2023

THE EFFECTS OF EXOGENOUS ENZYME SUPPLEMENTATION ON THE PERFORMANCE, BONE QUALITY, AND NUTRIENT UTILIZATION OF BROILER CHICKENS

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Digital Object Identifier: https://doi.org/10.13023/etd.2023.327

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food and Environment
at the University of Kentucky

By

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ABSTRACT OF THESIS

THE EFFECTS OF EXOGENOUS ENZYME SUPPLEMENTATION ON THE PERFORMANCE, BONE QUALITY, AND NUTRIENT UTILIZATION OF BROILER CHICKENS

Two experiments were conducted to examine the effects of Allzyme® Spectrum supplementation to a reduced nutrient diet on the growth performance, energy and nutrient digestibility and utilization, bone quality, and jejunal digesta viscosity in broiler chickens. Each experiment used 300 one-day old Cobb by-product breeder chicks with 5 treatments, 10 replicates, and 6 birds per replicate cage in a randomized complete block design. The only differences between the two experiments were experiment 1 lasted for 21 days using a corn-soybean meal-based diet and experiment 2 lasted for 22 days using a wheat-soybean meal-based diet. The 5 treatments included a positive control (PC) diet that met or exceeded nutrient Ca (0.8%) and avP (0.4%) and energy (3,050 kcal/kg) requirements of birds of this age, a negative control (NC) diet that consisted of a reduction of 90 kcal/kg ME and 0.15%-point less Ca and P, and the last 3 treatments were varying inclusion levels of Allzyme® Spectrum (150, 200, 250 g/ton, respectively) added to the NC diet. Allzyme® Spectrum (Alltech, Inc., Nicholasville, KY, USA) is an enzyme complex containing xylanase and phytase. Data were analyzed using the GLM procedures of SAS (v 9.4). Simple contrasts were used to compare the PC vs. NC diet and PC vs. enzyme supplemented diets. A post-hoc test was used to generate the coefficients for the polynomial contrasts. Orthogonal polynomial contrasts were used to compare increasing levels of enzyme supplementation on the performance, jejunal digesta viscosity, nutrient digestibility and utilization, and bone mineralization of chickens. In experiment 1, increasing levels of enzyme supplementation resulted in a quadratic increase ($P < 0.05$) in feed intake (FI), body weight gain (BWG) and feed efficiency (FE) d 9-21 and a linear increase ($P < 0.05$) in FI d 9-21 and 0-21 and FE d 0-9, 9-21, and 0-21. In experiment 2, FI of chickens from d 9-22 and 0-22 and FE d 9-22 linearly increased ($P < 0.05$) with increasing level of supplemental Allzyme® Spectrum. There was a quadratic effect ($P < 0.05$) of enzyme supplementation level on BWG d 9-22 and 0-22 and FE d 9-22 and 0-22. In experiment 2, increasing levels of enzyme supplementation resulted in both a quadratic and linear decrease ($P < 0.001$) in jejunal digesta viscosity. In both studies, increasing levels of enzyme supplementation resulted in a quadratic increase ($P < 0.05$) in dry matter and N digestibility and retention as well as, digestible and metabolizable energy and a linear increase ($P < 0.05$) in P and Ca digestibility. Increasing enzyme supplementation resulted in both a quadratic and linear increase ($P < 0.001$) in P and Ca utilization in experiment 1, however it only resulted in a linear increase ($P < 0.001$) in Ca and P utilization in experiment 2. In experiment 1, there was a quadratic increase ($P < 0.01$) in ileal digestibility of all the amino acids with increasing level of enzyme supplementation. In experiment 2, there was a quadratic increase ($P < 0.05$) in ileal digestibility of Arg, His, and Val and nonessential amino acids (excluding Glu: $P = 0.069$ and Tyr: $P = 0.088$) and a linear increase ($P = 0.001$) in ileal digestibility of Thr when comparing the enzyme supplemented diets. There was a quadratic increase ($P < 0.05$) with increasing levels of enzyme supplementation in bone

breaking strength and bone ash in both studies. In conclusion, Allzyme® Spectrum supplementation improved growth performance, energy and nutrient digestibility and utilization, and bone mineralization in broiler chickens and reduced jejunal digesta viscosity in 21- and 22-day-old broiler chickens fed wheat-based diets.

Keywords: broiler chicken, digestibility, enzyme, nutrient, energy

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06/13/2023

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DEDICATION

This thesis is dedicated in memory of my father, Mark J. Bauer, who lost his battle with cancer only a few months prior to seeing me graduate. Your love and support are what made this all possible. Everything I have ever accomplished has been because of you. You raised me to be the person I am today; I could never tell you enough how grateful I am for that.

ACKNOWLEDGMENTS

I would like to start out by thanking my major professor, Dr. Tayo Adedokun, for his constant guidance and support throughout this journey. Additionally, I would like to thank my committee members Dr. Merlin Lindemann, Dr. Anthony Pescatore, and Dr. Tuoying Ao for their beneficial guidance over the past two years. I'd like to extend a special thanks to Ms. Sydney Davis, Mr. Duncan Paczosa, and all the other graduate students for their constant assistance and support throughout my time at the University of Kentucky as a graduate student.

I also appreciate Mr. David Gillespie, Dr. Noel Inocencio, Ms. Velvet Barnett, and all technicians for their assistance both inside and outside the laboratory, your guidance is what helped make this possible. A big thank you goes to Mr. Mike Ford and all the staff at the Coldstream farm for aiding in the animal studies presented in this thesis. I would also like to thank members of the poultry group, Dr. Rebecca Delles, Dr. Jacqueline Jacob, Dr. Marquisha Paul, and Ms. Elizabeth Robinson for all their help with my studies.

Sincere gratitude is extended to all my family and friends who have supported me throughout this journey. A special thank you to my mother Mrs. Janet Bauer for your continuous love and support, without you I couldn't have achieved this. Finally, thank you to my father, Mr. Mark Bauer who pushed me to pursue a graduate degree. Thank you for always believing in me, even when I didn't believe in myself, no one wanted this for me more than you did, and I know you would be so proud.

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List of Abbreviations

BW = body weight

BWG = body weight gain

FI = feed intake

FE = feed efficiency

PC = positive control

NC = negative control

DM = dry matter

NSP = Nonstarch polysaccharide

AA = amino acids

BBS = bone breaking strength

ME = metabolizable energy

GE = gross energy

AME = apparent metabolizable energy

AMEn = apparent metabolizable energy corrected for nitrogen

ADE = apparent digestible energy

CHAPTER 1. Introduction

The poultry industry is one of the fastest growing agricultural industries in the world. There is a constant increase in demand for eggs and meat annually, as they are an excellent source of energy and protein. Approximately 70-80% of the total cost of raising poultry is attributed to feeding alone. Although in recent years, human population growth rates have slowed, there is still an annual increase in the world population (USDA, 2022). The USDA predicts that by 2031, the world population will be 8.5 billion, a 0.9% increase from 2022 (USDA, 2022). To keep pace with the continually growing population, farmers will need to find a way to produce quality livestock and poultry products, without competing with raw materials needed for human consumption. The feed-to-price ratio of broilers is expected to steadily rise over the next 10 years. The USDA estimates that by 2031, the feed-to-price ratio of broilers will rise over 34%. Therefore, there is a huge demand for products that can be added to poultry diets to increase the nutritional value of feed ingredients and consequently reduce feed costs.

Furthermore, environmental pollution is a huge concern in the poultry industry. Excess non-phytate phosphorus (P) is commonly added to poultry diets to offset the unavailability of phytate-bound P found in poultry plant-based feedstuff. Additionally, the high protein requirements of broiler chickens for rapid growth can cause large amounts of nitrogen to be excreted into the environment. This excess of nutrients in the soil then runs off from the fields into surrounding bodies of water. This then causes a spike in algae growth in the water. When algae forms, it uses up oxygen and blocks the sunlight from entering the water. This oxygen depleted water becomes a dead zone and can no longer support life. This is known as eutrophication, which is detrimental to the

ecosystems of lakes and coastal waters. Livestock and poultry manure are also responsible for significant global emissions of ammonia that is expelled into the environment. Thus, there is a large market for feed additives that can improve the nutrient availability in feeds to birds, subsequently eliminating the excess nitrogen and P excretion into the environment.

Current research is focused on using multi-enzymatic products to improve the nutrient availability of various feedstuffs to birds. Phytase is the most widely used enzyme in the poultry industry, due to its ability to increase the availability of phytate-bound P and mitigate the anti-nutritional effects of phytate (Naghshbandi and Moghimi, 2020). Phytate not only reduces the availability of P, but also reduces the digestibility of minerals, amino acids, and energy. The addition of phytase to poultry diets has been shown to alleviate these negative effects. Carbohydrase enzymes are commonly used for wheat, rye, and barley-based diets which contain high amounts of non-starch polysaccharides (NSP). The NSPs inhibit the absorption of nutrients by increasing intestinal viscosity. Carbohydrase enzymes degrade NSP, thus reducing intestinal viscosity and consequently allowing for better digestion and absorption of nutrients. Xylanase and β-glucanase are the most popular carbohydrases used, accounting for over 80% of the global market availability (Adeola and Cowieson, 2011). Other enzymes such as protease, which improve protein digestion, and tannase, which aids in alleviating the anti-nutritional effects of tannins, have started to gain popularity in the poultry industry as well (Abdulla et al., 2016). Research and development in the area of animal feed enzyme technology is ongoing, and it is likely that superior enzymes may be developed in the future to complement the conditions found in an animal's digestive tract. It is

expected that these developments will further enhance the overall effectiveness of enzymes used for animal feed nutrition.

CHAPTER 2. Literature Review

2.1 Feed Ingredients used in Poultry Diets

2.1.1 Cereal Grains

Globally, there is a huge reliance on traditional cereal grains and soybean meal to achieve the nutrient supply and balance needed in poultry diets. Cereal grains are traditionally added to poultry diets as a source of energy, however some cereals, such as wheat, make a large contribution to the crude protein content of the overall feed (Chadd, 2007). Cereals are grasses that produce edible starchy grains and many of these starches, excluding corn whose starch is highly digestible, contain anti-nutritive factors known as NSP. Energy is the most expensive component of feedstuffs for nonruminants, and cereals are used for this purpose worldwide (Choct and Hughes, 1997). Recently, there are concerns over the rising prices of common cereal grains due to competition in other sectors such as ethanol production, leaving many to look into alternative energy sources such as by-products (Chadd, 2007).

2.1.1.1 Corn

Corn, also known as maize, is the most routinely used grain in commercial poultry diets in the US. The majority of the corn grown in the US is hybrid corn, which accounts for about 95% of the corn grown in the US (USDA, 2022). Hybrid corn seed is acquired by crossing inbred lines obtained by self-pollination over several generations. Conventional breeding programs and biotechnology have been used to develop new varieties of dent corn. Among these is high oil corn, whose oil content is higher than conventional corn. This results in a corn variety with an increased energy and nutrient

density (Harper, 1998). Waxy corn is another newer variety of corn which gained its name due to the endosperm being wax-like when the kernel is opened. Waxy corn is almost exclusively amylopectin making it more digestible, although, waxy corn has a higher content of β-glucans, an anti-nutritive (Cromwell et al., 1968; Harper, 1998). Opaque-2 and floury-2 are new varieties of corn that contain higher amounts of certain essential amino acids. Opaque-2 corn has increased concentrations of tryptophan and lysine whereas floury-2 corn has higher amounts of methionine and lysine (Cromwell et al., 1968). While these two new varieties have superior amino acid profiles, their yields are inferior to other varieties of corn, so they are not widely grown (Cromwell et al., 1968).

The nutritional value of corn is variable depending on location, variety, growing conditions, and starch structure. Corn is a high-density source of available energy in poultry diets and is easily digestible. Corn is also highly palatable and low in fiber (Cowieson, 2005). Yellow dent corn is the most popular variety used in animal feed. While nutritional contents vary between varieties and growing locations, it averages an energy content of about 3,350 kcal/kg and a crude protein content of around 7.5% (Cowieson, 2005). Corn contributes roughly 20% of the protein and 65% of the metabolizable energy in broiler starter diets (Cowieson, 2005). The energy value of corn is generally considered the standard to which other feedstuffs are compared. However, corn is low in the essential amino acid, methionine, so it is typically fed alongside soybean meal to mitigate this deficiency. Majority of the P in corn is present as phytate-bound P. Due to birds not producing the enzyme phytase, this makes the phytate-bound P highly unavailable to birds. Aside from the addition of phytase to feed, there are also

varieties of corn that are low-phytate, which lessens the need for additional P supplementation.

Corn contains approximately 70% starch, 30% of which is amylose with the other 70% being amylopectin. Amylopectin is more digestible than amylose due to amylose forming a very compact physical structure (Buleon et al., 1998). Majority of corn starch is present in the endosperm. In corn, starch granules are rooted in the protein matrix that is usually hydrophobic and is either loosely or tightly packed depending on growing conditions and genetics. When it is tightly packed and higher in protein than starch, the endosperm is described as glassy whereas, when it is loosely packed it is described as floury (Cowieson et al., 2019). Thus, the digestion of starch requires solubilization of hydrophobic amylose which requires the enzyme, amylase.

Corn can be adversely affected by amylase inhibitors. Amylase inhibitors in grains serve as protection from insects, which can cause extensive damage to crops. Amylase inhibitors impede the digestion of starch and other carbohydrates by slowing digestion and reducing the absorption of glucose. Amylase inhibitors can cause an increased production of amylase in the gastrointestinal (GI) tract which can lead to pancreatic hyperplasia or hypertrophy (Cowieson, 2005). Some corn also contains starch that is described as “resistant to digestion” and is termed ‘resistant starch (RS).’ Resistant starch is usually classified into three categories: RS1, RS2, and RS3. RS1 is starch that evades digestion because of its encapsulation by or with other compounds such as carbohydrates or proteins. RS2 is caused by the conformation and structure of the native starch granules. RS3 is related to the effects of processing on starch. There has also been a fourth classification, RS4, which includes the development of unique chemical bonds through

chemical alteration (Cowieson, 2005). These various RS and amylase inhibitors can cause variations in the energy values of different corns.

2.1.1.2 Wheat

Wheat is a popular feed ingredient in poultry diets in parts of Canada and Europe. The use of wheat in poultry feed has become more popular due to its favorable prices and the availability of exogenous enzymes, which improve the feeding value of wheat in poultry. Wheat used in feed is de-husked, which reduces its fiber content. The energy content of wheat is slightly lower than corn, but wheat is higher in protein and consequently higher in amino acid concentrations. Thus, allowing wheat diets to require less protein supplementation when compared to corn.

Wheat's nutritional composition varies depending on year, growing location, moisture conditions, fertilization, and other agronomic factors. Of all cereal grains, wheat is considered the most variable regarding protein and available energy content. This variability in energy has been reported to be due to growing location. Variations as high as 1,400 kcal/kg have been reported (Ravindran and Amerah, 2009), making this an issue for nutritionists when developing diets.

Wheat varieties are categorized in three ways: hard or soft, red or white, and winter or spring. Varieties are considered soft wheat if they have a low gluten content, and higher starch content, whereas hard wheat has a higher protein and gluten content. Red and white wheat are classified based on the color of the grain. Red wheat is slightly higher in protein and gluten compared to its counterpart (Clark et al., 1923). Red wheat is also considered to have a more defined taste, often described as nutty or bitter. The final

classification is based on when the wheat is planted, winter or spring. Winter wheat has a higher yield potential due to its longer growing season. Winter wheat requires near freezing temperatures to trigger its reproductive stage. Spring wheat has a shorter growing season and can not withstand harsh, cold temperatures.

Wheat by-products are also commonly used in animal feed. Wheat bran is the hard outer layer of the wheat kernel and is high in fiber. Wheat bran is also higher in crude protein and lysine when compared to regular wheat, but it is significantly lower in energy. Wheat middlings are the small particles of wheat produced during the milling process for flour. Wheat middlings are higher in crude protein and fiber than regular wheat, but lower in both when compared to wheat bran. Although, wheat middlings have a higher energy content when compared to that of wheat bran (Slominski et al., 2003).

Nonstarch polysaccharides make up most of the cell wall contents and consist of the part of plant tissue that is not digestible. The NSP in wheat are arabinoxylans, β-glucans, and cellulose. Wheat contains, on average, 12% non-starch polysaccharides, 8% of which is arabinoxylans, 2% cellulose, and 1% β-glucans. Nonstarch polysaccharides are known to increase the viscosity of gut contents due to their ability to readily absorb water forming viscous solutions or gels. The viscous contents in the gut inhibit nutrient digestion by decreasing the integration of digestive enzymes with its contents (Ravindran and Amerah, 2009). As a result, NSP has long been considered as a limiting factor in the use of wheat in poultry diets. However, the increase in popularity of the use of carbohydrase enzymes, which mitigate the adverse effects of NSP, has made wheat a staple in poultry diets.

2.1.1.3 Barley

Barley is a cereal grain commonly used in Canada and Europe in poultry diets. Although barley is used as a main energy source, it is considered a low-energy grain due to its low starch and high fiber content. The energy content of barley varies widely, but is most dependent on growing location, cultivar, storage, and harvesting conditions (Jacob and Pescatore, 2012). Barley can be grown in a variety of climates, varying from subarctic to subtropical. Specific cultivars have been adapted for each individual climate.

Barley can be fed both hulled and dehulled. The proportion of kernel to hull varies widely between cultivars. The hull is a major contributor of crude fiber in the diet, so when the hull is removed, the crude fiber content is similar to that of wheat and corn (Jacob and Pescatore, 2012). There are two kinds of barley without hulls, hull-less and dehulled. Dehulled barley is hulled barley that had its hull removed. Hull-less barley looks similar to hulled barley during the growth phase, but close to maturity the hulls loosen and then are completely removed during harvesting. Hull-less barley is reported to be equal to wheat and better than hulled barley (Classen et al., 1988).

Barley's nutritive value and suitability as a feedstuff for poultry is limited greatly by its concentrations of NSPs. The main source of NSPs in barley are β-glucans. β-glucans are structural components of cell walls, and the structure of β-glucans varies depending on feedstuff. These variations in structure can account for β-glucans differences in their physiological role (Jacob and Pescatore, 2014). β-glucans are similar to other NSPs in how they greatly affect digesta viscosity. The β-glucans bind with water in the intestines, resulting in an increase in digesta viscosity. An increase in intestinal

viscosity affects the digestion and absorption of nutrients, resulting in poor bird performance. Fuente et al. (1995) reported that digesta viscosity accounts for up to 97% of the variation in apparent metabolizable energy (AME) among barley-based diets. Almirall et al. (1995) reported that broilers fed barley-based diets had an increased digesta viscosity and decreased digestive enzyme activities. β-glucanase is often added to barley diets due to its ability to hydrolyze the bonds in β-glucans, resulting in a decreased digesta viscosity and better nutrient digestion and absorption (Jacob and Pescatore, 2012). This has allowed barley to become more of a staple in poultry feeds.

2.1.2 Common Protein Sources

2.1.2.1 Soybean Meal

Soybean meal is the most commonly used source of protein in poultry diets worldwide (Ravindran et al., 2014). Soybeans are grown in over 35 countries as the major oilseed, with the biggest producers worldwide being the US, Brazil, Argentina, and China (Banaszkiewicz, 2011). The US is the main exporter of soybean seeds accounting for about 44% of exports and Argentine is the main exporter of soybean meal accounting for 37% worldwide (Banaszkiewicz, 2011). Approximately 90% of all soybeans harvested are used for animal feed (Banaszkiewicz, 2011). The seeds are subjected to various types of processing, so by-products of soybeans such as expellers and oilseed meal are used in animal feed as well.

Soybean meal is fed for its high protein and amino acid content. Thakur and Hurburgh (2007) reported that US soybean meal had the highest and most digestible protein and total amino content when compared to soybean meal from Argentina, Brazil,

China, and India. Ravindran et al. (2014) also reported that US soybean meal had the highest total content of Ca, AME, total digestible amino acids, and digestible crude protein. They also reported a specifically high content of certain amino acids including methionine, cysteine, arginine, lysine, threonine, and valine compared to the other countries. Soybean meal's amino acid profile complements that of most cereal grains, especially corn, due to its low arginine and lysine content (Ravindran et al., 2014).

The nutritive value of soybean is limited by trypsin and chymotrypsin inhibitors, these are a type of protease inhibitors. Trypsin inhibitors interfere with protein digestion and lower the nitrogen retention of animals, resulting in a decreased performance and increased nitrogen excretion (Banaszkiewicz, 2011). The activity of these inhibitors can be decreased by heating during processing of the meal, this can eliminate up to 90% of activity (Banaszkiewicz, 2011). Poultry are generally more sensitive to trypsin inhibitors compared to swine and cattle (Banaszkiewicz, 2011).

2.1.2.2 Canola Meal

Canola is also a name for cultivars of rapeseed that contain less than 2% of total fatty acids in the oil as erucic acid and less than 30 micromoles of alkenyl glucosinolates per gram of oil-free dry matter (DM) of the seed (Bell, 1993). Because of this, canola meal is superior to rapeseed meal, but it still sells for less than its competition, soybean meal (Bell, 1993). Canola meal generally contains less energy and protein than soybean meal, but it does have a higher concentration of B vitamins and essential minerals (Bell, 1993).

The amino acid composition compares favorably to that of soybean meal due to the higher presence of methionine and cysteine (Khajali and Slominski, 2012).

Canola meal is primarily used as a protein supplement, but its energy content is a critical factor in the formulation of feeds. The reduction of glucosinolate levels from specified plant breeding has led to higher metabolizable energy (ME) values in canola meal (Bell, 1993). Glucosinolates are plant secondary metabolites that have been shown to produce undesired effects in poultry (Fenwick and Curtis, 1980). Glucosinolates have been reported to decrease palatability, growth, and production (Fenwick and Curtis, 1980). The glucosinolate content of canola meal has been steadily declining and canola meal is now able to be fed up to 20% in broiler rations without any adverse side effects (Khajali and Slominski, 2012). Gopinger et al. (2014) reported that canola meal can be fed to broilers up to 16.7% with no adverse effects and up to 20% with no negative effects on crude protein digestibility, although they noted a linear decrease in the digestibility of DM and N-free extract with this increased inclusion.

Fiber, protein, and oil content also affect the ME values of canola meal, these factors can be influenced by a variety of things including seed variety, growing conditions, growing season, and hull type (Bell, 1993; Khajali and Slominski, 2012). The hulls are largely fiber, and they remain in the meal following oil extraction (Khajali and Slominski, 2012). Approximately 30% of the meal weight is fiber. Hull digestibility depends on the type of hull, yellow seed hull has a higher energy digestibility compared to the darker hulls (Bell, 1993). Protein levels in canola meal can vary depending on location and season, a higher protein content means more digestible protein, which can enhance ME values. Oil contents vary depending on the efficiency of the oil extraction

process and what by-products of the oil refining are added back into the meal (Khajali and Slominski, 2012). The higher the oil content of canola meal, the higher the ME values.

