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EFFECT OF EXOGENOUS ENZYMES ON APPARENT METABOLIZABLE ENERGY VALUE OF BARLEY IN SWINE AND BROILER CHICKENS

Brian L. Bryson

University of Kentucky, brian.bryson@uky.edu

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Brian L. Bryson, Student

Dr. Tayo Adedokun, Major Professor

Dr. David L. Harmon, Director of Graduate Studies

EFFECT OF EXOGENOUS ENZYMES ON APPARENT METABOLIZABLE
ENERGY VALUE OF BARLEY IN SWINE AND BROILER CHICKENS

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food and Environment at the
University of Kentucky

By

Brian L. Bryson
Lexington, Kentucky

Director: Dr. Tayo Adedokun, Assistant Professor of Animal and Food Sciences
Lexington, Kentucky
2018

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ABSTRACT OF THESIS

EFFECT OF EXOGENOUS ENZYMES ON APPARENT METABOLIZABLE ENERGY VALUE OF BARLEY IN SWINE AND BROILER CHICKENS

The objective of this thesis was to evaluate the effect of exogenous enzyme supplementation, phytase and xylanase-glucanase, on AME value of barley in poultry and swine. In the first study, 280 broilers were assigned 1 of 8 treatments. Barley inclusion in the diet resulted in decreased ($P < 0.05$) performance. There was a treatment \times phytase \times xylanase-glucanase interaction for dry matter retention with birds fed the corn-SBM-barley diet supplemented with phytase and xylanase-glucanase having higher ($P < 0.05$) DM retention compared to birds fed corn-SBM-based diet with only xylanase-glucanase supplementation. AME and AMEn of corn-SBM-based diets were greater ($P < 0.05$) than the corn-SBM-barley-based diets. Energy metabolizability and AMEn of barley significantly increased with xylanase-glucanase supplementation. In the second study, 24 pigs (12 pigs/phase) were assigned to 1 of 4 treatments with xylanase-glucanase and phytase. After a 7-d adaption period, urine and feces were quantitatively collected for 5 d. DE of the barley-based diet supplemented with xylanase-glucanase (3,578 kcal/kg) and phytase and xylanase-glucanase in combination (3,617 kcal/kg) were significantly different. Compared to control diets, exogenous enzymes either significantly improved or had a tendency to improve AME and AMEn value of barley in broilers, but not in growing pigs.

Keywords: apparent metabolizable energy, barley, broiler chicken, phytase, swine, xylanase

Brian L. Bryson

November 2, 2018

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By

Brian L. Bryson

Dr. Tayo Adedokun

Director of Thesis

Dr. David L. Harmon

Director of Graduate Studies

November 2, 2018

This thesis is dedicated to my family. Your prayers and support have not gone unnoticed and I am eternally grateful for you all. #GOML

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CHAPTER 1. Literature Review

1.1 Introduction

In any animal feeding operation, the best way to make a difference is to have maximum output with the least amount of input. This can be achieved by finding different ways to reduce production costs without negatively impacting animal performance. Dietary energy sources, the use of exogenous enzymes, and alternative feed ingredients are important topics in the poultry and swine industries because they enhance energy and nutrient utilization while at the same time reduce nutrient excretion into the environment. Researchers and nutritionists have to work together, consistently, to ensure the best results from the animal feeding operation; whether that is supplying the best energy source, supplementing enzymes to alleviate intestinal viscosity with the view to enhance digestion and absorption of nutrients, or researching the finest feed ingredients that are cheaper and allow maximal growth and performance. The future of swine and poultry nutrition is dependent on today's research and tomorrow's ideas.

The objective of this literature review is to discuss the important aspects of poultry and swine nutrition in regard to energy, exogenous enzymes, methodologies for energy utilization, and common feed ingredients used in poultry and swine diets.

1.2 Energy

Energy is described as a fundamental entity of nature that is transferred between parts of a system in the production of physical change within the system (Merriam-Webster). Simply put, it is the capacity to do work. In human and animal nutrition, energy is an essential component of life and one cannot survive without an adequate source of energy. Animal nutritionists evaluate energy as the oxidation of organic

compounds (NRC, 1994). According to the NRC (1994, 1998), energy can be expressed in terms of calories (cal), kilocalorie (kcal), megacalorie (Mcal), or joule (J). The NRC (1994, 1998) also states a calorie is the heat required to raise the temperature of 1 gram of water from 16.5° to 17.5° C. Furthermore, kilocalories, where 1,000 cal is equal to 1 kcal, are the common unit of energy used in poultry and swine. In these industries, the partitioning of nutrient and dietary energy is a simple, but complex system, however it explains the breakdown of energy as affected by feed chemical composition, physiological state of the animal, and the environment.

1.2.1 Gross Energy

The partitioning of energy begins with gross energy (GE), where GE is the total energy in a feed or feed ingredient. If feces are removed, it becomes digestible energy (DE). When urine and fermentation gas are subtracted from DE it becomes metabolizable energy (ME). Lastly, ME transitions into net energy (NE) of maintenance (NE_m) and production (NE_p) with the removal of heat increment energy (H_iE). Gross energy, also known as heat of combustion, is the amount of energy produced when a compound is completely oxidized (NRC, 1994) and it is determined using bomb calorimetry (Hossain et al., 2012).

1.2.2 Digestible Energy

As stated previously, DE is the GE of the feed consumed minus the GE of the feces. In swine, digestible energy can be measured because of the separation of feces and urine. In poultry, however, feces and urine are excreted as one and hence DE cannot be accurately measured in chicken excreta, therefore DE is not commonly used in the feed formulation.

Because DE isn't as important as ME in the poultry industry, the energy partitioning transitions from GE to ME. Metabolizable energy is described as the GE of the feed minus the GE of the excreta. This process is slightly different in swine where the DE minus urine and fermentation gases equals the ME. According to the NRC (1981, 1998), ME represents 92-98% of the DE. A factor that has had an impact on ME values has been correcting ME values for nitrogen retention, ME_n . According to the NRC (1994) adjusting for nitrogen retention has become a new norm in poultry nutrition. Hill and Anderson (1958) were the first to apply this correction for nitrogen balance into their research and it has generally been adopted since then.

1.2.3 Metabolizable Energy

Additionally, in poultry, ME is the standard measure of energy in describing energy requirements and diets for research studies (Lopez and Leeson, 2008). The partitioning of ME intake involves the following equation: $ME = HP + ER$ (Close, 1990) where HP is heat production and ER is energy retained. Heat production is the heat related to the utilization of ME intake for maintenance and productive process. Heat production can be measured by direct or indirect calorimetry. In pigs, direct calorimetry is determined by heat loss where the pig is enclosed in an insulated chamber. To build, maintain, and operate these chambers has proven to be very expensive over time (Velayudhan et al., 2015). The energy retained is from body tissues, such as fat (ERF) and protein (ERP) (Lopez and Leeson, 2005). Energy retention can be determined by indirect calorimetry (Farrell, 1974; Fuller, 1983), comparative slaughter method (MacLeod, 1991), or carbon-nitrogen balance technique (Adeola, 2001). Indirect calorimetry requires fewer animals, is less labor intensive, and takes less time (van

Milgen and Noblet, 2003). The method measures oxygen consumption and production of carbon dioxide and methane to estimate heat production. The comparative slaughter method is when a select number of the animals are killed, dried, and ground at the start of the experiment, then the energy content is measured by bomb calorimetry. Next, animals of same weight and age are fed for a select period. When the feeding period ends, the other select number of the animals are slaughtered, dried, and ground. The energy retention is the difference between carcass energy contents of the final and beginning slaughter groups (Patrick and Schaible, 1980; Adeola, 2001). Jadhao et al. (1999) conducted a study of carbon-nitrogen balance in White Leghorn and Rhode Island hens and Velayudhan et al. (2015) explained that this technique requires knowing the intake of carbon and nitrogen from measuring feed intake, and the output of carbon and nitrogen from collecting the feces, urine, or excreta voided. The carbon and nitrogen retained are calculated from the difference between intake and output.

1.2.4 Net Energy

The last step of the energy partitioning flow is net energy, which is derived from removing the energy lost as heat increment from ME. Net energy is articulated as the most accurate energy value of a feed (Noblet, 2007). Using net energy, in pigs, instead of DE or ME stems from NE/DE or NE/ME ratios being affected by fiber digestion (Carre et al., 2014). Net energy is not used in poultry because the fiber digestion is minimal, decreasing NE/ME ratios (Carre et al., 1990). Net energy is broken down into two systems – production and maintenance. Heat production includes basal metabolism, voluntary activity, product formation, digestion and absorption, thermal regulation, heat of fermentation and waste formation and excretion (NRC, 1981). Heat increment, net

energy for maintenance, and net energy for production operate under the theory that their values are constant values per weight of each feed (Latshaw and Moritz, 2009).

1.2.5 Sources of Energy in Diets

In broiler chicken and swine diets, carbohydrates in grains are the main source of energy (NRC, 1994; Infante-Rodriguez et al., 2016). Carbohydrates serve as a fuel to the body to keep it going, similarly to putting fuel in your car. Common carbohydrates in poultry and swine exist in cereal grains such as corn, sorghum, wheat, and barley. Cereal grains provide the majority of energy and contain starch, which is easily digested in diets (Moran, 1985). On the other hand, carbohydrates like cellulose, pentosans, and stachyose are poorly digested and do not add nutritional value to non-ruminants (NRC, 1994). According to Wagner and Thomas (1978), pentosans in barley interfered with nutrient utilization by increasing the viscosity of digesta. To offset this concern, Edney et al. (1989) supplemented rye and barley diets with exogenous enzymes to improve nutrient utilization.

Dietary macronutrients such as protein are important in providing energy because they have a low efficiency of energy utilization compared to fat and carbohydrates (Priyankarage et al., 2008). Musharaf and Latshaw (1999) reported that this is due to an increase in heat production during transamination, deamination, and protein turnover during protein metabolism. Protein is fed as a source of amino acids to build body protein (Patrick and Schaible, 1980). It is essential for protein requirements to be met when formulating diets because a deficiency in amino acids and energy results in a decline in performance. In broiler chickens between 0 and 3 weeks, the National Research Council (NRC, 1994) lists the recommended crude protein (CP) of starter diets at 23% with the

ME of 3,200 kcal/kg. In regard to finisher diets between 6 and 8 weeks, this value is decreased to 18% CP with 3,200 kcal/kg ME. Although these values are recommended, protein sources cost up to 50% of the total cost of production in feed (Banerjee, 1992) and with the high prices of imported protein sources and high temperature, optimal performance cannot be achieved under these conditions. On the other hand, diets containing protein above the requirements will cause an increase in nutrient excretion and this can be damaging to the environment (Kong and Adeola, 2014). Additionally, excess protein has been thought to decrease animal performance but the results are not consistent (Temim et al., 2000; Norgaard et al., 2014).

Fat is an important source of energy, in addition to carbohydrates and proteins. The available energy in fats is 2.25 times greater than in carbohydrates (Cho and Kim, 2012; Flanders and Gillespie, 2015). Lipids, another name for fat, are included to help cover the energy requirements for maximum growth performance. Liquid lipids, or plant oils, are mostly unsaturated fats. These unsaturated fats are common additives in poultry and swine diets as energy sources and to reduce caking of the feed. When soybean oil was included in the diet at 5%, Phillips and Ewan (1977) observed an increase in average daily gain (ADG) and a significant improvement in feed efficiency in young pigs. Additionally, DE, ME, and NE values of the diets increased as soybean oil was added to the basal diet. According to Frobish et al. (1970) and Lawrence and Maxwell (1983), fat inclusion significantly improved fatty acid digestibility in weaned pigs and also improved ADG and feed conversion ratio. In growing and finishing pigs, a consistent increase related to growth rate and feed:gain ratio was observed as feed intake decreased when fat was added to the diet (Cho and Kim, 2012). Dietary fat has the ability to improve

digestibility of other feed components (Lewis and Southern, 2001) while slowing the rate of passage of the digesta (Cunningham, 1962), proving its efficacy in the diet.

1.3 Enzymes

1.3.1 Purpose and History of Enzymes

Enzymes are highly effective biological catalysts capable of accelerating chemical reactions. Enzymes are protein molecules which have important implications for their stability during high-temperature feed manufacture (e.g. pelleting) and transit through the gastrointestinal tract (Ravindran, 2013). All animals use enzymes to digest feed.

