Effects of Supplemental Xylanase in Corn- and Wheat-Based Diets on Growth Performance and Cecal Volatile Fatty Acid Concentrations of Broilers during a Six-Week Production Period

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 4, 2018

Keywords: xylanase, corn, wheat, volatile fatty acid, broiler

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ABSTRACT

Supplemental xylanase has increasingly become an important part of diet formulation due to its ability to mitigate the anti-nutritive effects of arabinoxylans, increase energy utilization, and improve growth performance of broilers. Xylanase partially depolymerizes arabinoxylans, which can reduce intestinal viscosity, reduce nutrient encapsulation, and modulate intestinal microflora in broilers. Additionally, products of xylanase hydrolysis, arabinoxylo- and xylo-oligosaccharides, have been reported to stimulate a prebiotic effect, increasing broiler cecal fermentative capacity and volatile fatty acid (**VFA**) production. However, factors such as inclusion concentrations, cereal grain source, and bird age have been observed to affect xylanase's mode of action and magnitude of response. Therefore, 2 experiments were conducted to assess the energy-sparing effects of supplemental xylanase in corn- and wheat-based diets on Ross × Ross 708 male broiler growth performance and cecal VFA concentrations during a 6-week production period.

Experiment 1 evaluated effects of various supplemental xylanase concentrations (0, 12,000, and 24,000 BXU/kg) in corn- and wheat-based diets formulated with reduced apparent metabolizable energy concentrations on broiler growth performance and cecal VFA production from 1 to 40 d of age. From 1 to 26 d of age, cereal grain source ($P < 0.05$) affected broiler growth performance with birds receiving corn-based diets having increased body weight gain, increased feed intake, and lower feed conversion ratio than

those fed wheat-based diets. However, no dietary treatments ($P > 0.05$) differences were observed with cumulative growth performance. Cereal grain source ($P < 0.05$) influenced propionic, isobutyric, butyric, and isovaleric concentrations at 26 and 40 d of age with birds receiving corn-based diets having higher ($P < 0.05$) concentrations of propionic, isobutyric, and isovaleric, and lower ($P < 0.05$) concentrations of butyric than birds fed wheat-based diets. These results demonstrated that cereal grain source influenced broiler cecal VFA concentrations. However, supplemental xylanase did not influence broiler growth performance or cecal VFA concentrations.

Experiment 2 evaluated effects of age and supplemental xylanase in corn- and wheat-based diets on cecal VFA production during weekly intervals from 14 to 42 d of age. Cereal grain source and supplemental xylanase interacted ($P < 0.05$) to affect butyric (14 and 21 d of age) and total VFA (21 d of age) concentrations. Broilers fed corn-based diets with and without xylanase and wheat-based diet with xylanase exhibited higher concentrations of butyric and total VFA than those fed wheat-based without xylanase. Main effects of cereal grain source ($P < 0.05$) influenced propionic, isobutyric, butyric, isovaleric, valeric, and isocaproic acid concentrations at 14, 21, 28, 35, and 42 d of age. Broilers fed corn-based diets had higher ($P < 0.05$) concentrations of propionic, isobutyric, isovaleric, valeric, and isocaproic than birds fed wheat-based diets from 14 to 42 d of age. However, broilers fed wheat-based diets had higher ($P < 0.05$) concentrations of butyric acid at 28, 35, and 42 d of age compared with those fed corn-based diets. All individual and total VFA concentrations increased ($P < 0.05$) linearly from 14 to 42 d of age. Age and cereal grain interactive effects ($P < 0.05$) were observed with propionic, isobutyric, butyric, isovaleric, and valeric acid concentrations. These

results indicated that broiler cecal VFA concentrations are largely influenced by cereal grain source and bird age. However, inconsistent effects of xylanase supplementation on broiler growth performance and cecal VFA concentrations demonstrate that future research evaluating factors such as substrate availability, gastrointestinal environment and age, xylanase inhibitors, microflora composition, immunological and stress conditions, and health are needed.

ACKNOWLEDGMENTS

This body of work could not have been completed without the hard work and help of many different people. Therefore, I would like to begin by thanking my major professor, Dr. William Dozier, III. It has been a great honor and privilege to study under him due to his extensive knowledge and experience in both poultry research and industry. His integrity and commitment to excellence has been a great example to me. Moreover, he has been very gracious and patient with me throughout this entire process. Therefore, I sincerely acknowledge and appreciate all he has done for me. Likewise, I would like to thank my graduate committee members, Drs. Joseph Hess and Wilmer Pacheco for their kind and timely assistance and guidance. I would also like to acknowledge and express my gratitude to Drs. Mike Bedford, Brian Kerr, and Craig Wyatt for the help and support they provided.

Moreover, I would like to thank my colleagues, Dr. Kurt Perryman, Dr. Kate Meloche, Denise Landers, Ruben Kriseldi, Drew Wear, Henry Fuentes, Kenneth Smith, Sara Cloft, Stephanie Philpot, and Trevor Lee for their hard work and support throughout this process. I could not have asked for a better group of people to work with and I will always cherish their friendship. I would also like to thank Mitchell Pate, Steve Martin, and all the staff at the Auburn Poultry Research Farm. These individuals have all impacted my life in special ways, and I respect and appreciate their commitment to hard work. Their help and cooperation were integral to the integrity of this research.

Additionally, I would like to thank all the faculty and staff of the Auburn Poultry Science Department. This department is a great example of the Auburn family. Each individual has been more than gracious and helpful to me, and I will always appreciate and remember their kindness.

Last but not least, I would like to thank my church family at Trinity Presbyterian Church, Opelika, Alabama. Their prayers, fellowship, and wisdom have helped carry me through good and bad times, and I love and appreciate them all. Equally, I would like to thank my family and friends, Luther and Lanee McCafferty (grandfather and grandmother), H. L. Ford (grandfather), Barry McCafferty (father), Melinda Ford (mother), Kurt Fox (brother), Kristina Eaton (sister), Kayleen McCafferty (sister), Luke McCafferty (brother), Pastor Henry Lewis Smith (mentor/friend), Dr. Kimberly Key (mentor/friend), Pastor Bruce Bowers (mentor/friend), Dr. Roger Lien (mentor/friend), Steve and Becky Littrell (uncle and aunt), Danny and Sandra Mitchell (uncle and aunt), Dewey and Debbie Ford (uncle and aunt), Jim and Lori Woodburn (uncle and aunt) Dr. Jon Ford (uncle), Benjy McCafferty (uncle), all my cousins and extended family, and many others who have graciously helped me along the way. The love, support, and prayers of these individuals were foundational for this accomplishment. Therefore, I gratefully acknowledge and express my love for them all.

Finally, and truly, I would like to give all the glory and honor to the one eternal, triune, and living God—God the Father, God the Son, and God the Holy Spirit. For it is by His great mercy and steadfast love that I was granted this opportunity. *Soli Deo Gloria.*

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I. INTRODUCTION

The caloric value of plant-based feed ingredients used in broiler diets is limited by the presence of complex carbohydrates known as non-starch polysaccharides (**NSP**) (Choct, 2006). These NSP are relatively unavailable to endogenous digestive enzymes and have well defined anti-nutritive effects on digestion and absorption in broiler chickens (Bedford and Classen, 1992; Choct and Annison, 1990, 1992a). Dietary NSP inclusions have been observed to decrease nutrient utilization, growth performance, and gastrointestinal health of broilers (Choct and Annison, 1992a; Choct, 1997). Supplementation of dietary xylanase has been observed to mitigate these negative effects, and increase metabolizable energy through partial depolymerization of arabinoxylan NSP (Bedford and Classen, 1992; Annison, 1993).

Xylanase supplementation has increasingly become an integral part of diet formulation for broilers, particularly when diets are formulated with ingredients such as wheat, rye, barley, or triticale. These energy-contributing ingredients may be comprised of approximately 10 to 17% total NSP on a DM basis (Englyst, 1989; Choct, 1997). Total NSP are further classified into soluble (1.7 to 4.6% of DM) and insoluble (8.6 to 14.6% of DM) fractions (Englyst, 1989; Choct, 1997). Insoluble NSP fractions are considered to be relatively inert, functioning primarily as an energy diluent with increasing concentrations. However, soluble NSP at these concentrations have been observed to elicit notable viscosity-related effects (Choct, 1997). Consequently, these energy-contributing ingredients are commonly referred to as viscous cereals. Soluble NSP have a

high water-binding capacity, contributing to increases in intestinal digesta viscosity (Classen, 1996). Increased digesta viscosity limits endogenous enzyme and substrate interactions, and reduces intestinal passage rates (Choct and Kocher, 2000). Exogenous xylanases cleave these soluble NSP, which reduces their water-binding capacity and viscous-forming capabilities. Therefore, the observed energy-sparing effects of supplemental xylanase are often attributed to a reduction in intestinal digesta viscosity.

However, research has indicated that energy-sparing effects of supplemental xylanase extend beyond this primary mode of action. Specifically, increases in apparent metabolizable energy and nutrient digestibility have been observed in broilers fed diets with supplemental xylanase in the absence of altered digesta viscosity (Pettersson et al., 1990; Meng et al., 2005). Insoluble NSP fractions may physically encapsulate nutrients such as starch and protein, hindering endogenous enzyme access and decreasing subsequent digestion and absorption. Accordingly, xylanase supplementation increases energy utilization through this secondary mode of action by reducing the nutrient encapsulation effect of insoluble NSP (Bedford, 1995; Khadem et al., 2016).

In contrast to viscous cereals, corn-based diets have relatively low concentrations (approximately 5% of DM) of arabinoxylan NSP (Choct, 1997). However, increasing the potential to reduce diet costs through the energy-sparing effects of xylanase have led to increased supplementation in corn-based diets (Masey-O'Neill et al., 2014). Beneficial effects on broiler growth performance and nutrient digestibility have been observed with xylanase supplementation in corn-based diets (Zanella et al., 1999; Cowieson et al., 2010). However, the mode of action responsible for these observed responses remains

uncertain, as the current understanding of xylanase-mediated responses does not appear applicable to diets with low arabinoxylan NSP concentrations.

Although it has been suggested that these responses are a result of the nutrient encapsulation mode of action (Meng et al., 2005), other evidence indicates that these responses are more likely a result of a microbiota-mediated mode of action (Choct et al., 1996; Bedford and Cowieson, 2012). Particularly, xylanase supplementation increases the proportion of highly fermentable arabinoxylo-oligosaccharides (**AXOS**) and non-substituted xylo-oligosaccharides (**XOS**) fractions in the distal portion of the gastrointestinal tract (Morgan et al., 2017), which increases fermentative capacity and subsequent volatile fatty acid (**VFA**) production in the ceca. Increased VFA production has been observed to lower the pH of the distal gastrointestinal tract and promote proliferation of beneficial bacteria (Vahjen et al., 1998; Engberg et al., 2004). Furthermore, increased VFA production may also stimulate the ileal brake mechanism by increasing the secretions of the regulatory gastrointestinal hormones known as peptide YY (**PYY**) and glucagon-like peptide (**GLP-1**). Increased secretions of PYY and GLP-1 slows upper gastrointestinal motility and increases nutrient utilization (Tatemoto et al., 1982; Suzuki et al., 1983; Nauck et al., 1997; Spreckley and Murphy, 2015).

Current hypotheses propose that xylanase efficacy in corn-based diets is likely a result of the microbiota-mediated mode of action. However, limited data exists to support the proposed relationship between xylanase supplementation and VFA production, as well as age-specific effects on broiler fermentative capacity. Therefore, 2 experiments were conducted to assess the energy-sparing effects of xylanase supplementation in corn- or wheat-based diets on growth performance and VFA production in growing broilers.

Experiment 1 evaluated the effects of various supplemental xylanase concentrations in corn- or wheat-based diets on broiler growth performance and VFA production from 1 to 40 d of age. Experiment 2 further evaluated the effects of supplemental xylanase in corn- or wheat-based diets and the effects of age on VFA production during weekly intervals from 14 to 42 d of age. Data generated from these experiments should increase the understanding of xylanase efficacy in corn- and wheat-based diets, and provide a better understanding of broiler VFA production over time.

II. LITERATURE REVIEW

NON-STARCH POLYSACCHARIDES IN CORN AND WHEAT

Energy-contributing ingredients comprise approximately 60 to 70% of broiler diets, which represents a substantial proportion of dietary cost. These energy-contributing ingredients are mainly composed of complex carbohydrates that are broadly classified as either starch (55 to 70%) or non-starch polysaccharides (**NSP**) (8.0 to 13.2%) (Belitz et al., 2009). Starch is readily available and digested by endogenous amylase. However, broilers lack the necessary endogenous digestive enzymes to hydrolyze the β-1, 4 bond linkages of NSP (Choct, 1997). While these NSP compounds may be digested through microbial fermentation, the low fermentative capacity of poultry limits their nutritive value. Furthermore, dietary NSP have been observed to exert anti-nutritive effects on nutrient digestion and absorption of broilers (Choct and Kocher, 2000). The approximate concentrations of NSP of corn and wheat are 8.1 and 11.4%, respectively (Englyst, 1989; Choct, 1997). The NSP present in corn and wheat mainly consist of arabinoxylans, which are pentosan polysaccharides (arabinose and xylose sugars) on a linear β-1, 4- xylan backbone. Total NSP concentrations, substrate compositions, and substrate solubility usually vary among cereal grains. However, wheat typically exhibits greater concentrations of total NSP, arabinoxylans, and soluble arabinoxylans than corn (Englyst, 1989; Choct, 1997).

Soluble arabinoxylans account for approximately 1.9 and 22.2% of the total arabinoxylan content in corn and wheat, respectively (Englyst, 1989; Choct, 1997).

Although, soluble arabinoxylans comprise a minor proportion of the total NSP content in corn or wheat they are considered predominantly responsible for observed anti-nutritive effects in broilers (Bedford and Classen, 1992). Data have indicated that total NSP and insoluble arabinoxylan concentrations in corn and wheat may also interfere with nutrient digestion and absorption in broilers (Caprita et al., 2010; Meng et al., 2005). Nutritional strategies may be employed to partially reduce the anti-nutritive effects of arabinoxylans in broiler diets, but the application of exogenous xylanase to hydrolyze the β-1, 4 bond linkages of arabinoxylans has been established as one of the most efficacious methods to reduce the adverse effects and increase AME_n utilization of broilers (Ravindran, 2013).

XYLANASE SUPPLEMENTATION ON BROILER GROWTH PERFORMANCE AND ENERGY UTILIZATION

Xylanase supplementation in broiler diets has been a common nutritional strategy to mitigate the anti-nutritive effects of increased digesta viscosity associated with soluble NSP in viscous cereal grains (Bedford et al., 1991; Bedford and Classen, 1992a, b; Choct and Annison, 1992b). Bedford et al. (1991) determined that broilers fed rye-based diets supplemented with xylanase exhibited an 80% reduction in digesta viscosity, 11.7% increase in body weight gain (BWG), and a 6.3% decrease in feed conversion ratio (FCR) compared with the control-fed birds from 1 to 21 d of age. Annison (1992) reported increases in apparent metabolizable energy (AME) values ranging from 234 to 350 kcal/kg when broilers were fed wheat-based diets supplemented with various commercial xylanase products.

In addition to recovering broiler growth performance and energy utilization in diets formulated with highly viscous cereal grains (barley, rye, and triticale), xylanase supplementation has also been observed to improve these measures in the absence of viscosity-related effects. Increasing evidence has demonstrated the beneficial effects of xylanase supplementation on broiler performance and energy utilization even when diets are formulated with corn or wheat (Pettersson et al., 1990; Meng et al., 2005; Kiarie et al., 2014). Kiarie et al. (2014) observed increases of 7.7 and 8.0% in BWG and decreases of 4.2 and 4.8% in FCR at 21 and 42 d of age, respectively, when broilers were fed diets containing supplemental xylanase. Concomitantly, these authors observed a main effect of xylanase supplementation on nitrogen-corrected apparent metabolizable energy (**AME_n**) at 21 d of age, with xylanase increasing AME_n by 64 kcal/kg.

Liu and Kim (2016) observed similar responses on BWG and FCR with xylanase supplementation in wheat-based diets, but the magnitude of these responses were affected by inclusion concentration and age. These authors only observed increases of 1.7 and 1.2% for BWG and decreases of 2.1 and 8.0% for FCR at 18 and 35 d of age, respectively, in broilers fed diets containing the highest xylanase concentration (5,625 XU/kg) compared with those fed the diets containing the lowest xylanase concentration (1,875 XU/kg). Francesch et al. (2012) did not observe any xylanase effects on BWG, but increasing concentrations of supplemental xylanase (0, 100, 150, 200, 400, 4,000 FXU/kg) in wheat-based diets resulted in linear reductions in both feed intake (**FI**) (3.2 and 4.0%) and FCR (4.3 and 4.7%) at 21 and 35 d of age, respectively. Furthermore, these authors noted increases in AME_n of 62, 69, and 126 kcal/kg with the addition of xylanase, but these effects were also dependent on the type of wheat cultivar used in diet

formulation. Therefore, it is important to note that the magnitude of xylanase responses on growth performance and AME_n may diminish as the inherent digestibility of the dietary ingredients increases (Bedford and Cowieson, 2009; Cowieson, 2010).

The energy-sparing effects of xylanase supplementation on broiler growth performance and AME_n have led to increased flexibility in diet formulation. Specifically, allowing nutritionists to reduce dietary AME_n, which subsequently decreases supplemental fat inclusion. Xylanase supplementation has been documented to increase dietary AME_n of energy-contributing ingredients enabling adequate broiler growth performance when employing this formulation strategy (Cowieson, 2005; Olukosi and Adeola, 2008; Cowieson et al., 2010; Masey-O'Neill et al., 2012; Cowieson and Masey-O'Neill, 2013; Flores et al., 2017; Amerah et al., 2017). Masey-O'Neill et al. (2012) demonstrated that xylanase supplementation (16,000 BXU/kg) in corn-based diets formulated with a 100 kcal/kg reduction in AME_n was sufficient to maintain BWG, FI, and FCR of broilers similar to those fed the positive control diets at 21, 35, and 42 d of age. Furthermore, the addition of xylanase decreased FCR by 2.5 and 3.5% at 35 and 42 d of age, respectively. Moreover, Flores et al. (2017) observed that broilers fed corn-based diets formulated with a 150 kcal/kg reduction in AME_n with supplemental xylanase at either 10,000 or 20,000 XU/kg was able to maintain BWG, FI, and FCR similar to those fed the positive-control diets at 14, 27, and 41 d of age. These authors also observed similar ileal digestible energy (**IDE**) coefficients between broilers fed the reduced AME_n diets with xylanase supplementation and those fed the positive-control diets at 14, 21, and 42 d of age.

Conversely, the beneficial effects of xylanase supplementation on broiler growth performance and AME_n has been reported to be variable (Woyengo et al., 2008; Singh et al., 2012; Gehring et al., 2013a, b; Karimi et al., 2013; Pirgozliev et al., 2015). Singh et al. (2012) observed no beneficial effects on broiler growth performance with the addition of xylanase in corn-based diets formulated with AME_n reductions of 55 and 100 kcal/kg from 1 to 42 d of age. Similarly, Gehring et al. (2013a) observed no effects on growth performance of broilers fed corn-based diets with xylanase inclusions of 8,000, 16,000, or 32,000 BXU/kg from 1 to 25 d of age. Interestingly, Gehring et al. (2013b) also observed that xylanase supplementation in corn-based diets fed to young broilers (1 to 9 d of age) decreased IDE by 137 kcal/kg. These authors suggested that these negative effects were a result of xylanase increasing the pool of free phytate in the absence of adequate phytase activity, which exacerbated the anti-nutritive effects of phytate and decreased broiler energy utilization. These inconsistent responses on broiler growth performance and energy utilization indicate the highly interactive nature of xylanase supplementation and other factors, which intrinsically influence their efficacy (Bedford, 2002).

Factors such as cereal grain, extent of AME_n reduction, bird age, xylanase type, xylanase inclusion rate, and other exogenous enzymes may all contribute to the variation associated with xylanase responses (Adeola and Cowieson, 2011). In order to capitalize on the benefits of xylanase supplementation on growth performance and energy utilization, these factors must be accounted for in diet formulation. Furthermore, growth performance and energy utilization assays are good indicators of nutritional efficacy, but other less tangible physiological measures such as gastrointestinal health, immune responses, microflora shifts, and volatile fatty acid (**VFA**) production may be more

sensitive indicators of xylanase efficacy in broilers (Cowieson, 2010). Developing a more extensive understanding of the underlying effects of xylanase supplementation on gastrointestinal physiology is warranted to support the efficient integration of these enzymes into existing nutritional programs and dietary formulation strategies.

XYLANASE MODES OF ACTION

Xylanase, endo-1, 4-β-xylanase (EC 3.2.1.8), targets NSP substrates known as arabinoxylans, which are structural components of the endosperm and aleurone cell wall portions of cereal grains (Bedford and Morgan, 1996). Arabinoxylans are pentosan polysaccharides predominantly consisting of arabinose and xylose sugars on a linear β-1, 4-xylan backbone. These arabinose and xylose substituents are attached to the β-1, 4-xylan backbone through glycosidic linkages at the α-1, 2 and α-1, 3 positions (Perlin, 1951). Xylanase's mechanism of action is endo-hydrolysis of the β-1, 4-glycosidic linkages along the arabinoxylan backbone, resulting in arabinoxylo-oligosaccharides (**AXOS**) and non-substituted xylo-oligosaccharides (**XOS**) fractions (Morgan et al., 2017). This partial depolymerization of the arabinoxylan structure has been observed to enhance nutrient utilization through the following modes of action: viscosity reduction, nutrient encapsulation reduction, and microbiota mediation (Fengler and Marquardt, 1988; Pettersson and Aman, 1989; Choct et al., 1996; Bedford and Cowieson, 2012).

Viscosity Reduction

In cereal grains, structural arabinoxylans are present in both water soluble and water insoluble fractions to varying degrees. Soluble arabinoxylans constitute the fraction of arabinoxylans that are not anchored to the cell wall by alkali-labile ester-cross linkages (Mares and Stone, 1973). Soluble arabinoxylans account for approximately 1.9, 12.0,

22.2, and 38.6% of the total arabinoxylan content in corn, triticale, wheat, and rye, respectively (Englyst, 1989; Choct, 1997). While these soluble arabinoxylans only comprise a minor proportion of the total arabinoxylan content in cereal grains, their physiochemical properties in the broiler gastrointestinal tract are considered to be one of the main anti-nutritive characteristics of arabinoxylans (Bedford and Classen, 1992; Choct and Annison, 1992a, b). Specifically, soluble arabinoxylans form highly viscous solutions in the presence of water due to their large molecular weights and high-water binding capacity (Bedford and Classen, 1992; Nilsson et al., 2000). According to Choct (1997), soluble arabinoxylans are capable of absorbing water at approximately 10 times their respective weights. As a result, diets formulated with cereal grains containing high concentrations (< 5% of DM) of soluble arabinoxylans (triticale, wheat, and rye) have been observed to depress nutrient utilization and growth performance by increasing the intestinal digesta viscosity in broilers (Moran et al., 1969; Fengler and Marquardt, 1988; Bedford and Classen, 1992; Choct and Annison, 1992a, b). Increased intestinal digesta viscosity decreases the rate of diffusion among substrates and endogenous digestive enzymes and impedes effective nutrient and mucosal surface interactions (Antoniou et al., 1981; Edwards et al., 1988; Ikegami et al., 1990). Additionally, some evidence indicates that soluble arabinoxylans may directly form complexes with endogenous digestive enzymes and reduce their activity (Ikeda and Kusano, 1983).

Xylanase supplementation has been established as an effective strategy to mitigate the adverse effects of soluble arabinoxylans and to reduce intestinal digesta viscosity (Choct et al., 1996). Xylanase's partial depolymerization of arabinoxylan structure has been observed to decrease the concentration of high-molecular-weight carbohydrates (>

500 kDa) in the aqueous phase of the broiler intestinal digesta by 44.3% (Bedford and Classen, 1992). Bedford et al. (1991) demonstrated that xylanase supplementation (0.2% inclusion) in rye-based diets fed to broilers decreased foregut and hindgut digesta viscosity concentrations by 77.6 and 80.2%, respectively, compared with those fed the control diets. Concomitantly, these authors also observed beneficial effects on broiler growth performance measures with these reductions in intestinal digesta viscosity. Similarly, Choct et al. (1995) demonstrated that digesta viscosity in the small intestine was reduced by 49.0% when broilers were fed wheat-based diets supplemented with a xylanase cocktail (0.1% inclusion) compared with those fed the unsupplemented wheat-based diets. Furthermore, these authors observed that broilers fed the wheat-based diets with xylanase cocktail supplementation had intestinal digesta viscosity concentrations similar to those fed the corn-based control diets. Therefore, these data indicate that xylanase supplementation in broiler diets effectively reduces intestinal digesta viscosity to rather negligible concentrations.

Nutrient Encapsulation Reduction

Adverse nutritional effects of arabinoxylans have also been associated with the insoluble fraction (Meng et al., 2005). These adverse effects are primarily attributed to their physical properties and location in cereal grains. For example, insoluble arabinoxylans, along with other insoluble structural NSP (xylans, β-glucans, and cellulose), form the cell walls of cereal grains (Mares and Stone, 1973; Fincher, 1975) and represent the greatest proportion of NSP present in the endosperm (Bedford, 2002). Because these insoluble NSP are largely present in the endosperm and are relatively unavailable to the endogenous digestive enzymes of broilers, they can act as a physical

barrier between the endogenous digestive enzymes and nutrients when the endosperm cell wall is intact (Pettersson and Aman, 1988; Pettersson et al., 1989; Bedford and Schulze, 1998). This nutrient encapsulation effect renders highly available nutrients such as starch and protein inaccessible to digestion and absorption. Approximately 10.7 and 13.7% of the starch and 20.9 and 18.0% of the crude protein (**CP**) in corn- and wheat-based diets, respectively, are unabsorbed in the broiler small intestine (Zanella et al., 1999; Svihus and Hetland, 2001; Ravindran et al., 2005). Consequently, these unabsorbed nutrients are either fermented in the ceca, as a rather inefficient energy source, or voided in the feces (Noy and Sklan, 1995).

