EFFECTS OF VARYING NUTRIENT PROFILES AND ENZYME SUPPLEMENTATION IN BROILER DIETS ON GROWTH PERFORMANCE AND ENERGY DIGESTIBILITY

A Thesis

by

ASHLEY MARIE CAMPASINO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Approved by:

Chair of Committee, Jason Lee
Committee Members, Chris Bailey
Tryon Wickersham
Head of Department, John Carey

December 2012

Major Subject: Poultry Science

ABSTRACT

The current research program was designed to evaluate the impact of various dietary and ingredient nutrient profiles and exogenous enzyme inclusion on growth performance and energy utilization in broilers. Experiment one was designed to observe increasing levels of distillers' dried grains with Solubles (DDGS) and a non-starch polysaccharide degrading enzyme (NSPase) on energy digestibility. Experiment two evaluated the influence nutrient variation in corn and xylanase supplementation on growth performance and nutrient utilization.

In experiment one, DDGS concentration was increased from 0 to 15% in 5% increments and included the addition of an NSPase enzyme. Early broiler body weight and FCR was negatively affected with increasing DDGS concentration up to 15%. The decreased growth performance was associated with a decrease in energy and nitrogen utilization as a linear decrease was observed in IDE, INDC, and AME_n. Addition of the NSPase negated many of the negative effects on nutrient utilization as improvements in all digestibility measurements were observed. Interactions were observed with DDGS concentration and NSPase inclusion as the benefit of enzyme inclusion was augmented as DDGS level increase.

Experiment two evaluated different corn crops from six geographical locations in the US in an effort to determine the effects of xylanase inclusion and corn nutrient variation on growth performance and nutrient utilization. Significant differences were observed in growth performance, IDE, and AME_n in broilers when fed corn. Corn source impacted early body weights, and FCR throughout the experiment. Energy utilization

was also impacted on all measured parameters, following the starter phase (day 17) and at the conclusion of the trial (day 41) by corn source. Xylanase inclusion improved FCR during the finisher phase as well as the cumulative FCR (day 1-41). Digestibility data indicates that corn source impacts the xylanase effectiveness in young broilers, as improvements were not observed in all corn types. Xylanase inclusion, however, did increase IDE and AME_n in all corn sources on day 41 of age.

Data from this research program confirm that ingredient nutrient content, through the presence of ingredients with anti-nutritive properties or the incidence of varying nutrient profiles, does impact observed growth performance as related to reduced nutrient digestibility. Additionally, the inclusion of a dietary exogenous enzyme does improve energy and nitrogen digestibility in broilers, ultimately improving growth performance of broilers and improving efficiency.

DEDICATION

I would like to dedicate this to my family. Without your love and support I could not have been able to accomplish everything that I have. Thank you for always supporting and guiding me. To my parents, you have given me every opportunity for success, and assured that I have followed my heart to lead me here. To my sister, Jessica, you have always provided the perfect role model, showing me that with hard work and dedication anything can be achieved. I love you all, thank you for everything.

ACKNOWLEDGEMENTS

I would like to thank my committee chair and advisor, Dr. Jason Lee, and my committee members, Dr. Wickersham and Dr. Bailey, for all of their guidance, support, and advice throughout my time at Texas A&M. Dr. Bailey and Dr. Wickersham, thank you not only for welcoming me in your labs, but also for being readily available for advice and assistance. Jason, I would not have been able to do everything without you. Your support, direction, and counsel have helped me with some of my most important decisions; thank you.

Additionally, I would like to thank my coworkers, colleagues, and friends. Without the countless hours of hard work they have put in helping me I would not have been able to do it all. Thank you for being there for me when I needed it most.

Thank you to my family, for providing the support and guidance from hundreds of miles away. Your love and support has guided me through everything.

NOMENCLATURE

DDGS	Distillers' dried grains with solubles
NSP	Non-starch polysaccharides
NSPase	Non-starch polysaccharide degrading enzyme
AME	Apparent metabolizable energy
AME_n	Apparent metabolizable energy corrected for nitrogen
XAP	Xylanase-amylase-protease cocktail enzyme
IDE	Ileal digestible energy
IEDC	Ileal energy digestibility coefficient
INDC	Ileal nitrogen digestibility coefficient
FCR	Feed conversion ratio

TABLE OF CONTENTS

Page

ABSTRACT ii

DEDICATION iv

ACKNOWLEDGEMENTS v

NOMENCLATURE vi

TABLE OF CONTENTS vii

LIST OF TABLES ix

CHAPTER I INTRODUCTION 1

CHAPTER II REVIEW OF LITERATURE 6

- Use of Distiller's Dried Grains with Solubles 6
- Nutrient Variation in Crops 12
- Exogenous Enzyme Addition 16

CHAPTER III EFFECTS OF INCREASING DDGS LEVELS AND ENZYME INCLUSION ON GROWTH PERFORMANCE AND ENERGY DIGESTIBILITY IN YOUNG BROILERS 24

- Introduction 24
- Materials and Methods 26
- Summary of Experimental Results 31
- Discussion 34

CHAPTER IV EFFECTS OF NUTRIENT VARIABILITY IN CORN ASSOCIATED WITH GEOGRAPHICAL LOCATION AND XYLANASE INCLUSION ON BROILER PERFORMANCE AND ENERGY UTILIZATION 37

- Introduction 37
- Materials and Methods 39
- Summary of Experimental Results 46
- Discussion 52

Page

CHAPTER V CONCLUSIONS 55

REFERENCES 58

LIST OF TABLES

Page

Table 3.1 Dietary treatments fed to broilers with increasing concentrations of DDGS (0-15%) with and without the inclusion of an NSP degrading enzyme .. 27

Table 3.2 Dietary formulation and nutrient calculation of diets formulated for young broilers fed varying levels of DDGS with and without enzyme inclusion 28

Table 3.3 Body weight, mortality corrected feed conversion ratio (FCR), and energy digestibility of Young Broilers fed varying levels of DDGS and with or without enzyme inclusion..... 32

Table 4.1 Dietary treatment structure for male broilers fed corn from various geographical locations, with and without enzyme supplementation 40

Table 4.2 Dietary formulation and nutrient calculations of diets, based on percentages, formulated for male broilers fed corn from various geographical locations, with and without xylanse inclusion..... 41

Table 4.3 Xylanase recovery in experimental diets of varying nutrient profiles from corn of differing geographical locations 42

Table 4.5 Corn nutrient profile based on geographical location 43

Table 4.4 Body weight (mortality corrected), dietary phase FCR, and cumulative FCR of male broilers fed corn of varying nutrient profiles, with and without xylanase inclusion..... 47

Table 4.6 : Nutrient utilization including ileal digestible energy (IDE), ileal energy digestibility coefficient (IEDC), ileal nitrogen digestibility coefficient (INDC), apparent metabolizable energy (AME), and apartment metabolizable energy corrected for nitrogen (AME_n) of male broilers fed sources of corn varying in nutrient profiles, with and without xylanase inclusion on 17 and 41 days of age..... 50

Table 4.7 Linear regression relation between analyzed nutrient content of corn and observed digestibility and performance parameters in male broilers 53

CHAPTER I

INTRODUCTION

Demand for ethanol, a biofuel used as an alternative energy source, has increased exponentially in recent years, causing a redistribution of the usage of corn. As recently as 2002, 11% of US corn was being used for ethanol production; however in 2008 nearly 30% of US produced corn was directed to ethanol production (Donohue and Cunningham, 2009). This shift in usage directly increases cost of feed ingredients, resulting in greater feed production costs for livestock production. For example, between 2006 and 2008, it is estimated that feed costs in the poultry industry increased as much as $9.36 billion dollars in the United States alone (Donohue and Cunningham, 2009). As feed costs continue to rise, producers look towards various new methods concerning feed production as ways to increase feed efficiency and reduce diet costs.

One option for producers to reduce production costs is the inclusion of ingredients which are nutritionally cheaper and typically less desirable, such as distillers' dried grains with solubles (DDGS), a by-product of ethanol production. Although DDGS have a long history of incorporation in poultry diets, their inclusion level has typically been limited to inclusions of 5% or less (Lumpkins et al., 2004; Swiatkiewicz and Koreleski, 2008). Dramatic increases in ethanol production have led to increased DDGS availability for use in livestock diets (Loar et al., 2010).

The increase in DDGS supply has resulted in an increased inclusion of DDGS in poultry diets, despite studies which have reported negative linear trends between DDGS inclusion level and performance. Reductions in body weight gain have been observed

with inclusion levels as low as 7.5% (Loar et al., 2010). Other studies have reported inclusion levels should be as low as 6% in the starter phase, with potential for this level to be raised in susequent dietary phases (Lumpkins et al., 2004). Early studies, however, indicated that the DDGS inclusion could surpass 20%, assuming nutritional factors such as lysine and energy are kept constant (Waldroup et al., 1981; Parsons and Baker, 1983).

Part of the reasoning behind the varying recommendation levels results from the variance in nutrient content of DDGS. Although DDGS from more recently built ethanol plants are a more nutritionally consistent product due to the nearly exclusive usage of corn, nutritional variation is still observed (Lumpkins et al., 2004). In addition to concern over nutrient variability, producers dislike DDGS due the anti-nutritional properties associated with them, in the form of non-starch polysaccharides (NSPs). Monogastrics lack the capacity to digest NSPs and their inclusion increases intestinal viscosity and decreases digestion of other nutrients (Leeson and Summers, 2001).

The term NSP includes a group of carbohydrates previously referred to as fiber, which are typically found in the endosperm wall of cereal grains and associated with semi-structural materials (Leeson and Summers, 2001). Inclusion of NSPs, reduces digestibility of multiple components of the diet is often seen. According to Scott's Nutrition of the Chicken (2001), the addition of 4% NSPs, results in a 10% reduction in digestibility of starch, lipids, and protein. In particular, a decreased digestibility of fatty acids and monosaccharide occurs due to increased intestinal viscosity. This increases the thickness of the unstirred water layer adjacent to the mucosal villi in the intestine. Increased in intestinal viscosity leads to decreases in enzyme contact, nutrient uptake,

and net energy; even minor changes in intestinal viscosity have substantial impacts on nutrient digestibility and growth performance (Bedford, 1996). Numerous studies have shown depression in growth when NSPs are added into poultry diets (Antoniou and Marquardt, 1981; White et al., 1981; Ward and Marquardt, 1987), which has been directly associated with increased intestinal viscosity. Bedford and Classen (1992) observed a significant correlation between intestinal viscosity and production performance, including weight gain and feed conversion efficiency; furthermore, increase in two- or three-fold of high molecular weight carbohydrate complexes, due to the substitution of rye for wheat, resulted in 40- to 50-fold increases in intestinal viscosity.

Despite the risks to performance associated with DDGS, they still remain a readily available feedstuff, considered worth of dietary inclusion. Anti-nutritive properties, such as NSPs, are found in many dietary ingredients; furthermore nutrient variation is unavoidable with most crops and many dietary inclusions.

Similar to DDGS, corn has been known to have a substantial rate of variability of nutrients. As corn is the most widely grown crop in the US and the ingredient of highest inclusion in poultry diets, variation in the nutrient profile of corn is a large concern for producers.

Nutrient variation can arise for a variety of factors-most of which are extraneous, uncontrollable variables. These include things such as choice of fertilizer (Heckman et al., 2003; Mikkelsen, 2000), choice of cover crop (Reeves, 1994; Kabir and Koide, 2002), and geographical location (Heckman et al., 2003), which may lead to

differences in soil characteristics, such as acidity, alkalinity, salinity, anthropogenic processes, and erosion (Baligar et al., 2001). Furthermore, genotype has a significant impact on numerous factors, such as uptake of nutrients from the soil and root depth, which impacts the final nutrient profile of the crop (Bruetsch and Estes, 1976; Sattlemacher et al., 1994).