Supplementing the feed with particular enzymes improves the nutritional value of feed ingredients, which increases the efficiency of digestion. Ultimately, feed enzymes are used to improve feed efficiency, reduce feed cost, and create a better environment by reducing the volume of manure produced and phosphorus and nitrogen content excreted (Barletta, 2010).

Enzymes can be categorized into several classes. They can be categorized by the substrate they act upon and their origin. The enzymes classified by the substrates they act upon are phytases, which are phytate degrading enzymes; carbohydrases, fiber and starch degrading enzymes; and proteases, which consist of protein degrading enzymes. The second area of categorization of enzymes is the origin from which the enzymes are derived, which include exogenous and endogenous sources.

Research, in regard to the use of enzymes in poultry diets, has been ongoing since the 1920s. The first report of an enzyme product used in poultry diets was known as Protozyme, which derived from the fungus *Aspergillus oryzae* (Munir and Maqsood, 2013; Ravindran, 2013). The addition of an enzyme cocktail containing xylanase and β -

glucanase to an unpelleted poultry diet containing rye and wheat at various inclusion levels resulted in a significant increase in body weight gain and feed intake (Pettersson and Aman, 1989).

1.3.2 Exogenous Enzymes

The addition of exogenous enzymes has become the standard to improving digestibility and efficiency of nutrient utilization (Ravindran, 2013). According to Pariza and Cook (2010), all animals use enzymes in the digestion of food, either produced by the animal itself or by the microbes present in the digestive tract. However, the digestive process is not 100% efficient. Swine, for example, are unable to digest 15-25% of their feed, therefore the supplementation of the animal feed with proper enzymes increase the efficiency of digestion (Munir and Maqsood, 2013). Enzyme supplementation helps to reduce the amount of nutrient excretion, which if ignored, can result in extra cost to the farmer, feed supplier, and the environment (Sheppy, 2010). Exogenous enzymes are essential to reduction of feed cost. Because feed accounts for a vast majority of production costs, enzymes can reduce cost where other nutrients are poorly absorbed. When the price of corn, wheat, fat, and inorganic P increases, the use of enzymes in feed becomes more economically attractive, providing a bigger return on investment (Barletta, 2010).

Phytase is an enzyme that hydrolyzes phytate to inositol and inorganic phosphate (Bilal et al., 2015). On a more in-depth level, phytase is known as myo-inositol hexakisphosphate phosphohydrolase. It is essential for swine and poultry to have dietary phosphorus (P) for maintenance and growth. Therefore, an adequate amount must be included in the diet. Even with a sufficient amount of total P in the diet, a portion of the

total P comes from cereal grains and this P exists in a form that swine and poultry cannot digest. The majority of P, approximately 60%, is not available to non-ruminants because it is bound to phytate. In fact, phytate binds to many dietary cations such as Cu, Zn, Ca, Fe, Mg, and Mn, but also protein, fat, and vitamins, which may result in potential reductions in nutrient availability (Bohn et al., 2008). In the current market, trends have shown that hydrolytic enzymes have emerged as feed supplements to help improve the digestion and absorption of poorly available nutrients, such as phytate, from the animal diet (Barletta, 2010). According to Iqbal et al. (1994), the reason non-ruminant animals have a limited ability to digest phytate is due to the lack of significant endogenous phytase activity and low microbial population in the upper portion of the digestive tract. The main sites of phytate degradation by phytases are the stomach of pigs and the forestomach (crop, proventriculus, gizzard) in poultry where there is little degradation in the distal gastrointestinal tract (Selle et al., 2010). Phytases can be divided into two classes, depending on the carbon site off hydrolysis of phytic acid. The 3-phytase begins hydrolysis at the 3 position of the myo-inositol ring, while the 6-phytase begins hydrolysis at position 6 of the ring (Dersjant-Li et al., 2015). Furthermore, phytases have different origins of expression, pH optima, and temperature optima. The most common origins of phytase used in poultry feeds include *Aspergillus niger*, *Escheria coli*, and *Buttiauxella* spp. Optimal pH and temperature range is an essential factor affecting the phytase activity in the feed. Three commercial microbial phytase products were examined by Naves et al. (2012) and results show *A. oryzae* exhibited optimal activity at pH 4.0 and temperature 40 °C. The second expression, *A. niger* exhibited maximal activity at pH 5.0 and 45 °C. Lastly, *S. cerevisiae* presented its highest activity at pH 4.5 and temperature

between 50 and 60 °C. The researchers ultimately advised *A. niger* and *S. cerevisiae* exhibited the highest in-vitro activities that correspond with the optimal physiological conditions of broiler chickens. This finding would allow a higher rate of hydrolysis of phytate. Phytase is expressed as phytase units (FTU). One unit of phytase is defined as the quantity of enzyme which releases 1 µmole of inorganic phosphate per minute from 5 mmol/L sodium phosphate at pH 5.5 and 37 °C (Guggenbuhl et al., 2007). Natuphos® is a well-known commercially available phytase supplement added to diets to increase phosphorus digestion.

In a study conducted in pigs by Patras et al. (2006), although P adequate diets were used in the experiment, the apparent total tract digestibility (ATTD) of P was improved from 56.5 and 57.2% to 69.0 and 65.2%, respectively, by microbial phytase supplementation, proving its effectiveness. Proven on numerous occasions, supplementing microbial phytase improves the availability of phytate-bound P. Ballam et al. (1984) and Edwards and Veltmann (1983) reported that broiler chickens fed a corn-soybean meal (SBM) based diet had phytate-P utilization between 10 and 53% - adding exogenous phytase increased the P availability to 65% (Simons et al., 1990). However, there have often been studies where the addition of phytase to diets has been inconsistent (Johansen and Poulsen, 2003; Selle and Ravindran, 2008). The inconsistency in the relationship between microbial phytase and P digestibility can be linked to dietary phytate level, feed composition, or the Ca:P ratio in broilers and pigs of all ages. Qian et al. (1996) and Liu et al. (1998) reported that an increase in Ca:P ratio largely decreased P digestibility in corn-SBM diets fed to weanlings and finishing pigs. Sebastian et al. (1996) reported reduced Ca retention in broilers fed a low-P diet with increasing levels of

Ca (0.6, 1.0, and 1.25%). High levels of Ca in the diets of swine (Moore and Tyler, 1955) and poultry (Scheideler and Sell, 1987) decrease the availability of P absorbed. Calcium is capable of forming insoluble complexes with phytate-P, ultimately causing an obstruction with the phytase activity (Angel et al., 2002). Calcium-phytate complexes are mainly formed in the small intestine, where it has adverse influence on the efficacy of mucosal phytase. While these studies (Moore and Tyler, 1955; Scheideler and Sell, 1987) reported Ca negatively impacted P digestibility, the influence of Ca on phytase has a tendency to be positive.

Most calcium in pig diets are supplemented as inorganic calcium because of the low concentration of calcium in most plant-based feed ingredients (González-Vega et al., 2015). Brady et al. (2002), Liao et al. (2006), and Poulsen et al. (2010) reported that the inclusion of microbial phytase in swine diets increased the digestibility of calcium and phosphorus, but effects of phytase on the standardized tract total digestibility (STTD) of Ca in individual ingredients have yet to be reported. In growing pigs, the inclusion of microbial phytase helped to increase the ATTD and STTD, therefore it can be stated that some of the Ca in the feed ingredients were bound to phytate (González-Vega et al., 2015). In a study done by Guggenbuhl et al. (2007), the objective was to evaluate the effects of P and Ca on digestibility of 3 phytases in ten growing pigs. Calcium ATTD was significantly improved by each strain of phytase. Additionally, an increase of Ca in the diet may increase bone-ash content when Ca is limiting bone mineralization (Driver et al., 2005; Letourneau-Montminy et al., 2008).

A common source of calcium in the diets for swine and poultry is limestone. Limestone has a high acid-binding capacity (ABC), which can raise the pH of the gastric

phase of digestion (Selle et al., 2010). In a study done by MacDonald (1964), Ca levels increased, from 0.9 to 1.3%, with supplementation of limestone. Because of this, the digesta pH increased from 5.6 to 6.1 in the small intestine of the chickens. Ultimately, high dietary concentrations of Ca have the potential to reduce the extent of protein-phytase complex formation in the stomach by reacting with phytate or protein at acidic pH (Selle et al., 2010).

In essence, the use of exogenous phytase in non-ruminant animals' diets such as swine and poultry is important because phytase has the ability to cleave the phosphate groups from phytate, complex cations, and proteins, which increases availability (Kies et al., 2006). Nelson et al. (1968) were the first to show that phytate-P in diets could be hydrolyzed by a phytase. The use of phytase greatly increased after it was included in a number of publications after Nelson et al. (1968) and Rojas and Scott (1969) reported their results. In a corn-SBM-based diet, where the bioavailability of P was 15%, Cromwell et al. (1993) increased this percentage to 35 and 43% with the addition of 500 and 1000 FTU, respectively. Since the Nelson et al. (1968) publication, phytase has been relied upon heavily to increase P availability and performance, while reducing P excretion. To achieve success in poultry production, there has to be a reduction of cost and waste output, while increasing the animal performance. Phosphorus excretion plays an important role in poultry production, not only because the environmental pollution and waste of nutrients raises the production costs (Martins et al., 2013), but it also reduces feed cost because P is one of the most expensive nutrients in poultry and swine diets. Assuena et al. (2009) reported that the highest level of phytase inclusion resulted in the lowest P excreted. It was also stated that any level of phytase inclusion over 250 FTU

compromised live broiler performance. The results presented contradict other studies (Lan et al., 2002; Wu et al., 2004). In the literature mentioned previously, phytase nutritional matrix results were not considered in the formulation of experimental diets, which Assuena et al. (2009) attributes the difference in results. Because of phytase, researchers and nutritionists have been able to make phosphorus available to non-ruminants in a way that was once unavailable.

Carbohydrases are fiber and starch degrading enzymes. In poultry and swine, carbohydrases are known as non-starch polysaccharide (NSP) degrading enzymes, because they degrade the NSP in feed ingredients. Because of the degradation, xylanase and β -glucanase are common carbohydrases supplemented to the diet to improve performance and digestibility. Xylanase is an enzyme that originates from the hemi-cellulosic polysaccharide, xylan. Xylan being a polysaccharide, is made up of units of D-xylose, a pentose sugar. Xylans make up 30-35% of the cell wall material of annual plants, therefore it is considered an integral part of animal feed.

Xylanase has been used as a feed additive for over 20 years in pig and poultry diets, primarily to improve feed conversion ratio and weight gain (Paloheimo et al., 2010). According to a study done by Conte et al. (2003) xylanase inclusion improved broilers' feed conversion ratio, but there was not an influence on other performance parameters that were measured.

Soluble NSPs are the cause of highly viscous digesta and poor litter quality (Ward, 1996). The jejunal and ileal digesta viscosity could be affected by age and adaptation to the experimental diets (Petersen et al., 1999). Arabinoxylans and β -glucans are the most essential anti-nutritive NSP in cereal grains. While arabinoxylans are more

prevalent in wheat and rye diets and β -glucans in barley, β -glucans are more susceptible to bacterial degradation through the intestinal tract (Knudsen, 2014; Gonzalez-Ortiz et al., 2017). The digestive system of non-ruminants does not produce xylanase, the enzyme required to hydrolyze arabinoxylans (Barrera et al., 2004). Because of the issues related to NSPs, xylanase supplementation has been added to diets to decrease litter issues, such as wet litter, and increase nutrient uptake. Wu and Ravindran (2004) completed a study and reported that supplemented xylanase significantly lowered feed efficiency in whole wheat and ground wheat-based diets. Also, feed intake was significantly reduced ($P < 0.001$) in whole wheat-based diets with supplemented xylanase compared to the control and ground wheat-based diets with supplemented xylanase.

Cowieson et al. (2010) reported an improvement in DE content (3.2%) when broiler corn-SBM-based diets were supplemented with xylanase. Additionally, Aftab (2012) reported a ME improvement, from 2.2 to 5.3%, in broiler corn-SBM diets supplemented with xylanase. While this study by Cowieson et al. (2010) showed a positive effect of xylanase, this enzyme does not always prove to have a positive effect on digestibility, growth, or performance. Ideally, xylanase is used to break down NSPs and reduce the viscosity of digesta to improve pre-caecal nutrient digestibility (Bedford, 2000). However, nutrient digestibility improvement does not always explain the effects of the enzyme supplementation on performance. O'Shea et al. (2014) states that there is no consistent effect of carbohydrases, such as xylanase, on growth performance in pigs due to the extent of deficiency of limiting nutrients and the extent to which the enzyme increased digestible nutrient content (Adeola and Cowieson, 2011), an important challenge in using an exogenous enzyme to improve animal production.

