EVALUATION OF EXOGENOUS ENZYMES TARGETING NON-STARCH POLYSACCHARIDES IN REDUCED ENERGY DIETS ON BROILER GROWTH PERFORMANCE AND PROCESSING PARAMETERS

A Thesis

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ABSTRACT

Multiple experiments were conducted to investigate the inclusion of a cocktail NSPase and β-mannanase, separately and in combination, in reduced energy diets on broiler growth performance and processing yield. Each experiment contained a positive control (PC), negative control (NC) diet (-132 kcal/kg AME), and the inclusion of enzymes in the NC to evaluate enzyme effectiveness. The reduction in energy negatively impacted performance and processing parameters in all experiments. The inclusion of NSPase negated the negative effects of energy reduction in experiment 1. Experiment 2 evaluated increased pelleting temperature on NSPase activity. Body weight (BW) was increased ($P<0.05$) with the inclusion of NSPase pelleted at 80, 85, and 90 C throughout the experiment compared to NC; however, the treatment pelleted at 80 C outperformed the other NSPase pelleted treatments. The inclusion of NSPase pelleted at 80 C reduced ($P<0.05$) feed conversion ratio (FCR) compared to the NC throughout the experiment. At the conclusion of the trial, NSPase inclusion pelleted at 85 and 90 C yielded FCR similar to PC. The experimental design of experiment 3 and 4 included five dietary treatments including a PC, NC, NC supplemented with β-mannanase, NSPase, and β-mannanase/NSPase. Performance parameters were evaluated on d 14, 28, 42, and 47 and a subset of broilers were processed on day 48. Increases ($P<0.05$) in BW were observed with the inclusion of NSPase and β-mannanase/ NSPase on day 14 and with all treatment groups on day 28. An additive effect was observed with reduced FCR through day 28 with the combination of β-mannanase/ NSPase. In

experiment 4, performance was evaluated on days 14, 27, 35, and 41 and carcass yields determined on day 42. Increases in day 14 BW were observed with the inclusion of the NSPase alone and β-mannanase/NSPase to reach a similar weight as the PC. Inclusion of β-mannanase/NSPase increased ($P<0.05$) BW compared to the NC. Inclusion of the NSPase reduced ($P<0.05$) cumulative FCR through 41 days of age. Inclusion of β-mannanase/NSPase resulted in reduced ($P<0.05$) FCR in the finisher phase and cumulatively throughout the trial to levels of the PC. The combination of β-mannanase/NSPase did increase ($P<0.05$) WOG weight similar to observations in BW. These data confirm that enzyme supplementation in low energy diets improve performance and indicate that additive effects of a combination of enzymes could potentially be a cost saving strategy for producers.

DEDICATION

I would like to dedicate this to my family. You have always supported all of my endeavors and shown me so much love without the support and guidance of my family none of this would be possible. To my parents Tony and Debbie Klein, your support and guidance has always been amazing, you have raised me to be a good both spiritually and as a man and I thank you for that from the bottom of my heart. To my brother TJ you have always been a great role model for me showing me the right way to do things, you have always been there to give me advice and guidance along the way and I thank you for being my best friend. To my sister Elizabeth thanks for putting up with me in college and helping me along the way to become a good person, thanks for always being there when I needed to talk and listening even when you didn't want to. To my sister Victoria, thanks for always supporting me and providing me with the comedic relief you are so very good at, I am very grateful for my entire family and could not have done any of this without the love, support, and spiritual guidance of you all. I love all of you very much, thank you.

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NOMENCLATURE

BW	Body weight
FCR	Feed conversion ratio
NSP	Non-starch polysaccharide
MBM	Meat and Bone meal
SBM	Soybean meal
DDGS	Dried Distiller's grain with soluble
AME	Apparent Metabolizable Energy
AME_n	Apparent Metabolizable Energy corrected for Nitrogen
IDE	Ileal Digestible Energy
ME	Metabolizable Energy
VFA	Volatile Fatty Acids
P	Phosphorus
DM	Dry Matter
C	Celsius
CP	Crude Protein
PC	Positive Control
NC	Negative Control

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CHAPTER I

INTRODUCTION

The majority of broiler diets in the United States are corn and soybean meal based, making up about 85% of the total diet. However, since 2002, the US government has encouraged the production of ethanol and other biofuels. The majority of ethanol production in the United States comes from corn, as it is the most abundant crop available. In 2002, only 11% of available corn in the US was utilized in ethanol production; however, by 2008, approximately 30% of the corn crop was utilized for ethanol production (Donohue and Cunningham, 2009). This rise in ethanol production has led to a direct increase in the cost of feed ingredients. Feed ingredients as a percentage of live production cost increased from 51.8% in 2001 to 68.7% in 2008 leading to an overall increased cost of poultry feed in the US by 9.36 billion dollars since 2006 (Donohue and Cunningham, 2009).

Corn and soybean meal based diets contain varying levels of non-starch polysaccharides (NSP), which are complex high molecular weight carbohydrates found in the structure of plant cell walls (Bedford and Classen, 1993). Non-starch polysaccharides present a problem as they are not digestible by monogastric animals because they lack the digestive capacity of ruminant animals (Meng et al., 2005). Dietary NSPs represent a potential nutrient source if the proper enzyme for hydrolyzation is present. Research has shown that dietary NSPs increase intestinal viscosity which leads to a reduction in nutrient digestibility, and ultimately a reduction

in performance in regards to body weight and feed conversion ratio (Bedford and Morgan, 1995; Lázaro et al., 2003b).

One method used to increase the digestibility of grains high in NSPs is the supplementation of exogenous carbohydrases, which hydrolyze soluble NSP present in cereal grains. Published reports indicate carbohydrases are successful in improving nutrient utilization when used in combination with wheat, barley, rye, or oats; however, data suggests inconsistent results when corn and soybean meal are the primary ingredients (Maisonnier-Grenier et al., 2006). Cocktail carbohydrases (NSPase) hydrolyze NSPs into smaller particles that can be utilized by monogastric animals (Coppedge et al., 2012). NSPase inclusion has been reported to improve performance parameters when included in low energy broiler diets (Coppedge et al., 2012; Lázaro et al., 2003b; Meng et al., 2005). The observed increased intestinal viscosity of digesta associated with NSPs causes changes in the gut microflora and reduces nutrient utilization; however, the proper use of non-starch polysaccharide degrading enzymes can combat grains high in NSP (Choct et al., 1999). Additionally, inclusion of exogenous carbohydrases increase AME, IDE, and dry matter retention (Cowieson and Ravindran, 2008; Leslie et al., 2007; Meng et al., 2005; Olukosi et al., 2010). The majority of diets contain a variety of NSP; thus, perhaps the most effective means of maximizing nutrient utilization may be the use of cocktail carbohydrases that vary in specificity. Research has shown that cocktail carbohydrases preparations can increase starch digestibility, broiler performance, and improve feed conversions (Meng et al., 2005).

Corn and soybean meal are considered highly digestible ingredients, but research indicates there is room for improvement of their nutritional digestibility (Zanella et al., 1999). Corn contains inconsequential amounts of soluble NSP along with approximately 8% insoluble NSP, which does not create digesta viscosity problems in the form of arabinoxylans representing a potential source of nutrients (Choct, 2006). Soybean meal contains about 3% soluble NSP along with 16% insoluble NSP, primarily in the form of galactomannans, which also represent a potential source of nutrients (Irish and Balnave, 1993). Recent research suggests the dietary supplementation of a multi-enzyme complex of carbohydrases is efficient in reduction of P, energy, protein, and amino acid specifications of corn-soybean meal based diets (Francesch and Geraert, 2009).

The use of exogenous NSPase has been extensively researched and the efficacy of NSPase in broiler diets is commonly accepted (Bedford, 2000). The inclusion of xylanase, protease, or amylase improves FCR, increases AME, and starch digestibility in cereal grain diets (Choct et al., 1999; Kalmendal and Tauson, 2012; O'Neill et al., 2012). The inclusion of xylanase significantly increases early body weight gain through 24 days of age, reduces digesta viscosity, increases nutrient retention, and consequently improves performance of broilers (Esmaeilipour et al., 2011). However, these enzymes are heat sensitive and their activity may be reduced by the feed manufacturing process. Reports indicate pelleting temperature decreases the activity of the included enzymes which led to a reduction in broiler growth performance (Silversides and Bedford, 1999). The critical point of deactivation of these enzymes involved has always been considered

linear with activity of these exogenous enzymes decreasing as the temperature of pelleting increases (Samarasinghe et al., 2000; Spring et al., 1996).

β-mannans such as glucomannans and galactomannans in the plant cell wall (Zou et al., 2006) of soybean meal could potentially reduce nutrient bio-availability. β-mannanase supplementation is one way to combat the negative effects of the β-mannans by hydrolyzing β linkages. β-mannanase inclusion improves feed conversion ratio and reduces water: feed ratio, as well as dry fecal output of broilers by degrading β-mannans (Daskiran et al., 2004). β-mannanase increases AMEn (Zangiabadi and Torki, 2010), body weight gain, and improves feed conversion in diets that are primarily corn and soybean meal (Jackson et al., 2004), as well as diets containing fibrous ingredients (Lee et al., 2003; Zangiabadi and Torki, 2010). Additional benefits of β-mannanase includes a reduction of lesion development following *Eimeria* sp. and *Clostridium perfrigens* challenges (Jackson et al., 2003). This may be attributed to data indicating β-mannanase supplementation increases relative immune organ weights, increases concentration of serum Igm, and increases T-lymphocyte proliferation (Zou et al., 2006).

The objectives of the following experiments are to determine the effect of NSPase inclusion in reduced energy diets on broiler performance and processing yield, determining the effect of increasing pelleting temperature on enzyme activity, and evaluate if co-administration of NSPase and β-mannanase result in further improvements in growth performance as opposed to individual inclusion.

CHAPTER II

LITERATURE REVIEW

DIET COST

The majority of broiler diets in the United States are corn and soybean meal based, making up about 85% of the total diet. However, since 2002, the US government has encouraged the production of ethanol and other biofuels. The majority of ethanol production in the United States comes from corn grain as it is the most abundant crop available. In 2002, only 11% of available corn in the US was utilized for ethanol production; however, by 2008, approximately 30% of the corn crop was utilized in ethanol production (Donohue and Cunningham, 2009). This rise in ethanol production has led to a direct increase in the cost of feed ingredients. Feed ingredients as a percentage of live production costs increased from 51.8% in 2001 to 68.7% in 2008 leading to an overall increased costs of poultry feed in the US by 9.36 billion dollars since 2006 (Donohue and Cunningham, 2009).

These increases in ingredient and diet costs have led industry nutritionists to include less desirable ingredients such as wheat and Dried Distillers' Grain with Solubles (DDGS) in poultry diets. Additionally, the increases in diet cost has increased the importance of utilizing all potential nutrients present in the diet and reduce over formulation of nutrients in an effort to reduce diet cost. Less desirable ingredients such as wheat and DDGS contain higher concentrations of indigestible nutrients compared to

corn and soybean meal. In cereal grains, starch represents the greatest fraction of polymeric carbohydrate, while NSP comprise the greatest portion in protein-rich materials (Bach Knudsen, 2011). Common grains used in poultry diets were determined to have fiber concentrations of 108, 138, 221, 323, 233 (g/kg of DM) for corn, wheat, barley, corn DDGS, and soybean meal, respectively (Bach Knudsen, 1997).

NON-STARCH POLYSACCHARIDES

Corn and soybean meal are widely considered to be highly digestible ingredients; however, there is room for improvement of their nutritional value (Zanella et al., 1999). These cereal grains contain varying levels of NSP, which are complex high molecular weight carbohydrates found in the structure of plant cell walls (Bedford and Classen, 1993). Corn contains inconsequential amounts of soluble NSP and approximately 8% insoluble NSP, which do not yield digesta viscosity problems, in the form of arabinoxylans; however, they do represent a potential source of nutrients (Choct, 2006). Soybean meal contains about 3% soluble NSP along with 16% insoluble NSP primarily in the form of galactomannans which also represents a potential source of nutrients (Irish and Balnave, 1993). Other NSP present in corn and soybean meal based diets at lower levels include arabinose, xylose, rhamnose, and galactose (Coppedge et al., 2012). In cereal grains, starch represents the greatest fraction of polymeric carbohydrate, while NSP comprise the greatest portion in protein-rich materials (Knudsen, 2011). Common grains used in poultry diets were determined to have fiber concentrations of 108, 138, 221, 323, 233 (g/kg of DM) for corn, wheat, barley, corn DDGS, and soybean meal, respectively (Knudsen, 1997).

The presence of NSP in cereal grains can lead to a variety of problems for monogastric animals because they lack the digestive capacity of ruminant animals (Meng et al., 2005). These problems include an increase in intestinal viscosity and bacterial fermentation inhibiting the absorption of valuable nutrients such as starches, lipids, and proteins (Annison, 1993), which are most prevalent in grains with higher amounts of NSP such as barley, rye, wheat, and oats. This decrease in absorption of nutrients is achieved by reducing glucose and sodium transportation into the epithelial cells (Edwards et al., 1988), which leads to a reduction in the release rate of pancreatic enzymes, and bile acids (Larsen et al., 1993). While it is unclear if one mechanism is more important than the other, an experiment conducted by Smits et al. (1997), investigated the effects of feeding a non-fermentable carboxymethylcellulose mixture to broilers from 21-35 days of age at a low viscosity (LCMC) and high viscosity (HCMC) on broiler performance. Inclusion of HCMC caused a depression in body weight, an increase in water consumption, and an increase in viscosity, which was responsible for an observed decrease in starch and lipid digestion.

An increase of small intestinal viscosity in broilers impacts digesta retention time and substrate to enzyme interaction leading to decreases in digestibility. In an experiment performed by Choct, et al. (1999), broilers fed low ME wheat diets, variability in digestibility of starch in the duodenum was 37%, which is not a significant difference, likely due to the chicken's short duodenum where small amounts of nutrients are digested and absorbed. Variability in the jejunum and ileum decreased to 11% and 9% respectively when fed low ME wheat diets. The supplementation of enzymes further

reduced this variability in the jejunum and ileum to 9% and 1.5%. Additionally, high gut viscosity in the small intestine is not limited to the physical hindrance of nutrient digestion and absorption. A decreased rate of passage can drastically change the microbial balance in the gut by decreasing oxygen tension in the small intestine providing a stable environment for fermentative microflora to become established, leading to more volatile fatty acid (VFA) production in small intestine. Anaerobic bacteria counts in the small intestine of broilers has been shown to increase, specifically the number of *Clostridia* in the small intestine when fed diets with high amounts of water soluble NSP (Mathlouthi et al., 2002). This particular anti-nutritive property is typically associated with poorly digested grains resulting in nutrients evading digestion and absorption in upper small intestine. These nutrients enter the lower intestine where the presence of bacteria is much higher which utilize these undigested substrates leading to energy waste (Wagner and Thomas, 1978). Furthermore, colonization of the upper small intestine is inevitably associated with excess nutrients creating a more suitable environment for bacterial growth. Langhout et al. (1999) demonstrated all of these anti-nutritive properties of NSP using a corn based diet supplemented with high and low methylated citrus pectin. Inclusion created viscosity issues typically not associated with a corn based diet including decreased digestibility, changes in gut microflora, and alterations to gut morphology. The increase of viscosity reduced digestibility through inhibited enzymes substrate interaction, more competition for nutrients due to increases in anaerobic bacteria present in the intestine, and decreased villi length resulting in decreased contact area between potential nutrient and enterocyte leading to reduced

absorption of nutrients. Multiple experiments have reported a reduction in performance of broilers in terms of feed conversion ratio and body weight when fed diets high in NSP (Bedford and Morgan, 1995; Lázaro et al., 2003b). These anti-nutritive properties are a concern for producers because starch is the primary source of energy for broilers.

