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Effects of Feed Additive Strategies for Commercial Broiler Production and Gut Health

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By

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Effects of feed additive strategies for commercial broiler production and gut health

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The total removal of antibiotic growth promoters (AGP) from poultry feed is underway in the United States. Feed additive strategies will be utilized to maintain the efficient growth, health, and economic value found with current commercial broiler production. Experiment 1 investigated the effects of feeding an encapsulated butyric acid and zinc product (EBAZ) at 3 inclusions on d 0- 49 Ross x Ross 708 male and female broiler performance, blood chemistry, and cecal short-chain fatty acid content. These data suggest that EBAZ can be safely included at 0.5 g/kg and at 2.5 g/kg into diets for Ross x Ross 708 male and female broilers. The objective of the second experiment was to examine the efficacy of 2 commercially available carbohydrases in commercial diets on d 0-57 male broiler performance and processing. The resulting data demonstrates that the inclusion of xylanase (CE2) improved broiler performance thus, increased potential gross profits.

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CHAPTER I

LITERATURE REVIEW

World Population Increase and Poultry

Human life expectancy has steadily increased every year, due to advancements regarding disease prevention, as well as advancements in agriculture and medicine. This lengthening of life, coupled with an increase in birth rate, has led to a dramatic growth in population. In fact, the world's population will reach greater or equal to 9.6 billion in 2050, with an additional increase of 1.3 billion in the following fifty years (Gerland et al., 2014). With this increase in people, but not land, concerns exist for an increase in pollution, crime, and economic instability. Also of grave concern is having an adequate food supply to support this future world population (Gerland et al., 2014).

In order to provide an adequate food supply to this growing population, we must be strategic and proactive to improve agricultural production efficiency before 2050. This could be challenging considering recent increases in meat consumption, and in developed countries pork and poultry are credited with the majority of this meat being consumed (Delgado, 2003). Due to poultry's popularity throughout the world, efficient growth production, and relatively low cost, it may be the most favorable protein in 2050 (Delgado, 2003).

Poultry Production in the United States

The majority of broiler production takes place in the Southeastern states. For example, the five top broiler producing states in 2015 were Georgia, Alabama, Arkansas, North Carolina, and Mississippi (USDA poultry production and value). This region of the U.S. has increased its production of heavy broilers, which are used for further processed products and raised for longer periods of time in order reach a higher target weight of approximately 3.62 + kg (Lorenzi et al., 2014).

The United States consumes a large amount of poultry meat, while also producing the highest amount (Foreign Agricultural Service/USDA. October 2016). Per capita, Americans have consistently consumed more poultry meat than beef or pork since the year 2000 (Hood et al., 2012). This high level of consumption is supported by increased broiler production throughout the United States, such as the rise from 8.55 billion in 2014 to 8.69 billion head in 2015 with a value of $29.0 billion (USDA, 2015).

In 2015 all United States poultry sales were valued $48 billion; of this, broiler production represented $28.7 billion (USDA, 2015). This large and efficient production is achieved by popular vertical integration practices, which encompass the hatchery, feed mill, processing plants, and marketing. These companies partner with individual growers and provide the chicks, all feed necessary, as well as assign a technician to provide any assistance throughout the grow-out period. Contracted growers are expected to cover farm facility costs, care for birds using company specific welfare and management practices, and provide ideal growing environments until birds are ready for slaughter. Growers are encouraged to maintain good husbandry practices by receiving payments based on weight produced and feed efficiency.

Sustainability of Poultry

Animal agriculture is associated with the majority of the greenhouse gas emissions in the United States, but poultry only contribute to a small portion. Although production for poultry has increased, the nitrogen and phosphorus emissions were found to be lower in poultry meat and egg production in comparison to milk, beef, and pork on a feed efficiency basis (Flachowsky, 2002). This is a result of extensive selective breeding for efficient feed conversion and maximum growth, which in turn has made poultry production a more sustainable form of agriculture (Havenstein et al., 2003).

Poultry Production in Mississippi

The state of Mississippi lies in the southeastern portion of the United States and covers 46,914 of the 3,531,905 square miles that make up the United States (statesymbolsusa). Approximately 1,500 farms are located in this state that contribute to poultry production which is also the largest agricultural commodity (Hood et al., 2012). In 2015, Mississippi ranked 5th in broiler production by producing 723 million head with a value of $2.5 billion, demonstrating the importance of the state to the United States, and world's poultry meat needs (USDA, 2015). In addition, two major poultry companies, Sanderson Farms and Cal-Maine Foods are headquartered in the state, while 5 other major poultry companies (Wayne Farms LLC, Peco Foods, Inc., Tyson Foods, Inc., Marshall Durbin Company, and Koch Foods) are represented (Hood et al., 2012).

Poultry Nutrition and Feeding

As with all animal agriculture, maintaining adequate nutrition is key for efficient growth and performance throughout production. The largest portion of costs (60-70%) for

producing poultry comes from feed (Chiba, 2009). Nutritionists in the poultry industry commonly formulate on a least-cost basis in order to prevent profit loss, while also providing nutrient adequate diets fit for the production goals in mind (Chiba, 2009).

All 6 nutrient classes (protein, carbohydrates, water, fat, minerals, and vitamins) are acknowledged when poultry feed is formulated. In the United States, diets are prominently corn and soybean meal based, however feedstuffs and their inclusions can vary due to market and nutrient availability, price, nutrient requirements, bird age, production goals, preference and quantity of feed (Chiba, 2009).

As a rule of thumb, broiler diet energy increases and crude protein decreases with bird age (Ross Management Guidelines, 2014). Energy, used for production and maintenance, is primarily obtained through carbohydrates, fat and protein. Carbohydrates will be discussed further later on in this literature review.

High protein vegetable sources (soybean meal) or animal sources (animal by-products) provide essential amino acids. Amino acids, the building blocks of protein, are important for metabolic processes and muscle growth in broilers. Any deficiencies in amino acid requirements are met with the addition of crystalline amino acids to the feed (NRC, 1994). Broiler diets are often formulated using digestible amino acids, which can meet requirements for growth more precisely. Formulating this way leads to a reduction in diet costs by eliminating excess amino acid use and excretion (NRC, 1994).

Vitamin and mineral premixes generally include micro-ingredients, which are those with inclusion levels of less than 5% in completed diet. By adding via premix, it prevents deficiencies by providing minerals (Zn, Cl, Fe, Cu, Mn, Mg Ca, Co P, I Se, Na) and vitamins (A, D, E, K, Thiamine, Riboflavin, Panthothenic Acid, Niacin, Choline,

Vitamin B12, Folic Acid, and Biotin) that are crucial for poultry growth and development (Chiba, 2009).

Since the largest expense in poultry production is feed costs, integrators focus on providing nutrient sufficient feed at least-cost. By-products of grain processing and meat processing (corn distillers dried grains with solubles, meat and bone meal, feather meal) can be utilized in a strategy to reduce costs. However, these by-products tend to be highly variable in nutrient quality.

Carbohydrates

Carbohydrates found in poultry feed provide sources of high energy, to be used for production and maintenance in broilers. Most of these are supplied by cereal grains, like corn, wheat, rye, and barley (NRC, 1994). The type of grain used in formulations can vary due to price, as well as regional availability. Corn is the most popular form of grain for the United States, whereas wheat is common for European poultry diets. Each cereal grain can supply different amounts and types of polysaccharides based on their structure and cell wall.

The most important polysaccharide from cereal grains for poultry nutrition is starch, which is comprised of amylose and amylopectin; both contain α 1-4 glycosidic bonds that are broken down by amylase, but amylopection also contains α 1-6 at its branches (The Biochemistry of Plants Vol 14, 1988). Previous research demonstrates high starch digestibility and absorption for broilers, which has been associated with copious amounts of amylase enzyme produced endogenously (Osman, 1982; Hetland, 2002; Thomas et al., 2008).

Non-Starch Polysaccharides

Non-starch polysaccharides (NSPs) are considered the indigestible polysaccharides of carbohydrates for monogastric animals because monogastrics do not create the enzymes necessary to break β 1-4 bonds (Graminha et al., 2008). Type and ratio of NSPs can vary between cereal grains because of grain structure and tissue differences as Knudsen (2014) demonstrated when measuring the compositions of commonly used poultry cereal grains (corn, wheat, and barley). Total soluble NSP percentage was highest in barley at 26.1%, lowest in corn at 11.8%, and reasonably high in wheat at 21.7% (Knudsen, 2014). Soybean meal, a plant based protein source for poultry feed, also contains a portion of anti-nutritional factors including NSPs.

The presence of NSPs creates numerous digestibility and nutritional problems for broilers that seem to stem from high viscosity, especially diets including barley (Svihus and Gullord, 2002). Increased intestinal viscosity in broilers is often observed and associated with poor performance, short passage rates, decreased digestibility, and increased bacterial fermentation which is theorized to occur because of enzyme interference (Choct and Annison, 1990; Annison and Choct, 1991; Annison, 1993). Several factors are thought to contribute to floor raised broiler footpad lesions with one of these being sticky excreta. Nutritionally, this issue is a result of high intestinal viscosity caused by high concentrations of soluble NSPs in diets (Shepherd and Fairchild, 2010). Exogenous enzymes used to combat these effects and aid in bulky carbohydrate polysaccharide digestion are known as carbohydrases, and will be further discussed in Chapter 3.

Feed Additives

Feed additives in poultry are defined as low inclusion ingredients with an ability to stimulate various benefits in production such as enhanced efficiency, growth, health, and feed quality (Hashemi and Davoodi, 2010). There are several different forms of additives used, but for the purpose of providing information pertinent to this thesis, this literature review will be centered on antibiotic growth promoters (AGP), AGP alternatives, and exogenous enzymes.

Antibiotic Growth Promoters

Feed additives classified as antibiotic growth promoters (AGP) have gained a significant amount of controversy for their sub-therapeutic and quite liberal use in poultry feed since their approval in 1951 (Jones and Ricke, 2003). These have been routinely used as growth promoters in the poultry industry due to observations of low inclusion level effects on growth efficiency improvements over seventy years ago (Moore et al., 1946). The benefits of AGP for poultry production have been confirmed and explored through extensive research (March et al., 1978; Izat et al., 1990; Miles et al., 2006).

It is accepted that the cause of heavier weights and reduced feed conversion exhibited in birds fed AGPs is due to their effect on enteric microbial populations (Dibner and Richards, 2005). Jacobs and others (1953) reported this in early studies in which chicks given diets supplemented with various antibiotics did not show any growth benefit or microbial influence when raised in a sanitary setting. Ten years later, this notion was further explored and confirmed using treated air chambers (Coates et al., 1963). Though we know AGPs play a role in controlling microbiota in the gut, the specific mechanism on how these drugs function on bacteria to provide improved growth and mortality is still

unclear (Dibner and Richards, 2005; Niewold, 2007). However, recent research suggests that the addition of AGP results in decreased release of bile salt hydrolase, leading to increased weights in birds; bile salt hydrolase is a bacterial enzyme observed to reduce the efficiency of bile salts, making lipid digestion more difficult for broilers (Begley et al., 2006; Lin, 2014).

However, there has been an increase in concern about bacterial resistance within the last thirty years. This increase has been associated with human misuse, as well as the sub-therapeutic use of AGP in animal agriculture; with the latter getting the most attention and thus leading to consumer demands for major changes. Therefore, efforts by the Infectious Diseases Society of America have been directed by removing AGPs in agricultural food animals (Spellburg et al., 2009), for those that have human and animal application. This is because the probability of antibiotic resistance is greater when both food animals and humans are treated with the same class of drug. For instance when quinolones were given to broilers at sub-therapeutic doses, as well as in humans for treatment, Campylobacter was found to develop tendencies of resistance (Endtz et al., 1991). In order to prevent resistance, precautions like alternation, residue monitoring, and toxicology testing of all sub therapeutic antibiotics approved for use in poultry by the United States Food and Drug Administration (FDA) (Donoghue, 2003). Recently, in the 2017 revision of Veterinary Feed Directives, the FDA now requires a veterinarian prescription for the use of medically relevant antibiotics in animal agriculture (Veterinary Feed Directive, 2015). With the European Union elimination of antibiotic growth promoter use in poultry at the end of the twentieth century, other countries including the

United States have begun considering effective replacements in preparation for impending removal (Castanon, 2007).

AGP Alternatives

Total removal of AGPs from poultry feed in the United States is in the near future, with major poultry companies i.e. Perdue and Tyson Foods currently or in the process of raising birds without AGPs. This production change may initially cause detriments to feed efficiency, weight gain, and overall health in broilers. Thus, research into alternatives has become more prevalent. Some of these considered alternatives include probiotics, prebiotics, organic acids, minerals, and plant-based products. For this thesis, organic acids, specifically butyric acid and minerals such as zinc will be discussed in detail. While research conducted in Chapter 2 did not utilize antibiotic free diets, a potential AGP alternative was tested.

Organic Acids

Organic acids are used as an antibiotic alternative feed additive, as well as for the prevention of pathogenic contamination in processing facilities (Cherrington, 2008). They represent any organic structure "R-COOH"; however, those with antimicrobial acidification function have shorter carbon chains and tend to have a pKa (half dissociateschepd pH) ranging from 3 to 5 (Berge and Wierup, 2012; Khan and Iqbal, 2016). This ability to inhibit several types of bacteria has been observed in several in vitro studies (Sprong et al., 2001; Chaveerach et al.,2002; Van Immerseel et al., 2003; Van Immerseel et al., 2004b; Makras and De Vuyst, 2006).

Short-Chain Fatty Acids (SCFA)

Short-chain fatty acids (SCFA), are organic acids that consist of 6 carbons or less, produced by bacterial fermentation of carbohydrates (Scheppach, 1994). Propionate, acetate, and butyrate are the three most abundant SCFA that can be found endogenously within the hindgut due to anaerobic fermentation of carbohydrates (van der Wielen, 2000; Ricke, 2003). Several studies reveal that the host gains multiple benefits from the absorption of SCFAs. For instance, these organic acids play a role in metabolism (fat, glucose, cholesterol) for the body, as well as provide support for intestinal cells (Engelhardt et al., 1989; Hong et al., 2005).

SCFA can reduce the potency and proliferation of bacterial infections by their ability to infiltrate bacterial membranes and lower internal pH upon dissociation (Adil et al., 2011; van der Wielen et al., 2000). Lowering the pH weakens neutral pH pathogenic bacterial cells, not only because of its damaging effects on cell components, but also by rapid ATP depletion due to the cell's attempt to rid itself of protons (Davidson et al., 2013). These organic acids have antimicrobial and performance enhancing characteristics when used as feed additives in monogastric animals (Dibner and Richards, 2005).

Low gastrointestinal pH results in early dissociation of SCFAs before reaching the area of major pathogenic colonization in the hindgut, thus reducing their antimicrobial effectiveness (Flint and Garner, 2009). Therefore, microencapsulation, a method of covering a substance with intentions of a specific release, is a common practice for a variety of drugs and additives to combat undesirable release (Silva et al., 2014). Research conducted on the encapsulated/protected feed additive supplement for poultry has recorded successful dissociation further along the hindgut than when

compared to their unprotected counterparts (Hafeez et al., 2016; Van Immerseel et al., 2004; Jerzsele et al., 2012).

In the interest of improving gut health and decreasing food borne pathogen proliferation in poultry, research was done to test the effects of organic acids in vivo (Chapter 2). Previous work first focused on reducing *Salmonella* transmission through organic acid supplementation of feed (Hinton and Linton, 1988). Within several experiments, they observed an inhibition of *Salmonella* infection in the excreta and ceca of birds fed diets including formic or a combination of formic and propionic acids (Hinton and Linton, 1988). Similar results were also observed for another project that tested in chicks fed contaminated diets including a formic and propionic acid combination product (Iba and Berchieri, 1995).

In addition to lowered infections during grow out, the presence of *Salmonella* on broiler carcasses during processing may be reduced by including organic acids in feed. Reports of significantly reduced carcass *Salmonella* counts when birds were fed diets including calcium formate and a 0.4% inclusion of propionic acid, respectively (Izat et al., 1990a; Izat, et al., 1990b). Although not tested in this thesis, calcium formate is a salt form of short chain fatty acid, formate. Salt forms of short chain fatty acids can be less odorous and are easily added to feed.

When tested as a possible AGP alternative, SCFA inclusion has been shown to produce live performance results comparable to that of broilers in comparison to those fed diets including BMD (Samanta, 2010). When compared to feeding BMD a blend of orthophosphoric acid, formic acid, propionic acid, and calcium propionate, was effective in decreasing E. Coli and Clostridium in the feed, as well as improving live performance

when fed to broilers (Samanta, 2010). Izat and others found similar results, with a reduction in Salmonella for broilers dietary supplementation of propionic and formic acid reducing Salmonella (Izat et al., 1990b; Iba and Berchieri, 1995).

The majority of previous research on SCFA as feed additives in broilers include them at the manufacturer's recommended level. The highest inclusion levels were found in research conducted by Panda and cohorts (2009) and Cave (1982). In order to observe the effects of increasing doses on broiler gut health and processing characteristics, butyric acid was fed levels increasing by 2 g/kg until reaching the highest inclusion of 6 g/kg (Panda et al., 2009). This high level was observed to improve performance, but not significantly better than those fed 4 g/kg (Panda et al., 2009). To observe effects on feed intake, Cave (1984) studied levels of propionic acid inclusions of up to 100 g/kg into diets fed for 29 days to female Shaver Starbro chicks. Feed intake reduction severity increased with the increasing propionic acid inclusions (Cave, 1984). Both studies utilized antibiotic free diets.

Butyric Acid

Of the SCFAs, butyric acid, has garnered the most attention as a potential antibiotic alternative and gut health improving strategy in broilers. It is known to enhance gastrointestinal health through epithelial cell promotion and support of tight junction barrier in the intestinal tract, and observed to influence positive physiological changes in monogastric digestion (Sengupta et al., 2006; Dalmasso et al., 2008).

Butyric acid is also highly effective in vitro against E. Coli and Salmonella, two of the most prevalent foodborne pathogens affecting poultry meat (Kwon and Ricke, 1998). In a study supplementing butyric acid (2,4, and 6 g/kg) in broiler diets found that

butyric acid at 4 g/kg contributed to heavier body weights, and also reduced E. Coli counts comparable to those fed the antibiotic (Panda et al., 2009). The same experiment also found that all doses of butyric acid used resulted in longer intestinal villi and crypts (Panda et al., 2009).

There is a lack of research on the effects of SCFA and antibiotic combinations in poultry, reflecting the interest in SCFAs as primarily an antibiotic alternative. Nearly all studies performed include AGP only in control diets for a comparison to dietary treatments of butyric acid inclusion. However, Levy and others (2015) tested diets included BMD as well as an encapsulated butyric acid source in dietary treatments fed to Cobb 500 male broilers. Broiler performance improved along with higher inclusions of the encapsulated butyric acid (Levy et al., 2015).

The commercially available source of butyric acid used in this thesis was ButiPEARL™ Z (Chapter 1). ButiPEARL™ Z is a feed additive product that contains both butyric acid and zinc oxide. This product is protected using spray freezing, which according to the manufacturer will "allow for a targeted delayed release inside the gastrointestinal tract" (Kemin Industries). ButiPEARL™ Z is supplemented into broiler diets at a recommended level of 0.5 g/kg with the intentions of improving intestinal health.

Zinc

Zinc is one of the many minerals included in trace mineral premixes. Dietary zinc is necessary for poultry and other living organisms due to its extensive involvement within the body tissues; it plays a role in enzymes, hormones, growth, healthy immune

function, as well as intestinal health, strength and maintenance (Liu et al., 2012; Vallee and Falchuk, 1993; Rodriguez et al., 1996).

Research on zinc oxide supplementation in pigs has been shown to stimulate microorganism competiveness in the intestines of pigs (Katouli, 1999; Liu et al., 2012). When fed to broilers challenged with a Salmonella strain, zinc inclusion increased villus height, crypt depth, and higher body weight gain (Zhang et al., 2012). Additionally, the importance of zinc was further explored by Miyoshi and others (2016) through which the use of mouse colons and a zinc binder, they observed the degradation of two major protein constituents of the epithelial tight junction during zinc absence. Intestinal tight junction integrity is important for not only absorption of nutrients, but also the prevention of inflammation and pathogenic proliferation in monogastric animals (Berkes et al., 2003).

Animal feeds can supplement zinc in two different forms: organic and inorganic. Zinc oxide and zinc sulfate are considered inorganic source, while several zinc chelates or complexes with amino acids, proteinates, and polysaccharides are considered organic (Cao et al., 2000). Previous research using both forms of zinc report that organic sources are more bioavailable in poultry, explaining results of improved mineralization of tissue and bone, as well as better growth performance (Ao et al., 2009; Wedekind and Baker, 1990; Bun et al., 2011). The form of zinc used in Chapter 1 of this thesis is zinc oxide combined with butyric acid, which is another compound that is associated with strengthening the intestinal tight junctions (Kemin Industries; Zhang et al., 2012).

Exogenous Feed Enzymes

An enzyme is a protein based catalyst that encourage biological reactions by reducing the energy needed for initiation (Khattak et al., 2006). Enzymes are referred to as exogenous when they are not endogenously found within the body. Poultry, like many monogastric animals, cannot release all the nutrients from feed ingredients, due to a lack of enzymes necessary for digestion of fiber and other bound nutrients (Dida, 2016). Thus, the addition of exogenous enzymes in feed can aid in diet/nutrient utilization.

The first poultry involvement with enzyme supplementation occurred in the early 1900s, with the fungal based enzyme, protozyme (Holst, 1926). Nearly a century later, enzymes are routinely used in the poultry industry to degrade anti-nutrients, which liberates bound nutrients, maximizing nutritional availability to the bird. This can in turn decrease feed costs by providing more sustainable foodstuff options for poultry diets, in addition to several other benefits (Khattak et al., 2006). Feed enzymes are primarily produced using certain microorganisms such as fungus, bacteria or yeast (Khattak et al., 2006). Enzymes are known to have different activities with a substrate specific nature; this means positive broiler response to enzyme additions can be based on dietary formulations, which will be further explored in chapter 3 of this thesis.

Effective enzymes should be cost effective, efficient and thermostable. Since enzymes are proteins, they are susceptible to reduced efficacy or degradation when exposed to temperatures of above 80°C during the pelleting process (Silversides and Bedford, 1999). Preventative measures like protective outer coatings, genetic modification, and use of naturally thermophilic enzymes can be used to preserve enzyme efficacy through feed manufacture (Gilbert and Cooney, 1997).

There are three main categories of feed enzymes commonly used: carbohydrase, phytase, and protease. Proteases are primarily used for the degradation of plant sourced protein, such as soybean meal. The addition of an exogenous protease can increase amino acid digestibility, thereby promoting broiler growth and reducing excess nitrogen excretion when included into reduced crude protein diets (Angel et al., 2011). Exogenous carbohydrase and phytase enzymes will be further discussed in the remaining portion of this literature review.

Phytase

Phytate exists primarily in a cyclic chair formation with six phosphate groups and is found in many plant derived feedstuffs and provides storage for over half of the organic phosphorus (Pallauf and Rimbach, 1997; Nelson, 1967; Wyss et al. 1999). Unfortunately, phytate is considered an antinutrient to birds due to its binding of phosphorus, amino acids, and minerals in its chelated form (Nelson, 1967). This is because poultry have insufficient endogenous phytase levels, therefore exogenous additions of phytase are commonly included in commercial diets (Ravindran et al., 1995).

