibility to be positively correlated with IDE in a similar study evaluating the effects of corn source on ileal nutrient digestibility. Nutrient variability between the different corn sources influenced d 41 AME. Similar results were reported by Gehring et al. (2012) with differences in AMEn associated with variability between corn source.

Xylanase inclusion increased levels of butyrate in the cecum from 3.612 to 4.184 mM/g. Similar results have been observed with linear increases in levels of acetate and valerate in the ileum and levels of acetate in the cecum as enzyme levels increased in wheat-based diets (Wang et al., 2005). The same experiment reported an increase in total VFA content in the ileum and cecum with the inclusion of enzyme. In the current study, an increase in total VFA content was observed in the cecum with xylanase inclusion, but not to a level of statistical significance. On d 41, an interaction

between corn source and xylanase inclusion was observed regarding isovalerate percentage in the cecum. These data confirm that exogenous enzyme supplementation does influence VFA contents and percentage which agrees with other authors (Wang et al., 2005; Choct et al., 1999). The production of VFA in the large intestine is impactful not only for energy content, but also for the systemic effects that VFA absorption may cause. It is suggested that the absorption of VFA, such as butyrate, stimulates the release of certain gut hormones which may improve digestibility of nutrients in the proximal digestive tract (Singh et al., 2012, Cowieson and Masey O'Neill 2013, Masey O'Neill et al., 2014). These data confirm that nutrient variability of corn does impact broiler performance, nutrient utilization and VFA profiles in cecal contents.

The results of this experiment indicate the importance of rapid and accurate evaluation of corn nutrient content to maximize observed growth performance. Multiple growth parameters were impacted and varied depending on corn source. These data also indicate that other factors associated with corn source may in fact influence performance, as increased digestibility data did not always result in improvements in growth performance, especially in older broilers. Digestibility data following the starter phase tended to correlate with the observed BW related to corn source. Nutrient digestibility at the conclusion of the trial tended to be less predictable of the observed growth performance. Additionally, the impact or benefit of xylanase may have been limited or masked due to the varying diet nutrient content with equal corn source inclusion and the lack of AME reduction in the experimental diets. Following the starter phase, energy and nitrogen digestibility were increased by 1.3 and 1.1%, respectively,

with xylanase inclusion, but did not reach the level of statistical significance. Cecal butyrate levels were increased with xylanase inclusion. The increase in butyrate levels may indicate an alteration in gastrointestinal microbiota through the generation and change of oligosaccharides through polysaccharide hydrolyzation, as discussed by Masey O'Neill (2012).

The results of this study confirm the ability of exogenous enzymes to influence VFA production, as butyrate was increased with xylanase and an interaction was observed between corn source and xylanase in isovalerate percentage in the cecum. The production of VFA is not only important for energy, but also for systemic effects that VFA absorption may cause such as the release of certain gut hormones that may improve nutrient digestibility in the proximal digestive tract.

CHAPTER VI

EFFECTS OF CALCIUM AND PHOSPHORUS LEVEL IN A DIET CONTAINING PHYTASE ON MALE BROILER PERFORMANCE, BREAST MEAT YIELD, TIBIA CHARACTERISTICS, FECAL CHARACTERISTICS, AND LITTER CHARACTERISTICS

Introduction

In animals, Ca and P are essential nutrients for several biochemical pathways and skeletal formation, and the physiological roles of these macro-minerals are intricately linked (Wasserman, 1960). In poultry nutrition, the majority of diets are corn and SBM-based, and feed ingredients from plant sources are inadequate in meeting these mineral requirements. Therefore, Ca and P requirements are largely met by dietary inclusions of limestone, inorganic P supplements such as dicalcium phosphate, and if applicable, meat and bone meal. Considerable amounts of P are present in plant-sourced feed ingredients, but this P is mostly in the form of phytate-bound P which is largely unavailable to monogastric animals. Phytate, the mixed salt of phytic acid (myo-inositol hexaphosphate; IP_6) exists predominately in feed ingredients as IP_6 (Kasim and Edwards, 1998). Therefore, the polyanionic phytate molecule carries a maximum of 12 negative charges and has the potential to chelate 6 Ca molecules and form insoluble Ca-phytate complexes. Phytate limits the availability of Ca and P as a result of insoluble Ca-phytate complex formation. Insoluble Ca-phytate complexes are resistant to enzymatic hydrolysis by phytases (Taylor, 1965). Therefore, Ca can be a limiting factor of phytate

degradation. Even though Ca has the lowest affinity for phytate, it has the greatest impact because it is the mineral present in the diet at the highest concentration.

Exogenous microbial phytases are mainly active in the crop and proventriculus of poultry where acidic conditions increases substrate solubility and phytate is more susceptible to degradation (Campbell and Bedford, 1992). By enhancing the digestibility of phytate-P and utilizing lower dietary P levels, phytase reduces the excretion of undigested and excess P (Simons et al., 1990). The efficacy of exogenous phytase can be influenced by several factors, including substrate level from plant sources (Kim et al., 2002; Selle et al., 2003) and mineral-phytate complexes, and in particular Ca-phytates (Nelson, 1984; Angel et al., 2002). A study conducted by Lei et al. (1994) concluded that higher Ca levels and greater Ca:P ratios depressed exogenous phytase efficacy. Corn and SBM- based diets may be advantaged by relatively low Ca levels and small Ca:P ratios. In 42 d old broilers, Aksakal and Bilal (2002) found that phytase increased P retention by 8.5 percent in diets with a Ca:P of 2:1, but P retention was increased to 39.8 percent with Ca:P at 1:1. It has been suggested that Ca may inhibit phytase activity by competitive inhibition of the phytase competing for active sites with Ca (Qian et al., 1996). Exogenous phytase has the ability to enhance Ca absorption by the hydrolysis of phytase to lower phytate esters. Lower phytate esters have a reduced capacity to chelate Ca, therefore insoluble Ca-phytate complexes are diminished and Ca availability is enhanced (Selle et al., 2009).

