ABSTRACT

HERCHLER, MARISSA. Effects of Xylanase and Protease on Gut Health and Growth Performance of Newly Hatched Broiler Chickens. (Under the direction of Dr. Sung Woo Kim.)

Feed enzymes have been utilized in animal diets in order to reduce the anti-nutritional effects of major feedstuffs, such as corn, corn DDGS, and SBM. Reducing the effect of these anti-nutritional factors, such as NSP, using feed enzymes has been shown to reduce the cost of production by reducing the requirements or supporting the apparent digestibility of the nutrients. Research involving the additive effects of xylanase and protease on gut health and growth performance in broilers has been limited in corn-SBM diets, though previous trials have indicated little improvement in growth performance using diets with high NSP feedstuffs.

This study was to investigate the effects of supplemental xylanase and protease (BRI, Durham, NC) on gut health and growth performance of broiler chickens (288 day-old, male) fed experimental diets for 28 d. Treatments were based on 2 x 2 factorial arrangement with xylanase (0 and 15,000 XU/kg) and protease (0 and 300 U/g) as 2 factors with 9 cages/treatment and 8 birds/cage. Birds and feeders were weighed weekly for calculation of ADG, ADFI, and feed:gain (F:G). On d 7 and 28, 2 birds per pen were randomly chosen to collect blood and gut tissues for immunoglobulin G (IgG), malondialdehyde (MDA), protein carbonyl, morphology measurements. Ileal digesta were collected to determine viscosity and apparent ileal digestibility (AID). Digesta viscosity tended to decrease ($P = 0.059$) and decreased ($P < 0.05$) when both enzymes were used in wk 1 and 4, respectively, whereas it was not affected by using enzymes individually. Protease increased ($P < 0.05$) villus height (611 to 689 μm) in wk 4. Xylanase decreased ($P < 0.05$) concentrations of serum IgG (2.66

to 2.06 g/L) and ileal protein carbonyl (0.41 to 0.29 $\mu\text{mol/g}$ protein) in wk 1. Protease tended to decrease ($P = 0.083$) serum protein carbonyl (0.78 to 0.66 $\mu\text{mol/L}$) in wk 1. Protease decreased ($P < 0.05$) ileal protein carbonyl (0.39 to 0.31 $\mu\text{mol/g}$ protein) in wk 1, which was more effective ($P < 0.05$) with xylanase together. Protease decreased ($P < 0.05$) serum MDA (0.56 to 0.30 $\mu\text{mol/L}$) and ileal MDA (0.53 to 0.35 $\mu\text{mol/g}$ protein) in wk 4. Xylanase did not affect AID, whereas protease tended to increase AID of DM ($P = 0.064$; 74.7 to 76.5%), which was more effective ($P < 0.05$) with xylanase together. In wk 1, xylanase decreased ($P < 0.05$) F:G (1.213 to 1.173) and tended ($P = 0.063$) to decrease ADFI (20.1 to 19.4 g/d), whereas protease reduced ($P < 0.05$) ADG (17.2 to 16.0 g/d) and ADFI (20.1 to 19.4 g/d). Overall, protease increased ($P < 0.05$) F:G (1.504 to 1.528) whereas tended ($P = 0.089$) to reduce ADG (51.3 to 50.2 g/d), which was more effective ($P < 0.05$) with xylanase together. Mortality was not affected ($P > 0.10$) by the treatments. In conclusion, xylanase and protease benefited gut health by decreasing digesta viscosity, enhancing gut morphology, decreasing gut oxidative stress, and increasing nutrient digestibility, whereas these benefits were not related to growth performance.

Key Words: gut health, growth performance, xylanase, protease

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Effects of Xylanase and Protease on Gut Health and Growth Performance of Newly Hatched
Broiler Chickens

by
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BIOGRAPHY

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Chapter 1
Literature Review

Feedstuffs and Anti-nutritional Factors

One goal of the feed industry is to produce least-cost feed products in order to maximize both profit and nutritional value for optimum performance; however, the largest cost of livestock production are diets (Yegani and Korver, 2013; Sheppy, 2001). Diet costs can account for as much as 60 to 75% of the cost to raise and process broilers. Minimizing diet costs can certainly increase profit margins for the industry (Dosković *et al.*, 2013). As the global population continues to grow, there is a subsequent increase in the demand for affordable meat products, such as poultry, pork, or beef (Sheppy, 2001); therefore, it is becoming more important to ensure that animal diets provide the optimum digestible nutrients possible in order to raise healthy animals at the least cost to producers.

Major feedstuffs, such as corn and soybean meal (SBM), are important sources of protein, carbohydrates, amino acids, fat, fiber, vitamins, and minerals in animal diets (Yegani and Korver, 2013; Wang *et al.*, 2014), but they can present some nutritional problems. Soybean meal contains several anti-nutritional factors, such as, but not limited to glycinin and β -conglycinin. Corn and corn distillers dried grains with solubles (DDGS) contain arabinoxylans, which are non-starch polysaccharides (NSP). These NSP can prevent the digestion or absorption of carbohydrates and proteins in the gastrointestinal tract (Yegani and Korver, 2013). The NSP have been shown to decrease performance and nutrient digestion in animals including poultry (Choct *et al.*, 1996). Dietary supplementation of exogenous feed enzymes can also be used to help release nutrients, such as starch, proteins, and minerals, from feed that are not accessible by the animal's endogenous enzymes, and they can be used

to support the immature digestive systems of young animals (Sheppy, 2001). Being able to predict the development of the gastrointestinal tract as the birds age allows nutritionists to adjust the feed matrix for these changes. Exogenous feed enzymes can also reduce the feed costs by making nutrients more available within the feed. When dietary levels of metabolizable energy or crude protein can be increased by adding exogenous feed enzymes, nutritionists can decrease the amount of major feedstuffs being used, such as corn and SBM, thus reducing the overall cost of the diet (Dosković *et al.*, 2013).

Not only do feed enzymes reduce diet costs, they also may improve growth performance, gut health, and environmental conditions by improving digestive capacity, thus decreasing the moisture in excreta that adversely affect litter quality, and decreasing nutrient emission into the environment (Dosković *et al.*, 2013). During the first week after hatch, the gastrointestinal tract comprises the largest percentage of the total body weight as the bird partition much of their developmental resources to increase digestive capacity to supply nutrients needed to support subsequent growth (Croom *et al.*, 1999). During the first 3 weeks of age, lipase, amylase, and trypsin activities have been shown to be deficient in immature broiler gastrointestinal tracts (Kaczmarek *et al.*, 2014). The main limiting factor of decreased nutrient utilization during this period has been shown to be nutrient digestion, rather than nutrient absorption (Kaczmarek *et al.*, 2014).

The NSP are very complex structures that significantly increase the bulk and viscosity of intestinal digesta, which compromises the digestibility of nutrients, such as starch, protein, and fat by blocking their interactions in the mucosa (Choct *et al.*, 1996; Stefanello *et al.*,

2015). Feed enzymes help reduce digesta viscosity, which allows for increased nutrient absorption, resulting in an increase in body weight gain and feed: gain (F:G) (Dosković *et al.*, 2013). Ultimately, feed enzymes are being investigated as a means of improving the nutrient availability of major feedstuffs, such as corn and SBM in animal production (Cowieson, 2005).

Because corn has such a high energy value, it is one of the most frequently used feedstuffs in feed, globally (Summers, 2001). Corn provides about 65% of the metabolizable energy (ME) and 20% of the protein in poultry diets (Cowieson, 2005). Of the total amount of undigested energy in a typical corn/SBM diet, 18% comes from fat, 45% from protein, and 37% from starch (Cowieson, 2010). Corn encompasses approximately 10% NSP, which is primarily made up of arabinoxylan and β -galactomannan (Jo *et al.*, 2012). The corn kernel is comprised of the endosperm, germ, and hull fractions. Within the cell walls of the endosperm fraction, contains the majority of arabinoxylans; whereas the hull contains xylans and cellulose. These xylans are indigestible in the small intestine of broilers. Starch and proteins tend to evade digestion when there are high levels of NSP, and then travel to the large intestine to be fermented. There is limited energy yield in this process (Meng and Slominski, 2005).