Phytase is the term used for enzymes that can release phosphate from phytate; currently there are two classifications: 3- (EC 3.1.3.8) or 6-phytase (EC 3.1.3.26) (Haefner et al., 2005). Microorganisms tend to produce the 6-phytases that begin hydrolysis of phytate at the 6 position, while plant sources generally produce 3 phytase, which hydrolyzes at the third position (Wodzinski and Ullah, 1996).

Improved growth and increased Ca and P absorption has been reported in several studies of phytase inclusion in broilers (Huff et al., 1998; Sohail and Roland, 1999; Liu et al., 2008). In addition to improved performance, supplemental phytase is found to

facilitate bone development through the release of calcium and phosphorus in broilers, which can in turn reduce the incidence of fractures, which are high and costly for the heavy broiler industry (Angel et al., 2006).

The addition of phytase can also limit environmental phosphorus excretion (Bedford, 2000). Phytase inclusions can also result in less phosphorus excretion in litter, which is associated with eutrophication or excess mineralization of water. Eutrophication causes fresh and salt water ecosystem disruption algae overgrowth that depletes oxygen levels in the water fresh and salt water ecosystem disruption due to decreased oxygen levels resulting from algae overgrowth (Correll, 1998).

Amounts of supplemented phytase are measured using FTU/kg (phytase units); roughly 500 FTU/kg can be found in most commercial poultry diets and is known to improve broiler tibia ash, feed efficiency, body weights, phosphorus availability and absorption (Coweison et al., 2006). Further improvements have been documented with higher concentrations of phytase, which is referred to as super-dosing.

Walk and cohorts (2013) reported that supplementation of 500, 1,000 and 1,500 FTU/kg phytase in negative control diets resulted in weight gains similar to those fed positive control diets., but the highest dose (1500 FTU/kg) lead to better feed efficiency when compared to all other treatments (Walk et al., 2013). Greater degradation of phytate can also occur with super-dosing as reported by Shirley and Edwards (2003) in which phytase inclusions of 1,500, 3,000 and 6,000 and 12,000 FTU/kg resulted in phytate phosphorus loss of 65.2, 73.5, 84.9, and 94.8%, respectively. These growth improvements due to phytase super-dosing are thought to result from this further phytate dissemination, as well as calcium and phosphorus release (Cowieson, 2011).

Carbohydrase

As the name suggests, this enzyme class is primarily used for carbohydrate substrates to enhance the available energy of feed ingredients, especially aiding in the breakdown of non-starch polysaccharides (NSP). Because carbohydrates involve various polysaccharides and oligiosaccharides, there are numerous enzymes that can fall under the category of carbohydrase which includes: β-glucanases, xylanases, α-galactosidases, amylase, mannases, cellulases, and hemicellulases (Ravindran, 2013).

The inclusion of exogenous carbohydrase enzymes have been shown to alleviate some of the problems influenced by the presence of non-starch polysaccharides (Bedford and Classen, 1992). Research supports that the inclusion of carbohydrase enzymes can increase metabolizable energy for birds fed low energy wheat diets (Choct et al., 1991). These enzymes are helpful in a variety of diets, such as corn and soybean meal based, of which are considered very digestible (Zanella et al., 1999).

Xylanase

Xylanase enzymes are supplemented in broiler diets to act on xylans found in the major plant constituents of a poultry diet. The backbone of xylan can have several indigestible side groups, depending on plant type. Therefore, xylanase enzymes are normally classified depending on which of these is their substrate (Gilbert and Hazlewood, 1993; Kulkarni et al., 1999). This carbohydrase enzyme is produced by many microbial organisms, and it is usual for xylanase enzymes to exhibit several hydrolytic activities (Beg et al., 2001). Xylanases fed to poultry primarily function to reduce arabinoxylans, which are found within commonly used carbohydrates like wheat (Brillouet and Joseleau, 1987).

A large portion of carbohydrase research involves the use of xylanase, which has shown that the addition into poultry diets has a beneficial influence on broiler production in terms of gut viscosity reduction, litter quality improvements, improved performance, and increased gastrointestinal diversity (Choct et al., 1999; Choct, 2006; Engberg et al., 2004; Kiarie et al., 2014; Bedford and Schulze, 1998; Kalmendal, 2012).

Commercially Available Carbohydrases

Numerous commercially available carbohydrase enzymes are produced for poultry use. This thesis focuses on two of these: The first, is Superzyme™, is a multi-carbohydrase enzyme blend. According to the manufacturer, it is created through microbial fermentation, and is a mix of enzymes that possess multiple carbohydrase activities (Canadian Biosystems). The second, Hostazym® X, is produced by the Trichoderma strain of bacteria using surface fermentation (Huvepharma Inc.). It primarily has xylanase activity and is claimed to versatile with effectiveness in wheat, corn, and barley based diets (Huvepharma Inc).

Multi-carbohydrase/ Carbohydrase cocktails

Due to the large structural nature of most indigestible NSPs, the use of multiple carbohydrases may be able to more efficiently degrade these compounds, ultimately increasing the digestibility of various cereal grains (Ravindran, 2013). There are two ways multiple carbohydrases can be added into the feed. One is referred to as a "cocktail" and is a mixture of several different carbohydrase enzymes, each with one specific activity. The other is referred to as a multi-carbohydrase, meaning the enzyme has several different carbohydrase activities.

Meng and others (2005) demonstrated that the in vitro use of several carbohydrase enzymes were able to degrade wheat, soybean meal, canola meal, and pea NSP amounts when combined. Following this discovery, carbohydrase combinations were tested in vivo on broiler chicks. Results concluded that the addition of carbohydrase and combinations of them are effective in improving performance. The carbohydrase combinations resulted in improved FCR and starch digestibility when compared to those fed single carbohydrase enzymes (Meng et al., 2005). Similar results were seen with the use of two NSPase enzymes in reduced energy diets (Coppedge et al., 2012). As a result, FCR and breast weight was improved in low energy diets with the two NSPase inclusion (Coppedge et al., 2012).

The addition of xylanase, amylase, and protease has been shown to be effective on corn and soybean meal based diets. Amerah and cohorts (2017) supplemented these three enzymes in combination and individually into low energy diets. The combination carbohydrase supplementation resulted in birds with improved FCR comparable to the adequate energy control and lower than those birds fed diets with a singular enzyme (Amerah et al., 2017).

Carbohydrase and Phytase

Interactions between carbohydrase and phytase result in better broiler performance due to theorized synergistic or additive effects, allowing each to target substrates easier within NSP structures (Schramm et al., 2017). When used in combination, it is thought that the carbohydrase degrades the polysaccharides surrounding phytate phosphorus, allowing more exposure to the phytase enzyme (Selle et al., 2009).

The combination of xylanase and phytase on wheat-based diets is reported to enhance broiler feed efficiency, weight gain, lowered viscosity, and improved digestibility (Józefiak et al., 2010). Beneficial effects of xylanase and phytase supplementation are more exaggerated in diets made with wheat than those with corn; speculated to be due to the higher amounts of substrate or soluble NSP for xylanase to target (Kiarie et al., 2014).

However, carbohydrase's used in conjunction with phytases can also be helpful in diets consisting of corn and soybean meal, though these diets are considered more easily digestible. Some of these benefits include increased starch digestibility, increased body weights and feed efficiency, and AME increase has been observed in broilers consuming those diets which included phytase and a carbohydrase (Stefanello et al., 2016). Sparing effects on dietary AME, digestible amino acids, P, and Ca was reported by Francesch and Geraert (2009) when supplementing a multi-carbohydrase containing xylanase, β-glucanase, and phytase activity along with a 6-phytase in corn/soybean diets. Reports of no significant improvements with the combination in corn based diets have also been observed (Karimi et al., 2013; Juanpere et al., 2005). Gehring and others (2013) also reported that xylanase and phytase supplementation did not improve digestibility of protein in corn/soy diets and the authors speculated inconsistencies in corn diets to be a result of ingredient source.

Enzyme Matrix Values

Exogenous feed enzymes are added to diets in order to release certain bound nutrients for better bioavailability for the animal. Enzyme sparing effects on certain nutrients can provide potential diet cost savings by allowing diets to be formulated with

reduced nutrient specifications (Shelton et al., 2004). Nutrient value compensated by exogenous enzymes are expressed as a percent and these are referred to as matrix values (Kleyn, 2013).

Blood Biochemistry

As with humans, blood analyses can be performed with poultry to help understand and diagnose internal health. Parameters measured are linked with major organs and are used as indications of disease, stress, or toxicity. As stated in a rise in value of ALP is an indication of liver stress either from harmful substances or the inability of the liver to clear toxins (Basten, 2010). Below is a table of parameters and their significance to body. These are often measured in humans, as well as for chickens, as metabolic evidence of health.

Table 1.1 Blood Biochemistry Analysis Indications (adapted from Introduction to Clinical Biochemistry).

Parameter	***Organ Association***	***Non-Normal Numbers***
AST (Aspartate aminotransferase)	Liver	Liver injury, shock, hypoxia
ALT (Alanine aminotransferase)	Liver	Toxin, virus induced hepatitis
Albumin	Protein for metabolic functions	Metabolic disorder
ALP (Alkaline phosphatase)	Multiple organs	Blocked bile system
Sodium	Electrolytes	Water Intake
Potassium	Electrolytes	Cellular impulses or water intake
Urea	Cellular metabolism	Renal Issues
GGT (Gamma-glutamyl transferase)	Liver	Liver Damage

Conclusion

Due to the maximum and efficient growth achieved in broilers of the current time period, commercial poultry production is an instrumental facet of agriculture that is capable of supplying the world with necessary food now and in the year 2050. However,

the rise of bacterial resistance has led to the removal of routine antibiotic promoters (AGP) from poultry feed. These have been used for over half a century to elicit improved broiler growth and health. Therefore, in the midst of their removal, current nutrition research is heavily focused on finding alternatives, often using other feed additives.

An ideal feed additive AGP alternative should be able to modify the intestinal microflora or increase nutrient availability in the way that AGP do. Although very different from each other, short-chain fatty acids and exogenous enzymes are known to display those responses and ultimately improve intestinal characteristics, digestion, and ultimately performance. This thesis research will elaborate on their effects when fed to Ross x Ross 708 broilers in order to further understand these additives.

References

Adil, S., T. Banday, G. A. Bhat, M. S. Mir, and M. Rehman. 2010. Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. Vet. Med. Int.

Amerah, A. M., L. F. Romero, A. Awati, and V. Ravindran. 2017. Effect of exogenous xylanase, amylase, and protease as single or combined activities on nutrient digestibility and growth performance of broilers fed corn/soy diets. Poult. Sci. 96:807-816.

Angel, C. R., W. Saylor, S. L. Vieira, and N. Ward. 2011. Effects of a monocomponent protease on performance and protein utilization in 7- to 22-day-old broiler chickens. Poult Sci 90:2281-2286.

Angel, R., W. W. Saylor, A. D. Mitchell, W. Powers, and T. J. Applegate. 2006. Effect of dietary phosphorus, phytase, and 25-hydroxycholecalciferol on broiler chicken bone mineralization, litter phosphorus, and processing yields. Poult Sci 85:1200-1211.

Annison G. and Choct, M. 1991. Anti-nutritive activities of cereal non-starch polysaccharides in broiler diets and strategies minimizing their effects. World's Poultry Science Journal. 47:232-242.

Annison, G. 1993. The role of wheat non-starch polysaccharides in broiler nutrition. Crop and Pasture Science 44:405-422.

Ao, T., J. L. Pierce, R. Power, A. J. Pescatore, A. H. Cantor, K. A. Dawson, M. J. Ford. 2009. Effects of feeding different forms of zinc and copper on the performance and tissue mineral content of chicks. Poult. Sci.; 88: 2171-2175.

Basten, G. 2010. *Introduction to Clinical Biochemistry: Interpreting Blood Results*. Retrieved from http://web.mef.hr/web/images/pdf/i_clin_bio.pdf. Accessed May 2017.

Bedford, M. R. 2000. Exogenous enzymes in monogastric nutrition- their current value and future benefits. Anim. Feed Sci. Technol. 86:1-13.

Bedford, M. R. and Schulze, H.1998. Exogenous enzymes for pigs and poultry. Nutr. Res. Rev. 11:91-114.

Bedford, M. R.and Classen, H.L.1992. Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is effected through changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and food conversion efficiency of broiler chicks. J. Nutr. 122: 560-569.

Beg Q, Kapoor M, Mahajan L, Hoondal GS. Microbial xylanases and their industrial applications: a review. Appl. Microbiol. Biot. 2001. 56:326–338.

Begley, M., C. Hill, and C. G. M. Gahan. 2006. Bile salt hydrolase activity in probiotics. Appl. Environ. Microbiol.72: 1729-1738.

Berge, A. C., and M. Wierup. 2012. Nutritional strategies to combat Salmonella in mono-gastric food animals. Animal. 6:557-564

Berkes, J., V. K. Viswanathan, S. D. Savkovic, and G. Hecht. 2003. Intestinal epithelial responses to enteric pathogens: effects on the tight junction barrier, ion transport, and inflammation. Gut 52: 439-451.

Brillouet, J.M., and Joseleau, J.P. 1987. Investigation of the structure of a heteroxylan from the outer pericarp (beeswing bran) of wheat kernel. Carbohydr. Res.159:109.

Bun, S. D., Y. M. Guo, F. C. Guo, F. J. Ji, and H. Cao. 2011. Influence of organic zinc supplementation on the antioxidant status and immune responses of broilers challenged with Eimeria tenella. Poult Sci 90:1220-1226.

Canadian Bio-Systems, Inc. Calgary, Alberta, Canada

Cao, J., P.R. Henry, R. Guo, R. A. Holwerda, J. P. Toth, R. C., Littell, R. D., Miles, and C. B. Ammerman. 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. J. Anim. Sci. 78:2039-54.

Castanon, J. I R. 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poult. Sci. 86:2466-2471.

Cave, N. A. G. 1984. Effect of dietary propionic and lactic acids on feed intake by chicks. Poult. Sci. 63:131-134.

Chaveerach, P., Keuzenkamp, D. A., Urlings, H. A., Lipman, L. J., and Knapen, J. 2002. In vitro study on the effect of organic acids on Campylobacter jejuni/coli populations in mixtures of water and feed. Poult Sci 2002. 81:621-628.

Cherrington, C. A., M. Hinton, G. C. Mead, and I. Chopra. 2008. Organic acids: Chemistry, antibacterial activity and practical applications. Adv. Microbiol. Pys. 32:87-108.

Chiba, L. I. 2014. Animal Nutrition Handbook. Third Revision.

Choct M., R. J. Hughes, and M. R. Bedford. 1999. Effects of a xylanase on individual bird variation, starch digestion throughout the intestine, and ileal and caecal volatile fatty acid production in chickens fed wheat. 40:419-422.

Choct, M., 2005. Role of biotechnology in utilisation of alternative feed ingredients for monogastric animals. Nutritional biotechnology in the feed and food industries. Proceedings of Alltech's 21st Annual Symposium, Lexington, Kentucky, USA, 22-25 May 2005: 9-13.

Choct, M., 2006. Enzymes for the feed industry: past, present and future. World Poult. Sci. J. 62: 5-16.

Choct, M., and G. Annison. 1990. Anti-nutritive activity of wheat pentosans in broiler diets. Br. Poult. Sci. 31:811-821.

Coates, M. E., R. Fuller, G. F. Harrison, M. Lev, and S. F. Suffolk. 1963. Comparison of the growth of chicks in the Gustafsson germ-free apparatus and in a conventional environment, with and without dietary supplements of penicillin. Br. J. Nutr. 17:141–151.

Coppedge, J. R., L. A. Oden, B. Ratliff, B. Brown, F. Ruch, and J. T. Lee. 2012. Evaluation of nonstarch polysaccharide-degrading enzymes in broiler diets varying in nutrient and energy levels as measured by broiler performance and processing parameters. J Appl Poult Res 21:226-234.

Correll, D. 1998. The role of phosphorus in the eutrophication of receiving waters: A review. J. Environ. Qual. 27: 261-266.

Cowieson, A. J., M. Hruby, E. E. M. Pierson. 2006. Evolving enzyme technology: impact on commercial poultry nutrition. Nut. Res. Rev.19: 90-103.

Cowieson, A. J., P. Wilcock, and M. R. Bedford. 2011. Super-dosing effects of phytase in poultry and other monogastrics. World Poultry Sci. J. 67:225-235.

Dalmasso, G., H. T. Nguyen, Y.Yan, L. Charrier-hisamuddin, S. V. Sitaraman, and D. Merlin. 2008. Butyrate transcriptionally enhances peptide transporter PepT1 expression and activity. PLoS ONE 3: e2476.

Davidson P., T. Taylor, and S. Schmidt. 2013. Chemical Preservatives and Natural Antimicrobial Compounds, p 765-801.In Doyle M, Buchanan R (ed), Food Microbiology. ASM Press, Washington, DC.

Delgado C. L. 2003. Rising consumption of meat and milk in developing countries has created a new food revolution. J Nutr. 133:3907S–10S.

Dibner, J. J., and J. D. Richards. 2005. Antibiotic growth promoters in agriculture: History and mode of action. Poult. Sci. 84: 634-643.

Dida, M. F. 2016. Review Paper on enzyme supplementation in poultry ration. International J. of Bioorganic Chemistry. Vol. 1:1-7.

Donoghue, D. J. 2003. Antibiotic residues in poultry tissues and eggs: human health concerns? Poult. Sci. 82:618-621.

Endtz, H. G. J. Ruijs, B. Klingeren, W. H. Jansen, T. van der Reyden, R. P. Mouton 1991. Quinolone resistance in campylobacter isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. J Antimicrob Chemother. 27:199-208.

Engberg, R. M., M. S. Hedemann, S. Steenfeldt, and B. B. Jensen. 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poult. Sci. 83:925-938.

Engelhardt W., K. Ronnau, G. Rechkemmer, and T. Sakata. 1989. Absorption of short-chain fatty acids and their role in the hindgut of monogastric animals. Animal Feed Sci. and Tech. 23:45-53.

Flachowsky, G. 2002. Efficiency of energy and nutrient use in the production of edible protein of animal origin. J. Appl. Anim. Res. 22:1-24.

Flint, J. F., M. R. Garner. 2009. Feeding beneficial bacteria: A natural solution for increasing efficiency and decreasing pathogens in animal agriculture. J. Appl. Poult. Res. 18:367-378.

Foreign Agricultural Service/USDA. October 2016. Livestock and Poultry: World Markets and Trade. Accessed March 2016. Retrieved from https://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf

Francesch, M. and P. A. Geraert. 2009. Enzyme complex containing carbohydrases and phytase improves growth performance and bone mineralization of broilers fed reduced nutrient corn-soybean-based diets. Poult. Sci. 88:1915-1924.

Gehring, C. K., M. R. Bedford, and W. A. Dozier, III. 2013. Extra-phosphoric effects of phytase with and without xylanase in corn-soybean meal-based diets fed to broilers. Poult Sci. 92:979-991.

Gerland, P., A. E. Raftery, H. Ševcíková, N. Li, D. Gu, T. Spoorenberg, L. Alkeman, B. K. Fosdick, J. Chunn, N. Lalic, G. Bay, T. Buettner, G. K. Heilig, and J. Wilmoth, 2014. World Population Stabilization Unlikely This Century. Science. 346:234–237.

Gilbert, C., and G. Cooney. “Thermostability of Feed Enzymes and Their Practical Application in the Feed Mill." Enzymes in Farm Animal Nutrition. Ed. M. Bedford and G. Patridge. N.p.: CABI, 2010.249-59. Print.

Gilbert H.J., and G. P. Hazlewood. 1993.Bacterial cellulases and xylanases. J. Gen. Microbiol. 139: 187–194.

Graminha, E. B. N., A. Z. L. Gonclaves, R. D. R. B. Pirota, M. A. A. Balsalobre, R. Da Silva, and E. Gomes. 2008. Enzyme production by solid-state fermentation: Application to animal nutrition. Anim. Feed Sci. Technol., 144:1-22.

Haefner, S., A. Knietsch, E. Scholten, E. et al. Appl Microbiol Biotechnol. 2005. 68:588.

Hafeez A. K. Manner,C. Schieder, and J. Zentek. 2016. Effect of supplementation of phytogenic feed additives (powdered vs.encapsulated) on performance and nutrient digestibility in broiler chickens. Poult. Sci. 95:622-629.

Hashemi, S. R., and H. Davoodi. 2010. Phytogenics as new class of feed additive in poultry industry. Journal of Animal and Veterinary Advances. 9:2295-2304.

Havenstein, G., P. Ferket, and M. Qureshi. 2003. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1500-1508.

Hetland H., Svihus B., Olaisen V. 2002. Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broiler chickens, Br. Poult. Sci. 43:416-423

Hinton, M. and Linton, A. H. 1988. Control of salmonella infections in broiler chickens by the acid treatment of their feed. Vet Rec. 123: 416-421.

Holst, W.F. 1926. “Artificial” Enzymes and Poultry feeding. Poult Sci. 5:261-265.

Hong, Y. H., Y. Nishimura, D. Hishikawa, H. Tsuzuki, H. Miyahara, C. Gotoh, K. Choi, D. Feng, C Chen, H. G. Lee, K. Katoh, S. G. Roh, and S. Sasaki. 2005. acetate and propionate short chain fatty acids stimulate adipogenesis via GPCR43. Endocrinology. 146:5092-5099.

Hood, K., A. Myles, D. Peebles, and D. Thornton. 2012. The Poultry Industry and Its Economic Impact. Mississippi State University Extension Service. Accessed July 2016. Retrieved from http://msucares.com/pubs/publications/p2719.pdf.

Huff, W. E., P. A. Moore, Jr., P. W. Waldroup,A. L. Waldroup, J. M. Balog, G. R. Huff, N. C. Rath,T. C. Daniel,and V. Raboy. 1998. Effect of dietary phytase and high available phosphorus corn on broiler chicken performance. 1998. Poult. Sci. 77:1899-1904.

Huvepharma, Inc., Peachtree City, GA

Iba, A. M., and Berchieri Jr., A. 1995. Studies on the use of a formic acid-propionic acid mixture (Bio-add™) to control experimental Salmonella infection in broiler chickens. Avian Pathology. 24:303-311.

Izat, L., M. Colberg, M. A. Reiber, M. H. Adams, J. T. Skinner, M. C. Cabel, H. L. Stilborn, P. W. Waldroup; Effects of Different Antibiotics on Performance, Processing Characteristics, and Parts Yield of Broiler Chickens. Poult Sci 1990b. 69:1787-1791.

Izat, L., Tidwell, N.M., Thomas, R.A., Reiber, M.A., Adams, M.H.,Colberg, M. & Waldroup, P.W. 1990a. Effects of a buffered propionic acid in diets on the perfrormance of broiler chickens and on microflora in the intestine and carcass. Poult.Sci. 69:818-826.

Jacobs, R. L., J.F. Elam, G. W. Anderson, L.L. Gee, J. Fowler and J.R. Couch. 1953. Further evidence as to the possible mechanism involved in the growth-promoting responses obtained from antibiotics. J. Nutr. 51:507-513.

Jerzsele A., K. Szeker ,R. Csizinszky , E. Gere , C. Jakab ,J. J. Mallo ,and P. Galfi. 2012. Efficacy of protected sodium butyrate, a protected blend of essential oils,their combination, and Bacillus amyloliquefaciens spore suspension against artificially induced necrotic enteritis in broilers. Poult. Sci. 91:837-843.

Jones, F. T., and S. C. Ricke. Observations on the history of the development of antimicrobials and their use in poultry feeds. 2003. Poult. Sci. 82:613-617.