Including exogenous phytase at elevated levels in broiler diets presents the question as to what the appropriate adjustments in dietary Ca and P levels and Ca:P should be for optimum performance and nutrient utilization.. The ability of phytate to chelate Ca needs to be established so that adequate adjustments to dietary Ca levels can be made in diets supplemented with elevated levels of exogenous phytase. To maximize phytase activity, dietary Ca levels should be kept to a minimum in phytase-supplemented poultry diets but without compromising skeletal integrity and growth performance. The current study evaluated 3 levels of Ca and 2 levels of P in diets containing elevated levels of exogenous phytase to determine optimal mineral inclusion rates.

Materials and Methods

Experimental Design

The objective of the current study was to evaluate the effects of various Ca and P levels in a broiler feeding program containing a super-dose level of phytase[11] (4 lb/ton GraINzyme®; 4500 FTU/kg, analyzed in pelleted feed) on broiler performance and breast meat yield. The experimental design consisted of a 3 x 2 factorial of mineral reductions from a standard industry type feeding program. Ca levels were reduced 0.11, 0.13, and 0.15% in each phase of the feeding program, and P was reduced 0.12 and 0.15% in each phase compared to the industry standard. Thus, a total of 6 dietary treatments were utilized over a 49 d feeding period. Diets were fed to 1,260 Ross 708 male broilers with 10 replicate pens per treatment each containing 21 birds per replicate.

[11] GraINzyme® - Agrivida, Inc., Woburn, MA 01801

On d of hatch, chicks were randomly allotted to floor pens and dietary treatments based on initial average body weight. Chicks were provided age appropriate supplemental heat and given access to feed and water *ad libitum.* Chicks were placed in 1.67 m^2 rearing pens equipped with tube feeders and nipple drinkers with fresh pine shavings as bedding material. Animal care was provided in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC).

Experimental Diets

Corn and soybean meal (SBM)- based diets were formulated using Ca and aP industry type diet base line mineral levels as follows: starter 0.90% Ca and 0.45% aP, grower 0.84% Ca and 0.42% aP, finisher 0.76% Ca and 0.38% aP, and withdrawal 0.76% Ca and 0.38% aP. The basal diet (Table 6-1) includes a Ca concentration of 0.03% less than the lowest target value to account for the Ca contribution from monocalcium phosphate wich was used to meet target dose for aP. The starter was fed through d 14, the grower from d 15 to 28, the finisher from d 29 to 42, and the withdrawal from d 43 to 49.

Table 6-1. Ingredient profiles, calculated nutrient concentration, and analyzed nutrient concentration for the starter, grower, finisher, and withdrawal phases.

Basal Experimental Diets				
Ingredient (%)	**Starter**	**Grower**	**Finisher**	**Withdrawal**
Corn	1142.27	1542.35	2353.72	2158.02
Soybean meal	738.92	752.96	906.15	725.82
Fat animal/vegetable blend	46.21	67.44	123.37	110.76
DL-Methionine	5.56	6.36	8.08	6.67
Lysine HCL	2.65	4.03	5.83	5.63
L-Threonine	0.91	1.47	2.42	2.17
Limestone	22.57	26.28	34.13	30.75
Monocalcium phosphate	17.21	18.85	21.68	19.84
Salt	8.70	10.66	11.73	8.24
Sodium bicarbonate	--	--	4.88	7.31
Vitamins[1]	3.00	4.90	7.00	6.20
Minerals[2]	1.00	1.23	1.75	1.55
Salinomycin[3]	1.00	1.23	1.75	1.55
Calculated Nutrients				
Protein	22.50	20.00	18.00	17.00
Calcium	0.72	0.66	0.58	0.58
Phosphorus	0.55	0.51	0.45	0.45
Available phosphorus (aP)	0.30	0.27	0.23	0.23
Dig. Methionine	0.58	0.54	0.48	0.46
Dig. Lysine	1.20	1.07	0.95	0.90
Dig. Threonine	0.78	0.71	0.65	0.61
Dig. Tryptophan	0.19	0.17	0.16	0.15
Dig. Arginine	1.26	1.12	1.00	0.97
Dig. TSAA	0.89	0.81	0.74	0.70
Crude fat	4.64	5.25	6.15	6.27
Sodium	0.18	0.18	0.18	0.18
Analyzed Nutrients				
Moisture	12.61	13.13	12.73	13.75
Crude protein	22.0	19.2	18.7	17.5
Crude fat	4.49	2.01	5.73	5.74
Acid detergent fiber	3.2	2.3	2.9	2.8
Ash	4.66	5.00	4.32	4.32
Phosphorus	0.58	0.61	0.53	0.48
Calcium	0.79	0.93	0.66	0.84
Sodium	0.15	0.17	0.15	0.16

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 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.
[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] Sacox® - Huvepharma, Peachtree City, GA

During feed manufacturing, one large basal diet for each feeding phase was mixed, and included phytase at 4 lb/ton (GraINzyme®; 4500 FTU/kg, analyzed in pelleted feed). Limestone, monocalcium phosphate, and corn starch was then added to 6 batches of the basal diet so each treatment diet would have the desired calculated mineral levels. All diets were pelleted at 80°C with a conditioning time of 12 s. The starter diet was fed as a crumble, while the grower, finisher, and withdrawal diets were fed as a pellet.