High viscosity, microbial fermentation, and gut morphology is not a big issue in corn and soybean meal based diets. Corn and soybean meal are largely considered easily digestible as the concentration of water soluble NSP in these grains is significantly less when compared to the more viscous grains previously mentioned. However, there is room for improvement as there is the presence of NSP in these two grain sources, which are not readily digested by the bird due to the lack of digestive enzymes leading to variability in digestibility (Maisonnier-Grenier et al., 2006; Zanella et al., 1999). The length of time these nutrients are in the intestinal tract of the bird will always create problems as the contact time for these natural pancreatic enzymes and bile acids present is limited. Short contact time can lead to less absorption of these longer chain polysaccharides, representing a loss in digestible energy for the bird. Additionally, the inability for broilers to break down nutrients entrapped in the matrix of cell walls of feed results in another escape of valuable nutrients to the bird. Recent findings of variability in starch digestibility in corn and soybean diets fed to young chicks from 0-21 days (Choct et al., 1996; Wyatt et al., 1999), were determined to range between 84 and 90% which is lower than the previously thought 98%. This research, combined with the need to reduce diet cost, and the need to maximize nutrient utilization could potentially enable producers to cut cost.

EXOGENOUS ENZYME

Several methods were developed to combat the anti-nutritive effects associated with grains high in NSP. One such method involves the supplementation of exogenous carbohydrase enzymes to the diet to hydrolyze dietary soluble NSP, allowing for absorption of these valuable nutrients which represent a potential energy source for the bird (Cowieson and Adeola, 2005). Though this is not a new development for the poultry industry, recent pressure to reduce diet cost and maximize nutrient utilization has forced researchers to evaluate theses exogenous enzymes in singular form or in combination with several carbohydrases (cocktail carbohydrases).

Exogenous enzymes can be added to the diet to increase the feeding value of raw materials which leads to the targeted ingredient being used in abundance, and the utilization of high value ingredients such as fat is decreased (Bedford, 2000; Marquardt et al., 1994). An additional benefit of exogenous enzyme inclusion is to reduce the variation of nutrient quality of ingredients. This response to enzymes is most readily observed with the use of grains with the poorest quality. The result is reduced variation in subsequent bird performance leading to more uniformity within the flock along with better uniformity from flock to flock (Bedford, 2000). Finally, exogenous enzymes are supplemented to reduce the incidence of wet litter, typically associated with diets of the poorest quality containing barley, rye, oats, and triticale. Diets primarily formulated with these ingredients lead to the production of a very viscous wet manure (Bedford and Morgan, 1995; Bedford, 2000).

The additive characteristics of exogenous charbohydrases are most beneficial due to the flexibility it gives nutritionists when formulating diets, including the ability to reduce the amount of fat without sacrificing performance, which drastically reduces diet cost. The use of such enzymes in high NSP diets, such as wheat, rye, and barley, has been well established. Wang et al. (2005) investigated the effects of a xylanase and β-glucanase at various inclusion rates on broiler performance, feed digestibility, gut morphology, and VFA profile when fed a wheat based diet. A linear increase in average daily gain (ADG) was observed when enzyme supplementation was increased. Additionally, enzyme inclusion in this study reduced digestive organ weights and resulted in an increase in VFA production in the cecum as compared to the control diet. These findings support earlier reports by Choct et al. (1999) that evaluated the effects of xylanase on individual bird variation, starch digestion, and ileal and cecal VFA production in broilers fed a low AME wheat diet. A reduction in digesta viscosity was achieved through enzyme inclusion in each section of the gut with a decrease of 2.9 to 1.7 CP in the duodenum, 4.6 to 2.3 CP in the jejunum, and 14.0 to 3.9 CP in the ileum. Likewise, starch digestion was increased with the addition of xylanase from 73% to 79% in the jejunum and 96% to 98% in the ileum. Finally, VFA production was increased in the ceca with total VFA production increasing from 340 to 519 mmol. Multiple published reports have observed significant decreases in digesta viscosity, improved performance, reduced incidence of pasty vent (Esteve-Garcia et al., 1997), and enhanced retention of dry matter by 11%, energy by 10%, and crude protein by 15 % when fed a wheat based diet supplemented with xylanase.

Similar observations are reported with the addition of exogenous enzymes in diets primarily made up of grains high in NSP. Lázaro et al. (2003b) evaluated the influence of enzyme supplementation on performance and digestive parameters of broilers fed a rye based diet from 4-25 days. Enzyme inclusion improved body weight gain by 20.8% at 25 days, feed consumption by 4.9%, and FCR by 12.7% as compared to the control diet. Similarly, the addition of the enzyme reduced intestinal viscosity as compared to supplemented birds. These data are in agreement with published reports that have shown the inclusion of xylanase and β-glucanase in rye based diets increased bird performance (Langhout et al., 1997; Lázaro et al., 2003a). Additionally, Lázaro et al. (2003a) reported decreased viscosity, stained eggs, increased egg production, improved feed efficiency, and increased apparent nutrient digestibility in laying hens when fed a wheat-rye based diet.

Published reports indicate carbohydrases are successful in improving nutrient utilization when used in combination with wheat, barley, rye, or oats; however, data suggests inconsistent results when corn and soybean meal are the primary ingredients (Francesch and Geraert, 2009). Research performed by O'Neill et al. (2012) investigated the effects of xylanase on performance characteristics of broilers fed corn and soybean based diets with reduced energy. This reduction in energy was achieved by reducing the fat in the diet. As expected, this reduction in energy resulted in increased feed intake, FCR, and a decrease in body weight. The supplementation of xylanase to the reduced energy diet resulted in an increase in body weight and a decrease in FCR from day 0-35 and 0-42. Similarly, (Cowieson et al., 2010) investigated the use of

xylanase and glucanase in a diet composed of corn and soy that had a reduction in energy. Birds fed a reduced energy diet consumed 2.2% more feed through the duration of the trial, leading to a 6 point increase in FCR. The inclusion of xylanase in the reduced energy diet resulted in an observed improvement in FCR and body weight and no increase in feed intake, which was also supported by (Zanella et al., 1999)

This mechanism is result of an increase in feed efficiency through a significant increase in body weight gain without sacrificing feed intake. Additionally, the effects on FCR have been reported to develop over a period of time, not significant until 35 days (O'Neill et al., 2012), with ileal digestible energy reported as being significant at 42 days (Cowieson et al., 2010). These observations support the theory that xylanase has a developmental effect on the fermentative capacity of the ceca, perhaps selecting for development and maintenance of beneficial cecal flora by providing xylo-oligomers due to its interaction with fiber present in corn (O'Neill et al., 2012).

Still other reports, such as Leslie et al. (2007), determined the effects of phytase and glucanase on ileal digestibility of corn and soybean meal diets reported similar findings in regard to the inclusion of a glucanase. An increase in digestibility of corn and an increase in ileal digestible energy (IDE) of both corn and soybean meal was reported with an increase in corn IDE observed with an increase in age, but not with soybean meal. The age dependent effect on IDE of glucanase in corn is likely due to the increased access of starch granules within the endosperm of the cells with amylase. These findings suggest that there is value in utilizing these exogenous enzymes in diets

composed primarily of corn and soy, even though the level of soluble NSP present is low.

ENZYMATIC COCKTAIL STRATEGY

The majority of diets contain a variety of NSP, all of which have different substrates for different digestive enzymes. Perhaps the most effective means of maximizing nutrient utilization may be the use of cocktail carbohydrases (NSPase) that vary in specificity allowing for diverse enzymes to accommodate the variety of NSP present in these grains. Recent research suggests that inclusion of NSPase can increase starch digestibility and improve performance (Meng et al., 2005). The improvements were attributed to the degradation of cell wall polysaccharides by carbohydrases, which increased nutrient utilization. A number of carbohydrase preparations were supplemented including cellulase pectinase, xylanase, glucanase, galactomanase, and mannanase, singularly or in combinations. The results indicated the use of combinations of the enzymes resulted in a 37% higher degradation of NSP as compared to the control in wheat diets, and a 26% higher degradation of NSP in the soybean meal diet. The addition of all enzymes groups improved weight gain, AME, feed-to-gain ratio, and apparent ileal digestibility of starch and protein. This improvement is presumably due to the reduction in viscosity associated with these enzymes; however, the increase in performance of the combination groups is likely due the variety of enzymes allowing for more substrate to enzyme interaction of different NSP present.

Published reports indicate a multi-enzyme complex of carbohydrases is efficient in reducing the P, energy, protein, and amino acid specifications of corn-soybean meal

based diets (Francesch and Geraert, 2009). In a study performed by Coppedge et al. (2012), which investigated the use of a two NSPase enzymes on broiler performance fed a corn and soybean meal based diet with either a reduction in energy and protein of 4% (NC1), or reduction in energy of 4% (NC2),the reduction of nutrient concentration in both negative control diets depressed performance, while the inclusion of NSPase in the NC2 diet resulted in a decrease in FCR of 5% during starter phase. Improvement in performance parameters has also been reported when using a combination of β-glucanase, xylanase, and α-amylase in a corn and soybean meal based diet reduced in energy by 3%, resulting in restoration of growth performance similar to that of a control diet (Yu and Chung, 2004) .

Reports of improvement with cocktail carbohydrases have been inconsistent with multiple reports indicating minimal response (Kidd et al., 2001; Olukosi et al., 2007). In a broiler response study supplementing phytase and a mixture of carbohydrase and protease in a corn and soybean meal based diet deficient in AME and phosphorus with and without DDGS on broiler performance and digestibility, no interaction occurred between the carbohydrase mixture and phytase. Enzyme supplementation had no effect on growth parameters, while the coefficient of apparent ileal nitrogen digestibility was lower in diets containing DDGS. The addition of phytase increased the coefficient of apparent ileal dry matter digestibility in all diets independent of the DDGS, while improving the coefficient of apparent P retention in DDGS free diets (Olukosi et al., 2010). In a study of age related influence of a cocktail of xylanase, amylase and protease, and phytase individually and in combination in reduced energy and P diets

reported the influence of enzymes was more beneficial at a younger age, with decreasing contribution to nutrient retention as the bird aged (Olukosi et al., 2007). While observations indicated an increase in performance with the combination of phytase and the cocktail of enzymes, it suggests the increase due to the cocktail is marginal when compared to the benefits of the phytase (Olukosi et al., 2007). These findings support the theory that the additive benefit is most useful in young birds when the GI tract is not fully developed and the most energy is lost from lack of nutrient absorption. Phytase benefits will typically be more pronounced than carbohydrases as there is a mineral deficiency (poor skeletal development and weight gain) that will be more pronounced than an energy deficiency (increased feed conversion ratio).

Carbohydrases are typically heat sensitive and thus their activity could potentially be decreased during the feed manufacturing process. The optimal temperature to pellet commercial feeds efficiently, while maintaining the integrity of the enzyme and its digestive abilities to the bird, is of extreme importance for producers. Reports indicate that pelleting temperature decreases the activity of enzymes, which led to a reduction in broiler growth performance. Silversides and Bedford (1999) investigated the effect of pelleting temperatures on the recovery and efficacy of xylanase. The enzyme activity in supplemented diets, measured *in vitro,* was largely eliminated once temperatures exceeded 80° C with maximum broiler performance at 80° C, and 85° C showed reduced activity with increased temperatures. With enzyme supplementation, moderate heating is believed to result in the gelatinization of starch and cell wall destruction leading to enhanced nutrient availability, while more extreme

heating inactivates vitamins and enzymes (Pickford, 1992). The critical point of deactivation of these enzymes involved has always been considered linear with the activity decreasing as the pelleting temperature increases (Samarasinghe et al., 2000) and (Spring et al., 1996). Establishing the optimal pelleting temperature for manufacturers to maximize enzyme activity and influence is a priority of producers trying to cut costs.

GALACTOMANNANS AND β-MANNANASE

Other factors that could potentially reduce nutrient bio-availability are the anti-nutritive properties of the NSP in ingredients such as soybean meal, guar, and sesame meal. These occur in the form of β-mannans such as glucomannans and galactomannans in the plant cell wall (Zou et al., 2006). These NSP exhibit some of the same anti-nutritive properties as those found in other grains causing a depression in performance in regard to body weight and FCR (Knudsen, 2011). Galactomannans interfere with water and glucose absorption which was observed in pigs fed a diet containing guar gum (Knudsen, 1997).

These NSP, particularly β-mannans have also been shown to stimulate an innate system immune response, thus potentially stimulating a nonproductive energy draining innate immune response. The result is an increase in proliferation of macrophages and monocytes, and increased cytokine production, leading to an increase in disease symptoms while decreasing the efficiency of nutrient utilization (Hsiao et al., 2006). This is believed to be a result of the mannans various configurations that are surface components of numerous pathogens such as fungi, bacteria, and viruses which the innate

immune system recognizes as antigens or pathogens, including mannan (Hsiao et al., 2006).

β-mannanase is one way to combat the negative effects of the β-mannans; which works by hydrolyzing β-mannans into smaller particles that are readily utilized by monogastric animals. β-mannanase inclusion improved feed conversion ratio and reduced water to feed ratio as well as dry fecal output of broilers by degrading β-mannans (Daskiran et al., 2004). This experiment examined the effects of four dietary inclusion levels of guar gum each with and without the addition of a β-mannanase. The inclusion levels of guar gum included 0, 0.05, 1, and 2% with and without β-mannanase. Observed results included a severe reduction in BW and increased FCR with the highest inclusion of guar. The supplementation of β-mannanase improved BW and FCR in all dietary treatment groups during the first week, with BW of β-mannanase supplemented groups being fully restored to control levels after week two except in highest guar gum inclusion (2%) diets. Other reports suggest the ability of β-mannanase to hydrolyze guar gum reduces the digesta viscosity allowing for diets to contain various types of guar meal at higher concentrations than previously thought (Lee et al., 2003). Furthermore, research suggest that β-mannanase increased AMEn, body weight gain, and improved feed conversion in diets containing fibrous ingredients (Lee et al., 2003; Zangiabadi and Torki, 2010).