Studies have shown that poultry and swine benefit from processing conditions that provide only the minimum heat necessary to inactivate enzymes and denature proteins (Bell, 1993). Processing is the largest factor in the protein content and digestibility in canola meal (Gopinger et al., 2014). Excessive heat can lead to undesired browning reactions and reduced amino acid availability, especially lysine (Bell, 1993). Overheating during processing has been shown to reduce the apparent digestibility of lysine by 5% (Khajali and Slominski, 2012). Since lysine is a limiting amino acid in poultry, any reduction in lysine availability can seriously affect the value of canola meal use as a protein source.

Tannins can also affect the nutritional value of canola meal, although tannin content in canola meal is generally 1.5-3% (Bell, 1993). Tannins exist mainly in the hulls and are more prevalent in the darker hulls than the yellow (Khajali and Slominski, 2012). Tannins have been known to inhibit digestive enzymes, specifically ones that aid in the digestion of protein (Bell, 1993). The antinutritional effects of tannins can be offset by tannase, an enzyme that breaks down tannins (Bell, 1993; Khajali and Slominski, 2012).

2.2 The Role of Minerals in Poultry Production

Minerals, in regard to nutrition, are defined as elements on earth and in foods that are necessary for normal development and function. Minerals are the inorganic portions of tissue and feeds which are often classified into two categories: macro and trace

(micro). Macro minerals are required in larger amounts in the diet and are generally stated as a percentage of the diet, however trace minerals represent a much smaller portion of the diet and are usually stated as mg/kg or parts per million (ppm) of the diet. Minerals are necessary for building bones, energy production, as enzyme cofactors, maintaining homeostasis, and utilization of vitamins and various other nutrients (NRC, 1994).

Macro minerals for poultry production include P, Ca, K, Na, Mg, and Cl. P and Ca are vital for the development and maintenance of the skeleton. Potassium, magnesium, sodium, and chloride work with bicarbonates and phosphates to maintain homeostasis within the body (NRC, 1994).

P plays an essential role in the growth and development of poultry. Around 85% of the body's total P is stored in the bones (Applegate, 2003). P is also an essential mineral for energy, carbohydrate, amino acid, and fat metabolism. P is necessary for energy utilization and cell structure, in addition to its importance in bone formation. Digestible forms of P generally account for only 30 to 40 percent of the total P present in plants. The remaining P is in the form of phytate P which is poorly digested by poultry. Approximately 10% of phytate P present in wheat and corn is digested by birds (NRC, 1994). Supplemented non-phytate P and P from animal by-products are significantly more digestible (NRC, 1994).

Calcium is mainly used for bone formation in growing birds, but in layers it is used primarily for the formation of eggshells. Calcium also assists in blood clotting and as a secondary messenger within intracellular communications (NRC, 1994). The role of dietary calcium is closely related to that of P and the effect of vitamin D. 99% of Ca is

present in the bones and body ashes consist of over 70% Ca (Pelicia et al., 2009). Dietary calcium is absorbed throughout all the small intestine but is primarily absorbed in the duodenum and jejunum (Pelicia et al., 2009). The rate at which calcium is absorbed is quicker than any other ion second only to sodium (Pelicia et al., 2009). Diets deficient in calcium cause animals to increase calcium absorption levels, whereas calcium absorption is reduced when fed diets with adequate to high levels of calcium (Pelicia et al., 2009). Excessive calcium can inhibit the availability of various minerals including P, manganese, zinc, and magnesium. A calcium to P ratio of 2-to-1 is recommended for non-laying birds. For layers, it is recommended that they receive more calcium necessary for eggshell formation (NRC, 1994). Pelicia et al. (2009) reported that a diet containing 4.5% calcium was optimal for laying hen performance and eggshell quality.

Dolomitic limestone is a combination of magnesium and calcium carbonate which is used to neutralize acids and provides some additional calcium and magnesium for plant uptake. Since it is usually cheap to buy, some use it as a source of calcium for poultry. However, if fed in excess, dolomitic limestone can be harmful to birds. Many commercial limestones have high concentrations of calcium and low concentrations of magnesium, making them much safer sources of calcium to feed. Chlorophyll is the substance that gives plants their green color and is rich in magnesium, so generally, no additional supplementation of magnesium is required for birds (Schaible, 1941). Magnesium deficiency has been shown to reduce the antioxidant defense in the tissues (Liu et al., 2007). Other studies have reported that magnesium supplementation caused a decrease in lipid peroxidation in animal tissues (Liu et al., 2007). However, excessive magnesium has been shown to negatively affect bone calcification (Atteh and Leeson, 1983). In the

poultry industry, minimal attention has been paid to magnesium and its effects, and it is rarely included in mineral mixtures.

Dietary sodium and chloride are necessary for all animals and are supplied from the addition of salt to diets. Sodium and chloride aid in growth performance and eggshell production. Too much dietary salt can cause excessive drinking which can lead to issues with wet litter and ventilation (NRC, 1994).

Acid-base balance is influenced by potassium, sodium, and chloride proportions in the diet. The levels of potassium and sodium versus chloride are generally used to assess adequate dietary electrolyte balance. This is because chloride has an acid-producing effect, whereas potassium and sodium have an alkaline-producing effect. Potassium and sodium generally increase bicarbonate concentration and blood pH, whereas chloride has the opposite effects. A proper balance of these electrolytes is crucial for bone development, amino acid utilization, and eggshell quality (NRC, 1994).

The essential trace minerals for poultry include iodine, iron, copper, manganese, zinc, cobalt, and selenium. Cobalt does not require supplementation due to it being a part of vitamin B_{12}. Trace minerals are vital for bird development since they are cofactors of enzymes and components of larger molecules. They also play a role in many physiological functions such as growth, energy metabolism, reproduction, and immunity. Zinc, copper, and manganese are components and catalysts of the antioxidant enzyme superoxide dismutase (Alagawany et al., 2020). Due to soil concentrations of trace minerals varying by location, feeds grown in some areas may be deficient in specific minerals, such as iodine and selenium, and require supplementation. Interactions between

trace minerals can sometimes cause a deficiency in availability of another mineral (NRC, 1994).

2.3 Energy

Energy is simply defined as the ability to do work. Anything that can do work has energy. Doing work is also defined as causing or making change, meaning energy is transferred or transformed every time work is done. The law of conservation of energy states that energy cannot be created or destroyed, it can only transfer or transform from one form to another. This is in direct relation to the first law of thermodynamics that states that the total energy of a system remains constant. Dietary energy is a source of chemical energy, which is released during digestion.

There are several ways to express the energy value of a diet or feed ingredient including calorie (cal), kilocalorie (kcal), megacalorie (Mcal), and joules (J). The standard unit of energy is the cal. Calorie is the energy necessary to increase the temperature of 1 g of water through 1° C, due to the specific heat of water varying with temperature, 1 cal is expressed more accurately as 4.184 J (NRC, 1994). A kcal is equivalent to 1,000 cal and is commonly utilized by the poultry industry. A Mcal is equivalent to 1,000,000 cal or 1,000 kcal and is frequently utilized as a source for expressing other nutrient requirements relative to dietary energy. A joule is expressed as the amount of energy needed by a force of 1 newton acting through 1 meter and can similarly be expressed as 10^7 ergs, 1 erg is the amount of work needed to move a mass of 1 g by 1 cm/s (NRC, 1994).

2.3.1 Sources of Energy

Carbohydrates are the main source of energy in nonruminant diets and are contributed by cereal grains such as corn, wheat, sorghum, and barley. Most of the carbohydrates in these cereal grains are in the form of readily digestible starch. Some cereal grains, including wheat, barley, and rye, contain higher amounts of polysaccharides, such as cellulose, hemicellulose, oligosaccharides, and pentosans, which are poorly digested by poultry. Thus, many diets are supplemented with carbohydrase enzymes to alleviate these anti-nutritional effects.

Despite not being commonly thought of as a source of energy for poultry, protein is a significant contributor to the dietary energy requirements of birds. If the bird is not getting enough energy from the diet or stored fat, protein will then be broken down into ketones, which can be used for energy. If there is an abundance of protein from the diet, it is then broken down and stored as fat (Priyankarage et al., 2008).

Protein is an important source of amino acids for poultry. Protein requirements are more about the amino acids in the protein than the protein itself. Amino acids are essential for structural and protective tissues, as well as soft tissues, such as muscles and organs. An amino acid deficiency can result in a decrease in growth performance, especially of broilers. Essential amino acids require supplementation, as poultry cannot synthesize them quick enough or at all, to meet requirements. While nonessential amino acids do not require supplementation to the diet, supplementing them diminishes the need for the bird to synthesize them from the essential amino acids (NRC, 1994). Maynard et al. (2022) reported that the removal of valine, arginine, glycine and isoleucine negatively affected growth performance and carcass traits of broilers, suggesting that some

nonessential amino acids may be limiting. Many of the amino acids have interrelationships with one another meaning a deficiency in one amino acid can lead to a deficiency in another. Broilers require more protein than layers due to their rapid growth rates. Common sources of protein for poultry include soybean, canola, and sunflower meals and animal by-products. Animal proteins have a high level of protein/amino acids and moderate levels of energy making it a beneficial component of poultry feed. Soybeans are also a great source of energy and protein for nonruminant animals (Beski et al., 2015).

Fat is added to broiler diets to increase total energy, which improves feed efficiency and productivity. Fat in poultry diets can come from a variety of sources including tallow, feed-grade animal fat, and various oils. Besides providing energy, fats can also improve absorption of fat-soluble vitamins, palatability, and reduce dustiness of feed. Dietary fat has been shown to decrease the passage rate of digesta, resulting in better nutrient absorption and utilization of nonlipid constituents. The digestibility of varying fats is directly affected by the fatty acid profile of the fat. Unsaturated fats have proven to show better utilization and have a higher ME than saturated fats (NRC, 1994; Poorghasemi et al., 2013).

2.3.2 Different Classifications of Energy

2.3.2.1 Gross Energy (GE)

Gross energy, also referred to as the heat of combustion, is defined as the energy released as heat when a substance is entirely oxidized to water and carbon dioxide. Gross

energy is the total energy in a feed or feed ingredient. Gross energy is typically measured using 25 to 30 atmospheres of oxygen inside a bomb calorimeter (NRC, 1994).

2.3.2.2 Digestible Energy (DE)

Apparent ileal digestible energy (ADE) values are not typically used in poultry feed formulations due to the fact that birds excrete urine and feces together and separation of them is extremely difficult to measure digestibility. Digestible energy is defined as the GE of the feed consumed minus the GE of the excreta (NRC, 1994). Ileal digestibility is routinely used to measure amino acid bioavailability in nonruminants. Values for ileal digestibility can be expressed as apparent (AID), standardized (SID), or true (TID) ileal digestibility. AID is calculated by the quantity of intake minus the total ileal outflow. Apparent ileal digestibility values represent the net disappearance of amino acids from the digestive tract prior to the distal two-thirds of the ileum. The major issue with AID values is that individual feed ingredients are not always additive in mixed diets (Stein et al., 2007). True ileal digestibility values represent the proportion of dietary amino acids that disappear from the digestive tract prior to the distal two-thirds of the ileum, but do not include ileal endogenous amino acid losses. The issue with this is that TID values for feed ingredients aren't usually available. Standardized ileal digestibility is calculated similar to AID values except that the basal ileal endogenous amino acid losses are subtracted from the ileal outflow. This means that SID values distinguish between feed ingredients including varying levels of specific ileal endogenous amino acid losses and thus, the major disadvantages of AID and TID values are overcome using SID values (Stein et al., 2007).

2.3.2.3 Metabolizable Energy (ME)

Apparent ME (AME) is the GE of the feed minus the GE contained in feces, urine, and gaseous products. Because gaseous products are generally negligible for poultry, the AME is the GE of feed minus the GE of excreta. Metabolizable energy is subsequently defined as the GE of the feed consumed minus the GE contained in the urine, feces, and gaseous products of digestion for most other species. Metabolizable energy represents 92-98% of the DE (NRC, 1994). An animals' ME is the total energy they obtain from their diet. Metabolizable energy is the complete functional energy that can be divided up according to the bird's metabolic requirements (Zuidhof, 2019). Dietary energy value in poultry is expressed as ME, in most cases, ME is corrected to zero nitrogen retention (ME_n).

2.3.2.4 Net Energy (NE)

Net energy is defined as energy lost as heat increment subtracted from the ME. Net energy can include energy used for production (NE_p) or the energy used solely for maintenance (NE_m). For each feedstuff, the NE value varies according to its efficiency for maintenance or various production functions, there is no absolute NE value assigned to each individual feedstuff (NRC, 1994). NE is rarely used in poultry due to the low fiber content in poultry diets since fiber has a large impact on heat production. The swine industry uses NE over ME to formulate diets which results in considerable savings in feed costs (Carré et al., 2014; van der Klis and Jansman, 2019). They prefer the use of NE since NE/ME or NE/DE ratios are greatly affected by fiber digestion, and swine diets

contain a much higher fiber content when compared to poultry diets (Carré et al., 2014; van der Klis and Jansman, 2019).

2.4 The Role of Amino Acids in Poultry Production

Amino acids are the building blocks of proteins. They are generally classified as dietary essential and non-essential. Dietary essential amino acids must be supplemented to the diet since they cannot be synthesized at all or not at a rate that is fast enough to meet the metabolic requirements of the bird. Dietary non-essential amino acids can be synthesized in the body and do not require supplementation. Amino acids are vital for structural, protective, and soft tissues (NRC, 1994). The addition of crystalline amino acids can reduce the level of crude protein in poultry diets and the loss of nitrogen during protein metabolism. This helps alleviate the pollution from ammonia excretion. Kidd et al. (2004) reported that a high inclusion of essential amino acids improved broiler performance.

A good immune system is crucial for the health and performance of birds. Many of the essential amino acids play an important role in cytokine production and immune function, therefore during periods of intense immune stress, the demand for those essential amino acids is likely to increase due to the increased need in positive acute phase proteins. Additional supply of certain amino acids is needed for periods of increased synthesis of acute phase proteins, the specific amino acids required depend on the pathway that is activated in response to the inflammation (Obled, 2003). Almost all positive acute phase proteins are glycosylated and contain high amounts of threonine, serine, aspartate, and asparagine, making these amino acids possibly important for acute

phase protein production (Obled, 2003). Amino acids are also involved in the production of antibodies (Alagawany et al., 2020). Higher dietary levels of threonine and methionine have been shown to improve a birds' immune system (Yaqoob and Ali, 2018). Threonine works as a proteinogenic amino acid, as well as being a part of immunoglobulins. Proteinogenic amino acids are incorporated biosynthetically into proteins during translation. Tryptophan is also a proteinogenic amino acid, and it is a precursor of serotonin, which is a neurotransmitter involved in feed intake regulation (Kerr et al., 2005). Adequate amino acid supplementation is therefore vital for immune function in poultry.

There are ten amino acids classified as essential: methionine, lysine, threonine, tryptophan, arginine, leucine, isoleucine, histidine, valine, and phenylalanine. For broilers, methionine and lysine are the most limiting amino acids, followed by threonine. Glycine is essential for young birds, but not for older birds. Serine is considered non-essential for poultry and tyrosine and cysteine are considered semi-essential for poultry since they can be synthesized from phenylalanine and methionine, respectively (Alagawany et al., 2020).

Methionine is one of two sulfur amino acids with cysteine being the other. Methionine is involved in important biochemical processes as a methyl group donator. It is also involved in feather synthesis and muscle growth (Alagawany et al., 2020). Methionine is essential for the biosynthesis of important substances involved in growth. Methionine can be irreversibly converted to cysteine; therefore, if there is an ample supply of methionine, cysteine can be considered non-essential (Goulart et al., 2011). A deficiency in methionine reduces performance and protein content in the carcass of

broilers and an increase in body fat deposition (Moran, 1994). This agrees with Ahmed and Abbas (2011) who found that broilers fed above the NRC requirements for methionine exhibited an increase in breast weight and a reduction in abdominal fat.

Lysine is often considered the second limiting amino acid in poultry nutrition, specifically in corn-soybean-meal diets, but is considered the most limiting amino acid in regard to bird growth (Khwatenge et al., 2020). Lysine's main role is in protein synthesis; however, it also plays an important role in cell growth and maintenance. In the ideal protein diet, lysine is considered as a reference. This concept bases dietary concentrations of amino acids on fixed ratios to lysine, therefore changing the concentration of lysine will alter the requirements for all other amino acids (Alagawany et al., 2020). The interaction between lysine and protein plays an essential role in the effects on growth performance and carcass quality, therefore the dietary protein requirement is actually a requirement for the lysine that is in the protein. Lysine above the NRC requirement improved body weight gain in broilers (Nasr and Kheiri, 2011). Supplemental lysine in conjunction with methionine improved growth performance and meat yield in broilers (Zhai et al., 2016). In agreement, Belloir et al. (2019) found that lysine supplementation improved growth performance and carcass quality in broilers.

Threonine is considered a limiting amino acid for broiler chickens. Threonine is vital for the maintenance and synthesis of proteins, particularly mucin. Threonine influences energy metabolism and nutrient absorption, and research supports that adequate levels can enhance animal growth and immune function as well as help maintain intestinal health (Chen et al., 2018; Tang et al., 2021). Threonine is an essential nutrient for cell growth and proliferation, and it also functions as a nutritional modulator

that impacts the intestinal immune system through intricate signaling networks. Nutrient absorption is dependent on the soundness of the intestinal structure, threonine is utilized by the intestinal mucosa and is vital for maintaining intestinal mucosa integrity (Tang et al., 2021). Dozier et al. (2001) reported that broilers receiving inadequate threonine had less nitrogen retention and recovery of apparent metabolizable energy (AME) compared to those fed adequate levels.

Cysteine is considered a semi-essential amino acid for poultry because it can be synthesized from methionine and serine via transsulfuration (Alagawany et al., 2020). Cysteine is important in the function and structure of proteins as well as in aiding in oxidative stress (Alagawany et al., 2020). Cysteine, along with glycine and glutamic acid, is required for the synthesis of glutathione, which acts as an important antioxidant (Jha et al., 2019). Elwan et al. (2019) found that an *in ovo* injection of methionine and cysteine improved antioxidant status, embryotic development, and jejunum histomorphometry of post-hatch broilers subject to heat stress during incubation.

Due to the lack of key enzymes needed for synthesis of arginine, poultry have a unique need for dietary supplementation of arginine. Poultry do not have the ability to catalyze the first step needed to synthesize arginine from citrulline due to the absence of the enzyme carbamoyl phosphate synthetase (Bortoluzzi et al., 2018). Poultry also have a higher activity of kidney arginase compared to mammals, forcing the dietary supplementation of arginine to also account for this degradation (Bortoluzzi et al., 2018). Xu et al. (2018) reported that supplementing arginine above the NRC recommendations improved growth performance and immunity response of broilers. They also believe that

arginine may be utilized as an immunomodulating agent and possible growth promoter in broilers.

Glutamine serves as an important source of energy for the proliferation of intestinal mucosal and immune cells. It may also be considered an essential amino acid during periods of disease, inflammatory conditions, or surgery (Newsholme, 2001; Bortoluzzi et al., 2018). Glutamine has been reported to improve growth performance likely due to an improvement in small intestine morphology and villus height (Yi et al., 2001; Pelicia et al., 2015). Although not an essential amino acid under normal conditions, glutamine is vital for immunity responses and metabolism.

2.5 Digesta Viscosity

The addition of cereal grains that contain NSPs affect the birds' ability to digest starches, proteins, and lipids. Foregut viscosity accounts for more than half of the variability seen in performance of broilers fed wheat, rye, or barely based diets (Bedford, 1996). Viscosity is defined as the measure of a fluid's resistance to flow and is measured in centipoise units (cps). Bedford, M.R. (1996) found that the average foregut viscosity of corn is 2.4, wheat is 12, barley is 25, and rye is 250.

For cereal grains such as wheat and rye, arabinoxylans make up the majority of the NSPs. A gel-like viscosity is formed by the water-soluble portion of arabinoxylans in the intestinal tract. Arabinoxylans have long polymer chains that entangle between themselves, and these chains have a high capacity for holding water which can result in an increase in intestinal viscosity. Arabinoxylans also have a higher molecular weight, this directly correlates with an increased viscosity (Pirgozliev et al., 2019).

Meanwhile, for grains such as oats and barley, β-glucans make up a large amount of the NSP. β-glucans appear similar to cellulose on the molecular level, however, cellulose has a uniform structure, whereas β-glucans have β-1-3 linkages which distort the uniform structure, thus, allowing the microfibrils to cram closely together which makes β-glucans more soluble compared to cellulose. The long polymer chains of β-glucans form viscous solutions, thus increasing the intestinal viscosity (Masey O'Neill et al., 2014). The reduced feeding value of barley and rye can almost be entirely attributed to the issue of viscosity with these cereal grains (Bedford and Cowieson, 2012).

Nonstarch polysaccharides in these cereals trap nutrients within cells due to an increase in intestinal viscosity causing a reduction in digestion of these nutrients (Masey O'Neill et al., 2014). Friction caused by the NSPs can prolong the passage rate of digesta. This can cause a decrease in bird performance due to the decreased feed intake of the birds. There is a reduction of nutrient digestion due to highly viscous digesta decreasing the rate of enzyme diffusion into the digesta. Furthermore, the increase in digesta viscosity causes the digesta to have less contact with the brush border membrane enzymes, consequently decreasing nutrient digestibility and utilization (Jha and Mishra 2021).

Baker et al. (2021) describes a correlation between an increase in intestinal viscosity and an increase in pathogenic load in the small intestine, thus causing greater inflammation and oxidative stress to birds. Diets containing wheat, barley, and rye can be improved using an antibiotic, suggesting that the detrimental effects of these cereal grains can be partially attributed to the proliferation of pathogenic bacteria. It is suggested that the use of carbohydrase enzymes to offset the negative effects of NSPs could possibly

reduce the occurrence of these pathogenic bacteria (Bedford and Cowieson, 2012; Masey O'Neill et al., 2014). Since the anti-nutritional effects of these viscous cereal was largely mitigated through reduction of pathogenic microbiota, it is more likely that most of the anti-nutritive effects were due to the presence of the microbiota versus the rate of digestion (Bedford and Cowieson, 2012). This is supported by Feighner and Dashkevicz (1987) when they reported that rye-based diets increased the populations of bile-acid deconjugating enzyme bacteria. They noted that this effect could be mitigated by antibiotics (Bedford and Cowieson, 2012).

The use of carbohydrase enzymes has been used to offset the negative effects of NSPs since the late 1980s. It has been reported in innumerable studies that carbohydrase enzymes reduce the intestinal viscosity in birds (Esteve-Garcia et al., 1997; Engberg et al., 2004; Gonzalez-Ortiz et al., 2016; Munyaka et al., 2016; Arczewska-Wlosek et al., 2019; Aderibigbe et al., 2020). Xylanases are used to negate the anti-nutritional effects of arabinoxylans and β-glucanases are used to nullify the anti-nutritive effects of β-glucans. The reduction in viscosity leads to improvements in bird performance and nutrient digestibility (Raza et al., 2019). The reduction of digesta viscosity by enzyme supplementation plays a greater role in younger birds compared to their older counterparts. It is suggested that this is because younger birds have a more immature intestinal tract and it is assumed that younger birds produce less pancreatic enzymes needed to digest these NSPs (Smulikowska et al., 2002).