Bird Performance Data Collection

Pen BW and feed consumption (FC) were measured on days of dietary changes (d 14, 28, 42, and 49) for the calculation of mortality corrected FCR. On d 50, following an 8 hour feed withdrawal period, 3 birds per replicate were removed and processed to obtain carcass, breast and tender weights and yield. After weighing, the left and right *P. major* filets were palpated and scored for WS based on the scoring system of Kuttappan et al. (2012) and WB based on the scoring system of Tijare et al. (2016).

Bone, Fecal and Litter Mineral Analysis

On d 28, 3 birds per replicate were euthanized and the right tibias were collected and pooled per replicate pen for the determination of bone ash percent and weight. Tibia ash was determined on a fat- free dry matter basis. Bones were dried at 105°C for 24 h and then ashed at 600°C for 24 h. Bones were weighed pre and post ashing. Fecal samples were collected on d 45. A minimum of 8 defecations were collected per replicate pen. Litter samples were collected at the conclusion on the trial on d 50. Tibias, fecal and litter samples were analyzed for total Ca and P content on a dry matter basis using ICP (AOAC 2011.14).

R**esults**

During the starter phase a linear increase in BW was observed as Ca was reduced in the diet (Table 6-2). Diets containing the low Ca levels (0.15 reduction) yielded an increase (p=0.010) in d 14 BW compared diets with the high Ca levels (0.11 reduction). Available P did not impact BW on d 14. On d 28 an interaction was present between dietary Ca and aP (0.009). The diet containing the highest levels of Ca and aP had lower (p=0.003) BW by 26 g compared to diets containing the same level of Ca with the low aP level. The diet containing the highest levels of Ca and aP also had lower BW compared to diets containing moderate levels of Ca, regardless of aP level, and low Ca level and high aP. The diet containing low Ca and high aP yielded a heavier BW by 28 g compared to diets containing low Ca and low aP. Calcium and aP level did not impact BW during the remainder of the trial (d 42 and d 49).

Table 6-2. Body weight and weight gain of male broiler fed varying levels of calcium and phosphorus with the addition of phytase[1] at super-dose levels.

	Reductions		Body Weight (kg)				Weight Gain (kg)			
Treatment	**Ca**	**P**	**D 14 (g)**	**D 28**	**D 42**	**D 49**	**Starter (g)**	**Grower**	**Finisher**	**Withdrawal**
High Ca, High P	0.11	0.12	452	1.528[c]	2.954	3.625	410	1.076[b]	1.426	0.672
High Ca, Low P	0.11	0.15	457	1.554[ab]	3.002	3.683	415	1.096[ab]	1.448	0.681
Moderate Ca, High P	0.13	0.12	462	1.562[ab]	3.005	3.680	420	1.099[a]	1.444	0.675
Moderate Ca, Low P	0.13	0.15	458	1.568[ab]	3.005	3.670	416	1.110[a]	1.437	0.665
Low Ca, High P	0.15	0.12	467	1.579[a]	2.984	3.624	425	1.112[a]	1.405	0.640
Low Ca, Low P	0.15	0.15	462	1.551[bc]	2.997	3.684	419	1.089[ab]	1.446	0.688
			--	0.003	--	--	--	0.017	--	--
Main Effect Means										
--	**Ca**									
--	0.11	--	455[b]	1.541[b]	2.978	3.654	413[b]	1.086[b]	1.437	0.676
--	0.13	--	460[ab]	1.565[a]	3.005	3.675	418[ab]	1.105[a]	1.440	0.670
--	0.15	--	465[a]	1.565[a]	2.990	3.654	422[a]	1.101[ab]	1.425	0.664
--		**P**								
--	--	0.12	461	1.566	2.981	3.643	418	1.096	1.425	0.662
--	--	0.15	459	1.558	3.001	3.679	417	1.098	1.443	0.678
p-value										
Calcium			0.010	0.008	0.501	0.809	0.012	0.048	0.747	0.784
Phosphorus			0.559	0.868	0.300	0.240	0.499	0.673	0.269	0.282
Ca x P			0.194	0.009	0.559	0.567	0.189	0.017	0.503	0.288
SEM			0.001	0.005	0.012	0.021	0.001	0.004	0.009	0.011

[a,b] Main effect and treatment means differ significantly ($P \leq 0.05$)

[1]GraINzyme® - Agrivida, Inc., Woburn, MA 01801

During the starter phase, linear increases in weight gain were observed as Ca levels were reduced (p=0.012) in the diet (Table 6-2). Diets containing the low Ca levels (0.15 reduction) yielded an increase (p=0.001) in starter weigh gain by 9g compared to diets with the high Ca levels (0.11 reduction), with moderate Ca levels (0.13 reduction) yielding intermediate results. During the grower phase an interaction was present between Ca and aP level (p=0.017). Diets containing high Ca and high aP reduced (p=0.017) weight gain compared to moderate levels of Ca at both aP levels and the low

Ca and high aP diet. Weight gain was not impacted by Ca or aP during the finisher and withdrawal phases of production.