As mentioned previously, β -glucanase is another common carbohydrase supplemented to swine and poultry diets. High levels of β -glucans have been reported to reduce the nutritional value of barley in non-ruminants by increasing the viscosity of the intestinal fluid (Burnett, 1966). White et al. (1983) states that the increase in viscosity likely interferes with the digestive process by blocking the enzyme-substrate association. Increased viscosity also reduces the rate at which the released nutrients approach the mucosal surface for absorption (Thacker et al., 1992). When poultry and swine diets are supplemented with β -glucanase, the nutritive value of barley has the potential to increase significantly. In fact, Campbell et al. (1989) fed broiler chickens barley-based diets with or without supplemented β -glucanase and the results show that chickens fed the diet with supplemented β -glucanase were 35% heavier and 15% more efficient in feed utilization. In swine, these improvements are inconsistent. The influence of enzyme supplementation may be due to age of the pig, where young chickens and weanlings have less mature digestive tracts and endogenous enzyme secretion is much lower in comparison to older pigs (Bedford et al., 1992). Another attributing factor is that the NSP in the diet cannot be overcome by only one NSP degrading enzyme. Xylanase is frequently supplemented with β -glucanase to improve feed efficiency and nutrient digestibility. Yin et al. (2001) reported weanling pigs fed a barley based diet with supplemented xylanase and β -glucanase improved performance significantly. In addition to this study, Baidoo et al. (1998) reported that enzyme supplementation of xylanase and glucanase significantly improved nutrient utilization and performance of pigs fed diets containing hull-less barley. There were three phases of pigs weighing between 9-20, 20-40, and 40-60 kg and an improvement in ADG was observed in all three growth phases. When xylanase and

glucanase are in combination in poultry diets, it has also been reported to improve digestibility and performance. Mathlouthi et al. (2002) conducted a study to determine if supplementation of xylanase and glucanase improved conjugated bile acid fraction in intestinal contents in broilers fed a corn or rye-based diet. The addition of these exogenous enzymes to the rye-based diet improved weight gain, feed intake, feed efficiency, and decreased water intake. The digestibility of nutrients and AME were also significantly increased. The results in the literature confirm exogenous enzymes play an important role in the diets of non-ruminant animals. Although some results were inconsistent, it is essential to analyze the diet to understand what can be altered to allow the enzyme to hydrolyze the anti-nutritive factors in the feed ingredients. For maximum enzyme hydrolysis, there needs to be sufficient substrate availability to be acted upon.

1.4 Methodology

There are a few methods in swine and poultry nutrition used to determine nutrient and energy utilization. The quantitative feed and feces method, also known as total collection, the indicator method, and the direct and difference methods have been used widely in the industry in digestibility studies (Adeola, 2001; Dourado et al., 2010).

1.4.1 Total Collection Method

The total collection method is the process of measuring feed intake and fecal (or excreta) output to determine the amount of component ingested and emptied through feces (or excreta). Kong and Adeola (2014) calculated the digestibility and metabolizability of a component in a test feedstuff with the equation:

$$\text{Digestibility (\%)} = [C_{\text{input}} - C_{\text{output}}/C_{\text{input}}] \times 100$$

$$\text{Metabolizability (\%)} = [C_{\text{input}} - C_{\text{output}} - C_{\text{urine}}/C_{\text{input}}] \times 100$$

C_{input} and C_{output} are the amount of component ingested and emptied via feces, respectively, and C_{urine} is the amount of component voided in the urine. Total collection begins after a cluster of broiler chickens or individual pigs are adapted in their cage/crate and experimental diets. In swine, a color indigestible marker such as ferric oxide, chromic oxide, or indigo carmine is used to determine when to begin and end collection. The feces and urine are collected, separately, and measured daily quantitatively to determine daily output during the collection period. In poultry, the total collection method differs because feces and urine are voided as excreta. The total collection method is done by collecting all of the excreta at the same time daily, for a select number of days. Prior to excreta collection, feed is weighed back to allow accurate measure of nutrient utilization.

1.4.2 Indicator Method

The second method used in poultry and swine nutrition is the indicator method. The indicator method bypasses the strenuous routes of total collection and is less laborious; however, this relies heavily on accurate chemical analyses of the index marker (titanium or chromium). The ideal indicator has five conditions for which it must fulfill. The five conditions are 1) pass through the gastrointestinal tract at a constant rate as the digesta, 2) totally indigestible and unabsorbed, 3) has no pharmacological action on the gastrointestinal tract or the animal, 4) easily determined or analyzed, and 5) a natural constituent of the feed (Maynard, 1979). Jagger et al. (1992) expressed in order to be effective, the marker needs to have high and consistent recovery, as seen with lignin. Furthermore, Adeola (2001) stated that the amount of marker in the feed and the amount emptied should be constant over equal periods of time. Studies involving the indicator

method used markers such as chromic oxide, titanium dioxide, or acid insoluble ash (AIA) to determine ileal digestibility (Betancourt et al., 2012; Kim et al., 2012). The markers are commonly included at a rate between 0.1% and 0.5% of the diet. Because the indicator method is different, a separate formula is used to determine digestibility, or utilization, of nutrients and energy. The equation (Kong and Adeola, 2014) is as follows:

$$\text{Digestibility (\%)} = 100 - [(CI_{\text{input}} \times CC_{\text{output}} / CI_{\text{output}} \times CC_{\text{input}}) \times 100]$$

CI_{input} and CI_{output} represents the concentration of indicator compound in the feed and output (excreta or feces), respectively; CC_{input} and CC_{output} represents the concentration of component of interest in feed and output (excreta or feces), respectively.

1.4.3 Direct Method

The digestibility of a component, the nutrient, of a test feedstuff is determined by the direct or difference (indirect) method (Kong and Adeola, 2014). The direct method is utilized when the test diet is formulated so that all the nutrient of interest is provided by the test ingredient alone (Adeola, 2001). There is much simplicity to this method because only one test diet is needed when determining digestibility of ingredients with a high feed value. It is possible that the nutrient of interest is unable to stand as a test ingredient alone, therefore a basal diet can be formulated and a portion of the basal diet can be replaced by the test ingredient, which is the difference method.

1.4.4 Difference Method

Adeola (2001) states there are three approaches and calculations in the difference method. The first is to feed the basal diet to one group and determine the digestibility of this diet while another group is fed the basal diet with an added amount of the test

feedstuff and determine digestibility of the second diet. The digestibility of this approach is determined as follows:

$$\text{Digestibility of nutrient in test feedstuff, } A, \% = 100 \times [T \times t - (B \times b)/a]$$

In this equation, T equals the digestibility, %, of the component in the total diet (basal diet with added feedstuff), t is the proportion of the component in the total diet consumed; B is digestibility, %, of the component in basal diet; b is the proportion of component in basal diet consumed; and lastly, a is the quantity of component in test feedstuff added to the basal diet $t = b + a$. The second method, mentioned previously, is to feed a basal diet to one group while simultaneously feeding another group a diet with a portion of the basal diet replaced by the test feedstuff. The last approach is to feed a basal diet to one group while simultaneously feeding another group diets that have at least two proportions of the component in the basal diet replaced by the test feedstuff. The difference method has also been used to determine amino acid digestibility values in feedstuffs (Fan and Sauer, 1995). Kong and Adeola (2014) and Adeola (2001) described the determination of nutrient and energy digestibility using the total collection method, indicator method, and direct and difference method.

1.5 Important Feed Ingredients in the Industry

The profitability of any animal feeding operation relies heavily on feed ingredients availability, quality, and cost. Feed ingredients supply nutrients and energy for growth, performance, and overall well-being of the animal. Feed in poultry production accounts for 60-70% of the total cost of production (Yousaf, 2006) which shows just how important it is to any animal feeding operation. While every feed

ingredient cannot be discussed in this review, it is important to review the ingredients making up majority of diets and those that are essential to the diet.

1.5.1 Corn

Corn, also known as maize, is a cereal grain and a major source of energy in broiler chicken and swine diets. Classen (1996) reports that such cereal grains provide a great amount of the energy in poultry diets, mostly starch. Because of this fact, it is important to understand the use of ingredients with relatively high starch components. Digestion of corn starch is negatively affected by amylase inhibitors, amylose and amylopectin. With the assistance of enzymatic activity from α -amylase, maltase, and isomaltase, starch becomes easily digestible (Cowieson, 2005). Brown (1996) stated that corn starch has the potential to be resistant to digestion, giving it the name 'resistant starch'. There are three naturally occurring subcategories of resistant starch (RS); the first subcategory, RS1, occurs where the starch granules are physically inaccessible. The starch granules happen to be trapped in the food matrix, making them hard to digest. Under RS2, the second subcategory of resistant starch, the degree of resistance to digestion of a starch granule appears to be related to the structure and conformation of the native starch granules. Lastly, RS3 is exhibited during the process of formation of retrograding starch. The ubiquitous distribution of the resistant starches has shown to occur in various degrees in corn.

The nutritive value of corn is derived from the corn endosperm, a starch-protein matrix consisting of two types of endosperm; floury and vitreous. Floury endosperm is more open with less encapsulation by proteins, whereas vitreous is hard and tight (Rooney and Pflugfelder, 1986; Latham et al., 2016). A key issue with corn is that the

nutrient value is not consistent on a year-to-year basis. Many factors play a role in the inconsistency such as geographical location. According to Cowieson (2005) agronomic conditions, the harvesting process, and storage conditions have a long-standing effect on corn nutrient digestibility and safety, in terms of levels of mycotoxins. A study performed in China (Yin et al., 2017) examined the effect of storing corn in barns for up to five years. In their results, the researchers found that storing of corn for 5 years did not affect the digestibility of starch and CP, but the digestibility of histidine and arginine were significantly altered quadratically with the increase in storage time. The maximum storage time should be 4 years, before the digestibility of AA is negatively impacted. As stated by Cowieson (2005) differences in corn are unpredictable and ME between samples can differ by up to 400 kcal/kg. Mentioned at the start of this review, the determination of ME in broiler chickens is important because it measures the energy available to the chicken.

There is an assortment of corn sources used and evaluated in research within the swine and poultry industry. Yellow dent and NutriDense are two common types of corn used in diets. NutriDense is considered to provide greater nutrient density than yellow dent corn. According to Hastad et al. (2004), NutriDense contains a higher percentage of amino acids; 30% more lysine, 50% more sulfur-containing amino acids, 18% more threonine, and nearly 25% more tryptophan. NutriDense has created genetically modified low-phytate corn that contains 75% available P compared to yellow dent corn which only has 14% available P (Hastad et al., 2005; NRC, 1998).

1.5.2 Soybean meal

While corn contains about 8% CP, a key protein supplement used in animal nutrition is soybean meal. Soybean meal has high protein content, making it an excellent choice as a dietary supplement, on top of it being a relatively cheap source for protein (Sykes et al., 2010). Soybean meal generally has two ingredient compositions; regular (SBM-R), which contains 44-45% CP, and high protein (SBM-HP), which contains about 47% CP (de Coca-Sinova et al., 2010). The environment plays an important role in how effective SBM is in the diet. Digestibility can differ because of geographical location and how the SBM is processed, as previously discussed for corn. In an article written by van Kempen et al. (2006), the chemical composition of SBM varied based off of the origin of the soybeans. In addition to this fact, Mateos et al. (2009) gathered 262 SBM samples from USA (n=134), Argentina (n=77), and Brazil (n=51) and analyzed their chemical composition. The chemical composition of neutral detergent fiber (NDF) varied greatly with a low of 6.4% to a high of 15.8%; whereas the composition of lysine was higher in the United States than in Brazil or Argentina.

Soybean meal is widely used in the animal feeds because it is a relatively cheap component of the diet and research has proven its effectiveness for many decades. In pigs, oligosaccharides such as raffinose and stachyose affect the growth performance and health of newly weaned pigs (Pangeni et al., 2017). Such oligosaccharides resist digestion because they lack α -galactosidase, an enzyme capable of alleviating the detrimental effects of the saccharides (Gitzelmann and Auricchio, 1965). Raffinose and stachyose present in feedstuffs are also indigestible in poultry. Although poultry lacks the endogenous enzyme targeting α -1, 6-galactosyl bonds needed to digest the

oligosaccharides, the supplementation of α -galactosidase to a corn-SBM based diet has been proven to enhance performance of broilers in early stages of growth (Wang et al., 2005). Coon et al. (1990) conducted a study in adult roosters where raffinose and stachyose were removed from SBM by ethanol extraction, to provide an oligosaccharide-free diet, and their results showed an increase in true ME_n and reduction digesta transit time by 50%.