Additional research has suggested that β-mannanase increases AMEn, body weight gain, and improved feed conversion in diets that are corn: soybean meal based (Jackson et al., 2004). Birds were fed diets with the addition of β-mannanase at

inclusion levels of 0, 50, 80, and 110 MU/ton. Birds fed 80 MU/ton or more gained more weight than birds supplemented at lower levels. During the starter phase and throughout the course of the study, birds supplemented with β-mannanase at 80 MU/ton or greater yielded significantly improved feed conversions than those on lower inclusion rates. These results suggest the administration of β-mannanase in corn and soybean meal based diets improved weight gain and feed conversion ratio.

β-mannanase reduced lesion development following *Eimeria* sp. and *Clostridium perfrigens* challenges (Jackson et al., 2003). In this report two experiments were conducted to investigate the effects of β-mannanase on performance of broilers challenged with necrotic enteritis disease model involving *Eimeria* sp. and *Clostridium perfrigens.* In both experiments, the disease challenge in treatments without β-mannanase inclusion resulted in significant reductions in body weight, feed conversion, and the incidence of coccidial lesions ultimately caused significant mortality. In both experiments, the inclusion of β-mannanase resulted in significant improvements in overall performance and reduced lesion scores. This may be attributed to the fact that β-mannanase supplementation increased relative immune organ weights, increased concentration of serum Igm, and increased T-lymphocyte proliferation (Zou et al., 2006).

ALTERNATIVE FEED INGREDIENTS

Due to increases in ingredient costs, coupled with the rise in ethanol production in the United States, nutritionists have been forced to include less desirable ingredients including DDGS. DDGS is a by-product of ethanol production created in the fermentation process of cereal grains such as corn that can be a good choice for

producers to include in diet during economic hardship (Loar et al., 2012). Because of high nutrient variability and the low availability of some nutrients, it has typically been used in the poultry industry at lower inclusion rates. However, better methods of drying resulting in better nutrient quality in recent years has led to its inclusion in monogastric diets at higher inclusion levels (Świątkiewicz and Koreleski, 2008). DDGS have high levels of NSP; therefore, the use of these alternative ingredients in corn based diets could increase the digesta viscosity making the supplementation of theses exogenous carbohydrases even more important.

DDGS has been included in poultry diets at a level of 5% for many years; however, improved methods in manufacturing of ethanol combined with the drying process of DDGS allows for higher inclusion in broiler diets (Noll et al., 2001). No differences were observed in early body weight with inclusion of 15% DDGS in a starter ration of either high density formulated to contain 22% CP and 3050 kcal ME/kg or low density formulated to contain 20% CP and 3001 kcal ME/kg. In a separate experiment, performance was depressed during the starter diet with inclusion of 12% and higher, but only in the 18% inclusion at the duration of the trial. These results suggest that the use of DDGS from modern ethanol plants are highly acceptable feed ingredients in poultry diets with a recommended maximum level of 6% in the starter rising to between 12-15% in the grower and finisher (Lumpkins et al., 2004).

Similar results were observed by Gracia et al. (2003) who evaluated increasing levels of DDGS in broiler corn and soybean meal based diets for a 42 day trial with three phases of feeding each containing 0, 15, or 30% DDGS. The results agreed with

previous reports suggesting that diets with 15% DDGS formulated on a digestible amino acid basis can be effective, so long as the DDGS are equivalent to the standardized nutrient matrix. However, the inclusion of DDGS at 30% during the starter and grower resulted in depressed performance, but could be implemented in the finisher for a short period of time. These findings, coupled with the continued increase in the production of ethanol in the United States, have led to an abundance of DDGS available for the feed industry and continue to give nutritionists another alternative ingredient to add to poultry rations.

Therefore, the objective of these experiments was to evaluate the effects of a cocktail NSPase enzyme on broiler performance and processing parameters in a corn and soybean meal based diet reduced in energy to determine the additive effects in diets with negligible amounts of NSP. Furthermore, determining the most efficient pelleting temperature to manufacture these diets, and evaluating the impact on enzyme activity as pelleting temperature increases. Finally, to determine if an additive effect of co-administration of β-mannanase and NSPase is observed on broiler growth performance when fed reduced energy corn and soybean meal diets.

CHAPTER III

EFFECTS OF DIETARY INCLUSION OF A COCKTAIL NSPASE AND INCREASING PELLETING TEMPERATURE IN LOW ENERGY DIETS ON BROILER PERFORMANCE AND PROCESSING YIELD

INTRODUCTION

Recently, the poultry industry has been challenged with increases in ingredient costs, which has forced nutritionist to maximize nutrient utilization from these ingredients as well as include less desirable ingredients such as Dried Distiller's Grains with Solubles (DDGS). DDGS is a by-product of ethanol production created in the fermentation process of cereal grains such as corn. DDGS can be a good choice for producers to include in diets during economic hardship (Loar et al., 2012). Due to DDGS nutrient variability and the low availability of some nutrients, it has typically been used in the poultry industry at lower inclusion rates; however, better methods of drying resulted in better nutrient quality in recent years and has led to its inclusion in monogastric diets at higher levels (Świątkiewicz and Koreleski, 2008). The majority of diets used in the United States are corn and soybean meal based and contain varying levels of non-starch polysaccharides (NSP), which are complex high molecular weight carbohydrates found in the structure of plant cell walls (Bedford and Classen, 1993). Corn and soybean meal are considered highly digestible ingredients; however, research shows there is room for improvement of their nutritional value (Zanella et al., 1999). Corn contains inconsequential amounts of soluble NSP and approximately 8% insoluble

NSP, which does not yield digesta viscosity problems, in the form of arabinoxylans representing a potential source of nutrients (Choct, 2006). Soybean meal contains about 3% soluble NSP along with 16% insoluble NSP primarily in the form of galactomannans, which also represent a potential source of nutrients (Irish and Balnave, 1993). Non-starch polysaccharides present a problem as they are not digestible by monogastric animals because they lack the digestive ability of ruminant animals (Meng et al., 2005). Dietary NSPs represent a potential nutrient source if the proper enzyme for hydrolyzation is present. High levels of dietary NSPs increase intestinal viscosity which leads to a reduction in nutrient digestibility, and ultimately a reduction in performance in regards to body weight and feed conversion ratio (Bedford and Morgan, 1995), (Lázaro et al., 2003a). .

One method used to combat grains high in NSPs and to release potential energy from NSP is inclusion of exogenous carbohydrases, which hydrolyze soluble NSP present in cereal grains. Published reports indicate carbohydrases are successful in improving nutrient utilization when used in combination with wheat, barley, rye, or oats; however, data suggests inconsistent results when corn and soybean meal are the primary ingredients (Maisonnier-Grenier et al., 2006). Cocktail carbohydrases (NSPase) hydrolyze NSPs into smaller particles that can be digested by monogastric animals (Coppedge et al., 2012). NSPase inclusion has been reported to improve performance parameters when included in low energy broiler diets (Lázaro et al., 2003b); (Meng et al., 2005); (Coppedge et al., 2012). The observed increased intestinal viscosity of digesta associated with NSPs causes changes in the gut microflora and reduces nutrient

utilization; however, the proper use of non-starch polysaccharide degrading enzymes can combat grains high in NSP (Choct et al., 1999). Additionally, inclusion of exogenous carbohydrases increases AME, ileal digestible energy, and dry matter retention (Meng et al., 2005); (Leslie et al., 2007); (Cowieson and Ravindran, 2008); (Olukosi et al., 2010). The majority of diets contain a variety of NSP, thus the most effective means of maximizing nutrient utilization may be the use of cocktail carbohydrases that vary in specificity. Recent research suggest that inclusion of carbohydrases can increase starch digestibility and improve performance (Meng et al., 2005), while a multi-enzyme complex of carbohydrases is efficient in reducing the P, energy, protein, and amino acid specifications of corn-soybean meal based diets (Francesch and Geraert, 2009).

The use of exogenous NSPase has been extensively researched and the efficacy of NSPase in broiler diets is commonly accepted (Bedford, 2000). The inclusion of a xylanase, protease, or amylase improves FCR, increases AME, and starch digestibility cereal grain diets (Choct et al., 1999), (Kalmendal and Tauson, 2012), and (O'Neill et al., 2012). The inclusion of xylanase significantly increases early body weight gain through 24 days of age, reduces digesta viscosity, increases nutrient retention, and consequently improves performance of broilers (Esmaeilipour et al., 2011). However, these enzymes are heat sensitive and thus their activity may be decreased during the feed manufacturing process. Reports indicate that pelleting temperature decreases the activity of the included enzymes which led to a reduction in broiler growth performance (Silversides and Bedford, 1999). The critical point of deactivation of these enzymes involved has been always been considered linear with the activity of these exogenous

enzymes decreasing as the temperature of pelleting increases (Samarasinghe et al., 2000) and (Spring et al., 1996).

Therefore, the objective of these experiments was to evaluate the effects of a cocktail NSPase enzyme on broiler performance and processing parameters in a diet reduced in energy establishing an inclusion rate of 113g/ton. Furthermore, determining the most efficient pelleting temperature to manufacture these diets, and evaluating the impact on enzyme activity as pelleting temperature increases.

MATERIALS AND METHODS

Experiment 1

To evaluate the effects of a cocktail NSPase in a reduced energy diet on broiler performance and processing parameters, the experimental design was a complete block design with three dietary treatment groups. Each treatment contained eight replicates with 30 straight-run broilers placed per replicate pen for a total of 720 chicks, placed in floor pens for a 44 day assay period. Broilers were reared in floor pens that contained fresh pine shavings as bedding material, provided *ad libitum* access to dietary treatments and water, and provided age appropriate supplemental heat. Animal care was provided in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC). The diets were corn and soybean meal based; containing DDGS at inclusion levels up to 10%. Diets were formulated to be iso-nitrogenous and contained phytase[1] (250 FTU/kg). Dietary program consisted of four phases; a starter (0.68kg/bird), grower (1.45kg/bird), finisher (1.45kg/bird), and withdrawal (remaining

[1] Optiphos® PF, Enzyvia LLC Sheridan, IN

feed required). The starter was fed as a crumble with the remaining diet phases fed as a pellet. All diets were manufactured at a pelleting temperature of 80 C with a 25 second condition time. Dietary feed allocations per replicate pen were corrected for mortality. The three dietary treatments consisted of a positive control (PC) formulated to resemble an industry standard diet , a negative control (NC) diet with a reduction in energy of 132 kcal/kg AME as compared to the PC throughout the experiment, and a NC supplemented with a cocktail NSPase[2] (113g/ton) (Table 3-1). Samples were collected during feed manufacturing for nutrient analysis. Crude protein was determined using (AOAC, 2000) 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). Body weights (BW) and mortality corrected feed conversion ratios (FCR) were determined on days 14, 28, and 44. On day 45, following an 8 hour feed withdrawal, 6 male broilers per replicate pen were processed and deboned for calculation of carcass and breast yield. All carcasses were air chilled for 16 hours prior to debone to avoid influencing yield data based on differences associated with water uptake.

[2] Enspira®, Enzyvia LLC Sheridan, IN

Table 3-1: Dietary formulation, calculated nutrient content, and analysis of nutrients of positive control (PC) and negative control (NC) starter, grower, finisher and withdrawal diets fed to market broilers (Experiment 1).

	Starter		Grower		Finisher		Withdrawal	
Ingredient	PC	NC	PC	NC	PC	NC	PC	NC
Corn	57.12	60.41	58.33	61.59	57.29	60.58	57.34	60.63
Dried Distillers Grains with Solubles	4.00	4.00	10.00	10.00	15.00	15.00	15.00	15.00
Dehulled Soybean Meal (48%)	30.06	29.45	22.50	21.95	19.51	18.93	19.51	18.93
DL-Methionine (99%)	0.24	0.25	0.18	0.18	0.14	0.14	0.14	0.14
L-Lysine- HCl	0.19	0.21	0.26	0.27	0.03	0.04	0.03	0.04
Fat- AV Blend	2.70	0.00	2.89	0.18	3.28	0.58	3.28	0.58
Pork MBM	3.00	1.30	3.81	3.78	2.97	2.93	2.97	2.93
Limestone	1.46	1.47	1.28	1.30	1.10	1.11	1.10	1.11
Mono Calcium Phosphate	0.42	0.42	0.00	0.00	0.00	0.00	0.00	0.00
Sodium Chloride	0.43	0.43	0.39	0.39	0.37	0.37	0.37	0.37
Vitamins[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace Minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coban 90[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.00	0.00
Phytase [4]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Calculated Nutrient Concentration								
Protein	22.17	22.17	20.66	20.66	19.78	19.77	19.78	19.77
Lysine	1.29	1.29	1.18	1.18	0.92	0.92	0.92	0.92
Methionine	0.58	0.59	0.51	0.51	0.47	0.47	0.47	0.47
TSAA	0.92	0.93	0.82	0.82	0.77	0.77	0.77	0.77
Threonine	0.82	0.81	0.74	0.74	0.71	0.71	0.71	0.71
Calcium	1.13	1.13	1.04	1.04	0.88	0.88	0.88	0.88
Available Phosphorus	0.46	0.46	0.41	0.41	0.39	0.39	0.39	0.39
Total Phosphorus	0.57	0.57	0.51	0.52	0.51	0.51	0.51	0.51
Sodium	0.20	0.20	0.20	0.20	0.19	0.19	0.19	0.19
AME (kcal/kg)	3108	2977	3154	3023	3198	3067	3198	3067
Analyzed Nutrient Content								
Crude Protein	21.8	21.2	20.9	20.4	17.0	17.4	16.8	16.9
Crude Fat	5.19	3.90	6.43	3.99	6.45	3.77	5.91	4.34
Total Phosphorus	0.59	0.57	0.53	0.53	0.42	0.45	0.44	0.47
Acid Detergent Fiber	2.44	2.76	2.94	2.75	2.69	2.94	3.01	3.27

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

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

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

[4] Optiphos® PF, Enzyvia LLC Sheridan, IN

Experiment 2

This experiment was designed to compare the performance of broilers and processing yield of the broilers fed reduced energy diets with the inclusion of a cocktail NSPase (113g/ton) pelleted at three different temperatures. The experimental design was a complete block design with five dietary treatment groups. Each treatment included eight replicates with 35 male broilers per replicate for a total of 1,400 broilers, placed in floor pens for a 44 day assay period. Broilers were reared in floor pens with fresh pine shavings as bedding material, provided *ad libitum* access to dietary treatments and water, and provided age appropriate supplemental heat. Animal care was provided in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC). The diets were corn and soybean meal based containing DDGS at inclusion levels of 5% in the starter and increasing to 15% in the finisher and withdrawal diets. Diets were formulated to be iso-nitrogenous and contained phytase (250 FTU/kg). The dietary program consisted of four dietary phases: a starter (day 1-14), grower (day 14-28), finisher (day 28-37), and withdrawal (day 37-44), with the starter fed as a crumble and the other phases fed as a pellet. The five dietary treatments consisted of a PC that was formulated to be similar to an industry standard diet, a NC with a reduction in energy of 132 kcal/kg AME as compared to the PC throughout the experiment, a three remaining treatments that included the NC supplemented with NSPase and pelleted at 80, 85, and 90 C (Table 3-2). All diets had a conditioning time of 30 seconds and PC and NC diets were pelleted at 85 C. Dietary samples were collected during feed manufacturing for nutrient analysis. Crude protein was determined using (AOAC, 2000)

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). Body weights (BW) and mortality corrected feed conversion ratios (FCR) were determined on days 14, 28, 37, and 44 which coincided with dietary change. On day 45, following an 8 hour feed withdrawal, 6 male broilers per replicate pen were processed for whole bird (WOG) and fat pad measurements.

