Effect of Corn Quality and Enzyme Supplementation on Broiler Performance, Gastrointestinal Enzyme Activity, Nutrient Retention, Intestinal Mucin, and Jejunal Gene Expression

by

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(Abstract)

Three 2×2 factorial experiments (EXP) were conducted to investigate the underlying mechanism of corn quality and a supplemented cocktail enzyme of amylase, protease, and xylanase on broiler performance from 0 to 49 days of age. In each of the first two EXP, the four diets used consisted of (1) diet AR-/BR-; reduced dietary energy by increasing corn A matrix metabolizable energy (ME) 138 kcal/kg in EXP 1 or 125 kcal/kg ME with corn B in EXP 2; (2) diet AR+/BR+; AR-/BR- plus Avizyme 1502® (AZ); (3) diet AN-/BN-; normal energy diet; (4) diet AN+/BN+; AN-/BN- plus AZ. In EXP 3, four dietary treatments came from EXP 1 and 2 consisted of: AN-, AN+, BN-, and BN+. For each EXP, 1,440 male Ross 708 chicks were randomly assigned to one of the four dietary treatments (trts) with 9 replicates (reps)/trt and 40 chicks/ rep on day (d) 0. Body weight gain (BWG) and feed intake (FI) were determined on feed change days (d 14, 28, and 37) and on d 7 and 49. On d 28, subsets of birds were transferred to Petersime batteries to evaluate feed passage rate and nutrient retention. Digesta samples from gizzard, jejunum, and ileum as well as pancreatic tissue samples were collected for analyses of amylase, protease, and xylanase activities. Intestinal tissue samples were collected for determination of maltase, sucrase, and aminopeptidase N activities. Jejunal tissue on d 28 was also collected for total RNA isolation and a subsequent genome-wide microarray assay. On d 50, 54 birds per trt were processed to evaluate carcass yield. Interactions of ME and AZ were observed in both EXP 1 and 2. In EXP 1, interactions of ME and AZ on BWG were observed with higher values in birds fed AN- while lower in birds fed AN+, while opposite observations in EXP 2 with corn B diets. In both EXP 1

and 2, feed conversion ratio (FCR) in birds fed normal energy diets was better (P <0.05) as compared to birds fed reduced energy diets after d 28. Percent fatpad was increased (P <0.05) with dietary energy levels in EXP 1. Percent pectoral minor was increased (P <0.05) in birds fed AZ diets (4.80 vs. 4.62%). In EXP 3, interaction of dietary corn and AZ (P < 0.05) on BWG was noted during d 14 and 37. Birds fed corn B diets had better (P < 0.05) BWG and feed efficiency as compared to birds fed corn A diets until d 14. Nitrogen retention on d 30 was greater (P < 0.05) in birds fed AN- and BN+ as compared to birds fed AN+ and BN-. A 2.8% more apparent metabolizable energy with nitrogen correction (AMEn) in corn A diets was observed as compared to corn B diets. Corn A had higher amylase and xylanase activities as compared to corn B. Xylanase activity in jejunal and ileal digesta of birds fed corn A diets were higher (P < 0.05) as compared to that of corn B birds on d 7 and 49. Sucrase-isomaltase contributed 63, 80, and 74 % of the total maltase activity in duodenum, jejunum, and ileum. Sucrase activities in duodenum and jejunum were correlated (P < 0.05) with performance, whereas duodenal aminopeptidase N was negatively correlated (P < 0.05) with performance except period BWG. Pancreatic amylase and protease as well as gizzard protease and xylanase activities were correlated (P < 0.05) with performance. Number of jejunal genes regulated (P < 0.05) by corn variety was 77 as compared to those by enzyme supplementation in corn A diets (30 genes) and corn B diets (23 genes). Immune response and metabolism related genes were the most regulated genes in birds fed different corn diets without enzyme addition. In conclusion, enzyme supplementation improved broiler performance. Dietary formulation strategy using either reduced energy or normal energy in associated with enzyme supplementation should base on the feed ingredient quality. Corn quality may come from active components such as protease inhibitor and xylanase, and improved performance in birds fed high quality corn diets might also relate with minimal immune response and metabolic demand.

Key words: broiler, energy, corn, enzyme, performance

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Abbreviations used in this dissertation

EXP: Experiment

AN-: Diet of corn A and normal energy diet without enzyme

AN+: Diet of corn A and normal energy diet with enzyme (AN- plus Avizyme)

AN-/+: Corn A normal energy diets

AR-: Diet of corn A and reduced energy without enzyme

AR+: Diet of corn A and reduced energy with enzyme (AR- plus Avizyme)

AR-/+: Corn A reduced energy diets

BN-: Diet of corn B and normal energy diet without enzyme

BN+: Diet of corn B and normal energy diet with enzyme (BN- plus Avizyme)

BN-/+: Corn B normal energy diets

BR-: Diet of corn B and reduced energy without enzyme

BR+: Diet of corn B and reduced energy with enzyme (BR- plus Avizyme)

BR-/+: Corn B reduced energy diets

Trt: treatment

BWG: Body weight gain

FI: Feed intake

FCR: Feed conversion ratio (FI : BWG)

Eng: Energy

Enz: Enzyme

Cn: Corn

ME: Metabolizable energy

AMEn: Apparent metabolizable energy corrected with nitrogen retention

GIT: Gastrointestinal tract

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Chapter 1. Introduction

Corn has been utilized as a major energy feed ingredient in poultry diets because of its high available energy content and low soluble non-starch polysaccharides, which are an anti-nutrient factor (Iji *et al.,* 2003). In spite of this advantage, the nutritional value of corn varies widely as a result of climate changes during growth and harvest as well as processing and storage (Leeson *et al.,* 1993). Corn samples obtained from various locations, seasons, or years show different metabolizable energy (ME) as well as varied composition (Leeson and Summers, 1976; Maier, 1995; Collins *et al.,* 1998). Corn ME is mainly affected by the quality of corn starch, which is categorized into three classes: rapidly digestible starch, slowly digestible starch, and resistant starch (Englyst *et al.,* 1996). The more rapidly digestible the starch, the greater ME and quality the corn should have. Varied broiler nutrient retention and performance are reported as a result of birds being fed different varieties of corn. Retention of alanine and valine was different when birds were fed two varieties of corn (Song *et al.,* 2004). Birds fed heat-treated corn have improved feed efficiency, which comes from increased rapidly digestible starch (Iji *et al.,* 2003).

Besides heat-treating corn, supplementation of microbial enzymes has been shown to be a feasible alternative to reduce variation of corn ME (Cowieson, 2005) as well as to decrease environmental pollution (Francesch *et al.,* 2005). To optimize the use of microbial enzymes in corn soybean meal diets, corn is qualified by the degree of predicted ME released by microbial enzyme supplementation (Cowieson *et al.,* 2006a; Cowieson *et al.,* 2006b). Generally, to account for the uncertainty in both the bird's nutrient requirements and feed ingredients, two strategies for formulating diets are applied when supplementing enzymes: reducing energy level in the diet to utilize the respective energy released by enzyme addition (Cowieson and Adeola, 2005); or supplementing enzyme additives over the top of balanced diets. Higher ileal amino acid digestibility, body weight gain, and feed efficiency were reported when birds were fed corn soybean meal diets supplemented with exogenous enzymes (Zanella *et al.,* 1999; Cowieson *et al.,*

2006a). The increased nutrient availability with enzyme supplementation is associated with improved digestibility or retention of energy, protein, or other nutrients. Chicks consuming soybean meal diets supplemented with protease had higher body weight gain, apparent nitrogen retention, and apparent metabolizable energy without alteration of feed conversion ratio (Ghazi *et al.,* 1997a). Increased performance and digestibility of P, Ca, and amino acids were observed in birds fed an enzyme cocktail of amylase, protease, xylanase, and phytase (Cowieson *et al.,* 2006b). Performance improvement resulted from increased nutrient digestibility and reduced loss of endogenous secretions such as mucin (Cowieson *et al.,* 2003). Mucin is the main component of gastrointestinal mucus and is responsible for the protection and lubrication of the gut epithelium (Montagne *et al.,* 2000). Sialic acids are components of cellular mucin and can give an indication of endogenous secretion loss when determined in digesta (Cowieson *et al.,* 2004). Mucin secretion is mediated by dietary components (Enss *et al.,* 1994) and short chain fatty acids produced by bacterial fermentation (Barcelo *et al.,* 2000).