Inescapable nutritional variation in combination with anti-nutritional factors produces the need for producers to identify a method to decrease disparity between flocks and insure these discrepancies between feed sources do not lead to economic losses. One method which is frequently used is the addition of exogenous enzymes. Additions include individual or combinations of enzymes specially designed to increase digestibility of poorly digestible feedstuffs. Addition of an enzyme complex aids in digestion of multiple nutrients; an example of such complexes would be an NSPase enzyme cocktail, which is often used in cohorts with DDGS. Enzymes included in such cocktails may vary, however enzymes such as xylanase, β-gluconase, and α-galacotosidase are often included.

Addition of exogenous enzymes has been shown to improve feed conversion ratio (Gracia et al., 2003; Gao et al., 2007;Esmaeiliour et al, 2011; Kalmendal and Tauson, 2012; Masey O'Neill et al. 2012), improve nutrient and energy digestibility (Choct et al., 1999; Mathlouthi et al., 2002; Gao et al., 2007; Kalmendal and Tauson, 2012), and advantageous alterations in gut microflora (Mathlouthi et al., 2002; Gao et al., 2007). Despite a plethora of studies indicating positive effects of enzyme inclusion, contradicting studies have indicated inconsistent results with their usage. Their

supplementation does not always result in consistent improvements in performance parameters, such as body weight and feed conversion ratio; responses are widely varied and may be difficult to predict- even more so with the use of enzyme complexes (Cowieson et al., 2006; Gao et al., 2007; Choct et al., 1999).

Therefore, further evaluation of exogenous enzyme inclusion on growth performance and energy utilization in diets including DDGS and multiple sources of corn with varying nutrient profiles would be of value to the poultry industry.

CHAPTER II

REVIEW OF LITERATURE

Demand for ethanol, a biofuel used as an alternative energy source, has increased exponentially in recent years, redistributing corn use. As recently as 2002, 11% of US corn was being used for ethanol production; however in 2008 nearly 30% of corn was directed to ethanol production (Donohue and Cunningham, 2009). This shift in usage increases in feed costs for livestock production. For example, between 2006 and 2008, is it estimated that feed costs in the poultry industry increased as much as $9.36 billion dollars in the United States (Donohue and Cunningham, 2009). As feed costs continue to rise, producers look towards new methods to increase feed efficiency and reduce diet costs. One such option is to include ingredients which are nutritionally less desirable for poultry, due to the decreased digestibility which they cause, such as distillers' dried grains (DDG) and distillers' dried grains with solubles (DDGS). Additionally, producers may choose dietary exogenous enzyme inclusion, such as xylanase, which will act to increase the availability of typically non-digestible or poorly digestible ingredients or even formulating diets' energy contents to values which are lower than the accepted optimum (Dozier et al., 2011).

Use of Distillers' Dried Grains with Solubles

Distillers' dried grains with solubles (DDGS) have traditionally been a by-product of food-grade alcohol production. However, DDGS can also result as a derivative of fuel-grade ethanol production, which is the most common source in the US today. Historically, poultry producers have used DDGS in commercial diets at an

inclusion of five percent or less (Lumpkins et al, 2004; Swiatkiewicz and Koreleski, 2008). With a dramatic increase in ethanol production, an increase in DDGS production occurs as well (Loar et al, 2010). As a means to combat the rise in corn costs associated with the large amount of corn being diverted to ethanol production, producers have begun to utilize higher levels of DDGS, despite some anti-nutritive properties observed at high levels of inclusion.

Conflicting Recommendations for Inclusion Levels

A number of studies have examined DDGS inclusion in poultry diets in an effort to determine a optimum inclusion level based upon age. Lumpkins et al. (2004) concluded that 6% DDGS is a permissible inclusion level during the starter period. This level, however, can be safely raised to 12-15% during the grower and finisher periods with minimal negative consequences. Loar et al. (2010) reported a negative linear trend between body weight and DDGS inclusion during the grower phase. Birds receiving as low as 7.5% DDGS had reductions in final body weight.

A decline in body weight gain in correlation with an increase in feed conversion were observed by Wang et al. (2008) when the inclusion level surpassed 20% of the diet during the grower phase. Lumpkins et al. (2004) observed a decrease in performance during the starter period, when the inclusion of DDGS was 12%; a decrease in production was observed during the grower period as well with an 18% inclusion level. Parsons and Baker (1983) found that if lysine content is kept consistent, up to 20% of soybean meal can be replaced with DDGS. Finally, although it was likely using the

DDGS primarily from beverage production, Waldroup et al. (1981) found that if energy is kept constant, as much as a 25% of the diet can be included as DDGS.

Nutritional Variation of DDGS

Part of the reasoning behind the varying recommendation levels is due to the variance in nutrient content in the DDGS. Recently built ethanol plants produce a more nutritionally consistent DDGS product than older plants, partly due to nearly exclusive use of corn. Despite this, nutritional variation is still observed. Cromwell et al. (1993) evaluated nine different sources of DDGS evaluated nutritional parameters, including dry matter, crude protein, fat content, and concentrations of specific amino acids. A significant nutrient concentration range was reported, including dry matter content between 87.1-92.7%. Crude protein was range from 23.4 to 28.6%; although the average crude protein agreed with the NRC estimated crude protein, the variance between samples is reason for concern. Furthermore, fat content in this study was observed as low as 2.9% and as high as 12.8%, which would greatly affect the energy content in the diet and should be a considering in diet formulation for producers (Cromwell et al., 1993).

Similar studies have reported comparable variation in crude protein and fat content of DDGS from various sources. Caloric content of DDGS ranged from as low as 3661 to 3838 kcal/kg (Spiehs et al., 2002). Although the main objective of the study was to evaluate the variance in amino acid and energy digestibility of DDGS for different sources, Stein et al. (2006) was able to observe a difference in dry matter and crude protein as well as gross energy among their ten sources of DDGS. These ranges were

between 86.78-90.78% dry matter, 24.60-29.07% crude protein, and caloric values from 4705-4984 kcal/kg. With such a variation in nutritional content, it is simple to see why producers may be hesitant about utilizing elevated levels of DDGS. Despite similar inclusion levels of DDGS between diets, flocks may exhibit variable production performance due to variable nutrient concentrations.

Another concern for producers in incorporation of DDGS into the diet is the availability of nutrients, such as essential amino acids. It is thought that the production process of DDGS, which includes temperatures as high as 315°C (600°F) may lead to a decrease in amino acid availability (Lumpkins and Batal, 2005). Heat damage to the proteins may result in a loss of amino acids, particularly lysine, due to Maillard reactions between free amino acid groups and reducing carbohydrates (Kim et al., 2011). This reaction between simple sugars and amino acids during times of heating results in an alteration of the amino acids involved, rendering them unusable (Scaman, 2004). This is a major concern for lysine, which is one of the first two limiting amino acids in poultry. In poultry diets, absence of lysine would be directly observable in bird performance.

Several studies have specifically investigated amino acid digestibility within various DDGS sources. An early study performed by Waldroup et al. (1981) observed that the level of DDGS could be safely increased to 25% of the diet, as no detrimental effects on production were observed when the diets were supplemented with lysine. This observation indicates that the diets with high DDGS content have a marginal deficiency with lysine, considering that diets with substantial DDGS which were not supplemented with lysine resulted in production losses (Waldroup et al., 1981).

Dale and Batal (2006) evaluated nutritional contents on seventeen samples of DDGS coming from six different plants. Amino acid concentration was at similar levels to that of corn, although the lysine content had an observable variation. They observed a correlation between DDGS color, based on the Minolta Color Meter, and lysine content. As samples became darker, amino acid digestibility decreased- in particular, lysine (Dale and Batal, 2006). This observation of dissimilarity in coloration and amino acid digestibility is associated with differing production processes, confirming that inconsistencies seen in DDGS could impact poultry performance.

Further variation in DDGS nutritional content results in the proportions of wet distillers' grains (WDG) and the solubles in the final product. Since this process in not highly regulated, it can increase the variation between final products (Belyea et al. 1998). Upon testing samples for amino acid digestibility, inconsistencies were reported in methionine and cysteine digestibility (Kim et al., 2011). The samples had a variation in the ratio of WDG to solubles, which may be an explanation for the substantial variation, including the 16% variation in methionine content.

Non-starch Polysaccharides in DDGS

As published research reports discussed, nutrient variability in DDGS is a concern for poultry producers, although it may be unavoidable with their inclusion in poultry diets. However, it is not only the disparity between batches of DDGS that producers must consider with substantial levels of addition. The presence of anti-nutritional properties found in DDGS as a result of non-starch polysaccharides (NSPs) pose a problem for monogastrics. As a result of high levels of NSPs, increased intestinal

viscosity and decreased digestion of other nutrients are often seen (Leeson and Summers, 2001).

The term NSP includes a group of carbohydrates previously referred to as fiber. These include β-glucans, such as those found in barley, arabinoxylans, which are commonly found in wheat, and the raffinose group of oligosaccharides, which may be found in soybeans (Leeson and Summers, 2001). Other NSPs found in soybeans and soybean meals include xylans, xyloglucans, and arabinose (Meng and Slominski, 2005). The main source of NSPs in corn comes from arabinoxylans (Cowiseon and Adeola, 2005); therefore corn-soy diets based may contain arabinose, xylose, raffinose, rhammose, and galactose (Coppedge et al., 2012). All such compounds are typically found in the endosperm wall of cereal grains and are associated with semi-structural materials.

Presence of NSPs reduces digestibility of multiple dietary components. According to Scott's Nutrition of the Chicken, the addition of 4% NSPs results in a 10% reduction in digestibility of starch, lipids, and protein. In particular, a decreased digestion of fatty acids and monosaccharides occurs due to increased intestinal viscosity. This increases the thickness of the unstirred water layer adjacent to the mucosal villi in the intestine. Increased intestinal viscosity decreases in nutrient uptake, enzyme contact, and net energy and even minor changes in intestinal viscosity have substantial impacts on nutrient digestibility and growth performance (Bedford, 1996). Numerous studies have reported growth depression when NSPs are included in poultry diets (Antoniou and Marquardt, 1981; White et al., 1981; Ward and Marquardt, 1987), which has been

directly associated with increased intestinal viscosity. Bedford and Classen (1992) observed a significant correlation between intestinal viscosity and weight gain and feed conversion efficiency; furthermore, increase in two- or three-fold of high molecular weight carbohydrate complexes, due to the substitution of rye for wheat, resulted in 40- to 50-fold increases in intestinal viscosity.

Likewise, increases are observed in regards to retention time, endogenous enzyme secretions, and bile acid secretions. These changes are associated with the changes in viscosity and substrate interaction which occur in the intestine. Furthermore, NSPs in the diet can change gut microflora. Decreased rate of passage decreases the oxygen tension in the small intestine, produceing a favorable environment for fermentative microflora (Choct et al., 1999). Higher counts of anaerobic bacteria are observed in birds fed high levels of NSPs (Mathlouthi et al., 2012), which has been demonstrated to be as a consequence of increased intestinal viscosity (Langhout et al., 1999). Producers may also observe a change in consistency of excreta. Droppings become wet and sticky, which may lead to litter problems and potentially resulting in leg problems in broilers.

Nutrient Variation in Crops

Despite the risks and potential problems associated with DDGS, this readily available feedstuff is still considered a worthy dietary addition. Nutrient variation and anti-nutritional properties are not just limited to DDGS. Variation is found across all crops and dietary inclusions. Variation may arise due to soil conditions, handling and

processing techniques, climate, as well as other factors. Furthermore, as discussed previously, NSPs may come from other ingredients in the diet.

Similar to DDGS, corn has also been known to have a substantial variability in nutrient contents. As it is the largest inclusion in most commercial diets, variation in nutrient content is likely to be observed directly in production via feed conversion ratio and processing yields (Donohue and Cunningham, 2009). Additionally, corn is the most widely grown cereal crop in the U.S. (Cowieson, 2004). Geographical spread in production results in crops which are exposed to a variety of production conditions, producing variable nutrient profiles.

One of the sources of nutrient variation seen in crops used in poultry diets comes from agricultural practices of the producers. A study done by Heckman et al. (2003), examined nutrient removal of corn from the soil. This study observed that varying nutrient content in the grains was related to the nutrient contents found in the soil. Farmers must sustain fertility levels by replacing the nutrients which have been lost. This may be done by the addition of fertilizers in correlation of crop rotation. In the Mid-Atlantic region, for example, a majority of the manure is from the animal production industry; this contribution may result in excess levels of phosphorous (P) in the soil as compared to crops from other regions (Heckman et al., 2003). This was displayed directly in nutritional evaluations of six different regions; as the concentration of P and potassium (K) were observed to have as high as a twofold difference based on location (Heckman et al., 2003).

