Effects of Corn Varying in Extractable Salt-Soluble Protein Content with Phytase and Xylanase Supplementation on Growth Performance and Nutrient and Energy Digestibility of Broilers Fed Diets Adequate in Calcium and Non-Phytate Phosphorus

by

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ABSTRACT

Extractable salt-soluble protein content can be assayed rapidly to help identify corn that may have been improperly dried or stored. Research indicates a relationship between this variable and broiler performance. Identification of corn with submarginal nutritional value will be advantageous to nutritionists. However, it may be beneficial to identify strategies to mitigate losses in performance and economic returns. One potential strategy is to supplement diets with exogenous enzymes. Enzyme supplementation may reduce nutritional variability among sources of corn.

The first experiment evaluated the relationship between chemical composition of corn and nutrient and energy utilization in broilers from 28 to 30 d of age. Six of 12 sources of corn with similar proximate composition but with extractable salt-soluble protein content (PS) that varied from 25.7 to 49.2%, were selected for use in ileal digestibility assays. Ileal digestibility of N (IND; apparent), CF, and starch did not differ ($P > 0.05$) among sources of corn. Salt-soluble protein concentration was correlated with ileal digestible energy (IDE) among the corns (r = 0.5; $P \leq 0.001$). Ileal N and fat digestibility were correlated with IDE (r = 0.4 and 0.3, respectively; $P \leq 0.05$). Apparent ME_n ranged from 3,262 to 3,342 kcal/kg and was correlated with PS (r = 0.8; $P \leq 0.001$) and IDE (r = 0.36; $P \leq 0.05$). These results indicated that sources of corn with similar proximate composition may vary in their digestible energy content, and in such a situation PS may be used to differentiate sources of corn varying in IDE or AME_n.

The second and third experiments evaluated extra-phosphoric effects on amino acid (AA) and energy digestibility (experiments 2 and 3) and growth performance (experiment 3) of broilers fed diets (adequate in Ca and non-phytate P) supplemented with phytase and xylanase. In both experiments, factorial arrangements of treatments were evaluated consisting of 6 phytase (0, 1,000, 2,000, 4,000, 8,000, and 16,000 FTU/kg) and 2 xylanase (0 and 16,000 BXU/kg) concentrations in experiment 2 and 4 phytase (0, 500, 1,000, and 2,000 FTU/kg) and 4 xylanase (0, 8,000, 16,000, and 32,000 BXU/kg) concentrations in experiment 3. In each experiment, phytase and xylanase did not interact in their effects on AA and energy digestibility ($P > 0.05$) and only phytase main effects were observed. Broilers fed diets supplemented with phytase at 1,000 FTU/kg had increased ($P \leq 0.05$) apparent ileal digestibility (AID) of most amino acids (AA). No linear or quadratic effects of phytase were observed in experiment 2. In contrast, broilers fed diets supplemented with phytase in experiment 3 exhibited linear ($P \leq 0.05$) increases in AID of AA but not apparent ileal digestible energy. However, supplementation with 2,000 FTU/kg phytase increased ($P = 0.049$) ileal digestible energy by 36 kcal/kg compared with the basal diet. Broilers fed diets with 1,000 or 2,000 FTU/kg consumed more feed and grew faster (linear $P \leq 0.05$) than their counterparts fed diets supplemented with 0 or 500 FTU/kg of phytase.

The fourth experiment evaluated the potential interaction between sources of corn varying in PS and dietary supplementation with phytase and xylanase. The experiment consisted of a factorial arrangement of 16 treatments with 4 sources of corn (varying in PS) with or without phytase (0 or 1,000 FTU/kg) and xylanase (0 or 16,000 BXU/kg). Eight sources of corn were obtained in a similar manner as experiment 1 and were

evaluated for PS. Of the 8 sources, 4 with similar proximate composition but with varying PS were chosen for the experiment. Broilers were provided experimental diets from 1 to 9 and 24 to 29 d of age. From 10 to 23 d of age, broilers were fed diets that included the same enzyme concentrations as the experimental diets but with a common source of corn. From 1 to 9 d of age, main effects of corn, phytase, and xylanase were observed ($P \leq 0.05$). Among source of corn, Corn D (the source with the lowest PS content) had the lowest ($P \leq 0.001$) IND and IDE. Phytase increased digestibility of N and energy ($P \leq 0.001$); however, xylanase supplementation decreased digestibility of both ($P \leq 0.029$). From 23 to 29 d of age, broilers fed diets with either corn A or D had reduced IND with phytase and xylanase in combination compared with phytase or xylanase alone ($P \leq 0.05$). Supplementation with both enzmes reduced IDE of diets based on corn A or D compared with xylanase alone, or either enzyme alone, respectively ($P \leq 0.05$). In conclusion, corn sources varying in PS content interacted with phytase and xylanase supplementation on ileal nutrient and energy digestibility in broilers at 29 d of age. Phytase supplementation increased ileal digestibilities but had a more pronounced effect from 1 to 9 d of age. Further research should focus on clarifying the use of exogenous enzyme supplementation to mitigate effects of corn with sub-marginal nutrient value on nutrient utilization of broilers.

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I. INTRODUCTION

Corn can provide greater than 20 and 60% of the crude protein and apparent metabolizable energy (**AME_n**) in a commercial broiler diet, respectively. Variation in nutrient or energy content can result in deviations from calculated nutrient content with adverse economic consequences. Evaluating corn for digestible nutrient or energy content may reduce monetary losses arising from variability. However, many feed mills do not have the bin space to separate low or high AME_n corn or even the ability to thoroughly evaluate each incoming batch. There is a need for rapid assays to assess energy or nutrient density of corn, which may allow for adoption of new technology such as in-line formulation systems.

Extractable salt-soluble protein (**PS**) content is assessed by the wet-milling industry as an indicator of starch-extractability of corn (Métayer et al., 2009). Solubility of salt-soluble proteins decreases as corn is dried at increasing temperatures (Lasseran, 1973). Previous research has identified interactions between moisture content at harvest and drying temperature where corn harvested at higher moisture contents is more susceptible to loss of PS (Rivera et al., 1978a; Odjo et al., 2012). Métayer et al. (2009) demonstrated a relationship between PS content and nutritional value of corn fed to poultry. Rapid evaluation of PS content is possible using near-infrared reflectance spectroscopy (**NIR**) and may allow nutritionists to adjust nutrient matrix values of corn

as well as choose enzymes, which may improve nutrient utilization from corn that has been dried aggressively.

Diet supplementation with exogenous enzymes is one strategy to minimize monetary losses by lessening the effects of nutrient and energy variability of corn on broiler performance. Effects of supplementation of wheat-based diets with phytase and carbohydrase in combination has yielded sub-additive to synergistic responses in broilers (Cowieson and Adeola, 2005; Juanpere et al., 2005). The interactive effects of these enzymes may differ in diets based on corn because it contains less soluble non-starch polysaccharides and NDF than wheat (Knudsen, 1997).

The overall efficacy of phytase and xylanase supplemented in combination may depend on the accessibility or degradability of protein and starch in the corn endosperm. Phytate increases endogenous losses of amino acids and minerals as well as decreases activity and stability of several digestive enzymes. Thus, extra-phosphoric effects of phytase may minimize losses in performance and/or profit. The research reported herein focused on the combination of these factors with particular emphasis on diets adequate in Ca and non-phytate P and high concentrations of phytase to evaluate extra-phosphoric effects in 4 experiments. In the first experiment, the relationship between energy utilization and extractable salt-soluble protein content in various sources of corn were determined. The 2 following experiments were conducted to identify optimal concentrations of phytase and xylanase to maximize extra-phosphoric effects. The last experiment was a combination of the concepts learned in the preceding experiments. Optimal phytase and xylanase concentrations obtained from experiments 2 and 3 were supplemented to diets formulated with corn varying in extractable salt-soluble protein

content but with similar proximate composition. These experiments assessed ileal energy and nutrient digestibility as well as growth performance of broilers.

II. REVIEW OF THE LITERATURE

CHEMICAL KERNEL CHARACTERISTICS

Corn is the principal source of energy in many broiler diets worldwide. Differences in corn quality may result in over- or underestimation of apparent metabolizable energy (AME_n), translating to reduced broiler performance or poor feed conversion. In the U.S., corn is graded based on various attributes such as test weight per bushel (U.S. grade 2 = 24.5 kg), percentages of heat-damaged or broken kernels, and foreign material (USDA, 2004). Corn is further evaluated based on the endosperm type or the presence of live insects. Many of the factors that comprise U.S. corn grades are related to both chemical and physical characteristics, but indicators of potential nutrient availability are based on physical attributes only, such as weight or appearance (USDA, 2004). With significant drying damage, the appearance of corn kernels may be altered so that grading captures the associated reduction of nutrient availability. In contrast, corn with milder heat damage may be given the same grade as corn that appears identical but has a higher nutritional value.

Chemical attributes that may distinguish sources of corn that differ in nutritional value include protein solubility, zein content, amylose to amylopectin ratio, and vitreousness. These variables may be better predictors of nutritional value compared with more generalized designations such as bushel weight, which has been shown to be a poor estimator of feeding value (Leeson et al., 1993; Dale, 1994). Even though corn is generally regarded as a consistent ingredient, its feeding value can vary significantly

(Summers, 2001). The AME_n of corn for broilers may vary by more than 400 kcal/kg and is affected by genetics, agronomic conditions, proximate composition, pre- and post-harvest processing variables, and the presence of phytate and insoluble non-starch polysaccharides (Cowieson, 2005).

All of these factors may alter the ability of the bird to digest and utilize nutrients and may not be accounted for under the current grading system. Sources of corn with similar proximate composition may differ in nutritional value when protein and starch are considered according to their chemical characteristics rather than total content alone. In a study correlating physical and chemical (as total nutrient content) kernel traits with broiler performance, total Lys and Met contents of corn did not affect average daily gain of broilers in the starter phase (Moore et al., 2008). These authors inferred that differences in energy availability resulting from vitreousness or amylopectin content may have had a more profound effect than the content of amino acids on feed consumption and utilization. Thus, a thorough understanding of the kernel and the interrelationships of the starch and protein matrix are needed to identify high or low quality corn.

CORN KERNEL COMPOSITION

The majority of the corn kernel is comprised of endosperm (~80%) that can be divided into two classes, a central floury endosperm and a peripheral horny endosperm (Earl et al., 1946). Starch granules within the horny endosperm are polygonal and more tightly compressed by the protein matrix compared with the spherical, loosely packed starched granules of the floury endosperm (Whistler and Thornburg, 1957). Starch granules, as well as small protein bodies containing zein, are embedded within an amorphous continuous protein matrix (Duvick, 1961).

Vitreousness or hardness is associated with composition and distribution of endosperm protein. Corn with a higher proportion of horny endosperm is more vitreous or glassy due to increased protein synthesis and more mature protein bodies relative to the floury endosperm (Paiva, 1991; Dombrink-Kurtzman and Bietz, 1993). The endosperm contains nearly 80% of the total kernel protein (Wilson, 1987), comprised of 50 to 60% zein (protein bodies), 30% glutelin (matrix proteins), and 10 to 20% albumins, globulins, and free amino acids (Arruda et al., 1978). The content of zein, the primary glutelin-associated protein in dent corn hybrids (Boundy et al., 1967), may vary due to variety differences or the extent of maturity at harvest (Bressani and Conde, 1961). Protein bodies are larger and more abundant within the horny endosperm compared with the floury endosperm (Wolf et al., 1967). Because zein contains very little Lys and high concentrations of Pro, Glu, and Leu, its protein contribution is reflected in the amino acid profile of corn, as Lys content decreases and Leu content increases during kernel development (Bressani and Conde, 1961). This results in a lower biological value as zein and crude protein content increase with maturity (Mitchell et al., 1952; Bressani and Conde, 1961). The association of starch with protein bodies and matrix proteins reduces access to starch granules (Rooney and Pflugfelder, 1986), and thus, any impairment of protein solubility that limits proteolysis may impact both protein and starch digestion.

VITREOUSNESS

Vitreousness of corn is related to the proportion of horny to floury endosperm and research has shown that it can negatively affect starch digestion in ruminants (Philippeau et al., 1999). However, the effect of vitreousness for broilers is ambiguous. Flint-type corn with a harder endosperm and higher vitreousness has a lower starch digestion rate in

vitro compared with dent-type corn (Grbesa and Kiš, 2005). However, Kato et al. (2011) observed no effects of type of endosperm (flint, semi-dent, dent, etc.) on AME_n of corn in broilers. From 1 to 42 d of age, the type of corn endosperm in diets fed to broilers did not influence growth performance, although there were some large numerical differences that were not significant but favored corn with a harder endosperm (Benedetti et al., 2011). Collins et al. (2001) concluded that the type of endosperm (hard or soft) had little effect on broiler production and effects on feed intake and gain appeared to be influenced more by production environment. Other variables may affect nutritional value of corn on a more consistent basis than vitreousness because variability is typically low within a country or region due to a favored type of endosperm. For example, dent corn grown in the United States, dent or semi-flint corn in Europe, and flint corn in Brazil.

AGRONOMIC CONDITIONS

Agronomic factors such as growing region and fertilization have also been evaluated. O'Neill et al. (2012) fed diets to broilers containing a single genetic variant of corn grown in five distinct regions in China and did not observe differences in AME_n on growth performance. Additionally, increasing soil N fertility did not improve the nutritional value of corn for broilers from 10 to 21 d of age (Cromwell et al., 1983), despite a reduction in biological value associated with increased N fertilization due to an increase in synthesis of zein (Wang et al., 2008).

HARVEST MOISTURE AND DRYING

Harvest moisture and drying may exert considerable influence on nutritional value of corn. Improper drying alters the nature and solubility of protein (Peplinski et al., 1994), which may affect nutrient utilization by decreasing the availability of amino acids

and impairing α-amylase and water access to the starch granules (Altay and Gunasekaran, 2006). Drying may also affect the composition of *myo*-inositol esters, as Pontoppidan et al., (2007) observed a small but significant shift from phytate to *myo*-inositol penta*kis*phosphate following extrusion cooking of corn. Corn dried at temperatures higher than 30°C had reduced protein solubility in 0.5*M* NaCl (Wall et al., 1975). Thermal denaturation of protein permits formation of hydrogen and non-covalent hydrophobic bonds, as well as disulfide bridge formation, which cause protein aggregation and insolubility. Wall et al. (1975) extracted additional proteins with a buffered (pH 10.0) 0.5% sodium dodecyl sulphate solution and 0.6% β-mercaptoethanol, following extraction of saline and alcohol soluble proteins. These reagents disrupt hydrophobic and disulfide bonds, respectively, indicating bond formation with increasing drying temperature.

Changes in starch granule structure represent additional challenges in terms of nutrient digestion in poultry. Annealing may occur with starch granules in high moisture corn (>25% w/w) dried at high temperatures if the onset of gelatinization temperature is not exceeded (Tester and Debon, 1999). The annealing process is a physical reorganization of starch granules leading to a higher gelatinization temperature and narrowing of the gelatinization range (Gough and Pybus, 1971). Annealed starch as a result is less readily digested. Furthermore, gelatinization enthalpy increases with kernel moisture content up to a threshold of 70% and thus, moisture is a limiting factor for starch gelatinization in pelleted diets (Altay and Gunasekaran, 2006). Gelatinization of starch radically increases its rate of digestion by amylases and thus would be considered desirable in poultry feeds. Conditioned mash, however, contains only 13 to 15% moisture

and although pressure and frictional heat generated at the mash-die interface increase the extent of gelatinization, gelatinization typically does not exceed 25 to 30% (Skoch et al., 1981; Moritz et al., 2003). As a result most corn starch is present as native starch granules which are digested more slowly (Walker and Hope, 1963), affecting glycemic and insulin responses. Extent of gelatinization is highly correlated with the rate of in vitro digestion by α-amylase (r = 0.96) as well as plasma glucose and insulin (r = 0.88 and 0.90, respectively) in rats (Holm et al., 1998).

A limited amount of research has demonstrated a reduction in energy utilization in poultry following improper drying of corn. Carvalho et al. (2009) determined that AME_n of corn dried at ambient temperature, as well as at 80, 100, and 120°C in Avian male birds from 21 to 30 d of age decreased quadratically with increasing temperature. Intuitively, corn that is low in AME_n due to artificial drying may impart an overall deficient AME_n to a corn-soybean meal-based diet. This may be particularly troublesome with diets formulated to be marginal in energy due to high cost of energy rich ingredients. The loss in utilization of energy and most amino acids (Carvalho et al., 2009), likely translates to reduced broiler performance. Broilers that were fed corn-soybean meal-based diets formulated to contain corn dried at different temperatures responded in BW gain and feed conversion (Kaczmarek et al., 2007) such that those fed the corn dried at 140°C gained 354 g less from 1 to 35 d of age than their counterparts fed corn dried at 80°C ($P \leq 0.05$). The same birds also exhibited a 16 point (1.67 vs. 1.51 kg:kg) increase in feed conversion ratio when fed the high temperature dried versus the control corn. Bhuiyan et al. (2010) also evaluated the effects of corn dried at different temperatures on

broiler performance. At 21 d of age, birds fed diets with corn dried at 100°C weighed 42 g/bird less than those fed diets with corn dried at 80°C.

Time of harvest may also affect protein solubility and subsequent nutrient utilization, as early harvest corn has a higher moisture content but a lower proportion of zein and glutelin proteins compared with corn harvested at maturity (Bressani and Conde, 1961). Despite stabilization of kernel proximate composition from approximately 37 d after flowering onward (Bressani and Conde, 1961), early-harvest corn has a higher reducing sugar content and is more susceptible to thermal damage (e.g., Maillard reaction) compared with corn harvested at maturity (French and Kingsolver, 1964; Rivera et al., 1968a,b). It is also worth noting that storage time is critical as it generates more reducing sugars and so will result in greater damage during drying. These concepts are apparent by reductions in Lys content and availability following elevated drying temperatures in corn harvested early with higher moisture content (Wall et al., 1975; Rivera et al., 1978a). Such data run counter to the tenet that that heat combined with moisture positively affects enzymatic access to polymers for digestion (Moran, 1985). However, further exceptions to this tenet were provided by Rivera et al. (1978a) who observed a maturity × drying temperature interaction on total tract Lys digestibility in pigs. Corn harvested at 82% DM and dried at 60°C had a higher Lys digestibility than corn harvested at 63% DM, and the opposite was true when corn was dried at 40°C, which demonstrates there are exceptions to the rule that higher drying temperatures lead to lower nutrient digestibilities.

EXTRACTABLE SALT-SOLUBLE PROTEIN

Extractable salt-soluble protein content is used by the wet-milling industry to monitor starch-extractability with increasing temperatures. This occurs in two phases, as PS decreases rapidly during initial heating followed by a less rapid decrease ultimately reaching an asymptote (Mourad et al., 1996). Reduction of PS proceeds at a more rapid pace as moisture content increases (Odjo et al., 2012). Thus, corn harvested at a higher moisture content is more susceptible to the loss of PS. Solubility of most kernel proteins is negatively associated with drying temperature, with the exception of zein-like glutelins, which are not affected by drying temperatures up to 140°C (Malumba et al., 2009). Solubility indexes of albumins, globulins, total salt-soluble proteins, and zein, however, are all highly negatively correlated ($r \leq -0.95$) with drying temperature and starch yield from the wet-milling process. As a result Malumba et al. (2009) suggested PS as an assessment of corn suitability due its ability to identify processes where temperatures have exceeded those which provide optimal starch recovery in the wet-milling process of corn (Métayer et al., 2009). Lasseran (1973) determined the solubility of salt-soluble and alcohol-soluble proteins decreases as corn is dried at increasing temperatures.

Development of a standard method for determination of PS has provided a rapid and inexpensive assay for evaluating corn (method NF-V03-741; AFNOR, 2008). This method is a colorimetric protein assay similar to the Bradford method, but with a saline extraction step (Bradford, 1976). Absorption at 595 nm is compared with a standard curve constructed with bovine serum albumin. For rapid analysis of corn, PS content as well as vitreousness can be determined using near-infrared reflectance spectroscopy

(Ngonyamo-Majee et al., 2008; Cowieson, 2010). This technology may be beneficial for daily assessment of corn to prevent overestimation of corn $AME_{n,}$ which may negatively affect rate of growth and feed conversion. Additionally, prediction equations may be developed using these and other variables to predict AME_n of corn.

Métayer et al. (2009) evaluated AME_n in intact roosters and 1 to 38 d growth performance of broilers fed diets formulated with corn dried at 80, 110, or 140°C. Various drying temperatures resulted in PS values (equivalent mg of BSA) of 33, 25, and 12. Diets containing corn dried at 140°C decreased AME_n by 80 kcal/kg and increased feed conversion ratio of broilers by 3% (1.50 vs. 1.46) compared with corn dried at 80°C (Figure 2). Differences in growth performance are probably related to differences in nutritional value among sources of corn varying in PS. However, data are limited on the effects of PS on nutrient and energy utilization in broilers.

CHARACTERIZING THE SUBSTRATES

The gastrointestinal tract responds dynamically to the contents of the intestinal lumen (Mateos et al., 2002), and consequently demonstrates morphological or secretory changes according to the composition of the diet. Anti-nutritional factors that decrease nutrient utilization from feed may lead to changes in gross morphology of the digestive organs, including increased relative mass of the small intestine and pancreas (Bedford, 1996). Composition of the diet may also effect changes in the morphology of the gut mucosa (Mercurio and Behm, 1981), the rate of enterocyte turnover (Jacobs, 1983; Silva and Smithard, 2002), and the amount of pancreatic and mucosal secretions (Grossman et al., 1943; Cowieson et al., 2004a,b; Cowieson and Ravindran, 2007; Cowieson et al., 2008). Substrates for exogenous phytase and xylanase found in cereal grains and oilseed

meals include phytate, arabinoxylans, and xylans. In addition to being poorly digestible, phytate and xylan are known as antinutrients because they directly impede the digestive process and cause an overall reduction in nutrient utilization from feed.

PHYTATE

Approximately two-thirds of the total P in plant-derived feed ingredients exists as phytate (*myo*-inositol-1,2,3,4,5,6-hexa*kis*phosphate) P, and is largely unavailable to monogastric animals when fed in diets containing supplemental Ca (Gillis et al, 1957; Paullaf and Rimbach, 1997). Inorganic P, *myo*-inositol and various minerals are stored in developing seeds in the form of mixed K, Mg, and Ca phytates (Paullaf and Rimbach, 1997; Angel et al., 2002). Phytate is known as an antinutrient because it impairs nutrient utilization via inhibition of endogenous enzymes (Yoon et al., 1983; Liu et al., 2008), chelation of minerals (Maga, 1982), and hypersecretion and repartitioning of Na in the gastrointestinal tract (Cowieson et al., 2003; Cowieson et al., 2004b). Because phytate is a polyanionic molecule at gastric pH, it readily chelates mono and divalent mineral cations, and forms complexes with proteins and starch (Angel et al., 2002).

Phytate begins to be synthesized in corn kernels approximately 3 wk after pollination and increases rapidly with maturity (DeTurk et al., 1933; Early and DeTurk, 1944). Greater than 85% of the phytate in corn is concentrated in the germ (Hamilton et al., 1951; O'Dell et al., 1972). Phytate content of corn can vary considerably and corn having an abnormally high concentration of phytate could negatively affect nutrient utilization. Classen et al. (2010) reported phytate concentrations of 200 corn samples ranging from 0.2 to 0.9% with the mean being 0.6%. Li et al. (2000) observed increased P retention in birds fed diets containing low-phytate corn compared with commodity corn

(0.28 vs. 0.74% phytate, respectively), with no effects on growth performance. When non-phytate P concentration was not held constant (substitution of equal amounts of wild-type and low-phytate corn), birds fed diets containing low-phytate corn grew faster and had higher tibia ash concentration (Jang et al., 2003). This likely occurred due to a higher proportion of the P from the low phytate corn being of non-phytate origin and hence more available. Effects of phytate concentration on the nutritional value of corn could potentially be exacerbated by other factors affecting nutrient utilization in corn, particularly where protein and starch digestion are concerned.

The in vitro rate of wheat starch degradation as well as glycemic index in humans are negatively associated with phytate content (Yoon et al., 1983), and in broilers, Cowieson et al. (2004a) observed reduced digestion of corn starch in the presence of phytate. It may be that phytate interacts with starch via protein due to the close association of these nutrients (starch/protein envelope). Zein is extremely hydrophobic and may be more susceptible to interactions with phytate than other proteins. Cowieson and Cowieson (2011) determined phytate is likely to cause aggregation and insolubility of proteins by competing for hydrating water with other compounds in the water matrix. Thus, the ability of proteins to stay in solution in the presence of phytate is most dependent on surface polarity and hydrophobicity/-philicity, rather than the concentration of basic amino acids as reported previously (Cowieson and Cowieson, 2011).

Localization of Phytate in the Corn Kernel

Phytate is found in globoid crystals within discrete regions of cereal grains and oil seeds. The starchy endosperm of most cereal grains is essentially devoid of phytate, but corn differs in that approximately 3% of the total P in the endosperm is comprised of

phytate (Reddy, 2002). Regardless, the pool of phytate found in the endosperm is small and greater than 85% of the phytate in corn is concentrated in the germ (O'Dell et al., 1972).

Protein Interactions with Phytate

Hill and Tyler (1954) observed a reduction in phytate solubility towards pH 2.5 due to the presence of protein in vitro. For many years, it was assumed formation of protein-phytate complexes at low pH were based on electrostatic interaction between negatively charged phytate ions and proteins with a net positive charge (Okubo et al., 1975; Rajendran and Prakash, 1993). Recently, Cowieson and Cowieson (2011) provided convincing evidence for an alternative mechanism involving thermodynamics of water. These authors noted several inconsistencies exist between earlier research and the theory of direct electrostatic bond formation between phytate and protein. For example, interaction between phytate and protein should increase rather than decrease protein solubility, there should be a correlation with basic amino acid concentration or isoelectric point (**pI**) of proteins with solubility, and there should not be a direct influence of protein concentration.

Cowieson and Cowieson (2011) determined increasing phytate concentration decreased egg white lysozyme solubility in vitro. However, at phytate concentrations between 25 and 50 mM, solubility increased rapidly to approach 100% solubility at higher concentrations. Secondly, at a low phytate concentration (6 mM), increasing lysozyme concentration rapidly decreased solubility. Furthermore, in addition to lysozyme (pI 9.4), solubility assays with conalbumin (pI 6.9), ovalbumin (pI 5.2), ribonuclease A (pI 8.9), and carbonic anhydrase (pI 6.4) revealed no correlation between

pI and solubility in the presence of phytate. These results were attributed to a generic mass effect where phytate induces a hydration shell around itself and as a strong kosmotropic anion competes for hydrating water from proteins which causes their aggregation and insolubility.

Thus, the most important factor determining solubility is the polarity of the surface, which influences the propensity of proteins to give up water molecules comprising their own hydration shell. This relationship is of particular importance because the major storage proteins in cereal grains, known as prolamins (zein, kafirin, etc.), are extremely hydrophobic and susceptible to aggregation caused by phytate in this manner. In corn-soybean meal-based diets, corn may provide more than 20% of the dietary protein, with zein comprising more than 10% alone. Hydrophobic regions in other feed ingredient proteins, buried in the native conformation, may be exposed by thermal denaturation caused by drying, rendering, or pelleting (Koseki et al., 1989).

In the small intestine, ternary protein-phytate complexes may be formed with the presence of divalent cations via a cationic bridge. Zinc and Cu have a higher affinity for phytate than Ca; however, complexes involving Ca are primary due to the relative abundance of dietary Ca (Smith and Rackis, 1957; Okubo et al., 1975; Nosworthy and Caldwell, 1988; Champagne et al., 1990; Selle et al., 2000). Champagne (1988) proposed an equilibrium existing between Ca, protein, and phytate in which they exist separately, as a ternary compound, or as a Ca-phytate complex and free protein.

Endogenous Losses Stimulated by Phytate

The consequences of phytate's influence on protein solubility lead to secondary physiological responses that exacerbate the problem of reduced amino acid digestibility.

Phytate readily decreases the activation of pepsinogen and activity of pepsin (Woyengo et al., 2010; Liu and Cowieson, 2011). Furthermore, protein that has been made insoluble by phytate is less susceptible to attack by pepsin and solubilization by HCl (Vaintraub and Bulmaga, 1991). Several experiments have demonstrated inhibition of pancreatic enzymes including trypsin (Singh and Krikorian, 1982; Deshpande and Damodaran, 1989), chymotrypsin (Deshpande and Damodaran, 1989), carboxypeptidase A (Martin and Evans, 1989), amylase (Deshpande and Cheryan, 1984), and lipase (Knuckles, 1988; Liu et al., 2010). Inhibitory effects of phytase may occur through direct interaction or via chelation of Ca^{2+}, which leads to destabilization of trypsin (Caldwell, 1992) and amylase (Caldwell and Kung, 1952).

The antinutritive effects of phytate can be observed in the endogenous amino acid losses, which reflect the amino acid composition of endogenous digestive enzymes and mucin (e.g., Asp, Cys, Gly, Glu, Pro, Ser, Thr; Cowieson et al., 2004b; Cowieson et al., 2008). Evidence suggests pepsinogen and HCl are hyper-secreted in the proventriculus in response to phytate, based on the composition of endogenous amino acid flow (Cowieson et al., 2008; Woyengo et al., 2010). Tannic acid also reduces protein solubility and it has clearly been shown to increase secretion of pepsinogen and HCl (Mitjavila et al., 1973).

As the chyme leaves the gizzard, the duodenal mucosa is challenged with the higher H^+ concentration leading to compensatory hyper-secretion of mucin and $NaHCO_3$ (Cowieson et al., 2004b; Cowieson and Ravindran, 2007). Repartitioning of Na from digestive processes to $NaHCO_3$ may impede the normal Na-dependent transport of glucose and amino acids. Liu et al. (2008) fed corn-soybean meal-based diets containing 0.22 or 0.44% phytate to broilers from 1 to 3 wk of age and observed 4% reductions of

Na^+K^+-ATPase activity due to phytate in the duodenal and jejunal mucosae. Sucrase and maltase activities were also reduced in the duodenum, as well as amylase and sucrase in the jejunum. Changes in Na^+K^+-ATPase due to phytate have been associated with reduced blood glucose in chickens (Liu et al., 2008) and rats (Dilworth et al., 2005).

PHYTASE

Phytase catalyzes the step-wise hydrolysis of the phosphomonoester bonds of phytate to yield lower phosphate esters (**IP1-5** with phytate being **IP6**) of *myo*-inositol and inorganic phosphate (Greiner et al., 1993). The mechanism of phytate hydrolysis by phytase differs depending on the classification and source of phytase, as well as the expression system used for its production. Several distinct classes of enzymes with phytase activity have been identified (Mullaney and Ullah, 2003; Oh et al., 2004; Chu et al., 2004), although the majority of phytases used in animal feeds belong to the subfamily of high molecular weight histidine acid phosphatases (HAP; Piddington et al., 1993; Mitchell, et al., 1997). The HAP phytases can be further classified as either 3-phytases (EC 3.1.3.8) or 6-phytases (EC 3.1.3.26) depending on the stereospecificity of the initial hydrolytic attack (IUPAC-IUB, 1975).

Bacterial and fungal sources generally produce 3-phytases and preferentially hydrolyze the phosphomonoester bond at the third position, yielding IP5 as the initial hydrolysis product. In contrast, plants produce 6-phytases that preferentially dephosphorylate phytate at either the 6 or 4 positions, yielding 2 distinct positional isomers of IP5 (Quan et al., 2003). Phytate hydrolysis proceeds at different rates and with different orders of *myo*-inositol phosphate intermediates depending on the source and classification of phytase (Puhl et al., 2009).

