IMPACT OF XYLANASE AND OTHER EXOGENOUS ENZYMES, FED INDIVIDUALLY AND IN COMBINATION, WITH DIRECT FED MICROBIALS ON BROILER GROWTH PERFORMANCE

A Dissertation

by

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ABSTRACT

The aim of this research program was to evaluate the inclusion of exogenous enzymes, individually and in combination, with direct fed microbial products on broiler growth performance and nutrient digestibility over a series of experiments.

Inclusion of a thermotolerant xylanase product derived from *Pichia pastoris* significantly improved feed efficiency in broilers fed a corn-based diet with DDGS. When xylanase was supplemented a 10,000, 20,000, and 40,000 units, a clear dose response was observed with 20,000 unit supplementation showing consistent improvements in body weight (BW), ileal digestible energy (IDE), and feed conversion ratio (FCR) leading to improved feed efficiency and overall broiler performance.

Supplementation of a *Bacillus* based direct fed microbial (DFM) significantly increased broiler performance compared to the non-supplemented control through improvements in feed consumption (FC), BW, and body weight gain (BWG) which were comparable to antibiotic growth promoter (AGP) inclusion. Furthermore, inclusion of high levels (10, 15, and 20%) of distiller's dried grains with solubles (DDGS) significantly increased FCR resulting in reduced broiler performance. At the conclusion of the trial, increasing inclusion levels of DDGS did not impact DFM efficacy. However, DFM inclusion improved feed efficiency in DDGS containing diets regardless of level.

The inclusion of a feed additive containing a combination of xylanase, amylase, and protease (XAP) + DFM decreased finisher phase FCR as well as cumulative d 42 FCR and produced similar results to AGP inclusion. Foot pad lesion score was also significantly improved with XAP + DFM inclusion to levels similar with AGP inclusion. The inclusion XAP + DFM also improved caloric efficiency by reducing the amount of energy (kcal) needed to produce one kg of body weight gain. Finally, the inclusion of XAP + DFM resulted in improved broiler performance regardless of the presence or absence of AGP.

In totality, this research program confirms that the addition of xylanase is an effective way to increased nutrient digestibility and that combining effective exogenous enzymes with DFM can be an effective alternative to AGP inclusion. Additionally, the performance benefits of XAP + DFM inclusion can be realized regardless of the presence or absence of AGP.

DEDICATION

This dissertation is dedicated to my family and all of those who have played a part in shaping the person I have become and have helped me create my own path of success and achievement.

To my grandparents Rose, Frank, Frances and Franklin. Without the values and life lessons you instilled in me as a child, I would have never been able to accomplish what I have done thus far. Your years of hard work and tireless dedication have without a doubt make me the man I am. I love you all very much.

To my sisters, Hailey, Hannah, and Natalie. I know I've set the bar high for you but each one of you have the capabilities to surpass me if you put your mind to it. If you put your mind to it, you can accomplish anything. I promise.

To my father, Erasmo, and mother, Janet. Words cannot describe what you have done for me, and I will never be able to give back to you what you have given to me. There were times when I thought I wouldn't make it and that the right thing to do was to stop and come home, but your selfless love and support got me through that. I rarely use the word 'perfect' because there are very few things that are truly flawless, but both of you are the epitome of perfect parents; I could not ask to have better role models. I love you.

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NOMENCLATURE

ADF	Acid Detergent Fiber
AGP	Antibiotic Growth Promoter
AME	Apparent Metabolizable Energy
AOAC	Association of Agriculture Chemists
BMD	Bacitracin Methylene Disalicylate
BW	Body Weight
BWG	Body Weight Gain
BXU	Birchwood Xylanase Unit
CFU	Colony Forming Unit
d	Day
DDGS	Distillers Dried Grains with Solubles
DFM	Direct Fed Microbial
EU	European Union
FC	Feed Consumption (gram/bird/day)
FCR	Feed Conversion Ratio
FPD	Foot Pad Dermititis
FTU	Phytase Unit
ft	Feet
g	Gram
GRE	Glycopeptide Resistant Enterococci

hr	Hour
IACUC	Institutional Animal Car and Use Committee
IDE	Ileal Digestible Energy
kcal	Kilocalorie
kg	Kilogram
LO-DDGS	Low-Oil Distillers Dried Grains with Solubles
M	Molar
m	Meter
ME	Metabolizable Energy
mL	Milliliter
mM	Millimolar
NC	Negative Control
nm	Nanometer
nmol	Nanomole
NSP	Non-Starch Polysaccharides
pH	Potential of Hydrogen
PC	Positive Control
rRNA	Ribosomal Ribonucleic Acid
SBM	Soybean Meal
SCFA	Short Chain Fatty Acids
UV	Ultraviolet
VFA	Volatile Fatty Acids

VM	Virginiamycin
XA	Xylanase and Amylase
XAP	Xylanase, Amylase, Protease
XU	Xylanase Units
XYL10	Xylanase at 10,000 XU/kg
XYL20	Xylanase at 20,000 XU/kg
XYL40	Xylanase at 40,000 XU/kg

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Numerous factors contribute to the overall cost of raising a modern broiler with feed cost representing nearly 70% of total cost. The primary components of most broiler feeds in the United States include corn, which is utilized as a source of caloric energy in the form of starches, and soybean meal (SBM) as the primary source of dietary protein. Similarly, Europe and other countries throughout the world often use SBM to meet the dietary protein requirements for broilers. However, numerous other cereal grains (wheat, oats, rye, barley, etc.) are fed in order to fulfill energy requirements, typically based on geographic availability. Comparatively, non-viscous cereal grains such as corn have been shown to be more digestible than viscous cereal grains such as wheat, rye, and barley predominantly due to the difference in concentration of non-starch polysaccharides (NSP) in viscous grains versus non-viscous grains.

Non-starch Polysaccharides and Anti-Nutritive Factors

Non-starch polysaccharides are the major dietary fiber components which comprise the cell walls of the major cereal grains as well as SBM (Slominski, 2011). Due to the lack of sufficient endogenous enzymatic production, NSP are considered to have anti-nutritive properties as a result of their ability to impede digestion and absorption. Previous research has shown that total NSP content of corn and soybean was 9.32% and 29.02% of dry weight, respectively (Malathi

and Devegowda, 2001). Although the NSP content of SBM is much higher than corn, the NSP content of corn has a much greater impact since it typically constitutes approximately two-thirds of the total diet formulation. The anti-nutritive properties of NSP can potentially negatively impact broiler performance; however, NSP could also be utilized as a potential energy source through proper NSP degrading enzyme supplementation.

The general structures of viscous and non-viscous cereal grains are similar in that both contain some form of outer coating, which acts as a protective barrier and is composed of mostly cellulose, xylans, and significant amounts of lignin, and endosperm tissues containing the aleurone layer which is insoluble and is primarily composed of arabinoxylans and β-glucans (Knudsen, 2014). Arabinoxylans are long, complex polysaccharides composed of two sugars, arabinose and xylose, with β-(1-4) branched linkages. β-glucans are comparatively more simplistic in structure in that they are composed of only linear polymers of glucose with β-(1-3), (1-4) glycosidic linkages (Williams et al., 1997). The composition of arabinoxylans, β-glucans, and other NSP has also shown to vary amongst the various cereal grains. For instance, the viscous cereals are comprised mainly of soluble arabinoxylans (wheat) and β-glucans (barley and oats) while non-viscous cereals such as corn and sorghum contain mainly insoluble forms of arabinoxylan (Masey O'Neill et al., 2014). It is these qualities which contribute to the anti-nutritive properties of NSP in viscous and

non-viscous cereals, ultimately leading to impeded nutrient digestion and absorption and reduced broiler performance.

Historically, there have been two mechanisms attributed to the anti-nutritive aspects of NSP 1) increased water holding capacity of digesta leading to an increase in intestinal viscosity, and 2) encapsulation of nutrients due to the lack of sufficient endogenous enzyme secretion. The increases in intestinal viscosity and reduction in performance and nutrient utilization have been closely associated with feeding diets high in viscous cereals (wheat, rye, barley, etc.) composed of water-soluble and viscous arabinoxylans and β-glucans (Bedford and Schulze, 1998; Slominski, 2011). Previous research has shown a correlation between performance and intestinal viscosity as concentration of viscous cereals in the diet increase. A study conducted by Adeola and Bedford (2004) observed that feeding a high-viscous wheat-based diet (153 g/kg of NSP) compared to a low-viscous wheat-based diet (94 g/kg of NSP) significantly reduced performance as well as increased the viscosities of duodenal and ileal digesta by 45 and 56%, respectively. Similarly, Kiarie et al. (2014) confirmed through diet analysis that wheat-based diets contained more than double the soluble NSP content than corn-based diets. This increase in soluble NSP content has been shown to be responsible for increased digesta viscosity. Based on these analysis, the authors hypothesized that the increase in soluble NSP content was responsible for an increase in jejunal viscosity in wheat-based diets. Results such as those mentioned in the aforementioned studies have shown consistent results with

regards to soluble NSP content in viscous cereal grains. In contrast, studies which have focused on the impact of NSP content in corn-based, non-viscous, diets have focused more on the impact of encapsulated nutrients and the impact of insoluble NSP content rather than intestinal viscosity and soluble NSP content. Non-viscous cereals such as corn contain negligible amounts of β-glucans and a low content of soluble pentosans which attribute to the lack of issues associated with viscosity (Gracia et al., 2003). Instead, nutrient utilization is the primary area of focus. According to Cowieson (2010), a typical corn/SBM based diet contains approximately 400-450 kcal/kg of encapsulated and indigestible energy (18% from undigested fat, 45% from undigested protein, and 37% from undigested starch). An effective method of negating these anti-nutritive properties in corn/SBM based diets has been through the proper application of xylanase and other NSP degrading enzymes which aid in ameliorating the encapsulation of nutrients.

Effect of Xylanase on NSP in Viscous and Non-viscous Cereals

Xylanase is a type of hemicellulase that cleaves the complex xylan molecule present in the cell wall of plants into smaller fragments allowing for greater nutrient digestion and utilization (Kolenová et al., 2006). The main chain of xylan is composed of β-xylopryranose (xylose) residues connected by β-(1, 4) linkages (Bastawde, 1992). Furthermore, the xylan backbone of viscous cereals is characterized by arabinose sidechains which are attached to the xylose residues creating arabinoxylans (Figure 1). It is this property that imparts a

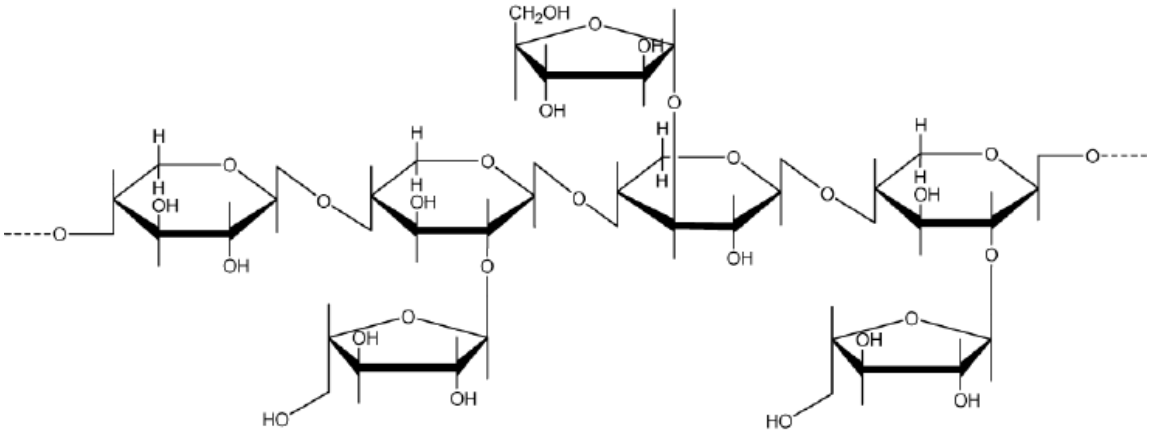

Figure 1. Arabinoxylan structure reprinted from (Miguel et al., 2013) Arabinoxylan molecule with linear xylan main chain connected by β-(1, 4) linkages and randomly attached arabinofuranose residues by either α-(1, 3) or α-(1, 2) linkages.

water soluble nature to NSP in these cereal grains (Ward, 1996). The increase in intestinal viscosity is due to the entanglement of these long polymers, resulting in an increase in water holding capacity of the digesta. Supplemental xylanase cleaves the β-linkages, breaking down the xylan backbone into individual xylose subunits allowing for endogenous enzyme digestion, access to nutrients which would be ordinarily inaccessible, and a reduction in intestinal viscosity.

The supplementation of xylanase in wheat-based diets, as well as other viscous cereals, and its impact on broiler performance has been reviewed extensively (Bedford and Classen, 1992; Bedford and Schulze, 1998). A 21-day study conducted by Wu et al. (2004) examined the impact of xylanase, individually and in combination with phytase, on broiler performance as well as digesta viscosity and gut morphology in a wheat-based diet. Performance results from the experiment concluded that the addition of xylanase individually into a wheat-based diet significantly improved BWG (16.5%), FC (10.8%), and FCR (4.9%) compared to the control with no addition of xylanase. Similarly, the addition of xylanase significantly reduced intestinal viscosity in the duodenum, jejunum, and ileum (28.6, 22.5, and 38.5%, respectively) as well as weights of the tissues (17.7, 15.8, and 14.6%, respectively). The authors hypothesized that improvements in performance as well as reductions in intestinal viscosity and small intestine tissue weights were directly correlated with the reduction in intestinal viscosity; this resulted in a reduction in pathogenic bacterial activity,

which can potentially stimulate intestinal tissue growth helping explain the increased intestinal tissue weight of the wheat based control fed birds. In a similar study, Zhang et al. (2014) examined the impact of supplemental xylanase into wheat-based diet on broiler performance, nutrient digestibility, NSP degradation, and concentrations of free sugars and oligosaccharides in the digesta. Results from this study, similar to those seen in the aforementioned experiment, showed that xylanase supplementation resulted in a significant improvement in BWG as well as FCR (5.8 and 5.3% respectively) compared to the wheat-based control diet. Additionally, ileal digestibility of crude protein (3.5%), starch (9.3%), soluble NSP (43.9%), and insoluble NSP (42.2%) as well as the concentrations of arabinose and xylose in digesta were significantly increased with xylanase supplementation compared to the control. The authors concluded that the significant increase in presence of arabinose and xylose in the digesta and improvements in nutrient digestibility were a result of xylanase supplementation, leading to a decrease in intestinal viscosity and an improvement in broiler performance.

As mentioned previously, the impact of xylanase supplementation in diets containing viscous cereals has produced more definitive and consistent results due to the impact the enzyme has on the water soluble properties of NSP in cereal grains with high levels of soluble NSP. Previous research has shown that the water soluble content of NSP in wheat, barley and rye to be 24, 25 and 46 g/kg respectively while corn is only 1 g/kg (Choct, 1997; Englyst, 1989). The lack

of water soluble NSP in corn is the principal component for the lack of impact xylanase supplementation has on intestinal viscosity in non-viscous cereal based diets as an increase in viscosity is not observed; instead the primary mode of action in non-viscous cereal grains is the release of encapsulated nutrients from the insoluble NSP fraction and a reduction in the cage effect leading to an improvement in broiler performance. Insoluble NSP is the main component of the endosperm cell wall of corn and previous research has indicated that xylanase can potentially increase access of endogenous and exogenous enzymes to protein, starches, and potential other nutrients within the endosperm cell by cleaving the β-linkages associated with xylan molecule's structure (Cowieson, 2005). There are numerous published reports discussing the impact of xylanase in combination with other NSP degrading enzymes in corn-based diets (Meng and Slominski, 2005; Olukosi et al., 2007). Recent advancements in enzyme technology have provided more consistent results with individual inclusion of xylanase in corn-based diets. Williams et al. (2014) and Latham et al. (2016) evaluated the impact of xylanase inclusion in low energy diets and determined that xylanase inclusion was able to significantly improve FCR compared to a low energy control diet. The impact of a thermostable xylanase in diets containing one variant of corn source harvested from five geographically different areas in China was examined by Masey O'Neill et al. (2012). Results from the study concluded that no differences were observed in nutrient content or digestibility; however, the inclusion of xylanase delivering 16,000 BXU/kg

significantly improved all cumulative performance parameters (FCR, BWG, FC) as well as IDE and apparent metabolizable energy (ME). A similar study conducted by Cowieson et al. (2010) evaluated the impact of xylanase individually and combination with glucanase in corn/SBM diets. Main effects from the experiment indicated that cumulatively through day 42, inclusion of xylanase delivering 16,000 BXU/kg as well as individual inclusion of glucanase and the combination of the two enzymes significantly reduced FCR compared to the low energy control. Based on the results from these studies, it can be inferred that the improvements in broiler performance were a result of increased nutrient utilization, evident from the improvements in FCR and IDE. This indicates that xylanase supplementation may provide access to nutrients which would otherwise be inaccessible due to the insoluble NSP matrix that composes the endosperm cell wall of the corn kernel.

Supplementation of xylanase and other exogenous enzymes have almost become routine within the poultry industry. However, from a historical point of view, inclusion of AGP were some of the first additives used to help maximize broiler performance.

Antibiotic Growth Promoters

Subtherapeutic inclusion of AGP has been utilized in poultry production for over 60 years, focusing primarily on improving performance and efficiency. Although the inclusion of AGP can have an impact on growth, the predominate impact is on efficiency due in part to the interactions the antibiotics have with the

gastrointestinal microbiota and the lack of effect on germ-free birds (Bedford, 2000a). AGP inclusion has been known to illicit improvements in efficiency through multiple modes of action including: competitive exclusion of enteric pathogens and non-indigenous microbes, stimulation of the intestinal immune system, and secretion of various short chain fatty acids (SCFA) and other nutrients from the microbiota that are then available for the host (Dibner and Richards, 2005).

Diet composition has been shown to be a factor that contributes to the response AGP inclusion has on the host animal. It has been suggested that nutritionists formulating diets for monogastric animals should take an approach similar to the one taken by ruminant nutritionists in that formulating diets should not only be done to meet or exceed the animals nutrient requirements but also to insure stability of the microbiota. Monogastric animals, such as poultry, do not possess a rumen which is a large fermentation chamber in ruminant animals that hosts vast amounts of bacterial species and is often the basis by which ruminant nutritionists make conclusions on how to properly feed the animal. Ruminant nutritionists often make dietary conclusions based on the impact the diet type will have on the microbiota located in the rumen because ensuring the stability of the microorganisms in the rumen is essential to the success of the animal. From a nutritional standpoint, monogastric animals are less dependent on the microbiota located in the ileum and ceca for survival as compared to ruminants and the bacteria there. However, diet type has shown to have an influence on microbiota

and digestive capacity of monogastric animals which may have an impact on growth performance. Diets containing poorly digested ingredients such as viscous cereal grains have been shown to increase the amount of available starch and protein which able to reach the lower intestine and thus has an impact on microbiota population density and species (Vahjen et al., 1998). Previous research has shown that feeding rye-based diets resulted in much larger anaerobic bacteria populations in the ileum than those based on corn (Wagner and Thomas, 1978). In response to these increases and fluctuations in bacteria populations brought on by these poorly digested ingredients, the bird responds by increasing the rate of digestive enzyme production and intestinal size increases to compete for the unabsorbed nutrients (Angkanaporn et al., 1994; Brenes et al., 1993). This in turn leads to an increase in the number of immature enterocytes along the villi which are incapable of fully utilizing nutrients that may be present and their digestive capacity is not yet fully realized. These changes in gastrointestinal morphology negatively impacts the microbiota and often results in intestinal disorders associated with the fluctuations and changes in bacteria species distribution (Bedford, 2000a). Historically, a strategy often employed to mitigate these fluctuations in bacterial species in response to feeding poorly digested ingredients was to utilize AGP as they would specially target the bacteria responsible for causing these intestinal disorders.