Performance improvement was observed inconsistently in birds fed diets with enzyme supplementation (McCracken *et al.,* 2001). Lack of improvement in performance with enzyme supplementation has been observed, while nutrient digestibility was still improved (Iji *et al.,* 2003; Troche *et al.,* 2007). Therefore, it seems that enzyme supplementation enhances nutrient digestibility no matter whether performance is improved or not. How enzyme supplementation increases nutrient digestibility becomes important. Amylase activity in the crop, pancreas, or small intestine of the poult has not been consistently changed by amylase and xylanase supplementation (Ritz *et al.,* 1995). Depressed pancreas secretion was observed when researchers administrated trypsin in the duodenum (Owyang *et al.,* 1986). Birds with early feed restriction and dietary exogenous protease and amylase addition to feed had increased sucrase, maltase, and lipase activities as compared to birds fed control diets (Pinheiro *et al.,* 2004). Components of dietary ingredients may also affect pancreatic secretion and subsequent intestinal

enzyme activity. When phenylalanine was injected into chick wing veins, increased trypsinogen and chymotrypsinogen secretion was observed (Yang *et al.,* 1989). Zinc oxide supplementation increased amylase, lipase, trypsin and total protease activity in rat pancreatic homogenates and small intestinal digesta (Szabo *et al.,* 2004).

Most balanced diet formulations are currently based on proximate nutrient values. Increasing evidence suggests that nutrient values of dietary ingredients are also affected by active components such as enzyme inhibitors. Trypsin inhibitor in soybean has been known to exist for a long time. Reduced performance was reported when birds were fed a normal raw soybean diet as compared to birds fed a diet containing soybeans with the trypsin inhibitor genetically removed (Palacios *et al.,* 2004). Increased pancreatic secretion was observed when the protease inhibitor camostat is applied in the duodenum (Adler *et al.,* 1988). Currently, soybean meal is processed to reduce or eliminate the inhibitor prior to being utilized in animal diets (Lusas, 2000). Although corn accounts for more than sixty percent of the final diet, little attention has been given to possible active components in raw corn, such as native enzymes and inhibitor(s). Indeed, a bi-functional Hageman factor inhibitor from corn was reported to inhibit animal protease and amylase activities (Behnke *et al.,* 1998).

In the present research over three experiments, birds were fed diets containing two varieties of corn with or without exogenous enzymes and dietary energy modification. Performance and gastrointestinal tract physiological parameters were analyzed. The objective of these experiments was to understand the underlying mechanisms of dietary effects of corn quality and enzyme supplementation.

Chapter 2. Review of literature

Corn and its quality

Corn classification

Corn has been defined as a grain that consists of 50% or more of whole kernels of shelled dent corn and/or shelled flint corn (*Zea mays L.*) and not more than 10% other grains for which standards have been established under the United States Grain Standards Act (USDA-GIPSA, 1996). Corn has been categorized into five general classes by kernel characteristics: dent corn, flint corn, pop corn, flour corn, and sweet corn. Flint corn has a hard starchy layer entirely surrounding the outer part of the kernel. Pop corn has a high proportion of hard starch that expands rapidly when heated, resulting in an explosive rupture of the epidermis and starch granules. Sweet corn has a high level of sugars in its endosperm. Most commercial corn is dent corn, which is a derivative of flint-flour crosses. Corn is primarily a source of animal feed, as well as of food and seed & industrial (FSI) including ethanol for fuel (EFF). Total US annual production of grain corn was 10.5 billion bushels in 2006. About 47.8, 28.3 (17.2 %, EFF), and 18.0 % were used for animal feed, FSI, and export in 2006, respectively (USDA-NASS, 2007).

There have been four factors in grading standards for corn: test weight, heat-damaged kernels, total damaged kernels, and broken corn and foreign material (USDA-GIPSA, 1999). Test weight is measured as pounds of corn per Winchester bushel. Heat-damaged kernels are evaluated as kernels and pieces of corn kernels materially discolored and damaged by heat. Total damage is defined as kernels and pieces of corn kernels that are badly ground-damaged, badly weather-damaged, diseased, frost-damaged, germ-damaged, heat-damaged, insect-bored, mold-damaged, sprout-damaged, or otherwise materially damaged. Broken corn and foreign material are measured as the sum of all material passing through a 4.76 mm round-hole sieve

plus all matter other than corn remaining on top of the sieve. The specific levels for corn grades from one to five are listed following as minimum test weight (lb/bu), heat-damaged kernels (%), total damaged kernels (%), and broken corn and foreign material (%), respectively: Grade one – 56, 0.1, 3.0, and 2.0; Grade two – 54, 0.2, 5.0, and 3.0; Grade three – 52, 0.5, 7.0, and 4.0; Grade four – 49, 1.0, 10.0, and 5.0; Grade five – 46, 3.0, 15.0, and 7.0 (USDA-GIPSA, 1999). Number 2 grade corn dominates the US market, whereas No. 3 corn dominates the U.S. export market.

Corn composition

Most yellow dent corn has been harvested at maturity when the moisture content of the kernel was between 22 to 25% and dried artificially to 15 to 16% moisture for storage and marketing. The chemical composition (%) of corn is moisture of 7 to 23 (average 16), starch of 61 to 78 (71.7), protein of 6 to 12 (9.6), fat of 3.1 to 5.7 (4.3), and ash of 1.1 to 3.9 (1.4) (Blessin *et al.,* 1963).

The corn kernel has been botanically classified as a caryopsis of dry, indehiscent, single-seeded fruit (Wolf *et al.,* 1952). A corn kernel is formed on the female inflorescence of the ear. About eight hundred kernels are on a developed hybrid ear and are removed from the inner cylinder cob of the ear by shelling. The corn is composed of a central pith core of large parenchyma cells which stores nutrients from the plant. It is surrounded by a tough fibrous layer containing vascular bundles that deliver nutrients into each developing kernel (Bonnett, 1954).

Corn kernels consist of three main parts: endosperm, germ, and pericarp. The endosperm consists of 82 to 84% of the kernel dry weight and constitutes 86 to 89% starch by weight (Bonnett, 1954; Blessin *et al.,* 1963). It is comprised of mostly elongate cells packed with round or polyhedral starch granules 3 to 25 μm in diameter (Schoch and Maywald, 1956).

Each granule is embedded in a continuous protein matrix of proteinacious phase. Endosperm cells wrapped in protein matrix become progressively smaller and thicker from the central fissure to the outer endosperm caused by inward pressure as the pericarp cap is pressed downward to replace the lost water. The germ is composed of the embryo and the scutelliu and makes up 10 to 12% of the kernel dry weight. The germ stores nutrients and hormones that are utilized by enzymes present during the initial stages of germination (Logan *et al.,* 2001). The germ deposits 81 to 85% of the total kernel oil mostly in triacylglyceride form (Earle *et al.,* 1946). The pericarp of the hull is the outermost covering of the kernel and makes up 5 to 6% of the kernel dry weight.

Most of the energy in corn is derived from the starch fraction that is found in the endosperm of the kernel. Starch granules are different in size and composition, depending on the type and variety of cereal as well as the age of the cells in the developing endosperm (South *et al.,* 1991). Starch has been classified into three categories: rapidly digestible starch (amylopectin) which is starch gelatinized by cooking or heating; slowly digestible starch (amylose) which is native starch granules that may be slowly but completely digested; and resistant digestible starch that is resistant to digestion (Englyst *et al.,* 1996). Three subcategories of resistant starch have been identified: (1) starch granules trapped in food matrix/grain are physically inaccessible, resulting in delayed the breakdown of the granules by digestive enzymes; (2) native starch granules are resistant to digestion due to their special structure and conformation, for example the gelatinization temperature of some starches is 70^{o}C while the gelatinization temperature for high amylose corn is around 170°C; and (3) retrograde starch material is formed during processing such as high temperature cooking followed by storage at lower temperatures over long periods of time (Brown, 1996). Retrograde and resistant starch can vary significantly with regard to enzyme susceptibility

(Eerlingen *et al.,* 1994). This type of starch usually passes undigested to the lower parts of the intestine (Brown, 1996).

Corn starch composed of 25 to 30% amylose and 70 to 75% amylopectin can be dissolved in water by heating beyond the gelatinization temperature (Marshall and Whelan, 1974). Upon cooling, the dispersed molecules of amylose and amylopectin can spontaneously reorganize and form crystallites that resist enzymatic hydrolysis. The amylose proportion increases with starch granule age and size. Some of the corn mutants differ in amylose concentration, resulting in wide variations in the digestive responses such as susceptibility to enzyme degradation and gelatinization. The digestibility of corn components was greatly influenced by the starch composition, especially the ratio between slow digested starch (amylose) and fast digested starch (amylopectin) (Englyst *et al.,* 1996).