STATISTICAL ANALYSIS

All data for both of the experiments were analyzed via a one-way analysis of variance (ANOVA) using the General Linear Procedure (SPSS V 18.0) with significant differences deemed at $P \leq 0.05$. Means were separated using Duncan's Multiple Range Test.

Table 3-2: Dietary formulation, calculated nutrient content, and analysis of nutrients of positive control (PC) and negative control (NC) starter, grower, finisher and withdrawal diets fed to market broilers (Experiment 2).

	Starter		Grower		Finisher		Withdrawal	
Ingredient	PC	NC	PC	NC	PC	NC	PC	NC
Corn	48.21	51.33	48.54	51.60	52.07	55.19	53.69	56.76
Dried Distillers Grains with Solubles	5.00	5.00	10.00	10.00	15.00	15.00	15.00	15.00
Dehulled Soybean Meal (48%)	38.90	38.42	33.33	32.90	24.96	24.47	23.13	22.69
DL-Methionine (99%)	0.22	0.22	0.15	0.14	0.07	0.06	0.06	0.05
Soy oil	4.32	1.68	4.88	2.25	5.02	2.38	5.44	2.82
Calcium Carbonate	1.65	1.66	1.63	1.64	1.64	1.65	1.56	1.57
Mono Calcium Phosphate	0.87	0.86	0.69	0.68	0.48	0.48	0.40	0.40
Sodium Chloride	0.52	0.52	0.48	0.48	0.46	0.46	0.46	0.46
Vit/Trace Minerals[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Coban 90[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.00	0.00
Phytase [3]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Calculated Nutrient Concentration								
Protein	23.84	23.85	22.50	22.53	20.06	20.06	19.30	19.32
Lysine	1.33	1.32	1.21	1.20	1.01	1.00	0.96	0.95
Methionine	0.58	0.57	0.49	0.49	0.39	0.39	0.37	0.37
TSAA	0.98	0.98	0.88	0.88	0.75	0.75	0.72	0.72
Threonine	0.92	0.92	0.87	0.87	0.77	0.77	0.74	0.74
Calcium	0.90	0.90	0.85	0.85	0.80	0.80	0.75	0.75
Available Phosphorus	0.45	0.45	0.42	0.42	0.38	0.38	0.36	0.36
Total Phosphorus	0.63	0.63	0.59	0.60	0.54	0.54	0.51	0.52
Sodium	0.22	0.22	0.21	0.21	0.21	0.21	0.21	0.21
AME (kcal/kg)	3058	2926	3102	2970	3146	3014	3190	3058
Analyzed Nutrient Content								
Crude Protein	25.5	23.4	24.4	22.4	22.3	23.9	20.8	20.4
Crude Fat	5.55	4.12	7.90	5.69	8.18	6.79	9.83	6.05
Total Phosphorus	0.76	0.72	0.56	0.62	0.49	0.61	0.55	0.56
Acid Detergent Fiber	2.16	4.11	1.70	3.60	1.80	3.50	1.80	3.30

[1] Vitamin and trace mineral premix added at this rate yields 7,715 IU vitamin A, 2,756 IU vitamin D_3, 17 IU vitamin E, 0.01102mg B_{12}, 6.614 mg riboflavin, 27.56 mg niacin, 6.614 mg d-pantothenic acid, 385.81 mg choline, 0.8267 mg menadion 0.6889 mg folic acid, 1.378 mg pyroxidine, 1.102 mg thiamine, 0.0331 mg biotin per kg diet. Trace mineral premix added at this rate yields 100 mg manganese, 100 mg zinc, 50 mg iron, 11.25 mg copper, 1.50 mg iodine, 0.15 mg selenium, per kg diet

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

[3]Optiphos® PF, Enzyvia LLC Sheridan, IN

RESULTS

Experiment 1

An energy response was observed as early as d 14 of age, with the NC fed broilers having a lower observed body weights (BW) ($P<0.05$) as compared to the PC fed broilers (Table 3-3). The inclusion of the NSPase in the NC diet increased BW as compared to the NC diet and to a level similar to the PC diet. Average male BW followed a similar trend on d 28 and 42 with the NC fed broilers having the lowest average observed BW which was lower ($P<0.05$) than the PC fed broilers. Inclusion of the NSPase in the NC diet increased ($P<0.05$) average male BW and reach a similar level of the PC fed broilers. No differences were observed in cumulative mortality throughout the experiment.

Table 3-3: Body weights (BW) determined on days 14, 28, and 44 of broilers fed a positive control (PC), negative control (NC), and negative control supplemented with a cocktail NSPase[1] (Experiment 1).

TRT	Day 14 BW (g)	Day 28 BW (kg)	Day 44 BW (kg)	Mortality
Positive Control (PC)	$444.4^{a} \pm 8.2$	$1.35^{a} \pm 0.02$	$2.80^{a} \pm 0.04$	5.8 ± 2.2
Negative Control (NC)	$409.5^{b} \pm 9.1$	$1.28^{b} \pm 0.02$	$2.71^{b} \pm 0.04$	3.8 ± 0.9
Negative Control + NSPase	$423.2^{ab} \pm 6.9$	$1.36^{a} \pm 0.02$	$2.84^{a} \pm 0.03$	4.1 ± 1.3

[a-c] Means in columns differ significantly at $P<0.05$.

[1] Enspira®, Enzyvia LLC. Sheridan IN. Inclusion rate of 113.5g/ton.

Mortality corrected feed conversion ratio (FCR) was also impacted with the reduction of energy during the experiment (Table 3-4). Through d 14, FCR was lower ($P<0.05$) in the NC diet compared to the other two treatment groups. Inclusion of the NSPase enzyme in the NC diet resulted in a lower ($P<0.05$) FCR during the second 2 wk period (d 15 to 28) as compared to NC fed broilers. The PC fed broilers were intermediate of the other two treatment groups during this period. No differences were observed in FCR for the period of d 29 to 44 or cumulative through 28 d of age (Table 3-4). The reduction of dietary energy negatively influenced cumulative FCR for the duration of the experiment with NC fed broilers having a 5 point increased in cumulative FCR as compared the PC fed broilers (Table 3-4). The inclusion of NSPase in the NC diet reduced ($P<0.05$) FCR as compared to the NC and was the lowest observed FCR at the conclusion of the trial (Table 3-4).

Table 3-4: Mortality corrected feed conversion ratio (FCR) of broiler chickens fed a positive control (PC), negative control (NC), and negative control supplemented with a cocktail NSPase[1] (Experiment 1).

TRT	FCR Day 1-14	FCR Day 15-28	FCR Day 28-44	FCR Day 1-28	FCR Day 1-44
Positive Control (PC)	$1.34^{a} \pm 0.03$	$1.71^{ab} \pm 0.05$	2.03 ± 0.04	1.64 ± 0.03	$1.84^{ab} \pm 0.03$
Negative Control (NC)	$1.26^{b} \pm 0.02$	$1.73^{a} \pm 0.04$	2.12 ± 0.06	1.62 ± 0.07	$1.89^{a} \pm 0.05$
Negative Control + NSPase	$1.31^{a} \pm 0.03$	$1.63^{b} \pm 0.02$	2.04 ± 0.03	1.58 ± 0.02	$1.80^{b} \pm 0.03$

[a-b] Means in columns differ significantly at $P<0.05$.

[1] Enspira®, Enzyvia LLC. Sheridan IN. Inclusion rate of 113.5g/ton.

Differences were not observed in carcass and breast weight or yield associated with the reduction in dietary energy between the PC and NC fed broilers (Table 3-5 and Table 3-6). However, inclusion of the NSPase in the NC diet increased ($P<0.05$) carcass weight as compared to the NC broilers (Table 3-5). The reduction in energy reduced ($P<0.05$) fat pad weight (Table 3-5) and yield (Table 3-6) of the NC fed broilers as compared to the PC. The inclusion of NSPase in the NC diet increased broiler fat pad weight (Table 3-5) and yield (Table 3-6) to a level similar to the PC diet. No significant differences were observed between any dietary treatments with regard to WOG, breast, or tender yield (Table 3-6).

Table 3-5: Processing parameters weight evaluated of 6 male broilers per replicate on day 45 following an 8 hour feed withdrawal fed a positive control (PC), negative control (NC), or negative control supplemented with NSPase[1] (Experiment 1).

TRT	Live WT	WOG WT	Breast WT	Tender WT	Fat Pad WT
Positive control (PC)	$3033.3^{ab} \pm 21.4$	$2159.2^{ab} \pm 16.7$	512.2 ± 5.84	115.0 ± 1.35	$35.8^{a} \pm 0.92$
Negative control (NC)	$2937.9^{b} \pm 21.4$	$2122.4^{b} \pm 16.7$	503.5 ± 5.84	114.1 ± 1.35	$30.2^{b} \pm 0.92$
Negative control + NSPase	$3073.3^{a} \pm 21.4$	$2201.0^{a} \pm 16.7$	523.8 ± 5.84	118.5 ± 1.35	$34.5^{ab} \pm 0.92$

[a-b] Means in columns differ significantly at $P<0.05$.

[1] Enspira®, Enzyvia LLC. Sheridan IN. Inclusion rate of 113.5g/ton.

Table 3-6: Processing parameters yield evaluated of 6 male broilers per replicate on day 45 following an 8 hour feed withdrawal fed a positive control (PC), negative control (NC), or negative control supplemented with NSPase[1] (Experiment 1).

TRT	WOG Yield (%)	Breast Yield (%)	Tender Yield (%)	Fat Pad Yield (%)
Positive control (PC)	72.0 ± 0.003	23.7 ± 0.002	5.3 ± 0.0005	1.7^{a} ± 0.0004
Negative control (NC)	72.3 ± 0.003	23.6 ± 0.002	5.4 ± 0.0005	1.4^{b} ± 0.0004
Negative control + NSPase	71.4 ± 0.003	23.7 ± 0.002	5.4± 0.0005	1.6^{ab} ± 0.0004

[a-b] Means in colums differ significantly at P<0.05.

[1] Enspira®, Enzyvia LLC. Sheridan IN. Inclusion rate of 113.5g/ton.

Experiment 2

Body weight was different due to dietary energy as early as 14 d of age. The PC fed broilers had the highest observed BW and was increased (P<0.05) compared to the NC fed broilers (Table 3-7). The inclusion of NSPase to the NC diet pelleted at all temperatures increased average male BW at d 14 to a level similar to that of the PC diet (Table 3-7). At the conclusion of the grower phase on day 28, the inclusion of the NSPase to the NC diet increased (P<0.05) BW at all pelleting temperatures as compared to the NC fed broilers. Broilers fed NSPase pelleted at 80 C had a higher average BW compared to the PC fed broilers. The effect of dietary energy did not negatively influence average male BW on d 37 with the PC and NC fed broilers having similar body weights. Inclusion of the NSPase to the NC diet increased (P<0.05) body weight compared to the NC fed broilers. The NSPase diet pelleted at 80 C resulted in an increase in average BW on d 37 as compared to NSPase inclusion diets pelleted at 85

and 90 C. At the conclusion of the trial on d 44, the reduction in dietary energy did not negatively influence BW with the PC and NC resulting in similar BW (Table 3-7). The inclusion of NSPase, pelleted at 80 C, increased ($P<0.05$) BW as compared to the NC diet and resulted in the highest observed body weight at the conclusion of the trial (Table 3-7). There was no significant difference regarding mortality throughout the experiment.

Table 3-7: Body weight (BW) and mortality of male broilers fed diets reduced in energy with the inclusion of a cocktail NSPase[1] pelleted at 80, 85, and 90 C(Experiment 2).

TRT	NSPase (g/ton)	Pellet Temp. (C)	Day 14 BW (g)	Day 28 (kg)	Day 37 (kg)	Day 44 (kg)	Morality (%)
Positive control (PC)		85	$511.6^{a} \pm 5.8$	$1.62^{bc} \pm 0.02$	$2.40^{c} \pm 0.02$	$2.99^{b} \pm 0.03$	5.4 ± 1.9
Negative control (NC)		85	$494.4^{b} \pm 4.7$	$1.58^{c} \pm 0.02$	$2.41^{c} \pm 0.01$	$3.00^{b} \pm 0.02$	3.2 ± 1.0
NC + NSPase	113	80	$508.2^{ab} \pm 3.3$	$1.68^{a} \pm 0.02$	$2.53^{a} \pm 0.02$	$3.11^{a} \pm 0.02$	4.3 ± 0.7
NC + NSPase	113	85	$502.0^{ab} \pm 3.9$	$1.65^{ab} \pm 0.01$	$2.46^{b} \pm 0.01$	$3.03^{b} \pm 0.02$	4.6 ± 1.2
NC + NSPase	113	90	$498.1^{ab} \pm 4.9$	$1.66^{ab} \pm 0.02$	$2.48^{b} \pm 0.01$	$3.04^{ab} \pm 0.02$	4.3 ± 0.9

[a-c] Means in columns differ significantly at $P<0.05$.

[1] Enspira®, Enzyvia LLC. Sheridan IN. Inclusion rate of 113.5g/ton.

