ABSTRACT

KOST, MARISSA MICHELLE. Response of Broilers to Low Energy Diets Supplemented with Exogenous Carbohydrases. (Under the direction of John T. Brake).

Ross 708 x 344 male broilers were used to determine the effects of energy level, carbohydrase supplementation, and enzyme dosages in broiler diets composed of various cereal grains. Three studies were conducted to determine the effects of carbohydrase supplementation on the live performance response and/or digestibility. Experiment 1 utilized a series of 9 diets formulated to contain a positive control (PC), a negative control (NC) with a low metabolizable energy (ME) density, and 7 NC diets amended with xylanase in doses from 125 to 2000 IU. All diets contained wheat, dried distillers grains with solubles (DDGS), corn, and soybean meal (SBM). In all formulations the dietary ME supplied by poultry fat was kept constant with a maximum of 1% fat added in the mixer and any additional added by post pellet liquid application (PPLA). A total of 2,304 male broilers were assigned to 9 treatments with 8 replicate pens of 32 birds each in Experiment 1. Weekly body weight (BW), feed intake, and mortality were recorded. Results showed no differences for BW, feed intake, or mortality. PC diet broilers exhibited improved FCR from 15-35 d and 0-35 d of age compared with those fed the NC and NC+xylanase diets, which confirmed the lower ME density of the NC diet and suggested a relative lack of ME releasing activity of the xylanase. However, xylanase did demonstrate a quadratic dose response that could be discerned by improved digestibility that somewhat correlated with improved FCR, with the major exception being the PC diet.

Experiments 2 and 3 evaluated the effects of supplementing β-glucanase into a diet containing corn, SBM, and DDGS. In Experiment 2, the 6 dietary treatments included: negative control (NC) basal starter and grower diets with 1% total fat added in the mixer, positive control (PC) starter and grower diets that contained 2.5% total added fat with 1% added in the mixer and 1.5% added by PPLA (PC+PPLA), positive control starter and grower diets that contained 2.5% fat added only in the mixer (PC+Mixer), and 3 enzyme supplemented diets added to the NC basal diet. The 3 enzyme supplemented diets were manufactured from an external control β-glucanase-based multigrain enzyme mixture (NC+DG2) or a β-glucanase enzyme at a high or low dosage (NC+HiGlu and NC+LoGlu). In Experiment 2, a total of 576 male broilers were assigned to 6 treatments with 6 replicate pens of 16 male broilers each. In Experiment 3, a common corn-SBM starter was fed followed by four grower dietary treatments that were manufactured by grinding, with a roller mill, the pelleted NC basal diet as well as the 3 NC+enzyme supplemented diets from Experiment 1. A total of 288 male broilers were assigned to 4 grower dietary treatments with 6 replicate pens of 12 male broilers each in Experiment 3. Results showed that supplementation of the NC with both sources of β-glucanases did not significantly improve overall FCR when compared to the NC diet in Experiment 2. Only the NC+LoGlu diet achieved the same overall FCR as the NC basal diet. In Experiment 3, a similar trend in FCR was observed during the grower phase as in Experiment 2. There were no differences in mortality among treatments. The data from both trials showed similar trend of results suggesting that β-glucanase supplementation in primarily corn-SBM diets needs to be further evaluated in order to conclude if its true efficacy with those feed ingredients.

Response of Broilers to Low Energy Diets Supplemented with Exogenous Carbohydrases

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LIST OF ABBREVIATIONS

AME	Apparent metabolizable energy
ANF	Anti-nutritional factor
BW	Body weight
d	Day
F	Fahrenheit
FCR	Feed conversion ratio
FI	Feed intake
g	Gram
NSP	Non-starch polysaccharide
PDI	Pellet durability index

INTRODUCTION

The price of feed ingredients has risen over the recent years resulting in a much larger portion of production costs coming from feed. This was in part, due to the major shift of corn utilization in the ethanol industry as opposed to animal feed production. The inclusion of alternative feed ingredients has been subsequently employed to overcome the rising costs of traditional feed ingredients. In the United States, typically corn and soybean meal (SBM) have constituted a large portion of broiler diets. Alternative feed ingredients such as distiller dried grains with solubles (DDGS) and wheat have gained more popularity for inclusion in corn-SBM diets.

Improving digestibility of traditional and alternative feed ingredients has driven the production and utilization of numerous feed additives. Various examples of feed additives include probiotics, prebiotics, antimicrobials, antioxidants, and exogenous enzymes, which was the focus of the current research. Exogenous enzymes such as carbohydrases have been reported to liberate "energy" (net apparent metabolizable energy, AME_n) and improve digestibility of feed ingredients that are typically unavailable for digestion and absorption.

The idea of reducing feed costs by omitting or reducing inclusion of expensive feed ingredients, such as fat, has also resulted in more interest and usage of exogenous enzymes in feed formulations. Determining the percentage fat that will work synergistically with the dosage of carbohydrase(s) has not been concluded. Carbohydrases should theoretically make up for the reduction in energy from removal of fat; but at certain low levels of fat, birds may not respond to the enzyme supplementation as expected.

Carbohydrase supplementation in primarily corn-SBM diets had often been neglected due to suspicions of little to no improvement in AME_n values. Several studies have shown various improvements in live performance and digestibility values. Although supplementation in corn-SBM diets has become more prevalent; the results remained somewhat inconsistent. The formulations of these corn-SBM diets in regards to substrate, fat level, and alternative ingredients when supplemented with carbohydrases have a major role in the efficacy of the exogenous enzyme to produce substantial improvements that reduce overall production costs.

LITERATURE REVIEW

Feed Expenditures and Production

Feed expenditures for food production in the United States have gradually increased over the past several years (USDA, 2015). Specifically, there was an 8% increase in total farm food production expenditures from 2013 to 2014. There were four expenditures that accounted for almost half (47.3%) of the total farm production expenditures in 2014. Out of those four, the leading one was feed with a total of 16% of the total farm production expenditures as reported by the USDA's National Agricultural Statistics Service (2015). For livestock farms, it was not surprising that the largest expenditure ($62.1 billion) was again feed at 31.8% of total expenditures.

Production of certain feed ingredients has also seen a gradual increase over the past several years (FAOSTAT, 2014). Specifically, global production of corn has increased substantially. In 2014, corn production was reported to be 1020 M MT, making it the most abundantly grown cereal crop ahead of wheat, which was 730 M MT. This surge in maize production was partly due to increased global demand for ethanol. Today, about one-third of total corn production has been for the ethanol industry, which has resulted in an overall increase in price of corn for animal feed and an abundance of corn byproducts.

Feed Ingredients

The reaction to the inflation of feed prices has led to the inclusion of alternative ingredients such as distillers dried grains with solubles (DDGS), a byproduct from the corn or wheat industry, which has not been traditionally included in poultry or swine diets in the United States. DDGS resulted from the dry milling of corn. It has been considered a medium-protein and high-energy ingredient consisting of a grain fraction and whole stillage from the yeast fermentation of grain to ethanol. Due to the high dry matter content of DDGS, it had a reasonably longer shelf life than other alternative feed ingredients.

Corn has been a common feed ingredient in poultry and swine diets for many years in the United States. Corn has been widely used due to its many favorable properties. For example, it had lower concentrations of anti-nutritional factors (ANFs) such as phytin, trypsin inhibitors, and lectins when compared to other cereal feed ingredients such as wheat, barley, or rye. Corn contains about 1g/kg of water soluble non-starch polysaccharides (NSPs), primarily arabinoxylan, compared with 24, 45, and 46 g/kg for wheat, barley, and rye, respectively.

Table I-1. Total pentosans, cellulose, pectin, and total non-starch polysaccharide (NSP) content of different feed ingredients (Malathi and Devegowda, 2001).

Ingredient	Total Pentosans	Cellulose	Pectin	Total NSP
	(%)			
Corn	5.35	3.12	1.00	9.32
Soybean meal	4.21	5.75	6.16	29.02
Sorghum	2.77	4.21	1.66	9.75

Corn has been considered a low viscous cereal grain due to its low concentration of NSPs. Its high starch content has a good energy value in addition to being easily digestible. The combination of corn and SBM has been demonstrated to have a favorable amino acid balance relative to animal requirement and to be well digested by poultry (Kocher et al., 2003).

Corn has typically provided about 20% of the protein in a broiler starter diet but the balance of amino acids in corn alone has been considered to be nutritionally poor (Cowieson, 2004). The nutrient composition of corn has also been shown to be variable in terms of starch, protein, fiber, fat, and amino acid content, which has further influenced the apparent metabolizable energy (AME_n) of the corn. Some studies have suggested that the presence of resistant starches in corn may actually limit the AME_n value (Williams et al., 1997). Nevertheless, starch has typically contributed around 60% of the AME_n content of poultry feeds so that even relatively small differences in starch digestibility could have a substantial impact on dietary AME_n content. Starch granules in corn have often varied in size ranging between 2-30 μm. The size of starch was important when determining the AME_n value of starch, with smaller granules having a relatively larger surface area and thus a greater potential for hydrolysis by endogenous amylase. During thermal processing, starch has also been reported to gelatinize to a varying extent based on its granule size, moisture content, and amylose to amylopectin ratio, as well as heat and time of processing (Cowieson, 2004).

The chemical composition and subsequent nutritional value of corn, as well as other feed ingredients, was influenced by various conditions can ultimately altered digestibility and overall responses to NSP enzymes in animal feed. Williams et al. (1997) reported that

considerable variation in NSP content was found according to genotype even within the same plant species. Certain environmental factors prior to harvest and storage conditions after harvest have also influenced NSP content (Williams et al., 1997; Cowieson, 2004). As described by Douglas et al. (2000), a 10% difference in BW gain and feed efficiency of broilers fed diets based on SBM collected from twelve different locations was observed. This same study reported a pronounced overall improvement in the digestible energy when SBM of poor quality or low digestible energy were supplemented with carbohydrase enzymes (Douglas et al., 2000; Aftab, 2012).

Fat Digestion and Absorption

Rising costs of feed ingredients, especially fat sources, has also stimulated the trend of adding exogenous enzymes to poultry diets in order to be able to reduce the normal AME_n caloric values. These lower energy poultry diets have been typically formulated by removing a portion of the added fat in order to achieve ~100 kcal/kg lower AME_n. As reported by Meng et al. (2004), several studies have shown that fat appears to experience the most severe impairment in digestion when in the presence of high digesta viscosity (Campbell et al., 1983; Ward and Marquardt, 1983; Choct and Annison, 1992). Reduced fat emulsification and hydrolysis has been shown to occur even in diets with medium viscosity (Pasquier et al., 1996). During the first few weeks post-hatching, the young chick's gastrointestinal tract was still immature and developing which resulted in inefficient fat absorption and emulsification. Digestion of fat has been described as the combined action of bile acids, lipase, colipase, and fatty acid binding protein. Activity of pancreatic lipase has increased with bird age, but bile

acids were essential to both emulsification and micelle formation in the intestines (Krogdahl, 1985; Maiorka et al., 2008). Maiorka et al. (2008) did not observe a difference in BW gain or feed conversion of birds fed different energy or oil levels until two weeks of age. This led the authors to conclude that 7-d-old birds did not regulate feed intake according to dietary energy level; but after 14 d of age, dietary energy level had a significant effect on feed intake, BW gain, and feed conversion after 14 d of age.

Carbohydrases have been a popular choice of exogenous enzyme with respect to energy manipulation of feed formulas due to carbohydrates being the main energy source in poultry diets. One study observed hyperlipidemic properties of xylanase addition in a wheat-SBM diet as adding xylanase appeared to have some emulsifying properties that increased lipid digestion and absorption (Karimi, 2013). Although xylanase displayed these properties, this study, as well as that of Barasch (2015), observed that adding xylanase to low-AME diets may have somewhat blocked intestinal fat digestion and absorption when compared to the effects of adding xylanase to normal-AME diets. Another study observed a decrease in fat digestibility as xylose levels increased in the diet possibly due to metabolic effects on cholesterol synthesis (Peng et al., 2004). The authors proposed that pentose sugars may either influence cholesterol synthesis or inhibit its secretion, supported by the increase in plasma cholesterol in relation to higher dietary levels of xylose. Different types of fat have also been shown to result in varying fat digestibility values when supplemented with carbohydrases (Meng et al., 2004).

Enzymes

Enzymes have been generally described as biological catalysts. Endogenous enzymes were those enzymes naturally produced and secreted in the body. Exogenous enzymes have been used to supplement naturally occurring endogenous digestive enzymes or to provide a digestive enzyme that was not endogenous to the body. Some examples of endogenous digestive enzymes in monogastric animals have included proteases, lipases, and amylases. In contrast, monogastrics typically have not had the ability to produce β-glucanases and pentosanases, other than in very minute quantities.

Exogenous Carbohydrases

The use of exogenous carbohydrase enzymes has been generally accepted to improve overall live performance in poultry when added to wheat and/or barley diets. There has been much less literature that has supported the use of carbohydrases in corn-SBM based diets due to the lower NSP concentration in corn when compared to wheat, as previously discussed. Nevertheless, it has become common to observe mixtures and combinations of multiple exogenous enzymes being used in broiler diets. The most common exogenous carbohydrases incorporated solely or as a mixture into animal feed have been amylases, β-glucanases, and xylanases. There have been reports of improvement in live performance when multiple enzymes were used in combination with both wheat and corn based diets. For example, improved live performance and diet digestibility were observed in studies when carbohydrases were used in combination with other enzymes such as amylase, proteases, and xylanases added to corn-soy diets (Zanella et al., 1999; Douglas et al., 2000; Café et al.,

2002). Furthermore, Stefanello (2015) discussed that other researchers had found increased digestibility of starch in the small intestine as a result of the addition of exogenous enzyme products into corn-SBM diets, leading to enhanced energy availability in poultry (Zanella et al., 1999; Yu and Chung, 2004; Meng and Slominski, 2005). Stefanello (2015) also reported that xylanase may have acted through an increased access of cell contents to endogenous enzymes following hydrolysis of cell wall arabinoxylans and also reduced the ANFs of various polysaccharides (Kocher et al., 2003; Meng et al., 2005; Francesch and Geraert, 2009).