Increased concentrations of insoluble arabinoxylans have also been observed to dilute dietary energy density, increase digesta rate of passage, and increase digesta water-holding capacity (Choct, 1997). The magnitude of these anti-nutritive effects can be partially mitigated by managing dietary NSP content and ingredient particle size (Kirwan et al., 1974; Rogel, 1985). However, due to the limited endogenous enzyme capacity of broilers, xylanase supplementation is required to further alleviate these anti-nutritive effects, especially in regards to nutrient encapsulation (Meng et al., 2005). Microscopic analysis of digesta contents collected from the terminal ileum of broilers fed wheat-based diets demonstrated substantial endosperm cell wall degradation with the addition of xylanase when compared with the unsupplemented control (Bedford and Autio, 1996). Likewise, microscopic evidence of endosperm cell wall degradation has also been observed when broilers were fed rye- or corn-based diets supplemented with or without xylanase (Bedford, 2002). These data indicated that supplemental xylanase degraded cell wall integrity of various cereal grains and reduced their nutrient encapsulation effect in

broilers, which ultimately increases nutrient availability. However, the magnitude of nutrient encapsulation reduction is difficult to quantify in broilers fed diets formulated with viscous cereal grains because xylanase is concomitantly altering the anti-nutritive effects of increased digesta viscosity. Therefore, when broilers are fed diets formulated with viscous cereal grains the beneficial effects of increased nutrient utilization are likely attributed to reductions in both viscosity and nutrient encapsulation.

The positive effects of nutrient encapsulation reduction has been demonstrated in broilers fed diets formulated with corn (Bedford, 2002). Meng and Slominski (2005) observed that the addition of a multi-carbohydrase xylanase cocktail in corn- and corn-soybean meal based diets increased NSP digestibility and AME_n of broilers by 63.4 and 124.5% and 106 and 72 kcal/kg, respectively, compared with birds receiving the unsupplemented control diets. Furthermore, these authors also observed that carbohydrase supplementation in the corn-based diets increased broiler apparent ileal starch digestibility by 2.8% and decreased ileal insoluble NSP concentrations by 13.0% when compared with those fed the unsupplemented control diets. Furthermore, Kiarie et al. (2014) observed that xylanase supplementation in either corn or wheat-based diets increased ileal CP digestibility in broilers by 4.4%. Collectively, these data indicated that supplemental xylanase effectively increases nutrient utilization by hydrolyzing the insoluble arabinoxylan constituents.

Microbiota Mediation

The aforementioned anti-nutritive effects of arabinoxylans have also been observed to negatively influence gastrointestinal health and growth performance of broilers through microflora alteration (Campbell et al., 1986, 1987; Choct et al., 1996).

Evidence demonstrated that microbiota mediation in broiler gastrointestinal tract was dependent upon the type of cereal grain used in diet formulation (Bedford and Cowieson, 2012). Poor broiler growth performance associated with feeding barley- or rye-based diets has been observed to be largely ameliorated with antibiotic supplementation, which indicates that these cereal grains negatively impact gastrointestinal tract health by indirectly altering microflora (Moran and McGinnis, 1968; MacAuliffe et al., 1976). Furthermore, the magnitude of increased intestinal digesta viscosity has been observed to be dependent upon microflora status (Campbell et al., 1983). Choct et al. (1997) reported that increased intestinal digesta viscosity presents a slow-moving, low-oxygen, and nutrient-rich environment, which allows for increased anaerobic bacterial proliferation in the small intestine. Particularly, increased incidences of necrotic enteritis from *C. perfringens* have been observed when broilers are fed diets containing high concentrations of NSP. According to Annett et al. (2002), the proliferation of *C. perfringens* on thioglycollate medium increased by 2.76, 4.78, and 4.88 logarithmic cycles, respectively, when digested corn, wheat, and barley media were used. These data indicated that increasing soluble NSP concentrations effectively enhances the development of anaerobic microflora, which may depress gastrointestinal health, nutrient utilization, and performance of broilers. Xylanase supplementation has been reported to minimize these negative effects on microflora alteration in the small intestine by reducing the concentrations of fermentative microorganisms (Bedford and Cowieson, 2012).

Xylanase supplementation has also been demonstrated to positively alter the microflora populations by increasing the presence of *Lactobacillus* or *Bifidobacterium* species in the broiler large intestine (Courtin et al., 2008; Eeckhaut et al., 2008).

Specifically, this microbiota mediation in the large intestine is due to increases in highly fermentable AXOS and XOS from arabinoxylan depolymerization. While these AXOS and XOS are still unavailable to endogenous digestive enzymes, they are highly available to certain fermentative bacterial species in the ceca (Bedford and Apajalahti, 2001). Increased concentrations of AXOS and XOS ultimately increase the fermentative capacity of broilers and subsequent VFA production (Choct et al., 1999; Wang et al., 2005). Increased concentrations of VFA have been observed to lower the pH of the gastrointestinal tract, increase metabolizable energy, and promote beneficial bacteria proliferation in broilers. Courtin et al. (2008) observed that broilers fed 0.5% AXOS in wheat-based diets increased beneficial ceca bifidobacteria concentrations by 1.35 logarithmic cycles compared with those receiving the unsupplemented control diets. Likewise, these authors observed that 0.25% and 0.50% AXOS supplementation in the corn- and wheat-based diets, respectively, each decreased FCR by 4.6% when compared with those fed the control diets from 1 to 21 d of age. Moreover, other research has demonstrated that broilers challenged with *S. enteritidis* and fed diets supplemented with 0.4% AXOS had lower percentages of *Salmonella*-positive cloacal swabs at 1, 3, 7, 11, and 15 d post-inoculation compared with those receiving the unsupplemented control diets (Eeckhaut et al., 2008). Therefore, these data indicated that AXOS and XOS from xylanase hydrolysis can alter the large intestine microbiota and enhance broiler gastrointestinal health and performance.

XYLANASE SUPPLEMENTATION AND THE ILEAL BRAKE MECHANISM

The efficacy of xylanase supplementation in broiler diets has been clearly linked to the enzyme's extrinsic modes of action on cereal grain arabinoxylans (Bedford et al.,

1991; Choct et al., 1996; Meng et al., 2005). However, research has also demonstrated that products of xylanase hydrolysis (AXOS and XOS) may indirectly affect intrinsic distal enteroendocrine control mechanism known as the ileal brake mechanism (Goodlad et al., 1987; Taylor, 1993; Masey-O'Neill et al., 2014). The ileal brake is a negative-feedback mechanism, which regulates various upper gastrointestinal functions based on the luminal nutrient content present in the distal portions of the ileum and large intestine (Read et al., 1984; Spiller et al., 1984; Taylor, 1989). Specifically, this negative-feedback mechanism is a physiological response that increases nutrient digestion and absorption in small intestine and reduces the percentage of unabsorbed nutrients in the large intestine (Taylor, 1993; Croom et al., 1999).

The ileal brake mechanism is predominantly regulated by the hormones peptide YY (**PYY**) and glucagon-like peptide (**GLP-1**), which have been observed to elicit strong inhibitory effects on upper gastrointestinal tract motility, gastric emptying rate, exocrine pancreatic secretions, and gastric acid secretions (Lundberg et al., 1982; Tatemoto, 1982; Savage et al., 1987; Jin et al., 1993; Wettergren et al., 1993). For example, low (0.4 pmol/kg/min) and high (1.1 pmol/kg/min) doses of intravenous PYY in humans decreased digesta transit time and gastric emptying rate by approximately 40 and 187% and 70 and 251%, respectively, when compared with those receiving the saline dose controls (Savage et al., 1987). Similarly, intravenous administrations of GLP-1 (0.9 pmol/kg/min) in humans has been observed to decrease gastric emptying rate, trypsin concentrations, and lipase concentrations by 50, 47, and 40%, respectively, when compared with those receiving the saline controls (Wettergren et al., 1993). Furthermore, Tatemoto (1982) demonstrated that single and continuous injections of PYY at 200

pmol/kg and 200 pmol/kg/hr in anesthetized cats decreased pancreatic secretions by 75 and 50%, respectively, when compared with non-injected controls. Also, Guo et al. (1987) observed that intravenous administrations of PYY at 200 and 400 pmol/kg/hr decreased the gastric acid secretions of dogs in a dose-dependent manner when compared with the non-administered controls. Therefore, the potency of PYY and GLP-1 as inhibitors of upper gastrointestinal function is consistent across a variety of species.

Peptide YY and GLP-1 are concomitantly produced by mucosal epithelial L-cells, which are primarily concentrated in the distal ileum and large intestine (Greeley et al., 1987). These regulatory peptides are usually secreted postprandially, in response to unabsorbed nutrients in the distal portions of the ileum and large intestine (Van Citters and Lin, 2006). Adrian et al. (1985) observed that human plasma PYY concentrations were directly proportional to calorie intake. Furthermore, these authors observed that lipid, protein, and carbohydrates were all PYY secretagogues, with lipids eliciting the strongest responses. In parallel, VFA products (acetate, butyrate, and propionate), produced from microbial fermentation of carbohydrates and glycoproteins in the large intestine have also been demonstrated as strong secretagogues of PYY and GLP-1 (Sleeth et al., 2010). Intraluminal infusions (10 mM) of acetate, acetoacetate, n-butyrate, and pyruvate in the distal colon of the rabbit increased PYY secretions by 368, 164, 195, and 139%, respectively, when compared with the saline-infused control group (Longo et al., 1991). Furthermore, these authors also observed that graded infusions of n-butyrate at 1.0, 3.3, and 10.0 mM concentrations linearly increased plasma PYY concentrations by 125, 161, and 195%, respectively. Similarly, colonic VFA infusions at 2 mM/h increased rat plasma PYY concentrations by 36, 145, and 173% at 30, 60, and 120 min post-

infusion compared with the saline-infused control (Cherbut et al., 1998). Therefore, these data establish that both fat and VFA are efficacious secretagogues of PYY and GLP-1, and likely contribute to the magnitude of intestinal absorption capacity (Croom et al., 1999).

Consequently, xylanase's ability to increase the proportion of highly fermentable AXOS and XOS in the distal portions of the gastrointestinal tract has been linked to increased ceca fermentative capacity and subsequent VFA production (Choct et al., 1996, 1999). Choct et al. (1999) observed that xylanase cocktail supplementation in wheat-based diets increased acetate, propionate, butyrate, valerate, and total VFA concentrations in the ceca by 61, 65, 110, 129, and 53%, respectively, when compared with those fed the control diet. As a result, increased circulating concentrations of PYY and GLP-1 may also be observed. Singh et al. (2012) observed that xylanase supplementation in corn-based diets increased serum PYY concentrations of broilers by 154% when compared with those fed the unsupplemented control diets. Therefore, greater secretions of PYY and GLP-1 may stimulate the ileal brake mechanism and ultimately decrease gizzard emptying rate and reduce intestinal transit time of broilers, allowing for more efficient nutrient utilization (Cowieson and Masey-O'Neill, 2013; Masey-O'Neill et al., 2014; Lee et al., 2017). This indirect mechanism may partially explain the energy-uplift associated with supplemental xylanase, particularly when diets are formulated with non-viscous cereals such as corn or sorghum.

FACTORS AFFECTING XYLANASE EFFICACY

Although the beneficial effects of xylanase supplementation have been well documented, xylanase responses may be variable due to their highly interactive nature

and other factors, which inherently affect efficacy (Ravindran, 2013). The magnitude of observed xylanase responses are mainly dependent upon factors such as dietary ingredients and quality, bird age, xylanase type, xylanase inclusion concentrations, and other exogenous enzymes (Adeola and Cowieson, 2011).

Dietary Ingredients and Quality

The response of xylanase increases when diets are formulated with arabinoxylan-rich ingredients such as rye, wheat, and triticale (Cowieson et al., 2006). These ingredients provide a greater response margin due to greater substrate availability. For example, the total arabinoxylan contents of wheat, rye, and triticale are approximately 35.8, 42.6, and 51.2%, respectively, greater than that of corn (Choct, 1997). Therefore, xylanase responses will likely decrease when broilers are fed diets formulated with cereal grains that contain less available substrate. For instance, the BWG response (increase over the control) with xylanase supplementation for corn- or wheat/rye-based diets is approximately 3.8 and 7.8%, respectively (Bedford et al., 1991; Olukosi et al., 2007a; Olukosi et al., 2007b; Cowieson and Ravindran, 2008a; Olukosi and Adeola, 2008; Olukosi et al., 2008; Tahir et al., 2008; Liu and Kim, 2017).

Other ingredient factors such as dietary lipid source and inclusion rate have been observed to affect xylanase responses (Danicke et al., 1997b, Langhout et al., 1997; Gehring et al., 2011). Danicke et al. (1997) observed greater responses to supplemental xylanase when broilers were fed diets formulated with tallow compared with those fed diets formulated soybean oil. Similarly, Langhout et al., 1997 observed that xylanase supplementation increased the digestibility of DM, crude fat, crude fiber, and AME_n when broilers were fed diets formulated with a blended animal lipid source. In contrast,

these authors did not observe a supplemental xylanase effect when broilers were fed diets formulated with soybean oil. Appropriate dietary lipid inclusion rates may also positively affect xylanase responses due to lipids ability to increase the digesta transit time. Mateos et al. (1982) observed that increasing supplemental fat (yellow grease) concentrations from 0, 5, 10, 15, 20, 25, and 30%, increased the digesta transit time by 26, 21, 34, 58, 57, 77 minutes when compared to the birds fed unsupplemented control. Furthermore, Noy and Sklan (1995) suggested that digesta transit time may be a limiting factor of nutrient absorption of young chicks due to the fact that it decreases with growth. Therefore, maintaining adequate dietary fat inclusions may be necessary to increase digesta transit time to optimize xylanase and arabinoxylan interactions.

Dietary inclusion of other feed additives that act as gastrointestinal microflora modulators also may affect responses to xylanase supplementation. Specifically, the strong antimicrobial activity of copper has led to increased pharmacological supplementation in broiler diets, which may decrease microflora populations in the distal gastrointestinal tract and reduce the prebiotic effect of xylanase (Bedford and Cowieson, 2012). Limited data exists to support this relationship, but negative interactive effects on broiler growth performance and nutrient digestibility have been observed with the combination of a xylanase-cocktail enzyme and copper sulfate at 250 mg/kg when compared to those fed diets formulated with additives individually (Marron et al., 2001). Therefore, interactions among xylanase and other feed additives may partly explain variation in xylanase efficacy.

Ingredient quality has also been demonstrated to affect xylanase responses (Cowieson et al., 2006). In terms of xylanase efficacy, ingredient quality can be assessed

on the concentration of anti-nutrients, such as soluble-NSP (Annison, 1993). Choct and Annison (1990) reported that wheat AME_n values were negatively correlated with soluble-NSP concentrations. Therefore, increasing concentrations of soluble-NSP ultimately increases the anti-nutritive properties of the cereal, which in turn, increases intestinal viscosity-related issues and decreases AME_n (Bedford et al., 1991; Bedford and Classen, 1993). Increased inclusions of various co-products such as, wheat bran or dried distiller's grains with solubles, may also affect dietary ingredient quality. As a result of the extracting processes, these co-products contain lower AME_n values and higher soluble-NSP concentrations than their ingredients of origin (Batal and Dale, 2006; Liu, 2011, Meloche et al., 2013). Furthermore, variations in processing conditions and cereal source can cause these products to be highly variable in nutrient content (Cromwell et al., 1993). Thus, increased dietary inclusions of low-AME_n cereal grains and/or co-products can lead to overestimation of dietary AME_n or increased anti-nutritive effects due to the variability in arabinoxylan concentrations. Therefore, a larger xylanase response may be expected when broiler diets are formulated with increased inclusions of these lower quality ingredients (Choct, 2006).

Bird Age

Bird age has also been observed to be one of the primary physiological factors to affect xylanase responses (Ravindran, 2013). Dynamic changes in nutrient digestion and absorption have been observed as the broiler advances in age, especially during the first few weeks post-hatch (Noy and Sklan, 1995). During this period, broilers are considered to have a limited absorptive capacity and lower endogenous enzyme concentrations because the gastrointestinal tract is still developing (Croom et al., 1999). Olukosi et al.

(2007) observed that apparent total tract retention of dry matter, energy, and CP dramatically increased by 42.3, 37.3, and 34.0% from 7 to 14 d of age, respectively. Broilers may be more responsive to supplemental xylanase during this early period of growth and development. At 14 d of age, Flores et al. (2017) observed that supplemental xylanase at 10,000 or 20,000 XU/kg in corn-based diets formulated with reduced AME_n (AME_n formulated 150 kcal/kg below PC) was capable of maintaining IDE similar to those fed PC diet. In contrast, Gehring et al., (2013b) observed that supplemental xylanase negatively affected IDE of corn-based diets fed to broilers from 1 to 9 d of age indicating that supplemental xylanase may have the potential to exacerbate the anti-nutrient (arabinoxylans, phytate, tannins, etc) concentrations of diets fed to young broilers. However, the anti-nutritive effects of arabinoxylans, particularly increased intestinal digesta viscosity, are considered to be more detrimental during the early period of growth as well (Cowieson et al., 2006). Yasar and Forbes (2000) observed the greatest intestinal digesta viscosity concentrations (11 kPa·s) in broilers at 14 d of age, but at 28 d of age the intestinal viscosity concentrations were reduced to < 4 kPa·s. Thus, indicating that xylanase responses may diminish as the broiler ages due to an increased ability to cope with these anti-nutritional factors. Conversely, the beneficial effects of xylanase on gastrointestinal microflora indicate that the magnitude of xylanase responses may continue to be evident in older broilers. However, the manifestations of these responses in older broilers may be more evident with varying rearing conditions that alter gastrointestinal health, immune responses, microflora shifts, and VFA production.

Xylanase Source

Broiler responses are affected by the type of xylanase used in diet formulation. Variations in xylanase origin (bacterial or fungal), activity (mono- or multi-component), kinetics (temperature, pH, molecular weight, maximal velocity (V_{max}), and Michaelis constant (K_m), form (liquid or powder) are some of the many factors that contribute to the range of observed xylanase responses (Paloheimo et al., 2011). Specifically, small differences in any of these properties can significantly result in different substrate affinities, which may partially explain the variety of different modes of action observed with xylanase supplementation (Bedford, 1997). Choct et al. (2004) observed that broilers fed diets formulated with commercial xylanases sourced from 3 fungal species exhibited varying effects on excreta moisture, intestinal digesta viscosity, and soluble NSP digestibility. Moreover, Kluepfel et al. (1992) observed large kinetic differences among 3 xylanases derived from a single bacterial organism. Furthermore, these authors also observed large individual variations in kinetic properties when these xylanases were subjected to xylans from different substrate sources (oat spelt and birchwood). Therefore, xylanase responses are attributable to both substrate source and isozyme present (Bedford, 1997).

Xylanase Concentrations

Broiler xylanase responses are also largely affected by dietary inclusion concentrations. In general, the rate of xylanase hydrolysis is directly proportional to its concentration (Ravindran, 2013). Therefore, maintaining adequate xylanase concentrations relative to dietary substrate concentrations are necessary to optimize catalytic efficiency. However, the lack of standardization in xylanase assay conditions

(pH concentrations, temperatures, substrates, and units of activity) among different enzyme manufacturers can increase the difficulty of dose-rate comparisons (Burrows, 2006). Moreover, feed processing conditions (conditioning temperatures and retention time), physiological status (bird age, health, and environment), and nutritional factors (fat percentage, nutrient density, cereal grain source, soluble-arabinoxylan concentrations) may also affect the broiler's sensitivity to varying xylanase inclusion rates (Cowieson and Ravindran, 2008b).

As a result, broilers do not typically respond to increasing xylanase concentrations in a linear dose-dependent manner (Ravindran, 2013). However, a non-phytase enzyme meta-analysis of 1,869 independent data-sets with approximately 346 dose titration experiments demonstrated that increasing enzyme concentrations increased broiler responses (Rosen, 2002). Likewise, Cowieson and Ravindran (2008b) demonstrated that graded supplemental xylanase-cocktail concentrations of 250 and 500 g/ton in corn-based diets decreased broiler FCR by 1.2 and 2.2%, respectively. Therefore, increasing supplemental xylanase concentrations may have the potential to increase response consistency by reducing the aforementioned sources of variations.

Other Exogenous Enzymes

Supplementation of other exogenous enzymes in combination with xylanase have also been observed to affect broiler performance (Cowieson et al., 2006). Variable broiler responses ranging from negative to positive and from sub-additive to additive have been observed with the addition of supplemental xylanase and other exogenous enzymes (Cowieson and Bedford, 2009). Although these combinations are not solely responsible for the wide range of observed responses, it has been demonstrated that the extent of an

enzyme efficacy is predominantly dependent upon either the most limiting nutrient, most abundant anti-nutrient, or the specific model used (Cowieson and Bedford, 2009). Also, the performance level of the broilers fed the control diets has been indicated as a major factor affecting the magnitude of xylanase responses (Rosen, 2002). Furthermore, Cowieson and Bedford (2009) suggested that the return from enzyme supplementation will diminish with each additional enzyme as the digestibility of the diet increases. Therefore, beneficial effects of xylanase and other enzyme combinations are more likely efficacious when diets are nutritionally marginal or contain a wide variety of lower quality feederstuffs. However, an understanding of the dietary substrates and the enzymes' mechanisms of action are required to fully capitalize on these responses (Adeola and Cowieson, 2011).

However, the synergistic effects of supplemental xylanase and phytase in wheat-based diets have been well established (Ravindran, 2013). Zyla et al. (1999) observed that the addition of only supplemental phytase (1,000 FTU/kg) in wheat-based diets increased broiler intestinal viscosities, but the addition of both phytase (graded concentrations 200, 400, 600, 800, and 1,000 FTU/kg) and xylanase (400 FXU/kg) sufficiently reduced intestinal viscosities of birds fed diets containing xylanase alone. Furthermore, these authors reported greater magnitude of toe ash in broilers fed diets containing both xylanase and phytase compared with those receiving only phytase supplemented diets. Being wheat-based diets, these synergistic effects may have been attributed to either phosphorus being the most limiting nutrient in the diet, non-phytate phosphorus or endogenous phytase encapsulation from insoluble arabinoxylans, or similar phytate and soluble arabinoxylan concentrations causing compounding anti-nutritive effects. In

contrast, Gehring et al. (2013b) observed negative responses on IDE with the addition of supplemental xylanase (16,000 BXU/kg) and phytase (1,000 FTU/kg) in corn-based diets. These authors observed that the combination of supplemental phytase and xylanase decreased IDE by approximately 198 kcal/kg when compared to diets containing only xylanase. Being corn-based diets, these negative effects may have been attributed to xylanase increasing the concentration of phytate phosphorus, in the absence of adequate phytase concentration, which exacerbated the anti-nutritive effect phytate phosphorus and decreased the IDE of the diet. Therefore, the highly interactive nature of xylanase and other exogenous enzymes can lead to distinct variations in observed broiler responses.

KNOWLEDGE GAPS IN THE LITERATURE

Exogenous xylanase efficacy is primarily attributed to viscosity reduction, nutrient encapsulation reduction, and microbiota mediation with broilers. The actual modes of action responsible for these beneficial effects are highly dependent upon the cereal grain source used in diet formulation. In the United States and Latin America, corn is the primary cereal grain used in broiler diet formulation, whereas wheat is the dominant dietary cereal grain used in Canada and Europe. Differences in corn and wheat nutrient composition, particularly arabinoxylan concentrations, indicate that the magnitude of broiler responses to xylanase supplementation may vary depending on the type of cereal grain used in diet formulation. Likewise, these variations in arabinoxylan concentrations may also affect optimal xylanase inclusion concentrations. Therefore, increased concentrations of supplemental xylanase may be necessary to optimize performance objectives of broilers fed diets containing higher arabinoxylan concentrations. Moreover, increasing evidence indicates that energy-sparing effects of

supplemental xylanase are a result of microbiota mediation in the broiler large intestine. Specifically, a prebiotic effect that increases the concentrations of highly-fermentable AXOS and XOS in the distal portions of the large intestine, which can increase ceca fermentative capacity and subsequent VFA production. Although microbiota mediation is generally accepted as a valid mode of action, data to support the proposed relationship between cereal grain source and xylanase inclusion concentrations on ceca VFA production in broilers is limited in the literature. Furthermore, the age-specific effects of cereal grain source and xylanase supplementation on ceca fermentative capacity have not been previously reported in the literature. Therefore, to address these knowledge gaps, the proposed research will evaluate the effects of feeding corn- and wheat-based diets with varying concentrations of supplemental xylanase on broiler growth performance and ceca VFA production from 1 to 40 d of age. Additionally, the second experiment will further evaluate the effects of supplemental xylanase in corn- or wheat-based diets and assess the effects of age on broiler VFA production during weekly intervals from 14 to 42 d of age.