Two of the most widely used AGP used in poultry production have been bacitracin methylene disalicylate (BMD) and virginiamycin (VM). Although these

products have been used subtherapeutically to improve performance and efficiency by suppressing the growth of gram-positive bacteria, the modes of action differ. Bacitracin methylene disalicylate is a branched, cyclic decapeptide produced by *Bacillus licheniformis* with the main mode of action including: inhibition of cell wall biosynthesis through dephosphorization of C_{55} – isopreyl pyrophosphatase which is responsible for the formation of the peptidoglycans located within the bacterial cell wall (Butaye et al., 2003; Kahn et al., 2005). Conversely, VM is a fermentation product produced from *Streptomyces virginiae* whose primary mode of action is the result of a synergistic interaction between two molecules (M_1 and S_1) (Cocito, 1979). Together, these molecules bind to the 50-S ribosomal subunit, decreasing its activity and suppressing bacterial protein synthesis (Chinali et al., 1981). An experiment conducted by Miles et al. (2006) explored the effect of BMD and VM on broiler performance and gastrointestinal growth parameters and morphology at various ages. The authors also noted that these AGP were used because it is understood that these products possess different modes of action, as mentioned previously. Performance results from the study indicated no significant difference in feed intake was observed cumulatively throughout the duration of the experiment between treatments fed AGP or control; however, AGP inclusion significantly increased average BW. Also, inclusion of VM significantly reduced FCR compared to the control with BMD being intermediate between the treatments. The impact of AGP on the various intestinal parameters measured on the duodenum and ileum (length,

weight, muscularis interna thickness, muscularis muscosae thickness, lamina propria core area, villus area, and lamina propria percentage of villus area) tended to be variable and in some instances displayed an interaction with sex, age of the bird, and treatment type. The authors concluded that there was no simple explanation for the complex relationship between the host animal and the microbiota, although results from the experiment did help verify that AGP inclusion is able to influence gastrointestinal microbial populations resulting in differing effects on gastrointestinal morphology. In a separate but similar study, Neumann and Suen (2015) compared the effects of feeding VM or BMD on broiler gastrointestinal microbiota. Analysis of jejunal and cecal bacterial microbiota was conducted through pyrosequencing of the V6-V8 region of the 16S rRNA gene and quantitative PCR. Summarization of the results suggested that VM had a more pronounced impact on broiler gastrointestinal tract bacterial communities relative to BMD primarily through significant enrichments in the genus *Faecalibacterium* in the ceca and *Lactobacillus* in both the jejunum and ceca. Results such as these described in the previous experiments help give validity as to the effectiveness of AGP in broiler diets.

In recent years however, there has been growing concern amongst consumers surrounding subtherapeutic inclusion of AGP and the fear of potential antibiotic resistance. Growing consumer pressure for increased antibiotic free production coupled with the 2006 complete ban of AGP in Europe has forced integrators to search for alternative products to AGP. One particular product that

has gained a large portion of the attention in the last decade has been the application of DFM.

Direct Fed Microbials

When discussing DFM type products, it is important to first understand the nomenclature that is used to describe these additives. Direct fed microbials are simply defined as products that contain live microorganisms administered into feed. However, due to the prophylactic benefit some of these products exhibit on the host, the terms DFM and probiotic are often used synonymously. If a DFM is also referred to as a probiotic, it is defined as a product that contains a live microorganism that, when administered in adequate amounts, confer a health benefit on the host (Hill et al., 2014). Appropriate supplementation of these products is believed to prevent or even attenuate the growth of potential enteric pathogens in poultry, resulting in enhanced bird performance. These qualities have prompted increased interest into the usage of DFM as potential alternatives to AGP (Khan, 2013).

Direct fed microbials are able to influence intestinal microbiota and morphology through various mechanisms and modes of action including: competitive exclusion, enhanced gastrointestinal tract and endogenous enzymatic function, and stimulation of the bird's immune system. Competitive exclusion is perhaps the most widely discussed and accepted mode of action for DFM and has been shown to illicit other responses within the bird's gastrointestinal tract. The effectiveness of the competitive exclusion concept lies

in the ability of the DFM to directly and indirectly compete for nutrients and physical attachment sites along the epithelium, potentially reducing colonization by enteric pathogens (Callaway et al., 2008). As a result, this creates an environment within the gastrointestinal tract that is suitable for native, beneficial bacteria and endogenous enzyme activity. Under these ideal conditions, various other positive effects occur including: a reduction in pH of the gastrointestinal tract to a level that is unsuitable for growth of pathogenic bacteria, production of SCFA and volatile fatty acids (VFA) which provides a potential source of energy for other tissues while also being toxic to some pathogenic bacteria, and enhanced epithelial cell function due to improvements in intestinal villi:crypt depth ratio which has been directly linked to increased nutrient digestibility (Ajuwon, 2015; Alloui et al., 2013; Awad et al., 2009; Callaway et al., 2008; Chichlowski et al., 2007; Ferreira et al., 2011). Previous research has shown that DFM are able to influence host immune system responses in multiple ways to include increased antibody production, up-regulation of cell-mediated immunity, enhancement of dendritic cell – T cell interaction, and augmented Toll-like receptor signaling (Galdeano et al., 2007; Ng et al., 2009). Although a clear interaction between DFM and the immune system of the host exists, there is still limited knowledge how the avian immune system is regulated in the gut and how the host differentiates between commensal and pathogenic bacteria (Chichlowski et al., 2007).

An assortment of bacterial species have been used as sources of DFM products including *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Escherichia*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, as well as a variety of yeast species (*Saccharomyces cerevisiae*) (Patterson and Burkholder, 2003). According to Simon et al. (2001), *Lactobacillus* and *Bifidobacterium* species have been used most extensively in humans while *Bacillus*, *Enterococcus*, and *Saccharomyces* yeast have been the most common organisms used in livestock. These products can further be divided into two classifications: (1) colonizing species, such as *Lactobacillus* and *Enterococcus* and (2) free flowing non-colonizing species such as *Bacillus* (spores) and *Saccharomyces cerevisiae* (Huyghebaert et al., 2011).

Recently, the amount of attention given to DFM products derived from *Bacillus* species has increased considerably due in part the resilient nature of the bacterial spore (Harrington et al., 2015). These spores have the ability to withstand an assortment of environment changes including heat, chemical exposure, changes in pH, UV radiation, and will remain in a dormant state until sufficient nutrients are presented (Hooge, 2003; Nicholson, 2002). The aforementioned properties are responsible for the interest in *Bacillus* species DFM products for use in broiler production. Previous research has shown the effectiveness of supplementing a *Bacillus* DFM on broiler performance (Hooge, 2003; Hooge et al., 2004; Jeong and Kim, 2014). A trial conducted by Harrington et al. (2015) examined how the supplementation of a *Bacillus subtilis* based DFM in reduced ME diets (approximately 60 kcal/kg reduction) would impact broiler

performance in comparison to industry-standard energy diets. Results from the study showed that supplementation of the *Bacillus subtilis* DFM significantly increased d 42 BWG and reduced mortality-adjusted FCR compared to the non-supplemented treatments. It was also determined through regression analysis that supplementation of the *Bacillus subtilis* DFM had an overall ME contribution of +62 kcal/kg feed. This energy contribution was then later associated with a financial saving in feed cost per kilogram of BW gain of $0.018/kg in a 2% ME reduced diet compared to the standard 100% ME diet. Although it was not quantified, it can be suggested that the energy contribution may have been a result of the production of SCFA and VFA. The production of these fatty acids could have stimulated epithelial cell function resulting in an improved villi:crypt depth ratio, thus improve nutrient utilization. The authors concluded that although supplementation of the *Bacillus subtilis* DFM improved broiler performance in low ME diets, further research is still needed to determine if similar results would occur in diets which contain different cereal grains. Another study conducted by Lee et al. (2010a) hypothesized that *Bacillus subtilis* DFM as an immunomodulating agent would increase the resistance of broilers to experimental coccidiosis (*Eimeria maxima*). Results from the study indicated that broilers challenged with *Eimeria maxima* significantly reduced BWG (18.8%) and increased lesion score severity compared to the non-infected control. The effects of the various *Bacillus subtilis* strains were variable throughout the study which was too be expected since it is assumed that different strains possess

different characteristics. In summary, supplementation of *Bacillus subtilis* DFM products significantly improved BW in *Eimeria maxima* infected treatments as well as increased serum nitric oxide levels (marker of innate immunity following an *Eimeria* infection). From these results, the authors concluded that supplementation of *Bacillus subtilis* DFM reduced the clinical signs of *Eimeria maxima* infection in broilers while also enhancing host innate and acquired immunity in a bacterial strain-dependent manner.

Increased consumer pressure for the removal of AGP from broiler production will undoubtedly create a void in diet formulation. These additives have traditionally been an essential component used to improve broiler performance through mitigation of enteric pathogen colonization. Currently, the industry is on the cusp of a major shift in how enteric pathogens are controlled. As a result, a number of nutritional strategies have been explored in order to help replace AGP. Some of these strategies include the use of exogenous enzymes, DFM products and probiotics, essential oils, prebiotics, synbiotics, phytobiotics, or combinations of these products (Bedford, 2000a; Roberts et al., 2015).

Combination of Xylanase and Other Exogenous Enzymes with DFM

One potential approach is the combination of a multi-enzyme complex containing xylanase, amylase, protease (XAP) and DFM products. From a physiologic standpoint, a synergistic effect may be achieved with the proper supplementation of these additives in combination. It has been hypothesized that xylanase can have a role in helping determine gut microbiota populations as

a result of the oligomers produced from the degradation process which can be used as a nutritional source for the native microbiota fermentation (Engberg et al., 2004). As mentioned previously, xylanase supplementation is effective in degrading the arabinoxylan backbone present in the cell wall of major cereal grains, releasing xylo-oligosaccharides as an end product. These xylo-oligosaccharides then have the ability to be hydrolyzed by beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* species located in the hindgut, resulting in increased synthesis of SCFA (Thammarutwasik et al., 2009; Wang et al., 2005). The production of these SCFA can result in positive effects on gut health and enhanced digestion and absorption in the small intestine through peptide YY production (regulatory peptide shown to reduce appetite) which results in delayed gastric emptying and duodenal transit rates (Singh et al., 2012).

Inclusion of supplemental α – amylase has also been shown to significantly improve broiler performance through increased nutrient digestibility and a reduction in endogenous amino acid losses (Gracia et al., 2003; Jiang et al., 2008). Amerah et al. (2016) reported that supplemental amylase, individually and in combination with xylanase and protease, significantly improved nitrogen digestibility compared to a negative control diet. Previous research has also shown that the weight of the pancreas as a percentage of BW decreases with α – amylase, indicating that secretion of pancreatic enzymes might be affected by the concentration of enzymes and substrates or products of their hydrolysis in the

lumen of the small intestine (Moran, 1985; Snook and Meyer, 1964). The authors speculated that the reduction in pancreas weight could have been related to less secretion of endogenous amylase due to the presence of exogenous amylase in the diet, sparing energy for growth of other tissues (Gracia et al., 2003).

Similar to supplemental amylase, protease has also been shown to improve broiler performance through significant improvements nutrient and amino acid digestibility. Cowieson et al. (2017) supplemented exogenous protease with and without ascorbic acid and explored the effect these products had on performance, nutrient digestibility, and gut physiology and morphology. Results from the study showed that supplementation of exogenous protease: significantly increased BWG, FCR, increased villus height and crypt depth, decreased epithelial thickness and goblet cell numbers in the jejunum, increased apparent coefficients of ileal nitrogen, essential amino acids, non-essential amino acids, and total amino acids. The authors speculated that the changes in gastrointestinal morphology with supplemental protease were indicative of enhanced gut integrity and suggest beneficial effects of protease that extend beyond increased amino acid recovery.

With the mechanisms of these enzymes taken into consideration and the impact DFM products have shown to have on gastrointestinal microbiota of broilers, it can be speculated that a synergistic effect may be achieved with the combination of these additives. However, unlike the numerous published studies

that have evaluated exogenous enzymes and DFM individually, there is limited published research regarding the combination of these products (Murugesan and Persia, 2015; Vandeplas et al., 2009). A study conducted by Dersjant-Li et al. (2015) evaluated the effect of a combination of multi-enzymes containing XAP and DFM comprised of three strains of *Bacillus amyloliquefaciens* on performance and welfare parameters in broilers raised under commercial production settings and determined the combination of the products resulted in improved feed efficiency early in the study while BWG was improved in the later stages. Similarly, XAP and DFM combination improved litter quality and reduced foot-pad lesion scores leading the authors to conclude that a positive benefit may be achieved through the supplementation of XAP and DFM.

The incorporation of exogenous enzymes as well as other feed additive products has become commonplace within the broiler industry. These products have been successful in not only maximizing performance of the animal but have also minimized overall cost of the formulation. However, nutritionists are constantly tasked in finding new strategies and technologies in order to meet the changing consumer preferences and demands. Therefore, the aim of the present research program was to evaluate, through a series of experiments: (1) the effect of a new form of thermotolerant xylanase on broiler performance and ileal digestible energy, (2) to determine the efficacy of a *Bacillus* based DFM on broiler performance, and (3) evaluate the effect of a feed additive containing XAP

and DFM in comparison to two known sources of AGP, and (4) in combination with AGP.

CHAPTER II

EVALUATION OF A THERMOTOLERANT XYLANASE ON BROILER GROWTH PERFORMANCE AND DIETARY ILEAL DIGESTIBLE ENERGY VALUE*

Introduction

The primary ingredients used in the majority of broiler diets fed in the United States include corn and SBM, with corn inclusion reaching in excess two-thirds of the total formulation. Due to gastrointestinal limitations and minimal endogenous enzyme production, broilers lack the digestive capacity to utilize the full nutritive value of the diet. In an effort to maximize nutrient utilization, the use of exogenous enzymes has become common in the broiler industry in order to improve the nutritive value of feedstuffs predominantly through solubilization of NSP (Slominski, 2011).

Non-starch polysaccharides are a major dietary fiber component comprised of both cellulosic and non-cellulosic polysaccharides within the cell wall of cereal grains (Slominski, 2011). Although NSP concentration in corn is less than that of SBM, the presence of NSP in corn is much more impactful due to the high inclusion rates within a typical corn/SBM diet (Yegani and Korver,

* Reprinted with the permission from "Evaluation of a thermotolerant xylanase on broiler growth performance and dietary ileal digestible energy value" by C. A. Flores, M. P. Williams, K. Smith, J. Pieniazek, R. Latham, J. J. Wang, J. Tyus, and J. T. Lee, 2017. *Journal of Applied Poultry Research*, 26, 60-71, Copyright [2016] by Oxford University Press.

2013). High concentrations of NSP (arabinoxylans, β-glucans, mannans, galactomannans) within the endosperm cell wall of these grains have the ability to impede nutrient absorption and energy utilization in the digestive tract, reducing overall broiler performance. Cowieson (2010) estimated that these anti-nutritive properties are responsible for approximately 400-450 kcal/kg of the diet not being digested when birds are fed a standard corn/soy ration with the energy range being partitioned at 18% fat, 45% protein, and 37% starch. An effective method of reducing the anti-nutritive properties of NSP is through proper supplementation of xylanase and other carbohydrases.

Xylanase is a type of hemicellulase that is effective in diets containing viscous (wheat) and non-viscous (corn) cereals and specifically acts on xylan, the major hemicellulosic polysaccharide present in plant cell walls (Kolenová et al., 2006). The β-(1, 4) linkages present along the backbone of xylan gives the molecule structure and aids in the entrapment of nutrients which directly impedes broiler performance by reducing the proportion of nutrients available for absorption and utilization. Degradation of these linkages breaks down the backbone of xylan into individual xylose units allowing for utilization of normally inaccessible nutrients. The impact of xylanase in wheat-based diets has been widely explored and has shown consistent results; however, xylanase supplementation into corn/SBM based diets has provided results which have not been as definitive. Multiple reports have confirmed that broiler performance, as well as IDE, is significantly improved with xylanase supplementation which may

be correlated with an increase in feed efficiency and utilization (Cowieson et al., 2010; Masey O'Neill et al., 2012; Romero et al., 2014).

Distillers' dried grains with solubles have become a commonly used alternative feed ingredient in broiler diets. Typical DDGS may have up to 12.55% oil content with low-oil DDGS (LO-DDGS), a by-product of oil extraction from DDGS, containing as little as 2.1% fat after the extraction process (Ganesan et al., 2009; Liu, 2010). In a study conducted by Loar et al. (2010) , it was concluded that DDGS may be included up to 15% of the diet without adverse effects on performance as long as appropriate nutrient profiles specific to the DDGS were used and diets were formulated on a digestible amino acid basis (Kiarie et al., 2014). Incorporation of DDGS into the diet also increases the overall concentration of NSP with reports indicating that DDGS may contain up to 12% insoluble arabinoxylans (Bach Knudsen, 2011). However, through proper xylanase supplementation, adverse nutritional effects associated with high NSP concentration may be alleviated, thus leading to improved nutrient utilization and broiler performance. Therefore, the objective of two experiments was to evaluate the effect of a new form of *Pichia pastoris*-based xylanase[1] in corn-soybean meal based diets with DDGS on broiler performance and IDE value. The working hypothesis used during both experiments was that the inclusion of xylanase would increase IDE leading to an improvement of growth performance.

[1] Xylamax™ - BioResource International, Inc., Durham, North Carolina, USA

Materials and Methods

Experimental Design

Experiment 1. On d of hatch, 1,260 Cobb 500 male broiler chicks were placed in floor pens to determine the effect of xylanase inclusion into a low energy corn/soy diet with DDGS to assess the impact on broiler growth performance and IDE. The experiment was conducted in a completely randomized block design and consisted of three experimental treatments with 12 replicates per treatment, each containing 35 chicks for a 42 d assay period.

Chicks were reared on used litter in 6 x 6 ft. (1.8m^2) floor pens, provided age appropriate heat and ventilation, and given access to feed and water *ad libitum*. The lighting program included continuous light through 3 d of age at 25 lux, 23 hr of light from d 4 to 7 at 25 lux, 20 hr of light from d 8 to 14 at 15 lux, 16 hr of light from d 15 to 28 at 10 lux, 18 hr of light from d 29 to 38 at 7 lux, and 23 hr of light for d 39 to 42 at 7 lux. On d 14, 28, and 42, at each dietary change, birds and remaining feed were weighed for determination of average BW and FC for the calculation of FCR.

Experiment 2. On d of hatch, 1,760 Cobb 500 male chicks were placed in floor pens to determine the effect of xylanase inclusion into a low energy corn/SBM diet with DDGS to evaluate the impact on broiler growth performance and IDE. The experiment was conducted in a completely randomized block design and consisted of four experimental treatments with 10 replicates per treatment, each containing 44 chicks for a 41 d assay period.

Chicks were reared on used litter in 6 x 6 ft. (1.8m^2) floor pens, provided age appropriate heat and ventilation, and given access to feed and water *ad libitum*. The lighting program was similar to that of the first experiment. On d 14, 27, and 41, at each dietary change, birds and remaining feed were weighed for determination of BW and FC for the calculation of FCR. Animal care for both experiments was provided in accordance with a protocol approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC).