Carbohydrases hydrolyze carbohydrates to release not only D-glucose, but also other sugars such as D-xylose and L-arabinose, which have been shown to have to have low nutritional values for both poultry and swine (Schutte, 1990; Schutte, 1991; Peng et al., 2004). Decreased absorptive capacity and/or increased urinary excretion may be the cause of the decreased energy value of pentose sugars when increasing in dietary levels (Peng et al., 2004). While L-arabinose was passively absorbed in the small intestines, D-xylose shared the same mechanism of transport as D-glucose. At a low concentration, the priority of absorption rates of sugars is D-glucose > D-xylose > L-arabinose. In contrast, at a high concentration, the priority of absorption was D-xylose > D-glucose > L-arabinose as described by Peng et al. (2004).

Non-Starch Polysaccharides

Many NSPs, such as D-xylose and L-arabinose, have ANFs that can cause negative effects during digestion. ANFs can be described as any compound that interfered with the intake, availability, or metabolism of nutrients in the animal. ANFs have been mainly found in legumes, but they were present in cereal grains as well. There have been two models proposed to describe the ANFs of NSPs. The first proposed model was encapsulation, where the NSPs coated a substrate and inhibited the access of digestive enzymes to the starch, fat, or protein substrate. The second proposed model was that the presence of NSPs in the intestinal lumen increases the viscosity of the intestinal contents that led to decreased nutrient absorption as well as increased endogenous enzyme secretions (Williams et al., 1997; Cowieson, 2010; Aftab, 2012; Zhang et al., 2012).

There has been growing focus regarding ANFs of NSPs in monogastric feeds due to the increasing popularity for inclusion of high NSP ingredients. These NSPs existed in a form that was indigestible to monogastrics such as pigs and poultry. It was well known that starch can be hydrolyzed by pancreatic α-amylase but NSPs were not able to be digested by pancreatic enzymes (Schutte, 1991; Williams et al., 1997) and could only be utilized after fermentation by gut bacteria usually at negligible amounts (Schutte, 1990). Thus, this fermentation process became an expenditure resulting in considerable energy losses. The low digestibility of NSPs may also cause a depression of the digestibility of other dietary components (Schutte, 1991; Williams et al., 1997; Cowieson, 2005). They have been shown to depress protein digestion and performance of both pigs and poultry (Huisman, 1990;

Schutte, 1991). Studies have also demonstrated a decreased average daily BW gain as well as a detrimental effect on feed conversion ratio (FCR) when there was an increased percentage of NSPs, such as xylose, in poultry feed (Schutte, 1990; Williams et al., 1997; Peng et al., 2004). The negative biological effects caused by ANFs have been further complicated due to its varying range of effects depending on the animal species and age as well.

Formulating Feed Using Carbohydrases

Ideally, feed should be formulated to enhance the action of the exogenous carbohydrase(s) chosen. There have been several factors to consider when including carbohydrase enzymes to feed in order to insure a certain efficacy. The most important factor was substrate concentration which was influential in the responsiveness of feed to enzyme supplementation. The balance of nutrients in the diet must also be appropriate for the improved digestion and absorption of nutrients to be transferred adequately into productivity and/or performance (Cowieson and Ravindran, 2008). Other factors have included presence of inhibitors, water content, optimal temperature, optimal pH, and specific degradation site in the molecule.

Post-Hatching Digestive Physiology

Vieira and Moran (1999) described the adaptation process of the hatching chick transitioning from maternal nutrition of a high fat yolk sac source to a high starch exogenous feed as a several day process. In the post-hatching chick, the composition of the exogenous feed has been reported to influence the secretion of endogenous enzymes (Vieira et al., 2015). An

increased endogenous enzyme secretion combined with an increased size of digestive organs has been reported to be necessary to accommodate the rapid increase in feed intake during this period (Vieira et al., 2015). This study also suggested that amylase secretion by the pancreas was low at 4 d of age but achieved a plateau between 7 and 21 d of age. These authors proposed that the immature pancreas during the post-hatching period might limit intestinal starch digestion, and, consequently, early growth rate. During the first weeks of life, the broilers' digestive capacity has been reported to not be fully developed as endogenous enzymatic activity was generally very low, and physiological development of the gastrointestinal tract was not complete (Williams et al., 1997; Aftab, 2012). This correlated to a lower digestibility of energy obtained from cereals and SBM in the initial 2 wk post-hatching compared to 3 wk of age (Aftab, 2012). The efficacies of carboyhydrases in the post-hatching and young chick have still not been clearly understood. Although, it was logical to assume that the starch substrate proportionally increased in exogenous feed as bird age increased so that it would be easier to demonstrate an apparent improvement in live performance or nutrient digestibility at an older age.

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CHAPTER 1

Abstract

The effects of xylanase supplementation on feed intake, body weight (BW), and feed conversion ratio (FCR) of male broilers reared to 41 d of age were evaluated in this study. A total of 2,304 Ross 344 × Ross 708 male broilers were assigned to 9 treatments with 8 replicate pens of 32 birds each in a complete block design. The 9 diets included a positive control (PC), a negative control (NC) with a low metabolizable energy (ME) density, and 7 NC diets amended with xylanase in doses from 125 to 2000 IU. All diets contained wheat, DDGS, corn, and soybean meal (SBM). The NC and NC+xylanase amended starter diets contained 1.5% added fat with 1% added in the mixer and 0.5% added by post pellet liquid application (PPLA), whereas the NC and NC+xylanase amended grower and finisher diets contained 1% fat added in the mixer only. The PC starter diet contained 4.05% total added fat with 1% added to the mixer and 3.05% added by PPLA. The PC grower and PC finisher diets contained 3% total fat (1% mixer + 2% PPLA). Male BW, feed consumption, and mortality did not differ ($P>0.05$). The PC diet broilers exhibited improved FCR ($P<0.01$) from 15-35 d and 0-35 d of age compared with those fed the NC and NC+xylanase diets, which confirmed the lower ME density of the NC diet and suggested a relative lack of ME releasing activity of the xylanase. However, the ileal digestibility values for the PC, NC, and NC+xylanse amended diets calculated on a dry matter basis differed significantly ($P<0.01$). Xylanase demonstrated a curvilinear dose response that could be discerned by inspection in this experiment as the NC+500 and NC+1000 diets exhibited an improved digestibility ($P<0.01$)

relative to the NC+2000, PC, and NC diets with the remaining diets being intermediate. The improvement relative to the NC suggested that these two diets were within the optimum enzyme activity dosage range. It was surmised that the nature of the dietary formulation may have created detrimental competition between hexoses and pentoses for absorption at greater xylanase inclusion and low dietary added fat may have minimized micelle formation required for optimum absorption. These circumstances may have precluded the ability to demonstrate a significant difference in live performance among the xylanase enzyme dosages.

Keywords: xylanase, wheat, corn, digestibility, PPLA

Introduction

The use of exogenous carbohydrase enzymes in corn based broiler diets has become increasingly popular in recent years as feed costs have increased. Continued popularity of corn in livestock diets, specifically poultry, along with the growth of the corn ethanol industry has resulted in greater quantities of distillers dried grains with solubles (DDGS) entering poultry feed formulations as a source of protein, phosphorus, and energy to replace more expensive ingredients such as corn and soybean mean (SBM). The global production of corn in 2014 was 1,021 million MT, far exceeding other cereal crops including wheat at 728 million MT (FAOSTAT Database, 2014). However, DDGS has been reported to possess poorer amino acid digestibility (Aldeola and Ilelegi, 2009), especially with respect to lysine and tryptophan (Baker, 2009), and increased non-starch polysaccharides (NSPs) that has historically limited its inclusion in poultry diets (Choct, 2006).

Soluble NSPs in a number of grains such as wheat have resulted in poor growth of broilers in addition to decreased nutrient utilization, wet litter, and stick droppings (Choct and Annison, 1990). The depression in digestion of nutrients due to NSPs has been measured as a decreased apparent metabolizable energy (AME), which probably led to the poor broiler live performance (Bedford and Schulze, 1998; Hetland et al., 2004). The purpose of using exogenous carbohydrases, such as xylanase, in broiler diets has been to digest cell walls and reduce gut viscosity that improved nutrient diffusion and absorption (Choct and Cadogan, 2001; Choct, 2006). The magnitude of response has been generally less for xylanases added to corn-based diets, which was probably due to the lower concentration of soluble NSPs in corn (1g/kg) as compared to wheat (24 g/kg) (Bedford, 2000; Choct, 2006). Hydrolysis of these NSPs has not only released glucose, but also released other sugars such as xylose and arabinose. The nutritive value of these other sugars has been reported to be very low in poultry (Schutte, 1990; Wagh and Waibel, 1966, 1967a). Studies have shown negative dose-dependent responses to increased levels of D-xylose and L-arabinose in poultry diets (Schutte, 1990; Peng et al., 2004).

The effects of these pentose sugars from a xylanase-extracted complex wheat and corn based diet have needed to be examined more closely. Thus, the objectives of this study were to determine whether supplementation of xylanase, in replacement of fat, would improve live performance and digestibility of broilers fed a complex, low energy diet despite potential negative implications of pentose sugars.

Materials and Methods

Bird Management and Husbandry. The care of the animals used in this experiment complied with the Guide for Care and Use of Animals in Agricultural Research and Teaching (FASS, 2010). In this experiment, 2,304 Ross 344 x 708 SF strain broiler chicks were hatched from eggs produced and incubated at the research site. The chicks were feather-sexed with the males separated and permanently identified with neck tags. The chicks were placed in a translucent curtain-sided, heated, and fan-ventilated broiler house. There were 32 chicks placed per pen in a manner as to account for any of the 16 breeder pen differences (2 broiler chicks per breeder pen) with 72 pens in total. Each pen was 1.2 m wide by 3.6 m long, which provided a stocking density of 7.41 chicks/m^2. Each pen was equipped with one Plasson bell-type drinker and two hanging tube feeders. Each pen was assigned to one of 9 dietary treatments with 8 replicate pens per treatment. The birds were raised on used litter that was covered with new wood shavings with *ad libitum* access to water and feed throughout the study. Each pen contained a supplemental chick font until 7 d of age in addition to three supplemental feeder flats to 7 d of age, two flats to 10 d of age, and one flat to 14 d. Feeders were shaken once per day from 0 to 14 d, twice per day from 14 to 35 d, and three times per day after 35 d to prevent variation in feed intake due to any feed flow characteristics. The lighting program began with 23 h of light from 0 to 7 d, 22 h of light to 14 d, 20 h of light to 21 d, and natural light from 22 d of age. The litter temperature at placement was 35 to 36°C and thereafter the house ambient temperature was maintained at 31 to 33°C from 2 to 7 d,

29.4°C from 8 to 14 d, 26.7°C from 15 to 21 d, and ambient thereafter. The birds had a budget of 0.9 kg starter feed/bird, 2.7 kg grower feed/bird, and 4.1 kg finisher feed/bird.

Experimental Diets and Feed Manufacturing. Diets were formulated to meet or exceed NRC requirements (NRC, 1994) on a total amino acid basis that reflected a similar study being conducted at Texas A&M University at the same time and sponsored by the same xylanase supplier with the same enzyme and were composed mainly of wheat, DDGS, corn, and SBM. The starter, grower, and finisher diets are depicted in Tables II-1, 2, and 3, respectively. The fine DDGS were obtained by grinding coarse DDGS using a 60 HP Roskamp Champion (Waterloo, IA) model 15 x 22 split screen hammermill equipped with a #4/4 size screen. Corn was ground using the same hammermill equipped with a #6/6 screen size (~550 μm). Diets were mixed using a 2-ton counterpoise mixer (Model TRDB126-0604Hayes & Stolz, Fort Worth, TX). Diets were then pelleted at 180-185°F using a 30 HP pellet mill (Model 1112-2, California Pellet Mill, Crawfordsville, IN) equipped with a 3.5 mm x 28 mm pellet die. Post pellet liquid fat application (PPLA) was carried out in a 500-pound double ribbon mixer (Model SRM-304, Scott Equipment Co., New Prague, MN). Pellet quality (Tables II-4, 5, and 6) was assessed on the day of feed manufacture by the tumbling-box method according to ASAE Standard S269.4 to determine pellet durability index (PDI). Modified PDI was determined by the addition of three 19.1 mm hexagonal nuts to the tumble-box chamber. Two samples were averaged to determine both standard and modified PDI values for each diet. An indigestible marker containing 0.5% titanium dioxide was added to the

finisher feed for determination of protein digestibility, dry matter, and apparent metabolizable energy (AME_n).

Data Collection. All data were recorded on a pen basis. Feed consumption and BW by pen were subsequently recorded at 14, 35, and 41 d of age. This approximately corresponded to the feeding periods of the starter, grower, and finisher diets. Mortalities were removed from their pen, weighed, and recorded twice daily. Adjusted feed conversion ratio (FCR) was calculated by adding the BW of the dead birds to the BW of live birds in each pen, respectively. At 42 d of age, ileal digesta was collected from within 2 cm of the posterior of Meckel's diverticulum to within 2 cm of the anterior of the ileal-cecal junction for protein and fat digestibility analysis.

