EVALUATION OF CORN EXPRESSED GLUCANASE AND HIGH AND LOW SPECIFIC ACTIVITY CORN EXPRESSED PHYTASE AT DIFFERENT INCLUSION RATES ON GROWTH PERFORMANCE OF BROILERS FED CORN-SOYBEAN MEAL BASED DIETS

A Thesis

by

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ABSTRACT

The objective of this research was to evaluate the effects of a corn expressed phytase and glucanase enzyme on performance, bone ash response, apparent metabolizable energy and apparent ileal digestibility at varying inclusion rates. Experiment one consisted of a standard energy, Ca, and available Phosphorus (aP) level diet positive control and a -0.15% Ca and aP reduced negative control. Dietary treatments consisted of either a high (PY1203) or a low (PY203) specific activity corn expressed phytase at various inclusion rates (0.175 kg/t, 0.5 kg/t, 1.0 kg/t, 2.0 kg/t, and 3.0 kg/t). Results from this study showed that FCR was maintained to the level of the PC at almost all inclusion rates. Additionally at 0.7 kg/t of PY1203, FCR was improved compared to all other treatments. Additionally, bone mineralization was maintained to that of the PC at almost all inclusion rates. This data indicates that the inclusion of high and low specific activity at varying inclusion rates can improve feed conversion and tibia bone mineralization. It can be concluded that birds were able to utilize the phytase enzyme effectively and gain nutritional benefits from it.

Experiment 2 consisted of corn-soybean diets that were supplemented with varying inclusion rates of AC1 (corn expressed glucanase) (0.175 kg/t, 0.35 kg/t, 0.5 kg/t, 0.75 kg/t, 1.00 kg/t, 1.5kg/t, or 2.00 kg/t.). The positive control was comprised of a standard ME diet while the negative control was reduced by 100 kcal/kg from the PC diet. Throughout the 42-day study, all dietary treatments performed similarly when evaluating growth performance (FCR, FC, and BW). When evaluating ileal digestibility, birds that were supplemented with 0.35 kg/t and 0.75 kg/t of AC1, outperformed all other dietary treatments. Additionally, all AC1 supplemented diets-maintained performance to that of the PC and outperformed the NC

when AME was evaluated. These findings may suggest that AC1 can improve feed efficiency with a larger energy reduction in diets.

Experiment 3 consisted of a nutrient adequate control, a nutrient reduced negative control and diets supplemented with corn expressed phytase or glucanase. At all inclusions of AC1 and PY1203, FCR was maintained to that of the PC. No differences were seen in body weights however when apparent ileal digestibility was evaluated, birds that were fed a high specific activity corn expressed phytase had an improved AID. At almost all levels of AC1 and PY1203, AME was maintained to that of the PC. This data showed that corn expressed phytase and glucanase maintained broiler performance and compensated for a reduction in avP, Ca, and ME at most levels.

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NOMENCLATURE

AC1	Grainzyme Carbohydrase
AID	Apparent Ileal Digestibility
AME	Apparent Metabolizable Energy
AMEn	Apparent Metabolizable Energy corrected for Nitrogen content
ANOVA	Analysis of Variance
aP	Available Phosphorus
avP	Available Phosphorus
BW	Body Weight
BWG	Body Weight Gain
C	Celsius
Ca	Calcium
CEP	Corn Expressed Phytase
Cu	Copper
d	Day
FCR	Feed Conversion Ratio
FI	Feed Intake
FTU	Phytase Units
g	Gram
GE	Gross Energy Content
GLM	Generalized Linear Model
h	Hour

IDE	Ileal Digestible Energy
Kg	Kilogram
L	Liter
lb	Pound
lb/t	Pound per ton
kg/t	Kilograms per ton
LSD	Least Significant Difference
ME	Metabolizable Energy
Mg	Milligram
mol	Mole
MORT	Mortality
NC	Negative Control
nPP	Nonphytate Phosphorus
NSPs	Non-starch Polysaccharides
P	Phosphorus
PC	Positive Control
pH	Potential of Hydrogen
U	Units
Zn	Zinc

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

Diets in the poultry industry have often been formulated with feed ingredients that contain non-starch polysaccharides (NSPs) and phosphate. NSPs increase intestinal viscosity which can reduce nutrient absorption and negatively impact growth performance as well as nutrient and mineral utilization (Mathlouthi et al., 2002). They are thought to cause an antinutritive, which adversely effects the digestion of other nutrients by imbibing water and causing feed to form a bolus that may reduce exposure to digestive enzymes (Choct et al., 1996; Leeson and Summer, 2001). Broilers do not have the ability to produce enzymes that aid in NSP digestion; therefore, diets must be supplemented with exogenous enzymes such as glucanase. In addition to glucanase supplementation, it is common that diets are supplemented with phytase. Phytase is the most commonly supplemented exogenous enzyme in monogastric formulations and is responsible for the removal of phosphates from phytic acids. This allows for phosphorus to be more available for growth promotion and reduces phosphorus pollution in the diet (Selle and Ravindran, 2007).

Previous literature has highlighted the benefits to using both of these enzymes on growth performance, digestibility and metabolizable energy. Nelson et al. (1971) were some of the first to discover the impacts of phytase when using an *Aspergillus ficuum* based phytase, they saw improvements in body weights and FCR at inclusions ranging from 950 to 7,600 FTU/kg. Additionally, it was shown that phytase can improve calcium and phosphorus digestibility. In a study conducted by Ravindran et al. (2008), it was shown that phytase improved Ca digestibility by 27% when feeding an *Escherichia coli* based phytase at 500 FTU/kg. Like phytase, glucanase has also been shown to improve digestibility and broiler

performance as well. This can be attribute to the enzymes ability to degrade cell wall and reduce intestinal viscosity. Edney et al. (2007) showed that the supplementation of glucanase, led to improvements in broiler performance as well as AME. Additionally, Rutherfur et al. (2007) showed that in a corn-soybean diet supplemented with an Escherichia coli derived glucnase, AME was improved.

Although the use of these enzymes has shown to improve performance, digestibility, and energy utilization, the way in which they are produced can be seen as unsafe. The production of glucanase and phytase is typically done using a microbial host (either a bacterium or a fungus). This has a led to the belief that the final product can be contaminated by host produced molecules being co-purified (Pariza, 2001). A new and safe method to producing enzymes has been engineered using transgenic corn. This process can create high concentrations of recombinant enzymes expressed in corn grain that can be fed at low inclusion rates Pen et al., 1993; Nyannor and Adeola, 2008; Nyannor et al., 2009, Gao et al., 2012; Denbow et al., 1998). This technology has led to the production of AC1, a glucanase enzyme similar to *Thermotoga maritima* that has showing to have improved thermal stability. AC1 has been shown to reduce intestinal viscosity when added to a high NSP (Ayers et al., 2018). Like AC1, a corn expressed phytase enzyme called Phy02 was also engineered. It is an *Escherichia coli* based phytase that has shown the ability to improve average daily gain, feed efficiency, and P digestibility in pigs (Lee et al., 2017; Knapp et al., 2018, Broomhead et al., 2018; Blavi et al., 2019).

The use of these corn expressed enzymes can be seen as a safer and cheaper alternative to microbial methods, due to the use of corn in animal and human diets. In the future these

enzymes can be used in poultry and swine as a cheaper alternative to exogenous enzymes that are being used now. In the future this technology can also be used to produce other enzymes such as amylase and xylanases.

CHAPTER II

LITERATURE REVIEW

Exogenous enzymes

Exogenous enzymes which include carbohydrase's (glucanase) and phytase, are used throughout the word as additives in non-ruminant diets. Carbohydrase enzymes are non-starch polysaccharide (NSP) enzymes that are used to improve digestibility by reducing intestinal viscosity caused by viscous grains such as corn, wheat, sorghum, and barley (Campbell and Bedford, 1992). Supplementation of NSP-degrading enzymes in diet formulations is common in the US poultry industry. An enzyme is selected based on the target substrate and overall performance purposes. Enzymes are used to either increase the value of feed ingredients in the diet or to reduce variation in nutrient quality (Bedford, 2000). The addition of these enzymes can diminish or limit the constraints of these grains and improve digestion (Bedford, 2000).

Phytase is used to target the substrate phytate. This substrate is the plant storage form of phosphate. In this form it is considered to have antinutritive effects on animals (Bedford, 2000). It is a poor form of phosphorous for non-ruminants, as they cannot efficiently utilize this compound (Maenz et al., 1997). If diets were not supplemented with phosphates, a phosphorous deficiency would result. Previously, non-ruminant diets were formulated with excess amounts of phosphorous. This excess was then excreted, causing phosphorus pollution in the environment. The need to reduce phosphorous pollution throughout Europe and the United States, led to the introduction of exogenous phytases. The use of this enzyme has allowed for non-ruminants to utilize plant phytate phosphorus. Additionally, this has led to the

reduction of phosphorus pollution (Bedford, 2000). Phytase has also been discovered to improve energy and amino acid utilization.

It is thought there are two modes of action in which NSP-degrading enzymes can improve digestibility (Cowieson, 2010). When used in non-viscous grain diets, enzymes degrade cell walls and allow for carbohydrates, proteins, and lipids to be more readily available (Annison, 1993; Bedford, 2018). When used in viscous grain diets, enzymes can increase digestibility by degrading soluble NSP which reduces the water holding capacity of NSPs and decreases digesta moisture (Almirall et al., 1995; Ward, 1996; Bedford, 2018). It is now thought that there is a third mode of action in NSP-degrading enzymes in which they can act as a probiotic. It is thought the addition of these enzymes produce fermentable substrates that can provide energy for intestinal microorganisms leading to increases energy recovery, volatile fatty acid production, and increased gizzard efficiency (González-Ortiz et al., 2016; Bedford, 2018).

β-Glucanase

Beta-glucanases which are known as endo-1, 3(4)-β-glucanase, are hydrolytic enzymes that catalyze the endo-hydrolysis of the backbone of cellulose, lichenin, and cereal β-D-glucans, and create smaller carbohydrate fragments that can be better digested by the bird (Bedford and Partridge, 2010) (Figure 1.). These enzymes are included in viscous grain-based diets because of their increased soluble NSP content. Additionally, these enzymes have been shown to reduce the anti-nutritive effects of beta-glucans and other water soluble NSP (Langhout et al., 1999). The viscous properties of soluble NSP can negatively impact FCR and growth rate (Mathlouthi et al., 2011). Previous literature has shown that these enzymes can

improve energy digestibility and performance in broilers when fed a corn-based diet. In a study by (Edney et al., 1989) it was shown that dietary supplementation of glucanase in corn-soybean based diets was effective in improving growth and feed conversion. Another study conducted by Rutherfur et al. (2007), showed that in corn-soybean diets supplemented with β-glucanase, apparent metabolizable energy (AME) was higher in corn-soy based diets as opposed to those that were not supplemented. Additionally, Munyaka et al. (2015) found that at an inclusion rate of 250 U kg−1 of β-glucanase, the ileal and cecal microbiota changed in broilers that were fed corn and wheat diets. Moharrery et al. (2015), reported that at an inclusion rate of 0.5 g kg−1 of β-glucanase, AME nitrogen corrected was improved. Overall, studies have shown that β-glucanase supplementation can mitigate the negative effects associated with NSPs such as β-glucan.

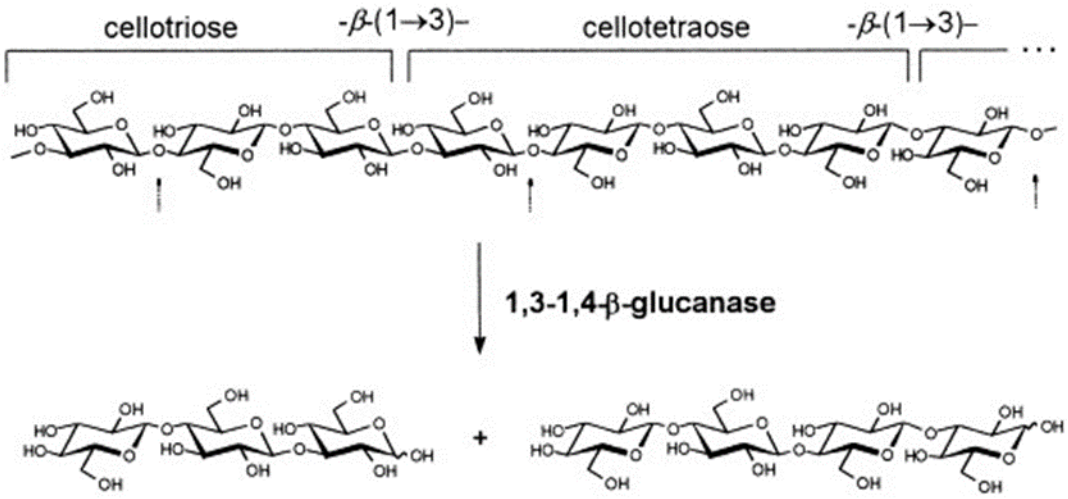

Figure 1 Structural features of β-glucans with enzymatic methods of cleavage for the molecule.Reprint from [Bacterial 1,3-1,4-β-glucanases: structure, function and protein engineering (Planas, 2000)].