Experimental Diets

Experiment 1. The three dietary treatments consisted of a control diet and two treatments consisting of an endo-β-1, 4-xylanase with increasing levels at 20,000 XU/kg (XYL20) and 40,000 XU/kg (XYL40), respectively. The xylanase was derived from a *Pichia pastoris* strain with an optimum enzyme activity temperature range of 50 to 60^{0}C. One unit of xylanase activity was defined as the amount of enzyme needed for the release of 1 nmol of reducing sugars (with xylose standard) per second from 0.5% xylan (Sigma X4252, from Beechwood) at 50^{0}C in 50 mM trisodium citrate buffer at pH 6.0. The control diet was formulated to a lower energy diet compared to the industry diets with an estimated reduction of 150 kcal/kg apparent metabolizable energy (AME). All diets were corn/SBM based with varying energy levels. Concentration of DDGS increased from 5% during the starter phase, 7.5% during the grower, and 10% during the finisher phase (Table 1). Titanium dioxide was included at 0.4% of the

Table 1. Experiment 1 ingredient profile, calculated and analyzed nutrient concentration of the control basal diet for the starter (d 0 to14), grower (d 15 to 28), and finisher (d 28 to 42) dietary phases

Ingredient Profile	Starter (%)	Grower (%)	Finisher (%)
Corn	56.82	60.64	60.74
Soybean Meal (48%)	32.80	26.89	24.74
DL - Methionine	0.22	0.19	0.14
L - Lysine HCL	0.19	0.19	0.06
L - Threonine	0.07	0.06	N/A
Animal/Vegetable blend fat	0.70	0.50	0.50
Limestone	1.52	1.53	1.53
Mono-calcium Phosphate	1.49	1.30	1.13
Sodium chloride	0.44	0.38	0.40
Sodium bicarbonate	N/A	0.06	0.03
Trace minerals[1]	0.05	0.05	0.05
Vitamins[2]	0.25	0.25	0.25
Coban 90[3]	0.05	0.05	0.05
LO Distillers Dried Grains w/ Solubles[4]	5.00	7.50	10.00
Titanium dioxide	0.40	0.40	0.40
Calculated Nutrient Concentration			
Protein	22.42	20.52	19.94
Crude Fat	3.59	3.68	3.87
Calcium	0.92	0.88	0.85
Available Phosphorous	0.45	0.41	0.38
Metabolizable Energy (kcal/kg)	2930	2970	2984
dig Methionine	0.53	0.48	0.41
dig TSAA	0.82	0.75	0.67
dig Lysine	1.17	1.03	0.88
dig Tryptophan	0.23	0.22	0.20
dig Threonine	0.78	0.70	0.62
dig Arginine	1.30	1.11	1.06
Sodium	0.20	0.20	0.20
Analyzed Nutrient Concentration			
Moisture	11.87	12.10	12.66
Dry Matter	88.13	87.90	87.34
Crude Protein	20.4	22.5	18.5
Crude Fat	2.86	3.66	3.46
Fiber	3.6	4.2	3.5
Ash	5.38	4.93	4.75

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

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

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

[4] Analyzed to contain 12.1% moisture, 28.4% protein, 4.95% crude fat, 10.0% ADF, and 4.20% Ash

diet and utilized as an indigestible marker for the determination of IDE. Three dietary phases were fed through the
duration of the trial including a starter (d 0 to 14, crumble), grower (d 15 to 28, pellet), and finisher (d 29 to 42, pellet). One large diet was manufactured and then subsequently divided among treatments. Xylanase was then added on top of the basal diet and then mixed for approximately 5 minutes in order to achieve a homogenous mixture. Pelleting temperature ranged between 83 and 85°C and a conditioning time of approximately 25 seconds was utilized. Temperature of the mash feed was continuously monitored with a probe strategically placed between the conditioner and the pellet dye.

Experiment 2. The four treatments consisted of a positive control (PC), a negative control (NC) formulated to an energy density 150 kcal/kg lower than PC, and the NC supplemented with either 10,000 XU/kg (XYL10) or 20,000 XU/kg (XYL20) per kg of finished feed (Table 2). Diets were corn-soybean meal based and contained DDGS. Titanium dioxide (0.4%) was added to all diets as an indigestible marker for determination of IDE. Dietary inclusion of DDGS increased in each successive phase from 5% in the starter
phase (d 0 to 14) to 7.5% in the grower phase (d 15 to 27) and then to 10% in the finisher phase (d 28 to 41). In each phase a single, larger batch of NC was divided and combined with the appropriate amounts of xylanase. Starter phase diets were fed in crumble form, while grower and finisher phase diets were

Table 2. Experiment 2 ingredient profile, calculated and analyzed nutrient concentration of the negative control (NC) and positive control (PC) basal diet for the starter (d 0 to 14), grower (d 15 to 27), and finisher (d 27 to 41) dietary phases

	Negative Control (%)			Positive Control (%)		
Ingredient Profile	Starter	Grower	Finisher	Starter	Grower	Finisher
Corn	57.80	62.80	66.32	54.40	59.01	62.53
Soybean Meal (48%)	32.59	25.19	18.86	32.91	25.87	19.54
DL - Methionine	0.22	0.19	0.15	0.22	0.19	0.15
L - Lysine HCL	0.19	0.25	0.26	0.19	0.23	0.24
L - Threonine	0.07	0.08	0.08	0.07	0.08	0.08
Animal/Vegetable blend fat	0.50	0.65	0.99	3.56	3.77	4.11
Limestone	1.54	1.40	1.40	1.54	1.39	1.39
Mono-calcium phosphate	0.90	0.72	0.68	0.91	0.73	0.69
Sodium chloride	0.44	0.33	0.19	0.44	0.34	0.20
Sodium bicarbonate	N/A	0.14	0.32	N/A	0.13	0.31
Trace minerals[1]	0.05	0.05	0.05	0.05	0.05	0.05
Vitamins[2]	0.25	0.25	0.25	0.25	0.25	0.25
Coban 90[3]	0.05	0.05	0.05	0.05	0.05	0.05
LO Distillers Dried Grains w/ Solubles [4]	5.00	7.50	10.00	5.00	7.50	10.00
Phytase[5]	0.01	0.01	0.01	0.01	0.01	0.01
Titanium Oxide	0.40	0.40	0.40	0.40	0.40	0.40
Calculated Nutrient Concentration						
Protein	22.53	20.13	18.07	22.40	20.13	18.07
Crude Fat	3.26	3.64	4.18	6.18	6.61	7.14
Calcium	0.92	0.82	0.80	0.92	0.82	0.80
AV Phosphorous	0.45	0.41	0.40	0.45	0.41	0.40
Metabolizable Energy (kcal/kg)	2910	2970	3020	3060	3120	3170
dig Methionine	0.53	0.48	0.42	0.53	0.48	0.42
dig TSAA	0.82	0.75	0.66	0.82	0.75	0.66
dig Lysine	1.17	1.03	0.89	1.17	1.03	0.89
dig Tryptophan	0.22	0.21	0.17	0.23	0.21	0.17
dig Threonine	0.78	0.70	0.62	0.78	0.70	0.62
dig Arginine	1.30	1.07	0.90	1.30	1.08	0.90
Sodium	0.20	0.20	0.20	0.20	0.20	0.20
Analyzed Nutrient Concentration						
Moisture	12.56	10.55	10.47	12.43	11.25	10.83
Dry Matter	87.44	89.45	89.53	87.57	88.75	89.17
Crude Protein	21.60	19.70	18.20	23.20	19.90	17.50
Crude Fat	4.26	6.19	4.61	5.57	3.59	5.53
Fiber	2.60	3.30	4.10	2.60	2.90	2.30
Ash	5.47	4.60	4.79	5.20	5.06	4.49

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

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

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

[4] Analyzed to contain 12.1% moisture, 28.4% protein, 4.95% crude fat, 10.0% ADF, and 4.20% Ash

[5] OptiPhos® PF- Huvepharma. Peachtree City, GA, USA

pelleted. Pelleting temperatures ranged between 83 to 85^{0}C with a 25 second conditioning time.

Similar to the first experiment, temperature of the mash feed was continuously monitored with a probe strategically placed between the conditioner and the pellet dye. Feed samples for both experiments were collected during feed manufacturing for nutrient analysis, which was conducted in triplicate. Crude protein was determined by combustion using an AOAC method (AOAC method 990.03) (AOAC), total phosphorous was determined by wet ash inductively coupled with plasma spectroscopy (AOAC method 985.01M), ADF was determined using an Ankom digestion unit (AOAC method 973.18) (Ankom Technology), and an ether extraction method was used to determine crude fat (AOAC method 920.39).

Ileal Digestible Energy

Ileal digestible energy was determined on multiple days of age during both experiments using titanium dioxide as an indigestible marker. Ileal contents were taken from 5 birds per replicate on d 14, 4 birds per replicate on d 28 during the first experiment and on d 27 during the second experiment, and 3 birds per replicate at the conclusion of each experiment for determination of IDE. Samples were then dried at 100°C for 24 hr and gross energy of ileal contents was determined using a Parr 6400 bomb calorimeter[2]. Ileal contents were removed four centimeters caudal to Meckel's Diverticulum and four centimeters dorsal to

[2] Parr Instrument Company, Moline, IL

the ileo-cecal junction and pooled per replicate pen. Ileal and feed samples were dried at 100°C for 24 hr. Samples were ground for gross energy and titanium concentration determination.

Titanium concentration was determined using a modified protocol outlined by Short et al. (Short et al., 1996). Half a gram of each dried sample was weighed and placed in an ashing oven at 450°C for 12 hr. Following ashing, each sample was titrated with 10 mL of 7.4 M sulfuric acid and boiled at 200°C for 3 hr until dissolved. Samples were then titrated with 10 mL of 30% hydrogen peroxide. Total sample volume of 100 mL was achieved using distilled water. Samples were analyzed for absorption using a Thermo Fisher Scientific Genesys 10S UV-Vis (Model 10S UV-Vis) Spectrophotometer[3] at 410 nm. Gross energy of feed and ileal samples was determined using a Parr 6400 bomb calorimeter. For both experiments, ileal contents were sampled and collected using the same procedure.

Statistical Analysis

All data was analyzed via one-way ANOVA using the General Linear Model (SPSS software) with means deemed significantly different at $P<0.05$. Further means determined to be different were analyzed via Fishers' LSD. The parameters evaluated during both experiments included BW, BWG, FC, mortality-corrected FCR, and IDE.

[3] Thermo Fisher Scientific, Waltham, MA

Results and Discussion

As shown in Table 3, no significant differences in FC were observed during the starter or grower phases during Experiment 1. During the finisher phase, broilers fed xylanase at both inclusion levels consumed at a significantly ($P<0.05$) lower rate than the control. No significant differences were observed cumulatively through d28 or d42. The inclusion of xylanase did not significantly impact BW or BWG throughout the experiment compared to the control diet.

No significant differences were observed in mortality corrected FCR throughout the starter or finisher phase. During the grower phase, the inclusion of xylanase did not impact FCR compared to the control diet; however XYL20 significantly ($P<0.05$) reduced FCR when compared to the XYL40. Cumulatively through d 28, XYL20 significantly reduced FCR compared to the control diet. Through d 42, XYL20 reduced FCR compared to the control and XYL40 yielded similar results to the inclusion of XYL20, however was not statistically different from the control diet. No significant differences were observed in weight corrected FCR through d 42.

No significant differences in IDE were observed during the starter phase through d 14. On d 28, XYL20 increased ($P<0.05$) IDE by 88 kcal/kg compared to the control diet, whereas XYL40 did not. At the conclusion of the trial on d 42, XYL20 and XYL40 yielded significantly ($P<0.05$) higher IDE values than the control, 174 and 180 kcal, respectively.

Table 3. Experiment 1, effect of xylanase at multiple levels compared to a control diet on male broiler body weight, feed consumption, body weight gain, mortality corrected feed conversion ratio, and ileal digestible energy.

	Treatment[1]			
Item	Control	XYL20	XYL40	PSEM
Body Weight (kg/bird)				
14 d	0.339	0.333	0.334	0.003
28 d	1.370	1.419	1.370	0.013
42 d	2.458	2.530	2.496	0.028
Feed Consumption (g/bd/d)				
Starter (0 to 14 d)	30.5	29.9	30.4	0.2
Grower (15 to 28 d)	110.0	112.8	112.7	1.0
Finisher (29 to 42 d)	228.5[a]	212.0[b]	213.4[b]	3.5
Cumulative Feed Consumption (g/bd/d)				
0 to 28 d	61.8	62.2	63.0	0.5
0 to 42 d	80.1	80.5	81.1	0.6
Body Weight Gain (kg)				
0 to 14 d	0.300	0.293	0.294	0.003
15 to 28 d	1.033	1.081	1.036	0.013
29 to 42 d	1.086	1.106	1.12	0.027
Cumulative BWG (kg)				
0 to 28 d	1.333	1.379	1.330	0.013
0 to 42 d	2.418	2.486	2.451	0.029
Mortality-Corrected Feed Conversion Ratio (FCR) (feed:gain)				
Starter (0 to 14 d)	1.355	1.345	1.350	0.009
Grower (15 to 28 d)	1.490[ab]	1.456[b]	1.496[a]	0.008
Finisher (29 to 42 d)	2.196	2.114	2.068	0.032
Cumulative Mortality Corrected FCR (feed:gain)				
0 to 28 d	1.454[a]	1.424[b]	1.459[a]	0.006
0 to 42 d	1.733[a]	1.689[b]	1.702[ab]	0.009
0 to 42 d[2]	1.749	1.678	1.704	0.018
Ileal Digestible Energy (IDE)				
14 d	3123	3074	3132	22
28 d	3040[b]	3128[a]	2989[b]	20
42 d	2930[b]	3104[a]	3110[a]	27

[a,b] Means with different superscripts within a row differ significantly at ($P < 0.05$).

[1] Control, with 150 kcal/kg reduction in energy; XYL20, control plus 20,000 units of xylanase; XYL40, control plus 40,000 units of xylanase

[2]Weight-corrected FCR, 2.5kg correction factor with 27g of BW equal to 1 point in FCR

During the starter phase of Experiment 2, the PC fed broilers yielded a significantly higher FC compared to the NC. The inclusion of xylanase at both inclusion rates did not influence FC when compared to the NC; however, XYL20 increased FC to levels that were comparable to the PC diet. Although no differences were observed in FC between the PC and NC diets during the grower phase, XYL20 significantly increased FC compared to the control diets with XYL10 yielding intermediate results. Cumulatively through d27, the NC significantly reduced FC compared to the PC diet while XYL20 increased ($P<0.05$) cumulative FC compared to the NC, yielding similar results to the PC. XYL10 yielded similar results to the PC diet, however was not statistically different from the NC. During the finisher phase, no significant difference in FC was observed between the PC and NC diet. However, XYL10 increased ($P<0.05$) FC compared to the NC. Cumulatively through d41, XYL20 significantly ($P<0.05$) increased consumption compared to the NC while XYL10 was intermediate.

The reduction of energy in the NC diet significantly ($P<0.05$) reduced BW and BWG compared to the PC on d 14 and d 27 (Table 4). On d 14, XYL20 increased BW and BWG to levels that were similar to the PC diet. XYL10 did not impact BW and BWG compared to the NC diet. On d 27, the inclusion of xylanase at both inclusion rates significantly ($P<0.05$) increased BW and BWG compared to the NC while yielding similar results to the PC. The reduction of energy in the NC diet significantly ($P<0.05$) reduced cumulative BWG through d

Table 4. Experiment 2, effect of xylanase at multiple levels compared to a standard US corn/SBM based and low energy diet on male broiler body weight, feed consumption, body weight gain, mortality corrected feed conversion ratio, and ileal digestible energy.

	Treatment[1]				
Item	PC	NC	XYL10	XYL20	PSEM
Body Weight (kg/bird)					
14 d	0.395[a]	0.378[b]	0.372[b]	0.392[a]	0.003
27 d	1.466[a]	1.417[b]	1.455[a]	1.475[a]	0.007
41 d	2.896	2.838	2.897	2.912	0.014
Feed Consumption (g/bd/d)					
Starter (0 to 14 d)	36.8[a]	35.2[b]	35.0[b]	35.8[ab]	0.3
Grower (15 to 27 d)	119.9[b]	120.2[b]	122.5[ab]	124.6[a]	0.8
Finisher (28 to 41 d)	191.5[ab]	190.0[b]	195.4[a]	194.5[ab]	1.0
Cumulative Feed Consumption (g/bd/d)					
0 to 27 d	69.8[a]	68.2[b]	69.2[ab]	70.3[a]	0.355
0 to 41 d	97.3[ab]	95.3[b]	96.9[ab]	97.8[a]	0.468
Body Weight Gain (kg)					
0 to 14 d	0.355[a]	0.338[b]	0.332[b]	0.351[a]	0.003
15 to 27 d	1.071[a]	1.039[b]	1.083[a]	1.083[a]	0.006
27 to 41 d	1.430	1.421	1.441	1.437	0.01
Cumulative BWG (kg)					
0 to 27 d	1.426[a]	1.376[b]	1.415[a]	1.434[a]	0.008
0 to 41 d	2.856[ab]	2.797[b]	2.856[ab]	2.871[a]	0.014
Mortality-Corrected Feed Conversion Ratio (FCR) (feed:gain)					
Starter (0 to 14 d)	1.450[ab]	1.454[ab]	1.472[a]	1.420[b]	0.009
Grower (15 to 27 d)	1.443[b]	1.489[a]	1.465[ab]	1.480[a]	0.006
Finisher (28 to 41 d)	2.037	2.038	2.043	2.057	0.013
Cumulative Mortality Corrected FCR (feed:gain)					
0 to 27 d	1.445[b]	1.480[a]	1.467[ab]	1.463[ab]	0.004
0 to 41 d	1.740	1.763	1.756	1.759	0.006
0 to 41 d[2]	1.671[b]	1.720[a]	1.677[ab]	1.679[ab]	0.008
Ileal Digestible Energy (IDE)					
14 d	3378[a]	3134[b]	3255[ab]	3272[a]	28
27 d	3365[a]	3199[b]	3285[ab]	3216[b]	23
41 d	3367[a]	3111[b]	3195[ab]	3277[ab]	33

[a,b] Means with different superscripts within a row differ significantly at ($P < 0.05$).

[1]Positive Control; Negative Control, with 150 kcal/kg reduction in energy; XYL10, NC plus 10,000 units of xylanase; XYL20, NC plus 20,000 units of xylanase

[2]Weight-corrected FCR, 3kg correction factor with 27g of BW equal to 1 point in FCR

27 compared to the PC diet. The inclusion of xylanase at both inclusion levels significantly ($P<0.05$) increased cumulative BWG through d 27 when compared to the NC diet to levels

that were similar to the PC. No significant differences in BW and BWG were observed between any of the treatments at the conclusion of the trial on d 41. Cumulatively through d 41, no significant difference observed between the PC and NC, however, XYL20 yielded a significantly ($P<0.05$) higher BWG than the NC.

During the starter phase, no significant differences in FCR were observed between the PC and NC diets. The inclusion of xylanase at both inclusion rates did not impact FCR compared to the control diets; however XYL20 significantly reduced starter FCR compared to XYL10. The NC significantly increased FCR compared to the PC during the grower phase. XYL10 reduced FCR to levels that were similar to the PC, however not statistically different from the NC diet. Cumulatively through d 27, a significant increase in FCR was observed with the NC compared to the PC diet. The inclusion of xylanase at both inclusion rates reduced FCR to levels comparable to the PC diet, however not significantly different than the NC. During the finisher phase, no significant differences were observed in FCR between the PC and NC or with the inclusion of xylanase at both inclusion rates. At the conclusion of the experiment on d 41, no differences were observed in cumulative FCR amongst any of the dietary treatments. A weight correction factor (3kg) was applied to cumulative FCR through d 41 in

order to normalize differences in BW with 27g of BW equal to one point of FCR. A significant ($P<0.05$) reduction in weight corrected FCR was observed between the PC (1.671) and NC (1.720) which corresponds with the 150 kcal/kg reduction in energy in the NC compared to the PC. XYL10 and XYL20 (1.677 and 1.679, respectively) produced similar results to the PC and resulted in approximately a four point reduction in FCR.