Józefiak, D. A. Ptak, S. Kaczmarek, P. Mackowiak, M. Sassek, B. A. Slominski. 2010. Multi-carbohydrase and phytase supplementation improves growth performance and liver insulin receptor sensitivity in broiler chickens fed diets containing full-fat rapeseed. Poult. Sci. 89: 1939-1946.

Juanpere, A., M. Pe´rez-Vendrell, E. Angulo, and J. Brufau. 2005. IRTA – Animal Nutrition, Apartat 415 Reus Tarragona 43280, Spain. Poult. Sci. 84:571–580R.

Kalmendal, R. Tauson. 2012. Effects of a xylanase and protease, individually or in combination, and an ionophore coccidiostat on performance, nutrient utilization, and intestinal morphology in broiler chickens fed a wheat-soybean meal-based diet. Poult Sci. 91:1387-1393.

Karimi, A., Y. Min, C. Lu, C. Coto, M. R. Bedford, P. W. Waldroup. 2013. Assessment of potential enhancing effects of a carbohydrase mixture on phytase efficacy in male broiler chicks fed phosphorus-deficient diets from 1 to 18 days of age. Poult Sci 92:192-198.

Kemin Industries, Inc., Des Moines, Iowa, USA.

Khan, S. H. and Iqbal, J. 2016. Recent advances in the role of organic acids in poultry nutrition. J. of Appl. Animal Res. 44: 359-369.

Khattak, F. M., T. N. Pasha, Z. Hayat and A. Mahmud 2006. Enzymes in poultry nutrition. J. Anim. Pl. Sci. 16:1-2.

Kiarie, E., L. F. Romero, and V. Ravindran 2014. Growth performance, nutrient utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or with supplemental xylanase. Poult Sci. 93:1186-1196.

Kleyn, Rick. 2013. Enzymes. Chicken Nutrition: A guide for nutritionists and poultry professionals. Context Publications. 251-72. Print.

Knudsen, K. 2014. Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. Poult. Sci. 93:2380-2393.

Kulkarni, N., A. Shendye, and M. Rao.1999. Molecular and biotechnological aspects of xylanases. FEMS Microbiol. Rev. 23:411-456.

Kwon, Y. M. and Ricke, S. C. 1998. Induction of acid resistance of Salmonella typhimurium by exposure to short-chain fatty acids. Appl. Environ. Microbiol. 64:6458-3463.

Levy A. W., J. W. Kessler, L. Fuller, S. Williams, G. F. Mathis, B. Lumpkins, and F. Valdez. 2015. Effect of feeding an encapsulated source of butyric acid (ButiPEARL) on the performance of male Cobb broilers reared to 42 d of age. Poult. Sci. 94:1864-1870.

Lin, J. 2014. Antibiotic growth promoters enhance animal production by targeting intestinal bile salt hydrolase and its producers. Frontiers in Microbiology. 5:1-4.

Liu, N., Y. J. Ru, A. J. Cowieson, F. D. Li, X. C. H. Cheng. 2008. Effects of Phytate and Phytase on the Performance and Immune Function of Broilers Fed Nutritionally Marginal Diets. Poult. Sci. 87:1105-1111.

M. Lorenzi, S. Mudalal, C. Cavani, M. Petracci; Incidence of white striping under commercial conditions in medium and heavy broiler chickens in Italy. J Appl Poult Res 2014. 23: 754-758.

M. Shepherd, B. D. Fairchild. 2010. Footpad dermatitis in poultry. Poult. Sci. 89:2043-2051.

Makras, L. and L. De Vuyst. 2006. The in vitro inhibition of Gram-negative pathogenic bacteria by bifidobacteria is caused by the production of organic acids. Int. Dairy J. 16:1049-1057.

March, B. E., R. Soong, and C. MacMillan. 1978 Growth rate, feed conversion, and dietary metabolizable energy in response to virginiamycin supplementation of different diets. Poult. Sci. 57:1346-1350.

Meng X, and Slominski, B. A., 2005. Nutritive values of corn, soybean meal, canola meal, and peas for broiler chickens as affected by a multicarbohydrase preparation of cell wall degrading enzymes. Poult. Sci. 84: 1242-1251.

Miles, R. D., G. D. Butcher, P. R. Henry, and R. C. Littell. 2006. Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quantitative morphology. Poult. Sci. 85:476-485.

Mississippi Department of Agriculture and Commerce. 2014 Annual report. Accessed June 2016. www.mdac.ms.gov/wp-content/uploads/mdac_annualrpt.pdf.

Miyoshi, Y., S. Tanabe, and T. Suzuki. 2016. Cellular zinc is required for intestinal epithelial barrier maintenance via the regulation of claudin-3 and occludin expression. Am J Physiol Gastrointest Liver Physiol. 311:105-116.

Moore, P. R., A. Evenson, T. D. Luckey, E. McCoy, C. A. Elvehjem, and E. B. Hart. 1946. Use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with the chick. J. Biol. Chem. 165:437-441.

Nelson, T. S., 1967. The utilization of phytate phosphorus by poultry—a review. Poult. Sci. 46: 862-871.

Niewold, T. A. 2007. The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. Poult. Sci. 86:605-609.

NRC. 1994. Poultry. Nutrient Requirements of 9th rev. ed. Natl. Acad. Press, Washington, DC.

Osman A. M. 1982. Amylase in chicken intestine and pancreas, Comp. Biochem. Physiol. B. 73:571-574.

Pallauf, J., & Rimbach, G. 1997. Nutritional significance of phytic acid and phytase.Archives of animal nutrition. 50:301.

Panda, A.K., S. V. Rama Rao, M. V. L. N. Raju, and G. Shyam Sunder. 2009. Effect of butyric acid on performance, gastrointestinal tract health and carcass characteristics in broiler chickens. Asian-Aust, J. Anim. Sci. 22:1026-1031.

Ravindran, V. Poultry feed availability and nutrition in developing countries-Feed supplements and additives. Poultry Development Review. Retrieved from http://www.fao.org/3/a-al704e.pdf. Accessed May 2017.

Ravindran, V., W. L. Bryden, and E. T. Kornegay. 1995. Phytates: occurance, bioavailability and implications in poultry nutrition. Poult. And Avian Biol. Rev. 6:125-143.

Ricke , S. C. 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poult. Sci. 82:632–639.

Rodriguez, P., Darmon, N., Chappuis, P., Candalh, C., Blaton, M.A., Bouchaud, C. and Heyman, M. 1996. Intestinal paracellular permeability during malnutrition in guinea pigs: effect of high dietary zinc. Gut, 39:416–422.

Samanta S., S. Haldar, and T. K. Ghosh. 2010. Comparative efficacy of an organic acid blend and bacitracin methylene disalicylate as growth promoters in broiler chickens:effects on performance, gut histology, and small intestinal milieu. Vet Med Int. 2010:1-8.

Scheppach, W. 1994. Effects of short chain fatty acids on gut morphology and function. Gut 35:35-38.

Schramm, V. G., J. F. Durau, L. N. E. Barrilli, J. O. B. Sorbara, A. J. Cowieson, A. P. Félix, and A. Maiorka. 2017. Interaction between xylanase and phytase on the digestibility of corn and a corn/soy diet for broiler chickens. Poult Sci 96:1204-1211.

Sengupta S., J. Muir, and P. R. Gibson. 2006. Does butyrate protect from colorectal cancer? Journal of Gastroenterology and Hepatology. 21:209–218.

Shelton, J. L., L. L. Southern, L. A. Gaston, and A. Foster. 2004. Evaluation of the nutrient matrix values for phytase in broilers. J. Appl. Poult. Res. 13:213-221.

Shirley, R. B. and Edwards, H. M. Jr. 2003. Graded levels of phyase past industry standards improves broiler performance. Poult. Sci. 82:671-680.

Silva P., L. Fries, C. Menezes, A. Holkem, C. Schwan, E. Wigmann, J. Bastos, C. Silva. 2014. Microencapsulation: concepts, mechanisms, methods and some applications in food technology. Ciência Rural, Santa Maria.44:1304-1311.

Silversides, F.G., Bedford, M.R., 1999. Effect of pelleting temperature on the recovery and efficacy of a xylanase enzyme in wheat-based diets. Poult. Sci. 78:1184–1190

Sohail, S. S. and D. A. Roland. 1999. Influence of supplemental phytase on performance of broilers four to six weeks of age. Poult. Sci. 78:550-555.

Spellburg, B., R. Guidos, D. Gilbert, J. Bradley, H. W. Boucher, W. M. Scheld, J. G. Bartlett, J. Edwards, Jr. the Infectious Diseases Society of America. 2009. The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. Clin Infect Dis. 46:155-64.

Sprong, R.C., Hulstein, M.F.E. and Van der Meer, R. 2001. Bactericidal activities of milk lipids. Antimicrobial Agents and Chemotherapy. 45:1298-1301.

Stefanello, C., S.L. Vieira, P. S. Carvalho, J. O. B. Sorbara, and A. J. Cowieson. 2016. Energy and nutrient utilization of broiler chickens fed corn-soybean meal and corn-based diets supplemented with xylanase. Poult. Sci. 95: 1881-1887.

Svihus, B. and M. Gullord. 2002. Effect of chemical content and physical characteristics on nutritional value of wheat, barley and oats for poultry. Animal Feed Sci. and Tech. 102:71 – 92.

The Biochemistry of Plants Vol 14: Carbohydrates. Structure of starch molecules and granules. CH5. Page 141.

Thomas D., V. Ravindran, and G. Ravindran. 2008. Nutrient digestibility and energy utilisation of diets based on wheat, sorghum or maize by the newly hatched broiler chick, Br. Poult. Sci. 49:429-435.

USDA. Poultry- Production and Value. 2015 Summary (April 2016). Accessed July 2016. Retrieved from http://usda.mannlib.cornell.edu/usda/current/PoulProdVa/PoulProdVa-04-28-2016.pdf.

V. Ravindran. 2013. Feed enzymes: The science, practice, and metabolic realities. J Appl Poult. Res. 22:628-636.

Vallee, B.L. and Falchuk, K.H. 1993. The biochemical basis of zinc physiology. Physiological Reviews. 73:79–118.

Van der Wielen, P. W. J. J., Biesterveld, S., Notermans, S., Hofstra, H., Urlings, B. A. P., and van Knapen, F. 2000. Role of Volatile Fatty Acids in Development of the Cecal Microflora in Broiler Chickens during Growth. Applied and Environmental Microbiology. 66:2536–2540.

Van Immerseel, F., De Buck, J., Boyen, F., Bohez, L., Pasmans, F., Volf, J., Sevcik, M., Rychlik, I., Haesebrouck, F. & Ducatelle, R. 2004. Medium-chain fatty acids decrease colonization and invasion shortly after infection with Salmonella Enteritidis in chickens through hilA suppression. Appl. and Environ. Microbiol. 70:3582-3587.

Van Immerseel, F., J. De Buck, G. Meulemans, F. Pasmans, P. Velge, E. Bottreau, F. Haesebrouck and R. Ducatelle. 2003. Invasion of Salmonella Enteritidis in avian intestinal epithelial cells in vitro is influenced by short-chain fatty acids. International Journal of Food Microbiology. 85:237-248.

Veterinary Feed Directive. Federal Register. Food and Drug Administration. 03 June 2015. Web. 24 May 2017.

Walk, C. L., M. R. Bedford, T. S. Santos, D. Paiva, J. R. Bradley, H. Wladecki, C. Honaker, and A. P. McElroy. 2013. Extra-phosphoric effects of superdoses of a novel microbial phytase. Poult. Sci. 92:719-725.

Wedekind, K.J. and D.H. Baker, 1990. Zinc bioavailability in feed-grade sources of zinc. J. Anim. Sci., 68:684-689.

Wodzinski, R. J., and A. H. J. Ullah. 1996. Phytase. Advances in Applied Microbiology. 42:263-302.

Wyss, M., R. Brugger, A. Kronenberger, R. Rémy, R. Fimbel, G. Oesterhelt, M. Lehmann, and A. P. G. M. van Loon. 1999. Biochemical characterization of fungal phytases (*myo*-inositol hexakisphosphate phosphohydrolases): Catalytic properties. Appl. Environ. Microbiol. 65:367-373.

Yuka M., S. Tanabe, and T. Suzuki. 2016. Cellular zinc is required for intestinal epithelial barrier maintenance via the regulation of claudin-3 and occludin expression. American Journal of Physiology - Gastrointestinal and Liver Physiology. 311:105-116.

Zanella, I., N.K. Sakomura, F. G. Silversides, A. Fiqueirdo, and M. Pack. 1999. Effect of enzyme supplementation of broiler diets based on corn and soybeans. Poult. Sci. 78:561-568.

Zhang B, Y. Shao, D. Liu , P. Yin , Y. Guo and J. Yuan. 2012. Zinc prevents Salmonella enterica serovar Typhimurium-induced loss of intestinal mucosal barrier of function in broiler chickens. Avian Pathology. 41:361-367.

CHAPTER II

EFFECTS OF AN ENCAPSULATED BUTYRIC ACID SOURCE WITH ZINC SUPPLEMENTATION ON BROILER PERFORMANCE, BLOOD CHEMISTRY AND CECAL FATTY-ACID ANALYSIS

Summary

Feed additives, such as butyric acid and zinc oxide, are considered beneficial to poultry production because of their gut health improving properties. Butyric acid, a short-chain fatty acid, and zinc oxide, an essential micronutrient, both have intestinal epithelial growth promoting characteristics. As such, inclusion of each into poultry diets has shown to improve broiler performance. However, research regarding effects of high inclusion of their combination is limited. This study was conducted to examine the effects of an encapsulated butyric acid source + zinc (EBAZ), ButiPEARL™ Z, at 3 inclusions on Ross x Ross 708 male and female broiler performance, blood chemistry, and cecal short-chain fatty acid content. The current study utilized a 2 (sex) x 3 (EBAZ inclusion; 0 g/kg, 0.5 g/kg, or 2.5 g/kg EBAZ) factorial arrangement of treatments with 8 replicates per treatment. Broilers were weighed as a pen on d0, 21, 35, and 49. On d49, 3 birds/pen were used for blood and cecal content collection; samples for both were pooled by pen. While d0-21 BWG per bird (P=0.013) and percent mortality (P=0.008) were significantly increased by the main effect of EBAZ inclusion at the 5X recommended dosage (2.5 g/kg), the recommended level (0.5 g/kg) resulted in similar results to the control (0 g/kg

EBAZ inclusion). In addition, d 0- 35 and d 0-49 percent mortality (P=0.0234; P=0.0147) was significantly highest with 5X EBAZ inclusion (2.5 g/kg) and lowest for the recommended level (0.5 g/kg), while the control (0 g/kg) exhibited intermediate level. No other measured variables were significant for EBAZ inclusion (P>0.05). For d 35-49 and d 0-49 feed intake per bird (P=0.0064; P=0.0060) was significantly greater at the 5X level for males consuming 2.5g/kg EBAZ than all other treatments. Day 49 glucose (P=0.02), sodium (P=0.05), phosphorus (P=0.009), and potassium (P=0.009) levels in blood serum increased as the level of inclusion of EBAZ increased in the diet, thought to arise from increased nutrient absorption and electrolyte transport. Inclusion of EBAZ significantly impacted ALP levels (P=0.004) with birds fed 2.5 g/kg EBAZ inclusion having the highest levels. Although, ALP is used to measure liver function, it is unlikely that EBAZ supplementation is the cause due to the bird performance observed. Although no added performance benefit was found in this study, these data suggest that EBAZ can be safely included at the recommended level of 0.5 g/kg and at 2.5 g/kg into diets for Ross × Ross 708 male and female broilers.

Keywords: Butyric acid, organic acids, broiler performance, blood chemistry, short-chain fatty acid analysis

Introduction

Short-chain fatty acids (SCFA) have been considered to maximize bird health and performance (Ricke, 2003). Butyric acid is a SCFA that can be found in numerous biological organisms and is known to be present in the lower gastrointestinal tract due to bacterial fermentation (Guilloteau et al., 2010; Pryde et al., 2002). Its presence in this location promotes intestinal health in monogastric animals by increasing villi length, as

well as supporting epithelial cell production, which ultimately leads to improved growth performance and more efficient absorption of nutrients even under challenging conditions (Kotunia et al., 2004;Leeson et al., 2005; Edmonds et al., 2016). Previous research has demonstrated that butyric acid also has bactericidal properties due to its ability to cross the membranes of pH specific pathogens and dissociate inside (Adil et al., 2011). When testing this characteristic, results find that it does reduce commonly found poultry pathogens, such as Salmonella typhimurium, Clostridium perfringens, and Salmonella enteritidis (Namkung et al., 2011; Van Immerseel et al., 2005).

Research on high inclusion levels of butyric acid effects on the health, physiological, and performance of broilers is relatively limited. To the author's knowledge, the highest reported experimental inclusion for broilers was 6 g/kg as investigated by Panda and others (2009), though the broiler strain utilized in this study was not stated. Also, gaps in the literature exist for research involving female broilers, as most research utilizes male broilers exclusively. It is important to also examine female response to potential feed additives considered for commercial use, as both sexes are used in broiler production.

Zinc has also been shown to provide favorable results when used for poultry. Both zinc oxide and zinc sulfate are used in commercial diets for inorganic zinc supplementation (Park et al., 2004). Reports have been made of the beneficial effects of zinc supplementation such as improved performance and increased intestinal strength and characteristics (Sahin et al., 2005; Hu et al., 2013). Though zinc function is complex and involved with numerous biological processes, its performance benefits have been linked to microbial population control in the hindgut (Højberg et al., 2005).

The feed additive tested in the current study contains both butyric acid and zinc oxide. This combination is thought to further promote healthy gastrointestinal tracts in broilers, which would lead to better growth performance. Although the effects of butyric acid supplementation on broiler health and performance has been researched, as Leeson and others (2005) states, research is deficient on its potential additive effects when given in higher inclusions. Even further, research on the combination of both butyric acid and zinc oxide and its inclusions are insufficient. Therefore, the objective of the current study was to investigate the effects of 0, 0.5, and 2.5 g/kg inclusions of an encapsulated butyric acid and zinc oxide product (EBAZ) on Ross x Ross 708 male and female broilers.

Materials and methods

Experimental Diet Preparation

Practical corn and soybean meal based diets with the inclusions of corn dried distiller's grains with solubles, and meat and bone meal were formulated to meet or exceed NRC recommendations (NRC, 1994) for each dietary phase (Table 1). Bacitracin methylene disalicylate (BMD; Zoetis, Parsippany, NJ) was included at 0.5 g/kg, as well as salinomycin (BioCox; Zoetis, Parsippany, NJ) included at 0.34 g/kg the starter and grower diets. One common basal mash diet, sans EBAZ inclusion, was prepared at the Mississippi State University Poultry Research Unit feed mill for the starter, grower, and finisher diets. Each basal batch was mixed in a vertical screw mixer (0.907 tonne, Jacobson) for 5 minutes dry, and 10 minutes following the addition of poultry fat.

The inclusion level of EBAZ was the only variant between experimental diets; these Treatments (trt) were created at the U.S Department of Agriculture (Poultry Research Unit, Starkville, MS), by replacing 2.5 g/kg of the basal diet with ground corn

or EBAZ, depending on treatment. The treatment structure was as follows: Trt 1 & 4 (2.5 g/kg ground corn), Trt 2 & 5 (0.5 g/kg EBAZ + 2.0 g/kg ground corn) and Trt 3 & 6 (2.5 g/kg EBAZ) and mixed with 99.75% of the basal diet. The EBAZ used contains approximately 200g of butyric acid and 100 g of zinc oxide in 1 kg of product. For each trt, EBAZ and/or corn was mixed ~11 kg of basal diet for 5 minutes in a small (11.34 kg capacity) horizontal mixer before added to the allotted basal diet. Next, diets were mixed for 4 minutes in a horizontal ribbon mixer (907 kg capacity) to ensure a homogenous mixture prior to pelleting.

All diets were pelleted using a 40 HP CPM with a 38.1× 4.76 mm pellet die. Duplicate samples were collected after cooling for each treatment throughout the run in order to form a representative sample for each treatment. The diets were offered over three dietary phases: starter (d 0-21), grower (d 21-35), and finisher (d 35-49). The starter diet was fed as crumbles, with pellets being introduced at d 21 and fed for the remainder of the study.

Bird Management

This study was conducted in agreement with the Mississippi State University Institutional Animal Care and Use Committee (IACUC #15-099). Ross x Ross 708 broiler chicks were obtained from a commercial hatchery (PECO, Gordo, AL) and sorted by sex on d 0. Fifteen male or female broilers were placed into each of 48 pens (24 pens of males + 24 pens of females) measuring 0.91 x 1.2 m with an area of 0.12m^2 per bird. Used litter (obtained from MSU commercial poultry houses, ~10 years old, 40 flocks) and a top covering of fresh pine shavings provided a 3 inch bedding for each pen. On d 0, the house temperature was set at 32°C then reduced ~4 °C every week, ultimately ending with an environment of 16°C on d 47. The lighting program was as follows: 24 hours of

light at 21.53 lux from d 1-8, 16 hours of light at 8 lux from d 9-18, 18 hours at 1 lux from d 19-32, and 20 hours of light at an intensity of 0.54 lux from d 33-49.

Evaporative cool cells, negative air pressure, 2 tunnel fans (121.92 cm), 2 stir fans (60.96 cm), and propane fueled forced air heating units (LB White upright heaters) were used to create ideal environmental conditions throughout the study. Water and feed were provided ad libitum, using a common drinker line (3 nipples/pen) and one tube-type feeder per pen, respectively. One feeder tray per pen functioned to provide feed for chicks during the first 7 days. On d 0, 21, 35, and 49 birds were weighed by pen and each feeder weight was recorded. Feed intake/bird (FI), body weight gain/bird (BWG), ending BW, FCR, and percent mortality were determined at the conclusion of each feeding phase. Necropsies were performed to investigate the cause of every mortality that occurred during the experimental period. Observations regarding animal health, general housing conditions, and activities performed inside the house were recorded multiple times daily.

Blood Serum and Cecal Content Collection

After pen weights were recorded on d 49, blood and cecal contents were collected from 3 randomly selected birds per pen (144 total). Disposable 5 mL syringes and needles were used to collect ~3 mL of blood from the brachial vein. Blood was then transferred to red top tubes (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ) labeled by pen number. Following blood collection, cervical dislocation was used to euthanize the birds in preparation for cecal content collection. Contents of the ceca were collected into 50 mL polypropylene conical tubes (Falcon tubes) labeled by pen number.

After allowing the collected blood to clot for approximately 2 hours, blood samples were centrifuged (Beckman Coulter, Inc. model J-6B) at 2°C and 1,900 × g for 10 min in order to obtain serum. Serum collected was pooled by pen to obtain 48 samples. Samples were stored in a – 20 °C freezer and were sent to a laboratory (Poultry Research and Diagnostic Laboratory, Pearl MS) for blood chemistry analysis (ACE Alera® Clinical Chemistry System, Alfa Wassermann, West Caldwell, NJ). The 18 parameters analyzed included: glucose, BUN, creatinine, sodium, potassium, chloride, calcium, phosphorus, total protein, albumin, AST, ALT, ALP, cholesterol, CK, amylase, magnesium, and GGT. Contents of the ceca were also pooled by pen to create 48 total samples. These were stored in a -20° C freezer, and were sent to a laboratory (Rumen Fermentation Laboratory, West Virginia University) for SCFA percentage analysis using gas chromatography.