The ratio of available energy to gross energy was higher for corn when compared with other commonly used feedstuffs (86.8, 78.9, 69.2, and 59.1 % for corn, wheat, barley, and oats, respectively) (Summers, 2001). The quality of corn produced by farmers varies greatly in all quality factors because of differences in soils, climate, insects, disease, hybrids, and management practices in regard to harvesting, drying, storing, etc. The variation of corn nutrients in Indiana was reported by sampling from different areas of the state (Maier, 1995). A wide alteration in nutrients was noted (based on 15% moisture): crude protein from 5.7 to 9.7 %, crude fat 2.6 to 4.9 %, starch 59.9 to 64.8, and density 1.2 to 1.31%. Corn also has variation in density or weight per bushel due to different growing seasons. There was a marked range in bushel weights and also composition values when corn samples were harvested during a wet, cold fall (Leeson and Summers, 1976). While bushel weights were decreased by approximately 40%, metabolizable energy varied by only 12% as determined with adult roosters. The lower bushel weight samples had a higher level of protein as compared to the more normal corn, while levels of lysine and methionine were not increased in the same proportion as protein

(Lilburn and Dale, 1989). Meanwhile, as protein level in different corn samples increased, the relative increase in non-essential amino acids was greater than that of the essential amino acids (Whitacre, 1987).

Corn in animal diets

Corn is one of the most important ingredients in poultry diets. It is relatively devoid of viscous non-starch polysaccharides, the principal anti-nutrient factors present in most feed cereals (Iji *et al.,* 2003). Due to the fact that corn has a highly available energy content and a low protein value, corn has been applied as a major energy source in a diet. Since the poultry diets, especially the broiler type, consist of 60 to 70 % corn, more than 20 % protein is attributed to corn. In spite of this advantage, the nutritive value of corn has been known to vary widely, as a result of climatic conditions during growth and harvest as well as post-harvest processing and storage (Leeson *et al.,* 1993). For broiler chickens, the terminal ileal digestibility of corn starch rarely exceeded 85% (Noy and Sklan, 1995). The undigested starch at the terminal ileum was assumed to be resistant to digestion by the animals and therefore supplementation of microbial enzymes in corn-based diets was necessary (Iji *et al.,* 2003). When birds were fed diets with a large amount of viscous soluble non-starch polysaccharide, increased fermentation levels were observed in the small intestine and this was detrimental to the performance and well-being of the birds (Choct *et al.,* 1996). Increased undigested corn starch in the ileum may have similar fermentation and adverse effects on bird growth.

To improve corn nutrition values, genetically-engineered corn with increased oil and/or protein concentrations has been developed. There was no difference in protein quality between the high oil corn and normal corn as measured by a net protein ratio test as well as other parameters (Han *et al.,* 1987). Broiler chicks fed the high oil corn had similar body weight gain at 22 days of age while feed efficiency was improved as compared to birds fed normal corn.

Similarly, laying hens fed the high oil corn had an improved ratio of feed to egg mass as compared to hens consuming normal corn. In another experiment, two high protein maize varieties contained approximately 11 % protein versus 9% in normal corn used in layer and broiler trials (Bond *et al.,* 1991). Layer hens fed higher protein corn for 6 months had better feed efficiency of feed to egg mass ratio as compared to hens fed normal corn, while egg production and egg size were similar. Broilers fed high protein corn on d 42 had greater body weight gain and feed conversion, possibly resulting from the beneficial effect of higher levels of methionine and several other essential amino acids in the high protein corn.

An alternative to increasing nutrient values of corn by genetic-engineering, improving utilization (digestibility and/or retention) of corn nutrients, has been explored for a long time. Several researchers have questioned apparent metabolizable energy (AME) and true metabolizable energy (TME) values used to indicate the energy utilized by the bird from corn. The corn AME for broiler chickens differed by 200 to 250 kcal/ kg from the first week post-hatch to the third week (Mahagna *et al.,* 1995). Similarly, the age of the bird was reported as an important factor when estimating available energy value of corn (Collins *et al.,* 1998).

Low ileal digestibility of both starch and fat was observed in young broiler chicks fed a corn soybean meal diet (Noy and Sklan, 1995). Amylase, trypsin and lipase activities in the pancreas were low on 4 days of age, while on 21 days of age they increased by 100-, 50- and 20-fold, respectively. Only 82 to 89% of fatty acids and starch were digested in the small intestine from d 4 to d 21. While nitrogen digestion was also not complete in the small intestine, it increased from around 78% on d 4 to 92% on d 21. Values used for available energy for the young chick show the possibility for improvement by the use of specific enzymes. When studying the AME and starch digestibility in the small intestine of turkey poults, there was a difference in AME for wheat and maize when measured in the small intestine compared to the colon (Persia and Lilburn, 1998). Microscopic examination of the ileal digesta showed large

undigested corn endosperm particles. About 5.21 % corn starch and 2.67% wheat starch reached the hind gut and underwent fermentation with poor energy utilization by the bird.

Another factor affecting the response of poultry to corn based diets is the type of corn grinding. One of the major approaches to improve the nutritive value of cereals for animal feeding is to grind the material. Grinding increases the surface area and enhances the opportunity to break down corn particles by digestive enzymes in the gut. Birds fed hammer-milled corn processed through a 9.53 mm screen had similar performance to birds consuming corn processed through a 6.35 or a 3.18 mm screen (Deaton *et al.,* 1995). Performance was greater in birds fed diets with medium size (0.769 mm geometric mean diameter) corn as compared to coarse or fine size (0.793 and 0.706 mm) corn diets. This improvement was partially attributed to lower geometric standard deviation in medium size corn (Nir *et al.,* 1994).

The glycemic index of foodstuffs has been used in human nutrition to manipulate glucose absorption rate in order to prevent metabolic disorders or to enhance athlete performance (Jenkins *et al.,* 2002). When fed diets that were iso-energetic but different in terms of starch digestion rate from d 9 to 30, birds consuming diets containing slowly digestible starch had better feed conversion than birds fed diets with rapidly digestible starch (Weurding *et al.,* 2003). Moreover, an interaction between starch digestion rate and amino acid content was observed. Supplementing casein and glutamine in a diet with slowly digestible starch did not improve the feed conversion ratio, but when casein and glutamine were added to a diet containing rapidly digestible starch, the feed conversion ratio was improved. These results might be related to the efficiency of protein assimilation which was affected by insulin levels (Vaugelade *et al.,* 1994). The energy supplying the intestinal wall might also be involved. Intestinal transport of absorbed nutrients coincides with their partial catabolism in the gut. The gastrointestinal tract consumes approximately 20% of all dietary energy to support digestive and absorptive processes. Therefore, metabolic activity of the small intestine also affects the

supply of nutrients to other tissues in the body (Cant *et al.,* 1996). Glutamine and glucose are major energy sources for the small intestine (Fleming *et al.,* 1997). It is speculated that rapidly digestible starch was almost entirely digested and assimilated in the upper small intestine and provided less glucose for its energy demands in the lower part (Weurding *et al.,* 2003). In this case, more amino acids might be oxidized for that purpose. Slowly digestible starch was partly digested in the lower small intestine and supplies glucose to this segment, thereby preventing amino acids from being oxidized for energy demand.

Dietary energy and protein, performance, and carcass yield

Dietary energy level, or energy to protein ratio, besides its effect on weight gain and feed efficiency of broiler chicks, has a great effect on the quality of broiler carcasses, such as on the yields of meat and fat content. Diets lower in protein than recommended (NRC, 1994) reduced the yields of meat and increased fattening (Moran and Bilgili, 1990; Bartov, 1996). When evaluating the effect of diets on carcass quality, energy to protein ratio seems to be more useful as compared to protein level alone. Although practical diets might markedly differ in protein and energy concentrations, they had the same energy to protein ratio and resulted in a similar degree of fatness (Bartov, 1979; Alao and Balnave, 1985). The optimal dietary protein level and lysine requirement for feed efficiency was higher as compared to that for body weight gain (Fancher and Jensen, 1989; Han and Baker, 1994).

The optimal dietary lysine level for feed efficiency was also similar to that for breast yield (Han and Baker, 1994) but was higher as compared to that for maximal growth rate (Bilgili *et al.,* 1992). The optimal level of total sulfate amino acids for feed efficiency was reported to be equal to that for breast yield and higher than that for maximum weight gain (Schutte and Pack, 1995). To reduce environmental concern with nitrogen excretion from birds and to decrease feed cost, a feed formulation strategy has been used to reduce dietary protein

level while supplementing with essential amino acids such as methionine and lysine. These diets usually supported adequate weight gain but increased fatness and reduced meat yield (Deschepper and De Groote, 1995). Negative effects on performance and meat yield were observed in birds fed diets with extremely high protein levels (Holsheimer and Veerkamp, 1992), but the effects of diets with moderately increased protein levels has not been reported. The yields of carcass and breast meat were increased by postponing slaughtering age (Holsheimer and Veerkamp, 1992). Reducing dietary protein (186 or 203 versus 217 g/kg) decreased performance and nitrogen retention but increased ME (Shafey and McDonald, 1991).