In feed ingredients, phytate predominantly exists in the thermodynamically favorable chair conformation and has 1 axial and 5 equatorial phosphate groups (Wyss et al., 1999). The axial 2-phosphate group of phytate is the most refractory to hydrolytic attack (Wyss et al., 1999; Lee et al., 2003; Quan et al., 2003) and thus far only the endogenous phytase from wheat (*Triticum aestivum* L. cv Nourin 61) is reported to dephosphorylate phytate completely to yield *myo*-inositol as the end product (Nakano et al., 2000). Given the appropriate reaction conditions, most phytases, regardless of class and source, yield IP_1 and 5 molecules of inorganic phosphate (Wyss et al., 1999).

Extra-Phosphoric Effects of Phytase

It is well accepted that beyond P adequacy, further positive responses to phytase supplementation can be attributed to extra-phosphoric effects (Cabahug et al., 1999; Ravindran et al., 2000). Phytase is typically given matrix values in diet formulation for non-phytate P and in certain cases Ca, Na, ileal digestible amino acids and energy. For example, a phytase with an activity of 2,500 FTU/g included at 0.02% of the diet would be given a matrix value of 650% to provide 1.3% non-phytate P. Higher inclusions increase nutrient and energy utilization, and subsequently feed intake and growth rate, independent of non-phytate P release. These benefits of phytase are extra-phosphoric effects and are directly related to the reduction of antinutritional effects of phytate.

Hydrolysis of phytate counteracts the negative effects of phytate on dietary nutrient utilization (McCance and Widdowson, 1942; Hill and Tyler, 1954; Singh and Krikorian, 1982) and loss of endogenous minerals and amino acids (Cowieson et al., 2004b; Cowieson and Ravindran, 2007; Onyango et al., 2009). Additionally, phytate has been demonstrated to inhibit the activity of many endogenous digestive enzymes.

According to Liu and Cowieson (2011) one explanation for the extra-phosphoric effects of phytase may be the restoration of optimal digestive enzyme kinetics. This process occurs as a result of various indirect effects of phytate, including hypersecretion of Na, chelation of Ca, and kosmotropic interaction with zymogens and enzymes, which reduce zymogen activation, enzyme activity, and enzyme stability (Evans and Pierce, 1981; Caldwell, 1992; Cowieson et al., 2004b; Selle et al., 2010; Cowieson and Cowieson, 2011; Liu and Cowieson, 2011).

The antinutritional effects of phytate on BW gain have been demonstrated by Cabahug et al. (1999). These authors fed broilers diets that were formulated to be adequate in non-phytate P with progressive concentrations of phytate. A 51% increase in phytate concentration (1.57 vs. 1.04%) resulted in a reduction in BW gain of 98 g/bird. Additionally, supplementation of diets adequate in non-phytate P with phytase at a concentration of 800 FTU/kg increased BW gain by 75 g/bird. This extraphosphoric effect on BW gain with phytase may be due to an increase in feed intake, as phytate appears to directly inhibit feed intake in broilers (Cowieson et al., 2011). Effects on growth performance may be mediated in part by increased nutrient and energy utilization. In 25 d old broilers, 400 FTU/kg of phytase increased apparent ileal Lys digestibility in diets with 0.45% non-phytate P, although this effect was apparent only in diets with 1.32% and not 1.04% phytate (Ravindran et al., 2000). Additionally, diets with 1.04% phytate fed to broilers had significantly higher AMEn when supplemented with phytase at 800 FTU/kg but not 400 FTU/kg (Ravindran et al., 2000).

Phosphorus adequacy can typically be restored without hydrolysis of a significant portion of the dietary phytate pool (≤60%; Shirley and Edwards, 2003; Rutherford et al.,

2004). However, Shirley and Edwards (2003) observed phytate-P disappearance of greater than 90% with phytase supplemented at 12,000 FTU/kg in diets fed to broilers. Commercial phytases preferentially attack the *myo*-inositol esters with 5 or 6 ortho-phosphate groups (Wyss et al., 1999). Higher concentrations of phytase than are typically used are required in order to hydrolize remaining IP5 and IP6 and significantly deplete the pool of IP3 and IP4. As the higher-phosphorylated esters are removed from the ingesta, the vast anti-nutritional effects of phytate are reduced accordingly.

The IP5 and IP6 esters exhibit an approximately 2 fold higher chelation capacity for dietary minerals compared with IP3 and IP4 (Luttrell, 1993; Persson et al., 1998). A large reduction in cation binding affinity occurs between IP5 and IP4 and once again between IP3 and IP2. For example, Luttrell (1993) determined binding affinity for Ca^{2+} was considerably lower for IP3 and IP4 compared with IP6 (6.3×10^3 and 6.2×10^4 vs. 1.9×10^5 l/mol, respectively) and was approximately 50% lower for IP2 vs. IP3 (9.0×10^3 vs. 6.3×10^3 l/mol, respectively).

One mechanism that has been largely overlooked for the extra-phosphoric effect may be restoration of an ideal Ca to non-phytate P ratio. Calcium is released disproportionately to P with standard concentrations of phytase (Cowieson et al., 2011). With high doses of phytase (> 2,000 FTU/kg), release of Ca and P may approach a plateau (Cowieson et al., 2011), which results in a 2:1 ratio of Ca and non-phytate P as demonstrated by broiler performance (Nelson et al., 1971) and plasma Ca and P (Shirley and Edwards, 2003).

ARABINOXYLANS

Substrate for β-1,4-endoxylanase in corn consists of various pentosan polysaccharides containing the pentose sugars, arabinose, and xylose. Pentosan polysaccharides consist of a β-1,4-xylopyranose backbone with arabinofuranosyl monomers bonded by 1,2- and 1,3-α-glycosidic bonds and comprise the bulk of hemicellulose (**AX**; Perlin, 1951). Classification of arabinoxylan is divided into the water-extractable (**WE-AX**) and water-unextractable (**WU-AX**) groups, which exhibit different physicochemical properties in the gastrointestinal tract environment. The WE-AX have high molecular weights and increase solution viscosity via arabinose-mediated solubility and polymer entanglement (Bedford, 1995), and oxidative gelation involving ferulic acid residues (Geissmann and Neukom, 1973; Meuser and Suckow, 1986). In contrast, WU-AX has a high water-holding capacity due to covalent and non-covalent interactions with other AX molecules, as well as cellulose and lignin (Meuser and Suckow, 1986).

Arabinoxylans limit nutrient digestion from wheat and rye because of increased digesta viscosity and sticky excreta due to WE-AX (Pawlik et al., 1990; Choct and Annison, 1992), as well as encapsulation of nutrients by WU-AX (Bedford, 1995). In contrast, digesta viscosity is unaffected in birds fed corn-based diets because only 3% of the total pentosans in corn consists of WE-AX (0.1% of the kernel; Kereliuk et al., 1995). Water-unextractable arabinoxylans comprise the bulk of the hemicellulose fraction in corn and represent a physical barrier to starch and protein within the endosperm.

Pelleting of broiler diets physically damages cell walls in plant-derived feed ingredients (Tovar et al., 1991). However, a portion of dietary nutrients invariably

remains encapsulated and may be released by the action of xylanase. The nutritive fractions of corn grain (endosperm and germ) are surrounded by 2 layers that are resistant to mechanical grinding of the gizzard (Bedford, 1995). The aleurone layer is a thin, single layer of cells with thick cell walls containing AX that immediately surrounds the endosperm and germ. Enclosing the aleurone layer is the pericarp, which consists of non-living cells, consisting primarily of cell-wall material (Moreau et al., 2000).

XYLANASE

Xylanases are classified as *O*-glycoside hydrolases (EC 3.2.1.x), that catalyze endohydrolysis of 1,4-β-D-xylosidic bonds in the arabinoxylan backbone (Collins et al., 2005). These enzymes are members of diverse glycoside hydrolase families (5, 7, 8, 10, 11, and 43) and possess unique physicochemical properties, including catalytic rate, pH optima, thermostability, etc. Members of families 5, 7, 10, and 11 catalyze hydrolysis via a retaining mechanism where 2 Glu residues in the active site are involved with formation of a glycosyl-enzyme intermediate, preceding general base catalysis with the retention of the β-conformation at the anomeric center (McCarter and Withers, 1994). In contrast, Glu and Asp are the catalytic residues in families 8 and 43, and catalysis proceeds with inversion of the anomeric center (McCarter and Withers, 1994).

Beneficial effects of xylanase in poultry diets are multi-faceted. These include a reduction in digesta viscosity and increased rate of passage (Wu et al., 2004a), increasing utilization of nutrients and energy (Annison, 1992; Hew et al., 1998), and positive effects on the gastrointestinal microfloral populations (Vahjen et al., 1998; Engberg et al., 2004). However, xylanases from different sources differ in substrate specificity toward arabinoxylans. For example, endoxylanase from *Aspergillus aculeatus* preferentially

degrades WE-AX, while endoxylanase from *Bacillus subtilis* exhibits greater activity towards WU-AX (Courtin and Delcour, 2001). The vast differences in commercial xylanases lead to discrepancies in the magnitude of responses observed. For example, Hew et al. (1998) fed broilers wheat-based diets supplemented with 1 of 2 xylanases. One enzyme yielded an approximately 50% higher increase in AME_n compared with the other (19 vs. 13%), although both enzymes increased AME_n and ileal amino acid digestibility.

PHYTASE AND XYLANASE IN COMBINATION

Research examining the supplementation of diets with phytase and xylanase in combination has focused on wheat-based diets because wheat contains more soluble non-starch polysaccharides than corn (Bach Knudsen, 1997). Phytate is deposited in aleurone particles associated with soluble fiber in wheat (Tanaka et al., 1974). In contrast, the majority of phytate (>85%) in corn is concentrated in the germ (O'Dell et al., 1972). Increased cellular permeability facilitated by xylanase supplementation may increase access to phytate and encapsulated nutrients (Selle et al., 2003), which may alter the magnitude of extraphosphoric effects on nutrient and energy digestibility. Thus, phytase and xylanase have a complimentary mode of action in the presence of sufficient substrate, leading to sub-additive to synergistic responses (Selle et al., 2003). Selle et al. (2009) evaluated ileal amino acid and energy digestibility in broilers fed wheat-soybean meal-based diets supplemented with phytase and xylanase. The combination of both enzymes resulted in a sub-additive increase in amino acid digestibility (8.6 vs. 4.8 and 5.5% with phytase or xylanase alone, respectively). In contrast, Selle et al. (2003) fed Lys-deficient (0.1% Lys) wheat-sorghum-soybean meal-based diets supplemented with phytase and xylanase to 28 d old broilers, and responses on amino acid digestibility were synergistic.

Peng et al. (2003) reported phytase and xylanase interacted in their effects on feed conversion ratio and phytate P digestibility, with xylanase affect being enhanced or dependent on phytase supplementation, respectively. These types of responses are common in wheat-based diets but in some cases xylanase has little influence on nutrient utilization or growth performance (Olukosi and Adeola, 2008). It may be that high concentrations of WE-AX are required to observe a phytase × xylanase interaction or that high activities of intrinsic phytase and xylanase mute the effects of exogenous sources (Olukosi and Adeola, 2008; Woyengo et al., 2008).

Wu et al. (2004b) demonstrated phytase and xylanase in combination increased AME_n, while a significant effect could not be detected due to either enzyme alone. This may be due partially to improved surface area for absorption as the combination increased villus height in the ileum and crypt depth in the jejunum and ileum. No further improvements in growth performance or gastrointestinal tract variables were observed with the combination of enzymes. In contrast, when the majority of the diet is comprised of corn, effects of xylanase on gut morphology may be absent. Lü et al. (2009) evaluated the phytase and xylanase supplementation of corn-wheat-soybean meal-based diets, which contained 20% wheat. Inclusion of 20% wheat was insufficient to demonstrate an effect of enzyme treatment on mucosal morphology or bacterial populations. However, the combination of phytase and xylanase were additive in their effects on AME_n and decreased digesta viscosity by 28%. Furthermore, phytase and xylanase interacted in their effects on feed conversion in broilers fed supplemented corn-soybean meal-wheat bran-based diets (25% wheat bran) from 1 to 21 d of age (Pourreza and Classen, 2001). Feed conversion was reduced by xylanase inclusion in the presence of 1,000 FTU/kg phytase

but not 500 FTU/kg. Use of xylanase alone in corn-soybean meal-based diets may depend on AME_n content being sufficiently reduced because the response is expected to be lower compared with that in diets based on viscous cereal grains. O'Neill et al. (2012) reported that xylanase supplementation resulted in reduced feed conversion during the periods from 1 to 35 and 1 to 42 d of age. Despite the majority of U.S. integrators using phytase in combination with one or more carbohydrases, the effects of phytase and xylanase in corn-soybean meal-based diets for broilers have not been evaluated thoroughly.

EXOGENOUS ENZYME SUPPLEMENTATION AND DRYING OF CORN

Experiments have examined the use of a combination of xylanase, amylase, and protease or these enzymes with phytase to improve the nutritional value of corn dried at high temperatures (Iji et al., 2003; Kaczmarek et al., 2007; Bhuiyan et al., 2010). Growth performance assessed at 21 or 28 d of age was not influenced by exogenous enzyme supplementation in corn artificially dried at temperatures up to 105°C (Iji et al., 2003; Bhuiyan et al., 2010). In general, ileal digestibility of protein, energy, and starch were unaffected by drying temperature or exogenous enzyme supplementation in these experiments. However, Bhuiyan et al. (2010) observed an interaction in the effects of these variables on ileal protein digestibility at 21 d of age, in which enzyme supplementation tended to decrease digestibility of diets based on corn dried at 80°C but increase digestibility when corn was dried at 90 or 100°C. In contrast, broilers fed diets supplemented with xylanase, amylase, and protease did not respond with corn dried at 80°C but had increased BW gain and reduced feed conversion ratio with corn dried at 140 or 120°C, respectively (Kaczmarek et al., 2007). The discrepancies between these

studies may have occurred because of the older broilers or higher drying temperatures evaluated by Kaczmarek et al. (2007) compared with other published research.

KNOWLEDGE GAPS IN THE LITERATURE

Corn supplies the majority of energy as well as a considerable quantity of the protein in broiler diets. Thus, variability in nutritional value due to improper drying, drought conditions, or other factors can have far reaching consequences on broiler production. Research has demonstrated adverse effects of excessive drying temperature on nutritional value of corn (Kaczmarek, 2007; Carvalho et al., 2009; Bhuiyan et al., 2010). However, these experiments evaluated corn dried at specific temperatures, which may not be known in a commercial setting. A need exists for rapid characterization of corn that has abnormally low nutritional value due to improper drying or other conditions.

One auspicious variable is PS, which can be evaluated rapidly using traditional chemical methods or by NIR. Data describing the relationship between PS and nutritional value of corn are lacking. However, Métayer et al. (2009) reported lower AME_n and higher feed conversion ratio in broilers fed diets containing corn dried at high temperatures with low PS compared with those having higher PS. Along with the need to further characterize this relationship, exogenous enzyme supplementation and corn with varying PS may interact in their effects on nutritional value. To the best of our knowledge, no published data on the potential interaction of corn with varying PS and exogenous enzyme supplementation exists in the literature. The need for research in this area is particularly important as more poultry producers are including carbohydrases in addition to phytase in order to reduce diet costs.

The use of exogenous enzymes is one strategy to improve consistency in nutritional value of corn (Cowieson, 2005). However, responses may be variable and partially dependant on the intrinsic digestibility of the basal diet (Kocher et al., 2003). This leads to the hypothesis that sources of corn, which have been subjected to various drying or storage conditions may interact with exogenous enzymes to influence nutrient digestibility. In particular, potential interactions between phytase and source of corn need to be evaluated because nearly all poultry diets are supplemented with phytase. By reducing the loss of endogenous protein and energy, phytase supplementation may lessen the impact of improper drying and reduce variability between adequate and submarginal quality of corn.

Interpretation of extra-phosphoric effects from many published experiments is confounded by the release of P. Typically, a large proportion of any observed nutrient digestibility or growth responses may be directly associated with the release of non-phytate P. Phytase is rarely added to a positive control diet that has been formulated to be adequate in non-phytate P. Even though broilers fed phytase-supplemented negative control diets often outperform a positive control (Cabahug et al., 1999; Cowieson et al., 2006). Nevertheless, in many experiments the point of P adequacy is unknown, making quantification of extra-phosphoric effects problematic. When graded concentrations of phytase are not supplemented to diets marginally deficient in non-phytate P, the point where non-phytate P becomes adequate and extra-phosphoric effects begin is uncertain. In fact, benefits of phytase supplementation likely occur due to release of P and extra-phosphoric effects simultaneously over a wide range of phytase concentrations. Extra-phosphoric effects have varied widely in peer-reviewed literature (Watson et al., 2006)

possibly because phytate concentration influences their occurrence and magnitude (Cabahug et al., 1999). To better understand the effects of phytase addition in broiler diets, it may be beneficial to study extra-phosphoric effects by using high concentrations of phytase to maximize dephosphorylation of phytate.

Invariably, a portion of the dietary phytate remains encapsulated by plant cell walls. The addition of carbohydrases may increase access to encapsulated phytate. Although phytate freed by carbohydrase would not otherwise be able to exert antinutritional effects, the hydrolysis of additional phytate may provide benefits such as supplying inositol (Hegsted et al., 1941; Cowieson et al., 2011). Supplementation of diets with phytase and carbohydrase in combination has produced mixed-results ranging from sub-additive to synergistic responses in broilers (Cowieson and Adeola, 2005; Juanpere et al., 2005) as well as swine (Oryschak et al., 2002; Kim et al., 2005). These experiments focused on wheat-based diets and the interactive effects of these enzymes may differ in diets based on corn. Responses in corn-soybean meal-based diets may be different in type or magnitude than in wheat-based diets because corn contains less soluble non-starch polysaccharides and NDF than wheat (Knudsen, 1997).

It is essential to produce diets that minimize differences between calculated and analyzed digestible nutrient content to maximize broiler performance. For this to occur, there is a need for 1) rapid evaluation of corn AME_n based on chemical kernel characteristics, e.g., PS; 2) understanding of extra-phosphoric increases in nutrient and energy digestibility with phytase supplementation; 3) the influence of xylanase on nutritional responses to phytase in corn-soybean meal-based diets; and 4) the interactive effects of corn varying in PS and exogenous enzyme supplementation. The research

reported herein addressed these knowledge gaps in the literature by evaluating the effects of PS on nutrient and energy utilization of corn (Chapter III), extra-phosphoric effects of phytase with xylanase in broilers fed diets adequate in Ca and non-phytate P (Chapter IV), and the interaction between sources of corn varying in PS and phytase and xylanase supplementation (Chapter V). Research reported in Chapter IV was conducted to identify the optimal concentrations of phytase and xylanase to be used in Chapter V.

REFERENCES

AFNOR (Association Française de Normalisation) 2008. Méthode Promatest d'évaluation de la dénaturation des proteins Thermosensibles. NF-V03-741. AFNOR, La Plaine Saint-Denis, France.

Altay, F. and S. Gunasekaran. 2006. Influence of drying temperature, water content, and heating rate on gelatinization of corn starches. J. Agric. Food Chem. 54:4235-4245.

Angel, R., N. M. Tamim, T. J. Applegate, A. S. Dhandu, and L. E. Ellestad. 2002. Phytic acid chemistry: influence on phytin-phosphorus availability and phytase efficacy. J. Appl. Poult. Res. 11:471-480.

Annison, G. 1992. Commercial enzyme supplementation of wheat-based diets raises ileal glycanase activities and improves apparent metabolisable energy, starch and pentosan digestibilities in broiler chickens. Anim. Feed Sci. Tech. 38:105-121.

Arruda, P., W. J. Da Silva, and J. P. F. Teixeira. 1978. Protein and free amino acids in a high lysine maize double mutant. Phytochem. 17:1217-1218.

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

Barrier-Guillot, B., Zuprizal, C. Jondreville, A. M. Chagneau, M. Larbier, and M. Leuillet. 1993. Effect of heat drying temperature on the nutritive value of corn in chickens and pigs. Anim. Feed Sci. Tech. 41:149-159.

Bedford, M.R. 1995. Mechanism of action and potential environmental benefits from the use of feed enzymes. Anim. Feed Sci. Tech. 53:145-155.

Bedford, M. R. 1996. Interaction between ingested feed and the digestive system in poultry. J. Appl. Poult. Res. 5:86-95.

Benedetti, M. P., J. R. Sartori, F. B. Carvalho, L. A. Pereira, V. B. Fascina, A. C. Stradiotti, A. C. Pezzato, C. Costa, and J. G. Ferreira. 2011. Corn texture and particle size in broiler diets. Braz. J. Poult. Sci. 13:227-234.

Bhuiyan, M. M., A. F. Islam, and P. A. Iji. 2010. Response of broiler chickens to diets containing artificially dried high-moisture maize supplemented with microbial enzymes. S. Afr. J. Anim. Sci. 40:348-362.

Boundy, J. A., J. H. Woychik, R. J. Dimler, and J. S. Wall. 1967. Protein composition of dent, waxy, and high-amylose corns. Cereal Chem. 44:160-169.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Bressani, R. and R. Conde. 1961. Changes in the chemical composition and in the distribution of nitrogen of maize at different stages of development. Cereal Chem. 38:76-84.

Cabahug, S., V. Ravindran, P. H. Selle, and W. L. Bryden. 1999. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus contents. I. effects on bird performance and toe ash. Br. Poult. Sci. 40:660-666.

Caldwell, M. L. 1992. Effect of calcium and phytic acid on the activation of trypsinogen and the stability of trypsin. J. Agric. Food Chem. 40:43-46.

Caldwell, M. L. and J. T. Kung. 1953. A study of the influence of a number of factors upon the stability and upon the activity of pancreatic amylase. J. Am. Chem. Soc.75:3132-3135.

Carvalho, D. C. O., L. F. T. Albino, J. G. de Vargas, Jr., R. S. Toledo, J. E. de Oliviera, and R. M. de Souza. 2009. True digestibility of amino acids and digestible amino acids values of corn samples submitted to different drying temperatures and storage periods. R. Bras. Zootec. 38:850-856.

Champagne, E. T. 1988. Effects of pH on mineral-phytate, protein-mineral-phytate, and mineral-fiber interactions. Possible consequences of atrophic gastritis on mineral bioavailability from high fiber foods. J. Am. Coll. Nutr. 7:499-508.

Champagne, E. T., M. S. Fisher, and O. Hinojosa. 1990. NMR and ESR studies of interactions among divalent cations, phytic acid, and N-acetyl-amino acids. J. Inorg. Biochem. 38:199-215.

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

Chu, H. M., R. T. Guo, T. W. Lin, C. C. Chou, H. L. Shr, H. L. Lai, T. Y. Tang, K. J. Cheng, B. L. Selinger, and A. H. J. Wang. 2004. Structures of Selenomonas ruminantium phytase in complex with persulfated phytate: DSP-phytase fold and mechanism for sequential substrate hydrolysis. Structure 12:2015–2024.

Classen, H. L., D. D. Maenz, and C. Caruthers. 2010. Ingredient considerations, total phytate concentrations and susceptibility of phytate to hydrolysis. Proc. 1st Int. Phytase Summit, Washington D.C.

Collins, N. E., and E. T. Moran, Jr. 2001. Influence of yellow dent corn hybrids having different kernel characteristics yet similar nutrient composition on broiler production. J. Appl. Poult. Res. 10:228-235.

Collins, T., C. Gerday, and G. Feller. 2005. Xylanases, xylanase families and extremophilic xylanases. FEMS Microbiol. Rev. 29:3-23.

Courtin, C. M. and J. A. Delcour. 2001. Relative activity of endoxylanases towards water-extractable and water-unextractable arabinoxylan. J. Cereal Sci. 33:301-312.

Cowieson, A. J. 2005. Factors that affect the nutritional value of maize for broilers. Anim. Feed Sci. Tech. 119:293-305.

Cowieson, A. J. 2010. Corn nutritional value, composition analyzed. Feedstuffs, June 7. p 30-32.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2003. The effect of phytase and phytic acid on endogenous losses from broiler chickens. Br. Poult. Sci. 44:23-24.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2004a. The effect of phytic acid and phytase on the digestibility of maize starch for growing chickens. Int. Poult. Sci. Forum, Atlanta, Georgia, USA, (Abst.): p. 31.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2004b. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. Br. Poult. Sci. 45:101-108.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2006. Supplementation of corn-soy-based diets with an *Eschericia coli*-derived phytase: effects on broiler chick

performance and the digestibility of amino acids and metabolizability of minerals and energy. Poult. Sci. 85:1389-1397.

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

Cowieson, A. J. and N. P. Cowieson. 2011. Phytate and the thermodynamics of water. Proceedings of the Aust. Poult. Sci. Symp, Sydney, New South Wales, February 14-16.

Cowieson, A. J. and V. Ravindran. 2007. Effect of phytic acid and microbial phytase on the flow and amino acid composition of endogenous protein at the terminal ileum of growing broiler chickens. Br. J. Nutr. 98:745-752.

Cowieson, A. J., V. Ravindran, and P. H. Selle. 2008. Influence of dietary phytic acid and source of microbial phytase on ileal endogenous amino acid flows in broiler chickens. Poult. Sci. 87:2287-2299.

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

Cromwell, G. L., M. J. Bitzer, T. S. Stahly, and T. H. Johnson. 1983. Effects of soil nitrogen fertility on the protein and lysine content and nutritional value of normal and opaque-2 corn. J. Anim. Sci. 57:1345-1351.

Dale, N. 1994. Relationship between bushel weight, metabolizable energy, and protein content of corn from an adverse growing season. J. Appl. Poult. Res. 3:83-86.

De Boland, A. R., G. B. Garner, and B. L. O'Dell. 1975. Identification and properties of "phytate" in cereal grains and oilseed products. J. Agric. Food Chem. 23**:**1186-1189.

DelCurto, T., R. C. Cochran, D. L. Harmon, A. A. Beharka, K. A. Jacques, G. Towne, and E. S. Vanzant. 1990. Supplementation of dormant tallgrass-prairie forage: I. Influence of varying supplemental protein and (or) energy levels on forage utilization characteristics of beef steers in confinement. J. Anim. Sci. 68:515-531.

Deshpande, S. S. and M. Cheryan. 1984. Effects of phytic acid, divalent cations, and their interactions on α-amylase activity. Food Sci. 49:516-519.

Deshpande, S. S. and S. Damodaran. 1989. Effect of phytate on solubility, activity and conformation of trypsin and chymotrypsin. Food Sci. 54:695-699.

DeTurk, E. E., J. R. Holbert, and B. W. Howk. 1933. Chemical transformations of phosphorus in the growing corn plant with results on two first generation crosses. J. Agr. Res. 46:121-141.

Dilworth, L. L., F. O. Omuruyi, and H. N. Asemota. 2005. Digestive and absorptive enzymes in rats fed phytic acid extract from sweet potato (*Ipomoea batatas*). Diabetol. Croat. 34:59-65.

Dombrink-Kurtzman, M. A. and J. A. Bietz, 1993. Zein composition in hard and soft endosperm of maize. Cereal Chem. 70:105-108.

Duvick, D. N. 1961. Protein granules of maize endosperm cells. Cereal Chem. 38: 374-385.

Earl, F. R., J. J. Curtis, and J. E. Hubbard. 1946. Composition of the component parts of the corn kernel. Cereal Chem. 77:721-723.

Early, E. B. and E. E. DeTurk. 1944. Time and rate of synthesis of phytin in corn grain during the reproductive period. J. Am. Soc. Agron. 36:803-814.

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

Evans, W. J. and A. G. Pierce. 1981. Calcium-phytate complex formation studies. J. Am. Oil Chem. Soc. 58:850-851.

Everts, H., R. A. Dekker, B. Smits, and J. W. Cone. 1996. The digestion of maize and native pea starch in the small intestine of pigs. Proc. Nutr. Soc. 55:59A.

French, R. C. and C. H. Kingsolver. 1964. The effect of excessive heat during artificial drying of corn on reducing sugar content and diastatic activity. Cereal Chem. 41:47-58.

Geissmann, T. and H. Neukom. 1973. On the composition of the water-soluble wheat flour pentosans and their oxidative gelation. Lebensmittel-Wissenschaft und-Technolog 6:54-62.

Gillis, M. B., K. W. Keane, and R. A. Collins. 1957. Comparative metababolism of phytate and inorganic P^{32} by chicks and poults. J. Nutr. 62:13-26.

Gough, B. M. and J. N. Pybus. 1971. Effect on the gelatinization temperature of wheat starch granules of prolonged treatment with water at 50°C. Starch/Staerke 23:210.

Grbesa, D. and G. Kiš. 2005. Relation between endosperm and *in vitro* kinetic of starch digestibility in maize hybrids for broiler chickens. Ital. J. Anim. Sci. 4 (SUPPL. 3):139-141.

Greiner, R., U. Konietzny, and K. -D. Jany. 1993. Purification and characterization of two phytases from Escherichia coli. Arch. Biochem. Biophys. 303:107-113.

Grossman, M. I., H. Greengard, and H. C. Ivy. 1943. The effect of dietary composition on pancreatic enzymes. Am. J. Physiol. 138:676-682.

Hamilton, T. S., B. C. Hamilton, B. Connor Johnson, and H. H. Mitchell**.** 1951. The dependence of the physical and chemical composition of the corn kernel on soil fertility and cropping system. Cereal Chem. 28:161-176.

Hegsted, D. M., G. M. Briggs, R. C. Mills, C. A. Evehjem, and E. B. Hart. 1941. Inositol in chick nutrition. Exp. Biol. Med. 47:376-377.

Heldt, J.S., R. C. Cochran, C. P. Mathis, B. C. Woods, K. C. Olson, E. C. Titgemeyer, T. G. Nagaraja, E. S. Vanzant, and D. E. Johnson. 1999. Effects of level and source of carbohydrate and level of degradable intake protein on intake and digestion of low quality tall grass- prairie hay by beef steers. J. Anim. Sci. 77:2846-2854.

Hew, L. I., V. Ravindran, Y. Mollah, and W. L. Bryden. 1998. Influence of exogenous xylanase supplementation on apparent metabolizable energy and amino acid digestibility in wheat for broiler chickens. Anim. Feed Sci. Tech. 75:83-92.

Hill, R. and C. Tyler. 1954. The influence of time, temperature, pH and calcium carbonate on the activity of the phytase of certain cereals. J. Agric. Sci. 44:306-310.

Holm, J., I. Lundquist, I. Björck, A. –C. Eliasson, and N. –G. Asp. 1988. Degree of starch gelatinization, digestion rate of starch in vitro, and metabolic response in rats. Am. J. Clin. Nutr. 47:1010-1016.

Iji, P. A., K. Khumalo, S. Slippers, and R. M. Gous. 2003. Intestinal function and body growth of broiler chickens on diets based maize dried at different temperatures and supplemented with a microbial enzyme. Reprod. Nutr. Dev. 43:77-90.

IUPAC–IUB. 1975. Enzyme nomenclature recommendation supplement 1: correction and additions. Biochem. Biophys. Acta. 429:1.

Jacobs, L. R. 1983. Effects of dietary fiber on mucosal growth and cell proliferation in the small intestine of the rat: a comparison of oat bran, pectin, and guar with total fiber deprivation. Am. J. Clin. Nutr. 37:954-960.

Jang, D. A., J. G. Fadel, K. C. Klasing, A. J. Mireles, Jr., R. A. Ernst, K. A. Young, A. Cook, and V. Raboy. 2003. Evaluation of low-phytate corn and barley on broiler chick performance. Poult. Sci. 82:1914-1924.