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III. EFFECTS OF CEREAL GRAIN SOURCE AND SUPPLEMENTAL XYLANASE CONCENTRATIONS ON BROILER GROWTH PERFORMANCE AND VOLATILE FATTY ACID PRODUCTION FROM 1 TO 40 DAYS OF AGE

ABSTRACT

An experiment was conducted to evaluate the effects of feeding diets varying in cereal grain source and supplemental xylanase concentrations on growth performance and cecal volatile fatty acid (VFA) production of Ross × Ross 708 male broilers from 1 to 40 d of age. One thousand five hundred thirty-six day-old chicks were randomly distributed into 64 floor pens (24 chicks/pen; 0.08 m2/bird) and fed 1 of 8 dietary treatments (TRT) with 8 replicates per TRT. Experimental TRT were of either corn- (TRT 1 to 4) or wheat-based (TRT 5 to 8) origins. The 4 dietary TRT for each cereal grain source consisted of a positive control (PC) reference diet and 3 reduced AMEn diets (AMEn reduced 66 kcal/kg below PC) with supplemental xylanase at either 0 (negative control), 12,000, or 24,000 BXU/kg. Birds and feed were weighed at 1, 14, 26, and 40 d of age to determine BW gain (BWG), feed intake (FI), and feed conversion ratio (FCR). At 26 and 40 d of age, cecal contents were collected and pooled per pen (7 birds/pen; 5 replicate pens/TRT) for VFA concentrations. No TRT differences ($P > 0.05$) in cumulative growth performance were observed. Likewise, no TRT differences ($P > 0.05$) in acetic or total VFA concentrations were observed at 26 or 40 d of age. However, cereal grain source ($P < 0.05$) influenced propionic, isobutyric, butyric, and isovaleric concentrations at 26 and

40 d of age with birds receiving the corn-based diets having higher ($P < 0.05$) propionic, isobutyric, and isovaleric concentrations, and lower ($P < 0.05$) butyric acid concentrations than those fed the wheat-based diets. These results indicate that dietary cereal grain source may influence individual cecal VFA concentrations. However, supplemental xylanase did not affect broiler growth performance or cecal VFA concentrations. Therefore, future research evaluating factors limiting xylanase responses on broiler growth performance and cecal VFA production is warranted.

INTRODUCTION

Xylanase supplementation has increasingly become an integral part of broiler diet formulation due to its ability to mitigate the anti-nutritional effects of non-starch polysaccharides (**NSP**) in cereal grains and increase AME_n (Masey-O'Neill et al., 2014). Supplemental xylanase has been demonstrated to alleviate the anti-nutritive effects of increased digesta viscosity associated with feeding diets formulated with viscous cereal grains such as rye, wheat, or triticale (Bedford et al., 1991; Choct and Annison, 1992b; Bedford and Schulze, 1998). However, research has reported that the beneficial effects of supplemental xylanase are multifaceted and its efficacy is projected to extend beyond this primary mode of viscosity reduction (Choct et al., 1996; Meng et al., 2005, Bedford and Cowieson, 2012). Specifically, positive effects on broiler growth performance and nutrient utilization have been observed with supplemental xylanase in non-viscous corn-based diets (Zanella et al., 1999; Cowieson and Bedford, 2009). However, contrasts in arabinoxylan content and composition among viscous and non-viscous cereal grains indicate that the nature of these responses are likely due to distinct but concurrent modes of action (Cowieson, 2010). Accordingly, corn and wheat are the primary cereal grains

used globally in broiler diets (Barletta, 2010). Therefore, understanding the effects supplemental xylanase in broilers fed corn- and wheat-based diets is important for both practical and mechanistic reasons. Likewise, aforementioned variations in arabinoxylan content and composition between corn and wheat may affect optimal xylanase inclusion concentrations.

Xylanase supplementation has been reported to positively alter the intestinal microbiota of the broiler by decreasing small intestinal fermentation, and enhancing the proportion of highly fermentable arabinoxylo- (**AXOS**) and xylo-oligosaccharides (**XOS**) in the distal portions of the gastrointestinal tract (Choct et al., 1996, 1999; Bedford and Cowieson, 2012). These AXOS and XOS may elicit a prebiotic effect and increase volatile fatty acid (**VFA**) production in the ceca, which has been reported to lower the pH of the gastrointestinal tract and promote beneficial bacterial proliferation (Courtin et al., 2008; Cowieson and Masey-O'Neill, 2013). Furthermore, increased ceca VFA production may increase the ileal brake mechanism, which slows gizzard emptying rate and decreases gastrointestinal transit time allowing for increased nutrient utilization (Cowieson and Masey-O'Neill, 2013; Lee et al., 2017). However, data supporting the proposed relationship between cereal grain source, supplemental xylanase concentrations, and cecal VFA concentrations are limited in the literature. Therefore, the objective of this study was to examine the effects various concentrations of supplemental xylanase in corn- and wheat-based diets on broiler growth performance and cecal VFA production during a 6-week production period.

MATERIALS AND METHODS

The use of live birds for this experiment was approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2016-2884).

Bird Husbandry

One thousand five hundred and thirty-six Ross × Ross 708 (Aviagen, Inc., Huntsville, AL) male chicks vaccinated for Marek's disease, Newcastle disease, and infectious bronchitis, were obtained from a commercial hatchery at day of hatch. Chicks were randomly distributed into 64 floor pens (24 chicks per pen; 0.08 m^2 per bird) in a solid-sided cross-ventilated house. The research facility was equipped with vent boards, exhaust fans, stir fans, evaporative-cooling pads, forced-air heaters, and electronic controller system (Evolution 3000, Hired Hand Manufacturing, Inc., Bremen, AL). Each pen was equipped with a nipple drinker line (6 nipples per pen) and a hanging pan-feeder. Chicks were reared on used litter and given ad libitum access to water and feed. House temperature was set at 33.0° C and was gradually reduced as the birds advanced in age with a final set point of 20° C at 40 d of age. Photoperiod was set at 23L: 1D from 1 to 7 d of age, and then set at 20L: 4D from 8 to 40 d of age. Light intensity was set at 30, 10, and 5 lux from 1 to 7, 8 to 14, and 15 to 40 d of age, respectively. Light intensity was verified at bird level (30 cm) using a photometric sensor (LI-250A Light Meter, LI-COR Bioscience, Lincoln, NE).

Dietary Treatments

Eight dietary treatments (**TRT**) were fed throughout the starter (1 to 14 d of age), grower (15 to 26 d of age), and finisher (27 to 40 d of age) phases (Tables 3.1, 3.2, and 3.3). Dietary TRT were of either corn- (TRT 1 to 4) or wheat-based (TRT 5 to 8) origins.

The 4 dietary TRT for each cereal grain source consisted of a positive control (**PC**) reference diet and 3 reduced AME_n (**$RAME_n$**) diets (AME_n reduced 66 kcal/kg below PC) with supplemental xylanase at either 0 (negative control; **NC**), 0.0075 (12,000 BXU/kg), or 0.015% (24,000 BXU/kg). The PC diets (TRT 4 and 8) were formulated to be adequate in all essential nutrients and contained no supplemental xylanase. The $RAME_n$ diets were similar in composition, but AME_n values were formulated 66 kcal/kg below the PC with 0.015% washed builder's sand. Xylanase was added to the $RAME_n$ diets at the expense of sand to achieve 0 (TRT 1 and 5; NC), 12,000 (TRT 2 and 6) and 24,000 BXU/kg (TRT 3 and 7). A mono-component xylanase (endo-1, 4-β-xylanase; EC 3.2.1.8) expressed by *Trichoderma reesei* (Econase XT; AB Vista Feed Ingredients, Marlborough, UK), which provides 160,000 BXU/g was used to achieve the desired dietary activity concentrations in the supplemented TRT. Moreover, all dietary TRT contained phytase (*Escherichia coli*-6-phytase; EC 3.1. 3. 26) expressed by *Trichoderma reesei* (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK), which provides 5,000 FTU/g to achieve activity concentrations of 500 FTU/kg. Phytase was formulated to provide 0.15, 0.165, and 0.03% of P, Ca, and Na, respectively. Feed form was provided as crumbles from 1 to 14 d of age and whole pellets thereafter. Enzymes were added to the mixer and conditioning/pelleting temperatures did not exceed 85°C. Approximately, 0.5% of the supplemental fat was added in the mixer and the remaining percentage was applied post-pelleting to ensure pellet quality. Pellet durability index (**PDI**) of the grower and finisher diets was measured using standard PDI procedure (method 269.4; ASAE, 2003a).

Representative subsamples of diets were lyophilized using a Virtis Pilot Lyophilizer (SP Industries, Warminster, Pa). Dried ground samples were analyzed for crude fat and nitrogen content. Crude fat was determined with a Soxtec solvent extraction system (model 2043, Foss North America, Eden Prairie, MN) via the hexane-submersion method (method 2003.06; AOAC, 2006). Nitrogen content was determined by the Dumas combustion method (method 990.03; AOAC, 2006) with a Rapid N Cube analyzer (Elementar Americas, Inc., Mt. Laurel, NJ) for CP determination.

A commercial laboratory determined phytase and xylanase activity concentrations of all experimental TRT using enzyme-linked immuno-sorbent assays (Quantiplate™ Kits, Envirologix, Inc., Portland, ME) specific for Quantum Blue and Econase XT (Enzyme Services and Consultancy, Ystrad Mynach, UK). In addition to dietary enzyme analyses, representative samples of the corn and wheat used in dietary TRT were analyzed for ingredient composition, neutral cellulase gammanase digestibility (**NCGD**), and viscosity by a commercial laboratory (Enzyme Services and Consultancy, Ystrad Mynach, UK). Ingredient composition (DM, ash, CP, fiber, starch, phytate-P, neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), and sugar) and NCGD were predicted by near-infrared reflectance spectrophotometer analysis (FOSS NIR Systems model 2500, Eden Prairie, MN) with a range of 400 to 2,500 nm using online Feed Quality Service calibrations (AB Vista Feed Ingredients, Marlborough, UK). Cereal grain viscosity measurements were determined by an in vitro digestion viscosity method described by Bedford and Classen (1993) and Svihus et al. (2000). Samples were digested in HCl/pepsin (45 min) and $NaHCO_3$/pancreatin (120 min) solutions at 40° C, centrifuged to separate the supernatant (1,800 × g for 10 min), and shear strength of the

supernatant was measured with a Brookfield digital viscometer (Model DV-II, Brookfield Engineering Laboratories, Stoughton, MA). Corn and wheat were analyzed for particle size using a sieve shaker (Tyler RoTap, Mentor, OH) according to the standard method (method S391.3; ASAE, 2003b). All of the aforementioned diet and ingredient analyses were performed in duplicate.

Measurements

Birds and feed were weighed at 1, 14, 26, and 40 d of age to determine, BW, BW gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**). Mortality was recorded daily and was used to adjust FCR based on bird days. At 26 and 40 d of age, 7 birds per pen (5 replicate pens per TRT) were randomly selected, weighed, and euthanized via CO_2 asphyxiation. Following asphyxiation, ceca were excised at the ileo-cecal junction. Cecal contents were gently squeezed into sterile sealed cups. Samples were pooled per pen, immediately placed on ice, and frozen at –20° C until further VFA analysis.

Determination of Cecal Volatile Fatty Acid Concentrations

Cecal VFA concentrations were determined using the method described by Weber et al. (2010). Cecal samples were thawed and thoroughly hand-mixed with sterile stir sticks for at least 30 s. Approximately 1.0 g of cecal digesta from each sample was placed into 15-mL polypropylene centrifuge tube and diluted with 5 mL of deionized water. Samples were mixed overnight at 1,200 rpm on a digital microplate shaker (Thermo Fisher Scientific Inc, Miami, OK). After mixing, samples were centrifuged at 4° C for 23 min at 21,000 × g to separate supernatant. Approximately 2.5 mL of clear supernatant was removed and placed into tubes and *o*-phosphoric acid was added to achieve a pH of 2.5. Exactly 1 mL of the pH-adjusted supernatant sample was placed into 20 mL gas

chromatography vials with 0.3 g of NaCl. Prepared samples were frozen and shipped to an external laboratory (USDA-ARS-MWA-NLAE, Ames, IA) for gas chromatography analysis (Agilent 7890A Gas Chromatograph, Agilent Technologies, Inc, Wilmington, DE). Samples were analyzed in duplicates and values were multiplied by 5 to adjust for the dilution factor. Total VFA concentrations are the summation of the following VFA metabolites: acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic, and heptanoic acid.

Statistical Analysis

Data were analyzed as a 2 × 3 factorial arrangement with 2 cereal grain sources (corn or wheat) and 3 xylanase concentrations (0, 12,000, 24,000 BXU/kg) with 2 PC diets. The design structure was a randomized complete block design with pen location as the blocking factor. Each TRT was represented by 8 and 5 replicate pens for the growth performance and VFA data, respectively. Pen was considered as the experimental unit. Analysis of variance was performed using PROC MIXED (SAS, 2011) by the following mixed-effects model:

$$Y_{ijk} = \mu\ldots + \rho_i + \alpha_j + \beta_k + (\alpha\beta)_{jk} + \varepsilon_{ijk}$$

where $\mu\ldots$ is the overall mean; the ρ_i are identically and independently normally distributed random block effects with mean 0 and variance σ^2_{ρ}; the α_j are fixed factor level effects corresponding to the j^{th} cereal grain source (corn or wheat) such that $\Sigma\alpha_j = 0$; the β_k are fixed factor level effects corresponding to the k^{th} xylanase concentration (0, 12,000, or 24,000 BXU/kg) such that $\Sigma\beta_k = 0$; the $(\alpha\beta)_{jk}$ are interaction level effects corresponding to either j^{th} cereal grain source and the k^{th} xylanase concentration such that $\Sigma_j(\alpha\beta)_{jk} = 0$ and $\Sigma_k(\alpha\beta)_{jk} = 0$; and the random error ε_{ijk} are identically and independently

normally distributed with mean 0 and variance σ^2. Pre-planned orthogonal contrasts between the PC and NC of each cereal grain source were used to detect differences in growth performance and VFA concentrations. Statistical significance was established at $P \leq 0.05$, and interaction and main effects were separated using Tukey's Honestly Significant Difference test (Tukey, 1953).

RESULTS AND DISCUSSION

Diet and Cereal Grain Analyses

Analyzed xylanase concentrations of the starter, grower, and finisher diets are reported in Tables 3.1, 3.2, and 3.3, respectively. Analyzed xylanase concentrations were in close agreement with their calculated values except for the starter wheat NC TRT, which contained a background concentration of 5,170 BXU/kg. However, all other unsupplemented control TRT had analyzed xylanase concentrations of < 2,000 BXU/kg. Likewise, dietary TRT formulated with 12,000 and 24,000 BXU/kg of supplemental xylanase had analyzed concentrations ranging from 9,100 to 13,500 BXU/kg and 20,100 to 26,200 BXU/kg, respectively. Good pellet quality was observed throughout the grower and finisher phases for all dietary TRT. The standard PDI values of the corn- and wheat-based diets ranged from 88.1 to 92.8% and 93.3 to 95.7%, respectively.

Cereal grain analyses of corn and wheat are reported in Table 3.4. Numerically, wheat had higher DM, ash, CP, crude fiber, NDF, and ADF, but lower EE, starch, sugar, and phytate-P contents compared with corn. Corn exhibited a slightly higher numerical NCGD (91.67%) than wheat (91.02%). In vitro digestion viscosity analysis demonstrated that wheat (6.0 mPa·s) had a greater viscosity concentration than corn (1.2 mPa·s). Particle size was approximately 655 and 665μm for the corn and wheat, respectively.

Growth Performance

1 to 14 d of age

From 1 to 14 d of age, no interactive or main effects ($P > 0.05$) of cereal grain or xylanase were observed on broiler growth performance (Table 3.4). However, broilers fed the corn NC had a lower BWG ($P = 0.04$) and a higher FCR ($P = 0.002$) than those fed the corn PC. Therefore, reducing the AME_n by 66 kcal/kg in the corn-based diets negatively affected broiler growth performance. Cowieson et al. (2010) observed similar responses when broilers were fed reduced AME_n (114 kcal/kg below PC) corn-based diets. These authors observed decreases in BWG and increases in FCR by 5.6 and 6.5%, respectively, when compared to those fed corn PC from 1 to 21 d of age. In the current research, no growth performances differences ($P > 0.05$) were observed between broilers fed the wheat PC and wheat NC. Although modern broilers tend to be less responsive to reductions in dietary AME_n during the starter phase (Dozier and Gehring, 2014), AME_n of the wheat may have been higher than formulated values causing an overestimation of AME_n during diet formulation. Therefore, the calculated reduction of 66 kcal/kg may not have been achieved. Furthermore, the low-level background concentration of xylanase in the wheat NC could have affected this response.

1 to 26 d of age

From 1 to 26 d of age, no interactive effects or xylanase main effects ($P > 0.05$) on broiler growth performance were observed. However, cereal grain main effects ($P < 0.05$) were observed on BWG, FI, and FCR (Table 3.5). Feeding diets formulated with corn subsequently increased BWG ($P = 0.001$), increased FI ($P = 0.013$), and decreased FCR ($P = 0.001$) by 6.0, 2.1, and 3.6%, respectively, when compared with those broilers

fed the wheat-based diets. These beneficial effects of feeding broilers corn-based diets compared with wheat-based diets have been well documented (Amerah et al., 2008; Abdollahi et al., 2010; Masey-O' Neil et al., 2014), and may be attributed to less variations in ileal starch digestibility and lower concentrations of anti-nutritional factors with corn-based diets (Abdollahi et al., 2010).

Masey-O'Neill et al. (2014) observed similar cereal grain effects from 1 to 28 d of age with corn-based diets increasing BWG, increasing FI, and decreasing FCR of broilers by 7.0, 3.3, and 3.9%, respectively, when compared with those fed wheat-based diets. Correspondingly, these authors did not observe any growth performance effects with supplemental xylanase at 16,000 or 32,000 BXU/kg inclusion concentrations from 1 to 28 d of age. However, these authors did observe interactive effects on apparent ileal digestibility energy (**AIDE**) at 28 d of age, broilers fed wheat-based diets supplemented with xylanase at 16,000 BXU/kg had a higher AIDE than those receiving the corn-based diet supplemented with 16,000 BXU/kg; but, the AIDE of the broilers receiving the corn- and wheat-based diets supplemented with 32,000 BXU/kg were not different. Therefore, the beneficial effects of supplemental xylanase on AIDE may not always be apparent with BWG, FI, and FCR of broilers.

1 to 40 d of age

From 1 to 40 d of age, no TRT differences ($P > 0.05$) on cumulative BWG, FI, and FCR were observed (Table 3.8). Cereal grain main effects observed from 1 to 26 d of age were mitigated during the finisher phase (27 to 40 d of age; Table 3.7) because broilers fed wheat-based diets consumed 3.2% more feed ($P = 0.02$) and gained 4.5% more BW ($P = 0.002$) than those fed the corn-based diets. This effect may have been due

to a reduction in the anti-nutritional effects of wheat because the broiler's ability to cope with these factors has been demonstrated to increase with age (Yasar and Forbes, 2000). Masey-O'Neill et al. (2014) did not observe interactive effects of cereal grain source and xylanase on BWG, FI, and FCR of broilers during a 49 d production period. However, these authors observed main effects of cereal grain source on BWG and FI with broilers receiving the corn-based diets having higher BWG and FI than those fed the wheat-based diets from 1 to 49 d of age. Moreover, these authors observed a main effect of xylanase with broilers receiving the diets supplemented with xylanase at 16,000 and 32,000 BXU/kg having lower FCR than those fed the unsupplemented diets from 1 to 49 d of age.

In the current research, the lack of xylanase responses may be attributed to the high performance of those fed the NC diets (Rosen, 2002) because all dietary xylanase concentrations were in close agreement with the calculated values. From 1 to 40 d of age, BWG and FI of the birds receiving the NC diets were approximately 10.1 and 9.5%, respectively greater than the BWG and FI objectives projected by primary breeder guidelines (Ross 708 Broiler Performance Objectives, 2014). Likewise, researchers have demonstrated that enzyme responses of broilers decrease as the inherent digestibility of the diet increases (Cowieson and Bedford, 2009). Although digestibility was not measured prior to experimentation, the low NDF fractions of both corn and wheat in concurrence with the above average growth performance led to small response margins with xylanase supplementation.

Other factors that may have contributed to lack of xylanase effects in the current research include raising broilers in a non-challenged environment and pharmacological

doses of dietary copper. Xylanase effects on broiler growth performance may be more prevalent when broilers are challenged by coccidiosis or other enteric diseases (Cowieson et al., 2006). Therefore, xylanase effects on growth performance may have been limited because the broilers used in the current experiment were not as challenged as those raised in commercial practice. Additionally, feeding pharmacological concentrations of dietary copper may have been observed to positively influence broiler growth responses (Pesti and Bakalli, 1996; Ewing et al., 1998; Skrivan et al., 2000). These positive effects on broiler growth performance have been proposed to be a result of microbial profile alteration in the gastrointestinal tract (Hawbaker et al., 1961; Hojberg et al., 2005). Therefore, the inclusion of supplemental dietary copper at 132 ppm throughout the starter, grower, and finisher diets may have negated or masked the beneficial effects of supplemental xylanase and influenced cumulative growth performance responses.

Cecal VFA Concentrations

26 d of age

Interactive effects ($P < 0.05$) on isobutyric and isovaleric acid concentrations were observed with broilers receiving the corn $RAME_n$ + 24,000 BXU/kg xylanase TRT having greater concentrations of isobutyric ($P = 0.03$) and isovaleric acid ($P = 0.01$) compared with those receiving wheat NC, wheat $RAME_n$ + 12,000 BXU/kg xylanase, and wheat $RAME_n$ + 24,000 BXU/kg xylanase TRT (Table 3.9). However, isobutyric and isovaleric acid concentrations of broilers receiving the corn RAME + 24,000 BXU/kg xylanase TRT were similar to those receiving the corn NC and corn RAME + 12,000 BXU/kg xylanase TRT. Therefore, interactive effects were more likely influenced by cereal grain source than supplemental xylanase. Cereal grain effects ($P < 0.05$) were also

observed with broilers fed the corn-based diets having greater ($P < 0.05$) concentrations of propionic, isobutyric, isovaleric, and caproic acids than those fed the wheat-based diets. In contrast, broilers receiving wheat-based diets had greater ($P < 0.05$) concentrations of butyric acid. Kiarie et al. (2014) reported cereal grain effects at 21 d of age, broilers fed wheat-based diets had higher concentrations of acetic, butyric, valeric, and isovaleric, but lower concentrations of propionic acid when compared with those fed the corn-based diets. However, it is important to note that these authors' experimental diets varied in co-products (type and concentrations) and supplemental fat concentrations. Therefore, VFA concentrations in the broiler ceca may be influenced by other nutritional factors in addition to cereal grain source.

40 d of age

At 40 d of age, no interactive effects were observed ($P > 0.05$) (Table 3.10). However, cereal grain main effects ($P < 0.05$) were noted, broilers provided corn-based diets had higher concentrations ($P < 0.05$) of propionic, isobutyric, and isovaleric acids when compared with those fed wheat-based diets. In contrast, broilers fed wheat-based diets exhibited a 34% higher ($P < 0.05$) concentration of butyric acid compared with those fed corn-based diets. The greater concentration of butyric acid in broilers fed wheat-based diets may have been due to greater concentrations of fermentable soluble NSP because butyric acid is typically associated with NSP fermentation (De Vuyst and Leroy, 2011), and wheat contained 0.17% more NDF on a numerical basis than corn (Table 3.4). A main effect of xylanase ($P < 0.05$) was observed with broilers receiving the $RAME_n$ +12,000 BXU/kg xylanase having greater ($P < 0.05$) concentrations of isovaleric acid than those fed the $RAME_n$ + 24,000 BXU/kg xylanase, but both

concentrations were similar ($P > 0.05$) when compared with the NC TRT. The main effect of xylanase on increasing isovaleric concentrations is not readily explained; however, isovaleric concentrations were variable in cecal contents of broilers fed corn- (5.58 mM/L) and wheat-based (1.55 mM/L) diets supplemented with xylanase at 24,000 BXU/kg. Hence, this variability with the highest xylanase concentration lowered isovaleric concentrations when compared with corn- and wheat-based diets containing supplemental xylanase at 12,000 BXU/kg.

Masey-O' Neil et al. (2014) observed contrasting cereal grain effects at 49 d of age with broilers fed wheat-based diets exhibiting higher acetic, butyric, caproic, and total VFA, but lower propionic concentrations when compared with those fed the corn-based diets. These authors also observed that supplemental xylanase concentrations increased acetic, butyric, isovaleric, caproic, and total VFA. Differences in cereal grain and xylanase responses on VFA concentrations may be attributed to variations in NSP concentrations and AA sources, which likely affected the composition and concentrations of undigested nutrients reaching the ceca and altered subsequent VFA concentrations. Although NSP are the preferred energy substrates for most ceca microorganisms, undigested fractions of dietary and endogenous AA are also readily deaminated and fermented to produce VFA, branched-chain VFA (**BCVFA**), and other metabolites (ammonia, amines, indoles, and cresol) (Apajalahti and Vienola, 2016). The products of branched-chain AA (**BCAA**; valine, leucine, and isoleucine) fermentation are strictly BCVFA (isobutyric, isovaleric, and 2-methylbutyric, respectively) (Macfarlane et al., 1992). Therefore, increased concentrations of BCVFA are typically associated with greater AA fermentation (Qaisrani et al., 2015; Apajalahti and Vienola, 2016). In the

current research, 2-methylbutryic acid was not measured, but the greater concentrations of isobutyric and isovaleric acid in broilers fed the corn-based diets may have indicated that greater concentrations of undigested BCAA were being fermented in the ceca. However, the undigested fraction of BCAA in corn (corn; 7.45% CP) and wheat (wheat, winter; 11.03% CP) are approximately 0.08 and 0.12%, respectively (AMINODat 5.0; Evonik Nutrition and Care, Hanau, Germany). The undigested AA fraction was calculated using standardized ileal digestible (**SID**) coefficients for poultry reported in AMNODat 5.0 (Evonik Nutrition and Care, Hanau, Germany) by the following equation (content = %): [(*total AA% of ingredient*) - (*SID coefficient* × *total AA% of ingredient*)]. Likewise, calculated intake of undigested AA from each contributing ingredient was determined by the following equation: [(*% undigested AA*) × (% *ingredient of complete diet*) × (*FI*)], and the calculated intake of undigested AA from each contributing ingredient (corn, soybean meal, and dried distillers grains with solubles) were summed to equal the total dietary intake of undigested AA. The calculated intake of undigested dietary BCAA during the starter ($P = 0.001$) (1 to 14 d of age), grower ($P > 0.05$) (15 to 26 d of age), and finisher ($P = 0.001$) (27 to 40 d of age) periods demonstrated that broilers receiving corn-based diets consumed 5.0, 1.6, and 9.5%, respectively, less undigested BCAA than those fed wheat-based diets. Therefore, observed differences in isobutyric and isovaleric acid concentrations were likely not attributable to total dietary concentrations of undigested BCAA.