Enzyme supplementation in animal diets

The availability of nutrients in feedstuff is often limited by the presence of anti-nutritional factors. Six anti-nutritional factors have been identified in vegetable proteins such as soybean meal (Huisman and Tolman, 1992). The first group of factors contains a depressive effect on protein digestion and on the utilization of protein such as protease inhibitors, lectins, phenolic compounds, and saponins. The second group of factors has a negative effect on the digestion of carbohydrates such as amylase inhibitors, phenolic compounds, and flatulence factors. The third group of factors consists of negative effects on the utilization of minerals such as glucosinolates, phytic acid, oxalic acid, and gossypol. The fourth group of factors inactivates vitamins, or causes an increase in the animal's vitamin requirements. The fifth group of factors stimulates the immune system that may cause a damaging hypersensitivity reaction such as antigen proteins. The sixth group of factors in feed has a toxic effect such as lectins and cyanide-containing compounds. Heat processing at appropriate temperature has been used to reduce or eliminate anti-nutritional factors in soybean meal (Lusas, 2000).

Exogenous enzymes may be supplemented in diet in addition to heat treatment to help reduce residual dietary anti-nutrition factors. Moreover, there are many other important

demands for the use of exogenous enzymes (Johnoson *et al.,* 1993). First, there is an increasing shift in the use of alternative feedstuff in formulating dies. Second, the use of enzymes has been known to be effective against particular dietary components. Third, novel by-products such as linseed meal have a depressing effect on growth. Fourth, there is introduction of excreta pollution control by the government, examples of which include phytase and protease reducing excretion of phosphorus and nitrogen (Francesch *et al.,* 2005). Fifth, there are the indirect physiological actions on problems of commercial importance, for example the reduction of sticky litter in poultry and post-weaning diarrhea in piglets (Inborr and Ogle, 1988). Sixth, there is trend toward reduction in animal performance due to restriction on the use of growth-promoting antibiotics (Sun *et al.,* 2005).

Amylase

The amylase family of enzymes has been used in a number of industrial processes such as food fermentation, textiles, paper industries, feed industries, etc. The main classes of amylases act on starch, which include α-amylase (EC 3.2.1.1), β-amylase (EC 3.2.1.2), and glucoamylase (EC 3.2.1.3). Alpha-amylase randomly splits α-1,4-glucosidic bonds between adjacent glucose units in linear amylose chains, while glucoamylase hydrolyzes single glucosidic residues from the non-reducing ends of amylose and amylopectin in a stepwise manner. Glucoamylase also hydrolyzes the α-1,6-linkages in the branching points of amylopectin at a slower rate as compared to the α-1,4-linkages.

Alpha-amylase has a molecular weight (MW) range of 50 kD, and requires Ca^{++} for stability and activity. The optimum pH varies depending on the enzyme source (6.0-7.0 for mammalian, 4.8-5.8 for *Apspergillus oryzea*, 5.85-6.0 for *Bacillus subtilis*, 5.5-7.0 for *B. licheniformis*). Optimum temperature for activity varies from 70 to 72^{o}C for the α-amylase produced by *B. subtilis*, and 90 °C for the enzyme from *B. licheniformis*. Commercially used α-

amylase, in particular for starch liquefaction, is most often obtained from *B. licheniformis* (Dawson and Alllen, 1984).

Birds fed a diet supplemented with α-amylase ate more, grew faster, and had better feed conversion than broilers fed the control diet (Gracia *et al.,* 2003). Also, α-amylase supplementation resulted in improved apparent fecal digestibility of organic matter and starch, increased AMEn of the diet, and reduced pancreas weight. Birds fed diets containing adherent *Lactobacillus* cultures, either as a single strain of *L. acidophilus* or a mixture of 12 *Lactobacillus* strains, had increased amylase activity in the small intestine, possibly due to extracellular secretion of amylase by the bacteria (Jin *et al.,* 2000). Some carbohydrate enzymes were effective in the *in vitro* test of cell wall polysaccharide solubilization, only a trend toward improved performance was noted when broiler chickens were fed semi-purified canola meal diets supplemented with these enzymes (Simbaya *et al.,* 1996). There might be differences between nutrients released by the enzymes and nutrients utilized by chickens, which was attributed to the discrepancy between high digestibility *in vitro* and utilization *in vivo*.

Protease

Physiologically, proteases have a major function in cellular catabolism of digestion and protein turnover of the immune system (Barrett, 1994a). Exopeptidases and endopeptidases are a complex group of enzymes capable of hydrolyzing the peptide bond in a protein molecule. Exopeptidases cleave the peptide bond proximal to the amino or carboxyl termini of the substrate, whereas endopeptidases cleave internal peptide bonds. Based on the functional group present at the active site, proteases are classified into four groups: serine (EC 3.4.21), cysteine (EC 3.4.22), aspartic (EC 3.4.23), and metalloprotease (EC 3.4.24). Serine proteases are characterized by the presence of a serine group in their active site; trypsin, chymotrypsin, and

carboxypeptidase of pancreatic proteases are in this group (Brenner, 1988). The serine proteases have conserved glycine residues in the vicinity of the catalytic serine residue to form the motif Gly-Xaa-Ser-Yaa-Gly. Aspartic acid proteases, also known as acidic proteases, are the endopeptidases that depend on aspartic acid residues for their catalytic activity (Barrett, 1995). Acidic proteases have been grouped into three families, pepsin, retropepsin, and enzymes from pararetroviruses. The activity of all cysteine proteases depends on a catalytic dyad consisting of cysteine and histidine with the presence of reducing agents such as HCN or cysteine (Barrett, 1994b). Based on their side chain specificity, cysteine proteases are categorized into four groups: papain-like, trypsin-like with preference for cleavage at the arginine residue, specific to glutamic acid, and others. Metalloproteases are characterized by the requirement for a divalent metal ion for enzyme activity (Barrett, 1995). Based on the specificity of their action, metalloproteases can be divided into four groups: neutral, alkaline, *Myxobacter* I, and *Myxobacter* II.

The proteolytic and lipolytic activities in broiler small intestine were not changed by supplementing either a single strain of *L. acidophilus* or a mixture of 12 *Lactobacillus* strains (Jin *et al.,* 2000). Piglets fed acid protease - treated SBM gained more weight over the first 7 days post-weaning as compared to piglets fed untreated SBM, and there were no changes in small intestine enzyme activities and villus height or crypt depth in response to the dietary treatments (Rooke *et al.,* 1998). No enteric disease was observed by histopathological examination in piglets fed either diets. Broiler chicks fed soybean meal diet treated with acid protease had greater body weight gain and dry matter feed intake without change of feed conversion ratio (Ghazi *et al.,* 1996b). The improvement of performance was due to the increased nitrogen digestibility by supplementing the protease (Ghazi *et al.,* 1996a). Enhanced protein hydrolysis of canola meal was observed for several protease enzymes evaluated by *in vitro* digestion experiments. Protein hydrolysis was most effective and exceeded those for

pancreatic enzymes acting alone when pancreatic enzymes were included in the incubation medium along with the protease enzymes (Simbaya *et al.,* 1996). The supplementation of the protease also improved broiler performance when added to semipurified canola meal diets. No improvement of performance on body weight gain and feed conversion ratio was observed when broilers were fed a corn-soybean diet supplemented with only protease (Marsman *et al.,* 1997). Inclusion of exogenous protease could partially hydrolyze the dietary protein and enhance protein utilization, and this enzyme might also depress endogenous protease secretion, which decreased the improvement in digestibility (Inborr, 1990). An improvement in broiler performance by including an acid protease (isolated from *Aspergillus* strains) in a soybean meal diet was observed (Ghazi *et al.,* 1997a), while broiler performance did not change when another alkali protease (isolated from *Bacillus* strains) was supplemented (Ghazi *et al.,* 1996b). Protein digestibility and intestinal protease activity did not change when broilers were fed plant-origin protease, while gizzard pH increased and ileum pH decreased on d 21 (Yu *et al.,* 2002).

Xylanase

Plant cell walls are mainly composed of cellulose 40 to 45%, hemicellulose 30 to 35%, and lignin 20 to 23%. Cellulose and hemicellulose are the major plant structural polysaccharides (Ladisch *et al.,* 1983). Cellulose is a linear polymer of glucose linked by β-1,4 -glycosidic bonds with simple primary and complex tertiary structures. Based on the main sugar residues present in the polymer backbone, hemicelluloses can be termed xylans, glucomannans, galactans or arabinans with xylans and glucomannans being the two main types of hemicelluloses (Timell, 1967). Xylans from annual plants, called arabinoxylans, are more heterogeneous as compared to xylans from perennial plants. The two major types of arabinoxylans are highly branched without uronic acid substitution in cereal endosperms and

much less branched substituted with uronic acid and/or with 4-*O*-methyl ether and galactose in lignified tissues. Arabinoxylans of gramineous plants contain acetic and phenolic acids (ferulic, *p*-coumaric) which are esterified to the backbone xylose units and the arabinose side groups, respectively (Hartley and Ford, 1989).