Soybean meal has other types, but the additional assortments tested are SBM-LO, SBM-HP, and SBM-Conventional (CV). Every source of SBM has different nutritional values, but they all supply high quality CP, some greater than others, but all prove to have the same end goal. Baker et al. (2011) used 120 Ross 308 male broiler chicks and ultimately found that SBM-HP and SBM-LO had a higher nutritional value than the SBM-CV due to increased concentration of digestible amino acids, however there was no difference in broiler chicken growth performance. Furthermore, their analyses proved SBM-HP and SBM-LO has a higher amino acid concentration than SBM-CV, therefore less SBM (HP or LO) will be needed in the diet in comparison to SBM-CV. As affirmed previously, sources of the same ingredient may differ analytically in composition, but with consistent research, nutritionists are able to judge which best suits their purpose. In the United States, corn and SBM make up a substantial proportion of poultry and swine diets because of their nutritional value and the energy and protein they supply to the animal.

1.5.3 Barley

Another feed ingredient used in the diet is barley. Barley began to be incorporated as an alternative grain source primarily as a cost saving source and availability. After

using barley in diets, nutritionists observed a problem with β -glucan content where solubilized β -glucan turned viscous and interfered with digestion (Brake et al., 1997). The enzyme, β -glucanase, is an exogenous enzyme needed to digest diets high in β -glucan content, however low β -glucan has shown not to have a significant effect interfering with digestion (Goodband and Hines, 1988).

Broiler chicken diets often contain hull-less barley or hulled barley. The difference between the two is hull-less barley has the hull removed during harvesting and hulled barley does not have the hull removed. Barley hull is made up of cellulose, lignin, and hemicellulose, and a small amount of protein (Bhatty, 1986). The crude fiber content in barley is mainly because of the hull and when the hull is removed the crude fiber content is comparable to corn (Jacob and Pescatore, 2012). Classen et al. (1988) reported that hull-less barley has higher AME values and more CP than hulled barley due to the dilution effect from the hull. The hulled barley also has lower CP, starch, and fiber than hullless barley (Edney et al., 1992).

In swine, the digestible energy of hulled vs. hullless barley was evaluated and studies found hullless barley had higher DE than hulled barley (Bhatty, 1986). In fact, when the hull was removed, DE increased by 10-15% than with the hull intact. Almost a century ago, Joseph (1924) proved hullless barley was far more superior to hulled barley and equal to corn in feeding value, but swine nutritionists did not follow suit after this discovery. Lastly, Wang et al. (2017) reported dehulled high and low fiber barley improved DE and ME in growing pigs compared to hulled high and low fiber barley. The major portion of NSP content in barley is in the hull, therefore when the hull is removed, digestibility increases significantly as seen in the results of the study.

1.6 Conclusion

The composition of poultry and swine diets ultimately will determine how the animal will perform. Other circumstances may have an impact, but the diet is where the majority of the cost of production comes from. In broilers and pigs, the AME and DE value of feed ingredients are greatly increased by the supplementation of exogenous enzymes. Additionally, because different methodologies are utilized to evaluate digestibility of energy and nutrients, it is essential to understand and distinguish which method is best for the research. The total collection method or indicator method are used to determine the digestibility of a component of interest in experimental diets. The total collection method is known to be more labor intensive because all fecal and urine samples (pigs) and excreta (chickens) are to be collected. The indicator method relies on an indigestible marker, such as TiO_2 that is incorporated in the diet. These techniques have positively impacted poultry and swine nutrition research. While researchers have initiated methods to reduce feed cost and improve performance and digestibility, more research will always be needed, especially to keep pace with improvements in breeding and genetics (crops and animals) as well as the development of new generations of exogenous enzymes.

Poultry and swine research is important because it allows researchers to meet the nutritional needs of the animals, become more environmentally sustainable (nutrient excretion reduced), enhance genetics for better efficiency, and ultimately ensure the optimal welfare of the animals (Scanen, 2007). Research has progressed over the last century because of awareness to the concerns mentioned previously. The NRC requirements of poultry and swine animals from 50 years ago are not the same as they are

today. Because of these changes, animals are raised more efficiently, which has led to a reduction in feed cost. For the future of the poultry and swine industry to be sustainable, it will be vital to keep improving on ways in which nutrient and energy could be used more efficiently by our animals.

CHAPTER 2 – Effect of phytase and xylanase-glucanase individually and in combination on apparent metabolizable energy value of barley in 21-day old broiler chickens

Abstract

A total of 280 14-d-old male broilers (Cobb 500) were assigned to 1 of 8 corn-SBM or corn-SBM-barley-based dietary treatments with or without phytase (Natuphos®E, 1000 FTU/kg) and xylanase-glucanase (X-G; Natugrain®TS, 560 TXU/kg and 250 TGU/kg) in a completely randomized design with 7 replicate cages/treatment and 5 birds/cage. Treatments were arranged in a $2 \times 2 \times 2$ factorial arrangement with 2 diet types (corn-SBM-based vs. corn-SBM-barley-based), 2 levels of phytase (0 vs. 1,000 FTU), and 2 levels of X-G (0 vs. 560 TXU). Excreta samples were quantitatively collected on d 19, 20, and 21. AME and AMEn of barley were determined using the difference method. There were no two- or three-way interactions between diet type, phytase, and xylanase-glucanase on birds' performance. Birds fed the corn-SBM-barley-based diets had lower ($P < 0.05$) body weight gain, feed intake, and feed efficiency compared to birds fed the corn-SBM-based diets. The inclusion of barley in the corn-SBM-based diets resulted in higher ($P < 0.05$) DM and N retention, but lower ($P < 0.05$) AME and AMEn. Xylanase-glucanase supplementation resulted in lower ($P < 0.05$) N retention in birds fed the corn-SBM-based diets. Xylanase-glucanase supplementation significantly improved energy metabolizability, AME, and AMEn of barley. Overall, birds fed the corn-SBM-based diet performed better than those fed a corn-SBM-barley-based diet. Phytase supplementation did not have an effect on AME and AMEn value of barley, however xylanase-glucanase supplementation increased AMEn value of barley. When added in combination, the supplementation of phytase and xylanase-glucanase showed a tendency to increase AME and AMEn value of barley.

Key words: Apparent metabolizable energy, barley, broiler, nitrogen retention

2.1 Introduction

Exogenous enzymes have been incorporated into the diets of broiler chickens to increase growth performance and nutrient and energy digestibility. Phytase is an enzyme used to liberate phytate-bound phosphorus in the feed. Phosphorus (P), an essential mineral in poultry diets, is derived from feed ingredients of plant origin and inorganic sources. A large fraction of P from plant-origin feed ingredients is in the form of phytate-P (Abdollahi et al., 2016). Phytate, also known as phytic acid, is the main storage form of

P in grains and cereals. However, broiler chickens are unable to digest phytate because they lack sufficient level of endogenous enzymes needed to break phytate down completely (Selle and Ravindran, 2007). The inability of poultry to digest phytate leads to large amounts of P being excreted into the environment. Broiler chickens need an adequate amount of P in their diet, but excessive P excretion is a major concern. To overcome this issue, inorganic P supplements such as mono- and di-calcium phosphate and phytase are added to the diet to meet this requirement. It has been proven that phytase reduces P excretion by up to 50% and improves the availability of phytate-bound minerals (Vohra et al., 2006; Manobhavan et al., 2016). Phytase utilization in poultry diets is effective because in addition to improving the bioavailability of phytate-P, it also improves Ca availability and energy metabolizability (Lu et al., 2009).

Xylanase is another exogenous enzyme used in poultry feeds to counteract the negative effects of non-starch polysaccharides (NSP). The presence of soluble NSP can lead to high viscosity and poor litter quality, therefore xylanase supplementation aids in allowing maximum energy digestibility while reducing litter complications (Gonzalez-Ortiz et al., 2017). Additionally, Bedford and Schulze (1998) acknowledged that majority of soluble pentosans in viscous grains lead to an increase in viscosity of the digesta in the gastrointestinal tract, causing a decrease in the nutritive value of the diet. Xylanase degrades soluble arabinoxylans, the main NSP in cereal grains. While the degree of degradation of these NSP is unknown, Choct and Annison (1992) reported a large reduction of the anti-nutritive effect when a depolymerized pentosan was added to a broiler diet. Although the primary purpose of phytase is to improve P digestibility, it is

important to evaluate the effect of both phytase and xylanase-glucanase independently and in combination on apparent ME values of barley.

The objective of this study was to investigate the contribution of exogenous enzymes, phytase and carbohydrase (xylanase-glucanase), individually or in combination to the apparent metabolizable energy (AME) value of a complete diet and barley in 21 day-old broiler chickens.

2.2 Materials and Methods

2.2.1 *Animal Care*

The experimental protocol used in this study was approved by the University of Kentucky Institutional Animal Care and Use Committee.

2.2.2 *Animals and Experimental Design*

A total of 280 day-old male broiler chicks (Cobb 500) were obtained from a commercial hatchery and tagged on day 0. The chicks were housed in heated battery cages from day 0 to 14. The battery cages were placed in an environmentally controlled room with 20 h light and proper ventilation. Cages were equipped with feeders, drinkers, and dropping trays for excreta collection. Room temperature was maintained at 31°C on day 0 and was gradually decreased and maintained at 25°C from d 14 to d 21. All birds were given *ad libitum* access to feed and water during the entire study (d 0-21). At the start of the experimental period, d 14, birds were weighed individually, allocated to cages in a completely randomized design, and transitioned from the common starter diet to the experimental diets. There were a total of 8 treatments and 7 replicates/treatment with 5 birds per replicate cage.

2.2.3 Enzymes and Experimental Diets

The two enzymes used in this study were supplied by BASF (Florham Park, New Jersey). The phytase used was Natuphos[®] E at 1,000 FTU/kg of diet. One phytase unit is defined as the quantity of enzyme which sets free 1 μ mol of inorganic P under conditions of the assay. The carbohydrase used was Natugrain[®] TS, a xylanase-glucanase combination. This was added to the diet at a rate of 560 TXU/kg. One thermostable endo-xylanase unit (TXU) is defined as the amount of enzyme which liberates 5 μ mol reducing sugars, measured as xylose equivalents, per minute under the reactions. Glucanase was added to the diet in combination with xylanase at a rate of 250 TGU/kg. Additionally, one thermostable beta-glucanase unit (TGU) is defined as the amount of enzyme which liberates 1 μ mol reducing sugars, measured as glucose equivalents per minute under the reaction conditions.

The ingredient composition of the experimental diets and their nutrient and energy contents are shown in Table 2.1. There were a total of eight diets where four corn-SBM diets were either unsupplemented, supplemented with phytase, xylanase-glucanase, or a combination of phytase and xylanase-glucanase. Each of the four corn-SBM diets were replaced with 30% barley to give rise to a total of eight diets. Barley replaced the energy yielding components of the control diet and was substituted at the same ratio across all diets. This was necessary to determine the AME and AMEn of barley using the difference method. Enzymes were added to the diet at the expense of corn, as a premix, at the rate of 10 g/kg of diet. Titanium dioxide (TiO₂) was added to all diets (5 g/kg).

2.2.4 Sample Collection

Excreta were collected daily via the total collection method (Ravindran et al., 1999; Mollah et al., 2007) on the last three days of the experiment, d 19, 20, and 21. Twenty-four hours before the first excreta collection, the collection trays were emptied, cleaned, and the wax-coated paper on the tray was replaced. Excreta was then collected every 24 h for a total of 72 hrs. The three collection periods began at 0700 h to allow for accurate measure of nutrient utilization. Excreta were stored in a -20 °C freezer until the end of experiment and subsequently dried in a forced-air oven at 55 °C for five days. After drying, excreta from the 3 collection days were combined for laboratory analysis. On the first and last days of collection, feed in the troughs were emptied into their respective buckets and weighed at the same time.