2.6 Bone Strength

A major issue for the broiler industry has been leg and gait disorders due to rapid growth. In severe cases, deformities impair the bird's ability to walk, resulting in death from starvation and dehydration, but milder deformities can also cause discomfort (Fleming, 2008). Lameness and bone deformities in poultry of market age can result in bone breakage during catch and transport, thus resulting in issues during processing (Rath et al., 2000).

Bone strength is determined by the ultimate load or stress at which the bone will break. The bone breaking strength (BBS) depends on its load at break, which is an accumulation of all forces and moments applied to it. In reality, BBS is the stress or load that’s normalized to represent the force that is applied per square area at the time of break. In some cases, BBS has been used interchangeably to refer to the number of loads applied to break the bone, especially when using the bones from small animals (Rath et al., 2000). Since this can cause some confusion, it is preferred to use the normalized value when comparing different bones and stages of maturity.

There are many factors that directly and indirectly affect bone strength. One of the biggest factors affecting bone strength is growth. As birds age, bone mass and strength increase due to bone strength being proportional to bone mass. Rath et al. (2000) reported that bone weight, diameter, and length plateaued at 25 weeks of age and remained relatively consistent through week 55 for laying hens. However, BBS, mineral content, bone ash, and density plateaued at 35 weeks of age. This indicated that the functional and physical potentials of bone take longer to mature than bone growth.

Gender is also influential in bone growth. Size and hormone differences can account for variances in bone growth and strength when comparing males and females (Rath et al., 2000). Appendicular skeletal growth has been known to be gender specific in mammals (Seeman, 2001). Rath et al. (2000) reported that male and female birds that are the same age showed different diaphyseal diameters, with female birds showing a consistently lower diameter compared to their male counterparts. However, a comparative study of male and female birds at 7 and 72 weeks old showed no significant differences in BBS based on gender (Rath et al., 2000).

Genetics may also play a role in bone strength. There are innumerable studies on humans and other mammals proving that bone density is an inheritable trait. Poultry are generally bred for meat production, so it is possible that the consequences of this on bone quality have been overlooked (Rath et al., 2000). Yair et al. (2017) reported that faster growing hatchlings that hatch from relatively small eggs have bone mechanical properties that are inferior when compared to slower growing hatchlings. They suggest that the faster-growing hatchlings from small eggs are at an increased risk for developing bone pathologies. Another study found that selecting birds for growth is negatively correlated with bone traits involved in health, integrity, and maturity of leg bones (Gonzalez-Ceron et al., 2015).

One of the most relevant factors to bone strength is nutrition. The calcium to P ratio is crucial for leg health of broilers. Bone contains around 70% mineral, 20% organic matter, and 10% water, with the majority of the mineral content being calcium and P (Rath et al., 2000). Adequate supplies of calcium, P, and vitamin D are essential for proper skeletal development, however, a severe deficiency in most nutrients can cause

skeletal developmental issues. Vitamin D is a calciotropic hormone that is involved in calcium absorption in the intestines, and it plays an important role in regulatory bone metabolism and strength. Because layers require a high amount of calcium, adequate vitamin D supplementation is vital for optimal bone health (NRC, 1994). Calcium deficiency is not usually an issue for poultry, although poult malabsorption syndrome can cause impairment of calcium absorption. High levels of phytate and cellulose in diets can impede calcium absorption as well. Due to the interaction between calcium and P, an imbalance of P metabolism can negatively influence skeletal integrity and strength (Rath et al., 2000). Rennie et al. (1997) reported that medullary bone volumes of laying hens were significantly increased by feeding oyster shell or fluoride. Boron has also been reported to improve bone strength and ash content (Wilson and Ruszler, 1997). Aluminum fed in excess to chickens has been shown to produce generalized growth depression and reduced bone strength (Johnson et al., 1992; Huff et al., 1996). Vitamins B_6, K, and C are essential to bone health due to their implication in the synthesis of matrix constituents and formulation of collagen crosslinks. Excess protein can cause a negative calcium balance and inhibit bone growth. Diets containing highly saturated fats can have negative effect on bone mineralization whereas low fat diets can increase cancellous bone mineral content and strength (Rath et al., 2000).

Stress and infection can be risk factors for bone integrity which can lead to bone weakness. Adverse conditions such as stress, infection, inflammation, and disease can influence growth and maturity of bones. Osteomyelitis and osteonecrosis are bone infections that can cause focal bone loss. Reo viruses can lead to “brittle bone syndrome” in poultry and avian leukosis has been reported to as a factor in avian osteopetrosis.

Immunosuppression can result in osteopenia, affecting trabecular and cancellous bone density (Rath et al., 2000).

Antinutrients are also a major factor affecting bone strength in poultry. Some sweet peas contain b-aminopropionitrile which impedes the enzyme lysyl oxidase, thus hindering collagen crosslinks and mineralization, leading to a decrease in bone strength. Diets contaminated with high amounts of mycotoxins can lead to bone fragility and adversely affect bone growth and strength (Rath et al., 2000). Mycotoxins indirectly affect bone integrity by influencing the metabolism of other factors, namely vitamin D, which is vital for optimal bone health.

2.7 Exogenous Enzymes

2.7.1 Purpose and History of Enzymes

Enzymes have become prevalent feed additives due to their ability to improve feed efficiency and reduce feed costs. Enzymes are biological catalysts composed of amino acids that regulate the rate of a specific chemical reaction in a specific substrate. The addition of enzymes improves nutrient availability which saves on feed ingredient costs. The supplementation of exogenous enzymes is estimated to save the global feed market about 3-5 billion USD every year (Lourenco et al, 2020).

The most widely used enzyme in the poultry industry are phytases that catalyze the hydrolysis of phytate (Adeola and Cowieson, 2011). The second most widely used enzymes in the poultry industry are carbohydrases, which cleave the NSPs in viscous cereals (Adeola and Cowieson, 2011; Pirgozliev et al., 2019). These two types of enzymes account for approximately 90% of the enzyme market (Adeola and Cowieson,

2011). Protease supplementation has become more relevant in the poultry industry for its ability to improve protein and amino acid digestibility. Proteases have become particularly relevant in young birds where there is a low relative activity of protease (Walk et al., 2018). Tannase is a new and upcoming enzyme being supplemented to broilers in order to improve the feeding value of field beans (Abdulla et al., 2016). Preliminary results have been promising but further research is required to study the effect of tannase in poultry formulations (Pirgozliev et al., 2019).

Enzymes can be produced commercially from microorganisms, plants, and animals, although enzymes from plants and animals are limited in production (Alabi et al., 2019). Plant cultivation is limited to regions where the plant is suitable to grow and is primarily seasonal. The total concentration of enzymes in plants is low, so producing enough plants to meet the enzyme productivity requirements is unfeasible. Enzymes produced from animals are mostly by-products of the meat industry, which already has other competition (Alabi et al., 2019). However, microorganisms can produce large amounts of enzymes and can produce these sufficient to meet market demands (Adeola and Cowieson, 2011). These microbes are not affected by seasonality, and it is possible to manipulate their genetics and environment to increase yields and improve the enzymes (Alabi et al., 2019). Phytases, carbohydrases, and proteases are naturally excreted by a range of different bacteria and fungi, who use them to meet their own metabolic needs (Adeola and Cowieson, 2011). They also produce a wide array of properties that make them suitable for specific applications. The most common strains for commercial production include *Aspergillus niger*, *Bacillus subtilis*, *Trichoderma longibrachiatum*, *Trichoderma viride*, and *Humicola insolens* (Alabi et al., 2019). The

genetically modified strains of these microbes are cultured on sugar and starch hydrolysis substrates using fermentation technology (Liu and Kokare, 2016). Most commercially available exogenous enzymes are obtained from these fermentation systems, which rely on genetically modified fungi or bacteria (Adeola and Cowieson, 2011).

Enzymes have been used historically for cheese making and brewing (Alabi et al., 2019). Modern enzymology began in 1870 when the use of refined rennin taken from the stomach of calves was used in cheese production (Choct et al., 1999). Knowledge and production technology regarding the mode of operation of enzymes has increased dramatically since then. Enzymes have now been used as feed additives in the agricultural industry for over 30 years (Alabi et al., 2019). The poultry industry especially has become very receptive to the use of enzymes due to their ability to improve nutrient utilization. The use of enzymes to help the bird absorb essential nutrients has been shown to be the most cost-effective and preferred method of improving feed utilization (Alabi et al., 2019). The nutritional value of industrial by-products by the addition of enzymes allows for a greater flexibility in diet formulation.

2.7.2 Carbohydrases

Poultry diets consist mainly of cereal grains, which contain substantial energy. Nevertheless, these cereal grains contain varying amounts of anti-nutritional factors such as NSP. Poultry are unable to digest these NSP as a consequence of the absence of vital endogenous enzymes, more specifically carbohydrases. Nonstarch polysaccharides can negatively affect bird health due to the increased chance of infection from competition from pathogenic bacteria within gut microbiota for digestible nutrients. Thus, there is a

market for a product to offset these negative effects and decrease production costs for producers. Enzymes have been used commercially for decades to improve bird performance and nutrient digestibility but have a limited ability to act on certain ingredients (Raza et al., 2019). Newer generations of carbohydrase enzymes have been proven to be effective in increasing the amount of energy available from feed ingredients by breaking down carbohydrates into easier to digest simple sugars (Pirgozliev et al., 2019).

Carbohydrases became commercialized for poultry diets in the late 1980's. They were originally used for their ability to minimize wet excreta, digestion, and metabolizable energy issues due to high fiber in the diets. Currently, over 80% of the carbohydrases used in the global market are accounted for by xylanase and glucanase (Adeola and Cowieson, 2011). Bacterial and fungal enzymes have been proven effective in degrading arabinoxylans and β-glucans present in viscous cereals. Carbohydrase enzymes have been known to produce varying effects. This is likely due to the variation in NSP chemical structure, as the NSP profile varies from feedstuff to feedstuff. Thus, an enzyme may achieve beneficial results in one feedstuff but may not be able to produce those results in another. This means that there is a need for a broad range of carbohydrase enzymes that can act on varying substrates (Adeola and Cowieson, 2011; Raza et al., 2019).

2.7.2.1 Xylanase

Xylanases are produced naturally by fungi, bacteria, yeast, marine algae, etc. but are not produced by mammals, and must be supplemented. Xylanolytic organisms have

been reported in extreme environments such as thermal springs, marines, Antarctic environments, and soda lakes (Chakdar et al., 2016). Xylanase hydrolyzes the β-1,4 glycosidic bonds of xylan into xylose. Xylans are the second most common plant cell polysaccharide in the world and account for one third of renewable organic carbon sources. Xylans differ between plant species and are essential for cell wall integrity as well as plant growth and development (Ahmed, 2013).

Xylanase breaks down NSP, specifically arabinoxylans, in cereal grains such as wheat, rye, oat, sorghum, and barley. These viscous cereals contain higher amounts of soluble NSP compared to non-viscous cereals such as corn and rice, which contain a higher amount of insoluble NSP. Arabinoxylans reduce the digestibility of starch, fat, and proteins in poultry diets. Xylanase has been shown to increase the concentrations of arabinose, galactose, and glucuronic acid in the digesta when added to wheat diets (Craig et al., 2020).

Xylanase supplementation has been shown to improve feed conversion ratio when added to both layer and broiler diets (Gonzalez-Ortiz et al., 2016; Gonzalez-Ortiz et al., 2017; Arczewska-Wlosek et al., 2019; Olukosi et al., 2020). There is some variability amongst xylanase's performance in young, immature birds, versus that of older, more mature birds. Arczewska-Wlosek et al. (2019) reported that although xylanase improved feed conversion ratio in broilers from d 1 to 21-days of age, it did not influence the feed conversion ratio in older birds. Taylor et al. (2018) found that adding xylanase to laying hen diets improved feed efficiency and increased calcium and dry matter digestibility.

Xylanase has been shown to do more than just improve growth performance. The inclusion of xylanase has also been shown to improve ileal energy digestibility in wheat

diets. Xylanase also improved dry matter and nitrogen retention (Gonzalez-Ortiz et al., 2016). Xylanase has been reported to increase chymotrypsin and lipase activities when supplemented to wheat diets (Engberg et al., 2004). Amerah et al. (2008) suggested that the particle size of wheat may influence the efficacy of xylanase. They reported xylanase did not affect average daily gain or feed intake but did have a significant interaction with coarse particle size in improving feed conversion ratio.

The efficacy of xylanase in poultry diets that are not primarily wheat-based is variable. It has been reported that xylanase does not significantly improve feed conversion ratio or digestibility in diets primarily composed of corn (Rabello et al., 2021, Yegani and Korver, 2013). This agrees with Singh et al. (2021) who reported that xylanase did not affect feed intake or feed conversion ratio in broilers fed a corn-based diet; however, they did report that xylanase increased the total short-chain fatty acids and the average daily gain in broilers. On the other hand, Morgan et al. (2022) reported that xylanase improved growth performance across wheat-, corn-, and barley-based diets. Xylanase also had a positive effect on NSP degradability and free oligosaccharide digestibility across all three diets. Jejunal digesta viscosity was decreased in broilers fed both corn and wheat diets; however, wheat diets had a greater digesta viscosity decrease (-31%) when compared to that of corn diets (-10%) (Munyaka et al., 2016). Xylanase has shown promising results in other diets that contain larger amounts of NSPs, like rye. Amerah et al. (2008) reported that xylanase significantly decreased relative weight and length of all gut components as well as reduced the viscosity of small intestine digesta in diets consisting of high concentrations of rye (20%). Research on the effects of xylanase

in primarily corn diets is conflicting, this is most likely due to the lower amount of arabinoxylans in corn.

Nonstarch polysaccharides has been reported to increase the viscosity of digesta in the small intestine, leading to a reduced absorption and digestibility of nutrients (Baker et al., 2021). Studies showed that arabinoxylans were not digested in the small intestine in broilers, which subsequently created a viscous 'chime' in the intestines. This viscous chime led to a proliferation of pathogenic bacteria, intestinal inflammation, and impairment of barrier function in the intestine (Baker et al., 2021). This correlation between an increase in digesta viscosity and an increase in pathogenic load within the small intestine, can lead to increased oxidative stress and inflammation (Baker et al., 2021). These negative effects can be alleviated by xylanase's ability to decrease digesta viscosity. It has been reported innumerable times that xylanase inclusion, especially in high wheat containing diets, reduces the viscosity of both jejunal and ileal digesta (Engberg et al., 2004; Taylor et al., 2018) Additionally, xylanases increase digesta passage rates and nutrient digestion in the small intestine, which restricts the growth of fermentative microorganisms. By decreasing host-enteric microbiota competition, nutrient utilization is enhanced (Choct et al., 1999; Raza et al., 2019)

In the late 1990's, xylanase inhibitors were first discovered within wheat (Baker et al., 2021). Xylanase inhibitors were found in barley, rye, and corn following the initial discovery. These inhibitors were found to only affect certain microbial xylanases belonging to glycoside hydrolase (GH) families 10 and 11, these inhibitors do not influence xylanases synthesized by plants or outside of the mentioned families (Baker et al., 2021).

2.7.2.2 β-glucanase

β-glucanase is a type of cellulase enzyme produced commonly by various bacteria, fungi, and actinomycetes. β-glucanase is not produced naturally by non-ruminants but can be found in rumen bacteria of ruminants. β-glucanase hydrolyzes glycosidic bonds in β-glucans and has been reported to reduce the anti-nutritional effects of β-glucans. β-glucans are NSP from cell walls of endosperm cells and are present in large amounts in barley and are also found in small amounts in soybean and canola meals. Barley diets supplemented with β-glucanase have the potential to significantly increase the nutritive value of barley.

β-glucanase has been shown to improve growth performance in broilers fed barley-based diets (Esteve-Garcia et al., 1997; Munyaka et al., 2016; Sun et al., 2019; Karunaratne et al., 2021). β-glucanase increased gross energy and crude protein digestibility in broilers fed barley-based diets (Sun et al., 2019). β-glucanase supplementation decreased intestinal viscosity in broiler chickens (Esteve-Garcia et al., 1997; Karunaratne et al., 2021). Józefiak et al. (2006) noted similar results, stating that β-glucanase supplementation decreased ileal viscosity, improved growth performance, increased lactic acid concentrations, and decreased the pH of crop contents in broilers. β-glucanase also improved apparent ileal digestibility in broilers (Perttila et al., 2001). Chen et al. (2021) reported that β-glucanase supplementation improved egg production in laying ducks and increased the activity of amylase and chymotrypsin in the duodenal digesta in addition to reducing digesta glucan content. β-glucanase has proven to be beneficial in alleviating the anti-nutritional effects of β-glucans in barley.

2.7.2.3 α-Amylase

Starch is a heterogenous structure which varies significantly in amylose and amylopectin composition. Both amylose and amylopectin are polymers made up of D-glucose molecules, they differ based solely on the type of chain that is formed. Amylose forms a linear chain polymer of D-glucose, whereas amylopectin forms a branch-chain polymer. Amylose consists of only α 1-4 glycosidic bonds, while amylopectin has both α 1-4 glycosidic and α 1-6 glycosidic bonds. Due to these α 1-6 glycosidic bonds, amylopectin is more soluble, amorphous, and readily digestible than amylose (Cowieson et al., 2019).

Corn is the most widely used starch source for poultry on a global scale and it contributes the majority of dietary energy. About 86% of the corn starch is found in the endosperm. Starch is classified as either normal, waxy, or amylo. Waxy corn has an amylopectin content of around 99% with the other 1% being amylose. Normal corn is around 75% amylopectin and 25% amylose, and high amylose corn is 25% amylopectin and 75% amylose (Cowieson et al., 2019).

Amylase is a starch digesting enzyme used to provide more available energy from increased starch digestion. Starch digestion in swine begins with salivary amylase in the mouth, but in poultry, starch digestion does not occur until pancreatic amylase encounters ingested starch polymers (Cowieson et al., 2019). α-amylase hydrolyzes amylose into maltose which is then degraded further into glucose by maltase. There is a limited amount of starch digestion that occurs in the crop and proventriculus due to α-amylase. Poultry can almost completely digest starch by the time it reaches the end of the ileum, however this can vary based on starch structure and solubility, age of the bird, and

various other factors. As birds mature, they develop an increased capacity to digest starches (Cowieson et al., 2019).

Gracia et al. (2003) reported that during the first 7-d period growth performance of birds fed amylase improved 9.4% and feed conversion by 4.2%. Cowieson et al. (2019) suggests that the first week, post-hatch, birds have a higher sensitivity to amylase as they require assistance from the enzyme to offset their immature pancreatic amylase production due to their intestinal tracts being less developed. Evidence supports that, when compared to their juvenile counterparts, older birds produce more pancreatic amylase (Krogdahl and Sell, 1988). However, this does not mean that older birds do not benefit from amylase supplementation. Gracia et al. (2003) reported quadratic interactions between age and amylase effect over a 42-d period in broilers fed corn/soy diets. Weight gain increases were noted from 0 to 7-d of 9.4%, from 0 to 21-d of 3.6%, and from 21-42-d of 5.5%. Feed conversion ratio decreases were also reported from 0 to 7-d of 5 points (1.13 vs 1.18), from 0 to 21-d of 0 points (1.41 vs 1.41), and from 21-42-d of 5 points (1.62 vs 1.67). This indicates that birds may not only have an amylase sensitivity post-hatch, but also in the grower/finisher phase when there is a high starch intake compared to metabolic weight (Cowieson et al., 2019). This data agrees with Vieira et al. (2015), who saw similar quadratic interactions between age and amylase supplementation in broilers fed corn/soy diets over a 40-d period. They reported that FCR of the broilers decreased 4.5, 2.6, 1.7, 0, 2, and 2.7 over six weeks respectively. Steffanello et al. (2017) observed similar age-dependent quadratic results when supplementing amylase to broilers fed corn/soy diets over a six-week period.

Amylase had a more substantial effect on FCR and egg quality in all corn diets compared to all wheat diets fed to laying hens (Olgun et al., 2018). Amylase supplementation in corn diets had a more profound effect on increasing AME when compared to a complete corn-soybean meal diet (Schramm et al., 2021). Amylase supplementation has also been shown to improve AMEn of the diet (Gracia et al., 2003). Aderibigbe et al. (2020) reported that α-amylase decreased jejunal viscosity while increasing total tract digestibility of starch and energy. Similarly, amylase improved energy utilization when added to corn-soybean meal diets. Evidence also suggests that increasing levels of amylase linearly increases ileal digestibility of resistant starch (Schramm et al., 2021). Córdova-Noboa et al. (2021) noted comparable results of improved energy utilization, broiler growth performance, and starch total tract digestibility when amylase was added to corn diets.

2.7.3 Phytase

Phytase was originally developed to reduce P pollution by increasing the digestibility of phytate-bound P leading to a reduction in the level of inorganic P that is added to the diet. The use of phytase eventually became popular for reducing the anti-nutritional effects of phytate and phytic acid. The first attempt at commercializing fungal phytase for animal feed nutrition was in 1962, although the project was not a success (Lei et al, 2013). The first successful attempt at commercialization of fungal phytase for animal feed was in 1991, by a German chemical company known as BASF. However, in 1999 bacterial phytases were found to be superior compared to fungal phytases, consequently leading to the popularity of bacterial phytases for animal feed nutrition (Lei

et al, 2013). Currently, there are few phytases on the market that are able to completely dephosphorylate phytic acid into myo-inositol due to the presence of the phosphate at position 2 on the ring. However, mucosal phosphatases and a few microbial esterases are able to cleave this phosphate, thus generating free myo-inositol in the GI tract of the animal (Adeola and Cowieson, 2011). Phytase is now the most widely used exogenous enzyme for nonruminants, accounting for approximately 60% of the enzyme market (Adeola and Cowieson, 2011)

About two thirds of the total P in poultry feed is present in the form of phytate, and poultry have a low capacity to utilize phytate-bound P. Poultry produce some phytase, but it is insufficient for hydrolyzing phytates (Mullaney and Ullah, 2003). Phytate is the molecule formed when phytic acid binds to a mineral, usually calcium or magnesium, thus reducing the nutrient availability. Phytate also reduces the digestibility of minerals, amino acids, and energy. Phytase hydrolyzes phytate into myo-inositol and phosphate groups, releasing a usable form of inorganic P (Mullaney and Ullah, 2003). Myo-inositol concentrations were increased in gizzard digesta when phytase was added to laying hen diets (Taylor et al., 2018). The addition of phytase reduced phytate but increased inositol concentrations in the gizzard and ileal digesta (Kriseldi et al., 2021). Inositol concentrations were also increased in broilers fed added phytase (Gautier et al., 2018). P digestibility and utilization was improved when phytase was added to both broiler and layer diets (Gautier et al., 2018; Taylor et al., 2018). Phytase allows nutritionists to make P more available in poultry and swine diets.

