EVALUATION OF EXOGENOUS ENZYME COMBINATIONS ON BROILER PERFORMANCE IN REDUCED ENERGY DIETS

A Thesis

by

TUCKER GREGORY ALLCORN

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Chair of Committee,	Jason Lee
Committee Members,	Craig Coufal
	Tri Duong
Head of Department,	David Caldwell

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ABSTRACT

Two studies were performed to evaluate the efficacy of supplementing exogenous enzyme combinations on broiler growth performance in reduced nutrient density corn-soybean meal diets. In experiment 1, 3,200 Cobb 500 broilers were allocated to 5 treatments with 16 replicates for 39 days. The experiment consisted of a nutritionally complete positive control, AME reduced negative control (NC), NC + Non-starch polysaccharide degrading enzyme (NSPase) containing xylanase, β-glucanase, α-galactosidase, NC + an enzyme combination of xylanase, amylase, protease (XAP), and NC + NSPase + XAP. Apparent metabolizable energy in the NC was reduced by 55 kcal/kg in the starter phase and 88 kcal/kg in the finisher and withdrawal phases. Energy reduction in the NC significantly decreased average BW and significantly increased FCR through the starter and finisher phase. Non-starch polysaccharide degrading enzyme inclusion increased average BW significantly compared to the negative control at levels similar to that of the positive control during the starter and finisher phases. Non-starch polysaccharide degrading enzyme significantly reduced FCR compared to the NC at levels that were similar to the positive control. Inclusion of XAP resulted in BW similar to the PC at d 14 and 27, and reduced ($P<0.05$) FCR from d 1to 27. The combined inclusion of NSPase + XAP resulted in no further benefit beyond individual inclusion of each enzyme combination. In experiment 2, 2,590 Cobb 500 broilers were allocated to 7 treatments with 10 replicates for 41 d. Treatments consisted of a nutritionally complete reference diet, and 6 AME reduced (-88 kcal/kg) treatments composing a 2 X 3 factorial

of phytase and XAP inclusion. Phytase was included at 600 (low) and 1,200 (high) FTU/kg, with XAP included at 1,200 (low), 1,800 (medium), and 2,400 (high) XU/kg. High phytase x low XAP and high phytase x medium XAP both resulted in similar cumulative FCR compared to the reference diet. Factorial analysis indicated high phytase compared to low phytase significantly reduced starter FCR and elevated finisher mortality. During the starter phase, medium XAP inclusion resulted in a significantly lower rate of feed consumption compared to the low XAP. Inclusion of medium XAP significantly reduced cumulative FCR from d 15 to 41 compared to low level XAP. These data indicate that supplementation of multiple enzyme preparation into a diet can influence growth performance; however, combinations of enzyme preparations similar in mode of action do not result in performance levels beyond that of individual preparation inclusion.

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NOMENCLATURE

ME	Metabolizable energy
SBM	Soybean meal
DDGS	Distiller's dried grain with solubles
NSP	Non-starch polysaccharide
BW	Body weight
FCR	Feed conversion ratio
CP	Crude protein
AA	Amino acid
aP	Available phosphorus
FTU	Phytase unit
BXU	Birch xylan unit
ADG	Average daily gain
NC	Negative control
PC	Positive control
NSPase	Non-starch polysaccharidase
XAP	Xylanase amylase protease
MBM	Meat and bone meal
MCP	Mono-calcium phosphate
AID	Apparent ileal digestibility
WOG	Without giblets

IACUC	Institute of Animal Care and Use Committee
ANOVA	Analysis of variance
U	Unit

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Broiler production represents the largest sector of the poultry industry in the U.S. with a projected 40.6 billion pounds of ready-to-cook product being produced in 2016. According to the National Chicken Council (2016), the broiler industry will use approximately 1.2 billion bushels of corn and 500 million bushels of soybean to manufacture over 55 million tons of mixed feed. Feed is the largest production expense for poultry integrators (Tahir et al., 2012), representing over 50% of production costs. Nutritionists are tasked with the difficult job of formulating the proper blend of ingredients to provide necessary nutrients in the most economical and feasible way to maximize production and limit feed costs.

DIETARY INGREDIENTS

Poultry feed is composed of a relatively small number of ingredients. Feed will regularly contain a cereal grain, oilseed meal, calcium source, inorganic phosphate, salt, vitamin premix, mineral premix, fat source, synthetic amino acids, and possibly by-products of plant or animal origin and various feed additives. The majority of U.S. poultry feed is corn and soybean meal based. Corn regularly accounts for over 50% of a mixed feed, and its primary nutrient contribution is energy. Corn contains 3340 kcal/kg of metabolizable energy (ME) according to Leeson & Summers (2001). Energy is one of the most expensive nutrients in a feed, and including corn into diets at such a large amount makes it a driving force in the price determination of a finished feed. Soybean

meal (SBM), the most commonly used oilseed meal in U.S. poultry diets, is included for its high protein content. The protein content can be variable depending on how the meal is processed, but typically contains 48% protein (Leeson and Summers, 2001). Phosphorus is often considered the third most expensive nutrient following energy and protein, and must be included to meet the available phosphorus requirement of poultry to ensure proper skeletal growth and other developmental needs. Much of the phosphorus found in plant ingredients is in the form of phytate. It is indigestible to the monogastric digestive tract of poultry, and therefore the phosphorus from plant ingredients rarely meets the animal's requirement in the absence of an exogenous phytate degrading enzyme. Therefore, inorganic phosphate is usually included as a bioavailable source of phosphorus to meet the bird's requirement of this nutrient. By-product ingredients from vegetable and animal sources can be an economical choice to include in feed. Corn distillers dried grains with solubles (DDGS) is reported to be an acceptable ingredient in poultry feeds at certain concentrations (Lumpkins et al., 2004, Loar et al., 2010). Depending on current markets, distiller's dried grains with solubles, can be an economical alternative ingredient commonly included as a substitute to a portion of corn and SBM. Bakery by-product meal is another alternative ingredient that can be used as an alternative energy source to spare corn, however the nutrient content can vary greatly from source to source depending on the initial bakery product and processing of that product. Animal by-product ingredients, such as meat and bone meal and fish meal are high protein sources that can be used in poultry feed as well. Continued production of corn ethanol in the U.S. has injected volatility to the price of corn, at times driving up

the cost of feed and ultimately the price of food at the consumer level. Government policy has increased demand of corn ethanol which has shifted massive amounts of grain from animal agriculture use to renewable fuel production (Aho, 2007). This increased demand of corn for ethanol production can result in low corn inventories, thus driving up the price of the grain. This may also leave the corn market more vulnerable to extreme price spikes which can occur from events such as drought (Aho, 2007). The cost of ingredients and nutrient availability from ingredients are some of the considerations nutritionists must account for when formulating poultry diets. All of these considerations mean that researchers are regularly exploring novel methods of improving nutrient availability as well as lowering the cost of feed.

DIETARY ALTERNATIVE INGREDIENTS

The concept of alternative ingredient inclusion is that a relatively small amount of a more economical possibly non-traditional ingredient would be included as an alternative to a higher priced ingredient with the caveat of not negatively impacting broiler performance. One such example is the use of corn DDGS as a partial replacement of corn and SBM. Distiller's dried grains with solubles, the by-product of corn ethanol production, have been reported as an acceptable ingredient at certain concentrations in poultry diets. Corn ethanol is popularly produced using a dry-grind method where corn is ground and mixed with water and fermented to produce ethanol. The remaining products from this fermented ground corn slurry are germ, fiber and protein which compose DDGS (Martinez-Amezcua et al., 2007). Historically, DDGS have been fed to ruminants as it is a high-fiber ingredient. Ethanol production has increased from the

renewable fuels standard program originating from the Energy Policy Act (2005), and thus DDGS production has increased as well. Increasing supply of this ingredient and improved processing technology has made DDGS an ingredient of greater interest to poultry integrators. Many researchers have investigated the applicability of DDGS in poultry feeds. Guney et al., (2013) concluded that up to 20% of low-oil DDGS may be included in broiler diets with no detrimental effects, and it is reported by Shim et al. (2011) that DDGS may be included up to 24% in broiler diets with no adverse performance effects. Lumpkins et al. (2004) reported slightly lower inclusion rates of 6% DDGS for starter phase and 12 to 15% DDGS for grower and finisher phase while still maintaining equivalent broiler performance. Based on the recommendations of the above reports, nutritionists have found DDGS to be an acceptable ingredient from a nutritive standpoint and have been including it in poultry diets.

EXOGENOUS ENZYMES

The digestive system of poultry as well as other monogastric animals lacks the ability to completely utilize the nutrients of some ingredients, and the inclusion of exogenous enzymes is practiced to improve access to nutrients otherwise unavailable. The inclusion of exogenous enzymes in poultry diets is a method of improving production efficiency by increasing nutrient availability with maintained performance and in some instances improved performance. There are several factors of plant based ingredients that decrease the availability of nutrients. Two important anti-nutritive structures of concern to nutritionists are non-starch polysaccharides (NSP) which are a component of dietary fiber found in the plant cell wall, and phytate a previously

mentioned storage form of phosphorus that is poorly available to poultry and can bind other nutrients as well. Non-starch polysaccharides found in poultry diets can include arabinoxylans and cellulose primarily from corn (Jaworski, 2015) as well as β-glucans, mannans, and pectins among others. Non-starch polysaccharides can alter digesta viscosity and decrease weight gain and increase feed conversion ratio (FCR) (Leeson and Summers, 2001). Digesta viscosity is a concern because when increased by the presence of NSP, nutrient digestion from regular physical transport is reduced and so is the likelihood of endogenous enzyme to substrate contact. Non-starch polysaccharides can encapsulate nutrients within ingredients as the cell wall of fiber poses a physical barrier to the monogastric digestive tract (Bach Knudsen, 2014). Fiber, being comprised of NSP, can ultimately reduce nutrient utilization in broilers and lead to lower ME values of feed (Bach Knudsen, 2014).