Soybean meal is another major feedstuff used globally for its high nutritional value and as an excellent source of protein (Wang *et al.*, 2014). When broilers are young, higher dietary protein is required for optimum growth than when they are older. Since soybean meal contains high levels of quality protein, it occupies a greater proportion of the starter diet than

in subsequent grower and finisher diets; however, this comes with greater amounts of antinutritional factors, such as NSPs, soybean agglutinin, soybean protease inhibitors, and the soybean allergenic proteins glycinin and β -conglycinin (Dasković *et al.*, 2013; Wang *et al.*, 2014). Unfortunately, the NSP in SBM cannot be completely utilized by monogastric animals, such as poultry or swine (Huisman *et al.*, 1998). In order to help reduce the allergenic effects of glycinin and β -conglycinin within soybeans, special processing techniques, such as extrusion, high pressure, heating, fermentation, or chemical processing have been suggested (Kim *et al.*, 2010; Wang *et al.*, 2014). High pressure processing of SBM changes the conformation of glycinin as it is dissociated into subunits and the groups become more hydrophobic and contain more sulfhydryl groups. At higher pressures (≥ 300 MPa), both glycinin and β -conglycinin can become denatured (Wang *et al.*, 2014). Even after processing, compounds such as β -galactomannans, are still present and can adversely affect digestibility and growth because there is an associated increased rate of passage of feed through the gastrointestinal tract (Kim *et al.*, 2003). Glycinin and β -conglycinin are major globulins within soybeans that account for up to 70% of the total seed globulins, in which glycinin accounts for approximately 40% and β -conglycinin accounts for approximately 30%. Both glycinin and β -conglycinin contain cysteine disulphide bonds that can impede digestion of SBM (Wang *et al.*, 2011). Exogenous protease supplemented to feed aid in breaking down polysaccharides and cysteine disulphide bonds into a more usable forms (Huisman *et al.*, 1998; Wang *et al.*, 2011). Protease breaks down cysteine disulphide bonds by hydrolyzing glycinin and β -conglycinin (Wang *et al.*, 2011), thus allowing the anti-

nutritional factors to be utilized in the gastrointestinal tract. Glycinin and β -conglycinin have been shown to increase serum IgG, decrease villus height, cause damage to intestinal morphology, and weaken immune function in early weaned pigs (Wang *et al.*, 2014).

Feed Enzymes

There are several different types of enzymes being used in feed production in order to reduce formula costs and promote health of animals that consume this feed. Some of the most common feed enzymes used today are phytase, gluconase, mannanase, xylanase, and protease. They fall into different categories, such as carbohydrases, lipases, proteases, oxidoreductases, or phosphatases, based on their mode of action (Munir and Maqsood, 2013). There are two proposed mechanisms by which feed enzymes are thought to work and increase production profitability in poultry. They can either support the apparent digestibility of nutrients, or reduce the nutrient requirements (Cowieson, 2010). Feed enzymes can be used individually or in combination, as cocktails. Feed enzymes or the combination of feed enzymes are chosen based on the nutritional problems the producer may encounter. Generally, supplemental feed enzymes allow for an increase in nutrient availability to the host animal by reducing the adverse effects of NSP or anti-nutritional factors within the feed ingredients. Consequently, growth performance and/or gut health is improved, especially in young birds with underdeveloped gastrointestinal tracts and digestive capacity. Feed enzymes are especially beneficial for monogastric animals, unlike ruminants that have microorganisms in the rumen that do this (Munir and Maqsood, 2013).

Use of Feed Enzymes

Of all the feed enzymes, phytases have been adopted most widely in the feed industry (Kiarie *et al.*, 2015). Phytase is a phosphatase that specifically hydrolyzes phytate and make its phosphorus more available (Munir and Maqsood, 2013). Phytase is an endogenous enzyme found mainly in the small intestine of the gastrointestinal tract, as well as the crop, proventriculus, and the gizzard of poultry. When used as an exogenous feed enzyme, phytase has been shown to improve the digestibility of phosphorous (P), calcium, amino acids, and energy. There have also been environmental advantages of dietary supplementation of phytase, as there is less P excretion in the feces if the total dietary phosphorus is respectively reduced to the degree P bioavailability is enhanced (Dersjant-Li *et al.*, 2014). Phytate is the predominant form of P in plant-sourced feedstuffs; it can also bind to starches, amino acids, and minerals within the diet and make them more indigestible. In phytate, P is bound as phosphate groups to an inositol ring and has the ability to chelate cations to form mineral-phytate complexes that are insoluble within diets (Karimi *et al.*, 2013). Young broilers show little phytase activity in their intestinal mucosa or duodenum, but the presence of endogenous phytase increases with growth, possibly due to the maturation of the gut and increased surface area of the intestinal mucosa. Lack of endogenous phytase can also negatively impact the utilization of P within the body (Morgan *et al.*, 2015).

Carbohydrases are the most common of the feed enzyme categories. Included in this category are amylase, glucosidase, lactase, mannanase, and xylanase (Munir and Maqsood,

2013). Exogenous carbohydrases are typically added to broiler diets containing cereal grains as a means of improving growth performance, nutrient utilization, and energy utilization (Stefanello *et al.*, 2015). Environmental conditions, such as droughts, have been shown to alter the composition of the endosperm in corn and sorghum that contain the anti-nutritive components, β -glucans and arabinoxylans. The increase in carbohydrates within cereal grains can decrease the digestibility of the grains by broilers (Yoder *et al.*, 2015).

β -glucanase is a carbohydrase used to hydrolyze β -glucans (Munir and Maqsood, 2013). β -glucans are found mainly in barley grains, oats, and rye, which all promote high viscosity of intestinal digesta (Bedford and Cowieson, 2012; Cox *et al.*, 2010; Mccleary, 2001), and within the cell walls of bacteria, fungi, and yeast (Cox *et al.*, 2010). Within the body, β -glucans increase the viscosity of digesta within the gastrointestinal tract, therefore reducing the nutrient absorption of the diet (Mccleary, 2001). β -glucans from fungi and yeast have been shown to increase immunity in avian species, which adversely affects growth performance. Immune response has been measured using IgG and IgA levels, where increased secretions of immunoglobulins help with the elimination of pathogens. The results of supplemental addition of β -glucans in broiler diets have been inconsistent in reporting performance. When performance is reduced, it is thought that more energy is allocated to immune function, rather than growth. Studies that found β -glucans improved performance, observed an increase in BW during the grower phase of broilers or pigs (Cox *et al.*, 2010).

β -mannans are found in both SBM and guar gum, though more prevalently in the latter. It has been exhibited in previous experiments involving guar gum that β -mannans are

highly anti-nutritional for monogastric species, such as swine and poultry (Jackson *et al.*, 2004). β -mannans are polysaccharides composed of repeating sugar units: mannose, galactose, and glucose. Soybean meal contains approximately 1.61% β -mannans (Karimi *et al.*, 2013). The NSPs form in plant cell walls as glucomannans and galactomannans (Zou *et al.*, 2006). Chickens do not have the ability to endogenously produce enzymes to make β -mannans nutritionally available, therefore, exogenous enzymes must be added to the diet to make them available (Karimi *et al.*, 2013). The hydrolysis and degradation of β -mannan by β -mannanase has been shown to improve F:G, ME, growth, and feed efficiency, as well as reduce the water:feed ratio in broilers (Zou *et al.*, 2006). In corn-SBM diets, β -mannanase helps to improve growth and feed efficiency, which increases as ME decreases. β -mannans are also responsible for increasing viscosity within the digestive system. β -mannanase can reduce the viscosity, which allows for an increase in nutrient uptake in the gastrointestinal tract. This has also been linked to an increase in energy metabolism (Jackson *et al.*, 2004). Previous studies have indicated better feed utilization, and thus better nutrient utilization when corn/SBM diets, with added guar meal, were supplemented with enzymes (Daskiran *et al.*, 2004). Jackson *et al.* (2004) suggested that β -mannanase could also be important in boosting the immune system of broilers and that there was a minimal amount of disease challenges during their experiment. In a study that measured performance with increased levels of β -mannans, with 2% added guar gum to simulate feed ingredients with high β -mannan content, the addition of β -mannanase aided in increasing BW in birds, as compared to those fed a basic corn-SBM based control diet (Daskiran *et al.*, 2004).