Kaczmarek, S., A. J. Cowieson, D. Józefiak, M. Bochenek, and A. Rutkowski. 2007. The effect of drying temperature and exogenous enzymes supplementation on the nutritional value of maize for broiler chickens. Proc. 16th Euro. Symp. Poult. Nutr., Strasbourg, France.

Kato, R. K., A. G. Bertechini, E. J. Fassani, J. A. Goncalves De Brito, and S. F. Castro. 2011. Metabolizable energy of corn hybrids for broiler chickens at different ages. Cienc. Agrotec. 35:1218-1226.

Kereliuk, G. R., F. W. Sosulski, and M. S. Kaldy. 1995. Carbohydrates of North American corn (Zea mays). Food Res. Int. 28:311-315.

Knuckles, B. E. 1988. Effect of phytate and other myo-inositol phosphate esters on lipase activity. Food Sci. 53:250-252.

Koseki, T., N. Kitabatake, and E. Doi. 1989. Irreversible thermal denaturation and formation of linear aggregates of ovalbumin. Food Hydrocolloid 3:123-134.

Lasseran, J.C. 1973. Incidence of drying and storing conditions of corn (maize) on its quality for starch industry. Starch 25:257-262.

Leeson, S., A. Yersin, and L. Volker. 1993. Nutritive value of the 1992 corn crop. J. Appl. Poult. Res. 2:208-213.

Li, T. -J., Q. -Z. Dai, Y. -L. Lin, J. Zhang, R. -L. Huang, Z. Ruan, Z. Deng, and M. Xie. 2008. Dietary starch sources affect net portal appearance of amino acids and glucose in growing pigs. Animal 2:723-729.

Li, Y. C., D. R. Ledoux, T. L. Veum, V. Raboy, and D. S. Ertl. 2000. Effects of low phytic acid corn on phosphorus utilization, performance, and bone mineralization in broiler chicks. Poult. Sci. 79:1444-1450.

Liu, N. and A. J. Cowieson. 2011. Effect of phytic acid and pH value on the activation of chicken pepsinogen in vitro. Pages 84-87 in 22nd Annual Aust. Poult. Sci. Symp. Sydney, NSW, Australia. February 14-16.

Liu, N., Y. J. Ru, F. D. Li, and A. J. Cowieson. 2008. Effect of diet containing phytate and phytase on the activity and messenger ribonucleic acid expression of carbohydrase and transporter in chickens. J. Anim. Sci. 86:3432-3439.

Liu, N., Y. Ru, J. Wang, and T. Xu. 2010. Effect of dietary sodium phytate and microbial phytase on the lipase activity and lipid metabolism of broiler chickens. Br. J. Nutr. 103:862-868.

Lü, M., D. Li, L. Gong, Y. Ru, and V. Ravindran. 2009. Effects of supplemental microbial phytase and xylanase on the performance of broilers fed diets based on corn and wheat. J. Poult. Sci. 46:217-223.

Luttrell, B. M. The biological relevance of the binding of calcium ions by inositol phosphates. J. Biol. Chem. 268:1521-1524.

Maga, J. A**.** 1982. Phytate: its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. J. Agric. Food Chem. 30:1-9.

Malumba, P., S. Janas, T. Masimango, M. Sindic, C. Deroanne, and F. Béra. 2009. Influence of drying temperature on the wet-milling performance and the proteins solubility indexes of corn kernels. J. Food Eng. 95:393-399.

Martin, C. J. and W. J. Evans. 1989. Phytic acid-enhanced metal ion exchange reactions: the effect on carboxypeptidase A. J. Inorg. Biochem. 35:267-288.

Mateos, G. G., R. Lazaro, and M. I. Gracia. 2002. The feasibility of using nutritional modifications to replace drugs in poultry feeds. J. Appl. Poult. Res. 11:437-452.

McCance, R. A. and E. M. Widdowson. 1942. Mineral metabolism of dephytinized bread. J. Physiol. 101:304-313.

McCarter, J. D. and S. G. Withers. 1994. Mechanisms of enzymatic glycoside hydrolysis. Curr. Opin. Struct. Biol. 4:885-892.

Meuser, F. and Suckow, P. Non-starch polysaccharides in Physics and Chemistry of Baking, J.M.V. Blanch, P.J. Frazier and T. Galliard, eds, The Royal Society of Chemistry, London (1986) pp 42–61.

Mercurio, K. C. and P. A. Behm. 1981. Effects of fiber type and level on mineral excretion, transit time, and intestinal histology. J. Food Sci. 46:1462-1477.

Métayer, J. P., A. –M. Debicki-Garnier, and F. Skiba. 2009. Le Promatest: un bon indicateur de la qualite du sechage et de la valeur alimentaire du mäis grain chez les volailles. Proc. Huitièmes Journées de la Recherche Avicole, St Malo, Normandy, France.

Mitchell, H. H., T. S. Hamilton, and J. R. Beadles. 1952. The relationship between protein content of corn and the nutritional value of the protein. J. Nutr. 48:461-476.

Mitchell, D. B., K. Vogel, B. J. Weimann, L. Pasamontes, and A. P. G. M. van Loon. 1997. The phytase subfamily of histidine acid phosphatases: isolation of genes for two novel phytases from the fungi Aspergillus terreus and Myceliophthora thermophila. Microbiol.143:245-252.

Mitjavila, S., G. de Saint Blanquat, and R. Derache. 1973. Effect de l'acide tannique sur la secretion gastrique chez le rat. Nutr. Metab. 15:163-170.

Moore, S. M., K. J. Stalder, D. C. Beitz, C. H. Stahl, W. A. Fithian, and K. Bregendahl. 2008. The correlation of chemical and physical corn kernel traits with production performance in broiler chickens and laying hens. Poult. Sci. 87:665-676.

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

Moran, E. T., Jr. 1985. Digestion and absorption of carbohydrates in fowl and events through perinatal development. J. Nutr. 115:665-674.

Moreau, R. A., V. Singh, A. Nunez, and K. B. Hicks. 2000. Phytosterols in the aleurone layer of corn kernels. Biochem. Soc. Trans. 28: 803-806.

Moritz, J.S., K. R. Cramer, K. J. Wilson, and R. S. Beyer. 2003. Feed manufacture and feeding of rations with graded levels of added moisture formulated at different energy densities. J. Appl. Poult. Res. 12:371-381.

Mourad, M., M. Hemati, and C. Laguerie. 1996. Kinetic study and modeling of the degradation of the maize kernels quality during drying in fluidized bed. Dry. Technol. 14:2307-2337.

Mullaney, E. J. and A. H. J. Ullah. 2003. The term phytases comprises several different classes of enzymes. Biochem. Biophys. Res. Commun. 312: 179-184.

Nakano, T., T. Joh, K. Narita, and T. Hayakawa. 2000. The pathway of dephosphorylation of myo-inositol hexakisphosphate by phytase from wheat bran of Triticum aestivum L. cv Nourin 61. Biosci. Biotechnol. Biochem. 64:995-1003.

Nauck, M. A., U. Niedereichholz, R. Ettler, J. J. Holst, C. Ørskov, R. Ritzel, and W. H. Schmiegel. 1997. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am. J. Physiol. 273(Endocrinol. Metab. 36):E981-E988.

Nelson, T. S., T. R. Shieh, R. J. Wodzinski, and J. H. Ware. 1971. Effect of supplemental phytase on the utilization of phytate phosphorus by chicks. J. Nutr. 101:1289-1294.

Ngonyamo-Majee, D., R. D. Shaver, J. G. Coors, D. Sapienza, C. E. S. Correa, J. G. Lauer, and P. Berzaghi. 2008. Relationships between kernel vitreousness and dry matter degradability for diverse corn germplasm I. Development of near-infrared reflectance spectroscopy calibrations. Anim. Feed Sci. Tech. 142:247-258.

Nosworthy, N., and R. A. Caldwell. 1988. The interaction of zinc(II) and phytic acid with soya bean glycinin. J. Sci. Food Agric. 44:143-150.

Oh, B. –C., W. –C. Choi, S. Park, Y. –o. Kim, and T. –K. Oh. 2004. Biochemical properties and substrate specificities of alkaline and histidine acid phytases. Appl. Microbiol. Biotechnol. 63:362-372.

O'Dell, B. L., A. R. De Boland, and S. R. Koirtyohann. 1972. Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. J. Agric. Food Chem. 20:718-721.

Odjo, S., P. Malumba, J. Dossou, S. Janas, and F. Béra. 2012. Influence of drying and hydrothermal treatment of corn on the denaturation of salt-soluble proteins and color parameters. J. Food Eng. 109:561-570.

Okubo, K., A. B. Waldrop, G. A. Iacobucci, and D. V. Myers. 1975. Preparation of low-phytate soyabean protein isolate and concentrate by ultrafiltration. Cereal Chem. 52:263-271.

Olukosi, O. A. and O. Adeola. 2008. Whole body nutrient accretion, growth performance and total tract nutrient retention responses of broilers to supplementation of xylanase and phytase individually or in combination in wheat-soybean meal based diets. J. Poult. Sci. 45:192-198.

O'Neill, H. V. M., N. Liu, J. P. Wang, A. Diallo, and S. Hill. 2012. Effect of xylanase on performance and apparent metabolisable energy in starter broilers fed diets containing one maize variety harvested in different regions of china. Asian Austral. J. Anim. 25:515-523.

O'Neill, H. V. M., G. Mathis, B. S. Lumpkins, and M. R. Bedford. 2012. The effect of reduced calorie diets, with and without fat, and the use of xylanase on performance characteristics of broilers between 0 and 42 days. Poult. Sci. 91:1356-1360.

Onyango, E. M., E. K. Asem, and O. Adeola. 2009. Phytic acid increases mucin and endogenous losses from the gastrointestinal tract of chickens. Br. J. Nutr. 101:836-842.

Oryschak, M. A., R. H. Simmins, R. T. Zjilstra. 2002. Effect of dietary particle size and carbohydrase and/or phytase supplementation on nitrogen and phosphorus excretion of grower pigs. Can. J. Anim. Sci. 82:533-540.

Paiva, E., A. L. Kriz, M. J. Peixoto, J. C. Wallace, and B. A. Larkins. 1991. Quantitation and distribution of γ-zein in the endosperm of maize kernels. Cereal Chem. 68:276-279.

Paullaf, J. and G. Rimbach. 1997. Nutritional significance of phytic acid and phytase. Arch. Anim. Nutr. 50:301–319.

Pawlik, J. R., A. I. Fengler, and R. R. Marquardt. Improvement of the nutritional value of rye by the partial hydrolysis of the viscous water-soluble pentosans following water-soaking or fungal enzyme treatment. Br. Poult. Sci. 31: 525-538.

Peng, Y. L., Y. M. Guo, and J. M. Yuan. 2003. Effects of microbial phytase replacing partial inorganic phosphorus supplementation and xylanase on the growth performance and nutrient digestibility in broilers fed wheat-based diets. Asian-Aust. J. Anim. Sci. 16:239-247.

Peplinski, A. J., J. W. Paulis, J. A. Bietz, and R. C. Pratt. 1994. Drying of high-moisture corn: Changes in properties and physical quality. Cereal Chem. 71:129-133.

Perlin, A. S. 1951. Structure of the soluble pentosans of wheat flours. Cereal Chem. 28:382–393.

Persson, H., M. Türk, M. Nyman, and A. –S. Sandberg. 1998. Binding of Cu^{2+}, Zn^{2+}, and Cd^{2+} to inositol tri-, tetra-, penta-, and hexaphosphates. J. Agric. Food Chem. 46:3194-3200.

Philippeau, C., F. Le Deschault De Monredon, and B. Michalet-Doreau. 1999. Relationship between ruminal starch degradation and the physical characteristics of corn grain. J. Anim. Sci. 77:238-243.

Piddington, C. S., C. S. Houston, M. Paloheimo, M. Cantrell, A. Miettinen-Oinonen, H. Nevalainen, and J. Rambosek. 1993. The cloning and sequencing of the genes encoding phytase (phy) and pH 2.5-optimum acid phosphatase (aph) from Aspergillus niger var. awamori. Gene. 133:55-62.

Pontoppidan, K., D. Pettersson, and A. –S. Sandberg. 2007. The type of thermal feed treatment influences the inositol phosphate composition. Anim. Feed Sci. Tech. 132:137-147.

Pourreza, J. and H. L. Classen. 2001. Effects of supplemental phytase and xylanase on phytate phosphorus degradation, ileal protein and energy digestibility of a corn-soybean-wheat bran diets in broiler chicks. J. Agric. Sci. Technol. 3:19-25.

Puhl, A. A., R. Greiner, and L. B. Selinger. 2009. Stereospecificity of myo-inositol hexakisphosphate hydrolysis by a protein tyrosine phosphatase-like inositol polyphosphatase from Megasphaera elsdenii. Appl. Microbiol. Biot. 82:95-103.

Quan, C. S., S. D. Fan, and Y. Ohta. 2003. Immobilization of Candida krusei cells producing phytase in alginate gel beads: an application of the preparation of myo inositol phosphates. Appl. Microbiol. Biotechnol. 62:41-47.

Rajendran, S. and V. Prakash. 1993. Kinetics and thermodynamics of the mechanism of interaction of sodium phytate with α-globulin. Biochem. 32:3474-3478.

Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolizable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci. 41:193-200.

Reddy, N. R. 2002. Occurrence, distribution, content, and dietary intake of phytate. Page 29 in Food Phytates. N. R. Reddy and S. K. Sathe, eds. CRC Press, Boca Raton, FL.

Rerat, A.A. 1985. Intestinal absorption of end products of digestion of carbohydrates and proteins in the pig. Arch. Anim. Nutr. 35:561-580.

Rivera, P. H., E. R. Peo, Jr., D. Flowerday, T. D. Crenshaw, B. D. Moser, and P. J. Cunningham. 1978a. Effect of maturity and drying temperature on nutritional quality and amino acid availability of normal and *opaque-2* corn for rats and swine. J. Anim. Sci. 46:1024-1036.

Rivera, P. H., E. R. Peo, Jr., B. D. Moser, T. Crenshaw, and P. J. Cunningham. 1978b. Effect of drying temperature on nutritional quality and availability of amino acids in normal and *opaque-2* corn for rats. J. Anim. Sci. 46:1275-1286.

Rooney, L. W., and R. L. Pflugfelder. 1986. Factors affecting starch digestibility with special emphasis on sorghum and corn. J. Anim. Sci. 63:1607-1623.

Rutherford, S. M., T. K. Chung, P. C. H. Morel, and P. J. Moughan. 2004. Effect of microbial phytase on ileal digestibility of phytate phosphorus, total phosphorus, and amino acids in a low-phosphorus diet for broilers. Poult. Sci. 83:61-68.

Selle, P. H., V. Ravindran, R. A. Caldwell, and W. L. Bryden. 2000. Phytate and phytase: consequences for protein utilization. Nutr. Res. Rev. 13:255-278.

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

Selle, P.H., V. Ravindran, G. Ravindran, P. H. Pittolo, and W. L. Bryden. 2003. Influence of phytase and xylanase supplementation on growth performance and nutrient utilization of broilers offered wheat-based diets. Asian-Aust. J. Anim. Sci. 16:394-402.

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

Skoch, E. R., K. C. Behnke, C. W. Deyoe, and S. F. Binder. 1981. The effect of steam conditioning rate on the pelleting process. Anim. Feed Sci. Tech. 6:83-90.

Silva, S. S. P., and R. R. Smithard.. 2002. Effect of enzyme supplementation of a rye-based diet on xylanase activity in the small intestine of broilers, on intestinal crypt proliferation and nutrient digestibility and growth performance of the birds. Br. Poult. Sci. 43:274–282.

Singh, M. and A. D. Krikorian. 1982. Inhibition of trypsin activity in vitro by phytate. J. Agric. Food Chem. 30:799-800.

Smith, A. K., and J. J. Rackis. 1957. Phytin elimination in soybean protein isolation. J. Am. Chem. Soc. 79:633-637.

Summers, J. D. 2001. Maize: Factors affecting its digestibility and variability in its feeding value. Pages 109-124 in Enzymes in Farm Animal Nutrition. M. R. Bedford and G. G. Partridge, ed. CABI, New York, NY

Suzuki, T., M. Nakaya, Z. Itoh, K. Tatemoto, and V. Mutt. 1983. Inhibition of interdigestive contractile activity in the stomach by peptide YY in Heidenhain pouch dogs. Gastroenterol. 85:114-121.

Tanaka, K., T. Yoshida, and Z. Kasai. 1974. Radioautographic demonstration of the accumulation of phytic acid in rice and wheat grains. Plant Cell Physiol. 15:147-151.

Tatemoto, K., M. Carlquist, and V. Mutt. 1982. Neuropeptide Y–a novel brain peptide with structural similarities to peptide YY, and pancreatic polypeptide. Nature 296:659-660.

Tester, R. F. and S. J. J. Debon. 1999. Annealing of starch – a review. Int. J. Biol. Macromol. 27:1-12

Tovar, J., A. Francisco, I. Bjork, and N. Asp. 1991. Relationship between microstructure and in vitro digestibility of starch in pre-cooked leguminous seed flours. Food Struct. 10:19-26.

USDA (United States Department of Agriculture). 2004. Grain Inspection Handbook Book II: Grain Grading Procedures. Federal Grain Inspection Service, USDA,Washington, DC.

Vahjen, W., K. Glaser, K. Schafer, and O. Simon. 1998. Influence of xylanase supplemented feed on the development of selected bacterial groups in the intestinal tract of broiler chicks. J. Agric. Sci. 130:489-500.

Vaintraub, I. A. and V. P. Bulmaga. Effect of phytate on the in vitro activity of digestive proteinases. J. Agric. Food Chem. 39:859-861.

Van der Meulen, J., J. G. M. Bakker, B. Smits, and H. De Visser. 1997. Effect of source of starch on net portal flux of glucose, lactate, volatile fatty acids and amino acids in the pig. Br. J. Nutr. 78:533-544.

Walker, G. J. and P. M. Hope. 1963. The action of some alpha-amylases on starch granules. Biochem. J. 86:452-462.

Wall, J. S., C. James, and G. L. Donaldson. 1975. Corn proteins: Chemical and physical changes during drying of grain. Cereal Chem. 52:779-790.

Wang, Z. -H., S. -X. Li, and S. Malhi. 2008. Effects of fertilization and other agronomic measures on nutritional quality of crops. J. Sci. Food Agric. 88:7-23.

Watson, B. C., J. O. Mathews, L. L. Southern, and J. L. Shelton. 2006. The effects of phytase on growth performance and intestinal transit time of broilers fed nutritionally adequate diets and diets deficient in calcium and phosphorus. Poult. Sci. 85:493-497.

Weurding, R. E., A. Veldman, W. A. G. Veen, P. J. Van der Aar, and M. W. A. Verstegen. 2001. Starch digestion rate in the small intestine of broiler chickens differs among feedstuffs. J. Nutr. 131:2329-2335.

Weurding, R. E., H. Enting, and M. A. Verstegen. 2003. The relation between starch digestion rate and amino acid level for broiler chickens. Poult. Sci. 82:279-284.

Whistler, R.L. and W. L. Thornburg. 1957. Development of starch granules in corn endosperm. J. Agric. Food Chem. 5:203-207.

Wilson, C.M. 1987. Proteins of the kernel. Pages 273-277 in Corn Chemistry and Technology. S. A. Watson and P. E. Ramstead, eds. Am. Assoc. Cereal Chem. St. Paul, MN.

Wiseman, J., N. T. Nicol, and G. Norton. 2000. Relationship between apparent metabolisable (ame) values and *in vivo*/*in vitro* starch digestibility of wheat for broilers. World Poultry Sci. J. 56:1-14.

Wolf, M. J., U. Khoo, and H. L. Seckinger. 1967. Subcellular structure of endosperm protein in high-lysine and normal corn. Science 157:556-557.

Woyengo, T. A., O. Adeola, C. C. Udenigwe, and C. M. Nyachoti. 2010. Gastro-intestinal digesta pH, pepsin activity and soluble mineral concentration responses to supplemental phytic acid and phytase in piglets. Livest. Sci. 134:91-93.

Woyengo, T. A., W. Guenter, J. S. Sands, C. M. Nyachoti, and M. A. Mirza. 2008. Nutrient utilisation and performance responses of broilers fed a wheat-based diet supplemented with phytase and xylanase alone or in combination. Anim. Feed Sci. Tech. 146:113-123.

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

Wu, Y. B., V. Ravindran, D. G. Thomas, M. J. Birtles, and W. H. Hendriks. 2004b. Influence of method of whole wheat inclusion and xylanase supplementation on the performance, apparent metabolisable energy, digestive tract measurements and gut morphology of broilers. Br. Poult. Sci. 45:385-394.

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

Würsch, P**.** 1986. Cell structure and starch nature as key determinants of the digestion rate of starch in legume. Am. J. Clin. Nutr. 43:25-29.

Yoon, J. H., L. U. Thompson, and D. J. A. Jenkins. 1983. The effect of phytic acid on in vitro rate of starch digestibility and blood glucose response. Am. J. Clin.Nutr. 38:835-852.

III. EFFECTS OF CORN SOURCE ON THE RELATIONSHIP BETWEEN IN VITRO ASSAYS AND ILEAL NUTRIENT DIGESTIBILITY

ABSTRACT

An experiment was conducted to determine the relationship between in vitro assays to estimate quality and ileal nutrient and energy digestibility of various corn sources. Twelve samples of corn were analyzed for gross energy (GE), N, moisture, crude fat (CF), salt-soluble protein content (PS) and vitreousness. Six of the 12 sources of corn had similar proximate composition but ranged in pairs as having low, moderate, or high quality based on protein solubility that varied from 25.7 to 49.2%. Experimental diets consisted of corn sources with 0.50% TiO_2. Five hundred and four (12 per pen; 0.039 m^2 per bird) Ross × Ross 708 male broiler chicks were randomly distributed to 42 pens (7 replicates per treatment) at 1 d of age. Broilers were fed common starter and grower diets from 1 to 27 d of age and experimental diets from 28 to 30 d of age. At 30 d of age, 8 birds per cage were sacrificed for digesta collection from 4 to 30 cm proximal to the ileocecal junction. Feed and digesta were analyzed for TiO_2, GE, N, CF, and starch content. Ileal digestibility of N (apparent), CF, and starch did not differ ($P > 0.05$) among sources of corn. Apparent ileal digestible energy (IDE) of the 6 corns averaged 3,323 kcal/kg. Salt-soluble protein concentration was correlated with IDE among the corns (r = 0.5; $P \leq 0.001$). Ileal N and fat digestibility were correlated with IDE (r = 0.4 and 0.3, respectively; $P \leq 0.05$). Apparent ME_n ranged from 3,262 to 3,342 kcal/kg and

was correlated with PS (r = 0.8; $P \leq 0.001$) and IDE (r = 0.36; $P \leq 0.05$). These results indicated that sources of corn with similar proximate composition may vary in their digestible energy content, and in such a situation PS may be used to differentiate those with wide-ranging IDE or AME_n. However, further research is required to investigate the relationship between PS and growth performance of broilers.

INTRODUCTION

Variation in nutrient content of corn has the potential to greatly affect the profitability of broiler production. For example, variation in AME_n may translate to economically important changes in feed conversion (Dozier et al., 2011). To prevent unforeseen reductions in growth performance, grading and analytical methods are used to minimize nutrient variability between calculated and analyzed values. In the present grading system, corn is graded based on bushel weight, damaged kernels, and foreign material (USDA, 2004) even though other factors may affect its feeding value (Summers, 2001).

Differences between corn samples can yield variability in AME_n of more than 400 kcal/kg (Cowieson, 2005). Grading methods used to evaluate corn such as bushel weight may be poor estimators of feeding value (Leeson et al., 1993; Dale, 1994), potentially ignoring inherent variation in nutrient content and digestibility. Furthermore, the USDA corn grading system is based primarily on physical characteristics and it may be useful to evaluate feed ingredients based on chemical attributes which may vary independently of proximate composition or appearance.

Nutrient digestibility of corn is affected by agronomic conditions, genetics, post-harvest processing, storage conditions, and antinutritional factors (Cowieson, 2005). However, corn is priced with disregard to variations in chemical quality due to the vast scale of analysis that would be required commercially (Moore et al., 2008) and the acceptance that nutrient value of feed ingredients may be constant based on broad-based quality designations (de Coca-Sinova et al., 2008). Assays have been developed to improve identification of corn quality, such as salt-soluble protein content (**PS**) and

vitreousness (Dombrink-Kurtzman and Bietz, 1993). These assays may identify differences in feeding quality unaccounted for under the current grading system. Salt-soluble protein and vitreousness assays may provide a means to assess differences in protein and starch accessibility related to the starch-protein interface, which should affect subsequent digestibility.

To our knowledge, there is a lack of peer-reviewed information regarding the combination of these techniques to predict nutrient digestibility of diverse samples of corn in broilers. The objective of this research was to evaluate the relationship between various analytical assays to predict quality of United States corn samples and ileal nutrient digestibility in broilers. Twelve sources of corn were analyzed for proximate composition and quality was evaluated based on PS and vitreousness. Six of the 12 sources of corn that had similar proximate composition but varied in predicted quality were fed to 28 d old broilers and ileal digestibility of N (apparent), starch, crude fat, and energy (apparent) were determined.

MATERIALS AND METHODS

Compositional and Quality Analysis

Twelve samples of corn originating from the midwest and southeast United States were analyzed for DM (method 930.15; AOAC, 2006), gross energy (**GE**), N, and crude fat (**CF**) (Table 1). Gross energy was determined using an isoperibol bomb calorimeter (model number 6300, Parr Instruments, Moline, IL) as described by the manufacturer's manual (Parr Instruments, 1948). Nitrogen content was determined via the Dumas method (method 990.03; AOAC, 2006) using a N analyzer (Rapid N Cube, Elementar Analysensyteme GmbH, Hanau, Germany) and crude protein was calculated by

multiplying percent N by a correction factor (6.25). Crude fat was determined by submerging samples in boiling hexane (method 2003.06; AOAC, 2006) in a fat extractor (Soxtec model number 2043, Foss North America, Inc., Eden Prarie, MN). In addition to proximate analyses, starch content was analyzed by the amyloglucosidase/α-amylase method (method 996.11; AOAC, 1999) as described by the test kit manufacturer (Megazyme International Ireland Ltd., Bray, Ireland), as well as crude fiber (method 962.09; AOAC, 2000) content, vitreousness and PS. Salt-soluble protein content was determined according to the official method (method NF-V03-741; AFNOR, 2008) as described by Janas et al. (2010). Vitreousness was determined by scanning corn samples with a near-infrared reflectance spectrophotometer (FOSS NIRSystems model 6500, Silver Springs, MD, USA) with a range of 400–2498 nm. Calibration equations used were developed by Ngonyamo-Majee et al. (2008) and had R^2 of >0.9 for prediction of vitreousness against values determined manually (Correa et al., 2002) and residual predictive value of 3.73. A residual predictive value between 3.1 and 4.9 is considered to be robust in predicting chemical composition of samples outside of the reference data set for screening purposes (Williams, 2001). Values obtained were used to select 6 sources of corn for determination of nutrient digestibility. Additionally, the mean particle size of the 6 corns was evaluated using a previously established method (method S319.2; ASAE, 1993).

Experimental Treatments

Corn sources with similar proximate composition but that differed based on values obtained from various analytical procedures were selected to provide the widest range of potential feeding quality. The 6 sources of corn evaluated for apparent ileal

energy and nutrient digestibility were selected primarily based on differences in PS because vitreousness was similar among all corn sources (Table 3). Corn sources that were selected varied widely in PS to provide 2 each of 3 quality designations (low, moderate, and high). Mean values of PS and vitreousness of the selected corns ranged from 25.7 to 49.2% and 57.8 to 59.8%, respectively.

Broiler Husbandry

All procedures relating to the use of live birds were approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2010-1717). Five hundred and four (12 per cage; 0.039 m^2 per bird) Ross × Ross 708 male broiler chicks (Aviagen North America, Huntsville, AL) were obtained from a commercial hatchery and randomly distributed to 42 battery cages (7 replicates per treatment) in a solid-sided facility at 1 d of age. Broilers were vaccinated for Marek's Disease, Infectious Bronchitis, and Newcastle disease at the hatchery. From 1 to 27 d of age all birds were provided common corn-soybean meal-based diets formulated with a single source of corn (0 to 17 d of age = AME_n, 3,075 kcal/kg; digestible Lys, 1.22%; digestible TSAA, 0.92%; digestible Thr, 0.83%; Ca, 0.90%; and non-phytate P, 0.45%; 18 to 27 d of age = AME_n, 3,140 kcal/kg; digestible Lys, 1.13%; digestible TSAA, 0.86%; digestible Thr, 0.74%, Ca, 0.80%; and non-phytate P, 0.40%) and reared with identical management practices until 27 d of age. The ambient temperature set point was 33°C at placement and was decreased as the birds increased in age, with a final set point of 24°C at 30 d of age. Photoperiod was 23 h of light and 1 h of darkness from 1 to 23 d of age, and 12 h L:D cycle from 24 to 30 d of age.

Nutrient Digestibility Assay

Each of the 6 sources of corn was ground through a hammer mill fitted with a 3 mm screen. Broilers were fed the experimental diets (corn mixed with 0.5% TiO_2 added as an inert marker) for 72 h (28 to 30 d of age), according to Leslie et al. (2007). Corn was fed as-is without supplemental vitamins or minerals. The duration of the experiment was assumed to permit interpretation of digestibility data free from confounding by potential nutritional deficiencies (Sullivan et al., 1974), as described by Leslie et al. (2007). Birds weighed >1.3 kg at the start of the experimental period and consumed approximately 100 g corn/bird/day. At 30 d of age, 8 birds per experimental unit were euthanized by CO_2 inhalation. The digesta contents of the terminal ileum [(a section spanning 4 to 30 cm upstream from the ileocecal junction (Rochelle et al., 2012)] were gently flushed with deionized water into sample cups and stored at -20°C until further analysis. From 29 to 30 d of age, feed intake was determined and all excreta were collected for determination of AME_n (Sibbald, 1979). Feed, digesta, and excreta were lyophilized in a Virtis Genesis Pilot Lyophilizer (SP Industries, Warminster, PA) and ground using a cyclone mill (Cyclotec model number 1093, Foss North America, Inc., Eden Prarie, MN; feed and excreta) or an electric coffee grinder (digesta). At 30 d of age, broilers yield approximately 1 g of dry digesta from the terminal ileum, and a coffee grinder was used to provide finely ground sample without significant loss.

Feed and digesta from each experiment were analyzed for TiO_2 by a method based on that of Leone et al. (1973). Briefly, 0.25 g of sample were added to glass test tubes and ashed at 580°C for 10 h; ashed samples diluted with 5 mL of H_2SO_4 and containing 0.8 g $NaSO_4$ were heated at 130°C for 72 h; contents of each tube were diluted to 50 mL with distilled deionized water and held for 12 h at 25°C; 3 mL of feed samples or 1 mL of

digesta samples (with 2 mL of 1.8 M H_2SO_4) were added to glass test tubes with 150 µL of H_2O_2; and after color development (30 min) absorbance was measured on a spectrophotometer at 410 nm. Feed was also analyzed for GE, N, CF, and starch content, and excreta were analyzed for N and GE content. Titanium dioxide content in feed samples was analyzed in quadruplicate, otherwise duplicate analyses were performed on digesta samples. In rate of passage research, TiO_2 may be retained in the ceca for 24 to 48 h and 100% is not recovered in less than 24 h (Dänicke et al., 1997). However, digesta was collected proximal to the ceca and originated from a feedstuff low in soluble non-starch polysaccharides, compared with diets based on rye as evaluated by Dänicke et al. (1997). The efficacy of the method used in the current study has been demonstrated previously. Rochelle et al. (2012) evaluated rate of passage of corn-soybean meal-based diets and recovered 100% of TiO_2 from excreta over a 12 h serial collection period using this method. Ileal N, starch, CF, and energy digestibility were calculated as (((diet nutrient or energy content/diet TiO_2 content)-(digesta nutrient or energy content/diet TiO_2 content))/(diet nutrient or energy content/diet TiO_2 content)) × 100.