Feed conversion ratios during the starter phase were influenced by dietary Ca and aP as an interaction was present between these mineral levels ($p=0.016$; Table 6-3). Broilers fed moderate and low Ca with high aP had lower ($p=0.005$) starter FCR compared to broilers with low aP levels at the same Ca concentrations. Calcium and aP levels influenced grower FCR as an interaction was present ($p=0.000$). Reducing aP in diets containing low levels of Ca increased FCR by 3.5 points compared to diets containing low Ca and high aP. A main effect difference was observed in Ca level during the finisher phase as moderate levels of Ca reduced ($p=0.020$) finisher FCR compared to diets containing high Ca levels. Broilers fed low levels of Ca yielded intermediate results. Mineral levels did not impact FCR during the withdrawal phase. An interaction was observed cumulatively through d 28 ($p<0.000$) and d 42 ($p=0.005$) between Ca and aP levels. Diets containing high Ca and the low level of Ca with low aP had a higher ($p<0.000$) cumulative FCR through d 28 and d 42 compared to diets containing moderate Ca and low Ca with high aP. A main effect difference was observed with Ca and aP levels regarding cumulative FCR at the conclusion of the trial (d 1-49). Broilers fed moderate levels of Ca had a lower ($p=0.006$) cumulative FCR by 0.016 compared to high Ca and by 0.01 compared to low Ca. High aP levels had higher ($p=0.042$) cumulative FCR compared to low aP levels at the conclusion of trial.

Table 6-3. Feed conversion ratio and cumulative FCR of male broiler fed varying levels of calcium and phosphorus with the addition of phytase[1] at super-dose levels.

	Reductions		Feed Conversion Ratio (FCR)				Cumulative FCR		
Treatment	Ca	P	Starter	Grower	Finisher	Withdrawal	D 1-28	D 1-42	D 1-49
High Ca, High P	0.11	0.12	1.194^{ab}	1.441^{ab}	1.669	1.985	1.372^{a}	1.504^{a}	1.582
High Ca, Low P	0.11	0.15	1.189^{abc}	1.442^{ab}	1.656	2.011	1.372^{a}	1.500^{a}	1.583
Moderate Ca, High P	0.13	0.12	1.176^{c}	1.430^{bc}	1.633	1.985	1.359^{b}	1.481^{b}	1.565
Moderate Ca, Low P	0.13	0.15	1.191^{ab}	1.417^{c}	1.646	2.015	1.355^{b}	1.483^{b}	1.569
Low Ca, High P	0.15	0.12	1.180^{bc}	1.415^{c}	1.651	2.027	1.349^{b}	1.480^{b}	1.567
Low Ca, Low P	0.15	0.15	1.202^{a}	1.450^{a}	1.659	1.998	1.380^{a}	1.504^{a}	1.586
p-value			0.005	0.000	--	--	0.000	0.000	--
Main Effects Means									
--	**Ca**								
High	0.11	--	1.191	1.442^{a}	1.663^{a}	1.998	1.372^{a}	1.502^{a}	1.583^{a}
Moderate	0.13	--	1.184	1.424^{b}	1.639^{b}	2.000	1.357^{b}	1.482^{c}	1.567^{b}
Low	0.15	--	1.191	1.432^{ab}	1.655^{ab}	2.013	1.365^{ab}	1.492^{b}	1.577^{a}
--		**P**							
High	--	0.12	1.183^{b}	1.429	1.651	1.999	1.360^{b}	1.488^{b}	1.572^{b}
Low	--	0.15	1.194^{a}	1.437	1.654	2.008	1.369^{a}	1.496^{a}	1.579^{a}
p-value									
Ca			0.215	0.010	0.020	0.804	0.002	0.000	0.006
P			0.009	0.094	0.660	0.654	0.007	0.043	0.042
Ca x P			0.016	0.000	0.264	0.404	0.000	0.005	0.137
SEM			0.002	0.003	0.004	0.015	0.002	0.002	0.002

[a,b] Main effect and treatment means differ significantly ($P \leq 0.05$)
[1] GraINzyme® - Agrivida, Inc., Woburn, MA 01801

During the starter phase a main effect difference was observed between Ca levels. Broilers fed diets containing low Ca levels had a higher (p=0.016) FC compared to diets containing high (0.7 g/bird/d) and moderate (0.5 g/bird/d) levels of Ca (Table 6-4). Available P level did not impact FC during the starter phase. During the grower phase broiler fed low aP diets increased (p=0.016) FC by 1.2 g/bird/d compared to high aP diets. Calcium and aP levels did not impact FC during the finisher and withdrawal

phases of production. A main effect difference was observed with aP level regarding cumulative FC through 28 and 42 d of age. Broilers fed diets with low aP had greater FC compared to high aP diets. A 0.7 g/bird/d difference was observed through d 28 with low aP compared to high aP diets. Through d 42, low aP diets had greater FC by 1.1 g/bird/d compared to diets with high aP levels. At the conclusion of the trial, Ca and aP did not impact FC.

Table 6-4. Dietary feed consumption of male broiler fed varying levels of calcium and phosphorus with the addition of phytase[1] at super-dose levels.