Fertilizer choice also has a substantial impact on nutrient profile of the final crop. As previously mentioned, the Mid-Atlantic region is known to have high levels of P due to the use of animal produced fertilizer, with a large majority being poultry manure (Heckman et al., 2003). However, it is not just P that is affected by animal manure. Other mineral accumulations may occur with the use of livestock manure as a fertilizer source, as a result of mineral supplementation in livestock diets. For example some soils have been shown to exhibit higher levels of copper (Cu) and zinc (Zn) (Mikkelsen, 2000). Furthermore, soil removes macronutrients at a quicker rate than micronutrients, all of which are ultimately displayed in the crops' nutrient profile (Heckman et al., 2003).

It is not only fertilizer choice which plays a role on the nutrient profile of crops; cover crop choice may also influence the final nutritive content of the product. Defined as crops which are grown specifically for the purpose of covering the ground to protect from erosion and soil nutrient loss, cover crops have a substantial impact on nutrient profile of the final product due to their role in soil quality (Reeves, 1994; Kabir and Koide, 2002). Choice of cover crop thus results in varying soil quality, which may change the availability of nutrients available for absorption. Furthermore, certain fungi may be beneficial to soil quality, and may ultimately increase host nutrient uptake; the quantity of these fungi varies with the variation of cover crop as well (Kabir and Koide, 2002).

Location is also an essential factor in the nutrient matrix of cereal. As previously discussed, some regions of the country have greater mineral contents in the soil than

others. Because of this, the availability of nutrients from the soil varies dependent upon the condition of the soil. This variance manifests itself directly in the nutritional content of the crop which is produced. Thus, it is plausible to assume that corn from the Mid-Atlantic region, in which soil has a higher P content due to the animal fertilizer used, would have a higher end P content than corn grown in the Mid-West, for example. Heckman et al. (2003), reported variation in nutrient profile of corn grown in different regions, even with the use of the same hybrid. Additionally, it is not merely minerals in the soil which have an influence on the final product. Factors such as acidity, alkalinity, salinity, anthropogenic processes, and erosion, which also vary dependent upon location, also play a significant role in the nutrients which are found in the ultimate product (Baligar et al., 2001).

Genotype of the crop additionally contributes to the nutrient content. In northern regions, where the growing seasons are shorter, farmers must choose a genotype which is more quickly maturing to accommodate for the climate and maximize yields. Difference in nutrient uptake of the plant may be explained in part by a difference in genotype. Bruetsch and Estes (1976) determined that genetic makeup may be responsible for the efficiency of uptake of phosphorus, iron, magnesium, potassium, calcium, and other elements by the plant. Additionally, nutrient harvest index is directly correlated to both genetic variation and soil fertility (Sattelmacher et al. 1994). Genotypes vary not only dependent upon traits, for example, shorter growing seasons require a quicker maturing crop for example, but farmers may also change genotype between growing seasons based on personal preference. Furthermore, genotype also

accounts for factors such as the depth which the roots reach in the soil; which correlates with the effectiveness of the plant for removing nutrients from the soil (Bruetsch and Estes, 1976).

There are many factors which may contribute to the variation in nutrient profiles between crops. Some of these are due to factors which may be controlled by humans- such as fertilization type and processing techniques. Other influences may be environmental, such as the soil conditions or the climate which the crop is grown, which dictates the length of the growing season. Even more variation may be brought on by non-predictable variables; including natural disasters, such as floods or droughts.

Exogenous Enzyme Addition

Producers also may turn to the supplementation of dietary exogenous enzymes as a mean to improve performance and nutrient utilization (Cowieson and Adeola, 2005). While exogenous enzymes are not novel, recent pressure to improve efficiency has lead to extensive research, in both single enzyme inclusion and in a "cocktail" form, with the combination of multiple enzymes. Although their use is prevalent, inconsistencies in production parameters, such as feed conversion ratio, nutrient retention, body weight gain and uniformity, have been reported (Choct et al., 1999; Coweison et al, 2006; Gao et al., 2007).

Exogenous dietary enzymes act as another method to decrease disparity between flocks and prevent nutritive discrepancies between feed sources from leading to economic losses. Additions may be of an enzyme specifically to increase digestion of a poorly or formerly indigestible aspect of the diet, or as an enzyme complex or cocktails.

Addition of an enzyme complex aids in digestion of multiple nutrients; an example of these would be a non-starch polysaccharide degrading (NSPase) enzyme cocktail, which is often used in cohorts with diets high in DDGS, wheat, or barley. Enzymes included in NSPase cocktails may vary, however may include enzymes such as xylanase, β-gluconase, and α-galacotosidase are often included.

The addition of exogenous enzymes into diets to improve animal performance has a long history. For poultry, the first study conducted investigating exogenous enzymes dates back to 1925, by Clickner and Follwell. Since then, both academia and industry have continued investigating usage of enzymes in poultry production. Although there are thousands of enzymes which have been discovered, only a few of these are available for commercial inclusion in diets (Leeson and Summers, 2001). Exogenous enzymes must withstand the pH of the poultry gastrointestinal tract before they can reach the site of degradation. Additionally, they may be exposed to the high temperatures and arduous processes associated with the production of the diets. Furthermore, the substrate interaction time for these enzymes is limited, due to the short retention time in poultry digestive tracts. With the aid of many years of research, today the most commonly used enzymes are those to degrade complex carbohydrates and those which promote the release of phosphorus from phytic acid (Leeson and Summers, 2001).

Addition of enzymes improves feed conversion ratio (Gracia et al., 2003; Gao et al., 2007; Esmaeiliour et al., 2011, Masey O'Neill et al. 2012; Kalmendal and Tauson, 2012), nutrient and energy digestibility (Choct et al., 1999;Mathlouthi et al., 2002; Gao et al., 2007; Kalmendal and Tauson, 2012), and provides advantageous alterations in gut

microflora (Mathlouthi et al., 2002; Gao et al., 2007). Despite a plethora of studies indicating positive effects with enzyme inclusion, contradicting studies have reported inconsistent results with dietary exogenous enzyme usage. Their inclusion does not always result in consistent improvements in performance parameters, such as body weight and feed conversion ratio and responses are widely varied and may be difficult to predict- especially with the use of enzyme cocktails (Choct et al., 1999; Cowieson et al., 2006; Gao et al., 2007).

Despite the reports of inconsistency with dietary exogenous enzyme inclusion, enzyme usage is increasing. As previously mentioned, the most commonly used are carbohydrate degrading enzymes as well as those which release phosphorus. Although these two classifications are the dominant enzymes in the industry, there are numerous others which are used today. Proteolytic enzymes and proteases exist for protein degradation, which may aid in amino acid digestibility. Lipases are used to improve fat digestion and may be beneficial due to the poor fat digestive capacity of young birds. Future research is necessary for the latter however, due to an association with decreased feed consumption, which is likely due to the increased energy digestibility (Leeson and Summers, 2001).

Slominski (2011) described five principles and reasons for dietary enzyme usage. Phytase is included in the diet to release phosphorus which would be otherwise unavailable to the bird, via phytate hydrolysis. Enzymes can also be used to break down cell walls, removing their encapsulating effect and thereby improve energy and amino acid availability to the animal. Also utilized are enzymes which improve hindgut

fermentation, specifically of NSPs, by solubilizing the cell walls. Enzymes also may be used hydrolyze carbohydrate-protein linkages, providing another mechanism to improve amino acid availability. Non-starch polysaccharide degrading enzymes, or NSPase enzymes, target NSPs, resulting in the eradication of anti-nutritive properties by enzymatic hydrolysis to prebiotic type components. This may result in improved gut development and health in young birds.

Xylanase Inclusion

Xylanase is an enzyme used either independently or in combination with other enzymes as part of an enzyme cocktail. By degrading the backbone of xylans, such as arabinoxylan, this enzyme breaks carbohydrates into more digestible monosaccharides. Additionally, xylanase may also be used to improve phosphorus utilization, by increasing cell wall permeability or liberating phytate which was previously bound (Esmaeilipour, 2011).

Birds supplemented with xylanase often exhibit improvements in growth performance. Xylanase inclusion on improved feed conversion ratioinnumerous studies (Choct et al., 1999; Gao et al., 2007;Esmaeiliour et al, 2011, Masey O'Neill et al. 2012, Kalmendal and Tauson, 2012;), with improvements over the control diets as high as six points (Masey O'Neill, 2012). Improvements to FCR and average daily gain (ADG) have been observed in wheat and wheat-barley based diets (Choct et al., 1999; Gao et al., 2007; Esmailipour et al., 2011; Kalmendal and Tauson, 2012), as well as corn-soy based diets (Masey O'Neill, 2012). These improvements range from strong trends ($P \leq 0.09$)

over control diets (Choct et al., 1999), to improvements in ADG as high as 4.5% (Esmailipour et al., 2011)

Improvements in FCR with xylanase inclusion can be attributed to increase an in nutrient retention. Esmaeilipour et al. (2011) observed an increase in retention of crude protein, dry matter and energy with xylanase inclusion. Kalmendal and Tauson (2012) also reported improvements in energy retention, with increases in apparent metabolizable energy-nitrogen corrected (AME_n) with the addition of xylanase. Additionally, Choct et al. (1999) observed improvements in AME with xylanase supplementation in wheat-based diets. Improvements to digestibility of fat (Mathlouthi et al., 2002; Gao et al., 2007; Kalmendal and Tauson, 2012), crude protein (Mathlouthi et al., 2002; Gao et al., 2007) and starch (Kalmendal and Tauson, 2012) have also been documented.

Other studies involving xylanase indicate the advantages with its use in relation to gut health. It is suspected that xylanase supplementation from an early age results in the selective promotion of advantageous microflora through provisions of xylo-oligomers (Masey O'Neill et al, 2012). Masey O'Neill et al. (2012) reported no improvement in performance data from day zero to day twenty-one; however, at day thirty five the improvement in FCR was significant. The authors suggest that the xylanase acted to help microflora development in the gut. Studies have indicated that xylanase inclusion in wheat-based diets may modify the total number of cecal anaerobes, which has been associated with an increase in bird performance (Mathlouthi and

Tauson., 2002). Gao et al. (2007) also reported trends in alterations of lactobacillus bacteria and coliform bacteria with xylanase supplementation.

Inconsistencies in Enzyme Usage

The largest factor when identifying inconsistencies regarding improvements in performance with xylanase inclusion appear to lie within the basis of the diet. Work by Masey O'Neill et al. (2012) found no effect between diet type, when using different energy levels and fat inclusions, and xylanase, which suggests its effectiveness despite the basis of this diet, however this meets conflicting reports. When feeding xylanase in wheat or wheat-barley based diets, improvements in FCR, average daily gain, and gain to feed ratios are frequent, but not universal (Mathlouthi et al., 2002; Esmailipour et al., 2011; Kalmendal and Tauson, 2012). Several studies using these NSP -rich diets have observed improvements, while other trials fail to produce differences in FCR (Gao et al., 2007). Additionally, when using corn-soy based diets, both improvements to FCR (Masey O'Neill, 2012) and lack thereof (Olukosi et al, 2007) have been published.

However, inconsistent results are not limited to the supplementation of xylanase. Supplementation of α-amylase improved growth performance, however no effects were observed between age of the broilers and enzyme inclusion (Gracia et al., 2003). Yu and Chung (2004), however, observed different results to the change in metabolizable energy with supplementation of a similar enzyme due varied grow out season of the broilers.

Non-starch Polysaccharide Degrading Enzymes

Improvements in starch digestion and decreased intestinal viscosity, conversely, can be expected consistently with inclusion of an NSPase enzyme (Choct et al., 1999; Mathlouthi et al., 2002; Gao et al., 2007; Kalmendal and Tauson, 2012). In the use of wheat or wheat-barley based diets, a decrease in intestinal viscosity is nearly a uniform result. Despite the consistent reduction in viscosity, disparity is observed in totality of the reduction. Studies have shown total tract reduction in viscosity (Choct et al., 1999; Mathlouthi et al, 2002); however other studies have published reductions were only observed in the jejunum (Gao et al., 2007; Esmailipour et al., 2011).