In contrast, cereal grain source differences ($P < 0.05$) in calculated intake of undigested Leu were observed from 1 to 26 d of age, broilers fed corn-based diets consumed 31.9 mg more ($P = 0.001$) undigested Leu than those fed wheat-based diets.

No calculated intake differences ($P > 0.05$) in undigested Leu from cereal grain source were observed from 1 to 40 d of age because during the finisher phase (27 to 40 d of age; Table 3.7) broilers fed wheat-based diets consumed 3.2% more feed than those fed corn-based diets. However, broilers fed corn-based diets numerically consumed 18.3 mg more undigested Leu than those fed wheat-based diets from 1 to 40 d of age. These greater concentrations of undigested Leu in broilers fed corn-based diets may have contributed to increased concentrations of isovaleric acid.

In addition, the calculated undigested Leu concentrations in the corn-based diets may have contributed to the increased concentrations of propionic and isobutyric. Zhang et al. (2013) demonstrated that in vitro ruminal BCVFA fermentation linearly increased with increasing concentrations of Leu. Similarly, certain ruminant microorganisms (*Ruminoccous albus, Ruminococcus flavefaciens,* and *Butyrivibio fibrisolvents*) require BCAA for growth and proliferation (Zhang et al., 2013). Accordingly, 19% of the broiler ceca microbiota composition is composed of *Ruminococcacea* (Apajalahti and Vienola, 2016). Therefore, it is plausible that these broiler ceca microorganisms may have similar requirements and responses to BCAA. The greater concentrations of propionic acid observed in the corn-based diets are typically associated with increased NSP fermentation, but straight-chain VFA such as propionic may also be a product of protein fermentation (Apajalahti and Vienola, 2016). However, increased concentrations of propionic acid are of interest because they have been observed to stimulate beneficial *Bifidobacterium* proliferation (Kaneko et al., 1994).

Dietary concentrations of resistant starch can also affect intestinal physio-chemical properties, post-ileal nutrient flow, and fermentation (Zijlstra, 2018). Cereal

grain sources containing high concentrations of amylose (> 60%) typically have increased concentrations of resistant starch (>49%) (Themeier et al., 2005). Compared with amylopectin, amylose polymers have less surface area and more intra-molecular hydrogen bonds, which reduces hydrolysis rate and digestibility (Regmi et al., 2011). Hence, broilers fed cereal grain sources rich in amylose likely have increased resistant starch concentrations, which may increases substrate availability in the ceca and subsequent VFA production (Zijlstra, 2018). Resistant starch types and concentrations can vary depending on particle size, processing conditions, and cereal grain sources (Englyst and Macfarlane, 1986). Moreover, corn has been reported have approximately 1.9 fold more resistant starch than wheat (Bednar et al., 2001). In the current experiment, corn and wheat had similar particle sizes and processing conditions, but variations in inherent resistant starch concentrations could have been one of the influencing factors affecting cereal grain source differences in cecal VFA concentrations.

The positive effects of including pharmacological dietary copper concentrations have been reported to be a result of gastrointestinal modulation (Hawbaker et al., 1961; Hojberg et al., 2005). Specifically, the bactericidal and bacteriostatic activities of copper indicate that it may have the potential to shift gastrointestinal microflora composition (Pang et al., 2009), which may negatively affect VFA production in broiler ceca. However, supplemental copper at 132 ppm did not negatively affect ceca fermentative capacity because VFA concentrations observed in the current experiment were comparable to similar experiments (Kiarie et al., 2014; Masey-O'Neill et al., 2014) that did not include pharmacological concentrations of supplemental copper in dietary TRT.

However, comparing cecal VFA concentrations and fermentative patterns from different experiments can be difficult due to variations in VFA flux-rate and ceca size.

Cecal VFA concentrations provide a broad understanding of the broiler's microflora and gastrointestinal health, but it is important to note that these are point-in-time measures whereas in vivo VFA concentrations are in a constant flux (Lee et al., 2017). Apajalahti (2017) observed that supplemental xylanase shifted broiler ceca microflora and increased butyrate production using ex vivo techniques, but these beneficial effects were not detected when measured in vivo. This author suggested that the rate of butyrate uptake by epithelial tissues was greater than the rate of production in vivo, so these effects may only be observed through ex vivo techniques. Therefore, it is concluded that butyrate values from residual cecal contents may be an unreliable indicator of production. However in the current experiment, the increased concentrations of butyric acid observed in broilers fed the wheat-based diets may indicate that fermentable NSP substrates in wheat are more conducive to butyric acid production than those found in corn.

In conclusion, cereal grain source and supplemental xylanase did not affect cumulative broiler growth performance. Feeding broilers corn-based diets subsequently increased concentrations of propionic, isobutyric, and isovaleric acid concentrations at 26 and 40 d of age indicating that broiler ceca fermentation may be affected by undigested Leu concentrations. However, supplemental xylanase did not affect broiler growth performance and cecal VFA concentrations. Therefore, future research evaluating the underlying factors affecting xylanase responses in broilers is warranted.

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Table 3.1 Ingredient and nutrient composition of diets fed to Ross × Ross 708 male broilers from 1 to 14 d of age

	Reduced AME_n[1]		Positive Control	
Ingredient, % "as-fed"	Corn	Wheat	Corn	Wheat
Corn	53.47	---	51.96	---
Wheat (11%)	---	52.14	---	50.67
Soybean meal (48%)	35.73	35.03	35.97	35.29
DDGS	5.00	5.00	5.00	5.00
Poultry oil	2.12	4.26	3.40	5.49
Dicalcium Phosphate	1.27	1.14	1.27	1.14
Calcium Carbonate	1.24	1.27	1.24	1.27
DL-Methionine	0.31	0.30	0.31	0.30
Vitamin Premix[2]	0.10	0.10	0.10	0.10
Mineral Premix[3]	0.10	0.10	0.10	0.10
NaCl	0.37	0.32	0.37	0.32
L-Lys·HCl	0.13	0.13	0.12	0.13
Choline	0.08	0.10	0.08	0.10
L-Threonine	0.04	0.06	0.04	0.06
Intellibond[4]	0.02	0.02	0.02	0.02
Sand	0.02	0.02	---	---
Phytase[5]	0.01	0.01	0.01	0.01
Xylanase[6]	---	---	---	---
Calculated Nutrient Content (%, unless otherwise indicated)				
AME_n, kcal/kg	2,959	2,959	3,025	3,025
Crude Protein	23.27	23.65	23.25	23.62
Digestible Lys	1.18	1.18	1.18	1.18
Digestible Met	0.61	0.59	0.62	0.60
Digestible TSAA	0.91	0.91	0.91	0.91
Digestible Thr	0.78	0.78	0.78	0.78
Digestible Val	0.92	0.92	0.92	0.92
Digestible Arg	1.36	1.36	1.36	1.36
Digestible Trp	0.24	0.26	0.24	0.26
Ca	1.00	1.00	1.00	1.00
Non-phytate P	0.48	0.48	0.48	0.48
Na	0.18	0.18	0.18	0.18

[1]Xylanase was added to the corn and wheat reduced AME_n ($RAME_n$) diets at the expense of sand to achieve the 3 experimental treatments containing either 0, 0.0075, or 0.015% xylanase, which provides 0, 12,000, and 24,000 BXU/kg of feed, respectively.

[2]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B12 (cyanocobalamin), 0.03 mg; folacin (folic acid), 2.7 mg: D-pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22.1 mg; niacin (niacinamide), 88.2 mg; thiamin (thiamin mononitrate), 5.5 mg; D-biotin (biotin), 0.18 mg; and pyridoxine (pyridoxine hydrochloride), 7.7 mg.

[3]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (stabilized ethylenediamine dihydriodide), 1.4 mg; Se (sodium selenite, cypress excel Se yeast), 0.3 mg.
[4]Intellibond C (Micronutrients, Inc., Indianapolis, IN) is a source of copper chloride that is 59.2% copper.
[5]Quantum Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides 5,000 FTU/g of phytase activity.
[6]Econase XT (AB Vista Feed Ingredients, Marlborough, UK) provides 160,000 BXU/g of xylanase activity. Xylanase (XYL) was included at 0, 0.0075, and 0.015%. Analyzed XYL activity concentrations: 1) Corn negative control (NC) ($RAME_n$ + 0.015% sand) = < 2000 BXU/kg; 2) Corn $RAME_n$ + 12,000 BXU/kg of XYL = 9,100 BXU/kg; 3) Corn $RAME_n$ + 24,000 BXU/kg of XYL = 20,100 BXU/kg; 4) Corn positive control (PC) = < 2,000 BXU/kg; 5) Wheat NC ($RAME_n$ + 0.015% sand) = 5,170 BXU/kg; 6) Wheat $RAME_n$ + 12,000 BXU/kg = 12,400 BXU/kg; 7) Wheat $RAME_n$ + 24,000 BXU/kg = 20,400 BXU/kg; 8) Wheat PC = < 2,000 BXU/kg.

Table 3.2 Ingredient and nutrient composition of diets fed to Ross × Ross 708 male broilers from 15 to 26 d of age

	Reduced AME_n[1]		Positive Control	
Ingredient, % "as-fed"	Corn	Wheat	Corn	Wheat
Corn	60.34	---	58.83	---
Wheat (11%)	---	58.84	---	57.37
Soybean meal (48%)	27.32	26.53	27.56	26.79
DDGS	7.00	7.00	7.00	7.00
Poultry oil	2.05	4.47	3.33	5.69
Dicalcium Phosphate	0.97	0.83	0.97	0.83
Calcium Carbonate	1.16	1.19	1.16	1.19
DL-Methionine	0.25	0.24	0.25	0.24
Vitamin Premix[2]	0.10	0.10	0.10	0.10
Mineral Premix[3]	0.10	0.10	0.10	0.10
NaCl	0.37	0.31	0.37	0.31
L-Lys·HCl	0.17	0.18	0.17	0.18
Choline	0.07	0.10	0.07	0.10
L-Threonine	0.06	0.08	0.06	0.08
Intellibond[4]	0.02	0.02	0.02	0.02
Sand	0.02	0.02	---	---
Phytase[5]	0.01	0.01	0.01	0.01
Xylanase[6]	---	---	---	---
Calculated Nutrient Content (%, unless otherwise indicated)				
AME_n, kcal/kg[7]	3,044	3,044	3,110	3,110
Crude Protein[8]	20.48	20.91	20.46	20.88
Digestible Lys	1.02	1.02	1.02	1.02
Digestible Met	0.52	0.50	0.52	0.50
Digestible TSAA	0.79	0.79	0.79	0.79
Digestible Thr	0.69	0.69	0.69	0.69
Digestible Val	0.80	0.80	0.80	0.80
Digestible Arg	1.14	1.14	1.14	1.14
Digestible Trp	0.20	0.22	0.20	0.22
Ca	0.88	0.88	0.88	0.88
Non-phytate P	0.42	0.42	0.42	0.42
Na	0.18	0.18	0.18	0.18

[1]Xylanase was added to the corn and wheat reduced AME_n diets at the expense of sand to achieve the 3 experimental treatments containing either 0, 0.0075, or 0.015% xylanase, which provides 0, 12,000, and 24,000 BXU/kg of feed respectively.

[2]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B12 (cyanocobalamin), 0.03 mg; folacin (folic acid), 2.7 mg: D-pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22.1 mg; niacin (niacinamide), 88.2 mg; thiamin (thiamin mononitrate), 5.5 mg; D-biotin (biotin), 0.18 mg; and pyridoxine (pyridoxine hydrochloride), 7.7 mg.

[3]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (stabilized ethylenediamine dihydriodide), 1.4 mg; Se (sodium selenite, cypress excel Se yeast), 0.3 mg.
[4]Intellibond C (Micronutrients, Inc., Indianapolis, IN) is a source of copper chloride that is 59.2% copper.
[5]Quantum Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides 5,000 FTU/g of phytase activity. Phytase was included in all dietary treatments (TRT) at 0.01%, thus the expected activity range was 400-600 FTU/kg.
[6]Econase XT (AB Vista Feed Ingredients, Marlborough, UK) provides 160,000 BXU/g of xylanase activity. Xylanase (XYL) was included at 0, 0.0075, and 0.015%. Analyzed XYL activity concentrations: 1) Corn negative control (NC) ($RAME_n$ + 0.015% sand) = < 2,000 BXU/kg; 2) Corn $RAME_n$ + 12,000 BXU/kg of XYL = 10,900 BXU/kg; 3) Corn $RAME_n$ + 24,000 BXU/kg of XYL = 23,000 BXU/kg; 4) Corn positive control (PC) = < 2,000 BXU/kg; 5) Wheat NC ($RAME_n$ + 0.015% sand) = < 2,000 BXU/kg; 6) Wheat $RAME_n$ + 12,000 BXU/kg = 13,500 BXU/kg; 7) Wheat $RAME_n$ + 24,000 BXU/kg = 23,700 BXU/kg; 8) Wheat PC = < 2,000 BXU/kg.

Table 3.3 Ingredient and nutrient composition of diets fed to Ross × Ross 708 male broilers from 27 to 40 d of age

	Reduced AME_n[1]		Positive Control	
Ingredient, % "as-fed"	Corn	Wheat	Corn	Wheat
Corn	63.98	---	62.47	---
Wheat (11%)	---	62.39	---	60.92
Soybean meal (48%)	21.97	21.14	22.21	21.40
DDGS	9.00	9.00	9.00	9.00
Poultry oil	2.23	4.80	3.51	6.02
Dicalcium Phosphate	0.65	0.49	0.65	0.50
Calcium Carbonate	1.02	1.05	1.02	1.05
DL-Methionine	0.21	0.20	0.21	0.20
Vitamin Premix[2]	0.10	0.10	0.10	0.10
Mineral Premix[3]	0.10	0.10	0.10	0.10
NaCl	0.36	0.30	0.36	0.30
L-Lys·HCl	0.21	0.21	0.20	0.21
Choline	0.08	0.11	0.08	0.10
L-Threonine	0.06	0.08	0.06	0.08
Intellibond[4]	0.02	0.02	0.02	0.02
Sand	0.02	0.02	---	---
Phytase[5]	0.01	0.01	0.01	0.01
Xylanase[6]	---	---	---	---
Calculated Nutrient Content (%, unless otherwise indicated)				
AME_n, kcal/kg[7]	3,114	3,114	3,180	3,180
Crude Protein[8]	18.84	19.30	18.82	19.26
Digestible Lys	0.93	0.93	0.93	0.93
Digestible Met	0.47	0.44	0.47	0.44
Digestible TSAA	0.72	0.72	0.72	0.72
Digestible Thr	0.62	0.62	0.62	0.62
Digestible Val	0.73	0.73	0.73	0.73
Digestible Arg	1.00	1.00	1.00	1.00
Digestible Trp	0.17	0.20	0.17	0.20
Ca	0.74	0.74	0.74	0.74
Non-phytate P	0.36	0.36	0.36	0.36
Na	0.18	0.18	0.18	0.18

[1]Xylanase was added to the corn and wheat reduced AME_n diets at the expense of sand to achieve the 3 experimental treatments containing either 0, 0.0075, or 0.015% xylanase, which provides 0, 12,000, and 24,000 BXU/kg of feed respectively.

[2]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B12 (cyanocobalamin), 0.03 mg; folacin (folic acid), 2.7 mg: D-pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22.1 mg; niacin (niacinamide), 88.2 mg; thiamin (thiamin mononitrate), 5.5 mg; D-biotin (biotin), 0.18 mg; and pyridoxine (pyridoxine hydrochloride), 7.7 mg.

[3]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (stabilized ethylenediamine dihydriodide), 1.4 mg; Se (sodium selenite, cypress excel Se yeast), 0.3 mg.
[4]Intellibond C (Micronutrients, Inc., Indianapolis, IN) is a source of copper chloride that is 59.2% copper.
[5]Quantum Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides 5,000 FTU/g of phytase activity. Phytase was included in all dietary treatments (TRT) at 0.01%, thus the expected activity range was 400-600 FTU/kg.
[6]Econase XT (AB Vista Feed Ingredients, Marlborough, UK) provides 160,000 BXU/g of xylanase activity. Xylanase (XYL) was included at 0, 0.0075, and 0.015%. Analyzed XYL activity concentrations: 1) Corn negative control (NC) ($RAME_n$ + 0.015% sand) = < 2000 BXU/kg; 2) Corn $RAME_n$ + 12,000 BXU/kg of XYL = 13,000 BXU/kg; 3) Corn $RAME_n$ + 24,000 BXU/kg of XYL = 24,900 BXU/kg; 4) Corn positive control (PC) = < 2000 BXU/kg; 5) Wheat NC ($RAME_n$ + 0.015% sand) = < 2000 BXU/kg; 6) Wheat $RAME_n$ + 12,000 BXU/kg = 12,500 BXU/kg; 7) Wheat $RAME_n$ + 24,000 BXU/kg = 26,200 BXU/kg; 8) Wheat PC = < 2000 BXU/kg.

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Table 3.4 Ingredient composition, neutral cellulase gammanase digestibility, particle size, and viscosity of the corn and wheat used in experimental diets

	Cereal grain[1]	
	Corn	Wheat
DM (%)	86.30	88.00
Ash (%)	1.22	1.87
Crude protein (%)	7.53	10.82
Ether extract (%)	3.98	2.41
Crude fiber (%)	1.35	2.19
Starch (%)	63.85	57.97
NDF (%)	8.09	8.26
ADF (%)	3.08	4.22
Sugar (%)	1.98	1.5
Phytate-P (%)	0.24	0.23
NCGD[2] (%)	91.67	91.02
Particle Size, (μm)[3]	655	665
Viscosity, (mPa·s)	1.2	6.0

[1]Values represent duplicate analyses on an as-fed basis. DM, ash, CP, ether extract, crude fiber, starch, NDF, ADF, sugar, phytate-P, and NCGD of the cereal grains were determined by near-infrared reflectance spectroscopy.
[2]NCGD = neutral cellulase gammanase digestibility. A coefficient that estimates the digestibility of the insoluble fiber fraction after cellulase and gammanase exposure.
[3]Particle size is the geometric diameter average (d_{gw}) of the cereal grain. The standard deviation (S_{gw}) and surface area of the corn and wheat were 1.84 and 1.78 μm and 84.0 and 80.8 cm^2/gram, respectively.

Table 3.5 Growth performance of Ross × Ross 708 male broilers fed diets varying in cereal grain source and supplemental xylanase concentrations from 1 to 14 d of age[1]

Cereal Grain	Dietary Treatment[2]	BW (kg)	BW Gain (kg)	Feed Intake (kg)	FCR[3] (kg:kg)	Mortality (%)
Corn	NC	0.434	0.394	0.495	1.256	1.6
	$RAME_n$[4] + 12,000 BXU/kg[5]	0.437	0.398	0.504	1.269	0.5
	$RAME_n$ + 24,000 BXU/kg	0.446	0.407	0.499	1.229	1.0
	PC	0.448	0.409	0.493	1.206	0.0
Wheat	NC	0.447	0.408	0.502	1.231	1.0
	$RAME_n$ + 12,000 BXU/kg	0.450	0.410	0.504	1.229	0.5
	$RAME_n$+ 24,000 BXU/kg	0.440	0.402	0.509	1.267	1.0
	PC	0.439	0.399	0.500	1.256	2.1
SEM		0.005	0.005	0.005	0.011	0.6
Cereal Grain Main Effects						
Corn		0.441	0.400	0.499	1.251	0.8
Wheat		0.444	0.407	0.505	1.242	1.2
SEM		0.003	0.003	0.003	0.007	0.3
Xylanase Main Effects						
	NC	0.440	0.401	0.499	1.244	1.3
	$RAME_n$ + 12,000 BXU/kg	0.443	0.404	0.504	1.249	0.5
	$RAME_n$ + 24,000 BXU/kg	0.443	0.404	0.504	1.248	1.0
	SEM	0.004	0.004	0.004	0.008	0.4
Analysis of Variance				*Probabilities*		
Cereal Grain × Xylanase[6]		0.11	0.12	0.69	0.51	0.90
Cereal Grain		0.11	0.09	0.24	0.36	0.73
Xylanase		0.82	0.80	0.57	0.90	0.47

Pre-planned orthogonal contrasts					
Corn NC vs. Corn PC	0.04	0.04	0.80	0.002	0.08
Wheat NC vs. Wheat PC	0.21	0.16	0.80	0.11	0.24

[1]Each value represents the least-square means of 8 replicate pens with each pen having 24 chicks at placement.

[2] Dietary treatment consisted of 4 possible treatments: 1) negative control (NC) ($RAME_n$+ 0.015% sand); 2) $RAME_n$ + 0.0075% Econase + 0.0075% sand; 3) $RAME_n$ + 0.015% Econase; or 4) positive control (PC).

[3]FCR = feed conversion ratio corrected for mortality.

[4]$RAME_n$ = Reduced AME_n

[5]Econase XT (AB Vista Feed Ingredients, Marlborough, UK) inclusion concentration, which provides 160,000 BXU/g of xylanase activity.

[6]Interaction effects include all dietary TRT except the PC diets, which were excluded from the analysis.

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

Table 3.6 Growth performance of Ross × Ross 708 male broilers fed diets varying in cereal grain source and supplemental xylanase concentrations from 1 to 26 d of age[1]

Cereal Grain	Dietary Treatment[2]	BW (kg)	BW Gain (kg)	Feed Intake (kg)	FCR[3] (kg:kg)	Mortality (%)
Corn	NC	1.368	1.327	1.891	1.423	3.3
	$RAME_n$[4] + 12,000 BXU/kg[5]	1.369	1.328	1.905	1.441	2.2
	$RAME_n$ + 24,000 BXU/kg	1.388	1.347	1.922	1.426	3.7
	PC	1.368	1.329	1.877	1.413	2.6
Wheat	NC	1.285	1.243	1.847	1.484	3.8
	$RAME_n$ + 12,000 BXU/kg	1.317	1.275	1.873	1.465	1.7
	$RAME_n$ + 24,000 BXU/kg	1.297	1.257	1.864	1.482	2.7
	PC	1.296	1.254	1.826	1.454	2.1
SEM		0.019	0.019	0.026	0.015	1.1
Cereal Grain Main Effects						
Corn		1.375	1.334	1.906	1.430	3.0
Wheat		1.300	1.259	1.861	1.477	2.6
SEM		0.011	0.011	0.014	0.007	0.6
Xylanase Main Effects						
	NC	1.326	1.285	1.869	1.454	3.6
	$RAME_n$ + 12,000 BXU/kg	1.343	1.302	1.889	1.453	1.9
	$RAME_n$ + 24,000 BXU/kg	1.342	1.302	1.893	1.454	3.2
	SEM	0.013	0.013	0.016	0.008	0.8
Analysis of Variance				*Probabilities*		
Cereal Grain × Xylanase[6]		0.53	0.58	0.83	0.23	0.64
Cereal Grain		0.001	0.001	0.013	0.001	0.89
Xylanase		0.58	0.58	0.47	0.99	0.50

Pre-planned orthogonal contrasts					
Corn NC vs. Corn PC	0.99	0.93	0.66	0.51	0.06
Wheat NC vs. Wheat PC	0.66	0.67	0.51	0.07	0.45

[1]Each value represents the least-square means of 8 replicate pens with each pen having 24 chicks at placement.

[2] Dietary treatment consisted of 4 possible treatments: 1) negative control (NC) ($RAME_n$+ 0.015% sand); 2) $RAME_n$ + 0.0075% Econase + 0.0075% sand; 3) $RAME_n$ + 0.015% Econase; or 4) positive control (PC).

[3]FCR = feed conversion ratio corrected for mortality.

[4]$RAME_n$ = Reduced AME_n

[5]Econase XT (AB Vista Feed Ingredients, Marlborough, UK) inclusion concentration, which provides 160,000 BXU/g.

[6]Interaction effects include all dietary TRT except the PC diets, which were excluded from the analysis.

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

Table 3.7 Growth performance of Ross × Ross 708 male broilers fed diets varying in cereal grain source and supplemental xylanase concentrations from 27 to 40 d of age[1]

Cereal Grain	Dietary Treatment[2]	BW Gain (kg)	Feed Intake (kg)	FCR[3] (kg:kg)	Mortality (%)
Corn	NC	1.470	2.548	1.733	1.6
	$RAME_n$[4] + 12,000 BXU/kg[5]	1.511	2.627	1.739	2.3
	$RAME_n$ + 24,000 BXU/kg	1.433	2.600	1.820	0.0
	PC	1.503	2.580	1.720	0.0
Wheat	NC	1.546	2.737	1.768	4.0
	$RAME_n$ + 12,000 BXU/kg	1.560	2.693	1.727	0.7
	$RAME_n$ + 24,000 BXU/kg	1.544	2.652	1.723	0.8
	PC	1.530	2.606	1.705	4.0
SEM		0.032	0.058	0.030	1.2
Cereal Grain Main Effects					
Corn		1.471	2.591	1.764	1.3
Wheat		1.550	2.694	1.739	1.8
SEM		0.022	0.039	0.018	0.7
Xylanase Main Effects					
	NC	1.508	2.642	1.750	2.8
	$RAME_n$ + 12,000 BXU/kg	1.535	2.660	1.733	1.5
	$RAME_n$ + 24,000 BXU/kg	1.488	2.625	1.771	0.4
	SEM	0.025	0.045	0.021	0.9
Analysis of Variance		*Probabilities*			
Cereal Grain × Xylanase[6]		0.53	0.38	0.10	0.27
Cereal Grain		0.001	0.02	0.32	0.61
Xylanase		0.25	0.81	0.45	0.17

Pre-planned orthogonal contrasts				
Corn NC vs. Corn PC	0.41	0.63	0.76	0.36
Wheat NC vs. Wheat PC	0.68	0.06	0.12	1.00

[1]Each value represents the least-square means of 8 replicate pens with each pen having 24 chicks at placement.