Statistical Analyses

Data were analyzed as a one-way treatment structure in a randomized complete block design. Cage location was the blocking factor. Each treatment was represented by 7 replicate cages. Analysis of variance was performed using PROC MIXED (SAS, 2009) by the following mixed-effects model:

$$Y_{ij} = \mu.. + \rho_i + \tau_j + \varepsilon_{ij}$$

where $\mu..$ 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 τ_j are fixed factor level effects corresponding to the j^{th} corn source (experiment 1 or 2, respectively) such that $\Sigma\tau_j = 0$; and the random error ε_{ij} are identically and independently normally distributed with mean 0 and variance σ^2. Linear relationships between quality variables and energy or nutrient digestibility were evaluated using Pearson's product-moment correlation via PROC CORR (SAS, 2009; Dowdy et al., 2004). Statistical significance was established at $P \leq 0.05$.

RESULTS AND DISCUSSION

The cost of providing dietary energy and protein is continually increasing and represents a large percentage of the cost of broiler production (Donahue and Cunningham, 2009; Angel et al., 2011). Variability in corn quality may affect feed conversion by altering utilization of nutrients and energy by broilers. The objective of the current research was to evaluate corn quality in vitro and in vivo and determine relationships between PS and vitreousness with nutrient and energy digestibility.

Corn Quality Screening Assay

The crude protein and CF content of the 12 corn sources were comparable with values published previously (Kasim and Edwards, 2000; Table 3.1). Corn sources with a similar proximate composition and diverse PS (25.7 to 49.2%) were selected in pairs to create low, moderate, and high quality classifications in order of increasing PS content, respectively. Proximate composition was similar among the selected corn sources with the exception of corn source 2 which had much higher CP (8.7 vs. ≤7.5%) compared with the other corns. Corns selected for the determination of nutrient digestibility had 65.6 to

66.8% starch, 3,863 to 3,924 kcal/kg gross energy, 7.1 to 8.7% CP, 3.0 to 3.3% CF, and 2.3 to 2.5% crude fiber (corrected to an 88% DM basis; Table 3.2). Mean particle size (Dgw) of the 6 corns averaged 976 μm following milling through a 3 mm screen. The effect on nutrient utilization was likely minimum because the difference between the highest and lowest corn particle sizes was only 84 μm (Table 3.3).

Salt-soluble protein content provides an indication of susceptibility of the protein matrix and embedded starch granules to enzymatic attack. While useful in this regard, PS does not identify cause and effect because values may vary due to several factors, such as content of the various protein fractions, drying at high temperature, moisture content and maturity at harvest (Wall et al., 1975; Odjo et al., 2012). Chemically, PS depends on the concentrations of albumins, globulins, glutelins, and zein, as well as sulfhydryl oxidation, denaturation, and protein aggregation caused by the drying process (Wall et al., 1975).

Corns with PS varying from 25.7 to 49.2% were obtained without major differences in vitreousness (Table 3.2). Vitreousness values varied by less than 5% among the 6 corn sources (mean 59.2%) indicating that differences in PS values may not have been related to varied protein composition or the abundance of either endospermic fraction. Instead variation in PS may have occurred due to chemical changes such as denaturation, functional group modification, and disulfide interchange induced by post-harvest drying (Wall et al., 1975). Blasel et al. (2006) determined that access to starch by amylase and amyloglucosidase was not compromised below 60% vitreousness. Since all corn sources in the current experiment fell below this range it is not likely that vitreousness had an appreciable effect on nutrient digestibility.

Ileal Energy and Nutrient Digestibility

In the current study, ileal digestibility of N, CF, and starch did not differ significantly ($P > 0.05$) among sources of corn (Table 3.4). Ileal digestible energy (**IDE**) and AME_n of the high quality corns were 115 and 56 kcal/kg higher, respectively, than the 2 low quality corns ($P \leq 0.02$). Salt-soluble protein content was positively correlated with IDE ($r = 0.48$; $P \leq 0.001$; Table 3.5) and AME_n ($r = 0.81$; $P \leq 0.001$) of the corn sources. Moreover, ileal N digestibility was positively correlated with IDE ($r = 0.38$; $P = 0.013$) which may reflect the relationship between protein solubility and energy utilization. These associations may have occurred because PS is related to the protein-starch interface and has been demonstrated to affect starch and protein separation in the wet-milling process (Malumba et al., 2009).

Starch granules and protein bodies within the endosperm enlarge during maturation becoming tightly associated with an amorphous continuous protein matrix (Duvick, 1961; Christianson et al., 1969). The content of zein, the primary glutelin-associated protein in dent corn hybrids (Boundy et al., 1967), may vary due to genetic strain differences or the extent of maturity at harvest (Bressani and Conde, 1961) and is negatively associated with biological value (Mitchell et al., 1952). Interaction with protein reduces access to starch granules (Rooney and Pflugfelder, 1986), and thus, any impairment of protein solubility that limits proteolytic hydrolysis may impact both protein and starch digestion.

Unexpectedly, differences in energy utilization from the 6 corn sources were obtained without detecting a change in ileal N, starch, or fat digestibility (Table 3.4). Apparent ileal N digestibility varied by 4 percentage points but was not different among the corns ($P = 0.573$). Rivera et al. (1978) reported no difference in true N digestibility (5

percentage point numerical difference) of corns dried at different temperatures and fed to rats, but linear reductions in availability of several amino acids, including Lys, Met, Ile, Val, and Trp, which vary in their GE content. Corn drying may have affected the interrelationship between fat, N, and energy as reflected in significant correlations between these variables. Apparent IDE was correlated with ileal digestibility of N and fat (r = 0.38 and 0.31; $P \leq 0.05$, respectively) and ileal fat digestibility was correlated with ileal N digestibility (r = 0.39; $P = 0.013$). Excessive drying temperature affects oil yield from wet-milled corn, irrespective of kernel damage (MacMasters et al., 1959; Weller et al., 1989). The germ contains mostly albumin, globulin, and glutelin proteins (Peri et al., 1983) and drying may have affected oil accessibility due to interactions with non-salt-soluble proteins, because ileal fat digestibility and salt-soluble protein content were not correlated ($P > 0.05$). The fact that ileal N digestibility and ileal starch digestibility exhibited a negative correlation (r = -0.41; $P \leq 0.001$) further indicates that proteins other than those of the salt-soluble fraction may have been damaged in some corns and influenced correlations with other nutrients. However, it should be noted that coefficients among ileal nutrient digestibility variables were rather low. Additional research should further explore the interrelationships among N, starch, and fat digestibility in dent corn, especially with respect to energy utilization.

Differences in the utilization of energy may have also been influenced by corn intake. Bhuiyan et al. (2010) reported a reduction in feed intake of 21 d old broilers fed diets formulated with corn dried at 100°C compared with 80 or 90°C. In the current study, it is likely that some differences in PS were related to drying temperature because PS is highly negatively correlated with drying temperature (r = -0.99; Malumba et al.,

2009) and because feed intake declined with PS ($P \leq 0.05$). A direct comparison between these studies cannot be made because we collected independent sources of corn that may or may not have varied widely in artificial drying scheme and Bhuiyan et al. (2010) did not measure PS. Regardless, Sibbald (1975) demonstrated that AME_n of wheat and corn oil in roosters increased with increasing intake and thus, corn intake may have influenced AME_n in a similar manner in the current study.

Corn provides the majority of AME_n in corn-soybean meal-based diets, and thus, it is intuitive that starch digestibility would be associated with utilization of energy. However, in the current study starch digestibility was not correlated with AME_n or IDE ($P > 0.05$). Any association between these variables may not always be observed. For example, AME_n of wheat fed to pigs has varied by nearly 500 kcal/kg, with little to no change in starch digestibility (Wiseman, 2006). Differential fermentation of undigested starch by cecal microflora may potentially remove the relationship between starch digestibility and AME_n by altering energy content more in some samples compared with others. Moreover, variables such as the rate of starch digestion may affect nutrient assimilation as well as the observed relationship between starch and energy utilization.

In broilers, the rate of starch digestion varies among feedstuffs (Weurding et al., 2001). Weurding et al. (2001) determined rates of starch digestion were different between hammer milled and roller milled corn (2.6 vs 3.1 k_d) although the extent of digestion was equal (97%). Studies in pigs also indicate that starch digestion may occur at different rates independent of ileal digestibility, affecting the utilization of dietary nutrients (Van der Meulen et al., 1997; Li et al., 2008). Diets containing 65% corn or pea starch were fed to gilts and net portal flux of glucose and amino acids were determined (Van der

Meulen et al., 1997). Although starch digestibility of corn and pea starch in pigs are reported to be similar (Everts et al., 1996), net portal glucose flux was higher for corn starch compared with pea starch, and net portal flux of most amino acids were higher for pea starch, despite a lower ileal amino acid digestibility (Everts et al., 1996). This effect may occur due to differences in insulin and glycemic responses, as well as the amount of fermentable substrate reaching the hindgut (Van der Meulen et al., 1997; Weurding et al., 2001) and subsequent release of enteric hormones such as peptide YY and glucagon-like peptide-1 that affect gastric emptying (Tatemoto et al., 1982; Suzuki et al., 1983; Nauck et al., 1997).

Another possibility is that the level of analysis affected the association between starch and AME_n. In the current study, starch digestibility was measured at the ileal level and AME_n was measured at the fecal level. Microbial fermentation of undigested starch may have increased AME_n of some corn samples more than others, removing the relationship between the two variables. Further research should evaluate the effects of corn quality on the cecal flora, enteric hormones, and the relationship between starch digestibility and AME_n to better understand the effects of nutrient variability on energy utilization.

In conclusion, these data indicated evaluation of different sources of corn by chemical indices rather than the current grading system may be beneficial in reducing nutrient variability between calculated and analyzed values. Because protein solubility is associated with energy utilization, PS may provide a tool to evaluate nutritional quality. Assessment of corn quality by these methods may serve to identify corns that have unusually high or low AME_n, thus providing a means to formulate broiler diets with

increased precision. However, the current data do not provide a means of predicting specific nutrient content or availability. Further research is needed to validate these methods in vivo. Results reported herein indicated that corn sources with similar composition varied in their digestible energy and AME_n content and that PS may be useful for identifying corn sources that differ in availability of energy.

REFERENCES

AFNOR, 2008. Méthode Promatest d'évaluation de la dénaturation des proteins Thermosensibles. NF-V03-741. AFNOR.

American Society of Agricultural Engineers. 1993. S319.2. Method of determining and expressing fineness of feed materials by sieving. American Society of Agricultural Engineers Yearbook of Standard. ASAE, St. Joesph, MI.

AOAC. 1999. Official Methods of Analysis. 16th ed., 5th Revision, Assoc. Off. Anal. Chem., Gaithersburg, MD.

AOAC. 2000. Official Methods of Analysis. 17th ed., Assoc. Off. Anal. Chem., Gaithersburg, MD.

AOAC. 2006. Official Methods of Analysis. 18th ed., Assoc. Off. Anal. Chem., Washington, DC.

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

Bhuiyan, M. M., A. F. Islam, and P. A. Iji. 2010. Response of broiler chickens to diets containing artificially dried high-moisture maize supplemented with microbial enzymes. S. Afr. J. Anim. Sci. 40:348-362.

Blasel, H. M., P. C. Hoffman, and R. D. Shaver. 2006. Degree of starch access: an enzymatic method to determine starch degredation potential of corn grain and corn silage. Anim. Feed Sci. Tech. 128:96-107.

Boundy, J. A., J. H. Woychik, R. J. Dimler, and J. S. Wall. 1967. Protein composition of dent, waxy, and high-amylose corns. Cereal Chem. 44:160-169.

Bressani, R., and R. Conde. 1961. Changes in the chemical composition and in the distribution of nitrogen of maize at different stages of development. Cereal Chem. 38:76-84.

Christianson, D. D., H. C. Nielson, U. Khoo, M. J. Wolf, and J. S. Wall. 1969. Isolation and chemical composition of protein bodies and matrix proteins in corn endosperm. Cereal Chem. 46:372-381.

Correa, C. E. S., R. D. Shaver, M. N. Pereira, J. G. Lauer, and K. Kohn. 2002. Relationship between corn vitreousness and ruminal in-situ starch degradability. J. Dairy Sci. 85:3008- 3012.

Cowieson, A. J. 2005. Factors that affect the nutritional value of maize for broilers. Anim. Feed Sci. Tech. 119:293-305.

Dale, N. 1994. Relationship between bushel weight, metabolizable energy, and protein content of corn from an adverse growing season. J. Appl. Poult. Res. 3:83-86.

Dänicke, S., O. Simon, H. Jeroch, and M. Bedford. 1997. Interactions between dietary fat type and xylanase supplementation when rye-based diets are fed to broiler chickens. 1. Physicochemical chime features. Br. Poult. Sci. 38:537-545.

De Coca-Sinova, A., D. G. Valencia, E. Jiménez-Moreno, R. Lázaro, and G. G. Mateos. 2008. Apparent ileal digestibility of energy, nitrogen, and amino acids of soybean meals of different origin in broilers. Poult. Sci. 87:2613-2623.

Dozier, W. A. III, C. K. Gehring, A. Corzo, and H. A. Olanrewaju. 2011. Apparent metabolizable energy needs of male and female broilers from 36 to 47 days of age. Poult. Sci. 90:804-814.

Duvick, D. N. 1961. Protein granules of maize endosperm cells. Cereal Chem. 38: 374-385. Dombrink-Kurtzman, M. A., and J. A. Bietz. 1993. Zein composition in hard and soft endosperm of maize. Cereal Chem. 70:105–108.

Dowdy, S. M., S. Wearden, and D. M. Chilko. 2004. Statistics for Research. 3rd ed. John Wiley and Sons Inc. New York, New York.

Everts, H., R. A. Dekker, B. Smits, and J. W. Cone. 1996. The digestion of maize and native pea starch in the small intestine of pigs. Proc. Nutr. Soc. 55:59A.

Janas, S., S. Boutry, P. Malumba, L. Vander Elst, and F. Béra. 2010. Modelling dehydration and quality degradation of maize during fluidized-bed drying. J. Food Eng. 100:527-534.

Kasim, A. B. and H. M. Edwards, Jr. 2000. Effect of sources of maize and maize particle sizes on the utilization of phytate phosphorus in broiler chicks. Anim. Feed Sci. Tech. 86:15-26.

Leeson, S., A. Yersin, and L. Volker. 1993. Nutritive value of the 1992 corn crop. J. Appl. Poult. Res. 2:208-213.

Leone, J. L. 1973. Collaborative study of the quantitative determination of titanium dioxide in cheese. J. Assoc. Off. Anal. Chem. 56:535-537.

Leslie, M. A., E. T. Moran, Jr., and M. R. Bedford. 2007. The effect of phytase and glucanase on the ileal energy of corn and soybean meal fed to broilers. Poult. Sci. 86:2350-2357.

Li, T. -J., Q. -Z. Dai, Y. -L. Lin, J. Zhang, R. -L. Huang, Z. Ruan, Z. Deng, and M. Xie. 2008. Dietary starch sources affect net portal appearance of amino acids and glucose in growing pigs. Animal 2:723-729.

MacMasters, M. M., M. D. Finker, M. M. Holzapfel, J. H. Ramser, and G. H. Dungan. 1959. A study of the effect of drying conditions on the suitability for starch production of corn artificially dried after shelling. Cereal Chem. 36:247-260.

Malumba, P., S. Janas, T. Masimango, M. Sindic, C. Deroanne, and F. Béra. 2009. Influence of drying temperature on the wet-milling performance and the proteins solubility indexes of corn kernels. J. Food Eng. 95:393-399.

Mitchell, H. H., T. S. Hamilton, and J. R. Beadles. 1952. The relationship between protein content of corn and the nutritional value of the protein. J. Nutr. 48:461-476.

Moore, S. M., K. J. Stalder, D. C. Beitz, C. H. Stahl, W. A. Fithian, and K. Bregendahl. 2008. The correlation of chemical and physical corn kernel traits with production performance in broiler chickens and laying hens. Poult. Sci. 87:665-676.

Nauck, M. A., U. Niedereichholz, R. Ettler, J. J. Holst, C. Ørskov, R. Ritzel, and W. H. Schmiegel. 1997. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am. J. Physiol. 273(Endocrinol. Metab. 36):E981-E988.

Ngonyamo-Majee, D., R. D. Shaver, J. G. Coors, D. Sapienza, C. E. S. CorreaJ. G. Lauer, and P. Berzaghi. 2008. Relationships between kernel vitreousness and dry matter degradability for diverse corn germplasm. I. Development of near infrared reflectance spectroscopy calibrations. Anim. Feed Sci. Technol. 142:247–258.

Odjo, S., P. Malumba, J. Dossou, S. Janas, and F. Béra. 2012. Influence of drying and hydrothermal treatment of corn on the denaturation of salt-soluble proteins and color parameters. J. Food Eng. 109:561-570.

Parr Instruments. 1948. Oxygen Bomb Calorimetry and Oxygen Bomb Combustion Methods. Manual 120. Parr Instruments, Moline, IL.

Peri, C., R. Barbieri, and E. M. Casiraghi, 1983. Physical, chemical and nutritional quality of extruded corn germ flour and milk protein blends. Int. J. Food Sci. Tech. 18:43-52.

Rivera, P. H., E. R. Peo, Jr., B. D. Moser, T. Crenshaw, and P. J. Cunningham. 1978. Effect of drying temperature on nutritional quality and availability of amino acids in normal and *opaque-2* corn for rats. J. Anim. Sci. 46:1275-1286.

Rochell, S. R., T. J. Applegate, E. J. Kim, and W. A. Dozier, III. 2012. Effects of diet type and ingredient composition on rate of passage and apparent ileal amino acid digestibility in broiler chicks. Poult. Sci. 91:1647-1653.

Rooney, L. W. and R. L. Pflugfelder. 1986. Factors affecting starch digestibility with special emphasis on sorghum and corn. J. Anim. Sci. 63:1607-1623.

SAS Institute. 2009. SAS User's Guide. Statistics. Version 9.2 Edition. SAS Inst. Inc., Cary, NC.

Sibbald, I. R. 1975. The effect of level of feed intake on metabolizable energy values measured with adult roosters. Poult. Sci. 54:1990-1997.

Sibbald, I. R. 1979. A bioassay for available amino acids and true metabolizable energy in feeding stuffs. Poult. Sci. 58:668-673.

Sullivan, J. F., R. E. Burch, and D. F. Magee. 1974. Enzymatic activity and divalent cation content of pancreatic juice. Am. J. Physiol. 226:1420–1423.

Summers, J. D. 2001. Maize: Factors affecting its digestibility and variability in its feeding value. Pages 109-124 in Enzymes in Farm Animal Nutrition. M. R. Bedford and G. G. Partridge, ed. CABI, New York, NY.

Suzuki, T., M. Nakaya, Z. Itoh, K. Tatemoto, and V. Mutt. 1983. Inhibition of interdigestive contractile activity in the stomach by peptide YY in Heidenhain pouch dogs. Gastroenterol. 85:114-121.

Tatemoto, K., M. Carlquist , and V. Mutt. 1982. Neuropeptide Y–a novel brain peptide with structural similarities to peptide YY, and pancreatic polypeptide. Nature 296:659-660.

USDA. 2004. Grain Inspection Handbook-Book II: Grain Grading Procedures. Federal Grain Inspection Serv., USDA,Washington, DC.

Van der Meulen, J., J. G. M. Bakker, B. Smits, and H. De Visser. 1997. Effect of source of starch on net portal flux of glucose, lactate, volatile fatty acids and amino acids in the pig. Br. J. Nutr. 78:533-544.

Wall, J. S., C. James, and G. Donaldson. 1975. Corn proteins: chemical and physical changes during drying of grain. Cereal Chem. 52:779-790.

Weller, C. L., M. R. Paulsen, and S. Mbuvi. 1989. Germ weight, germ oil content, and estimated oil yield for wet-milled yellow dent corn as affected by moisture content at harvest and temperature of drying air. Cereal Chem. 66:273-275.

Weurding, R. E., A. Veldman, W. A. G. Veen, P. J. van der Aar, and M. W. A. Verstegen. 2001. Starch digestion rate in the small intestine of broiler chickens differs among feedstuffs. J. Nutr. 131:2329-2335.

Williams, P.C. 2001. Implementation of near-infrared technology. Pages 145-169 in Near Infrared Technology in the Agricultural and Food Industries. P. C. Williams and K. Norris, ed. American Association of Cereal Chemists, St. Paul, MN.

Wiseman, J. 2006. Variations in starch digestibility in non-ruminants. Anim. Feed Sci. Tech. 130:66-77.

Table 3.1 Proximate composition of dent corn obtained from the United States[1,2,3]

Sample	1	2	3	4	5	6	7	8	9	10	11	12
Proximate Composition (%)												
Moisture	13.6	13.2	14.0	13.1	11.4	13.3	13.0	12.5	13.4	13.4	11.9	13.5
Gross Energy (kcal/kg)	3,863	3,865	3,903	3,914	3,890	3,916	3,897	3,924	3,882	3,891	3,891	3,901
Crude Protein[4]	7.33	8.87	6.88	7.56	8.91	7.53	6.77	7.21	7.81	7.27	8.05	8.22
Crude Fat	3.02	2.74	3.29	3.41	3.03	3.44	3.23	3.33	3.00	3.32	2.96	3.08

[1]Values for gross energy, crude protein, and crude fat are expressed on an 88% DM (as-fed) basis.
[2]Analyses were performed on duplicate samples.
[3]Summary statistics (mean, coefficient of variation): moisture (13.0, 5.7), dry matter (87.0, 0.9), gross energy (3,895, 0.5), crude protein (7.7, 9.1), crude fat (3.15, 6.8).
[4]6.25 × %N.

Table 3.2 In vitro predictors of quality and nutrient composition of corn selected to vary in salt-soluble protein content and to have similar proximate composition[1,2]

Sample[3]	PS[4] (%)	Vitreousness (%)	Starch (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)
8–High	49.2	58.5	65.9	7.36	3.27	2.35
2–High	46.6	59.8	65.7	8.72	3.04	2.46
10–Moderate	39.4	59.2	66.5	7.15	3.27	2.31
7–Moderate	33.4	59.7	65.6	7.19	3.34	2.33
1–Low	26.9	60.0	65.9	7.19	3.27	2.28
3–Low	25.7	57.8	66.8	7.05	3.25	2.27

[1]Analyses were performed on duplicate samples.

[2]Values for starch, crude protein, crude fat, and crude fiber content are expressed on an 88% DM (as-fed) basis.

[3]Sample number corresponds with Table 1 and high, moderate, and low terms are indicative of salt-soluble protein content.

[4]Salt-soluble protein content.

Table 3.3 Particle size distribution of corn selected to vary in salt-soluble protein content and to have similar proximate composition [1]

	Mean Particle Size[3] (µm ± SD)	3,360 µm[4] (%)	2,380 µm (%)	1,680 µm (%)	1,191 µm (%)	841 µm (%)	594 µm (%)	420 µm (%)	297 µm (%)	212 µm (%)	150 µm (%)
8–High	975 ± 1.6	0.5	1.7	5.9	15.5	58.9	4.8	5.1	4.9	1.3	1.5
2–High	954 ± 1.6	0.4	1.0	6.1	18.0	55.4	4.0	5.4	6.8	2.1	0.4
10–Moderate	925 ± 1.7	0.3	1.4	6.7	17.4	51.9	4.8	4.8	8.2	2.6	0.3
7–Moderate	989 ± 1.6	0.7	1.6	6.1	15.8	59.6	4.7	4.7	4.3	1.3	0.7
1–Low	1,009 ± 1.7	1.5	2.5	8.1	18.3	51.5	3.6	5.5	5.9	2.4	1.5
3–Low	1,004 ± 1.6	0.9	1.8	6.4	16.3	59.3	3.9	4.6	4.4	1.6	0.9

[1]Analyses were performed on duplicate samples.
[2]Sample number corresponds with Table 1 and high, moderate, and low terms are indicative of salt-soluble protein content.
[3]SD = Standard deviation.
[4]Diameter of screen openings that retained particles.

Table 3.4 Ileal nutrient digestibility, digestible energy, and AME_n of corn selected to vary in salt-soluble protein content and to have similar proximate composition[1,2]

Sample[3]	Corn Intake[4] (kg/bird)	Nitrogen (%)	Crude fat (%)	Starch (%)	IDE[5] (kcal/kg)	AME_n (kcal/kg)
8–High	0.234	77.2	63.1	91.3	3,408	3,342
2–High	0.228	75.9	69.4	93.6	3,358	3,297
10–Moderate	0.227	75.1	66.2	91.9	3,316	3,310
7–Moderate	0.221	75.0	67.5	90.5	3,322	3,314
1–Low	0.220	74.1	63.6	92.3	3,248	3,262
3–Low	0.217	74.0	64.1	91.5	3,287	3,265
SEM	0.006	1.4	2.5	0.8	34	23
			Probability			
Orthogonal contrasts						
8 and 2 vs. 1 and 3	0.041	0.079	0.361	0.510	0.001	0.018
8 vs. 3	0.043	0.121	0.787	0.896	0.014	0.021

[1]Analyses of corn (mixed with 0.5% TiO_2) were conducted on quadruplicate samples and analyses of digesta and excreta were conducted on duplicate samples on an as-fed basis. Nitrogen and energy digestibility were calculated on an apparent basis.
[2]Values are least-square means of 7 replicate cages of 8 birds per cage at 30 d of age.
[3]Sample number corresponds with Table 1 and high, moderate, and low terms are indicative of salt-soluble protein content.
[4]Determined from 29 to 30 d of age.
[5]Ileal digestible energy.

Table 3.5 Pearson's product-moment correlation (r-values) between in vitro assays and nutrient utilization[1,2]

Item	Item[3]						
	1	2	3	4	5	6	7
1. Salt-soluble protein	1.00						
2. Vitreousness	0.07	1.00					
3. AME_n	0.81**	0.04	1.00				
4. IDE	0.48**	-0.04	0.36*	1.00			
5. IND	0.23	0.03	0.21	0.38*	1.00		
6. IFD	0.03	0.10	0.04	0.31*	0.39*	1.00	
7. ISD	0.10	0.13	-0.11	0.23	-0.41**	-0.08	1.00

[1]Corns were primarily selected based on salt-soluble protein content.

[2]IDE = ileal digestible energy; IND = ileal N digestibility; IFD = ileal fat digestibility; ISD = ileal starch digestiblity.

[3]* = $P \leq 0.05$; ** = $P \leq 0.01$.

IV. EXTRA-PHOSPHORIC EFFECTS OF PHYTASE WITH AND WITHOUT XYLANASE IN CORN-SOYBEAN MEAL-BASED DIETS FED TO BROILERS

ABSTRACT

Two experiments were conducted to evaluate the extra-phosphoric effects of phytase on amino acid (AA) and energy digestibility (experiments 2 and 3) and growth performance (experiment 3) of broilers fed diets adequate in Ca and non-phytate P supplemented with xylanase. Ross × Ross 708 broiler chicks (864 males in experiment 2 and 1,152 females in experiment 3) were randomly distributed into battery cages (6 replicate cages per treatment) with 12 birds per cage at 1 d of age. In both experiments, factorial arrangements of treatments were evaluated consisting of 6 phytase (0, 1,000, 2,000, 4,000, 8,000, and 16,000 FTU/kg) and 2 xylanase (0 and 16,000 BXU/kg) concentrations in experiment 2 and 4 phytase (0, 500, 1,000, and 2,000 FTU/kg) and 4 xylanase (0, 8,000, 16,000, and 32,000 BXU/kg) concentrations in experiment 3. Treatments were provided from 27 to 32 d of age in experiment 2 and from 1 to 32 d of age in experiment 3. Digesta contents of the terminal ileum were collected at 32 d of age (experiments 2 and 3) and birds and feed were weighed at 1, 14, and 25 d of age in experiment 3. In each experiment, phytase and xylanase did not interact in their effects on AA and energy digestibility ($P > 0.05$) and only phytase main effects were observed. Broilers fed diets supplemented with phytase at 1,000 FTU/kg had increased ($P \leq 0.05$) apparent ileal digestibility (AID) of all AA with the exception of Ala and Met in experiment 2 and Ala,

His, and Met in experiment 3. In experiment 2, diets fed to broilers supplemented with higher concentrations of phytase did not further increase AID of any AA ($P > 0.05$) above the addition of 1,000 FTU/kg. Significant linear or quadratic effects of phytase were not ($P > 0.05$) observed. In contrast, broilers fed diets supplemented with phytase in experiment 3 exhibited linear ($P \leq 0.05$) increases in AID of AA but not apparent ileal digestible energy. However, supplementation with 2,000 FTU/kg phytase increased ($P = 0.049$) ileal digestible energy by 36 kcal/kg compared with the basal diet. Broilers fed diets with 1,000 or 2,000 FTU/kg consumed more feed and grew faster (linear $P \leq 0.05$) than their counterparts fed diets supplemented with 0 or 500 FTU/kg of phytase. The addition of 500 FTU/kg of phytase in diets fed to broilers did not affect ileal digestibility or growth performance ($P > 0.05$). Data obtained from these experiments demonstrated extra-phosphoric effects in broilers fed diets supplemented with 1,000 FTU/kg of phytase and diminishing returns with higher concentrations. The lack of xylanase response may have been related to the age of birds or environment (battery cages) used in these experiments.

INTRODUCTION

A common practice in evaluating phytase is to only supplement diets that are deficient in non-phytate P while comparing the observed response to that of a positive control. A large proportion of any observed nutrient digestibility or growth responses may be directly related to the release of non-phytate P. It is well accepted that beyond P adequacy further positive responses to phytase supplementation can be attributed to extra-phosphoric effects (Cabahug et al., 1999; Ravindran et al., 2000). However, when graded concentrations of phytase are supplemented to diets marginally deficient in non-phytate P, the point where non-phytate P becomes adequate and extra-phosphoric effects begin is unclear. Benefits of phytase supplementation likely occur due to release of P and extra-phosphoric effects simultaneously over a wide range of phytase concentrations. Significant extraphosphoric increases in BW gain have been reported at phytase concentrations ranging from as low as 300 to 800 FTU/kg (Cabahug et al., 1999; Keshavars, 2000; Watson et al., 2006).

Although extra-phosphoric effects of phytase occur intuitively, their interpretation from many published experiments is confounded by the release of P. Phytase is not typically added to a positive control diet that has been formulated to be adequate in non-phytate P, but when birds fed phytase-supplemented negative control diets outperform a positive control, extra-phosphoric effects are suggested (Cabahug et al., 1999; Cowieson et al., 2006). Nevertheless, in many studies it is impossible to know the point of P adequacy, leaving quantification of extra-phosphoric effects sparse in the peer-reviewed literature. Moreover, the magnitude of extra-phosphoric effects may depend partially on the molar concentrations of substrate and enzyme present. This is apparent by the

variability of response in peer-reviewed literature (Watson et al., 2006) and because phytate concentration has been demonstrated to greatly affect the occurrence of extra-phosphoric effects (Cabahug et al., 1999). To better understand the effects of phytase addition in broiler diets, it may be beneficial to study extra-phosphoric effects under theoretically optimal conditions, i.e., by maximizing dephosphorylation of phytate with high concentrations of phytase.