Xylanase was first introduced into the feed production industry in the late 1990s to reduce costs in wheat and corn based diets by increasing NSP availability and reducing feed intake (Cowieson *et al.*, 2010). The inclusion of xylanase in energy-reduced corn-SBM diets promotes the maintenance of broiler performance at similar levels as nutritionally adequate energy diets, thus allowing nutritionists to use xylanase as a cost-saving measure in diet formulation (Williams *et al.*, 2014). Xylanase is naturally produced by bacteria, fungi, and some insects, such as termites. Research looking into how termites degrade wood has led to extensive knowledge into how enzymes can be applied industrially. Plant cell walls contain hemicellulose, cellulose and lignin, which are found in wood and corn alike. Breaking down these cell wall polysaccharides by using xylanase can aid in increasing the digestibility of plant products by releasing the encased nutrients (Quian *et al.*, 2015, Zhang *et al.*, 2014).

Xylanase works in two ways: first, by reducing viscosity; second, by releasing monosaccharides. Monosaccharides are released when NSP are broken down, which subsequently releases starch within the endosperm. These starches interact with endogenous amylase, which releases glucose (Zhang *et al.*, 2014). In some studies, xylanase has been shown to increase P retention, but this has been seen more often in wheat than corn based diets. In wheat based diets, phytate-P interacts more closely with dietary arabinoxylan fibers, so the addition of xylanase facilitates access to the phytate-P due to its fiber-degrading capabilities (Cowieson and Ravindran, 2008). Most research involving xylanase has been in wheat based diets because there is a higher concentration of NSPs in wheat than corn (Cowieson *et al.*, 2010), however, xylanase has been shown to increase both energy

implementation and growth performance in broilers, even in corn based diets (Gehring *et al.*, 2013). Xylanase has been primarily used in feeds in order to help reduce the viscosity of digesta in the small intestine by allowing the partial hydrolysis of NSPs in the upper gastrointestinal tract (Zhang *et al.*, 2014). It has been suggested that the inclusion of xylanase in early diets aids in the development of healthy lower gut microbiomes and help in producing higher carcass yields (Williams *et al.*, 2014).

Proteases are used to hydrolyze proteins. Some important proteases are keratinase, pepsin, and, trypsin (Munir and Maqsood, 2013). Keratinase is a protease used in corn-SBM diets to hydrolyze proteins that contain cysteine disulphide bonds. Although the original purpose of dietary supplementation of keratinase was to improve digestibility of feather meal, it was subsequently found to improve performance, amino acid utilization, breast meat production, as well as villus structure and health in the gastrointestinal tract in broilers fed corn-SBM diets (Wang *et al.*, 2011). According to Guggenbuhl *et al* (2012), protease generally has positive effects when combined with other feed enzymes. Alone, this enzyme has been shown to increase both live performance and crude protein (CP) digestibility in broilers, as well as improve the nutritional value of SBM (Barekatin *et al.*, 2013). Protease mainly has effects on the growth and digestion of broilers when supplemented in the starter diet and fed during the first couple weeks after hatch; however, the effects of the enzyme decreases when supplemented in subsequent. As Dosković *et al.* studied, broilers fed supplemental protease in SBM based diets had higher body weights and metabolic energy with no changes in F:G. Higher body weights occurred because protease aids in nitrogen

retention (Dosković *et al.*, 2013). In an experiment using weaned pigs, Guggenbuhl *et al* (2012) found that apparent ileal digestibility (AID) of certain amino acids, as well as AID of CP was increased during the second phase of the study (day 29) with the use of an acid-stable protease (15,000 U/kg) in corn-SBM diets. Proteases have also been shown to be beneficial when used to improve the nutritional value of SBM after heat treatment. Heat treatment is applied when soybeans are being processed into SBM to reduce the activity of anti-nutritional factors in the final product, but excessive heat treatment will reduce the digestibility of essential amino acids, including lysine (Ghazi *et al.*, 2002).

Combinational Use of Feed Enzymes

The combinational use of feed enzymes has been studied extensively in wheat-based diets; however, there has also been some research on how enzyme blends improve the digestibility of corn-SBM diets (Jo *et al.*, 2012). Corn-SBM diets are more digestible than diets containing other grains and by-products, so the effect of supplemental enzymes purposed to improve digestibility often has little effect on growth performance or F:G because nutrients in the diets are not reduced substantially enough to allow for an effect to be observed. Consequently, further reductions in the dietary ME content could allow for enzymes to have a greater response (Yegani and Korver, 2013). In studies with swine, Jo *et al.* (2012) found there was a 9% increase in G:F when pigs were fed a barley-based diet supplemented with an enzyme blend of β -glucanase, xylanase, amylase, and pectinase. This opens opportunities to substitute corn and SBM with low-quality grains, such as rape seed meal, copra meal, wheat, barley, and palm kernel meal because they are cheaper. Moreover,

these innovative feed enzyme products help overcome the increased NSP and other antinutritional factors that are associated with these alternative feedstuffs, and thereby make the nutrients they contain more available to the animal (Jo *et al.*, 2012).

Commonly in broiler feeds, a blend of amylases, xylanases, and proteases are used to aid in ileal digestibility, bodyweight gain (BWG), and F:G (Parker *et al.*, 2007). Previous studies involving the use of xylanase, amylase, and protease in corn-SBM based broiler diets, showed a 3% increase in energy and amino acid digestibility, as well as a consistent level of performance with lower nutrient availability (Cowieson and Ravindran, 2008). Cowieson and Ravindran (2008) concluded in their study that the combinational use of xylanase, amylase, and protease aids in increasing the availability of energy and amino acid value in corn-SMB based broiler diets.

Barekatin *et al.* (2013) conducted an experiment using the factorial combination of dietary xylanase and protease supplementation to increase nutrient digestion in broilers fed diets containing sorghum DDGS. These researchers observed that the combination of xylanase and protease has a lower response on growth performance than either of the enzymes alone, indicating that protease may negatively affect xylanase activity. One suggested explanation for the lack of synergy between xylanase and protease is the degradation of xylanase by protease, which limits the effectiveness of xylanase (Barekatin *et al.*, 2013). There has been little research conducted based on the combination of these two feed enzymes, especially in corn-SBM based diets, therefore, there is little knowledge on the impact xylanase may have on the effects of protease, and vice versa.

Hypothesis and Objectives

Supplemental enzymes have been intensely studied since first being introduced in the feed industry. However, there is still much that is unknown regarding how exogenous enzymes work (Dosković *et al.*, 2013). Many enzyme studies have focused on wheat, barley, rice, or rye-based diets because of their high fiber and NSP concentrations (Jo *et al.*, 2012). Research into feed enzymes and their effects on nutrient availability in corn-SBM based diets with variable levels of NSP can aid nutritionists in developing diet formulations that can account for the expected levels of nutrient recovery (Cowieson and Ravindran, 2008). It is also important to maximize the response of feed enzymes in diets, even when NSP concentrations are low, as they are in corn, in order to make the maximum profit by investing in fewer nutritional resources (Cowieson and Ravindran, 2010). The goal of this study was to evaluate the response of broiler chicks fed a corn-SBM based diet supplemented with xylanase and protease. Even though previous studies have indicated there is potential for negative impacts on gut health and performance when combining these two feed enzymes in sorghum DDGS based diets, there is little research to indicate the same is true for corn-SBM based diets.