Phytate, a form of plant phosphorus, is a major anti-nutritive factor influencing digestibility in poultry diets. The phosphorus in phytate is not available to birds due to the monogsastric digestive system of chickens lacking the endogenous phytase enzyme. In several ingredients, including corn, soybean meal and wheat, an average of only 48.9% of total phosphorus was found to be in the form of non-phytate phosphorus (Tahir et al., 2012). The inability of birds to digest phytate phosphorus represents a problem in itself, as a bird may not be receiving its physiological requirement of phosphorus from ingredients whose main phosphorus contribution is from phytate. Adding to this issue, is the fact that phytate can bind other nutrients such as zinc, calcium, magnesium and iron, resulting in lowered bioavailability of these as well (Leeson and Summers, 2001). The

ability of phytate to bind other nutrients is due to its chemical structure, where negatively charged phosphate groups have the ability to chelate cations. The cations most commonly chelated to the phytate include the minerals listed earlier such as Zn, Ca, Mg, and Fe as well as Mn and Cu (Biehl et al., 1995, Sebastian et al., 1996, Leeson and Summers, 2001). Once bound to a phosphate group of phytate, the mineral is considered unavailable to the bird. The concept of including exogenous enzymes is that these anti-nutritive properties can be eliminated with the inclusion of phytase, releasing trapped nutrients and increasing the nutrient availability of the feed. This increased nutrient availability could result in improved performance, or maintained performance with a nutritionally marginal diet, allowing for reduced feed cost. To be effective in a nutritionally marginal diet, exogenous enzymes must be able to compensate for the reduced performance associated with the marginal diet in question. Karimi et al. (2013) reports that a diet containing reduced levels of available phosphorus negatively impacted growth performance and bone ash content. The affected performance parameters included body weight (BW) gain, feed intake, FCR, and mortality. Cowieson et al., (2006) reported similar impacts with a diet marginal in available phosphorus and calcium having lower BW gain than the control diet formulated to meet nutrient requirements. Francesch and Geraert, (2009) stated negative impacts on performance from reducing nutrient content as well. In this experiment, a diet marginal in ME, crude protein (CP), digestible amino acids (AA), available P, and calcium yielded a significant negative effect on growth performance. From d 0 through 21 in that experiment, average daily gain, average daily feed intake, and feed to gain ratio were all negatively impacted

compared to a control diet. These previously referenced articles highlight the effects from dietary reductions that exogenous enzymes must be able to overcome to allow for an impactful reduction in feed cost from the reduction of high cost nutrients.

One enzyme that has been widely explored is phytase which is used to target the substrate phytate or phytic acid. With phytase inclusion into poultry diets, the inherent available phosphorus content of the feed will increase as well as a possible increase in nutrients such as calcium, sodium, and potential amino acids.

Karimi et al., (2013) conducted an experiment to evaluate phytase in male broiler chicks. In that experiment phytase was supplemented alone at varying levels into diets deficient in available phosphorus. Inclusion rates were 0, 500, 1000, 1500, and 2000 phytase units (FTU)/kg. The authors observed a dose-dependent response on growth performance with improvements on body weight, feed intake, FCR, mortality and bone ash content. That same dose-dependent response was observed in phosphorus equivalency of phytase with values of 0.08, 0.11, 0.15, and 0.19% for inclusion rates of 500, 1,000, 1,500, and 2,000 FTU/kg, respectively. These data demonstrate the ability of phytase in low available phosphorus diets to improve growth performance and also illustrates the capacity of phytase to contribute to aP content of diets dependent on phytase dose.

The effect of varying levels of phytase on broiler performance and nutrient digestibility was evaluated by Cowieson et al., (2006). In that experiment, 6 levels of phytase being 150, 300, 600, 1200, 2400 and 24,000 FTU/kg were supplemented into a corn-soybean meal based diet deficient in available phosphorus. Broilers were grown to

16 d of age, with growth performance parameters being measured along with digestibility of amino acids, and metabolizability of minerals and energy. The effect of phytase was observed at inclusion rates greater than 150 FTU/kg, where weight gain, toe ash, and nutrient utilization improved compared to the P deficient negative control diet. With the inclusion of phytase at 24,000 FTU/kg, even further improvements were observed regarding toe ash and the utilization of specific nutrients. The authors conclude that the use of phytase in low P diets can improve performance and nutrient utilization of broilers to that of a nutritionally sound diet. Also, the authors note that high doses of phytase can further improve nutrient availability compared to lower levels of phytase.

Woyengo et al., (2010) evaluated growth performance and nutrient utilization of broilers fed diets supplemented with phytase at 600 FTU/kg into a low available P (0.26%) and Ca (0.89%) diet for broilers grown to 21 d. Phytase addition significantly improved BW gain and nutrient utilization. Specific improvements in nutrient utilization were a significant increase in ileal digestible P and metabolizable energy content of diet leading the authors to conclude that phytase supplementation improves broiler growth performance and nutrient utilization.

The effect of phytase on growth performance and nutrient retention was evaluated in a study which included a nutritionally sound corn-soybean meal diet as a positive control, a low phosphorus negative control, and the negative control supplemented with 600 FTU/kg of phytase for a 21 d experiment (Sebastian et al., 1996). Data from that study indicated that phytase addition significantly increased BW in broilers compared to the negative control to a level similar to the positive control.

Nutrient retention was significantly improved by phytase supplementation where P, Ca, Cu and Zn increased 12.5, 12.2, 19.3 and 62.3 percent, respectively. From these data, authors conclude that the addition of phytase to a low phosphorus diet can improve growth performance and increase nutrient retention.

The previously published reports illustrate the impact and consistency of response with the inclusion of phytase in poultry diets, and that though the specific improvement and the degree of improvement may be variable; the report of improved performance and nutrient availability is consistent. Phytase inclusion can compensate for a dietary reduction in nutrients while maintaining broiler performance. Aside from increasing nutrient availability, phytase allows for a reduction in inorganic phosphate in the diet, which can reduce the cost of feed. The proper implementation of phytase can be beneficial by improving production efficiency, and reducing the cost of feed without compromising performance.

CARBOHYDRASES

Various carbohydrase enzymes are being explored as well to target the NSP of poultry feed along with other poorly digestible structures. An important factor to consider when evaluating carbohydrases is the composition of feedstuffs and the availability of necessary substrate in the feed. Carbohydrases are often reported to be more effective in high NSP ingredients, as there is greater substrate for the enzyme to exert its benefits on. Carbohydrase use has been explored widely just as phytase, but results have been more variable. This is understandable as carbohydrase encompasses a group of enzymes, whose substrate availability can vary greatly depending on the

components of a specific feed, as well as within the same ingredient from different sources. Despite this variability, there have been instances of carbohydrases resulting in improved performance and increased nutrient availability.

The effect of various carbohydrases alone and in combination on NSP degradation of soybean meal was evaluated in an *in-vitro* study by Meng et al. (2005). The authors reported that cellulase was effective at reducing arabinose, xylose, and glucose. Pectinase was effective at reducing arabinose, galactose, and total NSP (arabinose, xylose, mannose, galactose, glucose, uronic acids, rhamnose, and fucose). A combination of xylanase + glucanase effectively reduced arabinose, xylose, galactose and total NSP. A combination of mannanase + cellulase reduced arabinose and glucose. That study illustrates the ability of carbohydrase preparations to potentially improve NSP degradation in broiler diets containing soybean meal when fed to broiler chickens.

In a study referenced earlier by Karimi et al. (2013), the inclusion of xylanase was evaluated in male broilers fed aP deficient diets. Increasing levels of xylanase were included at 0, 16,000 and 32,000 BXU/kg. When xylanase was included at 32,000 BXU/kg, BW and FCR were negatively impacted and the low inclusion rate of xylanase did not impact any evaluated parameter. That study demonstrates that xylanase in phosphorus deficient diets did not improve performance of male broilers.

Zhu et al. (2014) evaluated the effect of a cocktail carbohydrase on growth performance and digestive parameters of broilers in a factorial experiment of 2 ME levels and 2 enzyme inclusion levels where the enzyme preparation included xylanase, β-glucanase, and α-amylase. For the 21 d experiment, enzyme supplementation had no

effect on the growth parameters of average daily gain (ADG), feed intake, or FCR. Digestive parameters indicated that enzyme inclusion into a low energy diet significantly increased villus height and surface area in the ileum and jejunum. Furthermore, enzyme supplementation into the low ME diet significantly increased several digestive enzymes throughout the experiment including pancreatic amylase, trypsin, lipase, pepsin, and maltase. The authors concluded that inclusion a cocktail carbohydrase into low ME diets may improve the digestive capacity of the small intestine in broilers.

Various experiments have been conducted to determine the effects of combining multiple enzymes on broiler performance and nutrient digestibility. Francesch and Geraert (2009) evaluated the effects of carbohydrases plus phytase on growth performance and bone mineralization in broilers. Xylanase and β-glucanase were combined with phytase into a negative control (NC) diet marginal in AME, CP, digestible AA, aP and Ca. Supplementing the NC diet with combined enzymes significantly increased average daily feed intake and average daily gain. Enzyme supplementation resulted in improved feed:gain to a value that was lower than the positive control (PC), indicating that the addition of carbohydrases with phytase can reduce the specifications of P, energy, protein and amino acids in corn-soybean meal based broiler diets.

These studies evaluating the use of various carbohydrases demonstrate inconsistent results on growth performance. An *in-vitro* study supports the ability of non-starch polysaccharidase (NSPase) to improve nutrient availability and reduce NSP content of feed ingredients (Meng et al., 2005). In practice, when supplementing

exogenous enzymes in to poultry diets many more variables come in to consideration and this is primarily where the difference in results appears. Francesh and Geraert, (2009) suggested a possible greater efficacy may be achieved by combining multiple enzymes in poultry diets, where those authors combined carbohydrases with phytase.

One such combination of enzymes that has been explored in previous studies is the combination of xylanase, amylase and protease (XAP). This is a combination of enzymes that like carbohydrases, has yielded varying results. Several reports have combined this enzyme complex with additional phytase, while others have evaluated the combination in a reduced nutrient diet. In addition, others have explored the possibility of a dose response.

ENZYME COMBINATIONS

Olukosi et al. (2007) conducted an experiment evaluating the influence of XAP and phytase individually and in combination on broilers. Enzymes were supplemented into a corn-soybean meal based control diet deficient in P and ME. Both phytase and XAP individually and in combination significantly improved ileal digestible P. There was no effect of either enzyme supplement on ileal digestible energy. Phytase inclusion and phytase plus XAP inclusion significantly increased final BW at d 21, and the authors concluded that performance improvements appeared to be primarily associated with the inclusion of phytase.

A later study by Olukosi et al. (2015) reported that a blend of XAP is effective at increasing nutrient utilization and increasing solubilization of NSP components. In that experiment, increasing levels of protease and increasing levels of XAP were included

into a control diet marginally low in energy containing 10% DDGS. Certain levels of XAP inclusion resulted in significantly increased ileal digestibility of protein compared to the control diet. Protease inclusion alone improved nutrient utilization and NSP solubility; however, effects were greater with the combination of XAP. The authors concluded this increased effectiveness from the inclusion of xylanase and amylase with protease may be due to the close fiber-protein interactions in cereals and oilseeds. This link between protein and fiber can represent a limiting factor for the effectiveness of carbohydrase or protease alone, and the combination of enzymes can be expected to be additive in their effect on protein and carbohydrate hydrolysis (Olukosi et al., 2015).

The effect of XAP inclusion on broiler performance was evaluated by Café et al. (2002). This study compared the performance of broilers fed a nutritionally sound control diet to broilers fed the control plus XAP inclusion over a 49-d period. At d 16, 35, and 49 the inclusion of XAP improved BW of broilers. However, XAP addition did not improve FCR throughout the study. At d 16 and 42, XAP inclusion negatively influenced FCR resulting in a value significantly greater than the control. The authors do not suggest a reasoning for this negative impact on FCR, but cite other research (Zanella et al., 1999 and Douglas et al., 2000) on this enzyme combination reporting inconsistent results.