Laboratory Analyses. Feed and ileal digesta samples were analyzed for moisture, crude protein (CP) (N x 6.25), ash, and fiber by "proximate analysis" (Carolina Analytical Laboratory, Bear Creek, NC 27207). Titanium dioxide was determined by the method of Myers et al. (2004).

Calculations of apparent metabolizable energy corrected for N content (AMEn).

The AME_n values were determined using the following equation:

$$AME_n = GE_{diet} - [GE_{excreta} * (Marker_{diet} / Marker_{excreta})] - (8.22 * (CP_{excreta/6.25})$$

Where:

GE = gross energy [kcal/kg of sample (diet or excreta)]

Marker = concentration of marker in diet and excreta.

Calculation of ileal protein and fat digestibility. Ileal protein and fat digestibility were calculated using the following equation (Fan et al., 1994; Marty et al., 1994):

ADD = 100% - [(ID x AF) / (AD x IF)] * 100%

Where:

ADD = Crude protein or fat digestibility in diet (%)

ID = Marker concentration in diet (%)

AF = Crude protein or fat concentration in ileal digesta (%)

AD = Crude protein or fat concentration in diet (%)

IF = Marker concentration in ileal digesta (%)

Statistical Analyses. The experiment was analyzed as a randomized complete block design to identify main effects. The experiment was an arrangement of 9 diet types. The block design was based on location within the house. The experimental unit for the statistical analysis of the broiler live performance data was pen. All data was analyzed using SAS 9.4 with means partitioned by LSMeans and differences determined through the use of PROC GLM (SAS, 2012). Statements of statistical differences were based upon $P < 0.05$ unless otherwise indicated.

Results and Discussion

Dietary Analysis. The calculated and analyzed nutrient compositions of the diets are shown in Tables II-1, II-2, and II-3. The analyzed values approximated the formulated values and the difference in crude fat between the PC and NC diets reflected the addition of fat to achieve a greater ME density in the PC diet.

Pellet Quality. The pellet quality expressed as PDI for the starter diet before crumbling, grower, and finisher diets is shown in Tables II-4, II-5, and II-6. The NC diets were formulated with only 1% added poultry fat in the mixer as Auttawong (2015) reported that the optimum mixer fat addition was approximately 1.00-1.50% in order to maintain pellet quality, especially when diets were manufactured with any coarse corn as was the case in the present study. Thus, the NC diets were intended to produce pellets of adequate quality but relatively low ME value in order to provide the added enzyme an opportunity to demonstrate the ability to improve live performance through the release of additional ME. The addition of extra fat to produce the PC could have decreased pellet quality (Briggs et al., 1999) as fat has functioned as a lubricant in the pellet die and may have decreased die friction required for pellet binding so added fat in excess of 1% was added with PPLA (Plavnik et al., 1997) to avoid this confounding factor. As a result of these practices, large differences were not apparent except for the NC+250 finisher diet that exhibited a, for some undetermined reason, markedly decreased standard and modified PDI (Table II-6). However, this did not appear to negatively affect feed intake or BW but may have explained the slightly poorer than expected

FCR observed from 36-41 and 0-41 d of age (Table II-9) as well as the failure to demonstrate the expected step increase in ileal digestibility for the NC+250 when compared to the NC+125. The NC+250 should have been intermediate between the NC+125 and NC+500 diets in terms of digestibility (Table II-11) as well as have a similar FCR as those two diets (Table II-9), which failed to be the case. Thus, the data after 35 d of age for the NC+250 diet probably should be discounted.

Live Performance. Enzyme supplementation of diets did not significantly affect BW at 14, 35, or 41 d of age (Table II-7). Differences in feed consumption approached significance only during the 15 to 35 d growth period ($P<0.10$, Table II-8) where the higher ME (greater added fat) PC diet exhibited the lowest feed intake during this period when compared to all other dietary treatments. The reduced feed intake of the PC diet was explained by the additional fat providing more ME than did the NC diet and all NC amended diets. Brazilian investigators (Maiorka et al., 1997) demonstrated that the sensitivity of a broiler to increased dietary ME in terms of decreasing daily feed consumption did not become apparent until after 14 d of age, as was observed in the present study.

There was a significant enzyme effect on FCR during the 15 to 35 d, 0 to 35 d, and cumulative (0 to 41 d) growth periods ($P<0.01$, $P<0.01$, and $P<0.10$, respectively, Table II-9). During the 15 to 35 d and 0 to 35 d periods, the PC diet exhibited improved FCR compared to all other treatments as might be expected for a higher ME, higher fat diet (Maiorka et al., 1997). The lack of difference between the NC and NC+xylanase amended

diets suggested a relative lack of ME releasing activity of xylanase. Barasch et al. (Diss. NC St. Uni., 2015) demonstrated that xylanase functioned significantly better in the presence of 5.7% dietary fat as compared to 2% poultry fat at least partially due to altered micelle formation. Another study detected optimal interactions when xylanase was added to normal AME diets versus low AME diets (Karimi, 2013), which suggested that adding xylanase to low AME diets may have actually blocked intestinal fat digestion and absorption. The additional fat could have also been responsible for slowed gut passage rate, which would have provided more time for enzymes to function (Hunt and Knox, 1968; Li and Sauer, 1994). The percentage added fat in the current study for the NC diets was only 1% for the grower and finisher diets in an effort to main excellent pellet quality. This low added fat inclusion could have interfered with the ability of the enzyme to demonstrate an ability to release ME from the particular diets employed in the present study. Cumulatively at 41 d, the FCR of the PC approached significance compared to all other treatments (P<0.10). The NC diet amended with 250 IU, 2000 IU, and the 1000 IU reference xylanase (NC+1000REF) exhibited the numerically poorest FCR (P<0.10). The NC diet amended with 125 IU, 500 IU, 1000 IU, and 1500 IU of xylanase were intermediate to the PC and NC diets. Mortality was not significantly different between treatments for the duration of the study (Table II-10).

Ileal Digestibility. Mean ileal digestibility values calculated on a dry matter basis differed significantly (P<0.01) between treatments, which upon inspection described a curvilinear dose response (Table II-11) as the NC+500 and NC+1000 diets exhibited an improved digestibility (P<0.01) relative to the NC+2000, PC, and NC diets with the remaining diets

being intermediate. The improvement relative to the NC suggested that the NC+500 and NC+1000 diets were within the optimum enzyme activity dosage range. It was possible that the nature of the dietary formulation may have created detrimental competition between hexoses and pentoses for absorption at greater xylanase inclusions due to the fact the glucose and xylose have been shown to share the same transporter for absorption (Schutte, 1990; Schutte, 1991; Peng et al., 2004). Low dietary fat may have also minimized micelle formation required for optimum absorption of nutrients (Karimi, 2013; Barasch, 2015). These circumstances may have precluded the ability to demonstrate a significant difference in live performance among xylanase enzyme dosages. Obviously, the NC+1000REF xylanase exhibited less ME releasing activity than did the test xylanase in this study. The low percentage ileal digestibility of the PC diet may have been explained by the additional ME being fat *versus* carbohydrate in the NC-xylanase diets.

Diets supplemented with 250 IU, 500 IU, 1000 IU, and 1500 IU of xylanase exhibited the greatest percentage ileal digestibility when compared to the other treatments. The 500 IU, 1000 IU, and 1500 IU diets also exhibited numerically better FCR. The highest dose of 2000 IU of xylanase as well as the NC diet produced an obviously low percentage ileal digestibility. The reference xylanase at the 1000 IU dose as well as the PC diet produced the lowest percentage ileal digestibility. The low percentage ileal digestibility of the PC diet may be explained by the additional fat providing enough ME, thus decreasing the need for improved digestion of feed for nutrients.

Table II-1. Broiler starter diet fed from 0-14 d of age.

Ingredients	Starter Diet		
	PC[1]	NC[2]	NC+Xyl[3]
	(%)		
Wheat	30.00	30.00	30.00
Soybean meal (48% CP)	26.17	24.90	24.90
Corn	18.45	21.37	21.37
DDGS (28% CP)	15.00	15.00	15.00
Poultry fat	4.05	1.50	1.50
Limestone	1.01	1.02	1.02
Monocalcium phosphate (21% P)	1.87	1.86	1.86
Salt	0.49	0.49	0.49
DL-Methionine	0.20	0.20	0.20
L-Lysine	0.27	0.28	0.28
L-Threonine	0.16	0.16	0.16
Choline chloride (60%)	0.20	0.20	0.20
Vitamin premix[4]	0.05	0.05	0.05
Mineral premix[5]	0.20	0.20	0.20
Selenium premix[6]	0.03	0.03	0.03
Inert filler[7]	0.80	0.80	0.80
Sand[8]	1.00	1.89	1.89
Xylanase[9]	-	-	+
Coccidiostat[10]	0.05	0.05	0.05
Total	100.00	100.00	100.00
Calculated nutrient content			
Crude protein	22.00	22.00	22.00
Calcium	0.90	0.90	0.90
Available phosphorus	0.45	0.45	0.45
Lysine	1.25	1.25	1.25
Methionine	0.56	0.56	0.56
Threonine	0.85	0.85	0.85
Methionine + cysteine	0.91	0.91	0.91
Sodium	0.22	0.22	0.22
Metabolizable energy (kcal/g)	2.76	2.61	2.61

Table II-1. Continued

Analyzed nutrient content			
Moisture	12.57	12.54	12.48
Fat	3.63	3.78	3.93
Protein	21.97	21.77	22.64
Fiber	3.00	3.00	2.64
Ash	7.48	7.92	8.75
Phosphorus	0.77	0.71	0.78

[1]PC: Positive control of starter diet with 4.05% fat followed by grower and finisher diets with 3.00% fat inclusions.
[2]NC: Negative control of starter diet with 1.50% fat followed by grower and finisher diets with 1.00% fat inclusions.
[3]NC+Xyl: Xylanase enzyme (Verenium®) was added at 125 IU for NC+125, 250 IU for NC+250, 500 IU for NC+500, 1000 IU for NC+1000, 1500 IU for NC+1500, and at 2000 IU for NC+2000. A reference xylanase enzyme was added at 1000 IU for NC+1000REF.
[4]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.
[5]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt, 1 mg.
[6]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.
[7]Vermiculite was used as inert filler at similar density as corn.
[8]Sand was used as filler at similar density as monocalcium phosphate and limestone.
[9]Indicates the lack of (-) or addition of (+) xylanase enzyme in the diet at the specified dosages at expense of inert filler.
[10]Monensin sodium was included at 99 mg/kg.

Table II-2. Broiler grower diet fed from 15-35 d of age.

Ingredients	Grower Diet		
	PC[1]	NC[2]	NC+Xyl[3]
	(%)		
Wheat	33.00	33.00	33.00
Soybean meal (48% CP)	20.80	20.80	20.80
Corn	26.01	26.01	26.01
DDGS (28% CP)	13.00	13.00	13.00
Poultry fat	3.00	1.00	1.00
Limestone	0.94	0.94	0.94
Dicalcium phosphate (18.5% P)	1.60	1.60	1.60
Salt	0.50	0.50	0.50
DL-Methionine	0.13	0.13	0.13
L-Lysine	0.26	0.26	0.26
L-Threonine	0.14	0.14	0.14
Choline chloride (60%)	0.20	0.20	0.20
Vitamin premix[4]	0.05	0.05	0.05
Mineral premix[5]	0.20	0.20	0.20
Selenium premix[6]	0.03	0.03	0.03
Inert filler[7]	0.10	0.10	+
Sand[8]	-	2.00	2.00
Xylanase[9]	-	-	+
Coccidiostat[10]	0.05	0.05	0.05
Total	100.00	100.00	100.00
Calculated nutrient content			
Crude protein	20.14	20.14	20.14
Calcium	0.80	0.80	0.80
Available phosphorus	0.40	0.40	0.40
Lysine	1.11	1.11	1.11
Methionine	0.46	0.46	0.46
Threonine	0.77	0.77	0.77
Methionine + cysteine	0.79	0.79	0.79
Sodium	0.22	0.22	0.22
Metabolizable energy (kcal/g)	2.78	2.66	2.66

Table II-2. Continued

Analyzed nutrient content			
Moisture	11.78	11.99	11.86
Fat	3.80	3.66	3.74
Protein	19.92	20.17	20.21
Fiber	2.80	2.70	2.71
Ash	4.97	6.98	7.27
Phosphorus	0.60	0.61	0.64

[1]PC: Positive control of starter diet with 4.05% fat followed by grower and finisher diets with 3.00% fat inclusions.
[2]NC: Negative control of starter diet with 1.50% fat followed by grower and finisher diets with 1.00% fat inclusions.
[3]NC+Xyl: Xylanase enzyme (Verenium®) was added at 125 IU for NC+125, 250 IU for NC+250, 500 IU for NC+500, 1000 IU for NC+1000, 1500 IU for NC+1500, and at 2000 IU for NC+2000. A reference xylanase enzyme was added at 1000 IU for NC+1000REF.
[4]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.
[5]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt, 1 mg.
[6]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.
[7]Vermiculite was used as inert filler at similar density as corn.
[8]Sand was used as filler at similar density as dicalcium phosphate and limestone.
[9]Indicates the lack of (-) or addition of (+) xylanase enzyme in the diet at the specified dosages at expense of inert filler.
[10]Monensin sodium was included at 99 mg/kg.