Invariably, a portion of the dietary phytate remains encapsulated by plant cell walls. Dephosphorylation may potentially be maximized by the addition of carbohydrases to increase access to encapsulated phytate (Zyla et al., 1995). Increased cellular permeability facilitated by xylanase supplementation may increase access to phytate as well as encapsulated nutrients (Selle et al., 2003), which may alter the magnitude of extraphosphoric increases in nutrient and energy digestibility. Supplementation of diets with phytase and carbohydrase in combination has produced mixed-results ranging from sub-additive to synergistic responses in broilers (Cowieson and Adeola, 2005; Juanpere et al., 2005) as well as swine (Oryschak et al., 2002; Kim et al., 2005). These experiments focused on wheat-based diets and the interactive effects of these enzymes may differ in diets based on corn. Responses in corn-soybean meal-based diets may be different in type or magnitude than in wheat-based diets because corn contains less soluble non-starch polysaccharides and NDF than wheat (Knudsen, 1997).

Two experiments were conducted in order to evaluate extra-phosphoric effects of phytase in combination with xylanase in corn-soybean meal based diets fed to broilers. Experiment 2 evaluated extra-phosphoric effects with high concentrations of phytase and a wide range of xylanase concentrations and experiment 3 utilized a narrow range of

enzyme concentrations to determine optimal concentrations. To the best of our knowledge, extra-phosphoric effects of phytase in broilers have not been evaluated free of confounding by P and with potential xylanase interactions in corn-soybean meal-based diets.

MATERIALS AND METHODS

Bird Husbandry

All procedures relating to the use of live birds were approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2010-1717). In each of 2 experiments, Ross × Ross 708 broiler chicks (Aviagen North America, Huntsville, AL) were obtained from a commercial hatchery and randomly distributed to battery cages (12 per pen; 0.04 m^2 per bird; 864 males in experiment 2 and 1,152 females in experiment 3) in a solid-sided facility at 1 d of age. Broilers were vaccinated for Marek's Disease, Infectious Bronchitis, and Newcastle disease at the hatchery. The ambient temperature set point was 33°C at placement and was decreased as the birds increased in age, with a final set point of 24°C at 30 d of age. Photoperiod was 23L:1D from 1 to 25 d of age, and 12L:12D from 26 to 32 d of age.

Experimental Treatments

Experiment 1 consisted of a factorial arrangement of 12 treatments with 6 phytase (0, 1,000, 2,000, 4,000, 8,000, and 16,000 FTU/kg) and 2 xylanase (0 and 16,000 BXU/kg) concentrations. Based on results of experiment 1, the enzyme inclusions were adjusted to provide 4 phytase (0, 500, 1,000, and 2,000 FTU/kg) and 4 xylanase (0, 8,000, 16,000, and 32,000 BXU/kg) concentrations in a factorial arrangement of treatments in experiment 2. The purpose of the enzyme concentrations chosen was to

identify the optimal inclusions of each enzyme with regard to the extra-phosphoric effect. All experimental diets were analyzed at an external laboratory (Enzyme Services and Consultancy, Caerphilly, Wales) for phytase and xylanase activities. Phytase activity was determined using a method based on the AOAC/Engelen method (method 2000.12; AOAC, 2000; Engelen et al., 1994, 2001). Using phytic acid from rice as a substrate, feed samples were extracted for 30 minutes in 25mM Borate, pH 10.0, and analyses were conducted at pH 4.5 and 60°C. Phytase activity was determined based on the end-point determination of phosphate using a molybdate-vanadate color system. Xylanase activity in feed samples was determined using azurine-crosslinked wheat arabinoxylan (Xylazyme AX) tablets (Megazyme International Ireland Ltd., Bray, Ireland) according to McCleary (1992, 1995). Water-soluble dyed fragments released by the hydrolysis of xylazyme AX substrate by xylanase present in the sample at 50°C were quantitated by absorbance measured at 590 nm. In addition, basal diets were analyzed for phytate content according to the Megazyme method (method K-PHYT, Megazyme International Ireland Ltd., Bray, Ireland). Briefly, samples were extracted in 0.66 M HCl overnight, followed by enzymatic dephosphorylation with phytase to yield myo-inositol monophosphate and 5 molecules of orthophosphate. The inositol ring was completely dephosphorylated by the action of alkaline phosphatase. Orthophosphate groups released from phytate were reacted with ammonium molybdate (5% w/v) to yield 12-molybdophosphoric acid. Finally, 12-molybdophosphoric acid and 1 M H_2SO_4 with ascorbic acid (10% w/v) reacted to form molybdenum blue. The concentration of molybdenum blue formed in the reaction was proportional to the concentration of orthophosphate in the original sample. The orthophosphate concentration was determined

colorimetrically by measuring the increase in absorbance at 655 nm (Fiske and Subarrow, 1925) and phytate content was calculated based on phytate having 28.2% P.

Nutrient Digestibility Trials

In experiment 2, broilers were fed corn-soybean meal-based diets (0 to 17 d of age = AME_n, 3,075 kcal/kg; digestible Lys, 1.22%; digestible TSAA, 0.92%; digestible Thr, 0.83%; Ca, 0.90%; and non-phytate P, 0.45%; 18 to 27 d of age = AME_n, 3,140 kcal/kg; digestible Lys, 1.13%; digestible TSAA, 0.86%; digestible Thr, 0.74%, Ca, 0.80%; and non-phytate P, 0.40%) until 27 d of age. Experimental diets were provided from 28 to 32 d of age (Table 4.1). In experiment 3, broilers were fed experimental diets from d 1 through the remainder of the experiment (Table 4.2). The basis for feeding treatments from 1 d of age was to account the previous exposure of phytase supplementation on physiology of the gastrointestinal tract (Pirgozliev et al., 2009, 2011).

In each experiment, 8 birds per experimental unit were euthanized by CO_2 asphyxiation at 32 d of age. Digesta contents of the terminal ileum (a section spanning 4 to 30 cm upstream from the ileocecal junction) were gently flushed with deionized water into sample cups and stored at -20°C until being analyzed for TiO_2, amino acid, and energy content. Feed and digesta were lyophilized in a Virtis Genesis Pilot Lyophilizer (SP Industries, Warminster, PA) and ground using a cyclone mill (Cyclotec model number 1093, Foss North America, Inc., Eden Prarie, MN; feed) or an electric coffee grinder (digesta). Broilers yield approximately 1 g of dry digesta from the terminal ileum at 32 d of age, and thus, a coffee grinder was used to provide finely ground sample without significant loss.

Feed and digesta from each experiment were analyzed for TiO_2 content by a method based on that of Leone (1973) and used previously by Gehring et al. (2012). Briefly, 0.25 g of sample were added to glass test tubes and ashed at 580°C for 10 h; ashed samples diluted with 5 mL of H_2SO_4 and containing 0.8 g $NaSO_4$ were heated at 130°C for 72 h; contents of each tube were diluted to 50 mL with distilled deionized water and held for 12 h at 25°C; 3 mL of feed samples or 1 mL of digesta samples (with 2 mL of 1.8 M H_2SO_4) were added to glass test tubes with 150 μL of H_2O_2; and after color development (30 min) absorbance was measured on a spectrophotometer at 410 nm.

Feed and digesta were also analyzed for gross energy and amino acid content. Gross energy was determined using an isoperibol bomb calorimeter (model number 6300, Parr Instruments, Moline, IL) as described by the manufacturer's manual (Parr Instruments, 1948). Amino acid content was determined using an AA analyzer on hydrolysates obtained via acid hydrolysis (all AA with the exception of Met, Cys, and Trp), performic acid oxidation followed by acid hydrolysis (Met and Cys), and alkaline hydrolysis (Trp) (method 982.30 E(a,b,c); AOAC International, 2006). Titanium dioxide content in feed samples was analyzed in quadruplicate, otherwise duplicate analyses were performed on digesta samples. Apparent ileal digestibility (**AID**) of AA and energy were calculated using the following equation (content = %):

$$\frac{\left(\frac{\text{Diet AA or Energy Content}}{\text{Diet TiO}_2\text{ Content}}\right)-\left(\frac{\text{Digesta AA or Energy Content}}{\text{Diet TiO}_2\text{ Content}}\right)}{\left(\frac{\text{Diet AA or Energy Content}}{\text{Diet TiO}_2\text{ Content}}\right)}$$

to obtain an AID coefficient or the AID coefficient multiplied by the GE to obtain ileal digestible energy (**IDE**). Values were not standardized because it is well understood that phytase addition may alter endogenous losses of amino acids and minerals (Cowieson et

al., 2004, 2009; Cowieson and Ravindran, 2007). Therefore, endogenous losses are expected to vary between birds fed each concentration of phytase, making standardization with values from birds fed diets without phytase supplementation inappropriate.

Statistical Analyses

Data were analyzed as a factorial treatment structure in a randomized complete block design for both experiments. Cage location was the blocking factor. Each treatment was represented by 6 replicate cages. Analysis of variance was performed using PROC MIXED (SAS, 2009) by the following mixed-effects model:

$$Y_{ij} = \mu.. + \rho_i + \tau_j + \beta_k + (\tau\beta)_{jk} + \varepsilon_{ijk}$$

where $\mu..$ 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 τ_j are fixed factor level effects corresponding to the j^{th} phytase concentration such that $\Sigma\tau_j = 0$; the β_k are fixed factor level effects corresponding to the k^{th} xylanase concentration such that $\Sigma\beta_k = 0$; the $(\tau\beta)_{jk}$ are the interaction effects corresponding to the j^{th} phytase and k^{th} xylanase concentrations such that $\Sigma(\tau\beta)_{jk} = 0$; and the random error ε_{ijk} are identically and independently normally distributed with mean 0 and variance σ^2.

Because phytase concentrations were not evenly spaced (0, 1,000, 2,000, 4,000, 8,000, and 16,000 FTU/kg in experiment 1 and 0, 500, 1,000, and 2,000 FTU in experiment 2), concentrations were transformed [$\log_{10}$(FTU+1)] prior to analysis (Shirley and Edwards, 2003). Due to the log transformation, linear and quadratic regression of the responses was conducted by omitting the 0 point leaving approximately linear gradations of treatments. All other analyses conducted included the entire data set.

RESULTS AND DISCUSSION

There were no significant effects of xylanase supplementation or interaction between xylanase and phytase in either experiment ($P > 0.05$). Therefore, only the main effects of phytase supplementation are discussed. For experiment 2, analyzed activities of phytase and xylanase in experimental diets are reported in Table 4.3. Amino acid digestibility response to phytase supplementation (compared with the basal diet) was greatest for Ser (3.1 and 4.1%), Cys (2.7 and 3.2%), Ile (2.3 and 3.2%), Asp (2.2 and 3.2%), and Tyr (2.2 and 2.9%), with 1,000 and 2,000 FTU/kg supplemental phytase, respectively (Tables 4.4 and 4.5). The AA that tend to be affected the most by phytase supplementation included those that are associated with endogenous enzymes (Ala, Tyr, Ser, Gly) and mucin (Ser, Gly, Cys, and Thr), which are sources of endogenous AA loss affected by dietary phytate (Cowieson and Ravindran, 2007). The magnitude of the AA digestibility response to phytase supplementation in experiment 2 was greater than in experiment 3 which may reflect differences in sex of birds, dietary phytate or a number of other factors. Exogenous enzymes have the potential to increase digestibility of nutrients that remain unabsorbed in the terminal ileum (ileal undigested fraction) after feeding a basal diet. Methionine has limited potential for improvement with exogenous enzyme supplementation (Zanella et al., 1999; Cowieson and Ravindran, 2008) because digestibility at the terminal ileum is typically greater than 90% in a corn-soybean meal-based diet. In contrast, AA with a lower basal digestibility (e.g., Cys) have a larger ileal undigested fraction and potential for improvement. In experiment 2, birds fed diets supplemented with 1,000 FTU/kg had increased AID of all AA ($P \leq 0.05$) with the exception of Ala ($P = 0.085$). There was no further increase of AA digestibility with

higher concentrations of phytase ($P > 0.05$) and thus, linear and quadratic responses were not observed ($P > 0.05$). This likely occurred because the asymptote was reached at 1,000 FTU/kg and the linear portion of the response curves was between 0 and 1,000 FTU/kg.

In experiment 3, calculated phytase concentrations were narrowed to include 500 and did not exceed 2,000 FTU/kg, based on results of experiment 2. Analyzed values of phytase and xylanase activities in experimental diets are reported in Table 4.3. Inclusion of the 500 FTU/kg concentration resulted in detection of significant linear ($P \leq 0.05$) AA digestibility responses for all AA with the exception of Ala ($P = 0.072$), His ($P = 0.12$), and Met ($P = 0.37$). Compared with the basal diet, the greatest increases in AID in broilers fed diets with 1,000 or 2,000 FTU/kg of phytase occurred for Cys (1.2 and 1.6%), Ser (1.0 and 1.3%), Tyr (0.9 and 0.7%), Ile (0.7 and 0.9%), Thr (0.7 and 0.8%), and Asp (0.7 and 1.0%), respectively (Tables 6 and 7). Supplementation of diets fed to female broilers with 500 FTU/kg did not significantly increase AID of any AA ($P > 0.05$) compared with the basal diet.

The magnitude of the AA digestibility response to phytase supplementation differed numerically between experiments 2 and 3. Amino acid digestibility responses were not compared statistically because experiments 2 and 3 evaluated different treatments and male versus female broilers, respectively. However, numerically larger increases in ileal AA digestibility as a result of phytase supplementation in experiment 2 were likely influenced by the pool of substrate available. In experiment 2, the experimental grower diets had 0.99% phytate, compared with 0.84% phytate in the grower diets used in experiment 2 (Table 3). Cabahug et al. (1999) observed a 14% reduction in BW gain and a 1.5% increase in feed conversion ratio from 7 to 25 d of age, when broilers were fed

diets with 1.32 compared with 1.04% phytate. Diets with greater concentrations of phytate have a larger ileal undigested fraction of nutrients. Thus, it is expected that phytase responses will be greater with higher concentrations of phytate. Other factors such as previous exposure to phytase and solubility of phytate and protein may have also affected responses in the current study (Ravindran et al., 1999).

Data from the current studies indicated that increasing AID of AA in diets adequate in Ca and non-phytate P necessitates at least approximately 1,000 FTU/kg. Because conventionally between 500 and 800 FTU/kg is supplemented to poultry diets to restore available non-phytate P, 1,000 FTU/kg in the current study with adequate Ca and non-phytate P is analogous to at least 1,500 FTU/kg used in most phytase research. Adeola and Sands (2003) reviewed the effects of phytase on AA utilization in monogastric nutrition. The authors noted that phytase supplementation did not increase AID of AA for poultry in experiments with reduced Ca and non-phytate P conducted by Sebastian et al. (1997) and Namkung and Leeson (1999), and with adequate Ca and non-phytate P conducted by Zhang et al. (1999), in which 600, 1,150, and 600 FTU/kg were supplemented, respectively. However, it must be noted that there are significant differences between phytases and 1,000 FTU of the phytase used in the current study is equivalent to several thousand FTU of the products used in the quoted studies as judged by differences in their suggested P matrices. Such differences need consideration when extra-phosphoric effects are discussed as there is no correlation between FTU and such effects when phytases from different source organisms are considered.

In experiment 2, the greatest reduction of ileal undigested fraction occurred in birds fed diets with 2,000 FTU/kg (Figure 4.1), and in experiment 3, reduction was maximized

at 1,000 FTU/kg and decreased at 2,000 FTU/kg (Figure 4.3). Cowieson and Bedford (2009) reported the ileal undigested fraction is the most important factor in determining the mean response to enzyme supplementation. The ileal undigested fraction varied from 5% (Met) to 15 and 18% (Cys) in experiments 2 and 3, respectively. In either experiment, percent reduction of ileal undigested fraction did not differ ($P > 0.05$; Figures 4.1 and 4.3) between various classes of amino acids. However, further investigation may be warranted because the reduction of the undigested fraction of acidic, basic, and polar amino acids was numerically higher compared with that of non-charged/non-polar amino acids in each experiment. Percentage reduction of the ileal undigested fraction varied between the 2 experiments, with maximum reduction averaging 32% in experiment 2 and 14% in experiment 3. The fact that the magnitude of the response differed greatly in the 2 experiments indicates the response was specific (due to reduction in endogenous losses) rather than generic (due to reduction in viscosity or encapsulation) as noted with carbohydrase supplementation (Cowieson et al., 2010).

The energy response to phytase supplementation varied between experiments 2 and 3. In experiment 2, phytase supplementation up to 16,000 FTU/kg did not affect IDE. However, in experiment 3, phytase supplementation of diets with 2,000 FTU/kg increased ($P = 0.049$) IDE by 36 kcal/kg (Figure 4.2). There was no difference in IDE in birds fed diets with phytase included at either 500 or 1,000 FTU/kg. Apparent IDE was unaffected by xylanase supplementation of diets fed to broilers in both experiments ($P > 0.05$; Tables 4.4, 4.5, 4.6, and 4.7), likely because birds were grown to relatively young ages (older birds being more responsive in corn based diets) and diets based on corn lack significant amounts of substrate and are not associated with adverse digesta viscosity.

The lack of response in experiment 2 may have been related to higher endogenous energy losses compared with broilers fed phytase-supplemented diets from 1 d of age, as in experiment 3. Pirgozliev et al. (2009) demonstrated a 32% reduction in endogenous energy loss in birds which had previously been fed diets supplemented with phytase, suggesting the benefit of phytase may take time to build and time to wane. Thus the phytase may not have been fed long enough to effect a significant reduction in endogenous losses in experiment 2. This effect of phytase on endogenous energy losses and thus, the response observed for ileal digestible energy or AME_n is substantiated by previous research (Shirley and Edwards, 2003; Cowieson et al., 2006; Leslie et al., 2007). Ileal digestible energy was unaffected ($P > 0.05$) in broilers fed corn or soybean meal supplemented with phytase from 7 to 9, 14 to 16, or 21 to 23 d of age (Leslie et al., 2007). However, when broilers were fed diets containing phytase starting from 1 d of age, AME_n was increased ($P \leq 0.05$) at 16 d of age (Shirley and Edwards, 2003; Cowieson et al., 2006).

Increased AID of AA and energy observed in experiment 3 were reflected in increased BW gain and feed consumption from 1 to 14 and 1 to 25 d of age (Tables 8 and 9**)**. Female broilers fed diets containing 1,000 or 2,000 FTU/kg phytase consumed more feed and grew at a faster rate than those fed the basal diet ($P \leq 0.05$). Body weight gain (1 to 14 d) and feed intake (1 to 14 d and 1 to 25 d) responses were linear ($P \leq 0.05$) and proportional. Therefore feed conversion ratio was unaffected by phytase supplementation. In parallel with AID data, pre-planned orthogonal contrasts between 0 and 500 FTU/kg treatments indicated no effect ($P > 0.05$) of phytase below 1,000 FTU/kg.

Phytase supplementation of poultry diets results in curvilinear responses that reflect diminishing returns (Shirley and Edwards, 2003). The current studies indicated that concentrations between 1,500 and 2,000 FTU/kg were required to maximize AA, energy, and growth responses to phytase in broilers. Commercial phytases preferentially attack the myo-inositol esters (**IP1-6**) with 5 or 6 ortho-phosphate groups (Wyss et al., 1999). Higher concentrations of phytase than are typically used are required in order to significantly deplete the pool of IP3 and -4. As the higher-phosphorylated esters are removed from the ingesta, the vast anti-nutritional effects of phytate are reduced accordingly. Phytate acts as an anti-nutrient via chelation or complex formation with minerals, proteins, and possibly starch (McCance and Widdowson, 1942; Hill and Tyler, 1954; Badenhuizen, 1959), direct inhibition of pancreatic enzymes (Singh and Krikorian, 1982), destabilization of endogenous enzymes by chelating Ca (Deshpande and Damodaran, 1989), hyper-secretion of pepsin, HCl and consequently $NaHCO_3$ and mucin (Cowieson et al., 2004; Cowieson and Ravindran, 2007; Onyango et al., 2009), and indirect inhibition of amino acid and glucose absorption related to reduced Na availability or transporter expression (Dilworth et al., 2005; Liu et al., 2008).

Additionally, the IP5 and IP6 esters exhibit an approximately 2 fold higher chelation capacity compared with IP3 and IP4 (Luttrell, 1993; Persson et al., 1998). Sandberg et al. (1999) reported that IP5 directly inhibited Fe absorption in humans, whereas IP3 and IP4 sub-additively contributed to inhibition of Fe absorption but had no direct affect themselves. A large reduction in cation binding affinity occurs between IP5 and IP4 and once again between IP3 and IP2. For example, Luttrell (1993) determined that binding affinity for Ca^{2+} was considerably lower for IP3 and IP4 compared with IP6 (6.3×10^3

and 6.2 x 10^4 vs. 1.9 x 10^5 l/mol, respectively) and was approximately 50% lower for IP2 vs. IP3 (9.0 x 10^3 vs. 6.3 x 10^3 l/mol, respectively). Thus, abatement of antinutritional effects of phytate may require hydrolysis of 5 phosphate groups.

One mechanism that has been largely overlooked for the extra-phosphoric effect may be restoration of an ideal Ca to non-phytate P ratio. Ca is released disproportionately to P with standard concentrations of phytase (Cowieson et al., 2011). With high doses of phytase (> 2,000 FTU/kg), release of Ca and P may approach an asymptote (Cowieson et al., 2011), which results in a 2:1 ratio of Ca and non-phytate P as demonstrated by broiler performance (Nelson et al., 1971) and plasma Ca and P (Shirley and Edwards, 2003).

In conclusion, broilers fed corn-soybean meal-based diets adequate in Ca and non-phytate P exhibited increased AID of AA (experiments 2 and 3) and energy (experiment 2). In experiment 2, increased BW gain and feed intake of female broilers paralleled increases in ileal digestibility. Xylanase supplementation did not interact with phytase supplementation and did not affect any of the variables measured, possibly due to a lack of substrate, the age of the birds, or housing environment which did not provide an environmental challenge and the presence of an ionophore (experiment 2) which provides some antimicrobial activity. Extra-phosphoric increases in nutrient utilization and growth performance may be achieved with high concentrations of phytase from 1,500 to 2,000 FTU/kg in P-deficient diets fed to broilers with this phytase. The application of this nutritional strategy is dependent on feed ingredient prices and the relative efficacy and price of phytase supplementation.

REFERENCES

Adeola, O., and J. S. Sands. 2003. Does supplemental dietary microbial phytase improve amino acid utilization? A perspective that it does not. J. Anim. Sci. 81(E. Suppl. 2):E78–E85.

AOAC. 2000. Official Methods of Analysis. 17th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

AOAC. 2006. Official Methods of Analysis. 18th ed. Assoc. Off. Anal. Chem., Washington, DC.

Badenhuizen, N. P. 1959. Chemistry and biology of the starch granule. Pages XX in Protoplasmologia. Handbuch der Protoplasmaforschung. Springer-Verlag, Vienna, Austria.

Cabahug, S., V. Ravindran, P. H. Selle, and W. L. Bryden. 1999. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus contents. I. Effects on bird performance and toe ash. Br. Poult. Sci. 40:660-666.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2004. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. Br. Poult. Sci. 45:101-108.

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

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2006. Supplementation of corn-soy-based diets with an *Eschericia coli*-derived phytase: effects on broiler chick

performance and the digestibility of amino acids and metabolizability of minerals and energy. Poult. Sci. 85:1389-1397.

Cowieson, A. J. and V. Ravindran. 2007. Effects of phytic acid and microbial phytase on the flow and amino acid composition of endogenous protein at the terminal ileum of growing broiler chickens. Br. J. Nutr. 98:745-752.

Cowieson, A. J. and V. Ravindran. 2008. Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: growth performance and digestibility of energy, minerals and amino acids. Br. Poult. Sci. 49:37-44.

Cowieson, A. J., M. R. Bedford, P. H. Selle, and V. Ravindran. 2009. Phytate and microbial phytase: implications for endogenous nitrogen losses and nutrient availability. World Poultry Sci. J. 65:401-418.

Cowieson, A. J. and M. R. Bedford. 2009. The effect of phytase and carbohydrase on ileal amino acid digestibility in monogastric diets: complementary mode of action? World Poultry Sci. J. 65:609-624.

Cowieson, A. J., M. R. Bedford, and V. Ravindran. 2010. Interactions between xylanase and glucanase in maize-soy-based diets for broilers. Br. Poult. Sci. 51:246-257.

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

Deshpande, S. S. and S. Damodaran. 1989. Effect of phytate on solubility, activity and conformation of trypsin and chymotrypsin. J. Food Sci. 54:695-699.

Dilworth, L. L., F. O. Omoruyi, and H. N. Asemota. 2005. Digestive and absorptive enzymes in rats fed phytic acid extract from sweet potato (*Ipomoea batatas*). Diabetol. Croat. 34:59–65.

Engelen, A. J., F. C. van der Heeft, P. H. G. Randsdorp, and E. L. C. Smit. 1994. Simple and rapid determination of phytase activity. J. AOAC Int. 77:760-764.

Engelen, A. J., F. C. van der Heeft, P. H. G. Randsdorp, and W. A. C. Somer. 2001. Determination of phytase activity in feed by a colorimetric enzymatic method: Collaborative interlaboratory study. J. AOAC Int. 84:629-633.

Fiske, C. H. and Y. Subarrow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375-400.

Gehring, C. K., M. R. Bedford, A. J. Cowieson, and W. A. Dozier, III. 2012. Effects of corn source on the relationship between in vitro assays and ileal nutrient digestibility. Poult. Sci. 91:1908-1914.

Hill, R. and C. Tyler. 1954. The reaction between phytate and protein. J. Agric. Sci. 44:324-326.

Juanpere, J., A. M. Pérez-Vendrell, E. Angulo, and Brufau, J. 2005. Assessment of potential interaction between phytase and glycosidase enzyme supplementation on nutrient digestibility in broilers. Poult. Sci. 84:571-580.

Keshavarz, K. 2000. Reevaluation of nonphytate phosphorus requirement of growing pullets with and without phytase. Poult. Sci. 79:1143–1153.

Kim, J. C., P. H. Simmins, B. P. Mullan, and J. R. Pluske. 2005. The digestible energy value of wheat for pigs, with special reference to the post-weaned animal [Review]. Anim. Feed Sci. Tech. 122:257-287.

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

Leone, J. L. 1973. Collaborative study of the quantitative determination of titanium dioxide in cheese. J. AOAC 56:535–537.

Leslie, M. A., E. T. Moran, and M. R. Bedford. 2007. The effect of phytase and glucanase on the ileal digestible energy of corn and soybean meal fed to broilers. Poult. Sci. 86:2350-2357.

Liu, N., Y. J. Ru, F. D. Li, and A. J. Cowieson. 2008. Effect of diet containing phytate and phytase on the activity and messenger ribonucleic acid expression of carbohydrase and transporter in chickens. J. Anim. Sci. 86:3432-3439.

Luttrell, B. M. 1993. The biological relevance of the binding of calcium ions by inositol phosphates. J. Biol. Chem. 268:1521-1524.

McCance, R. A. and E. A. Widdowson. 1942. Mineral metabolism of dephytinized bread. J. Physiol. 101:304-313.

McCleary, B. V. 1992. Measurement of endo-1,4-β-D-xylanase. Pages 161-170 in Xylans and Xylanases. J. Visser, G. Beldman, M. A. Kusters-van Someren, and A. G. J. Voragen, A.G.J., eds. Elsevier Science Publishers, Amsterdam, The Netherlands.

McCleary, B. V. 1995. Measurement of polysaccharide-degrading enzymes in plants using chromogenic and colorimetric substrates. Pages 277-301 in New Diagnostics in Crop Sciences. J. H. Skerritt, and R. Appels, eds.CAB International, Wallingford, UK.

Namkung, H., and S. Leeson. 1999. Effect of phytase enzyme on dietary nitrogen-corrected apparent metabolizable energy and ileal digestibility of nitrogen and amino acids in broiler chicks. Poult. Sci. 78:1317-1319.

Nelson, T. S., T. R. Shieh, R. J. Wodzinski, and J. H. Ware. 1971. Effect of supplemental phytase on the utilization of phytate phosphorus by chicks. J. Nutr. 101:1289-1293.

Onyango, E. M., E. K. Asem, and O. Adeola. 2009. Phytic acid increases mucin and endogenous amino acid losses from the gastrointestinal tract of chickens. Br. J. Nutr. 101:836-842.

Oryschak, M. A., R. H. Simmins, R. T. Zjilstra. 2002. Effect of dietary particle size and carbohydrase and/or phytase supplementation on nitrogen and phosphorus excretion of grower pigs. Can. J. Anim. Sci. 82:533-540.

Persson, H., M. Turk, M. Nyman, and A. –S. Sandberg. 1998. Binding of Cu^{2+}, Zn^{2+}, and Cd^{2+} to inositol tri-, tetra-, penta-, and hexaphosphates. J. Agric. Food Chem. 46:3194-3200.

Pirgozliev, V., T. Acamovic, and M. R. Bedford. 2009. Previous exposure to dietary phytase reduces the endogenous energy losses from precision-fed chickens. Br. Poult. Sci. 50:598-605.

Pirgozliev, V., T. Acamovic, and M. R. Bedford. 2011. The effect of previous exposure to dietary microbial phytase on the endogenous excretions of energy, nitrogen and minerals from turkeys. Br. Poult. Sci. 52:66-71.

Ravindran, V., S. Cabahug, G. Ravindran, and W. L. Bryden. 1999. Influence of microbial phytase on apparent ileal amino acid digestibility of feed stuffs for broilers. Poult. Sci. 78:699-706.

Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary

phytic acid and non-phytate phosphorus level. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci. 41:193-200.

Sandberg, A. –S., M. Brune, N. –G., Carlsson, L. Hallberg, E. Skoglund, and L. Rossander Hulthén. 1999. Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. Am. J. Clin. Nutr. 70:240-246.

SAS Institute. 2009. SAS User's Guide. Statistics. Version 9.2 Edition. SAS Inst. Inc., Cary, NC.

Sebastian, S., S. P. Touchburn, E. R. Chavez, and P. C. Laguë. 1997. Apparent digestibility of protein and amino acids in broiler chickens fed a corn-soybean diet supplemented with microbial phytase. Poult. Sci. 76:1760-1769.

Selle, P. H., V. Ravindran, G. Ravindran, P. H. Pittolo, and W. L. Bryden. 2003. Influence of phytase and xylanase supplementation on growth performance and nutrient utilization of broilers offered wheat-based diets. Asian-Aust. J. Anim. Sci. 16:394-402.

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

Singh, M., and A. D. Krikorian. 1982. Inhibition of trypsin activity in vitro by phytate. J. Agric. Food Chem. 30:799-800.

Watson, B. C., J. O. Mathews, L. L. Southern, and J. L. Shelton. 2006. The effects of phytase on growth performance and intestinal transit time of broilers fed

nutritionally adequate diets and diets deficient in calcium and phosphorus. Poult. Sci. 85:493-497.

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

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

Zhang, X., D. A. Roland, G. R. McDaniel, and S. K. Rao. 1999. Effect of Natuphos phytase supplementation to feed on performance and ileal digestibility of protein and amino acids of broilers. Poult. Sci. 778:1567-1572.

Zyla, K., D. R. Ledoux, and T. L. Veum. 1995. Complete dephosphorylation of corn-soybean meal under stimulated intestinal conditions of the turkey. J. Agric. Food Chem. 43:288–294.