At the conclusion of the starter phase on d 14, the reduction in dietary energy in the NC diet negatively influenced FCR as compared to the PC diet. The NC diet resulted in the highest observed FCR (Table 3-8). The inclusion of NSPase pelleted at 80, 85, and 90 C in the NC diet reduced ($P<0.05$) FCR as compared to the NC diet

(Table 3-8). On day 28 at the conclusion of the grower phase, the reduction in dietary energy in the NC diet again negatively influenced FCR as compared to the PC diet. The NC diet resulted in highest observed FCR. The inclusion of NSPase pelleted at 80, 85, and 90 C in the NC diet reduced ($P<0.05$) FCR as compared to the NC diet, and also resulted in FCR reductions ($P<0.05$) compared to the PC diet (Table 3-8). Dietary energy did not influence finisher phase FCR as the PC and NC fed broilers had similar FCR. However, NSPase inclusion pelleted at 80 C yielded a lower FCR as compared to NSPase inclusion pelleted at 85 C (Table 3-8). There were no differences in regard to withdrawal FCR between any of the treatment groups (Table 3-8).

Table 3-8: Mortality corrected feed conversion ratio (FCR) of male broilers fed diets of a positive control (PC), negative control (NC) with reduction in energy of 132 kcal/kg, and a NC + a cocktail NSPase[1] pelleted at 80, 85, or 90 C at the conclusion of each dietary phase (Experiment 2).

TRT	NSPase (g/ton)	Pellet Temp. (C)	Starter	Grower	Finisher	Withdrawal[1]
Positive control		85	$1.135^{d} \pm 0.010$	$1.543^{b} \pm 0.011$	$2.022^{ab} \pm 0.020$	2.191 ± 0.037
Negative control		85	$1.244^{a} \pm 0.005$	$1.580^{a} \pm 0.009$	$1.950^{b} \pm 0.034$	2.188 ± 0.037
NC + NSPase	113	80	$1.186^{b} \pm 0.005$	$1.480^{c} \pm 0.017$	$1.935^{b} \pm 0.016$	2.263 ± 0.038
NC + NSPase	113	85	$1.179^{bc} \pm 0.004$	$1.485^{c} \pm 0.013$	$2.058^{a} \pm 0.006$	2.231 ± 0.044
NC + NSPase	113	90	$1.161^{c} \pm 0.009$	$1.474^{c} \pm 0.014$	$1.999^{ab} \pm 0.022$	2.326 ± 0.037

[a-d] Means in columns differ significantly at $P<0.05$.

[1] Enspira®, Enzyvia LLC. Sheridan IN. Inclusion rate of 113.5g/ton.

Cumulative FCR through d 28 was negatively influenced with the reduction in dietary energy as the NC fed broilers resulted in a higher ($P<0.05$) FCR as compared to the PC (Table 3-9). Inclusion of the NSPase pelleted at all evaluated temperatures reduced ($P<0.05$) observed FCR compared to the NC fed broilers (Table 3-9). Additionally, NSPase inclusion pelleted at 90 C resulted in a reduced ($P<0.05$) FCR compared to the PC fed broilers (Table 3-9). Similar results were observed in cumulative FCR at d 37 of age following the finisher diet with the NC fed broilers yielding the highest observed FCR which was increased ($P<0.05$) compared to the PC fed broilers (Table 3-9). The inclusion of NSPase pelleted at 85 C in the NC diet reduced ($P<0.05$) FCR as compared to the NC fed broilers to levels similar to PC fed broilers (Table 3-9). Additionally, inclusion of NSPase pelleted at 80 and 90 C in the NC diet further reduced ($P<0.05$) observed FCR to levels lower than PC fed broilers (Table 3-9). The reduction in dietary energy negatively affected FCR at the conclusion of the study with the NC resulting in the highest ($P<0.05$) observed FCR Inclusion of the NSPase in the NC diet reduced ($P<0.05$) FCR at all evaluated pelleting temperatures with the 80 C pelleting temperature resulting in an observed FCR lower ($P<0.05$) than the PC fed broilers (Table 3-9).

Table 3-9: Mortality corrected cumulative feed conversion ratio (FCR) of male broilers fed diets of a positive control (PC), negative control (NC) with reduction in energy of 132 kcal/kg, and a NC + a cocktail NSPase[1] pelleted at 80, 85, or 90 C(Experiment 2).

TRT	NSPase (g/ton)	Pellet Temp. (C)	Day 1-28	Day 1-37	Day 1-44
Positive control		85	$1.416^{b} \pm 0.009$	$1.613^{b} \pm 0.005$	$1.720^{b} \pm 0.004$
Negative control		85	$1.480^{a} \pm 0.005$	$1.640^{a} \pm 0.012$	$1.749^{a} \pm 0.010$
NC + NSPase	113	80	$1.395^{bc} \pm 0.013$	$1.580^{c} \pm 0.011$	$1.704^{c} \pm 0.011$
NC + NSPase	113	85	$1.395^{bc} \pm 0.008$	$1.608^{b} \pm 0.009$	$1.728^{b} \pm 0.010$
NC + NSPase	113	90	$1.384^{c} \pm 0.010$	$1.588^{c} \pm 0.008$	$1.724^{b} \pm 0.006$

[a-c] Means in columns differ significantly at $P<0.05$.

[1] Enspira®, Enzyvia LLC. Sheridan IN. Inclusion rate of 113.5g/ton.

At the conclusion of experiment 2, processing parameters were evaluated including carcass and fat pad weight and yield. Carcass without giblets (WOG) weight was increased ($P<0.05$) with NSPase inclusion pelleted at 80 C as compared to the NC and PC diet (Table 3-10). Fat pad weight with the inclusion of NSPase pelleted at 85 C in the NC diet resulted in lowest observed fat pad weight, which was lower than both the PC and NC diet. WOG yield followed WOG weights with no significant differences observed between the PC and NC diets (Table 3-10); however, inclusion of NSPase pelleted at all evaluated temperatures increased ($P<0.05$) WOG yield compared to both the PC and NC diet (Table 3-10). Fat pad yield was reduced in the NSPase included treatments pelleted at 85 and 90 C as compared to the PC diet (Table 3-10).

Table 3-10: Processing parameters evaluated including live weight, WOG, fat pad weight, WOG yield, and fat pad yield of male broilers fed diets of a positive control (PC), negative control (NC) with reduction in energy of 132 kcal/kg, and a NC + a cocktail NSPase[1] pelleted at 80, 85, or 90 C(Experiment 2).

TRT	NSPase (g/ton)	Pellet Temp (C)	Live WT (kg)	WOG (kg)	Fat Pad (g)	WOG Yield	Fat Pad Yield
Positive control		85	3.175	2.332[c]	24.0[a]	73.47[c]	1.03[a]
Negative control		85	3.191	2.364[bc]	24.0[a]	74.11[bc]	1.02[ab]
NC + NSPase	113	80	3.255	2.447[a]	22.3[a]	75.21[a]	0.91[ab]
NC + NSPase	113	85	3.182	2.374[bc]	17.7[b]	74.62[ab]	0.73[c]
NC + NSPase	113	90	3.212	2.390[b]	22.1[a]	74.41[ab]	0.90[b]
Pooled SEM			0.009	0.007	0.4	0.10	0.01

[a-c] Means in columns differ significantly at P<0.05.

[1] Enspira®, Enzyvia LLC. Sheridan IN. Inclusion rate of 113.5g/ton

DISCUSSION

These data confirm that reductions in dietary energy negatively impact broiler performance including observed reductions in average BW and increases in FCR. In both experiments, BW was negatively influenced with a reduction in dietary energy as the PC broilers exhibited increased BW as compared to the NC fed broilers throughout the entire grow-out period in experiment 1

Conversely, in experiment 2 BW was only reduced through 14 d of age. Other reports have shown linear reductions in BW associated with a reduction in dietary energy particularly in the first four weeks of life. In experiment 2 reduction in early

body weights were 494.4 g at day 14 and 1.58 kg average on day 28, similar to reductions observed by (Woyengo et al., 2010) with observed reduction in NC birds at 593.6 g thru 21 days, and O'Neill et al. (2012) who observed a reduction at 636 and 644 for the two NC diets thru 21 days. The reduction in dietary energy in the NC diet also negatively influenced FCR as compared to the PC diet throughout both experiments, significantly increasing cumulative FCR at the conclusion of both experiments. The negative influence of diets reduced in energy on FCR have previously been reported Cowieson and Adeola (2005) and Leeson et al. (1996),which is most likely attributed to the decrease in dietary energy which led to an increase in feed intake. The reduction in energy in the NC diet similarly influenced processing parameters with reductions in live weight, WOG, fat pad weight, and yield as compared to the PC diet, which was also reported by (Coppedge et al., 2012) .

The inclusion of NSPase in broiler diets has been researched extensively with an increase in the productive value of diet made up of corn and soybean believed to be attainable by adding these enzymes suggesting that the solubilization of cell wall NSP leads to improved overall energy utilization due to hydrolysis of certain types of carbohydrate and protein linkages leading to improved amino acid availability (Slominski, 2011). The inclusion of NSPase in the NC diet significantly increased average BW throughout experiment 1 as compared to the NC diet resulting in broiler body weights similar to that of the PC fed broilers at each day of evaluation on d 14, 28, and 44. (Olukosi et al., 2007) suggested the inclusion of a cocktail enzyme in a diet with a 115 kcal/kg reduction in energy, a 1.1 g/kg reduction in calcium, a 1.0 g/kg reduction

in phosphorus, and a 1.1 g/kg reduction in nonphytate phosphorus in a corn and soybean meal based diet increased body weight gain as compared to the NC diet, as was observed in experiment 1. The inclusion of NSPase in experiment 2 similarly resulted in an increase in BW as compared to the NC diet through 37 d of age at all evaluated pelleting temperatures. This increase in BW continued through the duration of the experiment in the NSPase treatment pelleted at the lowest evaluated temperature (80 C) as compared to the NC diet. Similar increased BW with the inclusion of a xylanase, cellulase or a combination has been reported by (Meng et al., 2005) in a wheat diet suggesting these carbohydrase preparations can target NSPs; however, the mechanisms of soybean meal and corn have not be as clear until more recent research. O'Neill et al. (2012) suggested the inclusion of xylanase in a reduced energy soybean meal and corn diet increased BW as compared to the NC diet through 42 days, similar to the observation in experiment 2.

The inclusion of NSPase in the reduced energy diet significantly reduced FCR as compared to the NC diet in both experiments through the duration of the trial. Cumulatively, FCR was reduced significantly as compared to the NC diet with the inclusion of NSPase in both experiments to a level similar to the PC diet. In experiment 2, NSPase inclusion at the lowest evaluated pelleting temperature resulted in a cumulative FCR lower than the PC diet. The reduction in FCR of diets supplemented with carbohydrases, and in viscous cereal diets such as wheat, has been researched extensively and accepted (Annison, 1993; Choct et al., 1999; Kalmendal and Tauson, 2012; Meng et al., 2005). Historically, improvements in performance of broilers with the inclusion of carbohydrase in corn and soybean meal based diets have been

inconsistent (Gracia et al., 2003; Meng and Slominski, 2005). Recent reports have indicated that NSPase inclusion in soybean meal and corn based diet can achieved reductions in FCR (Coppedge et al., 2012; Cowieson and Adeola, 2005; O'Neill et al., 2012).

The inclusion of NSPase in the NC diet significantly increased multiple processing parameters through both experiments including an increase in carcass weight and carcass yield as compared to the NC diet which resulted in yield similar to, or exceeding the PC diet. Fat pad weight and yield was increased with NSPase inclusion; however, it did not exceed that of the PC diet even in instances of increased BW (experiment 2) perhaps indicating that nutrient release associated with NSPase inclusion was utilized for tissue growth and not fat deposition. Similar results of increased yield and intermediate fat pad yield has previously been reported with the use of xylanase in a reduced energy diet by (Coppedge et al., 2012).

At the conclusion of the experiment in regard to BW and FCR, the inclusion of NSPase in the NC pelleted at 80 C yielded the best results of any treatment group, with the addition yielding the highest observed body weight and lowest observed cumulative FCR. While the addition of NSPase in the NC pelleted at 85 C and 90 C did not increase BW to levels as high as the inclusion of NSPase pelleted at 80 C, the increase was equivalent to the PC diet. Similarly, the inclusion of NSPase in the NC pelleted at 85 C and 90 C decreased cumulative FCR as compared to NC. These results suggest the optimum temperature to pellet NSPase at is 80 C to optimize enzyme activity, which is similar to the results reported by Silversides and Bedford (1999) who observed the most

favorable results in regard to FCR and BW with pelleting temperatures between 80 and 85 C. Likewise, these results suggest a linear decline in enzyme activity as pelleting temperature increases; however, the additive benefit is not completely lost in these higher temperature pelleted diets. This supports previous research which showed a decrease in enzyme activity when pelleting temperature approached 90 C, but not a complete loss in enzyme activity or additive benefit (Samarasinghe et al., 2000). The results from the processing parameters evaluated followed that of the performance data with the best results observed when the diet was pelleted at 80 C.

These data confirm the inclusion of a cocktail NSPase in reduced energy diets can improve growth performance of broilers. Furthermore, while increasing pelleting temperature did reduce xylanase activity, it did not eliminate the positive effects of inclusion; although, it did indicate that lower pelleting temperature can maximize NSPase effects.

CHAPTER IV

EFFECTS OF DIETARY INCLUSION OF A COCKTAIL NSPASE AND β-MANNANASE SEPARATELY AND IN COMBINATION IN LOW ENERGY DIETS ON BROILER PERFORMANCE AND PROCESSING PARAMETERS

INTRODUCTION

The majority of broiler diets used in the US poultry industry are corn and soybean meal based. Continued increases in ingredient cost have forced nutritionists to maximize nutrient utilization in poultry diets and include alternative ingredients such as Dried Distillers Grains' with Solubles (DDGS). Between the years of 2006 and 2008, it is estimated that feed costs in the poultry industry increased as much as $9.36 billion dollars in the United States alone (Donohue and Cunningham, 2009). The use of DDGS, until recently, was believed to only be acceptable in the diet at lower inclusion rates not exceeding 5% or 6%; however, new developmental methods of manufacturing ethanol has led to better development of its byproduct DDGS, which has led to acceptable inclusion rates approaching 15% in the grower and finisher phases (Świątkiewicz and Koreleski, 2008). The cereal grains used in poultry diets contain non-starch polysaccharides (NSP) which are major components of dietary fiber comprised of cellulose and non-cellulose polysaccharides. Non-starch polysaccharides are complex high molecular weight carbohydrates found in the structure of plant cell walls (Bedford and Classen, 1993). These NSPs contain anti-nutritive properties because chickens lack

the digestive capacity of ruminant animals and this results in a reduction in nutrient utilization (Meng et al., 2005), although NSP do represent a potential source of nutrients. Research has shown that diets high in NSPs can lead to increase intestinal viscosity (Bedford and Morgan, 1995), which leads to a reduction in nutrient digestibility (Lázaro et al., 2003b), and ultimately a reduction in performance in regards to body weight and feed conversion ratio (Annison, 1993; Choct et al., 1999).