2.2.5 Laboratory Analysis

Diets, barley, and excreta were ground to pass through a 2 mm screen using a mill grinder (Wiley Mill Grinder 3, Swedesboro, New Jersey, USA). All diets and excreta samples were analyzed for gross energy (GE), nitrogen (N), and dry matter (DM). Samples were analyzed in duplicate and analysis was repeated when coefficient of variation between duplicate samples was greater than 5%. The GE was determined using bomb calorimetry (Parr 6200 isoperibol bomb calorimeter; Parr Instrument Company, Moline, Illinois, USA) with benzoic acid as a calibration standard. Nitrogen was measured using the Dumas combustion method determined with an Elementar vario MAX CN analyzer (Elementar, Mt. Laurel, New Jersey, USA) with L-glutamic acid as a calibration standard. To determine DM, samples were placed in a drying oven (Precision Scientific Co, Chicago, Illinois, USA) at 110 °C for 24 h.

2.2.6 Calculations and Statistical Analysis

Apparent ME of the diet (AME_d) was calculated using this equation: $AME_d = GE_d \times EM_d$ where GE_d is the gross energy of the diet and EM_d is the percent (%) energy metabolizability, respectively. Apparent ME of the test ingredient (AME_{ti}) was calculated as follows: $AME_{ti} = EM_{ti} \times GE_{ti}$, where GE_{ti} and EM_{ti} are the gross energy and percent energy metabolizability of the test ingredient, respectively. The coefficient of energy metabolizability of the test ingredient (EM) was calculated using this equation: $EM = [EM_{td} - (EM_{rd} \times 1 - FC_{ti/td})] / FC_{ti/td}$, where EM_{td} is the coefficient of energy metabolizability of the test diet, EM_{rd} is the coefficient of energy metabolizability of the control diet, and $FC_{ti/td}$ is the fractional contribution of the test ingredient to the test diet (Olukosi et al., 2017).

All data were analyzed in a completely randomized design by ANOVA using the GLM procedure of SAS (SAS Institute, Cary, North Carolina, USA) appropriate for a factorial arrangement of treatments with a 2 (diet types, DT) x 2 (levels of phytase) x 2 (levels of xylanase-glucanase) for the diets and 2 (levels of phytase) x 2 (levels of xylanase-glucanase) for barley. The experimental data were expressed as means with standard deviations. Mean for the main and simple effects are reported. Any data that fell outside of the mean \pm 3 SD was removed from the data (outliers). Significant differences between means were determined by Tukey test. Level of significance was set at $P < 0.05$ and a P value between 0.05 and 0.1 was considered a trend.

2.3 Results

2.3.1 Analyzed Composition of Experimental Diets and Barley

The analyzed DM, crude protein (CP), and GE composition of the experimental diets are shown in Table 2.1. Average crude protein content and GE composition of the four diets without barley were greater than those diets replaced with barley (CP: 210.3 vs. 177.2 g/kg) (GE: 4,141 vs. 4,026 kcal/kg). Crude protein being lower in the barley-based diets was to be expected because the diets were formulated with less soybean meal than the control diets. Nearly double phytase was analyzed in the Ref-Bar-Phy-X-G diet compared to the Ref-Phy-X-G diet (990 vs. 500 FTU/kg). Additionally, the Ref-Phy-X-G diet had 300 more units of glucanase compared to the Ref-Bar-Phy-X-G diet.

The composition of barley is shown in Table 2.2. Analyzed barley DM was 878.1 g/kg and GE was 3,819 kcal/kg. Barley contained 100.1 g/kg CP and 163 g/kg crude fiber.

2.3.2 Growth Performance

The simple and main effects of exogenous enzyme supplementation to corn-SBM- and corn-SBM-barley-based diets are reported in Table 2.3. There was a significant difference between birds fed the corn-SBM-based and corn-SBM-barley-based diets, where birds that were fed the diets with added barley performed at a reduced level ($P < 0.05$) compared to birds fed the corn-SBM-barley based diets. At the end of the 7-d feeding trial, birds fed the corn-SBM-based diet were heavier ($P < 0.05$) and had a higher ($P < 0.05$) feed efficiency than birds fed the diets containing barley. Additionally, the two- and three-way interactions were not significant.

2.3.3 Retention and Energy of Experimental Diets

The simple and main effects of diet type and exogenous enzyme supplementation (phytase with or without xylanase-glucanase) on DM, N, and GE retention, and AME of the dietary treatments are reported in Table 2.4.

Dry matter retention was higher in ($P < 0.05$) in the corn-SBM-barley-based diets and a three-way interaction ($P = 0.039$) was observed. Corn-SBM-barley-based diets with xylanase-glucanase individually or in combination with phytase were greater ($P < 0.05$) than that of the corn-SBM-based diet with supplemental xylanase-glucanase individually. There was a tendency ($P = 0.054$) for a two-way interaction between diet type and xylanase-glucanase (DM retention).

The supplementation of xylanase-glucanase significantly decreased ($P < 0.05$) N retention; however, the addition of barley increased N retention (diet type). A two-way interaction ($P = 0.006$) between diet type and phytase was significant ($P = 0.001$), however the interaction between diet type and xylanase was considered a trend ($P < 0.066$).

For GE retention, an interaction ($P = 0.004$) was observed between diet type and xylanase-glucanase. Furthermore, phytase had a tendency to increase GE retention. Simple effects of diet type and phytase were reported for AME and AMEn values. Corn-SBM-based diets had higher AME and AMEn values and phytase supplementation increased ($P < 0.05$) AME and AMEn values. These effects led to a significant interaction between diet type and phytase. Two-way interactions between diet type and xylanase-glucanase and phytase and xylanase-glucanase were considered a trend ($P < 0.1$).

2.3.4 Energy Metabolizability, Apparent ME and MEn of Barley

The main and simple effect of phytase and xylanase-glucanase supplementation individually or in combination on energy utilizability (energy metabolizability), AME, and AMEn) of barley is reported in Table 2.5. Xylanase-glucanase supplementation increased ($P < 0.05$) EM, AME and AMEn values of barley. There was a two-way interaction ($P = 0.047$) for EM of barley with the supplementation of xylanase-glucanase to the corn-SBM-barley-based diet resulting in higher ($P = 0.047$) EM of barley. A tendency for significant two-way interactions were observed for EMn ($P=0.067$), AME ($P = 0.072$), and AMEn ($P = 0.069$) of barley (Table 2.5). There was a 2.5%-point increase in energy metabolizability of barley with xylanase-glucanase supplementation alone compared to no enzyme in the barley-based diet (81.3 vs 78.8%). Apparent ME, and AMEn increased by 1.5 and 2.7%-point, respectively, with xylanase-glucanase supplementation.

2.4 Discussion

The current study shows that feeding a corn-SBM-barley-based diet in place of a corn-SBM-based diet depresses broiler performance. Broilers fed diets with added barley consistently performed at a reduced level, which can be attributed to the barley, mainly the NSPs, in the diet. Enzymes, specifically a xylanase-glucanase combination, are utilized in poultry diets to improve the nutritional value of cereal grains such as barley, however the degree of response of the barley to the enzymes are quite variable (Classen, 1996). Xylanase and glucanase are commonly used in poultry diets containing wheat and barley because the arabinoxylans and beta-glucans have the potential to overcome the anti-nutritive effects of NSPs.

Phytase has an extensive history of improving body weight gain in corn-SBM-based diets (Huff et al., 1998). In this current study, there was no significant interaction on day 21 BW between birds fed diets with or without 1,000 FTU/kg of phytase. However, there was a depressing effect from barley on the corn-SBM-based diet. On average, broilers fed corn-SBM-barley-based diets had a higher feed intake than corn-SBM-based diets. Barley is known to have lower energy than other cereal grains, so it is necessary to consume more feed in order to meet nutrient requirements (Classen, 1996). While feed intake was higher in the barley-based diets, day 21 body weight and gain per bird were lowest. The low body weight and gain can be attributed to the NSPs, where size of molecules and the degree of digestion can affect digesta viscosity and passage rate of gut content (Choct, 1997). Yaghobfar and Kalantar (2017) reported significantly lower weight gain in barley-based diets compared to a corn-SBM-based diet.

Nitrogen retention improved in the corn-SBM-barley-based diets and diets with supplemented phytase, resulting in a significant interaction between the two effects (data not shown). Surprisingly, xylanase-glucanase supplemented decreased N retention. Gallardo et al. (2018) reported an improvement in N retention with supplementation of a multicarbohydrase including α -galactosidase, galactomannase, xylanase, and beta-glucanase. The lower endogenous and exogenous losses and increase in protein hydrolysis can be attributed to the N improvement (Adeola and Cowieson, 2011), but that was not observed in this current study. Cowieson and Ravindran (2008) reported 11% increase in N retention of corn-SBM diets supplemented with an enzyme cocktail containing xylanase, protease, and amylase. The supplemental exogenous phytase seemed to have improved N utilization better than this current study. The release of P by phytase

resulted in extra phosphoric effects, assisting in the breakdown of amino acids and resulting in better N retention.

Gross energy retention had a tendency to increase with supplementation of phytase individually. It is possible a higher concentration of phytase and xylanase-glucanase in the diet would aid in the increase in GE retention when enzymes were supplemented in combination. The greater break down on substrate will allow cleavage and release of anti-nutritive factors in the feed. There was a significant interaction between diet type and xylanase-glucanase resulting in increased GE retention of the corn-SBM-barley-based diet (data not shown). Esmailipour et al. (2012) also reported an increase in energy retention in broilers fed wheat-based diets. Reduction of digesta viscosity from xylanase supplementation could have played a factor in improving energy retention.

Apparent ME and AMEn of the corn-SBM-based diets were higher compared to the corresponding barley-based diets. On the other hand, AME was lowest in the unsupplemented barley-based diet. Apparent ME and AMEn of the barley-based diets improved with phytase supplementation which suggests a positive effect of the enzyme on degradation of the phytate present in the barley, allowing better utilization. The insignificant interaction between diet type, phytase, and xylanase could be attributed to not enough substrate for the enzymes to hydrolyze, or not enough enzyme supplemented in the dietary treatment. It is apparent that phytase was able to increase AME and AMEn of the diet individually, but in combination this was not the case except that there was a tendency for the combination of phytase and xylanase-glucanase supplementation to increase AMEn values of the diets (data not shown). These results differ from Gallardo et

al. (2018), where a multicarbohydrase (MC) and phytase combination significantly increased AMEn values of wheat-bran based diets, compared to phytase supplementation alone. Increase in AMEn with the MC supplementation could be related to total or partial arabinoxylan cleavage, which was not observed in the current study.

Tang et al. (2017) reported an increase ($P < 0.05$) in N retention of barley-based diets when 16,000 TXU/kg were supplemented to the diet. Enzyme addition consistently improved DM retention compared to unsupplemented barley-based diet. Esmailipour et al. (2012) reported significant increase in DM retention of wheat-soybean meal diets supplemented with 1000 TXU/g. Exogenous xylanase-glucanase in this study degraded the NSPs in the barley-based diets, which would explain the trend observed on the interaction treatment and xylanase-glucanase on DM retention.

While there was no significant effect of xylanase-glucanase on AME and AMEn of the diet, there was an interaction ($P < 0.05$) on AME and AMEn of barley. Energy metabolizability and AME increased greatly with supplementation of xylanase-glucanase. The NSPs in barley were degraded adequately to allow the exogenous enzyme to work on barley and increase the AME value. The interaction between phytase and xylanase-glucanase on energy metabolizability of barley was significant, while there was a tendency for the combination of phytase and xylanase-glucanase supplementation to result in higher AME and AMEn of barley compared to phytase supplementation alone. Similarities were found in what was reported by Ravindran et al. (1999) who reported that supplementation of phytase did not significantly improve AME value of barley. When Ravindran et al. (1999) supplemented glucanase and phytase in combination, AME of barley improved significantly by 5.9%. The author explained no significant

improvement in AME of barley could be because the level of substrate was too low to show an energy response. This explanation could also be the cause for the observation in the present study.

While enzymes are capable of improving broiler chickens' performance, barley cultivars also have a substantial impact. Ravindran et al. (2007) examined four barley cultivars representing conventional hulled normal starch barley, hull-less normal starch barley, and two hull-less waxy barleys. The hulled and hull-less normal starch barley diets with added β -glucanase were significantly higher in AMEn value in comparison to the hull-less waxy barley diets with added enzyme.