Romero et al. (2014) reported that XAP inclusion can improve apparent ileal digestibility (AID) of energy and protein in broiler diets. In this particular study, the impact of inclusion of xylanase and amylase with or without protease on AID of protein, starch, fat, and energy in corn- and wheat-based broiler diets containing phytase was

evaluated. The addition of xylanase + amylase and XAP both improved AID of fat at d 42 of age compared to the control. Both enzyme supplementations improved AID of protein at d 21 of age, however only XAP inclusion improved AID of protein at d 42 of age. At the conclusion of the experiment, XAP inclusion increased AID of energy by 152 kcal/kg. The addition of protease to xylanase and amylase positively influenced AID values more than the inclusion of xylanase and amylase.

Zanella et al. (1999) conducted 2 experiments to determine the effect of enzyme inclusion on nutrient digestibility and performance of broilers. The first experiment explored the effectiveness of a complex of xylanase, protease, and amylase included in nutritionally sound corn-soybean meal based diets to evaluate the impact on ileal digestibility of CP, starch, fat and ME, along with performance parameters. Enzymatic inclusion improved ileal digestibility of CP by 2.9%, and specifically improved digestibility of amino acids valine and threonine by 2.3% and 3.0%, respectively, while also increasing BW by 1.9% and improving feed:gain by 2.2%. The second experiment evaluated the same xylanase, protease, and amylase complex in a reduced energy diet compared to a control diet formulated to meet the dietary energy requirement. No differences in performance were observed prompting the authors to conclude that the enzyme complex improved nutrient utilization allowing for full compensation of the reduction in energy content between diets.

Hong et al., (2002) evaluated the effects of a commercial preparation of XAP on performance and digestibility of nutrients in White Pekin ducks. Enzyme preparation was included at 2 different levels (0.375 and 0.5 g/kg) into a corn-soybean meal diet

with wheat middlings to be compared to a control containing no added enzyme. Ducks were grown to 42 d with performance parameters being measured, along with ileal digestibility of energy, nitrogen, and amino acids. Enzyme inclusion significantly improved BW gain, feed efficiency, and amino acid digestibility and retention. However, ileal digestibility of energy was not influenced by enzyme inclusion at either level.

The previous series of published reports evaluating XAP document the variability observed in results when including this combination in broiler diets under differing conditions. One report found that improvements from enzyme inclusion appear to be primarily from phytase (Olukosi et al., 2007) when XAP is included in combination with phytase. In other instances, researchers have found the combined XAP to be more effective than individual enzymes and less complex combinations (Romero et al., 2014, Olukosi et al., 2015). Not only is it reported that this combination can improve performance, but it can compensate for dietary reductions in specific nutrients as well. Olukosi et al. (2015) suggest that this complex of enzymes may be effective at improving nutrient utilization because of the close interaction between protein and fiber in feed ingredients. Furthermore, negative impacts were reported when the enzyme complex was added to a nutritionally sound diet. Thus continued research needs to be completed to evaluate the most appropriate strategies for including these types of enzymatic products in poultry production.

The combination of many various enzymes has been explored to determine if there may be interactions or synergistic effects that would not be achieved from the inclusion of individual enzymes. Combinations may be more effective because of the

interactions between nutrients, as suggested earlier by Olukosi et al. (2015) referring to fiber-protein relationship in some ingredients. Supplementing with multiple enzymes can allow each enzyme greater access to its respective substrate.

Meng et al. (2005) evaluated the potential of NSP degradation and nutrient utilization from the combination of multiple carbohydrases. In this *in vitro* study, NSP of wheat and SBM were targeted with varying enzyme preparations that could contain cellulase, pectinase, xylanase, glucanase, galactanase and mannanase. Authors report that NSP degradation was greatest when enzymes were used in combination. In a follow up grow out experiment, all enzyme combinations significantly improved weight gain and feed to gain ratio. All enzyme combinations significantly increased ME, apparent ileal digestibility of starch and protein, and apparent total tract digestibility of NSP. The most complex enzyme combination (cellulase, pectinase, xylanase/glucanase, and mannanase/cellulase) was superior to other combinations in improving feed to gain ratio and protein digestibility, indicating that enzyme efficacy in broiler diets can be improved by the appropriate combination of carbohydrases.

Karimi et al. (2013) evaluated increasing levels of phytase in combination with increasing levels of xylanase in low phosphorus diets on growth performance of male broilers. As previously mentioned, the author reported phytase inclusion alone improving growth performance in a dose-dependent fashion, and xylanase inclusion alone having no effect, and negative effects at the greatest inclusion rate. Varying levels of phytase (0, 500, 1,000, 1,500, and 2,000 FTU/kg) were combined with varying levels of xylanase (0, 16,000 and 32,000 BXU/kg) in phosphorus deficient diets. Throughout

the 18 d, there were no interactions between phytase and xylanase, leading the authors to conclude that the addition of xylanase to phytase was not effective at improving the efficacy of phytase.

Woyengo et al. (2010) evaluated the addition of multicarbohydrase to a phytase supplemented diet low in available P and Ca. From the addition of phytase alone, significant improvements in BW and nutrient utilization were observed. The addition of a multicarbohydrase complex (cellulase, pectinase, mannanase, galactanase, xylanase, glucanase, amylase and protease) further increased d 21 BW. Multicarbohydrase included with phytase significantly improved ileal digestible P an additional 10.4% compared to phytase inclusion alone, and ME content of diet increased by an additional 74 kcal/kg. The authors concluded that supplementing a phytase containing diet with multicarbohydrase can further improve broiler performance and nutrient utilization, which conflicts with the reports of Karimi et al. (2013).

The applicability of the alternative ingredient DDGS and incorporating enzymatic inclusion with DDGS in broiler diets is well defined, as is the use of phytase to improve performance and nutrient utilization. It is apparent the combination of enzymes supplemented in broiler diets is still a subject with greatly varying results. Conflicting reports within the literature of the relationships, interactions, and impacts of combining multiple enzymes indicates that additional research needs to be conducted in this area. Therefore, the objective of the research described herein is to evaluate the effect of enzyme preparations individually and in combination on broiler growth performance parameters.

CHAPTER II

EXPERIMENT 1: EVALUATION OF COCKTAIL NSPASE AND XAP INCLUSION SEPARATELY AND IN COMBINATION IN REDUCED ENERGY BROILER DIETS ON MALE GROWTH PERFORMANCE

OVERVIEW

The purpose of this experiment was to evaluate the effect of a cocktail non-starch polysaccharidase (NSPase) and mixed enzyme blend containing xylanase, amylase, and protease (XAP) inclusion separately and in combination in energy reduced broiler diets on broiler performance and processing parameters. Criteria for evaluation included weight gain, feed intake, feed conversion ratio, without giblets (WOG) yield, fat pad yield, and mortality. Experimental diets were composed of two nutrient profiles: positive control (PC) and a negative control (NC) with a 55 kcal/kg metabolizable energy (ME) reduction during the starter phase and an 88 kcal/kg ME reduction for finisher and withdrawal phase. The experimental design consisted of five dietary treatments including the PC, NC, and the NC supplemented with NSPase, XAP, and the combination of NSPase and XAP. Enzyme inclusion was applied to the NC; cocktail NSPase was included at 113.5 g/ton, XAP was included at 226.8 g/ton. Dietary treatments were composed of 16 replicates, with each replicate containing 40 male broilers. In total, 3,200 chicks were placed for the 39 d experiment. Three dietary phases were implemented; starter from placement through d 14, finisher through d 27 and withdrawal through d 39. On d 14, 27 and 39, broilers were weighed on a per pen basis

and feed consumption was determined. On d 40, following 8 h of feed withdrawal, 7 broilers from each pen (560 total) were processed to obtain carcass and fat pad measurements. Reduction of dietary energy in the NC decreased ($p<0.05$) average broiler BW and increased ($p<0.05$) FCR through the starter and finisher phase. Inclusion of the cocktail NSPase increased ($p<0.05$) average broiler BW significantly compared to the NC at levels similar to that of the positive control during the starter and finisher phases. Throughout the experiment, inclusion of the cocktail NSPase significantly reduced ($p<0.05$) FCR compared to the NC at levels that were similar to the positive control. The inclusion of XAP resulted in BW similar to the PC at d 14 and 27, and reduced ($p<0.05$) FCR from d 1 to 27. The combined inclusion of NSPase + XAP resulted in no further benefit beyond individual inclusion of both enzymes. This data confirms the capacity of cocktail NSPase and XAP inclusion to positively influence performance parameters in broilers fed a reduced energy diet.

INTRODUCTION

Over the past decade, ingredient price volatility combined with access to new and improved technologies such as exogenous enzymes have resulted in poultry nutritionists increasing the use of exogenous carbohydrases in diets. The increased price of corn was primarily due to the use of corn for ethanol production which has shifted the use of corn away from the production of agriculture. To combat this price increase, poultry nutritionists focused on maximizing nutrient utilization to improve efficiency with the use of exogenous carbohydrases in diets that sometimes contain lower quality ingredients such as distiller's dried grains with solubles (DDGS). Increasing nutrient

utilization and feed efficiency can positively affect poultry production both economically and environmentally. If less feed is required for production and less indigestible nutrients are excreted into the environment, then environmental impact is reduced.

The presence of non-starch polysaccharides (NSP) in corn and soybeans, which are poorly digested by monogastric animals, represent a potential source of nutrients. However, NSP in feed ingredient also contain anti-nutritive properties (Meng and Slominski, 2005). Non-starch polysaccharides, which are a major component of dietary fiber, are composed of both cellulosic and non-cellulosic polysaccharides. In elevated concentrations NSP can increase intestinal viscosity. Increased viscosity reduces nutrient digestibility by decreasing enzyme to substrate contact and regular physical transport. Distiller's dried grains with solubles have become a common feed ingredient in poultry diets since it is both cost effective and readily available as a by-product from the production of corn ethanol. Distiller's dried grains with solubles have been shown to be an acceptable feed ingredient when included at certain concentrations (Lumpkins et al. 2004). However, DDGS are high in NSP and therefore it is a common practice to include exogenous enzymes when including DDGS into a broiler diet.

The inclusion of exogenous enzymes can be helpful in combatting the anti-nutritive factors of NSP and allow utilization of nutrients that would otherwise be unavailable (Bedford, 2000). The inclusion of an NSPase cocktail in broiler diets has been found to improve digestibility and broiler performance (Cowison and Adeola, 2005, Coppedge et al., 2012, Williams et al., 2014). The nature of a cocktail can vary

considerably in profile and enzyme presence. An enzyme cocktail of xylanase, amylase, and protease (XAP) is one profile shown to increase nutrient digestibility in broiler diets. Conflicting reports have been published regarding the efficacy of XAP. One author reported no effect on performance while improving ileal nitrogen and phosphorus digestibility compared to a nutritionally marginal NC (Olukosi et al., 2007). Another author reported the ability of the enzyme combination to fully compensate for nutrient reductions by resulting in growth performance parameters being similar to a nutritionally complete diet (Zanella et al. 1999). Café et al. (2002) reported XAP to have had a negative impact on FCR at 2 periods within the experiment, although ultimately suggested the combination to be effective at increasing net energy obtained from the diet. There is limited information available on the combination of multiple cocktail or multienzyme products. Therefore, this experiment was conducted to evaluate the inclusion of NSPase and XAP individually and in combination in low energy broiler diets containing DDGS.