[2] Dietary treatment consisted of 4 possible treatments: 1) negative control (NC) ($RAME_n$+ 0.015% sand); 2) $RAME_n$ + 0.0075% Econase + 0.0075% sand; 3) $RAME_n$ + 0.015% Econase; or 4) positive control (PC).

[3]FCR = feed conversion ratio corrected for mortality.

[4]$RAME_n$ = Reduced AME_n

[5]Econase XT (AB Vista Feed Ingredients, Marlborough, UK) inclusion concentration, which provides 160,000 BXU/g of xylanase activity.

[6]Interaction effects include all dietary TRT except the PC diets which were excluded from the analysis.

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

Table 3.8 Growth performance of Ross × Ross 708 male broilers fed diets varying in cereal grain source and supplemental xylanase concentrations from 1 to 40 d of age[1]

Cereal Grain	Dietary Treatment[2]	BW (kg)	BW Gain (kg)	Feed Intake (kg)	FCR[3] (kg:kg)	Mortality (%)
Corn	NC	2.838	2.800	4.446	1.588	4.7
	$RAME_n$[4] + 12,000 BXU/kg[5]	2.873	2.834	4.534	1.600	4.7
	$RAME_n$ + 24,000 BXU/kg	2.820	2.781	4.533	1.631	1.6
	PC	2.871	2.832	4.465	1.577	0.5
Wheat	NC	2.830	2.792	4.527	1.621	5.2
	$RAME_n$ + 12,000 BXU/kg	2.877	2.838	4.588	1.617	4.2
	$RAME_n$ + 24,000 BXU/kg	2.841	2.803	4.471	1.596	3.1
	PC	2.826	2.786	4.397	1.579	6.3
SEM		0.032	0.032	0.068	0.018	1.3
Cereal Grain Main Effects						
Corn		2.844	2.805	4.504	1.607	2.9
Wheat		2.850	2.811	4.523	1.611	4.7
SEM		0.021	0.021	0.047	0.011	0.8
Xylanase Main Effects						
	NC	2.834	2.795	4.487	1.605	5.0
	$RAME_n$ + 12,000 BXU/kg	2.875	2.836	4.561	1.608	4.4
	$RAME_n$ + 24,000 BXU/kg	2.831	2.792	4.502	1.614	2.3
	SEM	0.025	0.024	0.055	0.013	0.9
Analysis of Variance		*Probabilities*				
Cereal Grain × Xylanase[6]		0.90	0.89	0.57	0.19	0.20
Cereal Grain		0.82	0.81	0.67	0.76	0.06
Xylanase		0.31	0.31	0.55	0.89	0.09

Pre-planned orthogonal contrasts					
Corn NC vs. Corn PC	0.46	0.46	0.83	0.67	0.06
Wheat NC vs. Wheat PC	0.91	0.90	0.15	0.09	0.53

[1]Each value represents the least-square means of 8 replicate pens with each pen having 24 chicks at placement.

[2] Dietary treatment consisted of 4 possible treatments: 1) negative control (NC) ($RAME_n$+ 0.015% sand); 2) $RAME_n$ + 0.0075% Econase + 0.0075% sand; 3) $RAME_n$ + 0.015% Econase; or 4) positive control (PC).

[3]FCR = feed conversion ratio corrected for mortality.

[4]$RAME_n$ = Reduced AME_n

[5]Econase XT (AB Vista Feed Ingredients, Marlborough, UK) inclusion concentration, which provides 160,000 BXU/g of xylanase activity.

[6]Interaction effects include all dietary TRT except the PC diets, which were excluded from the analysis.

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

Table 3.9 Cecal volatile fatty acid concentrations (mM/L) of Ross × Ross 708 male broilers fed diets varying in cereal grain source and supplemental xylanase concentrations at 26 d of age[1]

Cereal Grain	Dietary Treatment[2]	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Isocaproic	Caproic	Heptanoic	Total
Corn	NC	201	9.21	2.80[ab]	18.95	5.09[ab]	1.23	0.010	0.022	0.0014	238
	$RAME_n$[3] + 12,000 BXU/kg[4]	197	10.40	3.20[ab]	19.08	5.70[ab]	1.22	0.009	0.019	0.0010	236
	$RAME_n$ + 24,000 BXU/kg	220	10.67	5.04[a]	17.56	8.54[a]	1.02	0.010	0.019	0.0020	263
	PC	230	11.00	3.92	19.13	6.95	1.41	0.010	0.023	0.0015	273
Wheat	NC	168	7.78	2.26[b]	19.77	3.89[b]	1.05	0.010	0.016	0.0008	203
	$RAME_n$ + 12,000 BXU/kg	225	7.35	2.47[b]	23.73	4.57[b]	1.09	0.011	0.018	0.0012	264
	$RAME_n$ + 24,000 BXU/kg	142	5.33	1.54[b]	22.26	2.55[b]	1.04	0.012	0.017	0.0008	175
	PC	198	8.80	1.42	21.90	2.78	1.28	0.021	0.021	0.0017	234
	SEM	33	1.83	0.68	1.94	1.11	0.14	0.003	0.003	0.0005	35
Cereal Grain Main Effects											
Corn		206	10.09	3.68	18.53	6.44	1.15	0.010	0.020	0.0015	246
Wheat		178	6.82	2.09	21.92	3.67	1.06	0.011	0.017	0.0011	214
SEM		24	1.33	0.50	1.26	0.87	0.11	0.002	0.002	0.0003	27
Xylanase Main Effects											
	NC	185	8.50	2.53	19.36	4.49	1.14	0.010	0.019	0.0011	221
	$RAME_n$ + 12,000 BXU	211	8.87	2.84	21.40	5.13	1.15	0.010	0.019	0.0011	250
	$RAME_n$ + 24,000 BXU	181	8.00	3.29	19.91	5.54	1.03	0.011	0.018	0.0014	219
	SEM	27	1.47	0.55	1.46	0.94	0.11	0.002	0.002	0.0004	29
Analysis of Variance						*Probabilities*					
Cereal Grain × Xylanase[5]		0.17	0.46	0.03	0.52	0.01	0.65	0.77	0.48	0.47	0.17
Cereal Grain		0.23	0.018	0.003	0.03	0.001	0.31	0.33	0.04	0.23	0.20
Xylanase		0.50	0.85	0.41	0.52	0.47	0.50	0.87	0.81	0.82	0.50

Pre-planned orthogonal contrasts										
Corn NC vs. Corn PC	0.42	0.37	0.14	0.94	0.11	0.19	0.96	0.74	0.93	0.38
Wheat NC vs. Wheat PC	0.98	0.42	0.02	0.27	0.01	0.67	0.006	0.44	0.37	0.95

[1]Each value represents the least-square means of pooled cecal digesta from 7 birds per pen with 5 replicate pens per treatment.

[2] Dietary treatment consisted of 4 possible treatments: 1) negative control (NC) ($RAME_n$+ 0.015% sand); 2) $RAME_n$ + 0.0075% Econase + 0.0075% sand; 3) $RAME_n$ + 0.015% Econase; or 4) positive control (PC).

[3]$RAME_n$ = Reduced AME_n

[4]Econase XT (AB Vista Feed Ingredients, Marlborough, UK) inclusion concentration, which provides 160,000 BXU/g.

[5]Interaction effects include all dietary TRT except the PC diets which were excluded from the analysis.

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

Table 3.10 Cecal volatile fatty acid concentrations (mM/L) of Ross × Ross 708 male broilers fed diets varying in cereal grain source and supplemental xylanase concentrations at 40 d of age[1]

Cereal Grain	Dietary Treatment[2]	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Isocaproic	Caproic	Heptanoic	Total
Corn	NC	163	9.65	3.89	17.55	6.00	1.32	0.008	0.017	0.0013	201
	$RAME_n$[3] + 12,000 BXU/kg[4]	141	11.06	4.47	14.28	7.46	1.18	0.008	0.015	0.0012	180
	$RAME_n$ + 24,000 BXU/kg	139	7.86	3.49	17.42	5.88	1.12	0.008	0.017	0.0021	175
	PC	214	11.71	3.99	20.60	6.64	1.50	0.009	0.086	0.0042	258
Wheat	NC	178	8.07	2.24	21.39	4.28	1.14	0.009	0.018	0.0028	215
	$RAME_n$ + 12,000 BXU/kg	181	6.21	2.12	22.72	3.79	1.06	0.006	0.013	0.0013	217
	$RAME_n$ + 24,000 BXU/kg	149	5.59	0.78	30.29	1.55	1.15	0.008	0.017	0.0018	188
	PC	202	8.13	1.77	23.39	3.20	1.03	0.007	0.015	0.0012	240
	SEM	27	1.32	0.60	3.11	0.69	0.13	0.001	0.002	0.0007	30
Cereal Grain Main Effects											
Corn		148	9.52	3.95	16.41	6.45	1.21	0.008	0.016	0.0016	185
Wheat		170	6.62	1.71	24.80	3.21	1.12	0.008	0.016	0.0020	206
SEM		15	0.76	0.34	2.46	0.40	0.10	0.002	0.002	0.0004	16
Xylanase Main Effects											
	NC	170	8.86	3.06	19.47	5.14[ab]	1.23	0.008	0.018	0.0021	208
	$RAME_n$ + 12,000 BXU	161	8.64	3.29	18.50	5.62[a]	1.12	0.007	0.014	0.0013	198
	$RAME_n$ + 24,000 BXU	144	6.73	2.13	23.85	3.71[b]	1.14	0.008	0.017	0.0019	182
	SEM	19	0.93	0.42	2.64	0.49	0.11	0.001	0.002	0.0005	20
Analysis of Variance						*Probabilities*					
Cereal Grain × Xylanase[5]		0.83	0.44	0.67	0.18	0.17	0.68	0.44	0.53	0.40	0.89
Cereal Grain		0.33	0.01	0.001	0.001	0.001	0.38	0.78	0.92	0.48	0.37
Xylanase		0.62	0.23	0.14	0.07	0.03	0.63	0.50	0.07	0.45	0.65

Pre-planned orthogonal contrasts										
Corn NC vs. Corn PC	0.21	0.29	0.91	0.35	0.56	0.29	0.58	0.03	0.09	0.19
Wheat NC vs. Wheat PC	0.54	0.97	0.59	0.54	0.33	0.54	0.22	0.91	0.48	0.56

[1]Each value represents the least-square means of pooled cecal digesta from 7 birds per pen with 5 replicate pens per treatment.

[2] Dietary treatment consisted of 4 possible treatments: 1) negative control (NC) ($RAME_n$+ 0.015% sand); 2) $RAME_n$ + 0.0075% Econase + 0.0075% sand; 3) $RAME_n$ + 0.015% Econase; or 4) positive control (PC).

[3]$RAME_n$ = Reduced AME_n

[4]Econase XT (AB Vista Feed Ingredients, Marlborough, UK) inclusion concentration, which provides 160,000 BXU/g.

[5]Interaction effects include all dietary TRT except the PC diets which were excluded from the analysis.

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

IV. EFFECTS OF SUPPLEMENTAL XYLANASE IN CORN- AND WHEAT-BASED DIETS ON CECAL VOLATILE FATTY ACID CONCENTRATIONS OF BROILERS FROM 14 TO 42 DAYS OF AGE

ABSTRACT

An experiment was conducted to evaluate the effects of supplemental xylanase in corn- or wheat-based diets on cecal volatile fatty acid (VFA) concentrations of Ross × Ross 708 male broilers during weekly intervals from 14 to 42 d of age. Day-old chicks (1,500) were randomly distributed into 60 floor pens (25 chicks/pen; 0.078 m2/bird) and fed 1 of 4 dietary treatments (TRT) throughout the starter (1 to 14 d of age), grower (15 to 28 d of age), and finisher (29 to 42 d of age) phases with 15 replicates per TRT. Dietary TRT consisted of a 2 × 2 factorial arrangement with 2 diet types (corn- or wheat-based) and 2 xylanase inclusions (0 or 16,000 BXU/kg) as the main factors. At 14, 21, 28, 35, and 42 d of age, cecal contents were collected (4 birds/pen) for VFA analysis. Main effects of cereal grain source ($P < 0.05$) affected propionic, isobutyric, butyric, isovaleric, valeric, and isocaproic acid concentrations at 14, 21, 28, 35, and 42 d of age. Broilers fed corn-based diets had higher ($P < 0.05$) propionic, isobutyric, isovaleric, valeric, and isocaproic concentrations than those fed wheat-based diets from 14 to 42 d of age. However, broilers fed wheat-based diets had higher ($P < 0.05$) butyric acid concentrations at 28, 35, and 42 d of age compared with those fed corn-based diets. Individual and total VFA concentrations increased ($P < 0.05$) linearly from 14 to 42 d of age. Age and cereal grain interacted ($P < 0.05$) to affect propionic, isobutyric, butyric,

isovaleric, and valeric acid concentrations. These results indicate that broiler cecal VFA concentrations are influenced by cereal grain source and age. In contrast, supplemental xylanase inconsistently influenced broiler cecal VFA concentrations. Therefore, future research evaluating factors affecting supplemental xylanase and cecal VFA production in broilers is warranted. Additionally, research investigating cereal grain source effects on cecal microflora development and fermentative patterns may be beneficial for optimizing cecal VFA production in broilers.

INTRODUCTION

In broilers, the beneficial effects of supplemental xylanase on growth performance and nutrient utilization are primarily attributed to viscosity reduction, nutrient encapsulation reduction, or microbiota mediation (Choct et al., 1996, 1999; Bedford, 2002; Bedford and Cowieson, 2012). The primary mode of action responsible for these beneficial effects may be dependent on the type of cereal grain used in diet formulation (Bedford and Schulze, 1998). In the United States and Latin America, corn is the primary cereal grain used in broiler diet formulation, whereas wheat is the dominant dietary cereal grain used in Canada and Europe (Barletta, 2010). Differences in corn and wheat nutrient composition, particularly arabinoxylan concentrations, indicate that the magnitude of broiler responses may vary with supplemental xylanase (Cowieson et al., 2010).

Energy-sparing effects of supplemental xylanase may be a result of microbiota mediation in the broiler small and large intestine (Choct et al., 1996, 1999). Accordingly, xylanase has been proposed to stimulate a prebiotic effect by increasing concentrations of highly-fermentable arabinoxylo- (**AXOS**) and xylo-oligosaccharides (**XOS**) in the distal

portions of the large intestine, which may increase ceca fermentative capacity and subsequent volatile fatty acid (**VFA**) production (Masey O'Neill et al., 2014). Cecal microbial concentrations have been observed to increase and vary as the broiler progresses in age (Bedford and Cowieson, 2012). Therefore, the prebiotic effect of xylanase may become more pronounced as birds' age. Although microbiota mediation is generally accepted as a valid mode of action, data to support the proposed relationship between diets varying in cereal grain source and xylanase inclusion on cecal VFA production in broilers is limited in the literature. Furthermore, the age-specific effects of diets varying in cereal grain source and xylanase inclusion on ceca fermentative capacity have not been previously reported in the literature. Therefore, the objective of this experiment was to evaluate the interactive effects of supplemental xylanase in corn- and wheat-based diets on broiler cecal VFA concentrations over time from 14 to 42 d of age.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Auburn University approved the use of live birds in this experimental protocol (PRN 2017-2999).

Bird Husbandry

One thousand five hundred Ross × Ross 708 (Aviagen, Inc., Huntsville, AL) male broiler chicks were obtained from a commercial hatchery at day of hatch and randomly distributed into 60 floor pens (25 chicks/pen; 0.078 m^2). Chicks were vaccinated for Newcastle disease, Marek's disease, and infectious bronchitis at the hatchery. The research facility was a solid-sided cross-ventilated house equipped with vent boards, exhaust fans, stir fans, evaporative-cooling pads, forced-air heaters, and an electronic controller system (Evolution 3000, Hired Hand Manufacturing, Inc., Bremen, AL). Each

floor pen contained new pine-shavings, a nipple drinker line (6 nipples per pen), and a hanging pan-feeder. Birds were provided ad libitum access to feed and water. Initial ambient house temperature was set at 33°C and was gradually decreased to 20°C as birds increased in age to ensure bird comfort. Photoperiod was 23 h of light and 1 h of darkness from 1 to 7 d of age, and 20 h of light and 4 h of darkness from 8 to 42 d of age. Light intensity was set at 30, 10, and 5 lux from 1 to 7, 8 to 14, and 15 to 42 d of age, respectively. Light intensity settings were verified at bird level (30 cm) using a photometric sensor (LI-250A Light Meter, LI-COR Bioscience, Lincoln, NE) for each intensity adjustment.

Dietary Treatments

Four dietary treatments (**TRT**) were fed throughout the starter (1 to 14 d of age), grower (15 to 28 d of age), and finisher (29 to 42 d of age) phases (Tables 4.1, 4.2, and 4.3, respectively). Dietary TRT consisted of a 2 × 2 factorial arrangement with 2 cereal grain sources (corn- or wheat-based) and 2 xylanase inclusion concentrations (0 or 16,000 BXU/kg). All dietary TRT were formulated to be adequate in all essential nutrients. Apparent metabolizable energy was formulated to be 66 kcal/kg below optimal concentrations. In the supplemented corn- and wheat-based TRT, xylanase was included at 100 g per tonne of feed, whereas in the unsupplemented diets, an equivalent amount of sand was added. The xylanase used was a mono-component xylanase (endo-1, 4-β-xylanase; EC 3.2.1.8) expressed by *Trichoderma reesei* (Econase XT providing 160,000 BXU/g; AB Vista Feed Ingredients, Marlborough, UK), supplemented to achieve 16,000 BXU/kg. All dietary TRT contained phytase (*Escherichia coli*-6-phytase; EC 3.1.3. 26) expressed by *Trichoderma reesei* (Quantum Blue; AB Vista Feed Ingredients,

Marlborough, UK), which provided 5,000 FTU/g to achieve activity concentrations of 500 FTU/kg. Phytase was formulated to provide 0.15, 0.165, and 0.03% of P, Ca, and Na, respectively. Feed form was provided as crumbles during the starter phase (1 to 14 d of age) and whole pellets thereafter (15 to 42 d of age). Enzymes were added in the mixer and conditioning/pelleting temperatures did not exceed 85° C. Approximately 0.5% of the supplemental fat was added in the mixer and the remaining percentage was applied post-pelleting to ensure pellet quality. Pellet durability indexes (**PDI**) of the grower and finisher diets were measured using standard PDI procedure (method 269.4; ASAE, 2003a).

Xylanase concentrations of all experimental TRT were determined by a commercial laboratory (Enzyme Services and Consultancy, Ystrad Mynach, UK) using enzyme-linked immuno-sorbent assays (Quantiplate™ Kits, Envirologix, Inc., Portland, ME) specific for Econase XT. In addition, representative sub-samples of corn and wheat used in dietary TRT were analyzed for nutrient composition, neutral cellulase gammanase digestibility (**NCGD**), and viscosity by a commercial laboratory (Enzyme Services and Consultancy, Ystrad Mynach, UK). Nutrient composition (DM, ash, CP, fiber, starch, phytate-P, neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), and sugar) and NCGD were predicted by near-infrared reflectance spectrophotometer analysis (FOSS NIR Systems model 2500, Eden Prairie, MN) with a range of 400 to 2,500 nm using online Feed Quality Service calibrations (AB Vista Feed Ingredients, Marlborough, UK). Cereal grain viscosity measurements were determined by an in vitro digestion viscosity method described by Bedford and Classen (1993) and Svihus et al. (2000). Samples were digested in HCl/pepsin (45 min) and $NaHCO_3$/pancreatin (120 min) solutions at 40° C,

centrifuged to separate the supernatant (1,800 × g for 10 min), and shear strength of the supernatant was measured with a Brookfield digital viscometer (Model DV-II, Brookfield Engineering Laboratories, Stoughton, MA). Corn and wheat samples were analyzed for particle size using a sieve shaker (Tyler RoTap, Mentor, OH) according to the standard method (method S391.3; ASAE, 2003b). All the aforementioned diet and cereal grain analyses were performed in duplicate.

Measurements

At 14, 21, 28, 35, and 42 d of age, 4 birds per pen (15 replicate pens per TRT) were randomly selected, weighed, and euthanized via CO_2 asphyxiation. Following asphyxiation, ceca were excised at the ileo-cecal junction. Cecal contents were gently squeezed into sterile sealed cups. Samples were pooled per pen, immediately placed on ice, and frozen at –20° C until further VFA analysis. Moreover, to ensure that birds were achieving acceptable performance, birds and feed were weighed at 1, 14, 28, and 42 d of age to determine BW and feed intake (Table 4.5). Feed conversion results were not included because weekly cecal sampling caused insufficient bird number by the end of experimentation. Live performance data were presented to provide an indication of growth performance, but caution should be exercised with interpretation because birds were removed weekly for necropsy.

Determination of Cecal Volatile Fatty Acid Concentrations

Cecal VFA concentrations were determined according to the method described by Weber et al. (2010). Cecal samples were thawed and thoroughly hand-mixed with sterile stir sticks for 30 s. Approximately 1.0 g of cecal digesta from each sample was placed into a 15-mL polypropylene centrifuge tube and diluted with 5 mL of deionized water.

Samples were mixed for 12 h at 1,200 rpm on a digital microplate shaker (Thermo Fisher Scientific Inc, Miami, OK). After mixing, samples were centrifuged at 4° C for 23 min at 21,000 × g to separate the supernatant. Approximately 2.5 mL of clear supernatant was removed and placed into tubes and *o*-phosphoric acid was added to achieve a pH of 2.5. Exactly 1 mL of the pH-adjusted supernatant sample was placed into 20 mL gas chromatography vials with 0.3 g of NaCl. Prepared samples were frozen and shipped to an external laboratory (USDA-ARS-MWA-NLAE, Ames, IA) for gas chromatography analysis (Agilent 7890A Gas Chromatograph, Agilent Technologies, Inc, Wilmington, DE). Samples were analyzed in duplicate and values were multiplied by 5 to adjust for the dilution factor. Total VFA concentrations are the summation of the following individual VFA: acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic, and heptanoic acid.

Statistical Analyses

Data were analyzed as a randomized complete block design with pen location as the blocking factor. Each TRT was represented by 15 replicate pens with pen considered as the experimental unit. Analysis of variance of growth performance and VFA data at each time point was performed using PROC MIXED (SAS, 2011) by the following mixed-effects model:

$$Y_{ijk} = \mu\ldots + \rho_i + \alpha_j + \beta_k + (\alpha\beta)_{jk} + \varepsilon_{ijk}$$

where $\mu\ldots$ is the overall mean; the ρ_i are identically and independently normally distributed random block effects with mean 0 and variance σ^2_{ρ}; the α_j are fixed factor level effects corresponding to the j^{th} cereal grain source (corn and wheat) such that $\Sigma\alpha_j = 0$; the β_k are fixed factor level effects corresponding to the k^{th} xylanase inclusion (0 and

16,000 BXU/kg) such that $\Sigma\beta_k = 0$; the $(\alpha\beta)_{jk}$ are interaction level effects corresponding to either j^{th} cereal grain source and the k^{th} xylanase inclusion such that $\Sigma_j(\alpha\beta)_{jk} = 0$ and $\Sigma_k(\alpha\beta)_{jk} = 0$; and the random error ε_{ijk} are identically and independently normally distributed with mean 0 and variance σ^2. Statistical significance was considered at $P \leq 0.05$, and interaction and main effects were separated using Tukey's Honestly Significant Difference test (Tukey, 1953).

Furthermore, to assess the effects of age on VFA concentrations, a repeated-measures analysis of variance was performed using PROC MIXED (SAS, 2011) by the following mixed-effects model:

$$Y_{ijkl} = \mu\ldots + \rho_i + \alpha_j + \beta_k + \gamma_l + (\alpha\gamma)_{jl} + (\beta\gamma)_{kl} + (\alpha\beta\gamma)_{jkl} + \varepsilon_{ijkl}$$

where $\mu\ldots$ is the overall mean; the ρ_i are identically and independently normally distributed random block effects with mean 0 and variance $\sigma^2_{\ \rho}$; the α_j are fixed factor level effects corresponding to the j^{th} cereal grain source (corn and wheat) such that $\Sigma\alpha_j = 0$; the β_k are fixed factor level effects corresponding to the k^{th} xylanase inclusion (0 and 16,000 BXU/kg) such that $\Sigma\beta_k = 0$; the γ_l are fixed factor level effects corresponding to the l^{th} sample day (14, 21, 28, 35, and 42 d of age) such that $\Sigma\ \gamma_l = 0$; the $(\alpha\gamma)_{jl}$ through $(\alpha\beta\gamma)_{jkl}$ are the interaction effects corresponding to either j^{th} cereal grain source, k^{th} xylanase inclusion, and l^{th} sample day such that $\Sigma(\alpha\gamma)_{jl}$ through $\Sigma(\alpha\beta\gamma)_{jkl} = 0$; and the random error ε_{ijkl} are identically and independently normally distributed with mean 0 and variance σ^2. Effects of xylanase inclusion were not significant ($P > 0.05$) and subsequently removed from the model. Linear, quadratic, and cubic polynomial contrasts were conducted to determine the relationship between cereal grain source and age on VFA concentrations. Statistical significance was established at $P \leq 0.05$, and interaction

and main effects were separated using Tukey's Honestly Significant Difference test (Tukey, 1953).

RESULTS

Diet and Cereal Grain Analyses

Analyzed xylanase concentrations of the starter, grower, and finisher diets are reported in Tables 4.1, 4.2, and 4.3, respectively. Analyzed xylanase concentrations were in close agreement with their calculated values. Analyzed xylanase concentrations in the unsupplemented control TRT ranged from < 2,000 to 2,500 BXU/kg. Dietary TRT formulated with 16,000 BXU/kg of supplemental xylanase had analyzed concentrations ranging from 14,400 to 16,200 BXU/kg. All dietary TRT exhibited good pellet quality throughout the grower and finisher phases. Pellet durability index values of the corn- and wheat-based diets ranged from 87.1 to 89.7% and 91.2 to 94.0%, respectively.