	Reductions		Feed Consumption g/bird/day				Cumulative FC g/bird/day		
Treatment	Ca	P	Starter	Grower	Finisher	Withdrawal	D 1-28	D 1-42	D 1-49
High Ca, High P	0.11	0.12	34.9	110.3	169.8	185.4	72.6	101.3	112.0
High Ca, Low P	0.11	0.15	35.2	113.0	171.9	190.7	73.9	103.1	114.3
Moderate Ca, High P	0.13	0.12	35.2	111.1	169.8	191.0	72.8	101.1	112.5
Moderate Ca, Low P	0.13	0.15	35.2	111.9	168.9	186.8	73.3	101.6	112.5
Low Ca, High P	0.15	0.12	35.7	112.2	166.6	183.5	73.7	101.1	111.5
Low Ca, Low P	0.15	0.15	35.8	112.4	170.0	190.5	74.0	102.3	113.3
Main Effect Means									
--	Ca								
High	0.11	--	35.0[b]	111.7	170.8	188.0	73.3	102.2	113.1
Moderate	0.13	--	35.2[b]	111.5	169.4	188.9	73.1	101.4	112.5
Low	0.15	--	35.7[a]	112.3	168.3	187.0	73.8	101.7	112.4
--		P							
High	--	0.12	35.2	111.2[b]	168.7	186.6	73.0[b]	101.2[b]	112.0
Low	--	0.15	35.4	112.4[a]	170.3	189.3	73.7[a]	102.3[a]	113.4
p-value									
Ca			0.016	0.369	0.418	0.902	0.091	0.499	0.748
P			0.503	0.016	0.338	0.436	0.015	0.047	0.113
Ca x P			0.856	0.115	0.541	0.370	0.250	0.627	0.478
SEM			0.106	0.400	0.954	2.303	0.227	0.396	0.609

[a,b] Main effect and treatment means differ significantly ($P \leq 0.05$)
[1] GraINzyme® - Agrivida, Inc., Woburn, MA 01801

At the end of the trial, broilers were processed for evaluation of carcass, breast, and tender weighs and yields. Calcium and aP did not impact live weight, carcass weight, or breast weight. Low aP levels increased ($p=0.026$) tender weight by 4 g compared to high aP diets (Table 6-5). Calcium and aP levels did not impact processing yield percentages of the carcass, breast, or tenders. Breast quality parameters of woody breast (WB) and white striping (WS) were also evaluated. An interaction was present between Ca and aP for WB incidence. Diets containing high Ca and low aP had a higher ($p=0.051$) incidence of WB compared to moderate Ca and low aP diets. Calcium and aP did not have an impact on the incidence of WS.

Table 6-5. Processing weights and yields and breast quality of male broilers fed varying levels of calcium and phosphorus with the addition of phytase[1] at super-dose levels.

	Reductions		Processing Weights (g)				Processing Yields (%)			Quality	
Treatment	Ca	P	Live	Carcass	Breast	Tender	Carcass	Breast	Tender	WB[2]	WS[3]
High Ca, High P	0.11	0.12	3653	2894	871	154	79.23	29.79	5.35	1.30[ab]	1.16
High Ca, Low P	0.11	0.15	3722	2972	889	161	79.36	29.87	5.40	1.53[a]	1.15
Moderate Ca, High P	0.13	0.12	3691	2930	856	153	79.40	29.20	5.22	1.29[ab]	1.20
Moderate Ca, Low P	0.13	0.15	3704	2943	864	156	79.44	29.30	5.30	1.25[b]	1.16
Low Ca, High P	0.15	0.12	3737	2978	868	153	79.69	29.12	5.17	1.50[ab]	1.18
Low Ca, Low P	0.15	0.15	3728	2961	873	156	79.42	29.44	5.32	1.30[ab]	1.25
p-value			--	--	--	--	--	--	--		--
Main Effect Means											
--	Ca										
High	0.11	--	3687	2933	880	158	79.29	29.83	5.38	1.41	1.16
Moderate	0.13	--	3697	2936	860	154	79.42	29.25	5.26	1.27	1.18
Low	0.15	--	3733	2969	870	158	79.55	29.28	5.24	1.40	1.21
--		P									
High	--	0.12	3694	2934	865	154[b]	79.44	29.35	5.24	1.36	1.18
Low	--	0.15	3718	2958	875	158[a]	79.40	29.54	5.34	1.36	1.19
p-value											
Ca			0.686	0.630	0.507	0.500	0.612	0.134	0.106	0.177	0.766
P			0.590	0.479	0.428	0.026	0.876	0.490	0.081	0.984	0.882
Ca x P			0.766	0.504	0.890	0.810	0.728	0.918	0.754	0.051	0.746
SEM			23.20	17.53	7.22	1.17	0.001	0.001	0.000	0.036	0.032

[a,b] Main effect and treatment means differ significantly ($P \leq 0.05$)
[1] GraINzyme® - Agrivida, Inc., Woburn, MA 01801
[2] Woody Breast
[3] White Striping

An interaction was present between Ca and aP regarding bone ash percent. Low aP levels in diets containing moderate Ca levels had a lower (p=0.003) bone ash by 3.02% compared to diets with moderate Ca and high aP (Table 6-6), and was also lower than all other dietary treatments. Calcium and aP did not impact ash weight on a g/bone basis. Tibia Ca and P percent was also determined on d 28. Dietary Ca or aP did not impact tibia Ca percentage. An interaction was present between Ca and aP regarding

tibia P percent. A decrease (p = 0.017) in tibia P percent was observed in diets containing low Ca with low aP compared to high Ca with low aP diets.

Table 6-6. Day 28 tibia characteristics of broilers fed varying levels of calcium and phosphorus with the addition of phytase[1] at super-dose levels.