Although not inescapable of variation involved with enzyme supplementation, enzyme cocktails provide another option to improve feed efficiency and bird performance. Despite variance in effectiveness, it is generally accepted that when enzymes are used in a combination, they provide more potent effects than when used separately (Olukosi et al., 2007). Previously mentioned, NSPase enzymes are a common inclusion in wheat and wheat-barley diets, as well as those containing high levels of DDGS

A significant amount of research has been published utilizing a combination of xylanase, amylase, and protease (XAP); additionally, this cocktail enzyme has been studied in amalgamation with phytase. Olukosi et al. (2007) reported an additive effect with enzymes inclusion regarding growth performance. Additionally, the authors reported increased nitrogen digestibility with both XAP alone and when included with

phytase. When compared to a reduced calorie diet, both the use of XAP and XAP supplemented in combination with phytase improved ileal digestibility of P.

The effect of xylanase and phytase was also studied by Kalmendal and Tauson (2012). With the addition of both enzymes individually, and as a combination, improvements on feed conversion, apparent digestibility of starch and fat, and apparent metabolizable energy were all improved compared to the control diets. Olukosi et al. (2007) reported no improvement between treatments when comparing the addition of enzymes separately and in combination. These two studies further show inconsistencies observed with the addition of multiple enzymes; however both studies showed numerous advantages with dietary enzyme inclusion.

As discussed, ingredient costs continue to rise, resulting in increased production costs. This increase forces producers to consider utilizing lower quality ingredients and exogenous enzyme supplementation in order to maximize nutrient utilization. The objective of this research program was to investigate the effects of NSPase inclusion in diets with increasing levels of DDGS, and corn sources from various geographical locations with xylanase supplementation on growth performance and energy utilization in broilers.

CHAPTER III

EFFECTS OF INCREASING DDGS LEVELS AND ENZYME INCLUSION ON GROWTH PERFORMANCE AND ENERGY DIGESTIBILITY IN YOUNG BROILERS

Introduction

Current interest in renewable energy production has exponentially increased the utilization of ethanol, a biofuel produced largely from corn. Increased utilization of ethanol has redistributed corn from livestock production to ethanol production. In 2002, 11% of the US corn crop was used in ethanol production; with the increase in production as much as 30% of US corn was being used for ethanol production in 2008 (Donohue and Cunningham, 2009). This reallocation has directly impacted the poultry industry by significantly increasing in diet cost. Between the years of 2006 and 2008 it is estimated that feed costs in the poultry industry increased as much as $9.36 billion dollars in the United States alone (Donohue and Cunningham, 2009). Increased diet costs raises overall production costs, thus producers have turned to higher inclusions of less desirable ingredients to reduce diet costs. One such ingredient is distillers' dried grains with solubles (DDGS), a by-product of ethanol production, which has become widely available as a result of the expansion of ethanol production (Loar et al., 2010).

Although DDGS have a long history of use in poultry diets; however they have been traditionally included at relatively low levels- typically five percent or less in starter diets (Lumpkins et al., 2004). These low levels are because of the nutrient variability observed in DDGS. Different sources of DDGS have been reported to vary

considerably in energy, dry matter content, fat, crude protein, and various amino acid concentrations and availability (Cromwell et al., 1993; Spiehs et al., 2002; Lumpkins and Batal, 2005; Stein et al., 2006; Dale and Batal, 2006).

Additionally, DDGS contain substantial levels of non-starch polysaccharides (NSPs), which negatively affect digestibility in monogastric animals. These structural and semi-structural carbohydrates include β-glucans, arabioxylans, and raffinose groups (Leeson and Summers, 2001); NSPs decrease digestibility by imbibing water and forming a viscous digesta which is directly related to performance. Bedford and Classen (1992) observed a significant correlation between intestinal viscosity and weight gain. Increased intestinal viscosity acts to decrease the interaction times between enzymes and substrates as well as nutrients and the absorptive structures, resulting in a depression in net energy (Bedford and Classen, 1992).

It is because of these variables that an agreed upon inclusion level is yet to be determined. Early studies indicated that as much as a 25% of the diet could be from DDGS source as long as constant energy was maintained (Waldroup et. al., 1981). More recent studies reported inclusion levels of between 12 and 15% during the grower and finisher phases do not negatively impact performance parameters (Lumpkins et al., 2004), however depressions in body weight gain and increased feed conversion ratio (FCR) have been reported with levels as high as 20% in the grower phase (Wang et al., 2008).

Inclusion of exogenous carbohydrases may reduce or eliminate the negative effects observed with high levels of NSPs. Multiple studies have reported the advantages

of enzyme inclusion with improvements in FCR (Esmaeiliour et al., 2011; Massey O'Neill et al., 2012; Kalmendal and Tauson, 2012; Mirzaie et al., 2012) and increases in energy retention (Adeola et al., 2010; Kalmendal and Tauson, 2012). The strategy of the inclusion of a multiple enzyme complex has been reported to result in sub-additive increases in performance as opposed to individual inclusion (Cowieson et al., 2010). The observation was confirmed by Coppedge et al. (2012) which reported significant increases in body weight with the inclusion of a multiple enzyme complex.

The objective of this study was to examine the effects of increasing DDGS concentrations with and without the addition of an NSP degrading enzyme complex (NSPase) on growth performance and energy utilization in young broilers.

Materials and Methods

General Procedures

For this study, a 4x2 factorial design was utilized, resulting in a total of eight treatments (Table 3.1). Four levels of DDGS were included, beginning with a control diet with no DDGS inclusion and increasing in 5% intervals to a highest inclusion rate of 15%. Each level of DDGS was then fed with and without the addition of an NSPase enzyme at the recommended inclusion level of the manufacturer[1] 113.5 grams per ton. This NSPase contained xylanase, β-glucanase, α-glactosidase, and cellulase[1].

[1] Enzyvia LLC, Sheridan, IN

Table 3.1: Dietary treatments fed to broilers with increasing concentrations of DDGS (0-15%) with and without the inclusion of an NSP degrading enzyme[1]

Treatment	DDGS Inclusion	Enzyme Inclusion
1	0%	-
2	5%	-
3	10%	-
4	15%	-
5	0%	113.5 g/ton
6	5%	113.5 g/ton
7	10%	113.5 g/ton
8	15%	113.5 g/ton

[1]Ensipra, Enzyvia LLC, Sheridan, IN

Two corn and soybean meal based diets (Table 3.2) were formulated with 0 and 15% DDGS levels, and mixed at 2:1 and 1:2 ratios to obtain the intermediate 5% and 10% DDGS level treatments. Diets were formulated to maintain consistent protein and supplemental fat levels, as well as similar energy to essential amino acid ratios (Table 3.2). Additionally, all diets included 0.5% of titanium dioxide as an external marker.

Table 3-2: Dietary formulation and nutrient calculations of diets formulated for young broilers fed varying levels of DDGS, with and without enzyme inclusion

	0% DDGS	15% DDGS
Corn	66.69	58.16
Dried Distillers' Grains with Solubles	0.00	15.00
Dehulled Soybean Meal	27.72	21.19
DL-Methionine	0.15	0.11
L-Lysine- HCl	0.09	0.23
Fat- AV Blend	1.00	1.00
Calcium Carbonate	1.47	1.53
Mono Calcium Phosphate	1.57	1.6
Sodium Chloride	0.46	0.34
Vitamins[1]	0.25	0.25
Trace Minerals[2]	0.05	0.05
Coban 90[3]	0.05	0.05
Titanium Dioxide	0.50	0.50
Nutrient Concentration		
Protein	20.20	20.20
Apparent Metabolizable Energy (kcal/kg)	3020	2985
Lysine	1.11	1.10
Methionine	0.46	0.43
TSAA	0.80	0.79
Tryptophan	0.24	0.21
Threonine	0.75	0.74
Arginine	1.31	1.19
Calcium	0.95	0.95
Available Phosphorus	0.45	0.45
Sodium	0.20	0.20

[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 monesin sodium, 90 g/lb (90g/ton inclusion: Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necatrix, Eimeria tenella, Eimeria acervulina, Eimeria brunette, Eimeria mivati,* and *Eimeria maxima*

Each treatment had a total of eight replicates, for a total of sixty-four pens. Five broilers were placed per pen, resulting in a total placement of 320 Ross 708 male broilers. Broilers were obtained on the day of hatch, banded, and sorted to achieve statistically equivalent initial body weights. Broilers were reared in battery brooders and provided *ad libitium* access to dietary treatments and water, and provided age appropriate supplemental heat. Care was provided in accordance with a Texas A&M University approved Animal Use Protocol (IACUC).

Body weights and feed consumption were taken on day 7, 14, and 21. Feed conversion ratios were calculated and adjusted for mortality.

Energy Utilization

On day 22 of age, all broilers were killed via carbon dioxide asphyxiation. Ileal contents were then manually collected from the portion of the small intestine four centimeters posterior to Meckel's Diverticulum to four centimeters anterior to the ileal-cecal junction, and pooled within each replicate pen. Additionally, fecal contents were collected, beginning twelve hours prior to termination. Immediately upon collection, all samples were dried at 105°C and ground for analysis.

Titanium concentration was determined using a modified protocol outline by Short et al. (1996). As such, half a gram of each dried sample was weighed and ashed. Following ashing, samples were titrated 10 mL of sulfuric acid, and boiled until dissolved. Samples were then titrated with 20 mL of hydrogen peroxide, and brought to

100 mL using distilled water. Samples were then analyzed using a Thermo Fisher Scientific Gensys 10S UV-Vis (Model G10S UV-Vis) Specphotometer[2] at 410 nm. Gross energy of feed, ileal, and fecal samples were determined using a Parr 6300 bomb calorimeter[3].

Nitrogen concentration of each dried sample was determined via combustion method, using an Elementar Rapid N Cube[4].

Ileal digestible energy (IDE) was calculated using the following equation (Scott, Neshiem, and Young, 1982):

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

Ileal energy digestibility coefficients (IEDC) and ileal nitrogen digestibility coefficients (INDC) were calculated as to compensate for differences in caloric intake, using the following equation (Scott, Neshiem, and Young, 1982):

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

Whereas 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.

Statistical Analysis

Data were analyzed using a 4x2 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

[2]Thermo Fisher Scientific, Waltham, MA
[3] Parr Instrument Company, Moline, IL
[4] Elementar Inc., Hanua, Germany

treatment means were deemed significant at P≤0.05 and separated using Duncan's Multiple Range Test.

Results

Growth Performance

Significant differences were observed with regard to body weight on all collection days regarding DDGS inclusion. On days 7 and 14 of age, a significant decrease (P≤0.05) in body weight was observed between the 15% inclusion level and the control and 5% DDGS treatments (Table 3.3). Body weights from day 21 showed a significant decrease in the 15% DDGS inclusion when compared to the 0 and 10% levels. Inclusion of 15% DDGS also resulted in an increased (P≤0.05) FCR as compared to all other treatment groups at 14 days of age (Table 3.3).

Although a significant difference was not observed regarding enzyme inclusion throughout the experiment as compared to control diets, a three point improvement in FCR was observed over the control groups through day 14 of age. Additionally, as much as a three percent increase in body weight was observed compared to the control groups (Table 3.3).

Nutrient Digestibility

Significant effects were observed regarding IDE with both factors (DDGS and enzyme inclusion). The inclusion of DDGS at 10 and 15% decreased IDE as compared to the control diet with 5% DDGS being intermediate. Enzyme inclusion increased (P≤0.05) IDE (74 kcal/kg) as compared to the control diet (Table 3.3).