Phytase has been shown to improve more than just P availability. When phytase was added to laying hen diets, it increased hen-day production and daily egg mass,

resulting in increased overall layer production (Taylor et al., 2018). When phytase was supplemented to broilers it improved growth performance, apparent nutrient digestibility and retention, and bone ash content (Gautier et al., 2018). Microbial phytase supplementation improved mineral retention in broilers (Viveros et al., 2002; Ptak et al., 2013; Gallardo et al., 2018) as well as increased apparent ileal digestibility of some amino acids, specifically alanine, aspartic acid, glycine, threonine, isoleucine, leucine, serine, and tyrosine, in addition to increasing AME (Selle et al., 2006). Zanu et al. (2020) reported that phytase supplementation was beneficial to mineral utilization and bone health to the point that they believe phytase can replace meat and bone meal in broiler diets. Phytase supplementation is proven effective in offsetting the negative effects of phytate.

Phytases are synthesized by microorganisms and occur naturally in bacteria, fungi, plants, and some animals. Transgenic microbial phytases have recently been produced in transgenic canola, alfalfa, and rice plants (Gonita et al, 2012). In 2001, research began to engineer a transgenic pig that could process phytates without supplementation, commonly known as the 'Enviropig.' More recently, Zhang et al. (2018) developed transgenic pigs that could digest both phytates and NSP eliminating the need for xylanase, phytase, and β-glucanase supplementation. They took genes from fungi and bacteria and interposed them into the genomes of pigs that express the enzymes in their salivary glands. The manure of the transgenic pigs contained 44% less P and 24% less nitrogen compared to non-transgenic pigs on the same diet. The transgenic pigs also had a 24% higher average daily gain compared to the non-transgenic pigs, meaning transgenic pigs attained slaughter weight almost a month earlier. Pollution reduction and

improved growth performance in these modified pigs may lead to transgenic animals being the future of enzymes.

Excessive P and nitrogen pollution has caused greater than 100,000 miles of rivers and streams, almost 2.5 million acres of lakes and ponds, and over 800 square miles of estuaries and bay to have substandard water quality in the United States alone (EPA, 2022). Nutrient pollution can travel thousands of miles to coastal areas where pollution has already caused massive dead zones (EPA, 2022). The low digestibility of phytate causes excessive excretion of P and other nutrients into manure. Local land base is often insufficient to accommodate the waste in an environmentally friendly manner. The low bulk density of poultry manure makes it not economically viable for transport over long distances. This causes repeated applications of P dense manure in farmlands near poultry houses. The excess P in the manure stays in the surface soil making it easier for the excess nutrients to runoff into watersheds which can lead to eutrophication (Chakraborty et al., 2021). Manure from broilers, cattle, and swine was collected and analyzed for various minerals. Turner (2004) found that broiler litter had over three times the amount of P compared to cattle manure. Swine manure had a slightly lower P content compared to broiler litter. Pillai et al. (2009) reported that the addition of phytase to layer diets decreased the total P and nitrogen in the manure but increased the amount of water-soluble P. They suggested the reduction in total nitrogen in the litter may indicate that nitrogen was better metabolized by the hens, and this may be associated with greater P availability. Applegate et al. (2003) found that the addition of phytase to broiler diets decreased both the total P and water-soluble P percent of dry matter.

Phosphorus is the third most expensive component in poultry feed. The addition of phytase saves on extra P supplementation costs. Williams (2006) found that there was a higher savings when phytase was supplemented to lower energy diets versus high-energy diets. The addition of phytase in laying hen diets reduced the production cost per egg and increased net profit per egg (Ponnuvel et al., 2014). Phytase not only improves the availability of phytate-bound P, but it also provides economic and environmental benefits as well.

2.7.4 Protease

Protease catalyzes proteolysis via hydrolysis, essentially breaking down proteins into amino acids. Proteases are categorized by the optimum pH in which they are effective. Acid proteases are active in pH range 2.5-3.5 and work in the gizzard and proventriculus. Neutral proteases work in the pH range of 6.5-7 and are active in the duodenum and jejunum. Basic or alkaline proteases are active in the 7.2-7.8 pH range and work in the ileum (Sipany, 2021).

The addition of protease to poultry diets is of interest to improve protein and amino acid digestibility and reduce anti-nutritional factors such as trypsin inhibitors, β-conglycinin, and glycinin. This may be particularly important for young birds where protease activity may be suboptimal (Walk et al., 2018). Trypsin inhibitors are found naturally in various legumes, including soybeans. Trypsin inhibitors are a type of serine protease inhibitor that reduces the effectiveness of trypsin. Trypsin is an enzyme involved in the breakdown of various proteins during digestion (Cohen et al., 2019). β-conglycinin and glycinin are the two most important soybean proteins. These proteins are not digested

but are then absorbed into the intestinal mucosa. This causes inflammation of the intestinal mucosa (Hill, 2003). Protease supplementation helps alleviate these anti-nutritional factors.

Proteases can improve growth performance, especially post-hatch due to the pancreatic protease activity being low from hatch to around 21 days (Jin et al., 1998). The digestive protease could be limiting the protein digestion due to the immaturity of the digestive systems of chicks. The addition of protease may aid pancreatic enzymes and improve the rate of protein breakdown in the intestines. Protease may also be able to reduce the need for additional amino acid supplementation (Lourenco et al., 2020).

Research into protease has been mostly focused on supplementation with other enzymes, while very little research has been done on protease supplementation alone. Cowieson et al. (2016) found that protease supplementation improved the gain to feed ratio. The addition of protease improved growth performance and apparent metabolizable energy in broilers (Jabbar et al., 2020). Maqsood et al. (2022) found that when protease was added to lower crude protein diets in broilers, growth performance, intestinal health, and carcass traits improved. Other studies have found that protease supplementation did not improve growth performance above that of a nutritionally adequate diet (Walk et al., 2019).

Environmental pollution is a huge issue in the agricultural production industry. Low protein feeds are being fed to poultry to attempt to reduce the amount of excess nitrogen that is excreted into the environment. Protease supplementation may be used to offset the effects of low protein diets on broiler growth. Wang et al. (2022) found that when protease was added to low protein diets it improved the growth performance of

broilers. Protease may be beneficial to improving protein and amino acid digestibility, and in turn helping to combat environmental pollution from poultry.

2.7.5 Tannase

Tannase is a hydrolytic enzyme that acts on hydrolysable tannins, breaking them down into gallic acid and glucose. Tannase functions differently in plants and microorganisms and is a crucial enzyme in the breakdown of gallotannins in many microorganisms (Srivastava and Kar, 2009). In plants, tannase is used to produce tannins. Tannins are known for their antimicrobial, antiviral, antiparasitic, antioxidant, and anti-inflammatory effects and are sometimes used to replace antibiotics (Huang et al., 2018).

Field beans have been considered as possible alternatives to soybeans due to their similar amino acid profile. Field beans offer a good alternative since they are significantly cheaper sources of protein compared to soybeans. The anti-nutritional factors present in field beans, including soluble NSP, oligosaccharides, and tannins, have caused many in the poultry industry to shy away from this protein source (Abdulla et al., 2016). The use of tannase is relatively new in poultry nutrition and recent research has suggested that tannase may be beneficial in alleviating some of these anti-nutritional factors. Tannase improved growth performance in broilers fed field beans (Abdulla et al., 2016). Ebrahimzadeh et al. (2018) reported the addition of tannase to corn-soybean diets improved the antioxidant status and immune responses of broilers. There is currently very little research into the use of tannase in poultry, but the recent research may prove promising.

2.8 Summary

The addition of enzymes to poultry diet has been one the major advances in the last fifty years (Khattak et al., 2006). The current global exogenous enzyme use in nonruminants is significantly greater than originally anticipated (Adeola and Cowieson, 2011). For many years nutritionists have known what it is, but not until the 1980s did they have the means to apply it. In fact, feed enzymes work on a simple principle, there are some compounds in plants that the animal either cannot digest, or which impede its digestive system, often because the animal does not produce the enzymes necessary to degrade these compounds (Khattak et al., 2006). It is then the nutritionist's responsibility to identify these indigestible compounds and provide an enzyme that will break them down. These enzymes then come from microorganisms that are selected specifically for the task and grown in controlled environments (Adeola and Cowieson, 2011).

The use of enzymes in poultry feed has become increasingly important due to the consistent rise in the price of common feed ingredients, which can become a major constraint on producers (Khattak et al., 2006). This forces nutritionists to utilize cheaper and unconventional feed ingredients, most of which have a higher proportion of anti-nutritional factors, such as NSPs. Since poultry does not naturally produce the enzymes needed to degrade these NSPs, supplementation of the enzymes (carbohydrase) that degrade these NSPs is vital.

It is vital to continue the effort to fully understand the use and limitations of the matrix values of different enzymes (Adeola and Cowieson, 2011). In the last decade, research has been conducted to study the effects of multi-enzymatic products on the performance of poultry. The economic and environmental benefits of enzymes have been

well established, the future of exogenous enzymes is promising (Khattak et al., 2006). However, further research into these multi-enzymatic products is required if enzymes are to reach their full potential in the poultry industry.

CHAPTER 3. The effects of Allzyme® Spectrum supplementation on the performance, digesta viscosity, energy and nutrient utilization and bone quality of broiler chickens fed corn-soybean meal diets with reduced ME, Ca, and avP

3.1 Abstract

A study was conducted to investigate the effect of Allzyme® Spectrum, a naturally fermented enzyme complex containing carbohydrase and phytase, on energy and nutrient digestibility of 21-d-old broiler chickens. Dietary treatments included a positive control (PC) group having a commercial equivalent energy and nutrient level and a negative control (NC) group with reduced metabolizable energy, calcium, and available phosphorus. The NC diet was mixed as one basal diet, before adding the varying inclusion levels of Allzyme® Spectrum (150, 200, 250 g/ton) to the remaining 3 treatments. Ten replicate cages of 6 chicks were randomly assigned to each of the 5 dietary treatments. Chickens had unrestricted access to water and feed during both the pre-starter (d 0 – 9) and starter periods (d 9 – 21). At the end of the trial, excreta samples were collected for the utilization assay of DM, N, Ca, P, and energy. Ileal digesta was collected from the distal two-thirds of the ileum for the digestibility determination of DM, N, Ca, energy, and amino acids (AA). Jejunal digesta was collected for digesta viscosity determination while the right tibia bones were collected for bone breaking strength (BBS) and bone ash analysis. Data were analyzed using the GLM procedures of SAS (v 9.4). Orthogonal polynomial contrasts were utilized to determine polynomial effects of increasing dietary supplemental levels of Allzyme® Spectrum on the growth performance, digesta viscosity, digesta nutrient utilization and digestibility, and bone mineralization of chickens. There was both a linear and quadratic increase ($P < 0.05$) in

feed intake (FI) 9-21 with increasing enzyme supplementation but there was a quadratic effect ($P = 0.002$) noted on FI 0-21 with increasing level of enzyme supplementation. There was a quadratic increase ($P < 0.05$) for daily body weight gain (BWG) d 9-21 and feed efficiency (FE) d 9-21 with increasing level of enzyme supplementation. Compared with those fed the PC diet, the chickens fed NC diet had lower ($P < 0.01$) utilization of DM, N, Ca, P and energy and lower ($P < 0.01$) apparent ileal essential and non-essential AA digestibility. Increasing level of Allzyme® Spectrum supplementation to the NC diet resulted in a quadratic increase ($P < 0.01$) in the utilization of DM, N, Ca, P and AME and apparent ileal essential and non-essential AA digestibility. Similar trend (quadratic increase) was observed for apparent ileal digestibility of DM, N, P, and digestible energy except for Ca where the enzyme effect was linear ($P < 0.05$). Bone breaking strength (BBS) and bone ash quadratically increased ($P < 0.001$) with enzyme supplementation. The results from this study indicate that the supplementation of Allzyme® Spectrum to corn-soybean meal-based diet results in an increase in nutrient utilization, apparent ileal AA digestibility, and bone quality.

Keywords: broiler chicken, digestibility, enzyme, nutrient, AMEn

3.2 Introduction

Corn is the most commonly used cereal grain in commercial poultry diets in the US. Corn is a high-density source of energy and is easily digestible by birds. Corn contributes roughly 65% of the metabolizable energy (ME) and 20% of the protein in broiler starter diets (Cowieson, 2005). Most of the phosphorus in corn is bound to phytate, therefore most poultry diets are supplemented with additional non-phytate phosphorus. There are some varieties of corn known as low-phytate corn, which have lesser amount of phytate bound phosphorus, thus lessening the need for additional phosphorus supplementation in the diet.

Corn contains roughly 70% starch, most of which is amylopectin with only 30% being amylose. Amylopectin is highly digestible, more so than amylose due to the compact structure of amylose. α-amylase is a starch digesting exogenous enzyme sometimes added to poultry diets to release more energy during starch digestion. It is especially supplemented to young birds who have an immature gastrointestinal (GI) tract which lacks enough endogenous pancreatic amylase required for starch digestion. Poultry develop an increased capacity to digest starches as they mature (Cowieson et al., 2019). Amylase has been reported to improve apparent metabolizable energy corrected for nitrogen (AMEn) of corn diets. (Gracia et al., 2003; Córdova-Noboa et al., 2021; Schramm et al., 2021).

Wheat is a popular energy supplying feed ingredient in parts of Canada and Europe often used in poultry diets. Wheat has become a more favorable feed ingredient due to the availability of commercially available enzymes which improve its feeding value. Wheat contains approximately 10% less ME compared to corn but can contribute

up to 18% of the protein in broiler diets when fed at high levels (Ravindran and Amerah, 2009). The higher protein content of wheat also allows for less amino acid supplementation.

Wheat contains a high amount of nonstarch polysaccharides (NSP) which are known to increase the viscosity of gut contents, thus decreasing nutrient utilization. Nonstarch polysaccharides readily absorb water which then forms viscous solutions that are often referred to as 'gel-like.' Nutrients are often trapped by NSPs causing an increase in intestinal friction consequently prolonging the passage rate of digesta. Highly viscous gut contents also have less contact with brush border membrane enzymes therefore decreasing nutrient digestibility and utilization (Jha and Mishra, 2021).

Carbohydrase enzymes have been used in poultry diets to mitigate the negative effects of NSPs. They were originally developed to minimize issues with wet litter but have since become popular for their ability to decrease digesta viscosity and improve nutrient digestibility. Since there are different types of NSPs, there are several exogenous carbohydrase enzymes capable of breaking down certain NSPs. Xylanase specializes in breaking down the NSP arabinoxylan, which is present in high amounts in wheat. Xylanase has been reported to improve growth performance and decrease digesta viscosity in wheat diets (Gonzalez-Ortiz et al., 2016; Gonzalez-Ortiz et al., 2017; Olukosi et al., 2020). There are conflicting reports on whether or not xylanase improves nutrient digestibility and reduces intestinal viscosity in primarily corn-based diets. Munyaka et al. (2016) reported that although xylanase showed a greater digesta viscosity reduction in wheat diets, it was also able to reduce the viscosity of corn diets.

Although the starches in corn and wheat differ, both cereal grains have been shown to have improved nutrient digestibility and utilization from the addition of phytase. Phytase is able to release phytate-bound phosphorus and other nutrients, thus making them more available to birds. Both cereal grains have their advantages and disadvantages, and the creation of multi-enzyme products allows nutritionists and producers to decide what works best in their program. The objective of this study was to examine the effects of dietary Allzyme® Spectrum on growth performance, energy and nutrient digestibility and utilization, and bone quality.

3.3 Materials and Methods

3.3.1 Animal Housing, Management, and Experimental Design

Experimental procedures and management of birds used in this study were approved by the Institutional Animal Care and Use Committee at the University of Kentucky. A total of 300 one-day-old male Cobb by-product breeder chicks were obtained from a commercial hatchery and fed a corn-soybean meal-based pre-starter diet (Table 3.1) from day 0-9 and were then moved to a corn-soybean meal-based starter diet (Table 3.2) for the remainder of the experiment (d 9-21). On day 0, birds were weighed as a group and randomly assigned to treatments. There was a total of 5 dietary treatments with 10 replicate pens of 6 chicks per cage. The birds were housed in battery cages (0.61 x 0.51 x 0.36 m) in an environmentally controlled room with 22 h of light and 2 h of darkness. All birds were given ad libitum access to feed and water for the duration of the experiment.

3.3.2 Experimental Diets

The enzyme used in this experiment was Allzyme® Spectrum and was supplied by Alltech Inc. (Nicholasville, KY). Allzyme® Spectrum contains 454,000 SPU/lb of Phytase (Aspergillus niger) and 4.2 million XU/lb of Xylanase (Trichoderma longibrachiatum). One Solid State Fermentation Phytase unit (SPU) is defined as the amount of enzyme that will release 1 mmol of inorganic P per minute under the conditions of the assay. One xylanase unit is the amount of enzyme that will release 1 mmol of xylans per minute under the conditions of the assay. Enzyme activity was determined prior to the addition of the enzyme in the diets.

There were 5 pre-starter and 5 starter diets. The PC diet for both the pre-starter and starter phase was a corn-soybean meal reference diet similar to that recommended by the Cobb500™ Broiler guide that met or exceeded the energy and nutrient requirements of birds of this age (Cobb-Vantress, 2022). The NC diet was a corn-soybean meal-based low nutrient diet consisting of 90 kcal less ME and 16.7% less Ca and 33.3% less avP than the PC diet. The NC diet and the enzyme supplemented diets were produced from a single basal diet and Allzyme® Spectrum was added individually to the respective diets. The other 3 diets were the NC diet supplemented with Allzyme® Spectrum at 150, 200, and 250 g/ton respectively. All starter diets contained titanium dioxide at 0.5% which serves as an indigestible marker for digestibility and utilization calculations.

3.3.3 Sample Collection

All birds and feed were weighed on days 0, 9, and 21 for each cage to determine performance parameters (body weight gain, feed intake, and feed efficiency). On day 21, all birds were euthanized by argon asphyxiation.

Excreta samples were collected from each cage on days 20 and 21 and were dried at 55 °C in a forced-air oven for 5 days. The dried samples were ground using a Wiley Mill Laboratory Standard (Model No. 3, Arthur H. Thomas Co., Philadelphia, PA, USA) fitted with a 1 mm screen and then stored in airtight plastic bags before being analyzed for dry matter, gross energy, titanium, Ca, P, and N.

On day 21, the right tibia was removed from two birds per pen with a bodyweight that was near to the average bodyweight of the cage and stored at – 20°C until processed for bone-breaking strength and bone ash determination. Furthermore, jejunal digesta samples were collected from the same two birds per pen and placed in a pre-labeled 15 mL centrifuge tube. The jejunal samples were frozen at -20 °C until viscosity measurement was done. On day 21, ileal digesta from the distal two thirds of the ileum was collected from the remaining four birds per pen by flushing with distilled water into clean pre-labeled plastic containers. Digesta samples from all birds in a cage were combined in the same plastic container and frozen at -20 °C. The ileal digesta samples were then freeze-dried and ground using a coffee grinder before storing in airtight bags in a refrigerator until they were analyzed for dry matter, gross energy, titanium, amino acid, Ca, P, and N content.

3.3.4 Sample Analysis

Excreta, ileal digesta, and jejunal digesta were all analyzed in duplicates, while all diets were analyzed in triplicate. If the coefficient of variation fell above 5%, those analyses were repeated for that pen. The dry matter contents of the diets, ileal digesta, and excreta were determined by drying the sample at 110 °C for 24 hours (AOAC International, 2006). Gross energy of the diets, ileal digesta, and excreta was determined using a bomb calorimeter (Parr adiabatic bomb calorimeter, model 6200, Parr Instruments, Moline, IL, USA) with the calibration standard being benzoic acid. Amino acid content of the diets and ileal digesta were determined at the Agricultural Experiment Station Chemical Laboratories, University of Missouri-Columbia (Columbia, MO). The Ca, P, N, and titanium contents of the diets, ileal digesta, and excreta were also analyzed at the Agricultural Experiment Station Chemical Laboratories. The Ca content was analyzed using Inductively Coupled Plasma and P content was analyzed using gravimetric method (AOAC International, 2006). The nitrogen contents of the diets, digesta, and excreta samples were analyzed at the Agricultural Experiment Station Chemical Laboratories by a combustion method (model FP2000, LECO, St. Joseph, MI; AOAC International, 2006; method 990.03), with EDTA as the internal standard. Concentrations of amino acids were analyzed using method 982.30 E(a,b,c) (AOAC, 2006). Concentrations of titanium in the diet, excreta, and ileal digesta samples was determined as described by Myers et al. (2004).

Jejunal digesta were used for viscosity determination by centrifuging the samples for 10 minutes at 5,000 RPM. The supernatant was removed and then centrifuged again

for 8 minutes at 10,000 RPM. That supernatant was then analyzed for viscosity using a viscometer (Vibro Viscometer SV-1A, A&D Weighing, Ann Arbor, MI).

The tibias were cleaned thoroughly during collection and, following thawing, all additional left-over tissue and fat was removed. The BBS was measured using an Instron Materials tester (model 4301, Instron Corp., Canton, MA) at a loading rate of 50 mm/min. The bones were then dried for 24 hours in a 105°C oven for 24 hours, (Precision Scientific Co., Chicago, IL). Bones were then completely soaked in a glass jar with petroleum ether for four extraction periods each lasting for 24 hours. Following the final extraction, after no color change of the petroleum ether solution was observed, bones were removed and allowed to dry for 24 hours under the hood at room temperature. They were then placed in the 105°C oven for 6 hours to ensure no moisture was left. Bones were then weighed by pen and placed in a porcelain crucible for ashing in a muffle furnace overnight. The resulting ash were weighed to determine bone ash percentage.

3.3.5 Calculations and Statistical Analysis

Although all the diets were individually analyzed for Ca, P, N, titanium, GE, and amino acids, the values of the NC and all the enzyme supplemented diets were averaged for these values because they were mixed from a single basal diet prior to the addition of the enzyme. The analyzed values for the PC diet were used for calculations.

Apparent ileal nutrient and energy digestibility (AID) and energy and nutrient utilization (TTU) of DM, energy, N, Ca, and P were calculated using the method of Kong and Adeola (2014).

AID or TTU (%) = 100 − [100(Ti/To)(No/Ni)]

In this equation, Ti is the titanium concentration in the feed, To is the concentration of titanium in the ileal digesta or excreta, No is the concentration of energy or nutrients in ileal digesta or excreta, and Ni is the concentration of energy or nutrients in the feed.

The AME and ADE were calculated using the equation below:

AME or $ADE, kcal/kg = Calculated$ energy utilization or ileal energy digestibility (%) $\times$ $GE\ of$ the $diet$ $(kcal/kg)$ on a DM basis.

The AME with nitrogen correction (AMEn) was obtained by correcting for nitrogen using Hill and Anderson (1958).