Cereal grain analyses of corn and wheat are reported in Table 4.4. Wheat numerically exhibited greater DM, ash, CP, crude fiber, ADF, and sugar concentrations, but numerically lower EE, NDF, starch, and phytate-P contents compared with corn. Wheat exhibited a slightly higher numerical NCGD (92.25%) than corn (91.49%). In vitro digestion viscosity analysis demonstrated that wheat (9.0 mPa·s) had a greater viscosity concentration than corn (1.2 mPa·s). Particle size was approximately 636 and 592 μm for the corn and wheat, respectively.

Cecal VFA Concentrations

14 d of age

Cereal grain source and supplemental xylanase interacted ($P < 0.05$) to affect cecal butyric acid concentrations of broilers (Table 4.6). Broilers receiving corn-based

diets with and without supplemental xylanase had 69 and 55%, respectively, higher (P = 0.02) concentrations of butyric acid when compared with those receiving the wheat-based diet without supplemental xylanase. However, broilers fed the wheat-based diet with supplemental xylanase had similar ($P > 0.05$) butyric acid concentrations when compared with corn-based diets with and without xylanase and the wheat-based diet without xylanase. Main effects of cereal grain source demonstrated that broilers fed corn-based diets had 3.0, 5.4, 6.0, 13.3, 5.2, 2.9, 3.5, and 1.2 fold higher ($P < 0.05$) concentrations of cecal propionic, isobutyric, isovaleric, valeric, isocaproic, caproic, heptanoic, and total VFA concentrations, respectively, than those fed wheat-based diets.

21 d of age

Interactive effects of cereal grain source and xylanase ($P < 0.05$) were observed with butyric acid and total VFA concentrations (Table 4.7). Broilers fed corn-based diets with and without supplemental xylanase had higher ($P < 0.05$) concentrations of butyric acid when compared with those receiving the wheat-based diets without supplemental xylanase. Broilers fed the wheat-based diet with supplemental xylanase exhibited similar ($P > 0.05$) concentrations of butyric acid when compared with those receiving corn-based diets with and without xylanase and the wheat-based diet without xylanase. Also, total VFA concentrations of broilers receiving the wheat-based diet with xylanase was 23% higher ($P < 0.05$) than those fed the wheat-based diet without xylanase. However, broilers fed corn-based diets with and without xylanase had similar ($P > 0.05$) total VFA concentrations when compared with wheat-based diets with and without xylanase. Cereal grain source main effects ($P < 0.05$) affected propionic, isobutyric, isovaleric, valeric, isocaproic, and heptanoic acid concentrations with broilers fed corn-based diets having

2.8, 3.1, 3.4, 3.1, 3.6, and 5.0 fold higher ($P < 0.05$) concentrations of propionic, isobutyric, isovaleric, valeric, isocaproic, and heptanoic acid, respectively, than those fed wheat-based diets.

28 d of age

Main effects of cereal grain source ($P < 0.05$) influenced acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, and total VFA concentrations (Table 4.8). Broilers fed corn-based diets had 1.1, 3.3, 3.5, 8.4, 1.9, 2.8, and 1.2 fold higher ($P < 0.05$) concentrations of acetic, propionic, isobutyric, isovaleric, valeric, isocaproic, and total VFA when compared with those fed wheat-based diets. In contrast, broilers fed wheat-based diets had 1.4 fold higher ($P < 0.05$) concentration of butyric acid than those fed corn-based diets.

35 d of age

Cereal grain source and xylanase interacted ($P < 0.05$) to affect propionic acid concentrations (Table 4.9). Broilers receiving the corn-based diet without supplemental xylanase had the highest ($P < 0.05$) concentration of propionic acid when compared with those fed the corn-based diet with xylanase and wheat-based diets with and without xylanase. Propionic concentrations of broilers receiving the corn-based diet with xylanase was 243 and 152% higher ($P < 0.05$) than birds fed wheat-based diets with and without xylanase, respectively. Cereal grain source main effects demonstrated that broilers fed corn-based diets had 4.5, 14.6, 2.0, 3.8, 1.5, and 1.1 fold higher ($P < 0.05$) concentrations of isobutyric, isovaleric, valeric, isocaproic, and total VFA when compared with those fed wheat-based diets. However, broilers fed wheat-based diets exhibited a 1.2 fold higher ($P < 0.05$) concentration of butyric acid than those fed corn-based diets.

42 d of age

No interactive or main effects of xylanase ($P > 0.05$) were observed at 42 d of age, but cereal grain source main effects ($P < 0.05$) affected propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, and caproic acid concentrations (Table 4.10). Broilers fed corn-based diets had 2.7, 4.5, 5.7, 2.1, 1.9, and 1.6 fold higher ($P < 0.05$) concentrations of propionic, isobutyric, isovaleric, valeric, isocaproic, and caproic acid when compared with those fed wheat-based diets. Conversely, broilers fed wheat-based diets exhibited a 1.5 fold higher ($P < 0.05$) concentration of butyric acid than birds fed corn-based diets.

Age Effects on Cecal VFA Concentrations

A repeated-measures analysis was conducted to assess the effects of age and dietary TRT on cecal VFA concentrations. Age, xylanase inclusion, and cereal grain source were all factor levels included into the initial mixed-effects model. However, xylanase inclusion did not affect ($P > 0.05$) VFA concentrations, so it was subsequently removed from the model. Moreover, polynomial contrasts (linear, quadratic, and cubic) were conducted to evaluate the relationship between age and cecal VFA concentrations from 14 to 42 d of age. Age increased acetic, propionic, isobutyric, butyric, isovaleric, and valeric acid concentrations in linear ($P < 0.05$), quadratic ($P < 0.05$), and cubic ($P < 0.05$) responses (Figures 4.1, 4.2, 4.3, 4.4, 4.5, and 4.6). Correspondingly, total VFA concentrations increased with age in both linear ($P < 0.05$) and cubic ($P < 0.05$) responses (Figure 4.7). In general, individual and total VFA concentrations increased from 14 to 28 d of age, but concentrations either decreased or remained constant from 28 to 35 d of age. However, individual and total VFA concentrations subsequently increased

from 35 to 42 d of age. These reductions in individual and total VFA concentrations from 28 to 35 d of age may have contributed to significant quadratic and cubic responses. However, corresponding mean F-values for linear, quadratic, and cubic contrasts were 602, 28, and 64, respectively. Therefore, a linear relationship best describes the effect of age on increasing VFA concentrations of broilers from 14 to 42 d of age.

Acetic Acid

No cereal grain source and age interactive effects or cereal grain source main effects ($P > 0.05$) were observed, but age main effects ($P < 0.05$) influenced acetic acid concentrations (Figure 4.1). From 14 to 21 d of age, acetic acid concentrations were similar ($P > 0.05$), but concentrations increased ($P < 0.05$) by 34% from 21 to 28 d of age. In contrast, acetic acid concentrations decreased ($P < 0.05$) by 11% from 28 to 35 d of age. However, from 35 to 42 d of age, concentrations increased ($P < 0.05$) by 44% with the highest concentration observed at 42 d of age.

Propionic Acid

Cereal grain source and age interacted ($P < 0.05$) to affect propionic acid concentrations (Figure 4.2). From 14 to 21 d of age, no differences ($P < 0.05$) in propionic acid concentrations were noted. From 21 to 28 d of age, propionic acid concentrations increased ($P < 0.05$) by 161% in broilers fed corn-based diets, whereas concentrations of birds fed wheat-based diets did not vary ($P > 0.05$). At 28 and 35 d of age, broilers fed corn-based diets exhibited 3.4 and 3.5 fold higher ($P < 0.05$) concentrations of propionic acid, respectively, when compared with those fed wheat-based diets. However, propionic acid concentrations increased ($P < 0.05$) from 35 to 42 d of age by 151 and 231% in broilers fed corn- and wheat-based diets, respectively.

Broilers fed corn-based diets had a 2.7 fold higher ($P < 0.05$) propionic acid concentration than those fed wheat-based diets at 42 d of age.

Isobutyric Acid

Interactive effects of cereal grain source and age ($P < 0.05$) were observed on isobutyric acid concentrations (Figure 4.3). From 14 to 21 and 21 to 28 d of age, isobutyric acid concentrations of broilers fed corn-based diets increased ($P < 0.05$) by 135 and 78%, respectively, whereas concentrations of those fed wheat-based diets did not differ ($P > 0.05$). Broilers fed corn-based diets exhibited 3.5 and 4.6 fold higher ($P < 0.05$) isobutyric acid concentrations compared with those fed wheat-based diets at 28 and 35 d of age, respectively. From 35 to 42 d of age, isobutyric acid concentrations increased ($P < 0.05$) by 141 and 144% in broilers fed corn- and wheat-based diets, respectively. At 42 d of age, broilers fed corn-based diets had a 4.5 fold higher isobutyric acid concentrations compared with those fed wheat-based diets.

Butyric Acid

Butyric acid concentrations were influenced by cereal grain source and age interactive effects ($P < 0.05$) (Figure 4.4). From 14 to 21 and 21 to 28 d of age, butyric acid concentrations increased by 71 and 53% in broilers fed wheat-based diets ($P < 0.05$), respectively, whereas concentrations of those fed corn-based diets did not differ ($P > 0.05$). As a result, broilers fed wheat-based diets had a 1.4 fold higher ($P < 0.05$) butyric acid concentrations compared with those fed corn-based diets at 28 d of age. However, from 28 to 35 d of age, butyric acid concentrations decreased ($P < 0.05$) by 30% in broilers fed wheat-based diets. Therefore, butyric acid concentrations were similar ($P < 0.05$) among cereal grain sources at 35 d of age. In contrast, broilers fed wheat-based

diets exhibited a 36% increase ($P < 0.05$) in butyric acid concentrations from 35 to 42 d of age, whereas concentrations of those fed corn-based diets remained similar ($P > 0.05$). At 42 d of age, broilers fed wheat-based diets had a 1.5 fold higher ($P < 0.05$) butyric acid concentration compared with those fed corn-based diets.

Isovaleric Acid

Cereal grain source and age interacted ($P < 0.05$) to affect isovaleric acid concentrations (Figure 4.5). No differences ($P > 0.05$) in isovaleric acid concentrations were observed from 14 to 21 d of age. However, from 21 to 28 and 28 to 35 d of age, isovaleric acid concentrations of broilers fed corn-based diets increased ($P < 0.05$) by 296 and 28%, respectively, but isovaleric acid concentrations of birds fed wheat-based diets did not vary ($P > 0.05$). From 35 to 42 d of age, isovaleric acid concentrations of broilers fed corn-based diets did not vary ($P > 0.05$). Correspondingly, concentrations of isovaleric acid of broilers fed wheat-based diets also remained constant from 35 to 42 d of age. However, isovaleric acid concentrations of broilers fed corn-based diets were 7.9, 14.6, and 5.7 fold higher than those fed wheat-based diets at 28, 35, and 42 d of age, respectively.

Valeric Acid

Valeric acid concentrations were affected by cereal grain source and age interactive effects ($P < 0.05$) (Figure 4.6). Valeric acid concentrations of broilers fed corn-based diets were 13.1 fold higher ($P < 0.05$) than those fed wheat-based diets at 14 d of age. From 14 to 21 d of age, valeric acid concentrations of broilers fed wheat-based diets increased ($P < 0.05$) by 786%, but concentrations of those fed corn-based diets did not differ ($P > 0.05$). From 21 to 28 and 28 to 35 d of age, valeric acid concentrations of

broilers fed corn-based diets remained constant ($P > 0.05$). Similarly, valeric acid concentrations of broilers fed wheat-based diets did not vary ($P > 0.05$) from 21 to 28 and 28 to 35 d of age. However, broilers fed corn-based diets had 1.9 and 2.0 fold higher valeric acid concentrations than those fed wheat-based diets at 28 and 35 d of age, respectively. From 35 to 42 d of age, valeric acid concentrations increased ($P < 0.05$) by 192 and 174% in broilers fed corn- and wheat-based diets, respectively. At 42 d of age, broilers fed corn-based diets had a 2.1 fold higher ($P < 0.05$) concentration of valeric acid compared with those fed wheat-based diets.

Total VFA

Cereal grain source and age did not interact ($P > 0.05$) to affect total VFA concentrations, but a main effect of age ($P < 0.05$) was observed (Figure 4.7). Total VFA concentrations increased ($P < 0.05$) by 17 and 45% from 14 to 21 and 21 to 28 d of age, respectively. In contrast, total VFA concentrations decreased ($P < 0.05$) by 19% from 28 to 35 d of age. However, an increase ($P < 0.05$) of 48% was observed in total VFA concentrations from 35 to 42 d of age. A main effect of cereal grain source ($P < 0.05$) was also observed with broilers fed corn-based diets exhibiting 10% more total VFA concentration from 14 to 42 d of age.

DISCUSSION

The current study displayed a complex array of interactions between grain source, bird age, and xylanase supplementation on VFA concentrations. Numerous factors can influence VFA production, including substrate availability (NSP, starch, and AA), the types and composition of microorganisms harbored in the gastrointestinal tract, the gastrointestinal environment of the bird affected by age, and immunological and stress

conditions, which can alter nutrient digestion and absorption. Quantifying and understanding these pronounced differences in individual VFA concentrations may have important implications for broiler production because increased VFA concentrations have been demonstrated to have beneficial effects on energy metabolism, microflora, and immune responses (Den Besten et al., 2013; Pan and Yu, 2014; Tan et al., 2014).

Cereal grain source and xylanase interacted to affect broiler cecal butyric (14 and 21 d of age), propionic (35 d of age), and total VFA (21 d of age). These interactive effects observed at 14 and 21 d of age may have demonstrated that the undigested fermentable substrates from corn-based diets with or without xylanase, effectively increased cecal butyric and total VFA concentrations in broilers. The lack of supplemental xylanase in wheat-based diets resulted in lower butyric and total VFA concentrations when compared with corn-based diets with and without xylanase and a wheat-based diet with xylanase, indicating that broiler cecal fermentation was limited. This reduction may have been attributed to increases in intestinal viscosity, which could have limited the flow of fermentable substrates into the ceca (Choct et al., 1996, 1999). Additionally, anti-nutritive effects of soluble arabinoxylans are considered to be more detrimental during the early period of growth (Cowieson et al., 2006). However, butyric and total VFA concentrations of broilers fed wheat-based diet with xylanase were comparable with those fed corn-based diets. Therefore, supplemental xylanase may have been able to modulate the gastrointestinal microflora and increase butyric and total VFA concentrations by reducing intestinal viscosity and increasing AXOS and XOS concentrations in the ceca (Choct et al., 1996; 1999; Masey-O'Neill et al., 2014). This is

a noteworthy finding because butyrate is important for gastrointestinal health (Rinttila and Apajalahti, 2013).

Interactive effects observed on propionic acid at 35 d of age are not readily explained. However, this difference is more likely representative of a cereal grain source effect rather than an interactive effect because broilers fed corn-based diets with and without xylanase had higher concentrations than those fed wheat-based diets with and without xylanase. Also, propionic acid concentrations among birds fed wheat-based diets with and without xylanase were similar. Therefore, these differences are not indicative of a true interactive effect. Moreover, the reduction in propionic acid concentrations between broilers fed the corn-based diet with xylanase and the corn-based diet without xylanase may have been due to variation because this was an isolated effect and was not observed at any other age.

Increased concentrations of propionic, isobutyric, isovaleric, valeric, and isocaproic observed at 14, 21, 28, 35, and 42 d of age in broilers fed corn-based diets compared with those fed wheat-based diets may have been attributed to less anti-nutritional effects. Cecal fermentative capacity depends on the microbial population as well as the type and concentration of fermentable substrates entering the ceca (Annison et al., 1968; Jozefiak et al., 2004). Although broilers were raised in the same environment, broilers fed wheat-based diets may have had an increased intestinal viscosity, which could have impeded substrate flow into the ceca (Choct et al., 1996, 1999) and delayed subsequent microflora development. Likewise, cereal grain analysis demonstrated that the wheat (9.0 mPa·s) used in diet formulation had a higher viscosity concentration than corn (1.2 mPa·s). Cereal grain source effects observed on butyric acid concentrations may

be indicative of this, because at both 14 and 21 d of age broilers fed corn-based diets exhibited higher concentrations than those fed wheat-based diets, but this effect was subsequently reversed at 28, 35, and 42 d of age with broilers receiving wheat-based diets exhibiting higher concentrations. Thus, anti-nutritional effects of increased intestinal viscosity may have been mitigated by 28 d of age because the broiler's ability to cope with anti-nutritional factors have been reported to increase with age (Yasar and Forbes, 2000). Additionally, increased concentrations of butyric acid in broilers fed wheat-based diets may have been due to lower ileal starch digestibility. Abdollahi et al. (2010) demonstrated that ileal starch digestibility coefficient of broilers fed wheat-based diets was 5.6% lower than those fed corn-based diets. Thus, broilers fed wheat-based diets may have greater concentrations of undigested starch reaching the ceca and large intestine. Accordingly, dominant cecal butyrate-producing bacterial families (*Lachnospiraceae* and *Runinococcaceae*) have been observed to be sensitive to dietary carbohydrate differences and can readily ferment starch to form butyric acid (Duncan et al., 2006; Rinttila and Apajalahti, 2013; Apajalahti and Vienola, 2016). Therefore, variations in ileal starch digestibility among cereal grains may be responsible for the observed differences in cecal butyric acid concentrations.

Additionally, cereal grain source effects observed on propionic, isobutyric, isovaleric, valeric, and isocaproic acid may emphasize the importance of other nutrient factors, which may influence cecal microflora and VFA production because corn contains 96 and 29% less soluble and total non-starch polysaccharide (**NSP**) concentrations than wheat, respectively (Choct, 2006). Likewise, cereal grain analysis indicated that the ADF concentration of corn was 6.6% less than that of wheat. Therefore, resistant starch may

have affected cecal microflora development and fermentative capacity because broilers receiving corn-based diets consumed less overall NSP substrate concentrations. Resistant starch concentrations are typically associated with cereal grains that contain high concentrations of amylose (>60%) (Themeier et al., 2005). Likewise, resistant starch concentrations of corn and wheat are reported to be 25.2 and 13.6%, respectively (Bednar et al., 2001). Therefore, broilers fed corn-based diets likely consumed about 1.9 fold more resistant starch than those fed wheat-based diets. Similar to AXOS and XOS, resistant starch is unavailable to endogenous amylase, and readily fermented by microflora in the distal large intestine (Zijlstra, 2018). In pigs, resistant starch has been reported to elicit a prebiotic effect and influence post-ileal nutrient flow, increase VFA production, and promote beneficial Bifidobacterium and Lactobacillus proliferation (Brown et al., 1997; Bird et al., 2007; Regmi et al., 2011). Therefore, it is plausible that variations in resistant starch concentrations between corn and wheat contributed to the observed cereal grain source differences in cecal VFA concentrations.

Physical characteristics such as ingredient particle size have been reported to affect nutrient digestion and utilization (Amerah et al., 2007). However, it is important to note that the ingredient particle size did not vary substantially between the corn (636 μm) and wheat (592 μm). Hence, it is unlikely that particle size influenced cereal grain source effects on individual VFA concentrations. Conversely, variations in AA concentrations between corn and wheat may have contributed to the cereal grain source effects because corn has an inferior AA profile compared with wheat (Faria Filho et al., 2005). Accordingly, the majority (50 to 60%) of protein in corn is made up of zein (protein bodies), which are deficient in Lys and Trp, and high in Pro, Glu, and Leu (Cowieson,

2005; Gehring et al., 2013). As a result, broilers fed corn-based diets may have had an altered composition and concentration of undigested AA reaching the ceca compared with those receiving wheat-based diets. Increased concentrations of undigested AA can increase putrefactive bacteria proliferation in the broiler ceca (Rinttila and Apajalahti, 2013; Apajalahti and Vienola, 2016), which may suppress microflora diversity, limit fermentative capacity, and affect individual VFA concentrations. Therefore, broilers fed wheat-based diets may have exhibited lower propionic, isobutyric, isovaleric, valeric, and isocaproic acid concentrations compared with those fed corn-based diet due to variations in cereal grain source AA profiles.

Increased concentrations of isovaleric acid in broilers fed corn-based diets may have been due to higher concentrations of undigested Leu because isovaleric acid is the fermentative product of Leu (Macfarlane et al., 1992). Moreover, Leu concentrations are greater in corn than in wheat (AMINODat 5.0, 2016). The percent of undigested Leu contributed by each cereal grain source (corn and wheat) was calculated using broiler SID AA coefficients reported in AMNODat 5.0 (Evonik Nutrition and Care, Hanau, Germany) by the following equation (content = %): [(*total Leu % of ingredient*) - (*SID coefficient of Leu* × *total Leu % of ingredient*)]. Moreover, the percent of undigested Leu from each contributing cereal grain was quantified on an intake basis by the following equation: [(*% undigested Leu*) × (*% cereal grain of complete diet*) × (*FI*)]. The calculated intake of undigested Leu in the current experiment demonstrated that broilers fed corn-based diets consumed 7.0 ($P = 0.001$), 39.2 ($P = 0.001$), and 70.4 ($P = 0.001$) mg more undigested Leu than those fed wheat-based diets from 1 to 14, 15 to 28, and 29 to 42 d of age, respectively. These greater calculated intakes of undigested Leu in broilers fed corn-

based diets can partially explain the increased concentrations of isovaleric acid and may indicate that Leu influences certain bacterial species, which affects cecal microflora development and profile. Isovaleric concentrations are consistent with those reported because broilers fed corn-based diets exhibited higher concentrations compared with those fed wheat-based diets (Kiarie et al., 2014; Masey-O'neil et al., 2014).

Data to support age effects on broiler cecal VFA concentrations are limited in the literature. In the current experiment, linear increases of cecal acetic, propionic, isobutyric, butyric, isovaleric, valeric, and total VFA concentrations from 14 to 42 d of age are consistent with data reported by Barnes et al. (1979). Moreover, cereal grain and age interactive effects on propionic, isobutyric, butyric, isovaleric, and valeric concentrations paralleled the cereal grain source effects observed at each time point with responses being more pronounced in broilers fed corn-based diets compared those fed wheat-based diets. Moreover, all individual (except isovaleric concentrations of broilers fed corn-based diets) and total VFA concentrations were observed to increase from 14 to 28 d of age, but concentrations either numerically remained constant or decreased from 28 to 35 d of age. Nevertheless, cecal VFA concentrations increased from 35 to 42 d of age. These effects on VFA concentrations at 35 d of age are not readily explainable, but a possible microflora shift may have occurred during this time. Choct et al. (2006) reported that a diet change can lead to alterations in the broiler microflora. In the current study, it is possible that the diet change at 28 d of age may have resulted in changes in the composition of gastrointestinal microorganisms, leading to a different pattern of fermentation in the distal gastrointestinal tract. Although ingredient source did not vary, dietary nutrient concentrations of the grower and finisher diets were different. Thus,

concentrations of nutrients reaching the ceca may have been altered, which could have limited fermentative capacity and VFA concentrations. However, this effect had a short duration because cecal VFA concentrations subsequently increased from 35 to 42 d of age, indicating possible microflora adaptation In agreement, Barnes et al. (1979) reported age reductions in acetic, butyric, propionic, valeric, and total VFA concentrations when broilers were fed diets with or without an antibiotic growth promoter (nitrovin) from 35 to 63 d of age.

Dietary xylanase responses on individual and total cecal VFA concentrations have varied in the literature (Singh et al., 2012; Masey-O'neill et al., 2014; Kiarie et al., 2014; Lee et al., 2017). These variable responses are likely attributed to xylanase's interactive nature with other factors, which inherently affects its efficacy (Ravindran, 2013). Factors such as dietary ingredients and quality, bird age, xylanase type, xylanase inclusion concentrations, other exogenous enzymes, and bacterial and coccidial challenges can influence the magnitude of response to supplemental xylanase (Adeola and Cowieson, 2011). Additionally, assessing xylanase's effects on cecal VFA concentrations is difficult because the rate at which cecal VFA concentrations are utilized by enterocytes may be greater than their rate of production (Apajalahti, 2017). Therefore, residual cecal VFA concentrations may be an unreliable measure when quantifying the effects of supplemental xylanase, but ex vivo techniques have reported beneficial effects on butyric acid production with xylanase (Apajalahti, 2017). Therefore, other techniques and methods may need to be developed and explored in order to better quantify the prebiotic effects of supplemental xylanase in broilers.

In conclusion, cereal grain source and supplemental xylanase effects were observed on cecal butyric (14 and 21 d of age) and total VFA (21 d of age) concentrations. In addition, feeding broilers corn-based diets increased propionic, isobutyric, isovaleric, valeric, and isocaproic acid concentrations compared with birds fed wheat-based diets throughout experimentation. As broilers advanced in age, individual and total VFA concentrations increased linearly from 14 to 42 d of age. However, minimal effects on broiler cecal VFA concentrations were observed with supplemental xylanase. Therefore, future research focused on the factors affecting supplemental xylanase and cecal VFA production in broilers is needed. Additionally, research evaluating cereal grain source effects on cecal microflora development and VFA concentrations is warranted.