It is thought that glucanase supplementation can increase nutrient digestibility by reducing digesta viscosity and modifying the microbial population in the digestive tract (Salih et al., 1991; Fuente et al., 1995; Hesselman and Åman, 1986; Perttilä et al., 2001; Choct et al., 1999; Józefiak et al., 2010). Viscous digetsa reduce the digestion and absorption of nutrients supplied by the diet (Almirall et al., 1995). Additionally, viscous digesta can stimulate the rapid growth of certain bacteria in the ileum, resulting in nutrient competition (Hübener et al., 2002).

A problem that has been associated with the early use of glucanase enzymes in poultry, is that in some cases it is not thermostable. Inborr and Bedford (1994) found that a feed and enzyme mixture at 85◦ C did not reduce enzyme activity compared to the same mixture at 75◦ C however, at 95◦ C they witnessed significant inactivation. This experiment used a β-glucanase feed enzyme product (Avizyme SX) at 3 levels of inclusion (0, 1, and 10 g/kg) to a barley- and wheat-based diet. In a similar study Esteve-Garcia et al. (1997) found that β-glucanase enzymes maintained over 80% activity when pelleting wheat and barley diets at 80◦ C. While incubating a *Trichoderma longibarachiatum* glucanase at 70, 80, and 100◦ C, Almiral land Esteve-garcia (1995), found that activity was decreased to 65,20, and 0% respectively. New advances have made it possible for glucanase enzymes to be thermostable at pelleting temperatures. Ayers et al. (2018) demonstrated a 90% recovery at a pelleting temperature of 90◦ C when using a corn expressed glucanase enzyme (AC1).

Glucanase enzymes are β-glucan-degrading enzymes and can be produced by a wide variety of sources (typically fungi or bacteria). These enzymes can be classified according to the type of β-glucosidic linkage that they cleave and the mechanism of substrate attack (Lisboa

de Marco and Felix, 2007). Substrates are hydrolyzed using one of two mechanisms (1) exo-β-glucanase hydrolyze the substrate by sequentially cleaving glucose residues from the nonreducing end, or (2) endo-β-glucanases cleave β-linkage at random sites along the polysaccharide chain, releasing smaller oligosaccharides. B-glucanase producing fungi such as *Trichoderma* are commonly used in the production of glucanase enzymes. These fungi release lytic enzymes that are responsible for the degradation of cell walls (Lima et al., 1997). Other organisms used to derive glucanase include *Aspergillus spp*. and *Bacillus spp.*

The use of β-glucanase in conjunction with other exogenous enzymes such as mannase, phytase, and xylanase has been attributed with performance benefits in the poultry industry. The mixture of these enzymes can improve nutrient availability and reduce the adverse effects of antinutritional factors seen in feed components (Munir and Maqsood, 2013). It is thought that the increase of digesta arabinoxylo-oligosaccharides in the broilers fed wheat-based diets (Morgan et al., 2017) can cause a prebiotic effect in broilers and result in arabinoxylo-oligosaccharides beneficially modulate digestive tract microbiota and the epithelial integrity through increasing microbial fermentation of these low molecular weight carbohydrates (De Maesschalck et al., 2015; Lee et al., 2017).

Glucanase impact on broiler performance

Previous literature has shown that the use of exogenous glucanase can improve broiler performance and can act as a prebiotic in chickens exposed to enteric disease (Karunaratne et al., 2021). Broiler performance is improved by the reduction in intestinal viscosity and degradation of cell walls (Choct et al., 2004; Bedford and Autio, 1996; Ravn et al., 2018). In a study conducted by Karunaratne et al. (2021), improvements in body weight gain were seen

when feeding graded levels of hulless barley (0, 30, and 60%) and a β-glucanase enzyme (Econase GT 200P) at 0, 0.01, and 0.1%). At 0.1% the lowest feed to gain ratio was seen (1.5) and body weights were improved when feeding 60% of hulless barley. In a similar study by (Mathlouthi et al., 2002), it was shown that broiler performance was improved when feeding corn or wheat and barley-based diets supplemented with xylanase and β-glucanase at 20 mg·kg–1. Improvements were seen weight gain (619 vs 605 g), feed intake (924 vs 899 g), and feed conversion ratio when compared to non-supplemented control diets. Similar results were seen in a study conducted by Gilani et al. (2021) when feeding a corn-soybean meal-based diet supplemented with 152 U beta-glucanase per kilogram. The results of their experiment showed that body weights were improved by 4.2%, average daily gain was improved by 5.4%, and FCR was reduced by 7.5 points from day 22-35. From the results seen in these studies, it can be concluded that the supplementation of glucanase can improve broiler performance by decreasing intestinal viscosity and degrading of cell walls.

Glucanase effects on apparent metabolizable energy

Glucanase has been shown to improve the nutritive value of non-starch polysaccharides included in poultry diets by reducing antinutritional effects (Preston et al., 2001; Choct et al., 2004). Studies have shown that glucanase supplementation can improve AME (Meng and Slominski, 2005; Saleh et al., 2005). It is uncertain as to which mechanism increases AME content. In a study conducted by Rutherfurd et al. (2007), it was shown that when feeding a corn-soy based diet supplemented with xylanase, amylase, and β-glucanase. They saw increases in AME (2,829 kcal/kg) in diets that were supplemented with enzymes when compared to unsupplemented diets (2,766 kcal/kg). Similar results were seen in a study by

Meng and Slominski (2005) when feeding a corn-soybean diet supplemented with 400 U/kg of glucanase, they saw a 2.4% increase in dietary AME. Similarly, results were seen by Rutherfurd et al. (2007) when feeding a corn-based diet supplemented with 140 fungal β-glucanase units/kg, they saw a 2.3% increase in AME. These results indicate that although the mechanism for which AME content is improved is unknown, the supplementation of glucanase in diets can improve AME.

Glucanase effects on apparent ileal digestibility

It is well understood that glucanase can improve ileal digestibility by reducing intestinal viscosity which allows for better mineral absorption. In an experiment by Perttila et al. (2001), when evaluating apparent ileal digestibility of broilers while feeding a semi-purified soyabean meal basal diet or a mixture of the basal diet and barley (50:50 on dry matter basis) supplemented with β-glucanase; AID of amino acids was improved in dried barley. Leslie et al. (2007), found that when feeding a corn-soybean diet supplemented with 0 or 500 units of glucanase/kg glucanase improved ileal-digestibility at all ages. Throughout the 23-day trial, glucanase supplemented had a higher IDE (3,210 kcal/kg) than all other diets. It is hypothesized that these results are due to an increase in amylase access to starch granules within the cells of the endosperm. The degradation of the cell wall allows for enzymes to have access to cell contents.

Phytase

Phytase (myo-inositol hexaphosphate phosphohydrolase) (Figure 2.) is the most commonly used exogenous enzyme in monogastric diet formulations. It is responsible for the removal of phosphates from phytic acids. Phytase sequesters orthophosphate groups from the

inositol ring of phytic acid (Figure 3.) to produce free inorganic phosphorus, along with a chain of intermediate myo-inositol phosphates via inositol pentaphosphate to inositol monophosphate (Kumar et al., 2012). This allows for phosphorus to be more available for growth promotion and reduces phosphorus pollution in the environment (Selle and Ravindran, 2007). The first source of phytase that was available commercially was produced from *Aspergillus niger* and was available in 1991 however, there are now other sources such as *Aspergillus ficuum*. It was first discovered in rice bran (Suzuki et al., 1907) and the blood of calves (McCollum and Hart, 1908). After it became available commercially, phytase started gaining traction globally. As a feed additive, it allows for phosphates to be more available to animals and reduces excretion/pollutants of phosphorus (Oh et al., 2004).

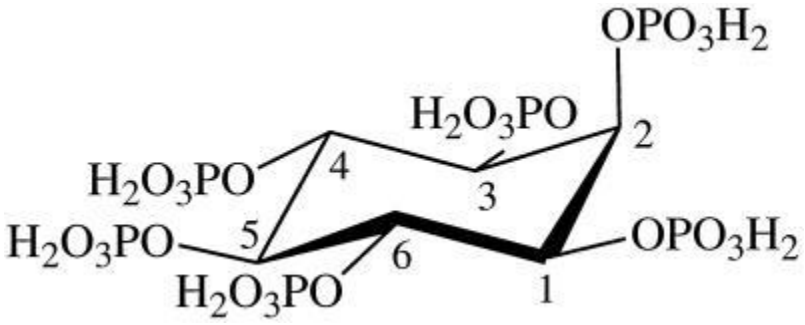

Figure 2. Energetically most favorable conformation of phytic acid (myo-inositol hexakisphosphate). The numbering of the carbon atoms is the numbering for the d-configuration (Wyss et al., 1999)

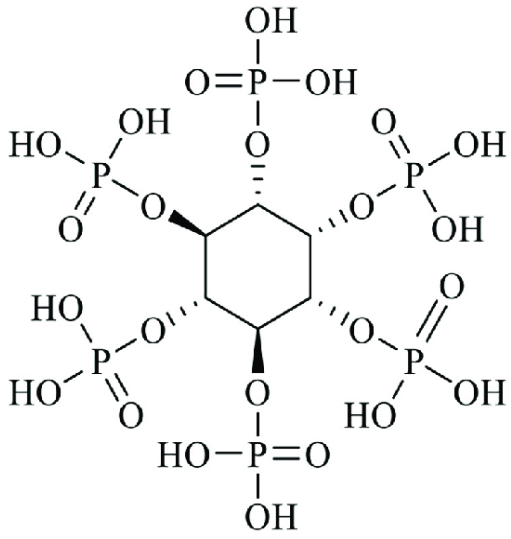

Figure 3 Structure of phytic acid (IP6, IUPAC)

The activity of phytase can be most commonly expressed as FTU, although there are several other abbreviations that are used such as FYT, PU and U. These abbreviations are all defined as the amount of phytase that liberates 1 μmol of inorganic-P per minute from 0.0015 mol/L sodium phytate at pH 5.5 and at a temperature of 37∘C (AOAC, 2000). By defining a measure of phytase activity there can be a reference point as to how the enzyme should behave under well-defined assay conditions (Nunes and Kumar, 2018). Phytase is often used at variable ranges with low, medium, and high specific activity. Low, medium and high specific activity range from 750 to 4500 FTU/kg. In some instances, it can be more beneficial to use a higher or lower specific activity based on the type of diet.

Phytases has been derived from many different sources such as plants, microbes, and endogenous sources. In plants, it can be found in rice, rapeseed, soybean, corn, wheat, and rye (Hayakawa et al., 1989; Houde et al., 1990; Hamada, 1996; Maugenest et al., 1997; Nakano et al., 1999; Weremko et al., 1997). It is most commonly found in cereal grains with rye, wheat, and barley having the highest FTUs/kg while plants such as oil seed and corn having the lowest. Cereals can be used in diets as a source of exogenous phytase to improve phosphorus retention (Weremko et al., 1997). Plant phytase is not commonly used as an exogenous source of phytase in broiler diets because the temperature at which feed is pelleted can reduce or even eliminate the activity of phytase (Weremko et al., 1997). The optimum temperature range for plant based phytase ranges from 40 to 60◦ C (Weremko et al., 1997); it has been reported that plant phytase can partially to totally inactivate when temperatures exceed 80◦ C (Jongbloed and Kemme, 1990).

Phytase is also produced microbially. These types of phytase are produced by fungi, bacteria, and yeast (Singh, 2008) and have a temperature range of 35 to 63◦ C (Wodzinksi and Ullah, 1996). The early phytases used in the industry were microbial phytases because they showed to be more tolerable to the gastrointestinal environment (Eeckhout and De Peape, 1991). One of the first enzymes that was used was derived from fungus although it was later shown that bacterial phytases were more effective (Rodriguez et al., 1999). The problem that occurred with fungal phytases was that they were thermolabile and active at a neutral pH (Lei et al., 2013). Another problem arose with bacterial phytase, as they displayed a higher specific activity and substrate affinity, but they were not stable at pelleting temperatures. A third generation of phytases was then produced that had an improved thermo-tolerance (Lei et al.,

2013). Advances in science and technology have led to microbial phytases that are more efficient. These advances have also led to a corn-expressed phytase that can be grown in genetically modified corn. This corn expressed phytase contains an engineered *Escherichia coli* phytase called Phy02 and has been shown to improve weight gain, feed efficiency and bone mineralization when young pigs were reduced Ca and P diets (Lee et al., 2017; Blavi et al., 2018; Knapp et al., 2018; Munoz Alfonso et al., 2018). This new form of corn expressed phytase has shown to improve broiler performance and bone mineralization and is available in low and high specific activity phytase (PY203 and PY1203). It can be used as a cheaper alternative to more expensive methods of phytase since the enzyme can be added to the corn. This technology will not only be a time saver, but it is also a more economical means to corn based diets.

Phytase impacts on performance

The addition of phytase to animal diets has been shown to have a positive effect on performance such as increased body weights, feed conversion, and energy utilization. This is achieved by phytases ability to reduce anti-nutritional effects of phytate and improve the digestibility of P, Ca, amino acids, and energy. The improvements that are seen in performance parameters can be attributed to an increase in feed consumption and the ability to better utilize nutrients and energy. Nelson et al. (1971) were some of the first people to discover the impacts of phytase on performance. In an experiment using corn-soybean meal-based diets and an *Aspergillus ficuum* based phytase, they saw improvements in body weight gain. In this study, activity ranged from 950 to 7,600 FTU/kg. A linear increase in body weights was seen in diets that were supplemented with phytase. The largest improvement was seen at 7,600 FTU/kg

where body weight gain was increased by 131% compared to the phytase-free control.