MATERIALS AND METHODS

Experimental Design

The evaluation of cocktail NSPase[1] and XAP[2] inclusion separately and in combination in reduced energy broiler diets containing DDGS was conducted in a randomized block design with 5 dietary treatments during a 39 d grow-out. The

[1] Enspira®, Enzyvia LLC, Sheridan, IN. Xylanase (2,700 U/g) from *A. niger* and *T. reesei*; also contains β-glucananse and α-galactosidase.
[2] Axtra® XAP, Danisco Animal Nutrition/DuPont, Marlborough, Wilshire, UK. 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.*

experimental design was composed of a positive control diet (PC), a negative control diet (NC) with an AME reduction of 55 kcal/kg for the starter phase, and 88 kcal/kg reduction for the finisher and withdrawal phase, and the NC supplemented with NSPase, XAP, and the combination of NSPase and XAP.

Experimental Diets

All diets were corn and soybean meal based with differing energy profiles and increasing levels of DDGS (5 to 15%) as broilers increased with age (Table 1). The PC diet was formulated similar to that of a typical broiler industry diet. Energy was reduced in the NC diet by 55 kcal/kg AME in the starter phase and 88 kcal/kg AME in the finisher and withdrawal phases. Exogenous enzymes were added to the NC diet prior to pelleting. Throughout all dietary phases, NSPase and XAP were included in the NC diet at 113.5 g/ton and 226.8 g/ton, respectively. The combination of NSPase and XAP were included in the NC diet at these same rates for all dietary phases (NSPase + XAP). All diets contained phytase[3] at 500 FTU/kg. The starter diet was fed from d 1 to 14 containing 5% DDGS, the finisher diet from d 15 to 27 containing 10% DDGS, and the withdrawal diet containing 15% DDGS was fed from d 28 to 39. All diets were manufactured as a pellet, and the starter diet was crumbled. In order to maintain enzyme activity, conditioning and pelleting temperature did not exceed 70°C and conditioning time was 12 s. Feed samples were obtained in duplicate during

[3] Phyzyme® XP 2500 TPT, Danisco Animal Nutrition/DuPont, Marlborough, Wilshire, UK. *Escherichia coli* derived phytase providing 500 FTU/kg.

Table 1. Calculated content of experimental diets fed to male broilers in Experiment 1.

	Day 1 to 14		Day 15 to 27		Day 28 to 39	
Ingredient (lbs/ton)	Positive Control	Negative Control	Positive Control	Negative Control	Positive Control	Negative Control
Corn	1183	1201	1211	1261	1234	1280
Soybean Meal	545	546	430	418	323	314
Meat & Bone	111	112	66.49	66.16	47	47
DDGS	100	101	200	200	300	300
Fat	26.6	5	53.88	16	57.5	19.88
Limestone	9.20	9.30	14.56	14.74	18.43	18.61
DL - methionine	5.49	5.58	4.25	4.20	3.02	2.95
Vitamin premix[1]	5.00	5.00	4.00	4.00	3.00	3.00
L-lysine HCl	4.24	4.39	5.18	5.36	5.41	5.51
Salt	3.90	3.91	3.69	3.59	3.51	3.43
Sodium sesquicarbonate	2.93	3.01	3.57	3.68	3.65	3.73
Threonine	1.49	1.38	1.23	1.09	1.02	0.86
Trace Mineral[2]	1.00	1.00	0.80	0.80	0.50	0.50
Coccidiostat[3]	1.00	1.00	1.00	1.00	--	--
Phyzyme 2500 TPT[4] (%)	0.02	0.02	0.02	0.02	0.02	0.02
Nutrient (%)						
Metabolizable energy (kcal/kg)	3003	2948	3113	3025	3157	3069
Protein	22.54	22.83	20.06	20.13	18.38	18.48
Calcium	0.92	0.93	0.79	0.79	0.76	0.76
Total Phosphorus	0.55	0.56	0.49	0.49	0.46	0.47
Sodium	0.18	0.18	0.18	0.18	0.18	0.18
DEB	213	214	195.00	193.00	179	179
Digestible Lysine	1.18	1.18	1.04	1.04	0.91	0.91
Digestible Methionine	0.63	0.60	0.51	0.51	0.44	0.43
Digestible M+C	0.92	0.93	0.81	0.81	0.72	0.72
Digestible Tryptophan	0.21	0.21	0.18	0.18	0.16	0.16
Digestible Threonine	0.77	0.77	0.68	0.68	0.62	0.61
Digestible Isoleucine	0.79	0.80	0.70	0.70	0.63	0.63
Digestible Valine	0.93	0.94	0.83	0.83	0.76	0.76

[1]Vitamin premix added at this rate yields per kg diet 11,023 IU vitamin A, 3,858 IU vitamin D3, 46 IU vitamin E, 0.0165 mg B12, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin. The carrier is ground rice hulls.

[2]Trace mineral premix at this rate yields per kg of diet 149 mg manganese, 125 mg zinc, 17 mg iron, 7 mg copper, 1.0 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium. The carrier is calcium carbonate and the premix contains less than 1% mineral oil

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

[4]*Escherichia coli* derived phytase providing 500 FTU/kg.

manufacture for nutrient analysis. Crude protein was determined using AOAC by combustion (AOAC 990.03), total phosphorus determined by wet ash ICP (AOAC 985.01M), acid detergent fiber determined using an ANKOM digestion unit (AOAC 973.18), and an ether extraction to determine crude fat (AOAC 920.39).

Animals and Management Practices

On d of hatch, 3,200 Cobb 500 male broiler chicks were allotted to floor-pens and dietary treatments based on initial BW to ensure statistically equivalent weights at d of age. Broilers were placed in 1.82 m x 1.82 m rearing pens equipped with tube feeders and nipple drinkers and fresh pine shavings provided as bedding material. Chicks were provided age appropriate supplemental heat and given access to feed and water *ad libitum.* All broilers were weighed on dates corresponding to dietary change (d 14, 27, and 39) to calculate average BW, feed consumption, and mortality corrected FCR. At termination of the experiment, 7 broilers from each replicate were randomly selected for processing to obtain carcass and fat pad data. Animal care was provided in accordance with an approved Institutional Animal Care and Use Committee (IACUC) protocol.

Termination of Trial

All broilers were weighed in bulk the evening of d 39 prior to processing on d 40. Prior to processing, broilers were placed on 8 h of feed withdrawal. Seven broilers from each replicate (112 broilers/treatment) were selected and weighed individually before processing. Eviscerated carcass and abdominal fat pad weights were obtained before emersion chilling for calculation and determination of corresponding yields.

STATISTICAL ANALYSIS

All data were analyzed via a one-way ANOVA with means deemed significantly different at $p<0.05$. Means were separated using Duncan's Multiple Range Test. Parameters subject to evaluation included BW, FCR, mortality and processing yields.

RESULTS

Average broiler BW was negatively influenced by the reduction in dietary energy as broilers fed the NC diet had lower ($p<0.05$) BW compared to the PC fed broilers as early as 14 d of age (Table 2). The addition of NSPase into the NC diet increased ($p<0.05$) BW on d 14 compared to the NC to a level that was similar to the PC. The combined inclusion of NSPase + XAP did not impact BW as compared to individual inclusion. On d 27, at the conclusion of the finisher phase, the energy reduced NC yielded significantly lower BW compared to the PC. All enzymatic treatments yielded BW similar to the PC; however, the addition of NSPase alone was the only treatment to yield a BW greater ($p<0.05$) than the NC. On d 39, all treatments were similar to one another with regards to average BW. At no time did the combination of XAP and NSPase outperform either enzyme when added individually. Throughout the experiment, no differences in mortality were observed between experimental treatments. Similar to BW, mortality corrected FCR was negatively impacted with the reduction in dietary energy as the NC fed broilers has a significantly higher FCR as compared to PC fed broilers (Table 3). During the starter phase, none of the enzymatic treatments were able to reduce FCR to levels similar to the PC. However, the inclusion of NSPase decreased ($p<0.05$) FCR compared to the NC. During the finisher phase of the

Table 2. Average body weight and mortality of male broilers fed diets reduced in energy and supplemented with an enzyme blend containing xylanase, amylase, and protease[1], a cocktail NSPase[2], or combination of both.

	Body Weight (Kg)				Mortality (%)
Treatment	Day 0	Day 14	Day 27	Day 39	Day 1-39
PC[3]	0.045	0.526[a]	1.639[a]	2.817	3.7
NC[4]	0.045	0.511[c]	1.599[b]	2.789	2.2
NC + XAP	0.045	0.517[abc]	1.608[ab]	2.795	3.4
NC + NSPase	0.045	0.522[ab]	1.636[a]	2.823	3.0
NC + XAP + NSPase	0.045	0.513[bc]	1.616[ab]	2.800	2.3
Pooled SEM	0.1	2	0.004	0.007	0.3
Pooled TRT CV (%)	1.0	2.9	2.4	2.3	10

[a-c] Means within columns with different superscripts differ significantly at $p<0.05$.
[1] 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*.
[2] Xylanase (2700 U/g) from *A. niger* and *T. reesei*; also contains β-glucananse and α-galactosidase.
[3] Positive control diet
[4] Negative control diet formulated to have a 55kcal/kg AME reduction in starter and 88kcal/kg AME reduction in finisher and withdrawal phases compared to PC.

Table 3. Mortality corrected feed conversion of male broilers fed diets reduced in energy and supplemented with an enzyme blend containing xylanase, amylase, and protease[1], a cocktail NSPase[2], or combination of both.

	Feed Conversion Ratio				
Treatment	Day 1 to 14	Day 14 to 27	Day 1 to 27	Day 27 to 39	Day 1 to 39
PC[3]	1.177[c]	1.494[b]	1.397[c]	1.958	1.631[c]
NC[4]	1.211[a]	1.535[a]	1.437[a]	1.979	1.670[a]
NC + XAP	1.196[ab]	1.528[ab]	1.426[b]	1.970	1.657[ab]
NC + NSPase	1.195[b]	1.500[b]	1.408[bc]	1.969	1.645[bc]
NC + XAP + NSPase	1.202[ab]	1.503[b]	1.413[bc]	1.934	1.632[c]
Pooled SEM	0.003	0.004	0.003	0.007	0.003
Pooled TRT CV (%)	2.1	2.5	1.8	3.2	1.7

[a-c] Means within columns with different superscripts differ significantly at $p<0.05$.
[1] 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*.
[2] Xylanase (2700 U/g) from *A. niger* and *T. reesei*; also contains β-glucananse and α-galactosidase.
[3] Positive control diet
[4] Negative control diet formulated to have a 55kcal/kg AME reduction in starter and 88kcal/kg AME reduction in finisher and withdrawal phases compared to PC.

experiment, the energy reduced NC again yielded an increased ($p<0.05$) FCR compared to the PC. The inclusion of NSPase and combination inclusion NSPase + XAP both significantly reduced ($p<0.05$) FCR compared to the NC, to level similar to the PC. Inclusion of XAP alone had no significant effect during the finisher phase. From d 1 to 27 FCR of the NC was significantly increased ($p<0.05$) compared to the PC. The inclusion of NSPase and NSPase + XAP significantly reduced ($p<0.05$) FCR compared to the NC to levels similar to the PC. The inclusion of XAP significantly reduced FCR compared to the NC, although not to a level similar to the PC. During the withdrawal phase of the experiment, no differences were observed. Regarding cumulative FCR (d 1 to 39), reducing dietary energy level negatively impacted FCR as the NC fed broilers had a significantly higher FCR compared to the PC. The addition of NSPase and NSPase + XAP to the NC diet significantly reduced ($p<0.05$) FCR compared to the NC to a level that was similar to the PC; however, the combination of XAP and NSPase did not outperform individual inclusions.