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Table 4.1 Ingredient and nutrient composition of diets fed to Ross × Ross 708 male broilers from 1 to 14 d of age

	Without Xylanase		With Xylanase[1]	
Ingredient, % "as-fed"	Corn	Wheat	Corn	Wheat
Corn	56.64	---	56.64	---
Wheat (11%)	---	54.72	---	54.72
Soybean meal (48%)	38.13	37.23	38.13	37.23
Vegetable oil	1.79	4.72	1.79	4.72
Dicalcium Phosphate	1.21	1.07	1.21	1.07
Calcium Carbonate	1.18	1.20	1.18	1.20
DL-Methionine	0.31	0.31	0.31	0.31
Vitamin Premix[2]	0.10	0.10	0.10	0.10
Mineral Premix[3]	0.10	0.10	0.10	0.10
NaCl	0.32	0.27	0.32	0.27
L-Lys·HCl	0.07	0.09	0.07	0.09
Choline	0.06	0.08	0.06	0.08
L-Threonine	0.05	0.06	0.05	0.06
Intellibond[4]	0.02	0.02	0.02	0.02
Sand	0.015	0.015	0.005	0.005
Phytase[5]	0.010	0.010	0.010	0.010
Xylanase[6]	---	---	0.010	0.010
Calculated Nutrient Content (%, unless otherwise indicated)				
AME_n, kcal/kg	2,959	2,959	2,959	2,959
Crude Protein	22.36	23.49	22.36	23.49
Digestible Lys	1.18	1.18	1.18	1.18
Digestible Met	0.61	0.59	0.61	0.59
Digestible TSAA	0.91	0.91	0.91	0.91
Digestible Thr	0.78	0.78	0.78	0.78
Digestible Val	0.92	0.92	0.92	0.92
Digestible Arg	1.38	1.38	1.38	1.38
Digestible Trp	0.24	0.27	0.24	0.27
Ca	1.00	1.00	1.00	1.00
Non-phytate P	0.48	0.48	0.48	0.48
Na	0.18	0.18	0.18	0.18

[1]Xylanase was added to each of the 2 basal diets at the expense of sand to achieve the corn- and wheat-based dietary treatments with analyzed xylanase activity concentrations of 14,400 and 15,300 BXU/kg, respectively.

[2]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B12 (cyanocobalamin), 0.03 mg; folacin (folic acid), 2.7 mg: D-pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22.1 mg; niacin (niacinamide), 88.2 mg; thiamin (thiamin mononitrate), 5.5 mg; D-biotin (biotin), 0.18 mg; and pyridoxine (pyridoxine hydrochloride), 7.7 mg.

[3]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (stabilized ethylenediamine dihydriodide), 1.4 mg; Se (sodium selenite, cypress excel Se yeast), 0.3 mg.
[4]Intellibond C (Micronutrients, Inc., Indianapolis, IN) is a source of copper chloride that is 59.2% copper.
[5]Quantum Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 500 FTU/kg of phytase activity.
[6]Econase XT (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 16,000 BXU of xylanase activity per 100 gram per tonne inclusion.

Table 4.2 Ingredient and nutrient composition of diets fed to Ross × Ross 708 male broilers from 15 to 28 d of age

	Without Xylanase		With Xylanase[1]	
Ingredient, % "as-fed"	Corn	Wheat	Corn	Wheat
Corn	64.54	---	64.54	---
Wheat (11%)	---	62.35	---	62.35
Soybean meal (48%)	30.68	29.64	30.68	29.64
Vegetable oil	1.76	5.10	1.76	5.10
Dicalcium Phosphate	0.96	0.80	0.96	0.80
Calcium Carbonate	1.06	1.09	1.06	1.09
DL-Methionine	0.25	0.25	0.25	0.25
Vitamin Premix[2]	0.08	0.08	0.08	0.08
Mineral Premix[3]	0.10	0.10	0.10	0.10
NaCl	0.32	0.26	0.32	0.26
L-Lys·HCl	0.10	0.12	0.10	0.12
Choline	0.05	0.08	0.05	0.08
L-Threonine	0.06	0.07	0.06	0.07
Intellibond[4]	0.02	0.02	0.02	0.02
Sand	0.015	0.015	0.005	0.005
Phytase[5]	0.010	0.010	0.010	0.010
Xylanase[6]	---	---	0.010	0.010
Calculated Nutrient Content (%, unless otherwise indicated)				
AME_n, kcal/kg	3,044	3,044	3,044	3,044
Crude Protein	19.45	20.73	19.45	20.73
Digestible Lys	1.02	1.02	1.02	1.02
Digestible Met	0.52	0.50	0.52	0.50
Digestible TSAA	0.79	0.79	0.79	0.79
Digestible Thr	0.69	0.69	0.69	0.69
Digestible Val	0.80	0.80	0.80	0.80
Digestible Arg	1.17	1.17	1.17	1.17
Digestible Trp	0.20	0.23	0.20	0.23
Ca	0.88	0.88	0.88	0.88
Non-phytate P	0.42	0.42	0.42	0.42
Na	0.18	0.18	0.18	0.18

[1]Xylanase was added to each of the 2 basal diets at the expense of sand to achieve the corn- and wheat-based dietary treatments with analyzed xylanase activity concentrations of 15,100 and 14,100 BXU/kg, respectively.

[2]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B12 (cyanocobalamin), 0.03 mg; folacin (folic acid), 2.7 mg: D-pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22.1 mg; niacin (niacinamide), 88.2 mg; thiamin (thiamin mononitrate), 5.5 mg; D-biotin (biotin), 0.18 mg; and pyridoxine (pyridoxine hydrochloride), 7.7 mg.

[3]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (stabilized ethylenediamine dihydriodide), 1.4 mg; Se (sodium selenite, cypress excel Se yeast), 0.3 mg.
[4]Intellibond C (Micronutrients, Inc., Indianapolis, IN) is a source of copper chloride that is 59.2% copper.
[5]Quantum Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 500 FTU/kg of phytase activity.
[6]Econase XT (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 16,000 BXU of xylanase activity per 100 gram per tonne inclusion.

Table 4.3 Ingredient and nutrient composition of diets fed to Ross × Ross 708 male broilers from 29 to 42 d of age

	Without Xylanase		With Xylanase[1]	
Ingredient, % "as-fed"	Corn	Wheat	Corn	Wheat
Corn	69.22	---	69.22	---
Wheat (11%)	---	66.88	---	66.88
Soybean meal (48%)	26.26	25.16	26.26	25.16
Vegetable oil	1.99	5.57	1.99	5.57
Dicalcium Phosphate	0.67	0.51	0.67	0.51
Calcium Carbonate	0.89	0.92	0.89	0.92
DL-Methionine	0.22	0.22	0.22	0.22
Vitamin Premix[2]	0.05	0.05	0.05	0.05
Mineral Premix[3]	0.10	0.10	0.10	0.10
NaCl	0.33	0.26	0.33	0.26
L-Lys·HCl	0.12	0.14	0.12	0.14
Choline	0.05	0.08	0.05	0.08
L-Threonine	0.06	0.07	0.06	0.07
Intellibond[4]	0.02	0.02	0.02	0.02
Sand	0.015	0.015	0.005	0.005
Phytase[5]	0.010	0.010	0.010	0.010
Xylanase[6]	---	---	0.010	0.010
Calculated Nutrient Content (%, unless otherwise indicated)				
AME_n, kcal/kg	3,114	3,114	3,114	3,114
Crude Protein	17.72	19.10	17.72	19.10
Digestible Lys	0.93	0.93	0.93	0.93
Digestible Met	0.47	0.44	0.47	0.44
Digestible TSAA	0.72	0.72	0.72	0.72
Digestible Thr	0.62	0.62	0.62	0.62
Digestible Val	0.73	0.73	0.73	0.73
Digestible Arg	1.04	1.05	1.04	1.05
Digestible Trp	0.18	0.21	0.18	0.21
Ca	0.74	0.74	0.74	0.74
Non-phytate P	0.36	0.36	0.36	0.36
Na	0.18	0.18	0.18	0.18

[1]Xylanase was added to each of the 2 basal diets at the expense of sand to achieve the corn- and wheat-based dietary treatments with analyzed xylanase activity concentrations of 16,100 and 16,200 BXU/kg, respectively.

[2]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B12 (cyanocobalamin), 0.03 mg; folacin (folic acid), 2.7 mg: D-pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22.1 mg; niacin (niacinamide), 88.2 mg; thiamin (thiamin mononitrate), 5.5 mg; D-biotin (biotin), 0.18 mg; and pyridoxine (pyridoxine hydrochloride), 7.7 mg.

[3]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (stabilized ethylenediamine dihydriodide), 1.4 mg; Se (sodium selenite, cypress excel Se yeast), 0.3 mg.
[4]Intellibond C (Micronutrients, Inc., Indianapolis, IN) is a source of copper chloride that is 59.2% copper.
[5]Quantum Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 500 FTU/kg of phytase activity.
[6]Econase XT (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 16,000 BXU of xylanase activity per 100 gram per tonne inclusion.

Table 4.4 Ingredient composition, neutral cellulase gammanase digestibility, particle size, and viscosity of the corn and wheat used in experimental broiler diets

	Cereal grain[1]	
	Corn	Wheat
DM	84.7	88.6
Ash	1.19	1.62
Crude protein	6.82	9.56
Ether extract	3.33	1.95
Crude fiber	1.27	1.92
Starch	63.19	61.42
NDF	8.61	7.58
ADF	3.12	3.34
Sugar	1.10	1.41
Phytate-P	0.24	0.23
NCGD[2]	91.49	92.25
Particle Size, (μm)[3]	636	592
Viscosity, (mPa·s)	1.2	9.0

[1]Values represent duplicate analyses on an as-fed basis. DM, ash, CP, ether extract, crude fiber, starch, NDF, ADF, sugar, phytate-P, and NCGD of the cereal grains were determined by near-infrared reflectance spectroscopy.

[2]NCGD = neutral cellulase gammanase digestibility. A coefficient that estimates the digestibility of the insoluble fiber fraction after cellulase and gammanase exposure.

[3]Particle size is the geometric diameter average (d_{gw}) of the grain. The standard deviation (S_{gw}) and surface area of the corn and wheat were 2.34 and 1.9 μm and 89.5 and 94.1 cm^2/gram, respectively.

Table 4.5 Body weight and feed intake of Ross × Ross 708 male broilers fed either corn- or wheat-based diets with or without supplemental xylanase[1]

		1 to 14 d of age[2]		1 to 28 d of age[3]		1 to 42 d of age[4]	
Cereal Grain	Xylanase	BW (kg)	FI[5] (kg)	BW (kg)	FI (kg)	BW (kg)	FI (kg)
Corn	Without	0.448	0.498[a]	1.722[a]	2.263	3.421	5.338
	With	0.449	0.491[ab]	1.696[ab]	2.273	3.357	5.302
Wheat	Without	0.437	0.483[b]	1.652[b]	2.228	3.299	5.220
	With	0.443	0.498[a]	1.680[ab]	2.215	3.334	5.173
	SEM	0.003	0.003	0.012	0.015	0.031	0.037
Cereal Grain Main Effects							
Corn		0.448	0.494	1.709	2.268	3.389	5.320
Wheat		0.440	0.491	1.666	2.222	3.317	5.197
SEM		0.003	0.002	0.009	0.011	0.022	0.026
Xylanase Main Effects							
	Without	0.442	0.498	1.687	2.245	3.360	5.279
	With	0.446	0.494	1.688	2.244	3.345	5.238
	SEM	0.003	0.002	0.009	0.011	0.022	0.026
Analysis of Variance		*Probabilities*					
Cereal Grain × Xylanase		0.41	0.001	0.03	0.44	0.12	0.88
Cereal Grain		0.007	0.25	0.001	0.003	0.02	0.002
Xylanase		0.26	0.22	0.95	0.93	0.63	0.27

[1]Xylanase = Econase XT (AB Vista Feed Ingredients, Marlborough, UK) which provides 160,000 BXU/g was included at 0.01% in the supplemented treatments to achieve 16,000 BXU/kg.

[2]Each value represents the least-square means of 15 replicate pens with each pen having approximately 25 birds.

[3]Each value represents the least-square means of 15 replicate pens with each pen having approximately 17 birds.

[4]Each value represents the least-square means of 15 replicate pens with each pen having approximately 9 birds.

[5]FI = Feed intake

[a-b] Means within a column for a given measurement not sharing a common superscript are different ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

Table 4.6 Cecal volatile fatty acid concentrations (mM/L) of Ross × Ross 708 male broilers fed either corn- or wheat-based diets with or without supplemental xylanase at 14 d of age[1]

Cereal Grain	Xylanase[2]	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Isocaproic	Caproic	Heptanoic	Total
Corn	Without	71	1.34	0.179	12.70[a]	0.227	0.656	0.0033	0.0077	0.0008	90
	With	77	1.21	0.184	11.64[a]	0.168	0.516	0.0029	0.0076	0.0007	92
Wheat	Without	63	0.44	0.036	7.53[b]	0.036	0.032	0.0002	0.0022	0.0003	72
	With	71	0.41	0.031	10.41[ab]	0.030	0.055	0.0009	0.0031	0.0001	85
	SEM	6	0.11	0.019	0.83	0.024	0.038	0.0004	0.0006	0.0001	6
Cereal Grain Main Effects											
Corn		74	1.28	0.182	12.17	0.197	0.586	0.0031	0.0077	0.0007	91
Wheat		67	0.42	0.034	8.97	0.033	0.044	0.0006	0.0027	0.0002	78
SEM		5	0.08	0.013	0.59	0.017	0.026	0.0003	0.0004	0.0001	6
Xylanase Main Effects											
	Without	67	0.89	0.107	10.12	0.132	0.344	0.0018	0.0049	0.0005	81
	With	74	0.81	0.108	11.02	0.099	0.286	0.0019	0.0054	0.0004	88
	SEM	6	0.08	0.013	0.59	0.017	0.027	0.0003	0.0005	0.0001	6
Analysis of Variance						*Probabilities*					
Cereal Grain × Xylanase		0.80	0.64	0.78	0.02	0.28	0.06	0.17	0.48	0.94	0.25
Cereal Grain		0.13	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.01
Xylanase		0.11	0.47	0.98	0.28	0.18	0.13	0.77	0.81	0.25	0.12

[1]Each value represents the least-square means of pooled cecal digesta from 4 birds per pen with 15 replicate pens per treatment.

[2] Xylanase = Econase XT (AB Vista Feed Ingredients, Marlborough, UK) was included at 0.01% in the supplemented treatments to achieve 16,000 BXU/kg.

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

Table 4.7 Cecal volatile fatty acid concentrations (mM/L) of Ross × Ross 708 male broilers fed either corn- or wheat-based diets with or without supplemental xylanase at 21 d of age[1]

Cereal Grain	Xylanase[2]	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Isocaproic	Caproic	Heptanoic	Total
Corn	Without	78	1.57	0.290	14.64[a]	0.383	0.719	0.0014	0.0327	0.0004	95[ab]
	With	75	1.45	0.318	14.30[a]	0.341	0.685	0.0016	0.0357	0.0005	94[ab]
Wheat	Without	66	0.55	0.085	10.71[b]	0.097	0.215	0.0033	0.0231	0.0001	78[b]
	With	80	0.54	0.109	13.73[ab]	0.119	0.231	0.0051	0.0276	0.0001	96[a]
	SEM	4	0.12	0.030	0.84	0.041	0.046	0.0028	0.0074	0.0001	4
Cereal Grain Main Effects											
Corn		77	1.51	0.304	14.47	0.362	0.702	0.0151	0.0342	0.0005	94
Wheat		73	0.54	0.097	12.22	0.108	0.223	0.0042	0.0254	0.0001	87
SEM		3	0.09	0.021	0.60	0.030	0.032	0.0021	0.0058	0.0001	3
Xylanase Main Effects											
	Without	72	1.06	0.187	12.68	0.240	0.467	0.0089	0.0279	0.0003	86
	With	77	1.00	0.214	14.02	0.230	0.458	0.0104	0.0317	0.0003	95
	SEM	3	0.09	0.021	0.60	0.030	0.032	0.0021	0.0058	0.0001	3
Analysis of Variance						*Probabilities*					
Cereal Grain × Xylanase		0.06	0.63	0.95	0.05	0.45	0.58	0.93	0.91	0.61	0.05
Cereal Grain		0.40	0.001	0.001	0.009	0.001	0.001	0.001	0.18	0.001	0.11
Xylanase		0.23	0.62	0.38	0.12	0.82	0.85	0.56	0.57	0.75	0.06

[1]Each value represents the least-square means of pooled cecal digesta from 4 birds per pen with 15 replicate pens per treatment.

[2] Xylanase = Econase XT (AB Vista Feed Ingredients, Marlborough, UK) was included at 0.01% in the supplemented treatments to achieve 16,000 BXU/kg.

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

Table 4.8 Cecal volatile fatty acid concentrations (mM/L) of Ross × Ross 708 male broilers fed diets of either corn- or wheat-based diets with or without supplemental xylanase at 28 d of age[1]

Cereal Grain	Xylanase[2]	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Isocaproic	Caproic	Heptanoic[3]	Total
Corn	Without	116	5.21	0.706	18.18	2.043	1.088	0.0384	0.0542	---	156
	With	109	4.15	0.812	18.95	2.184	1.104	0.0419	0.0502	---	149
Wheat	Without	93	1.43	0.205	25.00	0.213	0.567	0.0146	0.0759	---	124
	With	107	1.37	0.233	25.83	0.293	0.611	0.0138	0.0663	---	136
	SEM	6	0.37	0.068	1.98	0.280	0.069	0.0056	0.0121	---	10
Cereal Grain Main Effects											
Corn		113	4.68	0.759	18.57	2.114	1.096	0.0402	0.0522	---	152
Wheat		100	1.40	0.219	25.41	0.253	0.589	0.0142	0.0711	---	130
SEM		5	0.26	0.048	1.43	0.206	0.048	0.0042	0.0093	---	7
Xylanase Main Effects											
	Without	105	3.32	0.456	21.59	1.128	0.827	0.0265	0.0651	---	140
	With	108	2.76	0.522	22.39	1.239	0.857	0.0279	0.0582	---	143
	SEM	3	0.26	0.047	0.60	0.207	0.048	0.0042	0.0093	---	7
Analysis of Variance						*Probabilities*					
Cereal Grain × Xylanase		0.08	0.18	0.56	0.99	0.91	0.84	0.69	0.80	---	0.32
Cereal Grain		0.04	0.001	0.001	0.001	0.001	0.001	0.001	0.09	---	0.02
Xylanase		0.55	0.13	0.33	0.68	0.68	0.66	0.80	0.53	---	0.77

[1]Each value represents the least-square means of pooled cecal digesta from 4 birds per pen with 15 replicate pens per treatment.

[2] Xylanase = Econase XT (AB Vista Feed Ingredients, Marlborough, UK) was included at 0.01% in the supplemented treatments to achieve 16,000 BXU/kg.

[3] Heptanoic acid values were too low to be detected.

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

Table 4.9 Cecal volatile fatty acid concentrations (mM/L) of Ross × Ross 708 male broilers fed diets of either corn- or wheat-based diets with or without supplemental xylanase at 35 d of age[1]

Cereal Grain	Xylanase[2]	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Isocaproic	Caproic	Heptanoic[3]	Total
Corn	Without	97	5.15[a]	0.768	15.42	2.875	0.984	0.0216	0.0138	---	124
	With	98	3.70[b]	0.733	13.27	2.579	0.901	0.0198	0.0111	---	120
Wheat	Without	91	1.08[c]	0.169	17.25	0.172	0.432	0.0059	0.0045	---	105
	With	93	1.47[c]	0.164	18.44	0.202	0.527	0.0049	0.0124	---	115
	SEM	5	0.36	0.052	1.11	0.213	0.053	0.0026	0.0024	---	6
Cereal Grain Main Effects											
Corn		97	4.42	0.751	14.34	2.727	0.942	0.0207	0.0125	---	122
Wheat		92	1.27	0.167	17.85	0.187	0.479	0.0054	0.0085	---	110
SEM		5	0.26	0.039	0.84	0.153	0.035	0.0020	0.0017	---	5
Xylanase Main Effects											
	Without	94	3.11	0.469	16.33	1.524	0.708	0.0138	0.0092	---	115
	With	95	2.58	0.449	15.85	1.390	0.714	0.0124	0.0118	---	117
	SEM	4	0.26	0.039	0.84	0.153	0.036	0.0020	0.0018	---	5
Analysis of Variance						*Probabilities*					
Cereal Grain × Xylanase		0.77	0.01	0.75	0.13	0.46	0.07	0.87	0.051	---	0.13
Cereal Grain		0.21	0.001	0.001	0.002	0.001	0.001	0.001	0.11	---	0.01
Xylanase		0.74	0.15	0.67	0.66	0.54	0.90	0.54	0.29	---	0.54

[1]Each value represents the least-square means of pooled cecal digesta from 4 birds per pen with 15 replicate pens per treatment.

[2] Xylanase = Econase XT (AB Vista Feed Ingredients, Marlborough, UK) was included at 0.01% in the supplemented treatments to achieve 16,000 BXU/kg.

[3] Heptanoic acid values were too low to be detected.

Table 4.10 Cecal volatile fatty acid concentrations (mM/L) of Ross × Ross 708 male broilers fed diets of either corn- or wheat-based diets with or without supplemental xylanase at 42 d of age[1]

Cereal Grain	Xylanase[2]	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Isocaproic	Caproic	Heptanoic	Total
Corn	Without	133	10.77	1.815	14.76	2.560	2.765	0.0898	0.0923	0.0001	170
	With	147	12.02	1.806	16.49	2.820	2.730	0.0843	0.0988	0.0024	180
Wheat	Without	134	4.37	0.385	24.02	0.506	1.445	0.0436	0.0554	0.0001	169
	With	131	4.04	0.414	24.37	0.437	1.152	0.0502	0.0640	0.0001	162
	SEM	5	0.70	0.078	2.03	0.135	0.169	0.0114	0.0161	0.0008	7
Cereal Grain Main Effects											
Corn		140	11.40	1.811	16.63	2.690	2.747	0.0871	0.0956	0.0012	175
Wheat		133	4.20	0.399	24.19	0.472	1.299	0.0469	0.0597	0.0001	165
SEM		4	0.49	0.058	1.44	0.096	0.119	0.0105	0.0150	0.0008	5
Xylanase Main Effects											
	Without	134	7.57	1.100	19.39	1.533	2.105	0.0667	0.0738	0.0001	170
	With	139	8.03	1.110	20.43	1.628	1.941	0.0672	0.0814	0.0001	171
	SEM	4	0.48	0.058	1.44	0.096	0.119	0.0105	0.0018	0.0005	5
Analysis of Variance						*Probabilities*					
Cereal Grain × Xylanase		0.11	0.25	0.81	0.73	0.23	0.45	0.32	0.90	0.13	0.23
Cereal Grain		0.16	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.13	0.17
Xylanase		0.30	0.50	0.90	0.60	0.48	0.33	0.93	0.38	0.13	0.80

[1]Each value represents the least-square means of pooled cecal digesta from 4 birds per pen with 15 replicate pens per treatment.

[2] Xylanase = Econase XT (AB Vista Feed Ingredients, Marlborough, UK) was included at 0.01% in the supplemented treatments to achieve 16,000 BXU/kg.

[a-b] Means within a column for a given measurement not sharing a common superscript differ ($P \leq 0.05$) and were separated using Tukey's Honestly Significant Difference test.

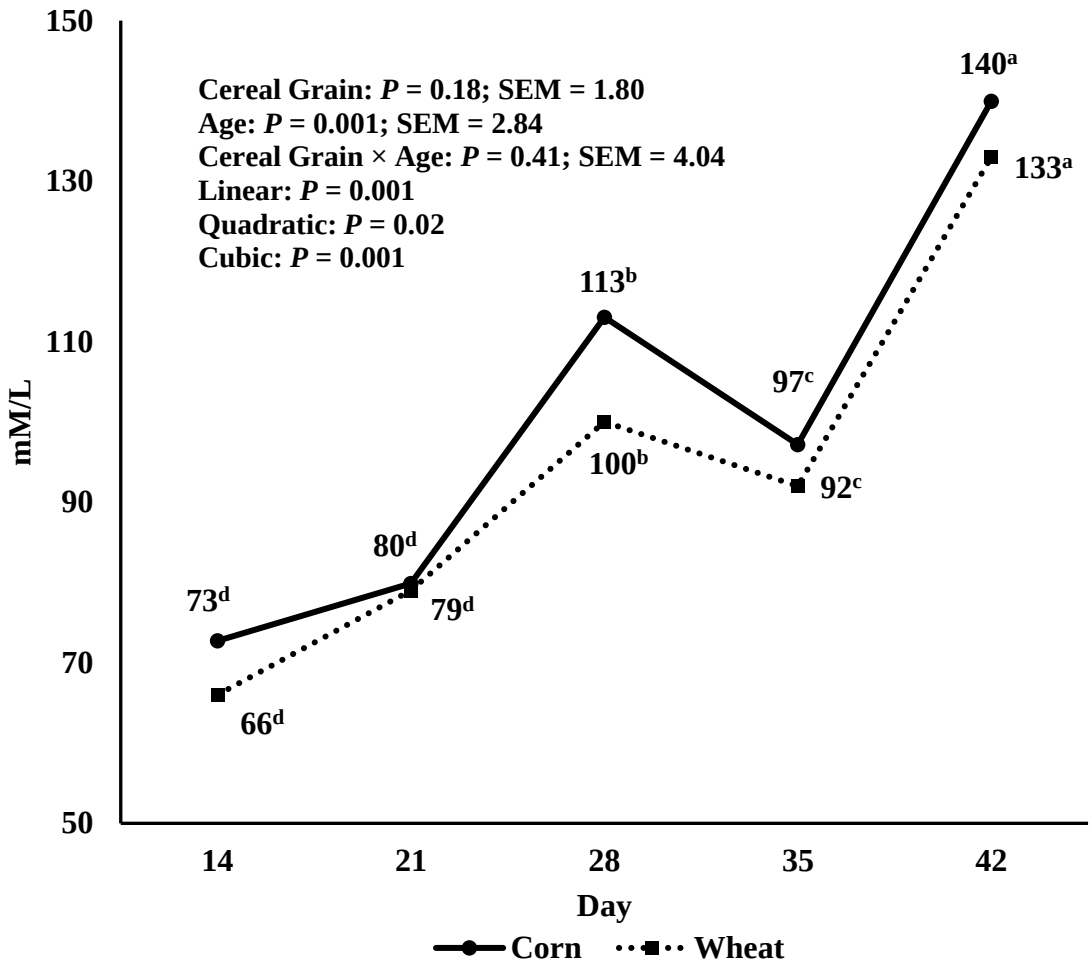

Figure 4.1 Cecal acetic acid concentrations of Ross × Ross 708 male broilers fed either corn- or wheat-based diets during a 42 d production period. Cecal contents were collected and pooled from 4 birds per pen (15 pens per treatment). Least-square means with different superscripts differ significantly ($P < 0.05$) and were separated using Tukey's Honestly Significant Difference Test.