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Chapter 2

Effects of Xylanase and Protease on Gut Health and Growth Performance of Newly Hatched Broiler Chickens

Abstract

This study was to investigate the effects of supplemental xylanase and protease (BRI, Durham, NC) on gut health and growth performance of broiler chickens (288 day-old, male) fed experimental diets for 28 d. Treatments were based on 2 x 2 factorial arrangement with xylanase (0 and 15,000 XU/kg) and protease (0 and 300 U/g) as 2 factors with 9 cages/treatment and 8 birds/cage. Birds and feeders were weighed weekly for calculation of ADG, ADFI, and feed:gain (F:G). On d 7 and 28, 2 birds per pen were randomly chosen to collect blood and gut tissues for immunoglobulin G (IgG), malondialdehyde (MDA), protein carbonyl, morphology measurements. Ileal digesta were collected to determine viscosity and apparent ileal digestibility (AID). Digesta viscosity tended to decrease ($P = 0.059$) and decreased ($P < 0.05$) when both enzymes were used in wk 1 and 4, respectively, whereas it was not affected by using enzymes individually. Protease increased ($P < 0.05$) villus height (611 to 689 μm) in wk 4. Xylanase decreased ($P < 0.05$) concentrations of serum IgG (2.66 to 2.06 g/L) and ileal protein carbonyl (0.41 to 0.29 $\mu\text{mol/g}$ protein) in wk 1. Protease tended to decrease ($P = 0.083$) serum protein carbonyl (0.78 to 0.66 $\mu\text{mol/L}$) in wk 1. Protease decreased ($P < 0.05$) ileal protein carbonyl (0.39 to 0.31 $\mu\text{mol/g}$ protein) in wk 1, which was more effective ($P < 0.05$) with xylanase together. Protease decreased ($P < 0.05$) serum MDA (0.56 to 0.30 $\mu\text{mol/L}$) and ileal MDA (0.53 to 0.35 $\mu\text{mol/g}$ protein) in wk 4. Xylanase did not affect AID, whereas protease tended to increase AID of DM ($P = 0.064$; 74.7 to 76.5%), which was more effective ($P < 0.05$) with xylanase together. In wk 1, xylanase decreased ($P < 0.05$) F:G (1.213 to 1.173) and tended ($P = 0.063$) to decrease ADFI (20.1 to 19.4 g/d),

whereas protease reduced ($P < 0.05$) ADG (17.2 to 16.0 g/d) and ADFI (20.1 to 19.4 g/d). Overall, protease increased ($P < 0.05$) F:G (1.504 to 1.528) whereas tended ($P = 0.089$) to reduce ADG (51.3 to 50.2 g/d), which was more effective ($P < 0.05$) with xylanase together. Mortality was not affected ($P > 0.10$) by the treatments. In conclusion, xylanase and protease benefited gut health by decreasing digesta viscosity, enhancing gut morphology, decreasing gut oxidative stress, and increasing nutrient digestibility, whereas these benefits were not related to growth performance.

Key Words: gut health, growth performance, xylanase, protease

Introduction

With an increase in the demand and cost to use corn to produce ethanol as an alternative fuel source (Williams *et al.*, 2014), producers want to ensure that their feeds are being optimally utilized, with as little nutritional waste possible. It is important for producers to provide their livestock with the maximum nutrients to raise healthy animals at the least cost. Three major feedstuffs in livestock feed are corn, corn distillers' dried grains with solubles (DDGS), and soybean meal (SBM). Corn and DDGS contain about 10% non-starch polysaccharides (NSP), which are made up of arabinoxylan and β -galactomannan (Jo *et al.*, 2012). The two major anti-nutritional components of SBM are glycinin and β -conglycinin, which make up about 70% of the total seed globulins (Wang *et al.*, 2014). These anti-nutritional factors can decrease performance and nutrient digestibility in monogastric animals (Choct *et al.*, 1996; Huisman *et al.*, 1998). Because these feed ingredients are included at such a high rate within diets, the effects of the anti-nutritional factors are multiplied, resulting in a significant decrease in performance and gut health.

Supplementation of feed enzymes in the diets of broilers has been investigated as a means to reduce endogenous losses and improve the availability of nutrients from major feed ingredients (Cowieson, 2005). Xylanase has been shown to improve energy digestion and growth performance in corn-based diets (Gehring *et al.*, 2013), as well as reduce digesta viscosity in the small intestine. Xylanase is used to alleviate the anti-nutritional effects of structural NSP of feedstuffs cell walls, and facilitate the nutrient release from viscous NSP complexes (Zheng *et al.*, 2014). Protease has been shown to improve the nutritional value of

SBM in animal diets, and improve CP digestibility and growth performance in broilers (Bareketain *et al.*, 2013). Protease primarily functions by breaking cysteine disulphide bonds and by hydrolyzing glycinin and β -conglycinin (Wang *et al.*, 2011).

Dietary supplementation of corn-SBM with a blend of xylanase and protease has not been extensively studied. Previous studies have demonstrated little improvement in growth performance when these two enzymes are combined; however, these studies were done in broilers fed diets containing wheat or sorghum DDGS that contain higher levels of NSP than in corn-SBM diets. It is hypothesized that xylanase and protease have additive beneficial effects on gut health and performance in monogastric animals, thus justifying dietary supplementation of corn-SBM diets with a blend of xylanase and protease. Using xylanase may allow other enzymes to access trapped nutrients, so xylanase supplementation may allow the supplemental protease to access glycinin and β -conglycinin and thereby reduce their effect on allergenic inflammation in the gastrointestinal tract. The goal of this research was to investigate the additive or synergistic effects of dietary xylanase and protease supplementation on gut health and growth performance of broiler chicks.

Materials and Methods

The experimental protocol involving animal husbandry and handling was approved by the Institutional Animal Care and Use Committee (IACUC) at North Carolina State University (Raleigh, NC). This experiment was conducted in Scott Hall at North Carolina State University (Raleigh, NC).

Animal Housing

A total of 288 day-old, male broiler chickens (Ross 708) were used. The birds were hatched at North Carolina State University Chicken Education Unit (CEU). On the day of hatch birds were weighed and randomly allotted to 36 cages (Alternative Design Manufacturing & Supply, Siloam Springs, AR); based on 4 dietary treatments (8 birds per cage). Feed and water were consumed *ad libitum* with one feed trough per cage and an adjustable double nipple-drinker within the cage. Birds were provided 23 h of light and 1 h of darkness. The temperature from hatching to 7 d was maintained at 29 to 32°C, 26 to 29°C from 7 to 10 d, 23 to 26°C from 10 to 14 d, and 22 to 23°C from 14 to 28 d. Drinkers and feed were checked and temperatures recorded daily.

Experimental Design and Diets

Birds were assigned to a completely randomized experimental design of treatments as a factorial arrangement of 2 dietary levels of xylanase (0 and 100 g/MT feed; 0.01%; 15,000 XU/kg feed) and 2 dietary levels of protease (0 and 500 g/MT feed; 0.05%; 300 U/g feed). Each of the 4 treatment combinations were replicated by 9 cages containing 8 chicks per cage. Experimental diets (Tables 1 and 2) were manufactured at North Carolina State

University Feed Mill Education Unit and formulated to meet or exceed the nutrient requirements specified for Ross 708 broiler chickens (Aviagen, 2014). Diets included corn (57%; particle size: 20%, 1,100 to 1,200 μm ; and 80%, 400 μm), soybean meal (32% in starter and 26% in grower feeds), poultry fat (2% in starter and 3% in grower feeds), and DDGS (5% in starter and 10% in grower feeds). The grower diet contained TiO_2 (0.5%) as an indigestible marker to calculate ileal digestibility of nutrients. Starter diet was fed from hatch to 14 d, followed by grower diet from 14 to 28 d. Treatment diets were mixed using a Scott mixer: the dry mix time for 180 seconds and the wet mix (after the addition of liquid fat) for 90 additional seconds. The feed was fed as a mash diet.