Hemicellulose can be converted to soluble sugars by enzymes, mainly of microbial origin termed hemicellulases (Suurnakki *et al.,* 1997). The two main enzymes involved in breakdown of the hemicellulose backbone are endoxylanases (xylanases) and endomannanases (mannanases) to hydrolyze xylan and mannan, respectively (Franco *et al.,* 2004). Xylobiose, xylotriose and substituted oligomers with two to four residues are released. Other hemicellulases, including β-xylosidase, β-mannosidase, α-L-arabinofuranosidase, α-D-glucuronidase, α-galactosidase, acetyl and phenyl esterases, remove side-chains and substituents (Shallom and Shoham, 2003). In general, xylanases specifically hydrolyze the internal β-1, 4 linkages of polymeric xylan and are called endoxylanases. Based on their action on different polysaccharides, endoxylanases have been classified as either specific or non-specific (Sunna and Antranikian, 1997). The specific endoxylanases break down xylans at only β-1, 4 linkages, while non-specific endoxylanases hydrolyze β-1, 4-linked xylans, β-1, 4 linkages of mixed xylans and other β-1, 4-linked polymers such as carboxymethyl cellulose. Xylanases are inhibited by the presence of high concentrations of their hydrolysis products. For example, endoxylanases are believed to be inhibited by high concentrations of xylobiose but not by xylose. In general, the activity of xylanases is neither activated nor inhibited by metal ions and reducing agents (Birsan *et al.,* 1998).

Cocktail of exogenous enzymes

To optimize the benefits of exogenous enzyme supplementation to the diets, strategies using combinations of carbohydrase, protease, xylanase, or phytase have been proposed and

applied in animal production. A combination of cellulase, pectinase, xylanase, glucanase, galactanase, and mannanase improved weight gain, feed efficiency, AMEn, apparent ileal digestibilities of starch and protein, and apparent total tract digestibility of non-starch polysaccharide in birds fed a wheat, wheat screening, SBM, canola meal, and peas-based diet (Meng *et al.,* 2005). Digestibility of P, Ca, and certain amino acids was improved by supplementing a combination of xylanase, amylase, protease and phytase (Cowieson *et al.,* 2006b). Birds fed a positive control diet with enzymes also had a positive response indicating that supplementation of xylanase, amylase, protease, and phytase over-the-top might be a nutritional and economical alternative to a reformulated approach.

Overall, crude protein digestibility was increased by 2.9%, valine by 2.3%, and threonine by 3.0% when broilers were fed a diet supplemented with 0.1% Avizyme1500, which contained 800 U/g xylanase from *Trichoderma longibrachiatum*, 6,000 U/g protease from *Bacillus subtilis*, and 2,000 U/g amylase from *Bacillus amyloliquifaciens* (Zanella *et al.,* 1999). The enzyme supplementation also improved body weight and feed conversion ratio by 1.9 and 2.2% on d 45, respectively. Pancreatic amylase, lipase, and chymotrypsin activities were greater when broilers were subjected to feed restriction and their diets were supplemented with a 0.06% combination of 25,000 U/g protease and 5,000 U/g from a mixture of dried *Aspergillus oryzae* and *Bacillus subtilis* fermentation products (Pinheiro *et al.,* 2004).

Chicks fed soybean meal diets supplemented with protease and/or α-galactosidase increased body weight gain, apparent nitrogen retention, and apparent metabolizable energy without alteration of feed conversion ratio (Ghazi *et al.,* 1997b). Similarly, supplementing proteases and/or α-galactosidase in broiler diets increased nitrogen retention and true metabolizable energy (Ghazi *et al.,* 1997a). A synergistic response in growth of broilers from d 4 to 11 was observed when phytase, carbohydrase, and protease were supplemented to a wheat/canola meal based diet deficient in available phosphorus (Simbaya *et al.,* 1996).

Enzyme supplementation and feeding management may affect bird feed passage rate and subsequent nutrient utilization. Birds under heat stress increased the rate of feed passage through the gut and altered digestion and subsequent feed utilization (Washburn, 1991). Time needed to recover 1% and 50% of a chromium oxide marker in feces and the mean retention time of the marker in the gastrointestinal tract was decreased when birds were fed rye diets supplemented with β -glucanase and xylanase (Lazaro *et al.,* 2003). Meanwhile, enzyme supplementation also reduced intestinal viscosity (Cowieson *et al.,* 2005). Birds fed low ME and phosphorus diets had decreased weight gain and feed efficiency (Olukosi *et al.,* 2007). A xylanase, amylase and protease cocktail did not improve performance in a low phosphorus, ME diet, but improved weight gain was observed with the addition of phytase in the diet. Ileal nitrogen digestibility was improved in birds fed xylanase or phytase individually, whereas there was no effect from the enzyme supplementation on ileal energy digestibility. Digestibility of ileal energy and protein in poults fed reduced dietary energy was lower than those of birds fed a normal energy corn-based diet (Troche *et al.,* 2007). When an enzyme cocktail of amylase, protease, and xylanase was supplemented in the reduced energy diet, improved energy and protein digestibilities was noted on d 42.

Intestinal enzymes

After polymer nutrients of starch and protein are digested into maltose/maltotriose and peptides, the smaller nutrients are further degraded by intestinal enzymes into mono-nutrients which are then absorbed by the intestine. Maltase, sucrase, and aminopeptidase are the most investigated intestinal enzymes in nutrition research. Increased pancreatic enzyme activities with age were associated with changed dietary composition (Nir *et al.*, 1993). Total intestinal activities of sucrase, maltase, aminopeptidase, and alkaline phosphatase in chickens were also

elevated with age due to the increase in surface area by an increase in villus height and intestinal length rather than an increase in the efficiency of individual cells (Iji *et al.*, 2001).

Maltase and sucrase

Four small intestinal mucosal α-1,4 exoglucosidases activities were usually assayed as maltase and identified as isomaltase, invertase or sucrase, maltase II, and maltase III (Dahlqvist and Telenius, 1969). Two maltase activities were associated with the sucrase and isomaltase activities in the small intestine. Two other maltase activities, not associated with any other identifiable activities, were called maltase-glucoamylase (Eggermont, 1969). Subsequent investigations revealed that these four maltases shared α-glucogenic activities for all α-1,4 glucoside substrates from G2 to G7 in length (Heymann and Gunther, 1994; Heymann *et al.,* 1995; Gunther and Heymann, 1998).

Cornish × Rock cross chickens fed a carbohydrate-free (CF) diet had longer intestines and larger intestinal areas as compared to chickens fed on a carbohydrate-containing (CC) diet (Biviano *et al.,* 1993). Maltase activity in chicks fed both diets increased from d 1 to d 17. After d17, chickens fed a CC diet had 1.9 fold higher maltase activities per unit intestinal area as compared to chickens fed a CF diet. Sucrase-isomaltase activity accounted for 70% of total maltase activity in the chicken small intestine. At low maltose/maltotriose concentration, maltase-glucoamylase was 10 times more active than sucrase-isomaltase, but at higher concentrations it experienced substrate inhibition whereas sucrase-isomaltase was not affected (Quezada-Calvillo *et al.,* 2007). As a result, maltase-glucoamylase contributed only 20% of mucosal α-glucogenic activity in the human, while sucrase-isomaltase contributed most of α -glucogenic activity at higher substrate concentrations. Chicken maltase activities in the brush border membrane of duodenum, jejunum, and ileum decreased from d 1 to 14 and increased from d 14 to 21 (Iji *et al.,* 2001). After early feed restriction, birds consuming diets

supplemented with amylase and protease had higher sucrase and maltase activities as compared to control birds (Pinheiro *et al.,* 2004). Inclusion of antibiotics or a probiotic mixture of multiple strains of *Lactobacillus* spp. and *Streptococcus faecium* in piglet feed resulted in increased sucrase and lactase (Collington *et al.,* 1990).

Aminopeptidases

Aminopeptidases act at a free N terminus of the polypeptide chain and liberate a single amino acid residue, a dipeptide, or a tripeptide. Increased tripeptidase but not dipeptidase was observed in piglets fed antibiotics or a probiotic mixture of multiple strains of *Lactobacillus* spp. and *Streptococcus faecium* (Collington *et al.,* 1990). Chicken aminopeptidase N activities in the brush border membrane of duodenum, jejunum, and ileum were reduced from d 1 to 21 (Iji *et al.,* 2001). Birds with feed restriction had higher aminopeptidase N as compared to birds fed *ad libitum* (Susbilla *et al.,* 2003).