Table 3.3: Body weight, mortality corrected feed conversion ratio (FCR), and energy digestibility of Young Broilers fed varying levels of DDGS and with or without enzyme[1] inclusion

		Body Weight			Feed Conversion Ratio						
Diet	**Enzyme**	**Day 7**	**Day 14**	**Day 21**	**Days 0-7**	**Days 0-14**	**Days 0-21**	**IDE**	**IEDC**	**AME_n**	**IDN**
0% DDGS	N	132.0	372.1	754.4	1.32	1.49	1.52	3281	0.778^{b}	3122^{a}	0.867^{a}
5%DDGS	N	133.3	386.0	736.7	1.29	1.45	1.52	3184	0.746^{c}	3102^{a}	0.845^{b}
10%DDGS	N	125.1	360.8	756.7	1.34	1.51	1.52	3202	0.752^{bc}	3029^{a}	0.831^{bc}
15% DDGS	N	116.4	336.3	689.2	1.49	1.70	1.60	3157	0.730^{c}	2829^{b}	0.815^{c}
0% DDGS	Y	136.0	381.1	761.0	1.33	1.49	1.53	3360	0.792^{a}	3039^{a}	0.869^{a}
5% DDGS	Y	135.8	372.1	761.5	1.25	1.47	1.55	3348	0.790^{a}	3153^{a}	0.869^{a}
10% DDGS	Y	130.9	373.7	776.1	1.37	1.49	1.52	3154	0.734^{c}	2855^{b}	0.822^{c}
15% DDGS	Y	121.2	347.4	707.6	1.32	1.56	1.55	3264	0.793^{a}	2993^{ab}	0.868^{a}
Main Effect Means											
Diet											
0% DDGS	-	134.0^{a}	376.6^{a}	757.7^{a}	1.32	1.49^{b}	1.53	3321^{a}	0.785	3083	0.868
5% DDGS	-	134.5^{a}	379.5^{a}	748.3^{ab}	1.27	1.46^{b}	1.53	3261^{ab}	0.766	3128	0.856
10% DDGS	-	128.0^{ab}	367.2^{ab}	766.4^{a}	1.35	1.50^{b}	1.52	3177^{b}	0.742	2955	0.827
15% DDGS	-	118.8^{b}	341.8^{b}	707.6^{b}	1.39	1.63^{a}	1.57	3207^{b}	0.760	2905	0.840
Enzyme											
-	Control	136.7	363.8	735.7	1.35	1.54	1.54	3206^{b}	0.751	3021	0.840
-	Enzyme	130.8	368.5	755.4	1.32	1.51	1.54	3280^{a}	0.776	3021	0.856
p-value											
Diet	-	0.029	0.031	0.026	0.215	0.019	0.558	0.005	0.001	0.001	>.001
Enzyme	-	0.294	0.249	0.146	0.333	0.36	0.976	0.012	>0.001	0.798	>.001
Diet x Enzyme		0.993	0.742	0.921	0.408	0.474	0.816	0.086	0.001	0.029	>.001

[a-c] Main effect and treatment means differ at ($P \leq 0.05$)

[1]Enspira, Enzyvia LLC, Sheridan, IN. Inclusion at 113.5 g/ton of complete feed

A significant interaction was observed between DDGS concentration and NSPase inclusion regarding IEDC, INDC, and AME_n, therefore statistical groups were made based on individual treatment means. In non-NSPase treatment groups, reductions ($P < 0.05$) in IEDC were observed in the 5 and 15% DDGS diets as compared to the control diet. Inclusion of the NSPase enzyme resulted in increases ($P < 0.05$) in IEDC in all dietary treatments except the 10% DDGS diets. Additionally, NSPase inclusion in the 5 and 15% DDGS containing diets increased ($P < 0.05$) IEDC as compared to the 0% DDGS diet without enzyme inclusion (Table 3.3).

In non-enzymatic treatments, reductions were observed in INDC with all DDGS inclusion rates as compared to the control diet; the 15% DDGS level had a lower ($P \leq 0.05$) N digestibility compared to the 5% DDGS diet. Inclusion of the NSPase increased N digestibility in the 5 and 15% DDGS treatment groups to a level comparable to the 0% DDGS diet (Table 3.3).

Inclusion of 15% DDGS reduced AME_n as compared to all other non-enzymatic treatment groups. Inclusion of the NSPase in the 15% DDGS diet increased AME_n to a level similar to the 0% DDGS control diet (Table 3.3).

A significant interaction was observed between DDGS concentration and NSPase inclusion with regards to ileal digestible nitrogen (IDN), and therefore statistical groups were made based on individual treatment means. In non-enzymatic treatments, reductions were observed in IDN with all DDGS inclusion rates as compared to the control diet with the 15% DDGS having a lower ($P < 0.05$) N digestibility compared to the 5% DDGS

diet. Inclusion of the NSPase increased N digestibility in the 5 and 15% DDGS treatment groups to a level comparable to the 0% DDGS diet (Table 3.3).

Discussion

As DDGS inclusion level increased, a general depression in growth performance was observed ($P \leq 0.05$). This agrees with previous research, which states high inclusion levels in starter diets may produce negative results (Lumpkins et al., 2004), and inclusion levels approaching 10% may lead to reductions in performance (Loar et al., 2010). These results also agree with previous studies (Wang et al., 2008), reporting a negative correlation between DDGS inclusion and body weight as well as a positive correlation between DDGS and FCR.

Additionally, though not deemed statistically significant, an improvement was observed in growth performance with enzyme supplementation. This accounts for as much as a 3% improvement in body weight in the enzyme treatments as well as a three point improvement in FCR. These improvements in growth parameters are in agreement with previous studies (Esmaeiliour et al, 2011, Masey O'Neill et al. 2012, Kalmendal and Tauson, 2012, Mirzaie et al., 2012) which have reported the benefits of enzyme supplementation in commercial poultry diets.

Referring to energy utilization, the trends were similar, although not identical, to those observed for growth performance. With IDE, the two high level DDGS inclusions were significantly lower than the control diet. Additionally, the enzyme treatments achieved a significantly higher IDE than the control treatments; agreeing with previous research examining enzyme supplementation and IDE (Coweison and Adeola, 2005;

Coweison et al., 2006; Bedford, 1996). The high DDGS inclusion level also had a significantly lower IEDC when compared to the control diet. The addition of an enzyme increased IEDC for the 0%, 5% and 15% diets to levels greater than the observed IEDC of their respective control treatments. A linear decrease was observed in INDC when comparing the non-enzyme increasing DDGS levels. With enzyme supplementation, however, the INDC of the 5% and 15% DDGS inclusions were brought to levels equivalent to the 0% control diet. This beneficial effect on nitrogen and protein has been demonstrated in previous research (Mathlouthi et al., 2002; Gao et al., 2007). Overall, these results agree with previous research, indicating that enzyme supplementation enhances energy utilization in broilers (Adeola et al, 2010). Discrepancies observed with the 10% inclusion level, although concerning, are also in agreement with previous research, indicating that responses to dietary exogenous enzyme supplementation, especially cocktail form, are widely varied and difficult to predict (Choct et al., 1999; Cowieson et al., 2006; Gao et al., 2007).

In conclusion, as DDGS inclusion increases, a depression in growth performance via body weights and FCR can be expected. Additionally, increases in DDGS levels above 10% in starter diets lead to a decrease in energy utilization and nitrogen digestibility, and decreases in IDE, IEDC, INDC, and AME_n should all be expected. However, negative effects of DDGS inclusion can be negated to some degree with the addition of an NSPase enzyme, which may enhance growth performance of market broilers.

CHAPTER IV

EFFECTS OF NUTRIENT VARIABILITY IN CORN ASSOCIATED WITH GEOGRAPHICAL LOCATION AND XYLANASE INCLUSION ON BROILER PERFORMANCE AND ENERGY UTILIZATION

Introduction

In livestock production, diet cost is the largest contributing factor to production. In 2008, feed ingredients accounted for 68.7% of live production costs (Donohue and Cunningham, 2009). Corn, which is both the most widely grown cereal crop in the US and makes up the majority of commercial US poultry diets, has recently increasec in cost, resulting from an exponential increase in ethanol production (Cowieson, 2004). In 2002, a mere 11% of US corn was directed towards ethanol production, however, by 2011 nearly 40% of US corn was diverted into ethanol production (Donohue and Cunningham, 2009). This redistribution in corn use, directly results in increasing dietary costs, and has caused producers to employ new methods to increase feed efficiency to reduce these costs. One method which may be utilized is the inclusion of dietary supplementation of exogenous enzymes, such as xylanase.

Xylanase is a carbohydrase enzyme which is included in poultry diets to improve feed conversion ratio and improve nutrient digestibility. Supplementation of xylanase has been shown to improve FCR in both wheat and wheat and barley diets (Choct et al., 1999; Gao et al., 2007; Esmailipour et al., 2011; Kalmendal and Tauson, 2012) as well as in corn-soy based diets (Matholuthi et al., 2003; Masey O'Neill et al, 2012). Improvements in FCR range from six point improvements (Masey O'Neill, 2012) to

merely strong trends ($P \leq 0.09$) of improvement when compared to the non-supplemented diets (Choct et al., 1999).

Exogenous enzyme inclusion has also been reported to improve energy utilization in addition to growth performance. Improvements with xylanase supplementation have been reported in AMEn (Kalmendal and Tauson, 2012), AME (Choct et al., 1999), and IDE (Bedford, 1996; Cowieson and Adeola, 2005; Cowieson et al., 2006). Moreover , dietary inclusion of exogenous enzymes has been shown to improve digestibility of other nutrients. For example, xylanase has been reported to improve digestibility of fat (Mathlouthi et al., 2002; Gao et al., 2007; Kalmendal and Tauson, 2012), crude protein (Mathlouthi et al., 2002; Gao et al., 2007) and starch (Kalmendal and Tauson, 2012).

Despite the reported benefits of enzyme inclusion, some published results do not indicate significant improvements. Discrepancies in xylanase activity lie heavily on basis of the diet in which they are supplemented. Masey O'Neill et al. (2012) observed no effect between diet type, with varying energy levels and fat inclusions with xylanase, suggesting effectiveness despite the basis of diet. This meets conflicting results from other studies. When feeding xylanase in wheat or wheat-barley based diets, improvements, although frequent, are not consistent (Mathlouthi et al., 2002;Esmailipour et al., 2011; Kalmendal and Tauson, 2012). Additionally, when using corn-soy based diets, both improvements to FCR (Masey O'Neill, 2012) and lack thereof (Olukosi et al., 2007) have been published.

Although enzymes are often advantageous to growth performance, another concern for producers is varying nutrient profiles. Nutrient variation is a concern for all

crops. Variation may arise from a variety of factors, including soil conditions, processing and handling techniques, genotypic variation, fertilizer choice, geographic location, acidity, alkalinity, anthropogenic processes, erosion, cover crop choice and so forth (Bruetsch and Estes, 1976; Reeves, 1994; Sattelmacher et al., 1996; Mikkelsen, 2000; Baligar et al., 2001; Kabir and Koide, 2002; Heckman et al, 2003). The varying nutrient profiles in corn, arising from the geographical span of its production, may potentially impact the observed performance of broiler flocks due to its significant inclusion in the diet.

The objective of this study was to investigate impacts of corn nutrient variation on observed performance parameters of broilers and determine if advantages associated with xylanase supplementation are dependent upon corn nutrient density.

Materials and Methods

General Procedures

A 6 x 2 factorial was utilized to determine the effect of multiple corn sources and xylanase inclusion on growth performance and nutrient utilization in broilers, yielding 12 treatment groups (Table 4.1). Each treatment was replicated 10 times, for a total of 120 pens. Within each replicate pen, initial placement included of 18 Cobb 500 males, resulting in a total of 2160 broilers. Diets were corn and soy based and identical within each dietary phase, less the source of corn and enzyme addition (Table 4.2). Xylanase[5] was added at a rate of 100 grams per metric ton. Broilers were reared on fresh pine

[5] Econase, AB Vista, Maraborough, UK

shavings and provided *ab libitum* access to feed and water, and provided care in accordance with a Texas A&M University approved Animal Use Protocol.