A significant reduction in IDE was observed in the NC compared to the PC at d 14, 27, and 41, due to the 150 kcal/kg reduction in energy in the NC diet. At d 14, the inclusion of xylanase at both inclusion rates increased IDE to levels that were comparable to the PC diet; however, XYL20 significantly increased IDE compared to the NC. On d 27, XYL10 increased IDE to levels that were comparable to the PC diet; however did not differ from the NC. At the conclusion of trial on d 41, the inclusion of xylanase at both inclusion rates increased IDE to levels similar to the PC diet however were not significantly different from the NC.

The potential performance improvements with xylanase supplementation and its impact on broilers fed various diet types (corn, wheat, etc.) has been extensively researched. The incorporation of wheat into diets has been noted to possess several advantages over corn in that wheat contains a higher crude protein content than corn (13% vs. 8.6%, respectively) and contains proportionally more lysine (Leeson, 1991). It does however have disadvantages including less metabolic energy and a higher concentration of NSP than corn which inhibit nutrient utilization and absorption. The major components of NSP

found in wheat are arabinoxylans composed of a backbone chain of β-(1,4)-linked xylose units with sidechains of arabinose attached (Crouch et al., 1997). Crouch et al. (1997) noted that the negative impact of arabinoxylans on performance is attributed to the water-soluble NSP fraction causing an entanglement of long polymers resulting in a high water-holding capacity and increased intestinal viscosity (Ward, 1996). Previous research has shown supplementation of xylanase-based feed additives, which degrade various NSPs in the cell wall causing a reduction in gut viscosity, is an effective way to mitigate the anti-nutritive properties of NSPs in wheat. This reduction in viscosity has been shown to increase BW and improve feed efficiency as well nutrient utilization leading to reduced FCR values and increased IDE (Engberg et al., 2004; Kalmendal and Tauson, 2012; Kiarie et al., 2014; Wu et al., 2004). The consistent improvements in broiler performance with xylanase supplementation in wheat based diets are potentially a result of the high concentration of NSP in wheat, allowing for adequate substrate. Consistent results with xylanase supplementation in corn-based diets however have not been observed.

The concept of introducing xylanase-based enzymes into corn-based diets began in the late 1990's following the success achieved in wheat-based diets (Cowieson et al., 2010). The mode of action by which xylanase acts on the arabinoxylans within the endosperm cell wall of corn is the same as it is in wheat. However, as mentioned previously, there have been notable and inconsistent results observed between xylanase supplementation in wheat and corn-based

diets, primarily due to the higher NSP concentration in wheat as compared to corn (Choct, 2006). Results in corn-based diets have been shown to vary in effectiveness, ranging from having no impact (Gehring et al., 2013; Kocher et al., 2003; Romero et al., 2014; Yegani and Korver, 2013) to significantly improving broiler performance (Cowieson et al., 2010; O'Neill et al., 2012; Wu et al., 2004).

When comparing the results from both experiments, significant improvements were observed in BW during the second experiment while improvements in FCR as well as IDE were observed during both studies. The inconsistencies in BW to the response of xylanase correlate with previous research in which xylanase inclusion showed no impact on BW (Cowieson et al., 2010; O'Neill et al., 2012; Yegani and Korver, 2013); however, there have been reports which indicate the effectiveness of xylanase resulting in increased broiler BW (Cowieson and Ravindran, 2008a; Wu et al., 2004). During the first experiment, no significant differences in BW were observed throughout the duration of the trial at either inclusion level (XYL20 or XYL40), but there were numerical increases with XYL20 supplementation. The increases in BW with XYL20 supplementation were again observed in second experiment. However, results were significant ($P<0.05$) at d 14 and 27 as well as cumulatively through d 27 and d 41. It is important to note that the low dose of xylanase in Experiment 1 (XYL20) was the high dose in Experiment 2, suggesting that there may have been a dose response to the level of xylanase supplemented. The impact of xylanase supplementation on FC was more prevalent during Experiment 2

compared to Experiment 1. During the first experiment, no significant differences in FC were observed throughout the duration of the trial with exception of the finisher phase in which both inclusion levels of xylanase (XYL20 and XYL40) significantly ($P<0.05$) increased FC compared to the control. During the second experiment, significant differences were observed during all phases of growth as well as cumulatively through d27 and 41. The PC consumed significantly ($P<0.05$) more feed than the NC during the starter phase as well as cumulatively through d 27, although this trend was lost throughout the remainder of the trial. This was an interesting phenomenon considering that it has been accepted that birds eat to meet their energy requirements. Based on these data, this does not seem to be the case. Based on that logic, the NC which was formulated to have a 150 kcal reduction in energy compared to the PC would have consumed more than the PC but that was not the case, suggesting that the birds may have been eating to meet another requirement other than energy. The incorporation of xylanase significantly affected feed consumption during all phases of the second experiment. Supplementation of XYL10 significantly ($P<0.05$) increased feed consumption compared to the NC during the finisher phase while supplementation of XYL20 significantly ($P<0.05$) increased feed consumption during the grower phase as well as cumulatively through d 27 and 41. Similar to the results seen with feed consumption, the impact of xylanase supplementation was more pronounced during the second experiment compared to the first experiment. The inclusion of XYL20 during the first experiment significantly

(P<0.05) reduced FCR during the grower phase and cumulatively through d 27. Cumulatively through d 42, a significant reduction in FCR was observed between XYL20 and the control which resulted in a four point reduction in FCR (1.73 vs. 1.69). During the grower phase and cumulatively through d 27 of the second experiment, the PC yielded a significantly lower FCR than the NC which was expected due to the 150 kcal/kg reduction in energy between the PC and NC. Cumulatively through d 41, supplementation of xylanase at both levels reduced weight corrected FCR values similar to the PC resulting in a four point reduction in FCR which was similar to the results seen in the first experiment. Supplementation with XYL20 during the first experiment significantly (P<0.05) increased IDE on d 28 and 42 compared to the control (88 kcal/kg, 176 kcal/kg respectively). Xylanase inclusion XYL40 also significantly increased IDE compared to the control on d42 (180 kcal/kg). During the second experiment, the PC consistently had significantly (P<0.05) higher IDE values than the NC throughout the duration of the trial which correlates with the 150 kcal/kg reduction in energy between the two treatments. Supplementation of xylanase increased IDE values and consistently produced results which were similar to that of the PC throughout the trial.

As mentioned previously, the effectiveness of xylanase in wheat-based diets as well as other viscous cereals is well established and has been confirmed to be a result of gut viscosity reduction. The mechanisms by which xylanase act within corn-based diets has been inconclusive. It has been suggested through

previous research that one effective mechanism of xylanase in corn-based diets could be a result of an increase in feed efficiency through the release of encapsulated nutrients, coupled with increases in BW and minimal effects on feed intake (Masey O'Neill et al., 2012). The significant improvements in IDE during both experiments with xylanase supplementation confirms the improvements in feed efficiency, and based on previous research, can help confirm the improvements in FCR that were observed during both studies (Cowieson et al., 2010; Zanella et al., 1999). These data also show a potential dose response to the level of xylanase supplemented and the resulting effect on broiler growth performance. Results from both studies indicate that XYL20, which was the low dose in Experiment 1 and the high dose in Experiment 2, produced consistent improvements in performance and nutrient utilization during both experiments while XYL40 and XYL10 produced inconsistent results.

CHAPTER III

EVALUATING THE EFFICACY OF A *BACILLUS*-BASED DIRECT FED MICROBIAL IN COMPARISON TO AN ANTIBIOTIC GROWTH PROMOTER IN DIETS CONTAINING LOW AND HIGH LEVELS OF DDGS ON BROILER GROWTH PERFORMANCE AND CARCASS YIELD

Introduction

Direct fed microbial products (DFM) are additives that contain live, viable microorganisms administered into feed. Due to the improvements these products have on gastrointestinal microbiota and morphology, DFM are often classified as probiotics, which are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host and ultimately improve overall bird performance (Hill et al., 2014). The use of DFM as an alternative to antibiotic growth promoters (AGP) has increased throughout the poultry industry primarily due to recently changing consumer preferences. Although other alternatives such as prebiotics, essential oils, and phytogenics are available, DFM are generally understood to be the best candidate as an alternative to AGP (Lee and Lillehoj, 2016). Direct fed microbials have also gained further attention as a prophylactic agent against enteric pathogens and bacterial diseases due to their presumed beneficial effects on native gastrointestinal microbiota and host immunity (Callaway et al., 2008).

Direct fed microbials can be derived from different, and in some instances, multiple bacterial species (*Lactobacillus, Bifidobacterium, Enterococcus, Streptococcus*, etc.). Recently, DFM products derived from *Bacillus* spp. have gained interest due largely to the resilient nature of the bacterial spore (Harrington et al., 2015). These spores are able to endure extreme temperature fluctuations which may be experienced during feed manufacturing and are resilient to UV radiation and various chemicals allowing for flexibility when it comes to product administration in the feed or in water (Harrington et al., 2015; Hooge, 2003). Another interesting property that *Bacillus* spores possess is the ability to withstand environments with very low and high pH, which allow the spores to pass through the host's digestive tract without being digested. According to Jeong and Kim (2014), the main mode of action of *Bacillus subtilis* C-3102 is their ability to create an anaerobic environment within the intestine after germination, which has been hypothesized to favor proliferation and growth of native lactobacilli. This can subsequently lead to competitive exclusion of pathogenic bacteria as well as the production of lactic acid to control and limit pathogenic bacteria in the intestine. In a study conducted by Li et al. (2016), the supplementation of *Bacillus subtilis* CGMCC 1.1086 modified native intestinal microbiota through enhancements of beneficial bacteria and inhibition of potential pathogens, which significantly improved broiler growth performance. Similarly, Flores et al. (2016) concluded that the inclusion of a DFM comprised of *Bacillus*

spores significantly improved performance of broilers by increasing starter phase BWG while also reducing finisher phase FCR.

Distiller's dried grains with solubles' have become a commonly used and acceptable feed ingredient for monogastric animals such as swine and poultry. During manufacturing, grain starches undergo various fermentation and drying processes, which produce ethyl alcohol and carbon dioxide, resulting in a DDGS product that has a high concentration of nutrients and is a rich source of crude protein and amino acids (Swiatkiewicz et al., 2016). There are concerns, however, with the bioavailability of amino acids and minerals when DDGS are fed due to variability which may arise during the heating process. In a study conducted by Fastinger et al. (2006), it was concluded that darker DDGS samples, which were most likely the result of excessive heating during processing, demonstrated a significant reduction in energy and lysine digestibility as well as other amino acids. Recently, it has been hypothesized that DDGS may contain a significant quantity of yeast biomass derived from brewer's yeast (*Saccharomyces cerevisiae*) which can potentially be beneficial for gastrointestinal health and development as well as immune system stimulation (Slominski, 2012) by providing a source of vitamins, microelements, and other biologically active substances (Swiatkiewicz and Koreleski, 2008). These biologically active substances may elicit a prebiotic effect on the host's gastrointestinal tract allowing for proliferation of beneficial bacteria supplied from DFM supplementation.

Therefore, the objective of the current studies was to evaluate the efficacy of a *Bacillus subtilis* based DFM on broiler growth performance and carcass yield in comparison to a known AGP and determine if the concentration of DDGS impacts DFM efficacy. The working hypothesis was that the inclusion of the DFM will improve broiler growth performance and yield, and that increasing DDGS content of the diet could potentially increase the efficacy of DFM with higher levels of fiber acting as a substrate for DFM growth.

Materials and Methods

Experimental Design

Animal care for both experiments was provided in accordance with a protocol approved by the Texas A&M University IACUC.

Experiment 1. On d of hatch, 1,050 Cobb 500 male broiler chicks were placed in floor pens to determine the effect of a *Bacillus*-based DFM in comparison to a known AGP on broiler growth performance and carcass yield. The experiment was conducted in a randomized complete block design with 10 replications of 35 chicks per pen for a 41 d evaluation period. On d of placement, all birds were spray vaccinated with a commercially available coccidiosis vaccine in a commercial spray cabinet and allowed to preen 1 h prior to randomization and placement. Chicks were reared on used litter in 1.8 m^2 floor pens, provided age appropriate heat and ventilation, and given access to feed and water *ad libitum*. The lighting program included continuous light through 3 d of age at 25 lux, 23 hr of light from d 4 to 7 at 25 lux, 20 hr of light from d 8 to 14 at 15 lux, 16

hr of light from d 15 to 28 at 10 lux, 18 hr of light from d 29 to 38 at 7lux, and 23 hr of light for d 39 to 42 at 7 lux. On d 14, 28, and 41, at each dietary change, birds and remaining feed were weighed for determination of average BW and FC for the calculation of mortality-adjusted FCR. At the conclusion of the trial and after an 8 hr feed withdrawal, 6 birds per replicate pen were selected to determine carcass without giblets **(WOG)** and breast meat weight and yield.

Experiment 2. On d of hatch, 1,680 Cobb 500 male broiler chicks were placed in floor pens to evaluate the effect of a *Bacillus*-based DFM on broiler growth performance in diets with a low and high inclusion rate of DDGS. This experiment was arranged as a randomized complete block 2 x 2 factorial with 10 replications per treatment each containing 42 chicks per pen for a 42 d evaluation period. On d of placement, all birds were spray vaccinated with a commercially available coccidiosis vaccine in a commercial spray cabinet and allowed to preen for 1 hr prior to randomization and placement. Chicks were reared on used litter in 1.8 m^2 floor pens, provided age appropriate heat and ventilation, and given access to feed and water *ad libitum*. The lighting program was similar to that of the first experiment. On d 14, 28, and 42, at each dietary change, birds and remaining feed were weighed for determination of average BW and FC for calculation of mortality-adjusted FCR.

Experimental Diets

Experiment 1. Three dietary treatments were fed through the duration of the trial which included: a non-medicated control comprised of a standard

corn/SBM diet with 5% DDGS inclusion and phytase, the control supplemented with BMD included at 50 g/ton, and the control supplemented with DFM[4] delivering $7.35x10^7$ CFU/kg. Three dietary phases were fed including a starter phase (d 0 to 14, crumble), grower phase (d 15 to 28, pellet), and finisher phase (d 29 to 41, pellet) (Table 5). One large basal diet was manufactured and then divided into the three treatments with pelleting temperature ranging between 80 and 85^0C with a 12 second conditioning time. Feed samples were collected during feed manufacturing for nutrient analysis, which was conducted in triplicate. Crude protein was determined by combustion using an AOAC method (AOAC method 990.03) (AOAC), total phosphorous was determined by wet ash inductively coupled with plasma spectroscopy (AOAC method 985.01M), ADF was determined using an Ankom digestion unit (AOAC method 973.18) (Ankom Technology), and an ether extraction method was used to determine crude fat (AOAC method 920.39).

Experiment 2. Four dietary treatments were fed throughout the second experiment. Two basal non-medicated control diets were manufactured and comprised of a standard corn/SBM diet with phytase, including DDGS at 2 different levels; low level of DDGS (5%, Starter; 7.50%, Grower; 10%, Finisher) and the high level of DDGS (10%, Starter; 15%, Grower; 20%, Finisher). Each control diet was fed individually or supplemented with a *Bacillus*-based DFM delivering $7.35x10^7$ CFU/kg, yielding a total of four treatments (Table 6). Three

[4] Novela™ – JBS United, Inc., Sheridan, IN, USA

Table 5. Experiment 1 ingredient profile, calculated and analyzed nutrient composition of the control basal diet for the starter (d 0 to 14), grower (d 15 to 28), and finisher (d 28 to 41) dietary phases.

Ingredient Profile, %	Starter	Grower	Finisher
Corn	59.35	63.59	69.34
Soybean Meal	28.90	25.07	19.46
DL-Methionine	0.32	0.24	0.18
Lysine HCL	0.29	0.22	0.20
L-Threonine	0.11	0.06	0.05
Soy Oil	1.19	1.36	1.39
Limestone	1.10	0.90	0.83
Monocalcium Phosphate	0.51	0.30	0.24
Salt	0.40	0.33	0.19
Sodium Bicarbonate	0.02	0.12	0.31
Trace Minerals[1]	0.05	0.05	0.05
Vitamins[2]	0.25	0.25	0.25
LO-Distiller's Dried Grains w/ Solubles	5.00	5.00	5.00
Meat and Bone Meal	2.50	2.50	2.50
Phytase[3]	0.01	0.01	0.01
Nutrient, %	**Starter**	**Grower**	**Finisher**
Protein	22.34	20.70	18.41
Crude Fat	4.25	4.54	4.73
Crude Fiber	2.79	2.74	2.64
Calcium	0.92	0.80	0.75
AV Phosphorous	0.45	0.40	0.38
Metabolizable Energy (kcal/kg)	3003	3058	3113
Digestible Methionine	0.62	0.53	0.44
Digestible TSAA	0.90	0.79	0.68
Digestible Lysine	1.20	1.05	0.90
Digestible Tryptophan	0.21	0.19	0.16
Digestible Threonine	0.80	0.70	0.61
Sodium	0.20	0.20	0.20
Analyzed Nutrient, %[4]	**Starter**	**Grower**	**Finisher**
Moisture	13.15	12.84	13.34
Dry Matter	86.65	87.16	86.66
Crude Protein	21.70	20.20	17.00
Crude Fat	4.44	4.58	4.76
Fiber	2.80	1.50	2.80
Ash	5.58	4.32	4.16

Table 5. Continued

[1]Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[2]Vitamin premix added at this rate yields 22,045 IU vitamin A, 7,716 IU vitamin D3, 91 IU vitamin E, 0.04 mg B12, 11.9 mg riboflavin, 91.8 mg niacin, 40.4 mg d-pantothenic acid, 261.1 mg choline, 2.9 mg menadione, 3.50 mg folic acid, 14.3 mg pyroxidine, 5.87 mg thiamine, 1.10 mg biotin per kg diet. The carrier is ground rice hulls.

[3]OptiPhos®PF – Huvepharma. Peachtree City, GA.

[4]Nutrient analysis conducted by Midwest Laboratories, Inc., Omaha, Nebraska

Table 6. Experiment 2 ingredient profile, calculated and analyzed nutrient composition of the control basal diets for the starter (d 0 to 14), grower (d 15 to 28), and finisher (d 28 to 41) dietary phases.