Table II-3. Broiler finisher diet fed from 36-41 d of age.

Ingredients	Finisher Diet		
	PC[1]	NC[2]	NC+Xyl[3]
	(%)		
Wheat	32.00	32.00	32.00
Soybean meal (48% CP)	17.20	17.20	17.20
Corn	30.15	30.15	30.15
DDGS (28% CP)	13.00	13.00	13.00
Poultry fat	3.00	1.00	1.00
Limestone	0.95	0.95	0.98
Dicalcium phosphate (18.5% P)	1.64	1.64	1.64
Salt	0.50	0.50	0.50
DL-Methionine	0.09	0.09	0.09
L-Lysine	0.26	0.26	0.26
L-Threonine	0.09	0.09	0.09
Choline chloride (60%)	0.20	0.20	0.20
Vitamin premix[4]	0.05	0.05	0.05
Mineral premix[5]	0.20	0.20	0.20
Selenium premix[6]	0.03	0.03	0.03
Inert filler[7]	0.10	0.10	+
Sand[8]	-	2.00	2.00
Xylanase[9]	-	-	+
Titanium dioxide[10]	0.50	0.50	0.50
Coccidiostat[11]	0.05	0.05	0.05
Total	100.00	100.00	100.00
Calculated nutrient content			
Crude protein	18.5	18.5	18.5
Calcium	0.80	0.80	0.80
Available phosphorus	0.40	0.40	0.40
Lysine	1.01	1.01	1.01
Methionine	0.40	0.40	0.40
Threonine	0.67	0.67	0.67
Methionine + cysteine	0.71	0.71	0.71
Sodium	0.22	0.22	0.22
Metabolizable energy (kcal/g)	2.80	2.68	2.68

Table II-3. Continued

Analyzed nutrient content			
Moisture	12.61	11.89	12.02
Fat	4.90	3.85	3.90
Protein	19.01	18.59	18.86
Fiber	2.80	2.90	2.77
Ash	5.80	7.34	7.89
Phosphorus	0.65	0.60	0.68

[1]PC: Positive control of starter diet with 4.05% fat followed by grower and finisher diets with 3.00% fat inclusions.
[2]NC: Negative control of starter diet with 1.50% fat followed by grower and finisher diets with 1.00% fat inclusions.
[3]NC+Xyl: Xylanase enzyme (Verenium®) was added at 125 IU for NC+125, 250 IU for NC+250, 500 IU for NC+500, 1000 IU for NC+1000, 1500 IU for NC+1500, and at 2000 IU for NC+2000. A reference xylanase enzyme was added at 1000 IU for NC+1000REF.
[4]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.
[5]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt, 1 mg.
[6]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.
[7]Vermiculite was used as inert filler at similar density as corn.
[8]Sand was used as filler at similar density as dicalcium phosphate and limestone.
[9]Indicates the lack of (-) or addition of (+) xylanase enzyme in the diet at the specified dosages at expense of inert filler.
[10]Titanium dioxide was included as an indigestible marker to determine digestibility.
[11]Monensin sodium was included at 99 mg/kg.

Table II-4. Broiler starter diet standard and modified pellet durability indexes (PDIs).

Dietary Treatments	Standard PDI[11]	Modified PDI[12]
[1]NC+125	91.1 [bc]	78.9 [ABC]
[2]NC+250	90.9 [c]	78.0 [BC]
[3]NC+500	91.7 [abc]	79.6 [ABC]
[4]NC+1000	90.7 [c]	75.3 [CD]
[5]NC+1500	92.9 [a]	81.5 [AB]
[6]NC+2000	92.5 [a]	82.3 [A]
[7]NC+1000	92.1 [ab]	77.7 [BC]
[8]PC	91.8 [abc]	77.4 [C]
[9]NC	90.9 [c]	73.5 [D]
SEM[10]	0.06	0.67
P-value	0.015	0.010

[a-c] Means in a column that possess the same superscripts do not differ significantly ($P \leq 0.05$).
[A-D] Means in a column that possess the same superscripts do not differ significantly ($P \leq 0.01$).
[1] NC+125: Starter, grower, and finisher diets supplemented with 125 IU of xylanase to the negative control.
[2] NC+250: Starter, grower, and finisher diets supplemented with 250 IU of xylanase to the negative control.
[3] NC+500: Starter, grower, and finisher diets supplemented with 500 IU of xylanase to the negative control.
[4] NC+1000: Starter, grower, and finisher diets supplemented with 1000 IU of xylanase to the negative control.
[5] NC+1500: Starter, grower, and finisher diets supplemented with 1500 IU of xylanase to the negative control.
[6] NC+2000: Starter, grower, and finisher diets supplemented with 2000 IU of xylanase to the negative control.
[7] NC+-1000: Starter, grower, and finisher diets supplemented with 1000 IU of a reference xylanase to the negative control.
[8] PC: Positive control of starter diet with 4.05% fat followed by grower and finisher diets with 3.00% fat inclusions.
[9] NC: Negative control of starter diet with 1.50% fat followed by grower and finisher diets with 1.00% fat inclusions.
[10] Standard error of mean (SEM) for n=2 samples of 9 dietary treatments.
[11] Standard PDI was determined by the tumbling-box method according to ASAE Standard S269.4.
[12] Modified PDI was determined by the addition of three 19.1 mm hexagonal nuts to the tumble-box chamber.

Table II-5. Broiler grower diet standard and modified pellet durability indexes (PDIs).

Dietary Treatments	Standard PDI[11]	Modified PDI[12]
[1]NC+125	88.5 [xyz]	68.1 [xyz]
[2]NC+250	86.2 [yz]	67.5 [yz]
[3]NC+500	85.1 [z]	65.5 [z]
[4]NC+1000	88.2 [xyz]	70.1 [wxyz]
[5]NC+1500	90.4 [x]	73.8 [w]
[6]NC+2000	90.0 [xy]	72.8 [wx]
[7]NC+1000	88.8 [xyz]	71.0 [wxy]
[8]PC	90.9 [x]	70.9 [wxy]
[9]NC	87.4 [xyz]	68.9 [xyz]
SEM[10]	0.70	1.04
P-value	0.096	0.055

[w-z] Means in a column that possess the same superscripts do not differ approaching significance ($P \leq 0.10$).
[1] NC+125: Starter, grower, and finisher diets supplemented with 125 IU of xylanase to the negative control.
[2] NC+250: Starter, grower, and finisher diets supplemented with 250 IU of xylanase to the negative control.
[3] NC+500: Starter, grower, and finisher diets supplemented with 500 IU of xylanase to the negative control.
[4] NC+1000: Starter, grower, and finisher diets supplemented with 1000 IU of xylanase to the negative control.
[5] NC+1500: Starter, grower, and finisher diets supplemented with 1500 IU of xylanase to the negative control.
[6] NC+2000: Starter, grower, and finisher diets supplemented with 2000 IU of xylanase to the negative control.
[7] NC+-1000: Starter, grower, and finisher diets supplemented with 1000 IU of a reference xylanase to the negative control.
[8] PC: Positive control of starter diet with 4.05% fat followed by grower and finisher diets with 3.00% fat inclusions.
[9] NC: Negative control of starter diet with 1.50% fat followed by grower and finisher diets with 1.00% fat inclusions.
[10] Standard error of mean (SEM) for n=2 samples of 9 dietary treatments.
[11] Standard PDI was determined by the tumbling-box method according to ASAE Standard S269.4.
[12] Modified PDI was determined by the addition of three 19.1 mm hexagonal nuts to the tumble-box chamber.

Table II-6. Broiler finisher diet standard and modified pellet durability indexes (PDIs).

Dietary Treatments	Standard PDI[11]	Modified PDI[12]
[1]NC+125	88.5 [A]	69.9 [AB]
[2]NC+250	73.1 [B]	52.2 [C]
[3]NC+500	88.2 [A]	69.0 [B]
[4]NC+1000	90.1 [A]	70.3 [AB]
[5]NC+1500	93.0 [A]	75.3 [A]
[6]NC+2000	91.3 [A]	72.7 [AB]
[7]NC+1000	90.9 [A]	72.4 [AB]
[8]PC	92.1 [A]	72.6 [AB]
[9]NC	90.4 [A]	75.0 [A]
SEM[10]	1.54	1.59
P-value	<0.001	<0.001

[A-C] Means in a column that possess the same superscripts do not differ significantly ($P \leq 0.01$).
[1] NC+125: Starter, grower, and finisher diets supplemented with 125 IU of xylanase to the negative control.
[2] NC+250: Starter, grower, and finisher diets supplemented with 250 IU of xylanase to the negative control.
[3] NC+500: Starter, grower, and finisher diets supplemented with 500 IU of xylanase to the negative control.
[4] NC+1000: Starter, grower, and finisher diets supplemented with 1000 IU of xylanase to the negative control.
[5] NC+1500: Starter, grower, and finisher diets supplemented with 1500 IU of xylanase to the negative control.
[6] NC+2000: Starter, grower, and finisher diets supplemented with 2000 IU of xylanase to the negative control.
[7] NC+-1000: Starter, grower, and finisher diets supplemented with 1000 IU of a reference xylanase to the negative control.
[8] PC: Positive control of starter diet with 4.05% fat followed by grower and finisher diets with 3.00% fat inclusions.
[9] NC: Negative control of starter diet with 1.50% fat followed by grower and finisher diets with 1.00% fat inclusions.
[10] Standard error of mean (SEM) for n=2 samples of 9 dietary treatments.
[11] Standard PDI was determined by the tumbling-box method according to ASAE Standard S269.4.
[12] Modified PDI was determined by the addition of three 19.1 mm hexagonal nuts to the tumble-box chamber.

Table II-7. Broiler male body weight as affected by age and addition of xylanase to starter, grower, and finisher diets.

Dietary Treatments	Age (d)			
	0	14	35	41
	(g)			
[1]NC+125	37.6	433	2213	2893
[2]NC+250	37.7	439	2211	2901
[3]NC+500	37.6	442	2234	2923
[4]NC+1000	37.8	437	2213	2879
[5]NC+1500	37.8	434	2194	2873
[6]NC+2000	37.8	436	2210	2859
[7]NC+1000	37.9	428	2202	2879
[8]PC	37.7	434	2205	2925
[9]NC	37.9	429	2175	2854
SEM[10]	0.05	1.41	7.19	9.96
P-Value	0.846	0.387	0.827	0.665

[1] NC+125: Starter, grower, and finisher diets supplemented with 125 IU of xylanase to the negative control.
[2] NC+250: Starter, grower, and finisher diets supplemented with 250 IU of xylanase to the negative control.
[3] NC+500: Starter, grower, and finisher diets supplemented with 500 IU of xylanase to the negative control.
[4] NC+1000: Starter, grower, and finisher diets supplemented with 1000 IU of xylanase to the negative control.
[5] NC+1500: Starter, grower, and finisher diets supplemented with 1500 IU of xylanase to the negative control.
[6] NC+2000: Starter, grower, and finisher diets supplemented with 2000 IU of xylanase to the negative control.
[7] NC+-1000: Starter, grower, and finisher diets supplemented with 1000 IU of a reference xylanase to the negative control.
[8] PC: Positive control of starter diet with 4.05% fat followed by grower and finisher diets with 3.00% fat inclusions.
[9] NC: Negative control of starter diet with 1.50% fat followed by grower and finisher diets with 1.00% fat inclusions.
[10] Standard error of mean (SEM) for n=2 samples of 9 dietary treatments.

Table II-8. Broiler male feed intake as affected by age and addition of xylanase to starter, grower, and finisher diets.

Dietary Treatments	Age (d)				
	0 - 14	15 - 35	36 - 41	0 - 35	0 - 41
	(g/bird)				
[1]NC+125	629	2989 [x]	1300	3628	4989
[2]NC+250	634	2977 [x]	1342	3627	5061
[3]NC+500	626	3003 [x]	1308	3640	5006
[4]NC+1000	626	2965 [x]	1304	3601	4970
[5]NC+1500	619	2938 [x]	1273	3562	4928
[6]NC+2000	629	2952 [x]	1309	3587	4939
[7]NC+1000	611	2999 [x]	1307	3627	4964
[8]PC	616	2872 [y]	1298	3501	4916
[9]NC	626	2940 [x]	1350	3571	5012
SEM[10]	2.7	9.9	11	11	20
P-Value	0.601	0.088	0.867	0.118	0.784

[x,y] Means in a column that possess the same superscripts do not differ approaching significance ($P \leq 0.10$).
[1] NC+125: Starter, grower, and finisher diets supplemented with 125 IU of xylanase to the negative control.
[2] NC+250: Starter, grower, and finisher diets supplemented with 250 IU of xylanase to the negative control.
[3] NC+500: Starter, grower, and finisher diets supplemented with 500 IU of xylanase to the negative control.
[4] NC+1000: Starter, grower, and finisher diets supplemented with 1000 IU of xylanase to the negative control.
[5] NC+1500: Starter, grower, and finisher diets supplemented with 1500 IU of xylanase to the negative control.
[6] NC+2000: Starter, grower, and finisher diets supplemented with 2000 IU of xylanase to the negative control.
[7] NC+-1000: Starter, grower, and finisher diets supplemented with 1000 IU of a reference xylanase to the negative control.
[8] PC: Positive control of starter diet with 4.05% fat followed by grower and finisher diets with 3.00% fat inclusions.
[9] NC: Negative control of starter diet with 1.50% fat followed by grower and finisher diets with 1.00% fat inclusions.
[10] Standard error of mean (SEM) for n=2 samples of 9 dietary treatments.