Growth Performance

Birds and feeders were weighed on d 7, 14, 21, and 28 for calculation of body weight (BW), average daily feed intake (ADFI), average daily gain (ADG), and feed:gain (F:G). The F:G was calculated as ADFI divided by ADG. Dead birds were removed from cages and weighed for subsequent mortality calculations and adjusted growth performance.

Sample Collection and Processing

On d 7 and 26, 72 birds (2 birds per cage) were randomly selected for sampling using the random number generator in Excel software (Microsoft Corporation, Redmond, WA). Using 22 gauge, 1 inch needles and 3 or 5 mL syringes blood was collected by heart puncture of chicks sampled on d 7, and by wing vein or jugular vein on d 26. Birds were immediately euthanized by cervical dislocation following blood collection on d 7, in accordance with IACUC protocol, whereas birds blood sampled on d 26 were returned to their respective

cages for the remainder of the study. Blood was transferred to a 10 mL BD Vacutainer for the collection of serum. Tubes sat at room temperature (21°C) for 5 h to allow clotting and were then centrifuged for 10 min at 3,000 x g for serum collection. Serum samples were stored at -80°C until further analysis.

On d 7, tissue samples (2 cm before Meckel's Diverticulum to Meckel's Diverticulum) were collected from the jejunum of 2 randomly selected birds in each pen and used for histological measurements. The jejunum tissues samples were pooled by cage. On d 28, birds were euthanized by cervical dislocation, and a 2 cm portion of the ileum immediately following Meckel's Diverticulum, was collected for histological measurements. These tissues from the 2 randomly selected birds in each cage were pooled. At both 7 and 28 days, the lumen of the 2 cm gut sections were gently flushed with 10% buffered formaldehyde solution and then transferred into a 50 mL vial containing 45 mL 10% buffered formaldehyde solution (formalin) for subsequent histological preparation. These fixed gut tissues sections were cut into cross sections and sent to the North Carolina State University Histopathology Laboratory (College of Veterinary Medicine, Raleigh, NC) for hematoxylin and eosin (H & E) staining and slide preparation. Villus height, villus width, and crypt depth were measured using an Olympus CX31 microscope (Waltham, MA). The same person performed all analysis of intestinal morphology.

On d 7, the ileum, from Meckel's diverticulum to a point 2 cm above the ileo-ceca junction, was used to collect digesta contents and mucosa scraping. The digesta was squeezed out of the tissue into a 1.5 mL conical tube for subsequent viscosity analysis, and

then placed on ice. Viscosity was measured using a Brookfield DV2T Viscometer (Middleboro, MA). The mucosa was sampled by opening the tissue and using a glass slide to collect the mucosa. Mucosa samples were then transferred to 1.5 mL conical tube and flash frozen in liquid nitrogen. Mucosa samples were stored in the -80°C freezer until further analysis of protein concentration, then protein carbonyl, Immunoglobulin G (IgG), and malondialdehyde (MDA). This process was duplicated for the birds sampled on d 28, using a portion of the ileum from 2 to 10 cm below the Meckel's Diverticulum.

Additional digesta samples were obtained on d 28 from the ileum at an area from 5 to 10 cm above the ileo-ceca junction for AID calculation. The digesta samples were weighed, then frozen at -20°C until being freeze-dried (24D x 48, Virtis, Gardiner, NY). These were then reweighed to calculate dry matter content before being processed for nutrient digestibility. Digesta samples were finely ground for titanium dioxide, dry matter, lipid, gross energy, and crude protein digestibility analyses.

IgG, Protein Carbonyl, and Malondialdehyde (MDA) Measurement

Mucosa samples (500 mg) were suspended in 0.5 mL PBS and homogenized (Tissuemiser, Thermo Fisher Scientific Inc., Rockford, IL). Serum and Mucosa samples were diluted to 40,000x in deionized water and PBS, respectively, for protein carbonyl concentration analysis according to OxiSelect™ Protein Carbonyl ELISA Kit (Cell BioLabs, Inc., San Diego, CA) and plates were read at 450 nm on a BioTech Synergy HT plate reader (BioTek Instruments, Inc., Winooski, VT).

For IgG analysis, serum was diluted to 8000x and mucosa was diluted to 80x with water and PBS, respectively, then analyzed using Chicken IgG ELISA Quantitation Set (Bethyl Laboratories, Inc., Montgomery, TX) and plates were read at 450 nm on a BioTech Synergy HT plate reader (BioTek Instruments, Inc., Winooski, VT).

For MDA analysis, serum and mucosa samples were utilized at their original concentrations using an OxiSelect™ TBARS Assay Kit (MDA Quantitation) (Cell BioLabs, Inc., San Diego, CA) and plates were read at 540 nm on a BioTech Synergy HT plate reader. For the final MDA concentration, values were divided by the protein concentration values, which were measured using a Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL) and read on a BioTech Synergy HT plate reader at 562 nm (BioTek Instruments, Inc., Winooski, VT).

Chemical Analysis

Titanium dioxide was quantified in ground feed and digesta samples as described in Myers et al. (2004) and used to calculate dry matter, lipid, gross energy, and crude protein digestibility. Dry matter was quantified by weighing digesta samples prior to and after freeze drying, as described in Passos *et al.* (2015). Gross energy was quantified in ground feed and digesta samples using a Parr 6200 Calorimeter (Parr Instrument Co., Moline, IL) as described in Passos et al. (2015). Lipid was quantified using a modified ether extract method (AOAC, 2006) as described in Passos *et al.* (2015). Crude protein was quantified in ground feed and digesta samples using TruSpec N Nitrogen Determinator (LECO Corp., St. Joseph, MI) as described in Passos et al. (2015).

Apparent Ileal Digestibility

Titanium dioxide was calculated using a standard curve and used to calculate AID of dry matter, lipid, gross energy, and crude protein. Dry matter digestibility was calculated using the associated titanium dioxide levels in the feed and digesta as follows:

$$\text{Dry Matter (\%)} = 100 - \left(100 * \frac{\text{TiO}_2 \text{ feed}}{\text{TiO}_2 \text{ digesta}} * \frac{\text{DM digesta}}{\text{DM feed}} \right)$$

Lipid digestibility was quantified using the equation:

$$\text{Lipid (\%)} = 100 - \left(100 * \frac{\text{TiO}_2 \text{ feed}}{\text{TiO}_2 \text{ digesta}} * \frac{\text{Lipid in digesta}}{\text{Lipid in feed}} \right)$$

Gross energy digestibility was calculated using the equation:

$$\text{Gross Energy (\%)} = 100 - \left(100 * \frac{\text{TiO}_2 \text{ feed}}{\text{TiO}_2 \text{ digesta}} * \frac{\text{GE digesta}}{\text{GE feed}} \right)$$

Crude protein digestibility was quantified using the equation:

$$\text{Crude Protein (\%)} = 100 - \left(100 * \frac{\text{TiO}_2 \text{ feed}}{\text{TiO}_2 \text{ digesta}} * \frac{\text{CP digesta}}{\text{CP feed}} \right)$$

Statistical Analysis

This experiment was in a completely randomized design based on a 2 x 2 factorial arrangement of treatments. Therefore, all data were analyzed with one-way ANOVA and a Mixed procedure of SAS 9.4 (SAS Institute Inc, Cary, NC). Significance was observed with probability levels of $P < 0.05$ and tendencies were observed with probability levels of $0.05 \leq P < 0.10$.

Results

Viscosity

Viscosity (Table 3) was not affected by the xylanase or protease treatments alone. The combinational treatment tended ($P = 0.059$) to decrease viscosity during wk 1 and reduced ($P < 0.05$) viscosity during wk 4.

Histology

Xylanase had no effects on villus height, crypt depth, or villus width (Table 4) throughout the duration of the experiment. Protease tended ($P = 0.071$) to decrease jejunal villus width (79 to 72 μm) in wk 1 and increased ($P < 0.05$) ileal villus height (611 to 689 μm) in wk 4. The combinational treatment tended to decrease ($P = 0.091$) ileal crypt depth in wk 4.