A potential method for combating the anti-nutritive properties of NSPs, such as increases in intestinal viscosity, and improving nutrient utilization of NSPs, is the inclusion of exogenous enzymes such as a cocktail carbohydrase. Non-Starch degrading enzymes (NSPase) hydrolyze bonds that are indigestible by monogastric animals allowing for increases in digestibility. (Bedford and Classen, 1993; Coppedge et al., 2012; Mathlouthi et al., 2002). Increased intestinal viscosity causes alterations in the gut microflora and can reduce nutrient utilization; however, the proper use of non-starch polysaccharide degrading enzymes can combat these negative effects (Choct et al., 1999). NSPase inclusion in broiler diets have been reported to improve growth performance (Coppedge et al., 2012; Lázaro et al., 2003b; Meng et al., 2005). These improvements in growth performance can be attributed to increases AME, ileal digestible energy, and dry matter retention (Cowieson and Ravindran, 2008; Leslie et al., 2007; Meng et al., 2005; Olukosi et al., 2010) associated with exogenous enzyme inclusion (Cowieson and Ravindran, 2008; Leslie et al., 2007; Meng et al., 2005; Olukosi et al., 2010). The majority of diets contain a variety of NSP, thus the most effective means of enzyme use may be a cocktail carbohydrase approach that vary in

specificity. Research has shown that cocktail carbohydrases preparations can increase starch digestibility, broiler performance, and improve feed conversions (Meng et al., 2005).

β-mannans such as glucomannans and galactomannans in the plant cell wall of soybean meal, guar, and sesame meal could potentially reduce nutrient bio-availability (Zou et al., 2006) β-mannanase supplementation which hydrolyzes β-mannans is one way to combat the negative effects of β-mannans. β-mannanase inclusion improves feed conversion ratio and reduces water to feed ratio, as well as reduces dry fecal output of broilers by degrading β-mannans (Daskiran et al., 2004). β-mannanase inclusion in broiler diets also increases AMEn (Zangiabadi and Torki, 2010), body weight gain, and improves feed conversion (Jackson et al., 2004; Lee et al., 2003; Zangiabadi and Torki, 2010). β-mannanase inclusion reduces lesion development following *Eimeria* sp. and *Clostridium perfrigens* challenges (Jackson et al., 2003) indicating beneficial immunological impacts. Additionally, β-mannanase supplementation increases relative immune organ weights, increases concentration of serum Igm, and increases T-lymphocyte proliferation (Zou et al., 2006). Therefore, the objective of these two experiments were to determine if an additive effect of co-administration of β-mannanase and NSPase would be observed on broiler growth performance when fed reduced energy diets. The two experiments were identical in experimental treatments; however, they varied with regards to ingredient profile, duration of trial, NSPase used, time of year, and bird strain to determine if observed outcomes were similar.

MATERIALS AND METHODS

Experiment 3

To evaluate the effects of a cocktail NSPase and β-mannanase in low energy diets in combination and separately on broiler performance and processing parameters, the experimental design was a randomized complete block design containing five dietary treatments with eight replicate pens per treatment group each containing 28 Cobb 500 straight run broilers (total of 1120 chicks). Chicks were wing banded and allotted to floor pens based on day old chick weight for a 48- day assay period. Broilers were reared in floor pens which contained fresh pine shavings as bedding material, provided *ad libitum* access to dietary treatments and water, and provided age appropriate supplemental heat. Animal care was in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC). The diets were corn and soybean meal based and contained DDGS up to 10% and meat and bone meal up to 5% (Table 4-1). Diets were formulated to be iso-nitrogenous and contained phytase[3] (250 FTU/kg). The dietary phases consisted of a starter (1.5 lbs.), grower (3.2 lbs.), finisher (3.2 lbs.), and withdrawal (remaining feed required), with diets being fed as a crumble during the starter phase and pellet throughout the grower, finisher, and withdrawal phases. The five treatment groups consisted of a positive control (PC) diet which was formulated to an industry standard diet, negative control (NC) diet that had a reduction in energy of 132 kcal/kg compared to the PC diet. Three treatment groups consisted of

[3] Optiphos® L, Enzyvia LLC Sheridan, IN

Table 4-1: Dietary formulation, calculated nutrient content, and analysis of nutrients of positive control (PC) and negative control (NC) starter, grower, finisher and withdrawal diets fed to market broilers(Experiment 3).

	Starter		Grower		Finisher		Withdrawal	
Ingredient	PC	NC	PC	NC	PC	NC	PC	NC
Corn	58.08	61.33	65.94	69.92	65.47	69.42	65.04	69.23
Dried Distillers Grains with Solubles	6.08	5.14	2.33	1.15	10.00	8.91	10.0	10.0
Dehulled Soybean Meal (48%)	26.34	26.27	21.99	21.91	16.22	16.03	15.93	14.38
DL-Methionine (99%)	0.27	0.26	0.24	0.24	0.19	0.19	0.19	0.20
L-Lysine- HCl	0.28	0.28	0.33	0.33	0.29	0.29	0.28	0.32
Fat- A/V Blend	2.50	--	2.85	0.15	3.45	0.75	4.20	1.45
Pork MBM	4.22	4.18	4.38	4.33	2.41	2.44	2.43	2.43
Limestone	1.17	1.30	1.00	1.00	1.11	1.10	1.10	1.12
Mono Calcium Phosphate	0.30	0.31	0.11	0.13	--	--	--	--
Sodium Chloride	0.41	0.01	0.29	0.29	0.21	0.21	0.20	0.16
Vitamins[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace Minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coban 90[3]	0.05	0.05	0.05	0.05	0.05	0.05	--	--
L-Threonine 98	0.02	0.02	--	0.01	--	0.01	0.01	0.03
Sodium Bicarb	--	0.56	0.19	0.20	0.30	0.31	0.31	0.37
Phytase[4]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Calculated Nutrient Concentration								
Protein	22.80	22.80	20.50	20.50	18.19	18.20	18.01	17.67
Lysine	1.29	1.29	1.20	1.20	1.02	1.02	1.00	1.00
Methionine	0.60	0.59	0.54	0.53	0.48	0.48	0.48	0.49
TSAA	0.92	0.92	0.83	0.83	0.75	0.75	0.75	0.75
Threonine	0.80	0.80	0.70	0.70	0.63	0.63	0.63	0.63
Calcium	0.95	1.00	0.85	0.85	0.75	0.75	0.75	0.75
Available Phosphorus	0.35	0.35	0.30	0.30	0.25	0.25	0.25	0.25
Total Phosphorus	0.62	0.62	0.55	0.55	0.49	0.49	0.49	0.49
Sodium	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Crude Fat	5.24	2.77	5.48	2.80	6.41	3.74	7.14	4.51
AME (kcal/kg)	3108	2977	3154	3023	3198	3067	3240	3108
Analyzed Nutrient Content								
Crude Protein	22.5	23.7	18.3	20.2	18.0	18.2	17.6	18.1
Crude Fat	4.54	3.81	5.12	3.66	6.41	3.99	6.99	5.17
Total Phosphorus	0.65	0.60	0.54	0.59	0.51	0.51	0.48	0.50
Acid Detergent Fiber	3.37	3.56	2.35	2.25	2.84	3.35	3.05	2.61

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

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

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

[4] Optiphos® L, Enzyvia LLC Sheridan,

the NC + β-D-mannanase[4], NC + cocktail NSPase[5] inclusion, and the NC + β-D-mannanase/cocktail NSPase inclusion. NSPase was added pre-pelleting into the mixer and β-mannanase was spray applied post pelleting. Samples were collected during feed manufacturing for nutrient analysis. Crude protein was determined using (AOAC, 2000) 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). Body weights and feed conversion ratios (FCR) were determined on d 14, 28, 42, and 47. On day 48, following an eight hour feed withdrawal period, five male and five female broilers from each pen were processed and deboned for carcass and breast yields (40 male and 40 female per treatment/400 total or 35% of total placement). All carcasses were air chilled for 16 hours prior to debone to avoid influencing yield data based on differences in carcass water uptake

Experiment 4

The experimental treatment groups were identical to the previous experiment; however, each of the eight replicates contained 40 sexed Ross 708 chicks per replicate placed at a 1:1 male to female ratio. Chicks were wing banded and allotted to dietary treatment based on chick weight (total of 1,600 chicks). Broilers were reared in floor pens which contained fresh pine shavings as bedding material, provided *ad libitum* access to dietary treatments and water, and provided age appropriate supplemental heat.

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[4] Hemicell® L, ChemGen Corp. Gaithersburg, MD
[5] Enspira®, Enzyvia LLC Sheridan, IN

Animal care was in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC). All diets were corn and soybean meal based containing DDGS at 2.5% and MBM at 5% and formulated to be iso-nitrogenous (Table 4- 2). All diets contained phytase (250 FTU/kg). The five dietary treatments were similar as experiment 1 with a positive control (PC) diet, a negative control (NC) diet with a reduction in energy of 132 kcal/kg compared to the PC diet, NC + β-mannanase, NC + NSPase, and NC + β-mannanase/cocktail NSPase. Three dietary phases included starter (d1-14), grower (d 14-27), and finisher (d27-41) were used, and diets were fed as a crumble (starter) and pelleted (grower and finisher) form. Antibiotic growth promoters (AGP) were used in the form of Bacitracin Methylene Disalicylate (BMD) during the starter and grower phases at a concentration of 50 g/ton, and Virginiamycin during the finisher phase at a concentration of 20 g/ton. Samples were collected during feed manufacturing for nutrient analysis. Crude protein was determined using (AOAC, 2000) 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

Table 4-2: Dietary formulation, calculated nutrient content, and analysis of nutrients of positive control (PC) and negative control (NC) starter, grower, finisher and withdrawal diets fed to market broilers(Experiment 4).

	Starter		Grower		Finisher	
Ingredient	PC	NC	PC	NC	PC	NC
Corn	60.01	36.16	62.25	65.55	67.06	70.35
Dried DistillersGrains with Solubles	2.50	2.50	2.50	2.50	2.50	2.50
Dehulled Soybean Meal (48%)	27.09	26.56	25.16	24.54	20.60	19.99
Pork MBM	5.00	5.00	5.00	5.00	5.00	5.00
DL-Methionine (99%)	0.27	0.27	0.19	0.19	0.17	0.17
Fat Blended A/V Blend	2.65	--	3.00	0.30	3.15	0.45
L-Threonine 98	0.01	--	--	--	--	--
Lysine HCL	0.25	0.26	0.17	0.19	--	0.013
BMD 50[1]	0.05	0.05	0.05	0.05	--	--
3-Nitro-20[2]	0.025	0.025	0.025	0.025	--	--
Statfac 20[3]	--	--	--	--	0.05	0.05
Sodium Bicarb	--	0.16	--	--	0.09	0.11
Mono Calcium Phosphate	0.21	0.20	--	--	--	--
Sodium Chloride	0.42	0.31	0.42	0.42	0.41	0.40
Vitamins[4]	0.25	0.25	0.25	0.25	0.25	0.25
Trace Minerals[5]	0.05	0.05	0.05	0.05	0.05	0.05
Coban 90[6]	0.05	0.05	0.05	0.05	--	--
Phytase [7]	0.013	0.013	0.013	0.013	0.013	0.013
Calculated Nutrient Concentration						
Protein	22.87	22.88	21.97	21.97	19.93	19.93
Lysine	1.29	1.29	1.18	1.18	0.92	0.92
Methionine	0.60	0.60	0.51	0.51	0.47	0.47
TSAA	0.92	0.92	0.82	0.82	0.75	0.76
Threonine	0.80	0.79	0.76	0.76	0.69	0.69
Calcium	1.00	1.00	0.85	0.85	0.75	0.75
Available Phosphorus	0.35	0.35	0.30	0.30	0.30	0.30
Total Phosphorus	0.61	0.61	0.56	0.57	0.54	0.55
Sodium	0.20	0.20	0.20	0.20	0.22	0.22
Crude Fat	5.24	2.69	5.63	3.03	5.87	3.27
AME (kcal/kg)	3108	2977	3154	3023	3198	3067
Analyzed Nutrient Content						
Crude Protein	22.0	23.1	20.9	20.6	18.3	18.6
Crude Fat	6.90	3.88	6.45	3.90	6.67	3.79
Total Phosphorus	0.58	0.63	0.57	0.56	0.52	0.55
Acid Detergent Fiber	4.74	5.07	4.78	4.34	3.30	3.42

[1] Bacitracin Methylene Disalicylate active ingredient Bacitracin Methylene Disalicylate 50g/lb (50 /ton inclusion: Alpharma Inc., Fort Lee, NJ) for increased rate of weight gain and improved feed efficiency.

[2] 3-Nitro-20 active ingredient 3-Nitro-4hydroxyphenylarsonic acid 20g/lb (10g/ton inclusion: Alpharma Inc., Fort Lee, NJ) for growth promotion, improved feed efficiency and improved pigmentation.

[3] Stafac 20 active ingredient Virginiamycin 20g/lb (20g/ton inclusion: Philbro Animal Health, Fairfield, NJ) for increased rate of weight gain and improved feed efficiency.

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

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

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

[7] Optiphos® L, Enzyvia LLC Sheridan, IN

973.18), and an ether extraction to determine crude fat (AOAC 920.39). Body weights and feed conversion ratios (FCR) were determined on days 14, 27, and 41 to evaluate performance. On day 42, following an eight hour feed withdrawal period, eight male and eight female broilers from each replicate pen were removed and processed for carcass and fat pad measurements (64 male and 64 females per treatment/640 total or 40% of total placement).

STATISTICAL ANALYSIS

All data for both of the experiments were analyzed via a one-way ANOVA, and means were deemed significantly different at $P \leq 0.05$. Means were separated using Duncan's Multiple Range Test. All processing data were analyzed via a 5 (dietary treatment) x 2 (sex) factorial Analysis of Variance using the General Linear Model Procedure (SPSS V 18.0). Main effect means were deemed significantly different at $P \leq 0.05$ and separated using Duncan's Multiple Range Test when appropriate. In instances when a significant interaction were present between dietary treatment and sex, data were analyzed using a one-way Analysis of Variance and individual treatment means determined statistically different a $p \leq 0.05$ and separated using Duncan's Multiple Range Test. All percentage data including processing yields and mortality were subjected to an arcsin transformation prior to analysis.