Enzyme inclusion had no impact on feed consumption during the starter and finisher phases (Table 4). During the withdrawal period, NSPase + XAP reduced ($p<0.05$) FC compared to the NC and NSPase inclusion alone. Similar effects are observed regarding total feed consumption with inclusion of NSPase + XAP resulting in significantly reduced ($p<0.05$) values compared to the NC and NSPase inclusion alone. At no point was there a difference in feed consumption between the PC and NC.

The processing parameters of carcass weight, fat pad weight, WOG yield and fat pad yield were not significantly impacted by any of the dietary treatments.

DISCUSSION

The inclusion of exogenous enzymes in poultry diets has become common practice as a means of improving productive performance in broilers (Cowieson and Adeola, 2005). These improvements can recover negative effects that are associated with a reduction in dietary energy (Coppedge et al., 2012). Dietary energy content be reduced by substituting fat with corn and by dilution with the use of alternative high fiber ingredients, such as DDGS used in this experiment. Both methods of energy reduction can be practiced as a means of decreasing feed cost, which can represent over 50% of total production expenses. Based on the results of this experiment, the energy reduction in the NC negatively affected broiler performance throughout the study. O'Neill et al. (2012) reported similar findings with an energy reduction of 100 kcal/kg increasing FCR. The reduction in energy decreased early BW during the first 2 phases of the feeding period and increased FCR for the entirety of the experiment. The inclusion of NSPase in the NC improved growth performance (increased BW and decreased FCR) of broilers to levels that were similar to the PC. This improvement in broiler performance supports the ability of NSPase to eliminate negative effects from dietary energy reduction. Throughout the experiment XAP inclusion did not impact average BW compared to the NC. The inclusion of XAP led to an improvement in FCR compared to the NC during the finisher phase. The combined inclusion of NSPase + XAP in the NC resulted in no difference from the NC for average BW. The addition of NSPase + XAP positively influenced FCR throughout the experiment by reducing FCR level similar to

Table 4. Feed consumption of male broilers fed diets reduced in energy and supplemented with an enzyme blend containing xylanase, amylase, and protease[1], a cocktail NSPase[2], or combination of both.

	Feed Consumption (kg/bird)			
Treatment	Starter	Finisher	Withdrawal	Total
PC[3]	0.570	1.531	2.227[ab]	4.329[ab]
NC[4]	0.565	1.542	2.297[a]	4.404[a]
NC + XAP	0.570	1.529	2.246[ab]	4.346[ab]
NC + NSPase	0.571	1.541	2.281[a]	4.394[a]
NC + XAP + NSPase	0.565	1.527	2.168[b]	4.261[b]
Pooled SEM	0.001	0.005	0.013	0.018
Pooled TRT CV (%)	2.562	3.216	5.304	3.846

[a,b] Means within columns with different superscripts differ significantly at $p<0.05$.
[1] 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*.
[2] Xylanase (2700 U/g) from *A. niger* and *T. reesei*; also contains β-glucananse and α-galactosidase.
[3] Positive control diet
[4] Negative control diet formulated to have a 55kcal/kg AME reduction in starter and 88kcal/kg AME reduction in finisher and withdrawal phases compared to PC.

Table 5. Processing parameters and yield of male broilers fed diets reduced in energy and supplemented with an enzyme blend containing xylanase, amylase, and protease[1], a cocktail NSPase[2], or combination of both.

	Processing Parameters				
Treatment	Live Wt (g)	WOG (g)	Fat Pad (g)	WOG %	Fat Pad %
PC[3]	2827	2101	31.2	74.3	1.48
NC[4]	2845	2126	30.5	74.7	1.41
NC + XAP	2835	2113	29.8	74.5	1.41
NC + NSPase	2839	2121	30.1	74.7	1.42
NC + XAP + NSPase	2818	2127	31.5	75.5	1.48
Pooled SEM	9.3	6.5	0.3	0.08	0.01
Pooled TRT CV (%)	2.9	2.9	9.9	2.1	9.6

[1] 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*.
[2] Xylanase (2700 U/g) from *A. niger* and *T. reesei*; also contains β-glucananse and α-galactosidase.
[3] Positive control diet
[4] Negative control diet formulated to have a 55kcal/kg AME reduction in starter and 88kcal/kg AME reduction in finisher and withdrawal phases compared to PC

the PC. However, the combination of NSPase and XAP at no time resulted in a benefit beyond individual enzyme inclusion. This could be due to a major component of each enzyme product being xylanase. The combination of enzyme products may have contributed more xylanase than there was necessary substrate for the enzyme to act on in the feed. In that instance, the inclusion of more enzyme would not lead to greater improvements. It could only be economical to include one of the enzyme preparations from the data generated in this experiment. Including both preparations would add unnecessary cost to the feed with no added benefit.

The use of a cocktail NSPase, such as the one used in this experiment, has repeatedly shown positive results regarding broiler performance in reduced energy diets. Coppedge et al. (2012) observed decreases of 2 to 4% in FCR in diets containing NSPase through the starter and grower phases The NSPase used in that study was similar to the one used in the current experiment, containing xylanase, α-galactosidase, and β-glucanase as well as β-mannanase. Similar results were observed in this experiment with a reduction in FCR for the NSPase treatment compared to the NC. Another study, using a multi-enzyme complex containing carbohydrase and phytase included into energy reduced diets (-65 kcal/kg and -85 kcal/kg) resulted in reduced FCR, supporting the use of enzyme supplementation to eliminate the negative effects associated with reduced dietary energy (Francesh and Geraert, 2009). The authors reported significant improvements in FCR and weight gain during the first 2 phases of the experiment (Francesh and Geraert, 2009). Furthermore, the inclusion of cocktail NSPase resulted in reduced FCR compared to the NC for the entirety of the experiment. Klein et al. (2015)

reported improved FCR in broilers supplemented with cocktail NSPase similar to the one used in the current experiment when included in a reduced ME diet. Campasino et al. (2015) reported improvements in FCR during the grower phase, and BW comparable to a PC when including NSPase into a reduced ME diet. Results from this experiment correspond with those previously mentioned supporting the use of cocktail NSPase to improve growth performance in broilers fed reduced energy diets.

Similar to the results found early in this experiment regarding the effects of XAP inclusion, Olukosi et al. (2007) concluded there to be no significant improvements in performance parameters from the inclusion of XAP in poultry diets. However, contradicting results have been reported in other studies. In one experiment the inclusion of XAP was found to improve both feed-to-gain ratio, which was also reported during the early phases of this experiment, as well as body weight gain (Cowieson and Adeola, 2005). These inconsistencies in results from the inclusion of XAP indicate that perhaps additional factors may be related to efficacy such as ingredient profile or nutrient concentration. However, it has been suggested that the unsuccessful results of XAP inclusion could be due to the conventional nonspecific enzyme preparations (Cowieson and Adeola, 2005), and also to the nutritional variety of feed ingredients from different sources and their substrate availability (Yegani and Korver, 2013).

The basis of evaluating a combination of NSPase and XAP as based upon reports where enzyme combinations were shown to be effective in significantly improving weight gain and feed-to-gain ratio (Meng et al., 2005, Klein et al., 2015, Williams et al., 2014). In this experiment, the inclusion of cocktail NSPase + XAP in

the NC resulted in decreased FCR during the finisher phase, d 1 to 27, and cumulative d 1 to 39, at levels that were similar to the PC. Again, at no evaluation time point did the combination outperform the 2 enzymes when added individually. The approach of using an enzyme combination can potentially be more beneficial as compared to using a specific enzyme complex as there may be greater necessary substrate availability with a greater variety of enzymes in the combination. However, in this study, both enzymes used were a cocktail or an enzyme blend with a major component of each being xylanase. The duplication of components between the 2 enzyme products could be the reason for lack of a combination effect which has been reported by Klein et al. (2015) and Williams et al. (2014). However, the enzyme combinations used in those studies included a cocktail NSPase and a β-mannanase which target different substrates in different ingredients. It is notable that the combined inclusion of NSPase and XAP decreased feed consumption compared to the NC and resulted in the lowest consumption value in the withdrawal phase and also from d 1 to 39. It is possible that this late impact is related to the relatively higher level of DDGS included in the withdrawal phase. Where earlier in the experiment the duplication from combining enzyme products was not effective there may now have been sufficient substrate from DDGS included at 15%. The increased level of DDGS would increase the level of NSP compared to earlier feeding periods. It is possible that this increase of NSP led the level of xylanase that was included from combined products to cross a threshold from excessive to effective. This theory could possibly translate into other parameters with broilers grown to a greater age.

In conclusion, a reduction in dietary energy resulted in negative impacts on broiler growth performance as demonstrated by increased FCR throughout the experiment, and decreased BW in the early phases of the experiment. The inclusion of cocktail NSPase and XAP individually in the energy reduced diet eliminated the negative effects on growth performance, thus resulting in BW and FCR measurements that were similar to the PC. The combination of a cocktail NSPase with XAP did improve growth performance, however not to a level beyond individual inclusion.

CHAPTER III

EXPERIMENT 2: EVALUATION OF MULTIPLE LEVELS OF PHYTASE AND XYLANASE, AMYLASE, PROTEASE INCLUSION ON BROILER GROWTH PERFORMANCE

OVERVIEW

An experiment was conducted to determine the effect of two levels of phytase (600 and 1200 FTU/kg) and three levels of a multienzyme (xylanase, amylase, and protease - XAP) product inclusion (1200 U/kg, 1800 U/kg, and 2400 U/kg) in a reduced nutrient corn-soybean meal diets containing DDGS on broiler growth performance. The experimental design consisted of seven treatments including a reference control diet, and the remaining six treatments composing a two by three factorial of the varying levels of enzymes included in a reduced energy (-88 kcal/kg ME) reduced available phosphorus (-0.12%) diet. Each treatment included 10 replicates with 37 male chicks per treatment group (2590 total placement). Dietary program consisted of a three phase program, starter (5% DDGS), grower (10% DDGS), and finisher (15% DDGS). Broilers were weighed and feed consumption determined on d 15, 28, and 41. At the conclusion of the experiment average body weight of each treatment was similar to the reference diet. One-way analysis indicated that the individual treatment of high phytase x low XAP and high phytase x medium XAP, both resulted in similar cumulative FCR as compared to the reference diet while all other individual treatments failed to reach a similar FCR of the reference diet. Factorial analysis confirmed that 1200 FTU/kg of phytase reduced

($p<0.05$) starter FCR although had an elevated level of mortality during the finisher phase compared to the 600 FTU/kg level. During the starter phase, mid level of XAP inclusion resulted in a lower ($p<0.05$) rate of consumption compared to the low level of XAP inclusion. Inclusion of the mid level of XAP reduced observed ($p<0.05$) FCR for the combined FCR for the grower and finisher phases (d 15-41) as compared to the low XAP level. These data confirm that the use of combination inclusion of phytase and multienzyme can compensate for reductions in dietary available phosphorus and metabolizable energy.