	Reductions		**Tibia Characteristics**			
Treatment	**Ca**	**P**	**Ash %**	**Ash Wt (g/bone)**	**Tibia Ca %**	**Tibia P %**
High Ca, High P	0.11	0.12	52.34[a]	1.623	36.896	17.621[ab]
High Ca, Low P	0.11	0.15	52.19[a]	1.671	36.799	17.784[a]
Moderate Ca, High P	0.13	0.12	52.38[a]	1.670	36.756	17.525[b]
Moderate Ca, Low P	0.13	0.15	49.36[b]	1.581	36.733	17.664[ab]
Low Ca, High P	0.15	0.12	52.19[a]	1.652	36.596	17.690[ab]
Low Ca, Low P	0.15	0.15	52.94[a]	1.641	36.500	17.526[b]
p-value			0.003	--		0.017
Main Effect Means						
--	**Ca**					
High	0.11	--	52.26[a]	1.647	36.848	17.703
Moderate	0.13	--	50.87[b]	1.638	36.745	17.595
Low	0.15	--	52.57[a]	1.691	36.548	17.603
--		**P**				
High	--	0.12	52.30	1.655	36.749	17.612
Low	--	0.15	51.50	1.633	36.677	17.655
p-value						
Ca			0.020	0.931	0.551	0.131
P			0.119	0.422	0.752	0.375
Ca x P			0.010	0.121	0.988	0.009
SEM			0.28	0.015	0.118	0.029

[a,b] Main effect and treatment means differ significantly (P≤0.05)
[1]GraINzyme® - Agrivida, Inc., Woburn, MA 01801

Fecal samples were collected on d 45 to determine moisture, fecal Ca and fecal P content (Table 6-7). Dietary Ca or aP did not impact fecal moisture percent. Fecal Ca and P percent was greater (p = 0.036 and p = 0.019, respectively) in diets containing high aP levels compared to low aP diets. Dietary Ca level did not impact fecal Ca or P percent. Litter samples were collected at the conclusion of the trial to determine litter Ca and P percent. As expected, high and moderate levels of Ca resulted in higher (p =

0.055) litter Ca percent compared to low levels of dietary Ca. Dietary aP did not impact litter Ca. High levels of dietary aP resulted in greater (p = 0.025) litter P by 0.14% compared to low aP diets. Dietary Ca did not influence litter P percentage.

Table 6-7. Day 45 fecal characteristics and day 50 litter characteristics of broilers fed varying levels of calcium and phosphorus with the addition of phytase[1] at super-dose levels.

	Reductions		**Fecal Characteristics**			**Litter Characteristics**	
Treatment	**Ca**	**P**	**Moisture %**	**Fecal Ca %**	**Fecal P %**	**Litter Ca %**	**Litter P %**
High Ca, High P	0.11	0.12	82.080	1.868	1.236	2.148	1.289
High Ca, Low P	0.11	0.15	82.351	1.817	1.213	1.903	1.084
Moderate Ca, High P	0.13	0.12	82.471	1.763	1.289	1.912	1.312
Moderate Ca, Low P	0.13	0.15	82.197	1.602	1.173	1.998	1.286
Low Ca, High P	0.15	0.12	82.600	2.147	1.439	1.941	1.310
Low Ca, Low P	0.15	0.15	82.450	1.559	1.041	1.648	1.121
Main Effect Means							
--	**Ca**						
High	0.11	--	82.216	1.843	1.225	2.026[a]	1.187
Moderate	0.13	--	82.334	1.683	1.231	1.953[a]	1.300
Low	0.15	--	82.525	1.853	1.240	1.776[b]	1.121
--		**P**					
High	--	0.12	82.384	1.926[a]	1.321[a]	2.002	1.304[a]
Low	--	0.15	82.333	1.659[b]	1.142[b]	1.850	1.164[b]
p-value							
Ca			0.707	0.455	0.985	0.055	0.211
P			0.868	0.036	0.019	0.100	0.025
Ca x P			0.130	0.182	0.108	0.068	0.341
SEM			0.155	0.067	0.042	0.046	0.031

[a,b] Main effect and treatment means differ significantly (P≤0.05)
[1]GraINzyme® - Agrivida, Inc., Woburn, MA 01801

Discussion

Recent publications have investigated the negative effects of high levels of dietary Ca on broiler performance. A study was conducted by Akter et al. (2016) to

determine the effect of different levels of Ca and aP on phytase activity and its impact on broiler performance. Three levels of Ca and 2 levels of aP were evaluated with or without microbial phytase. The results concluded that increasing Ca level reduced feed intake and BW gain on d 10 and d 24, especially in phytase-supplemented diets. Calcium can form insoluble complexes with phytate P, thus limiting the availability of Ca and P and hindering phytase activity (Angel et al., 2002). Even though Ca has the lowest affinity for phytate, it has the greatest impact because of the high concentrations of Ca in poultry diets. Increases in intestinal pH and low apparent ileal P digestibility in broilers fed diets containing high levels of dietary Ca have been reported (Sebastian et al., 1996; Adeola and Walk, 2013). This may be a possible explanation for the reduction in average BW in the current study in diets containing high Ca levels (0.11 reduction) compared to low levels of Ca (0.15 reduction). On d 28, the diet containing the highest level of Ca and aP had lower ($p=0.003$) BW by 26 g compared to diets containing moderate levels of Ca, regardless of aP level, and low Ca and high aP.