RESULTS AND DISCUSSION

Experiment 3

Early body weight was negatively impacted with the reduction in dietary energy with the NC fed broilers having the lowest observed BW at d 14 of age which was lower

(P<0.05) than PC fed broilers which yielded the highest observed BW (Table 4-3). The inclusion of NSPase and β-D-mannanase/NSPase in combination in the NC diet increased body weight (P< 0.05) through 14 days of age as compared to the NC. Similar observations were observed on d 28 of age with NC broilers having the lowest body weight which was reduced (P<0.05) compared to the PC fed broilers. Inclusion of β-D-mannanase, NSPase, and β-D-mannanase/NSPase in combination in the NC diet increased body weight (P< 0.05) compared to the NC and were comparable to the PC diet. No differences were observed in body weight between any of the treatment groups on day 42 of age. At the conclusion of the experiment, a 70 gram difference (P> 0.05) was observed between the positive and NC diets (Table 4-3).

Table 4-3. Body weight of straight-run market broilers fed diets reduced in energy and supplemented with a cocktail NSPase[1] and β-mannanase[2] separately and in combination.

Treatment	Day 0 BW	Day 14 BW	Day 28 BW	Day 42 BW	Day 47 BW
Positive Control	45.6 ± 0.1	507.4^{a} ± 8.6	1.61^{a} ± 0.01	2.81 ± 0.03	3.31 ± 0.03
Negative Control	45.6 ± 0.1	465.5^{c} ± 4.0	1.54^{b} ± 0.01	2.78 ± 0.01	3.24 ± 0.02
β-mannanase	45.6 ± 0.1	475.8^{bc} ± 5.5	1.58^{a} ± 0.02	2.79 ± 0.02	3.28 ± 0.02
NSPase	45.6 ± 0.1	494.7^{a} ± 6.5	1.59^{a} ± 0.01	2.81 ± 0.03	3.30 ± 0.02
β-mannanase + NSPase	45.6 ± 0.1	491.1^{ab} ± 5.3	1.59^{a} ± 0.02	2.81 ± 0.04	3.32 ± 0.04

[a-c] Means in same column differ significantly a

[1] Enspira®, Enzyvia LLC Sheridan, IN. Inclusion rate of 113.5g/ton

[2] Hemicell®, ChemGen Corp. Gaithersburg, MD. Inclusion rate of 362.9g/ton

The reduction in dietary energy negatively influenced mortality corrected feed conversion ratio (FCR) during through 14 d of age, as the NC fed broilers had a higher ($P<0.05$) FCR than the PC fed broilers (Table 4-4). The inclusion of β-mannanase/NSPase in combination reduced FCR to a level comparable to the PC diet, while individual inclusion did not reach the level of the PC. Similar observations were observed through 28 d of age as the reduction of dietary energy increased ($P<0.05$) FCR in the NC fed broilers compared to the PC fed broilers. Inclusion of NSPase or β-D-mannanase alone did not reduce FCR to a level similar to the PC broilers; however, β-D-mannanase/NSPase in combination did reduce FCR to a level similar to the PC fed broilers. Following d 28 of age, cumulative FCR was not affected ($P>0.05$) due to a reduction in dietary energy although a 3 point difference was observed between the PC and NC diets (Table 4-4).

Table 4-4. Cumulative mortality corrected feed conversion and mortality of straight-run market broilers fed diets reduced in energy and supplemented with a cocktail NSPase[1] and β-mannanase[2] separately and in combination(Experiment 3).

Treatment	FCR Day 1 to 14	FCR Day 1 to 28	FCR Day 1 to 42	FCR Day 1 to 47	Total Mortality
Positive Control	$1.24^{c} \pm 0.01$	$1.44^{c} \pm 0.02$	1.75 ± 0.02	1.85 ± 0.02	6.3 ± 1.6
Negative Control	$1.35^{a} \pm 0.01$	$1.51^{ab} \pm 0.01$	1.78 ± 0.01	1.88 ± 0.01	4.9 ± 2.2
β-mannanase	$1.35^{a} \pm 0.03$	$1.50^{ab} \pm 0.03$	1.76 ± 0.03	1.88 ± 0.03	7.1 ± 1.9
NSPase	$1.32^{ab} \pm 0.01$	$1.52^{a} \pm 0.02$	1.79 ± 0.02	1.89 ± 0.02	8.4 ± 2.6
β-mannanase + NSPase	$1.28^{bc} \pm 0.01$	$1.46^{bc} \pm 0.01$	1.76 ± 0.01	1.87 ± 0.01	6.7 ± 2.2

[a-c] Means in same column differ significantly at $P<0.05$

[1] Enspira®, Enzyvia LLC Sheridan, IN. Inclusion rate of 113.5g/ton

[2] Hemicell®, ChemGen Corp. Gaithersburg, MD. Inclusion rate of 362.9g/ton

The reduction in energy in the NC diet negatively impacted processing parameters including a decrease ($P<0.05$) in live weight and carcass weight compared to the PC (Table 4-5). Processed broilers fed the NC diet with the inclusion of all enzyme treatments resulted in increased live and carcass weight to levels similar to the PC diet. An interaction was observed in fat pad and tenderloin weight as dietary and enzyme effect were only observed in male broilers. A reduction ($P<0.05$) in fat pad weight was observed in male broilers in the NC diet compared to the PC diet (Table 4-5). Inclusion of all enzyme treatments resulted in similar fat pad weights of male broilers similar to the NC diet. The inclusion of β-D-mannanase/NSPase and NSPase alone in the NC diet increased main effect breast meat weight ($P< 0.05$) compared to the NC. The inclusion of NSPase in the NC diet increased ($P<0.05$) tenderloin weights in male broilers when compared to the NC diet (Table 4-5). No differences were observed in regards to female fat pad weights between any dietary treatment groups, suggesting that even the low energy diet was sufficient and met the female broilers energy requirements.

Table 4-5. Processing parameters including live weight, carcass weight without giblets (WOG) weight, fat pad weight, breast weight, and tender weight of broilers fed reduced energy diet with addition of a cocktail NSPase[1] and β-mannanase[2] separately and in combination.

TRT	Sex	Live Wt. (g)	WOG Wt. (g)	Fat Pad Wt. (g)	Breast Wt. (g)	Tender Wt. (g)
PC	Male	3693.9	2666.8	58.3^{a}	661.3	143.5^{b}
	Female	2997.5	2182.9	51.9^{abcd}	531.7	125.2^{cd}
NC	Male	3648.1	2593.5	45.2^{d}	647.1	140.3^{b}
	Female	2915.4	2119.7	51.0^{abcd}	513.1	121.3^{d}
β-mannanase	Male	3641.7	2631.9	49.5^{bcd}	656.7	141.3^{b}
	Female	2977.4	2162.0	48.4^{bcd}	524.4	126.3^{cd}
NSPase	Male	3771.4	2740.7	46.7^{cd}	697.5	152.2^{a}
	Female	3018.3	2212.6	56.5^{ab}	543.1	128.9^{cd}
NC+ β-mann/NSPase	Male	3671.3	2653.0	48.3^{bcd}	656.3	138.5^{b}
	Female	3037.5	2235.6	54.4^{abc}	560.1	130.8^{c}
Main Effects						
TRT						
PC		3332.1^{a}	2415.4^{ab}	55.0	594.0^{abc}	134.0
NC		3235.3^{b}	2326.6^{c}	48.4	571.6^{c}	129.6
β-mannanase		3314.7^{a}	2400.6^{b}	49.0	591.6^{bc}	133.9
NSPase		3378.9^{a}	2465.5^{a}	51.9	617.1^{a}	140.0
NC+ β-mann/NSPase		3354.4^{a}	2444.3^{ab}	51.4	608.2^{ab}	134.6
Sex						
Male		3686.0^{a}	2658.5^{a}	49.8	663.9^{a}	143.1
Female		2988.8^{b}	2182.4^{b}	52.4	534.5^{b}	126.4
p-value						
Treatment		0.029	0.001	0.089	0.007	0.005
Sex		<0.001	<0.001	0.107	<0.001	<0.001
Treatment x Sex		0.472	0.430	0.022	0.151	0.030
Pooled SEM		21.8	15.6	0.9	5.0	0.9

[a-d] Means in same column differ significantly at P<0.05.
[1] Enspira®, Enzyvia LLC Sheridan, IN. Inclusion rate of 113.5g/ton
[2] Hemicell®, ChemGen Corp. Gaithersburg, MD. Inclusion rate of 362.9g/ton

While no main effect differences were observed in breast and tenderloin yield in broilers between the dietary treatments, a statistical difference was observed in carcass yield (Table 4-6). The reduction in energy in the NC diet negatively impacted carcass yield as the NC diet produced the lowest observed carcass yield. The inclusion of

NSPase, and the combination of β-mannanase/NSPase in the NC diet increased carcass yield ($P< 0.05$) when compared to the NC. While no differences were observed in fat pad yield of females, a difference was observed ($P<0.05$) of males between the PC and NC diet (Table 4-6) indicating that the NC was sufficient to meet the requirement for females. The inclusion of β-mannanase, NSPase, and combination of β-D-mannanase + NSPase in the NC diet did not result in an increase in male fat pad weight (Table 4-6).

Experiment 4

Similar to experiment 3, d 14 BW was negatively impacted with the reduction in dietary energy with average BW of the NC fed broilers being lower ($P<0.05$) than the PC fed broilers which yielded the heaviest BW (Table 4-7). The inclusion of NSPase and the combination of β-mannanase/NSPase in the NC diet increased d 14 BW to a level similar to the PC diet. Similarly, d 27 BW was negatively affected with a reduction in dietary energy as the PC fed broilers had a higher ($P<0.05$) BW as compared to the NC fed broilers (Table 4-7).

Table 4-6. Processing parameters including carcass yield, breast yield, tenderloin yield, and fat pad yield of broilers fed reduced energy diet with addition of a cocktail NSPase[1] and β-mannanase[2] separately and in combination.

TRT	Sex	Carcass Yield (%)	Breast Yield (%)	Tenderloin Yield (%)	Fat Pad Yield (%)
PC	Male	72.2	24.8	5.4	2.21[a]
	Female	72.8	24.4	5.8	2.37[a]
NC	Male	71.2	24.9	5.4	1.74[b]
	Female	72.7	24.2	5.7	2.39[a]
β-mannanase	Male	72.3	25.0	5.4	1.88[b]
	Female	72.6	24.2	5.9	2.23[a]
NSPase	Male	72.7	25.4	5.6	1.70[b]
	Female	73.3	24.6	5.8	2.54[a]
NC+ β-mann/NSPase	Male	72.3	24.7	5.2	1.82[b]
	Female	73.6	25.0	5.9	2.44[a]
Main Effects					
TRT					
PC		72.5[ab]	24.6	5.6	2.29
NC		72.0[b]	24.5	5.6	2.11
β-mannanase		72.4[ab]	24.6	5.6	2.05
NSPase		73.0[a]	25.0	5.7	2.15
NC+ β-mann/NSPase		72.9[a]	24.9	5.5	2.13
Sex					
Male		72.1[b]	25.0[a]	5.4[b]	1.88[b]
Female		73.0[a]	24.5[b]	5.8[a]	2.40[a]
p-value					
Treatment		0.015	0.559	0.443	0.249
Sex		<0.001	0.025	<0.001	<0.001
Treatment x Sex		0.310	0.388	0.318	0.023
Pooled SEM		0.1	0.1	0.03	0.04

[a-b] Means in same column differ significantly at P<0.05.

[1] Enspira®, Enzyvia LLC Sheridan, IN. Inclusion rate of 113.5g/ton

[2] Hemicell®, ChemGen Corp. Gaithersburg, MD. Inclusion rate of 362.9g/ton

Table 4-7. Body weight of straight-run market broilers fed diets reduced in energy and supplemented with a cocktail NSPase[1] and β-mannanase[2] separately and in combination.

Treatment	Day 14 BW (g)	Day 27 BW (kg)	Day 35 BW (kg)	Day 41 BW (kg)	Mortality
Positive Control	$443.2^{a} \pm 5.4$	$1.37^{a} \pm 0.01$	$2.10^{a} \pm 0.01$	$2.55^{a} \pm 0.03$	$5.0^{a} \pm 0.9$
Negative Control	$423.4^{bc} \pm 4.5$	$1.32^{c} \pm 0.01$	$2.05^{b} \pm 0.01$	$2.51^{ab} \pm 0.02$	$3.1^{ab} \pm 0.9$
β-mannanase	$409.7^{c} \pm 4.9$	$1.29^{d} \pm 0.02$	$2.00^{c} \pm 0.01$	$2.46^{b} \pm 0.02$	$1.8^{b} \pm 0.7$
NSPase	$430.4^{ab} \pm 5.9$	$1.33^{bc} \pm 0.01$	$2.06^{b} \pm 0.01$	$2.51^{ab} \pm 0.02$	$2.8^{ab} \pm 0.7$
β-mannanase + NSPase	$437.8^{ab} \pm 5.3$	$1.35^{ab} \pm 0.01$	$2.10^{a} \pm 0.01$	$2.56^{a} \pm 0.03$	$4.7^{ab} \pm 0.8$

[a-c] Means in same column differ significantly at P<0.05.

[1] Enspira®, Enzyvia LLC Sheridan, IN. Inclusion rate of 113.5g/ton

[2] Hemicell®, ChemGen Corp. Gaithersburg, MD. Inclusion rate of 362.9g/ton

As with d 14 BW, individual inclusion of each enzyme did not reach the level of the PC diet, however inclusion of β-D-mannanase/NSPase in combination did increase (P<0.05) BW as compared to the NC diet to similar levels of the PC fed broilers (Table 4-7). On d 35, BW continued to be affected by dietary energy level with NC broilers having lower (P<0.05) BW compared to PC broilers (Table 4-7). Again, individual inclusion of each enzyme did not result in BW similar to the PC diet; however, β-mannanase/NSPase fed in combination increased (P<0.05) BW compared to the NC and was similar to the PC diet (Table 4-7). Similar to experiment 1, BW was not affected at the conclusion of the experiment on day 41 (Table 4-7). Final BW was similar between the PC and NC fed broilers although a 40 g difference was present (P = 0.12). Inclusion of β-D-mannanase resulted in the lowest observed cumulative mortality during the

experiment which was lower (P<0.05) than the PC fed broilers with more than a 3% decrease in total mortality (Table 4-7).