Statistical Analysis

All variables measured were analyzed as a 2 sex (male or female) x 3 EBAZ inclusion (0 g/kg, 0.5 g/kg, or 2.5 g/kg EBAZ) factorial arrangement within a randomized complete block design. Blocking was performed using location within the house and each treatment was replicated eight times. The experimental unit was one pen. Data was analyzed using the GLM procedure (SAS, 2014). Significant differences between treatments were determined at $P \leq 0.05$.

Results

Live Performance

Sex × EBAZ interactions

Significant Sex × EBAZ inclusion interactions were observed for FI/bird during the d 35-49 (P=0.0064) and d 0-49 (P=0.006) feeding phases (Table 6; Table 7). From d 35-49, males given diets including 2.5 g/kg EBAZ consumed the highest amount of feed. Additionally, females receiving diets with 0 g/kg, 0.5 g/kg, and 2.5 g/kg EBAZ exhibited a similar feed intake to males receiving diets of 0 and 0.5 g/kg EBAZ. During d 0-49, males fed diets of 2.5 g/kg EBAZ consumed the highest amount, while females given diets including 0 g/kg and 2.5 g/kg EBAZ exhibited the lowest feed intake; when both sexes were given diets including 0.5 g/kg EBAZ, an intermediate amount of feed was consumed (Table 7). No significant Sex × EBAZ interactions were established for BWG, ending BW, FCR, or percent mortality during d 0-21, d 21-35, d 0-35, d 35-49, and d 0-49 (P>0.05).

Sex effects

The main effect of sex was found to be significant for FI, BWG, and ending BW during d 0-21, d 21-35, d 0-35, d 35-49, and d 0-49 (P<0.05; Tables 3-7, respectively). As expected, male broilers displayed higher consumption and increased BWG, BW as compared to females (Ross 708 Broiler Performance Objectives, 2014). Sex also had a significant effect on FCR with lower FCR exhibited for male birds as compared to females for d 0-49 (P=0.0097). However, female FCR was significantly lower by 6 points in d0-35, as compared to males (P=0.0004; Table 3).

EBAZ effects

The main effect of EBAZ inclusion was significant for BWG during the first 21 days (P=0.0129; Table 3). Birds fed diets containing EBAZ at 2.5 g/kg had lower BWG as compared to those fed EBAZ at 0 g/kg and 0.5 g/kg. While EBAZ inclusion did not influence d 0-21 FI, during d 35-49 data demonstrated that birds fed diets containing 2.5 g/kg EBZA inclusion consumed more than those fed diets containing 0 and 0.5 g/kg EBZA (P=0.0438; Table 6). In addition, a tendency (P=0.0528) was exhibited for ending BW, with birds consuming 2.5 g/kg EBAZ weighing more than those consuming 0.5 g/kg and 0.0 g/kg EBAZ. Overall, trends were also demonstrated for BWG and ending BW in which birds fed diets with 2.5g/kg EBAZ inclusion were heaviest (P=0.053). EBZA inclusion was significant for percent mortality during d 0-21, d 0-35, and d 0-49 ($P \leq 0.05$; Tables 3, 5, and 7, respectively). From d 0-21, birds fed diets containing 2.5 g/kg EBZA experienced higher mortality of nearly 5% compared to those fed 0 g/kg and 0.5 g/kg EBAZ. Percent mortality continued to be the highest for d 0-35 (P=0.0234) and d 0-49 (P=0.0147) with birds fed 2.5 g/kg EBAZ inclusion. However, during these times 2.5 g/kg and 0 g/kg inclusion mortality was statistically similar, but the mortality was significantly different and lower for those fed 0.05 EBAZ when compared to the 2.5 g/kg inclusion. Inclusion of EBAZ was not significant for percent mortality during d 21-35 and d 35-49 (P>0.05; Tables 4 and 6).

Blood Chemistry Analysis

Blood serum chemistry parameters measured at the end of the experimental period on d 49 and are shown in Tables 9, 10, and 11.

Sex × EBAZ interactions

Significant interactions were observed between sex and EBAZ inclusion for albumin (P=0.022). The highest concentrations were observed in females fed diets including 0.5 g/kg and 2.5 g/kg EBAZ inclusion as well as in males fed diets including 0 g/kg EBAZ inclusion. The lowest albumin levels were displayed by females consuming diets with 0 g/kg EBAZ inclusion, whereas males consuming diet with 0.5 g/kg and 2.5 g/kg had intermediate levels. Significant sex × EBAZ inclusion were also observed for serum ALP levels (P=0.006). Males given 2.5 g/kg EBAZ inclusion displayed the highest ALP, while males given 0.5 g/kg EBAZ displayed the lowest. Males given 0.0 g/kg EBAZ exhibited intermediate levels of serum ALP along with all female birds, regardless of EBAZ inclusion.

Sex effects

The main effect of sex had a higher concentrations of blood serum glucose (P=0.0035), total protein (P=0.004), and magnesium (P=0.038) in males versus females. However, female potassium (P=0.004) and CK (P=0.032) concentrations were significantly higher than those in males. Sex did not affect BUN, creatinine, sodium, chloride, calcium, phosphorus, AST, ALT, amylase, cholesterol, and GGT serum levels (P>0.05).

EBAZ effects

Inclusions of EBAZ had a significant effect on blood serum glucose (P=0.022) and sodium (P=0.05) with both increasing along with increased EBAZ inclusion. In addition, potassium (P=0.009) and phosphorus (P=0.009) levels were significantly higher

with EBAZ inclusion, as compared to the control (0 g/kg). The main effect of EBAZ inclusion resulted in significantly higher ALP (P=0.004) blood concentration at 2.5 g/kg and the 0.5 g/kg inclusion resulted in the lowest. Feeding 0 g/kg EBAZ resulted in an intermediate level of serum ALP. Inclusions of EBAZ did not affect BUN, creatinine, calcium, total protein, AST, ALT, ALP, cholesterol, CK, amylase, magnesium, and GGT blood serum levels (P>0.05).

Cecal SCFA analysis

Cecal contents collected on d 49 were analyzed and the results for short-chain fatty acid (SCFA) analysis performed on the cecal contents of D49 broilers can be found in Table 8. No significant Sex × EBAZ interactions were found for any of the SCFA (P>0.05). The main effect of sex displayed significant increases in propionic (P= 0.003) isobutyric (0.0003), 2-methyl butyric (0.0002), and valeric (0.001) in all cases for males. Sex did not significantly affect acetic and butyric percentages in cecal contents (P>0.05).The main effect of EBAZ inclusion did not significantly influence SCFA percentages of cecal contents at any level of inclusion (P>0.05).

Discussion

Performance

This experiment was conducted to determine the safety and effects of feeding diets containing 0 g/kg, manufacturer recommended (0.5 g/kg), or a 5× inclusion (2.5 g/kg) of EBAZ to Ross × Ross 708 male or female broilers. Live performance variables were measured in an attempt to observe any positive or negative effects of EBAZ

inclusion as compared to the control of 0 g/kg EBAZ. Also, it was of interest if males or females were equally sensitive to EBAZ.

As expected, the males primarily outperformed females throughout the experimental period (Ross 708 Broiler Performance Objectives, 2014). Sexual dimorphism for growth performance in male and female broilers has been widely observed in the past, so this finding was expected (Mignon-Grasteau et al., 1998; Maniatis et al., 2013). However, females exhibited better feed efficiency during d 0-35, which seems to be driven by the larger difference in FI than the difference in ending BW.

In the current study, the addition of EBAZ did not contribute to performance improvements for FCR, BWG, or ending BW, but did encourage feed consumption. These results are in agreeance with those of Aghazadeh and cohorts (2012) in which butyric acid increased feed intake without contributing to better FCR or BWG. Contrary to our findings, Sarvari and others (2015) reported that a zinc oxide (ZnO) and an organic acid blend (1g/kg ZnO + 3g/kg OA) improved FCR in 42d Ross 308 broilers. Though it must be noted, that the organic acid blend used did not contain butyric acid and in the current study, the product tested consisted of butyric acid and ZnO.

The interaction observed for males fed 2.5 g/kg EBAZ was potentially driven by the high presence of zinc oxide and butyric acid. Coated and uncoated butyric acid inclusions ranging from 0.3 g/kg to 2.2 g/kg have been shown to promote better growth performance and processing qualities in broilers (Qaisrani et al., 2015; Leeson et al., 2005; Kaczmarek et al., 2016) and at the exaggerated inclusion level (2.5g/kg EBAZ), butyric acid comprised 0.5 g/kg of the EBAZ of for the current study. Additionally, various zinc sources at 0.06, 0.12, and 0.18 g/kg have been shown to improve weight gain

and feed consumption in broilers and the amount of ZnO included at the 2.5 g/kg level of EBAZ is 0.25 g/kg (Liu et al., 2012). These improvements suggest better nutrient utilization, most likely through enhanced intestinal characteristics. Intestinal morphology was not examined in the present study, but other research has found evidence that organic acid inclusion leads to deeper crypts, longer villi, and improved broiler performance (Adil et al., 2010; Kum et al., 2010; Qaisrani et al., 2015).

Mortality

The main effect of EBAZ inclusion influenced percent mortality for d 0-35, as well as overall (d 0-49). This contradicts reports by Levy and others (2015) in which treatments of 0.1, 0.2, 0.3, 0.4, and 0.5 g/kg of ButiPEARL, an encapsulated butyric acid source, had no effect on overall mortality, even though diets used also contained AGPs like in the current study. However, it should be noted that this product was only tested with a much lower range of inclusions as compared to the 5× dose tested in the current study. The highest mortality percentages in the present study occurred from d 0-21, indicating that younger birds may not tolerate the addition of EBAZ at the high dose, 2.5 g/kg. These results concur with those of Cave (1984) in which mortality rose with increasing levels of propionic acid in diets fed to female Shaver Starbro chicks. As for the zinc component, there are reports that inclusions of zinc oxide at 1 g/kg, 2 g/kg, and 4 g/kg can be associated with poor growth and organ detriment in broiler chicks (Dewar et al., 1983). However, it is doubtful the presence of zinc oxide was the cause due to the lower level used in the current study (0.25 g/kg ZnO in 2.5 g/kg EBAZ), as well as the absence of lesions described by Dewar and others (1983) in necropsies performed in the present study.

High mortalities were also observed for chicks given 0 g/kg EBAZ in the current study. Though statistically similar, those fed 0.5 g/kg EBAZ were numerically lower throughout the study. One possible explanation for this could be that the birds experienced a challenge despite the use of practical diets including antibiotic and anticoccidial. In support of this, necropsies in 9 of 28 (32%) mortalities revealed yellowish caseous exudate in the pericardium of the heart, indicative of the bacterial infection, Colibacillosis (Diseases of Poultry, MSU Extension). Although uncertain, it is possible that this challenge occurred from the used litter utilized as pen bedding in this study, because recycled poultry litter is known to be an ideal environment for microbial growth, including those species involved with poultry diseases (Lu et al., 2003). As previously mentioned, the litter used in the current study was used for approximately 10 years (40+ flocks) without replacement. Perhaps the severity of the challenge plus the absence of EBAZ (0.0 g/kg) left chicks susceptible, while the highest inclusion (2.5 g/kg) of EBAZ may have stressed the chicks further. It seems as though the recommended inclusion (0.5 g/kg) of EBAZ prevented additional mortality. Interestingly, Panda and cohorts (2009) reported that butyrate addition at 4 g/kg, which is much greater than the inclusion in the present study (0.1 and 0.25 g/kg butyric acid in 0.5 and 0.25 g/kg EBAZ, respectively) decreased E. coli and suggested that infections like, Colibacillosis could be prevented with butyric acid supplementation.

Blood serum

Blood serum glucose, sodium, potassium, chloride, phosphorus, and ALP concentrations were influenced by EBAZ inclusion. Sodium, chloride, and potassium serum concentration increased as inclusions of EBZA increased. Though not certain, this

could be due to the contribution of SCFAs protons, aiding in electrolyte transport of sodium (Cummings, 1981). However it is unknown whether these factors were influenced by butyric acid and/or zinc oxide. Kaya et al. (2014) reported similar higher serum glucose, phosphorus and ALP levels with higher organic acid mixture (propionic, citric, lignosulphonic acid) supplementation for laying hens and speculated that this was a result of better mineral absorption due to better intestinal environment.

With the addition of Zn (Sarvari et al., 2015) noted increased blood plasma ALP levels in broilers given 0.1 g/kg. The zinc portion of EBAZ at the highest inclusion (2.5g/kg EBAZ; 0.25 g/kg ZnO) may be the cause for high ALP blood concentration given these past research findings. Literature for humans shows that the serum parameter ALP is often evaluated as an indication of liver health (Thapa and Walia, 2007). Though a reference number for ALP levels in Ross × Ross 708 broilers, or poultry in general is lacking, this increase is an indication of possible liver stress.

For a serum albumin level, an interaction between sex and EBAZ inclusion was observed present study for females consuming EBAZ inclusions (P=0.0222; Table 10). This may be partially explained by two observations in previous research. The first, noted higher albumin blood levels for females than males when evaluating sex effects on blood parameters (Panigrahy et al., 2017; Ahmed et al., 2014). The second, being that organic acid (formic, malic, tartic, citric, lactic and orthophosphoric acid mix) supplementation has previously been reported to increase albumin levels in broilers, though only males were used in this study (Savari et al., 2015).

Cecal SCFA

Dietary treatments did not influence the percentage of SCFA in the cecal contents of male and female broilers in this study (P>0.05). Research conducted by Janczyk and others (2014) revealed that addition of dietary zinc oxide at 3.1 g/kg after 28 days reduced valeric and butyric acid in the cecum of pigs. Although microflora population was not assessed, the lack of change in SCFA percentage shows that EBAZ inclusion, recommended (0.5 g/kg) or exaggerated (2.5 g/kg), may not influence the microbial population in the ceca responsible for volatile fatty acid production in the settings tested in the current experiment.

In the present study, male broilers had increased SCFA percentages, whereas the females had lower percentages regardless of EBAZ inclusion. Volatile fatty acids are produced by bacteria upon fermentation of nutrients such as carbohydrates and protein (Berge and Wierup, 2012). Although no studies were found on the effects on feed intake and SCFA concentrations, it is possible that the higher feed consumption observed in male broilers supplied more nutrients for more bacterial fermentation and increased production. However, sex could be a contributing factor. Shastri and cohorts (2015) reported that when testing oligofructose inclusion in rat diets higher SCFA in males than females given the same diet; it was speculated that this was a result of sex differences in protein metabolism, indicating different sources of nitrogen for microbial support.

Conclusions

Though unintended, a bacterial challenge occurred despite the use of practical diet formulations with BMD 50 (bacitracin methylene disalicylate) included at 0.5 g/kg and Salinomycin (BioCox 60) included at 0.34 g/kg. The recommended level of EBAZ

(0.5g/kg) resulted in lower mortality throughout the study demonstrating that EBAZ addition can improve bird health. Although highest serum ALP levels were found in birds consuming the exaggerated EBAZ inclusion (2.5 g/kg), it is unlikely that the addition of EBAZ causes toxicity as demonstrated by the trends observed for improved BW and BWG in d 0-49. Blood serum analysis can be helpful for initial health indications, but it cannot be used reliably as an assessment of bird health for this study due to insufficient relevant reference values for male and female broilers. In conclusion, the results from this study suggest that EBAZ can be supplemented up to 2.5 g/kg without any detrimental effects on the growth of male and female Ross × Ross 708 broilers.

Table 2.1 Experimental Diet Formulations

	Inclusion Rate %		
Ingredient	**Starter**	**Grower**	**Finisher**
Corn	54.924	58.6199	63.0979
Corn DDGS	4.000	7	8
SBM	30.210	23.3313	18.7797
ProPlus 55[1]	3.500	5	5
Poultry Fat	3.292	2.7257	2.1204
Limestone: Calcium Carbonate	1.097	0.8521	0.6523
Monocalcium Phosphate	1.116	0.7239	0.6287
Salt, NaCl	0.243	0.2075	0.1902
Selenium Premix 0.06%	0.022	0.0242	0.0243
Sodium Bicarbonate	0.305	0.2972	0.3134
L-Lys HCl, 78.8% Feed Grade	0.266	0.2782	0.3177
DL-Met, 99%	0.322	0.2714	0.2374
L-Thr, 98.0 g/kg Feed Grade	0.098	0.0847	0.088
BMD-50	0.050	0.05	0.05
Salinomycin - BioCox 60	0.034	0.034	0.00
Vitamin-Trace Min PM_Nutrablend 3000[2]	0.273	0.25	0.25
Corn or EBA+Z[3]	0.250	0.250	0.250

[1]H.J Baker's ProPlus 55Animal Protein Concentrate

[2]Guarantees per one (1) lb. of diet: 1,400,000 IU vitamin A, 500,000 ICU vitamin D_3, 3,000 IU vitamin E, 2 mg vitamin B_{12}, 250 mg vitamin B_6, 1,200 mg riboflavin, 5,000 mg niacin, 1,200 mg d-pantothenic acid, 150 mg menadione, 125 mg folic acid, 70,000 mg choline, 200 mg thiamine, 6 mg biotin, 18,143 mg Zn (from $ZnSO_4$), 18,143 mg (from $MnSO_4$), 9,071 mg Fe (from $FeSO_4$), 2,041 mg Cu (from $CuSO_4$), 272 mg I (from ethylenediamine dihydroiodide), 27 mg Se (from Na_2SeO_3)

[3] ButiPEARL Z (Kemin AgriFoods North America, Des Moines, IA).

Table 2.2 Proximate Analysis for Experimental Diets.

Analysis	Unit	0 g/kg EBAZ[1]	0.5 g/kg EBAZ[2]	2.5 g/kg EBAZ[3]
Starter[4]				
Moisture	%	12.7	12.6	12.6
Protein	%	20.7	20.6	20.5
Fat	%	5.68	5.73	5.47
Fiber	%	2.09	1.82	2.16
Ash	%	5.46	5.37	5.17
Grower[5]				
Moisture	%	12.1	12.1	12.2
Protein	%	19.6	20.1	20.2
Fat	%	5.7	5.76	5.9
Fiber	%	2.1	2.20	2.32
Ash	%	5.51	5.56	5.55
Finisher[6]				
Moisture	%	12.3	11.9	12.3
Protein	%	19.5	19.5	19.3
Fat	%	5.47	5.52	5.34
Fiber	%	3.25	2.82	2.76
Ash	%	5.67	5.71	5.72

Table 2.3 Starter (d 0-21) comparisons of dietary treatments on broiler performance.

Sex	EBAZ inclusion[1]	D0-21 Feed Intake/bird[2] (kg)	D0-21 BWG/bird[3] (kg)	D21 Ending Body Weight[4] (kg)	D0-21 FCR[5]	D0-21 Percent Mortality (%)
Male	0 g/kg	1.040	0.801	0.843	1.313	0.834
	0.5 g/kg	1.029	0.782	0.822	1.315	0.834
	2.5 g/kg	1.019	0.775	0.815	1.324	5.834
Female	0 g/kg	0.966	0.719	0.759	1.342	1.668
	0.5 g/kg	0.980	0.732	0.771	1.340	0.834
	2.5 g/kg	0.966	0.694	0.745	1.354	5.001
SEM[6]		0.0105	0.0087	0.0098	0.0074	1.151
Marginal Means-Sex						
Male		**1.029[a]**	**0.786[a]**	**0.827[a]**	**1.317[b]**	2.500
Female		**0.970[b]**	**0.715[b]**	**0.758[b]**	**1.345[a]**	2.501
SEM		**0.0061**	**0.0050**	**0.0057**	**0.0043**	0.6643
Marginal Means-EBAZ inclusion						
0 g/kg		1.003	**0.760[a]**	0.801	1.328	**1.251[b]**
0.5 g/kg		1.005	**0.757[a]**	0.797	1.327	**0.834[b]**
2.5 g/kg		0.992	**0.735[b]**	0.780	1.339	**5.418[a]**
SEM		0.0074	**0.0062**	0.0070	0.0052	**0.8152**
Main Effects and Interactions						
Sex		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.7061
EBAZ inclusion		0.4621	**0.0129**	0.0966	0.2355	**0.0081**
Sex x EBAZ inclusion		0.4513	0.1442	0.2733	0.9294	0.8613

[1]0 g/kg EBAZ inclusion (2.5 g/kg ground corn), 0.5g/kg EBAZ inclusion (0.5 g/kg EBAZ + 0.2% ground corn), 2.5g/kg EBAZ inclusion (2.5 g/kg EBAZ)
[2] Feed Intake is based on a per bird basis.
[3] Body Weight Gain per bird basis
[4] Average Ending Bird Weight
[5] Feed Conversion Ratio (Feed:Gain) was adjusted with mortality weight
[6]Standard Error of the Mean
a-b Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 2.4 Grower (d 21-35) comparisons of dietary treatments on broiler performance.

Sex	EBAZ inclusion[1]	D21-35 Feed Intake/bird[2] (kg)	D21-35 BWG/bird[3] (kg)	D35 Ending Body Weight[4] (kg)	D21-35 FCR[5]	D21-35 Percent Mortality (%)
Male	**0 g/kg**	2.038	1.111	1.932	1.704	1.667
	0.5 g/kg	1.980	1.090	1.912	1.828	0.000
	2.5 g/kg	2.035	1.176	1.993	1.734	0.893
Female	**0 g/kg**	1.774	1.043	1.802	1.738	0.893
	0.5 g/kg	1.822	1.067	1.838	1.711	0.000
	2.5 g/kg	1.789	1.092	1.837	1.639	0.000
SEM[6]		0.0318	0.0290	0.0297	0.0395	0
Marginal Means-Sex						
Male		**2.018[a]**	**1.126[a]**	**1.946[a]**	1.755	0.853
Female		**1.795[b]**	**1.067[b]**	**1.826[b]**	1.696	0.298
SEM		**0.0184**	**0.0168**	**0.0172**	0.0228	0
Marginal Means-EBAZ inclusion						
0 g/kg		1.906	1.077	1.867	1.721	1.280
0.5 g/kg		1.901	1.079	1.875	1.769	0.000
2.5 g/kg		1.912	1.134	1.915	1.686	0.446
SEM		0.0225	0.0205	0.0210	0.0280	0
Main Effects and Interactions						
Sex		**<0.0001**	**0.0207**	**<0.0001**	0.0777	0.3426
EBAZ inclusion		0.9438	0.0985	0.2405	0.1178	0.1997
Sex x EBAZ inclusion		0.2195	0.5517	0.3792	0.1468	0.7918

[1]0 g/kg EBAZ inclusion (2.5 g/kg ground corn), 0.5g/kg EBAZ inclusion (0.5 g/kg EBAZ + 0.2% ground corn), 2.5g/kg EBAZ inclusion (2.5 g/kg EBAZ)
[2] Feed Intake is based on a per bird basis.
[3] Body Weight Gain per bird basis
[4] Average Ending Bird Weight
[5] Feed Conversion Ratio (Feed:Gain) was adjusted with mortality weight
[6]Standard Error of the Mean
a-b Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 2.5 Comparisons of dietary treatments on broiler performance during d 0-35

Sex	EBAZ inclusion[1]	D0-35 Feed Intake/bird[2] (kg)	D0-35 BWG/bird[3] (kg)	D35 Ending Body Weight[4] (kg)	D0-35 FCR[5]	D0-35 Percent Mortality (%)
Male	**0 g/kg**	3.088	1.892	1.932	1.629	2.500
	0.5 g/kg	3.009	1.872	1.912	1.610	0.833
	2.5 g/kg	3.103	1.953	1.993	1.568	6.667
Female	**0 g/kg**	2.740	1.762	1.802	1.560	3.333
	0.5 g/kg	2.803	1.799	1.838	1.538	0.833
	2.5 g/kg	2.755	1.798	1.837	1.531	5.00
SEM[6]		0.0383	0.0297	0.0297	0.0180	1.4650
Marginal Means-Sex						
Male		**3.067[a]**	**1.906[a]**	**1.946[a]**	**1.602[a]**	3.333
Female		**2.766[b]**	**1.786[b]**	**1.826[b]**	**1.543[b]**	3.056
SEM		**0.0221**	**0.0172**	**0.0172**	**0.0104**	0.8458
Marginal Means-EBAZ inclusion						
0 g/kg		2.914	1.827	1.867	1.594	**2.917[ba]**
0.5 g/kg		2.906	1.836	1.875	1.574	**0.833[b]**
2.5 g/kg		2.929	1.876	1.915	1.549	**5.833[a]**
SEM		0.0271	0.0210	0.0210	0.0127	**1.0380**
Main Effects and Interactions						
Sex		**<0.0001**	**<.0001**	**<.0001**	**0.0004**	0.8457
EBAZ inclusion		0.8265	0.2397	0.2405	0.0599	**0.0234**
Sex x EBAZ inclusion		0.1172	0.3804	0.3792	0.5514	0.7658

[1]0 g/kg EBAZ inclusion (2.5 g/kg ground corn), 0.5g/kg EBAZ inclusion (0.5 g/kg EBAZ + 0.2% ground corn), 2.5g/kg EBAZ inclusion (2.5 g/kg EBAZ)
[2] Feed Intake is based on a per bird basis.
[3] Body Weight Gain per bird basis
[4] Average Ending Bird Weight
[5] Feed Conversion Ratio (Feed:Gain) was adjusted with mortality weight
[6]Standard Error of the Mean
a-b Values within columns with different superscripts differ significantly (P < 0.05)

Table 2.6 Finisher (d 35-49) comparisons of dietary treatments on broiler performance.