INTRODUCTION

Feed represents the largest portion of production expenses in the poultry industry (Tahir, et al., 2012). As a result, researchers are exploring multiple methods of improving broiler production efficiency and are evaluating more cost effective alternatives to commonly used ingredients. Two popular ingredients in the U.S. domestic market that can account for the majority of a poultry diet are corn and soybean meal (SBM). These ingredients are included primarily for their contributions to energy and protein content of the diet. Energy and protein can be considered 2 of the most expensive nutrients in a poultry diet (Tahir, et al., 2012). One economical alternative ingredient that has demonstrated value in poultry diets DDGS. A considerable amount of previous research confirms our ability to include DDGS as a partial replacement of corn and SBM in broiler diets without sacrificing bird performance (Lumpkins, et al., 2004, Loar, et al., 2010, Shim, et al., 2011). The main nutritional components of DDGS remaining after ethanol production are protein, fat, and fiber, which can be 2 to 3 times the value of the

original grain (NRC, 1994). The increased amount of fiber can lead to an increased amount of non-starch polysaccharides (NSP) (Kiarie et al., 2014). Non-starch polysaccharides are a major component of fiber that can reduce the digestibility of other nutrients and have been correlated to reducing energy content (Leeson and Summers, 2001).

The use of exogenous enzymes in poultry diets is being widely explored in order to utilize otherwise unavailable nutrients within feed ingredients. Ingredients of poultry diets, even those considered high quality such as corn and soybean meal contain components that have anti-nutritive properties which do not allow all nutrients to be utilized by the monogastric digestive system of chickens. For example, phytate can compose 50% of plant P, and is poorly digestible to chickens and can chelate to other nutrients, thus reducing their availability to the bird. The inclusion of exogenous phytase has been reported to effectively improve nutrient utilization and growth performance (Sebastian, et al., 1996, Pieniazek, et al., 2016). Pieniazek et al. (2016) reported phytase supplementation to compensate for reductions in available P by improving BW, FCR and amino acid digestibility. Phytase inclusion can also contribute to available P content of diets. Karimi et al. (2014) reported P equivalency ranging from 0.08 to 0.19 from phytase included at 500 to 2,000 FTU/kg.

Some major NSP found in cereal grains can include cellulose, arabinoxylan, and β-glucan (Bach Knudsen, 2014). Oligosaccharides, a group of NSP found in soybean meal, are indigestible to poultry since poultry lack endogenous α-galactosidase. Non-starch polysaccharides can completely encapsulate nutrients and chelate metal ions

resulting in reduced starch, protein, and lipid digestion (Leeson and Summers, 2001). The use of NSP degrading exogenous enzymes to improve nutrient availability and growth performance has yielded varying results. Several studies indicate that the use of exogenous enzymes has improved nutrient availability and growth performance (Cowieson and Adeola, 2005, Kiarie et al., 2014), while others do not (Kaczmarek et al. 2014). Kiarie et al. (2014) reported improved growth performance and AME when supplementing xylanase in corn and wheat diets. Phytase plus XAP supplementation in broiler feed improved feed to gain ratio, BW gain and ileal digestible energy as reported by Cowieson and Adeola (2005).

It is reported that there can be benefits from including XAP with phytase and therefore, it was theorized that there would be an optimal inclusion rate of each enzyme product that would result in the greatest benefit. An experiment was conducted to evaluate the potential interaction between phytase and a multienzyme complex containing xylanase, amylase, and protease (XAP) at multiple levels when included in nutritionally marginal broiler diets.

MATERIALS AND METHODS

Experimental Design

To evaluate multiple levels of phytase[4] and multienzyme[5] inclusion on broiler performance an experiment was conducted using a complete randomized block design with 7 dietary treatments containing 10 replicates per treatment during a 41 d

[4] Axtra®PHY 10,000 TPT, Danisco Animal Nutrition/DuPont, Marlborough, Wilshire, UK.
[5] Axtra® XAP, Danisco Animal Nutrition/DuPont, Marlborough, Wilshire, UK.

experiment. The phytase and multienzyme used in this experiment are commercially available products. The experimental design consisted of a reference diet and six energy-reduced enzymatic treatments composing a 2 X 3 factorial of varying levels of phytase and XAP. The energy reduction for the enzymatic treatments was 88 kcal/kg throughout the experiment.

The Experimental Diets

Diets were corn and soy bean meal based containing animal protein, soy oil, and increasing levels of DDGS from 5 to 10 to 15% in the starter, grower and finisher periods, respectively (Table 6). The reference diet was formulated to be similar to that of a typical industry broiler diet. Diets were formulated to be equal regarding the amount of digestible amino acids present in the feed. The reference diet contained phytase at 600 FTU/kg and did not contain XAP. All enzymatic treatments were formulated with a reduction of 88 kcal/kg ME compared to the reference diet. The reduced energy enzymatic treatments were manufactured as one large basal diet that were then divided into sub batches and individual treatments with the addition of enzymes as a premix of enzyme and corn starch at an inclusion rate of 500 g/ton. Two different levels of phytase were included, being 600 FTU (referred to as low phytase) and 1,200 FTU (high phytase), and three varying levels of XAP were included at 1,200 (low XAP), 1,800 (mid XAP), and 2,400 U/kg of feed (high XAP). These 2 inclusion rates of phytase and three inclusion rates of XAP created a 2 X 3 factorial of enzymatic treatments. The six enzymatic treatments were low phytase X low XAP, low phytase X mid XAP, low phytase X high XAP, high phytase X low XAP, high phytase X mid XAP, and high

Table 6. Calculated content of experimental diets fed to male broilers in Experiment 2.

	Starter d 1 to 15		Grower d 15 to 28		Finisher d 28 to 41	
	Reference Diet	-88 kcal/kg	Reference Diet	-88 kcal/kg	Reference Diet	-88 kcal/kg
Ingredient (%)						
Corn	55.87	57.66	59.60	61.75	61.80	63.80
SBM	31.64	31.62	22.60	22.56	15.72	15.72
DDGS	5.00	5.00	10.00	10.00	15.00	15.00
MBM	3.00	3.00	3.00	3.00	3.00	3.00
Soy Oil	2.25	0.50	2.84	0.71	2.77	0.73
Limestone	0.92	0.92	0.79	0.79	0.86	0.86
MCP	0.35	0.35	0.15	0.15	0.00	0.00
Salt	0.36	0.36	0.34	0.34	0.32	0.32
DL-Met	0.23	0.23	0.20	0.20	0.13	0.13
L-Lysine	0.12	0.12	0.23	0.23	0.26	0.26
Choline 60	0.08	0.08	0.10	0.10	0.00	0.00
Trace min.[1]	0.05	0.05	0.08	0.08	0.08	0.08
Vitamins[2]	0.25	0.25	0.05	0.05	0.05	0.05
Threonine	0.00	0.00	0.05	0.05	0.04	0.04
Phytase[3] (FTU/kg)	600	600	600	600	600	600
Nutrient (%)						
ME (kcal/kg)	3008	2925	3086	2997	3130	3041
Crude Protein	22.29	22.40	20.00	19.80	18.00	18.00
Av. Phosphorus[4]	0.44	0.44	0.40	0.40	0.37	0.37
Calcium[5]	0.90	0.90	0.78	0.78	0.76	0.76
Lysine	1.31	1.31	1.18	1.18	1.03	1.03
Methionine	0.61	0.61	0.55	0.55	0.47	0.47

[1]Trace mineral premix at this rate yields per kg of diet 149 mg manganese, 125 mg zinc, 17 mg iron, 7 mg copper, 1.0 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium. The carrier is calcium carbonate and the premix contains less than 1% mineral oil

[2]Vitamin premix added at this rate yields per kg diet 11,023 IU vitamin A, 3,858 IU vitamin D3, 46 IU vitamin E, 0.0165 mg B12, 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.

[3] *Buttiauxella spp.* Phytase. Additional 600 FTU/kg added for high phytase treatments.

[4]Value includes contribution from phytase of 0.12%

[5]Value includes contribution from phytase of 0.11%

phytase X high XAP. The starter diet was fed from placement to d 15, grower was fed to d 28, and the finisher phase was fed to termination of the experiment at d 41. All diets were processed at 82°C, with the starter fed as a crumble and the grower and finisher fed as a pellet.

Animals and Management Practices

On d of hatch, 2,590 Cobb 500 male broiler chicks were transported from the hatchery to the research facility. Upon arrival, chicks were wing banded for identification, weighed, and allocated to floor pens based on chick weight. Each pen contained 37 chicks for a stocking density of 0.075 m^2/bird. Floor pens measured 1.52 m X 1.82 m and contained used litter mixed with fresh pine shavings for bedding material. Each pen was equipped with a 13.6 kg capacity tube feeder and nipple type watering system with *ad libitum* access to feed and water. Broilers were weighed on a per pen basis and feed consumption was measured at the conclusion of each dietary phase. Age appropriate environmental conditions were maintained inside the facility by automated control systems. All animals were raised in accordance with an approved Institutional Animal Care and Use Committee (IACUC) protocol.

STATISTICAL ANALYSIS

Data from enzymatic treatments was subjected to a 2 X 3 factorial analysis with main effect means deemed significantly different at $p \leq 0.05$. For all comparison back to the reference diet, data from individual treatments were analyzed via a one-way ANOVA with means deemed significantly different at $p \leq 0.05$. Means were separated using Duncan's Multiple Range Test. Evaluated parameters included: average body

weight (BW), feed consumption (FC), mortality corrected feed conversion ratio (FCR), average daily gain (ADG), and mortality.

RESULTS

At the conclusion of the starter phase on d 15, no significant differences in average BW were observed between main effects or individual treatments (Table 7). On d 28, following the grower phase, no differences were observed in main effects of phytase or XAP. However, one-way analysis did indicate that inclusion of high phytase x high XAP was the only treatment not similar to the reference diet, and was significantly lower compared to the high phytase x low XAP diet. At termination of the experiment on d 41, all enzymatic treatments were able to reach a statistically similar BW to the reference diet. For the analysis of main effects and interactions, no main effect differences or interactions were observed on BW for either phytase and XAP levels.

An early response was observed in the starter phase for FCR, as the inclusion of high phytase regardless of XAP level resulted in similar FCR compared to the reference diet, while low phytase treatments were significantly higher than the reference diet (Table 8). During the grower phase, the lowest FCR was observed in the reference diet while the only enzymatic treatment that reached a similar level of the reference diet was the high phytase X low XAP. No differences in FCR were observed in the finisher phase of the experiment. Regarding main effects and interactions, the inclusion of high phytase in the starter phase significantly reduced FCR compared to the low phytase treatment.