Following the commercialization of phytase as a feed additive in 1991, there have been many studies that have investigated the effects of phytase on broiler performance. These studies used microbial phytases such as *Aspergillus niger*, *Aspergillus ficuum*, and *Escherichia coli*. Kornegay et al. (1996) conducted a study that evaluated the effects of an *Aspergillus niger* phytase ranging from 0-1,200 FTU/kg when added to corn-soybean-based diets that contain three levels of non-phytate-P (2.0, 2.7, and 3.4 g/kg). Improvements were seen in body weighs and feed consumption, with the largest improvements seen at 1,000, 800, and 600 FTU/kg. Similar results were seen in a study conducted by Adedokun et al. (2004), when using an *Escherichia coli* phytase. Improvements in body weight and feed intake were seen in birds that were fed 656 and 1081 FTU/kg. Body weights were improved by 31 and 34% and feed in take was improved by 19 and 24% respectively when compared to a phytase free control.

Improvements in feed conversion ratio were seen in a study conducted by Selle et al. (2007). In this study, broilers were fed a corn-wheat-based diet supplemented with an *Aspergillus niger* phytase at 500 FTU/kg. Body weights were improved by 29 grams per bird, feed intake was improved by 16 grams per bird and FCR was improved by 0.04 when compared to a phytase free control. Similar results were seen in a study conducted by Lu et a. (2009) where broilers were fed an *Escherchia coli* phytase at 500 FTU/kg. This study used a corn-wheat-based diet and saw improvements in body weights (3.1 g/day), feed consumption (3 g/day), and feed conversion ratio (0.07) when compared to a non-supplemented diet. The improvements seen in these studies may be attributed to this enzymes ability to free up bound phosphorous which allows for better absorption.

Phytase impacts on mineral availability

The use exogenous phytase can lead to increased phytate degradation and improve mineral availability and utilization, this can promote bone quality and growth as Ca and P are required for the formation of new tissues (Dersjant-Li et al., 2020; Williams et al., 2000). Since phosphorus makes up a large component of the bird's skeleton, bone measurements are often considered to be good criteria for estimating phosphorus availability (De Groote and Huyghebaert, 1997). The most commonly used indicator of bone mineralization in birds is tibia ash because of the increased availability of P, Ca, Zn, and Cu (Sebestian et al., 1996). Previous literature has shown that phytase can improve tibia ash in broilers by allow for improved mineral availability and utilization. In a study conducted by Shirley and Edwards (2003), they found that when supplementing phytase from 0 to 12,000 FTU/kg, tibia ash and tibia weight were improved. Tibia ash was improved from 26 to 42% and tibia weight was increased from 0.200 to 0.601 g/tibia. Similar findings were seen by Pieniazek et al. (2017), when using phytase ranging from 0 to 2,000 FTU/kg. They found that tibia weight was improved by 478.4 g/tibia. In a study by Brenes et al. (2003) that evaluated Ca, P, and Zn contents in tibia ash; improvements were seen in all three elements. Broilers were fed a phytase supplemented diet that ranged from 200 to 600 FTU/kg. Increases up to 4% were seen in tibia ash.

Along with improvements in tibia ash, phytase has also been shown to improve P and Ca digestibility in poultry (Emiola et al., 2007; Olukosi et a., 2007; Afsharmanesh et al., 2008; Selle et al., 2009). In a study conducted by Ravindran et al. (2006), when feeding an Escherichia coli derived phytase at 500 FTU/ kg, ileal Ca digestibility was improved by 27%. This study used a corn-soybean meal-based diet that contained 7.8 g/kg Ca. Rutherford et al.,

(2004) saw improvements P digestibility (11.8%) when feeding a low P diet containing 750 FTU/kg *Peniophora lycii* phytase. Improvements in apparent ileal digestibility were seen by Dersjant-Li et al. (2020) when feeding 500 and 1,000 FTU/kg of phytase. They saw an improvement of 26.2 and 33.2 percent in apparent ileal digestibility of phosphorus when feeding a diet with a reduction in Ca and available P (2.0 and 1.9 g/kg). Similar improvements were seen by Santos et al. (2008), when evaluating the digestibility of Ca and P in broilers that were fed an AME, Ca, and P reduced diet supplemented with 500 FTU/kg phytase. They saw a 22.7 and 36.9% increase in Ca and P digestibility respectively. Similar improvements were seen in a study conducted by Selle et al. (2009) when feeding a low P wheat-based diet supplemented with 500 FTU/kg *Escherichia coli* based phytase. They saw improvements in Ca and P digestibility (11 and 14%, respectively). From the results seen in these studies it can be concluded that the ability of phytase to increase mineral availability leads to improvement in apparent ileal digestibility.

Phytase impacts on apparent metabolizable energy

Phytase has been shown to not only improve P digestibility and utilization but also enhance energy utilization from lipids by reducing the soap formation from mineral-phytate complexes (Selle et al., 2007; Dersjant-Li et al., 2015; Ravindran et al., 2006). This effect was first reported by Rojas and Scott (1969) in a study using *Aspergillus ficcum* derived phytase in cottonseed meal and soybean meal. They found that AME yields for broiler chicks were improved when diets were supplemented with phytase. Ravindran et al., (2001) found that AME was increased by the addition of 750 FTU/kg however when levels exceeded 750 FTU/kg, AME was decreased. Similar results were seen by Cowieson et al. (2006) when they

supplemented diets with phytase, they saw an increase in AMEn (120 kcal/kg) when compared to a non-phytase supplemented control. In a study conducted by Pieniazek et al. (2017) they observed that an increase in AME as phytase inclusion level increased. Diets were supplemented with phytase at 250, 500, and 2,000 FTU/kg.

Previous literature has indicated that phytase may have additional extra-phosphoric effects. One of these effects is the removal of adverse effects of phytate on starch digestion (Truong et al., 2014; 2015). In a study conducted by Liu et al. (2014) it was reported that when using a phytase derived from *Buttiauxella sp.* at 1,000 FTU/kg, starch digestibility was improved by 2.56% for corn, sorghum, and wheat in the proximal ileum. Additionally, it was also increased by 19% for wheat-based diets in the proximal jejunum. Similar results were seen by Truong et al. (2015) when feeding the same type of phytase at 500 FTU/kg. Starch digestibility was improved in the proximal jejunum by 17.6% in corn-wheat-based diets. Based on these results, it can be concluded that the addition of phytase enzymes can improve starch digestion in the jejunum and ileum.

Corn expressed enzymes

Exogenous enzymes have traditionally been produced using microbial processes and are provides as either granulated or liquid formulation. The determination of whether or not an enzyme is safe to use or not depends on the enzyme itself along with the microbial production host and processes associated with the production and purification of the enzyme (Pariza et al., 2010). Fungal and bacterial hosts have traditionally been used in microbial enzyme production which has led to the concern that other host produced molecules may be co-purified which may

lead to contamination of the final product (Pariza, 2001). An alternative to the traditional method of producing exogenous enzymes has been discovered using transgenic corn. This process involves the production of high concentration of recombinant enzymes within transgenic corn grain, for the use of feed additives at low inclusion rates (Pen et al., 1993; Nyannor and Adeola, 2008; Nyannor et al., 2009, Gao et al., 2012; Denbow et al., 1998). The use of corn as production is thought to be a safe because of its long history of use in human and animal diets. The reduction in risks associated with production allows for an enzyme to be considered safe and to be used as a feed additive.

The use of a corn expressed glucanase enzyme has been engineered by using genetic transformation to express endo-β-1,4-glucanase activity in corn grain. This new enzyme (AC1) is very similar to *Thermotoga maritima* and has additional amino acid changed that were introduced to improve thermal stability. AC1 has multiple activities such as endo-cellulase, cellobiohydrolase (exo-cellulase), β-xylosidase, and β-1,3-glucanase, but its primary activity is endo-1,4-β-glucanase. The AC1 gene encodes a 37.7 kDa protein and is stable at and above 80°C, the growth temperature of *T. maritima* (Rab, 2015). Studies by Ayers et al. (2018) have shown that AC1 was effective in reducing intestinal viscosity, when added to a high NSP diet. Additionally, it has been shown that AC1 was thermostable, with a 90% recovery following pelleting at 90°C. Jasek et al. (2018) demonstrated improvements in body weight when 100 β-glucanase units (β-Glu-U) per kg were added to a reduced energy, corn-soybean meal diet.

Additionally, a novel corn expressed phytase has also been developed that contains an engineered *Escherichia coli* phytase called Phy02 and has been reported to be safe for use in both poultry and swine (Ligon, 2016; Broomhead et al., 2018; Blavi et al., 2019. Previous

studies have shown that corn expressed phytase can improve average daily gain, feed efficiency, bone mineralization and P digestibility in young pigs that were fed Ca and P deficient diets (Lee et al., 2017; Knapp et al., 2018, Broomhead et al., 2018; Blavi et al., 2019). Since this is a relatively new enzyme, its efficacy compared to inorganic phosphate in poultry remains unknown. In a study by Wang et al. (2021) it was shown that corn express phytase was effective in releasing P from phytate and improved broiler performance as well as bone mineralization when feeding 4,5000 FTU/kg of corn expressed phytase.

CHAPTER III

COMPARING THE EFFICACY OF VARIETIES, LOW AND HIGH PHYTASE EXPRESSING, AT EQUIVALENT FTY/KG IN BROILERS

Introduction

Diets for broilers are typically based on cereals and oil seeds in which 70-80% of the phosphorus content is bound in the form of phytate (Taylor and Coleman 1979; Selle and Ravindran, 2007). In this form, it is poorly available to poultry and other non-ruminant species, except for in low Ca diets (Tanim et al. 2004). For this reason, diets are commonly supplemented with inorganic phosphates to mitigate for the limited supply of phosphorus from plant ingredients; as a result, feed costs are increased along with excretion of phosphorus (P) (Broz et al., 1994; Denbow et al., 1995). To reduce these effects and reduce the excretion of unwanted P into the environment, phytase enzymes have been developed from fungal and microbial sources and are now added to poultry diets. (Dersjant-Li and Kwakernaak, 2019). By adding this type of enzyme, phytate-bound P can be released, and P excretion can be reduced. Microbial phytase is the most common form supplemented in poultry diets. The process of making microbial phytase involves fermentation which results in a higher cost. Corn expressed phytase can be used as an alternative to microbial phytase and lower feed costs. The process of post harvesting corn expressed phytase does not require heat which preserves phytase activity and makes corn an ideal crop for expressing phytase.

One experiment by (Smith et al., 2019) showed that birds that were fed phytase at 3,000 FTU/kg had a lower FCR when compared to those that were fed the standard 500 FTU/kg.

Additionally, birds that were fed 3,000 FTU/kg and 1,500 FTU/kg of phytase had higher body weights than those that were fed the standard 500 FTU/kg.

The postharvest processing of corn does not require the use of heat which in turn preserves phytase activity. This makes corn an ideal plant to express phytase and to be supplemented in poultry diets. The novel corn-expressed phytase (CEP; Grainzyme, Agrivida Inc.) contains an engineered Escherichia coli phytase called Phy02 and is safe and effective in poultry diets (Ligon, 2016 and Broomhead et al. 2018). Since CEP is a new form of phytase, its effects relative to inorganic phosphate remain unknown. It is thought that bone mineralization is more sensitive and prevalent because Phosphorus (P) is a major component of the bird's skeleton (Zyla et al., 2004 and Gautier et al., 2018). It has been shown in previous studies by Nyannor et al; that the addition of corn expressed phytase to a P-deficient diet in weanling pigs, improved growth performance.

The objectives of this experiment are to determine if performance and bone ash response is equivalent between high and low specific activity (PY 1203 and 203) corn expressed phytase varieties when fed at equal phytase dose levels. It is though that both low and high specific activity corn expressed phytase varieties will improve broiler performance and bone mineralization in calcium and aP reduced diets.

Materials and Methods

Animal Husbandry, Diet, and Experimental Design

A total of 2,640 Cobb 500 male broiler chicks were used in this experiment. Day-old broilers were equally housed at 22 birds per replicate pen, pens were blocked in a random complete block design. There were 12 replicate pens per treatment. Each pen was lined with re-used litter as bedding and equipped with one tube feeder and a nipple drinker line. Pens were blocked and treatments were assigned at random to one of ten dietary treatments (Table 1). The birds were fed a three-phase diet consisting of a starter (day 1-14, crumble), grower (day 15-28, pellet), and finisher (day 29-42, pellet) as seen in Table 2. Pelleting temperatures were kept at 85 C. Birds were allowed ad libitum access to feed and water. Bird management was in accordance with guidelines outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (2010), all procedures were approved by Texas A&M University animal care and use committee.