Table II-9. Broiler male feed conversion ratio adjusted for mortality (FCR) as affected by age and addition of xylanase to starter, grower, and finisher diets.

Dietary Treatments	Age (d)				
	0 - 14	15 - 35	36 - 41	0 - 35	0 - 41
	(g:g)				
[1]NC+125	1.50	1.68 ^{A}	1.83	1.65 ^{A}	1.69 ^{xy}
[2]NC+250	1.49	1.68 ^{A}	1.92	1.64 ^{A}	1.71 ^{x}
[3]NC+500	1.47	1.68 ^{A}	1.87	1.64 ^{A}	1.69 ^{xy}
[4]NC+1000	1.47	1.67 ^{A}	1.89	1.63 ^{A}	1.69 ^{xy}
[5]NC+1500	1.48	1.67 ^{A}	1.85	1.63 ^{A}	1.68 ^{xy}
[6]NC+2000	1.50	1.67 ^{A}	2.04	1.63 ^{A}	1.72 ^{x}
[7]NC+1000	1.48	1.68 ^{A}	1.91	1.64 ^{A}	1.70 ^{x}
[8]PC	1.46	1.62 ^{B}	1.81	1.59 ^{B}	1.64 ^{y}
[9]NC	1.51	1.68 ^{A}	1.98	1.65 ^{A}	1.72 ^{x}
SEM[10]	0.005	0.003	0.025	0.003	0.006
P-Value	0.360	<0.001	0.451	<0.001	0.079

^{x,y} Means in a column that possess the same superscripts do not differ approaching significance ($P \leq 0.10$).

^{A,B} Means in a column that possess the same superscripts do not differ significantly ($P \leq 0.01$).

[1] NC+125: Starter, grower, and finisher diets supplemented with 125 IU of xylanase to the negative control.

[2] NC+250: Starter, grower, and finisher diets supplemented with 250 IU of xylanase to the negative control.

[3] NC+500: Starter, grower, and finisher diets supplemented with 500 IU of xylanase to the negative control.

[4] NC+1000: Starter, grower, and finisher diets supplemented with 1000 IU of xylanase to the negative control.

[5] NC+1500: Starter, grower, and finisher diets supplemented with 1500 IU of xylanase to the negative control.

[6] NC+2000: Starter, grower, and finisher diets supplemented with 2000 IU of xylanase to the negative control.

[7] NC+-1000: Starter, grower, and finisher diets supplemented with 1000 IU of a reference xylanase to the negative control.

[8] PC: Positive control of starter diet with 4.05% fat followed by grower and finisher diets with 3.00% fat inclusions.

[9] NC: Negative control of starter diet with 1.50% fat followed by grower and finisher diets with 1.00% fat inclusions.

[10] Standard error of mean (SEM) for n=2 samples of 9 dietary treatments.

Table II-10. Broiler male mortality as affected by age and addition of xylanase to starter, grower, and finisher diets.

Dietary Treatments	Age (d)				
	0 - 14	15 - 35	36 - 41	0 - 35	0 - 41
			(%)		
[1]NC+125	0.78	1.56	1.56	2.34	3.91
[2]NC+250	1.56	2.34	2.34	3.91	6.25
[3]NC+500	0.78	1.56	1.56	2.34	3.91
[4]NC+1000	1.56	1.56	1.56	3.13	4.69
[5]NC+1500	0.78	0.78	2.34	1.56	3.91
[6]NC+2000	0.39	0.78	1.17	1.17	2.34
[7]NC+1000	0.78	2.73	0.78	3.52	4.30
[8]PC	1.56	1.95	3.13	3.52	6.64
[9]NC	0.78	0.78	2.34	1.56	3.91
SEM[10]	0.21	0.26	0.30	0.30	0.44
P-Value	0.856	0.578	0.735	0.302	0.442

[1] NC+125: Starter, grower, and finisher diets supplemented with 125 IU of xylanase to the negative control.
[2] NC+250: Starter, grower, and finisher diets supplemented with 250 IU of xylanase to the negative control.
[3] NC+500: Starter, grower, and finisher diets supplemented with 500 IU of xylanase to the negative control.
[4] NC+1000: Starter, grower, and finisher diets supplemented with 1000 IU of xylanase to the negative control.
[5] NC+1500: Starter, grower, and finisher diets supplemented with 1500 IU of xylanase to the negative control.
[6] NC+2000: Starter, grower, and finisher diets supplemented with 2000 IU of xylanase to the negative control.
[7] NC+1000: Starter, grower, and finisher diets supplemented with 1000 IU of a reference xylanase to the negative control.
[8] PC: Positive control of starter diet with 4.05% fat followed by grower and finisher diets with 3.00% fat inclusions.
[9] NC: Negative control of starter diet with 1.50% fat followed by grower and finisher diets with 1.00% fat inclusions.
[10] Standard error of mean (SEM) for n=8 pens of 32 broilers per pen.

Table II-11. Broiler male ileal digestibility as affected by addition of xylanase to starter, grower, and finisher diets.

Dietary Treatments	Ileal Digestibility[11]
	—— (%) ——
[1]NC+125	63.13 [BC]
[2]NC+250	63.53 [ABC]
[3]NC+500	67.52 [A]
[4]NC+1000	66.00 [AB]
[5]NC+1500	63.93 [ABC]
[6]NC+2000	61.23 [CD]
[7]NC+1000	57.65 [D]
[8]PC	58.48 [D]
[9]NC	61.73 [CD]
SEM[10]	1.49
P-value	<0.001

[A-D] Means in a column that possess the same superscripts do not differ significantly ($P \leq 0.01$).

[1] NC+125: Starter, grower, and finisher diets supplemented with 125 IU of xylanase to the negative control.

[2] NC+250: Starter, grower, and finisher diets supplemented with 250 IU of xylanase to the negative control.

[3] NC+500: Starter, grower, and finisher diets supplemented with 500 IU of xylanase to the negative control.

[4] NC+1000: Starter, grower, and finisher diets supplemented with 1000 IU of xylanase to the negative control.

[5] NC+1500: Starter, grower, and finisher diets supplemented with 1500 IU of xylanase to the negative control.

[6] NC+2000: Starter, grower, and finisher diets supplemented with 2000 IU of xylanase to the negative control.

[7] NC+-1000: Starter, grower, and finisher diets supplemented with 1000 IU of a reference xylanase to the negative control.

[8] PC: Positive control of starter diet with 4.05% fat followed by grower and finisher diets with 3.00% fat inclusions.

[9] NC: Negative control of starter diet with 1.50% fat followed by grower and finisher diets with 1.00% fat inclusions.

[10] Standard error of mean (SEM) for n=2 samples of 9 dietary treatments.

[11] Digestibility determined with the addition of titanium dioxide, an indigestible marker, at a level of 0.5% in the finisher feed only.

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CHAPTER 2

Abstract

The effects of β-glucanase on feed intake, body weight (BW), feed conversion ratio (FCR), and mortality of male broilers reared to 39 d of age were evaluated in Experiment 1. A total of 576 Ross 344 x Ross 708 male broilers were assigned to 6 treatments with 6 replicate pens of 16 male broilers each. The 6 dietary treatments included negative control (NC) basal starter and grower diets with 1% total fat added in the mixer, positive control (PC) starter and grower diets that contained 2.5% total added fat with 1% added in the mixer and 1.5% added by post-pellet liquid application (PC+PPLA), positive control starter and grower diets that contained 2.5% fat added only in the mixer (PC+Mixer), and 3 enzyme supplemented diets added to the NC basal diet. The 3 enzyme supplemented diets were manufactured from either an external control β-glucanase-based multigrain enzyme mixture (NC+DG2) or a β-glucanase enzyme at either a high or low dosage (NC+HiGlu and NC+LoGlu). All diets contained corn, soybean meal (SBM), and distillers dried grains with solubles (DDGS).

In Experiment 2, a total of 288 Ross 344 x Ross 708 male broilers were assigned to 4 grower dietary treatments with 6 replicate pens of 12 male broilers each and reared to 40 d of age. All treatments were fed a common starter diet containing corn and SBM. Four grower dietary treatments were manufactured by grinding, with a roller mill, the pelleted NC basal diet as well as the 3 NC+enzyme supplemented diets from Experiment 1.

In Experiment 1, birds that received the NC+PPLA and NC+DG2 exhibited ($P<0.01$) an improved FCR when compared to the NC and NC+HiGlu diets from 15-28 d of age. Supplementation of the NC with both sources of β-glucanases did not significantly improve overall FCR when compared to the NC diet. Only the NC+LoGlu diet achieved the similar ($P<0.05$) cumulative FCR as the NC basal diet. There were no significant differences in mortality among treatments. Feed intake was affected from 15-28 d of age, where the NC+Mixer birds exhibited a reduced ($P<0.01$) feed intake when compared to the NC and all 3 NC+enzyme diets while the NC+PPLA exhibited a reduced ($P<0.01$) feed intake when compared to the NC, NC+DG2, and NC+HiGlu diets. Numerically, the NC, NC+DG2, and NC+HiGlu had the greatest cumulative BW, which was directly related to their increased feed intake from 15-28 d. Overall, both sources and dosages of β-glucanase enzyme did not demonstrate an improved broiler live performance as compared to added fat.

In Experiment 2, there were no significant differences in BW observed among treatments. Birds that received the NC basal diet as well as the NC+LoGlu diet approached a significant ($P<0.10$) improvement in FCR when compared to the NC+HiGlu diet from 15-28 d of age. This was similar to what was observed in Experiment 1 during the grower phase. There were no significant differences in mortality observed. We speculate that the length of storage for the diets in Experiment 2 may have reduced enzyme activity as well as the ability to observe a significant live performance response.

Keywords: Glucanase, PPLA, corn, added fat, pellet quality

Introduction

Many cereal grains used in poultry feeds contain non-starch polysaccharides (NSPs), which have been observed to be not easily digested and absorbed by the growing broiler chick. Multiple studies have shown that appropriate enzyme supplementation can increase diet metabolizable energy (ME) of diets containing high levels of wheat or barley (Leeson and Caston, 2000). It has been determined that hydrolyzed β-d-glucan was responsible for the improved growth from these exogenous enzymes (Lesson and Caston, 2000). Recently, diets have also routinely contained distillers dried grains with solubles (DDGS) that has also been reported to be not easily digested. The addition of carbohydrases to corn and soybean meal (SBM) based broiler diets, when formulated to have a 3% reduction in dietary ME, has been accomplished without compromising the feed efficiency of broilers reared under either hot or cool season conditions (Yu and Chung, 2004). As corn and SBM have been mainly used in U.S. poultry diets along with the recent addition of large quantities of DDGS, the objective of this study was to investigate the effects of a β-glucanase enzyme on live performance of broilers fed reduced energy, corn-SBM-DDGS based diets.

Materials and Methods

Bird Management and Husbandry. The care of the animals used in this experiment complied with the Guide for Care and Use of Animals in Agricultural Research and Teaching (FASS, 2010). Two experiments were conducted with Ross 344 x 708 SF strain broiler chicks (Aviagen Inc., Huntsville, AL) that were hatched from eggs produced and incubated at the research site. The chicks were feather-sexed with the males separated and permanently

identified with neck tags. The chicks were placed in a heated and fan-ventilated enclosed broiler house where each 1.2 m in width by 3.8 m in length pen was equipped with one Plasson bell-type drinker and one hanging tube feeder. The birds were raised on used litter that was covered with new wood shavings. Each pen contained a supplemental chick font until 7 d of age in addition to three supplemental feeder flats to 7 d of age, two flats to 10 d of age, and one flat to 14 d. Small amounts of feed were moved out of the tube feeders onto the feeder flats once daily to 7 d of age. Water and feed were provided for *ad libitum* consumption and feeders were shaken once per day from 0 to 14 d, twice per day from 14 to 35 d, and three times per day after 35 d to prevent variation in feed intake due to any feed flow characteristics. The lighting program began with 23 h of light from 0 to 7 d, 22 h of light to 14 d, 20 h of light to 21 d, and 16 h from 22 d of age. The brooding temperature at reception of 35.0-36.1°C on the litter, which was determined by an infrared device, was maintained through the first night before being decreased to approximately 32°C the following morning. The litter temperature was maintained from 2 to 7 d at 31.0 to 32.5°C, 8 to 14 d at 28 to 30°C, 15 to 21 d at 26 to 28°C, and at 22 to 25°C thereafter. The birds had a budget of 0.9 kg starter feed/bird and thereafter consumed approximately 2.7 kg grower feed/bird.

In Experiment 1 that started on 12 November 2013, there were 16 chicks placed in each of 36 pens in a manner as to account for any differences in the 16 broiler breeder source pens (1 broiler chick per breeder pen) with 576 chicks in total. Each pen was assigned to one of 6 dietary treatments as described below with six replicate pens per treatment. In Experiment 2

that started on 19 March 2014, there were 12 chicks placed in each of 24 pens in a manner as to account for any of the broiler breeder pen differences with 288 chicks in total. These birds were otherwise managed in a manner similar to those of Experiment 1.