Supplementing with high levels of phytase may improve the bird's utilization efficiency of dietary Ca and P, and indirectly improve amino acid utilization via a reduction in gastric pH and improved pepsin efficacy. High dietary Ca may result in an unfavorable pH in the proventriculus and gizzard, as the optimum pH for pepsin activity is pH 2.8 (Bohak, 1969). Guinotte et al. (1995) reported an increase in gizzard pH reduced Ca solubility in broilers, and higher pH has been implicated in Ca-phytate interactions in the gastrointestinal tract (Selle et al., 2009). High dietary Ca may influence gastrointestinal pH and directly reduce protein digestion through reduced

pepsin efficacy or indirectly influence Ca-phytate precipitation. Reducing dietary Ca in the presence of phytase may spare pepsin efficacy and improve AA digestibility through a reduction in proximal gastric pH (Walk et al., 2012). In the current study, broilers fed diets containing moderate Ca levels and low Ca with high aP resulted in improved FCR through 28 and 42 d of age compared to diets containing high Ca and low Ca with low aP. These improvements in FCR with moderate and low Ca levels may be due to removing Ca chelators, such as phytate, improving the bird's macro-mineral utilization and amino acid digestibility through a reduction in gastric pH levels.

A study conducted by Walk et al. (2012) evaluated 2 levels of Ca and 3 levels of phytase to determine the impacts on broiler performance, tibia ash percent, gastrointestinal pH, and nutrient digestibility. Reducing dietary Ca reduced broiler tibia ash, however, broiler performance was not influenced. Powell et al. (2011) reported an improvement in tibia ash percent as dietary Ca level was reduced from 1.33% to 0.67%. In the current study, moderate levels of dietary Ca (0.13 reduction) with low aP levels reduced tibia ash by 3.02% compared to diets containing the same level of Ca with high aP levels. The moderate Ca and low aP diet also reduced tibia ash compared to high Ca and low Ca diets at both dietary aP levels, indicating that the level of dietary P and Ca:P ratio are important when determining tibia ash percent. It was observed in the current study that diets containing low aP levels resulted in lower tibia P percent in low Ca diets compared to diets containing high levels of Ca.

Increasing concerns regarding environmental pollution from poultry litter has increased pressure on the poultry industry to pay closer attention to nutrient excretion

and its ultimate fate in the environment. Phosphorus is considered a major issue because it is recognized as a direct cause of eutrophication that leads to the impairment of water quality (Summers, 1997). A study was conducted by Coto et al. (2007) to evaluate the effect of various levels of Ca and aP on excretion of Ca and P in young broilers. As expected, the total P in the excreta increased as dietary P level increased. In the current study, as high dietary aP increased fecal P content by 0.18% compared to low aP levels. This finding highlights the importance of formulating feeds close to the P requirement to reduce the potential for negative environmental impacts. Coto et al. (2007) observed increases in Ca in the excreta when the dietary aP increased. Similar results were observed in the current study with high dietary aP increasing fecal Ca compared to low aP diets. This is supported by Viveros et al. (2002) who state that at higher levels of aP, more Ca is bound to phytate which cannot be retained, and is therefore excreted.

Litter samples were collected at the conclusion of the trial to determine litter Ca and P content. According to Leytem et al. (2007), increasing dietary Ca reduces the proportion of soluble inorganic phosphorus due to the formation of Ca:P complexes that increase the amount of Ca:P precipitates in the litter. In the current study, high and moderate levels of dietary Ca resulted in greater litter Ca content. The results of the current study indicate that high dietary aP increased fecal Ca output; however, this increase in fecal Ca was not detected in litter samples. As expected, high dietary aP levels increased litter P content compared to low levels of aP. The results of this study indicate the importance of using accurate mineral matrix values when formulating diets containing super-dose levels of phytase.

CHAPTER VII

CONCLUSION

The inclusion of exogenous enzymes in broiler feeding programs can be a useful tool to improve bird performance and profitability through enhanced digestion and utilization of dietary ingredients. Supplementation with enzymes increases the range of feed ingredients that can be used within the dietary formulation. Enzymes can also reduce the constraints usually set on the inclusion rate of poorly digested ingredients. The nutritive value between batches of ingredients can be enhanced with enzyme supplementation, thus improving the degree of precision diet formulation. The methods of enzyme supplementation discussed in the current set of experiments can provide useful information to nutritionist in the poultry industry when formulating broiler diets.

In Chapter III, xylanase inclusion at 2 levels was evaluated in reduced energy broiler diets to determine the impact on broiler growth performance. Reducing dietary energy in the NC2 diet by 132 kcal/kg reduced d 15 BW by 15.6 g compared to the PC diet. Xylanase inclusion at 60 g/ton (X1) in the NC1 and NC2 diets improved BW to levels that were comparable to the PC diet. On d 23, xylanase (X1) inclusion in the NC2 diet increased BW compared to the reduced energy NC2 control. A linear increase was observed in FCR through the starter phase and cumulative FCR through d 23 as dietary energy levels were reduced. The inclusion of xylanase (X1) in the NC1 diet yielded the lowest observed FCR during these phases. Xylanase inclusion reduced FCR during the withdrawal phase to levels that were comparable to the PC diet. The inclusion of xylanase (X1 and X2) in both reduced energy diets reduced cumulative FCR to levels

that were similar to the PC diet. The elevated inclusion of xylanase at 100 g/ton (X2) in the NC1 diet increased WOG yield when compared to the lower inclusion of xylanase (X1). The PC diet had a greater fat pad weight and yield compared to the NC2 diet. The inclusion of xylanase at both inclusion rates in the NC2 diet reduced fat pat yield when compared to the PC diet. Factorial analysis conducted on the two NC diets evaluating enzyme inclusion indicated the inclusion of xylanase reduced cumulative FCR throughout the experiment. Increasing xylanase inclusion (X2) in the finisher and withdrawal phases reduced FCR when compared to the control diets. The results of this study confirm the ability of xylanase inclusion in reduced energy broiler diets to improve growth performance. The results also indicate the ability of xylanase to improve carcass yield without increasing fat pad yield as the energy from the xylanase is being converted into muscle tissue instead of fat. Including xylanase in broiler diets can potentially reduce waste and increase sustainability by more efficient conversion of feed to muscle.