Table 1: Treatment Designations, Phytase levels of treatments, and Grainzyme product used

Treatments	Abbreviations
Positive Control	PC
Nergative Control	NC
NC+0.5 kg/t 203	0.5 kg 203
NC+1.0 kg/t 203	1.0 kg 203
NC+2.0 kg/t 203	2.0 kg 203
NC+3.0 kg/t 203	3.0 kg 203
NC+0.175 kg/t 1203	0.175 kg 1203
NC+0.355 kg/t 1203	0.355 kg 1203
NC+0.705 kg/t 1203	0.705 kg 1203
NC+1.06 kg/t 1203	1.06 kg 1203

Table 2: Diet formulations of starter, grower and finisher diets feeding high and low specific activity corn expressed phytase

Ingredient:	Starter PC	Starter NC	Grower PC	Grower NC	Finisher PC	Finisher NC
Fat, Animal	1.121	1.121	1.657	1.657	2.300	2.300
Calcium Carbonate	1.409	1.319	1.331	1.241	1.215	1.125
Mono-Dical Phos.	1.491	0.777	1.401	0.687	1.250	0.536
Salt, Plain	0.448	0.448	0.449	0.449	0.450	0.450
L-Lysine	0.154	0.154	0.147	0.147	0.137	0.137
DL-Methionine	0.303	0.303	0.266	0.266	0.241	0.241
L-Threonine	0.082	0.082	0.070	0.070	0.078	0.0789
Soybean Meal	34.455	34.455	29.289	29.289	25.512	25.512
Corn	60.234	59.934	65.085	64.785	68.512	68.212
Choline Chloride	0.05	0.05	0.05	0.05	0.05	0.05
Salinomycin	0.05	0.05	0.05	0.05	0.05	0.05
TAMU Trace Mineral Premix	0.05	0.05	0.05	0.05	0.05	0.05
TAMU Vitamin Premix	0.15	0.15	0.15	0.15	0.15	0.15

Performance and Sampling Parameters

Mortalities were collected, recorded, and weighed each day. All birds and feed were weighed on day 14, 28, and 42 for the determination of body weight gain (BWG), feed intake (FI) and the calculation of mortality adjusted feed conversion ratio (FCR). On day 28, five birds per replicate were euthanized, with left tibias removed for the determination of bone ash. All connective tissue, muscle, and fibulas were removed from each collected tibia before analysis. Left tibias were dried in a Forced Air Oven (VWR 89511-410, Radnor, PA) for 12 hours at 100 °C. The dried tibias were then defatted in diethyl ether for 6-8 h and allowed to dry under a chemical hood for 12 hours upon the completion of defatting procedures so all

ether could evaporate from the bones. Defatted tibias were dried again at 100 °C for 12 hours, then ashed at 600 °C in ceramic crucibles for 24 hours. All crucibles and tibias were weighed before and after ashing to determine tibia mineral content.

Statistical Analysis

All data was analyzed via One-Way ANOVA using the GLM model (Minitab Software) with treatment means deemed significantly different at $P \leq 0.05$. Treatment means that were determined to be significant were further separated using Fishers LSD Test.

Results

Body Weights

Treatment means for body weights can be seen in Table 3 and 4. The 203 and 1203 birds did not differ in body weight at any age ($P > 0.05$); however, they did differ from the PC and NC. The 203 (0.5 kg) and 1203 (0.5 kg) fed birds had higher body weights than NC birds (0.41 kg, ± 0.24 $P < 0.05$). The PC, 203, and 1203 birds weighed more (1.6, 1.8, and 1.8 kg, respectively ± 0.011) than the NC birds on day 28 (1.5 kg ± 0.011, $P < 0.05$). At day 42, the 203 (3.4 kg ± 0.024) and 1203 birds (3.4 kg ± 0.024) weighed more ($P > 0.05$) than the NC birds (3.1 kg ± 0.024) with the PC being intermediate (3.3 kg). There was also an effect of phytase level on body weights at all ages. The PC and NC both weighed less than all the birds fed phytase supplemented diets at day 14 and d28. At day 42 the NC was lower than all phytase groups while the PC was intermediate. There were also differences between body weights within the phytase supplemented treatments. Generally, throughout the trial the High birds weighed the most with the Low birds weighing the least and the other two groups being intermediate.

Table 3: Average Body Weight of birds fed high and low specific corn expressed phytase

Treatment:	D14 BW	D28 BW	D42 BW
Positive Control (PC)	0.410^{d}	1.646^{d}	3.280^{cd}
Negative Control (NC)	0.410^{d}	1.499^{e}	3.087^{d}
NC + 1.0lb/t 203	0.482^{c}	1.740^{c}	3.310^{bc}
NC + 2.0lb/t 203	0.505^{b}	1.818^{ab}	3.486^{ab}
NC + 4.0lb/t 203	0.504^{b}	1.826^{ab}	3.411^{abc}
NC + 6.0lb/t 203	0.508^{ab}	1.813^{b}	3.449^{abc}
NC + 0.35lb/t 1203	0.498^{bc}	1.761^{c}	3.358^{abc}
NC + 0.71lb/t 1203	0.493^{bc}	1.778^{bc}	3.436^{abc}
NC + 1.41lb/t 1203	0.522^{ab}	1.812^{b}	3.452^{abc}
NC + 2.12lb/t 1203	0.502^{b}	1.867^{a}	3.544^{a}
SEM	*0.004*	*0.011*	*0.024*
P-value	*0.000*	*0.000*	*0.001*

$^{a-d}$ Means within rows with different superscripts differ significantly at P<0.05

Table 4: Average bird weight (kg) of birds fed 203 and 1203 from d0-42

Treatment:	D0 BW	D14 BW	D28 BW	D42 BW
PC	0.0925	0.410^{b}	1.646^{b}	3.280^{bc}
NC	0.0930	0.410^{b}	1.499^{c}	3.087^{c}
203	0.0930	0.500^{a}	1.799^{a}	3.414^{ab}
1203	0.0933	0.504^{a}	1.805^{a}	3.448^{a}
SEM	*0.001*	*0.004*	*0.011*	*0.024*
P-value	*0.089*	*0.000*	*0.000*	*0.000*

$^{a-d}$ Means within rows with different superscripts differ significantly at P<0.05

Feed Consumption

The means for feed consumption can be seen in Table 5-7. The 203 and 1203 did not differ in feed consumption from day 0-42 (P > 0.05) however; they did differ from the NC and PC. The 203 (116.251 g/bird/day ± 0.005) and 1203 (116.021 g/bird/day ± 0.005) consumed more feed that the PC (109.336 g/bird/day ± 0.005) and the NC (103.727g/bird/day ± 0.005) (P <0.05). On day 14, 203 (41.216 g/bird/day ± 0.005) and 1203 (42.432 g/bird/day ± 0.005) birds consumed more feed than the PC and NC (33.506 and 32.263 g/bird/day ± 0.005 respectively). On day 28, 203 and 1203 (136.877 and 136.852 g/bird/day ± 0.005 respectively) birds

consumed more feed that the PC and NC (128.958 and 124.19 g/bird/day ± 0.005 respectively) ($P > 0.05$). There were no differences seen in feed consumption during the finisher phase (d 28-42). There was an effect on phytase level and feed consumption, at day 14 and 28, 203 and 1203 birds consumed more feed than the NC and PC ($P < 0.05$). At day 42, the NC consumed the least amount of feed with the PC being the intermediate. 203 and 1203 birds consumed the most amount of feed.

Table 5: Average feed consumption (g/bird/day) from d0-42 comparing 203 and 1203

Treatment	D0-14 FC	D14-28 FC	D0-28 FC	D28-42 FC	D0-42 FC
PC	33.506^{c}	128.958^{b}	78.373^{b}	212.329	109.336^{b}
NC	32.263^{c}	124.419^{c}	75.619^{b}	202.861	103.727^{c}
203	41.216^{b}	136.877^{a}	86.446^{a}	213.233	116.251^{a}
1203	42.432^{a}	136.852^{a}	86.479^{a}	213.326	116.021^{a}
SEM	*0.427*	*0.616*	*0.504*	*1.408*	*0.719*
P-value	*0.000*	*0.000*	*0.000*	*0.182*	*0.000*

$^{a-d}$ Means within rows with different superscripts differ significantly at P<0.05

Table 6: Average feed consumption (g/bird/day) from d0-42

Treatment:	D0-14 FC	D14-28 FC	D0-28 FC	D28-42 FC	D0-42 FC
Positive Control	33.506^{e}	128.958^{e}	78.373^{d}	212.329	109.336^{c}
Nergative Control	32.263^{e}	124.419^{f}	75.619^{d}	202.861	103.727^{d}
NC+0.5 kg/t 203	38.888^{d}	134.720^{bcd}	85.311^{bc}	206.661	114.917^{ab}
NC+1.0 kg/t 203	41.697^{bc}	137.614^{bc}	86.405^{b}	219.188	117.413^{ab}
NC+2.0 kg/t 203	41.573^{bc}	138.051^{b}	86.948^{ab}	213.329	116.551^{ab}
NC+3.0 kg/t 203	42.707^{abc}	137.122^{bc}	87.121^{ab}	213.754	116.124^{ab}
NC+0.175 kg/t 1203	40.762^{cd}	134.456^{cd}	85.787^{b}	210.217	115.435^{ab}
NC+0.355 kg/t 1203	40.763^{cd}	131.175^{de}	82.695^{c}	212.866	112.623^{bc}
NC+0.705 kg/t 1203	44.683^{a}	137.875^{bc}	87.991^{ab}	209.223	116.567^{ab}
NC+1.06 kg/t 1203	43.517^{ab}	143.903^{a}	89.441^{a}	220.998	119.457^{a}

$^{a-d}$ Means within rows with different superscripts differ significantly at P<0.05

Table 7: Average feed consumption form d0-42 comparing FTU

Treatment	D0-14 FC	D14-28 FC	D0-28 FC	D28-42 FC	D0-42 FC
PC	33.506^{c}	128.958^{c}	78.373^{d}	212.329^{abc}	109.336^{b}
NC	32.263^{c}	124.419^{d}	75.619^{d}	202.861^{c}	103.727^{c}
Low	39.825^{b}	134.588^{b}	85.549^{bc}	208.439^{bc}	115.176^{a}
Mlow	41.230^{b}	134.395^{b}	84.550^{c}	216.027^{ab}	115.018^{a}
Mhigh	43.128^{a}	137.963^{a}	87.470^{ab}	211.276^{abc}	116.559^{a}
High	43.112^{a}	140.513^{a}	88.281^{ab}	217.376^{a}	117.790^{a}
SEM	*0.427*	*0.616*	*0.504*	*1.408*	*0.719*
P-value	*0.000*	*0.000*	*0.000*	*0.069*	*0.000*

$^{a-d}$ Means within rows with different superscripts differ significantly at P<0.05

Feed Conversion

The means for mortality adjusted FCR can be seen in Table 8 and 9. Overall, from day 0-42, all diets outperformed the NC and performed similarly to the NC ($P < 0.05$). There were no differences seen in the starter or finisher phase ($P > 0.05$). On day 28, all phytase supplemented diets outperformed the NC ($P < 0.05$) and performed similarly or better than the PC ($P > 0.05$). At all activity levels of phytase, the NC was outperformed ($P < 0.05$).

Table 8: Feed conversion from d0-42 comparing dietary treatments

Treatment:	D0-14 FCR	D14-28 FCR	D0-28 FCR	D28-42 FCR	D0-42
Positive Control	1.245	1.482^{bcd}	1.426^{bc}	1.887	1.615^{b}
Nergative Control	1.193	1.633^{a}	1.517^{a}	1.850	1.668^{a}
NC+0.5 kg/t 203	1.200	1.509^{bcd}	1.428^{bc}	1.882	1.619^{b}
NC+1.0 kg/t 203	1.215	1.478^{bcd}	1.408^{bcd}	1.904	1.605^{b}
NC+2.0 kg/t 203	1.215	1.468^{cd}	1.401^{cd}	1.903	1.611^{b}
NC+3.0 kg/t 203	1.230	1.480^{bcd}	1.413^{bc}	1.861	1.601^{b}
NC+0.175 kg/t 1203	1.237	1.492^{bc}	1.420^{bc}	1.872	1.611^{b}
NC+0.355 kg/t 1203	1.226	1.442^{d}	1.384^{d}	1.809	1.569^{c}
NC+0.705 kg/t 1203	1.248	1.475^{bcd}	1.413^{bc}	1.811	1.582^{b}
NC+1.06 kg/t 1203	1.258	1.490^{bc}	1.429^{b}	1.858	1.614^{b}
SEM	*0.005*	*0.006*	*0.004*	*0.019*	*0.005*
P-value	*0.068*	*0.000*	*0.000*	*0.978*	*0.010*

$^{a-d}$ Means within rows with different superscripts differ significantly at P<0.05

Table 9: Feed conversion ratio from d0-42 FTU comparison

Treatment	D0-14 FCR	D14-28 FCR	D0-28 FCR	D28-42 FCR	D0-42 FCR
PC	1.245	1.482^{bc}	1.426^{b}	1.887	1.615^{b}
NC	1.193	1.633^{a}	1.517^{a}	1.850	1.668^{a}
Low	1.218	1.500^{b}	1.424^{b}	1.877	1.615^{b}
Mlow	1.221	1.460^{c}	1.396^{c}	1.857	1.587^{b}
Mhigh	1.232	1.472^{bc}	1.407^{bc}	1.857	1.597^{b}
High	1.244	1.485^{bc}	1.421^{b}	1.859	1.607^{b}
SEM	*0.005*	*0.006*	*0.004*	*0.019*	*0.005*
P-value	*0.103*	*0.000*	*0.000*	*0.997*	*0.004*

[a-d] Means within rows with different superscripts differ significantly at P<0.05

Bone Ash

The means of tibia bone ash weight and percentage are shown in Table 10. Treatments that were supplemented with PY 1203 and 203 except for 0.5 kg 203 and 0.175 kg 1203, increased tibia ash content when compared to the NC and performed similarly or better than the PC ± 0.005 (P < 0.05). When comparing the PC and NC versus the level of FTUs in the phytase supplemented diets, the PC outperformed the NC (P < 0.05). At all FTU levels tibia ash increased when compared to the NC and performed similarly to the PC ± 0.005 (P < 0.05).