Sex	EBAZ inclusion[1]	D35-49 Feed Intake/bird[2] (kg)	D35-49 BWG/bird[3] (kg)	D49 Ending Body Weight[4] (kg)	D35-49 FCR[5]	D35-49 Percent Mortality (%)
Male	0 g/kg	**2.589[b]**	1.360	3.319	1.876	0.000
	0.5 g/kg	**2.507[b]**	1.351	3.215	1.936	0.000
	2.5 g/kg	**2.836[a]**	1.429	3.449	1.950	1.795
Female	0 g/kg	**2.521[b]**	1.224	3.025	2.013	1.667
	0.5 g/kg	**2.598[b]**	1.247	3.086	2.029	0.833
	2.5 g/kg	**2.534[b]**	1.247	3.085	2.033	0.000
SEM[6]		0.0579	0.0290	0.0492	0.0574	0
Marginal Means-Sex						
Male		2.644	**1.380[a]**	**3.328[a]**	**1.921[b]**	0.598
Female		2.551	**1.239[b]**	**3.065[b]**	**2.025[a]**	0.833
SEM		0.0334	**0.0166**	**0.0284**	**0.0331**	0
Marginal Means-EBAZ inclusion						
0 g/kg		2.555	1.292	3.172	1.944	0.833
0.5 g/kg		2.553	1.299	3.150	1.982	0.417
2.5 g/kg		2.685	1.338	3.267	1.991	0.897
SEM		0.0409	0.0204	0.0348	0.0406	0
Main Effects and Interactions						
Sex		0.0559	**<0.0001**	**<0.0001**	**0.0348**	0.7128
EBAZ inclusion		0.0438	0.2351	0.0528	0.6998	0.7985
Sex x EBAZ inclusion		**0.0064**	0.3961	0.0605	0.8880	0.0804

[1]0 g/kg EBAZ inclusion (2.5 g/kg ground corn), 0.5g/kg EBAZ inclusion (0.5 g/kg EBAZ + 0.2% ground corn), 2.5g/kg EBAZ inclusion (2.5 g/kg EBAZ)
[2] Feed Intake is based on a per bird basis.
[3] Body Weight Gain per bird basis
[4] Average Ending Bird Weight
[5] Feed Conversion Ratio (Feed:Gain) was adjusted with mortality weight
[6]Standard Error of the Mean
[a-b] Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 2.7 Overall (d 0-49) comparisons of dietary treatments on broiler performance.

Sex	EBAZ inclusion[1]	D0-49 Feed Intake/bird[2] (kg)	D0-49 BWG/bird[3] (kg)	D49 Ending Body Weight[4] (kg)	D0-49 FCR[5]	D0-49 Percent Mortality (%)
Male	**0 g/kg**	**5.703^{b}**	3.279	3.319	1.721	2.500
	0.5 g/kg	**5.517^{bc}**	3.175	3.215	1.738	0.833
	2.5 g/kg	**5.939^{a}**	3.409	3.449	1.725	8.333
Female	**0 g/kg**	**5.261^{d}**	2.985	3.025	1.760	5.000
	0.5 g/kg	**5.401^{cd}**	3.046	3.086	1.760	1.667
	2.5 g/kg	**5.289^{d}**	3.045	3.085	1.735	5.000
SEM[6]		0.0781	0.0492	0.0492	0.0103	1.7556
Marginal Means-Sex						
Male		5.720	**3.288^{a}**	**3.328^{a}**	**1.728^{b}**	3.889
Female		5.317	**3.026^{b}**	**3.065^{b}**	**1.752^{a}**	3.889
SEM		0.0451	**0.0284**	**0.0284**	0.0059	1.0135
Marginal Means-EBAZ inclusion						
0 g/kg		5.482	3.132	3.172	1.741	**3.75^{ab}**
0.5 g/kg		5.459	3.111	3.150	1.749	**1.25^{b}**
2.5 g/kg		5.614	3.227	3.267	1.730	**6.667^{a}**
SEM		0.0552	0.0348	0.0348	0.0073	**1.2413**
Main Effects and Interactions						
Sex		<0.0001	**<0.0001**	**<0.0001**	**0.0097**	1.000
EBAZ inclusion		0.1152	0.0530	0.0528	0.1897	**0.0147**
Sex x EBAZ inclusion		**0.0060**	0.0610	0.0605	0.4274	0.2450

[1] 0 g/kg EBAZ inclusion (2.5 g/kg ground corn), 0.5g/kg EBAZ inclusion (0.5 g/kg EBAZ + 0.2% ground corn), 2.5g/kg EBAZ inclusion (2.5 g/kg EBAZ)
[2] Feed Intake is based on a per bird basis.
[3] Body Weight Gain per bird basis
[4] Average Ending Bird Weight
[5] Feed Conversion Ratio (Feed:Gain) was adjusted with mortality weight
[6] Standard Error of the Mean
[a-b] Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 2.8 Short-chain fatty analysis on the cecal contents of d 49 broilers.

Sex	EBAZ inclusion[1]	Acetic (%)	Propionic (%)	Isobutyric (%)	Butyric (%)	2-Methyl Butyric (%)	Valeric (%)
Male	**0 g/kg**	0.444	0.098	0.012	0.119	0.006	0.017
	0.5 g/kg	0.415	0.097	0.011	0.098	0.006	0.016
	2.5 g/kg	0.400	0.102	0.011	0.103	0.006	0.158
Female	**0 g/kg**	0.403	0.090	0.011	0.097	0.006	0.014
	0.5 g/kg	0.406	0.085	0.010	0.092	0.005	0.014
	2.5 g/kg	0.401	0.078	0.010	0.090	0.005	0.014
SEM		0.0138	0.0055	0.0005	0.0085	0.0004	0.0003
Marginal Means-Sex							
Male		0.419	**0.099[a]**	**0.011[a]**	0.107	**0.006[a]**	**0.016[a]**
Female		0.403	**0.085[b]**	**0.010[b]**	0.093	**0.005[b]**	**0.014[b]**
SEM		0.0080	**0.0032**	**0.0003**	0.0049	**0.0002**	**0.0005**
Marginal Means-EBAZ inclusion							
0 g/kg		0.424	0.094	0.011	0.108	0.006	0.015
0.5 g/kg		0.411	0.091	0.011	0.095	0.006	0.015
2.5 g/kg		0.400	0.090	0.010	0.096	0.005	0.015
SEM		0.0098	0.0039	0.0003	0.0060	0.0003	0.0006
Main Effects and Interactions							
Sex		0.1699	**0.0031**	**0.0010**	0.0650	**0.0042**	**0.0009**
EBAZ inclusion		0.2785	0.7435	0.1962	0.2739	0.6312	0.6793
Sex x EBAZ inclusion		0.3245	0.3820	0.8253	0.6336	0.4446	0.5716

Sampling was performed on 3 random birds per pen (144 total birds). Cecal samples were pooled to create 48 total samples. These were frozen, and sent to the a laboratory (Rumen Fermentation Laboratory, West Virginia University) and analyzed using gas chromatography

[1]0 g/kg EBAZ inclusion (2.5 g/kg ground corn), 0.5g/kg EBAZ inclusion (0.5 g/kg EBAZ + 0.2% ground corn), 2.5g/kg EBAZ inclusion (2.5 g/kg EBAZ)

[1] Standard Error of the Mean

[a-b] Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 2.9 Blood serum panel analysis on d 49 broilers.

Sex	EBAZ inclusion[1]	Glucose (mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
Male	**0 g/kg**	215.625	1.000	0.125	140.929	3.819	107.502
	0.5 g/kg	218.028	1.000	0.118	140.641	4.205	107.302
	2.5 g/kg	223.000	1.000	0.133	145.088	4.483	111.038
Female	**0 g/kg**	193.250	1.000	0.105	130.913	4.161	101.600
	0.5 g/kg	209.125	1.000	0.111	139.638	4.881	108.40
	2.5 g/kg	215.028	1.000	0.122	141.991	4.610	109.787
SEM[2]		5.054	0	0.0118	2.9443	0.1506	2.2604
Marginal Means-Sex							
Male		**218.884[a]**	1.000	0.125	142.219	**4.169[b]**	108.614
Female		**205.801[b]**	1.000	0.113	137.514	**4.551[a]**	106.596
SEM		**2.9181**	0	0.0068	1.7003	**0.0870**	1.3054
Marginal Means-EBAZ inclusion							
0 g/kg		**204.438[b]**	1.000	0.115	**135.921[b]**	**3.990[b]**	**104.551[b]**
0.5 g/kg		**213.576[ab]**	1.000	0.114	**140.140[ab]**	**4.543[a]**	**107.851[ab]**
2.5 g/kg		**219.014[a]**	1.000	0.127	**143.54[a]**	**4.546[a]**	**110.412[a]**
SEM		3.5756	0	0.0084	**2.0819**	**0.1066**	1.5984
Main Effects and Interactions							
Sex		**0.0035**	-	0.2298	0.0614	**0.0041**	0.2869
EBAZ inclusion		**0.0222**	-	0.5129	**0.0500**	**0.0009**	**0.0492**
Sex x EBAZ inclusion		0.2891	-	0.8379	0.2977	0.1988	0.3093

Sampling was performed on 3 random birds per pen (144 total birds). After allowing the collected blood to clot for approximately 2 hours, blood samples were centrifuged (Beckman Coulter, Inc. model J-6B) at 2°C and 1,900 × g for 10 min in order to obtain serum. Serum collected was pooled by pen to obtain 48 samples. Samples were stored in a – 20 degree Celsius freezer and were sent to a laboratory for blood chemistry analysis (Pearl Diagnostic Lab, Pearl MS).

[1]0 g/kg EBAZ inclusion (2.5 g/kg ground corn), 0.5g/kg EBAZ inclusion (0.5 g/kg EBAZ + 0.2% ground corn), 2.5g/kg EBAZ inclusion (2.5 g/kg EBAZ)

[1] Standard Error of the Mean

[a-b] Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 2.10 Blood serum analysis panel on d 49 broilers.

Sex	EBAZ inclusion[1]	Calcium (mg/dl)	Phosphorus (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	AST (U/L)	ALT (U/L)
Male	**0 g/kg**	9.890	6.443	3.161	**1.337[a]**	364.875	0
	0.5 g/kg	9.200	6.910	3.246	**1.317[ab]**	359.500	0
	2.5 g/kg	9.838	6.975	3.275	**1.313[ab]**	424.375	0
Female	**0 g/kg**	8.838	6.113	2.950	**1.213[b]**	339.000	0
	0.5 g/kg	9.450	6.575	3.100	**1.363[a]**	469.750	0
	2.5 g/kg	9.640	6.734	2.995	**1.397[a]**	435.150	0
SEM[2]		0.3497	0.1829	0.0824	0.0375	45.9331	0
Marginal Means-Sex							
Male		9.642	6.776	**3.227[a]**	1.322	382.917	0
Female		9.309	6.473	**3.015[b]**	1.324	414.633	0
SEM		0.2019	0.1056	**0.0476**	0.0217	26.6297	0
Marginal Means-EBAZ inclusion							
0 g/kg		9.364	**6.278[b]**	3.055	1.275	351.938	0
0.5 g/kg		9.325	**6.743[a]**	3.173	1.340	414.625	0
2.5 g/kg		9.739	**6.854[a]**	3.135	1.355	429.763	0
SEM		0.2474	**0.1293**	0.0583	0.0265	32.7789	0
Main Effects and Interactions							
Sex		0.2549	0.0536	**0.0040**	0.9481	0.4262	-
EBAZ inclusion		0.4433	**0.0090**	0.3584	0.0986	0.2287	-
Sex x EBAZ inclusion		0.1852	0.9596	0.7298	**0.0222**	0.2982	-

Sampling was performed on 3 random birds per pen (144 total birds). After allowing the collected blood to clot for approximately 2 hours, blood samples were centrifuged (Beckman Coulter, Inc. model J-6B) at 2°C and 1,900 × g for 10 min in order to obtain serum. Serum collected was pooled by pen to obtain 48 samples. Samples were stored in a – 20 degree Celsius freezer and were sent to a laboratory for blood chemistry analysis (Pearl Diagnostic Lab, Pearl MS).

[1]0 g/kg EBAZ inclusion (2.5 g/kg ground corn), 0.5g/kg EBAZ inclusion (0.5 g/kg EBAZ + 0.2% ground corn), 2.5g/kg EBAZ inclusion (2.5 g/kg EBAZ)

[2] Standard Error of the Mean

[a-b] Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 2.11 Blood serum analysis panel on d 49 broilers.

Sex	EBAZ inclusion[1]	ALP (U/L)	Cholesterol (mg/dl)	CK (U/L)	Amylase (U/L)	Magnesium (mg/dl)	GGT (U/L)
Male	**0 g/kg**	**2474.095^{bc}**	114.353	40165.000	513.665	2.001	19.168
	0.5 g/kg	**2012.875^{c}**	109.375	36187.880	507.379	1.992	19.786
	2.5 g/kg	**4195.000^{a}**	109.125	55492.500	560.625	2.125	19.125
Female	**0 g/kg**	**3126.500^{b}**	102.625	47001.250	510.625	1.875	17.125
	0.5 g/kg	**2222.500^{bc}**	110.250	73143.750	546.125	2.038	20.000
	2.5 g/kg	**2533.702^{bc}**	102.285	68921.703	572.005	1.933	19.080
SEM[2]		337.8707	4.9497	10322.9776	34.1949	0.0509	0.7751
Marginal Means-Sex							
Male		2893.990	110.951	**43948.460^{b}**	527.223	**2.040^{a}**	19.360
Female		2627.567	105.053	**63022.245^{a}**	542.918	**1.949^{b}**	18.735
SEM		195.0693	2.8584	**28583.037**	19.7424	**0.0294**	0.4476
Marginal Means-EBAZ inclusion							
0 g/kg		2800.297	108.489	43583.125	512.145	1.938	18.147
0.5 g/kg		2117.688	109.813	54665.815	526.752	2.015	19.893
2.5 g/kg		3364.351	105.705	62207.102	566.315	2.029	19.102
SEM		239.3836	3.5052	7302.827	24.192	0.0360	0.5481
Main Effects and Interactions							
Sex		0.3527	0.1603	**0.0315**	0.5877	**0.0380**	0.3356
EBAZ inclusion		0.0035	0.7133	0.2062	0.3002	0.1783	0.0984
Sex x EBAZ inclusion		**0.0057**	0.4386	0.3261	0.8233	0.0732	0.3012

Sampling was performed on 3 random birds per pen (144 total birds). After allowing the collected blood to clot for approximately 2 hours, blood samples were centrifuged (Beckman Coulter, Inc. model J-6B) at 2°C and 1,900 × g for 10 min in order to obtain serum. Serum collected was pooled by pen to obtain 48 samples. Samples were stored in a – 20 degree Celsius freezer and were sent to a laboratory for blood chemistry analysis (Pearl Diagnostic Lab, Pearl MS).

[1]0 g/kg EBAZ inclusion (2.5 g/kg ground corn), 0.5g/kg EBAZ inclusion (0.5 g/kg EBAZ + 0.2% ground corn), 2.5g/kg EBAZ inclusion (2.5 g/kg EBAZ)

[2] Standard Error of the Mean

[a-b] Values within columns with different superscripts differ significantly ($P < 0.05$)

References

Ahmed, A.S., J. M. Alhamada and Z.M. Hakami. 2014. Evaluation of some blood parameters of hajar 1 and hajar 2 saudi chicken lines over the first 30 weeks of age. Asian J. of Poult. Sci. 8:115-122.

Abdel-Fattah, S.A., M.H. El-Sanhoury, N.M. El-Mednay, and F. Abdel-Azeem. 2008. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. Int. J. Poult. Sci. 7:215-222.

Adil, S., T. Banday, G. A. Bhat, M. S. Mir, and M. Rehman. 2010. Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. Vet. Med. Int. 2010:1-7.

Aghazadeh, A.M., & TahaYazdi, M. 2012. Effect of butyric acid supplementation and whole wheat inclusion on the performance and carcass traits of broilers. South African Journal of Animal Science, 42:241-248.

Berge, A. C., and M. Wierup. 2012. Nutritional strategies to combat Salmonella in mono-gastric food animals. Animal 6:557-564.

Cave, N. A. G. 1984. Effect of dietary propionic and lactic acids on feed intake by chicks. Poult. Sci. 63:131-134.

Cummings, J. H. 1981. Short chain fatty acids in the human colon. Gut. 22:763–779.

Dewar, W.A., P. A. Wright, R. A. Pearson, and M. J. Gentle. 1983. Toxic effects of high concentrations of zinc oxide in the diet of the chick and the laying hen. Brit. Poult. Sci. 24:397-404.

Diseases of Poultry. Mississippi State University Extension. Retrieved from http://extension.msstate.edu/agriculture/livestock/poultry/diseases-poultry. Accessed May 2017.

Edmonds, M. S., S. Johal, and S. Moreland. 2014. Effect of supplemental humic and butyric acid on performance and mortality in broilers raised under various environmental conditions. J. Appl. Poult. Res. 23:1-8.

Guilloteau, P., Martin, L., Eeckhaut, V., Ducatelle, R., Zabielski, R., and Van Immerseel, F. 2010. From the gut to the peripheral tissues: The multiple effects of butyrate. Nutrition Research Reviews, 23:366-384.

Højberg, O., Canibe, N., Poulsen, H. D., Hedemann, M. S., and Jensen, B. B. 2005. Influence of dietary zinc oxide and copper sulfate on the gastrointestinal ecosystem in newly weaned piglets. Applied and Environmental Microbiology. 71:2267–2277.

Hu, C. H., Z. C. Qian, J. Song, Z. S. Luan, and A. Y. Zuo. 2013. Effects of zinc oxide-montmorillonite hybrid on growth performance, intestinal structure, and function of broiler chicken. Poult. Sci. 92:143-150

Janczyk, P., K. Busing, B. Dobenecker, K. Nockler, and A. Zeyner. 2015. Effect of high dietary zinc oxide on the caecal and faecal short chain fatty acids and tissue zinc and copper concentration in pigs is reversible after withdrawal of the high zinc oxide from the diet. Journal of Animal Physiology and Nutrition. 99:13-22.

Kaya, H., A. Kaya, M. Gul, S. Çelebi, S. Timurkaan, and B. Apaydin. 2014. Effects of supplementation of different levels of organic acids mixture to the diet on performance, egg quality parameters, serum traits and histological criteria of laying hens. Europ. Poult. Sci.78.

Kotunia A., J. Wolinski, D. Laubitz, M. Jurkowska, V. Rome, P. Guilloteau, and R. Zabielski. Effect of sodium butyrate on the small intestine development in neonatal piglets fed by artificial sow. J Physiol Pharmacol. 2004. 55:59-68.

Kum, S., U. Eren, A. G. Onol, and M. Sandikci. 2010. Effects of dietary organic acid supplementation on the intestinal mucosa in broilers. Rev. Med. Vet. 10:463-468.

Leeson, S., H. Namkung, M. Antongiovanni, and E. H. Lee. 2005. Effect if butyric acid on the performance and carcass yield of broiler chickens. Poult. Sci. 84:1418-1422.

Levy A. W., J. W. Kessler, L. Fuller, S. Williams, G. F. Mathis, B. Lumpkins, and F. Valdez. 2015. Effect of feeding an encapsulated source of butyric acid (ButiPEARL) on the performance of male Cobb broilers reared to 42 d of age. Poult. Sci. 94:1864-1870.

Lu, J., S. Sanchez, C. Hofacre, J. Maurer, B. G. Harmon, and M. Lee. 2003. Evaluation of broiler litter with reference to the microbial composition as assessed by using 16s rrna and functional gene markers. Appl. Environ. Microbiol. 69:901-908.

Maniatis G., N. Demiris, A. Kranis, G. Banos, A. Kominakis. 2013. Genetic analysis of sexual dimorphism of body weight in broilers. J. Appl. Genetics. 54:61-70.

Mignon-Grasteau, S., C. Beaumont, J.P. Poivey, and H. de Rochambeau. 1998. Estimation of the genetic parameters of sexual dimorphism of body weight in 'label' chickens and Muscovy ducks. Genet. Sel. Evol. 30:481-491.

Namkung, H., H. Yu, J. Gong, and S. Leeson. 2011. Antimicrobial activity of butyrate glycerides toward Salmonella Typhimurium and Clostridium perfringens. Poult. Sci. 90:2217-2222.

NRC - National Research Council. Nutrient requirements of poultry. 9th edition. Washington: National Academic. 1994.

Panda, A.K., S. V. Rama Rao, M. V. L. N. Raju, and G. Shyam Sunder. 2009. Effect of butyric acid on performance, gastrointestinal tract health and carcass characteristics in broiler chickens. Asian-Aust, J. Anim. Sci. 22:1026-1031.

Panigrahy, K. K., K. Behera, L. M. Mohapatra, A. P. Acharya, K. Sethy, S. Panda, and S. K. Gupta. 2017. Sex-related differences in hemato-biochemical indices of adult Vanaraja chickens during summer and winter seasons. Veterinary World. 10:176–180.

Park, S. Y., S. G., Birkhold, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2004. Review on the role of dietary zinc in poultry nutrition, immunity, and reproduction. Biological Trace Element Research. 101:147-163.

Pryde, S. E., S.H. Duncan, G.L. Hold, C.S. Stewart, and H.J. Flint. 2002. The microbiology of butyrate formation in the human colon. FEMS Microbiol. Lett., 217:133-139.