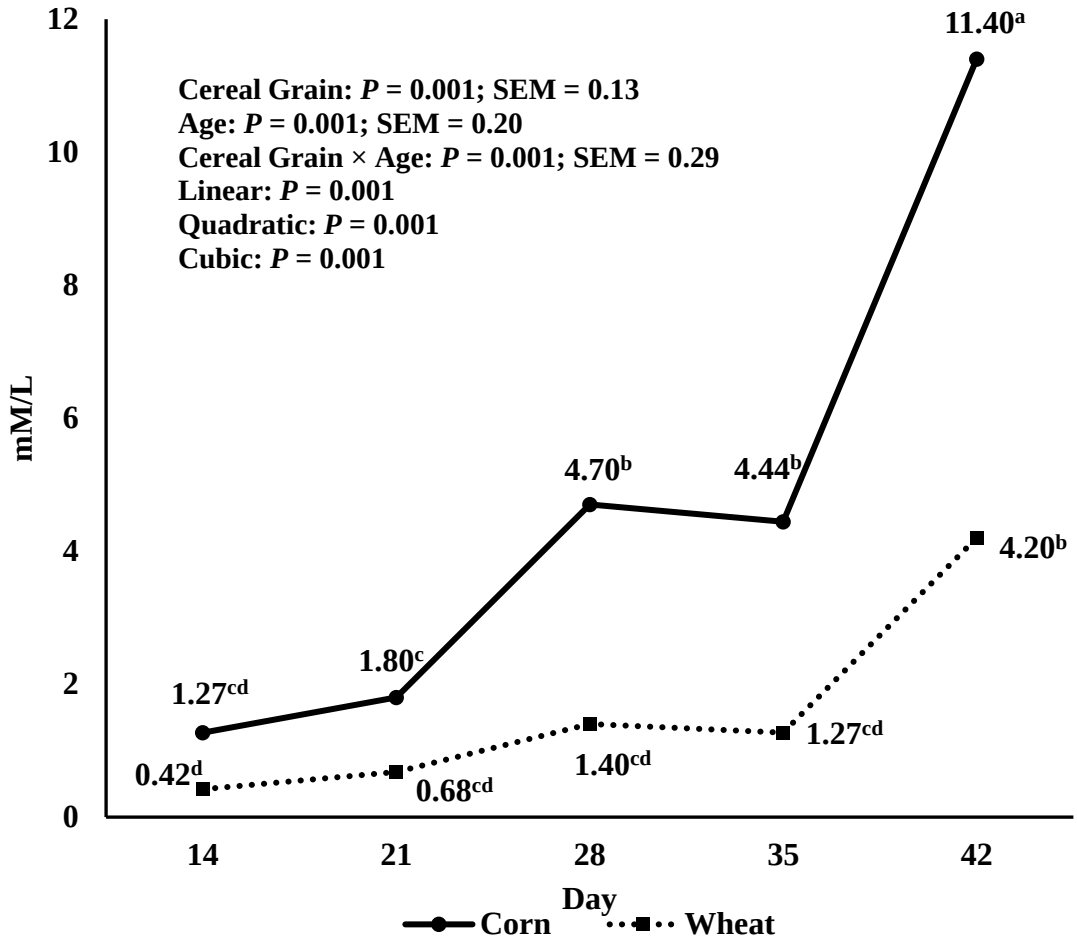

Figure 4.2 Cecal propionic acid concentrations of Ross × Ross 708 male broilers fed either corn- or wheat-based diets during a 42 d production period. Cecal contents were collected and pooled from 4 birds per pen (15 pens per treatment). Least-square means with different superscripts differ significantly ($P < 0.05$) and were separated using Tukey's Honestly Significant Difference Test.

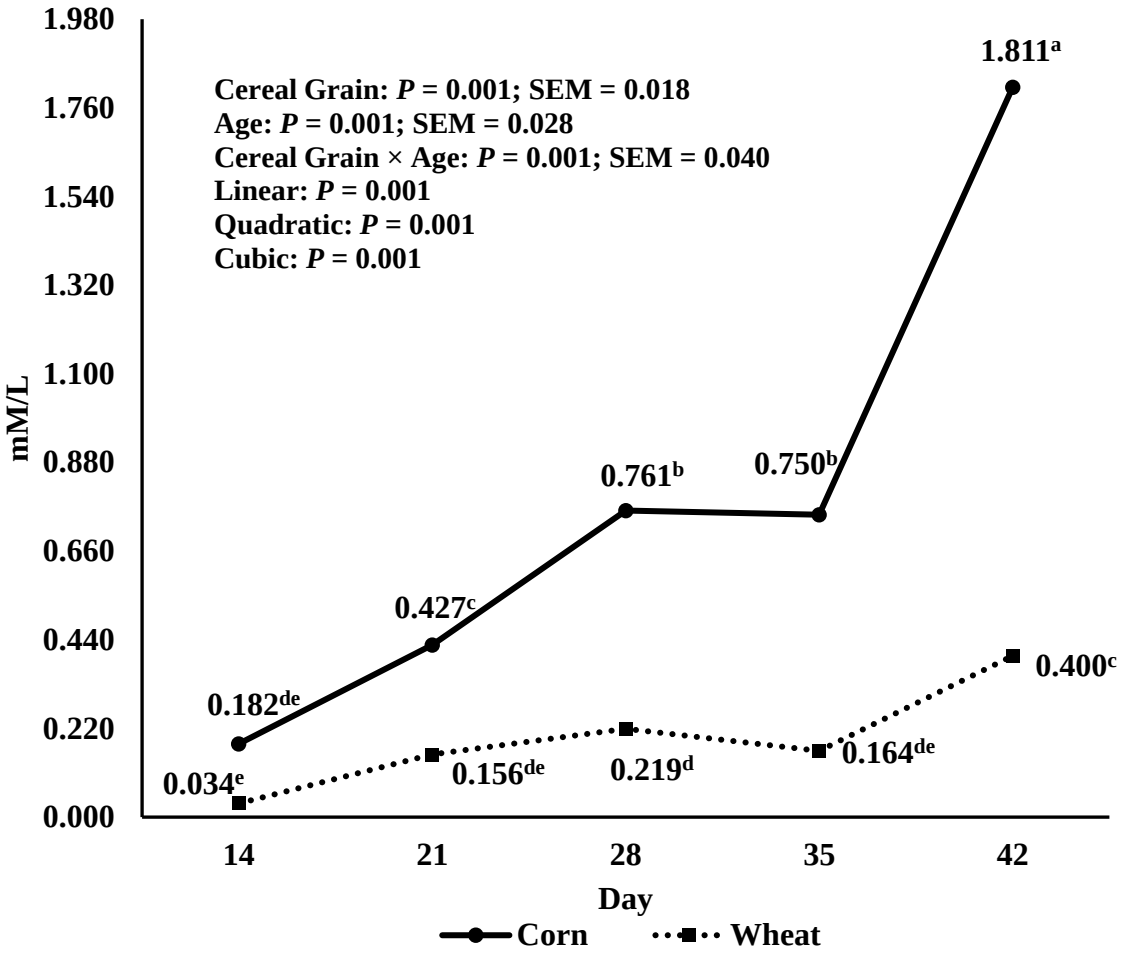

Figure 4.3 Cecal isobutyric acid concentrations of Ross × Ross 708 male broilers fed either corn- or wheat-based diets during a 42 d production period. Cecal contents were collected and pooled from 4 birds per pen (15 pens per treatment). Least-square means with different superscripts differ significantly ($P < 0.05$) and were separated using Tukey's Honestly Significant Difference Test.

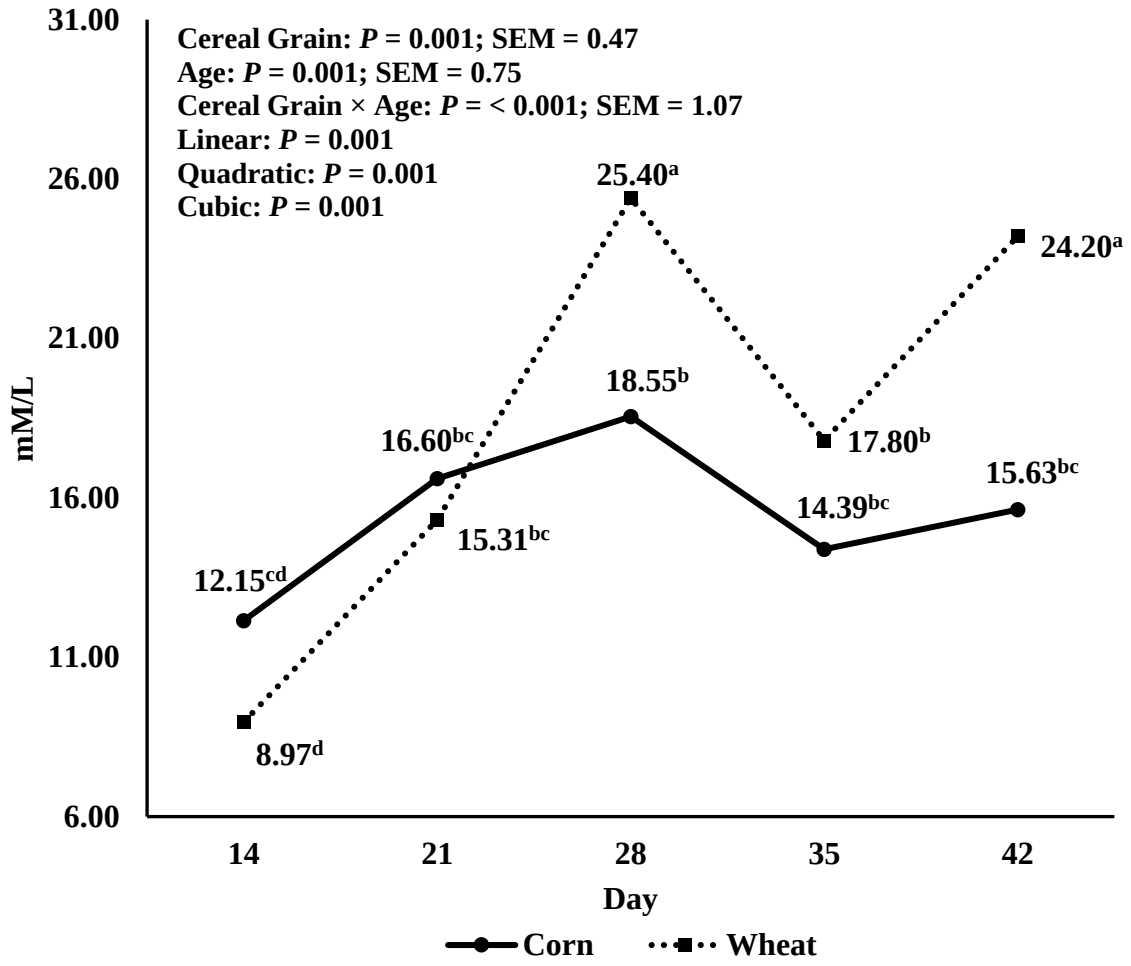

Figure 4.4 Cecal butyric acid concentrations of Ross × Ross 708 male broilers fed either corn- or wheat-based diets during a 42 d production period. Cecal contents were collected and pooled from 4 birds per pen (15 pens per treatment). Least-square means with different superscripts differ significantly ($P < 0.05$) and were separated using Tukey's Honestly Significant Difference Test.

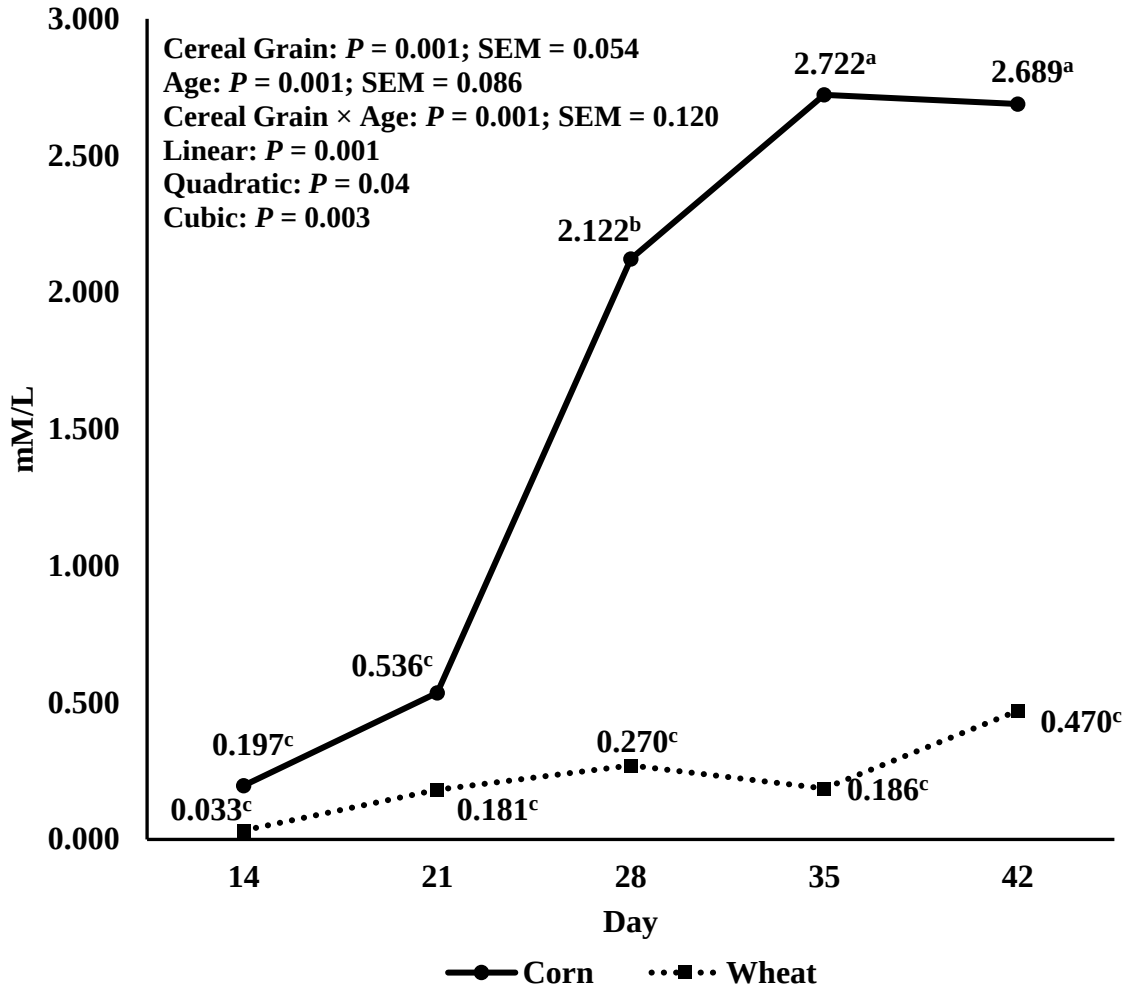

Figure 4.5 Cecal isovaleric acid concentrations of Ross × Ross 708 male broilers fed either corn- or wheat-based diets during a 42 d production period. Cecal contents were collected and pooled from 4 birds per pen (15 pens per treatment). Least-square means with different superscripts differ significantly ($P < 0.05$) and were separated using Tukey's Honestly Significant Difference Test.

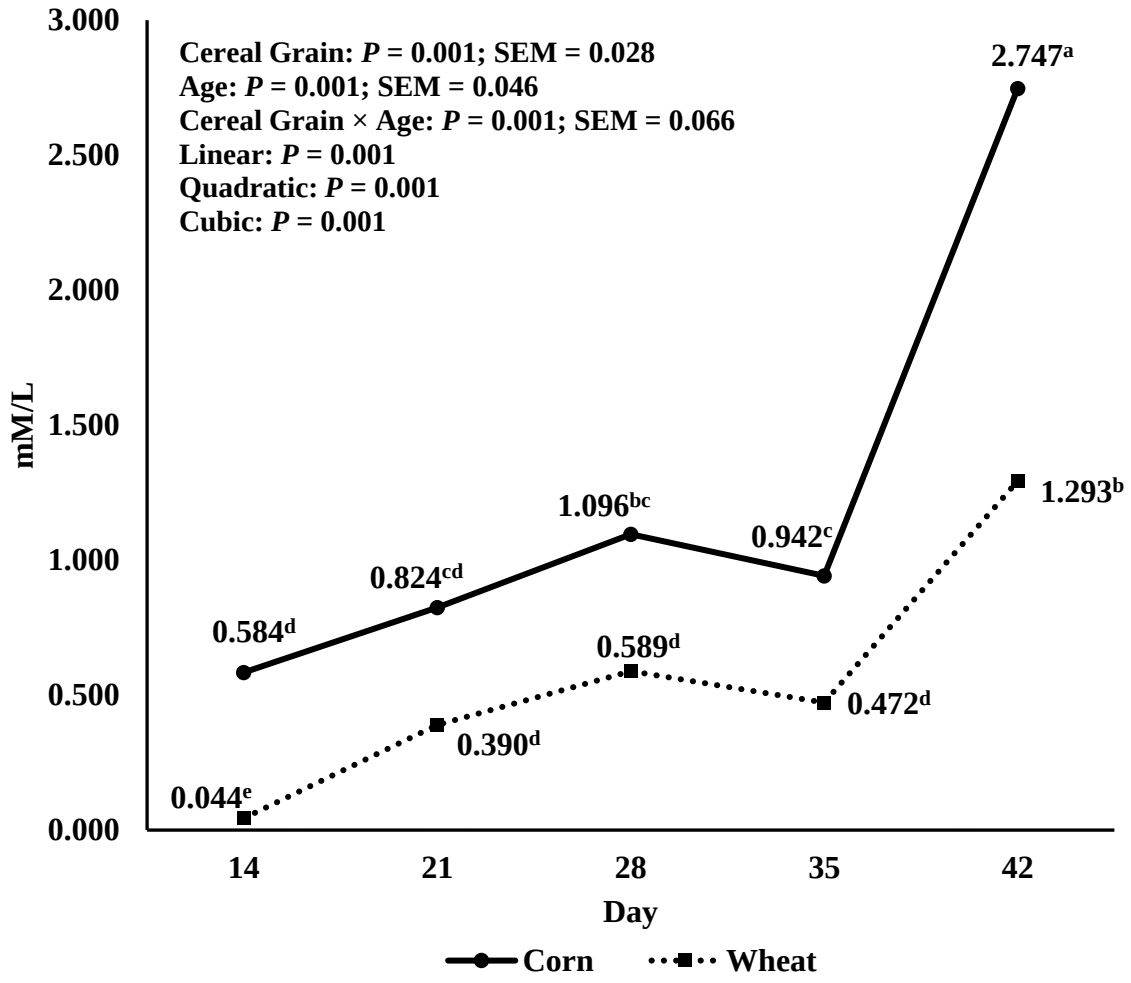

Figure 4.6 Cecal valeric acid concentrations of Ross × Ross 708 male broilers fed either corn- or wheat-based diets during a 42 d production period. Cecal contents were collected and pooled from 4 birds per pen (15 pens per treatment). Least-square means with different superscripts differ significantly ($P < 0.05$) and were separated using Tukey's Honestly Significant Difference Test.

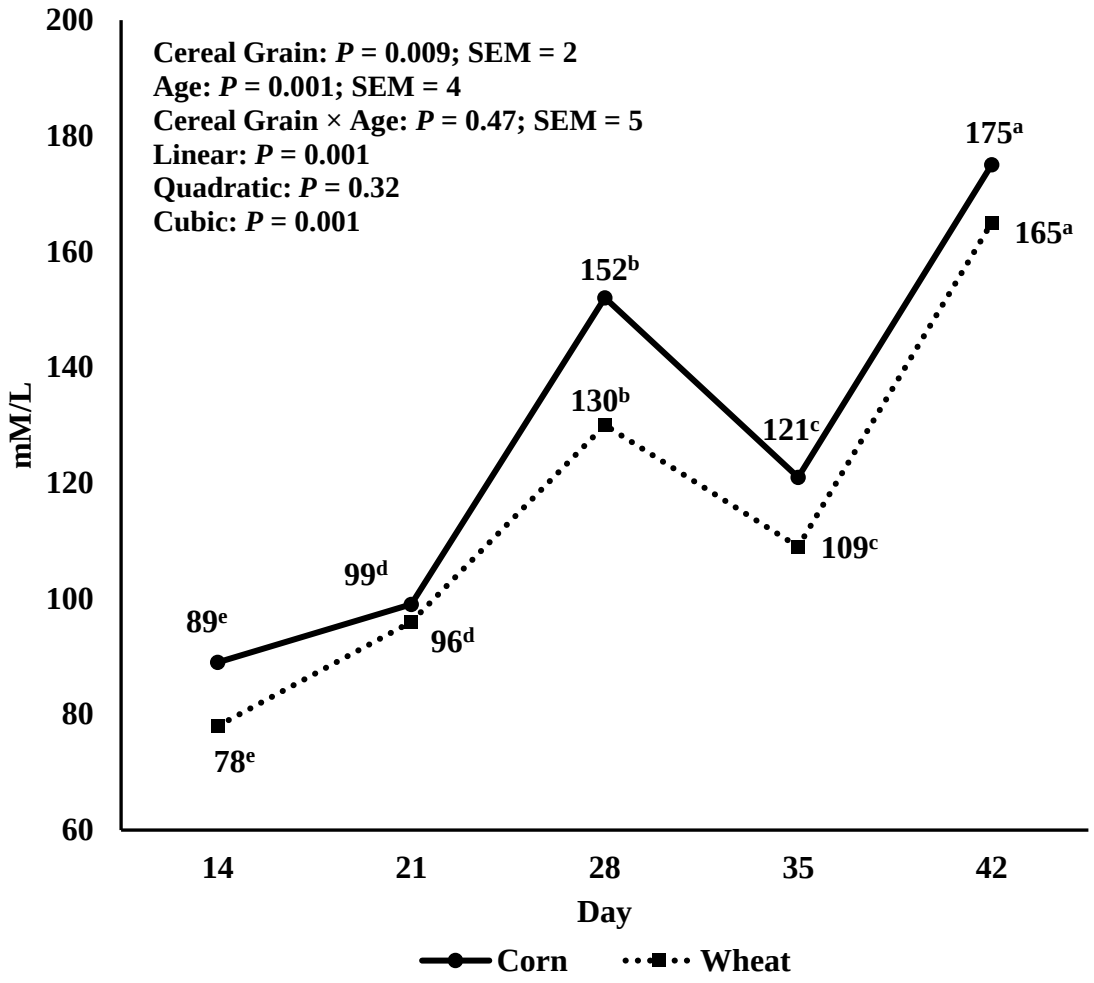

Figure 4.7 Cecal total volatile fatty acid concentrations of Ross × Ross 708 male broilers fed either corn- or wheat-based diets during a 42 d production period. Cecal contents were collected and pooled from 4 birds per pen (15 pens per treatment). Least-square means with different superscripts differ significantly ($P < 0.05$); superscripts refer to main effects of age and were separated using Tukey's Honestly Significant Difference Test

V. CONCLUSIONS

The first experiment was conducted to evaluate effects of various concentrations of supplemental xylanase in corn- and wheat-based diets on broiler growth performance and cecal VFA concentrations during a 6-week production period. Cereal grain source and supplemental xylanase did not affect cumulative broiler growth performance. Broilers fed corn-based diets had increased concentrations of propionic, isobutyric, and isovaleric acid compared with those fed wheat-based diets at 26 and 40 d of age. Conversely, broilers fed wheat-based diets had increased butyric acid concentrations compared with birds fed corn-based diets at 26 and 40 d of age. However, supplemental xylanase concentrations did not affect broiler cecal VFA concentrations at 26 and 40 d of age.

The second experiment was conducted to evaluate the effects of age and supplemental xylanase in corn- and wheat-based diets on cecal VFA production during weekly intervals from 14 to 42 d of age. Cereal grain source and supplemental xylanase interacted to affect cecal butyric (14 and 21 d of age) and total VFA (21 d of age) concentrations. In addition, broilers fed corn-based diets had increased propionic, isobutyric, isovaleric, valeric, and isocaproic acid concentrations compared with birds fed wheat-based diets at 14, 21, 28, 35, and 42 d of age. However, broilers fed wheat-based diets exhibited greater butyric acid concentrations at 28, 35, and 42 d of age. Individual and total VFA concentrations linearly increased as the broiler advanced in age.

Overall, these results provide evidence that supplemental xylanase in corn- or wheat-based diets may be inconsistent in altering broiler growth performance and cecal VFA concentrations. However, cereal grain source affected individual cecal VFA concentrations. These responses were likely attributed to variations in cereal grain source AA profile, ileal starch digestibility, and resistant starch concentrations because corn has an inferior AA profile for all essential AA except Leu, higher ileal starch digestibility coefficient, and higher resistant starch concentrations than wheat. Hence, broilers fed corn-based diets may have had reduced concentrations of AA and starch, but increased concentrations of resistant starch reaching the ceca, which may have altered microflora development and pattern of fermentation. Therefore, ileal AA, starch digestibility, and resistant starch concentrations of cereal grain sources may be important for modulating cecal microflora development and subsequent fermentative capacity of broilers. Also, linear age increases in cecal VFA concentrations may indicate that microflora proliferation continues throughout the production period. Thus, early establishment of optimal microflora may be a key factor for managing broiler gastrointestinal health and increasing VFA production. However, inconsistent effects of supplemental xylanase on broiler growth performance and cecal VFA concentrations indicates that future research investigating factors such as substrate availability, gastrointestinal environment and age, xylanase inhibitors, microflora composition, immunological and stress conditions, and health are needed.