Pancreatic enzyme secretion regulation

The pancreas is the major organ synthesizing and secreting digestive enzymes made by the pancreatic acinar cells of animal and human. This secretion was physiologically regulated by the vagal nerve whose postganglionic neurons release acetylcholine and by gastrointestinal hormones such as cholecystokinin (CCK) (Owyang and Logsdon, 2004). Digestive enzyme synthesis and cell growth are also regulated by nutrients and the hormones, neurotransmitters, and growth factors they release.

Intra-duodenal proteases inhibit exocrine pancreatic secretion *in vivo*. Pancreatic exocrine secretion in rats was regulated by a feedback partly by intra-luminal trypsin (Ihse *et al.,* 1979). This negative feedback regulation of pancreatic enzyme secretion was regulated by

the release of CCK (CCK-22) from the intestine (Folsch *et al.,* 1987). The feedback regulation was also neurally mediated, involving a cholinergic pathway (Louie *et al.,* 1986).

Suppression of pancreatic secretion was also observed in a patient by intra-duodenal trypsin as well as by bile-pancreatic juice (Ihse *et al.,* 1977). In the presence of a soybean trypsin inhibitor (type 1-S) associated with bile-pancreatic juice, the intra-duodenal infusion caused a marked stimulation of secretion. These results showed that trypsin in the upper intestine had a negative feedback regulation of the pancreatic secretion in man. However, pancreatic secretion and plasma levels of CCK and secretin did not change after duodenal perfusion of a serine protease inhibitor, camostat (Adler *et al.,* 1988). Meanwhile, duodenal deliveries of lipase and amylase activities were increased due to stimulation of pancreatic secretion when intra-luminal protease activity decreases. The effects might in part be independent of CCK because plasma CCK concentrations were not affected (Layer *et al.,* 1990). Pancreatic enzymes at normal therapeutic levels did inhibit postprandial pancreatic secretion in healthy humans, while no significant effect on bile acid secretion, gastro-duodenal motility and hormone release were observed (Dominguez-Munoz *et al.,* 1997). In contrast, intra-jejunal perfusion of pancreas-biliary juice in the absence of intra-luminal nutrients did not change pancreatic secretion (Krawisz *et al.,* 1980) or even increase pancreatic secretion of amylase and chymotrypsin as well as plasma CCK when active or inactive pancreatic extracts were applied (Mossner *et al.,* 1989).

Intra-duodenal perfusion of a protease inhibitor (camostat) resulted in increased basal and stimulated exocrine pancreatic secretion without changes in plasma CCK level *(Adler et al.,* 1988; Layer *et al.,* 1990), The stimulatory effect of camostat on enzyme secretion was not inhibited by the infusion of the CCK receptor antagonist loxiglumide but suppressed by atropine – an antagonist of the cholinergic receptor (Adler *et al.,* 1989), suggesting that the stimulated secretion is independent of CCK but dependent on the cholinergic system. In studies

of camostat-induced growth in mice, activation of extracellular signal regulated kinases (ERKs), Jun N-terminal kinases (JNKs), and mammalian target of rapamycin (mTOR) pathways were shown to occur with different time courses (Tashiro *et al.,* 2006). Cholinergic analogs interact directly with muscarinic M3 receptors on acinar cells. A study using knockout mice whose individual muscarinic receptors were genetically deleted showed that both M1 and M3 receptors were present on acinar cells and mediated amylase secretion from dispersed pancreatic acini (Gautam *et al.,* 2005). In the inter-digestive state, exocrine pancreatic secretion fluctuated cyclically and was closely coordinated with the gastrointestinal motor migration complex and possibly involving the cholinergic system (Keane *et al.,* 1980). Pancreatic enzymes did not appear to play a major physiological role in the regulation of inter-digestive motility (Dominguez-Munoz *et al.,* 1997).

Pancreatic acinar cells from rodents possessed primarily specific CCK1 receptors while human acinar cells were mostly CCK2 receptors which binded both gastrin and CCK with high affinity (Owyang and Logsdon, 2004). Low levels of CCK1 receptor (CCK1R) in human pancreas were also detected by quantitative polymerase chain reaction (Galindo *et al.,* 2005). CCK1Rs resided on vagal afferent endings, therefore CCK receptors and receptor-mediated effects in the rodent pancreas could be viewed as a model of CCK-induced vagal stimulation in humans (Owyang and Logsdon, 2004).

Dietary components can regulate the synthesis of digestive enzymes primarily at the transcriptional level. Pancreatic secretagogues could regulate synthesis of digestive enzymes at the translational level primarily by the phosphatidylinositol3-kinase (PI3K)– protein kinase B (PKB)–mTOR pathway (Sans and Williams, 2002). Enhanced translation was induced by increased initiation through the supply of eukaryotic translation initiation factor 4E (eIF4E) and phosphorylation of ribosomal protein S6. Pancreatic protein synthesis in mice increased in response to refeeding a diet (Sans *et al.,* 2004a). This was accomplished by activation of the

translational machinery downstream of mTOR. An increase in trypsin synthesis in humans has also been demonstrated following enteral but not parenteral (intravenous) feeding (O'Keefe S *et al.,* 2006). Amino acids, particularly branched chain amino acids, could activate mTOR and its downstream effector S6K without change of plasma CCK in mice with a genetic deletion of CCK and in isolated acini (Sans *et al.,* 2006). CCK has also been shown to enhance the rate of translation elongation through effects on elongation factor 2 phosphorylation (Sans *et al.,* 2004b). In a study, following partial pancreatectomy in mice, pancreatic regeneration was blocked by inhibiting PI3K with chemical inhibitors wortmannin or with p85alpha siRNA (Watanabe *et al.,* 2005). Amylase, lipase, trypsin and total protease activity in rat pancreas and small intestinal digesta were increased with ZnO (Szabo *et al.,* 2004).

Mucin yield and bacterial population in animal intestine

The gut of monogastric animals, including that of poultry, is inhabited by microbial populations, mostly bacterial, throughout the life of the host. The acid secreted in the proventriculus and the swift flow of contents in the duodenum and jejunum ensure that the more proximal regions contain only transient bacterial cells in the healthy host (Guarner and Malagelada, 2003). Gastric juices and small-intestine secretions (bile and pancreatic fluids) amplify the hostile nature of the upper gastrointestinal (GI) tract to microbial colonization. The lactobacilli proliferate on the epithelial surface of the avian crop (Fuller and Brooker, 1974). The flow of digesta is slower in the ileum as compared to the upper gastrointestinal tract, and conditions are thus more favorable for microbial colonization.

To prevent colonization by microbes in the gastrointestinal tract, animals have developed several approaches to exclude the microbes, such as production of immunoglobulin A and mucin. Mucin is comprised of polymeric glycoproteins (Pigman *et al.,* 1973) and forms a mucus layer that covers the epithelium of the gastrointestinal tract, lungs, and urogenital tract.

Mucins have been divided into two groups: secreted and membrane-associated (Dekker *et al.,* 2002). The secreted mucins are high molecular weight and size with 50 to 80% O-linked carbohydrates and therefore are able to form visco-elastic gels (Gendler and Spicer, 1995). Membrane-associated mucins have many similar structural properties to secreted mucins as well as additional properties as one of membrane components. The mucin protein backbone consists of a central domain which is rich in serine, threonine, proline, alanine, glycine, and cysteine (Montagne *et al.,* 2004). A great number of threonine and serine residues provide attachment sites for the oligosaccharide chains. Five monosaccharides commonly found on mucins are comprised of N-acetylgalactosamine, N-acetylglucosamine, galactose, fucose, and sialic acids (Forstner and Forstner, 1994). Depending on the monosaccharide composition, mucins are classified into neutral, sulfated acid, and non-sulfated acid mucins. The proportion of the three mucins varies along the gastrointestinal tract during postnatal development and in response to dietary treatments (Lien *et al.,* 1996).

Mucin monomers are bound together end -to-end by disulfide bridges to form large, flexible, hydrated, viscous mucus layers. The mucus layers are loaded with cells, bacteria, nutrients, protective factors, and wastes. The mucus layer lubricates the gastrointestinal epithelium and protects it from mechanical damage by dietary ingredients (Enss *et al.,* 1994). Mucus also protects the epithelia from corrosion by the acidic gastric juice and from proteolysis by digestive enzymes (Forstner and Forstner, 1994). Visco-elasticity of mucus can block motility of bacteria. The viscous drag of rat epididymal mucus was comparable with that of mucus from other mucosal surfaces and this visco-elasticity was just high enough to block *Escherichia coli* as well as sperm from swimming (Usselman and Cone, 1983). Indeed, most intestinal bacterial reside on the outer, luminal surface of the mucus layer. The mucus layer created a digestion area in which enzymes were immobilized near the epithelium surface for easy hydrolysis and absorption of nutrients (Montagne *et al.,* 2004). The mucus layer provided

a selective diffusion barrier that filters the nutrients to be absorbed and prevents larger compounds from reaching the epithelial lining (Montagne *et al.,* 2000).