Table 4.1 Ingredient and nutrient composition of basal diet fed to male broilers from 27 to 32 d of age, experiment 2

Item	
Ingredient, %	
Corn	66.55
Soybean meal (48%)	26.60
Poultry oil	2.94
Deflourinated phosphate	1.32
TiO_2	0.50
Variable[3]	0.50
Calcium carbonate	0.38
NaCl	0.25
Vitamin Premix[1]	0.25
Mineral Premix[2]	0.25
DL-Met	0.23
L-Lys·HCl	0.13
L-Thr	0.06
BioCox 60[3]	0.05
Calculated nutrient composition, % unless	
AME_n, kcal/kg	3,150
Crude Protein	17.6
Digestible Lys	0.91
Digestible TSAA	0.71
Digestible Thr	0.61
Digestible Val	0.71
Digestible Ile	0.64
Ca	0.65
Non-phytate P	0.33
Na	0.18

[1]Vitamin premix provides per kg of diet: Vitamin A (Vitamin A acetate), 8,000 IU; Vitamin D (cholecalciferol), 2,000 IU; Vitamin E (DL-alpha tocopherol acetate), 8 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B_{12} (cyanocobalamin), 0.02 mg; folic (folic acid), 0.5 mg: D-pantothenic acid (calcium pantothenate) 15 mg; riboflavin (riboflavin), 5.4 mg; niacin (niacinamide), 45 mg; thiamin (thiamin mononitrate), 1 mg; D-biotin (biotin), 0.05 mg; and pyridoxine (pyridoxine hydrochloride), 2.2 mg; choline (choline chloride), 500 mg.
[2]Mineral premix provides per kg of diet: Mn (manganous oxide), 65 mg; Zn (zinc oxide), 55 mg; Fe (iron sulfate monohydrate), 55 mg; Cu (copper sulfate pentahydrate), 6 mg; I (calcium iodate), 1 mg; Se (sodium selenite), 0.3 mg.
[3]The variable portion of the diet contained Econase XT or Econase XT and Quantum phytase (from AB Vista, Marlborough, Wiltshire, UK) if needed, and the remainder
consisted of washed builders sand.
[3]BioCox 60 provided 60 g/907 kg of Salinomycin from Alpharma, Fort Lee, NJ, USA.

Table 4.2 Ingredient and nutrient composition of basal diets fed to Ross × Ross 708 female broilers from 1 to 14 and 15 to 32 d of age, experiment 3

Item	1 to 14 d	15 to 32 d
Ingredient, %		
Corn	53.14	59.04
Soybean meal (48%)	37.10	30.99
Poultry oil	5.00	5.57
Dicalcium phosphate	1.76	1.54
Calcium carbonate	1.08	0.98
NaCl	0.41	0.41
Variable[1]	0.60	0.65
DL-Met	0.28	0.24
Vitamin Premix[2]	0.25	0.25
Mineral Premix[3]	0.25	0.25
L-Thr	0.07	0.06
L-Lys·HCl	0.02	0.02
TiO_2	0.50	0.50
Calculated nutrient composition, % unless otherwise noted		
AME_n, kcal/kg	3,025	3,140
Crude Protein	22.0	19.53
Digestible Lys	1.18	1.02
Digestible TSAA	0.87	0.78
Digestible Thr	0.77	0.68
Digestible Val	0.91	0.89
Digestible Ile	0.80	0.81
Ca	0.90	0.80
Non-phytate P	0.45	0.40
Na	0.18	0.18

[1]The variable portion of the diet contained Econase XT or Econase XT and Quantum phytase (from AB Vista, Marlborough, Wiltshire, UK) if needed, and the remainder
consisted of washed builders sand.
[2]Vitamin premix provides per kg of diet: Vitamin A (Vitamin A acetate), 8,000 IU; Vitamin D (cholecalciferol), 2,000 IU; Vitamin E (DL-alpha tocopherol acetate), 8 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B_{12} (cyanocobalamin), 0.02 mg; folic (folic acid), 0.5 mg: D-pantothenic acid (calcium pantothenate) 15 mg; riboflavin (riboflavin), 5.4 mg; niacin (niacinamide), 45 mg; thiamin (thiamin mononitrate), 1 mg; D-biotin (biotin), 0.05 mg; and pyridoxine (pyridoxine hydrochloride), 2.2 mg; choline (choline chloride), 500 mg.
[3]Mineral premix provides per kg of diet: Mn (manganous oxide), 65 mg; Zn (zinc oxide), 55 mg; Fe (iron sulfate monohydrate), 55 mg; Cu (copper sulfate pentahydrate), 6 mg; I (calcium iodate), 1 mg; Se (sodium selenite), 0.3 mg.

Table 4.3 Analyzed phytase and xylanase activities in diets fed to broilers

Experiment 2			Experiment 3 Starter			Experiment 3 Grower		
Treatment	Phytase (FTU/kg)	Xylanase (BXU/kg)	Treatment	Phytase (FTU/kg)	Xylanase (BXU/kg)	Treatment	Phytase (FTU/kg)	Xylanase (BXU/kg)
1	165	2,000	1	≤50	≤1,000	1	≤100	≤1,000
2	947	2,000	2	661	≤500	2	518	≤1,000
3	2,000	2,100	3	1,350	≤500	3	827	≤1,000
4	5,110	2,200	4	1,940	≤500	4	2,170	≤1,000
5	9,740	2,000	5	≤100	9,210	5	≤100	12,900
6	18,100	2,200	6	562	13,200	6	599	12,700
7	≤50	22,300	7	936	10,500	7	957	7,800
8	897	26,100	8	2,040	11,600	8	2,320	10,800
9	2,390	27,100	9	≤50	20,400	9	≤50	20,400
10	4,300	29,600	10	539	22,400	10	965	15,900
11	7,870	23,000	11	1,203	22,600	11	1,080	20,100
12	16,540	24,200	12	2,110	20,200	12	2,390	23,000
			13	≤50	46,900	13	≤100	42,600
			14	690	45,700	14	654	43,800
			15	1,120	45,800	15	1,210	47,000
			16	2,680	57,000	16	2,202	53,000

[1]Analyzed phytate content in experiment 2 grower diet and experiment 3 starter and grower diets were 0.99, 1.02 and 0.84%, respectively.

Table 4.4 Apparent ileal digestibility of indispensible amino acids in diets fed to Ross × Ross 708 male broilers from 27 to 32 d of age and formulated to contain adequate Ca and non-phytate P with or without supplementation of phytase and xylanase, experiment 2[1]

Phytase (U/kg)	Xylanase (U/kg)	Arg (%)	Cys (%)	His (%)	Ile (%)	Leu (%)	Lys (%)	Met (%)	Phe (%)	Thr (%)	Val (%)
0		92.6	78.8	87.3	82.5	86.0	89.2	94.0	86.5	81.3	82.7
1,000		94.1	80.9	88.6	84.4	87.6	90.5	94.7	88.2	82.6	84.2
2,000		94.1	81.3	88.8	85.1	87.9	91.0	94.6	88.6	83.3	85.0
4,000		94.1	81.0	88.7	85.1	88.1	90.9	94.7	88.8	83.1	84.9
8,000		93.8	80.6	88.5	84.8	87.9	90.8	94.6	88.6	82.8	84.6
16,000		93.8	81.3	88.7	85.0	88.0	90.8	94.5	88.8	82.8	84.8
SEM		0.3	0.5	0.4	0.4	0.4	0.3	0.3	0.4	0.5	0.4
	0	93.7	80.5	88.3	84.4	87.5	90.4	94.5	88.2	82.6	84.3
	16,000	93.8	80.8	88.6	84.6	87.7	90.6	94.6	88.3	82.7	84.5
	SEM	0.2	0.3	0.2	0.3	0.3	0.2	0.2	0.2	0.4	0.3
Source of Variation[3]		Probability (Pr > F)									
Main effect of phytase		≤0.001	0.005	0.005	≤0.001	≤0.001	≤0.001	0.128	≤0.001	0.017	≤0.001
Main effect of xylanase		0.538	0.517	0.309	0.461	0.461	0.501	0.359	0.525	0.788	0.531
phytase × xylanase		0.899	0.275	0.231	0.341	0.311	0.501	0.410	0.276	0.320	0.341
0 vs. 1,000 U/kg phytase		≤0.001	0.004	0.004	0.003	0.003	0.015	0.014	≤0.001	0.031	0.009
0 vs. 2,000 U/kg phytase		≤0.001	≤0.001	≤0.001	≤0.001		≤0.001	0.023	≤0.001	0.001	≤0.001
1,000 vs. 2,000 U/kg		0.891	0.529	0.499	0.246	0.513	0.123	0.784	0.387	0.228	0.153
1,000 vs. 4,000 U/kg		0.977	0.869	0.671	0.205	0.279	0.269	0.888	0.213	0.334	0.169
2,000 vs. 4,000 U/kg		0.389	0.318	0.432	0.729	0.954	0.478	0.900	0.943	0.419	0.490
Linear effect of phytase[5]		0.176	0.934	0.942	0.375	0.446	0.649	0.439	0.234	0.967	0.466
Quadratic effect of		0.732	0.821	0.755	0.342	0.487	0.213	0.813	0.532	0.277	0.252

[1]Values are least-square means of 12 (phytase concentrations) or 48 (xylanase concentrations) replicate battery cages with 12 birds per cage at 1 d of age (mean initial BW = 0.045 kg).

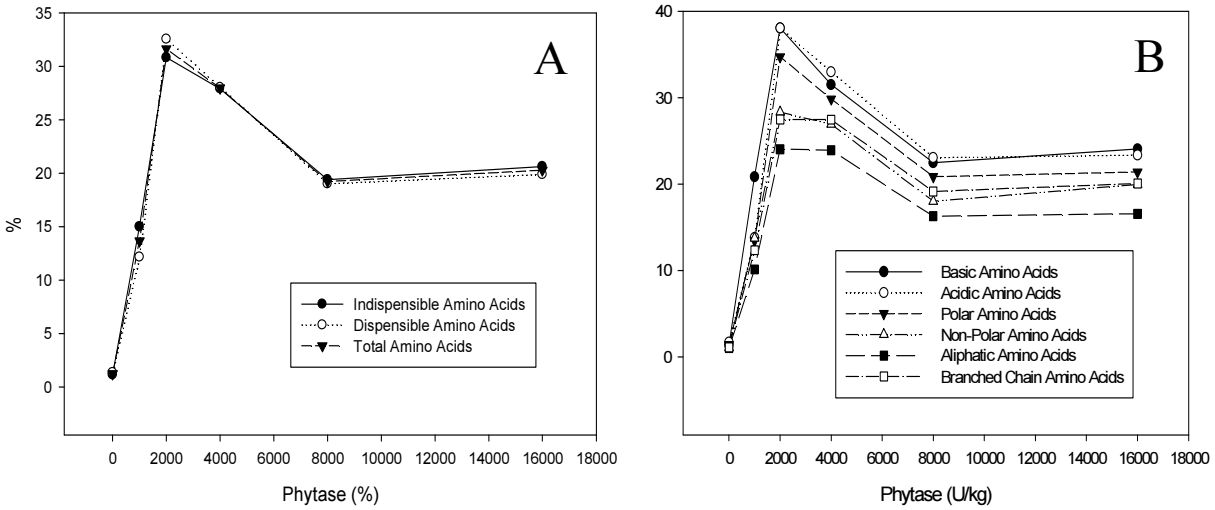
[2]Pooled standard error.
[3]Phytase levels were $\log_{10}$ transformed prior to analysis.
[4]Phytase and xylanase levels did not interact ($P > 0.05$).
[5]Linear and quadratic effects of phytase were analyzed without the zero point so that levels were evenly spaced.

Table 4.5 Apparent ileal digestibility of dispensible amino acids in diets fed to Ross × Ross 708 male broilers from 27 to 32 d of age and formulated to contain adequate Ca and non-phytate P with or without supplementation of phytase and xylanase, experiment 2[1]

Phytase (FTU/kg)	Xylanase (FTU/kg)	Ala (%)	Asp (%)	Glu (%)	Gly (%)	Pro (%)	Ser (%)	Tyr (%)	AA[2] (%)
0		85.5	84.8	89.5	81.8	84.7	83.6	85.5	85.8
1,000		86.4	86.7	90.8	82.9	86.1	86.2	87.4	87.3
2,000		86.7	87.5	91.3	83.5	86.5	87.0	88.0	87.8
4,000		86.9	87.4	91.4	83.4	86.5	87.0	87.9	87.8
8,000		86.7	87.0	91.1	83.1	86.4	86.4	87.7	87.5
16,000		86.7	87.2	91.3	83.2	86.7	86.5	87.8	87.7
SEM		0.4	0.4	0.3	0.4	0.4	0.5	0.4	0.4
	0	86.4	86.7	90.9	82.8	86.1	86.1	87.4	87.3
	16,000	86.6	86.8	91.0	83.1	86.1	86.1	87.3	87.4
	SEM	0.3	0.2	0.2	0.3	0.3	0.4	0.3	0.2
Source of Variation[4]		Probability (Pr > F)							
Main effect of phytase		0.110	≤0.001	≤0.001	0.030	0.003	≤0.001	≤0.001	≤0.001
Main effect of xylanase		0.315	0.875	0.736	0.400	0.986	0.848	0.831	0.576
phytase × xylanase		0.369	0.274	0.399	0.270	0.316	0.448	0.294	0.316
0 vs. 1,000 FTU/kg phytase		0.085	≤0.001	0.002	0.038	0.010	≤0.001	≤0.001	0.001
0 vs. 2,000 U/kg phytase		0.024	≤0.001	≤0.001	0.002	≤0.001	≤0.001	≤0.001	≤0.001
1,000 vs. 2,000 FTU/kg phytase		0.576	0.129	0.210	0.269	0.365	0.199	0.292	0.292
1,000 vs. 4,000 FTU/kg phytase		0.341	0.211	0.167	0.397	0.373	0.212	0.361	0.299
2,000 vs. 4,000 FTU/kg phytase		0.981	0.340	0.597	0.391	0.711	0.316	0.586	0.530
Linear effect of phytase[5]		0.672	0.720	0.383	0.997	0.355	0.917	0.755	0.730
Quadratic effect of phytase		0.417	0.238	0.342	0.386	0.712	0.161	0.373	0.418

[1]Values are least-square means of 12 (phytase concentrations) or 48 (xylanase concentrations) replicate battery cages with 12 birds per cage at 1 d of age (mean initial BW = 0.045 kg).
[2]Total including indispensable amino acids.
[3]Pooled standard error.
[4]Phytase levels were $\log_{10}$ transformed prior to analysis.
[5]Linear and quadratic effects of phytase were analyzed without the zero point so that levels were evenly spaced.

Figure 4.1 Percent improvement of digestibility of ileal undigested amino acids by addition of phytase to diets adequate in Ca and non-phytate P fed to Ross × Ross 708 male broilers from 27 to 32 d of age, experiment 2[1]



A) Indispensible amino acids = Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val and dispensible amino acids = Ala, Asp, Cys, Glu, Gly, Pro, Ser, Tyr. B) Basic amino acids = Arg, His, and Lys, acidic amino acids = Asp and Glu, polar amino acids = Asp, Glu, His, Lys, Ser, Thr, and Phe, non-polar amino acids = Ala, Arg, Cys, Gly, Ile, Leu, Met, Phe, Pro, and Val, aliphatic amino acids = Gly, Ala, Ile, Leu, Val, and Pro, and branched chain amino acids = Ile, Leu, and Val. There were no differences between amino acid groupings for any concentration of phytase ($P > 0.05$).

Table 4.6 Apparent ileal digestibility of indispensible amino acids in diets formulated to contain adequate Ca and non-phytate P with or without supplementation of phytase and xylanase and fed to Ross × Ross 708 female broilers from 27 to 32 d of age, experiment 3[1]

Treatment	Phytase (U/kg)	Xylanase (U/kg)	Arg (%)	Cys (%)	His (%)	Ile (%)	Leu (%)	Lys (%)	Met (%)	Phe (%)	Thr (%)	Val (%)
	0		92.7	81.3	89.1	86.3	87.9	89.5	94.6	87.8	83.0	84.6
	500		92.7	81.3	89.1	86.3	87.8	89.4	94.6	87.8	82.8	84.3
	1,000		93.2	82.3	89.4	86.9	88.5	89.9	94.8	88.3	83.6	85.1
	2,000		93.3	82.6	89.5	87.1	88.6	90.1	94.7	88.5	83.7	85.2
	SEM		0.6	0.4	0.3	0.5	0.4	0.4	0.2	0.4	0.4	0.6
		0	93.0	82.1	89.4	86.9	88.4	89.9	94.8	88.4	83.5	85.3
		800	92.8	81.6	89.1	86.5	87.9	89.5	94.5	87.8	83.0	84.4
		16,000	93.1	82.2	89.3	86.8	88.4	89.9	94.8	88.2	83.4	85.0
		32,000	93.1	81.6	89.2	86.5	88.1	89.7	94.6	87.9	83.1	84.5
		SEM	0.6	0.4	0.3	0.5	0.4	0.4	0.2	0.4	0.4	0.6
Source of Variation[3]			Probability (Pr > F)									
Main effect of phytase			0.008	≤0.001	0.215	0.002	0.004	0.007	0.183	0.021	0.006	0.049
Main effect of xylanase			0.463	0.258	0.553	0.429	0.317	0.231	0.348	0.150	0.434	0.055
phytase × xylanase			0.854	0.677	0.425	0.425	0.437	0.551	0.772	0.606	0.366	0.564
0 vs. 500 U/kg phytase			0.763	0.984	0.923	0.993	0.818	0.823	0.921	0.971	0.524	0.391
500 vs. 1,000 U/kg phytase			0.045	0.013	0.162	0.010	0.007	0.031	0.053	0.063	0.006	0.031
1,000 vs. 2,000 U/kg phytase			0.543	0.426	0.790	0.691	0.937	0.425	0.294	0.489	0.885	0.768
Linear effect of phytase[4]			0.008	0.002	0.117	0.005	0.009	0.004	0.368	0.015	0.007	0.018

[1]Values are least-square means of 24 replicate battery cages with 12 birds per cage at 1 d of age (mean initial BW = 0.045 kg).
[2]Pooled standard error.
[3]Phytase levels were $\log_{10}$ transformed prior to analysis.
[4]Linear effects of phytase were analyzed without the zero point so that levels were evenly spaced.

Table 4.7 Apparent ileal digestibility of dispensible amino acids in diets formulated to contain adequate Ca and non-phytate P with or without supplementation of phytase and xylanase and fed to Ross × Ross 708 female broilers from 27 to 32 d of age, experiment 3[1]

Treatment	Phytase (FTU/kg)	Xylanase (BXU/kg)	Ala (%)	Asp (%)	Glu (%)	Gly (%)	Pro (%)	Ser (%)	Tyr (%)	AA[2] (%)
	0		87.2	86.1	90.4	84.0	86.8	86.6	87.7	87.4
	500		87.0	86.0	90.4	83.9	86.8	86.5	87.6	87.3
	1,000		87.6	86.7	90.9	84.6	87.4	87.5	88.5	87.9
	2,000		87.6	87.0	91.0	84.6	87.5	87.7	88.3	88.1
	SEM		0.3	0.5	0.3	0.4	0.3	0.5	0.6	0.4
		0	87.5	86.7	90.8	84.4	87.3	87.1	88.4	87.9
		800	87.1	86.2	90.4	84.1	86.9	86.8	87.6	87.4
		16,000	87.6	86.5	90.8	84.4	87.3	87.4	88.1	87.8
		32,000	87.2	86.4	90.7	84.1	87.0	86.9	88.0	87.6
		SEM	0.3	0.5	0.3	0.4	0.3	0.5	0.6	0.4
Source of Variation[4]			Probability (Pr > F)							
Main effect of phytase			0.125	0.010	0.020	0.033	0.009	≤0.001	0.011	0.006
Main effect of xylanase			0.351	0.599	0.335	0.604	0.390	0.214	0.124	0.285
phytase × xylanase			0.591	0.518	0.701	0.589	0.455	0.634	0.852	0.667
0 vs. 500 FTU/kg phytase			0.507	0.613	0.967	0.772	0.948	0.858	0.788	0.783
500 vs. 1,000 FTU/kg phytase			0.048	0.024	0.062	0.031	0.024	0.003	0.007	0.013
1,000 vs. 2,000 FTU/kg phytase			0.965	0.489	0.483	0.868	0.691	0.511	0.632	0.713
Linear effect of phytase[5]			0.072	0.003	0.012	0.030	0.012	≤0.001	0.032	0.007

[1]Values are least-square means of 24 replicate battery cages with 12 birds per cage at 1 d of age (mean initial BW = 0.045 kg).
[2]Total including indispensible amino acids.
[3]Pooled standard error.

[4]Phytase levels were $\log_{10}$ transformed prior to analysis.
[5]Linear effects of phytase were analyzed without the zero point so that phytase concentrations were evenly spaced.

Figure 4.2 Main effect of phytase supplementation on apparent ileal digestible energy of diets formulated to contain adequate Ca and non-phytate P and fed to Ross × Ross 708 female broilers from 27 to 32 d of age, experiment 3[1]

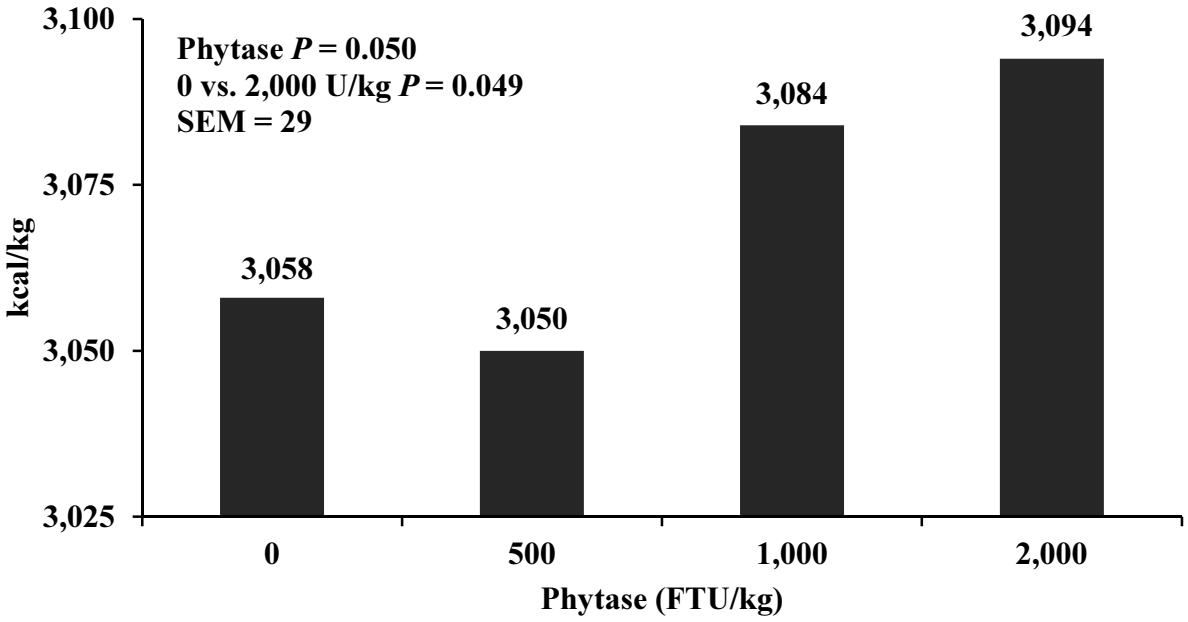
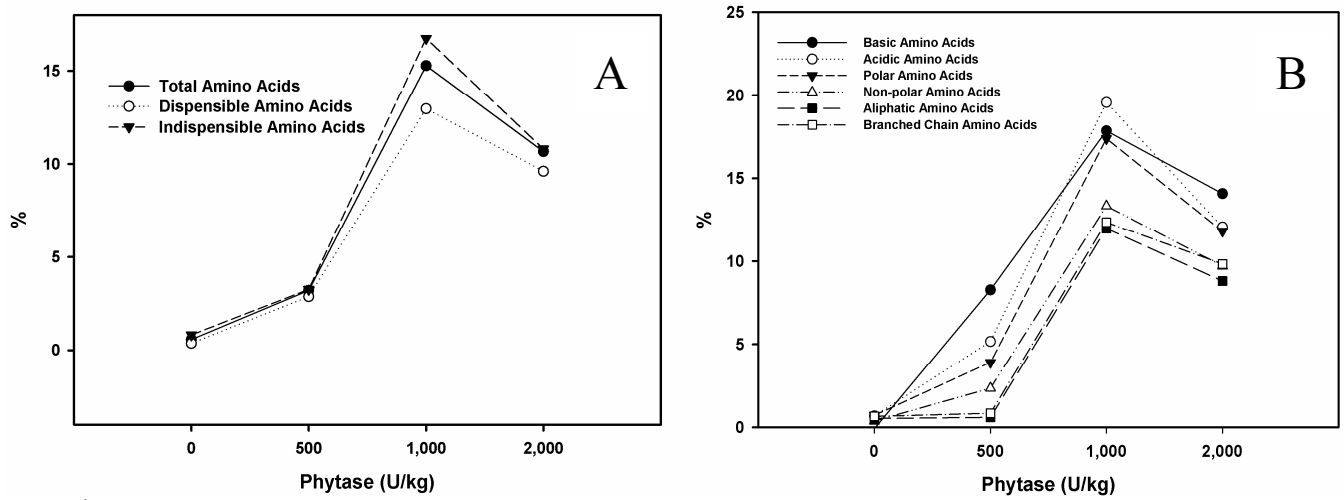

3,100
3,075
3,050
3,025
kcal/kg
Phytase P = 0.050
0 vs. 2,000 U/kg P = 0.049
SEM = 29
3,058
3,050
3,084
3,094
0
500
1,000
2,000
Phytase (FTU/kg)

Figure 4.3 Percent improvement of digestibility of ileal undigested amino acids by addition of phytase to diets adequate in Ca and non-phytate P fed to Ross × Ross 708 female broilers from 15 to 32 d of age, experiment 3[1]



[1]Amino acid groupings are as follows: Total = Ala, Arg, Asp, Cys, Gly, Glu, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Tyr, and Val; Indispensible = Arg, Cys, His, Ile, Leu, Lys, Met, Phe, Thr, and Val; Dispensible = Ala, Asp, Glu, Gly, Pro, Ser, and Tyr; Basic = Lys, Arg, and His; Acidic = Asp and Glu; Polar = Asp, Glu, His, Lys, Phe, Ser, and Thr; Non-polar = Ala, Arg, Cys, Gly, Ile, Leu, Met, Phe, Pro, Val; Aliphatic = Ala, Gly, Ile, Leu, Met, Phe, Pro, and Val; Branched chain = Ile, Leu, and Val. There were no differences between amino acid groupings for any concentration of phytase ($P > 0.05$).

Table 4.8 Growth performance of Ross × Ross 708 female broilers fed adequate Ca and non-phytate P diets supplemented with phytase and xylanase from 1 to 14 d of age, experiment 3[1]

Treatment	Phytase (U/kg)	Xylanase (U/kg)	BW (kg)	BW Gain (kg)	Feed Intake (kg)	FCR[2]	Mortality (%)
	0		0.421	0.376	0.449	1.173	3.1
	500		0.426	0.381	0.457	1.190	2.1
	1,000		0.433	0.389	0.463	1.174	3.8
	2,000		0.442	0.398	0.471	1.183	1.0
	SEM		0.005	0.005	0.005	0.012	0.9
		0	0.431	0.386	0.465	1.194	2.4
		8,000	0.427	0.382	0.454	1.175	3.1
		16,000	0.429	0.384	0.453	1.170	2.4
		32,000	0.436	0.391	0.467	1.180	2.1
		SEM	0.005	0.005	0.005	0.012	0.9
Source of Variation[4]				Probability (Pr > F)			
main effect of phytase			0.001	≤0.001	0.025	0.676	0.142
main effect of xylanase			0.414	0.376	0.135	0.460	0.867
phytase × xylanase			0.439	0.441	0.869	0.903	0.485
0 vs. 500 U/kg phytase			0.318	0.317	0.275	0.294	0.410
0 vs. 1,000 U/kg phytase			0.023	0.021	0.066	0.960	0.582
0 vs. 2,000 U/kg phytase			≤0.001	≤0.001	0.003	0.522	0.102
phytase linear effect[5]			0.005	0.004	0.057	0.676	0.384

[1]Values are least-square means of 24 replicate battery cages with 12 birds per cage at 1 d of age (mean initial BW = 0.045 kg).
[2]Feed conversion ratio corrected for bird d.
[3]Pooled standard error.

[4]Phytase levels were $\log_{10}$ transformed prior to analysis.
[5]Linear and quadratic effects of phytase were analyzed without the zero point so that levels were evenly spaced.

Table 4.9 Growth performance of Ross × Ross 708 female broilers fed adequate Ca and non-phytate P diets supplemented with phytase and xylanase from 1 to 25 d of age, experiment 3[1]

Treatment	Phytase (U/kg)	Xylanase (U/kg)	BW (kg)	BW Gain (kg)	Feed Intake (kg)	FCR[2]	Mortality (%)
	0		1.197	1.151	1.655	1.412	3.1
	500		1.212	1.165	1.659	1.416	2.4
	1,000		1.229	1.182	1.688	1.408	4.5
	2,000		1.248	1.202	1.713	1.408	1.4
	SEM		0.008	0.008	0.012	0.006	1.1
		0	1.221	1.174	1.687	1.416	2.8
		8,000	1.215	1.168	1.660	1.409	3.1
		16,000	1.222	1.174	1.673	1.405	3.1
		32,000	1.229	1.182	1.696	1.413	2.4
		SEM	0.008	0.008	0.012	0.006	1.1
Source of Variation[4]				Probability (Pr > F)			
main effect of phytase			≤0.001	≤0.001	0.002	0.710	0.191
main effect of xylanase			0.590	0.591	0.154	0.584	0.957
phytase × xylanase			0.412	0.420	0.297	0.559	0.380
0 vs. 500 U/kg phytase			0.166	0.158	0.769	0.648	0.635
0 vs. 1,000 U/kg phytase			0.002	0.003	0.048	0.604	0.343
0 vs. 2,000 U/kg phytase			≤0.001	≤0.001	≤0.001	0.585	0.237
phytase linear effect[5]			≤0.001	≤0.001	0.002	0.305	0.472

[1]Values are least-square means of 24 replicate battery cages with 12 birds per cage at 1 d of age (mean initial BW = 0.045 kg).
[2]Feed conversion ratio corrected for bird d.
[3]Pooled standard error.

[4]Phytase levels were $\log_{10}$ transformed prior to analysis.
[5]Linear effects of phytase were analyzed without the zero point so that levels were evenly spaced.

V. INTERACTIVE EFFECTS OF PHYTASE AND XYLANASE SUPPLEMENTATION WITH EXTRACTABLE SALT-SOLUBLE PROTEIN CONTENT OF CORN IN DIETS WITH ADEQUATE CALCIUM AND NON-PHYTATE PHOSPHOROUS FED TO BROILERS

ABSTRACT

The objective was to determine the effects of extractable salt-soluble protein content of corn (PS) and exogenous enzyme supplementation on N, starch, and energy digestibility in broilers fed diets adequate in Ca and non-phytate P. Broilers were randomly distributed into floor pens (6 replicate pens per treatment) with 28 birds per pen at 1 d of age. Treatments consisting of 4 sources of corn varying in PS (A, 58.1; B, 54.2; C, 53.7; and D, 30.6 mg BSA equivalent values) with or without phytase (0 and 1,000 FTU/kg) and xylanase (0 and 16,000 BXU/kg) were randomly assigned to each pen. Different sources of corn were provided from 1 to 9 and 24 to 29 d of age. However, enzyme treatments were provided throughout the experiment. From 1 to 9 d of age, no interactions were observed. Ileal N digestibility (IND) and ileal digestible energy (IDE) of diets based on corn D were lower ($P \leq 0.05$) than that of diets based on corn A, B, or C. Phytase increased ($P \leq 0.01$) IND and IDE by 5 and 16%, respectively, and xylanase exerted the opposite effect ($P \leq 0.03$). From 24 to 29 d of age, phytase and xylanase in combination resulted in reduced ($P \leq 0.05$) IND of diets based on corn D compared to the basal diet in broilers. Broilers fed diets based on corn A or D had lower (3-way

interaction; $P \leq 0.05$) IDE when phytase and xylanase were supplemented in combination compared with either enzyme alone. In conclusion, responses to exogenous enzyme supplementation are not constant and are influenced by the source of ingredients as well as the age of broilers. The magnitudes of the responses to phytase on nutrient and energy digestibility were greater at 9 compared with 29 d of age. Future research is needed to focus on further evaluating phytase and xylanase in corn-soybean meal-based diets for broilers.