Experimental Diets and Feed Manufacturing. Diets were formulated to meet or exceed NRC requirements (NRC, 1994) on a total amino acid basis and were composed mainly of corn, SBM, and DDGS. Feed was produced at the North Carolina State University Feed Mill Educational Unit (Raleigh, NC) following current Good Manufacturing Practices. The fine DDGS were obtained by grinding coarse DDGS using a 60 HP Roskamp Champion (Waterloo, IA) model 15 x 22 split screen hammermill equipped with a #4/4 size screen. Fine corn was ground using the same hammermill equipped with a #4/4 screen size for the starter diets and a #6/6 screen size (~550 μm) for the grower diets. Coarse corn (5% of total corn inclusion) was ground with a two-pair roller mill (Model C128829, RMS, Harrisburg, SD) roller mill with 0/100 gap openings. Dry ingredients were mixed using a counterpoise mixer (Model TRDB126060, Hayes & Stolz, Fort Worth, TX) to produce the mash diets. The mash diets were steam conditioned at 88°C for 45 seconds and pelleted using a 30 HP pellet mill (Model PM1112-2, California Pellet Mill Co., Crawfordsville, IN) equipped with a ring die (3.5 mm x 36 mm). Pellets were cooled with ambient air in a counterflow cooler (Model VK09x09KL, Geelen Counterflow USA Inc., Orlando, FL). Post pellet liquid fat application (PPLA) was carried out in a Scott Equipment Co. (New Prague, MN) model SRM-304 500-pound double ribbon mixer.

The 6 dietary treatments employed in Experiment 1 shown in Tables III-2 and III-3 included negative control (NC) basal starter and grower diets with 1% total fat added in the mixer, positive control (PC+PPLA) starter and grower diets that contained 2.5% total added fat with 1% added in the mixer and 1.5% added by post-pellet liquid application (PC+PPLA), positive control starter and grower diets that contained 2.5% fat added only in the mixer (PC+Mixer), and 3 enzyme supplemented diets added to the NC basal diet. The 3 enzyme supplemented diets were manufactured with either an external control β-glucanase-based multigrain enzyme mixture (NC+DG2) or a β-glucanase enzyme at either a high or low dosage (NC+HiGlu and NC+LoGlu).

Experiment 2 was conducted as described for Experiment 1 with the following exceptions. All birds were fed a common crumbled starter diet containing corn and SBM without DDGS as shown in Table III-4. The corn-SBM-DDGS based NC and 3 NC+enzyme supplemented grower diets that had been stored following Experiment 1 were ground using a roller mill adjusted to produce a uniform crumble from the existing pellets and fed following the initial 0.9 kg fresh common starter feed/bird.

Pellet quality shown in Table III-5 was assessed on the day of manufacture by the tumbling-box method according to ASAE Standard Method S269.4 (ASABE, 2007). Modified PDI was determined by the addition of three 19.1 mm hexagonal nuts to the tumble-box chamber. Two samples were averaged to determine both standard and modified pellet durability index

(PDI) values for each diet. Mash and pellet samples were taken from each starter and grower diet for proximate analysis.

Data Collection. During the two experiments feed intake and BW by pen were recorded at placement, 14, 28, and 39 d of age in Experiment 1 and at placement, 14, 28, and 40 d of age in Experiment 2. The change from the starter to the grower diets took place just after the 14 d data collection. Grower feed was added on top of the starter feed so that all birds received their budgeted amount of starter feed as they gradually phased into consumption of the grower feed. Mortalities were removed from their pen, weighed, and recorded twice daily. Adjusted feed conversion ratio (FCR) was calculated by adding the BW of the dead birds to the BW of live birds in each pen.

Laboratory Analyses. Feed samples were analyzed on an “as is” basis for moisture, crude protein (CP) (N x 6.25), ash, and fiber by "proximate analysis” performed by Carolina Analytical Services, LLC (Bear Creek, NC 27207).

Statistical Analyses. Experiment 1 was a one-way arrangement of 6 diet types. Experiment 2 was a one-way arrangement of 4 diet types. Experiments 1 and 2 were analyzed as a randomized complete block design to identify main effects. The block design was based on location within the house. The experimental unit for the statistical analysis of the broiler live performance data was pen. All data was analyzed using SAS 9.4 with means partitioned by LSMeans and differences determined through the use of PROC GLM (SAS, 2012).

Statements of statistical differences were based upon $P < 0.05$ unless otherwise indicated.

Results and Discussion

The analyzed compositions of the diets in Experiment 1 and 2 are shown in Tables III-3 and III-4. The difference in metabolizable energy between the positive and negative control diets reflected the removal of fat to achieve a lower energy density in the negative control diet.

The analyzed PDIs of the grower diets in Experiments 1 and 2 are shown in Table III-5. The NC+DG2 diet exhibited the best standard and modified PDI overall. The PC+PPLA diet also achieved a similar standard PDI as the NC+DG2 diet when compared to all other diets. This is likely due to the fact that PPLA of fat has been shown to not negatively affect pellet quality when compared to adding fat in the mixer.

The effect of diet on BW in Experiment 1 is shown in Table III-6. No differences were observed other than at 14 d of age where BW for the NC and NC+HiGlu diets were greater ($P \leq 0.05$) than for PC+PPLA and PC+Mixer diets with NC+DG2 and NC+LoGlu diets intermediate. This was during the initial starter period when crumbled diets had been fed and broilers have been observed to be quite sensitive to dietary protein (Plumstead, 2005). Thus, any reduction in feed intake during this time could have slowed BW gain due to reduced consumption of protein.

The effect of diet on feed intake in Experiment 1 is shown in Table III-7. Broilers consuming the higher ME PC+PPLA and PC+Mixer diets exhibited numerically reduced feed intake to

14 d of age as discussed above. Further, broilers consuming NC, NC+DG2, and NC+HiGlu diets exhibited greater feed intake than those consuming the PC+Mixer diet with the NC+LoGlu and PC+PPLA diets intermediate ($P \leq 0.01$) from 15 to 28 d. The PC+Mixer diet exhibited the lowest feed intake of all treatments but did not differ from the PC+PPLA diet. It has been observed that feed intake regulation as a result of increased dietary ME developed over the first 3 wk of broiler life (Maiorka et al., 2008) so it was expected that feed intake would be lower in the diets with greater ME by this age. These 15-28 d differences were generally reflected in the 28 d cumulative data where the NC+DG2 and NC+HiGlu diets consumed a greater amount of feed than did the PC+PPLA diet with NC and NC+LoGlu diets intermediate ($P \leq 0.05$). Again, the PC+Mixer diet consumed the least amount of feed but was not statistically different from PC+PPLA and NC+LoGlu diets. The PC+Mixer diet effect was probably due to the combined effects of increased ME and reduced pellet quality due to the fat being added in the mixer relative to PPLA as was the case for the comparison of PC+PPLA versus PC+Mixer. Even though fat addition has been a common practice to increase the metabolizable energy content of broiler diets it has been demonstrated that increased oil content decreased pellet quality (Briggs et al., 1999) as fat functioned as a lubricant in the pellet die and may have decreased die friction required for good pellet binding.

The effect of diet on FCR in Experiment 1 is depicted in Table III-8. From 0-14 d the NC diet exhibited an improved FCR that approached significance ($P \leq 0.10$) as compared to PC+Mixer and NC+DG2 diets with PC+PPLA, NC+HiGlu, and NC+LoGlu diets

intermediate. These data demonstrated an initial negative effect of ME either from added fat (PC+Mixer), which could have been poor crumble quality, or from multigrain enzyme (NC+DG2). From 15 to 28 d of age the additional fat advantage shifted to the PC+PPLA diet as well as the multigrain enzyme NC+DG2 diet that produced improved FCR as compared to the NC and NC+HiGlu with PC+Mixer and NC+LoGlu diets intermediate ($P \leq 0.01$). From 29 to 39 d of age the NC+DG2 diet produced a poorer FCR than all other diets for an unexplained reason. Cumulatively to 28 d of age, there were no effects on FCR. Cumulatively to 39 d of age, the PC+PPLA diet performed the best ($P \leq 0.05$) but did not differ from the NC, PC+Mixer, and NC+LoGlu diets. The poorest FCR was exhibited by the NC+DG2 diet probably due to the residual effects of the 29-39 d period. Otherwise, the NC+HiGlu diet was statistically similar ($P \leq 0.05$) to the NC, PC+Mixer, and NC+LoGlu diets. The poorest FCR being in the NC diet was expected at certain times as it possessed a lower ME with no additional fat or benefit of enzyme supplementation. Indeed, the PC+PPLA diet produced an improved FCR relative to the NC diet probably due to the added ME in the absence of poorer pellet quality as demonstrated by the comparison to PC+PPLA at 39 d of age. Plavnik et al. (1997) explained that energy supplied by fat could decrease broiler BW gain if pellet quality and associated feed consumption were reduced. Hence, high levels of fat required for high energy diets has often been applied post-pellet in order to avoid reduction in pellet quality associated with excessive fat added in the mixer. However, PPLA capability has been generally absent in USA feed mills. These data suggested that none of the enzyme supplementations produced the advertised results under the conditions of this study.

The NC, NC+DG2, NC+HiGlu, and NC+LoGlu diets were formulated with only 1% added poultry fat in the mixer. Based upon the data of Auttawong (2015) in the same feed mill as utilized in the present studies, the optimum mixer fat addition was approximately 1.50% or less in order to maintain pellet quality, especially when diets were manufactured with any coarse corn. Thus, 1% added fat in the mixer for the basal diet (NC) was intended to produce pellets of adequate quality but relatively low ME value in order to provide the added enzymes with an opportunity to demonstrate the ability to improve live performance through the release of additional ME. As a means to comparatively demonstrate such ME release, PC+PPLA and PC+Mixer diets were formulated with 1.5% greater added fat, which increased formulated dietary ME by 0.13 kcal/g of diet. Further, in order to be able to discern effects of pellet quality on live performance the additional fat was applied by PPLA (PC+PPLA) or in the mixer (PC+Mixer). This should have theoretically produced better pellet quality with the PC+PPLA diet at the same ME as the PC+Mixer diet. Improved pellet quality at the same ME and added fat level would be expected to improve FCR and/or reduce feed intake while achieving a similar BW.

In a previous study with the "multigrain" (Ronozyme®MultiGrain, DSM, Heerlen, Netherlands) enzyme used in the NC+DG2 diet, a reduction of ME of 4.5% in starter, grower, and finisher diets, which was 0.90-1.37 kcal/g feed depending upon diet, significantly reduced live performance to 48 d of age as measured by BW gain and FCR (Univ. of Ga., 2013). The reduction in ME was accomplished by reducing soybean oil as shown in the table of major ingredients from that study shown below. These experimental

diets were of interest since they contained much more SBM than the diets of the present study, which suggested a lower protein SBM with greater amounts of high fiber hulls, and wheat midds that would not normally be utilized in commercial broiler diets in the USA. These particular ingredients probably provided appropriate substrate for the multigrain enzyme mixture. Further, the fat source in these diets was soybean oil that has been recently utilized in only a limited manner in a few global locations due to relatively high cost. The positive control diets contained greater than 4% oil in all cases while the negative control diets contained less than 2.63% in all cases. This was an obviously carefully designed experiment.

Table III-1. Composition of positive and negative control experimental diets[1] (DSM Rox UGA Study 2013)

	Diets[1]					
	Starter		Grower		Finisher	
Ingredients	Positive	Negative	Positive	Negative	Positive	Negative
	(%)					
Corn	45.46	45.46	49.51	49.51	59.44	59.44
Soybean meal	40.19	40.19	36.02	36.02	26.54	26.54
Soybean oil	3.99	2.34	4.31	2.63	4.02	2.31
Wheat midds	3.00	3.00	3.00	3.00	3.00	3.00
Meat and bone meal	2.00	2.00	2.00	2.00	2.00	2.00
DDGS	2.00	2.00	2.00	2.00	2.00	2.00

Given that final BW and cumulative feed consumption to 39 d did not differ in Experiment 1, the differences in cumulative FCR to 39 d must be examined in order to discern treatment

effects (Table III-8). As expected, the numerically best FCR was exhibited by the PC+PPLA and PC+Mixer diets that had the greater added fat and greatest dietary ME. The NC diet did not have the 1.5% additional fat relative to the PC+PPLA and PC+Mixer diets. Therefore, the NC diet would have been expected to exhibit a poorer FCR relative to PC+PPLA and PC+Mixer diets. In fact, this effect that had been apparent ($P \leq 0.01$) from 15 to 28 d of age was also apparent ($P \leq 0.05$) cumulatively. This diminished effect may have been the effect of the large broilers in this study not being able to consume sufficient feed to maintain their maximum growth trajectory or an absence of appropriate dietary substrate to produce the additional ME required for growth after 28 d of age. The cumulative feed consumption for the NC versus PC+PPLA diet was 137 g greater, which was calculated to actually be only 17.8 kcal. By comparison, the difference in feed intake from 15 to 28 d between the NC and PC+PPLA diets was 101 g, which was calculated to be 121 kcal. At the smaller 28 d BW the effect of the difference in ME required for maintenance of the smaller PC+PPLA broilers would be evident but less so when similar BW was achieved at 39 d. Broilers that were smaller, probably due to initially reduced protein intake, at 14 d (PC+PPLA and PC+Mixer diets) but achieved similar 39 d BW would normally be expected to exhibit an improved FCR, as was apparent, due to reduced maintenance requirements during the period from 14 to 39 d of age. By comparison, on a cumulative basis, none of the NC+enzyme diets consistently produced improved live performance relative to the three controls.

The effect of diet on male mortality in Experiment 1 is shown in Table III-7. Mortality was increased in broilers consuming the NC+DG2 diet from 29 to 39 d of age as compared to the

NC diet with the PC+PPLA, PC+Mixer, and NC+HiGlu intermediate ($P \leq 0.05$). The NC+LoGlu diet experienced no mortality during this age period but was not different from the NC, NC+HiGlu, and PC+PPLA diets. No overall cumulative differences in mortality were observed.