Table 10: D28 Tibia bone ash (%)

Treatment:	D28 ASH
Positive Control	52.318^{a}
Nergative Control	47.954^{d}
NC+0.5 kg/t 203	51.209^{bc}
NC+1.0 kg/t 203	51.64^{abc}
NC+2.0 kg/t 203	51.746^{abc}
NC+3.0 kg/t 203	52.152^{ab}
NC+0.175 kg/t 1203	51.087^{c}
NC+0.355 kg/t 1203	51.635^{abc}
NC+0.705 kg/t 1203	51.551^{abc}
NC+1.06 kg/t 1203	51.891^{abc}
SEM	*0.158*
P-value	*0.000*

[a-d] Means within rows with different superscripts differ significantly at P<0.05

Discussion

Traditionally broiler diets consist of grains and cereal in which phosphorus is bound in the form of phytate (Taylor and Coleman 1979; Selle and Ravindran, 2007). In this form, phosphorus is poorly available for utilization by broilers (Tanim et al. 2004). Phytase hydrolyzes phytate substrates to release phosphorous in a free form which animals can absorb efficiently, resulting in a lower demand for supplemental inorganic phosphorus (Geurrand, 2018). Furthermore, phytate reduces the ability for broilers to absorb minerals, increases endogenous losses, which characterizes it as an anti-nutrient (Cowson et al., 2011). Other studies have shown that at high inclusion rates, phytase can improve FCR (Pirgozlev et al., 2011). In this current experiment, a corn based phytase was used in a corn-soybean meal-based diets to evaluate the effects of high and low specific activity (PY 1203 and 203) corn expressed phytase on broiler performance and bone ash. The hypothesis is that corn expressed phytase could be used as a cheaper alternative to microbial phytase if similar results can be observed because corn is already used in the majority of poultry diets, and it is an ideal plant to express phytase.

When comparing body weights throughout this current study, birds that were fed 4500 FTU of phytase, had a higher body weight than all other treatments. Similar results were observed in an experiment conducted by Wang and Kim in 2014. They found that at higher levels of CEP, treatments outperformed the NC. Wang and Kim (2014) also found that body weight gain was less sensitive to the dietary nPP requirement than bone mineralization. Additionally, all dietary treatments that were fed PY1203 had higher average body weights than the PC and NC. Similar results were observed by Nyannor and Adeola (2007) when evaluating growth performance.

In the current study, FCR was lower in all CEP treatments compared to the NC during the grower phase, overall, during the first 28 days of the grow out and overall, at day 42. All CEP levels performed similarly to the PC. These findings were different from the findings from a study conducted by Wang and Kim (2014) as they did not see and improvement in FCR at any FTU level or inclusion rate of CEP. The results of this current study indicate that CEP could be used to maintain FCR similar to a non-deficient diet or even improve FCR. Similar results have been observed in microbial produced phytase studies, however, CEP could be a less expensive means of providing phytase to the diet of chickens.

In this current study, tibia ash was evaluated on day 28, both PY 203 and PY 1203 increased tibia ash content when compared to the NC. PY 1203 and 203 performed similarly or better than the PC at all inclusion rates except for 0.50 kg/MT of PY203 and 0.18kg/MT of PY1203. When tibia ash was evaluated comparing FTU levels, all FTU levels tibia ash content increased when compared to the NC. All FTU levels performed similarly to the PC. This aligns with findings by Wang and Kim (2015) which found that the addition of CEP increased ash weight and percentage. Kim (2015) also found that the at 4,500 FTU/kg phytase, bone ash % was increased the most. This was consistent with finding in this current experiment. Similar findings by Nelson and Walker (1964) suggest that tibia ash can be an accurate way to measure P bioavailability. Nelson and Walker (1964) found that the supplementation of phytase improved tibia ash percentage and saw linear and quadratic responses. In a study conducted by Broomhead et al. (2018), it was found that the addition of 4,000 FTU per kg corn expressed phytase (Grainzyme) led to improved growth performance and bone ash weight when compared to even PC diets. Additionally, in a study conducted by Alfonso et al. (2018), it was

found that when Grainzyme was added to a Ca and P deficient diet, growth performance was improved.

In summary, the reduction in dietary Ca and aP negatively affected broiler performance in this study. Both high specific activity (PY 1203) and low (PY 203) specific activity corn expressed activity improved body weight, feed conversion ratio, and bone ash when compared to the NC illustrating its effectiveness similar to microbial produced phytase. Similar findings were observed in growth performance in experiments using microbial phytase (Cowieson et al., 2004 and Pieniazek et al., 2017). Additionally, similar findings were found when evaluating tibia bone ash percentage (Shirley and Edwards, 2003; Dilger et al., 2004). Based on similar studies using microbial produced phytase (Cowieson and Adeola, 2005) phytase can be improve Ca and P utilization and may enable the formulation of lower-cost diets by using corn expressed phytase. An increase in bone mineralization was also expected because phosphorus is a large component in a bird's skeleton and phytase has been illustrated to do this (Selle and Ravidran, 2007). Overall, birds that were fed 3000-4500 FTU of phytase outperformed other dietary treatments. When looking at both PY 203 and 1203, it was observed that at a lower inclusion PY1203 performed similarly or better than almost all performance parameters. This indicates that it may be possible to feed less of PY 1203 and get the same performance benefits, less corn will be needed which would lead to a less expensive diet. In conclusion, the addition of CEP proved to improve broiler performance and bone mineralization. Additionally, the use of PY 1203 and CEP can be used as a cheaper alternative to more expensive methods of phytase since phytase can be added into via corn and less will be needed when using PY1203. Making this technology not only a time saver but also a more economical means to feed poultry and still have efficient growth and bone formation.

CHAPTER IV

EVALUATING GRAINZYME™ AC1 GLUCANASE SUPPLEMENTATION IN CONJUNCTION WITH PHYTASE IN BROILERS

Introduction

Poultry diets use high proportions of corn and soybean meal due to their perceived high nutritional quality and abundance (Pieniazek et al., 2017). In cereal grains and oil seeds, phosphorous can be found in the form of phytate (Taylor and Coleman, 1979). In this form, it is poorly available and cannot be hydrolyzed by poultry (Tanim et al., 2004). Phytase is a subgroup of phosphatases that are capable of initiating phytate dephosphorylation (Selle and Ravindran, 2007). Phytase supplementation in Ca- and avP-reduced diets liberates the phytate bound P, decreasing the formation of Ca-phytate complexes (Bello, 2018). Phytase can improve weight gain, bone ash percentages, and nutrient utilization in broilers (Shirley and Edwards 2003; Dilger et al., 2004).

To reduce these effects and reduce the excretion of unwanted P into the environment, phytase enzymes have been developed from fungal and microbial sources and are now added to poultry diets. (Dersjant-Li and Kwakernaak, 2019). By adding this type of enzyme, phytate-bound P can be released, and P excretion can be reduced. Microbial phytase is the most common form supplemented in poultry diets. The process of making microbial phytase involves fermentation which results in a higher cost. Corn expressed phytase can be used as an alternative to microbial phytase and lower feed costs. The process of post harvesting corn expressed phytase does not require heat which preserves phytase activity and makes corn an ideal crop for expressing phytase. The novel corn-expressed phytase (CEP; Grainzyme,

Agrivida Inc.) contains an engineered *Esherichia coli* phytase called Phy02 and is safe and effective in poultry diets (Ligon, 2016 and Broomhead et al. 2018). Since CEP is a new form of phytase, its effects relative to inorganic phosphate remain unknown. It is thought that bone mineralization is more sensitive and prevalent because Phosphorus (P) is a major component of the bird's skeleton (Zyla et al., 2004 and Gautier et al., 2018).

Exogenous glucanase enzymes have been used in diets due to their ability to reduce viscosity in the small intestine, allowing for better mineral absorption and digestion. (Annison, 1993). Glucanase allows birds to digest non-starch polysaccharides (NSP) more efficiently by reducing the anti-nutritive effects of beta-glucans and other water soluble NSP. (Langhout et al., 1999). Glucanase can improve growth, feed conversion and apparent metabolizable energy (AME). (Edney et al., 1989). Another study (Rutherfur et al., 2007) using microbial glucanase showed that in diets supplemented with β-glucanase, apparent metabolizable energy (AME) was higher in corn-soy based diets as opposed to those that were not supplemented. Moharrery et al. (2015), reported that at an inclusion rate of 0.5 g kg−1 of β-glucanase, AME nitrogen corrected was improved.

The most common form of glucanase that is supplemented in diets is in the microbial form. In this form, fungal and bacterial hosts are used in the production of microbial enzymes which can potentially lead to the contamination of the product (Pariza, 2001). Genetically modified corn can be used as an alternative to microbial enzymes. In the process of making genetically modified corn, high concentrations of recombinant enzymes are produced within transgenic corn grain to be used as feed additives at low inclusion rates (Pen, 1993; Nyannor, 2008; Gao, 2012). The corn expressed glucanase that was used in this experiment was AC1.

AC1 expresses a recombinant carbohydrase, with endo-β-1,4-glucanase activity (Broomhead et al., 2019).

The objective of this study was to compare the efficacy of corn-expressed glucanase, (Grainzyme® Glucanase AC1]) and high specific activity (Grainzyme® PY1203, [PY1203]), at different inclusion rates in broilers. It can be expected that the addition of corn expressed phytase and glucanase will improve broiler performance, AID, and AME. Additionally, both enzymes can be used as a cheaper alternative since corn is already used in poultry diets.

Materials and Methods

Animal Husbandry, Diet, and Experimental Design

A total of 2,592 Cobb 500 male broiler chicks were used in this experiment. Birds were equally housed at 24 birds per replicate treatment, across a total of 108 pens (0.91 x 1.83 m). The birds were randomly assigned to floor pens and one of nine dietary treatments (Table 11). Each pen was lined with used litter as bedding and equipped with one tube feeder and a nipple drinker line. The birds were fed a three-phase diet consisting of a starter (d 0-14, crumble), grower (d 14-28, pellet), and finisher (d 28-42, pellet) as seen in Table 12. Birds were allowed ad libitum access to feed and water. Bird management was in accordance with guidelines outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (2010), all procedures were approved by Texas A&M University animal care and use committee (IACUC 2018-0181)

Table 11: Dietary treatment description and abbreviation for birds fed AC1

Treatment	Abbreviation
Positive Control (PC)	PC
Negative Control (NC)	NC
NC + 0.175 kg/t Grainzyme 1203 + 0.175 kg/t AC1	0.175 kg/t AC1
NC + 0.175 kg/t Grainzyme 1203 + 0.35 kg/t AC1	0.35 kg/t AC1
NC + 0.175 kg/t Grainzyme 1203 + 0.5 kg/t AC1	0.5 kg/t AC1
NC + 0.175 kg/t Grainzyme 1203 + 0.75 kg/t AC1	0.75 kg/t AC1
NC + 0.175 kg/t Grainzyme 1203 + 1.00 kg/t AC1	1.00 kg/t AC1
NC + 0.175 kg/t Grainzyme 1203 + 1.5 kg/t AC1	1.5 kg/t AC1
NC + 0.175 kg/t Grainzyme 1203 + 2.00 kg/t AC1	2.00 kg/t AC1

Table 12: Diet formulations for starter, grower and finisher phases

Ingredient:	Starter PC	Starter NC	Grower PC	Grower NC	Finisher PC	Finisher NC
DDGS	7.000	7.000	12.000	12.000	18.000	18.000
Fat, Animal	2.766	0.250	2.816	0.299	4.105	1.588
Calcium Carbonate	1.344	1.347	1.305	1.309	1.214	1.218
Mono-Dicalcium Phosphorous	0.678	0.674	0.563	0.560	0.330	0.326
Salt, Plain	0.396	0.396	0.360	0.360	0.315	0.315
L-Lysine	0.111	0.117	0.218	0.224	0.182	0.188
DL-Methionine	0.272	0.268	0.240	0.236	0.186	0.182
L-Threonine	0.045	0.044	0.065	0.064	0.022	0.022
Soybean meal	34.882	34.443	25.228	24.782	21.445	21.000
Corn	52.171	55.125	56.870	59.832	53.866	56.826
Choline Chloride	0.050	0.050	0.050	0.050	0.050	0.050
Salinomycin	0.050	0.050	0.050	0.050	0.050	0.050
Phytase (1203)	0.018	0.018	0.018	0.018	0.018	0.018
TAMU Mineral Premix	0.050	0.050	0.050	0.050	0.050	0.050
TAMU Vitamin Premix	0.150	0.150	0.150	0.150	0.150	0.150
Sand	0.018	0.018	0.018	0.018	0.018	0.018

Performance and Sampling Parameters

Mortalities were collected, recorded, and weighed each day. All birds were weighed on day 14, day 28, and day 42 for performance parameters: Average bird weight (BW), Average feed consumption (FC), Feed conversion ratio (mortality adjusted, FCR), and Mortality (MORT) by phase (Starter, day 0-14; Grower, day 14-28; Finisher, day 28-42) and Overall (day 0-42).