Data were analyzed using the PROC GLM procedure of SAS 9.4 v 4 (2011 SAS Institute Inc., Cary, NC) appropriate for a randomized complete block design. PROC IML was used to generate the coefficients used for orthogonal polynomial contrasts. Orthogonal polynomial contrasts were utilized to determine polynomial effects of increasing dietary supplemental levels of Allzyme® Spectrum. Simple contrasts, between the PC and NC diets and the PC and enzyme supplemented diets were also performed. Prior to statistical analysis, all data was subjected to an outlier test, and all outliers (data that fell outside the Mean±3SD) were removed. Significant differences between means were determined using Tukey's Honest Significant Difference with the level of significance at $P < 0.05$. Digestibility and utilization data is presented on a DM basis.

3.4 Results

3.4.1 Analyzed Composition of Experimental Diets

The pre-starter and starter diets were analyzed for N, GE, Ca, and P contents and the starter diet was also analyzed for titanium. The ingredient composition of the diet, and the formulated and analyzed nutrient contents of the diets are reported in Tables 3.1 and 3.3 (pre-starter diet) and Tables 3.2 and 3.4 (starter diets). The crude protein contents of the diets were determined by multiplying the determined N content by 6.25. In the starter diets, the analyzed Ca was lower in the PC diet (7.3 vs 8.4 g/kg) than the formulated value. In general, the analyzed nutrient values in the pre-starter and starter diets were close to the formulated values (Tables 3.3 and 3.4).

3.4.2 Growth Performance and Viscosity

The simple and polynomial contrasts of increasing level of exogenous enzyme supplementation on performance are reported in Table 3.5 and 3.6. The simple and orthogonal effects of exogenous enzyme supplementation on jejunal digesta viscosity are reported in Figure 3.1. There was both a linear and quadratic increase ($P < 0.05$) in FI (d 9-21) with increasing enzyme supplementation but only a linear increase ($P = 0.002$) noted on FI for the entire duration of the study (d 0-21). Increasing enzyme supplementation resulted in a quadratic increase ($P = 0.003$) for BWG d 9-21. There was also a quadratic increase ($P = 0.004$) on FE (d 9-21) with increasing exogenous enzyme supplementation where the 200g/ton Allzyme® Spectrum level had the highest FE at 0.66 (Table 3.6).

The simple and polynomial contrasts of increasing level of exogenous enzyme supplementation on jejunal digesta viscosity are reported in Figure 3.1. There were no orthogonal or simple contrasts ($P > 0.05$) effect of enzyme supplementation on digesta viscosity.

3.4.3 Energy, Nutrient, and Amino Acid Digestibility

The simple and orthogonal effects of exogenous enzyme supplementation on apparent ileal nutrient, energy, and amino acid digestibility are reported in Table 3.7, 3.8, and 3.9, respectively. There was a quadratic increase ($P < 0.001$) in DM and N digestibility with increasing level of enzyme supplementation. Enzyme supplementation resulted in a linear increase ($P < 0.001$) for Ca and P digestibility. Birds fed the enzyme supplemented diets had a higher ($P < 0.001$) Ca and P digestibility compared to birds fed the PC diet. Increasing levels of enzyme supplementation resulted in a quadratic increase ($P = 0.001$) in P digestibility. Phytate forms an insoluble complex with Ca in the small intestine and by hydrolyzing that phytic acid bond, the digestibility of Ca and P is improved in diets supplemented with phytase. Increasing levels of enzyme supplementation resulted in a quadratic increase ($P < 0.001$) in ADE. There was no difference ($P = 0.403$) in ADE between the PC diet and the enzyme supplemented diets.

The NC diet had a lower ($P < 0.01$) apparent ileal AA digestibility compared to the PC diet; however, enzyme supplementation to the NC diets restored apparent ileal AA digestibility to that of the PC diet. There was a quadratic increase ($P < 0.01$) for digestibility of all the essential and non-essential amino acids with increasing level of enzyme supplementation. The 250 g/ton Allzyme® Spectrum diet resulted in the highest

digestibility ($P < 0.05$) of all essential amino acids. The 250g/ton Allzyme® Spectrum diet also resulted in the highest digestibility ($P < 0.05$) for all the non-essential amino acids excluding serine and tyrosine, where 150 g/ton Allzyme® Spectrum diet had the highest digestibility. This improvement is likely due to phytase breaking down the phytate-bound protein as phytate will non-selectively bind to proteins, which can inhibit certain digestive enzymes like α-amylase and trypsin, therefore reducing protein and amino acid digestibility.

3.4.4 Energy and Nutrient Utilization

The simple and orthogonal contrasts of increasing levels of exogenous enzyme supplementation on DM, N, Ca, P, AME, and AMEn are reported in Table 3.10. Dry matter and N retention quadratically increased ($P < 0.001$) with enzyme supplementation. Chickens fed the enzyme supplemented diets had a higher ($P = 0.045$) N retention compared to birds fed the PC diet. The improved N utilization can be attributed to phytase cleaving the phytate-bound proteins. Enzyme supplementation to the NC diet resulted in an improvement ($P < 0.05$) in DM, N, Ca, and P utilization compared to that of the PC diet. In this study, Ca utilization was higher in birds fed the NC diets versus those fed the PC diet. This is due to the bird needing to digest and utilize more Ca since there is less being provided in the diet. Increasing enzyme supplementation resulted in both a quadratic and linear increase ($P < 0.001$) in P and Ca utilization. The birds given enzyme supplemented diets had a significantly higher P and Ca utilization percentage ($P < 0.001$) when compared to birds fed the PC diet. Increasing levels of enzyme supplementation resulted in a quadratic increase ($P < 0.001$) in AME and AMEn.

3.4.5 Bone Quality

The simple and orthogonal contrasts of exogenous enzyme supplementation on BBS and bone ash of broiler chickens fed increasing level of Allzyme® Spectrum are reported in Table 3.11. Bone breaking strength quadratically increased ($P < 0.001$) with increasing level of enzyme supplementation, where the 250 g/ton Allzyme® Spectrum diet had the highest BBS of 17.7 kg/f. There was also a quadratic increase ($P < 0.001$) on bone ash percentage, where the 200 g/ton Allzyme® Spectrum diet had the highest bone ash percentage of all the treatments at 51.9%. For both BBS and bone ash, there was no significant difference ($P = 0.090$ and 0.610, respectively) between the PC diet and enzyme supplemented diets, but the PC resulted in higher ($P < 0.001$) BBS and bone ash compared to the NC diet. The improvement in BBS and bone ash could be attributed to phytase effect on phytate-bound mineral complexes, by allowing those nutrients to be more available to the bird.

3.5 Discussion

The current study shows that feeding a corn-soybean-meal-based diet supplemented with Allzyme® Spectrum improves broiler growth performance and nutrient and energy digestibility and utilization. Broilers given the enzyme supplemented diets consistently performed better than those fed the reduced nutrient diet (NC). This can be attributed to the presence of NSPs and phytate, whose negative effects are mitigated by carbohydrase and phytase enzymes, respectively. Broilers given the enzyme supplemented diets also performed equivalently to or better than those fed the PC diet.

Corn is not high in arabinoxylans, only having an arabinoxylan content of 3.9% compared to wheat which is 25% (Choct, 1997). Soybean meal has a low arabinoxylan content of 3.8% with almost 1/5 of it being highly soluble (Choct, 1997). This means that corn is not greatly affected by the carbohydrase enzyme, xylanase, and we can assume that most of the effects of this enzyme complex in this study were due to the activities of phytase in the enzyme complex.

Corn is notorious for having high levels of phytate, with up to 90% of the P present in the form of phytate. This represents approximately 75% of overall levels of the total P found in the kernel (He et al., 2017). Phytase has been proven to mitigate the negative effects of phytate and make P and Ca more available to the bird. Phytate forms an insoluble complex with Ca in the small intestine and by hydrolyzing that phytic acid bond, the digestibility of Ca is improved in diets supplemented with phytase (Lalpanmawia et al., 2014). The improvement in digestibility of Ca and P in this current study agrees with Cowieson (2005) who reported a 36% improvement in P digestibility and a 38.9% improvement in Ca digestibility in birds fed a corn-soybean-meal-based NC diet compared to those fed a diet supplemented with 2,400 FTU of phytase. Walters et al. (2019) also reported improvements in Ca and P digestibility in birds fed corn-based diets supplemented with phytase. In this study, Ca utilization was higher in birds fed the NC diets versus those fed the PC diet. This is due to the bird needing to digest and utilize more Ca since there is less being provided in the diet. However, Ca digestibility was not significantly different between the enzyme supplemented diets and the PC diet due to the fact that there was adequate Ca provided in the PC diet, but in the enzyme supplemented

diets they are able to digest more Ca due to its better availability from the action of phytase enzyme.

The lack of significant improvement in growth performance in this study agrees with Singh et al. (2021) who reported that xylanase did not significantly affect growth performance parameters in broilers fed a corn-based diet. This is also in agreement with both Yegani and Korver (2013) and Rabello et al. (2021) who both reported no significant improvement of feed conversion ratio in broilers fed diets primarily composed of corn.

In this study, there was no significant overall treatment effect of enzyme supplementation on the jejunal digesta viscosity. This is not surprising due to the fact that the digesta viscosity of birds fed corn-soybean-meal-based diets is generally low to begin with. Munyaka et al. (2016) found that adding xylanase to corn diets did significantly decrease jejunal digesta viscosity from 2.00 to 1.81 mPa. Kiarie et al. (2014) reported that supplementing xylanase to corn diets resulted in a much lower reduction in viscosity compared to supplementing in wheat diets. Generally, the digesta viscosity of broilers fed wheat-based diets is 1.5-2 times that of corn-based diets (Kiarie et al., 2014; Munyaka et al., 2016).

The improvement in N utilization is likely due to phytases ability to liberate proteins and AA from phytate. This agrees with Gonzalez-Ortiz et al. (2016) who reported that the inclusion of xylanase and phytase improved N retention in broilers. The efficacy of enzyme supplementation on ileal digestibility of AA is variable with some reporting positive benefits, such as Gallardo et al. (2018) who reported that the inclusion of phytase and carbohydrase enzyme improved ileal digestibility of AA. Walters et al.

(2019) also reported an increase in apparent digestibility of AA when phytase was added to low-P corn-based diets. However, others reported no beneficial effects phytase on apparent ileal digestibility of AA when compared with either the NC or PC diets (Woyengo et al., 2010). This is likely due to phytases action on phytate by liberating the proteins and AA as mentioned above.

The improvement of AME and ADE from enzyme supplementation observed in this study corresponds with many other published articles. The improved energy digestibility and utilization in the enzyme supplemented diets can be attributed to phytase's ability to increase protein and AA digestibility. AME of the birds fed the enzyme supplemented diets improved from those fed the NC diet and were equal to those fed the PC diet which agrees with Peniazek et al. (2017) who noted a significant increase in the AME of NC diet to enzyme supplementation and found that the inclusion of enzymes to the NC diet increased AME equal to that of the PC diet. Pirgozliev et al. (2008) reported that AME of birds supplemented with phytase improved 14-18.3% compared to birds fed unsupplemented diets. Similar increases were observed in this study with increases of almost 14% in AME of the enzyme supplemented diets when compared to the NC diet. Cowieson (2005) reported a similar increase of AMEn from the NC diet (2.8 vs. 5%) to the phytase-supplemented diet; he also reported a significant improvement of AME from the PC diet to the phytase supplemented diets.

The improvement of BBS and bone ash percentage in this current study was expected since phytase hydrolyzes phytate-bound mineral complexes, thus allowing these minerals to be more available to the birds. This agrees with Leyva-Jimenez et al. (2019) who reported that the addition of phytase improved bone quality including ash and bone

breaking strength of broilers. This also agrees with Walters et al. (2019) who showed that adding phytase to low P diets improved tibia quality parameters compared to birds fed the NC diet.

The ability of exogenous enzymes to optimize nutrient digestion and utilization provides significant economic and environmental benefits. This study showed that supplementing enzymes to a reduced energy and nutrient corn-soybean-meal-based diet improved nutrient and energy digestibility and utilization equal to or better than that of the PC (or commercial) diet. This means that producers can feed diets with less-than-optimal nutrients or ingredients, alongside enzyme supplementation, and receive similar or better results than those feeding diets containing adequate level of nutrients and energy without enzyme supplementation. Energy is the most expensive component of broiler chicken diets, followed by protein and P, respectively. The inclusion of Allzyme® Spectrum to the reduced energy and nutrient diets improved both energy and nutrient digestibility and utilization, thus allowing for producers to save money by adding less energy-producing feedstuffs and non-phytate P to the diets. This agrees with Williams (2006), who reported higher savings when phytase was added to lower energy diets compared to high energy diets. Excessive P and N can cause eutrophication in surrounding bodies of water, thus causing detrimental environmental damage (Chakraborty et al., 2021). Allzyme® Spectrum ability to optimize N and P digestibility and utilization reduces the amount of P and N that get excreted by birds and ultimately into the environment. This agrees with Applegate et al. (2003) who found that adding phytase to broiler diets decreased total P concentration in excreta. This is also in

agreement with Pillai et al. (2009) who reported less total P and N in excreta, when phytase was added to broiler chicken diets.

3.6 Conclusion

The results of this study showed that Allzyme® Spectrum supplementation improves growth performance, energy and nutrient digestibility and utilization, and bone quality 21-d old broilers fed a reduced nutrient, corn-based diet. This study provides evidence that supplementing exogenous phytase and xylanase to a reduced nutrient diet can improve bird performance equivalent to that of birds being fed a commercial diet, thus allowing producers to feed lower-cost diets without having to sacrifice bird performance.

Table 3.1: Ingredients composition of the experimental pre-starter diets fed to broiler chickens from day 0-9 (on as-fed basis).

Diet	PC	NC
Ingredient, g/kg		
Corn	624.2	593.0
Soybean meal (47%CP)	333.2	322.1
Wheat bran	0.0	52.3
Soy oil	2.8	0.0
L-Lysine HCl	3.20	3.30
DL-Methionine	2.90	2.90
Salt (NaCl)	4.10	4.10
Limestone	10.6	11.7
Dicalcium phosphate	16.7	8.3
Vitamin-mineral premix[1]	1.50	1.50
Choline	0.8	0.8
Total	1000.0	1000.0

PC= positive control, NC= negative control

[1] Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: 32 mg of iron from iron sulfate; 8 mg of copper from copper sulfate,; 51 mg of manganese from manganese oxide; zinc oxide, 60 mg; iodine (EDDI), 1.48 mg; sodium selenate, 0.24 mg; vitamin A (retinyl acetate), 8,820 IU; vitamin D3 (cholecalciferol), 2,822 IU; vitamin E (dl-α-tocopheryl acetate), 26 IU; vitamin K activity, 0.73 mg; thiamine, 1.76 mg; riboflavin, 6.17 mg; pantothenic acid, 14 mg; niacin, 44 mg; pyridoxine, 4 mg; folic acid, 0.88 mg; biotin, 0.18 mg; vitamin B12, 0.02 mg; choline, 383 mg.

Treatments B-E were mixed as one basal diet prior to the addition of Allzyme® Spectrum to diets C-E, at 150, 200, and 250 g/ton respectively. Allzyme® Spectrum contains 454,000 SPU/lb of Phytase (Aspergillus niger) and 4.2 million XU/lb of Xylanase (Trichoderma longibrachiatum). One Solid State Fermentation Phytase unit (SPU) is defined as the amount of enzyme that will release 1 mmol of inorganic P per minute under the conditions of the assay. One xylanase unit is the amount of enzyme will release 1 mmol of xylans per minute under the conditions of the assay. The calculated enzyme activity for each enzyme containing diet is 150 SPU and 1,395 XU, 200 SPU and 1,860 XU, and 250 SPU and 2,325 XU for the 150, 200, and 250 g/ton

Table 3.2: Ingredients composition of the experimental starter diets fed to broiler chickens from day 9-21 (on as-fed basis).

Diet	PC	NC
Ingredient, g/kg		
Corn	665.50	656.00
Soybean meal (47%CP)	283.5	274.0
Wheat bran	0.0	34.9
Soy oil	9.0	0.0
L-Lysine HCl	3.70	3.80
DL-Methionine	2.90	2.90
L-Threonine	0.40	0.50
Salt (NaCl)	3.90	3.90
Limestone	9.8	11
Dicalcium phosphate	14.3	6
Vitamin-mineral premix[1]	1.50	1.50
Choline	0.8	0.8
Titanium dioxide	5	5
Total	1000.0	1000.0

PC= positive control, NC= negative control

[1] Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: 32 mg of iron from iron sulfate; 8 mg of copper from copper sulfate,; 51 mg of manganese from manganese oxide; zinc oxide, 60 mg; iodine (EDDI), 1.48 mg; sodium selenate, 0.24 mg; vitamin A (retinyl acetate), 8,820 IU; vitamin D3 (cholecalciferol), 2,822 IU; vitamin E (dl-α-tocopheryl acetate), 26 IU; vitamin K activity, 0.73 mg; thiamine, 1.76 mg; riboflavin, 6.17 mg; pantothenic acid, 14 mg; niacin, 44 mg; pyridoxine, 4 mg; folic acid, 0.88 mg; biotin, 0.18 mg; vitamin B12, 0.02 mg; choline, 383 mg.

Treatments B-E were mixed as one basal diet prior to the addition of Allzyme® Spectrum to diets C-E, at 150, 200, and 250 g/ton respectively. Allzyme® Spectrum contains 454,000 SPU/lb of Phytase (Aspergillus niger) and 4.2 million XU/lb of Xylanase (Trichoderma longibrachiatum). One Solid State Fermentation Phytase unit (SPU) is defined as the amount of enzyme that will release 1 mmol of inorganic P per minute under the conditions of the assay. One xylanase unit is the amount of enzyme will release 1 mmol of xylans per minute under the conditions of the assay. The calculated enzyme activity for each enzyme containing diet is 150 SPU and 1,395 XU, 200 SPU and 1,860 XU, and 250 SPU and 2,325 XU for the 150, 200, and 250 g/ton

Table 3.3: Formulated and analyzed energy and nutrient contents of the experimental pre-starter diets fed to broiler chickens from day 0-9

Nutrients and energy	Positive Control	Negative Control
Formulated		
Crude protein, g/kg	212.6	212.8
MEn, kcal/kg	2,975	2,885
Ca, g/kg	9.0	7.5
P, g/kg	6.8	5.7
Non-phytate P, g/kg	4.5	3.0
Lys, g/kg	13.9	13.9
Met, g/kg	5.9	5.9
Met + Cys, g/kg	9.5	9.7
Thr, g/kg	7.9	8.0
Trp, g/kg	2.6	2.6
Val, g/kg	9.8	9.8
Analyzed		
Crude protein, g/kg	205	208
Gross energy, kcal/kg	3,947	3,897
Ca, g/kg	9.9	7.7
P, g/kg	6.9	5.7
Lys, g/kg	14.4	14.3
Met, g/kg	5.8	5.5
Met + Cys, g/kg	8.8	8.6
Thr, g/kg	7.6	7.6
Trp, g/kg	2.4	2.4
Val, g/kg	9.9	9.8

The negative control diet and diets containing supplemental exogenous enzymes were mixed from a single basal diet.

Table 3.4: Formulated and analyzed energy and nutrient contents of the experimental starter diets fed to broiler chickens from day 9-21.

Nutrients and energy	Positive Control	Negative Control
Formulated		
Crude protein, g/kg	192.8	192.9
MEn, kcal/kg	3,057	2,967
Ca, g/kg	8.0	6.5
P, g/kg	6.2	4.9
Non-phytate P, g/kg	4.0	2.5
Lys, g/kg	13.0	12.9
Met, g/kg	5.4	5.4
Met + Cys, g/kg	8.7	8.8
Thr, g/kg	7.5	7.6
Trp, g/kg	2.3	2.3
Val, g/kg	8.9	8.8
Analyzed		
Crude protein, g/kg	191.9	188.2
Gross energy, kcal/kg	3,943	3,908
Ca, g/kg	7.3	6.7
P, g/kg	6.0	5.0
Lys, g/kg	13.8	13.6
Met, g/kg	4.9	5.2
Met + Cys, g/kg	7.8	8.1
Thr, g/kg	7.6	7.6
Trp, g/kg	2.2	2.1
Val, g/kg	9.1	9.0

The negative control diet and diets containing supplemental exogenous enzymes were mixed from a single basal diet.