IgG, Protein carbonyl, and MDA

Xylanase had no effects on protein carbonyl concentrations (Table 5) in serum, however, xylanase reduced ($P < 0.05$) ileal mucosa protein carbonyl (0.41 to 0.29 $\mu\text{mol/g}$ protein) in wk 1. Protease tended ($P = 0.083$) to decrease concentrations of protein carbonyl (0.78 to 0.66 $\mu\text{mol/L}$) in blood serum during wk 1. Protease reduced ($P < 0.05$) ileal mucosa protein carbonyl (0.39 to 0.31 $\mu\text{mol/g}$ protein) concentrations during wk 1, which was more effective ($P < 0.05$) with xylanase and protease together. The combinational treatments had no effects on serum protein carbonyl concentrations in wk 1 or wk 4. There were no treatment effects during wk 4 on protein carbonyl concentrations in neither the blood serum nor the ileal mucosa.

Xylanase reduced ($P < 0.05$) serum IgG (2.66 to 2.06 g/L) concentration (Table 5) during wk 1. Protease did not affect serum or ileal mucosa IgG concentrations in wk 1 or wk 4. The combinational treatment had no effects on serum or ileal mucosa IgG concentration.

None of the treatments had an effect on serum or ileal mucosa MDA (Table 5) concentrations in wk 1. Xylanase had no effect on serum or ileal MDA concentrations, whereas protease reduced ($P < 0.05$) MDA concentrations in both serum (0.55 to 0.30 $\mu\text{mol/L}$) and ileal mucosa (0.53 to 0.35 $\mu\text{mol/g}$ protein) in wk 4. The combinational treatment tended to decrease ($P = 0.087$) MDA concentrations of ileal mucosa in wk 4.

Apparent Ileal Digestibility

Xylanase had no effect (on AID (Table 6). Protease tended ($P = 0.064$) to increase AID of dry matter (74.73 to 76.48 %), which was more effective ($P < 0.05$) with the addition of xylanase, and tended ($P = 0.084$) to increase AID of lipids (52.14 to 57.00 %). The combinational treatment tended ($P = 0.080$) to increase AID of gross energy. AID of crude protein was not effected by any of the treatments.

Live Performance

Xylanase did not affect BW or ADG (Table 7) during this study; however, xylanase tended ($P = 0.063$) to decrease ADFI (16.6 to 16.5 g/d), which was more effective ($P < 0.05$) when supplemented with protease, and decreased ($P < 0.05$) F:G (1.213 to 1.173) in wk 1.

Protease supplementation reduced ($P < 0.05$) BW (165 to 157 g), ADG (17.2 to 16.0 g/d), and ADFI (20.1 to 19.4 g/d), which were more effective ($P < 0.05$), especially when in combination with xylanase. Protease tended ($P = 0.054$) to increase F:G (1.174 to 1.212) in

wk 1. From wk 1 to 2, protease reduced ($P < 0.05$) ADFI (34.3 to 33.6 g/d), especially when in combination with xylanase ($P < 0.05$). Protease reduced ($P < 0.05$) BW (165 to 157 g) and ADG (67.5 to 65.0 g/d) in wk 3 and tended ($P = 0.089$) to reduce BW overall. Protease increased ($P < 0.05$) F:G (1.514 to 1.569) in wk 3, especially in combination with xylanase, as indicated by the significant ($P < 0.05$) interaction effect. From wk 3 to 4, protease increased ($P < 0.05$) F:G (1.608 to 1.642), which was more effective ($P < 0.05$) with xylanase together. Overall, protease tended ($P = 0.089$) to reduce ADG (51.3 to 50.2 g/d) and increased ($P < 0.05$) F:G (1.504 to 1.528), which was more effective ($P < 0.05$) with xylanase together. In wk 4, the combination of xylanase and protease tended ($P = 0.097$) to effect ADG and increased ($P < 0.05$) F:G. Mortality was not affected by the dietary treatments (Table 8).

Discussion

Digesta viscosity is influenced by the feedstuffs that compose the diet, especially those with high levels of NSP (Amerah *et al.*, 2008). High viscosity within the gastrointestinal tract can slow the rate of passage of digesta and hinder digestion and absorption of nutrients (Choct *et al.*, 1999). Xylanase has been shown to reduce the effects of NSP, especially arabinoxylans, which are found in highly viscous cereal grains, such as corn (Romero *et al.*, 2014). The NSP are partially depolymerized by feed enzymes, such as xylanase, which can decrease the molecular size of particles, thus decreasing viscosity of the digesta (Choct *et al.*, 1999).

This study indicated that the combination of dietary xylanase and protease supplementation reduced digesta viscosity in broilers fed a corn-SBM based diet. These results correspond with the AID data, where DM and GE digestibility were increased by the addition of xylanase to the protease treatment. It is possible that the combination of xylanase and protease allowed enzymes to access NSP that would not have been accessible by either of the supplemental feed enzymes alone.

Gastrointestinal morphology can also be impacted by the amount of NSP present in diets. Diets with high NSP can negatively impact villus height and the ratio of villus height to crypt depth in pigs fed wheat based diets (Passos *et al.*, 2015). Villus height corresponds to the ability of the enterocytes to absorb nutrients; enterocytes increase their absorptive capacity as they rise from the base of the villi to the top. Crypt depth can be increased by damage to the enterocytes by bacteria (Parsaie *et al.*, 2007). This study indicated that xylanase alone did not affect villus height, crypt depth, or villus width in broilers fed corn-SBM diets. Similarly, Passos *et al.* (2015) found there was no effect of xylanase on gastrointestinal morphology in pigs fed corn-SBM diets. On the other hand, we observed protease decreased villus width measured at wk 1 and increased villus height at wk 4, whereas the combination of xylanase and protease tended to decrease crypt depth at wk 4, indicating an improvement in morphology. This corresponds with the results of the protein oxidation tests for IgG and protein carbonyl in the serum and ileum of the broilers at wk 1 or wk 4, indicating increased gastrointestinal health.

Glycinin and β -conglycinin, 2 anti-nutritional factors present in SBM, have the potential to impair gastrointestinal morphology and immunity in monogastric animals fed corn-SBM diets (Wang *et al.*, 2011). Immunoglobulin G (IgG) in serum and ileal mucosa was used to quantify immune function in broiler chicks at wk 1 and wk 4 of age. Feeding SBM, especially in early weaned pigs, can result in increased serum IgG (Wang *et al.*, 2014). Wang *et al.* (2011) observed no changes in serum IgG levels when keratinase was added to corn-SBM pig diets. Contrary to previous experiments, the data indicated that there were no effects on IgG levels in the serum or mucosa by either protease or the combination of protease and xylanase. On the other hand, xylanase decreased serum IgG of chicks measured at wk 1 of age.

Protein carbonyls are formed when amino acid residues or protein backbones are oxidized. There is often an increase in protein carbonyl, which is used as an indicator of protein oxidation, when SBM is heat treated with soy protein isolate or wheat peptides. When these amino acids are changed, there can be modifications in protein function, resulting in increased viscosity, solubility, emulsification, and water-holding capacity. This leads to reduced nutritional value of SBM (Wu *et al.*, 2014). This study indicated that xylanase, protease, and the interaction between these 2 enzymes decreased ileum mucosa levels of protein carbonyl at wk 1. The interaction between xylanase and protease further decreased serum protein carbonyl as compared to xylanase or protease alone. According to this data, there may have been recovery within the gastrointestinal tract to decrease protein carbonyl and IgG levels without the aid of enzyme supplementation after wk 1.