For this current study, the energy yielding components of the corn-SBM diets were replaced with 30% barley in order to estimate the AME value of barley. Fuente et al. (1995) examined the effect of xylanase and β -glucanase on AME of diets with increasing levels (30, 40, 50, and 60%) of barley fed to broilers at different ages. A significant interaction was observed between the 10-d old and 30-d old chickens, showing a difference in response to the addition of enzymes to the barley inclusion levels for AMEn. Young chickens had an increase in AMEn values from 30 to 40% barley supplemented with enzyme addition (3,276 to 3,317 and 3,346 kcal/kg), then the value decreased significantly as barley inclusion level increased. Older chickens' AMEn value remained constant from 30 to 40% barley supplemented with enzyme addition and decreased thereafter; showing the most significant effect at 30 or 40%, much like this current study where barley was substituted at an inclusion level of 30%.

2.5 Conclusion

It can be concluded that enzyme addition tends to improve AME and AMEn value of barley. The addition of xylanase-glucanase alone proved to have the most impact on AME value of barley. Furthermore, the results of this study are in line with existing information in the literature that the supplementation of exogenous enzymes to a barley-based diet improves nutrient utilization and AME value of the barley. Therefore, the use of an enzyme preparation containing a combination of phytase, xylanase, and glucanase would further improve energy utilization when supplemented at a higher dosage and possibly reduce the quantity of the wet litter. Any barley inclusion level over 30% is at risk of further reducing broiler performance and AME value. For that reason it can be suggested to replace barley at a maximum of 30%, or increase enzyme dosage to allow an increase level of degradation of the target substrate.

Table 2.1. Composition of the experimental diets (on as-is basis).

<i>Ingredients, g/kg</i>	Corn-SBM	Corn-SBM-Phy	Corn-SBM-X-G	Corn-SBM-Phy-X-G	Corn-SBM-Bar	Corn-SBM-Bar-Phy	Corn-SBM-Bar-X-G	Corn-SBM-Bar-Phy-X-G
Corn	567.8	567.8	567.8	567.8	383	383	383	383
Soybean meal, 48%CP	320	320	320	320	219.24	219.24	219.24	219.24
Barley	0	0	0	0	300	300	300	300
Soy oil	45	45	45	45	30.83	30.83	30.83	30.83
Di-calcium phosphate	19	19	19	19	19	19	19	19
Limestone	12	12	12	12	12	12	12	12
Titanium dioxide	5	5	5	5	5	5	5	5
Vitamin-mineral premix ¹	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
L-Lysine HCl	2	2	2	2	2	2	2	2
DL-Methionine	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
L-Threonine	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Salt	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
Corn	20	10	10	0	20	10	10	0
Phytase premix ²	0	10	0	10	0	10	0	10
Xylanase premix ³	0	0	10	10	0	0	10	10
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
<i>Analyzed Nutrients & Energy</i>								
GE, kcal /kg	4,105	4,134	4,178	4,145	4,016	4,034	4,016	4,038
ME, kcal/kg ⁴	3,174	3,174	3,174	3,174	3,098	3,098	3,098	3,098
CP, g/kg	203.8	220.6	206.9	210	176.3	181.9	177.5	173.1
DM, g/kg	966	963	966	967	966	966	966	965
Ca, g/kg	10	10	10	10	9.79	9.79	9.79	9.79
P, g/kg	7.41	7.41	7.41	7.41	7.25	7.25	7.25	7.25
Non-phytate P, g/kg ⁴	4.29	4.29	4.29	4.29	4.45	4.45	4.45	4.45
Ca:P ⁴	1.3	1.3	1.3	1.3	1.4	1.4	1.4	1.4
Phytase, FTU/kg	<60	700	<60	500	100	710	110	990
Xylanase, TXU/kg	<100	<100	690	810	<100	<100	700	680
Glucanase, TGU/kg	<100	<100	660	390	<100	<100	370	450

¹Vitamin-mineral premix was formulated to supply the following at 0.25% per kilogram of diet: 11,025 IU of vitamin A; 3,528 IU of vitamin D; 33 IU of vitamin E; 0.91 mg of vitamin K; 2.21 mg of thiamin; 7.72 mg of riboflavin; 55 mg of niacin; 18 mg of pantothenate; 5 mg of vitamin B-6; 0.22 mg d-biotin; 1.10 mg of folic acid; 478 mg of choline; 0.03 of vitamin B-12; 75 mg of Zn; 40 mg of Fe; 64 mg of Mn; 10 mg of Cu; 1.85 mg of I; and 0.30 mg of Se

² Phytase (Natuphos[®] E, BASF Animal Nutrition) premix was formulated to supply 1,000 FTU/kg. Added at expense of ground corn

³ Xylanase-glucanase (Natugrain[®] TS, BASF Animal Nutrition) premix was formulated to supply 560 TXU/kg and 250 TGU/kg. Added at expense of ground corn

⁴Calculated values

Bar = barley, Phy = phytase, X-G = xylanase-glucanase

Table 2.2 Analyzed proximal composition and gross energy value of barley (g/kg, on as-is basis)

Item	Barley
Dry matter	878.1
Crude protein	100.1
Gross energy, kcal/kg	3,819
Crude fat	8.9
Crude fiber	16.3
Ash	15.3
Acid detergent fiber	23.3
Neutral detergent fiber	96.5

Table 2.3. Simple and main effect of exogenous enzymes supplementation to corn-soybean meal- and corn-soybean meal-barley based diets on the performance of broilers¹.

Diet type (DT)	Phy	X-G	d14 BW	d 21 BW	Gain/bird	FI/bird	Gain:Feed
Corn-SBM			399	869	470	643	731
Corn-SBM-Bar			396	829	433	666	650
	0		399	847	448	651	689
	1000		396	851	455	659	691
		0	396	851	454	657	692
		560	398	847	449	652	689
Corn-SBM ²	0	0	416	883	466	650	718
Corn-SBM	0	560	394	860	466	636	733
Corn-SBM	1000	0	382	865	483	647	747
Corn-SBM	1000	560	402	867	465	640	726
Corn-SBM-Bar ²	0	0	387	817	430	659	653
Corn-SBM-Bar	0	560	397	827	430	657	654
Corn-SBM-Bar	1000	0	400	837	437	671	651
Corn-SBM-Bar	1000	560	400	834	434	676	642
SD			23.41	43.02	26.13	31.25	26.35
DT			0.728	0.001	<.0001	0.010	<.0001
Phy				0.724	0.354	0.349	0.779
X-G				0.757	0.424	0.617	0.608
DT*Phy				0.417	0.867	0.361	0.205
DT*X-G				0.558	0.602	0.484	0.937
Phy*X-G				0.806	0.472	0.698	0.132
DT*Phy*X-G				0.428	0.591	0.990	0.371

¹Bar = barley, Phy = phytase, X-G = xylanase-glucanase, BW = body weight, FI = feed intake, DT = diet type

²n=7, except for corn-SBM-Bar diet with 1,000 FTU and 560 TXU supplementation where n=6

Table 2.4. Simple and main effect of exogenous enzymes supplementation to corn-soybean meal- and corn-soybean meal-barley-based diets on dry matter, nitrogen, and energy retention and AME values of broiler chickens¹.

Diet type (DT)	Phy	X-G	DM Ret, %	N Ret, %	GE Ret, %	AME, kcal/kg	AMEn, kcal/kg
Corn-SBM			78.5	75.2	80.8	3,463	3,392
Corn-SBM-Bar			79.5	75.9	80.6	3,363	3,305
	0		78.9	75.3	80.5	3,399	3,334
	1000		79.1	75.8	80.9	3,427	3,363
		0	78.9	75.9	80.5	3,398	3,334
		560	79.1	75.2	80.9	3,428	3,363
Corn-SBM	0	0	78.8 ^{ab}	75.1	80.9	3,438	3,370
Corn-SBM	0	560	78.0 ^b	73.9	80.2	3,468	3,395
Corn-SBM	1000	0	78.5 ^{ab}	76.5	81.0	3,478	3,407
Corn-SBM	1000	560	78.7 ^{ab}	75.3	80.9	3,468	3,398
Corn-SBM-Bar	0	0	78.7 ^{ab}	75.8	79.6	3,309	3,251
Corn-SBM-Bar	0	560	79.9 ^a	76.3	81.2	3,379	3,322
Corn-SBM-Bar	1000	0	79.5 ^{ab}	76.2	80.6	3,367	3,308
Corn-SBM-Bar ²	1000	560	79.7 ^a	75.2	81.2	3,397	3,339
SD			0.87	0.91	0.87	36.90	38.44
DT			0.001	0.012	0.655	<.0001	<.0001
Phy			0.289	0.052	0.075	0.008	0.011
X-G			0.378	0.006	0.925	0.387	0.419
DT*Phy			0.830	0.001	0.137	0.006	0.009
DT*X-G			0.054	0.066	0.004	0.063	0.054
Phy*X-G			0.892	0.195	0.600	0.061	0.090
DT*Phy*X-G			0.039	0.155	0.107	0.962	0.895

¹Phy = phytase, X-G = xylanase-glucanase, DM = dry matter, N = nitrogen, GE = gross energy, AME = apparent metabolizable energy, AMEn = apparent metabolizable energy corrected for N, DT = diet type

²n=7, except for corn-SBM-Bar diet with 1,000 FTU and 560 TXU supplementation where n=6

Table 2.5. Simple and main effect of exogenous enzyme supplementation on energy metabolizability and AME values of barley in 21-d-old broiler chickens¹.

Ingredient	Phy	X-G	EM, %	EMn, %	AME, kcal/kg	AMEn, kcal/kg
Barley	0		80.1	79.6	3,249	3,224
Barley	1000		80.2	79.7	3,257	3,234
Barley		0	79.5	78.6	3,229	3,187
Barley		560	80.8	80.7	3,277	3,272
Barley	0	0	78.8 ^b	77.8	3,202	3,156
Barley	0	560	81.3 ^a	81.3	3,296	3,292
Barley	1000	0	80.1 ^{ab}	79.3	3,255	3,218
Barley ²	1000	560	80.3 ^{ab}	80.1	3,259	3,251
SD			1.42	1.75	60.07	70.58
Phy			0.817	0.821	0.717	0.705
X-G			0.027	0.004	0.051	0.005
Phy*X-G			0.047	0.067	0.072	0.069

¹Bar = barley, Phy = phytase, X-G = xylanase-glucanase, EM= energy metabolizability of barley, EMn = energy metabolizability of barley corrected for N, AME = apparent metabolizable energy, AMEn = apparent metabolizable energy corrected for N

²n=7, except for corn-SBM-Bar diet with 1,000 FTU and 560 TXU supplementation where n=6

Chapter 3 – Effect of supplementation of phytase and xylanase-glucanase individually or in combination on apparent metabolizable energy value of barley in growing pigs

Abstract

A total of 24 crossbred pigs were split into two phases (12 pigs/phase) and assigned to 1 of 4 corn-SBM or corn-SBM-barley-based dietary treatments with or without phytase (Natuphos[®] E, 1,000 FTU) and xylanase-glucanase (X-G, Natugrain[®] TS, 560 TXU and 250 TGU) in a completely randomized block design. Pigs were adapted to metabolism crates and dietary treatments for 7 d. Urine and feces were quantitatively collected for 5 d post adaption period. Data were analyzed using proc GLM procedure of SAS with phase as the blocking factor. The effect of phase was not significant. Individual means were compared using orthogonal contrasts selected before start of the experiment. Contrasts of dry matter, nitrogen, and energy digestibility and retention were not different. Digestible energy value was higher ($P = 0.002$) in the control corn-SBM-based diet in comparison to the corn-SBM-barley-based diet (3,641 vs. 3,562 kcal/kg). Digestible energy of the diet supplemented with X-G alone (3,578 kcal/kg) and X-G and phytase in combination (3,617 kcal/kg) were different ($P = 0.016$). AME and AMEn of Ref-Bar-Phy-X-G (3,572 and 3,441 kcal/kg) was higher ($P < 0.05$) compared to Ref-Bar-X-G diet (3,524 and 3,394 kcal/kg). Xylanase-glucanase supplementation alone did not increase AME and AMEn of barley. A combination of both X-G and phytase supplementation increased AME (1.5%) and AMEn (1.5%) of barley, but results were not significant. Results showed that a combination of X-G and phytase increases AME and AMEn of barley, but not significantly.