Qaisrani, N., M. M. van Krimpen, R. P. Kwakkel, M. W. A. Verstegen, and W. H. Hendriks. 2015. Diet structure, butyric acid, and fermentable carbohydrates influence growth performance, gut morphology, and cecal fermentation characteristics in broilers. Poult. Sci. 2015. 94:2152-2164.

Ricke , S. C. 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poult. Sci. 82:632–639.

Ross 708 Broiler Performance Objectives. 2012. Aviagen North America, Huntsville, AL.

Sahin, K., M. O. Smith, M. Onderci, N. Sahin, M. F. Gursu, and O. Kucuk. 2005. Supplementation of zinc from organic or inorganic source improves performance and antioxidant status of heat-distressed quail. Poult. Sci. 84: 882-887.

Sarvari, BG, Seyedi, AH, Shahryar, HA, Sarikhan, M, and Ghavidel, SZ. 2015. Effects of dietary zinc oxide and a blend of organic acids on broiler live performance, carcass traits, and serum parameters. Revista Brasileira de Ciência Avícola, 17:39-45.

Thapa, B. R. and Walia, A. 2007. Liver function tests and their interpretation. Indian J. Pediatr. 74:663-671.

Van Immerseel, F., F. Boyen, I. Gantois, L. Timbermont, L. Bohez, F. Pasmans, F. Haesebrouck, and R. Ducatelle. 2005. Supplementation of coated butyric acid in the feed reduces colonization and shedding of Salmonella in poultry. Poult. Sci. 84:1851-1856.

CHAPTER III

DETERMINING THE EFFICACY OF TWO CARBOHYDRASE ENZYMES IN COMMERCIAL DIETS ON D 0-57 BROILER PERFORMANCE AND PROCESSING

Abstract

Exogenous enzymes, including carbohydrases are commonly included in today's commercial poultry diets for their multiple beneficial effects on poultry production. However, enzyme performance is variable due to types and dietary constituents. Therefore, carbohydrases effectiveness can be expected to differ with diet fluctuations that commonly occur in commercial broiler diets. The objective of this study was to examine the efficacy of commercially available carbohydrases using 2 different commercial diets (CD). The current study utilized a 2 × 2 factorial arrangement with variations in commercial diets (CD; CDA or CDB) and carbohydrase enzyme (CE; CE1 - multi-carbohydrase enzyme or CE2 - xylanase enzyme). Data was analyzed as a 2 × 2 factorial arrangement and analysis was performed using SAS version 9.4. Both CDA and CDB were similar in most ingredients, with inclusions of corn, soybean meal, a meat and bone meal blend, a phytase enzyme, an antibiotic, and an anticoccidial; however, CDB diets also included wheat. Matrix values for ingredients differed, but were consistent for enzymes tested. On d of hatch, Ross × Ross 708 male chicks were randomly distributed into 36 pens (23 birds/pen; 9 replications/treatment). Individual weights were recorded on

d 14, 28, 43, and 56. Performance variables measured included: average BW, BW gain, feed conversion ratio (FCR), feed intake, mortality and d 57 processing (4 birds/pen). On d 15, 29, and 46, 1 bird per pen was randomly selected and sacrificed for gastrointestinal sampling. These sampling were performed by excising the gizzard and small intestine from each bird to record lengths, weights, and pH. The main effect of CE was significant for FI (P= 0.0087) and FCR (P=0.0387) during d 14-28, with lower intake and improved FCR by 2 pts observed with CE2 inclusion. Overall, the main effect of CD was significant for BW gain, BW, and FCR, demonstrating improvements for birds fed CDB (P<0.0001). These influences on performance translated to potential profit differences using an economic analysis, in which ingredient costs and enzyme costs were set equal between CD. The analysis utilized feed intake, feed costs, and potential profit based on chicken parts produced for each treatment. Enzyme influences on performance translated to potential profit differences in which inclusions of CE2 resulted in increased monetary gain, whereas formulations with CE1 increased diet costs for both CD and was found to reduce potential profit for CDA and CDB by $0.169 and $0.081/bird, respectively.

KEYWORDS:commercial diets, exogenous feed enzymes, broiler performance, economic analysis.

Introduction

Poultry diet formulations utilized within the industry are generated with the intentions of achieving production goals, ranging from low FCR to maximum breast and tenderloin yield. In addition to this, emphasis is often held on formulating with least-cost objectives due to costs associated with feed ingredients and feed manufacture. Therefore, it is unusual for two integrators to feed the same diets. Additionally, formulations within

integrators are frequently altered, as the feed ingredients are generally adjusted/changed in pursuit of economic advantages and profitable production goals.

These formulation modifications often include substitutions of cereal grains. Although cereal grains harbor energy yielding carbohydrates like starch, this exchange may be problematic due to cereal grains also containing indigestible structural constituents known as non-starch polysaccharides (NSP). These complex carbohydrates vary in amounts and types, depending on source and can be found in other plant-based feedstuffs such as soybean meal, corn distillers dried grains with solubles, wheat middlings, etc. (Klyen, 2013). Non-starch polysaccharides cannot be hydrolyzed by the endogenous enzymes found in poultry and other monogastric animals, thereby causing several disadvantages in broiler production such as poor performance, decreased absorption and digestibility, short feed passage rates, and foot-pad dermatitis (Dida, 2016; Choct and Annison, 1990; Choct and Annison, 1991; Shepherd and Fairchild, 2010).

Exogenous enzymes, such as carbohydrases are strategically used in poultry feed to help enhance energy availability, digestion and utilization, which allows for the use of lower priced ingredients that are known to contain higher NSP levels than corn (Kleyn, 2013). There are numerous commercially available carbohydrases, but they differ in microorganism origin, substrates, preparation, and production, meaning they cannot be expected to yield the same results throughout commercial diet fluctuations (Angel and Sorbara, 2014; Polizeli et al., 2005). Therefore, it is important to applicably test the efficacy of different carbohydrase products using practical dietary formulations to better predict enzyme response with least-cost diet fluctuations.

This study partnered with two poultry integrators in order to determine the best carbohydrase for their current diet formulations. Experimental commercial diets varied due to integrator preference, whereas one integrator wanted to use practical corn and soybean meal diets, while another wanted to include wheat. Two commercial carbohydrase enzymes were tested in this stud. The first was a multi-carbohydrase enzyme, thought to target multiple complex carbohydrates that can be found within multiple feedstuffs including corn, wheat, soybean meal, and meat and bone meal utilized in the commercial diets. The second was a primary xylanase enzyme, whose substrate is arabinoxylans, which is the main NSP comprising cereal grains. The objective of this study was to compare the performance of birds fed two commercial diets (CD) that included commercially available carbohydrase enzymes (CE1 or CE2) at manufacturer matrix values and inclusion recommendations.

Materials and methods

Birds and House Management

This study was conducted in agreement with the Mississippi State University Institutional Animal Care and Use Committee (IACUC # 15-099). On day of hatch, Ross x Ross 708 male broiler chicks were obtained from a local hatchery (Peco Foods, Gordo, AL) and were equally distributed into 36 pens (23 birds/pen; 1.22 x 1.52 m). Water and feed were provided *ad libitum* in each pen using one drinking line (4 nipples/pen) and one tube-type feeder. Supplemental feed was also offered for the first 7 days in an additional feed tray. Pen bedding of 4 inches, consisted of used litter (~10 years old, 40 flocks) obtained from MSU commercial poultry houses. Stocking density increased throughout the study due to the removal of birds for gastrointestinal sampling. The

stocking density for each floor pen was as follows: 0.081m2 per bird (day 0 to day 14), 0.085m2 per bird (day 15 to day 28), 0.088m2 per bird (day 29 to day 43), and 0.093m2 per bird (day 44 to day 56).

Birds were housed within a newly renovated solid-walled experimental house located at the Mississippi State University poultry research farm. This facility was equipped with an Edge Controller (Cumberland, Assumption, IL). Environmental conditions were monitored daily and achieved through the use of cool cells (12 m), 3 infrared brooding heaters, and 4 fans (1.2 m). Temperature, ventilation, and lighting set points were made appropriate for bird age. The target temperature program used began at 32.2°C on d 0, dropped to 31.1°C on d 4, then from d 7 the target temperature dropped approximately 4 - 5 °C every 7 days ultimately reaching 18.3°C at d 35, to the end of study. Slight temperature adjustments were made as needed, based on bird observations. Illumination throughout the experimental house was provided by LED bulbs. The lighting program was as follows: 24 hours of full intensity lighting from d 0-7, from d 7-10 full intensity with 4 hours of dark, gradual dimming began d 10-11 with 4 hours of dark, and from d 18- 57 lights were at full dim (2.69 lux) with a dark period of 4 hours.

Commercial Diet Formulations

This study involved four treatments using variations of two different commercial diets (CD; CDA or CDB) and two different carbohydrase enzymes (NSP; CE1 - multi-carbohydrase enzyme or CE2 - xylanase enzyme). Diets were formulated upon the nutrient specifications and ingredients used by each integrator involved and obtained through direct communication with the coordinating nutritionist. All ingredients used were sourced by Mississippi State University, with the exception of integrator specific

micro-mix ingredients, which were obtained directly from each integrator's feed mill. Enzyme treatments were chosen based on integrator interest. Both CDA and CDB formulations utilized in this study were proprietary and were formulated with the coordinating integrator nutritionist approval. Due to differences in formulation strategies among integrators and enzyme matrix values, diets were not identical and therefore varied in: available/digestible nutrients, ingredients utilized, additives and supplements. The calculated values of diets are in Table 3.1.

Enzymes/Experimental Diet Preparation

Enzyme treatments were chosen based on integrator interest. Manufacturer recommended inclusion levels and matrix values were used for both enzymes. CE1, was a commercial multi-carbohydrase with activities of xylanase (2,400 U/g), amylase (24,000 U/g), protease (2,400 U/g), invertase (1,400 U/g), cellulose (1,000 U/g), glucanase (300 U/g), and mannanase (120 U/g) was added at 0.03% with a matrix value of 110 kcal/kg (Superzyme, Canadian Bio-Systems Inc., Alberta, Canada). CE2 was a commercial xylanase produced by Trichoderma longibrachiatum containing endo-1,4-β-xylanase and secondary activities of β-glucanase, α-amylase, and protease(15000 EPU/g) included at 0.01% with a matrix value of 77 kcal/kg (Hostazym X, Huvepharma, Inc., Peachtree City, GA). The unit EPU refers to the amount of released xylan at pH 4.7 and 50°C with one enzyme unit. A phytase enzyme (1500 FTU or phytase units) was also included at 0.1 g/kg with the matrix values 0.15 g/kg Na and 1.5 g/kg for both Ca and P in CDA and included at 0.3 g/kg with the matrix values 1.32 g/kg Ca, 1.2 g/kg AP, and 0.24 g/kg Na in CDB (Quantum Blue, AB Vista, Plantation, FL). Diets were proprietary, but both CDA and CDB utilized corn, soybean meal, a meat and bone meal (ProPlus 57,

H.J. Baker, Shelton, CT), an antibiotic (BMD-50, Zoetis, Parsippany, NJ), and an anticoccidial (Salinomycin 60 Premix, Bio Agri Mix, Ontario, Canada or Zoamix 25%, Zoetis, Parsippany, NJ). However, CDB also included wheat in the formulations (10-25%). CE1 was included at 0.03% with a matrix value of 110 kcal/kg, while CE2 was included at 0.01% with a matrix value of 77 kcal/kg.

Feed Manufacture

The treatment structure was as follows: treatment 1 (CDA + CE1), treatment 2 (CDA + CE2), treatment 3 (CDB + CE1), and treatment 4 (CDB + CE2). Initially, basal batches (CDA and CDB) without enzyme inclusions, were batched at the Mississippi State University Poultry Research Unit feed mill. Each of the 4 basal batches was mixed using a vertical screw mixer (0.907-tonne, Jacobson) for 5 minutes dry and an additional 10 minutes following the addition of fat. All diets were then transported to the U.S. Department of Agriculture (Poultry Research Unit in Starkville, MS) for pelleting. Before pelleting, carbohydrase enzymes were added to complete each treatment upon the removal of basal diet. This was then mixed for five minutes in a small horizontal mixer (11.34 kg capacity) before being added to the remaining coordinating batch to mix in a (907 kg capacity) horizontal mixer (907 kg capacity) for 4 minutes. Immediately following, a representative unconditioned mash sample was collected for each treatment.

Diets were steam conditioned for 10 seconds prior to pelleting in a 40 HP CPM pellet mill with a 38.1 × 4.76 mm pellet die. Steam temperature was monitored and did not rise above 80°C throughout the pelleting process. After the cooler, duplicate samples of finished feed treatments were collected throughout the run. Diets were offered as crumbles for the starter (d 0-14) phase, and pellets for the grower (d 14-28), finisher (d

28-43), and withdrawal (d 43-56) feeding phases. Unconditioned mash and finished feed samples were retained for enzyme retention and are currently under analysis.

Live Performance

Individual bird weights were recorded on d 14, 28, 43, and 56 immediately after the weighing of each pen's feeder. Performance variables such as body weight gain (BWG per bird), Average Body Weight (Avg BW), Feed Intake (FI per bird), Percent Mortality, and mortality corrected feed conversion ratio (FCR) were calculated from d 0-14, 14-28, 28-43, and 43-56. Additionally, weight adjusted FCR (Adjusted FCR) was performed to determine enzyme influence on d 0- 57 FCR with unbiased body weights. Similar to the technique used by Liang (2013) this adjusted FCR was calculated by assuming a 4.3 kg body weight for all birds using 7 points.

Processing

Four, randomly chosen, birds per pen were weighed and tagged following the d 56 weigh day. After 12 hours without feed, tagged broilers were cooped and processed on d 57 at the Mississippi State University poultry processing plant. Fresh, hot carcass and fat pad weights were recorded immediately after carcass removal from processing shanks. Carcasses were then chilled in ice water for 3 hours prior to deboning. Weights were recorded for the deboned parts: boneless skinless breast, tenders, total breast (breast + tender), thighs, drumsticks, and wings. Yields for the all weights were calculated using live bird weight and carcass weight.

Gastrointestinal Sampling

Immediately following weigh days on d 14, 28, and 43, one random bird per pen was tagged and weighed for gastrointestinal tract sampling. Before weighing in new phase diets, selected birds were moved from floor pens to battery cages (61 cm × 46 cm) within a solid room and placed back on their original feeding phase diets. Birds were grouped by treatment with 3 battery cages for every treatment (3 birds/cage) until d 43 when 5 battery cages for treatment (4 with 2 birds/cage + 1 with 1 bird/cage) were used to accommodate for bird size. Feed and water were supplied ab libitum through the use of 2 nipple drinkers and 1 feeder in each cage. The battery cage room was climate controlled using a central heat and air unit and two small stir fans (14 inches) for ventilation. The battery room lighting and temperature followed the same schedule described for the floor-pen experimental solid-walled poultry house.

On day of sampling, which took place on d 15, 29, and 46 the chosen birds were weighed and euthanized using carbon dioxide. Following euthanasia, the duodenum, jejunum, and ileum (meckel's diverticulum to ileo-cecal junction), were removed section lengths were measured in centimeters. Digesta content was removed by gently squeezing the duodenum, jejunum, ileum, and gizzard prior to weighing; section weights were also recorded as a percentage of live weight.

Starch Digestibility

To determine starch digestibility, finisher d 28- 43 pelleted diets were ground and titanium dioxide was added at 0.3% and mixed thoroughly using a small horizontal mixer (44.1 kg capacity). These diets were fed to broilers moved to the battery room on d 43 until ileum digesta was collected on d 46 during gastrointestinal sampling. Ileum digesta

samples (108 total; 1 bird/pen) were frozen in a -20°C before freeze drying (FreeZone Freeze Dry System, Labconco, Kansas City, MO). After freeze drying, digesta samples were ground using a 20mm sieve and remained in the enclosed plastic sample storage cups with lids. Feed samples with titanium dioxide inclusion and ground digesta samples were sent to a laboratory (ATC Scientific, North Little Rock, AR) and analyzed for starch using Ewer's procedure (Mitchell, 1990) and titanium dioxide content, The formula used to calculate digestibility is below:

$((Starch_{diet}/TiO^2_{diet})-(Starch_{digesta}/TiO^2_{digesta}))/ (Starch_{diet}/TiO^2_{diet}) \times 100$

Economic Analysis

To calculate potential cost savings/profit for each of the enzymes tested, an economic analysis was performed in the following manner:

1. Diet costs were calculated using current ingredient costs obtained through Feedstuffs (Feedstuffs.com/ingredient-prices), USDA (ers.usda.gov), and/or MSU suppliers (Nutra Blend, LLC, Neosho, MS and Ware Milling, Inc., Houston, MS). Enzyme costs were estimated and set at $7,000/ton.
2. Production cost per bird was calculated using these estimated diet costs, and feed intake per bird.
3. Potential gross profit was determined using amount of processing meat parts produced per enzyme treatment and chicken part prices obtained from Georgia Dock (agr.georgia.gov)
4. Comparisons were made between the estimated production cost and potential gross profit for each diet and tested enzyme to determine a general cost for each enzyme, depending upon CD.

Statistical Analysis

This study utilized a 2 carbohydrase enzyme (CE1 or CE2) x 2 commercial diet (CDA or CDB) factorial arrangement within a randomized complete block design. Each treatment was replicated 9 times and pen location within the house was considered to be

the blocking factor. The experimental unit was one floor pen containing birds, and the experimental period was from d 0- 57. All of the measured variables were analyzed using the GLM procedure in SAS (Version 9.4). Significance was set at $P \leq 0.05$.

Results

Live Performance

Performance variables during d 0-14, d 14-28, d 28-43, d 43-56, and d 0-56 can be found in Tables 3.2, 3.3, 3.4, 3.5, and 3.6, respectively.

CE × CD Interactions

A significant CE × CD interaction occurred for d 0-14 CV in which birds fed CE1+CDB demonstrated better uniformity and birds fed CE2+CDB demonstrated the least uniformity (P=0.0231). Intermediate CV values were demonstrated for CE1+CDA and CE2+CDA. A trend for CE × CD interaction was observed for the variable Avg BW during d 0-14 with birds receiving CDB diets with CE1 inclusion achieving higher weights (P=0.081). No other significant CE × CD interactions occurred for any other live performance variables throughout the study (P>0.05).

CE effects

The main effect of CE was not significant for any performance variables measured during d 0-14. However, FI/bird and FCR during d 14-28 was significant with CE with a higher feed intake with CE1, while feed efficiency was exhibited with CE2 with an improved FCR by 1 pt (P=0.0087 and P=0.0387, respectively; Table 3.3). BWG, Avg BW, and percent mortality were not affected by CE during d 14-28 (P>0.05). Though d 28-43 BWG, Avg BW, FI/bird, and percent mortality were not influenced

(P>0.05), CE was found significant for FCR with a 3 pt improvement for birds fed CE2 in comparison to those fed CE1 (P=0.0072; Table3.4). No significance was observed for CE during d 43-56 and d 0-56 (P>0.05).

CD effects

The main effect of CD was found to be significant for BWG, Avg BW, and FCR throughout each phase of growth and d 0-57 (P≤0.05). During d 0-14 BWG and Avg BW were highest in birds fed CDB diets (P=0.001 and P=0.0003, respectively). In addition, d 0-14 FCR was improved by 3pts (P<0.0001; Table 3.2). The variables FI/bird, percent mortality, and CV were not affected by CD during d 0-14 (P>0.05). The main effect of CD was significant for d 14-28 BWG, Avg BW, and FI/bird with higher values obtained with CDB (P<0.0001). In addition, birds fed CDB also had significantly improved FCR by 5 pts (P<0.0001; Table 3.3). The variables percent mortality and CV were not significantly affected by CD during d 14-28 (P>0.05). During d 28-43, CD was found to be significant for BWG and Avg BW with birds exhibiting higher weights with CDB (P=0.0088 and P=<0.0001, respectively). During d 28-43, FCR was also influenced by CD with a 5 pt improvement observed for birds fed CDB (P=0.0001; Table 3.4). The variables FI/bird, percent mortality, and CV were not significant affected by CD during d 28-43 (P>0.05). The main effect of CD was significant for BWG and Avg BW during d 43-56 with birds fed CDB exhibiting higher weights (P=0.007 and P<0.0001, respectively; Table 3.5). In addition, FCR was significantly affected by CD with a 3 pt improvement displayed in birds fed CDB (P=0.003). Percent mortality, FI/bird, and CV were not significantly affected by CD during d 43-56 (P>0.05). Overall (d 0-56), CD significantly affected BWG and Avg BW with higher weights achieved with CDB

(P<0.0001; Table 3.6). The main effect of CD was found significant for FCR and weight adjusted FCR with 10 and 15 pt improvements, respectively observed for birds fed CDB (P<0.0001). The variables FI/bird and percent mortality were not significantly affected by CD during d 0-56 (P>0.05).

Processing

Processing characteristic results can be found as live weight yield (Table 3.7), carcass weight yield (Table 3.8) or average weight (Table 3.9).

CE × CD Interactions

No significant CE × CD interactions were found for any yields relative to live weight, carcass weight, or average weights of processing characteristics (P>0.05).

CE effects

The main effect of CE enzyme was found to be significant for fat yield relative to live weight (P=0.050; Table3.7), as well as relative to carcass weight (P=0.043; Table 3.8). Higher abdominal fat yields were exhibited in birds fed CE2, as compared to those fed CE1. The main effect of CE was not found to be significant for any other additional processing characteristics (P>0.05).

CD effects

The main effect of CD was found to be significant for tender yield relative to live weight and carcass weight (P=0.029; P=0.018, respectively). For both, tender yields were higher in birds fed CDA than in birds fed CDB. No other significant CD influence was observed for carcass yield, total breast yield, fat yield, wing yield, breast yield, thigh yield, or drumstick yield relative to carcass or live weight (P>0.05; Table 3.7). Average

weights of carcass (P=0.0002), total breast (P=0.015), wing (P=0.005) and thigh (P=0.003) were significantly increased with CE with higher weights exhibited in those fed CDB in comparison to those fed CDA (Table 3.9).

Gastrointestinal Sampling

Gastrointestinal sampling results for d 15, d 29, and d 46 can be found in tables 3.10-3.12, respectively.

CE × CD Interactions

Although no significant d 15 gastrointestinal sampling CE x CD interactions were observed, a strong trend was demonstrated for jejunum % BW in which birds consuming CE1+CDA and CE2+CDB had the highest yields, while those fed CE1+CDB were lowest (P=0.095; Table 3.10). No CE x CD interactions were observed for any of the measured variables during d 15 gastrointestinal sampling (P>0.05). A significant CE x CD interaction was observed for d 29 ileum length with longer ileums observed in birds fed CE2 +CDA, whereas CE1+ CDA and CE2+CDB were the shortest (P=0.027; Table 3.11). Intermediate and similar ileum lengths were found in birds consuming CE1+CDB. No CE x CD interactions were observed for any other measured variables for d 29 gastrointestinal sampling (P>0.05). A significant CE × CD interaction occurred for d 46 duodenum weight, which was highest in birds fed CE2+CDB in comparison to those fed CE1+CDA, CE1+CDB, and CE2+CDA (P=0.001; Table 3.12). In addition, a significant CE × CD interaction demonstrated highest d 46 duodenum % BW in birds fed CE2 + CDA, while lowest % BW were found with CE1+CDA, CE1+CDB, CE2+CDB (P=0.001). The main effect of CD was found to be significant for d 46 jejunum length

with CE1+CDB & CE2+CDA longest, CE2+CDB shorter and those fed CE1+ CDA intermediate (P=0.006). Ileum length was also influenced by an interaction between CE × CD with CE2+CDA the longest, CE1+CDA and CE2+CDB the shortest for d 46 (P=0.019). No CE x CD interactions were observed for any other measured variables during d 46 gastrointestinal sampling (P>0.05).