INTRODUCTION

Corn provides greater than 20 and 60% of the crude protein and apparent metabolizable energy (**AME_n**) in diets for broilers, respectively. Consequently, growth performance can be impacted by variations in nutrient and energy availability. Identification of corn having submarginal nutritional quality may help nutritionists alleviate problems associated with variability. Rapid assays for variables such as extractable salt-soluble protein content (**PS**) may be a means to mitigate nutrient and energy variability. Rapid evaluation of PS content is possible using near-infrared reflectance spectroscopy and may allow nutritionists to adjust nutrient matrix values of corn in real-time.

A relationship between PS content and nutritional value of corn fed to poultry has been established (Métayer et al., 2009; Gehring et al., 2012). Corn that is dried at high temperatures or with high initial moisture content has a lower concentration of PS compared with corn that is dried under optimal conditions (Lasseran, 1973). Accessibility of protein and starch in the corn endosperm is affected by starch-protein interactions, which can be exacerbated by artificial drying (Holm and Björk, 1988). Thus, enzymes such as phytase and xylanase may potentially enhance the nutritional quality of improperly dried corn by increasing accessibility and solubility of these nutrients.

Limited research has examined the use of a combination of xylanase, amylase, and protease, or these enzymes with phytase to improve the nutritional value of corn dried at high temperatures (Iji et al., 2003; Kaczmarek et al., 2007; Bhuiyan et al., 2010). Broilers fed diets supplemented with xylanase, amylase, and protease did not respond with corn dried at 80°C but had increased BW gain and reduced feed conversion ratio

with corn dried at 120 or 140°C, respectively (Kaczmarek et al., 2007). Thus, the effects of enzyme supplementation depends on the source of corn in some cases. However, PS content was not evaluated and it is unclear whether sources of corn varying in PS content interact with exogenous enzyme supplementation in the same manner.

It is impractical to know drying or storage conditions of each source of corn. Therefore, variables such as PS may potentially be used as a tool to predict nutritional quality of corn. Additionally, it may be beneficial to supplement exogenous enzymes with corn once the PS content is known to improve nutrient and energy utilization. Phytase effects on nutrient digestibility are well accepted. However, corn sources varying in PS content or the addition of xylanase may alter the amount or nature of phytate available for hydrolysis.

To the best of our knowledge, the effects of exogenous enzyme supplementation to diets based on corn varying in PS have not been evaluated. The magnitude of extra-phosphoric effects associated with nutrient digestibility and endogenous losses may be particularly affected by nutrient accessibility and solubility. Therefore, the current experiment evaluated the interactive effects of phytase and xylanase supplementation to diets based on corn varying in PS with adequate Ca and non-phytate P on nutrient and energy digestibility in broilers.

MATERIALS AND METHODS

All procedures relating to the use of live birds were approved by the Auburn University Institutional Animal Care and Use Committee.

Bird Husbandry

Two thousand six hundred and eighty-eight Ross × Ross 708 male broiler chicks (Aviagen North America, Huntsville, AL) were obtained from a commercial hatchery and randomly distributed to battery cages (28 per pen; 0.04 m^2 per bird) in a solid-sided facility at 1 d of age. Broilers were vaccinated for Marek's Disease, Infectious Bronchitis, and Newcastle disease at the hatchery. The ambient temperature set point was 33°C at placement and was decreased as the birds increased in age, with a final set point of 24°C at 29 d of age. Photoperiod was 23L:1D from 1 to 9 d of age, and 20L:4D from 10 to 29 d of age. Broilers and feed were weighed at 23 and 29 d of age. Mortalities were recorded daily and used to correct feed conversion ratio.

Sources of Corn

Eight samples of corn obtained from broiler integrators in the southeastern United States were analyzed for DM (method 930.15; AOAC, 2006), N, starch, ether extract, PS, and vitreousness (Table 5.1). Nitrogen content was determined via the Dumas method (method 990.03; AOAC, 2006) using a N analyzer (LECO FP-228, LECO Corp., St. Joseph, MI) and crude protein was calculated by multiplying percent N by a correction factor (6.25). Ether extract was determined by submerging samples in boiling hexane (method 2003.06; AOAC, 2006) in a fat extractor (Soxtec model number 2043, Foss North America, Inc., Eden Prarie, MN).

In addition to proximate analyses, starch content was analyzed by the amyloglucosidase/α-amylase method (method 996.11; AOAC, 1999) as described by the test kit manufacturer (Megazyme International Ireland Ltd., Bray, Ireland), as well as PS and vitreousness. Extractable salt-soluble protein content was determined according to

the official method (method NF-V03-741; AFNOR, 2008) as described by Janas et al. (2010). Vitreousness was determined by scanning corn samples with a near-infrared reflectance spectrophotometer (FOSS NIRSystems model 6500, Silver Springs, MD, USA) with a range of 400 to 2,498 nm. Calibration equations used were developed by Ngonyamo-Majee et al. (2008) and had R^2 of >0.9 for prediction of vitreousness against values determined manually (Correa et al., 2002) and residual predictive value of 3.73. A residual predictive value between 3.1 and 4.9 is considered to be robust in predicting chemical composition of samples outside of the reference data set for screening purposes (Williams, 2001). Values obtained were used to select 4 sources of corn for inclusion in experimental diets.

Experimental Treatments

Sixteen dietary treatments consisted of a factorial arrangement of 4 sources of corn varying in PS (58.1, 54.2, 53.7, and 30.6 BSA equivalent values), 2 concentrations of phytase (0 or 1,000 FTU per kg of feed), and 2 xylanase concentrations (0 or 16,000 BXU per kg of feed; Tables 5.2 and 5.3). Broilers were fed experimental diets containing 1 of 4 sources of corn only from 1 to 9 and 24 to 29 d of age because of a limited volume of corn. However, enzyme supplementation was continued throughout the 1 to 29 d period because previous exposure to phytase affects physiology of the gastrointestinal tract (Pirgozliev et al., 2009, 2011). From 10 to 16 and 17 to 23 d of age, broilers were provided starter and grower diets formulated to include a common source of corn (Table 5.2, corn 8; PS = 53.7).

Enzyme concentrations were chosen based on the results of previous experiments which demonstrated extra-phosphoric effects on N and energy digestibility with 1,000

FTU/kg phytase and no differences in these variables with xylanase ranging from 8,000 to 32,000 BXU/kg (Gehring et al., 2011; 2012b). Thus, the concentration of xylanase in the current study was chosen based on the concentration recommended by the manufacturer (AB Vista Feed Ingredients, Marlborough, UK).

Enzyme and Phytate Analysis

All experimental diets were analyzed at an external laboratory (Enzyme Services and Consultancy, Caerphilly, Wales) for phytase and xylanase activities. Phytase activity was determined using a method based on the AOAC/Engelen method (method 2000.12; AOAC, 2000; Engelen et al., 1994, 2001). Using phytic acid from rice as a substrate, feed samples were extracted for 30 minutes in 25mM Borate, pH 10.0, and analyses were conducted at pH 4.5 and 60°C. Phytase activity is determined based on the end-point determination of phosphate using a molybdate-vanadate color system. Xylanase activity in feed samples was determined using azurine-crosslinked wheat arabinoxylan (Xylazyme AX) tablets (Megazyme International Ireland Ltd., Bray, Ireland) according to McCleary (1992, 1995). Water-soluble dyed fragments released by the hydrolysis of xylazyme AX substrate by xylanase present in the sample at 50°C were quantitated by absorbance measured at 590 nm.

In addition, basal diets were analyzed for phytate content according to the Megazyme method (method K-PHYT, Megazyme International Ireland Ltd., Bray, Ireland). Briefly, samples were extracted in 0.66 M HCl overnight, followed by enzymatic dephosphorylation with phytase to yield myo-inositol monophosphate and 5 molecules of orthophosphate. The inositol ring was completely dephosphorylated by the action of alkaline phosphatase. Orthophosphate groups released from phytate were

reacted with ammonium molybdate (5% w/v) to yield 12-molybdophosphoric acid. Finally, 12-molybdophosphoric acid and 1 M H_2SO_4 with ascorbic acid (10% w/v) reacted to form molybdenum blue. The concentration of molybdenum blue formed in the reaction was proportional to the concentration of orthophosphate in the original sample. The orthophosphate concentration was determined colorimetrically by measuring the increase in absorbance at 655 nm (Fiske and Subarrow, 1925) and phytate content was calculated based on phytate having 28.2% P.

Nutrient Digestibility

Gastrointestinal and microbiological maturity may affect responses to exogenous enzymes especially with regard to potentially impaired access to substrate. Both the mucosal surface and cecal microflora mature post-hatch with immature and mature states prior to and following approximately 3 wk of age, respectively (Huhtanen and Pensack, 1965; Noy and Sklan, 1995). Therefore, ileal N and energy digestibilities of diets containing 1 of 4 sources of corn varying in PS and with exogenous enzymes were evaluated at 9 and 29 d of age.

At 9 and 29 d of age, 8 birds per experimental unit were euthanized by CO_2 asphyxiation. Digesta contents of the terminal ileum (a section spanning 4 to 30 cm upstream from the ileocecal junction) were gently flushed with deionized water into sample cups and stored at -20°C until being analyzed for TiO_2, N, and energy content. Feed and digesta were lyophilized in a Virtis Genesis Pilot Lyophilizer (SP Industries, Warminster, PA) and ground using a cyclone mill (Cyclotec model number 1093, Foss North America, Inc., Eden Prarie, MN; feed) or an electric coffee grinder (digesta). Broilers yield approximately 1 g or less of dry digesta per bird from the terminal ileum

prior to 30 d of age, and thus, a coffee grinder was used to provide finely ground sample without significant loss.

Feed and digesta from each experiment were analyzed for TiO_2 content by a method based on that of Leone et al. (1973) and used by Gehring et al. (2012). Briefly, 0.25 g of sample were added to glass test tubes and ashed at 580°C for 10 h; ashed samples diluted with 5 mL of H_2SO_4 and containing 0.8 g NaSo4 were heated at 130°C for 72 h; contents of each tube were diluted to 50 mL with distilled deionized water and held for 12 h at 25°C; 3 mL of feed samples or 1 mL of digesta samples (with 2 mL of 1.8 M H_2SO_4) were added to glass test tubes with 150 μL of H_2O_2; and after color development (30 min) absorbance was measured on a spectrophotometer at 410 nm. Control samples consisting of 0.035 g of TiO_2 consistently yielded recoveries of 100 ± 1% indicating that the method is capable of sufficiently solubilizing and detecting TiO_2.

Feed and digesta were also analyzed for N, starch (only at 29 d of age because of limitations with sample size), and gross energy content. Gross energy was determined using an isoperibol bomb calorimeter (model number 6300, Parr Instruments, Moline, IL) as described by the manufacturer's manual (Parr Instruments, 1948). Nitrogen and starch content in feed and digesta were analyzed using the same methods as those used to analyze the sources of corn, as described previously. Titanium dioxide content in feed samples was analyzed in quadruplicate, otherwise duplicate analyses were performed on digesta samples. Ileal digestibility of N (**IND**), starch (**ISD**) and energy were calculated using the following equation (content = %):

$$\frac{\left(\frac{\text{Diet N, Starch, or Energy Content}}{\text{Diet TiO}_2\text{ Content}}\right) - \left(\frac{\text{Diet N, Starch, or Energy Content}}{\text{Diet TiO}_2\text{ Content}}\right)}{\left(\frac{\text{Diet N, Starch, or Energy Content}}{\text{Diet TiO}_2\text{ Content}}\right)}$$

to obtain an AID coefficient or the AID coefficient multiplied by the GE to obtain ileal digestible energy (**IDE**).

Statistical Analyses

Data were analyzed as a factorial treatment structure in a randomized complete block design. Pen location was the blocking factor. Each treatment was represented by 6 replicate pens. Interactive and main effects were evaluated. Statistical significance was considered at $P \leq 0.05$. Means were separated by Tukey's HSD test (Snedecor and Cochran, 1980) when a significant interaction or main effect with more than 2 factor levels was observed. Letter groupings were derived from Tukey pair-wise comparisons in PROC MIXED using pdmix800 macro in SAS software (Saxton, 1988). Analysis of variance was performed using PROC MIXED (SAS, 2009) by the following mixed-effects model:

$$Y_{ij} = \mu.. + \rho_i + \tau_j + \beta_k + \gamma_l + (\tau\beta)_{jk} + (\tau\gamma)_{jl} + (\beta\gamma)_{kl} + (\tau\beta\gamma)_{jkl} + \varepsilon_{ijkl}$$

where $\mu..$ 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 τ_j are fixed factor level effects corresponding to the j^{th} source of corn such that $\Sigma\tau_j = 0$; the β_k are fixed factor level effects corresponding to the k^{th} phytase concentration such that $\Sigma\beta_k = 0$; the γ_l are fixed factor level effects corresponding to the l^{th} xylanase concentration such that $\Sigma\ \gamma_l = 0$; the $(\tau\beta)_{jk}$ through $(\tau\beta\gamma)_{jkl}$ are the interaction effects corresponding to the j^{th} source corn, k^{th} phytase concentration, and l^{th} xylanase concentration, such that $\Sigma(\tau\beta)_{jk}$ through $\Sigma(\tau\beta\gamma)_{jkl} = 0$; and the random error ε_{ijkl} are identically and independently normally distributed with mean 0 and variance σ^2. Relationships between nutrient and energy

digestibility and growth performance were evaluated using Pearson's product-moment correlation via PROC CORR (SAS, 2009; Dowdy et al., 2004).

RESULTS AND DISCUSSION

Sources of Corn

Eight sources of corn were collected from commercial broiler integrators in the southeastern United States. Samples of corn were analyzed for proximate composition, PS, and vitreousness prior to initiating the experimentation. Of the 8 sources that were evaluated, 4 were selected that had similar proximate composition and vitreousness (58.0 to 61.5%) but with varying PS (31 to 58 mg BSA equivalent value). These sources of corn had 74.8 to 75.7% starch, 8.7 to 9.1% CP, and 3.9 to 4.1% ether extract (corrected to an 88% DM basis; Table 5.1).

Salt-soluble protein is highly susceptible to thermal denaturation during the drying process. Denaturation occurs rapidly and with less variability compared with other protein fractions (Malumba et al., 2009). Thus, PS provides an indication of the thermal exposure that a source of corn has been subjected to. Gehring et al. (2012b) determined that corn varying in PS differed in IDE and AME_n when fed to broilers. The majority of corn evaluated in the current study had a smaller range in PS content (Table 5.1). However, corn 7 had much lower PS compared with the other 7 sources (31 vs. 47 to 58 mg BSA equivalents). The low value may be associated with chemical changes such as denaturation, functional group modification, and disulfide interchange as a result of artificial drying (Wall et al., 1975). Experimental diets were formulated to contain 1 of 4 sources of corn designated A, B, C, and D (sources 1, 3, 4, and 7, respectively; Table 5.1) that had similar proximate composition but varied in PS content.

Analyzed Phytase and Xylanase Activities in Experimental Diets

Analyzed phytase and xylanase activities indicated that exogenous enzymes were only present in the intended diets (Table 5.3). In general, phytase activities were in good agreement with the calculated 1,000 FTU/kg but some treatments did approximate 1,500 FTU/kg (2,6, and 14 in the starter, and 8 in the grower phase). Xylanase activities were slightly higher than the intended 16,000 BXU/kg with higher values in diets having a higher intrinsic xylanase activity (those based on corn sources 1 and 4). Analyzed phytate content was similar among all diets and did not likely play a role in reponses to enzyme supplementation.

Ileal Nitrogen and Energy Digestibility, 9 d of Age

No significant interactions were observed; therefore, only main effects will be discussed. Broilers fed diets based on corn D had decreased ($P > 0.05$) IDN and IDE compared with those based on corn C (Table 5.4). Nitrogen and energy digestibility of diets based on corn sources A, B, and C did not differ ($P > 0.05$) possibly due to the extractable salt-soluble protein content of the corn. Extractable salt-soluble protein content ranged from 51 (corn C) to 58 mg BSA equivalent value (corn A) among these sources; however, corn D had a PS value of 31. In the current study, corn sources were obtained from broiler integrators and the drying and storage conditions are not known. However, the solubility of salt-soluble proteins decreases in close association with increasing drying temperatures (Lasseran, 1973; Malumba et al., 2009). Thermal denaturation of protein is associated with the formation of hydrogen and non-covalent bonds as well as sulfhydryl oxidation, which causes protein aggregation and insolubility (Wall et al., 1975). Nutrient and energy utilization may be decreased via protein

denaturation by impairing endogenous enzyme and water access to protein and starch in the endosperm (Altay and Gunasekaran, 2006). Furthermore, AME_n is negatively related to PS values (Métayer et al., 2009, Gehring et al., 2012b). Although the relationship between AME_n and IDE may be affected by the extent of microbial fermentation of undigested starch in some corn sources compared with others (Gehring et al., 2012b).

Supplementation of diets with 1,000 FTU/kg phytase increased IND (5%; $P \leq 0.05$) and IDE (16%). Phytase supplementation of diets fed to broilers likely increased nutrient and energy utilization in the current study by reducing the antinutritional effects of phytate. Phytate impairs nutrient utilization by inhibiting endogenous enzymes (Yoon et al., 1983; Liu et al., 2008), chelating minerals (Maga, 1982), and stimulating hypersecretion and repartitioning of Na in the gastrointestinal tract (Cowieson et al., 2003; Cowieson et al., 2004). Diets were formulated to be adequate in Ca and non-phytate P and thus, improvements in nutrient utilization were observed largely independent of P release. Extra-phosphoric increases in N and energy utilization have been demonstrated previously (Ravindran et al., 2000). These authors noted greater responses on AME_n in adequate Ca and non-phytate P diets and inferred that this may be due to the dilution effect of inorganic phosphate sources or the formation of insoluble soaps (Fedde et al., 1960; Leeson, 1993; Ravindran et al., 2000). Phytase consistently reduced ($P \leq 0.05$) the ileal undigested fractions of N and GE regardless of corn source.

The effect of phytase on ileal undigested fractions was greater for that of GE compared with N. This may be because the effect of phytase on individual amino acids depends on their inherent digestibility. Thus, phytase typically yields a larger increase in digestibility with amino acids having lower basal digestibility (e.g., Cys and Thr)

compared with highly digestible amino acids such as Met. Because IND is a crude measurement, overall reduction of undigested N may not reflect the response with specific amino acids.

Xylanase supplementation decreased IND (64 vs. 61%; $P = 0.05$) and IDE (2,073 vs. 1,936 kcal/kg; $P = 0.03$) in broilers. Xylanase is expected to facilitate acess to encapsulated nutrients via endo-hydrolysis of arabinoxylans. Typically, this results in increased nutrient and energy digestibility of wheat-based diets in combination with reduced digesta viscosity (Bedford and Schulz, 1998; Selle et al., 2003; Cowieson et al., 2010). However, Pourreza and Classen (2001) reported a reduction in IND with increased supplemental xylanase (2,700 vs. 5,400 BXU/kg) from 80.5 to 77.6%, respectively, in corn-soybean meal-wheat bran-based diets. This occurred in parallel with reduced phytate P degradability with or without phytase supplementation. It may be that xylanase increases the pool of free phytate, and without sufficient phytase activity, exacerbates antinutritive effects on nutrient digestibility.

Ileal Nitrogen, Starch, and Energy Digestibility, 29 d of Age

Significant 2 and 3-way interactions were observed on IND and IDE from 24 to 29 d of age (Table 5.5; $P \leq 0.001$). In contrast with responses at 9 d of age, PS content did not affect IND. The response to exogenous enzyme supplementation on IND depended on the source of corn in the diets. Broilers fed diets based on corn A or D had reduced ($P \leq 0.05$) IND with phytase and xylanase in combination compared with either enzyme alone. Additionally, phytase and xylanase in combination reduced ($P \leq 0.05$) IND of diets based on corn D compared with the unsupplemented diet. In contrast,

enzyme supplementation did not affect ($P > 0.05$) IND of diets based on corn B and IND was increased ($P \leq 0.05$) 3% with either enzyme in diets based on corn C.

A 3-way interaction occured ($P \leq 0.001$) for intakes of N, GE, and starch. Phytase increased ($P \leq 0.05$) N intake of broilers fed diets based on corn D compared with xylanase alone or the combination of enzymes. Although IND was not affected ($P > 0.05$) for diets based on corn B, N intake increased ($P \leq 0.05$) with phytase supplementation and again with xylanase supplementation. Broilers fed diets based on corn A and corn D with phytase and xylanase in combination exhibited reduced ($P \leq 0.05$) IDE compared with xylanase alone or with either enzyme, respectively (3-way interaction). In contrast, xylanase supplementation to diets based on corn C resulted in decreased ($P \leq 0.05$) IDE (3,073 kcal/kg) compared with phytase supplementation (3,250 kcal/kg). If xylanase enables the release of previously encapsulated phytate, it may have subdued the phytase response. In previous experiments with diets adequate in Ca and non-phytate P, 1,000 FTU/kg was the minimum concentration at which a significant response was observed (Gehring et al., 2011; 2012a). In diets based on corn C, excess phytate released by xylanase may have made a portion of the protein and starch refractory to hydrolysis and thus, reduced IDE. Regardless of corn source, there were no differences in GE intake between diets supplemented with phytase and xylanase and those with xylanase alone ($P > 0.05$). Phytase significantly increased ($P \leq 0.05$) GE intake of broilers fed diets based on corn D compared with xylanase alone or the combination of enzymes.

Iji et al. (2003) reported that IND and IDE was not affected by drying temperature or xylanase, amylase, and protease supplementation. However, Bhuiyan et al. (2010) reported a corn × enzyme interaction on IND in broilers fed diets with corn dried at 80,

90, or 100°C with phytase, xylanase, amylase, and protease. Enzyme supplementation reduced IND in diets containing corn dried at 80°C while increasing digestibility in diets containing corn dried at higher temperatures. Another possibility is an adverse reaction between phytase and xylanase in combination with certain sources of corn. For example, Olukosi et al. (2007) observed phytase × enzyme cocktail interactions on Ca and P digestibility where the combination of enzymes yielded a reduced response compared with phytase alone. These authors attributed this response to attenuation of phytase activity in the presense of xylanase, amylase, and protease.

Only broilers fed diets based on corn B with both enzymes and those fed diets based on corn C with phytase differed significantly in ileal starch digestibility (**ISD**) (Table 5.5; corn × xylanase; $P = 0.050$). The former diet resulted in 87.1% starch digestibility compared with the latter having been supplemented with phytase alone (98.7%). Although there were few differences in the extent of starch digestion, differences in IDE may have occurred due to variation in the synchrony of starch and protein digestion. It is known that variation in starch composition affects the rate of hydrolysis as well as the appearance of glucose and amino acids in the portal circulation (Liu et al., 2008). Weurding et al. (2001) determined that the rates of starch digestion differed between roller- and hammer-milled corn even though ileal digestibility did not vary. The intestinal mucosa is responsible for a disproportionate amount of dietary amino acid catabolism and energy expenditure compared with other tissues (Wu, 1998; Liu et al., 2008). Extensive first-pass catabolism of amino acids and utilization of energy is essential for the maintainance and competency of the mucosa (Stoll et al., 1998). Thus,

differences in accessibility of starch and protein within the endosperm among sources of corn may translate to varying absorptive capacity.

Although phytase main effects on IND and IDE were not significant ($P > 0.05$), this occurred due to the influence of xylanase. Analysis of the data with the 8 treatments containing xylanase removed resulted in main effects of phytase on IND (78.0 vs. 79.4%; $P \leq 0.001$) and IDE (3,143 vs. 3,227 kcal/kg; $P \leq 0.001$). This is consistent with ileal digestibility data at 9 d of age, in which phytase increased ($P \leq 0.05$) IND and IDE, as well growth performance from 23 to 29 d of age. Parralel extra-phosphoric increases in ileal nutrient and energy digestibility and growth performance of broilers has also been demonstrated with previous experiments (Gehring et al., 2012a).

Changes in ileal undigested fractions may have influenced IDE by altering the amount of fermentable substrate made available to cecal microflora. For example, starch undigested fraction of 13% represents a 160% increase in fermentable starch compared with an undigested fraction of 5%. The range in fermentable material was greatest for starch; however, N and GE undigested fractions ranged from 19.5 to 23.8% and 18.4 to 24.6%. These differences represented approximately 20 to 30% differences in the amount of fermentable substrate escaping the ileum.

Broiler Growth Performance, 24 to 29 d of age

Phytase supplementation increased ($P \leq 0.04$) 29 d BW and BW gain but corn and enzyme supplementation did not interact ($P > 0.05$) to affect growth rate (Table 5.6). Increased BW and growth rate can be attributed to phytate hydrolysis with associated reduction in antinutritive effects (Cabahug et al., 1999). Although, broiler growth was influenced by phytase, the most pronounced effects of corn source and enzyme

supplementation occurred with feed intake. Phytase and xylanase supplementation in combination or xylanase alone increased feed intake of broilers fed diets containing corn B. Phytase alone increased feed intake of broilers compared with the unsupplemented diet in diets based on corn C or D. Alternatively, intake of diets based on corn A, which had the highest PS content (58 mg BSA equivalent value) was not affected by exogenous enzyme supplementation (corn × phytase × xylanase; $P \leq 0.001$). These responses led to 2-way interactions (corn × xylanase and phytase × xylanase; $P \leq 0.05$) on feed conversion ratio. There were no significant differences among treatments in feed conversion ratio with the exception of the diet based on corn B with 16,000 BXU/kg xylanase (1.313 kg:kg) and the diet based on corn C with 1,000 FTU/kg phytase and 16,000 BXU/kg xylanase (1.243 kg:kg; $P = 0.002$). The corn × xylanase interaction occurred ($P = 0.002$) because broilers fed diets based on corn B with xylanase had higher feed conversion ratio than those without xylanase (1.402 vs. 1.280 kg:kg). The phytase × xylanase interaction occurred ($P = 0.037$) because xylanase supplementation alone yielded an increase in feed conversion ratio of 2% whereas phytase and xylanase in combination resulted in reduced feed conversion by 1.5% compared with the unsupplemented diet.

One factor that may have affected growth performance and nutrient digestibility responses in the current study is corn drying temperature. Rapid drying at high temperatures reduces solubility of protein (Wall et al., 1975; Peplinski et al., 1994), which may decrease the availability of amino acids and impair α-amylase and water access to the starch granules (Altay and Gunasekaran, 2006). Thermal denaturation of protein induces the intra- and inter-molecular formation of hydrogen and non-covalent

hydrophobic bonds, as well as sulfhydryl oxidation, which causes protein aggregation. Kaczmarek et al. (2007) dried sources of corn at temperatures ranging from 80 to 140°C. Supplementation of diets with xylanase, amylase, and protease resulted in a significant corn × enzyme supplementation interaction where enzyme supplementation increased 1 to 35 d BW of broilers but with a less pronounced effect in diets containing corn dried at 80°C compared with higher temperatures. Notably, the interaction was not significant from 1 to 14 d of age and in a study conducted by Bhuiyan et al. (2010) corn × enzyme supplementation interaction on BW was significant at 21 d of age but not at 7 d of age.

Correlation Among Nutrient Digestibility and Growth Performance Variables

Ileal nutrient and energy digestibilities were generally not well correlated with growth performance variables (Table 5.7). However, feed consumption was highly correlated with intakes of N, starch, and GE ($P \leq 0.001$). Ileal digestible energy as measured at 9 or 29 d of age was not correlated ($P > 0.05$) with 23 to 29 d growth performance. This is in contrast with results of Masey O'Neill et al. (2012). These authors found significant positive correlations between IDE and AME_n with BW and BW gain. One possible reason for this discrepancy may be the origin of the corn sources. In the current study, corn may have differed with varying genetics while the sources used by Masey O'Neill et al. (2012) were the same cultivar and varied only in growing region. Scott et al. (1999) reported negative correlations between AME_n at 8 or 16 d of age with feed conversion ratio in broilers fed wheat-based diets without enzyme supplementation. The association was not significant at 16 d of age with the addition of a non-starch polysaccharide degrading enzyme. This may be related to a reduction in bird to bird variability with enzyme addition. Additionally, Rose et al. (1996) found no association

between AME_n and feed conversion ratio in broilers fed diets based on wheat. Maissonier et al. (2001) reported strong correlations between ISD and IND and ISD and ileal fat digestibility. Although no correlation was observed in the current study between ISD and digestibility of N or GE, IND and IDE were highly correlated at 9 and 29 d of age (r = 0.77 and 0.84, respectively; $P \leq 0.001$). Higher variability with starch digestibility (compared with IND or IDE) may have removed their association.

Ileal starch digestibility at 29 d of age was correlated with 9 d IND and IDE (r = 0.33 and 0.56; $P \leq 0.001$). This infers that broilers that were better able to digest nutrients at 9 d of age retained an advantage in terms of starch digestion at 29 d of age. It is unclear why only ISD and not IND or IDE were correlated with these variables. Broilers are more susceptible to antinutritients while their gastrointestinal tract is developing compared with older broilers (Masey O'Neill et al., 2012). However, Scott et al. (1999) reported a large reduction in the statistical association between energy utilization, nitrogen retention, and growth performance. It may be that enzyme supplementation affected the correlations between some variables more than others.

Dietary Enzyme Efficacy and Gastrointestinal Tract

Interactions between source of corn and exogenous enzyme supplementation may be influenced by maturity in terms of gastrointestinal tract development and microfloral diversity. Broiler chicks develop full capacity for starch digestion rapidly after emergence from the shell (Moran, 1985). However, it may take 2 wk for mature enterocytes to replace the population of embryonic cells completely (Moran, 2007). During this time, maturation of the mucosal surface coincides with increased digestive enzyme competency, which is apparent by lower nutrient digestibility prior to 3 wk of age (Noy

and Sklan, 1995). Accordingly, the cecal microflora matures with increasing diversity as birds age (Huhtanen and Pensack, 1965). Cecal microflora of chicks immediately post-hatch may represent only 50% of the species diversity present in chicks greater than 3 wk of age (Hume et al., 2003). Results from the current study may infer that interactive effects of source of corn and exogenous enzyme supplementation may become more important after a certain level of gastrointestinal competency has been achieved.

Research has demonstrated that a single factor does not determine nutritional value of corn and it is the nature and interaction of nutrients that influences digestibility of starch and protein (Masey O'Neill et al., 2012). The current research was designed to evaluate differences in response to exogenous enzymes with sources of corn from broiler integrators in an applied manner. Enzyme interactions with corn source indicated that PS does influence accessibility to protein and starch in the endosperm. However, responses were not directly associated with PS content and it is likely that other factors influenced response to exogenous enzymes. It is unclear from the current study what these factors may be because, in practice, low crude protein and vitreousness, or high starch content can diminish the effects of low PS content. However, nutrient composition and vitreousness were similar among sources A, B, C, and D. The N and energy utilization responses to exogenous enzyme supplementation were more pronounced at 9 compared with 29 d of age. Juvenile broilers are likely to benefit more from reduction of antinutrients due to a reduced capacity for endogenous enzymes and shunting of energy from growth to enzyme synthesis (Olukosi et al., 2007).