The dietary program consisted of three dietary phases with a starter (day 0-17), grower (day 18-34) and finisher diet (day 35-41). During diet manufacturing, one premix consisting of all ingredients except corn was mixed to avoid nutrient variability associated with all ingredients less corn sources. An equal amount of the premix was then mixed with the same amount of each corn source. All diets were pelleted at 70°C; the starter diet was fed as a crumble while the grower and finisher diets were fed as a pellet. Feed samples were collected during feed manufacturing and sent to the manufacturer for enzyme recovery analysis (Table 4.3) Body weights and feed consumptions were collected at the end of each dietary phase (days 17, 34, and 41) to calculate FCR, which was adjusted for mortality. Starter and finisher diets contained 0.5% titanium dioxide as an external marker (Table 4.2)

Table 4.1: Dietary treatment structure for male broilers fed corn from various geographical locations, with and without enzyme supplementation

Treatment	Corn Source	Corn ID	Xylanase[1] g/metric ton
1	Iowa	A	100
2	Iowa	A	-
3	North Dakota	B	100
4	North Dakota	B	-
5	Nebraska	C	100
6	Nebraska	C	-
7	South Dakota	D	100
8	South Dakota	D	-
9	Texas	E	100
10	Texas	E	-
11	Minnesota	F	100
12	Minnesota	F	-

[1]Econase, AB Vista, Maraborough, UK

Table 4.2: Dietary formulation and nutrient calculations of diets, based on percentages, formulated for male broilers fed corn from various geographical locations, with and without xylanase inclusion

	Starter	Grower	Finisher
Corn	61.56	64.90	71.00
De hulled Soybean Meal	32.55	29.70	23.94
Fat- AV Blend	1.50	1.30	1.10
DL-Methionine	0.255	0.23	0.23
L-Lysine-HCl	0.16	0.14	0.21
Calcium Carbonate	1.57	1.46	1.36
Mono Calcium Phosphate	1.56	1.40	1.29
Sodium Chloride	0.46	0.40	0.18
Vitamins[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
TiO_2	0.05	-	0.05
Nutrient Concentration			
Protein	21.34	20.20	18.00
ME kcal/kg	3000	3024	3074
Lysine	1.25	1.15	1.05
Methionine	0.58	0.54	0.52
TSSA	0.93	0.88	0.82
Tryptophan	0.25	0.24	0.22
Arginine	1.40	1.31	1.11
Calcium	0.95	0.87	0.80
Available Phosphorus	0.45	0.41	0.38
Sodium	0.20	0.20	0.18

[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 monesin 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*.

Table 4.3: Xylanase recovery in experimental diets of varying nutrient profiles from corn of differing geographical locations

	Xylanase Recovery		
Corn Source	Starter	Grower	Finisher
A	12510	15000	17395
B	16250	16200	19800
C	17750	15450	21000
D	16300	9075	15700
E	15350	18700	16600
F	11380	14650	12600

Corn Samples

Six different sources of corn were obtained from locations within the United States, to provide nutrient variation of the same ingredient. Corn was obtained from Iowa, North Dakota, Nebraska, South Dakota, Texas, and Minnesota (Table 4.1). Prior to the start of the study, a sample of each corn was analyzed for proximate analysis as to assure an appropriate variation range was achieved (Table 4.4). Proximate and physiochemical analyses were conducted using Near Infrared Reflectance spectroscopy which was carried out using a Foss 6500 NIR spectrophotometer[6]. 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).

[6] FOSS NIR Systems, Inc,, Maryland USA

Table 4.4: Corn nutrient profiles from geographical location

Source	Starch	Protein	Oil	Fiber	Other	Moisture	PSI	Vitreousness
A	78.46	7.89	3.59	2.42	7.65	14.46	39.84	55.29
B	77.19	8.36	3.62	2.53	8.3	13.43	38.35	59.01
C	78.33	7.81	3.66	2.42	7.79	15.01	40.16	56.83
D	77.15	8.27	3.6	2.53	8.46	13.83	37.76	57.32
E	76.14	9.03	3.57	2.61	8.66	12.39	45.51	59.06
F	78.5	7.76	3.64	2.41	7.69	15.17	35.98	56.33

Nutrient Utilization

On days 17 and 41, ileal and fecal contents were collected and pooled within replicate pens. On day 17 (five broilers per replicate pen) and 41 (three broilers per replicate pen) were euthanized via carbon dioxide asphyxiation. Ileal contents were manually extracted from 2 cm posterior to Meckel's diverticulum to 4 cm anterior to 4 cm anterior the ileal-cecal junction. Fecal collections were conducted using stainless steel pans, which were placed in each pen to allow for collection of clean fecal material. A minimum of 8 defecations were collected per replicate pen. Ileal and fecal collections were thoroughly homogenized and stored at -20°C prior to analyses. Moisture concentrations were determined on a portion of each sample by oven drying at 105°C for 24 hours. The dried 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, half a gram of each dried sample was weighed and ashed. Following ashing, samples were titrated 10 mL of sulfuric acid (7.4 M), and boiled until dissolved. Samples were then titrated with 20 mL of 30% hydrogen peroxide, and brought to 100mL using distilled water. Samples were then analyzed for absorption using

a Thermo Fisher Scientific Gensys 10S UV-Vis (Model G10S UV-Vis) Spectrophotometer[7] at 410 nm.

Gross energy of feed, ileal, and fecal samples were determined using a Parr 6300 bomb calorimeter[8]. Nitrogen concentration of each dried sample was determined via combustion method, using an Elementar Rapid N Cube[9].

Ileal digestible energy (IDE) was calculated using the following equation (Scott, Neshiem, and Young, 1982):

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

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

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

Whereas 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.

Statistical Analysis

Data were analyzed as a 6x2 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.

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

Results

Growth Performance

Differences in body weight were observed early during growout following the starter and grower periods, while differences were observed in FCR through the duration of the entire experiment with regard to corn source. Broilers fed corn source A had significantly heavier ($P \leq 0.05$) body weights than those fed corn B, E, and F in the starter period. Additionally, during this period corn D broilers exhibited increased ($P \leq 0.05$) body weights compared to broilers fed B and E corn sources, which yielded the lightest body weights in the starter phase. Following the grower phase, broilers fed diets containing corn sources A, C, and D had increased ($P \leq 0.05$) body weights as compared to broilers fed diets with corn sources B and F. At the conclusion of the trial (day 41), no significant differences ($P < 0.05$) were observed in body weight due to differences in corn source. Broilers fed corn A, however, exhibited the highest observed body weight and broilers fed corn source F exhibited the lowest body weights; a similar trend was observed as which occurred at the conclusion of the grower phase. Inclusion of xylanase did not significantly impact ($P < 0.05$) body weight at any time point during the experiment.

Regarding FCR, significant differences were observed among corn sources during all dietary phases (Table 4.5). Through the starter period, D and E corn sources yielded the lowest observed FCR, which were significantly lower than broilers fed corn B. During the grower phase, broilers fed corn source E exhibited the lowest FCR, which was

statistically reduced compared to broilers fed corn C and F. Broilers fed corn source F exhibited the poorest FCR during this dietary phase, and was increased ($P \leq 0.05$) compared to all other corn sources. During the finisher phase, corn source C resulted in increased ($P \leq 0.05$) FCR in broilers compared to broilers fed source B and E. Cumulative FCR throughout the grower phase identified broilers fed corn source F had the highest FCR as compared to broilers fed all other sources of corn. Broilers fed corn source C had an elevated FCR compared to those which were fed corn source D and E, while those fed source E corn yielded the lowest observed FCR. The FCR for E was significantly lower ($P \leq 0.05$) than sources A, B, D, and F. Cumulative FCR for the entire trial identified corn source F broilers produced the highest ($P \leq 0.05$) observed FCR as compared to all other corn sources. Additionally, source E has the lowest ($P \leq 0.05$) FCR compared to the remaining sources. Source C corn resulted in an elevated ($P \leq 0.05$) FCR as compared to broilers fed corn B, D, and E.

Regarding xylanase inclusion, no effect was observed during the starter and grower phases as compared to the non-xylanase-supplemented diets. A reduction in FCR ($P \leq 0.05$) was however detected during the finisher phase in the broilers supplemented with xylanase over the non-supplemented broilers. Additionally, cumulative FCR was improved ($P \leq 0.05$) in xylanase supplemented broilers compared with the non-supplemented control.

Table 4.5: Body weight (mortality corrected), dietary phase FCR, and cumulative FCR of male broilers fed corn of varying nutrient profiles, with and without xylanase[1] inclusion

		Body Weight			Dietary Phase FCR			Cumulative FCR	
Corn Source	**Xylanase**	**Day 17 (g)**	**Day 34 (kg)**	**Day 41 (kg)**	**Starter**	**Grower**	**Finisher**	**Day 1-34**	**Day 1-41**
A	Y	687.8	2.088	2.817	1.363	1.634	2.107	1.528	1.664
A	N	689.0	2.099	2.806	1.371	1.637	2.198	1.533	1.686
B	Y	672.5	2.064	2.830	1.382	1.617	2.057	1.524	1.651
B	N	664.7	2.016	2.732	1.399	1.635	2.142	1.542	1.681
C	Y	683.3	2.089	2.793	1.367	1.659	2.150	1.544	1.683
C	N	673.8	2.053	2.734	1.371	1.648	2.224	1.540	1.695
D	Y	687.2	2.065	2.749	1.350	1.630	2.150	1.519	1.663
D	N	681.3	2.072	2.782	1.355	1.633	2.152	1.523	1.669
E	Y	666.8	2.014	2.740	1.358	1.620	2.100	1.516	1.651
E	N	670.0	2.090	2.799	1.342	1.601	2.104	1.500	1.642
F	Y	669.3	2.017	2.744	1.377	1.678	2.158	1.556	1.704
F	N	672.9	2.006	2.734	1.381	1.692	2.191	1.568	1.717
Main Effect Means									
A		688.4^{a}	2.093^{a}	2.811	1.367^{ab}	1.636^{bc}	2.153^{ab}	1.531^{bc}	1.675^{bc}
B		668.6^{c}	2.040^{b}	2.781	1.391^{a}	1.626^{bc}	2.100^{b}	1.533^{bc}	1.666^{c}
C		678.5^{abc}	2.071^{a}	2.763	1.369^{ab}	1.654^{b}	2.187^{a}	1.542^{b}	1.689^{b}
D		684.3^{ab}	2.069^{a}	2.765	1.353^{b}	1.632^{bc}	2.151^{ab}	1.521^{cd}	1.666^{c}
E		668.4^{c}	2.05^{ab}	2.768	1.350^{b}	1.611^{c}	2.102^{b}	1.508^{d}	1.647^{d}
F		671.1^{bc}	2.012^{b}	2.739	1.379^{ab}	1.685^{a}	2.175^{ab}	1.562^{a}	1.711^{a}
-	Control	675.3	2.057	2.765	1.370	1.639	2.168^{a}	1.533	1.681^{a}
-	Enzyme	677.8	2.058	2.780	1.366	1.638	2.119^{b}	1.530	1.668^{b}
p-value									
Diet	-	0.007	0.048	0.320	0.044	≤0.001	0.045	≤0.001	≤0.001
Enzyme	-	0.506	0.992	0.419	0.643	0.882	0.020	0.557	0.017
Diet x Enzyme		0.85	0.210	0.110	0.918	0.787	0.639	0.455	0.317

a-e Main effect and treatment means differ significantly at (P≤0.05)

[1]Econase, AB Vista, Maraborough, UK

Nutrient Digestibility

A significant interaction was observed for day 17 IDE and IEDC between corn source and enzyme inclusion, as enzyme inclusion did not significantly improve these parameters in all corn sources, therefore statistical groups were made based on individual treatment means (Table 4.6) Corn source D and E has a lower IDE as compared to sources B, C, and F. Corn source F had the highest observed IDE, which was elevated ($P \leq 0.05$) as compared to corn sources A, C, D, and E. Similar resulted were observed in IEDC; corn source F produced the highest ($P \leq 0.05$) digestibility coefficient as compared to all other corn sources. Sources D and E had reduced IEDC as compared to all other corn sources. An improvement ($P \leq 0.05$) in IDE and IEDC with xylanase inclusion was observed in diets containing corn from sources B and D, however increases were not observed in other corn sources.