Digestibility and AME

On day 28 and 42, intestinal contents were collected from the ileum (between the Meckel's diverticulum and the ileocecal junction) by gently finger-stripping the intestinal segment from 4 birds/pen and pooled. On day 28, samples were analyzed for ileal digestibility. Nutrient digestibilities were calculated according to, using the ratio of the weight of the excreta

to the weight of the feed intake during the main balance period. The digestibilities for a given nutrient were calculated as follows:

Digestibility (%)Totalcollection

= ([nutrientdiet – (excreta feed × nutrientexcreta)]/ [nutrientdiet]) × 100

On d42, samples were analyzed for AME and AME corrected for nitrogen. The AME contents of the experimental diets were calculated from their respective excreta/feed ratios, as well as their corresponding gross energy (GE) contents, using the following equation:

AME (kcal/kg diet) Totalcollection

= GEdiet – (GEexcreta × excreta feed)

The total AMEn intake of each dietary treatment was calculated using 8.73 as the nitrogen correction factor using the following equation (Titus 1955):

Amend intake= [GE intake - GE excretion] - [8.73 x (N intake – N excretion)]

Statistical Analysis

All data was analyzed via One-Way ANOVA using the GLM model (Minitab Software) with treatment means deemed significantly different at ($P< 0.05$). Treatment means that were determined to be significant were further separated using Fishers LSD Test.

Results

Body Weights

The means for body weights are shown in Table 13. There were no differences in bodyweights at the end of the 42-day trial ($P > 0.05$), however on day 14, birds that were fed 2.00 kg/t AC1 had a lower body weight than PC, NC, 0.175 kg/t AC1, 0.35 kg/t AC1, and 0.5 kg/t AC1 ($P > 0.05$). All other treatments preformed similarly ($P > 0.05$).

Table 13: Average bird weight (kg.) from d0-42

Treatment:	D0 BW	D14 BW	D28 BW	D42 BW
Positive Control (PC)	0.041	0.513^{ab}	1.696	3.216
Negative Control (NC)	0.041	0.505^{bc}	1.657	3.261
NC + 0.175 kg/t Grainzyme 1203 + 0.175 kg/t AC1	0.041	0.516^{a}	1.671	3.240
NC + 0.175 kg/t Grainzyme 1203 + 0.35 kg/t AC1	0.041	0.518^{a}	1.667	3.219
NC + 0.175 kg/t Grainzyme 1203 + 0.5 kg/t AC1	0.041	0.513^{ab}	1.645	3.235
NC + 0.175 kg/t Grainzyme 1203 + 0.75 kg/t AC1	0.042	0.511^{abc}	1.627	3.173
NC + 0.175 kg/t Grainzyme 1203 + 1.00 kg/t AC1	0.041	0.509^{abc}	1.658	3.219
NC + 0.175 kg/t Grainzyme 1203 + 1.5 kg/t AC1	0.041	0.505^{bc}	1.665	3.242
NC + 0.175 kg/t Grainzyme 1203 + 2.00 kg/t AC1	0.041	0.503^{c}	1.685	3.239
SEM	0.000	0.001	0.008	0.017
P-value	0.055	0.029	0.621	0.983

[a-d] Means within rows with different superscripts differ significantly at P<0.05

Feed Consumption

The means for feed consumption are shown in Table 14. There were no differences seen between treatments during the grower or finisher phase ($P > 0.05$). During the starter phase, birds that were fed 2.00 kg/t AC1 consumed less feed than those that were fed 0.175 kg/t AC1 ($P < 0.05$). All other treatments had similar feed consumption.

Table 14: Average feed consumption (g/bird/day) from d0-42

Treatment:	D0-14 FC	D14-28 FC	D0-28 FC	D28-42 FC	D0-42 FC
Positive Control (PC)	39.882[bc]	131.193	83.697	195.727	106.770
Negative Control (NC)	40.658[abc]	136.825	86.986	202.003	110.200
NC + 0.175 kg/t Grainzyme 1203 + 0.175 kg/t AC1	41.745[a]	137.291	87.216	197.143	111.406
NC + 0.175 kg/t Grainzyme 1203 + 0.35 kg/t AC1	40.925[ab]	133.869	86.332	197.539	109.915
NC + 0.175 kg/t Grainzyme 1203 + 0.5 kg/t AC1	41.018[ab]	136.704	86.726	199.422	109.221
NC + 0.175 kg/t Grainzyme 1203 + 0.75 kg/t AC1	40.599[abc]	131.719	85.181	195.248	109.297
NC + 0.175 kg/t Grainzyme 1203 + 1.00 kg/t AC1	40.699[abc]	135.296	85.817	194.422	108.410
NC + 0.175 kg/t Grainzyme 1203 + 1.5 kg/t AC1	40.466[abc]	135.436	86.624	202.659	111.000
NC + 0.175 kg/t Grainzyme 1203 + 2.00 kg/t AC1	39.526[c]	136.669	86.817	202.090	111.693
SEM	0.160	0.659	0.318	1.101	0.482

P-value	0.008	0.230	0.210	0.500	0.330

[a-d] Means within rows with different superscripts differ significantly at P<0.05

Feed Conversion Ratio

The means for FCR are shown in Table 15. From day 0-42, there were no differences seen between treatments (P > 0.05). During the starter phase, PC outperformed NC (P < 0.05). Birds that were fed 0.35 kg/t AC1 and 0.75 kg/t AC1 outperformed NC but not PC (P < 0.05). All other dietary treatments performed similarly to the NC (P > 0.05).

Table 15: Average feed conversion ratio from d0-42

Treatment:	**D0-14 FCR**	**D14-28 FCR**	**D0-28 FCR**	**D28-42 FCR**	**D0-42 FCR**
Positive Control (PC)	1.154[c]	1.571[d]	1.449[c]	1.785	1.588
Negative Control (NC)	1.217[a]	1.664[ab]	1.534[a]	1.732	1.619
NC + 0.175 kg/t Grainzyme 1203 + 0.175 kg/t AC1	1.191[ab]	1.665[ab]	1.525[ab]	1.763	1.628
NC + 0.175 kg/t Grainzyme 1203 + 0.35 kg/t AC1	1.181[b]	1.632[bc]	1.499[b]	1.746	1.605
NC + 0.175 kg/t Grainzyme 1203 + 0.5 kg/t AC1	1.192[ab]	1.696[a]	1.545[a]	1.733	1.624
NC + 0.175 kg/t Grainzyme 1203 + 0.75 kg/t AC1	1.188[b]	1.657[abc]	1.519[ab]	1.766	1.622
NC + 0.175 kg/t Grainzyme 1203 + 1.00 kg/t AC1	1.192[ab]	1.660[abc]	1.522[ab]	1.742	1.617
NC + 0.175 kg/t Grainzyme 1203 + 1.5 kg/t AC1	1.216[a]	1.641[bc]	1.518[ab]	1.772	1.627
NC + 0.175 kg/t Grainzyme 1203 + 2.00 kg/t AC1	1.196[ab]	1.615[cd]	1.493[b]	1.805	1.627
SEM	0.003	0.006	0.005	0.010	0.003
P-value	0.000	0.000	0.000	0.750	0.090

Ileal Digestibility

The means for Ileal digestibility are shown in Table 16. When evaluating ileal digestibility for calcium content, NC and PC performed similarly. Birds that were fed 0.35 kg/t AC1 outperformed all other dietary treatments ($P < 0.05$). Birds that were fed 0.175 kg/t AC1 and 1.00 kg/t AC1 were outperformed by PC ($P < 0.05$). All other dietary treatments performed similarly to the PC.

When evaluating ileal digestibility for phosphorus content, the PC outperformed the NC ($P < 0.05$). Birds that were fed 0.35 kg/t AC1, 0.5 kg/t AC1, 0.75 kg/t AC1, and 2.00 kg/t AC1 outperformed the NC and performed similarly to the PC ($P < 0.05$). All other dietary treatments performed similarly to the NC ($P > 0.05$).

Table 16: Average ileal digestibility % d28 and d42

Treatment:	Calcium (%)	Phosphorous (%)
Positive Control (PC)	54.750^{bc}	63.840^{ab}
Negative Control (NC)	48.040^{cd}	55.980^{cd}
NC + 0.175 kg/t Grainzyme 1203 + 0.175 kg/t AC1	42.690^{d}	61.320^{bc}
NC + 0.175 kg/t Grainzyme 1203 + 0.35 kg/t AC1	63.240^{a}	68.160^{a}
NC + 0.175 kg/t Grainzyme 1203 + 0.5 kg/t AC1	61.620^{ab}	66.630^{ab}
NC + 0.175 kg/t Grainzyme 1203 + 0.75 kg/t AC1	58.360^{ab}	68.1270^{a}
NC + 0.175 kg/t Grainzyme 1203 + 1.00 kg/t AC1	43.110^{d}	52.740^{d}
NC + 0.175 kg/t Grainzyme 1203 + 1.5 kg/t AC1	47.770^{cd}	61.180^{bc}
NC + 0.175 kg/t Grainzyme 1203 + 2.00 kg/t AC1	55.720^{b}	62.340^{ab}
SEM	1.095	0.850
P-value	0.000	0.000

$^{a-d}$ Means within rows with different superscripts differ significantly at P<0.05

Apparent Metabolizable Energy

Means for AME and AME N-Corrected are shown in Table 6. When evaluating AME, birds that were fed the PC outperformed the NC and all other dietary treatments ($P < 0.05$). Birds that were fed 0.175 kg/t Grainzyme 1203 + 2.00 kg/t AC1, outperformed all other treatments except for the PC ($P < 0.05$). At all inclusion rates of AC1, the NC was outperformed ($P < 0.05$). When AME was corrected for Nitrogen, birds that were fed 2.00 kg/t AC1 and PC performed similarly and outperformed all other dietary treatments ($P < 0.05$). Birds that were fed 0.175 kg/t AC1 performed similarly to NC. At all other inclusion rates of AC1, the NC was outperformed ($P < 0.05$).

Table 17: Apparent metabolizable energy (kcal/kg, D42)

Treatment:	AME	AME (N-Corrected)
Positive Control (PC)	3763.954[a]	3422.155[a]
Negative Control (NC)	3465.913[g]	3111.508[c]
NC + 0.175 kg/t Grainzyme 1203 + 0.175 kg/t AC1	3537.801[f]	3118.241[c]
NC + 0.175 kg/t Grainzyme 1203 + 0.35 kg/t AC1	3564.830[def]	3230.735[b]
NC + 0.175 kg/t Grainzyme 1203 + 0.5 kg/t AC1	3579.807[de]	3216.994[b]
NC + 0.175 kg/t Grainzyme 1203 + 0.75 kg/t AC1	3537.351[ef]	3242.344[b]
NC + 0.175 kg/t Grainzyme 1203 + 1.00 kg/t AC1	3637.250[c]	3265.902[b]
NC + 0.175 kg/t Grainzyme 1203 + 1.5 kg/t AC1	3603.073[cd]	3228.811[b]
NC + 0.175 kg/t Grainzyme 1203 + 2.00 kg/t AC1	3689.932[b]	3377.756[a]
SEM	9.857	14.860
P-value	0.000	0.000

[a-d] Means within rows with different superscripts differ significantly at P<0.05

Discussion

Most poultry diets contain cereal grains which contain non-starch polysaccharides (NSPs). The presence of NSPs such as beta glucans in cereal grains can negatively affect nutrient utilization and reduce growth performance in broiler performance in broilers (Munyaka et al., 2016). Glucanase enzymes are used in poultry diets due to their ability to degrade plant cell walls and release nutrients from grain endosperm (Zarghi, 2018). It was expected that the use of corn expressed glucanase enzymes would have decrease intestinal viscosity and enhance nutrient digestibility; this in turn will improve utilization of fibrous feed ingredients. (Jozefiak et al., 2010). It was expected that the inclusion of a corn expressed glucanase enzyme (AC1) in a reduced energy corn-soy bean meal diet will in turn improve nutrient utilization and improve broiler performance.

Despite the assumption that improved performance would be observed the current experiment did not observe any differences in body weights, feed consumption, or FCR when all treatments were evaluated at the end of the study. Similar findings were found in a study by (Jasak et al., 2018). Additionally, Bi and Chung (2004) had similar findings when evaluating FC and FCR, with no differences between dietary treatments. While Cowieson et al. (2004) did observe improvements in FCR and body weight gain when including glucanase through d 21, but when FCR was evaluated on d 42, no differences were found in that study as well. In a study conducted by Ayres et al., (2010) it was found that in a high fiber diet supplemented with AC1, FCR was decreased while body weights were increased. It is possible in this current study and previous studies that the energy reduction in the NC diets was not large enough, but this would need to be confirmed in future research. Another possibility is that birds, while not

statistically different ate a little more than PC so maybe they behaviorally made up for the deficiency so glucanase didn't have a chance to be effective on weight or FCR.

However, in this current study, the addition of AC1 did improve calcium and phosphorus digestibility. At all levels of AC1 except for 0.175 kg/t AC1, 1.00 kg/t AC1, and 1.5 kg/t AC1, the NC was outperformed ($P < 0.05$). At all inclusion levels of AC1 except for 1.00 kg/t AC1, the NC was outperformed, and performance was similar to the PC ($P < 0.05$). In a study conducted by Sun et al., (2018), similar results were seen using microbial phytase when calcium and phosphorus digestibility were analyzed. Additionally, in a study conducted by Kim et al., (2021) similar results were observed when using microbial glucanase.