Key words: Apparent metabolizable energy, barley, energy retention, nitrogen retention, pig, swine

3.1 Introduction

Exogenous enzymes have been incorporated in the diets of swine to increase growth performance and nutrient and energy digestibility. Phytase is an enzyme supplemented to release phytate-bound phosphorus in the feed. Phosphorus (P) is an essential mineral in swine diets, aiding in bone formation and other physiological functions in the body (González-Vega and Stein, 2014). In cereal grains, about 60-75% of the total P is bound to phytate (phytate-P), which is poorly digested by the pig and other non-ruminant animals (NRC, 2012). Phytate is the main storage form of P in cereal grains

and pigs are unable to digest phytate because they lack sufficient level of endogenous phytase needed to break it down completely (Humer et al., 2015). The inability of pigs to digest phytate leads to large amounts of P being excreted into the environment, which poses a major environmental concern. Microbial phytase has potential environmental benefits where P excretion can be reduced up to 40% (Omogbenigun et al., 2003). When phytase is supplemented in the diet, P excretion decreases significantly, as Sands et al. (2001) reported a 28% decreased in fecal P excretion when 600 FTU/kg phytase was supplemented to the diet. Studies have also confirmed that microbial phytase supplementation can result in major bioavailability of phytate-P (Jongbloed et al., 1992; Cromwell et al., 1993). In addition to improving the bioavailability of phytate-P, phytase utilization also improves absorption and retention of other essential minerals such as Ca, Mg, Cu, Fe, and Zn (Adeola et al., 1995).

Xylanase is an exogenous enzyme that has been used in swine diets to counteract the negative effects of non-starch polysaccharides (NSP) in cereal grains. The presence of soluble NSP such as arabinoxylans and β -glucans lead to a reduction in nutrient digestibility and an increase in digesta viscosity (Woyengo et al., 2008). With the supplementation of xylanase, NSP are degraded while nutrient digestibility greatly improves (Moehn et al., 2007).

The objective of this study was to investigate the contribution of exogenous enzymes, phytase and carbohydrase (xylanase-glucanase), individually or in combination on apparent metabolizable energy (AME) value of barley in growing pigs.

3.2 Materials and Methods

3.2.1 Animal Care

The experimental protocol used in this study was approved by University of Kentucky's Institutional Animal Care and Use Committee.

3.2.2 Animals and Experimental Design

A total of 24 crossbred (Duroc × Yorkshire × Landrace) growing barrows with an average initial BW of 28.57 ± 1.98 kg were obtained from C. Oran Little Swine Research facility and were transported to University of Kentucky Garrigus building for the duration of the experiment. Following the procedure from Jang et al. (2014), the experiment was conducted in two phases with each phase having 12 pigs (total of 24 pigs for the 2 phases). Before the experiment began, pigs were weighed individually after a few days of acclimatization to the new environment. In each phase, 12 pigs were allotted to an individual, adjustable, stainless steel metabolism crate (1.27m × 0.45m) in a completely randomized block design and assigned to 1 of 4 treatments. The temperature was maintained at 24 °C for the duration of the study. Pigs had a 7-d adaption period to the experimental diet and metabolism crate, followed by a 5-d total collection (TC) of feces and urine. After the first collection period, Phase 1 pigs were removed and Phase 2 pigs were housed in the crates. The process of adaption and sample collection remained unchanged for Phase 2. All pigs were euthanized at the end of the study.

3.2.3 Enzymes and Experimental Diets

The two enzymes used in this study were supplied by BASF Animal Nutrition (Florham Park, New Jersey, USA). The phytase used was Natuphos® E at 1,000 FTU/kg of diet. One phytase unit is defined as the quantity of enzyme which sets free 1 μmol of

inorganic P under conditions of the assay. The xylanase used was Natugrain[®] TS, a xylanase-glucanase combination and was added to the diet at a rate of 560 TXU/kg of diet. One thermostable endo-xylanase unit (TXU) is defined as the amount of enzyme which sets free 5 µmol reducing sugars, measured as xylose equivalents per minute under the reactions. In the xylanase-glucanase enzyme combination, 250 TGU/kg was added to the diet. Additionally, one thermostable beta-glucanase unit (TGU) is defined as the amount of enzyme which sets free 1 µmol reducing sugars, measured as glucose equivalents per minute under the reaction conditions.

The ingredient composition of the experimental diets and their nutrient composition are shown in Table 3.1. There were a total of four diets – an unsupplemented corn-SBM-based diet and three corn-SBM-barley-based diets that were either unsupplemented, supplemented with xylanase-glucanase alone, or supplemented with a combination of phytase and xylanase-glucanase. The barley-based diets contained 30% barley, which replaced the energy yielding components of the corn-SBM-based diet in a way that their ratios were similar across all experimental diets. This was necessary to determine the AME and AMEn of barley using the difference method. The enzymes were added to the diet at the expense of corn, as a premix at the rate of 10g/kg of diet. Titanium dioxide (TiO₂) was added to all diets at the rate of 5 g/kg of diet (for AME determination using index method).

3.2.4 Adaptation and Collection Procedures

The study consisted of a 7-d adaptation period followed by a 5-d collection period. Pigs were fed experimental diets twice a day (each meal half of daily allotment), at 0700 and 1700 h, for 12 d. Experimental diets were fed in gruel form (feed:water, 1:1

wt/vol). Initially, pigs were fed at 4% of body weight (BW), which was then reduced to 3.5% after the first three meals to ensure that all pigs were able to finish the meal in a timely manner. Rejected feed was dried in a forced-air oven at 55 °C for 12 h, weighed, and discounted from the amount initially offered. Following each feeding, water was provided in the metabolism crate feeder to provide ad libitum access in between meals.

To mark the beginning and end of the TC period, 4 grams of an indigestible marker, indigo carmine, was added to the morning meal on d 7 and 12. All feces were collected daily, from screens placed under the metabolism crate, and subsequently weighed and stored in a freezer at -20 °C until the end of the collection periods. Total urine was collected daily from a urine bucket placed under the crate. The buckets were emptied every morning following fecal collection and 10 mL of 37% formaldehyde solution was added to arrest any microbial activity in the bucket. Every morning, the weight and volume of daily urine produced was determined and recorded. A 30 % urine sub-sample was kept and stored at -20 °C until processed.

3.2.5 Sample Preparation

Feces were dried in a forced-air oven at 55 °C for 5 d and were subsequently weighed and ground through a 2-mm screen (Wiley Mill Grinder 3, Swedesboro, New Jersey, USA). Following the grinding process, feces were stored in a plastic bag until laboratory analysis was performed. Urine was thawed for 12 hours and filtered through glass wool three times to remove any particulate matter in the urine. After filtration, a known volume was measured, weighed, and dried in a forced-air oven at 55 °C for 2 d. The weight of the dried urine sample was determined after which it was ground using a mortar and pestle.

3.2.6 Laboratory Analysis

All diets, barley, fecal, and urine samples were analyzed for gross energy (GE), nitrogen (N), and dry matter (DM). Samples were analyzed in duplicate and analysis was repeated when coefficient of variation between duplicate samples was greater than 5%. The GE was determined using bomb calorimetry (Parr 6200 isoperibol bomb calorimeter; Parr Instrument Company, Moline, Illinois) with benzoic acid as a calibration standard. Nitrogen in urine was determined using the Dumas combustion method determined with an Elementar vario MAX CN analyzer (Elementar, Mt. Laurel, New Jersey, USA) with L-glutamic acid as a calibration standard. To determine DM, samples were placed in a drying oven (Precision Scientific Co, Chicago, Illinois, USA) at 110 °C for 24 h. University of Missouri Experiment Station Chemical Laboratory (Columbia, MO) analyzed nitrogen content in feces (model FP2000, Leco, Corp., St. Joseph, MI) with EDTA as the calibration standard. Neutral detergent fiber was analyzed in Ankom Fiber Analyzer using the Ankom's proprietary 200 Filter Bag Technique (Ankom Technology, Macedon, NY) at the University of Missouri Experiment Station. Crude fat was determined by the ether extract method.

3.2.7 Calculations and Statistical Analysis

The coefficient of energy retention, as a percentage, was calculated as: $En_{ret} = GE_i - (GE_f + GE_u)$, where GE_i is gross energy intake, GE_f is gross energy of feces, and GE_u is gross energy of the urine. Apparent ME of the diet (AME_d) was calculated using this equation: $AME_d = GE_d \times EM_d$ where GE_d is the gross energy of the diet and EM_d is the percent (%) energy metabolizability, respectively. Apparent ME of the test ingredient (AME_{ti}) was calculated as follows: $AME_{ti} = EM_{ti} \times GE_{ti}$, where GE_{ti} and EM_{ti} are the

gross energy and percent energy metabolizability of the test ingredient, respectively. The coefficient of energy metabolizability of the test ingredient (EM_{ti}) was calculated using this equation: $EM_{ti} = [EM_{td} - (EM_{rd} \times 1 - FC_{ti/td})] / FC_{ti/td}$, where EM_{td} is the coefficient of energy metabolizability of the test diet, EM_{rd} is the coefficient of energy metabolizability of the control diet, and $FC_{ti/td}$ is the fractional contribution of the test ingredient to the test diet.

Data were analyzed according to a completely randomized block experimental design using the proc GLM procedure of SAS (SAS Institute, Cary, North Carolina, USA). The effect of phase was determined by including phase in the class variable. Any data that fell outside of mean ± 3 SD was removed from the data (outliers). The experimental data were expressed as means with standard deviations. Individual means were also compared using orthogonal contrasts. Specific contrasts of interest were chosen prior to data analysis. Level of significance was set at $P < 0.05$ and a P value between 0.05 and 0.1 was considered to show a trend.

3.3 Results

3.3.1 Analyzed Composition of Experimental Diets and Barley

The analyzed composition of DM, crude protein (CP, $N \times 6.25$), AME, Ca, P, and enzymes in the experimental diets are shown in Table 3.1. Analyzed crude protein content of the corn-SBM-based diet was higher than the three other diets (153 vs. 137, 140, 139 g/kg). It was expected for CP to be lower in the barley-based diets because the diets were formulated with less soybean meal than the corn-SBM diet. Analyzed Ca levels in the diets were between 6.5 and 7.8 g/kg. Analyzed P levels were between 5.3 and 5.9 g/kg.

The composition of barley is shown in Table 3.2. Analyzed barley DM was 878.1 g/kg and GE was 3,819 kcal/kg. Barley contained 100.1 g/kg CP and 163 g/kg crude fiber.

3.3.2 Digestibility, Retention, and Energy of Experimental Diets

Tables 3.3 and 3.4 show the effect of enzyme supplementation on total tract digestibility, retention, DE, AME, and AMEn values of corn-SBM or corn-SBM-barley-based diets fed to growing pigs. No significant effects were observed for DM, N, or energy digestibility and retention. Digestible energy (DE) was higher ($P = 0.002$) in the corn-SBM-based diet compared to the corn-SBM-barley-based diet without enzyme supplementation (3,641 vs. 3,562 kcal/kg). Orthogonal contrasts showed a difference ($P = 0.016$) in the DE between the corn-SBM-barley-based diets with xylanase-glucanase alone compared to phytase and xylanase-glucanase in combination (1.1% increase). Apparent ME of the corn-SBM-based diet decreased ($P = 0.017$) by 2.2% when barley replaced 30% of the energy yielding portion of the diet without enzyme supplementation. Orthogonal contrasts showed that a combination of xylanase-glucanase and phytase resulted in higher ($P = 0.037$) AME and AMEn ($P = 0.030$) compared to xylanase-glucanase supplementation alone.

3.3.3 Energy metabolizability, Apparent ME, and MEn of Barley

Enzyme supplementation did not influence energy metabolizability of barley (EMti), AME, or AMEn of barley (Table 3.5). Although not significant, the barley-based diet with phytase and xylanase-glucanase in combination had the highest EMti, AME, and AMEn compared to the unsupplemented and xylanase alone barley-based diets.

3.4 Discussion

The composition of the barley used in this study is as shown in Table 3.2. The barley used in the current study had lower CP, ash, acid detergent fiber, and neutral detergent fiber compared to the swine NRC (NRC, 2012) values. Bhatta (1986) reported similar CP values (10.0% vs. 10.5%), but with higher crude fiber and ash values compared to the current study (4.0 and 2.2% vs. 1.6 and 1.5%).