Table 3.5: Effect of increasing level of Allzyme Spectrum® supplementation on performance of 21-d-old broiler chickens fed corn-soybean-meal based diets

				Feed intake, g/b/d			Body weight gain, g/b/d		
Treatment	Diet	Allzyme Spectrum®	IBW, g/b	d 0-9	d 9-21	d 0-21	d 0-9	d 9-21	d 0-21
A	PC	0	40.7	21.3_z	74.2^a	52.6^a	17.6_z	47.6^a	35.2
B	NC	0	40.4_z	21.2	68.3^c	48.6^b	17.3_z	43.1^b	32.1
C	NC	150	40.8	22.2	70.0^c	50.4^{ab}	18.3	$45.0^{ab}{}_z$	33.5
D	NC	200	41.1_z	20.7	$70.1^{bc}{}_z$	48.1^b	16.9	$46.7^a{}_z$	32.2
E	NC	250	40.7	22.2_z	$73.3^{ab}{}_z$	$52.3^a{}_z$	17.5	47.1^a	34.8
Pooled standard deviation			0.63	1.32	2.43	2.77	1.71	3.11	3.38
P-value			0.187	0.069	0.002	0.001	0.446	0.015	0.147
			Probability						
PC vs NC			0.309	0.844	<0.001	0.003	0.684	0.002	0.047
PC vs Enzyme supplemented diets			0.448	0.435	0.018	0.029	0.964	0.235	0.176
Linear effect of enzyme supplementation			0.204	0.863	0.004	0.002	0.737	0.230	0.061
Quadratic effect of enzyme supplementation			0.035	0.262	0.024	0.564	0.503	0.003	0.504

[a-c] Means with different superscripts within the same column differ ($P < 0.05$)

[z]n=10 replicate cages with 6 birds/replicate, except for subscript z where n =9

PC = Positive Control, NC = Negative Control, IBW = Initial body weight

Pre-starter diet fed from d 0-9 and starter diet fed from d 9-21

SEM = SD/√n

Table 3.6: Effect of increasing level of Allzyme Spectrum® supplementation on feed efficiency and jejunal digesta viscosity of 21-d-old broiler chickens fed corn-soybean-meal based diets

			Feed efficiency			
Treatment	Diet	Allzyme Spectrum®	d 0-9	d 9-21	d 0-21	Viscosity, mPa
A	PC	0	0.831	0.641^{abc}	0.668	2.1
B	NC	0	0.808$_z$	0.628$^{c}_z$	0.666$_z$	2
C	NC	150	0.826	0.632^{bc}	0.663	2.1
D	NC	200	0.813	0.661^{a}	0.681$_z$	1.8
E	NC	250	0.824$_z$	0.652^{ab}	0.684$_z$	2.0^{a}
Pooled standard deviation			0.04	0.02	0.03	0.25
P-value			0.628	0.02	0.339	0.239
		Probability				
PC vs NC			0.182	0.228	0.827	0.616
PC vs Enzyme supplemented diets			0.471	0.402	0.433	0.251
Linear effect of enzyme supplementation			0.352	0.281	0.524	0.098
Quadratic effect of enzyme supplementation			0.617	0.004	0.225	0.197

[a-c] Means with different superscripts within the same column differ significantly ($P < 0.05$)

[z]n =10 replicate cages with 6 birds/replicate, except for subscript z where n =9

PC = Positive Control, NC = Negative Control

Pre-starter diet fed from d 0-9 and starter diet fed from d 9-21

SEM = SD/√n

Table 3.7: Effect of increasing level of Allzyme Spectrum® supplementation on apparent ileal nutrient and energy digestibility of 21-d-old broiler chickens fed corn-soybean-meal based diets (DM basis)

Treatment	Diet	Allzyme Spectrum®	DM, %	N, %	Ca, %	P, %	Energy, %	ADE, kcal/kg
A	PC	0	79.7[a]	86.5[a]	46.1[b]z	55.6[c]z	81.3[a]z	3561[a]z
B	NC	0	74.3[b]	83.1[b]	49.0[b]	47.3[d]	76.7[b]	3328[b]
C	NC	150	80.1[a]	87.1[a]	63.8[a]z	61.5[b]z	81.8[a]	3550[a]z
Dz	NC	200	79.0[a]	86.9[a]	62.1[a]	64.3[ab]	80.9[a]	3510[a]
Ez	NC	250	80.1[a]	87.5[a]	57.2[a]	67.8[a]	81.7[a]	3549[a]
Pooled standard deviation			1.95	1.67	9.05	5.37	1.73	75.26
P-value			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
			Probability					
PC vs NC			<0.001	<0.001	0.451	0.002	<0.001	<0.001
PC vs Enzyme supplemented diets			0.909	0.271	<0.001	<0.001	0.777	0.403
Linear effect of enzyme supplementation			0.716	0.462	<0.001	0.001	0.882	0.235
Quadratic effect of enzyme supplementation			<0.001	<0.001	0.210	<0.001	<0.001	<0.001

[a-c]Means with different superscripts within the same column differ significantly ($P < 0.05$)

[z]n=10 replicate cages with 6 birds/replicate, except for subscript z where n =9
PC = Positive Control, NC = Negative Control, ADE = apparent digestible energy
Pre-starter diet fed from d 0-9 and starter diet fed from d 9-21
SEM = SD/√n

Table 3.8: Effect of increasing level of Allzyme Spectrum® supplementation on apparent ileal digestibility of essential amino acids of 21-d-oldbroiler chickens fed corn-soybean-meal based diets (DM basis)

Treatment	Diet	Allzyme Spectrum®	Arg, %	His, %	Ile, %	Leu, %	Lys, %	Met, %	Phe, %	Thr, %	Trp, %	Val, %
A	PC	0	91.6[a]	90.0[a]	87.1[a]	88.8[b]	91.4[a]	94.5[a]	88.6[a]	83.7[a]	89.7[a]	86.3[a]
B	NC	0	89.1[b]	87.0[b]	84.4[b]	86.0[c]	89.6[b]	93.4[b]	85.5[b]	79.3[b]	86.1[b]	83.1[b]
C	NC	150	92.0[a]	90.4[a]	88.4[a]	89.7[ab]	92.1[a]	95.0[a]	89.56[a]	85.0[a]	89.6[a]	87.3[a]
D_z	NC	200	91.8[a]	90.3[a]	88.1[a]	89.4[ab]	92.0[a]	95.0[a]	89.3[a]	84.0[a]	89.4[a]	87.0[a]
E_z	NC	250	92.3[a]	90.8[a]	88.9[a]	90.0[a]	92.4[a]	95.3[a]	89.9[a]	85.6[a]	89.6[a]	87.7[a]
Pooled standard deviation			1.30	1.16	1.93	1.56	1.35	0.98	1.55	1.94	1.66	1.92
P-value			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
					Probability							
PC vs NC			<0.001	<0.001	0.004	<0.001	0.005	0.014	<0.001	<0.001	<.0001	<0.001
PC vs Enzyme supplemented diets			0.383	0.230	0.058	0.119	0.141	0.118	0.102	0.143	0.830	0.181
Linear effect of enzyme supplementation			0.654	0.481	0.147	0.257	0.278	0.233	0.228	0.306	0.771	0.352
Quadratic effect of enzyme supplementation			<0.001	<0.001	0.001	<0.001	0.002	0.006	<0.001	<0.001	<0.001	<0.001

[a-c]Means with different superscripts within the same column differ significantly ($P < 0.05$)
[z]n=10 replicate cages with 6 birds/replicate, except for subscript z where n =9
Pre-starter diet fed from d 0-9 and starter diet fed from d 9-21
SEM = SD/√n

Table 3.9: Effect of increasing level of Allzyme Spectrum® supplementation on apparent ileal digestibility of non-essential amino acids of broiler chickens fed corn-soybean-meal based diets (DM basis)

Treatment	Diet	Allzyme Spectrum®	Ala, %	Asp, %	Cys, %	Glu, %	Gly, %	Pro, %	Ser, %	Tyr, %
A	PC	0	88.4[a]	87.4[a]	80.7[a]	91.8[a]	84.7[a]	87.8[a]	87.2[a]	88.5[a]
B	NC	0	85.2[b]	84.3[b]	74.6[b]	89.7[b]	80.2[b]	84.5[b]	83.1[b]	84.6[b]
C	NC	150	89.0[a]	88.4[a]	81.3[a]	92.5[a]	85.1[a]	88.6[a]	88.1[a]$_z$	88.9[a]
D_z	NC	200	88.7[a]	88.1[a]	80.5[a]	92.3[a]	84.7[a]	88.2[a]	87.4[a]	88.7[a]
E_z	NC	250	89.3[a]	88.7[a]	81.8[a]	92.7[a]	85.4[a]	88.7[a]	88.0[a]	89.4[a]
Pooled standard deviation			1.62	1.64	2.58	1.23	1.96	1.59	1.50	1.55
P-value			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
					Probability					
PC vs NC			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PC vs Enzyme supplemented diets			0.352	0.120	0.619	0.126	0.645	0.254	0.192	0.227
Linear effect of enzyme supplementation			0.577	0.256	0.965	0.251	0.909	0.439	0.595	0.436
Quadratic effect of enzyme supplementation			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a-c]Means with different superscripts within the same column differ significantly ($P < 0.05$)

[z]n=10 replicate cages with 6 birds/replicate, except for subscript z where n =9

PC = Positive Control, NC = Negative Control

Pre-starter diet fed from d 0-9 and starter diet fed from d 9-21

SEM = SD/√n

Table 3.10: Effect of increasing level of Allzyme Spectrum® supplementation on apparent nutrient and energy utilization of 21-d-old broiler chickens fed corn-soybean-meal based diets (DM basis)

Treatment	Diet	Allzyme Spectrum®	DM, %	N, %	Ca, %	P, %	Energy, %	AME, kcal/kg	AMEn, kcal/kg
A	PC	0	76.4[b]	61.9[a]	58.3[b]	58.9[b]	78.7[a]	3446[a]	3351[a]
B	NC	0	66.8[c]	51.7[b]	49.5[c]	45.0[c]	69.7[b]	3024[b]	2853[b]
C	NC	150	77.1[ab]	65.0[a]	66.8[a]$_z$	64.0[a]	79.0[a]	3429[a]	3305[a]
D	NC	200	77.3[ab]	64.7[a]	69.0[a]$_z$	65.3[a]$_z$	79.1[a]	3436[a]	3312[a]
E	NC	250	77.6[a]$_z$	65.3[a]	69.5[a]	64.6[a]	79.5[a]$_z$	3451[a]$_z$	3336[a]
Pooled standard deviation			1.17	4.13	4.63	3.24	1.06	46.15	54.31
P-value			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
					Probability				
PC vs NC			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PC vs Enzyme supplemented diets			0.031	0.045	<0.001	<0.001	0.172	0.657	0.101
Linear effect of enzyme supplementation			0.065	0.081	<0.001	<0.001	0.316	0.497	0.060
Quadratic effect of enzyme supplementation			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a-c]Means with different superscripts within the same column differ significantly ($P < 0.05$)

[z]n=10 replicate cages with 6 birds/replicate, except for subscript z where n =9

PC = Positive Control, NC = Negative Control, AME = apparent metabolizable energy, AMEn = AME corrected for nitrogen

Pre-starter diet fed from d 0-9 and starter diet fed from d 9-21

SEM = SD/√n

Table 3.11: Effect of increasing level of Allzyme Spectrum® supplementation on the tibia quality of 21-d-old broiler chickens fed corn-soybean-meal based diets

Treatment	Diet	Allzyme Spectrum®	Bone breaking strength, kg/f	Bone ash, %
A	PC	0	18.4[a]	51.5[a]
B	NC	0	12.8[b]	48.5[b]
C	NC	150	17.6[a]	51.8[a]
D	NC	200	17.5[a]	51.9[a]$_z$
E	NC	250	17.7[a]	51.6[a]
Pooled standard deviation			1.23	1.20
P-value			<0.001	<0.001
		Probability		
PC vs NC			<0.001	<0.001
PC vs Enzyme supplemented diets			0.090	0.610
Linear effect of enzyme supplementation			0.091	0.488
Quadratic effect of enzyme supplementation			<0.001	<0.001

[a-c]Means with different superscripts within the same column differ significantly ($P < 0.05$)

[z]n=10 replicate cages with 6 birds/replicate, except for z where n= 9

PC = Positive Control, NC = Negative Control

Pre-starter diet fed from d 0-9 and starter diet fed from d 9-21

SEM = SD/√n

CHAPTER 4. The effects of Allzyme® Spectrum supplementation on the performance, digesta viscosity, energy and nutrient utilization and bone quality of broiler chickens fed wheat-soybean meal diets with reduced ME, Ca, and avP

4.1 Abstract

The objective of this study was to investigate the effect of dietary Allzyme® Spectrum on the performance, bone quality, apparent metabolizable energy, and nutrient digestibility of broiler chickens fed wheat-soybean meal-based diets with low ME, calcium (Ca), and available phosphorus (avP). Allzyme® Spectrum (Alltech, Inc., Nicholasville, KY, USA) is an enzyme complex containing xylanase and phytase. Growth performance, tibia quality, nutrient and energy digestibility and utilization of broiler chickens fed wheat-soybean meal-based diets with reduced ME, Ca, and avP (negative control; NC) plus 3 inclusion levels of Allzyme® Spectrum (150, 200, 250 g/ton) during the pre-starter (d 0-9) and starter (d 9-22) phases were evaluated in this study. A positive control (PC) diet that met or exceeded nutrient Ca (0.8%) and avP (0.4%) and energy (3,050 kcal/kg) requirements of birds of this age was fed to the PC group. The NC diet consisted of a reduction of 90 kcal/kg ME and 16.7% less Ca and 33.3% less avP. A total of 300 d-old male broiler chicks were assigned to the 5 treatments in a randomized complete block design with 10 replicate cages of 6 chicks per treatment. Data were analyzed using the GLM procedures of SAS (v 9.4). Simple contrasts were used to compare the PC and the NC diets as well as the PC vs. the NC diets containing increasing levels of supplemental enzymes (150, 200, 250 g/ton Allzyme® Spectrum). Orthogonal polynomial contrasts were utilized to determine the polynomial effects of increasing supplemental levels of Allzyme® Spectrum on the performance, jejunal digesta viscosity, nutrient digestibility

and utilization, and bone quality of chickens. The chickens fed the NC diet had lower ($P < 0.01$) body weight gain (BWG), feed intake (FI), and feed efficiency (FE) compared to those fed the PC diet for the entire 22d growing period, while the chickens' fed diets supplemented with 150, 200, 250 g/ton Allzyme® Spectrum had the equivalent BWG, FI, and FE compared to those fed the PC diet. Feed intake of chickens from d 9-22 and 0-22 and FE d 9-22 linearly increased ($P < 0.05$) with increasing level of supplemental Allzyme® Spectrum. There was a quadratic effect ($P < 0.05$) of enzyme supplementation level on BWG d 9-22 and 0-22 and FE d 9-22 and 0-22. There was both a linear and quadratic decrease ($P < 0.001$) in jejunal digesta viscosity with enzyme supplementation to the NC diet. There was also a reduction ($P < 0.001$) in jejunal digesta viscosity in the enzyme supplemented diets (2.9-3.1 mPa) compared to the digesta from birds fed the PC diet (5.2 mPa). Birds fed the NC diet had lower ($P < 0.05$) apparent ileal digestibility and utilization of DM, N, and digestible and metabolizable energy but higher ($P < 0.05$) Ca digestibility and utilization compared with those fed the PC diet. Increasing the level of Allzyme® Spectrum supplementation to the NC diet resulted in a quadratic increase ($P < 0.05$) in energy and nitrogen digestibility and utilization and linear increase ($P < 0.05$) in Ca digestibility and utilization. Increasing the level of enzyme supplementation resulted in a quadratic increase ($P = 0.005$) in P digestibility and a linear increase ($P < 0.001$) in P utilization There was a quadratic increase ($P < 0.05$) in the ileal digestibility of nonessential amino acids when comparing the enzyme supplemented diets excluding Glu ($P = 0.069$) and Tyr ($P = 0.088$). There was no difference ($P > 0.05$) in the ileal digestibility of amino acids when comparing the PC diet to the enzyme supplemented diets excluding Thr ($P < 0.001$) where Thr digestibility for the enzyme supplemented

diets were higher (87.2 vs. 85.0%). There was a quadratic increase ($P < 0.05$) in apparent ileal digestibility of Arg, His, and Val with increasing enzyme supplementation and there was a linear increase ($P = 0.001$) in ileal digestibility of Thr. Increasing the level of enzyme supplementation resulted in both a quadratic and linear increase ($P < 0.001$) in BBS and bone ash (percentage). Results from this study showed that the supplementation of Allzyme® Spectrum to a wheat-soybean meal-based diet resulted in improved growth performance, energy and nutrient digestibility and utilization, bone quality, apparent ileal nonessential and some essential AA digestibility, as well as reduced digesta viscosity.
Keywords: broiler chicken, amino acids, digestibility, enzyme, nutrient

4.2 Introduction

Exogenous enzymes play a vital role in poultry nutrition. Enzymes not only allow for better utilization of nutrients in feedstuffs, but they also, potentially, decrease both feed costs and pollution from excessive excretion of nutrients. With global populations continually rising, there is an increased demand for animals and animal by-products at an affordable price (USDA, 2022).

Phytases are the most widely used enzyme in poultry nutrition (Naghshbandi and Moghimi, 2020). Phytases popularity comes from its ability to increase the availability of phytate-bound phosphorus and mitigate the negative effects of phytate. Poultry are only able to utilize about one third of the phytate-bound phosphorus in feedstuffs nutrition (Naghshbandi and Moghimi, 2020). Phytate also reduces the digestibility of nutrients in birds due to phytic acid binding to certain minerals. Phytase has been reported to improve overall bird performance and nutrient digestibility and absorption.

Phytase was originally developed to reduce phosphorus pollution. The low digestibility of phytate-bound phosphorus causes excessive excretion of phosphorus and other nutrients into the environment. Excessive phosphorus and nitrogen runoff into surrounding bodies of water can cause eutrophication which is detrimental to the environment (Chakraborty et al., 2021). The addition of phytase to poultry diets allows for better nutrient absorption and, thus, less nutrient excretion into the environment.

Phytase also serves as a way to reduce poultry feed costs. Phosphorus is the third most expensive component of poultry diets. The inclusion of phytase allows for less additional supplementation of available phosphorus sources. This is increasingly important with the recent increase in feedstuff prices.

Carbohydrase enzymes are commonly added to wheat diets due to their high amounts of NSPs. The predominant NSP in wheat is arabinoxylans. The water-soluble portion of arabinoxylans causes a gel-like viscosity to form in the intestinal tract, thus, increasing intestinal viscosity. Consequently, arabinoxylans trap nutrients within cells which reduces the digestion of those nutrients. NSPs can also prolong the passage rate of digestion because they increase friction in the intestinal tract (Ravindran and Amerah, 2009). These factors, in conjunction with highly viscous digesta inhibiting the rate of enzyme diffusion from brush border membrane enzymes, cause a decrease in feed intake of birds and consequently decreased bird performance.

The carbohydrase enzyme that specifically breaks down arabinoxylans is xylanase. It has been reported that xylanase decreased digesta viscosity and improved bird performance of broilers when added to wheat diets. Due to this decrease in intestinal viscosity, xylanase also improves nutrient absorption and utilization. An improvement in nutrient availability also means a reduction in total feed costs. The objective of this study was to examine the effects of dietary Allzyme® Spectrum on growth performance, energy and nutrient digestibility and utilization, and bone quality.

4.3 Materials and Methods

4.3.1 Animal Housing, Management, and Experimental Design

Experimental procedures and management of birds utilized in this study were approved by the Institutional Animal Care and Use Committee at the University of Kentucky. A total of 300 one-day-old male Cobb by-product breeder chicks were attained from a commercial hatchery and fed a wheat-soybean-meal-based pre-starter diet (Table

4.1) from day 0-9 and were then switched to a wheat-soybean-meal-based starter diet (Table 4.2) for the rest of the experiment (d 9-22). All birds were given *ad libitum* access to feed and water for the duration of the experiment. The birds were housed in cages (0.61 x 0.51 x 0.36 m) in an environmentally controlled room with 22 h of light and 2 h of darkness. On day 0, birds were weighed as a pen and then randomly assigned to treatments. This study consisted of five dietary treatments, each with 10 replicate pens of 6 chicks per cage.

4.3.2 Experimental Diets

The enzyme product used in this experiment was Allzyme® Spectrum and was supplied by Alltech Inc. (Nicholasville, KY). Allzyme® Spectrum contains 4.2 million XU/lb of Xylanase (*Trichoderma longibrachiatum*) and 454,000 SPU/lb of Phytase (*Aspergillus niger*). One xylanase unit is the amount of enzyme will release 1 mmol of xylans per minute under the conditions of the assay. Enzyme activity was determined prior to the addition of the enzyme in the diets. A Solid-State Fermentation Phytase unit (SPU) is defined as the amount of enzyme that will release 1 mmol of inorganic P per minute under the conditions of the assay.

There were 5 pre-starter and 5 starter diets used in this study. The PC diet for both the pre-starter and starter was a wheat-soybean meal reference diet comparable to that recommended by the Cobb500™ Broiler guide that met or exceeded the energy and nutrient requirements of birds of this age (Cobb-Vantress, 2022). The NC diet was a wheat-soybean meal-based reduced nutrient diet consisting of 90 kcal less ME and 16.7% less Ca and 33.3% less avP than the PC diet. The NC diet and the enzyme supplemented

diets were mixed from a single basal diet, divided into different portions to which different levels of exogenous enzymes were added to the respective diets. The remaining 3 diets were the NC diet supplemented with Allzyme® Spectrum at 150, 200, and 250 g/ton respectively. All starter diets contained titanium dioxide at 0.5% which served as an indigestible marker for digestibility and utilization calculations.

4.3.3 Sample Collection

All birds and feed were weighed on days 0, 9, and 22 for each pen to determine growth performance parameters (body weight gain, feed intake, and feed efficiency). On day 22, all birds were euthanized by argon asphyxiation.

On days 21 and 22, excreta samples were collected from each pen and then dried for 5 days at 55 °C in a forced-air oven. The dried samples were ground using a Wiley Mill Laboratory Standard (Model No. 3, Arthur H. Thomas Co., Philadelphia, PA, USA) fitted with a 1 mm screen and then stored in airtight plastic bags before being analyzed for dry matter (DM), gross energy (GE), Ca, N, P, and titanium.

On day 22, the right tibia was removed from two birds per pen with a bodyweight that was near to the average bodyweight of the cage and stored at – 20°C until measured for bone-breaking strength (BBS) and bone ash determination. Additionally, jejunal digesta samples were collected from the same two birds as the right tibia and placed in a pre-labeled 15 mL plastic tube. The jejunal samples were frozen at -20 °C until measured for viscosity. Ileal digesta from the distal two thirds of the ileum was collected from the remaining birds in the pen by flushing with distilled water into clean pre-labeled plastic containers. All ileal digesta samples from each pen were combined in a single container

before being frozen at -20 °C. The ileal digesta samples were then freeze-dried and ground using a coffee grinder before storing in airtight bags in a refrigerator until they were analyzed for GE, DM, AA, titanium, Ca, P, and N.

4.3.4 Sample Analysis

Diets were analyzed in triplicate while excreta, ileal digesta, and jejunal digesta were all analyzed in duplicate. If the coefficient of variation fell above 5%, those analyses were repeated for that pen. The DM contents of the diets, ileal digesta, and excreta were dried at 110 °C for 24 hours (AOAC, 2006). GE of the diets, ileal digesta, and excreta was determined using a bomb calorimeter (Parr adiabatic bomb calorimeter, model 6200, Parr Instruments, Moline, IL, USA) with the calibration standard being benzoic acid. N of the diets, excreta, and ileal digesta were analyzed using the LECO analyzer (model FP2000, LECO, St. Joseph, MI; AOAC International, 2006; method 990.03), with EDTA as the internal standard. Titanium content of the diets and excreta were analyzed using the Short et al. (1996) method and then determined using a UV-visible spectrophotometer (UV-1800, Shimadzu Scientific, Kyoto, Japan) at a wavelength of 410 mm. The Ca and P of the diets, ileal digesta, and excreta along with the AA of the diets and ileal digesta were determined at the Agricultural Experiment Station Chemical Laboratories, University of Missouri-Columbia (Columbia, MO). The Ca was analyzed using Inductively Coupled Plasma and P was analyzed using gravimetric method (AOAC, 2006). Concentrations of AA were analyzed using method 982.30 E(a,b,c) (AOAC, 2006). Concentrations of titanium in the ileal digesta samples was determined as

described by Myers et al. (2004) at the Agricultural Experiment Station Chemical Laboratories, University of Missouri-Columbia (Columbia, MO).

Jejunal digesta were used for viscosity determination by first centrifuging the samples for 10 minutes at 5,000 RPM. The supernatant was then removed and centrifuged again for 8 minutes at 10,000 RPM. That supernatant was then analyzed for viscosity using a viscometer (Vibro Viscometer SV-1A, A&D Weighing, Ann Arbor, MI).

The tibias were cleaned thoroughly during collection and, following thawing, all additional left-over tissue and fat was removed. The BBS was measured using an Instron Materials tester (model 4301, Instron Corp., Canton, MA) at a loading rate of 50 mm/min. Following BBS determination, the bones were dried for 24 hours in a 105°C oven for 24 hours, (Precision Scientific Co., Chicago, IL). The dried bones were then completely soaked in a glass jar with petroleum ether for four extraction periods each lasting for 24 hours, for a total of 96 hours. After no color change of the petroleum ether solution was observed following the final extraction, bones were then removed and allowed to dry for 8 hours under the hood at room temperature. They were then placed in the 105°C oven overnight to ensure there was no moisture left. These dried, de-fatted bones were then weighed by pen and placed in a porcelain crucible for ashing in a muffle furnace overnight. The porcelain crucible containing the bones were then weighed back to determine bone ash percentage.