	Low DDGS Control			High DDGS Control		
Ingredient Profile, %	**Starter**	**Grower**	**Finisher**	**Starter**	**Grower**	**Finisher**
Corn	55.69	60.58	64.27	52.82	56.02	57.23
Soybean Meal	33.61	26.37	20.16	30.97	22.61	16.03
DL - Methionine	0.27	0.22	0.16	0.27	0.22	0.15
Lysine HCL	0.17	0.21	0.23	0.24	0.31	0.32
L - Threonine	0.07	0.08	0.07	0.08	0.10	0.09
Fat, AV Blended	2.02	2.25	2.43	2.49	3.00	3.58
Limestone	1.54	1.36	1.31	1.57	1.41	1.36
Monocalcium Phosphate	0.89	0.67	0.58	0.84	0.59	0.47
Salt	0.44	0.36	0.23	0.42	0.31	0.19
Sodium Bicarbonate	N/A	0.09	0.27	N/A	0.13	0.27
Trace Minerals[1]	0.05	0.05	0.05	0.05	0.05	0.05
Vitamins[2]	0.25	0.25	0.25	0.25	0.25	0.25
LO – Distiller's Dried Grains w/ Solubles	5.00	7.50	10.00	10.00	15.00	20.00
Phytase[3]	0.01	0.01	0.01	0.01	0.01	0.01
Nutrient, %						
Protein	22.85	20.50	18.50	22.85	20.57	18.90
Crude Fat	4.70	5.17	5.54	5.28	6.07	6.86
Crude Fiber	2.83	2.80	2.78	2.95	2.98	3.04
Calcium	0.92	0.80	0.75	0.92	0.80	0.75
AV Phosphorus	0.45	0.40	0.38	0.45	0.40	0.38
Metabolizable Energy (kcal/kg)	2992	3058	3102	2992	3058	3102
Digestible Methionine	0.58	0.51	0.43	0.59	0.52	0.44
Digestible TSAA	0.88	0.78	0.68	0.88	0.78	0.68
Digestible Lysine	1.18	1.04	0.90	1.18	1.04	0.90
Digestible Tryptophan	0.23	0.19	0.16	0.22	0.18	0.15
Digestible Threonine	0.79	0.71	0.63	0.79	0.71	0.63
Sodium	0.20	0.20	0.20	0.20	0.20	0.20
Analyzed Nutrient , %[4]						
Moisture	12.69	12.13	11.08	12.31	12.04	11.21
Dry Matter	87.31	87.87	88.92	87.69	87.96	88.68
Crude Protein	21.40	20.40	18.20	22.30	20.50	18.60
Crude Fat	4.71	4.79	5.85	5.62	6.14	7.42
Fiber	2.90	3.50	4.20	4.30	3.30	3.90
Ash	5.05	4.31	4.19	5.15	4.65	4.37

[1]Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.
[2]Vitamin premix added at this rate yields 22,045 IU vitamin A, 7,716 IU vitamin D3, 91 IU vitamin E, 0.04 mg B12, 11.9 mg riboflavin, 91.8 mg niacin, 40.4 mg d-pantothenic acid, 261.1 mg choline, 2.9 mg menadione, 3.50 mg folic acid, 14.3 mg pyroxidine, 5.87 mg thiamine, 1.10 mg biotin per kg diet. The carrier is ground rice hulls.
[3]Optiphos®PF – Huvepharma. Peachtree City, GA.
[4]Nutrient analysis conducted by Midwest Laboratories, Inc., Omaha, Nebraska

dietary phases were fed including a starter phase (d 0 to 14, crumble), grower phase (d 15 to 28, pellet), and finisher phase (d 29 to 42, pellet). Feed sample collection and analysis were similar to that of the first experiment.

Statistical Analysis

All data from Experiment 1 was analyzed via One-Way ANOVA using the GLM model (SPSS Software) with treatment means deemed significantly different at $P \leq 0.05$. Treatment means that were determined to be significant were further separated using Duncan's Multiple Range Test.

All data from Experiment 2 was analyzed via 2x2 factorial using the GLM model (SPSS Software) for all data. Main effects were deemed significantly different at $P \leq 0.05$ and in the instance of a significant interaction, treatment means were then subject to One-Way ANOVA.

Results and Discussion

Increasing consumer concern surrounding antimicrobial resistance has resulted in a shift for researchers and industry producers to find viable alternatives to AGP. Some of these potential replacements include phytogenics, prebiotics, and DFM. Phytogenics are plant-derived feed additives that have shown to possess various anti-microbial properties and the ability to influence gastrointestinal morphology, which may improve performance (Windisch et al., 2008). Contrastingly, prebiotics are non-digestible oligosaccharides that selectively stimulate the host's indigenous microbiota thereby potentially reducing the likelihood of enteric pathogen colonization and ultimately improving broiler

performance (Alloui et al., 2013; Callaway et al., 2008). The application of DFM and probiotics are not new concepts, but have generated the majority of attention as the best potential candidate as a potential alternative to AGP due to the beneficial effect these additives have on the host's native microbiota (Lee and Lillehoj, 2016).

The objective of the first experiment was to evaluate the efficacy of a *Bacillus subtilis* based DFM on broiler performance and processing yield. Broiler response to DFM supplementation showed consistent improvements in performance throughout the trial (Table 7). During the starter and grower phases, as well as cumulatively through d 28 and d 41, average FC was significantly increased with DFM supplementation compared to the control yielding similar results to AGP inclusion (BMD). These increases in consumption lead directly to improvements in BW. At d 14, 28, and 41, broilers supplemented with the *Bacillus*-based DFM produced significantly heavier birds compared to the control, similar to AGP inclusion. Similar trends were observed with BWG wherein DFM supplementation significantly improved BWG throughout the experiment compared to the control. When evaluating FCR however, no differences were observed from placement to termination. These results from the current experiment agree with previous publications regarding the supplementation of a *Bacillus subtilis* based DFM. A study conducted by Hooge et al. (2004) investigated the effect of *Bacillus subtilis* C-3102 spores on broiler performance and concluded over three separate experiments that

Table 7. Experiment 1, effect of a *Bacillus*-based DFM compared to a known AGP on broiler body weight, feed consumption, body weight gain, mortality-corrected feed conversion ratio, and processing parameters.

	Treatment			
	Control	BMD	DFM	P-value
Body Weight (kg/bird)				
14 d	0.406[b]	0.469[a]	0.500[a]	< 0.001
28 d	1.500[b]	1.605[a]	1.611[a]	< 0.001
41 d	2.898[b]	2.995[a]	3.041[a]	0.001
Feed Consumption (g/bird/d)				
Starter (0 to 14 d)	32.1[b]	37.1[a]	37.5[a]	< 0.001
Grower (15 to 28 d)	121.2[b]	126.1[a]	127.4[a]	0.006
Finisher (29 to 41 d)	193.5	194.9	197.8	0.274
Cumulative Feed Consumption (g/bird/d)				
0 to 28 d	74.9[b]	80.8[a]	81.6[a]	< 0.001
0 to 41 d	111.3[b]	115.9[a]	117.4[a]	< 0.001
Body Weight Gain (kg)				
0 to 14 d	0.364[b]	0.427[a]	0.430[a]	> 0.001
15 to 28 d	1.094[b]	1.136[a]	1.139[a]	0.018
29 to 41 d	1.798[b]	1.859[a]	1.902[a]	0.002
Cumulative BWG (kg)				
0 to 28 d	1.458[b]	1.563[a]	1.569[a]	< 0.001
0 to 41 d	2.485[b]	2.526[ab]	2.570[a]	0.047
Mortality Corrected Feed Conversion Ratio (FCR) (feed:gain)				
Starter (0 to 14 d)	1.247	1.230	1.235	0.103
Grower (15 to 28 d)	1.526	1.550	1.558	0.353
Finisher (29 to 41 d)	1.808	1.821	1.803	0.676
Cumulative Mortality Corrected FCR (feed:gain)				
0 to 28 d	1.459	1.464	1.472	0.736
0 to 41 d	1.626	1.630	1.627	0.929
Average Live Weight (kg)	2.917	2.925	3.035	0.110
Average Chill Weight (kg)	2.218[b]	2.286[ab]	2.348[a]	0.008
Average Fat Pad Weight (g)	29.5	32.8	33.0	0.058
Carcass Yield (%)	76.10[b]	78.30[a]	77.40[a]	0.004
Fat Pad Yield (%)	1.33	1.43	1.41	0.220
Breast Yield (%)	29.4	29.1	29.0	0.746

[a,b] Means in the same rows differ significantly at $P \leq 0.05$

supplementation of *Bacillus subtilis* spores increased BW, which is in agreement with the current study. Similarly, supplementation of a DFM, comprised of three different *Bacillus* strains, increased average BW of broilers during the starter phase when compared to the control as well as broilers fed BMD (Flores et al., 2016). It is commonly thought that the impact of DFM supplementation on broiler growth performance is more pronounced early on in the bird's life due in part to the early colonization of beneficial bacteria and enrichment of native microbiota. This ideology is confirmed in the current study, as broilers supplemented with a *Bacillus subtilis*-based DFM exhibited heavier average BW than the control at d 14 and these results were then carried for the remainder of the trial. When evaluating processing parameters, the improvements in BW led directly to a significant increase in average carcass yield of broilers supplemented with DFM compared to the control and were also comparable to AGP inclusion. Previous research has shown that DFM supplementation can lead to improvements in carcass weight as well as breast meat percentage compared to the non-supplemented control group (Falaki et al., 2011).

The objective of the second experiment was to determine the impact of varied levels of DDGS on the efficacy of the same *Bacillus subtilis* based DFM used in Experiment 1. It was hypothesized that higher levels of fiber present in the DDGS would act as a substrate for the DFM and increase efficacy, thus improving broiler performance. A similar idea was explored by Slominski (2012), however the proposed mode of action was quite different. He suggested since

DDGS are a co-product of brewer's yeast (*Saccharomyces cerevisiae*) fermentation (6.2% and 5.6% residual yeast biomass content of wheat and corn DDGS, respectively), they could potentially contain a significant amount of yeast stimulation of the immune system. Additionally, the presence of yeast cells in DDGS can potentially provide a rich source of vitamins and other biologically active substances (nucleotides, mannanoligosaccharides, beta-1,3/1,6-glucan, inositol, glutamine, and nucleic acids) which may provide a source of fermentable substrates for gastrointestinal tissues and cells, which could prompt a prebiotic effect on the host (Swiatkiewicz and Koreleski, 2008).

In the current study, no differences in BW or BWG were observed throughout the trial with respect to DFM supplementation or DDGS inclusion level (Table 8). A significant interaction between DFM supplementation and DDGS inclusion level was observed for BW at d 28 (P=0.020) as well as for grower phase BWG (P=0.014) and cumulative BWG through d 28 (P=0.020) however, no significant differences were observed after one-way analysis. Feed consumption was not affected by DFM supplementation throughout the study; however, inclusion of high levels of DDGS significantly increased cumulative FC through d 42 (P=0.032) (Table 9). A significant interaction between DFM supplementation and DDGS inclusion level was observed for grower phase FC (P=0.019) although no significant differences were observed after one-way analysis. Supplementation with the DFM reduced starter phase FCR (P=0.007) and increased FCR during the finisher phase (P=0.014) with the inclusion of the

Table 8. Experiment 2, effect of a *Bacillus*-based DFM in low and high DDGS diets on broiler body weight and body weight gain (kg).

TREATMENT		Day 14 (kg)	Day 28 (kg)	Day 42 (kg)	Starter BWG (kg)	Grower BWG (kg)	Finisher BWG (kg)	D0-28 BWG (kg)	D0-42 BWG (kg)
Low DDGS[1]	**Control**	0.404	1.454	2.671	0.367	1.050	1.217	1.417	2.634
Low DDGS	**DFM**	0.404	1.432	2.645	0.366	1.039	1.214	1.394	2.608
High DDGS[2]	**Control**	0.405	1.419	2.618	0.368	1.014	1.199	1.382	2.581
High DDGS	**DFM**	0.408	1.454	2.701	0.373	1.044	1.248	1.416	2.664
	Interaction, One-Way ANOVA		0.228			0.221		0.240	
Main Effects*									
DDGS									
	Low	0.404	1.443	2.658	0.367	1.039	1.216	1.406	2.621
	High	0.408	1.436	2.660	0.370	1.029	1.223	1.399	2.622
DFM									
	Control	0.405	1.436	2.645	0.368	1.032	1.208	1.399	2.608
	DFM	0.407	1.443	2.673	0.370	1.036	1.231	1.405	2.636
P-value, DDGS		0.281	0.571	0.963	0.291	0.301	0.735	0.578	0.966
P-value, DFM		0.595	0.588	0.313	0.652	0.663	0.315	0.613	0.314
P-value, DDGS*DFM		0.461	0.020	0.061	0.471	0.014	0.251	0.020	0.061

[1] Low DDGS – 5% (Starter), 7.5% (Grower), 10% (Finisher)
[2] High DDGS – 10% (Starter), 15% (Grower), 20% (Finisher)

Table 9. Experiment 2, effect of a *Bacillus*-based DFM in low and high DDGS diets on broiler feed consumption (grams/bird/day) and mortality-corrected feed conversion ratio (feed:gain).

TREATMENT		Starter FC	Grower FC	Finisher FC	D0-28 FC	D0-42 FC	Starter FCR	Grower FCR	Finisher FCR	D0-28 FCR	D0-42 FCR
Low DDGS[1]	**Control**	37.2	117.6	181.5	68.9	91.3	1.285	1.544	1.879	1.471	1.644
Low DDGS	**DFM**	36.6	114.6	179.2	67.9	90.1	1.265	1.546	1.859	1.466	1.636
High DDGS[2]	**Control**	38.3	115.0	181.4	69.4	92.2	1.302	1.579	1.923	1.500	1.681
High DDGS	**DFM**	37.5	116.3	188.3	69.1	93.2	1.265	1.550	1.912	1.469	1.658
	Interaction, One-Way ANOVA		0.369							0.078	
	Main Effects*										
	DDGS										
	Low	36.9	116.1	180.4	68.4	90.7[b]	1.275	1.545	1.869[b]	1.468	1.640[b]
	High	37.9	115.6	184.9	69.2	92.7[a]	1.283	1.564	1.917[a]	1.484	1.670[a]
	DFM										
	Control	37.8	116.3	181.4	69.2	91.8	1.293[a]	1.562	1.901	1.485	1.662[a]
	DFM	37.1	115.4	183.8	68.4	91.7	1.265[b]	1.548	1.886	1.467	1.646[b]
	P-value, DDGS	0.240	0.598	0.150	0.144	0.032	0.392	0.144	0.014	0.078	0.002
	P-value, DFM	0.403	0.308	0.451	0.235	0.906	0.007	0.294	0.401	0.052	0.046
	P-value, DDGS*DFM	0.895	0.019	0.138	0.508	0.237	0.403	0.237	0.803	0.020	0.292

[a,b] Means in the same columns differ significantly at P≤0.05

[1] Low DDGS – 5% (Starter), 7.5% (Grower), 10% (Finisher)

[2] High DDGS – 10% (Starter), 15% (Grower), 20% (Finisher)

high level DDGS. At the conclusion of the trial, high levels of DDGS inclusion increased (P=0.002) FCR while DFM supplementation reduced FCR (P=0.046) on d 42. These results were in agreement with previous research in which DFM supplementation had no impact on BW and BWG (Lee et al., 2010b; Salim et al., 2013; Waititu et al., 2014). Although no differences in BW or BWG were observed in the current study with DDGS inclusion, previous research in our laboratory has shown that increasing levels of DDGS can negatively impact BW and BWG (Campasino et al., 2015). Similarly, a study conducted by Loar et al. (2012) examined the effect of feeding broilers 0 or 8% DDGS during a pre-finisher phase fed from d 0 to 28 and then fed a diet containing one of five increasing levels of DDGS (0, 7, 14, 21, and 28%) from d 28 to 42. Cumulative d 42 results from that study indicated that inclusion of DDGS at 8% during the pre-finisher phase decreased BWG as well as increased FCR. When evaluating cumulative performance through d 42, an almost linear decrease in BWG and feed intake was observed as DDGS inclusion was increased from 0 to 28% (Loar et al., 2012).

Although no differences in FCR were observed during Experiment 1, improvements in FCR during Experiment 2 for the starter phase as well as cumulatively through d 42 were observed with DFM supplementation. Similar reductions in FCR have been previously reported with *Bacillus subtilis* supplementation (Harrington et al., 2015; Hooge et al., 2004). Harrington et al. (2015) explored the effect of *Bacillus subtilis* on broiler performance in varying

ME diets (100% ME, 98% ME, 96% ME, 94% ME). Results from the aforementioned study revealed that supplementation of *Bacillus subtilis* into each of the varying ME diets reduced FCR compared to the non-supplemented diet. Furthermore, it was determined based on regression analysis that *Bacillus subtilis* supplementation had a ME contribution of +62 kcal/kg of feed which could explain the improvements in feed efficiency. These results also correlated with Hooge et al. (2004) in which over the course of three experiments, it was concluded that *Bacillus subtilis* supplementation reduced cumulative d 42 FCR compared to the control.

Inclusion of high levels of DDGS increased FCR during the finisher phase and cumulatively through d 42 during Experiment 2 which negatively impacted broiler performance. These results correspond with a series of experiments conducted by Wang et al. (2007a; 2007b) which explored the effect of various inclusion levels of DDGS on broiler performance and carcass characteristics. It was concluded from both experiments that diets containing up to 15% DDGS, when formulated on a digestible amino acid basis, can be included into broiler diets without having any adverse effects on performance or processing parameters. This was a particularly interesting conclusion because it corresponds directly with the results from Experiment 2. Inclusion of DDGS did not have any impact on broiler performance during the starter or grower phases (10% and 15% DDGS, respectively, for the high level); however, FCR was

increased during the finisher phase (20% DDGS) as well as cumulatively through d 42.

The presence of significant interactions during Experiment 2 do indicate that ingredient profile of the diet can impact expected performance improvements associated with DFM inclusion. During the grower phase of production, performance separation between the control and DFM supplemented diets was elevated in the high DFM diet. For example, FCR through 28 d of age was improved from 1.500 to 1.469 with DFM supplementation in the high DDGS diet as compared to 1.471 to 1.466 with DFM supplementation in the low DDGS diet. This response indicates the potential to impact DFM efficacy with ingredient profile adjustments. This may have been due to the potential increase in substrate (mannanoligosaccharides and other biologically active substances) provided through the inclusion of high levels of DDGS; thus, the efficacy of DFM may have been increased due to the utilization of these substrates. Further research is needed in order explore this outcome. In summation, the inclusion of a *Bacillus* DFM improved broiler performance and carcass yield. Similarly, supplementation of the *Bacillus* DFM improved feed efficiency of broiler fed diets containing varying levels DDGS while a potential relationship between DFM efficacy and DDGS inclusion level.

CHAPTER IV

DIRECT FED MICROBIAL AND ITS COMBINATION WITH XYLANASE, AMYLASE, AND PROTEASE ENZYMES IN COMPARISON WITH AGP ON BROILER GROWTH PERFORMANCE AND FOOT PAD LESION DEVELOPMENT*

Introduction

Historically, antibiotics have been used to prevent potential pathogens from creating a negative economic impact for integrators. However, the use of antibiotics has led to concerns regarding drug resistant bacteria, drug residues in the body of the bird, and an imbalance of normal gut microflora (Andremont, 2000; Barton, 2000; Burgat, 1991; Sorum and Sunde, 2001). Increasing public concerns regarding subtherapeutic usage of AGP have resulted in the ban of these products in European commercial animal feed. The first country to completely eliminate the use of antimicrobials for growth promotion was Sweden in 1986 (Aarestrup, 1995). Similarly, in 1995, avoparcin was banned in Denmark in response to the reports that its use created an animal reservoir of glycopeptide-resistant enterococci (GRE) and that GRE were a potential risk to public health. As a result, in 1997, the Commission of the European Union

* Reprinted with the permission from "Direct-fed microbial and its combination with xylanase, amylase, and protease enzymes in comparison with AGPs on broiler growth performance and foot-pad lesion development" by C. Flores, M. Williams, J. Pieniazek, Y. Dersjant-Li, A. Awati, J. T. Lee, 2016. *Journal of Applied Poultry Research*, 25, 328-337, Copyright [2016] by Oxford University Press.

banned avoparcin in all European Union (EU) member countries, and, in 1999, the four remaining antibiotics used for growth promotion were banned on the basis of the 'Precautionary Principle'; these four antibiotics included bacitracin (a polypeptide), spiramycin and tylosin (macrolides), and virginiamycin (a streptogramin combination) (Casewell et al., 2003). The banning of these products in the EU and the likelihood of the same bans being implemented in the United States has led to the growing use of non-antimicrobial products as an alternative to antibiotics to achieve the desired interaction with the intestinal microflora; these include both exogenous enzymes (Bedford, 2000a) and DFM (Patterson and Burkholder, 2003).