Endogenous loss of protein with mucin increased when animals were fed high fiber diets because of increased small intestinal cytokines and goblet cell activities (Cassidy *et al.,* 1981). Incorporation of radiolabeled tracers into intestinal mucin glycoproteins were greater when rat diets were supplemented with water-insoluble dietary fibers like 10% cellulose or wheat bran (Vahouny *et al.,* 1985). Supplementation of 5% citrus fiber in rat diets increased small intestinal mucin secretion due to the insoluble fraction (cellulose and lignin) of the citrus fiber preparation (Satchithanandam *et al.,* 1990). Luminal mucin secretion quantified by an ELISA was not changed in the small intestine of rats fed 20% cellulose, rice bran, or psyllium (Satchithanandam *et al.,* 1996). Supplementing cellulose had no effects on jejunal mucin secretion in rats fed an elemental diet or administered total parenteral nutrition (Frankel *et al.,* 1995). The stimulatory effect of dietary fibers on mucin secretion in the gastrointestinal tract might be related to bulk-forming properties (Enss *et al.,* 1994; Schmidt-Wittig *et al.,* 1996) and short chain fatty acids produced by bacterial fermentation (Barcelo *et al.,* 2000). Small intestinal mucins were secreted in proportion to the setting volumes (in water) of dietary indigestible components, and chronic consumption of indigestible components was required for the appearance of enhanced mucin secretion (Tanabe *et al.,* 2005).

Recovery of mucin in ileal digesta can reveal endogenous output of protein and carbohydrate. Mucin accounted for 5 to 11% of endogenous protein in pigs fed balanced or protein-free diets (Lien *et al.,* 1997). Although mucin represented a small proportion of endogenous amino acids in ileal digesta, mucin contributed to endogenous threonine, serine, and proline in ileal digesta as 28 to 35%, 13 to 16%, and 7 to 24%, respectively. Mucin from the small intestine was estimated to be 73% of total mucin at the terminal ileum, while the rest originated from the stomach. In calves, mucin protein accounted for 19% of the total basal

endogenous losses of crude protein at the ileum, while 40% loss of lysine was associated with mucin secretion (Montagne *et al.,* 2000).

Mucins can only be slowly digested by host endogenous enzymes, and most mucins would be undigested and shed in feces if there were no commensal microbes that specialize in degrading mucins. Although germfree rats secreted less mucus, their ceca became filled with mucus (Szentkuti *et al.,* 1990). After these animals were provided with commensal bacteria, the excess mucus in the cecum was digested and the rate of mucus secretion increased. Some commensal bacteria without the ability to degrade mucin, such as lactobacilli, could adhere directly to epithelial cell surfaces without causing irritation (Ruseler-van Embden *et al.,* 1995).

Carbohydrates and proteins from mucus are fermented by microflora in the large intestine. The commensal and pathogenic microbes that degraded mucus slowly began to colonize the human gut over the first several months to 2 years of human life (Midtvedt *et al.,* 1994). These bacteria clung to specific glycans on mucin and cleaved specific sugars from the tips of the glycans (Neutra and Forstner, 1987). These microbes also acted cooperatively and each supplied glycosidases other strains lack. This process was totally symbiotic as long as the host secreted mucus fast enough.

Chapter 3. Performance, meat yield, and nutrient retention of broilers subjected to varied dietary energy and an enzyme cocktail of amylase, protease, and xylanase with two varieties of corn

Performance, carcass yield, and nutrient retention of broilers subjected to varied dietary energy and an enzyme cocktail of amylase, protease, and xylanase with two varieties of corn

Abstract Three 2 × 2 factorial experiments (EXP) using corn A and B were conducted to investigate the effect of diets containing two varieties of corn (Cn) with or without exogenous enzymes and dietary energy (ME) modification on broiler performance from 0 to 49 days of age. In each of EXP 1 and 2, four dietary treatments (trt) consisted of: (1) diet AR-/BR-, reduced dietary energy by increasing corn matrix ME by 138 kcal/kg with corn A in EXP 1 or by 125 kcal/kg ME with corn B in EXP 2; (2) diet AR+/BR-, AR-/BR- plus Avizyme 1502® (AZ); (3) diet AN-/BN-, normal energy; (4) diet AN+/BN+, AN-/BN- plus AZ. In EXP 3, four dietary treatments were came from the two EXP with normal energy diets, which consisted of AN-, AN+, BN-, and BN+. In each EXP, 1,440 Ross 708 male chicks were randomly assigned to one of the four dietary trts (9 reps/trt and 40 chicks/ rep) on d 1. Body weight (BW) and feed intake (FI) were recorded on d 7, feed changes (d 14, 28, 37), and d 49. On d 28, subsets of birds were transferred to Petersime batteries to determine feed passage rate and nutrient retention. P On d 50 and 51, 54 birds per trt were processed to evaluate meat yield. Sialic acids in ileum digesta were determined in EXP 3. Generally, interactions of ME and AZ were observed in both EXP 1 and 2. In EXP 2, interactions for BWG were observed with improvements noted in birds fed BN+ while lower BWG in birds fed BR+. The opposite was noticed using corn A in EXP1. In both EXP 1 and 2, FCR of birds consuming normal energy diets was better (P <0.05) as compared to birds fed reduced energy diets after d 28. Percent fatpad was increased (P <0.05) with dietary energy levels in EXP 1. Percent pectoral minor was increased (P <0.05) in birds fed diets with enzyme addition (4.80 vs. 4.62%) in EXP 1. Interaction (P < 0.05) of dietary corn and enzyme on BWG was observed on d 14 and 37 in EXP 3. Birds fed corn B diets had better (P < 0.05) BWG and feed efficiency as compared to birds fed corn A diets until d 14 in EXP 3. A 2.8% more AMEn in corn A diets was observed as compared to corn B diets. Nitrogen retention on d 30 was greater

($P < 0.05$) in birds fed AN- and BN+ as compared to birds fed AN+ and BN- in EXP 3. In conclusion, enzyme supplementation improved broiler performance, and a dietary formulation strategy of supplementing exogenous enzyme in association with reduced or normal energy diets should base on the feed ingredient quality. The improved feed efficiency might result from greater nitrogen and energy retention.

Key Words: broiler, corn, enzyme, energy, performance

Introduction

Corn is a primary raw material for animal feed, food starch, plant seed, and industrial fuel ethanol. Corn has been applied as a major energy source in poultry diets because it has high available energy content and low non-starch polysaccharides, anti-nutrient factors (Iji *et al.,* 2003). In spite of this advantage, the nutritive value of corn has been shown to be quite variable, resulting from altered climate during growth and harvest as well as processing and storage (Leeson *et al.,* 1993). The main factor affecting corn metabolizable energy (ME) is the quality of corn starch, which can be categorized into three classes: rapidly digestible starch, slowly digestible starch, and resistant starch (Englyst *et al.,* 1996). The more rapidly the starch can be digested, the greater ME and quality. Varied nutrient retention and broiler performance are reported as a result of birds being fed different varieties of corn. Retention of alanine and valine was altered when birds were fed two varieties of corn *(Song et al.,* 2004). Birds fed corn heated at 95 °C for 24 h prior to consuming exibited increased body weight, as a result of increased rapidly digestible starch (Iji *et al.,* 2003).

As an alternative to heat-treatment of corn, supplementing microbial enzymes has been reported to be a feasible approach to reduce the variation of corn energy (Cowieson, 2005). Microbial enzymes are often added to farm animal diets to increase nutrient utilization and improve performance (Ghazi *et al.,* 2002) as well as reduce nutrient excretion decreasing environmental pollution (Francesch *et al.,* 2005). The increased nutrient availability with enzyme supplementation is associated with improved digestibility or retention of energy, protein, or other nutrients. Chicks fed soybean meal based diets supplemented with protease had increased body weight gain, apparent nitrogen retention, and apparent metabolizable energy without affecting feed conversion (Ghazi *et al.,* 1997a). Improved ileal amino acid digestibility, body weight gain, and feed conversion were observed when birds were fed corn-soybean meal based diets supplemented with exogenous enzymes (Zanella *et al.,* 1999; Cowieson *et al.,* 2006a). Increased performance and digestibility of P, Ca, and amino acids were reported in birds fed

diets supplemented with a combination of amylase, protease, xylanase, and phytase (Cowieson *et al.,* 2006b).