The effect of the ground broiler grower diets on male BW in Experiment 2 is shown in Table III-10. The 40 d BW observed was similar to that of Experiment 1 and no treatment differences were apparent. Numerically, the NC+LoGlu exhibited higher cumulative BW when compared to the other enzyme treatments.

The effect of the ground broiler grower diets on feed consumption by males in Experiment 2 is depicted in Table III-11. No treatment differences were apparent.

The effect of the ground broiler grower diets on FCR of males in Experiment 2 is shown in Table III-12. Overall FCR was exhibited the same trend that was observed in Experiment I (Table III-8) but differences that approached significance ($P<0.10$) were only apparent from 15 to 28 d of age where the NC and NC+LoGlu diets were improved relative to the NC+HiGlu diet with the NC+DG2 diet intermediate. As discussed by Aftab (2012), a lesser response in respect to improved digestibility of starch (and AME) may be expected in mash as compared to pelleted diets following supplementation of carbohydrase enzymes (Bedford, 1996). The more complete digestion of starch in mash diets would leave much less room for

improvement when compared to pelleted diets. This may explain the lack of significant differences between treatments cumulatively.

The effect of the ground broiler grower diets on male mortality in Experiment 2 is shown in Table II-13. Cumulative mortality was much lower than in Experiment 1 (Table III-0) but did not differ by treatment.

The observed differences in feed consumption in Experiment 1 (Table III-7) and PDI (Table III-5) raised questions about whether effects were due to feed manufacturing or enzyme activity. To address this question, 4 of the diets (NC, NC+DG2, NC+HiGlu, and NC+LoGlu) from Experiment 1 were ground to produce crumbles that should have had little difference in feed acceptability. The two diets with greater fat addition, PC+PPLA and PC+Mixer, were excluded from this subsequent comparison in Experiment 2. This excluded the two diets that differed in feed intake from the basal NC diet and the 3 enzyme amended NC+DG2, NC+HiGlu, and NC+LoGlu diets of Experiment 1. As expected, in the absence of feed manufacturing and dietary ME differences the 4 remaining diets produced no differences with respect to any variable in Experiment 2. These data confirmed the general conclusions drawn from the data in Experiment 1. The only exception was the aberrant FCR of the NC+DG2 diet from 29 to 39 d of age in Experiment 1, which was probably some error.

These results were somewhat paradoxical in that earlier experiments and practical experience with feed enzymes suggested a ME releasing effect (Yu and Chang, 2004; Zhang et al., 2012)

However, it must be remembered that these initial experiences (FAOSTAT, 2014) occurred when dietary added fat was generally greater than at present owing to the lower cost of fat at that time. The USDA's ERS revealed that the feed and residual use of fats and oils dramatically decreased from 2007 to 2008 (2015). This trend continued into 2009 where it leveled off and has remained for the past several years. Pellet quality could have been relatively compromised at that time (Plavnik et al., 1997) due to the greater amounts of added fat in some diets, certainly USA diets. Furthermore, Barasch et al. (Diss. NC State Uni., 2015) demonstrated that the effect of feed enzymes such as xylanase and phytase were significantly increased in the presence of a greater percentage dietary fat (5.7%) as compared to a lower fat inclusion (2%) using poultry fat as used in the present study. The mechanism for this observed difference may have been altered micelle formation required for absorption of all nutrients as well as slowed gut passage rate (Krogdahl, 1985; Li and Sauer, 1994), which would have allowed more time for enzymes to function. In the present study, the percentage coarse corn was only 5%, which was apparently insufficient to produce the slowed gut passage rate necessary for optimum enzyme function. In the present studies, the fat inclusion was relatively low in all diets and ranged from 1.0 for the negative controls to 2.5% for the positive controls. The inhibitory effect of lipids on gut passage rate (Krogdahl, 1985) may have increased the digestibility of other nutrients by extending the time of exposure to enzymes and absorptive sites in the NC+PPLA and NC+Mixer diets.

As feed costs have increased and alternative uses for animal fat developed over the past few years the amount of fat added to poultry diets subsequent to the initial widespread

introduction of feed enzymes have decreased dramatically in an effort to reduce overall feed costs. Utilizing different types of fats has also resulted in varying enzyme effect on fat digestibility (Meng et al., 2004). Apparently, the extra-caloric effects of added fat (Krogdahl, 1985; Li and Sauer, 1994) were forgotten until experiments such as those of the present study were conducted.

Table III-2. Broiler starter diet fed from 0-14 d of age in Experiment 1.

	Starter Diet		
Ingredients	PC[1]	NC[2]	NC+β-Glu[3]
	(%)		
Corn	54.89	54.89	54.89
Soybean meal (48% CP)	32.81	32.81	32.81
DDGS (28% CP)	5.00	5.00	5.00
Poultry fat	2.50	1.00	1.00
Limestone	0.81	0.81	0.81
Dicalcium phosphate (18.5% P)	1.99	1.99	1.99
Salt	0.50	0.50	0.50
DL-Methionine	0.20	0.20	0.20
L-Lysine	0.13	0.13	0.13
L-Threonine	0.12	0.12	0.12
Choline chloride (60%)	0.20	0.20	0.20
Vitamin premix[4]	0.05	0.05	0.05
Mineral premix[5]	0.20	0.20	0.20
Selenium premix[6]	0.05	0.05	0.05
Inert filler[7]	0.50	2.00	+
β-Glucanase[8]	-	-	+
Coccidiostat[9]	0.05	0.05	0.05
Total	100.00	100.00	100.00
Calculated nutrient content			
Crude protein	22.00	22.00	22.00
Calcium	0.90	0.90	0.90
Available phosphorus	0.45	0.45	0.45
Lysine	1.25	1.25	1.25
Methionine	0.55	0.55	0.55
Threonine	0.85	0.85	0.85
Methionine + cysteine	0.90	0.90	0.90
Sodium	0.21	0.21	0.21
Metabolizable energy (kcal/g)	2.92	2.80	2.80

[1,2]Poultry fat was added at 1% in the mixer for all diets. PC+PPLA diet and C had an additional 1.5% fat added in the diet with post-pellet liquid application (PPLA) or mixer addition, respectively.

[3]β-Glucanase enzyme was added at 0.025 kg (0.01% of diet) for NC+DG2 diet (Roxazyme G2®) and 0.30 kg (0.14% of diet) for NC+HiGlu and NC+LoGlu (Agrivida®) in the starter diets.

[4]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.

Table III-2. Continued

[5]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt, 1 mg.
[6]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.
[7]Vermiculite was used as inert filler at similar density as corn.
[8]Indicates the lack of (-) or addition of (+) β-glucanase enzyme in the diet at the specified dosages for diets D-F at the expense of inert filler, respectively.
[9]Monensin sodium was included at 99 mg/kg.

Table III-3. Broiler grower diet fed from 15-39 or 40 d of age in Experiments 1 and 2.

Ingredients	Grower Diet		
	PC[1]	NC[2]	NC+β-Glu[3]
	(%)		
Corn	58.53	58.53	58.53
Soybean meal (48% CP)	26.63	26.63	26.63
DDGS (28% CP)	8.00	8.00	8.00
Poultry fat	2.50	1.00	1.00
Limestone	0.76	0.76	0.76
Dicalcium phosphate (18.5% P)	1.69	1.69	1.69
Salt	0.50	0.50	0.50
DL-Methionine	0.13	0.13	0.13
L-Lysine	0.13	0.13	0.13
L-Threonine	0.08	0.08	0.08
Choline chloride (60%)	0.20	0.20	0.20
Vitamin premix[4]	0.05	0.05	0.05
Mineral premix[5]	0.20	0.20	0.20
Selenium premix[6]	0.05	0.05	0.05
Inert filler[7]	0.50	2.00	+
β-Glucanase[8]	-	-	+
Coccidiostat[9]	0.05	0.05	0.05
Total	100.00	100.00	100.00
Calculated nutrient content			
Crude protein	20.00	20.00	20.00
Calcium	0.80	0.80	0.80
Available phosphorus	0.40	0.40	0.40
Lysine	1.10	1.10	1.10
Methionine	0.47	0.47	0.47
Threonine	0.75	0.75	0.75
Methionine + cysteine	0.80	0.80	0.80
Sodium	0.21	0.21	0.21
Metabolizable energy (kcal/g)	2.99	2.86	2.86
Analyzed nutrient content			
Moisture	14.07	13.85	14.09
Fat	5.37	3.59	3.53
Protein	19.78	21.03	20.81
Fiber	2.58	2.57	2.58
Ash	5.67	6.86	6.83

Table III-3. Continued

[1,2]Poultry fat was added at 1% in the mixer for all diets. PC+PPLA diet and C had an additional 1.5% of fat included in the diet with post-pellet liquid application (PPLA) or mixer addition, respectively.
[3]β-Glucanase enzyme was added at 0.109 kg (0.012% of diet) for NC+DG2 diet (Roxazyme G2®) and 1.2 kg (0.132% of diet) for NC+HiGlu and NC+LoGlu (Agrivida®) in the grower diets.
[4]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.
[5]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt, 1 mg.
[6]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.
[7]Vermiculite was used as inert filler at similar density as corn.
[8]Indicates the lack of (-) or addition of (+) β-glucanase enzyme in the diet at the specified dosages for diets D-F at the expense of inert filler, respectively.
[9]Monensin sodium was included at 99 mg/kg.

Table III-4. Common broiler starter diet fed from 0-14 d of age in Experiment 2.

Ingredients	Starter Diet
	(%)
Corn	59.59
Soybean meal (48% CP)	35.11
Poultry fat	1.00
Limestone	0.78
Dicalcium phosphate (18.5% P)	2.02
Salt	0.50
DL-Methionine	0.24
L-Lysine	0.10
L-Threonine	0.11
Choline chloride (60%)	0.20
Vitamin premix[1]	0.05
Mineral premix[2]	0.20
Selenium premix[3]	0.05
Coccidiostat[4]	0.05
Total	100.00
Calculated nutrient content	
Crude protein	22.00
Calcium	0.90
Available phosphorus	0.45
Lysine	1.26
Methionine	0.58
Threonine	0.84
Methionine + cysteine	0.92
Sodium	0.21
Metabolizable energy (kcal/g)	2.85

Table III-4. Continued

Analyzed nutrient content	
Moisture	12.00
Fat	2.45
Protein	21.24
Fiber	2.90
Ash	5.89

[1]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.
[2]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt, 1 mg.
[3]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.
[4]Monensin sodium was included at 99 mg/kg.

Table III-5. Broiler grower diet standard and modified PDIs in Experiments 1 and 2.

Dietary Treatments	Standard PDI[8]	Modified PDI[9]
	(%)	(%)
[1]NC	83.1 [C]	56.6 [CD]
[2]PC+PPLA	89.1 [A]	66.5 [B]
[3]PC+Mixer	82.7 [C]	52.9 [D]
[4]NC+DG2	90.4 [A]	75.2 [A]
[5]NC+HiGlu	77.6 [D]	52.6 [D]
[6]NC+LoGlu	85.2 [B]	60.4 [C]
SEM[7]	0.11	0.91
P-value	<0.001	<0.001

[A,B] Means in a column that possess the same superscripts do not differ significantly ($P \leq 0.05$).
[1] NC: Starter and grower basal diets.
[2] PC+PPLA: Starter and grower basal diets with 1.50% fat added via post-pellet liquid application (PPLA).
[3] PC+Mixer: Starter and grower basal diets with 1.50% fat added in the mixer.
[4] NC+DG2: Starter and grower basal diets with an external control enzyme, Ronozyme® *MultiGrain*.
[5] NC+HiGlu: Starter and grower basal diets with high level of β-glucanase.
[6] NC+LoGlu: Starter and grower basal diets with low level of β-glucanase.
[7] Standard error of mean (SEM) for n=2 samples of each dietary treatment.
[8] Standard PDI was determined by the tumbling-box method according to ASAE Standard S269.4.
[9] Modified PDI was determined by the addition of three 19.1 mm hexagonal nuts to the tumble-box chamber.

Table III-6. Broiler male body weight (BW) as affected by age and addition of β-glucanase to starter and grower diets in Experiment 1.

Dietary Treatments	Age (d)			
	0	14	28	39
	(g)			
[1]NC	46.3	473 [a]	1661	2922
[2]PC+PPLA	46.8	448 [b]	1607	2867
[3]PC+Mixer	45.6	443 [b]	1580	2884
[4]NC+DG2	45.8	460 [ab]	1650	2913
[5]NC+HiGlu	46.5	473 [a]	1657	2910
[6]NC+LoGlu	46.0	459 [ab]	1628	2863
SEM[7]	0.14	2.95	9.33	9.77
P-Value	0.157	0.025	0.109	0.378

[a,b] Means in a column that possess the same superscripts do not differ significantly ($P \leq 0.05$).
[1] NC: Starter and grower basal diets.
[2] PC+PPLA: Starter and grower basal diets with 1.50% fat added via post-pellet liquid application (PPLA).
[3] PC+Mixer: Starter and grower basal diets with 1.50% fat added in the mixer.
[4] NC+DG2: Starter and grower basal diets with an external control enzyme, Ronozyme® *MultiGrain*.
[5] NC+HiGlu: Starter and grower basal diets with high level of β-glucanase.
[6] NC+LoGlu: Starter and grower basal diets with low level of β-glucanase.
[7] Standard error of mean (SEM) for n=6 pens of 16 broilers per pen.