Corn source F had the highest observed INDC, which was significantly higher ($P \leq 0.05$) than all other sources of corn (Table 4.6). Calculated AME and AME_n on day 17 were higher ($P \leq 0.05$) in broilers fed sources B, C, and F as compared to corn sources A, D, and E. Day 17 AME and AME_n were increased ($P \leq 0.05$) with xylanase inclusion as compared to non-supplemented diets.

On day 41, significant interactions were not observed between corn source and xylanase inclusion, however main effected differences ($P \leq 0.05$) were observed regarding both corn source and xylanase inclusion (Table 4.6). Broilers fed corn source D had increased ($P \leq 0.05$) IDE as compared to sources B, C, and E. Additionally, corn source

A, and F produced higher (P≤0.05) IDE values than sources B and E. Inclusion of xylanase increased (P≤0.05) day 41 IDE by 69 kcal/kg.

The results for IEDC differed from IDE with corn source A and F having increased (P≤0.05) digestibility coefficients compared to corn source B, C, D, and E. Corn source E had the lowest observed IEDC, which wad decreased (P≤0.05) compared to corn source A, B, and F. Similar to IDE, xylanase inclusion increased (P≤0.05) IEDC by 1.6%.

Corn source D was determined to have the highest (P≤0.05) day 41 AME and AME_n values, which corn source B had the lowest (P≤0.05) values. Corn source E produced AME and AME_n values higher (P≤0.05) than source B, however it was reduced as compared to corn source A, C, D, and F. Similar to observed results on day 17, inclusion of xylanase increased (P≤0.05) AME and AME_n on day 41, by an average of 47 kcal/kg. Referring to INDC, corn source F had the highest value and was increased (P≤0.05) as compared to values for corn sources B, D, and F. Corn sources B and D had the lowest INDC values, and were decreased (P≤0.05) compared to A, C, and E corn sources. Xylanase inclusion increased (P≤0.05) INDC on day 41 by 2.7%.

Table 4.6: Nutrient utilization including ileal digestible energy (IDE), ileal energy digestibility coefficient (IEDC), ileal nitrogen digestibility coefficient (INDC), apparent metabolizable energy (AME), and apartment metabolizable energy corrected for nitrogen (AME_n) of male broilers fed sources of corn varying in nutrient profiles, with and without xylanase[1] inclusion on 17 and 41 days of age

		Day 17					Day 41				
Corn Source	**Xylanase**	**IDE**	**IEDC**	**INDC**	**AMEn**	**AME**	**IDE**	**IEDC**	**INDC**	**AMEn**	**AME**
A	N	3149de	0.740^{cd}	0.824	3176	3201	3155	0.735	0.732	3343	3362
A	Y	3174^{d}	0.746^{cd}	0.836	3237	3266	3239	0.754	0.781	3393	3415
B	N	3275^{bc}	0.758^{bc}	0.822	3406	3429	3094	0.732	0.698	3223	3238
B	Y	3365^{a}	0.777^{a}	0.838	3433	3458	3127	0.739	0.734	3258	3277
C	N	3229^{cd}	0.751^{bcd}	0.836	3363	3387	3144	0.731	0.789	3355	3378
C	Y	3226^{cd}	0.750^{bcd}	0.831	3363	3393	3192	0.743	0.769	3397	3418
D	N	3077^{e}	0.717^{e}	0.815	3202	3227	3206	0.720	0.705	3382	3399
D	Y	3292^{abc}	0.767^{ab}	0.841	3320	3347	3304	0.742	0.738	3473	3492
E	N	3080^{e}	0.720^{e}	0.849	3182	3211	3083	0.718	0.743	3273	3293
E	Y	3148de	0.735^{de}	0.834	3240	3267	3135	0.730	0.777	3297	3318
F	N	3349^{ab}	0.785^{a}	0.869	3410	3444	3153	0.747	0.722	3356	3371
F	Y	3351^{ab}	0.786^{a}	0.871	3392	3429	3260	0.773	0.749	3394	3411
Main Effect Means											
A		3161	0.743	0.830^{b}	3206^{b}	3233^{b}	3197^{ab}	0.745^{a}	0.756^{ab}	3368^{b}	3388^{b}
B		3320	0.768	0.830b	3419^{a}	3443^{a}	3110^{c}	0.736^{b}	0.716^{c}	3241^{d}	3258^{d}
C		3228	0.750	0.833^{b}	3363^{a}	3390^{a}	3167^{bc}	0.737^{bc}	0.779^{a}	3375^{b}	3397^{b}
D		3185	0.742	0.828^{b}	3261^{b}	3287^{b}	3254^{a}	0.731^{bc}	0.721^{c}	3428^{a}	3446^{a}
E		3114	0.727	0.842^{b}	3211^{b}	3239^{b}	3109^{c}	0.724^{c}	0.760^{ab}	3285^{c}	3305^{c}
F		3350	0.786	0.870^{a}	3401^{a}	3436^{a}	3207^{ab}	0.760^{a}	0.735^{bc}	3375^{b}	3391^{b}
-	Control	3193	0.745	0.836	3290^{b}	3316^{b}	3139^{b}	0.730^{b}	0.731^{b}	3321^{b}	3339^{b}
-	Enzyme	3259	0.760	0.842	3331^{a}	3360^{a}	3208^{a}	0.746^{a}	0.758^{a}	3367^{a}	3387^{a}
p-value											
Diet	-	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	0.001	0.006	≤0.001	≤0.001	≤0.001
Enzyme	-	0.734	≤0.001	0.210	0.032	0.025	0.002	0.003	0.002	≤0.001	≤0.001
Diet x Enzyme		≤0.001	0.002	0.117	0.357	0.389	0.917	0.905	0.250	0.641	0.643

a-e Main effect and treatment means differ significantly at (P≤0.05)

[1]Econase, AB Vista, Maraborough, UK

Discussion

Differences were observed in body weight, FCR, and nutrient digestibility throughout the duration of the experiment with regard to corn source. This indicates that geographical location may impact on nutrient profile of the crop (Bruetsch and Estes, 1976; Reeves, 1994; Sattelmacher et al., 1996; Mikkelsen, 2000; Baligar et al., 2001; Kabir and Koide, 2002; Heckman et al, 2003), and changes in nutrient profiles are observed directly in the production of the bird.

With regards to body weight, significant increases were not observed in the particular trial with xylanase inclusion, however, a trend of increased body weight with xylanase inclusion was observed, similar to trends previously demonstrated (Choct et al., 1999). Improvements in FCR were observed with dietary xylanase inclusion ($P \leq 0.05$). Improvements occurred during later stages of production, indicating enzyme usage may be more beneficial in a mature gastrointestinal tract. This improved FCR agrees with previously published research, indicating xylanase inclusion is effective at lowering FCR when compared to control groups (Choct et al., 1999; Gao et al., 2007; Esmailipour et al., 2011; Kalmendal and Tauson, 2012; Masey O'Neill et al., 2012).

With regards to energy utilization, significant differences between corn source and enzyme inclusion were observed, in addition to interactions between corn source and xylanase inclusion on day 17. Differences were observed in day 17 IDE and IEDC between corn sources as corn source F produced the highest IDE and IEDC values, while corn source E produced the lowest. Increases in IDE and IEDC with xylanase supplementation were only observed in corn sources B and D.

On day 17, no interactions were present between corn source and xylanase inclusion regarding determined INDC, AME and AME_n values, however significant differences between corn sources were observed. Source F resulted in the highest INDC values, although this source had some of the lowest crude protein values. Xylanase supplementation was beneficial to both AME and AME_n values at 17 days of age, with a significant increase observed in with its presence. This agrees with previous research evaluating xylanase and metabolizable energy values, which reported higher values with xylanase inclusion (Choct et al., 1999; Kalemndal and Tauson, 2012).

Xylanase inclusion resulted in increases in all evaluated nutrient utilization parameters. Previous research with xylanase inclusion reported similar results in these parameters (Bedford, 1996; Choct et al., 1999; Coweison and Adeola, 2005; Coweison et al., 2006; Kalmendal and Tauson, 2012).

Differences in IDE were as great as 145 kcal/kg between corn sources; similar differences in IDE between sources of corn with different nutrient profiles have been reported previously (Gehring et al., 2012). Additionally, AME_n differences, which were as great as 143 kcal/kg, also agree with previous research examining various corn sources and metabolizable energy values, where variability can be as high as 400 kcal/kg between different sources (Cowieson, 2005; Gehring et al., 2012). The observed differences in IDE and AMEn values are directly related to analyzed nutrient content of each corn sample confirming that initial nutrient profile will impact digestibility and observed growth performance of broiler flocks (Table 4.7).

Table 4.7 Linear regression relation between analyzed nutrient content of corn and observed digestibility and performance parameters in male broilers

		Slope	P-Value
Day 17			
	IDE and Vitreousness	-0.8699	0.0243
	IDE and PSI	-0.2144	0.0485
	AMEn and Oil	0.8147	0.0483
	AMEn and Vitreousness	-0.9132	0.0110
	AME and Oil	0.8181	0.0466
	AME and Vitreousness	-0.9160	0.0103
Day 41			
	FCR and Starch	0.8629	0.0269
	FCR and Protein	-0.8759	0.0221
	FCR and Fiber	-0.8745	0.0226
	FCR and Moisture	0.9288	0.0074
	IEDC and Fiber	-0.8201	0.0457

These data confirm that ingredient nutrient variation associated with geographical location of production does impact observed growth parameters and nutrient digestibility in marker broilers. Additionally, xylanase inclusion does improve nutrient utilization in mature broilers, resulting in improved growth performance regardless of nutrient variability.

CHAPTER V

CONCLUSION

The cost of corn, which is the largest component in poultry diets, has increased drastically due to the expansion of the ethanol industry. Accordingly, producers have turned to various methods to control diet costs,. These methods include using increased inclusion of less desirable ingredients, such as dried distillers' grains with solubles, and of exogenous enzymes to improve efficiency and reduce the impact of ingredient variability, and ultimately, reduce diet cost.

Increased ethanol production augments availability of DDGS, all the while increasing corn cost. Despite being used at lower inclusion levels for years, the current increase in supply has resulted in a need to identify efficient inclusion levels. In agreement with previous research (Batal et al., 2004), a general depression in body weight was observed as DDGS content increased ($P \leq 0.05$). Furthermore, an increase in FCR was observed in conjunction with increased levels of DDGS, with significance on day 14. Energy utilization was negatively affected by higher inclusion rates of DDGS. A linear decrease was observed with regards to IDNC when DDGS inclusion increased. Additionally, general depressions in IDE and IEDC were observed at the higher inclusion levels as well.

Exogenous enzyme supplementation with a cocktail based NSPase improved energy utilization and performance in diets with various levels of DDGS. During the first experiment, NSPase supplementation resulted in a 3% improvement in body weight as well as a three point improvement in FCR over the control diet. Referring to energy

utilization, enzyme supplementation led to a significantly higher IDE when compared to the control. Significant interactions occurred with regards to IDEC and IDNC. Inclusion of an NSPase enzyme compensated for reductions due to DDGS inclusion at 5%, and 15%.

In the second experiment, significant impacts on both growth performance and nutrient utilization were observed due to nutrient variability in corn. Significant differences on broiler body weight were observed during the starter and grower phases, and impacts were observed on FCR throughout the experiment. These growth effects were associated with differences observed in nutrient utilization including ileal digestibility of energy and nitrogen in broilers on day 17 and 41.

Positive effects were observed on growth performance with the inclusion of xylanase, however, not until the finisher phase of the experiment for FCR. Treatments receiving xylanase exhibited reduced FCR during this phase when compared to the control diets. Effects of xylanase on nutrient utilization were similar those for growth performance. Xylanase inclusion did not positively impact ileal digestibilities in all corn types on day 17, however significant increases in all digestibility parameters were observed with xylanase inclusion at day 41 of the experiment.