Table 7. Average body weight of broilers fed varying levels of phytase and XAP.

	Body weight (kg)		
Treatment	Day 15	Day 28	Day 41
Reference Diet[1]	0.469	1.561[a]	2.871
600 U/kg Phytase[2] + 1200 U/kg XAP[3]	0.471	1.543[ab]	2.868
600 U/kg Phytase + 1800 U/kg XAP	0.423	1.533[ab]	2.846
600 U/kg Phytase + 2400 U/kg XAP	0.467	1.540[ab]	2.842
1200 U/kg Phytase + 1200 U/kg XAP	0.473	1.567[a]	2.906
1200 U/kg Phytase + 1800 U/kg XAP	0.473	1.535[ab]	2.893
1200 U/kg Phytase + 2400 U/kg XAP	0.474	1.519[b]	2.837
ANOVA			
Pooled SEM	0.002	0.006	0.017
Treatment, P-value	0.638	0.013	0.554
Main Effects and Interactions			
Phytase			
600 U/kg	0.467	1.539	2.848
1200 U/kg	0.473	1.540	2.879
XAP			
1200 U/kg	0.472	1.555	2.882
1800 U/kg	0.468	1.534	2.869
2400 U/kg	0.471	1.529	2.839
P-value			
Phytase	0.118	0.890	0.258
XAP	0.635	0.098	0.243
Phytase x XAP	0.690	0.209	0.609

[a,b] Means in columns with different superscripts differ at $p<0.05$.

[1] Reference diet formulated to meet nutrient requirements of birds depending on phase. Enzymatic treatments have 88kcal/kg AME_n reduction.

[2] *Buttiauxella spp.* Phytase. Recovery analysis indicates phytase activity of 706 and 1,446 FTU/kg for inclusion rates 600 and 1,200 FTU/kg, respectively

[3] 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*. Recovery analysis indicates xylanase activity of 1,692, 1,741, and 2,304 U/kg for inclusion rates 1,200, 1,800 and 2,400 U/kg, respectively.

Table 8. Feed conversion ratio corrected for mortality of each phase for broilers fed varying levels of phytase and XAP.

	Corrected FCR		
Treatment	Starter	Grower	Finisher
Reference Diet[1]	1.273[c]	1.468[c]	1.871
600 U/kg Phytase[2] + 1200 U/kg XAP[3]	1.330[a]	1.511[b]	1.881
600 U/kg Phytase + 1800 U/kg XAP	1.318[ab]	1.496[b]	1.884
600 U/kg Phytase + 2400 U/kg XAP	1.325[ab]	1.496[b]	1.888
1200 U/kg Phytase + 1200 U/kg XAP	1.298[abc]	1.491[bc]	1.876
1200 U/kg Phytase + 1800 U/kg XAP	1.289[bc]	1.517[ab]	1.833
1200 U/kg Phytase + 2400 U/kg XAP	1.297[abc]	1.539[a]	1.888
ANOVA			
Pooled SEM	0.005	0.004	0.008
Treatment, P-value	0.011	<0.001	0.303
Main Effects and Interactions			
Phytase			
600 U/kg	1.324[x]	1.501	1.884
1200 U/kg	1.295[y]	1.515	1.865
XAP			
1200 U/kg	1.314	1.501	1.878
1800 U/kg	1.303	1.507	1.858
2400 U/kg	1.312	1.517	1.888
P-value			
Phytase	0.003	0.038	0.248
XAP	0.652	0.144	0.258
Phytase x XAP	0.978	0.001	0.255

[a-c] Means in columns with different superscripts differ at $p<0.05$.
[x,y] Means in columns with different superscripts differ at $p<0.05$.
[1] Reference diet formulated to meet nutrient requirements of birds depending on phase. Enzymatic treatments have 88kcal/kg AME_n reduction.
[2] *Buttiauxella spp.* Phytase. Recovery analysis indicates phytase activity of 706 and 1,446 FTU/kg for inclusion rates 600 and 1,200 FTU/kg, respectively
[3] 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*. Recovery analysis indicates xylanase activity of 1,692, 1,741, and 2,304 U/kg for inclusion rates 1,200, 1,800 and 2,400 U/kg, respectively.

There were no differences in ADG regarding individual treatment comparison or among main effects during the starter phase (Table 9). Throughout the grower phase, the inclusion of high phytase X high XAP reduced average daily gain compared to the reference diet and the high phytase X low XAP while all other enzymatic treatments were similar to the reference diet. No differences in ADG were observed between treatments for the finisher phase of the experiment. No main effect differences were observed with either phytase or XAP inclusion level for ADG during any dietary phase.

Feed consumption was influenced by enzymatic inclusion however only during the starter phase (Table 10). During this phase, the reference diet yielded the lowest observed FC and was similar to low phytase X mid XAP, high phytase X mid XAP, and high phytase X high XAP while all other enzymatic treatments had a significantly higher rate of feed intake. During the starter phase, the addition of mid XAP fed broilers consumed at a rate lower than that of broilers fed the low XAP dose while no impact was observed with phytase level. For the remaining phases of the experiment, there were no significant differences in FC between individual treatments or main effects

Cumulative mortality corrected FCR from d 1 to 28 including the starter and grower phases indicated that none of the enzymatic treatments were able to reach a level similar to the reference diet (Table 11). However, during the grower and finisher phases of the experiment (d 15 to 41), all enzymatic treatments were statistically similar to the reference diet with the exception of the high phytase X high XAP. Cumulative FCR for the entirety of the experiment period was influenced by enzymatic inclusion as the high

Table 9. Average daily gain of each phase for broilers fed varying levels of phytase and XAP.

	Average Daily Gain (kg/bird)		
Treatment	Starter	Grower	Finisher
Reference Diet[1]	0.029	0.083[a]	0.099
600 U/kg Phytase[2] + 1200 U/kg XAP[3]	0.029	0.081[ab]	0.101
600 U/kg Phytase + 1800 U/kg XAP	0.028	0.082[ab]	0.099
600 U/kg Phytase + 2400 U/kg XAP	0.028	0.082[ab]	0.098
1200 U/kg Phytase + 1200 U/kg XAP	0.029	0.083[a]	0.100
1200 U/kg Phytase + 1800 U/kg XAP	0.029	0.081[ab]	0.101
1200 U/kg Phytase + 2400 U/kg XAP	0.029	0.080[b]	0.098
ANOVA			
Pooled SEM	0.000	0.000	0.001
Treatment, P-value	0.664	0.024	0.630
Main Effects and Interactions			
Phytase			
600 U/kg	0.028	0.082	0.099
1200 U/kg	0.029	0.081	0.100
XAP			
1200 U/kg	0.029	0.082	0.100
1800 U/kg	0.028	0.081	0.100
2400 U/kg	0.029	0.081	0.098
P-values			
Phytase	0.162	0.332	0.792
XAP	0.907	0.372	0.242
Phytase x XAP	0.421	0.028	0.398

[a,b] Means in columns with different superscripts differ at $p<0.05$.

[1] Reference diet formulated to meet nutrient requirements of birds depending on phase. Enzymatic treatments have 88kcal/kg AME_n reduction.

[2] *Buttiauxella spp*. Phytase. Recovery analysis indicates phytase activity of 706 and 1,446 FTU/kg for inclusion rates 600 and 1,200 FTU/kg, respectively

[3] 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*. Recovery analysis indicates xylanase activity of 1,692, 1,741, and 2,304 U/kg for inclusion rates 1,200, 1,800 and 2,400 U/kg, respectively.

Table 10. Feed consumption on a gram/bird/day basis of each phase for broilers fed varying levels of phytase and XAP.

	Feed Consumption (g/bird/day)		
Treatment	Starter	Grower	Finisher
Reference Diet[1]	36.7[c]	123.4	188.3
600 U/kg Phytase[2] + 1200 U/kg XAP[3]	38.7[a]	125.0	191.6
600 U/kg Phytase + 1800 U/kg XAP	37.6[abc]	123.8	190.6
600 U/kg Phytase + 2400 U/kg XAP	38.0[ab]	123.9	189.1
1200 U/kg Phytase + 1200 U/kg XAP	37.9[ab]	126.1	193.8
1200 U/kg Phytase + 1800 U/kg XAP	37.4[bc]	124.9	192.4
1200 U/kg Phytase + 2400 U/kg XAP	37.6[abc]	123.7	191.6
ANOVA			
Pooled SEM	0.1	0.6	1.258
Treatment, P-value	0.023	0.493	0.404
Main Effects and Interactions			
Phytase			
600 U/kg	38.1	124.5	190.2
1200 U/kg	37.7	124.9	192.6
XAP			
1200 U/kg	38.3[x]	126.0	192.5
1800 U/kg	37.5[y]	124.3	191.5
2400 U/kg	37.8[xy]	123.8	190.3
P-value			
Phytase	0.132	0.689	0.292
XAP	0.049	0.146	0.621
Phytase x XAP	0.646	0.838	0.990

[a-c] Means in columns with different superscripts differ at $p<0.05$.

[x,y] Means in columns with different superscripts differ at $p<0.05$.

[1] Reference diet formulated to meet nutrient requirements of birds depending on phase. Enzymatic treatments have 88kcal/kg AME_n reduction.

[2] *Buttiauxella spp.* Phytase. Recovery analysis indicates phytase activity of 706 and 1,446 FTU/kg for inclusion rates 600 and 1,200 FTU/kg, respectively

[3] 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*. Recovery analysis indicates xylanase activity of 1,692, 1,741, and 2,304 U/kg for inclusion rates 1,200, 1,800 and 2,400 U/kg, respectively.

Table 11. Cumulative feed conversion ratio for periods of days 1-28, 15-41, and 1-41 for broilers fed varying levels of phytase and XAP.

	Cumulative Corrected FCR		
Treatment	Day 1-28	Day 15-41	Day 1-41
Reference Diet[1]	1.423[b]	1.685[b]	1.624[c]
600 U/kg Phytase[2] + 1200 U/kg XAP[3]	1.473[a]	1.713[ab]	1.657[a]
600 U/kg Phytase + 1800 U/kg XAP	1.458[a]	1.706[ab]	1.650[ab]
600 U/kg Phytase + 2400 U/kg XAP	1.457[a]	1.708[ab]	1.651[ab]
1200 U/kg Phytase + 1200 U/kg XAP	1.456[a]	1.700[b]	1.640[abc]
1200 U/kg Phytase + 1800 U/kg XAP	1.462[a]	1.691[b]	1.631[bc]
1200 U/kg Phytase + 2400 U/kg XAP	1.473[a]	1.729[a]	1.661[a]
ANOVA			
Pooled SEM	0.003	0.004	0.003
Treatment, p-value	0.001	0.033	0.010
Main Effects and Interactions			
Phytase			
600 U/kg	1.463	1.708	1.652
1200 U/kg	1.464	1.706	1.644
XAP			
1200 U/kg	1.465	1.705[xy]	1.647
1800 U/kg	1.460	1.699[y]	1.640
2400 U/kg	1.465	1.718[x]	1.656
P-value			
Phytase	0.824	0.866	0.257
XAP	0.768	0.100	0.134
Phytase x XAP	0.112	0.110	0.166

[a-c] Means in columns with different superscripts differ at $p<0.05$.