CE effects

The main effect of CE was not significant for any d 15 or d 29 gastrointestinal sampling variables (P>0.05; Tables 3.10 and 3.11, respectively). The main effect of CE was found significant for d 46 duodenum weight and duodenum % BW (P=0.0002; Table 3.12). Duodenums were higher and longer with CE2 supplementation. All other d 46 gastrointestinal sampling variables were found not significant (P>0.05).

CD effects

The main effect of CD influenced gizzard yield for d 15 with yields found with birds receiving CDA (P=0.009; Table 3.10). Although not significant, a trend was observed for d 15 BW with birds fed CDB heavier than those fed CDA (P=0.062). A trend for CD was also observed for d 15 gizzard weight with those from birds fed CDA weighing more than those from birds fed CDB (P=0.0867).The main effect of CD was found significant on d 29 sampling for duodenum weight and duodenum %BW with those with CDA observed to be heavier than those with CDB (P=0.003 and P=0.001, respectively). Jejunum % BW on d 29 was significantly influenced by CD with larger jejunum yields found with CDA, while those with CDB were lower (P=0.036; Table 3.11). In addition, CD was found significant for d 29 ileum weight and ileum % BW with

heavier weights and yields found with CDA, whereas those with CDB were lower (P=0.021 and P=0.042, respectively). Gizzard pH on d 29 sampling was significantly affected by CD with higher pH found in those birds fed CDA and lower pH found in those birds fed CDB (P=0.004). Although not significant, a strong trend was demonstrated for jejunum length with longer jejunums found in birds consuming CDA in comparison to those fed CDB (P=0.055). The variables BW, duodenum length, jejunum length, jejunum weight, ileum length, ileum pH, gizzard weight and gizzard yield were not significantly affected by the main effect of CD for d 29 gastrointestinal sampling (P>0.05). Jejunum % BW on d 46 was significantly affected by CD with higher yields found in birds fed CDA, whereas those fed CDB has lower yields (P=0.022; Table 3.12). The main effect of CD was found to be significant for ileum weight and ileum % BW with higher values found with those fed CDA in comparison to those fed CDB (P=0.008 and P=0.001, respectively). Although not significant, strong trends were demonstrated for duodenum length (P=0.070), jejunum length (P=0.070) and jejunum weight (P=0.098) in which birds fed CDA displayed higher values, whereas birds fed CDB had lower values.

Starch Digestibility

The results for d 46 starch digestibility can be found in Table 3.13.

CE × CD Interactions

No significant CE × CD Interactions were observed for starch digestibility on d 46 (P>0.05).

CE effects

Though not significant, a trend was demonstrated for d 46 starch digestibility with CE2 displaying higher starch disappearance than that of CE1 (P=0.061).

CD effects

The main effect of CD was not significant for d 46 starch digestibility (P>0.05).

Economic Analysis

The results of the economic analysis for CDA and CDB can be found in Figures 3.1 and 3.2, respectively.

The economic analysis used for the current study utilizes feed cost on a feed intake basis in conjunction with realistic ingredient and chicken part monetary values in order to demonstrate practical integrator benefits. As a result of supplementation of CE2, both CDA and CDB exhibit higher potential gross profits for 1 million birds. However, CE1 supplementation in CDA resulted in slightly higher amount of breast meat, causing the total chicken part profit to rise.

Discussion

Live Performance/Processing

Within the poultry industry, it is common for integrators to have varied feed formulations due to a variety of factors: company production goals, location, market, etc. Limitations regarding feed costs and feed ingredient availability can also influence formulation differences between companies (Kleyn, 2013). Therefore, while CDA and CDB effects were measured in this study, it was not the main objective of this study to

compare differences between CD, as they were expected to demonstrate performance differences.

To explain CE differences and interactions between CE and CD, it is important to consider the dietary constituents and specifications of each CD, as it has been speculated that enzyme effectiveness is largely dependent on substrate availability (Angel and Sorbara, 2014). In the present study, CDA diets contained higher energy throughout all feeding phases and higher digestible amino acid levels during d 14-28, whereas CDB contained higher digestible amino acid levels during d 43-56. The major ingredient variant between the two diets was a small portion of wheat included in CDB diets at 0.10 kg/kg-0.25kg/kg. As a reminder, the matrix values for each CE were utilized, which were 110 kcal/kg and 77 kcal/kg for CE1 and CE2, respectively. Additionally, the matrix values assigned to the phytase enzyme (0.15 g/kg Na, 1.5 g/kg Ca, 1.5 g/kg P) were also used with both CDA and CDB.

Carbohydrase enzymes mainly act upon the two main NSPs found in plant fiber, arabinoxylans and β-glucans; the amounts of these vary between ingredient sources, with higher total NSPs and soluble arabinoxylans found in wheat, whereas corn contains less than 1% soluble arabinoxylans (Kleyn, 2013; Knudsen, 2014). Arabinoxylans are the main substrate of xylanases, which may explain why its supplementation has been reported to perform more effectively in wheat-based broiler diets as compared to corn-based diets (Wu et al., 2004a; Choct and Annison, 1990).

When included in corn/soy diets, xylanase and other NSPase enzymes contribute inconsistent results. Kiarie and others (2014) reported that xylanase (1250 EPU) and phytase (500 FTU) supplementation in a corn or wheat diet, both improved bird

performance when compared to the non-enzyme inclusion control, but the wheat-based diet resulted in increased performance in comparison to the corn diet. Overall, supplementation of xylanase did result in BWG and FCR improvement and it was speculated that the difference is due to variations of NSP between corn and wheat (Kiarie et al., 2014). Similar results were observed in the current study, with improved growth performance for birds fed CDB, although it should be noted that the CDB utilized in the current study was corn/soy based with wheat inclusion, instead of heavily wheat-based.

Multiple enzyme use is theorized to be more effective for various dietary formulations because of their possible synergistic or additive interactions. In the present study, regardless of CD, the use of the multi-carbohydrase (CE1) resulted in significantly better BW CV during d 0-14 (Table 3.2). Additionally birds receiving CE1 exhibited a higher FI during d 14-28 (Table 3.3), which contributed to numerically higher BWG and BW for these birds throughout the study. Previous research has reported similar findings in that the combination of an enzyme cocktail (xylanase, amylase, and protease) plus a phytase enzyme in corn/soy based diets improved broiler weight gain (Cowieson and Adeola, 2005). The only significant influence on growth performance with CE inclusion was observed from early in the experimental period. In agreement, Olukosi and others, 2007 observed improved performance in younger birds when feeding diets supplementing phytase and an enzyme cocktail in combination and it was speculated that exogenous enzyme blends are more beneficial for an immature gastrointestinal tract.

Although CDB had lower energy levels, the resulting improved growth performance and efficiency observed throughout the study, indicate that both CE1 and CE2 were able to release energy in the diet. Previous research has shown that xylanase

supplementation in various poultry diets improves energy digestibility (Choct et al., 1995; Nian et al., 2011; Liu et al., 2012; Pirgozliev et al, 2015). Similar results can also be found with multi-carbohydrase supplementation (Francesch and Geraert, 2009; Józefiak et al., 2010).

Diets CDB had lower energy specifications throughout the study compared to diets CDA, but the resulting performance indicates this was not an issue. Poultry are known to adapt their consumption to meet their energy needs (Richards and Proszkowiec-Weglarz, 2007), therefore the consistently lower FI and improved FCR from d 14-57 observed in birds fed diets with CE2 in the current study is likely due to the ability of xylanase to increase energy availability. In further evidence of this notion, CE2 inclusion, regardless of CD resulted in increased fat pad yields on d 57, which is indicative of increased dietary energy (Tables 7 and 8). However, in contrast to our results, Campasino and others (2015) did not observe increased fat pad weights with NSPase (xylanase, beta-glucanase, and alpha-galactosidase) supplementation in low energy diets.

Gastrointestinal Sampling

Gastrointestinal characteristics are hard to make sense of in regards to the performance differences observed in the current study. CE and CD interacted to contribute to longest ileums for d 29 and d 46 in birds receiving CE2 +CDA, whereas those receiving CE1+CDA and CE2+CDB had significantly shorter lengths (Tables 3.11 and 3.12). Though none of our diets were solely wheat-based, Wang and others (2005) also reported elongated ileums for broilers fed wheat diets supplemented with xylanase and B-glucanase on d 21 and d 42. However, Engberg and cohorts (2004) in which xylanase supplementation reduced jejunum and ileum weights. Additionally, birds fed

CE2+CDA displayed higher jejunum length and duodenum yields, while the heaviest duodenum sections were found in birds fed CE2+CDB (Table 3.12). Research associated longer and heavier intestinal sections with high dietary NSP levels (Viveros et al., 1994; Smits et al., 1997) and although NSP levels of the diets were not analyzed, maybe the addition of CE2 was not as effective at hydrolyzing NSP las well as the addition of CE1 in CDA.

Starch Digestibility

Starch digestibility was measured on d 46 to evaluate the effective release of energy from each treatment, and as a result a strong trend was demonstrated for broilers fed diets including CE2 (P=0.0613; Table 13). Digestion of starch provides broilers with glucose for energy used for maintenance and production and compared to NSPs, starch is highly digestible with ranges found above 0.9, even in diets containing whole grains (Svihus, 2014). In the current study, the improved FCR and increased fat yields discussed earlier are most likely a result of the higher digestibility observed with CE2 inclusion. The authors speculate that perhaps the amylase activity in CE2 is more potent than the amylase in CE1, given that amylase targets the high amounts of amylose found in the two starch variants: amylose and amylopectin (Moran, 1982). Similar findings were reported by Stefanello and others (2016) in which the addition of amylase and xylanase increased starch digestibility of corn-based diets in the ileum.

Economic Analysis

The economic analysis conducted for the present study utilized chicken part pricing and ingredient pricing at the current period of time. These are subject to

fluctuation in price due to demand, production, and consumers, therefore this economic analysis is not meant to be a concrete determination of monetary enzyme benefits. In order to visualize enzyme effects in an economic setting, all ingredient and enzyme prices were set equal. Therefore, the resulting possible profits are the result of ingredient inclusion difference, feed intake, growth performance, and processing characteristics of CE use. The ability of CE2 to maintain high BW and lower FCR is apparent with potential gross profit using 1 million birds being $80,956 and $168,563 more in CDA and CDB, respectively than the potential profit using CE1. The use of CE1 results in a reduction of in CDA and CDB resulted in a profit reduction of $0.081/bird and $0.169/bird, respectively. However, due to variations in commercial integrator objectives, the use of CE1 resulted in higher breast meat yields for CDA. It seems as though market values should be accounted for when deciding which commercial carbohydrase fits best.

Conclusions

Live performance, gastrointestinal sampling, and processing were all influenced by the main effect of CD. However, variations should be expected due to integrator production goals and ingredient preferences. For the purpose of this study, it is important to evaluate the main effect CE, as well as any CE × CD interactions. In the current study, CE significantly affected FI during d 14-28 and FCR d 14-43, with higher FI observed with CE1, while improved FCR was observed with CE2 supplementation. The lack of significance in all other performance variables indicates that either CE can be utilized by both integrators for similar performance outcomes. Though both CE1 and CE2 demonstrated improved energy utilization through similar starch digestibility, CE2 may be more useful for low energy diets as evident from decreased FI and increased fat yields.

However, the economic analysis revealed that the lowered FI and improved FCR exhibited with CE2 inclusion offer more potential gross profits than the use of CE1. In conclusion, integrators who frequently substitute small proportions of corn for wheat or feed corn-based diets can effectively benefit from supplementation of CE2.

Table 3.1 Analyzed nutrient composition of experimental diets

	Starter				Grower				Finisher				Withdrawal			
	Treatment															
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
CP	22.814	21.514	22.911	22.893	19.513	19.631	20.619	20.602	17.227	16.605	20.287	20.294	15.828	15.878	19.888	19.897
AME	1404.3	1404.3	1329.8	1344.8	1439.0	1430.0	1350.0	1365.0	1445.0	1440.0	1385.0	1400.0	1463.2	1460.2	1395.0	1410.0
CA	0.919	0.923	0.877	0.877	0.893	0.857	0.551	0.551	0.850	0.821	0.718	0.718	0.798	0.770	0.718	0.718
P	0.582	0.569	0.594	0.595	0.573	0.566	0.529	0.528	0.559	0.540	0.614	0.613	0.517	0.522	0.588	0.587
Na	0.180	0.179	0.196	0.196	0.216	0.221	0.196	0.196	0.301	0.272	0.206	0.206	0.301	0.299	0.176	0.176

1 (CDA + CE1), treatment 2 (CDA + CE2), treatment 3 (CDB + CE1), and treatment 4 (CDB + CE2).

Table 3.2 Live performance from d 0-14 of male broilers fed 2 different carbohydrase enzymes in varying commercial diets

Carbohydrase Enzyme	Commercial Diet	BWG[1] (kg/bird)	D14 Avg[2] BW[3] (kg)	FI[5]/bird (kg)	Percent Mortality[6]	FCR[7]	CV (%)
CE1	**CDA**	0.416	0.462	0.500	0.483	1.201	**9.101[ab]**
	CDB	0.441	0.491	0.515	1.932	1.171	**7.828[b]**
CE2	**CDA**	0.415	0.461	0.504	1.450	1.209	**8.182[ab]**
	CDB	0.428	0.474	0.499	0.483	1.171	**9.287[a]**
SEM[8]		0.005	0.005	0.007	0.754	0.007	0.486
Carbohydrase Enzyme							
CE1		0.428	0.474	0.507	1.208	1.186	8.482
CE2		0.422	0.468	0.502	0.966	1.190	8.735
SEM		0.004	0.003	0.005	0.533	0.005	0.343
Commercial Diet							
CDA		**0.416[b]**	**0.461[b]**	0.502	0.966	**1.205[a]**	8.669
CDB		**0.434[a]**	**0.481[a]**	0.507	1.208	**1.171[b]**	8.558
SEM		0.004	0.003	0.005	0.533	0.005	0.343
P-Values							
Carbohydrase		0.159	0.089	0.491	0.752	0.686	0.5860
CD		**0.0006**	**0.0003**	0.547	0.752	**<0.0001**	0.865
Carbohydrase x CD		0.134	0.081	0.162	0.122	0.706	**0.023**

[1]Body Weight Gain
[2]Average
[3]Body Weight
[4]Feed Intake is based on a per pen basis
[5]Feed Intake is based on a per bird basis
[6]Mortality percentage is based on a beginning pen number of birds per pen (excluding birds used for sampling), thus if 1 bird dies in a pen (20 birds) the resulting mortality percentage would be 5%
[7]Feed Conversion Ratio (Feed:Gain) was adjusted with mortality weight
[8]Standard error of the mean
a-b Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 3.3 Live performance from d 14-28 of male broilers fed 2 different carbohydrase enzymes in varying commercial diets

Carbohydrase Enzyme	Commercial Diet	BWG[1] (kg/bird)	D28 Avg[2] BW[3] (kg)	FI[5]/bird (kg)	Percent Mortality[6]	FCR[7]	CV (%)
CE1	CDA	1.040	1.501	1.604	1.515	1.576	10.783
	CDB	1.135	1.621	1.696	1.010	1.525	10.850
CE2	CDA	1.035	1.487	1.567	0.505	1.555	10.346
	CDB	1.122	1.596	1.656	1.539	1.506	11.881
SEM[8]		0.009	0.012	0.013	0.726	0.009	0.500
Carbohydrase Enzyme							
CE1		1.085	1.558	**1.647[a]**	1.263	**1.549[a]**	10.816
CE2		1.084	1.545	**1.614[b]**	1.022	**1.532[b]**	11.092
SEM		0.007	0.009	0.009	0.513	0.007	0.354
Commercial Diet							
CDA		**1.038[b]**	**1.495[b]**	**1.587[b]**	1.010	**1.565[a]**	10.376
CDB		**1.128[a]**	**1.608[a]**	**1.675[a]**	1.275	**1.516[b]**	11.217
SEM		0.007	0.009	0.009	0.513	0.007	0.354
P-Values							
CE		0.134	0.102	**0.009**	0.743	**0.039**	0.562
CD		**<0.0001**	**<0.0001**	**<0.0001**	0.719	**<0.0001**	0.128
CE x CD		0.820	0.564	0.799	0.300	0.899	0.161

[1]Body Weight Gain
[2]Average
[3]Body Weight
[4]Feed Intake is based on a per pen basis
[5]Feed Intake is based on a per bird basis
[6]Mortality percentage is based on a beginning pen number of birds per pen (excluding birds used for sampling), thus if 1 bird dies in a pen (20 birds) the resulting mortality percentage would be 5%
[7]Feed Conversion Ratio (Feed:Gain) was adjusted with mortality weight
[8]Standard error of the mean
a-b Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 3.4 Live performance from d 28-43 of male broilers fed 2 different carbohydrase enzymes in varying commercial diets

Carbohydrase Enzyme	Commercial Diet	BWG[1] (kg/bird)	D43 Avg[2] BW[3] (kg)	FI[5]/bird (kg)	Percent Mortality[6]	FCR[7]	CV (%)
CE1	CDA	1.756	3.257	2.956	0.529	1.758	9.843
	CDB	1.778	3.377	2.950	1.085	1.720	8.386
CE2	CDA	1.758	3.262	2.912	1.058	1.744	9.243
	CDB	1.832	3.412	2.903	1.058	1.675	8.831
SEM[8]		0.016	0.020	0.024	0.665	0.011	0.644
Carbohydrase Enzyme							
CE1		1.766	3.314	2.953	0.807	**1.740[a]**	9.115
CE2		1.793	3.337	2.907	1.058	**1.706[b]**	9.055
SEM		0.011	0.014	0.017	0.470	0.008	0.455
Commercial Diet							
CDA		**1.757[b]**	**3.260[b]**	2.935	0.794	**1.752[a]**	9.591
CDB		**1.805[a]**	**3.395[a]**	2.927	1.071	**1.697[b]**	8.610
SEM		0.011	0.014	0.017	0.470	0.008	0.455
P-Values							
CE		0.329	0.567	0.069	0.709	**0.007**	0.905
CD		**0.009**	**<0.0001**	0.743	0.680	**0.0001**	0.163
CE x CD		0.395	0.723	0.980	0.680	0.129	0.428

[1] Body Weight Gain
[2] Average
[3] Body Weight
[4] Feed Intake is based on a per pen basis
[5] Feed Intake is based on a per bird basis
[6] Mortality percentage is based on a beginning pen number of birds per pen (excluding birds used for sampling), thus if 1 bird dies in a pen (20 birds) the resulting mortality percentage would be 5%
[7] Feed Conversion Ratio (Feed:Gain) was adjusted with mortality weight
[8] Standard error of the mean
a-b Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 3.5 Live performance from d 43-56 of male broilers fed 2 different carbohydrase enzymes in varying commercial diets

Carbohydrase Enzyme	Commercial Diet	BWG[1] (kg/bird)	D56 Avg[2] BW[3] (kg)	FI[5]/bird (kg)	Percent Mortality[6]	FCR[7]	CV (%)
CE1	CDA	1.266	4.523	3.277	1.754	3.036	10.239
	CDB	1.421	4.782	3.331	4.591	2.722	11.841
CE2	CDA	1.236	4.402	3.163	1.725	2.952	9.605
	CDB	1.382	4.794	3.294	3.421	2.720	10.923
SEM[8]		0.050	0.049	0.046	1.252	0.075	0.895
Carbohydrase Enzyme							
CE1		1.343	4.645	3.302	3.173	2.888	11.040
CE2		1.314	4.610	3.232	2.573	2.821	10.107
SEM		0.035	0.035	0.032	0.885	0.053	0.633
Commercial Diet							
CDA		**1.252[b]**	**4.467[b]**	3.223	1.740	**2.999[a]**	9.922
CDB		**1.401[a]**	**4.789[a]**	3.311	4.006	**2.721[b]**	11.290
SEM		0.035	0.035	0.032	0.885	0.053	0.633
P-Values							
CE		0.513	0.290	0.216	0.636	0.491	0.398
CD		**0.007**	**<0.0001**	0.114	0.083	**0.003**	0.119
CE x CD		0.917	0.199	0.426	0.653	0.794	0.876

[1]Body Weight Gain
[2]Average
[3]Body Weight
[4]Feed Intake is based on a per pen basis
[5] Feed Intake is based on a per bird basis
[6] Mortality percentage is based on a beginning pen number of birds per pen (excluding birds used for sampling), thus if 1 bird dies in a pen (20 birds) the resulting mortality percentage would be 5%
[7] Feed Conversion Ratio (Feed:Gain) was adjusted with mortality weight
[8] Standard error of the mean
a-b Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 3.6 Live performance from d 0-56 of male broilers fed 2 different carbohydrase enzymes in varying commercial diets

Carbohydrase Enzyme	Commercial Diet	BWG[1] (kg/bird)	D56 Avg[2] BW[3] (kg)	FI[5]/bird (kg)	Percent Mortality[6]	FCR[7]	Adjusted FCR
CE1	CDA	4.326	4.523	8.336	4.444	1.940	1.905
	CDB	4.574	4.782	8.460	8.889	1.856	1.790
CE2	CDA	4.211	4.402	8.226	5.000	1.947	1.948
	CDB	4.586	4.794	8.323	6.667	1.830	1.761
SEM[8]		0.047	0.049	0.059	1.647	0.015	0.019
Carbohydrase Enzyme							
CE1		4.443	4.645	8.398	6.667	1.898	1.847
CE2		4.409	4.610	8.272	5.833	1.888	1.847
SEM		0.033	0.035	0.042	1.165	0.011	0.013
Commercial Diet							
CDA		**4.272[b]**	**4.466[b]**	8.281	4.722	**1.944[a]**	**1.924[a]**
CDB		**4.580[a]**	**4.789[a]**	8.396	7.778	**1.842[b]**	**1.770[b]**
SEM		0.033	0.035	0.048	1.165	0.011	0.013
P-Values							
CE		0.290	0.290	0.066	0.618	0.469	0.730
CD		**<0.0001**	**<0.0001**	0.057	0.076	**<0.0001**	**<0.0001**
CE x CD		0.199	0.199	0.937	0.408	0.239	0.074

[1]Body Weight Gain
[2]Average
[3]Body Weight
[4]Feed Intake is based on a per pen basis
[5]Feed Intake is based on a per bird basis
[6]Mortality percentage is based on a beginning pen number of birds per pen (excluding birds used for sampling), thus if 1 bird dies in a pen (20 birds) the resulting mortality percentage would be 5%
[7]Feed Conversion Ratio (Feed:Gain) was adjusted with mortality weight
[8]Standard error of the mean
a-b Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 3.7 Processing Characteristics on d 57 reported as average yield relative to d 56 live weight (%)