In conclusion, sources of corn varying in PS content and exogenous phytase and xylanase supplementation interacted in their effects on ileal N and energy digestibility of

broilers at 29 d but not 9 d of age. Magnitude of phytase responses was greater during the first wk post-hatch compared with broilers at 4 wk of age. Responses with exogenous enzymes can not be assumed to be constant and are influenced by the source of corn used as well as the age of broilers. Further research is needed to better understand the interactive effects of PS and enzyme supplementation on nutrient digestibility and growth performance of broilers.

REFERENCES

AFNOR (Association Française de Normalisation) (2008) Méthode Promatest d'évaluation de la dénaturation des proteins Thermosensibles. NF-V03-741. AFNOR, La Plaine Saint-Denis, France.

Altay, F. and S. Gunasekaran. 2006. Influence of drying temperature, water content, and heating rate on gelatinization of corn starches. J. Agric. Food Chem. 54:4235-4245.

AOAC. 1999. Official Methods of Analysis. 16th ed., 5th Revision, Assoc. Off. Anal. Chem., Gaithersburg, MD.

AOAC. 2000. Official Methods of Analysis. 17th ed., Assoc. Off. Anal. Chem., Gaithersburg, MD.

AOAC. 2006. Official Methods of Analysis. 18th ed., Assoc. Off. Anal. Chem., Washington, DC.

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

Bhuiyan, M. M., A. F. Islam, and P. A. Iji. 2010. Response of broiler chickens to diets containing artificially dried high-moisture maize supplemented with microbial enzymes. S. Afr. J. Anim. Sci. 40:348-362.

Cabahug, S., V. Ravindran, P. H. Selle, and W. L. Bryden. 1999. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus contents. I. effects on bird performance and toe ash. Br. Poult. Sci. 40:660-666.

Correa, C. E. S., R. D. Shaver, M. N. Pereira, J. G. Lauer, and K. Kohn. 2002. Relationship between corn vitreousness and ruminal in-situ starch degradability. J. Dairy Sci. 85:3008- 3012.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2003. The effect of phytase and phytic acid on endogenous losses from broiler chickens. Br. Poult. Sci. 44:23-24.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2004. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. Br. Poult. Sci. 45:101-108.

Cowieson, A. J., M. R. Bedford, and V. Ravindran. 2010. Interactions between xylanase and glucanase in maize-soy-based diets for broilers. Br. Poult. Sci. 51:246-257.

Dowdy, S. M., S. Wearden, and D. M. Chilko. 2004. Statistics for Research. 3rd ed. John Wiley and Sons Inc. New York, New York.

Engelen, A. J., F. C. van der Heeft, P. H. G. Randsdorp, and E. L. C. Smit. 1994. Simple and rapid determination of phytase activity. J. AOAC Int. 77:760-764.

Engelen, A. J., F. C. van der Heeft, P. H. G. Randsdorp, and W. A. C. Somer. 2001. Determination of phytase activity in feed by a colorimetric enzymatic method: Collaborative interlaboratory study. J. AOAC Int. 84:629-633.

Fedde, M. R., P. E. Waibel, and R. E. Burger. 1960. Factors affecting the absorbability of certain dietary fats in the chick. J.Nutr.70:447-452.

Fiske, C. H. and Y. Subarrow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375-400.

Gehring, C. K., M. R. Bedford, and W. A. Dozier, III. 2012a. Effects of phytase and xylanase supplementation on ileal digestibility and growth performance of female

broilers from 1 to 32 d of age. Page 6 in International Poultry Scientific Forum Abstracts. US Poultry & Egg Association, Tucker, GA.

Gehring, C. K., M. R. Bedford, A. J. Cowieson, and W. A. Dozier, III. 2012b. Effects of corn source on the relationship between in vitro assays and ileal nutrient digestibility. Poult. Sci. 91:1908-1914.

Gehring, C. K., M. R. Bedford, and W. A. Dozier, III. 2011. Effects of phytase and xylanase supplementation of diets fed to male broilers on nutrient digestion from 28 to 32 d of age. Poult. Sci. 90(E-Suppl. 1):36. (Abstr.)

Holm, J. and I. Björk. 1988. Effects of thermal processing of wheat on starch: II. enzymic availability. J. Cereal Sci. 8:261-268.

Huhtanen, C. N. and J. M. Pensack. 1965. The development of the intestinal flora of the young chick. Poult. Sci. 44:825–830.

Hume, M. E., L. F. Kubena, T. S. Edrington, C. J. Donskey, R. W. Moore, S. C. Ricke, and D. J. Nisbet. 2003. Poultry digestive microflora biodiversity as indicated by denaturing gradient gel electrophoresis. Poult. Sci. 82:1100-1107.

Iji, P. A., K. Khumalo, S. Slippers, and R. M. Gous. 2003. Intestinal function and body growth of broiler chickens on diets based maize dried at different temperatures and supplemented with a microbial enzyme. Reprod. Nutr. Dev. 43:77-90.

Janas, S., S. Boutry, P. Malumba, L. Vander Elst, and F. Béra. 2010. Modelling dehydration and quality degradation of maize during fluidized-bed drying. J. Food Eng. 100:527-534.

Kaczmarek, S., A. J. Cowieson, D. Józefiak, M. Bochenek, and A. Rutkowski. 2007. The effect of drying temperature and exogenous enzymes supplementation on the

nutritional value of maize for broiler chickens. Proc. 16th Euro. Symp. Poult. Nutr., Strasbourg, France.

Lasseran, J.C. 1973. Incidence of drying and storing conditions of corn (maize) on its quality for starch industry. Starch 25:257-262.

Leeson, S. 1993. Recent advances in fat utilization by poultry. Pages 170-181 in Recent Advances in Animal Nutrition in Australia. D. J. Farrell, ed. University of New England Armidale, Australia.

Leone, J. L. 1973. Collaborative study of the quantitative determination of titanium dioxide in cheese. J. Assoc. Off. Anal. Chem. 56:535-537.

Liu, N., Y. J. Ru, F. D. Li, and A. J. Cowieson. 2008. Effect of diet containing phytate and phytase on the activity and messenger ribonucleic acid expression of carbohydrase and transporter in chickens. J. Anim. Sci. 86:3432-3439.

Maga, J. A**.** 1982. Phytate: its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. J. Agric. Food Chem. 30:1-9.

Malumba, P., S. Janas, T. Masimango, M. Sindic, C. Deroanne, and F. Béra. 2009. Influence of drying temperature on the wet-milling performance and the proteins solubility indexes of corn kernels. J. Food Eng. 95:393-399.

Masey O'Neill, H. V., N. Liu, J. P. Wang, A. Diallo, and S. Hill. 2012. Effect of xylanase on performance and apparent metabolisable energy in starter broilers fed diets containing one maize variety harvested in different regions of China. Asian-Aust. J. Anim. Sci. 25:515-523.

McCleary, B. V. 1992. Measurement of endo-1,4-β-D-xylanase. Pages 161-170 in Xylans and Xylanases. J. Visser, G. Beldman, M. A. Kusters-van Someren, and A. G. J. Voragen, A.G.J., eds. Elsevier Science Publishers, Amsterdam, The Netherlands.

McCleary, B. V. 1995. Measurement of polysaccharide-degrading enzymes in plants using chromogenic and colorimetric substrates. Pages 277-301 in New Diagnostics in Crop Sciences. J. H. Skerritt, and R. Appels, eds.CAB International, Wallingford, UK.

Moran, E. T., Jr. 2007. Nutrition of the developing embryo and hatchling. 86:1043-1049.

Moran, E. T., Jr. 1985. Digestion and absorption of carbohydrates in fowl and events through prenatal development. J. Nutr. 115:665-674.

Ngonyamo-Majee, D., R. D. Shaver, J. G. Coors, D. Sapienza, C. E. S. Correa, J. G. Lauer, and P. Berzaghi. 2008. Relationships between kernel vitreousness and dry matter degradability for diverse corn germplasm I. Development of near-infrared reflectance spectroscopy calibrations. Anim. Feed Sci. Tech. 142:247-258.

Noy, Y. and D. Sklan. 1995. Digestion and absoroption in the young chick. Poult. Sci. 74:366-373.

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

Peplinski, A. J., J. W. Paulis, J. A. Bietz, and R. C. Pratt. 1994. Drying of high-moisture corn: Changes in properties and physical quality. Cereal Chem. 71:129-133.

Pirgozliev, V., T. Acamovic, and M. R. Bedford. 2009. Previous exposure to dietary phytase reduces the endogenous energy losses from precision-fed chickens. Br. Poult. Sci. 50:598-605.

Pirgozliev, V., T. Acamovic, and M. R. Bedford. 2011. The effect of previous exposure to dietary microbial phytase on the endogenous excretions of energy, nitrogen and minerals from turkeys. Br. Poult. Sci. 52:66-71.

Pontoppidan, K., D. Pettersson, and A. –S. Sandberg. 2007. The type of thermal feed treatment influences the inositol phosphate composition. Anim. Feed Sci. Tech. 132:137-147.

Pourreza, J. and H. L. Classen. 2001. Effects of supplemental phytase and xylanase on phytate phosphorus degredation, ileal protein and energy digestibility of a corn-soybean-wheat bran diets in broiler chicks. J. Agric. Sci. Technol. 3:19-25.

Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorous levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci. 41:193-200.

Rose, S. P., M. S. Uddin and M. R. Bedford. 1996. Growth of broiler chickens fed different wheat varieties. Proceedings of the WPSA World Congress 1996, New Delhi, India.

SAS Institute. 2009. SAS User's Guide. Statistics. Version 9.2 Edition. SAS Inst. Inc., Cary, NC.

Saxton, A.M. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. Pages 1243-1246 in Proc. 23rd SAS Users Group Intl., SAS Institute, Cary, NC.

Scott, T. A., F. G. Silversides, H. L. Classen, M. L. Swift and M. R. Bedford. 1999. Prediction of the performance of broiler chicks from apparent metabolizable energy and protein digestibility values obtained using a broiler chick bioassay. Can. J. Anim. Sci. 79:159-164.

Selle, P. H., V. Ravindran, G. Ravindran, P. H. Pittolo, and W. L. Bryden. 2003. Influence of phytase and xylanase supplementation on growth performance and nutrient utilization of broilers offered wheat-based diets. Asian-Aust. J. Anim. Sci. 16:394-402.

Snedecor, G. W., and W. G. Cochran. 1980. Statistical methods, 7th edn. Iowa State Univ. Press, Ames.

Stoll, B., D. G. Burrin, H. Yu, J. Henry, F. Jahoor, and P. F. Reeds. 1998. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. J. Nutr. 128:606–614.

Wall, J. S., C. James, and G. L. Donaldson. 1975. Corn proteins: Chemical and physical changes during drying of grain. Cereal Chem. 52:779-790.

Weurding, R. E., A. Veldman, W. A. G. Veen, P. J. Van der Aar, and M. W. A. Verstegen. 2001. Starch digestion rate in the small intestine of broiler chickens differs among feedstuffs. J. Nutr. 131:2329-2335.

Williams, P.C. 2001. Implementation of near-infrared technology. Pages 145-169 in Near Infrared Technology in the Agricultural and Food Industries. P. C. Williams and K. Norris, ed. American Association of Cereal Chemists, St. Paul, MN.

Wu, G., 1998. Intestinal mucosal amino acid catabolism. J. Nutr. 128:1249-1252.

Yoon, J. H., L. U. Thompson, and D. J. A. Jenkins. 1983. The effect of phytic acid on in vitro rate of starch digestibility and blood glucose response. Am. J. Clin.Nutr. 38:835-852.

Table 5.1 Proximate composition and extractable salt-soluble protein content of corn obtained from the southeastern United States[1,2]

Source[3]	PS[4] (%)	Vitreousness (%)	Starch (%)	Crude protein (%)	Ether Extract (%)
1*	58.1	58.0	75.2	8.8	4.1
2	56.2	58.6	75.8	8.4	4.1
3*	51.3	58.5	74.8	9.1	3.9
4*	55.3	61.5	75.5	9.0	4.1
5	54.7	51.5	75.6	8.2	4.0
6	57.3	56.1	75.3	8.5	4.1
7*	31.0	60.6	75.7	8.7	4.0
8	47.2	64.4	76.1	10.0	3.9

[1]Analyses were performed on duplicate samples.

[2]Values for starch, crude protein, and crude fat content are expressed on an as-fed basis.

[3]Numbers with a * were selected for the in vivo experiment and designated as A (source 1), B (source 4), C (source 3), and D (source 5).

[3]Extractable salt-soluble protein content (samples were analyzed in duplicate by the wet-chemistry method).

Table 5.2 Ingredient and nutrient composition of basal diets fed to Ross × Ross 708 male broilers from 1 to 16 and 17 to 29 d of age

Item	1 to 16 d of age	17 to 29 d of age
Ingredient, %		
Corn[1]	53.14	59.04
Soybean meal (48%)	37.10	30.99
Poultry oil	5.00	5.57
Dicalcium phosphate	1.76	1.54
Calcium carbonate	58.1 1.08	0.98
NaCl	0.41	0.41
Variable[2]	0.60	0.65
DL-Met	0.28	0.24
Vitamin Premix[3]	0.25	0.25
Mineral Premix[4]	0.25	0.25
L-Thr	0.07	0.06
L-Lys·HCl	0.02	0.02
TiO_2	0.50	0.50
Calculated nutrient composition, % unless otherwise noted		
AME_n, kcal/kg	3,025	3,140
Crude Protein	22.0	19.5
Digestible Lys	1.18	1.02
Digestible TSAA	0.87	0.78
Digestible Thr	0.77	0.68
Digestible Val	0.91	0.89
Digestible Ile	0.80	0.81
Ca	0.90	0.80
Non-phytate P	0.45	0.40
Na	0.18	0.18

[1]Corn consisted of sources A, B, C, or D (corresponding with Table 5.1) during the experimental periods of 1 to 9 and 24 to 29 d of age. Interim diets had identical diet formulations and enzyme supplementation but with a common U.S. grade 2 source of corn.
[2]The variable portion of the diet contained Econase xylanase or Econase and Quantum phytase (from AB Vista, Marlborough, Wiltshire, UK) if needed, and the remainder consisted of washed builders sand.
[3]Vitamin premix provides per kg of diet: Vitamin A (Vitamin A acetate), 8,000 IU; Vitamin D (cholecalciferol), 2,000 IU; Vitamin E (DL-alpha tocopherol acetate), 8 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B_{12} (cyanocobalamin), 0.02 mg; folic (folic acid), 0.5 mg: D-pantothenic acid (calcium pantothenate) 15 mg; riboflavin (riboflavin), 5.4 mg; niacin (niacinamide), 45 mg; thiamin (thiamin mononitrate), 1 mg; D-biotin (biotin), 0.05 mg; and pyridoxine (pyridoxine hydrochloride), 2.2 mg; choline (choline chloride), 500 mg.
[4]Mineral premix provides per kg of diet: Mn (manganous oxide), 65 mg; Zn (zinc oxide), 55 mg; Fe (iron sulfate monohydrate), 55 mg; Cu (copper sulfate pentahydrate), 6 mg; I (calcium iodate), 1 mg; Se (sodium selenite), 0.3 mg.

Table 5.3 Analyzed phytase and xylanase activities and phytate content in diets fed to broilers

	1 to 16 d of age				17 to 29 d of age		
Treatment[1]	Phytase (FTU/kg)	Xylanase (BXU/kg)	Phytate (%)	Treatment	Phytase (FTU/kg)	Xylanase (BXU/kg)	Phytate (%)
Corn A (58.1 PS[2])							
1	≤50	1,700		1	≤50	2,600	
2	1,400	2,600	0.26	2	987	2,900	0.25
3	≤50	22,700		3	≤50	25,500	
4	1,340	23,600		4	1,080	26,600	
Corn B (51.3 PS)							
5	≤50	≤500		5	≤50	≤500	
6	1,400	≤500	0.25	6	1,030	763	0.25
7	≤50	18,300		7	≤50	20,100	
8	1,200	18,100		8	1,480	20,600	
Corn C (55.3 PS)							
9	≤50	2,300		9	≤50	3,300	
10	834	2,500	0.25	10	1,380	3,000	0.27
11	≤50	21,800		11	≤50	24,600	
12	1,310	22,700		12	849	22,000	
Corn D (31.0 PS)							
13	≤50	700		13	≤50	≤500	
14	1,460	1,200	0.26	14	873	750	0.27
15	≤50	19,800		15	≤50	17,300	
16	1,330	20,000		16	921	21,100	

[1]Treatments designate a 4 (corn source varying in extractable salt-soluble protein content) × 2 (with or without phytase) × 2 (with or without xylanase) factorial arrangement. Corn letter corresponds with Table 5.1.
[2]PS = extractable salt-soluble protein content of corn used in diets fed to broilers.

Table 5.4 Ileal nitrogen digestibility and digestible energy of diets comprised of corn varying in extractable salt-soluble protein content with or without phytase and xylanase supplementation fed to Ross × Ross 708 male broilers from 1 to 9 d of age[1,2]

Corn[3]	Phytase (FTU/kg)	Xylanase (BXU/kg)	N UF[4] (%)	Nitrogen (%)	GE UF (%)	IDE[5] (kcal/kg)
A			38.8[b]	62.9[ab]	57.2[a]	1,907[ab]
B			38.1[b]	61.9[ab]	55.8[a]	1,968[ab]
C			33.1[b]	66.9[a]	48.1[b]	2,296[a]
D			42.5[a]	57.5[b]	58.2[a]	1,847[b]
SEM			1.8	2.4	2.1	94
	0		40.2	60.6	58.1	1,859
	1,000		36.1	63.9	51.5	2,150
	SEM		1.5	1.5	1.9	83
		0	37.0	63.8	53.2	2,073
		16,000	39.2	60.8	56.4	1,936
		SEM	1.5	1.5	1.9	83
			Probabilities			
Analysis of Variance						
Corn			≤0.001	≤0.001	≤0.001	≤0.001
Phytase			0.02	0.034	≤0.001	≤0.001
Xylanase			0.08	0.050	0.023	0.029

[1]Analyses of feed were conducted on quadruplicate samples and analyses of digesta were conducted on duplicate samples. Nitrogen and energy digestibility were calculated on an apparent basis.
[2]Values are least-square means of 6 replicate cages of 20 birds per cage at 9 d of age. Means were separated via Tukey's Honestly Significant Difference test with $\alpha = 0.05$. Means within a column within a factor that do not share a common letter are significantly different.
[3]Corn letter corresponds with Table 5.1. Corn A, B, C, and D had extractable salt-soluble protein content values of 58.1, 54.2, 53.7, and 30.6 (BSA-equivalent values), respectively.
[4]UF = Undigested fraction.
[5]Ileal digestible energy.

Table 5.5 Ileal nitrogen and starch digestibility and digestible energy of diets comprised of corn varying in extractable salt-soluble protein content with or without phytase and xylanase supplementation fed to Ross × Ross 708 male broilers from 24 to 29 d of age[1,2]

Corn[3]	Phytase (FTU/kg)	Xylanase (BXU/kg)	N Intake (kg/bird)	N UF[4] (%)	Nitrogen (%)	Starch Intake (kg/bird)	Starch UF (%)	Starch (%)	GE Intake (kcal/bird)	GE UF (%)	IDE[5] (kcal/kg)
A	0	0	0.017^{bcd}	21.9^{bcd}	78.1^{bcd}	0.185^{bc}	4.1^{ab}	95.9^{ab}	2,063^{bcd}	22.3^{bcdef}	3,157^{abcd}
A	1,000	0	0.017^{bcde}	20.7^{cde}	79.3^{abc}	0.181^{bcd}	6.0^{ab}	94.0^{ab}	2,027^{bcd}	20.9^{bcdef}	3,216^{abcd}
A	0	16,000	0.017^{defg}	20.0^{de}	80.0^{ab}	0.174^{cd}	6.8^{ab}	93.2^{ab}	1,923^{def}	18.4^{f}	3,290^{ab}
A	1,000	16,000	0.016^{defg}	23.7^{b}	76.3^{d}	0.176^{bcd}	3.8^{ab}	96.2^{ab}	1,962^{def}	24.0^{bc}	3,097^{cd}
B	0	0	0.015^{fg}	22.7^{bc}	77.3^{cd}	0.158^{e}	4.5^{ab}	95.5^{ab}	1,812^{f}	24.3^{b}	3,089^{d}
B	1,000	0	0.017^{cde}	22.8^{bc}	77.2^{cd}	0.172^{cde}	4.7^{ab}	95.3^{ab}	1,961^{def}	22.6^{bcde}	3,139^{abcd}
B	0	16,000	0.020^{a}	23.8^{b}	76.2^{d}	0.207^{a}	10.7^{ab}	89.3^{ab}	2,353^{a}	23.9^{bcd}	3,066^{d}
B	1,000	16,000	0.019^{b}	23.2^{b}	76.8^{d}	0.191^{ab}	12.9^{b}	87.1^{b}	2,178^{abc}	20.7^{bcdef}	3,191^{abcd}
C	0	0	0.015^{g}	22.8^{bc}	77.2^{cd}	0.170^{de}	6.2^{ab}	93.8^{ab}	1,840^{ef}	24.0^{bcd}	3,108^{bcd}
C	1,000	0	0.017^{bcde}	19.5^{e}	80.5^{a}	0.186^{bc}	1.3^{a}	98.7^{a}	2,005^{cde}	20.1^{def}	3,250^{abc}
C	0	16,000	0.017^{defg}	21.8^{bcde}	78.2^{abcd}	0.183^{bcd}	6.9^{ab}	93.1^{ab}	1,977^{def}	24.6^{b}	3,073^{d}
C	1,000	16,000	0.017^{cdef}	20.5^{cde}	79.5^{abc}	0.182^{bcd}	2.7^{ab}	97.3^{ab}	1,966^{def}	21.6^{bcdef}	3,191^{abcd}
D	0	0	0.016^{fg}	20.6^{cde}	79.4^{abc}	0.182^{bcd}	5.5^{ab}	94.5^{ab}	1,929^{def}	20.2^{cdef}	3,220^{abcd}
D	1,000	0	0.018^{bc}	19.5^{de}	80.5^{ab}	0.205^{a}	4.6^{ab}	95.4^{ab}	2,184^{ab}	18.8^{ef}	3,302^{a}
D	0	16,000	0.016^{defg}	22.2^{bcd}	77.8^{bcd}	0.179^{bcd}	7.2^{ab}	92.8^{ab}	1,906^{def}	23.7^{bcd}	3,107^{cd}
D	1,000	16,000	0.016^{efg}	27.6^{a}	72.4^{e}	0.181^{bcd}	3.0^{ab}	97.0^{ab}	1,950^{def}	29.0^{a}	2,904^{e}
SEM			0.000	0.5	0.5	0.003	2.1	2.1	36	0.9	38
Probabilities											
Analysis of Variance											
Corn			≤0.001	≤0.001	≤0.001	0.005	0.045	0.045	≤0.001	0.029	0.031
Phytase			0.002	0.395	0.395	0.005	0.101	0.101	0.003	0.256	0.143
Xylanase			0.001	≤0.001	≤0.001	0.008	0.032	0.032	0.006	≤0.001	≤0.001
Corn × Phytase			≤0.001	≤0.001	≤0.001	0.004	0.201	0.201	0.005	≤0.001	≤0.001
Corn × Xylanase			≤0.001	≤0.001	≤0.001	≤0.001	0.050	0.050	≤0.001	≤0.001	≤0.001

Phytase × Xylanase	≤0.001	≤0.001	≤0.001	≤0.001	0.467	0.467	≤0.001	≤0.001	≤0.001
Corn × Phytase × Xylanase	≤0.001	≤0.001	≤0.001	0.001	0.556	0.556	0.001	≤0.001	≤0.001

[1]Analyses of feed were conducted on quadruplicate samples and analyses of digesta were conducted on duplicate samples. Nitrogen and energy digestibility were calculated on an apparent basis.
[2]Values are least-square means of 6 replicate cages of 20 birds per cage at 30 d of age. If a significant interaction was observed, means were separated via Tukey's Honestly Significant Difference test with $\alpha = 0.05$. Means within a column that do not share a common letter are significantly different.
[3]Corn letter corresponds with Table 5.1. Corn A, B, C, and D had extractable salt-soluble protein content values of 58.1, 54.2, 53.7, and 30.6 (BSA-equivalent values), respectively.
[4]UF = Undigested fraction.
[5]Ileal digestible energy.

Table 5.6 Growth performance of Ross × Ross 708 male broilers fed diets comprised of corn varying in extractable salt-soluble protein content with or without phytase and xylanase supplementation from 24 to 29 d of age[1,2]

Corn[3]	Phytase (FTU/kg)	Xylanase (BXU/kg)	29 d BW (kg/bird)	BW Gain (kg/bird)	Feed Intake (kg/bird)	FCR[4] (kg:kg)	Mortality (%)
A	0	0	1.195	0.369	0.499^{bcd}	1.350^{ab}	0.0
A	1,000	0	1.217	0.382	0.488^{cd}	1.295^{ab}	0.0
A	0	16,000	1.167	0.363	0.468^{def}	1.295^{ab}	3.3
A	1,000	16,000	1.203	0.373	0.473^{def}	1.297^{ab}	0.0
B	0	0	1.176	0.364	0.440^{f}	1.271^{ab}	1.7
B	1,000	0	1.201	0.372	0.478^{de}	1.286^{ab}	1.7
B	0	16,000	1.222	0.379	0.577^{a}	1.325^{a}	1.7
B	1,000	16,000	1.222	0.381	0.532^{ab}	1.350^{ab}	0.0
C	0	0	1.133	0.351	0.445^{ef}	1.313^{ab}	1.7
C	1,000	0	1.184	0.369	0.488^{cd}	1.329^{ab}	1.7
C	0	16,000	1.158	0.359	0.480^{def}	1.340^{ab}	0.0
C	1,000	16,000	1.217	0.377	0.477^{def}	1.243^{b}	1.7
D	0	0	1.218	0.377	0.493^{def}	1.313^{ab}	0.0
D	1,000	0	1.214	0.376	0.531^{abc}	1.356^{ab}	0.0
D	0	16,000	1.173	0.364	0.463^{def}	1.282^{ab}	1.7
D	1,000	16,000	1.216	0.377	0.471^{def}	1.257^{b}	0.0
SEM[5]			0.036	0.012	0.009	0.041	1.4
					Probabilities		
Analysis of Variance							
Corn			0.314	0.329	≤0.001	0.234	0.801
Phytase			0.039	0.040	0.007	0.217	0.369
Xylanase			0.713	0.695	0.004	0.731	0.764
Corn × Phytase			0.714	0.721	0.006	0.371	0.633

Corn × Xylanase	0.320	0.349	≤0.001	0.002	0.489
Phytase × Xylanase	0.687	0.712	≤0.001	0.037	0.369
Corn × Phytase × Xylanase	0.871	0.849	0.001	0.120	0.633

[1]Analyses of feed were conducted on quadruplicate samples and analyses of digesta were conducted on duplicate samples. Nitrogen and energy digestibility were calculated on an apparent basis.
[2]Values are least-square means of 6 replicate cages of 20 birds per cage at 30 d of age. If a significant interaction was observed, means were separated via Tukey's Honestly Significant Difference test with $\alpha = 0.05$. Means within a column that do not share a common letter are significantly different.
[3]Sample number corresponds with Table 5.1. Corn 1, 3, 4, and 7 had extractable salt-soluble protein content values of 58.1, 54.2, 53.7, and 30.6 (BSA-equivalent values), respectively.
[4]Feed conversion ratio corrected for bird days.

Table 5.7. Pearson's product-moment correlation (r-values) between nutrient digestibility and growth performance variables [1,2]

	Item[3]											
Item	9 d IND	9 d IDE	29 d N intake	29 IND	29 d Starch intake	29 d ISD	29 d GE intake	29 d IDE	29 d BW	29 d BWG	29 d FI	29 d FCR
9 d IND	1.00											
9 d IDE	0.77**	1.00										
29 d N intake	-0.13	-0.07	1.00									
29 IND	0.04	-0.01	-0.06	1.00								
29 d Starch intake	-0.18	-0.07	0.85**	-0.10	1.00							
29 d ISD	0.33**	0.56**	-0.19	0.09	-0.12	1.00						
29 d GE intake	-0.17	-0.10	0.93**	-0.15	0.93**	-0.18	1.00					
29 d IDE	0.01	-0.09	0.03	0.84**	-0.03	-0.01	-0.06	1.00				
29 d BW	-0.02	0.03	0.26*	-0.18	0.26*	-0.10	0.29*	-0.08	1.00			
29 d BWG	-0.18	0.03	0.26*	-0.18	0.26*	-0.10	0.29*	-0.07	0.99**	1.00		
29 d FI	-0.18	-0.11	0.97**	-0.14	0.93**	-0.19	0.99**	-0.04	0.29**	0.29*	1.00	
29 d FCR	-0.07	-0.03	0.47**	0.08	0.44**	-0.03	0.48**	0.04	-0.57**	-0.57**	0.48**	1.00

[1]Sources of corn were primarily selected based on extractable salt-soluble protein content.

[2]IND = ileal N digestibility; IDE = ileal digestible energy; ISD = ileal starch digestibility; BWG = BW gain; FI = feed intake; FCR = feed conversion ratio.

[3]* = $P \leq 0.05$; ** = $P \leq 0.01$.

VI. CONCLUSIONS

Corn provides the majority of energy and a considerable portion of the dietary protein making it economically important to maximize recovery of these nutrients. Identification of potential nutritional value of corn, improvement of nutritional value, and reduction of variability among sources of corn is of utmost importance. Four experiments were conducted to address these needs.

The first experiment was designed to determine the relationship between chemical composition of corn and nutrient and energy utilization in broilers. It was concluded that PS was positively correlated with IDE and AME_n. Furthermore, assessment of corn quality by rapid-determination of PS content may be utilized to identify sources of corn that have unusually high or low AME_n. This strategy may assist nutritionists in formulating diets with greater precision to reduce monetary losses associated with poor quality corn.

The second and third experiments examined AA and energy digestibility effects of high concentrations of phytase (>750 FTU/kg) with and without xylanase supplementation in diets adequate in Ca and non-phytate P in broilers. Broilers fed diets supplemented with phytase exhibited increased AID of AA and energy. In experiment 3, increased BW gain and feed intake of female broilers paralleled increases in ileal digestibility of AA and energy. Xylanase supplementation did not interact with phytase supplementation and did not affect any of the variables measured, possibly due to a lack of microbial fermentative capacity either due to bird age or housing environment. The

studies concluded that extra-phosphoric effects may be achieved with diets if high concentrations of phytase (1,500 to 2,000 FTU/kg) are supplemented. The application of this nutritional strategy is dependent on feed ingredient prices, phytase efficacy, and price of phytase supplementation.

The fourth experiment evaluated the interactive effects of corn varying in PS content with phytase and xylanase supplementation in diets fed to broilers. At 9 d of age, no interactions were observed. Phytase increased IND and IDE at 9 d of age and the effect of phytase was more pronounced than at 29 d of age. Two and 3-way interactions were observed for ileal N and energy digestibility of broilers at 29 d of age. In many cases, opposing digestibility responses were observed in broilers fed diets based on different corn sources or differing in the supplemented enzymes. In conclusion, corn sources with similar proximate composition can vary greatly in nutritional value, especially with the addition of exogenous enzymes. Phytase supplementation with 1,000 FTU/kg above P adequacy can be used to improve amino acid digestibility of broiler diets. Xylanase response on nutrient and energy digestibility was transient and equivocal. Future research should further evaluate the use of xylanase in corn-based diets to clearly characterize the response.