Direct fed microbial products are additives that contain live, viable microorganisms administered into feed. Due to the improvements these products have on gastrointestinal microbiota and morphology, DFM are often classified as probiotics, which are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host and ultimately improve overall bird performance (Hill et al., 2014). Once administered into the feed, DFMs can beneficially influence the intestinal microflora, ultimately improving host health and welfare. The modes of action by which DFM act include: competitive exclusion of pathogenic bacteria by competing for mucosal attachment and nutrients, lowering the gut pH through acid fermentation which provides a suitable environment for the beneficial bacteria to thrive and flourish, stimulating the immune system associated with the gut which enhances the

birds natural immune responses against pathogens, and increasing epithelial integrity which can lead to improved nutrient digestibility (Salim et al., 2013). The mechanism which is most commonly associated with DFM supplementation is competitive exclusion, which refers to the physical blocking of opportunistic pathogen colonization by probiotic bacteria via their ability to physically colonize environmental niches within the intestinal tract such as intestinal villus and colonic crypts which are favorite colonization sites of enteric pathogens (Duggan et al., 2002). Furthermore, several studies have documented that *Lactobacillus* and *Bacillus* DFMs decreased the levels of harmful enteric pathogenic bacteria (*Clostridium spp*. and *Escherichia coli*) and increased the levels of beneficial lactic acid producing bacteria in the normal microbiota (Chichlowski et al., 2007; Li et al., 2009; Teo and Tan, 2007).

The use of exogenous enzymes in broiler feed has steadily increased over the last few decades; some of which include the combination of XAP which act on various insoluble NSP and other anti-nutritional factors commonly found in a commercial corn/SBM broiler diet. These enzymes hydrolyze indigestible bonds in the cell wall of the plant into smaller fragments and indigestible protein, allowing for improved digestibility. Because many of the grains used in broiler diets contain a variety of NSP, the use of products containing a mixture of multiple exogenous enzymes ranging in specificity may be the most effective practice in degradation of NSP (Coppedge et al., 2012). Supplementation of xylanase can have a beneficial effect on the gut microflora through reduction in

viscosity and production of small oligomers that can be used by the beneficial bacteria in the lower gut (Bedford, 2000a; Bedford, 2000b). Exogenous amylase can be used to increase the hydrolysis of starch and improve starch digestibility which results in more energy being released for the bird (Bedford and Partridge, 2001; Gracia et al., 2003). Furthermore, exogenous proteases are used to increase the hydrolysis of proteins in the feed, including hydrolysis of trypsin inhibitors (Caine et al., 1998) resulting in improved digestibility of protein and amino acids (Cowieson and Adeola, 2005; Yu et al., 2007) as well as reducing indigestible protein content and substrates available for pathogenic bacteria. Previous research has shown that the combination of XAP has the potential to have a greater impact on broiler performance than the combination of xylanase and amylase (XA) without protease. Over the course of four experiments, Romero et al. (2013) concluded that nitrogen-corrected apparent metabolizable energy was higher in broilers fed XAP over those fed XA which suggested that inclusion of protease increased large intestine and cecal digestion. Similarly, supplementation of XAP with phytase improved nutrient digestibility and broiler performance in a diet marginally low in metabolizable energy, calcium, and phosphorus (Cowieson and Adeola, 2005).

In order to support a thriving environment within the gut, it is essential that the beneficial bacteria are provided with adequate amounts of substrate in order to flourish; this can be accomplished with the inclusion of carbohydrases and protease which through the degradation process provide substrates for

beneficial bacteria to utilize. Therefore, the combination of DFMs and XAP may have a synergistic effect that could result in better gut health and overall broiler performance (Dersjant-Li et al., 2014). The objective of the current experiment was to evaluate the effect of a DFM and its combination with XAP on broiler growth performance as compared to AGPs. The working hypothesis is that the combination of XAP and DFM will improve broiler growth performance similar to that observed with AGP inclusion.

Materials and Methods

Experimental Design and Diets

On d of hatch, 1,600 straight-run Ross 708 broilers were feather sexed and placed at a 50:50 male: female ratio for this study. The experiment was carried out in a completely randomized block design with 5 experimental treatments and 8 replications per treatment during a 42 d assay period. One basal diet was mixed and then subsequently divided amongst the 5 treatments. The design consisted of a corn/SBM NC with 10% wheat inclusion and 5% DDGS with 500 FTU/kg inclusion of phytase (Axtra® PHY, Danisco Animal Nutrition/DuPont, Marlborough, Wilshire, UK). Matrix values were provided by the manufacturer for phytase contribution including available phosphorus, calcium, sodium, ME, and digestible amino acids. The second treatment consisted of the NC with the inclusion of a DFM (Enviva® Pro, providing 150,000 CFU/g of three *Bacillus spp.* strains, Danisco Animal Nutrition/DuPont, Marlborough, Wilshire, UK) (DFM150). The third diet consisted of the NC with

the inclusion of a feed additive (Syncra® AVI, Danisco Animal Nutrition/DuPont, Marlborough, Wilshire, UK) that contained DFM at 75,000 CFU/g in combination with xylanase, amylase and protease (provides 2,000 U/kg endo-xylanase from *T. reesei*, 200 U/kg alpha-amylase from *B. licheniformis*, and 4,000 U/kg serine protease from *B. subtilis,* Axtra® XAP, Danisco Anima Nutrition/DuPont, Marlborough, Wilshire, UK) (XAP + DFM75). The remaining two treatments consisted of the NC with AGP inclusion; BMD 50, Zoetis, Florham Park, NJ 07932) and VM (Stafac 20, Phibro Animal Health, Ridgefield Park, NJ) at 50 g/ton and 20 g/ton, respectively (Table 10). Three dietary phases were fed throughout the duration of the trial including a starter phase, fed d 0 through 10, grower d 11 through 21, and finisher d 22 to 42. All birds were fed a mash diet from placement to termination and were allowed access to feed and water ad libitum. Feed samples were collected during feed manufacturing for nutrient analysis, which was conducted in triplicate. Crude protein was determined by combustion using an AOAC method (AOAC method 990.03) (AOAC), total phosphorous was determined by wet ash inductively coupled with plasma spectroscopy (AOAC method 985.01M), ADF was determined using an Ankom digestion unit (AOAC method 973.18) (Ankom Technology), and an ether extraction method was used to determine crude fat (AOAC method 920.39). Animal care throughout the duration of the trial was provided in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC).

Table 10. Ingredient profile, calculated and analyzed nutrient concentration of the negative control (NC) basal diet. The basal diet was supplemented with XAP + DFM75 (xylanase, amylase, and protease), DFM150, BMD, or VM. The dietary program consisted of a starter (d 0 to 10), grower (d 11 to 21), and finisher (d 22 to 42) dietary phases

Ingredient Profile	Starter	Grower	Finisher
Corn	47.85	52.03	60.03
Soybean Meal (48%)	31.00	24.89	17.99
DL - Methionine	0.31	0.27	0.20
L - Lysine HCL	0.29	0.25	2.10
L - Threonine	0.10	0.08	0.05
A/V Fat	0.61	2.10	1.49
Wheat	10.00	10.00	10.00
Limestone	1.35	0.91	0.68
Mono-calcium Phosphate	0.65	0.29	0
Salt	0.34	0.25	0.10
Sodium Bicarbonate	N/A	0.07	0.28
Trace Minerals[2]	0.05	0.05	0.05
Vitamins[3]	0.25	0.25	0.25
Choline Chloride 60 %	0.10	0.10	0.10
Coban 90[4]	0.05	0.05	0.05
LO Distillers Dried Grains w/ Solubles	5.00	5.00	5.00
Meat and Bone Meal	2.04	3.00	3.52
Phytase[5]	0.01	0.01	0.01
Calculated Nutrient Concentration			
Protein	23.34	21.15	18.66
Crude Fat	3.62	5.31	4.99
Calcium	1.05	0.90	0.80
AV Phosphorous	0.50	0.45	0.40
Metabolizable Energy (kcal/kg)	2905	3030	3080
dig Methionine	0.63	0.56	0.47
dig TSAA	0.94	0.84	0.72
dig Lysine	1.27	1.10	0.91
dig Tryptophan	0.23	0.20	0.17
dig Threonine	0.83	0.73	0.61
dig Arginine	1.35	1.20	1.02
Sodium	0.20	0.19	0.19
Analyzed Nutrient Concentration			
Moisture	12.22	12.20	13.15
Dry Matter	87.78	87.80	86.85
Crude Protein	23.80	22.10	20.20
Crude Fat	3.21	4.85	4.87
Fiber	2.80	3.00	3.20
Ash	5.04	4.72	4.23

Table 10. Continued
[1]Xylanase was analyzed and reported as 2400 U/kg for the starter, 1909 U/kg for the grower, and 2460 U/kg for the finisher.
[2]Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.
[3]Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.
[4]Active drug ingredient monensin sodium, 90 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necatrix, Eimeria tenella, Eimeria acervulina, Eimeria brunetti, Eimeria mivati, and Eimeria maxima*.
[5] Phytase was added at 500FTU/kg in all diets (Axtra® PHY **–** Danisco Animal Nutrition/ DuPont Industrial Biosciences, Marlborough, UK)

Performance Parameter Measurements

All broilers and feeds were weighed on d 10, 21, and 42 with measurements being calculated for average BW, mortality adjusted FCR, cumulative FCR, and FC. Chicks were reared on used litter in 6 x 6 ft. (1.8 m^2) floor pens and were provided age appropriate temperature. The lighting program included continuous light through 3 d of age at 25 lux, 23 hr. of light from d 4 to 7 at 25 lux, 20 hrs. of light from d 8 to 14 at 15 lux, 16 hr. of light from d 15 to 28 at 10 lux, 18 hr. of light from d 29 to 38 at 7 lux, and 23 hr. of light for d 39 to 42 at 7 lux.

Foot Pad Lesion Scores

On d 42, 6 birds per pen (3 males and 3 females) were randomly selected for determination of foot pad lesion. The methodology for foot pad lesion scoring was followed according to the protocol established by Bilgili et al. (2006) with a score of zero showing no signs of lesion, a score one showing minor redness to the foot pad, and a score of two showing visible lesions present on the surface of the foot pad.

Statistical Analysis

All data were subject to one-way ANOVA using the GLM model (SPSS Software) with means deemed significantly different at $P \leq 0.05$. Further, means determined to be different were separated using Duncan's Multiple Range Test.

Results and Discussion

The effectiveness of NSP degrading enzymes is dependent on the types of ingredients and substrate in the diet and is well documented that use of NSP degrading enzymes in commercial broiler can improve feed efficiency and reduce anti-nutritive factors associated with NSPs (Cowieson and Adeola, 2005; Masey O'Neill et al., 2012; Meng et al., 2005). They do so primarily through two diet-dependent mechanisms: reduction of intestinal viscosity in wheat-based diets and increased nutrient digestibility by limiting the amount of encapsulated nutrients in corn-based diets (Bedford, 1995; Bedford and Morgan, 1996; Crouch et al., 1997; Engberg et al., 2004; Min et al., 2009). More specifically, the combination of XAP has been shown to impart beneficial effects on broiler performance. Cowieson and Adeola (2005) concluded that supplementation of XAP and phytase improved performance and nutrient digestibility when compared to a negative control diet. Additionally, results from a study conducted by Olukosi et al. (2007) determined that XAP supplementation with phytase increased final BW as well as reduced FCR compared to a negative control diet. It is widely accepted that XAP supplementation beneficially influences broiler performance through increased nutrient digestibility; however, DFMs impact broiler performance through improvements in gastrointestinal health and by balancing the gut microflora. Aside from competitive exclusion and stimulating the immune system associated with the gut, inclusion of DFM into broiler diets have been documented to produce bacteriocins, increase the

production of short-chain fatty acids, reduce epithelial cell apoptosis, and stimulate the intraepithelial lymphocytes (Fuller, 1977; Ng et al., 2009; Nurmi and Rantala, 1973; Salim et al., 2013). Thus, it can be assumed that inclusion of both XAP and a DFM in combination may synergistically improve feed efficiency when compared to using the products separately (Momtazan et al., 2011). Momtazan et al. (2011) hypothesized that this improvement in feed efficiency with the inclusion of XAP and DFM may be a result in the reduction of intestinal pathogen levels within the gut; enzymes, by degrading the anti-nutritive factors and reducing the viscosity of the digesta particularly in diets containing an ingredient profile high in dietary NSP (wheat, barley, rye, etc), and DFM, by increasing the beneficial microflora, producing antimicrobial components and short chain fatty acids, and lowering the pH of the digestive tract.

Performance

On d 10, birds fed DFM150 had the heaviest average BW and were heavier ($P \leq 0.05$) than birds fed the NC, the combination of XAP+DFM75, as well as those fed BMD (Table 11). Virginiamycin treatment birds exhibited intermediate results to that of the birds fed the NC and NC with DFM150 supplementation. No differences on BW were observed on d 21 or 42. These data correspond with previous studies in which BW was increased ($P \leq 0.05$) during the early stages of growth but not in the later stages with DFM supplementation (Salim et al., 2013; Yeo and Kim, 1997). According to Yeo and Kim (1997), this improvement in growth performance in the early stage of the

Table 11. Effect of direct fed microbial (DFM150) and its combination with XAP (xylanase, amylase, and protease (XAP + DFM75)) supplementation on broiler growth performance as compared to antibiotic growth promoters (0 to 42 d)

Item	Treatment					PSEM
	NC[1]	DFM150[2]	XAP + DFM75[3]	BMD[4]	VM[5]	
Body Weight (kg/bird)						
10 d	0.174[bc]	0.187[a]	0.173[c]	0.177[bc]	0.183[ab]	0.002
21 d	0.664	0.683	0.677	0.668	0.670	0.004
42 d	2.234	2.306	2.295	2.282	2.273	0.122
Feed Consumption (g/bd/d)						
Starter (0 to 10 d)	18.8	19.67	18.7	18.1	18.9	0.225
Grower (11 to 21 d)	72.6	73.6	74.4	72.6	71.1	0.560
Finisher (22 to 42 d)	145.9	148.2	148.7	147.8	146.9	0.933
Body Weight Gain (kg)						
0 to 10 d	0.141[b]	0.153[a]	0.139[b]	0.144[b]	0.148[ab]	0.002
11 to 21 d	0.49	0.496	0.504	0.491	0.488	0.004
22 to 42 d	1.57	1.623	1.618	1.614	1.604	0.01
0 to 21 d	0.631	0.649	0.643	0.634	0.636	0.004
0 to 42 d	2.06	2.119	2.122	2.105	2.091	0.012
Mortality Corrected Feed Conversion Ratio (FCR) (feed:gain)						
Starter (0 to 10 d)	1.352	1.307	1.374	1.303	1.296	0.014
Grower (11 to 21 d)	1.623	1.628	1.614	1.615	1.603	0.013
Finisher (22 to 42 d)	1.948[a]	1.915[b]	1.896[b]	1.907[b]	1.929[ab]	0.006
0 to 21 d	1.565	1.557	1.567	1.551	1.535	0.012
0 to 42 d	1.841[a]	1.811[ab]	1.805[b]	1.806[b]	1.816[ab]	0.006

[a,b,c] Means with different superscripts within a row differ significantly at ($P \leq 0.05$).
[1] Negative Control, without supplementation; [2] NC + DFM150 (providing 150000CFU/g three Bacillus strains); [3] XAP + DFM75, NC plus the combination of xylanase, amylase, protease (XAP), and 75000CFU/g three *Bacillus* strains; [4]BMD, NC plus 50 g/ton of bacitracin methylene disalicylate; [5]Virginiamycin (VM), control plus 20 g/ton.

bird's life might be attributed to the build-up of a well-balanced microflora provided by the DFM within the diet. Although the differences in BW during the current trial were limited to the starter phase only, previous research has shown that differences in BW from placement to termination have been observed. Over the course of three separate trials, Hooge et al. (2004) observed an increase in BW with DFM supplementation comprised of *Bacillus subtilis* spores compared to the control diet while Nayebpor et al. (2007) observed an increase in BW at d21, 28, and 42 compared to the control. Although there have been reports of increased broiler performance with DFM supplementation, there are others with conflicting results in which supplementation with DFM had no effect on BW, however these inconsistencies may have been attributed to other factors (dietary composition, health status, environmental challenge) which may have impacted the efficacy of the DFM (Lee et al., 2010b; Waititu et al., 2014).

With regards to feed consumption, no differences were observed throughout the trial which agrees with previous studies that evaluated the individual inclusion of enzymes (Cowieson and Ravindran, 2008a; Meng et al., 2005) and DFM (Nayebpor et al., 2007; Waititu et al., 2014). Although during the current trial XAP and DFM were used in conjunction with each other, no differences were observed in feed consumption as reported in previous research. Miles et al. (2006) observed that feed intake was not impacted with the inclusion of VM or BMD within diets which is in agreement with the

observations in this study in which the supplementation of BMD and VM individually did not impact FC.

No differences were observed in FCR during the starter and grower phases. However, during the finisher phase, a reduction ($P \leq 0.05$) in FCR was observed in birds fed DFM150, the combination of XAP+DFM75, and BMD compared to the NC, with the VM fed broilers being intermediate. Similar to the results in the current study, Lei et al. (2014) observed that DFM supplementation can reduce FCR during the finisher phase as compared to the NC. Furthermore, previous research has shown that inclusion XAP can improve FCR and overall broiler performance (Cowieson and Ravindran, 2008a; Meng et al., 2005). The use of AGP's has been shown to reduce FCR in the later stages when compared to a control diet which is in agreement with the current study (Mountzouris et al., 2010).

No differences were found in mortality corrected FCR during starter and grower phases, however, in finisher phase, DFM150, the combination of XAP+DFM75, as well as BMD showed lower mortality corrected FCR compared to negative control. Cumulatively, no differences in mortality corrected FCR were observed from placement through d 21, however, from placement to termination on d 42, a similar trend to that of the finisher phase FCR was seen. Birds fed the combination of XAP+DFM75 and BMD exhibited a lower ($P \leq 0.05$) FCR compared to the NC, DFM and VM. This corresponds with previous research where the inclusion of carbohydrases with and without protease

(Cowieson and Adeola, 2005; Masey O'Neill et al., 2012) and DFM (Awad et al., 2009; Jin et al., 1998) reduced FCR when compared to the control. In the current study, built up litter was used that could increase the challenge to the birds which simulated the commercial farming conditions. Under this condition, DFM seems to be more effective in improving body weight gain during the starter phase and the combination of XAP+DFM75 is more effective in improving feed efficiency in the finisher phase. This benefit could derive from the different modes of action for enzymes and DFM function cooperatively within the gut. Inclusion of xylanase can potentially increase the digestion of non-starch polysaccharides which could provide the substrates needed for the DFM to flourish. Amylase can improve the breakdown of starch while protease may reduce the levels of indigestible nitrogen, which could potentially limit the availability of substrate for pathogenic bacteria. If the mechanisms of XAP and DFM are able to reach their full potential, logically the combination of these products may help improve intestinal health and microbial balance of the gut which may result in improved broiler growth performance and feed efficiency.