[x,y] Means in columns with different superscripts differ at $p<0.05$.

[1] Reference diet formulated to meet nutrient requirements of birds depending on phase. Enzymatic treatments have 88kcal/kg AME_n reduction.

[2] *Buttiauxella spp*. Phytase. Recovery analysis indicates phytase activity of 706 and 1,446 FTU/kg for inclusion rates 600 and 1,200 FTU/kg, respectively

[3] 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*. Recovery analysis indicates xylanase activity of 1,692, 1,741, and 2,304 U/kg for inclusion rates 1,200, 1,800 and 2,400 U/kg, respectively.

phytase X low XAP and high phytase X mid XAP dietary treatments resulted in similar FCR as compared to the reference diet. No main effect differences were observed in any cumulative FCR period regarding phytase inclusion. However, the mid level of XAP inclusion yielded a lower FCR for the evaluation period of d 15 to 41 (grower and finisher periods) as compared to the high XAP inclusion rate although no difference was observed when evaluating the entire experimental period.

During the starter, grower, and finisher phases there were no differences in mortality between any of the individual treatment groups (Table 12). No differences were observed in main effects on mortality rate associated with XAP inclusion level, however, a main effect of phytase inclusion on mortality during the finisher phase was observed as the high level of phytase yielded mortality rates significantly greater than the low inclusion of phytase although this difference did not persist when evaluating mortality for the entire experimental period.

DISCUSSION

The goal of this experiment was to evaluate the effect of varying levels of phytase and XAP on broiler growth performance in nutritionally marginal broiler diets. Data generated during this experiment demonstrated that the use of certain levels of these combined enzyme products may be more efficacious at improving growth performance in broilers.

Previous studies have indicated similar results when combining phytase with an XAP. Olukosi, et al. (2007) evaluated XAP and phytase individually and in combination on broiler performance during a 21-d experiment. Enzymatic treatments were formulated

Table 12. Mortality (%) of broilers fed varying levels of phytase and XAP.

	Mortality (%)			
Treatment	Starter	Grower	Finisher	Total
Reference Diet[1]	2.4	0.8	1.7	4.9
600 U/kg Phytase[2] + 1200 U/kg XAP[3]	3.2	2.8	0.0	6.0
600 U/kg Phytase + 1800 U/kg XAP	3.8	1.1	0.6	5.4
600 U/kg Phytase + 2400 U/kg XAP	3.2	0.3	0.3	3.8
1200 U/kg Phytase + 1200 U/kg XAP	4.6	2.0	0.9	7.3
1200 U/kg Phytase + 1800 U/kg XAP	2.4	1.4	1.1	4.9
1200 U/kg Phytase + 2400 U/kg XAP	2.2	1.4	0.9	4.3
ANOVA				
Pooled SEM	0.4	0.3	0.2	0.5
Block, p-value	0.929	0.159	0.408	0.606
Treatment, p-value	0.753	0.188	0.089	0.646
Main Effects and Interactions				
Phytase				
600 U/kg	3.423	1.4	0.3[b]	5.0
1200 U/kg	3.063	1.6	1.0[a]	5.5
XAP				
1200 U/kg	3.919	2.4	0.5	6.6
1800 U/kg	3.108	1.2	0.9	3.5
2400 U/kg	2.703	0.9	0.6	4.0
P-value				
Block	0.855	0.107	0.349	0.708
Phytase	0.716	0.715	0.032	0.708
XAP	0.592	0.073	0.575	0.219
Phytase x XAP	0.471	0.387	0.876	0.811

[a,b] Means in columns with different superscripts differ at $p<0.05$.

[1] Reference diet formulated to meet nutrient requirements of birds depending on phase. Enzymatic treatments have 88kcal/kg AME_n reduction.

[2] *Buttiauxella spp*. Phytase. Recovery analysis indicates phytase activity of 706 and 1,446 FTU/kg for inclusion rates 600 and 1,200 FTU/kg, respectively

[3] 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*. Recovery analysis indicates xylanase activity of 1,692, 1,741, and 2,304 U/kg for inclusion rates 1,200, 1,800 and 2,400 U/kg, respectively.

to be deficient in metabolizable energy and P. The inclusion of XAP alone did not improve performance although the inclusion of phytase alone did improve broiler weight gain compared to the NC. The effect on performance when using supplements in combination resulted in a sub-additive effect with improvements of final BW, weight gain and feed-to-gain compared to the NC. The authors indicated that the improvement in performance appeared to be mainly from the use of phytase. The current experiment yielded results similar to Olukosi et al. (2007) in that certain combinations of phytase and XAP improved early performance regarding feed consumption (FC) and FCR. These data indicate that phytase may be more responsible for the improvement in growth performance as the high phytase inclusion improved FCR beyond that of the low inclusion rate during the starter phase. Subsequently at the conclusion of the experiment, the only treatments that were able to reach the level of the reference diet were enzymatic treatments that included the high level of phytase. However, the level of XAP does seem to impact growth performance as the high inclusion rate did not reach a similar level to the reference diet.

The inclusion of XAP in corn-soybean meal based diets and its impact on broiler performance has been previously evaluated (Café, et al., 2002). However, in that particular experiment XAP was included in a nutritionally sound diet with broilers grown to 49 d of age while in the experiment described here XAP was included in a lower energy diet. The inclusion of XAP improved body weight of broilers at d 16, 35, and 49 (Café, et al., 2002), however, it negatively impacted FCR at d 16 and 42. In the current experiment, the inclusion of XAP in combination with phytase in a nutritionally

marginal diet yielded FCR values similar to the reference diet of adequate nutritional density. In the starter phase of this experiment, all high phytase containing treatments, regardless of XAP inclusion rate, resulted in FCR similar to the reference diet and in the grower phase the inclusion of high phytase X low XAP yielded a FCR similar to the reference diet. Interestingly, differences were observed between the different levels of XAP included. Observations included a reduction in starter feed consumption as XAP level increased as well as an increase in cumulative FCR (d 15 to 41), indicating the dose of XAP is an important factor to consider. These data indicate the effectiveness of enzyme inclusion on FCR may be greater when included in reduced nutrient diets or when combined with phytase.

Throughout the grower phase of this experiment, several parameters with the inclusion of high phytase X high XAP were negatively influenced including reduced average BW and ADG and increased FCR. It appears that to some extent, there may be a threshold limit to the level of enzyme inclusion one could expect to see positive results from in this scenario. Negative impacts from XAP inclusion were reported by Café et al. (2002) as well. In that experiment, XAP inclusion in nutritionally complete diets negatively impacted feed:gain compared to a control diet when measured at d 0-16 and d 0-42, though the author concluded the use of XAP can result in improved performance as observed on other parameters and at other phases of that experiment.

Francesh and Geraert (2009) reported that the use of combined non-starch polysaccharidase (NSPase) and phytase suggests reduced amino acid, phosphorus, energy and protein specifications of corn-soybean meal diets as supplementation with

the enzyme complex improved average daily gain and feed:gain. Their results are similar to the current experiment, where at certain concentrations the inclusion of phytase and XAP into a reduced ME and P diet resulted in growth performance similar to that of the nutritionally sound reference diet indicating that the included enzymes compensated for the reduction in dietary available phosphorus and metabolizable energy. This idea was supported by Woyengo et al. 2010, who reported a benefit from the addition of multicarbohydrase to a phytase-supplemented diet on 21 d BW gain and FCR compared to phytase inclusion alone. A phytase or XAP alone treatment was not included as multiple reports indicated the positive benefit of the combination (Francesh and Geraert, 2009; Olukosi et al., 2007; Woyengo et al., 2010). This experiment focused on the relationship between phytase and XAP inclusion related to multiple inclusion rates to identify an optimum level of the combination to maximize performance. There were periods during this experiment where phytase and XAP inclusion influenced parameters with a main effect. However, due to the negative effect the high inclusion of XAP seemed to have at times it is suggested that the majority of growth improvement can be attributed to phytase inclusion. The level of phytase and XAP to be included in poultry diets is an important consideration to the ultimate performance of a flock. In addition to performance, enzyme product inclusion is an important economic consideration that can impact production efficiency by adding to the cost of feed.

CHAPTER IV

CONCLUSION

In the two experiments, varying enzyme preparation combinations and doses of enzyme preparation combinations were evaluated for effects on growth performance of broilers. Previous research (Olukosi et al., 2015, Romero et al., 2014) on the subject suggests that enzyme combination can be more effective than individual or less complex enzyme combinations at improving growth performance and nutrient utilization. However, several past studies indicate varying results when testing this hypothesis.

In experiment 1, a cocktail of xylanase, β-glucanase, and α-galactosidase and a combination of xylanase, amylase, and protease were included into reduced energy diets individually and in combination. Supplementation of NSPase increased BW in the starter and finisher phases and reduced FCR throughout the experiment. Including XAP improved BW at d 14 and 27 and improved FCR from d 1 to 27. From the data of that experiment, it can be concluded that individual enzyme product combinations can be effective at improving broiler performance in low energy diets and compensate for nutrient reductions. However, supplementing these 2 enzyme product combinations that have similar modes of action together does not yield any further benefit superior to individual inclusion.

In Experiment 2, 2 varying levels of phytase were included with 3 varying levels of xylanase, amylase, and protease to determine if combining enzyme preparations of differing modes of action could compensate for reductions specifically in energy. At the

conclusion of the experiment, BW of all treatments was similar to the reference diet. Inclusion of high phytase X low XAP and high phytase X mid XAP resulted in cumulative FCR similar to the reference diet. High phytase had a main effect of reducing starter FCR. Factorial analysis indicates mid XAP reduced starter feed consumption compared to low XAP. Mid XAP reduced d 15 to 41 FCR compared to low XAP. The data from Experiment 2 indicates that a combination of phytase and XAP can be effective at improving performance in diets containing a lower level of energy; however, dose seems to be an important factor as the two highest doses of enzyme were not the most effective. An important consideration when including an enzyme product or combination of products is the economic impact that can be expected on production efficiency. The effective inclusion of enzymes is beneficial when cost-effective. The price of including enzymes should be worth a gain in growth performance or should be cheaper than nutrients that can be compensated for. An improvement in growth performance can translate into heavier BW which can lead to more marketable product. Also, performance improvements can result in reduced production costs from more efficient feed conversion, meaning less feed consumed to meet target BW. The value of marketable product gained or feed saved should be greater than the cost of including the enzyme product to be considered economical. Including enzyme products to compensate for nutrient reductions can be economical in the instance that the cost of the nutrients removed is greater than the cost to include the enzyme product. In the situation of nutrient reductions included enzymes must be able to compensate by maintaining expected growth performance. In totality, the data from the research herein indicate that

supplementation of enzyme products into a reduced energy diet can influence growth performance. However, combinations of enzyme products with similar mode of action do not result in performance levels beyond that of individual inclusion.

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