Chapter IV focused on evaluating the inclusion of NSPase and β-mannanase and confirms the importance of enzyme specificity targeting substrates in dietary ingredients. The reduction of dietary energy in the NC diet reduced male broiler BW throughout the trial compared to the PC diet. The inclusion of enzymes increased BW compared to the NC diet throughout the trial. During the grower 2, finisher, and withdrawal phases, the inclusion of β-mannanase and NSPase increased weight gain compared to the NC, however the intermittent application of β-mannanase and NSPase increased weight gain greater than the PC diet. Reducing dietary energy in the NC diet had a negative impact on FCR throughout the trial. The inclusion of β-mannanase and NSPase improved

cumulative FCR throughout the trial. Energy reductions in the NC diet decreased all processing parameters evaluated. Individual enzyme inclusion increased individual bird live weight to levels that were comparable to that of the PC diet. The intermittent application of β-mannanase and cocktail NSPase resulted in a further increase in live weight when compared to the PC diet. The separate inclusion of β-mannanase and cocktail NSPase did not influence fat pad yield compared to the NC. When including exogenous enzymes in poultry diets, fewer calories are utilized to yield similar carcass weights without increasing fat pad yields. Again, this can potentially reduce the amount of waste produced and increase sustainability as poultry feed is utilized more efficiently. The intermittent application of β-mannanase and cocktail NSPase resulted in an increase in fat pad yield compared to the NC diet. The results of the experiment indicate the importance of substrate availability in feed ingredients when supplementing with exogenous enzymes.

Evaluating nutrient variabilities between corn sources in Chapter V indicated the importance of accurate evaluation of nutrient content of dietary ingredients. Early BW differences were observed between corn sources through 18 d of age. On d 31, corn source had an impact on body weight, resulting in a range of 91 g. An interaction was present between corn source and enzyme, with xylanase inclusion negatively impacting broiler BW when fed corn source A. During the finisher phase, broilers fed corn source A yielded the lowest observed FCR. Xylanase inclusion reduced FCR during the finisher phase of production. On d 18, corn source influenced nitrogen and energy digestibility on all evaluated parameters. Nutrient variability resulted in a range of 152

kcal/kg for IDE between corn sources. Variability in corn source resulted in a range of 4.4 % for INDC between sources, with corn B yielding the lowest at 79.7 % and corn D yielding the highest at 84.1 %. The range of AME for the 6 corn sources was 176 kcal/kg. On d 41, nutrient variability between corn sources resulted in a main effect difference in energy and nitrogen digestibility on all evaluated parameters. Nutrient variability associated with corn source resulted in a range of 213 kcal/kg for IDE. Nitrogen digestibility was influenced by nutrient variability, resulting in a range of 6.1% for INDC. The nutrient variability resulted in a range of 4.3% for IEDC between corn sources. The range of AME for the 6 corn sources was 194 kcal/kg. At d 18, the inclusion of xylanase increased the concentration of butyrate in the ceca. The inclusion of xylanase increased total VFA concentration in the ceca; however, not at a significant level. On d 41, an interaction between corn source and xylanase inclusion was observed in isovalerate percentage in the cecal contents. The results confirm that nutrient variability does impact broiler performance and nutrient digestibility. It is important to have a complete understanding of nutrient composition when including exogenous enzymes in broiler diets.

When evaluating phytase inclusion in broiler diets, the results in Chapter VI affirm the importance of accurate mineral matrix values. During the starter phase, a linear increase in BW was observed as Ca was reduced in the diet. On d 28, the diet containing the highest level of Ca and aP had the lowest BW by 26 g compared to diets containing moderate levels of Ca, regardless of aP level, and low Ca and high aP. Broilers fed diets containing moderate Ca levels at both levels of aP and low Ca with

high aP resulted in improved FCR through 28 and 42 d of age compared to diets containing high Ca at both aP levels and low Ca with low aP. During the starter phase, broilers fed diets containing low Ca levels had greater feed consumption compared to diets containing high and moderate levels of Ca. During the grower phase broilers fed low aP diets also had greater feed consumption compared to high aP diets. Available P level impacted feed consumption through 28 and 42 d of age. In general, broilers fed diets with low aP resulted in greater feed consumption compared to high aP diets. Moderate levels of dietary Ca (0.13 reduction) with low P levels had lower tibia ash by 3.02% compared to diets containing the same level of Ca with high aP levels. The moderate Ca and low P diet also had lower tibia ash compared to high Ca and low Ca diets at both dietary aP levels, suggesting that the level of dietary aP and Ca:P ratio are important when determining tibia ash percent. High dietary aP resulted in greater fecal Ca compared to low aP diets. High and moderate levels of dietary Ca yielded greater litter Ca content. To maximize phytase activity, dietary Ca levels should be kept to a minimum without compromising skeletal integrity.

For accurate formulation to the nutrient specifications of the diet, it is vital to have an adequate understanding of the nutritional value of raw ingredients. The results of the current experiments quantified improvements in nutrient digestibility and performance by utilizing various exogenous enzymes to target specific substrates in diets varying in nutrient content and density.

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