Foot Pad Lesion Scores

Foot pad dermatitis (FPD) is a common animal welfare concern that affects the plantar surface of the footpad in growing broilers. Foot pad dermatitis is known by multiple names, such as pododermatitis and contact dermatitis, all of which refer to a condition that is characterized by inflammation and necrotic lesions, ranging from superficial too deep on the plantar surface of the footpads

and toes (Shepherd and Fairchild, 2010). Factors that may affect the onset of FPD include nutritional deficiencies and more commonly litter moisture. Shepherd and Fairchild (2010) noted that deficiencies of amino acids and vitamins such as methionine, cystine, riboflavin, and biotin in the diet increased the incidence of FPD. Increased litter moisture has also been shown to increase the incidence of FPD by softening the footpad (Mayne, 2005). This results in extensive ulcers which may ultimately lead to abscesses and thickening of underlying tissue (Greene et al., 1985). During the current study, a reduction ($P \leq 0.05$) in foot pad lesion scores was seen in birds fed the combination of XAP+DFM75 compared to the NC (Figure 2). This was in agreement with a previous study conducted by Dersjant-Li et al. (2015) in which the combination of XAP and DFM reduced average foot pad lesion scores compared to the control and was a result of increased litter quality which was attributed to decreased water intake and increased dry matter in the litter. The combination also exhibited similar results to that of both AGP treatments and DFM150 indicating that all treatments can potentially reduce the likelihood of foot pad dermatitis. According to Awati et al. (2014), DFM supplementation has been shown to reduce litter ammonia concentration which may help explain the reduction in foot pad scores seen between NC and the feed additive treatment containing XAP and DFM.

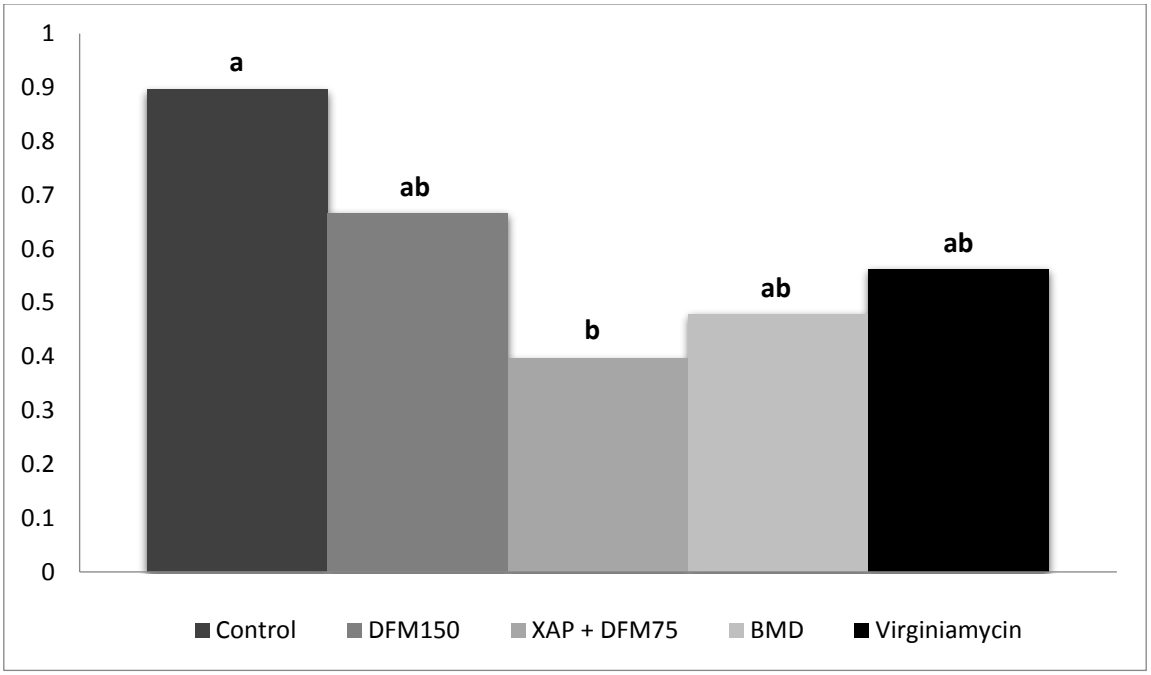

Figure 2. Effect of diet type on foot pad dermatitis.
Effect of direct-fed microbials (DFM150) and its combination with XAP (xylanase, amylase, and protease (XAP + DFM75)) supplementation on foot pad dermatitis scores compared to AGPs (42 d). [a,b] Means with different superscripts within a row differ significantly at $P \leq 0.05$. Negative Control, without supplementation; DFM150: control + DFM150, providing 150000CFU/g three Bacillus strains; XAP + DFM75: control plus a combination of xylanase, amylase, protease (XAP), and 75000CFU/g three *Bacillus* strains; BMD, control plus 50 g/ton of bacitracin methylene disalicylate; Virginiamycin, control plus 20 g/ton.

Caloric Conversion

Caloric conversion was measured to evaluate feed efficiency throughout the duration of the trial and was calculated as:

$$\text{Calorie Conversion} = \frac{\text{kcal of energy consumed}}{\text{kg of body weight gain}}$$

A reduction ($P \leq 0.05$) in caloric conversion (120 kcal less energy was used per kg BWG) was observed in birds fed the feed additive containing the combination of XAP+DFM75 (5521 kcal/kg BWG) compared to the NC (5641 kcal/kg BWG). The treatments containing DFM150, BMD, and VM were intermediate, showing numerically lower caloric conversion ratio compared to NC (Figure 3). The reduction necessary for the production of a kg of BWG observed with the inclusion of DFM150 (101 kcal/kg BWG) and the combination of XAP+DFM75 (120 kcal/kg BWG) compared to the NC correlates with previous research where DFM (Dersjant-Li et al., 2014) or the combination of XAP+DFM (Dersjant-Li et al., 2015) supplementation resulted in a lower caloric conversion ratio. Similar to the trends seen with the foot pad scores, both DFM and the combination of XAP+DFM75 treatment exhibited similar results to that of both AGP suggesting that the inclusion of both DFM150 or the combination DFM75 with XAP can be as effective as AGPs in converting raw energy to BW. Overall, the inclusion of DFM150 increased BWG in the early stages while reducing FCR in the later stages. Similarly, inclusion of the feed additive containing XAP +

DFM75 improved feed efficiency, foot pad lesion scores, and caloric efficiency to levels similar to AGP inclusion.

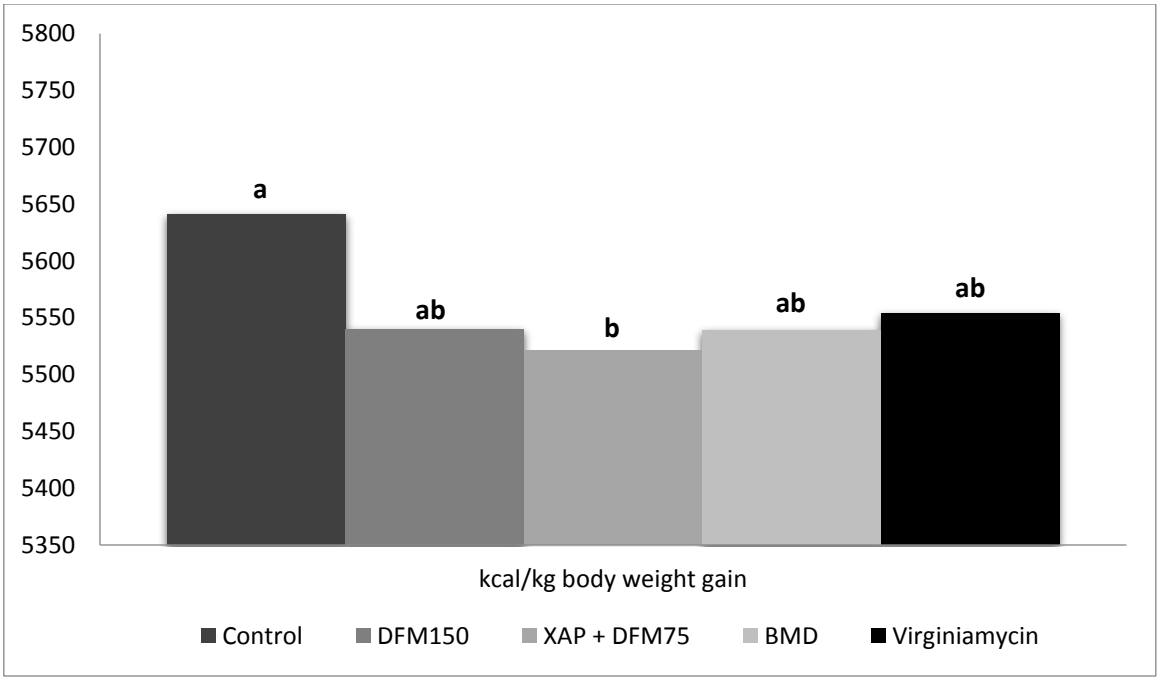

Figure 3. Effect of diet type on calorie conversion.
Effect of direct-fed microbials (DFM150) and its combination with XAP (xylanase, amylase, and protease (XAP + DFM75)) on calorie conversion (kcal of energy consumed/kg of body weight gain) compared to AGPs treatments (42 d). [a,b] Means with different superscripts within a row differ significantly ($P \leq 0.05$). Negative Control, without supplementation; DFM: control + DFM150, providing 150000CFU/g three Bacillus strains; XAP + DFM75, control plus a combination of xylanase, amylase, protease (XAP) and 75000CFU/g three *Bacillus* strains ; BMD, control plus 50 g/ton of bacitracin methylene disalicylate; Virginiamycin, control plus 20 g/ton.

CHAPTER V

EFFECT OF DIRECT FED MICROBIAL AND ENZYME BLEND COMBINATION ON GROWTH PERFORMANCE IN BROILERS FED U.S. COMMERCIAL DIETS, WITH OR WITHOUT AGP

Introduction

Historically, AGP have been a commonly used additive in broiler diets to promote broiler performance by reducing infection by gastrointestinal pathogens. However, growing public concern over the development of antibiotic resistant microorganisms resulting from the use of AGP in animal feeds (Salim et al., 2013) has led to changes worldwide throughout the animal feeding industry. These concerns led to the first ban on the use of AGP as animal feed additives in Sweden in 1986 (Casewell et al., 2003); followed by bans on AGPs in Denmark (1995) and Germany (1996). Eventually by 1999, the EU enacted regulations which removed all AGP from the Community Register of authorized feed additives by 2006 (Castanon, 2007). The most recent country to implement a ban on antibiotics as growth promoters was South Korea in 2012 with more countries expected soon to follow (Lee et al., 2015). There is a need for an alternative to AGP to not only keep the risk of enteric disease at a minimum, but also to satisfy the demand and increasing consumer popularity for products deemed “antibiotic-free”.

Direct-fed microbial products are products that contain live, viable microorganisms administered into feed. Due to the improvements on gastrointestinal microbiota and morphology, DFM are often classified as probiotics which are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host ultimately improving overall bird performance (Hill et al., 2014). The most widely accepted and documented modes of action by which DFM facilitate improvements on bird performance is through competitive exclusion of enteric pathogens (Lee et al., 2010b; Salim et al., 2013). Direct fed microbial cultures limit the available resources that enteric pathogens (*Clostridium* spp. and *Eimeria* spp.) utilize and as a result, create a suitable environment within the gastrointestinal tract for beneficial bacteria colonization allowing the bird to maximize its ability to grow efficiently, thereby improving broiler performance (Awad et al., 2009; Fritts et al., 2000; Kabir, 2009).

The anti-nutritive properties of NSP present a nutritional challenge when feeding broilers. Monogastric animals have limited digestive enzymes in their gastrointestinal tract and as a result, the application of non-starch polysaccharide degrading enzymes have become common throughout the industry in an effort to utilize the full nutritive value of the diet. The effectiveness of these enzymes has been shown to have various results depending on diet type (reduction of digesta viscosity in wheat-based diets and limiting the encapsulation effect in corn-based diets). Recently, DFM have been combined

with exogenous enzymes (Dersjant-Li et al., 2014; Dersjant-Li et al., 2015; Momtazan et al., 2011) to evaluate the combination of these products in broiler diets. Dersjant-Li et al. (Dersjant-Li et al., 2015) determined that the combination of XAP and *Bacillus*-based DFM spores improved broiler performance by increasing feed efficiency as well as through improvements in litter conditions. Therefore, the objective of the current study was to evaluate the effect of a feed additive containing XAP in combination with *Bacillus*-based DFM spores on broiler growth performance, in the presence or absence of AGP. It is hypothesized that the inclusion of the feed additive will improve broiler growth performance regardless of the presence of AGP inclusion.

Materials and Methods

Experimental Design and Diets

The current study was conducted as a randomized block design consisting of 2,160 day old Cobb 500 male broiler chicks assigned to 6 experimental treatments with 9 replications per treatment for a 42 d assay period. Animal care throughout the duration of the trial was provided in accordance with a protocol approved by the Texas A&M University IACUC. The experiment was arranged as a 3 (AGP) x 2 (feed additive) factorial experiment consisting of a corn/soy based control diet formulated on a digestible amino acid basis with 10% wheat inclusion, 5% DDGS, and 500 FTU/kg inclusion of phytase, control + BMD at 50g/ton, and control + VM at 20g/ton. Full matrix values were provided for phytase contribution including available phosphorous,

calcium, sodium, metabloziable energy, and digestible amino acids (Axtra® PHY - according to the manufacturer's recommendation). These treatments were then supplemented with or without a feed additive (Syncra® AVI, Danisco Animal Nutrition/DuPont) that contained a *Bacillus* DFM (3 strains of *Bacillus* at 75,000 CFU/g) in combination with XAP (provides 2,000 U/kg endo-xylanase from *T. reesei*, 200 U/kg alpha-amylase from *B. licheniformis*, and 4,000 U/kg serine protease from *B. subtilis*) (XAP + DFM) resulting in the remaining three treatments: control + feed additive, BMD + feed additive, and VM + feed additive. Three dietary phases were fed throughout the duration of the trial including a starter phase, fed (d 0 to 10) as a crumble, grower (d 11 to 21) as a pellet, and finisher (d 22 to 42) as a pellet (Table 12). During feed manufacturing, one large basal diet was manufactured and divided in the equal portions prior to the addition of the test product. Premixes including AGP, feed additive, and sand were included 900 g/ton. All diets were pelleted at 65ºC with a 15 second conditioning time. All birds were allowed access to feed and water *ad libitum* through the duration of the trial. Feed samples were collected during feed manufacturing for nutrient analysis, which was conducted in triplicate. Crude protein was determined by combustion using an AOAC method (AOAC method 990.03) (AOAC), total phosphorous was determined by wet ash inductively coupled with plasma spectroscopy (AOAC method 985.01M), ADF was determined using an Ankom digestion unit (AOAC method 973.18) (Ankom

Table 12. Ingredient profile, calculated and analyzed nutrient concentration of the control basal diet for the starter (d 0 to 10), grower (d 11 to 21), and finisher (d 22 to 42) dietary phases.

Feed Composition (%)	**Starter**	**Grower**	**Finisher**
Corn	47.85	52.88	60.03
Soybean Meal	31.00	24.78	17.99
Wheat	10.00	10.00	10.00
DL - Methionine	0.31	0.27	0.20
Lysine HCL	0.29	0.25	0.21
L - Threonine	0.10	0.08	0.05
Fat, Blended	0.61	1.75	1.49
Limestone	1.35	0.89	0.68
Monocalcium Phosphate	0.65	0.33	N/A
Salt	0.34	0.28	0.10
Sodium Bicarbonate	N/A	0.03	0.28
Trace Minerals[1]	0.05	0.05	0.05
Vitamins[2]	0.25	0.25	0.25
Choline	0.10	0.10	0.10
Coban 90[3]	0.05	0.05	0.05
LO - DDGS	5.00	5.00	5.00
MBM	2.04	3.00	3.52
Phytase[4]	0.01	0.01	0.01
Nutrient, %	**Starter**	**Grower**	**Finisher**
Protein	23.34	21.17	18.66
Crude Fat	3.62	5.00	4.99
Crude Fiber	3.04	2.94	2.85
Calcium	1.05	0.90	0.80
AV Phosphorous	0.49	0.45	0.39
Metabolizable Energy (kcal/kg)	2928	3060	3119
Digestible Methionine	0.63	0.56	0.47
Digestible TSAA	0.94	0.84	0.72
Digestible Lysine	1.27	1.10	0.91
Digestible Tryptophan	0.23	0.20	0.17
Digestible Threonine	0.83	0.73	0.61
Digestible Arginine	1.35	1.20	1.02
Sodium	0.20	0.19	0.19
Analyzed Nutrient %	**Starter**	**Grower**	**Finisher**
Moisture	12.98	12.25	12.49
Dry Matter	87.02	87.75	87.51
Crude Protein	21.7	19.7	18.9
Crude Fat	4.60	4.86	4.98
Fiber	3.4	3.6	3.1
Ash	4.88	4.68	4.47

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

[2] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

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

[4] Phytase was added at 500FTU/kg in all diets (Axtra® PHY – Danisco Animal Nutrition/ DuPont Industrial Biosciences, Marlborough, UK

Technology), and an ether extraction method was used to determine crude fat (AOAC method 920.39).

Performance Parameter Measurements

All broilers and feed were weighed on d 10, 21, and 42 and average BW and FC were measured and used to calculate mortality-adjusted FCR. In an effort to standardize the data for accurate comparison of treatment means, a BW correction factor was applied to cumulative d 42 FCR with one point of FCR being equivalent to 27 g of BW. Chicks were reared on used litter in 6 x 6 ft. (1.8m^2) floor pens and were provided age appropriate temperature. The lighting program was continuous light through 3 d of age at 25 lux, 23 hrs. of light from d 4 to 7 at 25 lux, 20 hrs. of light from d 8 to 14 at 15 lux, 16 hrs. of light from d 15 to 28 at 10 lux, 18 hrs. of light from d 29 to 38 at 7 lux, and 23 hrs. of light for the remaining 3 d at 7 lux.

Foot Pad Lesion Scores

On d 42, 6 birds per pen were randomly selected for determination of foot pad lesion. The methodology for foot pad lesion scoring was according to the protocol established by Bilgili et al. (2006).

Statistical Analysis

All data was analyzed using a 3 (AGP) x 2 (feed additive) factorial ANOVA using the GLM (SPSS software). Main effect differences were deemed significant at $P \leq 0.05$ and in the instance of a significant interaction, a one-way

ANOVA was used to determine individual treatment differences as separated using Duncan's Multiple Range Test.

Results

No differences or interactions were observed between feed additive supplementation or with AGP inclusion with regards to FC or mortality during the starter, grower, and finisher phases (Table 13). No differences in BW or BWG were observed on d 10 and 21; however, a 1.6% increase ($P \leq 0.05$) in final BW was observed with supplementation of the feed additive compared to the control on d 42 (Table 14). On d 42, a main effect was observed with feed additive supplementation yielding an increase ($P \leq 0.05$) in BWG compared to the control. A main effect was also observed for cumulative BWG through 21 and 42 d yielding an increase ($P \leq 0.05$) with feed additive supplementation compared to the control. Throughout the experiment, no significant differences or interactions were observed with AGP inclusion with regards to BW or BWG.

No differences or interactions in FCR were observed during the starter phase with feed additive supplementation or with AGP inclusion (Table 15). However, an interaction between AGP and feed additive administration was observed during the grower phase. Inclusion of BMD and supplementation of VM + feed additive significantly ($P \leq 0.05$) reduced FCR compared to the control and VM inclusion. Supplementation of the feed additive into the control also significantly ($P \leq 0.05$) reduced FCR compared to VM inclusion. During the

Table 13. Feed consumption (g/bird/day) and mortality (%) of male broilers fed a diet containing DFM, feed additive containing an enzyme blend (XAP) and DFM, AGP individually, or in combination.