4.3.5 Calculations and Statistical Analysis

Although all the diets were individually analyzed for Ca, P, N, titanium, GE, and amino acids, the values of the NC and all the enzyme supplemented diets were averaged for these values because they were mixed from a single basal diet prior to the addition of the enzyme. The analyzed values for the PC diet were used for PC calculations.

Apparent ileal nutrient and energy digestibility (AID) and energy and nutrient utilization (TTU) of DM, energy, N, Ca, and P were calculated using the method of Kong and Adeola (2014).

AID or TTU (%) = 100 − [100(Ti/To)(No/Ni)]

In this equation, To is the concentration of titanium in the ileal digesta or excreta, Ti is the titanium concentration in the feed, No is the concentration of energy or nutrients in ileal digesta or excreta, and Ni is the concentration of energy or nutrients in the feed. The AME and ADE were calculated using the equation below:

AME or ADE ($kcal/kg$) = $Calculated$ energy utilization or ileal energy digestibility (%) × $GE\ of$ the $diet$ ($kcal/kg$) on a DM basis.

The AME corrected for nitrogen (AMEn) was obtained using the Hill and Anderson (1958) method.

Data was analyzed using the PROC GLM procedure of SAS 9.4 v 4 (2011, SAS Institute Inc., Cary, NC) appropriate for a randomized complete block design. PROC IML was used to generate the coefficients used for orthogonal contrasts. Orthogonal polynomial contrasts were utilized to determine polynomial effects of increasing dietary supplemental levels of Allzyme® Spectrum. A simple contrast between the PC and NC diets was also performed. Additionally, a contrast between the PC and the Allzyme®

Spectrum supplemented diets was also performed. Prior to statistical analysis, all data was subjected to an outlier test, and all outliers (data that fell outside Mean±3SD) were removed. Significant differences between means was determined using Tukey's Honest Significant Difference and the level of significance was $P < 0.05$. Digestibility and utilization data is presented on a DM basis.

4.4 Results

4.4.1 Analyzed Composition of Experimental Diets

The pre-starter and starter diets were analyzed for N, GE, Ca, and P contents and the starter diets were also analyzed for titanium. The ingredient composition of the diet, and the formulated and analyzed energy and nutrient contents of the diets are reported in Table 4.1 and 4.3 (pre-starter diet) and Table 4.2 and 4.4 (starter diets). The crude protein contents of the diets were determined by multiplying the N content of the by 6.25. The analyzed Ca in the starter diets was 26% higher than the formulated value (10.6 vs 8.4 g/kg) for the PC diet while the analyzed Ca for the NC diets was 15% higher than the formulated value (7.7 vs 6.7 g/kg). In general, the analyzed nutrient values in the pre-starter and starter diets were close to the formulated values (Tables 4.3 and 4.4).

4.4.2 Growth Performance and Viscosity

The simple and orthogonal effects of exogenous enzyme supplementation on performance are reported in Table 4.5 and 4.6. The chickens fed the NC diet had lower ($P<0.01$) body weight gain (BWG), feed intake (FI), and feed efficiency (FE) compared to those fed the PC diet for the entire 22d growing period, while the chickens

fed the diets supplemented with 150, 200, 250 g/ton Allzyme® Spectrum had the equivalent BWG, FI, and BWG compared to those the fed PC diet. Feed intake of chickens from d 9-22 and 0-22 and FE d 9-22 linearly increased ($P < 0.05$) with increasing level of supplemental Allzyme® Spectrum. There was a quadratic increase ($P < 0.05$) of enzyme supplementation level on BWG d 9-22 and 0-22 and FE d 9-22 and 0-22, where the 150 g/ton Allzyme® Spectrum had the highest BWG d 0-22.

The simple and orthogonal effects of exogenous enzyme supplementation on jejunal digesta viscosity are reported in Figure 4.1. There was both a linear and quadratic decrease ($P < 0.001$) in jejunal digesta viscosity between the enzyme supplemented diets and the NC diet, where the 200 g/ton Allzyme® Spectrum diet had the lowest viscosity of all treatments (2.9 mPa). There was a significant reduction ($P < 0.001$) in jejunal digesta viscosity in the enzyme supplemented diets (2.9-3.1 mPa) compared to the PC diet (5.2 mPa).

4.4.3 Energy, Nutrient, and Amino Acid Digestibility

The simple and orthogonal effects of exogenous enzyme supplementation on performance are reported in Table 4.7, 4.8, and 4.9. Enzyme supplementation improved ($P < 0.05$) Ca and P digestibility of enzyme supplemented birds when compared to the PC diet. There was also a quadratic increase ($P < 0.01$) in DM, N, and P digestibility and ADE for birds fed the enzyme supplemented diets. Birds fed the NC diet had lower ($P < 0.05$) apparent ileal digestibility of DM, N, and digestible energy but higher ($P < 0.05$) Ca digestibility compared with those fed PC diet. There was a linear increase ($P = 0.002$) in Ca digestibility in the enzyme supplemented diets. There was no

difference ($P > 0.05$) in DM and N digestibility and ADE for birds fed the PC diet versus those fed the enzyme supplemented diets.

There was a quadratic increase ($P < 0.05$) on the ileal digestibility of nonessential amino acids when comparing the enzyme supplemented diets excluding Glu ($P = 0.069$) and Tyr ($P = 0.088$). There was no significant difference ($P > 0.05$) in the ileal digestibility of amino acids when comparing the PC diet to the enzyme supplemented diets excluding Thr ($P < 0.001$) where enzyme supplementation resulted in higher ($P < 0.05$) apparent ileal digestibility compared to the PC diet (87.2 vs. 85.0%). There was a quadratic increase ($P < 0.05$) in ileal digestibility of Arg, His, and Val for enzyme supplemented diets and there was a linear increase ($P = 0.001$) in ileal digestibility of Thr. Although there was not a significant effect on the apparent ileal digestibility of all the amino acids, there was a numerical improvement in apparent ileal digestibility of all amino acids with enzyme supplementation.

4.4.4 Energy and Nutrient Utilization

The simple and orthogonal effects of exogenous enzyme supplementation on performance are reported in Table 4.10. Increasing enzyme supplementation resulted in a quadratic increase ($P < 0.001$) in DM and N retention and AME and AMEn. Increasing enzyme supplementation also resulted in a linear increase ($P < 0.001$) in Ca and P utilization. Birds fed the NC diet had lower ($P < 0.05$) utilization of DM, N, and metabolizable energy but higher ($P < 0.05$) Ca and P utilization compared with those fed the PC diet. Birds fed the enzyme supplemented diets had a higher ($P < 0.001$) DM, N, Ca, and P utilization compared to the birds fed the PC diet. The birds fed the enzyme

supplemented diets did not have a significant difference ($P = 0.495$) in AMEn when compared to birds fed the PC diet. The AME and nutrient utilization improvement in the enzyme supplemented diets in this study can be associated with the partial or total cleavage of arabinoxylans in the wheat.

4.4.5 Bone Mineralization

The simple and orthogonal contrasts of exogenous enzyme supplementation on BBS and bone ash are reported in Table 4.11. The BBS quadratically increased ($P < 0.001$) with enzyme supplementation. Birds fed the PC diet did have a higher ($P = 0.003$ and $P = 0.013$) BBS and bone ash compared to birds fed the enzyme supplemented diets. There was a quadratic increase ($P < 0.001$) in percent bone ash between the enzyme supplemented diets, where the 250 g/ton Allzyme® Spectrum diet had the numerically highest bone ash percentage of all the enzyme supplemented treatments at 53%. Exogenous enzyme supplementation resulted in linear increase ($P < 0.01$) in both the BBS and percent bone ash.

4.5 Discussion

The current study shows that feeding a wheat-soybean-meal-based diet supplemented with Allzyme® Spectrum to broiler chickens improves growth performance and nutrient and energy digestibility and utilization. Birds fed the enzyme supplemented diets had an overall better performance than those fed the reduced nutrient diet (NC). This can be attributed to the presence of NSPs and phytate, whose negative effects were offset by the carbohydrase and phytase enzymes, respectively, in the enzyme

product that was supplemented. Birds given the enzyme supplemented diets also performed equivalently to or better than those birds fed the PC diet.

Wheat is high in arabinoxylans, a form of hemi-cellulose NSPs. Wheats soluble arabinoxylan content is 17 times that of corn; the only cereal grain with a higher soluble arabinoxylan content is rye (Choct, 1997). Xylanase has been shown to offset the anti-nutritive factors of arabinoxylans by hydrolyzing the β-1,4 glycosidic bonds of xylan into xylose. Alongside phytase, the exogenous enzyme responsible for hydrolyzing phytate, these enzymes can improve the nutritional value of wheat-based diets.

Xylanase is most known for its ability to reduce digesta viscosity in wheat-based diets. The reductions of digesta viscosity (-51%) in this current study are in accordance with Engberg et al. (2004) who reported a very similar decrease (-50%) in digesta viscosity when xylanase was added to wheat-based diets. Munyaka et al. (2016) also reported a decrease (-31%) in digesta viscosity of broilers fed wheat diets supplemented with xylanase. Likewise, Anwar et al. (2023) reported a greater reduction in digesta viscosity in birds fed wheat-based diets supplemented with both xylanase and phytase compared to birds supplemented with only xylanase (-37% vs. -56%). The reduction in digesta viscosity seen in this study can be attributed to xylanase's ability to break down arabinoxylans.

The improved growth performance of the birds fed enzyme supplemented diets in this study is consistent with published manuscripts (Gonzalez-Ortiz et al., 2016; Gonzalez-Ortiz et al., 2017; Arczewska-Wlosek et al., 2019; Olukosi et al., 2020). Anwar et al. (2023) reported birds fed diets supplemented with xylanase and phytase had an improved FI, BWG, and feed conversion ratio (FCR) compared to those birds who did

not receive enzyme supplemented diet. The improvements in growth performance seen in this study can be attributed to phytases' ability to cleave phytate bound proteins and nutrients, making them more available to the bird and xylanases' ability to reduce digesta viscosity also allows for better absorption and utilization of nutrients due to brush border membrane enzymes having more contact with the substrate.

The AME and nutrient utilization improvement in the enzyme supplemented diets in this study can be associated with the partial or total cleavage of arabinoxylans in the wheat. The reduction of digesta viscosity due to the inclusion of Allzyme® Spectrum allows for better absorption and utilization of nutrients in the gastrointestinal tract. An improvement in digestible and metabolizable energy due to enzyme supplementation is well documented (Walters et al., 2019; Anwar et al., 2023). The improvement in AMEn (8%) in this study is comparable to the improvement (5%) in AMEn that Selle et al. (2009) reported when feeding xylanase and phytase in combination to broilers being fed low-P wheat-based diets. Similarly, Gallardo et al. (2018) reported similar increases (9.7%) in AME due to phytase and xylanase. There is speculation that the improvement in energy may be independent of its effect on AA digestion. It has been suggested that Ca phytate may increase the formation of metallic soaps in the gut lumen, resulting in reduced digestion of saturated fats (Adeola and Cowieson, 2011; Cowieson et al., 2017).

Nonstarch polysaccharides are known to bind multivalent cations (Gallardo et al., 2018), so the addition of phytase and xylanase would allow for better availability of these cations to the birds, thus improving digestibility and utilization. Phytase has also been shown to improve mineral digestibility and utilization due to its ability to hydrolyze mineral-phytate complexes. This is likely why we see an improvement in P and Ca

digestibility and utilization in this current study. The results of this current study agree with Gallardo et al. (2018) who reported similar findings on Ca (5% vs. 8%) and P (10% vs. 13%) retention when xylanase and phytase were added to wheat-based broiler diets. Selle et al. (2009) also reported an improvement in Ca and P retention with phytase and xylanase supplementation. Walters et al. (2019) reported an improvement in Ca and P digestibility when phytase was added to low-P diets.

Improvements in DM digestibility are consistent with reports from other researchers (Anwar et al., 2023). The improvement (13%) in N digestibility and retention in this study can be associated with lower exogenous and endogenous losses of N due to the action of the carbohydrase and phytase enzymes on NSPs and phytate. Both phytate and NSPs are known to bind to proteins so the addition of exogenous enzymes can increase dietary protein hydrolysis. Selle et al. (2009) saw an 11% increase in N retention in broilers being supplemented with phytase and xylanase. However, they reported that N retention improved more (10% vs. 6%) when only phytase was supplemented compared to when only xylanase was supplemented. This is likely due to phytases' ability to cleave phytate-bound proteins. Gallardo et al. (2018) reported similar improvements (8%) in N retention when broilers were fed wheat diets supplemented with phytase and xylanase.

There are variable results reported regarding improvement on apparent amino acid digestibility with exogenous enzyme supplementation. Selle et al. (2006) observed that improved AA digestibility was associated with reduction of endogenous AA losses and no increase in dietary AA digestibility. It has been suggested that the negative effect of phytate on AA digestibility may be due to increased losses of endogenous AA from the intestine rather than a direct impact on dietary protein utilization (Cowieson et al., 2017;

Gallardo et al., 2018). Woyengo et al. (2008) reported an improvement in apparent AA digestibility in pigs fed wheat-based diets supplemented with phytase and xylanase. Cowieson et al. (2017) reported that Met and Glu showed lower responses to phytase addition compared to other AA. Similar studies have shown improvement in apparent AA digestibility in wheat diets with enzyme supplementation (Gallardo et al., 2018; Anwar et al., 2023). It has been suggested that NSPs impair the digestibility of AA by encapsulating the AA in grains, thereby preventing digestion and absorption of those AAs (Anwar et al., 2023). The improvement in apparent digestibility of some AA in this current study can be attributed to xylanases ability to degrade NSPs thus enhancing protein digestibility.

Bone quality parameters have been shown to improve when enzymes are supplemented to reduced nutrient diets (Lalpanmawia et al., 2014; Leyva-Jimenez et al., 2019; Walters et al., 2019). Al-Qahtani et al. (2020) reported a comparable improvement (23%) in BBS in broilers fed wheat-based diets supplemented with xylanase and phytase. The improvement in BBS and percent bone ash is likely due to phytase hydrolyzing the phytate-bound minerals and making them more available to the bird. Xylanase reducing digesta viscosity and allowing for better absorption of nutrients also contributes to improved BBS and bone ash percentages.

The addition of xylanase and phytase to broiler diets allows birds to optimize nutrient digestion and absorption. This means less additional P and protein can be added to diets, thus reducing feed costs and providing economic benefits to producers. This also allows nutritionists to use cheaper ingredients and more by-products in broiler diets, subsequently providing more economic benefits to producers. (Williams, 2006). It also

means because nutrients are better absorbed that less N and P is being excreted into the environment, therefore providing environmental benefits as well (Chakraborty et al., 2021).

4.6 Conclusion

Allzyme® Spectrum supplementation in this study resulted in an improvement in growth performance, energy and nutrient digestibility and utilization, and tibia quality, while also reducing jejunal digesta viscosity in 22-d old broilers fed a wheat-based, reduced nutrient diet. This study shows that the use of phytase and xylanase in wheat-based diets offers the ability for producers to feed lower quality diets while still maintaining performance standards equivalent to that of birds fed a commercial diet. The use of exogenous phytase and xylanase shows potential as a useful tool to provide a more sustainable, lower cost diet to broilers while also enhancing the digestibility and utilization of nutrients, thus leading to a reduced amount of P and N pollution to the environment.

Table 4.1: Ingredients composition of the experimental pre-starter diets fed to broiler chickens from day 0-9 (on as-fed basis).

Diet	PC	NC
Ingredient, g/kg		
Wheat (hard red)	712.4	722.20
Soybean meal (47%CP)	231.4	222.1
Wheat bran	0.0	20.6
Soy oil	14.0	0.0
L-Lysine HCl	5.20	5.20
DL-Methionine	2.60	2.60
L-Threonine	1.40	1.40
Salt (NaCl)	3.40	3.40
Limestone	10.6	11.8
Dicalcium phosphate	16.7	8.4
Vitamin-mineral premix[1]	1.50	1.50
Choline	0.8	0.8
Total	1000.0	1000.0

PC= positive control, NC= negative control

[1] Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: 32 mg of iron from iron sulfate; 8 mg of copper from copper sulfate,; 51 mg of manganese from manganese oxide; zinc oxide, 60 mg; iodine (EDDI), 1.48 mg; sodium selenate, 0.24 mg; vitamin A (retinyl acetate), 8,820 IU; vitamin D3 (cholecalciferol), 2,822 IU; vitamin E (dl-α-tocopheryl acetate), 26 IU; vitamin K activity, 0.73 mg; thiamine, 1.76 mg; riboflavin, 6.17 mg; pantothenic acid, 14 mg; niacin, 44 mg; pyridoxine, 4 mg; folic acid, 0.88 mg; biotin, 0.18 mg; vitamin B12, 0.02 mg; choline, 383 mg.

Treatments B-E were mixed as one basal diet prior to the addition of Allzyme® Spectrum to diets C-E, at 150, 200, and 250 g/ton respectively. Allzyme® Spectrum contains 454,000 SPU/lb of Phytase (Aspergillus niger) and 4.2 million XU/lb of Xylanase (Trichoderma longibrachiatum). One Solid State Fermentation Phytase unit (SPU) is defined as the amount of enzyme that will release 1 mmol of inorganic P per minute under the conditions of the assay. One xylanase unit is the amount of enzyme will release 1 mmol of xylans per minute under the conditions of the assay. The calculated enzyme activity for each enzyme containing diet is 150 SPU and 1,395 XU, 200 SPU and 1,860 XU, and 250 SPU and 2,325 XU for the 150, 200, and 250 g/ton

Table 4.2: Ingredients composition of the experimental starter diets fed to broiler chickens from day 9-22 (on as-fed basis).

Diet	PC	NC
Ingredient, g/kg		
Wheat (hard red)	759.90	779.20
Soybean meal (47%CP)	174.3	164.4
Wheat bran	0.0	14.0
Soy oil	20.9	4.6
L-Lysine HCl	6.00	6.00
DL-Methionine	2.30	2.30
L-Threonine	1.90	1.90
Salt (NaCl)	3.20	3.20
Limestone	9.8	10.9
Dicalcium phosphate	14.4	6.2
Vitamin-mineral premix[1]	1.50	1.50
Choline	0.8	0.8
Titanium dioxide	5	5
Total	1000.0	1000.0

PC= positive control, NC= negative control

[1] Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: 32 mg of iron from iron sulfate; 8 mg of copper from copper sulfate,; 51 mg of manganese from manganese oxide; zinc oxide, 60 mg; iodine (EDDI), 1.48 mg; sodium selenate, 0.24 mg; vitamin A (retinyl acetate), 8,820 IU; vitamin D3 (cholecalciferol), 2,822 IU; vitamin E (dl-α-tocopheryl acetate), 26 IU; vitamin K activity, 0.73 mg; thiamine, 1.76 mg; riboflavin, 6.17 mg; pantothenic acid, 14 mg; niacin, 44 mg; pyridoxine, 4 mg; folic acid, 0.88 mg; biotin, 0.18 mg; vitamin B12, 0.02 mg; choline, 383 mg.

Treatments B-E were mixed as one basal diet prior to the addition of Allzyme® Spectrum to diets C-E, at 150, 200, and 250 g/ton respectively. Allzyme® Spectrum contains 454,000 SPU/lb of Phytase (*Aspergillus niger*) and 4.2 million XU/lb of Xylanase (*Trichoderma longibrachiatum*). One Solid State Fermentation Phytase unit (SPU) is defined as the amount of enzyme that will release 1 mmol of inorganic P per minute under the conditions of the assay. One xylanase unit is the amount of enzyme will release 1 mmol of xylans per minute under the conditions of the assay. The calculated enzyme activity for each enzyme containing diet is 150 SPU and 1,395 XU, 200 SPU and 1,860 XU, and 250 SPU and 2,325 XU for the 150, 200, and 250 g/ton

Table 4.3: Formulated and analyzed energy and nutrient contents of the experimental pre- starter diets fed to broiler chickens from day 0-9.

Nutrients and energy	Positive Control	Negative Control
Formulated		
Crude protein, g/kg	220.3	220.4
MEn, kcal/kg	2,975	2,885
Ca, g/kg	9.0	7.5
P, g/kg	7.5	6.2
Non-phytate P, g/kg	4.5	3.0
Lys, g/kg	13.7	13.6
Met, g/kg	5.4	5.4
Met + Cys, g/kg	9.4	9.5
Thr, g/kg	8.4	8.4
Trp, g/kg	2.7	2.7
Val, g/kg	9.3	9.3
Analyzed		
Crude protein, g/kg	212.5	213.5
Gross energy, kcal/kg	3,938	3,888
Ca, g/kg	12.4	10.6
P, g/kg	6.5	5.4
Lys, g/kg	13.6	13.7
Met, g/kg	5.6	5.2
Met + Cys, g/kg	9.1	8.7
Thr, g/kg	8.2	8
Trp, g/kg	2.3	2.3
Val, g/kg	9.6	9.4

The negative control diet and diets containing supplemental exogenous enzymes were mixed from a single basal diet.

Table 4.4: Formulated and analyzed energy and nutrient contents of the experimental starter diets fed to broiler chickens from day 9-22.

Nutrients and energy	Positive Control	Negative Control
Formulated		
Crude protein, g/kg	199.8	201.1
MEn, kcal/kg	3,056	2,966
Ca, g/kg	8.0	6.5
P, g/kg	6.9	5.6
Non-phytate P, g/kg	4.0	2.5
Lys, g/kg	12.8	12.7
Met, g/kg	4.9	4.9
Met + Cys, g/kg	8.6	8.7
Thr, g/kg	8.0	8.0
Trp, g/kg	2.4	2.4
Val, g/kg	8.3	8.3
Analyzed		
Crude protein, g/kg	199.6	201.4
Gross energy, kcal/kg	3,964	3,927
Ca, g/kg	10.6	7.7
P, g/kg	6.0	4.4
Lys, g/kg	12.9	12.7
Met, g/kg	4.7	4.5
Met + Cys, g/kg	8.2	7.8
Thr, g/kg	7.6	8.3
Trp, g/kg	2.1	2.1
Val, g/kg	8.6	8.5

The negative control diet and diets containing supplemental exogenous enzymes were mixed from a single basal diet.

Table 4.5: Effect of increasing level of Allzyme Spectrum® supplementation on performance of 22-d-old broiler chickens fed wheat-soybean-meal based diets.