Table III-7. Broiler male feed intake as affected by age and addition of β-glucanase to starter and grower diets in Experiment 1.

Dietary Treatments	Age (d)				
	0-14	15-28	29-39	0-28	0-39
	(g/bird)				
[1]NC	771	1829 [A]	2264	2608 [ab]	4904
[2]PC+PPLA	746	1728 [BC]	2183	2492 [bc]	4767
[3]PC+Mixer	760	1707 [C]	2216	2475 [c]	4831
[4]NC+DG2	790	1823 [A]	2277	2622 [a]	5092
[5]NC+HiGlu	791	1848 [A]	2266	2656 [a]	4981
[6]NC+LoGlu	773	1799 [AB]	2250	2572 [abc]	4822
SEM[7]	5	11	13	17	39
P-Value	0.160	0.004	0.287	0.023	0.190

[a,b,c] Means in a column that possess the same superscripts do not differ significantly ($P \leq 0.05$).

[A,B,C] Means in a column that possess the same superscripts do not differ significantly ($P \leq 0.01$).

[1] NC: Starter and grower basal diets.

[2] PC+PPLA: Starter and grower basal diets with 1.50% fat added via post-pellet liquid application (PPLA).

[3] PC+Mixer: Starter and grower basal diets with 1.50% fat added in the mixer.

[4] NC+DG2: Starter and grower basal diets with an external control enzyme, Ronozyme® *MultiGrain*.

[5] NC+HiGlu: Starter and grower basal diets with high level of β-glucanase.

[6] NC+LoGlu: Starter and grower basal diets with low level of β-glucanase.

[7] Standard error of mean (SEM) for n=6 pens of 16 broilers per pen.

Table III-8. Broiler male feed conversion ratio adjusted for mortality (FCR[1]) as affected by age and addition of β-glucanase to starter and grower diets in Experiment 1.

Dietary Treatments	Age (d)				
	0-14	15-28	29-39	0-28	0-39
	(g:g)				
[2]NC	1.71 ^{y}	1.55 ^{A}	1.80 ^{b}	1.59	1.68 ^{abc}
[3]PC+PPLA	1.75 ^{xy}	1.49 ^{C}	1.79 ^{b}	1.56	1.65 ^{c}
[4]PC+Mixer	1.80 ^{x}	1.50 ^{BC}	1.77 ^{b}	1.58	1.66 ^{bc}
[5]NC+DG2	1.80 ^{x}	1.49 ^{C}	2.00 ^{a}	1.58	1.73 ^{a}
[6]NC+HiGlu	1.75 ^{xy}	1.57 ^{A}	1.84 ^{b}	1.62	1.71 ^{ab}
[7]NC+LoGlu	1.77 ^{xy}	1.54 ^{AB}	1.80 ^{b}	1.60	1.69 ^{abc}
SEM[8]	0.01	0.01	0.01	0.02	0.01
P-Value	0.098	0.003	0.027	0.136	0.049

$^{x-z}$ Means in a column that possess the same superscripts do not differ approaching significance ($P \leq 0.10$).

$^{a-c}$ Means in a column that possess the same superscripts do not differ significantly ($P \leq 0.05$).

$^{A-C}$ Means in a column that possess the same superscripts do not differ significantly ($P \leq 0.01$).

[1] FCR was calculated by adding the weights of the dead birds to the weights of live birds in each pen, respectively.
[2] NC: Starter and grower basal diets.
[3] PC+PPLA: Starter and grower basal diets with 1.50% fat added via post-pellet liquid application (PPLA).
[4] PC+Mixer: Starter and grower basal diets with 1.50% fat added in the mixer.
[5] NC+DG2: Starter and grower basal diets with an external control enzyme, Ronozyme® *MultiGrain*.
[6] NC+HiGlu: Starter and grower basal diets with high level of β-glucanase.
[7] NC+LoGlu: Starter and grower basal diets with low level of β-glucanase.
[8] Standard error of mean (SEM) for n=6 pens of 16 broilers per pen.

Table III-9. Broiler male mortality as affected by age and addition of β-glucanase to starter and grower diets in Experiment 1.

Dietary Treatments	Age (d)				
	0-14	15-28	29-39	0-28	0-39
	(%)				
[1]NC	1.04	1.04	1.04 [bc]	2.08	3.13
[2]PC+PPLA	1.04	2.08	3.13 [abc]	3.13	6.25
[3]PC+Mixer	0.00	1.04	5.21 [ab]	1.04	6.25
[4]NC+DG2	0.00	1.04	6.25 [a]	1.04	7.29
[5]NC+HiGlu	1.04	2.08	2.08 [abc]	3.13	5.21
[6]NC+LoGlu	0.00	0.00	0.00 [c]	0.00	0.00
SEM[7]	0.30	0.43	0.62	0.54	0.87
P-Value	0.700	0.738	0.048	0.484	0.195

[a-c] Means in a column that possess the same superscripts do not differ significantly ($P \leq 0.05$).
[1] NC: Starter and grower basal diets.
[2] PC+PPLA: Starter and grower basal diets with 1.50% fat added via post-pellet liquid application (PPLA).
[3] PC+Mixer: Starter and grower basal diets with 1.50% fat added in the mixer.
[4] NC+DG2: Starter and grower basal diets with an external control enzyme, Ronozyme® *MultiGrain*.
[5] NC+HiGlu: Starter and grower basal diets with high level of β-glucanase.
[6] NC+LoGlu: Starter and grower basal diets with low level of β-glucanase.
[7] Standard error of mean (SEM) for n=6 pens of 16 broilers per pen.

Table III-10. Broiler male body weight (BW) as affected by age and addition of β-glucanase to ground crumble grower diets following a common starter fed to 14 d of age in Experiment 2.

Dietary Treatments	Age (d)				
	0	14	28	35	40
	(g)				
[1]NC	44.0	459	1611	2393	3013
[2]NC+DG2	44.0	459	1552	2327	2937
[3]NC+HiGlu	45.0	474	1594	2353	2990
[4]NC+LoGlu	44.4	471	1610	2388	3007
SEM[5]	0.25	3.6	12.5	14.4	16.5
P-Value	0.525	0.309	0.329	0.345	0.380

[1] NC: Common starter diet followed by a ground grower basal diet.
[2] NC+DG2: Common starter diet followed by a ground grower basal diet with an external control enzyme, Ronozyme® *MultiGrain*.
[3] NC+HiGlu: Common starter diet followed by a ground grower basal diet with high level of β-glucanase.
[4] NC+LoGlu: Common starter diet followed by a ground grower basal diet with low level of β-glucanase.
[5] Standard error of mean (SEM) for n=6 pens of 12 broilers per pen.

Table III-11. Broiler male feed intake as affected by age and addition of β-glucanase to ground crumble grower diets following a common starter fed to 14 d of age in Experiment 2.

Dietary Treatments	Age (d)				
	0-14	15-28	29-35	0-28	0-40
	(g/bird)				
[1]NC	673	1755	1305	2438	4544
[2]NC+DG2	683	1702	1311	2385	4613
[3]NC+HiGlu	704	1776	1278	2480	4574
[4]NC+LoGlu	706	1740	1285	2446	4527
SEM[5]	8	13	50	17	23
P-Value	0.403	0.272	0.247	0.308	0.590

[1] NC: Common starter diet followed by a ground grower basal diet.
[2] NC+DG2: Common starter diet followed by a ground grower basal diet with an external control enzyme, Ronozyme® *MultiGrain*.
[3] NC+HiGlu: Common starter diet followed by a ground grower basal diet with high level of β-glucanase.
[4] NC+LoGlu: Common starter diet followed by a ground grower basal diet with low level of β-glucanase.
[5] Standard error of mean (SEM) for n=6 pens of 12 broilers per pen.

Table III-12. Broiler male feed conversion ratio adjusted for mortality (FCR[1]) as affected by age and addition of β-glucanase to ground crumble grower diets following a common starter fed to 14 d of age in Experiment 2.

Dietary Treatments	Age (d)					
	0-14	15-28	15-40	0-28	0-35	0-40
	(g:g)					
[2]NC	1.62	1.53	1.52	1.56	1.59	1.53
[3]NC+DG2	1.65	1.56	1.59	1.58	1.64	1.60
[4]NC+HiGlu	1.64	1.59	1.54	1.60	1.63	1.55
[5]NC+LoGlu	1.66	1.53	1.51	1.56	1.59	1.53
SEM[6]	0.02	0.01	0.02	0.01	0.01	0.01
P-Value	0.874	0.115	0.260	0.171	0.140	0.267

[1] FCR was calculated by adding the weights of the dead birds to the weights of live birds in each pen, respectively.
[2]NC: Common starter diet followed by a ground grower basal diet.
[3] NC+DG2: Common starter diet followed by a ground grower basal diet with an external control enzyme, Ronozyme® *MultiGrain*.
[4] NC+HiGlu: Common starter diet followed by a ground grower basal diet with high level of β-glucanase.
[5] NC+LoGlu: Common starter diet followed by a ground grower basal diet with low level of β-glucanase.
[6] Standard error of mean (SEM) for n=6 pens of 12 broilers per pen.

Table III-13. Broiler male mortality as affected by age and addition of β-glucanase to ground crumble grower diets following a common starter fed to 14 d of age in Experiment 2.

Dietary Treatments	Age (d)					
	0-14	15-28	29-35	0-28	0-35	0-40
	(%)					
[1]NC	0.00	1.39	0.00	1.39	1.39	1.39
[2]NC+DG2	0.00	0.00	1.39	0.00	1.39	1.39
[3]NC+HiGlu	0.00	0.00	0.00	0.00	0.00	0.00
[4]NC+LoGlu	1.39	0.00	0.00	1.39	1.39	1.39
SEM[5]	0.35	0.35	0.35	0.49	0.60	0.60
P-Value	0.413	0.413	0.413	0.582	0.801	0.801

[1] NC: Common starter diet followed by a ground grower basal diet.
[2] NC+DG2: Common starter diet followed by a ground grower basal diet with an external control enzyme, Ronozyme® *MultiGrain*.
[3] NC+HiGlu: Common starter diet followed by a ground grower basal diet with high level of β-glucanase.
[4] NC+LoGlu: Common starter diet followed by a ground grower basal diet with low level of β-glucanase.
[5] Standard error of mean (SEM) for n=6 pens of 12 broilers per pen.

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SUMMARY

The overall objective of this research was to evaluate various carbohydrase enzymes for their potential as a feed additive in low energy broiler diets. Supplementation of both wheat and corn based diets reduced in energy with different carbohydrases at various dosages was evaluated in our trials. Efficacy of the enzyme was determined based on quantifiable responses of the broilers for live performance and digestibility since these parameters were of more practical interest to the industry. Any improvements in feed efficiency would be advantageous due to the large percentage of feed cost in total production costs.

Quantifying an expected contribution from an enzyme when included in the diet was also of interest leading to the evaluation of ileal digestibility. One proposed mode of action of carbohydrases to improve digestibility was to reduce gut viscosity allowing other nutrients more access to be digested and absorbed properly. This should have theoretically translated directly into an improved digestibility of diets supplemented with carbohydrase. Being able to attribute some type of digestibility value that correlated into an "energy" value would have been extremely useful when formulating feed with carbohydrase addition. Based on the research conducted, while ileal digestibility may be the most accurate method of digestibility, its values did not directly correlate with the live performance. The ileal digestibility values calculated from Experiment 1 did result upon closer inspection with fat digestion and absorption when diets were supplemented with carbohydrases.

Typically, fat has been removed from the diet to create a lower energy feed that is further supplemented with an exogenous carbohydrase to make up for the energy deficit. It

would be desirable to have an optimal balance exist between total percentage of fat in the diet and carbohydrase inclusion. However, the desire to create inexpensive broiler feed has often led to overlooking the "extra-caloric" effects of fat in the diet. Fat has been shown to decrease gut passage rate often leading to increased time of exposure for nutrients to be digested and absorbed. The release of additional nutrients as a result of from exogenous enzyme supplementation coupled with decreased gut passage rate has often translated directly into improved live performance. Additionally, different types of fats (plant versus animal sources of fat) have been shown to have varying degrees of digestibility when supplemented with various exogenous enzymes. Further research may need to be conducted to gain a better insight into enhanced enzyme effect due to fat inclusion levels.

As expected, different cereal grains should have varying values regarding release of "energy" when supplemented with carbohydrases. It was well known that wheat had a greater concentration of NSPs when compared to corn. One should expect wheat to have more "energy" liberated than corn when it was supplemented with specific carbohydrases. When formulating feed and removing fat to lower the energy density of the diet, it was important to have a realistic "energy" value for improvement after supplementation. Carbohydrase supplementation in wheat diets has been shown to produce larger uplifts in AME when compared to supplementation in corn diets. In our current research, it was possible that the fat inclusion level chosen for the NC diets to be supplemented with carbohydrases was actually too low. Based on AME reduction, the carbohydrase may not have been able to liberate the required AME from the diet to produce live performance values greater than those observed with the PC diets that had a higher overall energy density. This was especially

apparent in primarily corn based diets whose uplift in AME did not match the roughly 100 kcal reduction in energy from fat removal as typically used in the industry.

In all three studies conducted, the supplementation of both wheat and corn based reduced energy diets did not result in live performance values similar to the respective normal energy diets in each of those trials. Proper feed formulation was apparently pertinent to success with carbohydrase supplementation in wheat and especially corn-based diets. There should be potential for inclusion of carbohydrases in corn-based diets if there was an appropriate environment to promote optimal efficacy of the enzyme.