As in experiment 3, dietary energy level negatively impacted observed early FCR as the NC fed broilers had an elevated (P<0.05) FCR as compared to the PC fed broilers following the starter period (Table 4-8). While inclusion of both enzymes individually in the NC diet did lower FCR, neither reached the level of the PC diet; however, the combination of β-mannanase/NSPase to the NC diet did reduced FCR to a level comparable to the PC diet (Table 4-8). Dietary energy level continued to effect FCR during the grower phase and cumulatively through 27 d of age with the NC diet having an increased (P<0.05) FCR compared to the PC diet (Table 4-8). Enzyme inclusion did not impact grower phase or cumulative FCR through d 27 FCR. FCR was again increased (P<0.05) in the finisher phase of production in the NC diet as compared to the PC diet (Table 4-8). Inclusion of individual exogenous enzymes in the NC diet reduced FCR to a level similar to the PC diet; however, inclusion of β-mannanase/NSPase in combination reduced (P<0.05) FCR compared to the NC diet to the same level of the PC (Table 4-8). Cumulative FCR through d 35 of age was elevated (P<0.05) in the NC diet compared to the PC diet (Table 4-8). Inclusion of NSPase and the combination of β-mannanase/NSPase in the NC diet resulted in a reduction in FCR when compared to the NC diet (P< 0.05), although it did not reach the level of the PC diet (Table 4-8). At the conclusion of the trial (d 1-41), the PC diet yielded the lowest observed FCR (P<0.05) (Table 4-8). The addition of NSPase to the NC diet resulted in a reduction in FCR (P<

0.05), additionally the inclusion of the combination of β-mannanase/NSPase in the NC diet resulted in a further reduced FCR to a level comparable to the PC diet (Table 4-8).

Table 4-8. Mortality corrected feed conversion ratio and cumulative feed conversion ratio of broilers fed diets reduced in energy and supplemented with a cocktail NSPase[1] and β-mannanase[2] separately and in combination (Experiment 4).

TRT	FCR Starter	FCR Grower	FCR Finisher	FCR Day 1-27	FCR Day 1-35	FCR Day 1-41
Positive Control	$1.25^{b} \pm 0.01$	$1.46^{b} \pm 0.01$	$1.99^{b} \pm 0.02$	$1.40^{b} \pm 0.01$	$1.54^{c} \pm 0.01$	$1.68^{c} \pm 0.01$
Negative Control	$1.30^{a} \pm 0.01$	$1.51^{a} \pm 0.01$	$2.06^{a} \pm 0.01$	$1.45^{a} \pm 0.01$	$1.58^{a} \pm 0.01$	$1.74^{a} \pm 0.01$
β-mannanase	$1.28^{a} \pm 0.01$	$1.50^{a} \pm 0.01$	$2.02^{ab} \pm 0.01$	$1.43^{a} \pm 0.01$	$1.57^{ab} \pm 0.01$	$1.72^{ab} \pm 0.01$
NSPase	$1.28^{a} \pm 0.01$	$1.50^{a} \pm 0.02$	$2.01^{ab} \pm 0.02$	$1.43^{a} \pm 0.01$	$1.56^{b} \pm 0.01$	$1.71^{b} \pm 0.01$
β-mannanase + NSPase	$1.27^{ab} \pm 0.01$	$1.50^{a} \pm 0.01$	$1.99^{b} \pm 0.02$	$1.43^{a} \pm 0.01$	$1.56^{b} \pm 0.01$	$1.70^{bc} \pm 0.01$

[a-c] Means in same column differ significantly at P<0.05.
[1] Enspira®, Enzyvia LLC Sheridan, IN. Inclusion rate of 113.5g/ton.
[2] Hemicell®, ChemGen Corp. Gaithersburg, MD. Inclusion rate of 362.9g/ton

During the starter phase, a difference was observed in consumption per bird between all treatment groups and the β-mannanase treatment (P< 0.05) (Table 4-9). Again, a difference was observed during the grower phase between the β-mannanase treatment group and the PC, NC, and combination of β-mannanase/NSPase treatment groups (P<0.05). The NC treatment group consumed the most feed per bird during the finisher phase, although no statistical differences were observed. The β-mannanase treatment group consumed the least amount of feed throughout the duration of the trial with consumption being reduced (P<0.05) compared to the NC and combination of β-mannanase/NSPase treatment groups (Table 4-9).

Table 4-9. Consumption per bird for each dietary phase and cumulative for the experiment of birds fed a reduce energy diet supplemented with a cocktail NSPase[1] and β-mannanase[2] separately and in combination.

TRT	Starter (g)	Grower (kg)	Finisher (kg)	Day 1-41 (kg)
Positive Control	$500^{a} \pm 4$	$1.37^{a} \pm 0.01$	2.39 ± 0.02	$4.25^{ab} \pm 0.04$
Negative Control	$494^{a} \pm 5$	$1.36^{a} \pm 0.01$	2.44 ± 0.02	$4.29^{a} \pm 0.04$
β-mannanase	$473^{b} \pm 6$	$1.32^{b} \pm 0.01$	2.37 ± 0.03	$4.16^{b} \pm 0.04$
NSPase	$499^{a} \pm 5$	$1.35^{ab} \pm 0.01$	2.37 ± 0.02	$4.22^{ab} \pm 0.04$
β-mannanase + NSPase	$504^{a} \pm 7$	$1.35^{a} \pm 0.01$	2.42 ± 0.04	$4.30^{a} \pm 0.05$

[a-c] Means in same column differ significantly at P<0.05.

[1] Enspira®, Enzyvia LLC Sheridan, IN. Inclusion rate of 113.5g/ton.

[2] Hemicell®, ChemGen Corp. Gaithersburg, MD. Inclusion rate of 362.9g/ton

A difference was observed in live weight and carcass weight (P< 0.05) between the PC and NC (Table 4-10). The addition of the combination of β-mannanase/NSPase in the NC diet resulted in an increase in live and carcass weight compared to the NC (P< 0.05) to a level similar to that of the PC. Again, a difference was observed (P<0.05) in fat pad weights between the PC and NC diets; however, an interaction was not observed between treatment and sex as in experiment 1. The inclusion of NSPase in the NC diet resulted in lowest observed (P<0.05) fat pad weight of any treatment group and was reduced compared to the PC (Table 4-10). No significant differences were observed in fat pad yield between the PC and NC diet; however, the PC diet produced the highest yield of any treatment (Table 4-10). The inclusion of NSPase in the NC diet resulted in a fat pad yield lower (P<0.05) than the PC. As expected, males had higher weight and yields of all evaluated parameters with the exception of fat pad weights (Table 4-10).

Table 4-10. Processing parameters of male female and straight-run broilers fed diets reduced in energy and supplemented with a cocktail NSPase[1] and β-mannanase[2] separately and in combination.

TRT	Sex	Live Wt. (g)	WOG Wt. (g)	Fat Pad Wt. (g)	WOG Yield (%)	Fat Pad Yield (%)
PC	Male	2798.9	2183.9	36.2	78.0	1.66
	Female	2300.0	1822.0	36.1	79.3	1.98
NC	Male	2725.1	2127.4	34.0	78.2	1.61
	Female	2239.4	1779.3	32.6	79.4	1.83
β-mannanase	Male	2673.1	2087.4	34.2	78.1	1.64
	Female	2255.0	1759.6	34.1	78.1	1.94
NSPase	Male	2756.7	2158.2	32.2	78.4	1.49
	Female	2253.0	1779.2	32.0	78.9	1.80
NC+ β-mann/NSPase	Male	2767.8	2168.4	34.7	78.3	1.60
	Female	2333.7	1848.3	34.2	79.0	1.86
Main Effects						
TRT						
PC		2553.3^{a}	2007.2^{a}	36.2^{a}	78.6	1.81^{a}
NC		2478.4^{b}	1950.5^{bc}	33.3^{b}	78.8	1.72^{ab}
β-mannanase		2462.5^{b}	1923.5^{c}	34.2^{ab}	78.1	1.79^{a}
NSPase		2502.9^{b}	1964.1^{b}	32.1^{b}	78.7	1.65^{b}
NC+ β-mann/NSPase		2552.4^{a}	2010.9^{a}	34.5^{ab}	78.7	1.73^{ab}
Sex						
Male		2744.8^{a}	2145.3^{a}	34.3	78.2^{b}	1.60^{b}
Female		2276.1^{b}	1797.3^{b}	33.8	79.0^{a}	1.88^{a}
p-value						
Treatment		<0.001	<0.001	0.010	0.242	0.032
Sex		<0.001	<0.001	0.513	<0.001	<0.001
Treatment x Sex		0.203	0.534	0.979	0.276	0.911
Pooled SEM		11.9	9.3	0.4	0.1	0.02

[a-c] Means in same columns with different superscripts are different at P <0.05
[1] Enspira®, Enzyvia LLC Sheridan, IN. Inclusion rate of 113.5g/ton.
[2] Hemicell®, ChemGen Corp. Gaithersburg, MD. Inclusion rate of 362.9g/ton.

The results of the two experiments suggest that low energy diets have a negative impact on broiler performance and processing yields. A significant difference in body weights in both experiments between the PC and NC diets was observed. The reduction in energy resulted in an increased in FCR in the NC diet in both experiments as well.

Processing parameters followed the performance data with significant differences observed between the PC and NC in live weights and carcass weights in both experiments. Previous research has reported similar results in reduced energy diets resulting in decreased broiler performance (O'Neill et al., 2012). The addition of each enzyme individually to the low energy diet had positive results in both experiments on multiple parameters. However, the combination of β-mannanase/NSPase in the low energy diet resulted in more consistent and increased improvements in body weight and FCR. The combination enzyme treatment significantly increased body weight in experiment 1 through 28 days, and throughout the entire experiment in experiment 2. The greater impact in experiment 2 could be related to multiple factors including: the use of a different NSPase, dietary ingredient profile, and the use of growth promoters which were used in experiment 2, but not experiment 1. This supports previous research by O'Neill et al. (2012), and Coppedge et al. (2012) that showed increases in body weights of broilers fed low energy diets with xylanase inclusion. The inclusion of NSPase in the low energy diet resulted in a significant increase in body weight gained in both experiments particularly thru day 28 when compared to the low energy diet. These results agree with previous research by Olukosi et al. (2007) who found that chicks benefited more from enzyme addition at a younger age and that the contribution of enzymes to nutrient retention decreased with age.

While the addition of the β-mannanase and NSPase separately in the low energy diet reduced cumulative FCR; the inclusion of the combination of β-mannanase/NSPase additive and sub-additive reductions in FCR as compared to individual inclusion. The

observed effects are thought to be related to NSPase used and/or dietary ingredient profile, as these differed between the two experiments. In experiment 1, the combination treatment group significantly reduced FCR through 28 days to a level comparable to the PC diet, while in experiment 2, cumulative FCR for days 1-41 for the combination of β-mannanase/NSPase in the low energy diet resulted in a significant reduction in FCR compared to the NC diet which was similar to the PC. In experiment 2, the inclusion of β-mannanase in the NC diet did not result in an improvement in BW or FCR throughout the experiment, an unexpected observation as previous research, such as experiment 1, along with published reports, have indicated an improvement in BW and FCR with the addition of β-mannanase in broiler diets (Jackson et al., 2004; Zou et al., 2006). Inclusion of the NSPase alone in experiment 2 improved FCR in the NC diet. Cowieson and Adeola (2005), and O'Neill et al. (2012) also reported similar reductions in FCR with the inclusion of NSPase in nutrient deficient diets.

While no significant differences were observed in mortality between the PC and NC diets in the experiments, the addition of β-mannanase in the low energy diet in experiment 2 resulted in a significant reduction in mortality compared to the PC treatment group, and had the lowest mortality of any dietary treatment. Jackson et al. (2003) observed that the inclusion of β-mannanase in broiler diets resulted in an increase in immunological activity, as well as significant reduction in coccidial lesion scores in the GI tract of the bird following a *Eimeria sp.* and *Clostridium perfringens* challenge leading to an overall increase in performance, perhaps attributing to the reduction in mortality in experiment 2. The lack of performance response in experiment 2 with

inclusion of β-mannanase may be associated with a lack of environmental challenge as broilers were reared on fresh pine shavings as opposed to used litter similar to industry conditions. A described mechanism of action for β-mannanase is associated with immunological activity reducing inflammation in the GI tract (Jackson et al., 2003) caused by the presence of these NSP which can trigger the innate immune system causing an nonproductive immune response (Hsiao et al., 2006).

The addition of the β-mannanase and NSPase, individually or in combination in the low energy diet, resulted in a significant increase in live weights and carcass weights compared to the NC diet in experiment 1. Experiment 3 had similar results with the exception of the β-mannanase treatment, which was related to the reduced rate of feed consumption observed during experiment 4, which was an unexpected observation. The addition of NSPase and the combination of β-mannanase/NSPase in the low energy diet resulted in a significant increase in breast weight compared to the NC diet. Coppedge et al. (2012) observed an increase in breast and tender weight with the inclusion of NSPase in a corn and soy diet similar to results observed in experiment 1. Fat pad weight was lower in broilers fed the reduced energy diet in experiment 2, regardless of sex; however, reduced fat pad weight associated with dietary energy level were only observed in male broilers. The inclusion of β-mannanase, NSPase, and the combination resulted in fat pad weights and yields similar to that of the low energy diet indicating that energy release associated with enzyme inclusion was at least partially for tissue development and not all lipid deposition. These experiments suggest that the co-administration of an NSPase and β-mannanase enzyme is an effective strategy to combat

diets low in dietary energy, and an additive effect may be observed when using multiple enzymes.

CHAPTER V

CONCLUSION

Continued increases in ingredient costs, specifically corn due to the growth of the ethanol industry have force nutritionist to maximize the utilization of dietary nutrients. As corn makes up a vast majority of the diets used in the United States, the importance to maximize its use to the bird will continue to be a high priority for the poultry industry. The use of alternative ingredients such as DDGS will continue to be an effective strategy with the supplementation of exogenous enzymes to combat the NSP present in corn, soybean meal, and DDGS.

In all experiments, the reduction in energy in the NC diet resulted in a decrease in broiler growth performance, while inclusion of the NSPase improved growth performance in the reduced energy diets. Reducing the energy level of the diet primarily through the removal of fat and replacing with corn can significantly reduce the cost of the diet. Additionally, increasing the concentration of higher fiber ingredients such as DDGS can also reduce diet cost. Supplementation of the diet with NSPase will allow for both reduction of fat and increasing alternative feed ingredient concentration without negatively influencing performance. However, diet manufacturing temperature needs to be considered when using an NSPase, as xylanase recovery is reduced as pelleting temperature increases.

The addition of NSPase in broiler diets target the NSP found in corn and DDGS while the addition of β-mannanase would target the NSP found in soybean meal.

Simultaneous inclusion of an NSPase and β-mannanase resulted in improved growth performance as compared to broilers fed each exogenous enzyme individually.

These data suggest that the use of an exogenous cocktail NSPase is an effective method to reduce the costs of diets while not sacrificing performance. Furthermore, the maximum manufacturing temperature for this enzyme appears to be 80 C to optimize enzyme recovery. Enzyme activity and effectiveness decreases as temperature increases; however, the benefit of this enzyme is not completely lost when manufactured at higher temperatures. Lastly, these data suggest that the combination of enzymes NSPase/β-mannanase in low energy diets is more effective than individual inclusion at improving broiler performance and processing parameters when fed a corn and soybean meal based diet; therefore, they provide a cost saving strategy to poultry nutritionists.

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