Malondialdehyde is used as an indicator of oxidative stress. The MDA can become elevated when various diseases are present or when there is an excess of oxygen free radicals. Lipid peroxidation can also be measured with MDA concentration, as MDA is the final product of lipid peroxidation (Wu *et al.*, 2015). This study indicated that protease decreased MDA concentration in both the serum and mucosa tissue, and the combination of xylanase and protease tended to decrease MDA concentrations in the ileal mucosal tissue at wk 4 of the experiment. This decrease in MDA concentration corresponds with the tendency to increase lipid digestibility in the ileum with the inclusion of protease in the diet and indicates an increase in gastrointestinal health.

Apparent ileal digestibility of dry matter was improved with the inclusion of protease, and enhanced the effects when combined with xylanase. The interaction between xylanase and protease also increased the digestibility of gross energy, and protease alone increased the digestibility of lipid. Guggenbuhl *et al.* (2012) found that the inclusion of protease in corn-SBM diets did not affect AID in newly weaned pigs after wk 2; however, during the second phase (wk 3 to 4) it was found that there was an improvement in AID of crude protein digestibility, but no improvement in AID of energy digestibility. It was also reported that the inclusion of protease has led to inconsistent results in both AID and performance in broilers (Guggenbuhl *et al.*, 2012). These inconsistencies could have been due to differences in feed ingredients or variations of NSP within the feed ingredients.

Xylanase is used to reduce the adverse effects of NSP, commonly found in corn and DDGS, on gastrointestinal health, leading to an increase in performance (Gehring *et al.*, 2013;

Zhang *et al.*, 2014). Protease, on the other hand, has been used to improve performance and gastrointestinal health by reducing the amount of anti-nutritional factors present in SBM (Barekatin *et al.*, 2013). During wk 1, we saw that xylanase improved F:G while decreasing ADFI. This indicates there was less feed being ingested by the birds, while still maintaining BW and ADG. Protease decreased ADG, and ADFI during wk 1, which corresponded to an increase in F:G. This occurred because the birds had to eat more feed to maintain their BW. The interaction between xylanase and protease enhanced the negative impact protease had on BW, ADG, and ADFI during the first week. Protease also decreased BW in the grower (wk 3 to 4) phase of the study, decreased overall ADG, and increased wk 3 to 4 and overall F:G, especially when in combination with xylanase as indicated by a significant interaction effect.

Xylanase has been found to improve BW, as well as F:G at wk 3 (Zhang *et al.*, 2014). Previously, studies have indicated that the dietary addition of xylanase or protease, individually can increase performance indicators in broilers (Stefanello *et al.*, 2015; Wang *et al.*, 2011). According to Wang *et al.* (2008), studies investigating the effects of keratinase supplementation in corn-SBM diets, have resulted in significant improvement of growth performance. In their study, keratinase increased BW and improved F:G in SBM or cottonseed meal based diets (Wang *et al.*, 2008). Dosković *et al.* (2013) found that protease led to a higher BW due to increased nitrogen retention.

Little research has investigated the combinational effects of xylanase and protease in corn-SBM based diets; however, Kalmendal and Tauson (2012) found that there was no significant change in performance when xylanase with or without protease treatments were

added to wheat-SBM diets, as compared to the control treatment. Barekatain *et al.* (2013) found that the interaction between xylanase and protease resulted in little significance in growth performance in broilers fed sorghum DDGS based diets, similar to this study, where the interaction led to few improvements in performance.

In our study, broilers were fed nutritionally adequate diets and had no nutritional or pathogenic challenges. This lack of challenges within the gastrointestinal system allowed the feed enzymes to improve gut health, but there were not enough nutritional or pathogenic deficits to show improvement in growth, as well. For future studies investigating the combination of xylanase and protease in corn-SBM diets, it may be useful to incorporate some of these challenges into the system in order to test whether or not growth performance will correlate with gut health. In conclusion, xylanase and protease benefited gut health by decreasing digesta viscosity, enhancing gut morphology, decreasing gut oxidative stress, and increasing nutrient digestibility, however these benefits were not related to any improvement in growth performance.

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Table 1. Ingredient and calculated chemical composition of experimental starter¹ diets on an as fed basis for 28 d

Xylanase, 15,000 U/g Protease, 300 U/g	Starter			
	0		15,000	
	0	300	0	300
Ingredient %				
Yellow corn	57.30	57.25	57.30	57.24
Soybean meal	31.75	31.75	31.75	31.75
DDGS, corn	5.00	5.00	5.00	5.00
L-Lys HCl	0.13	0.13	0.13	0.13
DL-Met	0.18	0.18	0.18	0.18
L-Thr	0.10	0.10	0.10	0.10
Dical P (6-01-080)	1.98	1.98	1.98	1.98
Limestone, ground (6-02-632)	0.82	0.82	0.82	0.82
Vitamin premix ²	0.05	0.05	0.05	0.05
Mineral premix ³	0.20	0.20	0.20	0.20
Salt	0.50	0.50	0.50	0.50
Choline chloride, 60%	0.20	0.20	0.20	0.20
Selenium premix	0.05	0.05	0.05	0.05
Poultry fat	1.73	1.73	1.73	1.73
Xylamax	0.00	0.00	0.01	0.01
Versazyme	0.00	0.05	0.00	0.05
TiO ₂	0.00	0.00	0.00	0.00
Total	100.00	100.00	100.00	100.00
Calculated composition				
ME, kcal/kg	2,976	2,974	2,959	2,974
Digestible Lys, %	1.10	1.10	1.10	1.1
Digestible Met + Cys, %	0.78	0.78	0.78	0.78
Digestible Trp, %	0.25	0.25	0.25	0.25
Ca, %	0.89	0.89	0.89	0.89
Available P, %	0.44	0.44	0.44	0.44
Analyzed content				
Xylanase, EPU/kg	0	0	14,961	15,712
Protease, EPU/kg	0	315	0	333

¹Starter diets were fed from d 0 to 14 and switched to grower diets on d 14²Vitamin premix (NCSU-90) provided per kilogram of diet: Vitamin A, 6,600 IU; cholecalciferol, 2,000 IU; Vitamin E, 33 IU; Vitamin B12, 19.8 µg; riboflavin, 6.6 mg; niacin, 55 mg; pantothenic acid, 11 mg; Vitamin K, 2 mg; folic acid, 1.1 mg; thiamine, 2 mg; pyridoxine, 4 mg; biotin, 126 mg.³Trace mineral (TM-90) premix provided in milligrams per kilogram of diet: Manganese, 120; zinc, 120; iron, 80; copper, 10; iodine, 2.5; cobalt, 1.0. Selenium premix as either sodium selenite or organic selenium (Sel Plex 50) was provided to each diet at a level to assure a concentration of either 0.1 or 0.3 ppm.

Table 2. Ingredient and calculated chemical composition of experimental grower¹ diets on an as fed basis for 28 d

Xylanase, 15,000 U/g Protease, 300 U/g	Grower			
	0		15,000	
	0	300	0	300
Ingredient %				
Yellow corn	56.9	56.85	56.89	56.84
Soybean meal	25.92	25.91	25.92	25.91
DDGS, corn	10.01	10.01	10.01	10.01
L-Lys HCl	0.20	0.20	0.20	0.20
DL-Met	0.15	0.15	0.15	0.15
L-Thr	0.10	0.10	0.10	0.10
Dical P (6-01-080)	1.64	1.64	1.64	1.64
Limestone, ground (6-02-632)	0.79	0.79	0.79	0.79
Vitamin premix ²	0.05	0.05	0.05	0.05
Mineral premix ³	0.20	0.20	0.20	0.20
Salt	0.50	0.50	0.50	0.50
Choline chloride, 60%	0.20	0.20	0.20	0.20
Selenium premix	0.05	0.05	0.05	0.05
Poultry fat	2.79	2.79	2.79	2.79
Xylamax	0.00	0.00	0.01	0.01
Versazyme	0.00	0.05	0.00	0.05
TiO ₂	0.50	0.50	0.50	0.50
Total	100	100	100	100
Calculated composition				
ME, kcal/kg	3,032	3,031	3,032	3,030
Digestible Lys, %	1.02	1.02	1.02	1.02
Digestible Met + Cys, %	0.72	0.72	0.72	0.72
Digestible Trp, %	0.22	0.22	0.22	0.22
Ca, %	0.79	0.79	0.79	0.79
Available P, %	0.40	0.40	0.40	0.40
Analyzed content				
Xylanase, EPU/kg	0	0	14,460	13,943
Protease, EPU/kg	0	355	0	353