Treatment		[1]Starter	[2]Grower	[3]Finisher	Total Feed Consumption	[1]Starter	[2]Grower	[3]Finisher	Total Mortality
Control	Control	26.7	91.2	200.3	120.3	1.1	0.6	4.0	5.0
BMD[4]	Control	26.0	91.5	199.8	117.5	0.0	1.5	2.9	3.9
VM[5]	Control	26.2	89.9	193.9	118.0	0.5	0.6	2.5	3.3
Control	Feed Additive[6]	26.2	90.9	193.0	118.8	0.8	1.9	1.3	3.6
BMD[4]	Feed Additive[6]	26.0	91.5	199.8	117.5	0.0	1.5	2.9	3.9
VM[5]	Feed Additive[6]	26.0	89.3	195.7	117.7	0.3	0.6	3.3	3.6
Main Effects*									
AGP									
	BMD	25.9	90.3	195.8	117.5	0.3	1.2	2.1	3.2
	Virginiamycin	26.1	89.6	194.8	117.8	0.4	0.8	2.9	3.5
	Control	26.4	91.1	196.6	119.6	1.0	1.2	2.7	4.3
Feed Additive[6]									
	Feed Additive[6]	26.1	90.6	196.2	118.0	0.4	1.3	2.5	3.7
	Control	26.2	90.1	195.3	118.6	0.7	0.7	2.6	3.6
P-Value, AGP		0.138	0.138	0.786	0.139	0.192	0.384	0.884	0.655
P-Value, Feed Additive		0.419	0.385	0.688	0.526	0.237	0.163	0.917	0.937
P-Value, AGP*Feed Additive		0.396	0.094	0.022	0.771	0.914	0.527	0.165	0.423
Pooled SEM		0.1	0.3	1.1	0.5	0.2	0.2	0.5	0.5

[1]Starter phase: day 0 to 10; [2]Grower phase: day 11 to 21; [3]Finisher phase: day 22 to 42;
[4]BMD – Bacitracin Methylene Disalicylate (50g/ton);[5]VM – Virginiamycin (20g/ton); [6]Feed Additive – XAP + DFM comprised of xylanase, amylase and protease and 3 *Bacillus* strains

Table 14. Table 14 Body weight (g) and body weight gain (BWG) (g) of male broilers fed a diet containing DFM, feed additive containing an enzyme blend and DFM, AGP individually, or in combination.

Treatment		Day 0	Day 10	Day 21	Day 42	[1]Starter BWG	[2]Grower BWG	[3]Finisher BWG	D0-21 BWG	D0-42 BWG
Control	Control	37.7	264	955	3137	227	690	2182	917	3099
BMD[4]	Control	37.7	260	949	3152	223	689	2203	911	3114
VM[5]	Control	37.7	266	949	3142	229	683	2193	911	3104
Control	Feed Additive[6]	37.5	266	959	3169	229	693	2210	922	3131
BMD[4]	Feed Additive[6]	37.7	265	961	3225	227	696	2263	924	3187
VM[5]	Feed Additive[6]	37.5	262	952	3185	225	693	2229	918	3147
Main Effects*										
AGP										
BMD		37.7	262	955	3188	225	693	2233	917	3151
Virginiamycin		37.6	264	952	3163	227	688	2211	915	3126
Control		37.6	265	957	3153	228	691	2196	919	3115
Feed Additive[6]										
Feed Additive[6]		37.6	264	959	3193[a]	227	694	2234[a]	921[a]	3155[a]
Control		37.7	263	951	3144[b]	226	687	2193[b]	913[b]	3106[b]
P-Value, AGP		0.952	0.158	0.637	0.691	0.140	0.552	0.593	0.632	0.691
P-Value, Feed Additive		0.503	0.540	0.063	0.013	0.454	0.063	0.029	0.058	0.013
P-Value, AGP*Feed Additive		0.928	0.065	0.730	0.667	0.070	0.671	0.747	0.737	0.668
Pooled SEM		0.09	0.001	2	14	1	2	13	2	14

[a,b] Superscripts indicate significant differences between treatments (P≤0.05)
[1]Starter phase: day 0 to 10; [2]Grower phase: day 11 to 21; [3]Finisher phase: day 22 to 42 [4]BMD – Bacitracin Methylene Disalicylate (50g/ton)
[5]VM – Virginiamycin (20g/ton);[6]Feed Additive – XAP + DFM comprised of xylanase, amylase and protease and 3 *Bacillus* strains

Table 15. Feed conversion ratio (FCR), body weight corrected FCR, and average foot pad lesion score of male broilers fed a diet containing DFM, feed additive containing mixed enzymes and DFM, AGP individually or in combination.

Treatment		[1]Starter	[2]Grower	[3]Finisher	D0-21	D0-42	[4]BW Corrected D42	Avg. Foot Pad Score
Control	Control	1.183	1.445[ab]	1.875	1.38	1.663	1.51	2.28
BMD[5]	Control	1.163	1.422[c]	1.824	1.357	1.625	1.467	1.78
VM[6]	Control	1.148	1.448[a]	1.831	1.371	1.636	1.482	2.20
Control	Feed Additive[7]	1.156	1.425[bc]	1.829	1.358	1.625	1.461	2.17
BMD[5]	Feed Additive[7]	1.147	1.428[abc]	1.807	1.357	1.612	1.427	1.76
VM[6]	Feed Additive[7]	1.162	1.413[c]	1.806	1.352	1.612	1.442	1.76
Main Effects*								
AGP								
	BMD	1.155	1.425	1.815[b]	1.357	1.619[b]	1.447	1.77[b]
	Virginiamycin	1.155	1.431	1.818[b]	1.362	1.624[b]	1.462	1.98[ab]
	Control	1.169	1.435	1.852[a]	1.369	1.644[a]	1.486	2.22[a]
Feed Additive[7]								
	Feed Additive[7]	1.155	1.422[b]	1.814[b]	1.356[b]	1.616[b]	1.443[b]	1.90
	Control	1.165	1.438[a]	1.843[a]	1.369[a]	1.641[a]	1.465[a]	2.08
P-Value, AGP		0.264	0.355	0.016	0.079	0.015	0.114	0.006
P-Value, Feed Additive		0.196	0.006	0.005	0.002	>0.001	0.001	0.087
P-Value, AGP*Feed Additive		0.077	0.019	0.485	0.092	0.332	0.935	0.257
Pooled SEM		0.004	0.003	0.007	0.002	0.004	0.009	0.060

[a,b,c] Superscripts indicate significant differences between treatments (P≤0.05)

[1]Starter phase: day 0 to 10; [2]Grower phase: day 11 to 21; [3]Finisher phase: day 22 to 42; [4]One point of FCR, equivalent to 27g of BW [5]BMD – Bacitracin Methylene Disalicylate (50g/ton); [6]VM – Virginiamycin (20g/ton); [7]Feed Additive – XAP + DFM comprised of xylanase, amylase and protease and 3 *Bacillus* strains

finisher phase, a reduction (P≤0.05) in FCR was observed with the feed additive supplementation compared to the control as well as with BMD and VM inclusion compared to control. Cumulatively through d 21 and d42, feed additive supplementation reduced (P≤0.05) FCR compared to the control. Similarly, a reduction (P≤0.05) in cumulative d 42 FCR was also observed with BMD and VM inclusion compared to the control. A reduction (P≤0.05) in BW-corrected FCR on d 42 was also observed with feed additive supplementation compared to control.

Broilers fed the control diet exhibited increased (P≤0.05) incidence and severity in average foot pad lesions compared to birds fed diets with BMD inclusion (Table 15). Broilers fed diets with VM were intermediate between control and BMD.

Discussion

The objective of the current experiment was to evaluate the effect of a feed additive containing XAP in combination with a *Bacillus*-based DFM on broiler growth performance, in the presence or absence of AGP. Although the usage of AGP worldwide was begun to decline due to concerns of antibiotic resistance, the inclusions of BMD, VM, and other AGP have proven to be successful at improving broiler performance. When administered into feed, these products modify different aspects of bacterial cellular metabolism, resulting in impaired growth or death of the microorganism (Ferket, 2004) as well

as reduce the number of opportunistic pathogens and subclinical infection leading to increased feed efficiency (Dibner and Richards, 2005). Although there was no effect with AGP on BW and feed consumption, both BMD and VM significantly ($P<0.05$) reduced finisher phase and d 42 cumulative FCR compared to the control diet which correspond to results seen in the literature (Hooge et al., 2003; Miles et al., 1984).

The improvements in BW, BWG, and FCR with feed additive supplementation in the absence of a feed intake response suggest that the improvements in broiler performance were not a result of FC but feed efficiency. Previous research has shown that improvements in broiler performance with DFM (Jeong and Kim, 2014; Mountzouris et al., 2010; Waititu et al., 2014; Zhang and Kim, 2014) and XAP (Coppedge et al., 2012; Cowieson and Ravindran, 2008a; Meng et al., 2005)) supplementation may be achieved without impacting feed intake. Although DFM and XAP were administered individually during these studies and not in combination as in the current experiment, the combination of these products may improve broiler performance due to the mechanisms and modes of action by which these products interact and coexist within the gastrointestinal tract of the bird.

Direct fed microbials have demonstrated the ability to alter host immune response and intestinal microbiota through multiple modes of action (Chichlowski et al., 2007; De Vuyst and Leroy, 2007; Salim et al., 2013). One of the most noteworthy mechanisms by which DFMs confer their beneficial

gastrointestinal influence is through competitive exclusion. The colonization of the intestinal villi and crypts by beneficial bacteria limit mucosal attachment sites and nutrients available for potential enteric pathogens, such as *Salmonella* spp. and *Clostridium* spp. (Duggan et al., 2002). Direct fed microbials have also been shown to produce VFA within the gastrointestinal tract, ultimately lowering the pH, which provides a suitable environment for the beneficial bacteria. Marteau et al. (2004) noted that the formation of these weak organic acids alters the environmental pH of the gastrointestinal tract below levels which are needed for the survival of non-indigenous pathogenic bacteria. Volatile fatty acids also serve as energy sources to the bird, are readily available for uptake in the small intestine and colon, stimulate electrolyte and water absorption within the intestinal tract, and play a key role in the development and proliferation of epithelial cells (Chichlowski et al., 2007). The production of SCFA from DFM supplementation has been noted to promote the proliferation and functional maturation of intestinal epithelial cells which may lead to improvements in nutrient absorption. Further development and maturation of intestinal epithelial cells with *Bacillus*-based DFM supplementation has shown to lead to increased villi height, indicating that the epithelium is functionally active, and decreased crypt depth, indicating a reduction in cellular regeneration due to intestine damage (Jayaraman et al., 2013; Lei et al., 2015). The relationship between villi height and crypt depth is considered to be one of the most important parameters when evaluating intestinal health and recovery and are directly correlated to

increased nutrient digestion and absorption. When taking these various mechanisms into consideration, it is plausible to assume that with DFM supplementation improvements within the gastrointestinal tract may be achieved. These gastrointestinal improvements may then create a suitable environment for efficient exogenous NSP-degrading enzyme activity.

Unlike ruminants, monogastric animals, such as broilers, lack the necessary endogenous enzyme production to effectively utilize the complete nutritive value of the diet. In order to mitigate the effect of NSP, which comprise the endosperm cell wall of major cereal grains, the effective supplementation of an enzyme blend, such as XAP, is essential. Cowieson and Ravindran (2008b) concluded the combination of a xylanase, amylase, and protease improved the overall nutritive value of a corn/soybean-based diet in a dose dependent manner while Olukosi et al. (2007) improved overall performance of broilers fed a corn/SBM diet that was marginally deficient in ME and phosphorus with supplementation of XAP and phytase. Because modern diets contain various sources of carbohydrates (corn, wheat, DDGS, rye, etc.) with varying NSP content, supplementation with of exogenous enzymes blends may be the most effective way to enhance NSP degradation. The effectiveness of xylanase supplementation has shown to be a result of two mechanisms: reduction of intestinal viscosity in broilers fed wheat-based diets and the release of encapsulated nutrients from within the endosperm cell wall of corn-based diets. Increased intestinal viscosity is generally a concern associated with feeding

wheat-based diets due to the much higher concentration of water-soluble NSP and conversely is typically not an issue seen in corn/SBM based diets because of the lower NSP content (Yegani and Korver, 2013). Hydrolysis of arabinoxylans and degradation of the xylose-linked β, 1-4 bonds with xylanase supplementation in corn-based diets reduces the encapsulating effect, allowing access to nutrients which would normally be inaccessible to the bird (Bedford, 1995; Cowieson, 2005). These nutrients are then able to be absorbed which can potentially lead to an increase in protein, starch, and energy utilization thereby increasing broiler performance (Slominski, 2011). In a study conducted by Gracia et al. (2003), supplementation of exogenous α-amylase in a corn/soybean meal based diet increased hydrolysis and digestibility of starch and decreased pancreas weight which was determined to be a result of less secretion of endogenous amylase due to presence of exogenous amylase in the intestine. Additionally, protease supplementation has been shown to significantly increase protein hydrolysis, solubility, and digestibility through improved digestion of crude protein leading to significant improvements in broiler performance (Fru-Nji et al., 2011). Furthermore, exogenous protease supplementation may increase endogenous peptidase production reducing the nutritional requirement for amino acids and ME (Cowieson, 2005). Caine et al. (1998) showed that the effect of trypsin inhibitors decreased with protease supplementation allowing for additional protein digestion, pancreatic secretion of trypsin, and utilization of amino acids. The influence of these enzymes on

intestinal microbiota through the coordinated digestion of dietary carbohydrates and NSP is a result of two main mechanisms which were described by Choct et al. (1996) and Bedford (1996; 2000b): removal of fermentable starch and protein through accelerated digestion and formation of fermentable oligosaccharides as a result of the de-polymerization of insoluble fiber. The sugars that are released are fermented by intestinal bacteria resulting in the formation of SCFA which can then be used as energy yielding substrates by broilers (Romero et al., 2014).

These metabolic mechanisms are what link XAP and DFM supplementation and may result in the potential synergistic interaction. The improvements in carbohydrate degradation and utilization with XAP supplementation results in the formation of fermentable substrates (oligosaccharides) that beneficial bacteria (which have populated the intestinal mucosa through DFM supplementation) are able to utilize, thus creating an environment within the gut for efficient enzymatic activity. The synergistic effect of XAP and DFM administration on improving broiler performance has been reported previously (Dersjant-Li et al., 2014; Momtazan et al., 2011). The performance results from these experiments are in agreement with the current study in which significant improvements in BW, BWG, and FCR were observed with feed additive supplementation compared to the control diet. Similarly, Flores et al. (2016) observed that the supplementation of XAP and DFM significantly improved cumulative FCR through d 42, reduced incidence of foot pad lesions, and improved caloric conversion (kcal of energy consumed/kg of

body weight gain) compared to the control diet while producing similar results to BMD and VM treated birds. In summary, supplementation of XAP + DFM improved broiler performance compared to the control diet. Similarly, inclusion of BMD and VM significantly improved FCR compared to the control diet. Improvements in broiler performance were observed at the termination of the study with the inclusion of XAP + DFM irrespective of the presence of AGP.

CHAPTER VI

CONCLUSION

Supplementation of exogenous enzymes, such as xylanase, have become valuable tools when formulating modern broiler diets whether they contain viscous or non-viscous cereal grains. Proper usage of these additives have provided nutritionists a method to effectively utilize a larger portion of the nutrients in ingredients which would normally be inaccessible due to insufficient endogenous enzyme production.

Furthermore, increased concerns regarding antibiotic resistance and pressure from consumers has forced broiler producers to slowly begin phasing out the use of AGP for subtherapeutic purposes. For decades, AGP have been successful at improving broiler performance by mitigating enteric pathogen colonization within the gastrointestinal tract. The removal of these products will undoubtedly create a void in diet formulation, potentially allowing for greater enteric pathogen colonization. Although many products are currently available as potential alternatives to AGP, DFM have shown to be some of the most promising.

When evaluating the impact of a novel thermotolerant xylanase derived from *Pichia pastoris* on broiler growth performance and IDE (Chapter II), a significant improvement in feed efficiency was observed in broilers fed a corn-based diet containing DDGS. When evaluating the results from both Experiment

1 and 2, a clear dose response was observed with xylanase supplementation. Inclusion of xylanase at 20,000 units (XYL20) produced the most consistent improvements in BW and IDE and over the course of both experiments, showed improvements in FCR which lead to improvements in feed efficiency and overall broiler performance.

The efficacy of a *Bacillus*-based DFM in comparison to a known AGP on broiler growth performance and processing yield (Experiment 1) and in the presence of high and low DDGS (Experiment 2) was explored in Chapter III. Throughout the duration of Experiment 1, the supplementation of DFM consistently improved broiler performance (BW and BWG) compared to the control and produced similar results to BMD. Broilers supplemented with DFM produced a significantly higher carcass yield compared to the control and was similar to BMD. During Experiment 2, a few significant interactions were present between DDGS level and DFM. The nature of the interaction was an elevated level of improvement in performance with DFM supplementation in the high DDGS diet indicating the potential for a higher level of substrate with elevated DDGS level increasing efficacy. Overall, supplementation of the *Bacillus*-based DFM significantly increased broiler performance compared to the non-supplemented control through improvements in FC, BW, and BWG which were comparable to AGP inclusion. Also, inclusion of high levels of DDGS significantly increased FCR which reduced broiler performance; however,

increasing DDGS inclusion levels did not impact DFM efficacy, as DFM inclusion improved feed efficiency in DDGS containing diets, regardless of level.

When evaluating the effect of a DFM and its combination with XAP on broiler growth performance and foot pad lesion development in comparison to AGP (Chapter IV), supplementation of XAP+DFM75 produced the lowest FCR which was significantly different compared to the control and similar to BMD. Similarly, supplementation of XAP+DFM75 produced the best average foot pad lesion score and caloric conversion value and was significantly different from the control with DFM, BMD, and VM being intermediate. Overall, inclusion of DFM150 increased BW and BWG in the starter phase and reduced finisher phase FCR. Inclusion of XAP+DFM75 decreased finisher and cumulative FCR, improved foot pad lesion scores, and improved caloric efficiency by reducing the amount of energy (kcal) needed to produce one kg of BWG.

When investigating the impact of a feed additive, comprised of DFM and XAP, with and without AGP (Chapter V), supplementation of the additive improved BW, BWG, and FCR compared to the control diet. Furthermore, inclusion of both BMD and VM reduced cumulative FCR through d 42 compared to the control. Inclusion of BMD also reduced foot pad lesion development compared to the control with VM being intermediate. Based on previous research, the incorporation of the additive was expected to improve broiler performance; however, the continued improvement in performance with additive supplementation in conjunction with an AGP was an interesting observation

which was not initially expected as the inclusion of XAP+DFM improved broiler growth performance regardless of the presence of AGP.

In summation, these data indicate that xylanase supplementation has the ability to improve broiler growth performance as well as IDE values by as much as 180 kcal/kg by ameliorating anti-nutritive factors such as the encapsulation effect associated with NSP in the diet. This ultimately allows access to nutrients which normally be inaccessible to the bird through endogenous enzyme production allowing for greater nutrient utilization and improved broiler performance. Furthermore, results from these studies indicated that a *Bacillus*-based DFM and a feed additive containing exogenous enzymes and *Bacillus*-based DFM can improve broiler performance, carcass yield, foot pad lesion score, and caloric efficiency. The combination of exogenous enzymes and *Bacillus*-based DFM exhibited similar results in performance, foot pad lesion scores, and caloric efficiency compared to AGP inclusion. In an era absent of AGP, these results indicate that supplementing an additive consisting of exogenous enzymes and DFM can be potentially used as an alternative to AGP usage, ushering the continued sustainability and profitability of the broiler industry.

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