Combined, these data indicated that increasing levels of DDGS inclusion in young broiler diets will reduce growth performance and nutrient utilization. Furthermore, variation in corn nutrient profiles in unavoidable, and this variation may account for differences in growth performance, as observed in body weight and FCR, as well as energy utilization observed in production facilities. Dietary exogenous enzymes in both single enzyme and cocktail enzyme forms, however, have the ability to compensate for these effects in both growth performance and energy utilization, as seen in both of the current experiments. These data confirm that exogenous enzyme supplementation is an effective method to improve nutrient utilization and growth performance in broiler chickens, and should be used as a cost saving strategy by poultry producers to combat increasing feed production costs.

REFERENCES

Antonoiu, T. and R. R. Marquardt. 1981. Influence of rye pentosans on the growth of chicks. Poultry Science, 1981 60:1898-1904.

Baligar, V.C., N. K. Fageria, and Z. L. He. 2001. Nutrient Use Efficiency in Plants. Communications in Soil Science and Plant Analysis, 32:921-950.

Batal, A.B. and N.M. Dale. 2006. True metabolizable energy and amino acid digestibility of distillers dried grains with solubles. Journal of Applied Poultry Research, 15:89-93.

Bedford, M.R. 1996. The Effect of Enzymes on Digestion. Journal of Applied Poultry Research, 5:370- 378.

Bedford M. R. and H. L Classen. 1992. Reduction of Intestinal Viscosity through Manipulation of Dietary Rye and Pentosanase Concentration is Effected through Changes in the Carbohydrate Composition of the Intestinal Aqueous Phase and Results in Improved Growth rate and Food Conversion Efficiency of Broiler Chicks. Journal of Nutrition, 122:560-569.

Belyea, R.S., S. Eckhoff, M. Wallig, and M. Tumbleson. 1998. Variatiability in the nutritional quality of distillers solubles. Bioresource Technology, 66:207-212.

Bruetsch, T. F. and G. O. Estes. 1976. Genotype Variation in Nutrient Uptake Efficiency in Corn. Agronomy Journal, 68:521-523.

Choct, M. 2006. Enzymes for the feed industry: past, present and future. Would's Poultry Science Journal, 62:5-15.

Choct, M., R. J. Hughes, and M. R. Bedford. 1999. Effects of a xylanase on individual bird variation, starch digestion throughout the intestine, and ileal and caecal volatile fatty acid production in chickens fed wheat. British Journal of Poultry Science, 40:419–422.

Coppedge, J. R., L.A. Oden, B. Ratliff, B. Brown, F. Ruch, and J. T. Lee. 2012. Evaluation of nonstarch polysaccharide-degrading enzymes in broiler diets varying in nutrient and energy levels as measure by broiler performance and processing parameters. Journal of Applied Poultry Research, 21:226-234.

Cowieson, A. J. 2004. Factors that affect the nutritional value of maize for broilers. Animal Feed Science and Technology. 119: 293-305.

Cowieson, A. J., and O. Adeola. 2005. Carbohydrases, protease, and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. Poultry Science, 84:1860–1867.

Cowieson, A. J., D. N. Singh, O. Adeola. 2006. Prediction of ingredient quality and the effect of a combination of xylanase, amylase, protease and phytase in the diets of broiler chicks. 2. Energy utilization. British Poultry Science. 47: 490-500.

Coweieson, A.J., D.N. Singh, and O. Adeola. 2006. Prediction of ingredient quality and the effect of a combination of xylanase, amylase, protease and phytase in the diets of broiler chicks. 2. Energy and nutrient utilization. British Poultry Science. 47:490-500.

Coweison, A.J., M.R. Bedford, V. Bavindran. 2010. Interactions between xylanase and glucanase in maize-soy-based diets for broilers. British Poultry Science. 51:246:257.

Cromwell, G. L., K. L. Herkelman, and T. S. Stahly. 1993. Physical, chemical, and nutritional characteristics of distillers dried grains with solubles for chicks and pigs. Journal of Animal Science, 71:679-686

Donohue, M., and D. L. Cunningham. 2009. Effects of grain and oil seed prices on the costs of US poultry production. Journal of Applied Poultry Research. 18:325-337

Dozier III, W.A., A. Corzo and H.A. Olanrewaju, 2011. Apparent metabolizable energy needs of male and female broilers ranging from thirty-six to forty-seven days of age. Poultry Science, 90: 804-814.

Esmaeilipour, O, M. Shivazad, H. Moravej, S. Aminzadeh, M. Rezaian, and M.M van Krimpen. 2011. Effects of xylanase and citric acid on the performance, nutrient retention, and characteristics of gastrointestinal tract of broilers fed low-phosphorus wheat-based diets. Poultry Science, 90:1975-1982.

Gao, F., Y. Jiang, G. H. Zhou and Z. K. Han. 2007. The effects of xylanase supplementation on growth, digestion, circulating hormone and metabolite levels, immunity and gut microflora in cockerels fed on wheat-based diets. British Journal of Poultry Science, 48:480-488.

Gehring, C.K., M. R. Bedford, A. J. Cowieson, and W. A. Dozier. 2012. Effects of corn source on the relationship between in vitro assasys and ileal nutrient digestibility. Poultry Science, 91:1908-1914.

Gracia, M. I., M. J. Aranibar, R. Lazaro, P. Medel, and G. C. Mateos. 2003. α-Amylase supplementation of broiler diets based on corn. Poultry Science, 82:436-442.

Heckman, J.T., J.T Sims, D.B. Beegle, F.J Coale, S.J Herbert, T.W. Bruulsema, and W. J. Bamka. 2003. Nutrient Removal by Corn Grain Harvest. Agronomy Journal. 95: 587-591.

Kabir, Z. and R. T. Koide. Effect of autumn and winter mycorrhizal cover crops on soil properties, nutrient uptake, and yield of sweet corn in Pennsylvania, USA. Plant and Soil, 238: 205-215.

Kalmendal, R and R. Tauson. 2012. Effects of a xylanase and protease, individually or in combination, and an ionophore coccidiostat on performance, nutrient utilization, and intestinal morphology in broiler chickens fed a wheat-soybean-meal based diet. Poultry Science, 91:1387-1393.

Langhout, D. J., J. B. Schutte, P. Van Leeuwen, J. Wiebenga, S. Tamminga. 1999. Effect of dietary high- and low-methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestine wall of broiler chicks. British Journal of Poultry Science. 40:340-347

Leeson, Steven, and John D. Summers. Scott's Nutrition of the Chicken. 4th ed. Guelph, Ont.: University, 2001. Print.

Loar, R.E., J.S. Moritz, J. R. Donaldson, and A. Corzo. 2010. Effect of feeding distillers dried grains with solubles to broilers from 0 to 28 days posthatch on broiler performance, feed manufacturing efficiency, and selected intestinal characteristics. Poultry Science, 89:2242-2250.

Lumpkins, B.S., A.B. Batal, and N.M. Davis. 2004. Evaluation of Distillers Dried Grains with Solubles as a Feed Ingredient for Broilers. Poultry Science. 83:1891-1896

Lumpkins, B.S. and A.B. Batal. 2005. The Bioavaility of Lysine and Phosphorous in Distillers Dried Grains with Solubles. Poultry Science. 84:581-586.

Masey O'Neill, H.V., G. Mathis, B.S. Lumpkins, and M.R. Bedford. 2012. The effect of reduced calorie diets, with and without fat, and the use of xylanase on performance characteristics of broilers between 0 and 42 days. Poultry Science, 91:1356-1360.

Matholuthi, N. S., M. A. Mohamed, and M. Larbier. 2003. Effect of enzyme preparation containing xylanase and beta-glucanase on performance of laying hens fed wheat/barley or maize/soybean meal-based diets. British Poultry Science., 44:60-66.

Mathlouthi, N., S. Mallet, L. Saulinier, B. Quemener, and M. Larbier. 2002. Effects of xylanase and beta-glucanase addition on performance, nutrient digestibility and physic-chemical conditions in the small intestine contents and caecal microflora of broiler chickens fed a wheat and barley-based diet. Animal Res., 51:395-406.

Meng, X. and B. A. Slominski. 2005. Nutritive Values of Corn, Soybean Meal, Canola Meal, and Peas for Broiler Chickens Affected by a Multicarbohydrase Preparation of Cell Wall Degrading Enzymes. Poultry Science, 84:1242-1251.

Mikkelsen, R. L. 2000. Nutrient Management for Organic Farming: A Case Study. Journal of Natural Resources and Life Sciences Education, 29:88-92.

Mirzaie, S., M. Zaghari, S. Aminzadeh, M. Shivazad, and G. G. Mateos. 2012. Effects of wheat inclusion and xylanase supplementation of the diet on productive performance, nutrient retention, and endogenous intestinal enzyme activity of laying hens. Poultry Science. 91:413-425.

Olukosi, O. A., A. J. Cowieson, and O. Adeola. 2007. Age-Related Influence of a Cocktail Xylanase, Amylase, and Protease or Phytase Individually or in Combination in Broilers. Poultry Science, 86:77-86.

Pan, C.F., F. A. Ignasan, W. Guenter, and R. R. Marquardt. 1998. The effect of enzyme and Inorganic phosphorus supplements in wheat- and rye- based diets on laying hen performance, energy, and phosphorus availability. Poultry Science, 77:83-89.

Parsons, C.M., and D. H. Baker. 1983. Distillers dried grains with solubles as a protein source of the chick. Poultry Science, 62:2445-2451.

Piotrowski, C., R. Garcia, S. Flanagan, T. dos Santos, P. Philips, R. Ten Doeschate, and P. Cambt. 2011. Development of near infra-red reflectance spectroscopy calibration for the prediction of nutrients to assess the quality of the maize. In: proceedings of 9ieme Journess de la Recerche Avicole 2011, Tours, France, p. 114

Reeves, D.W. 1994. Cover Crops and Rotations. Advances in Soil Science-Crop Residue Management, Lewis Publishers, CRC Press, Boca Raton, FL, USA, pp125-172.

Sattelmacher, B., W. J. Horst, and H. C. Becker. Factors that contribute to genetic variation for nutrient efficiency of crop plants. Z. Pflanzenernaehr. Bodenk., 157:215-224.

Scaman, Christine. "Maillard Reaction." Chemistry of Food Sciences. Faculty of Food Sciences, UBC, 29 Oct. 2004. Web. 8 June 2012.

Scott, M.L, M. C. Nesheim, and R. J. Young. Nutrition of the Chicken. 3rd Edition. Ithaca, NY, 1982. Print.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. Determination of titanium dioxide as an inert marker in chicken digestibility studies. Animal Feed Science Technology, 59:215-221.

Slominski, B. A. 2011. Recent Advances in research on enzymes for poultry diets. Poultry Science, 90:2013-2023.

Spiehs, M. J., M. H. Whitney, and G. C. Shurson. 2002. Nutrient for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. Journal of Animal Science, 80:2639-2645.

Stein, H. H., M.L. Gibson, C. Pedersen and M. G. Boersma. 2006. Amino acid and energy digestibility in ten samples of distillers dried gains with solubles fed to growing pigs. Journal of Animal Science. 84:853-860.

Swiatkiewicz, S. and J. Koreleski. 2008. The use of distillers dried grains with soluble (DDGS) in poultry nutrition. World's Poultry Science Journal, 64:257-266.

Waldroup, P.W. J.A. Owen, B.E. Ramsey and D. L. Whelchel. 1981. The use of high levels of distillers dried grains with solubles in broiler diets. Poultry Science, 60:1479-1484.

Wang, Z., S. Cerrate, C. Coto, F.Yan and P.W. Waldroup. 2208. Evaluation of High Levels of Distillers Dried Grains with Solubles (DDGS) in Broiler Diets. International Journal of Poultry Science, 7:990-996.

Ward, A. T. and R.R. Marquardt. 1987. Antinutritional activity of a water soluble pentosan-rich fraction from rye. Poultry Science, 66L1665-1674.

White, W.B., H. R. Bird, M. L. Sunde, W. C. Burger, and J. A. Marlett. 1981. The viscosity interaction of barley beta-glucan with trichoderma viride cellulose in the chick intestine. Poultry Science, 60:1043-1048.

Yu, B. and T. K. Chung. 2004. Effects of Multiple-Enzyme Mixtures on Growth Performance of Broilers Fed Corn-Soybean Meal Diets. Journal of Applied Poultry Research, 13:178:182.