Carbohydrase Enzyme	Commercial Diet	Carcass Yield[1]	Total Breast Yield[2]	Fat Yield[3]	Wing Yield[4]	Breast Yield[5]	Tender Yield[6]	Thigh Yield[7]	Drumstick Yield[8]
CE1	**CDA**	76.046	26.804	1.120	7.565	22.435	4.369	11.955	9.431
	CDB	75.378	26.299	1.152	7.549	22.050	4.249	12.386	9.396
CE2	**CDA**	75.692	26.504	1.264	7.692	21.898	4.606	12.641	9.790
	CDB	76.242	26.886	1.309	7.694	22.653	4.233	12.390	13.890
SEM[8]		0.661	0.496	0.073	0.130	0.428	0.106	0.296	2.146
Carbohydrase Enzyme									
CE1		75.712	26.551	**1.136[b]**	7.557	22.243	4.309	12.170	9.413
CE2		75.967	26.695	**1.287[a]**	7.693	22.276	4.420	12.515	11.840
SEM		0.468	0.351	0.052	0.092	0.303	0.075	0.210	1.517
Commercial Diet									
CDA		75.869	26.654	1.192	7.629	22.166	**4.488[a]**	12.298	9.611
CDB		75.810	26.593	1.231	7.621	22.352	**4.241[b]**	12.388	11.643
SEM		0.468	0.351	0.052	0.092	0.303	0.075	0.209	1.517
P-Values									
CE		0.703	0.774	**0.050**	0.308	0.939	0.307	0.256	0.269
CD		0.930	0.903	0.601	0.957	0.669	**0.029**	0.763	0.353
CE x CD		0.366	0.380	0.933	0.946	0.196	0.247	0.261	0.345

[1]Carcass (kg)/Body Weight (kg)*100
[2](Breast (kg) + Tender (kg))/Body Weight*100
[3]Fat Pad (kg)/Body Weight (kg) * 100
[4]Wings (kg)/Body Weight (kg)*100
[5]Breast (kg)/Body Weight (kg)*100
[6]Tenders (kg)/Body Weight (kg)* 100
[7]Thighs (kg)/Body Weight (kg)*100]
[8]Drumsticks *kg)/Body Weight (kg)*100
[9] Fisher's Least Significant Difference
[10]Standard error of the mean
a-b Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 3.8 Processing Characteristics on d 57 reported as average yield relative to carcass weight (%)

Carbohydrase Enzyme	Commercial Diet	Total Breast Yield[1]	Fat Yield[2]	Wing Yield[3]	Breast Yield[4]	Tender Yield[5]	Thigh Yield[6]	Drumstick Yield[7]
CE1	CDA	35.239	1.473	9.947	29.494	5.745	11.955	9.431
	CDB	34.867	1.525	10.031	29.232	5.635	12.386	9.396
CE2	CDA	35.025	1.670	10.163	28.939	6.086	12.641	9.790
	CDB	35.259	1.718	10.094	29.705	5.554	12.390	13.890
SEM[8]		0.566	0.091	0.172	0.497	0.126	0.296	2.146
Carbohydrase Enzyme								
CE1		35.053	**1.499[b]**	9.989	29.363	5.690	12.170	9.413
CE2		35.142	**1.694[a]**	10.129	29.322	5.820	12.515	11.840
SEM		0.400	0.064	0.122	0.351	0.089	0.209	1.517
Commercial Diet								
CDA		35.132	1.572	10.055	29.216	**5.916[a]**	12.298	9.611
CDB		35.063	1.621	10.063	29.468	**5.595[b]**	12.388	11.643
SEM		0.400	0.064	0.122	0.351	0.089	0.209	1.517
P-Values								
CE		0.877	**0.043**	0.425	0.935	0.313	0.256	0.269
CD		0.904	0.589	0.964	0.616	**0.018**	0.763	0.353
CE x CD		0.597	0.987	0.660	0.311	0.108	0.261	0.345

[1](Breast (kg) + Tender (kg))/Carcass Weight*100
[2]Fat Pad (kg)/Carcass Weight (kg) * 100
[3]Wings (kg)/ Carcass Weight (kg)*100
[4]Breast (kg)/ Carcass Weight (kg)*100
[5]Tenders (kg)/ Carcass Weight (kg)* 100
[6]Thighs (kg)/ Carcass Weight (kg)*100]
[7]Drumsticks *kg)/ Carcass Weight (kg)*100
[8] Fisher's Least Significant Difference
[9]Standard error of the mean
a-b Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 3.9 Processing characteristics on d 57 reported as average weight

Carbohydrase Enzyme	Commercial Diet	Carcass[1]	Total Breast[2]	Fat[3]	Wing[4]	Breast[5]	Tender[6]	Thigh[7]	Drumstick[8]
CE1	CDA	3.574	1.259	0.053	0.355	1.054	0.205	0.561	0.443
	CDB	3.798	1.325	0.058	0.380	1.111	0.214	0.624	0.473
CE2	CDA	3.523	1.234	0.059	0.358	1.020	0.214	0.588	0.455
	CDB	3.784	1.335	0.065	0.381	1.125	0.210	0.614	0.681
SEM[8]		0.055	0.032	0.004	0.006	0.027	0.006	0.013	0.101
Carbohydrase Enzyme									
CE1		3.686	1.292	0.055	0.367	1.082	0.210	0.593	0.458
CE2		3.654	1.285	0.062	0.370	1.072	0.212	0.601	0.568
SEM		0.039	0.022	0.003	0.004	0.019	0.004	0.009	0.072
Commercial Diet									
CDA		**3.548**[b]	**1.247**[b]	0.056	**0.356**[b]	**1.037**[b]	0.210	**0.575**[b]	0.449
CDB		**3.791**[a]	**1.330**[a]	0.061	**0.381**[a]	**1.118**[a]	0.212	**0.619**[a]	0.578
SEM		0.039	0.022	0.003	0.004	0.019	0.004	0.009	0.072
P-Values									
CE		0.569	0.815	0.086	0.733	0.715	0.671	0.526	0.286
CD		**0.0002**	**0.015**	0.132	**0.001**	**0.007**	0.695	**0.003**	0.217
CE x CD		0.738	0.593	0.972	0.925	0.386	0.257	0.181	0.345

[1]Body Weight (kg)
[2]Breast (kg) + Tender (kg)
[3]Fat Pad (kg)
[4]Fisher's Least Significant Difference
[5]Standard error of the mean
a-b Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 3.10 Gastrointestinal characteristics on d 15 of male broilers fed 2 different carbohydrase enzymes in varying commercial diets

Enzyme	Diet	Variable												
		BW[1]	Duodenum			Jejunum			Ileum			Gizzard		
		(kg)	Length (cm)	Weight (g)	% BW[2]	Length (cm)	Weight (g)	% BW[3]	Length (cm)	Weight (g)	% BW[4]	pH	Weight (g)	% BW[5]
CE1	CDA	0.552	23.778	5.806	1.052	47.632	10.828	1.963	45.889	7.128	1.292	2.689	14.302	2.565
	CDB	0.568	23.392	6.139	1.083	48.501	9.820	1.777	44.000	6.894	1.233	2.544	13.506	2.382
CE2	CDA	0.536	23.667	5.878	1.100	48.444	9.833	1.838	45.847	6.939	1.293	2.457	14.443	2.722
	CDB	0.562	23.444	6.234	1.110	49.889	10.256	1.926	48.222	6.972	1.305	2.426	13.556	2.419
SEM[8]		0.011	0.633	0.307	0.051	1.977	0.466	0.079	1.966	0.287	0.041	0.110	0.460	0.083
Carbohydrase Enzyme														
CE1		0.560	23.625	5.972	1.068	47.750	10.327	1.855	44.944	7.003	1.255	2.617	13.847	2.473
CE2		0.548	23.556	6.015	1.099	49.167	10.044	1.858	46.938	6.968	1.255	2.435	14.025	2.567
SEM		0.008	0.448	0.217	0.036	1.400	0.330	0.056	1.393	0.203	0.029	0.006	0.326	0.059
Commercial Diet														
CDA		**0.544**	**23.722**	5.842	**1.076**	48.000	10.331	1.901	45.625	7.033	1.292	2.575	14.356	**2.644**[a]
CDB		**0.565**	**23.438**	6.153	**1.090**	49.000	10.024	1.807	46.111	6.932	1.246	2.485	13.535	**2.400**[b]
SEM		0.008	0.448	0.217	0.036	1.398	0.329	0.056	1.393	0.203	0.029	0.006	0.326	0.059

Table 3.0 (Continued)

P-Values													
CE	0.341	0.964	0.788	0.483	0.587	0.557	0.886	0.307	0.850	0.385	0.128	0.840	0.265
CD	0.062	0.641	0.275	0.695	0.569	0.538	0.551	0.904	0.732	0.590	0.438	0.087	**0.009**
CE x CD	0.641	0.900	0.970	0.840	0.887	0.141	0.095	0.298	0.652	0.405	0.615	0.924	0.483

[1]Body Weight
[2]Duodenum (lb)/Body Weight (lb) * 100
[3]Jejunum (lb)/Body Weight (lb) * 100
[4]Ileum (lb)/Body Weight (lb) * 100
[5]Gizzard (lb)/Body Weight (lb) * 100
[6]Standard Error of the Mean
[a-b] Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 3.11 Gastrointestinal characteristics on d 29 of male broilers fed 2 different carbohydrase enzymes in varying commercial diets

Enzyme	Diet		Variable												
		BW	Duodenum			Jejunum			Ileum				Gizzard		
		(kg)	Length (cm)	Weight (g)	% BW[2]	Length (cm)	Weight (g)	% BW[3]	Length (cm)	Weight (g)	% BW[4]	pH	pH	Weight (g)	% BW[5]
CE1	CDA	1.691	33.638	13.111	0.783	62.444	24.043	1.414	**63.944[b]**	17.239	1.023	5.463	2.917	29.611	1.758
	CDB	1.668	31.944	12.156	0.693	61.700	21.610	1.240	**69.389[ab]**	15.098	0.915	5.423	2.503	29.185	1.769
CE2	CDA	1.696	31.889	14.393	0.830	71.667	23.694	1.398	**74.500[a]**	117.872	1.053	5.442	3.020	27.778	1.637
	CDB	1.741	32.355	12.596	0.705	61.050	22.261	1.264	**63.278[b]**	16.312	0.946	5.618	2.735	27.341	1.589
SEM[8]		0.037	0.832	0.598	0.036	2.742	1.160	0.067	3.508	0.736	0.049	0.268	0.103	1.210	0.083
Carbohydrase Enzyme															
CE1		1.678	32.765	12.628	0.745	61.625	22.791	1.337	66.667	16.307	0.977	5.420	2.697	29.194	1.756
CE2		1.719	32.059	13.348	0.773	67.059	22.978	1.337	69.029	17.185	1.007	5.551	2.899	27.485	1.610
SEM		0.026	0.588	0.423	0.025	1.939	0.821	0.047	2.481	0.521	0.035	0.191	0.073	0.856	0.059
Commercial Diet															
CDA		1.693	32.735	**13.688[a]**	**0.807[a]**	67.056	23.838	**1.401[a]**	69.222	**17.556[a]**	**1.038[a]**	5.453	**2.970[a]**	28.694	1.696
CDB		1.704	32.088	**12.351[b]**	**0.705[b]**	61.267	21.941	**1.258[b]**	66.324	**15.835[b]**	**0.936[b]**	5.546	**2.589[b]**	27.857	1.660
SEM		0.026	0.588	0.423	0.025	1.947	0.820	0.047	2.481	0.522	0.035	0.192	0.073	0.859	0.060

Table 3.11 (Continued)

P-Values														
CE	0.315	0.436	0.172	0.427	0.142	0.899	0.953	0.535	0.229	0.546	0.765	0.135	0.153	0.105
CD	0.768	0.474	**0.035**	**0.010**	0.055	0.114	**0.036**	0.422	**0.021**	**0.042**	0.813	**0.004**	0.730	0.841
CE x CD	0.375	0.212	0.496	0.640	0.094	0.675	0.770	**0.027**	0.701	0.999	0.711	0.551	0.996	0.728

1Body Weight
2Duodenum (lb)/Body Weight (lb) * 100
3Jejunum (lb)/Body Weight (lb) * 100
4 Ileum (lb)/Body Weight (lb) * 100
5Gizzard (lb)/Body Weight (lb) * 100
6Standard Error of the Mean
a-b Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 3.12 Gastrointestinal characteristics on d 46 of male broilers fed 2 different carbohydrase enzymes in varying commercial diets

Enzyme	Diet		Variable												
		BW[1]	Duodenum			Jejunum			Ileum				Gizzard		
		(kg)	Length (cm)	Weight (g)	% BW[2]	Length (cm)	Weight (g)	% BW[3]	Length (cm)	Weight (g)	% BW[4]	pH	pH	Weight (g)	% BW[5]
CE1	CDA	3.460	35.462	**14.644**[b]	**0.424**[b]	**64.183**[ab]	29.839	0.857	**62.778**[b]	23.639	0.679	6.663	2.773	35.718	1.033
	CDB	3.598	33.444	**15.896**[b]	**0.442**[b]	**68.835**[a]	29.735	0.835	**69.704**[ab]	21.386	0.597	6.663	2.642	35.711	0.989
CE2	CDA	3.597	35.147	**32.639**[b]	**0.887**[a]	**75.443**[a]	33.925	0.986	**75.443**[a]	27.644	0.791	6.748	2574	39.931	1.098
	CDB	3.622	33.556	**17.072**[a]	**0.470**[b]	**55.333**[b]	27.844	0.770	**65.889**[b]	20.944	0.578	6.719	3.126	35.767	0.988
SEM[8]		0.092	0.928	2.036	0.052	3.931	1.766	0.047	3.216	1.526	0.036	0.176	0.173	1.515	0.045
Carbohydrase Enzyme															
CE1		3.534	34.188	14.974	0.425	67.188	29.841	0.843	65.647	22.735	0.644	6.698	2.706	35.906	1.016
CE2		3.610	34.353	23.834	0.652	64.875	30.838	0.855	71.000	24.294	0.673	6.766	2.927	37.244	1.027
SEM		0.065	0.657	1.440	0.037	2.780	1.249	0.033	2.274	1.080	0.025	0.127	0.123	1.072	0.032
Commercial Diet															
CDA		3.524	35.200	22.469	0.626	70.933	31.959	**0.908**[a]	69.353	**25.642**[a]	**0.727**[a]	6.742	2.701	37.533	1.060
CDB		3.620	33.500	16.259	0.449	61.706	28.785	**0.797**[b]	67.294	**21.309**[b]	**0.590**[b]	6.714	2.892	35.739	0.987
SEM		0.065	0.659	1.440	0.037	2.785	1.249	0.033	2.274	1.080	0.025	0.127	0.123	1.076	0.032

Table 3.12 (Continued)

P-Values														
CE	0.397	0.916	0.0002	0.0002	0.785	0.545	0.517	0.188	0.258	0.217	0.735	0.446	0.184	0.500
CD	0.389	0.070	0.003	0.001	0.070	0.098	**0.022**	0.691	**0.008**	**0.001**	0.944	0.263	0.190	0.110
CE x CD	0.544	0.825	**0.001**	**0.001**	**0.006**	0.118	0.062	**0.019**	0.161	0.084	0.949	0.083	0.195	0.487

[1]Body Weight
[2]Duodenum (lb)/Body Weight (lb) * 100
[3]Jejunum (lb)/Body Weight (lb) * 100
[4]Ileum (lb)/Body Weight (lb) * 100
[5]Gizzard (lb)/Body Weight (lb) * 100
[6]Standard Error of the Mean
a-b Values within columns with different superscripts differ significantly ($P < 0.05$)

Table 3.13 Starch digestibility on d 46 for male broilers fed 2 different carbohydrase enzymes in varying commercial diets

Carbohydrase Enzyme	Commercial Diet	Starch Digestibility[1] (%)
CE1	**CDA**	96.979
	CDB	97.660
CE2	**CDA**	98.367
	CDB	98.073
SEM[2]		0.443
Carbohydrase Enzyme		
CE1		97.456
CE2		98.210
SEM		0.313
Commercial Diet		
CDA		97.7636
CDB		97.868
SEM		0.314
P-Values		
CE		0.0613
CD		0.6828
CE x CD		0.295

[1]$((Starch_{diet}/TiO^2_{diet})-(Starch_{digesta}/TiO^2_{digesta}))/ (Starch_{diet}/TiO^2_{diet}) \times 100$

[2]Standard Error of the Mean

a-b Values within columns with different superscripts differ significantly ($P < 0.05$)

CDA Economic Analysis		
	CE1	**CE2**
Breast	247.841	239.839
Wings	148.103	149.429
Tenders	74.643	77.946
Thighs	42.988	45.075
Drumstick	25.610	26.319
Total per bird - Gross Chicken Part Profit (cents)	539.185	538.608
Total Feed Costs per bird (cents) Total Feed Costs per bird (dollars)	201.115 2.011	192.441 1.924
Gross bird profit in cents (profit processing-feed costs per bird)	338.070	346.166
Gross bird profit dollar amount (profit processing-feed costs per bird)	3.381	3.462
Potential Gross Profit for 1 million birds		
	$ 3,380,703.84	$ 3,461,660.10

Figure 3.1 Economic Analysis using 2 different carbohydrase enzymes in varying commercial diets

CDB Economic Analysis		
	CE1	**CE2**
Breast	261.284	264.591
Wings	158.709	159.088
Tenders	77.946	76.460
Thighs	47.823	47.057
Drumstick	27.342	39.439
Total per bird – Gross Chicken Part Profit (cents)	573.103	586.635
Total Feed Costs per bird (cents) Total Feed Costs per bird (dollars)	190.486 1.905	187.161 1.872
Gross bird profit in cents (profit processing-feed costs per bird)	382.617	399.474
Gross bird profit dollar amount (profit processing-feed costs per bird)	3.826	3.995
Potential Gross Profit for 1 million birds		
	$ 3,826,171.60	$ 3,994,735.20

Figure 3.2 Economic analysis using 2 different carbohydrase enzymes in varying commercial diets

References

Angel, R, J. O. B. Sorbara; Why is it important to understand substrates if we are to optimize exogenous enzyme efficacy? Poult. Sci. 2014. 93:2375-2379.

Bach, K. E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Tech. 67:319-338.

Campasino, A., M. Williams, R. Latham, C.A. Bailey,B. Brown, and J.T. Lee. 2015. Effects of increasing dried distillers' grains with solubles and non-starch polysaccharide degrading enzyme inclusion on growth performance and energy digestibility in broilers. J. Appl. Poult. Res. 24:135-144.

Choct, M. and G. Annison. 1992. Anti-nutritive effect of wheat pentosans in broiler chickens. Roles of viscosity and gut microflora. Br. Poult. Sci. 33:821-834.

Choct, M., and G. Annison. 1990. Anti-nutritive activity of wheat pentosans in broiler diets. Br. Poult. Sci. 31:811-821.

Choct, M., R. J. Hughes, R. P. Trimble, K. Angkanaporn, and G. Annison. 1995. Non-starch polysaccharide-degrading enzymes increase the performance of broiler chickens fed wheat of low apparent metabolizable energy. J. Nutr. 125:485-492.

Cowieson, A. J. 2010. Strategic selection of exogenous enzymes for corn/soy-based poultry diets. J. Poult. Sci. 47:1-7.

Cowieson, A. J. and Adeola, O. 2005. Carbohydrases, protease, and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. Poult. Sci. 84:1860-1867.

Dida, M. F. 2016. Review Paper on Enzyme Supplementation in Poultry Ration. International Journal of Bioorganic Chemistry.1:1-7.

Engberg, R. M., M. S. Hedemann, S. Steenfeldt, and B. B. Jensen. 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poult. Sci. 83:925-938.

Francesch, M. and P. A. Geraert. 2009. Enzyme complex containing carbohydrases and phytase improves growth performance and bone mineralization of broilers fed reduced nutrient corn-soybean-based diets. Poult. Sci. 88:1915-1924.

Józefiak, D., A. Ptak, S. Kaczmarek, P. Maćkowiak, M. Sassek, B. A. Slominski. 2010. Multi-carbohydrase and phytase supplementation improves growth performance and liver insulin receptor sensitivity in broiler chickens fed diets containing full-fat rapeseed. Poult Sci. 89: 1939-1946.

Kiarie, E., L. F. Romero, V. Ravindran. 2014. Growth performance, nutrient utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or with supplemental xylanase. Poult Sci. 93:1186-1196.

Kleyn, R. 2013. Enzymes. Chicken Nutrition: A guide for nutritionists and poultry professionals. Context Publications. 251-272. Print.

Liang, Y., M. T. Kidd, S. E. Watkins, and G. T. Tabler. 2013. Effect of commercial broiler house retrofit: A 4-year study of live performance. J Appl. Poult. Res. 2022:211-216.

Liu, D., G. Shuangshuang and Y. Guo. 2012. Xylanase supplementation to a wheat-based diet alleviated the intestinal mucosal barrier impairment of broiler chickens challenged by Clostridium perfringens. J. Avian Pathology. 41:291-298.

Moran, E. T., Jr. 1982. Starch digestion in fowl. Poult. Sci. 61:1257-1267.

Nian, F., Y. M. Guo, Y. J. Ru, A. Peron, and F. D. Li. 2011. Effect of xylanase supplementation on the net energy for production, performance, and gut microflora of broilers fed corn/soy-based diet. Asian-Aust. J. Anim. Sci. 24:1282-1287.

Olukosi, O. A., A.J. Cowieson, and O, Adeola. 2007. Age-related influence of a cocktail of xylanase, amylase, and protease or phytase individually or in combination in broilers. Poult. Sci. 86:77-86.

Pirgozliev, V., S. P. Rose, T. Pellny, A. M. Amerah, M. Wickramasinghe, M. Ulker, M. Rakszegi, Z. Bedo, P. R. Shewry, and A. Lovegrove. 2015b. Energy utilization and growth performance of chickens fed novel wheat inbred lines selected for different pentosan levels with and without xylanase supplementation. Poult. Sci. 94:232–239.

Polizeli, M. L., A. C. Rizzatti, R. Monti, H. F. Terenzi, J. A. Jorge, D. S. Amorim. 2005. Xylanases from fungi: properties and industrial applications. Appl. Microbiol Biotechnol. 67:577-591.

Richards, M.P., Proszkowiec-Weglarz, M. 2007. Mechanisms regulating feed intake, energy expenditure, and body weight in poultry. Poultry Science. 86:1478-1490.

Selle, P. H., V. Ravindran, and G. G. Partridge. 2009. Beneficial effects of xylanase and/or phytase inclusions on ileal amino acid digestibility, energy utilisation, mineral retention and growth performance in wheat-based broiler diets. Anim. Feed Sci. Tech. 153:303-313.

Shepherd, E. M. B. D. Fairchild; Footpad dermatitis in poultry. Poult Sci 2010. 89:2043-2051.

Smits, C.H.M., Veldman, A., Verstegen, M.W.A. and Beynen, A.C. 1997. Dietary carboxymethylcellulose with high instead of low viscosity reduces macronutrient digestion in broiler chickens. Journal of Nutrition 127:483-48.

Stefanello, C., S.L. Vieira, P. S. Carvalho, J. O. B. Sorbara, and A. J. Cowieson. 2016. Energy and nutrient utilization of broiler chickens fed corn-soybean meal and corn-based diets supplemented with xylanase. Poult. Sci. 95:1881-1887.

Svihus, B. 2014. Starch digestion capacity of poultry. Poult. Sci. 93:2394-2399.

Viveros, A., Brenes, A., Pizarro., M. and Castano, M. 1994. Effect of enzyme supplementation of a diet based on barley, and autoclave treatment, on apparent digestibility, growth performance and gut morphology of broilers. Animal Feed Science and Technology 48:237-251.

Wang, Z. R., S. Y. Qiao, W. Q. Lu, and D. F. Li. 2005. Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology, and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. Poult. Sci. 84:875-881.

Wu, Y. B., V. Ravindran, D. G. Thomas, M. J. Birtles, and W. H. Hendriks. 2004. Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolisable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. Br. Poult. Sci. 45:76-84.