In this current study, AME was improved at all inclusion rates of AC1 when compared to the NC. A similar trend was seen when AME was corrected for nitrogen Where at all inclusion rates except for 0.175 kg/t AC1 the NC was outperformed

Based on these results it can be concluded that at almost all levels of inclusion of AC1, AME and AME N-corrected was improved. It was also apparent that the reduction in energy in the NC, negatively impacted broiler performance in the starter and grower phase. Similar results were found in trials conducted by (Jasek et al., 2018; Bi and Chung, 2004; and Cowieson et al., 2010). These results could be a result of rearing conditions and possibly length of trial.

Based on the composition of AC1, it is capable of hydrolyzing and ameliorating some of the anti-nutritive components commonly found in broiler diets (Jasek et al., 2018). This should result in improved digestion and absorption, improving broiler performance. This could also explain why differences were seen in AME and ileal digestibility. This study showed that the inclusion of AC1 in an energy, Ca, and aP reduced diet can improve AME and ileal

digestibility. Further studies will need to be conducted to see if broiler performance is improved. A larger reduction in energy may be needed to see changed in performance parameters. If further studies show improved broiler performance, AC1 would be a feasible additive to poultry diets as it can be grown in the corn that is already being used in diets. It can be a cheaper alternative to other glucanase products since there is not an expensive microbial process related to its cost.

CHAPTER V

EFFECTS OF A CORN-EXPRESSED GLUCANASE AND HIGH SPECIFIC ACTIVITY CORN-EXPRESSED PHYTASE AT DIFFERENT INCLUSION RATES ON GROWTH PERFORMANCE OF BROILERS FED CORN-SOYBEAN MEAL BASED DIETS WITH REDUCED AVAILABLE PHOSPHORUS, CALCIUM, AND METABOLIZABLE ENERGY

Introduction

Poultry diets use high proportions of corn and soybean meal due to their perceived high nutritional quality and abundance (Pieniazek et al., 2017). In cereal grains and oil seeds, phosphorous can be found in the form of phytate (Taylor and Coleman, 1979). In this form, it is poorly available and cannot be hydrolyzed by poultry (Tanim et al., 2004). Phytase is a subgroup of phosphatases that are capable of initiating phytate dephosphorylation (Selle and Ravindran, 2007). Phytase supplementation in Ca- and avP-reduced diets liberates the phytate bound P, decreasing the formation of Ca-phytate complexes (Bello, 2018). Phytase can improve weight gain, bone ash percentages, and nutrient utilization in broilers (Shirley and Edwards 2003; Dilger et al., 2004).

To reduce these effects and reduce the excretion of unwanted P into the environment, phytase enzymes have been developed from fungal and microbial sources and are now added to poultry diets. (Dersjant-Li and Kwakernaak, 2019). By adding this type of enzyme, phytate-bound P can be released, and P excretion can be reduced. Microbial phytase is the most common form supplemented in poultry diets. The process of making microbial phytase involves fermentation which results in a higher cost. Corn expressed phytase can be used as an alternative to microbial phytase and lower feed costs. The process of post harvesting corn expressed phytase does not require heat which preserves phytase activity and makes corn an

ideal crop for expressing phytase. The novel corn-expressed phytase (CEP; Grainzyme, Agrivida Inc.) contains an engineered Escherichia coli phytase called Phy02 and is safe and effective in poultry diets (Ligon, 2016 and Broomhead et al. 2018). Since CEP is a new form of phytase, its effects relative to inorganic phosphate remain unknown. It is thought that bone mineralization is more sensitive and prevalent because Phosphorus (P) is a major component of the bird's skeleton (Zyla et al., 2004 and Gautier et al., 2018).

Exogenous glucanase enzymes have been used in diets due to their ability to reduce viscosity in the small intestine, allowing for better mineral absorption and digestion. (Annison, 1993). Glucanase allows birds to digest non-starch polysaccharides (NSP) more efficiently by reducing the anti-nutritive effects of beta-glucans and other water soluble NSP. (Langhout et al., 1999). Glucanase can improve growth, feed conversion and apparent metabolizable energy (AME). (Edney et al., 1989). Another study (Rutherfur et al., 2007) using microbial glucanase showed that in diets supplemented with β-glucanase, apparent metabolizable energy (AME) was higher in corn-soy based diets as opposed to those that were not supplemented. Moharrery et al. (2015), reported that at an inclusion rate of 0.5 g kg−1 of β-glucanase, AME nitrogen corrected was improved.

The most common form of glucanase that is supplemented in diets is in the microbial form. In this form, fungal and bacterial hosts are used in the production of microbial enzymes which can potentially lead to the contamination of the product (Pariza, 2001). Genetically modified corn can be used as an alternative to microbial enzymes. In the process of making genetically modified corn, high concentrations of recombinant enzymes are produced within transgenic corn grain to be used as feed additives at low inclusion rates (Pen, 1993; Nyannor, 2008; Gao, 2012). The corn expressed glucanase that was used in this experiment was AC1.

AC1 expresses a recombinant carbohydrase, with endo-β-1,4-glucanase activity (Broomhead et al., 2019).

The objective of this study was to compare the efficacy of corn-expressed glucanase, (Grainzyme® Glucanase AC1]) and high specific activity (Grainzyme® PY1203, [PY1203]), at different inclusion rates in broilers. It can be expected that the addition of corn expressed phytase and glucanase will improve broiler performance, AID, and AME. Additionally, both enzymes can be used as a cheaper alternative since corn is already used in poultry diets.

Materials and Methods

Animal Husbandry, Diet, and Experimental Design

A total of 2,160 Cobb 500 male broiler chicks were used in this experiment. Day-old broilers were equally housed at 24 birds per replicate pen, pens were blocked in a random complete block design. There were 9 replicate pens per treatment. Each pen was lined with re-used litter as bedding and equipped with one tube feeder and a nipple drinker line. Pens were blocked and treatments were assigned at random to one of ten dietary treatments (TABLE 18). The birds were fed a three-phase diet consisting of a starter (d 1-14, crumble), grower (d 15-28, pellet), and finisher (d 29-42, pellet) as seen in Table 19. Pelleting temperatures were kept at 85 C. Birds were allowed ad libitum access to feed and water. Bird management was in accordance with guidelines outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (2010), all procedures were approved by Texas A&M University animal care and use committee (IACUC 2018-0181).

Table 18: Treatment description of birds fed Grainzyme 1203 and AC1 glucanase

Treatment	Description
1	PC
2	NC
3	NC + 0.35 lb/t Grainzyme 1203 + 1.0 lb/t Grainzyme AC1 Glucanase
4	NC + 0.35 lb/t Grainzyme 1203 + 2.0 lb/t Grainzyme AC1 Glucanase
5	NC + 0.35 lb/t Grainzyme 1203 + 3.0 lb/t Grainzyme AC1 Glucanase
6	NC + 0.35 lb/t Grainzyme 1203 + 4.0 lb/t Grainzyme AC1 Glucanase
7	NC + 1.00 lb/t Grainzyme 1203
8	NC + 3.00 lb/t Grainzyme 1203
9	NC + 6.00 lb/t Grainzyme 1203

Table 19: Diet formulations for birds fed Grainzyme 1203 and AC1 glucanase

Ingredient:	Starter PC	Starter NC	Grower PC	Grower NC	Finisher PC	Finisher NC
DDGS	7.000	7.000	12.000	12.000	18.000	18.000
Fat, Animal	2.766	0.250	2.816	0.299	4.105	1.588
Calcium Carbonate	1.344	1.347	1.305	1.309	1.214	1.218
Mono-Dicalcium Phosphorous	0.678	0.674	0.563	0.560	0.330	0.326
Salt, Plain	0.396	0.396	0.360	0.360	0.315	0.315
L-Lysine	0.111	0.117	0.218	0.224	0.182	0.188
DL-Methionine	0.272	0.268	0.240	0.236	0.186	0.182
L-Threonine	0.045	0.044	0.065	0.064	0.022	0.022
Soybean meal	34.882	34.443	25.228	24.782	21.445	21.000
Corn	52.171	55.125	56.870	59.832	53.866	56.826
Choline Chloride	0.050	0.050	0.050	0.050	0.050	0.050
Salinomycin	0.050	0.050	0.050	0.050	0.050	0.050
Phytase (1203)	0.018	0.018	0.018	0.018	0.018	0.018
TAMU Mineral Premix	0.050	0.050	0.050	0.050	0.050	0.050
TAMU Vitamin Premix	0.150	0.150	0.150	0.150	0.150	0.150
Sand	0.018	0.018	0.018	0.018	0.018	0.018

Performance and Sampling Parameters

All birds and feed were weighed on day 14, 28, and 42 for the determination of body weight gain (BWG), feed intake (FI) and the calculation of mortality adjusted feed conversion ratio (FCR). On d28, four birds per pen, were randomly selected, euthanized by cervical dislocation, and intestinal contents were collected from the ileum (between the Meckel's diverticulum and the ileocecal junction) by gently finger-stripping the intestinal segment. On D28, samples were analyzed for apparent ileal digestibility (AID). On D42, samples were analyzed for AME and AME corrected for nitrogen.

Statistical Analysis

All data was analyzed via One-Way ANOVA using the GLM model (Minitab Software) with treatment means deemed significantly different at $P \leq 0.05$. Treatment means that were determined to be significant were further separated using Fishers LSD Test.

Results

Body Weights

The means for body weights can be seen in Table 20. On day 14, the PC and NC performed similarly along with all other dietary treatments. On day 28, the PC and NC performed similarly along with all other dietary treatments. On Day 42, all dietary treatments performed similarly.

Table 20: Average bird weights of birds fed Grainzyme 1203 and AC1 glucanase from d0-42

Treatment	d0 BW	d14 BW	d28 BW	D42 BW
PC	0.039	0.483	1.503[b]	3.002[bc]
NC	0.039	0.484	1.477[b]	2.954[c]
NC + 0.35 lb/t Grainzyme 1203 + 1.0 lb/t Grainzyme AC1 Glucanase	0.039	0.482	1.443[c]	2.960[c]
NC + 0.35 lb/t Grainzyme 1203 + 2.0 lb/t Grainzyme AC1 Glucanase	0.039	0.488	1.494[b]	3.022[abc]
NC + 0.35 lb/t Grainzyme 1203 + 3.0 lb/t Grainzyme AC1 Glucanase	0.039	0.486	1.470[bc]	3.004[bc]
NC + 0.35 lb/t Grainzyme 1203 + 4.0 lb/t Grainzyme AC1 Glucanase	0.039	0.481	1.483[b]	2.975[bc]
NC + 1.00 lb/t Grainzyme 1203	0.039	0.499	1.540[ab]	3.104[ab]
NC + 3.00 lb/t Grainzyme 1203	0.039	0.492	1.594[a]	3.145[a]
NC + 6.00 lb/t Grainzyme 1203	0.039	0.493	1.535[ab]	3.044[abc]
SEM	4.58E-05	1.80E-03	9.71E-03	1.65E-02
P-value	0.492	0.277	0.008	0.05

[a-d] Means within rows with different superscripts differ significantly at P<0.05

Feed Consumption

The means for feed consumption can be seen in Table 21. From d0-28, the PC and NC performed similarly to one another along with all other dietary treatments. From d0-42, the PC and NC both performed similarly along with all other dietary treatments.

Table 21: Average feed consumption (g/bird/day) of birds fed Grainzyme 1203 and AC1 glucanase from d0-42

Treatment	FC d0-14	FC d14-28	FC d0-28	FC d28-42	FC d0-42
PC	37.15[d]	119.34[bc]	76.30	187.69	99.26
NC	38.73[bcd]	118.65[bc]	77.26	194.00	102.61
NC + 0.35 lb/t Grainzyme 1203 + 1.0 lb/t Grainzyme AC1 Glucanase	37.24[cd]	119.19[bc]	77.30	184.13	101.06
NC + 0.35 lb/t Grainzyme 1203 + 2.0 lb/t Grainzyme AC1 Glucanase	39.76[ab]	123.59[abc]	79.20	188.06	103.16
NC + 0.35 lb/t Grainzyme 1203 + 3.0 lb/t Grainzyme AC1 Glucanase	38.92[bcd]	118.57[c]	77.13	189.99	101.66
NC + 0.35 lb/t Grainzyme 1203 + 4.0 lb/t Grainzyme AC1 Glucanase	38.91[bcd]	123.49[abc]	79.34	184.47	102.85
NC + 1.00 lb/t Grainzyme 1203	39.23[bc]	125.14[ab]	80.51	189.61	104.92
NC + 3.00 lb/t Grainzyme 1203	39.18[bc]	128.10[a]	80.41	192.96	102.69
NC + 6.00 lb/t Grainzyme 1203	41.47[a]	124.93[abc]	80.61	186.59	102.57
SEM	0.26	0.81	0.48	1.05	0.62
P-value	0.002	0.027	0.202	0.331	0.701

[a-d] Means within rows with different superscripts differ significantly at P<0.05

Feed Conversion Ratio

The means for feed conversion ratio can be seen in Table 22. From d0-28, all dietary treatments performed similarly. From d0-42, the PC outperformed the NC; all AC1 and Grainzyme 1203 supplemented diets outperformed the NC and performed similarly to the PC.