Park et al. (2016) conducted a study with growing pigs and reported DE and AME concentrations in barley were not affected by an enzyme cocktail containing xylanase, mannanase, and protease. This current study showed that enzyme supplementation of corn-SBM-barley-based diets did not have a significant effect on the DM, N, and energy digestibility and retention. The results were comparable across treatments, suggesting that the level of exogenous enzymes may not be enough to trigger an effect on digestibility and retention of energy. Secondly, since the diets used in the current study contained adequate level of energy, the efficiency with which the pigs would utilize any extra energy as a result of the exogenous enzyme supplementation would be minimal. However, DE, AME (48 kcal/kg), and AMEn (47 kcal/kg) were significantly higher in the diet with xylanase-glucanase and phytase in combination in comparison to xylanase-glucanase alone. Furthermore, this result shows that despite the diet containing sufficient level of energy, exogenous enzyme supplementation enhanced energy utilization. These findings suggest that there may not have been enough substrate for xylanase-glucanase to act on, which can affect the magnitude of enzyme response. When xylanase and phytase are supplemented to a wheat-based diet, studies have reported an increase in energy, digestibility, amino acids, and P digestibility (Nortey et al., 2007). Oryschak et al. (2002)

reported enzyme synergy between phytase and xylanase because xylanase is able to disrupt the cell wall matrix and hydrolyze carbohydrates, while allowing phytase to access phytate binding to proteins and starch.

Apparent ME, MEn, and EMti of barley increased when xylanase-glucanase and phytase were supplemented in combination compared to xylanase-glucanase alone. Energy metabolizability of barley increased nearly 4% and apparent ME and MEn increased nearly 130 kcal/kg when the barley-based diets contained both enzymes in combination, rather than xylanase-glucanase alone. The improvement in values of AME, and AMEn can be attributed to phytase supplemented in the diet in combination, breaking down phytate-bound content in barley and allowing for greater energy utilization than xylanase-glucanase can alone.

In broiler chickens, Fuente et al. (1998) reported a 2% average increase in AMEn values due to exogenous enzyme supplementation. The study evaluated barley storage time and enzyme addition to barley-based diets and a linear relationship was established. It was concluded that an increase in barley storage time from 0 to 32 weeks also increased dietary AMEn values. Kocher et al. (1997) reported an increase in AME values and reduced variability of 11 barley varieties that were unsupplemented or supplemented with beta-glucanase. On the average, AME of the 11 barley diets that were supplemented with exogenous enzyme were nearly 600 kcal/kg higher (3,299 vs. 2,712 kcal/kg) than unsupplemented diets.

Poultry and swine are both non-ruminant animals. However, unlike chickens, pigs tend to be less responsive to enzyme supplementation (Graham and Balnave, 1995), as seen in the results mentioned previously. Several factors can be considered for pigs'

inability to improve performance and AME with the supplementation of exogenous NSP-degrading enzymes. The primary factor being the presence of sufficient substrate for the enzyme to act on. Glucanase acts on beta-glucans, which are more prevalent in barley. Xylanase acts on arabinoxylans, which are dominant in wheat. Because the xylanase-glucanase combination contained more xylanase activity than glucanase, it is possible it was less effective for a barley-based diet. To improve AME value of barley, it can be suggested to increase the level of supplemented enzymes to the diet to better break down beta-glucans and arabinoxylans present in the feed.

3.5 Conclusion

It can be concluded that exogenous enzyme supplementation did not significantly affect performance or AME value of barley. The addition of xylanase alone and xylanase and phytase in combination confirm other studies reported in the literature that pigs are less responsive to enzyme supplementation, unlike broiler studies. Several factors can be considered to evaluate this study in a different approach such as the quality of barley in the diet, the length of the study, and the relative amount of enzyme incorporated in the diet. Further research is needed to evaluate how to increase AME value of barley with the incorporation of exogenous enzymes in the dietary treatment.

Table 3.1. Composition of the experimental diets.

<i>Ingredients, g/kg</i>	Corn-SBM	Corn-SBM-Bar	Corn-SBM-Bar-X-G	Corn-SBM-Bar-Phy-X-G
Corn	751.8	509.3	509.3	509.3
Soybean meal, 48%	165	113.2	113.2	113.2
Barley	0	300	300	300
L-Lysine HCl	9.0	9.0	9.0	9.0
DL-Methionine	1.8	1.8	1.8	1.8
L-Threonine	0.7	0.7	0.7	0.7
Soy oil	18.0	12.4	12.4	12.4
Di-calcium phosphate	11.5	11.5	11.5	11.5
Salt	6.4	6.4	6.4	6.4
Limestone	8.3	8.3	8.3	8.3
Titanium dioxide	5.0	5.0	5.0	5.0
Vitamin-Min Premix ¹	1.0	1.0	1.0	1.0
Trace mineral premix ²	1.50	1.50	1.50	1.50
Corn	20	20	10	0
Phytase premix ³	0	0	0	10
Xylanase-glucanase premix ⁴	0	0	10	10
Total	1,000	1,000	1,000	1,000
<i>Analyzed Nutrients & Energy Composition</i>				
DM, g/kg	954.2	956.1	954.2	955.4
CP, g/kg	153.1	136.9	140.0	138.8
ME, kcal/kg ⁵	3,366	3,236	3,236	3,236
Ca, g/kg	6.68	7.78	7.38	6.52
P, g/kg	5.93	5.79	5.70	5.27
Non-phytate P, g/kg ⁵	3.13	3.22	3.22	3.22
Ca:P ⁵	1.2	1.2	1.2	1.2
Phytase, FTU/kg	<60	140	120	800
Glucanase, TGU/kg	<100	<100	340	320
Xylanase, TXU/kg	<100	<100	570	670

¹ Vitamin premix supplied the following per kilogram of diet: 11,000 IU of vitamin A; 1,100 IU of vitamin D; 77 IU of vitamin E; 2.2 mg of vitamin K; 1.65 mg of thiamin; 8.25 mg of riboflavin; 30.25 mg of niacin; 27.50 mg of pantothenic acid; 4.95 mg of vitamin B-6; 0.36 mg of biotin; 4.95 mg of folic acid; and 0.03 of vitamin B-12

² Trace mineral premix supplied the following per kilogram of diet: 50 mg as manganese sulfate monohydrate; 100 mg of Fe as ferrous sulfate monohydrate; 125 mg of Zn as zinc sulfate monohydrate; 20 mg of Cu as copper sulfate; 0.35 mg of I as calcium iodate; and 0.30 mg of Se as sodium selenite.

³ Phytase (Natuphos® E, BASF Animal Nutrition) premix was formulated to supply 1,000 FTU/kg of diet. Added at the expense of ground corn

⁴ Xylanase-glucanase (Natugrain® TS, BASF Animal Nutrition) premix was formulated to supply 560 TXU/kg and 250 TGU/kg. Added at the expense of ground corn

⁵ Calculated values

Table 3.2. Analyzed proximal composition and gross energy value of barley (g/kg, on as-is basis).

Item	Barley
Dry matter	878.1
Crude protein	100.1
Gross energy, kcal/kg	3,819
Crude fat	8.9
Crude fiber	16.3
Ash	15.3
Acid detergent fiber	23.3
Neutral detergent fiber	96.5

Table 3.3. Effect of exogenous enzyme supplementation on digestibility and retention of corn-SBM or corn-SBM-barley based diets¹.

Diet	Phy	X-G	Digestibility			Retention			
			DM, %	N, %	En, %	DM, %	N, %	En, %	En corr. for N, %
1. Corn-SBM	No	No	90.09	86.73	88.72	87.93	76.61	87.39	83.81
2. Corn-SBM-Bar	No	No	90.75	85.62	89.36	88.65	73.53	87.96	84.76
3. Corn-SBM-Bar-X-G	No	Yes	90.16	85.09	88.71	87.95	74.56	87.39	84.15
4. Corn-SBM-Bar-Phy-X-G	Yes	Yes	91.43	86.46	89.47	89.09	75.21	88.34	85.12
SD			0.97	1.51	0.89	1.21	3.46	1.25	1.15
						----- Probability -----			
Contrasts									
Diets 1 vs. 2			0.278	0.242	0.263	0.341	0.161	0.460	0.195
Diets 2 vs. 3			0.327	0.569	0.255	0.355	0.631	0.460	0.397
Diets 3 vs. 4			0.239	0.349	0.835	0.531	0.411	0.603	0.590

¹Bar = barley, Phy = phytase, X-G = xylanase-glucanase, DM = dry matter, N = nitrogen, En = energy

²n=5 (Corn-SBM and Corn-SBM -Bar-X-G); n = 6 (Corn-SBM-Bar and Corn-SBM-Bar-Phy-X-G)

Table 3.4. Effect of exogenous enzyme supplementation on DE and AME of corn-SBM or corn-SBM-barley-based diets¹.

Diet	Phy	X-G	DE, kcal/kg	AME, kcal/kg	AMEn, kcal/kg
1. Corn-SBM	No	No	3641	3586	3440
2. Corn-SBM-Bar	No	No	3562	3506	3378
3. Corn-SBM-Bar-X-G	No	Yes	3578	3524	3394
4. Corn-SBM-Bar-Phy-X-G	Yes	Yes	3617	3572	3441
SD			36.12	50.23	46.25
----- Probability -----					
Contrasts					
Diets 1 vs. 2			0.002	0.017	0.043
Diets 2 vs. 3			0.469	0.553	0.587
Diets 3 vs. 4			0.016	0.037	0.030

¹Bar = barley, Phy = phytase, X-G = xylanase-glucanase, DE = digestible energy, AME = apparent metabolizable energy, AMEn = apparent metabolizable energy corrected for N

²n=5 (Corn-SBM and Corn-SBM-Bar-X-G); n = 6 (Corn-SBM-Bar and Corn-SBM-Bar-Phy-X-G)

Table 3.5. Effect of exogenous enzyme supplementation individually and in combination on the AME values of barley in growing pigs¹.

Diet	Phy	X-G	EMti, %	AME, kcal/kg	EMnti, %	AMEn, kcal/kg
2. Corn-SBM-Bar	No	No	88.68	3,387	86.47	3,302
3. Corn-SBM-Bar-X-G	No	Yes	86.64	3,309	84.34	3,221
4. Corn-SBM-Bar-Phy-X-G	Yes	Yes	90.04	3,439	87.79	3,353
SD			4.94	188.48	4.55	173.75
----- Probability -----						
Contrasts						
Diets 2 vs. 3			0.509	0.509	0.456	0.456
Diets 3 vs. 4			0.278	0.278	0.234	0.234

¹Bar = barley, Phy = phytase, X-G = xylanase-glucanase, AME = apparent metabolizable energy, AMEn = apparent metabolizable energy corrected for N, EMti = coefficient of energy metabolizability test ingredient, n = number of replicates

²n=5, except Corn-SBM-Bar where n=6

CHAPTER 4 – Summary and Conclusions

4.1 Experiment 1

This experiment showed that broilers fed barley-based diets exhibit a reduction in growth performance, even with enzyme supplementation, in comparison to a corn-SBM-based diet. Although performance was depressed with barley-based diets, DM and N retention were higher, on average, for these diets. Additionally, exogenous enzymes, phytase and xylanase-glucanase, had a tendency to increase AME value of barley when supplemented in combination. Lastly, when xylanase-glucanase was supplemented alone, AME and AMEn value of barley improved significantly compared to an unsupplemented diet. With a higher inclusion of exogenous enzymes, it can be suggested AME value of barley may be further improved.

4.2 Experiment 2

This experiment showed that differences were not seen across diets in DM, N, or energy digestibility and retention. Differences were seen in digestible energy between the unsupplemented corn-SBM-based and corn-SBM-barley-based diets. Digestible energy, AME, and AMEn of the barley-based diet with both phytase and xylanase-glucanase was significantly higher than xylanase-glucanase alone. Finally, the barley-based diet with phytase and xylanase-glucanase had a tendency to improve energy metabolizability, AME, and AMEn value of barley.

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VITA

Brian Bryson was born and raised in Cincinnati, Ohio to Leslie Bryson and Gary Brown. Brian grew up in Cincinnati and graduated from St. Xavier High School in 2012. He then moved to Morehead, KY where he attended Morehead State University with a Black Achiever's scholarship. At Morehead State, Brian was a club soccer player, supervisor of Intramural Sports, and Vice President of the Xi Alpha chapter of Alpha Phi Alpha Fraternity, Inc. Brian graduated from Morehead State with a Bachelor's in Animal Science in May 2016.

Brian then moved to Lexington, Kentucky in August 2016. He was awarded the Lyman T. Johnson Fellowship and began a Master's program in Poultry/Swine Nutrition under the supervision of Dr. Tayo Adedokun. In June 2018, Brian was awarded 'Outstanding Master's Student in the Department of Animal and Food Sciences'.