				Feed intake, g/b/d			Body weight gain, g/b/d		
Treatment	Diet	Allzyme Spectrum®	IBW, g/b	d 0-9	d 9-22	d 0-22	d 0-9	d 9-22	d 0-22
A	PC	0	40.8	21.5$_z$	75.7^a	53.3^a	17.7	48.8^a	35.9^a
B	NC	0	40.4	20.0	67.7^b	47.8^b	15.2	38.9^b	29.0^b
C	NC	150	40.7$_z$	21.5	76.2^a	53.6^a	17.9	47.2^a	35.1^a
D$_z$	NC	200	39.9	20.4	75.0^a	52.2^a	16.4	47.6^a	34.6^a
E	NC	250	41.3	21.3	76.2$^a{}_z$	53.1^a	16.9	47.9$^a{}_z$	34.8^a
Pooled standard deviation			0.355	0.584	1.483	1.093	0.704	1.171	0.896
P-value			0.120	0.260	0.001	0.002	0.065	<0.001	<0.001
			Probability						
PC vs NC			0.409	0.076	<0.001	0.001	0.018	<0.001	<0.001
PC vs Enzyme supplemented diets			0.591	0.485	0.978	0.773	0.472	0.391	0.328
Linear effect of enzyme supplementation			0.418	0.157	<0.001	<0.001	0.089	<0.001	<0.001
Quadratic effect of enzyme supplementation			0.247	0.568	0.083	0.070	0.111	0.049	0.026

$^{a-c}$ Means with different superscripts within the same column differ significantly ($P < 0.05$)

^{z}n=10 replicate cages with 6 birds/replicate, subscript z where n =9

PC = Positive Control, NC = Negative Control, IBW = Initial body weight

Pre-starter diet fed from d 0-9 and starter diet fed from d 9-22

SEM = SD/√n

Table 4.6: Effect of increasing level of Allzyme Spectrum® supplementation on feed efficiency and jejunal digesta viscosity of 22-d-old broiler chickens fed wheat-soybean-meal based diets.

Treatment	Diet	Allzyme Spectrum®	Feed efficiency			Viscosity, mPa
			d 0-9	d 9-22	d 0-22	
A	PC	0	$0.820^{a}{}_{z}$	0.644^{a}	0.673^{a}	5.2^{b}
B	NC	0	$0.758^{b}{}_{z}$	0.574^{b}	0.606^{b}	6.2^{a}
C	NC	150	0.832^{a}	$0.620^{a}{}_{z}$	$0.656^{a}{}_{z}$	3.1^{c}
D	NC	200	0.811^{a}	0.634^{a}	$0.660^{a}{}_{z}$	2.9^{c}
E	NC	250	0.791^{a}	0.629^{a}	0.656^{a}	3.0^{c}
Pooled standard deviation			0.02	0.01	0.01	0.38
P-value			0.016	<0.001	<0.001	<0.001
			Probability			
PC vs NC			0.008	<0.001	<0.001	0.0783
PC vs Enzyme supplemented diets			0.638	0.112	0.086	<0.001
Linear effect of enzyme supplementation			0.041	<0.001	<0.001	<0.001
Quadratic effect of enzyme supplementation			0.006	0.158	0.046	<0.001

[a-c] Means with different superscripts within the same column differ significantly ($P < 0.05$)

[z]n =10 replicate cages with 6 birds/replicate, except for subscript z where n =9

PC = Positive Control, NC = Negative Control

Pre-starter diet fed from d 0-9 and starter diet fed from d 9-22

SEM = SD/√n

Table 4.7: Effect of increasing level of Allzyme Spectrum® supplementation on apparent ileal nutrient and energy digestibility of 22-d-old broiler chickens fed wheat-soybean-meal based diets (DM basis)

Treatment	Diet	Allzyme Spectrum®	DM, %	N, %	Ca, %	P, %	Energy, %	ADE, kcal/kg
A	PC	0	78.3[a]	87.7[a]	61.7[b]	59.3[bc]	77.1[a]	3454[a]
B	NC	0	71.9[b]	86.2[b]	71.2[a]	56.4[c]	69.8[b]	3139[b]
C	NC	150	78.4[a]	88.4[a]	75.4[a]$_z$	62.3[ab]	76.6[a]	3404[a]
D	NC	200	79.0[a]	88.3[a]	73.2[a]	65.5[a]	77.4[a]	3434[a]
E	NC	250	78.7[a]	88.5[a]	76.2[a]	66.0[a]	77.2[a]	3426[a]
Pooled standard deviation			1.35	1.20	9.25	5.39	0.46	60.83
P-value			<0.001	<0.001	0.008	<0.001	<0.001	<0.001
			Probability					
PC vs NC			<0.001	0.011	0.027	0.232	<0.001	<0.001
PC vs Enzyme supplemented diets			0.442	0.091	<0.001	0.011	0.991	0.157
Linear effect of enzyme supplementation			0.370	0.162	0.002	0.021	0.927	0.228
Quadratic effect of enzyme supplementation			<0.001	0.003	0.337	0.005	<0.001	<0.001

[a-c]Means with different superscripts within the same column differ significantly ($P < 0.05$)

[z]n=10 replicate cages with 6 birds/replicate, except for subscript z where n =9

PC = Positive Control, NC = Negative Control, ADE = apparent digestible energy

Pre-starter diet fed from d 0-9 and starter diet fed from d 9-22

SEM = SD/√n

Table 4.8: Effect of increasing level of Allzyme Spectrum® supplementation on apparent ileal digestibility of essential amino acids of 22-d-oldbroiler chickens fed wheat-soybean-meal based diets (DM basis)

Treatment	Diet	Allzyme Spectrum®	Arg, %	His, %	Ile, %	Leu, %	Lys, %	Met, %	Phe, %	Thr, %	Trp, %	Val, %
A	PC	0	88.0[a]	87.8[a]	87.8[ab]	87.7[ab]	90.5	93.6$_z$	89.2[ab]	85.0[b]	88.5	85.6[a]
B$_z$	NC	0	86.3[b]	86.1[b]	86.6[b]	86.4[b]	89.8	92.2	87.9[b]	84.7[b]	87.6	83.9[b]
C	NC	150	88.1[a]$_z$	88.1[a]$_z$	89.0[a]	88.8[a]	91.3	93.2	90.1[a]	87.4[a]	89.1	86.1[a]$_z$
D$_z$	NC	200	88.4[a]	88.2[a]	88.3[ab]	88.2[a]	90.9	92.9	89.5[a]	86.9[a]	89.0	86.3[a]
E	NC	250	88.6[a]	88.4[a]	89.0[a]	88.7[a]$_z$	91.5	93.3	90.2[a]	87.3[a]$_z$	89.4	86.7[a]
Pooled standard deviation			1.72	1.61	1.84	1.85	1.80	1.10	1.49	1.87	1.74	1.82
P-value			0.032	0.027	0.042	0.049	0.264	0.089	0.013	0.004	0.160	0.022
						Probability						
PC vs NC			0.035	0.026	0.153	0.122	0.391	0.009	0.070	0.943	0.246	0.045
PC vs Enzyme supplemented diets			0.606	0.490	0.178	0.216	0.272	0.275	0.172	<0.001	0.276	0.307
Linear effect of enzyme supplementation			0.709	0.587	0.363	0.355	0.458	0.200	0.377	0.001	0.405	0.449
Quadratic effect of enzyme supplementation			0.017	0.014	0.165	0.117	0.362	0.080	0.082	0.146	0.158	0.020

[a-c]Means with different superscripts within the same column differ significantly ($P < 0.05$)

[z]n=10 replicate cages with 6 birds/replicate, except for subscript z where n =9
PC = Positive Control, NC = Negative Control
Pre-starter diet fed from d 0-9 and starter diet fed from d 9-22
SEM = SD/√n

Table 4.9: Effect of increasing level of Allzyme Spectrum® supplementation on apparent ileal digestibility of nonessential amino acids of 22-d-oldbroiler chickens fed wheat-soybean-meal based diets (DM basis)

Treatment	Diet	Allzyme Spectrum®	Ala, %	Asp, %	Cys, %	Glu, %	Gly, %	Pro, %	Ser, %	Tyr, %
A	PC	0	83.8[a]	85.0[a]	86.2[a]	93.8[ab]	84.2[a]	92.8[a]	86.2[a]	88.5[ab]
B_z	NC	0	81.7[b]	83.0[b]	82.3[b]	93.2[b]	81.9[b]	91.7[b]	84.1[b]	87.2[b]
C	NC	150	84.2[a]$_z$	86.1[a]	85.9[a]	94.6[a]	84.5[a]$_z$	93.5[a]	87.2[a]	89.8[a]
D_z	NC	200	84.5[a]	85.4[a]	85.8[a]	94.3[a]	84.8[a]	93.2[a]	86.7[a]	89.1[a]
E	NC	250	84.8[a]	86.0[a]	85.5[a]	94.6[a]	84.9[a]	93.4[a]	87.3[a]	89.7[a]
Pooled standard deviation			2.20	1.92	1.79	0.90	1.82	0.85	1.99	1.75
P-value			0.032	0.005	<0.001	0.006	0.004	<0.001	0.009	0.014
					Probability					
PC vs NC			0.040	0.022	<0.001	0.126	0.007	0.010	0.030	0.095
PC vs Enzyme supplemented diets			0.395	0.252	0.452	0.058	0.462	0.054	0.233	0.122
Linear effect of enzyme supplementation			0.501	0.445	0.555	0.140	0.548	0.114	0.390	0.240
Quadratic effect of enzyme supplementation			0.018	0.032	<0.001	0.069	0.003	0.004	0.029	0.088

[a-c]Means with different superscripts within the same column differ significantly ($P < 0.05$)

[z]n=10 replicate cages with 6 birds/replicate, except for subscript z where n =9
PC = Positive Control, NC = Negative Control
Pre-starter diet fed from d 0-9 and starter diet fed from d 9-22
SEM = SD/√n

Table 4.10: Effect of increasing level of Allzyme Spectrum® supplementation on apparent nutrient and energy utilization of 22-d-old broiler chickens fed wheat-soybean-meal based diets (DM basis)

Treatment	Diet	Allzyme Spectrum®	DM, %	N, %	Ca, %	P, %	Energy, %	AME, kcal/kg	AMEn, kcal/kg
A	PC	0	$72.8^{b}{}_{z}$	63.1^{b}	65.6^{c}	61.8^{c}	$76.6^{b}{}_{z}$	$3352^{a}{}_{z}$	$3249^{a}{}_{z}$
B	NC	0	68.3^{c}	60.8^{c}	69.9^{b}	68.4^{b}	$71.8^{c}{}_{z}$	$3099^{c}{}_{z}$	$2989^{b}{}_{z}$
C	NC	150	74.8^{a}	69.6^{a}	72.7^{a}	75.2^{a}	77.2^{a}	3329^{b}	3244^{a}
D	NC	200	74.9^{a}	68.2^{a}	72.3^{a}	$73.6^{a}{}_{z}$	$77.3^{a}{}_{z}$	$3337^{ab}{}_{z}$	$3249^{a}{}_{z}$
E	NC	250	74.8^{a}	68.8^{a}	74.4^{a}	75.2^{a}	77.1^{a}	3325^{b}	3237^{a}
Pooled standard deviation			0.62	3.79	3.65	1.81	1.55	21.04	22.05
P-value			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
			Probability						
PC vs NC			<0.001	0.044	0.012	<0.001	<0.001	<0.001	<0.001
PC vs Enzyme supplemented diets			<0.001	<0.001	<0.001	<0.001	0.002	0.009	0.495
Linear effect of enzyme supplementation			<0.001	<0.001	<0.001	<0.001	0.001	0.044	0.833
Quadratic effect of enzyme supplementation			<0.001	<0.001	0.598	0.262	<0.001	<0.001	<0.001

$^{a\text{-}c}$Means with different superscripts within the same column differ significantly ($P < 0.05$)

^{z}n=10 replicate cages with 6 birds/replicate, except for subscript z where n =9

PC = Positive Control, NC = Negative Control, AME = apparent metabolizable energy, AMEn = AME corrected for nitrogen

Pre-starter diet fed from d 0-9 and starter diet fed from d 9-22

SEM = SD/√n

Table 4.11: Effect of increasing level of Allzyme Spectrum® supplementation on the tibia quality of 22-d-old broiler chickens fed wheat-soybean-meal based diets.

Treatment	Diet	Allzyme Spectrum®	Bone breaking strength, kg/f	Bone ash, %
A	PC	0	17.7^{a}	$53.4^{a}{}_{z}$
B	NC	0	11.2^{c}	50.1^{b}
C	NC	150	15.6^{b}	$51.4^{b}{}_{y}$
D	NC	200	15.4^{b}	$51.2^{b}{}_{z}$
E	NC	250	15.4^{b}	53.0^{a}
Pooled standard deviation			1.98	1.62
P-value			<0.001	<0.001
		Probability		
PC vs NC			<0.001	<0.001
PC vs Enzyme supplemented diets			0.003	0.013
Linear effect of enzyme supplementation			0.006	0.002
Quadratic effect of enzyme supplementation			<0.001	0.011

[a-c]Means with different superscripts within the same column differ significantly ($P < 0.05$)

[z]n=10 replicate cages with 6 birds/replicate, except for y & z where n= 8&9, respectively

PC = Positive Control, NC = Negative Control

Pre-starter diet fed from d 0-9 and starter diet fed from d 9-22

SEM = SD/√n

CHAPTER 5. CONCLUSION

Poultry is one of the fastest growing agriculture commodities and approximately 70% of the cost of raising poultry is due to feed cost. Energy providing ingredients are the most expensive to add to poultry diets, followed by protein, and phosphorus components, respectively. Phytase is also a vital component for the reduction of P and N pollution. The ability of phytase to allow the bird to better absorb P and N means that less is being excreted into the environment. Therefore, poultry producers are looking for ways to save money and combat pollution and this can be done by the inclusion of feed additives that can improve the nutrient availability of feedstuffs to birds.

Two studies were conducted evaluating an enzyme complex containing phytase and xylanase in either corn-SBM- or wheat-SBM-based diets for broiler chickens. In the first experiment, we examined the effects Allzyme Spectrum, an enzyme complex containing phytase and xylanase when supplemented to corn-soybean-meal-based diets containing reduced energy, Ca, and avP and their effects on performance, jejunal digesta viscosity, bone mineralization, and energy and nutrient digestibility and utilization. The results indicated that the enzyme supplementation improved performance, bone quality, apparent ileal amino acid (AA) digestibility, and DM, N, Ca, and P digestibility and utilization, as well as AMEn and ADE. This was expected since corn is generally high in phytate and phytase breaks down the bounds in phytate, thus making not only the bound P but also the cations and AA bound to phytic acid more available to the bird. However, there was no improvement in jejunal digesta viscosity. This is not surprising due to the already low digesta viscosity of birds fed corn-soybean meal-based diets.

In the second experiment we investigated the effects of Allzyme Spectrum enzyme containing phytase and xylanase when supplemented to wheat-soybean meal-based diets with reduced energy and nutrient on growth performance, jejunal digesta viscosity, bone mineralization, and energy and nutrient digestibility and utilization. These results revealed that enzyme supplementation improved growth performance, bone mineralization, apparent ileal digestibility of nonessential and some essential AA, and energy, DM, N, Ca, and P digestibility and utilization while also reducing jejunal digesta viscosity. The effect of exogenous enzyme supplementation on digesta viscosity is not surprising due to xylanase reducing digesta viscosity by breaking down the NSPs, thus improving nutrient digestion and absorption.

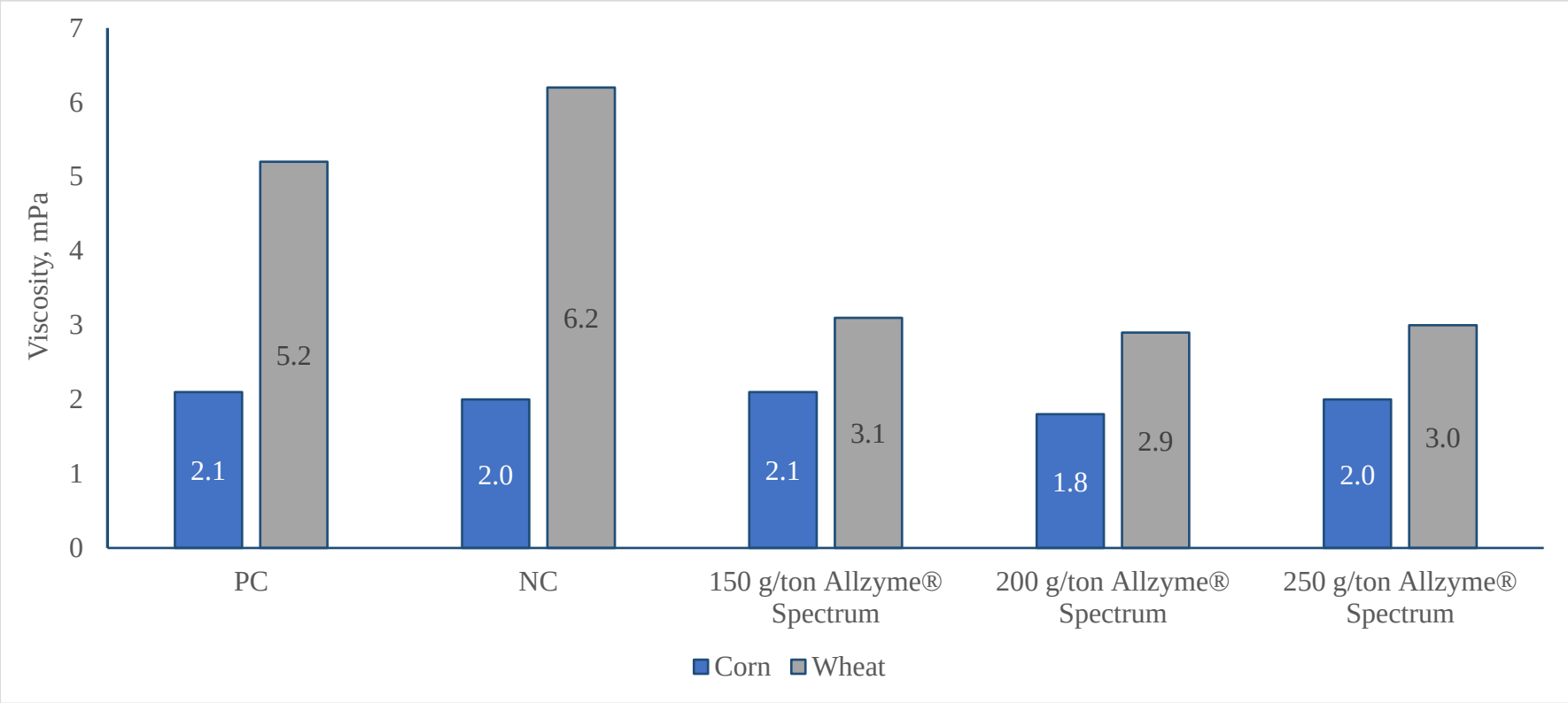
In both studies enzyme supplementation improved energy and nutrient digestibility and utilization to the level that was similar or better than that of the positive control diet (PC). This is important because it shows that supplementing these enzymes to reduced nutrient diets still allowed the birds to perform at a level that is similar to birds on the PC diet. This could potentially allow producers to save on feed costs by utilizing poor quality feed ingredients. This also means that since the bird is utilizing more of the nutrients, diets given to the bird could be less dense in nutrient and energy and could result in less nutrient being excreted into the environment, thus reducing environmental pollution.

Figure 5.1 shows the effects of the phytase and xylanase supplementation in this study and its effects on digesta viscosity. The vast impact that the exogenous enzyme supplementation had on reducing the digesta viscosity of birds fed wheat-soybean meal-based diets is evident. Even though the supplementation of the enzyme was able to

reduce the digesta viscosity of the birds fed the wheat-soybean meal-based diets, it still was not able to reduce the digesta viscosity to that of the birds fed the corn-soybean meal-based diets. Hopefully, an enzyme or a combination of enzymes will be able to further reduce the viscosity of digesta in wheat-based diets to levels similar to that of corn-based diets. If this is achieved, it may lead to a situation in which broiler chickens would be able to better utilize nutrients and energy in wheat-based diets.

Looking ahead, further research into the effects of utilizing multi-enzyme complex containing more than two enzymes targeting different but specific substrate is needed if enzymes are to reach their full potential. The demand and use of enzymes in poultry diet will continue to increase, hence it is essential that we continue to research the matrix values of varying enzymes so we can fully understand their applications and limitations in poultry production.

Figure 5.1: Jejunal digesta viscosity of birds fed corn vs. wheat-soybean meal-based diets supplemented with a phytase and xylanase containing enzyme product.



APPENDICES

APPENDIX 1: JEJUNAL VISCOSITY PROTOCOL

Sample preparation:

1. Thaw samples in refrigerator
2. Mix sample thoroughly
3. Use a 15 mL Centrifuge tube and add 6-8 mL of sample into the tube
4. Spin the centrifuge tubes in large centrifuge (Sorvall ST 16 Centrifuge, ThermoFisher Scientific, Germany)
    a. Make sure to balance the samples in the centrifuge
5. Spin at highest RPM (~5000) for about 10 minutes
    a. If samples are super viscous, increase this time
6. Pipette out supernatant into 2 mL centrifuge tubes
7. Spin these tubes in small centrifuge (Centrifuge 5430 R, Eppendorf AG, Germany) for 8 minutes at 10,000 RPM
    a. Again, if samples are extremely viscous increase time
8. Pipette out supernatant into a new 2 mL centrifuge tube
9. Repeat steps 6-8 until the new 2 mL centrifuge tube is full
    a. If you do not have enough supernatant from the original 15 mL tube, centrifuge the 15 mL tube for an additional 10 minutes, if this doesn't result in enough, add additional sample to 15 mL tube and centrifuge again

Using the viscometer:

1. Run viscometer with a standard to ensure calibration
    a. Brookfield Viscosity Standard was used viscosity is 965 mPa*s at 25 degrees Celsius (Brookfield Viscosity Standard, Brookfield Engineering Laboratories, INC., Middleboro, MA)
2. Prior to sample prep warm up water bath to correct temperature of samples
    a. About 40°C
    b. You may have to keep bath warmer than sample temp as the samples lose heat quickly, adjust accordingly
3. Add 4-6 sample tubes (the finished 2 mL centrifuge tubes) to the water bath, make sure to close the lids tightly
    a. Allow a few minutes for samples to acclimate
4. Immediately place the sample in the holder of the viscometer (Vibro Viscometer SV-1A, A&D Weighing, Ann Arbor, MI), approximately the 2 mL cup holds 1.8 mL at the upper-level gauge
    a. Samples lose heat quickly and it is harder to warm them up than it is to cool them down
5. Confirm the protector is in the protective position
6. Pinch the grips, and gently lower the sensor plats to align with upper-level gauge in the sample cup
7. Turn the knob to adjust height direction as needed

8. Let the temperature gauge level out the temperature reading if it is not within +/- 0.5°C, then adjust accordingly:
    a. If the sample is too cold, remove the sample from machine for 30 seconds then check the temperature again
    b. If the sample is too hot, remove the sample and place the cup bottom in water bath
        i. This will take about 30 seconds to one minute, then check temperature again
    c. Repeat if needed
9. Using the RsVisco program, select run, this will run for the allotted time you've chosen and stop on its own
10. Remove sample when reading is finished
11. Clean and dry cup and repeat with next sample

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