Table 22: Average FCR of birds fed Grainzyme 1203 and AC1 glucanase from d0-42

Treatment	FCR d0-14	FCR d14-28	FCR d0-28	FCR d28-42	FCR d0-42
PC	1.16[b]	1.67	1.51	1.75[a]	1.61[bc]
NC	1.21[a]	1.71	1.55	1.85[b]	1.69[a]
NC + 0.35 lb/t Grainzyme 1203 + 1.0 lb/t Grainzyme AC1 Glucanase	1.16[b]	1.75	1.56	1.69[a]	1.62[bc]
NC + 0.35 lb/t Grainzyme 1203 + 2.0 lb/t Grainzyme AC1 Glucanase	1.18[ab]	1.74	1.56	1.72[a]	1.64[bc]
NC + 0.35 lb/t Grainzyme 1203 + 3.0 lb/t Grainzyme AC1 Glucanase	1.21[a]	1.72	1.55	1.73[a]	1.63[bc]
NC + 0.35 lb/t Grainzyme 1203 + 4.0 lb/t Grainzyme AC1 Glucanase	1.19[ab]	1.73	1.57	1.73[a]	1.64[b]
NC + 1.00 lb/t Grainzyme 1203	1.16[b]	1.70	1.53	1.69[a]	1.60[c]
NC + 3.00 lb/t Grainzyme 1203	1.19[ab]	1.68	1.53	1.75[a]	1.62[bc]
NC + 6.00 lb/t Grainzyme 1203	1.22[a]	1.71	1.56	1.71[a]	1.63[bc]
SEM	0.01	0.01	0.01	0.01	0.00
P-value	0.029	0.406	0.292	0	0.003

[a-d] **Means within rows with different superscripts differ significantly at P<0.05**

Ileal Digestibility

The means for ileal digestibility can be seen in Table 23. On day 28, the PC outperformed the NC. Birds fed 3.0 kg/MT PY1203 improved (P<0.05) AID Ca compared with NC. Additionally, when evaluating phosphorus content, the PC outperformed the NC. Birds fed 3.0 kg/MT PY1203 improved (P<0.05) AID P compared with NC.

Table 23: Apparent ileal digestibility of birds fed Grainzyme 1203 and AC1 on d28

Treatment	Calcium (%)	Phosphorous (%)
PC	60.86[a]	75.85[a]
NC	47.89[d]	64.92[bcd]
NC + 0.35 lb/t Grainzyme 1203 + 1.0 lb/t Grainzyme AC1 Glucanase	51.74[bcd]	62.8[d]
NC + 0.35 lb/t Grainzyme 1203 + 2.0 lb/t Grainzyme AC1 Glucanase	50.61[cd]	64.8[bcd]
NC + 0.35 lb/t Grainzyme 1203 + 3.0 lb/t Grainzyme AC1 Glucanase	52.03[bcd]	67.43[bcd]
NC + 0.35 lb/t Grainzyme 1203 + 4.0 lb/t Grainzyme AC1 Glucanase	53.85[bc]	64.13[cd]
NC + 1.00 lb/t Grainzyme 1203	54.92[bc]	68.78[bc]
NC + 3.00 lb/t Grainzyme 1203	52.74[bcd]	69.4[bc]
NC + 6.00 lb/t Grainzyme 1203	56.58[ab]	70.43[ab]
SEM	0.676	0.727
P-value	0	0

[a-d] Means within rows with different superscripts differ significantly at P<0.05

AME-N Corrected

The means for AME-N corrected can be seen in Table 24. On day 42, when AME was corrected for nitrogen, the PC outperformed the NC. AME was maintained in treatments 4, 5, 8, and 9.

Table 24: AME and AMEn for birds fed Grainzyme 1203 and AC1 glucanase from d0-42

Treatment	AME	AMEn
PC	3768.23[ab]	3622.253
NC	3461.53[c]	3393.701
NC + 0.35 lb/t Grainzyme 1203 + 1.0 lb/t Grainzyme AC1 Glucanase	3614.4[bc]	2965.609
NC + 0.35 lb/t Grainzyme 1203 + 2.0 lb/t Grainzyme AC1 Glucanase	3655.28[ab]	3024.117
NC + 0.35 lb/t Grainzyme 1203 + 3.0 lb/t Grainzyme AC1 Glucanase	3676.64[ab]	3441.826
NC + 0.35 lb/t Grainzyme 1203 + 4.0 lb/t Grainzyme AC1 Glucanase	3620.49[bc]	3436.809
NC + 1.00 lb/t Grainzyme 1203	3621.72[bc]	3457.311
NC + 3.00 lb/t Grainzyme 1203	3799.97[a]	3157.925
NC + 6.00 lb/t Grainzyme 1203	3671.19[ab]	3188.593
SEM	20.65152	38.64403
P-value	0.01	0.1

[a-d] Means within rows with different superscripts differ significantly at P<0.05

Discussion

In this current experiment, it was shown that AC1 and Grainzyme 1203 supplemented diets made up for the reduction in Ca and aP at almost all dose levels and maintained growth performance. When FCR was evaluated on day 42, it was shown that diets supplemented with AC1 and PY1203, performed similarly to the PC and outperformed the NC. Similar results were seen in a study by Ayres et al., (2010) when diets were supplemented with AC1 an improved FCR was seen however, in their study, they also saw an improvement in body weights as well. These results are like the findings in this study as almost all supplemented diets had an improved FCR however, like Ayres et al., they also saw an improvement in body weights. In a study using AC1, (Jasek et al., 2018) did not see improvements in FCR or body weights throughout the duration when reducing ME by 100 kcal. It can be speculated that he reduction in energy was not large enough to see an impact on growth performance. In this study when feed consumption was evaluated, there were no differences seen between dietary treatments. Similar findings were observed by Bi and Chung (2004) as they did not see a difference in feed consumption.

When evaluating AID on day 28 of this study, it was shown that birds fed 3.0 kg/MT PY1203 improved ($P<0.05$) AID P and AID C compared with NC. It can be hypothesized that the use of phytase allowed for in improved Ca and P utilization which led to improved AID (Cowieson and Adeola, 2005). The increase in Ca and P can be expected because both are large components in a bird's skeleton and phytase has been illustrated to do this (Selle and Ravidran, 2007).

In this study, when AME was evaluated on day 42, at almost all inclusion rates of AC1 and PY1203, the NC was outperformed ($P < 0.05$). A similar trend was seen when AME was

corrected for nitrogen. Based on the composition of AC1, it is capable of hydrolyzing and ameliorating some of the anti-nutritive components commonly found in broiler diets (Jasek et al., 2018). This could explain the differences in AME and AID. In a study by Santos et al., (2008) when using microbial phytase, it was shown that AME, AID, and mineral absorption were all improved. The results from this study show that both PY1203 and AC1 can improve broiler performance and can make up for a reduction in ME. Additionally, it showed that both enzymes improved nutrient utilization and improved broiler performance.

The use of PY 1203, AC1 and CEP can be used as a cheaper alternative to more expensive methods of phytase since phytase can be added into via corn and less will be needed when using PY1203. Making this technology not only a time saver but also a more economical means to feed poultry and still have efficient growth, nutrient utilization, and bone formation.

In conclusion, reduction in dietary Ca and aP negatively affected broiler performance. The combination of AC1 and PY1203 compensated for reduction in aP, Ca and ME at most dose levels. Inclusion of AC1 and PY1203 maintained growth performance of broilers through 42 days of age. Changing the loading rates of either enzyme had no discernible effect at the rates tested. In conclusion, both high specific activity phytase (PY1203) and corn-expressed glucanase (AC1) compensated for the reduction in avP, Ca, and ME at most dose levels. Additionally, at almost all dose-levels growth performance was maintained through day 42. It can be speculated that the reduction in ME was large enough to allow glucanase to have an impact on performance. Future studies using a larger reduction, may show that corn expressed glucanase does improve growth performance. The improvements in digestibility and AME in this study, indicate that it in theory should also influence growth.

CHAPTER VI

CONCLUSIONS AND DISCUSSION

The use of exogenous enzymes has been a common practice in formulation of diets since the late 1900s when the commercialization of phytase occurred. The production of these enzymes involves microbial processes that are considered to be unsafe in the eyes of many. The use of fungal and bacterial hosts for the production of enzymes has led to the concern that other host produced molecules may be co-purified which may lead to contamination of the final product (Pariza, 2001). An alternative to the traditional method of producing exogenous enzymes has been discovered by the use of transgenic corn that can hold a high concentration of enzyme and be fed at low inclusion rates (Pen et al., 1993; Nyannor and Adeola, 2008; Nyannor et al., 2009, Gao et al., 2012; Denbow et al., 1998). Corn is considered to be safe because of its common use in human and animal diets.

The corn expressed glucanase used in these studies (AC1) has endo-β-1,4-glucanase activity that is expressed in corn grain, it is similar to *Thermotoga maritima* with additional amino changes that were introduced to improve thermal stability. Studies by Ayers at el. (2018) showed that AC1 was effective in reducing intestinal viscosity, when added to a high NSP diet. Additionally, Jasek et al. (2018) demonstrated improvements in body weight when 100 β-glucanase units (β-Glu-U) per kg were added to a reduced energy, corn-soybean meal diet. These findings align with the findings in the experiment that was discussed in chapter III, as it was shown that AC1 can improve ileal digestibility and AME. These results can be explained by the composition of AC1 and its ability to hydrolyze and ameliorate some of the anti-nutritive components commonly found in broiler diets (Jasek et al., 2018). Previous literature suggests that the use of an exogenous glucanase enzyme can improve broiler

performance. In a study by Karunaratne et al. (2021), improvement in bodyweights were seen when feeding graded levels of hulless barley supplemented with a β-glucanase enzyme. An improvement in feed to gain ratio (1.5 versus 1.67) was also seen. Additionally, Mathlouthi et al. (2002) found that feeding a corn or wheat and barley-based diet supplemented with a and β-glucanase at 20 mg·kg–1, weight gain (619 vs 605 g), feed intake (924 vs 899 g) and feed conversion ratio were all improved. The results from these studies show the ability of microbial and corn expressed glucanase to improve AME, AID, and broiler performance as well as reduce intestinal viscosity.

The corn expressed phytase (PY1203) enzyme that was used in this study was an Escherichia coli phytase called Phy02 and has been reported to be safe for use in both poultry and swine (Ligon, 2016; Broomhead et al., 2018; Blavi et al., 2019). Previous experiments show that corn expressed phytase can improve average daily gain, feed efficiency, bone mineralization and P digestibility in young pigs that were fed Ca and P deficient diets (Lee et al., 2017; Knapp et al., 2018, Broomhead et al., 2018; Blavi et al., 2019). Its efficacy compared to microbial phytase has yet to be determined. The data from chapter II and chapter IV indicate that bone ash and AID can be improved by the supplementation of corn expressed phytase. This aligns with data seen in previous experiments. Shirley and Edwards (2003) found when supplementing phytase from 0 to 12,000 FTU/kg, tibia ash and tibia weight were improved. Tibia ash was improved from 26 to 42% and tibia weight was increased from 0.200 to 0.601 g/ tibia. Similarly, Pieniazek et al. (2017), when using phytase ranging from 0 to 2,000 FTU/kg. They found that tibia weight was improved by 478.4 g/tibia. Ravindran et al. (2008) found that when feeding an *Escherichia coli* derived phytase at 500 FTU/ kg, ileal Ca digestibility was improved by 27%. Rutherford et al., (2004) saw improvements P digestibility (11.8%) when

feeding a low P diet containing 750 FTU/kg *Peniophora lycii* phytase. Additionally, data from similar studies showed that phytase has the ability to improve broiler performance. Nelson et al. (1971) saw an increase in body weights by 131% when supplementing phytase at 7,600 FTU/kg. Similarly, Kornegay et al. (1996) saw improvements in body weights, FCR, and feed consumption when feeding a microbial based phytase at 1,000, 800, and 600 FTU/kg.

In conclusion, the addition of corn expressed phytase has been shown to improve broiler performance, AID, and AME. Although, corn expressed glucanase did not show an improvement in growth performance in these studies, it has been shown to aid in performance in other studies. Jasek et al. (2018), saw similar results as the ones in this study with no improvements in growth performance parameters. One explanation for this could be that there was not a large energy reduction in these diets to see the benefits of using supplementing glucanase. Both studies only used a 100-kcal reduction in ME, perhaps a reduction of 500 kcal or 1000 kcal would allow for glucanase to have an impact on performance parameters. Additionally, the corn expressed enzymes used in these experiments can be used as cheaper and more economical alternatives to microbially produced enzyme by allowing for the safe production of exogenous enzymes using transgenic corn. These enzymes can be utilized in the industry in years to come to help reduce feed costs. Additionally, it can be concluded that the super dosing of phytase can lead to an improvement in growth performance, digestibility, and energy utilization however, there can be a threshold for super dosing. The results from chapter V showed no improvements when using over 1.5 kg/t of Grainzyme 1203. This could indicate that birds may only be able to utilize phytase at certain loading rate and do not see performance benefits past this threshold. In the future, studies investigating this hypothesis may show that birds can only utilize a certain dose of phytase.

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