¹Grower diets were fed from d 14 to 28²Vitamin premix (NCSU-90) provided per kilogram of diet: Vitamin A, 6,600 IU; cholecalciferol, 2,000 IU; Vitamin E, 33 IU; Vitamin B12, 19.8 µg; riboflavin, 6.6 mg; niacin, 55 mg; pantothenic acid, 11 mg; Vitamin K, 2 mg; folic acid, 1.1 mg; thiamine, 2 mg; pyridoxine, 4 mg; biotin, 126 mg.³Trace mineral (TM-90) premix provided in milligrams per kilogram of diet: Manganese, 120; zinc, 120; iron, 80; copper, 10; iodine, 2.5; cobalt, 1.0. Selenium premix as either sodium selenite or organic selenium (Sel Plex 50) was provided to each diet at a level to assure a concentration of either 0.1 or 0.3 ppm.

Table 3. Viscosity of ileal digesta (cP¹) in broilers fed experimental diets for 28 d

Xylanase, 15,000 U/g								
	0		15,000			<i>P</i> value ²		
Protease, 300 U/g	0	300	0	300	SEM	Xyl	Prot	Int
Wk 1, cP	3.00	3.35	4.40	2.87	0.43	0.314	0.208	0.059
Wk 4, cP	2.98	3.77	4.43	2.34	0.56	0.989	0.202	0.009

¹cP = centipoise (1 cP = 1/100 dyne s/cm²)

² Xyl: effect of xylanase; Prot: effect of protease; and Int: interaction

Table 5. IgG, Protein carbonyl, and MDA measured from blood serum and ileal mucosal tissue in broilers fed experimental diets for 28 d

Xylanase, 15,000 U/g		0		15,000		SEM	<i>P</i> value ¹		
Protease, 300 U/g		0	300	0	300		Xyl	Prot	Int
IgG									
Serum, g/L									
Wk 1		2.80	2.51	1.96	2.15	0.19	0.003	0.797	0.203
Wk 4		2.21	2.04	2.26	2.08	0.21	0.408	0.834	0.955
Ileum, mg/g protein									
Wk 1		0.20	0.26	0.21	0.20	0.03	0.501	0.430	0.326
Wk 4		0.20	0.17	0.15	0.09	0.04	0.111	0.217	0.617
Protein carbonyl									
Serum, μmol/L									
Wk 1		0.80	0.70	0.76	0.62	0.07	0.404	0.083	0.776
Wk 4		0.70	0.64	0.68	0.66	0.03	0.899	0.262	0.520
Ileum, μmol/g protein									
Wk 1		0.41	0.40	0.37	0.21	0.02	<0.001	<0.001	0.001
Wk 4		0.19	0.18	0.19	0.19	0.01	0.699	0.492	0.927
Malondialdehyde									
Serum, μmol/L									
Wk 1		0.43	0.37	0.45	0.52	0.07	0.225	0.918	0.361
Wk 4		0.58	0.30	0.53	0.30	0.07	0.760	0.002	0.711
Ileum, μmol/g protein									
Wk 1		0.55	0.81	0.60	0.66	0.11	0.598	0.147	0.362
Wk 4		0.61	0.33	0.45	0.37	0.06	0.295	0.004	0.087

¹ Xyl: effect of xylanase; Prot: effect of protease; and Int: Xyl X Prot interaction

Table 6. Apparent ileal digestibility (AID, %) of DM, GE, lipid, and crude protein in broilers fed experimental diets for 28 d

Feed experimental diets for 28 d									
Xylanase, 15,000 U/g		0		15,000		<i>P</i> value ¹			
Protease, 300 U/g		0	300	0	300	SEM	Xyl	Prot	Int
AID, %									
Dry matter		75.78	75.56	73.68	77.39	0.90	0.881	0.064	0.038
Gross energy		75.66	71.85	72.06	75.91	2.10	0.914	0.992	0.080
Lipid		54.34	57.58	49.93	56.40	2.70	0.311	0.084	0.557
Crude protein		85.04	83.79	83.83	84.78	1.16	0.929	0.899	0.353

¹ Xyl: effect of xylanase; Prot: effect of protease; and Int: Xyl X Prot interaction

Table 7. Growth performance of broiler chickens fed experimental diets for 28 d¹

Xylanase, 15,000 U/g		0		15,000		SEM	<i>P</i> value ²		
Protease, 300 U/g		0	300	0	300		Xyl	Prot	Int
BW, g									
Initial		45	45	45	45	0	0.862	0.656	0.629
Wk 1		163	159	167	154	2	0.799	0.002	0.044
Wk 2		437	429	437	430	5	0.906	0.132	0.944
Wk 3		903	882	916	886	10	0.403	0.018	0.613
Wk 4		1460	1455	1499	1446	17	0.385	0.089	0.160
ADG, g/d									
Wk 1		16.8	16.4	17.5	15.5	0.3	0.796	0.002	0.045
Wk 2		39.2	38.5	38.6	39.4	0.6	0.788	0.875	0.234
Wk 3		66.5	64.8	68.5	65.2	0.9	0.218	0.009	0.391
Wk 4		79.7	81.8	83.3	80.0	1.6	0.579	0.707	0.097
Wk 1 to 2		28.0	27.4	28.0	27.5	0.4	0.908	0.134	0.941
Wk 3 to 4		73.1	73.3	75.9	72.6	1.0	0.325	0.136	0.094
Overall		50.6	50.4	51.9	50.0	0.6	0.385	0.089	0.160
ADFI g/d									
Wk 1		20.0	20.2	20.2	18.5	0.4	0.063	0.042	0.017
Wk 2		48.4	47.9	48.6	47.9	0.4	0.794	0.114	0.703
Wk 3		102.2	100.7	102.0	103.2	1.3	0.379	0.937	0.292
Wk 4		137.1	135.9	137.6	138.8	1.8	0.344	0.996	0.490
Wk 1 to 2		34.2	34.0	34.4	33.2	0.2	0.205	0.007	0.031
Wk 3 to 4		119.7	118.3	119.8	121.0	1.4	0.309	0.968	0.350
Overall		76.9	76.2	77.1	77.1	0.7	0.459	0.612	0.613
F:G									
Wk 1		1.190	1.235	1.158	1.188	0.063	0.044	0.054	0.706
Wk 2		1.236	1.246	1.262	1.218	0.016	0.967	0.308	0.109
Wk 3		1.537	1.554	1.490	1.584	0.011	0.453	<0.001	0.001
Wk 4		1.723	1.665	1.654	1.738	0.021	0.922	0.541	0.002
Wk 1 to 2		1.221	1.242	1.229	1.209	0.013	0.341	0.955	0.121
Wk 3 to 4		1.637	1.615	1.579	1.668	0.012	0.842	0.008	<0.001
Overall		1.522	1.513	1.485	1.542	0.009	0.609	0.008	0.001

¹Each mean represents 9 cages of 8 birds (d0 to d7) or 6 birds (d8 to d 28).² Xyl: effect of xylanase; Prot: effect of protease; and Int: Xyl X Prot interaction

Table 8. Effects of feed enzyme supplementation on mortality in broilers experimental diets for 28 d

Xylanase, 15,000 U/g	0		15,000		SEM	<i>P</i> value ¹		
	0	300	0	300		Xyl	Prot	Int
Protease, 300 U/g	0	300	0	300	SEM	Xyl	Prot	Int
Mortality, %	0.22	0.11	0.00	0.22	0.12	0.641	0.641	0.167

¹ Xyl: effect of xylanase; Prot: effect of protease; and Int: Xyl X Prot interaction