The improvement in body weight and feed conversion came from increased nutrient digestibility, which partially resulted from a reduction in the loss of endogenous secretions such as mucin (Cowieson *et al.,* 2003). Mucin is the main component of gastrointestinal mucus and is responsible for the protection and lubrication of gut epithelium (Montagne *et al.,* 2000). Sialic acid is a component of mucin and can be used as an indication of endogenous secretion loss when determined in digesta (Cowieson *et al.,* 2004). Mucin secretion was partially mediated by dietary components (Enss *et al.,* 1994) and short chain fatty acids produced by bacterial fermentation (Barcelo *et al.,* 2000).

However, there have been inconsistent observations in performance associated with enzyme supplementation (McCracken *et al.,* 2001). Several explanations have been proposed to explain these discrepancies, including that enzyme addition provided dietary nutrients over bird requirement; alteration of dietary ingredient quality affected enzyme efficacy; or inclusion of antimicrobials in the diet interfered with enzyme effects (Troche *et al.,* 2007). The nutrient requirements of modern poultry are debated, but most nutritionist agree that minimum nutrient levels are met by the specifications in Nutrient Requirements of Poultry by the National Research Council (NRC). Corn samples from different geographical locations, seasons, or years have variable metabolizable energy (ME) as well as a different composition of amino acids, starch, and oil (Leeson and Summers, 1976; Maier, 1995; Collins *et al.,* 1998). To optimize the use of microbial enzymes in corn soybean meal based diets, corn is qualified by the degree of predicted ME released by microbial enzyme supplementation (Cowieson *et al.,* 2006a). When birds are fed high quality corn, they can digest and utilize the corn energy. Therefore, reducing the energy level in the diet is necessary when supplemented with microbial enzymes. On the other hand, when birds are fed low quality corn, birds may not digest the corn efficiently to release enough energy for their utilization. Including microbial enzymes to normal energy diets

is necessary to compensate for reduced efficiency of bird digestion. Additionally, when formulating diets, the ME value is obtained either from book values or estimated with equations based on proximate values (WPSA, 1989), which can result in a difference between the real ME and estimated. To account for the uncertainty in both bird nutrient requirements and feed ingredients, two strategies of formulating diets have been applied when supplementing enzymes: reducing energy level in the diets to utilize the respective energy released by enzyme addition (Cowieson and Adeola, 2005); or adding enzyme additives over the top of balanced diets without reduction of energy levels.

In the following experiments, corn was labeled high or low quality based on energy released by pancreatic enzymes and microbial enzymes *in vitro*. The two corn samples were then used in each experiment in which diets with reduced or normal ME were combined with an enzyme cocktail consisting of amylase, protease, and xylanase. The objective of the experiments was to investigate different qualities of corn with or without dietary enzyme supplementation in association with ME modification on broiler performance, carcass yield, and nutrient retention.

Materials and Methods

Animals and diets

Prior to trial initiation, two varieties of corn (A and B) were obtained and analyzed for proximate composition, amino acids, and estimated ME released by 0.05% Avizyme 1502®[1] with software Avicheck™ as described previously (Cowieson *et al.*, 2006a). Soybean meal and corn gluten meal were analyzed for dry matter, crude protein, crude fat, crude fiber, crude ash, calcium, phosphorus, and sodium. Amino acid profiles in soybean meal and corn gluten were estimated by AminoDat® 3.0[2]. Metabolizable energy in corn, soybean meal, and corn gluten was calculated by an ME estimating equation (WPSA, 1989). In each experiment (EXP), 1,440 Ross

[1] Danisco Animal Nutrition, IL
[2] Degussa GmbH Bennigsenplatz, Düsseldorf, Germany

708 male broiler chicks (total 2,880 chicks) were placed on fresh pine shavings in 36 pens with 9 replicates (pens) per treatment and 40 chicks per replicate (0.078 m^2 / chicks at day of age). A four-period feeding design was used with starter (d 0 to 14), grower (d 14 to 28), finisher (d 28 to 37), and withdrawal (d 37 to 49) diets. Below is the detailed dietary formulation in the three EXP.

In EXP 1, chicks were assigned to one of four dietary treatments using corn A. The four diets consisted of: diet AR- (negative basal diet): corn A with increased corn matrix energy of 138 kcal ME/kg based on determined ME release by Avizyme 1502® supplementation, resulting in reductions of 74, 84, 94, and 100 kcal ME/kg feed in starter, grower, finisher, and withdrawal diets, respectively (Table 1); diet AR+: AR- plus 0.05% Avizyme 1502®; diet AN- (positive basal diet): corn A with normal matrix energy; diet AN+: AN- plus 0.05% Avizyme 1502®.

In EXP 2, chicks were fed one of four corn B diets: diet BR- (negative basal diet): corn B with increased corn matrix energy of 125 kcal ME/kg, resulting in decrease of 68, 78, 88, and 93 kcal ME/kg feed in starter, grower, finisher, and withdrawal diets, respectively; diet BR+: BR- plus 0.05% Avizyme 1502®; diet BN- (positive basal diet): corn B with normal matrix energy; diet BN+: BN- plus 0.05% Avizyme 1502®.

Chicks fed normal energy diets in EXP 1 and 2 were used to form EXP 3 in order to investigate the effect of corn quality and enzyme supplementation. The four diets consisted of AN-, AN+, BN-, and BN+.

All diets were formulated using corn, soybean meal and corn gluten meal according to the nutrient requirements of the Ross 708 male broiler[3]. Diets were mixed at the Virginia Tech Turkey Research Farm and transported to a local commercial feed mill to be either pelleted or crumbled. Feed samples were collected at d 7, 14, 28, and 49 and analyzed for recovered amylase activity

[3] Ross 708 male broiler nutrition specifications, 2006

Table 1. Composition and calculated nutrients of basal diets during four feeding periods.

	Starter (d 1 to 14)				Grower (d 14 to 28)				Finisher (d 28 to 37)				Withdrawal (d 37 to 49)			
	Experiment 1		Experiment 2		Experiment 1		Experiment 2		Experiment 1		Experiment 2		Experiment 1		Experiment 2	
Diets[1]	AR-	AN-	BR-	BN-	AR-	AN-	BR-	BN-	AR-	AN-	BR-	BN-	AR-	AN-	BR-	BN-
Ingredient, %	----------%----------															
Corn A	55.69	53.75			60.49	58.38			68.23	65.85			72.18	69.66		
Corn B			57.67	55.78			62.64	60.59			70.66	68.34			74.74	72.29
Soybean meal	34.67	35.03	33.47	33.84	29.71	30.10	28.41	28.81	22.66	23.09	21.18	21.64	19.64	20.09	18.07	18.56
Corn gluten, 60% CP	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Yellow grease	3.37	4.95	2.72	4.23	3.94	5.66	3.24	4.88	3.31	5.26	2.52	4.37	2.76	4.82	1.92	3.88
Dicalcium phosphate	1.94	1.94	1.95	1.95	1.83	1.83	1.84	1.84	1.73	1.73	1.74	1.74	1.48	1.48	1.49	1.49
Limestone	0.99	0.99	0.75	0.76	0.82	0.82	0.56	0.57	0.78	0.78	0.49	0.50	0.70	0.70	0.38	0.40
Salt	0.27	0.28	0.38	0.38	0.31	0.32	0.43	0.43	0.30	0.31	0.43	0.43	0.27	0.27	0.40	0.41
Lysine	0.31	0.31	0.30	0.30	0.25	0.25	0.25	0.24	0.32	0.32	0.31	0.31	0.35	0.35	0.34	0.34
D.L. methionine	0.29	0.29	0.28	0.29	0.21	0.21	0.21	0.21	0.20	0.21	0.20	0.20	0.22	0.22	0.22	0.22
Threonine	0.06	0.06	0.07	0.07	0.02	0.02	0.04	0.03	0.07	0.07	0.08	0.08	0.10	0.10	0.11	0.11
Choline Chloride	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
0.06 % Selenium	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix[3]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Coban[4]	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.08	0.08	0.08	0.08	How	about	these	????
Calculated value																
ME, kcal/kg $\times 10^{-2}$	29.68	30.42	29.70	30.42	30.57	31.41	30.63	31.41	31.02	31.96	31.08	31.96	31.18	32.18	31.25	32.18
Dry matter, %	87.64	87.86	88.20	88.38	87.61	87.84	88.21	88.41	87.45	87.71	88.13	88.35	87.30	87.57	88.02	88.25
Protein, %	22.00	22.00	22.00	22.00	20.00	20.00	20.00	20.00	17.50	17.50	17.50	17.50	16.50	16.50	16.50	16.50
Fat, %	5.68	7.19	5.33	6.75	6.35	7.99	5.97	7.52	5.91	7.75	5.47	7.22	5.46	7.41	5.00	6.85
Lysine, %	1.35	1.35	1.35	1.35	1.18	1.18	1.18	1.18	1.06	1.06	1.06	1.06	1.01	1.01	1.01	1.01
Methionine, %	0.62	0.62	0.62	0.62	0.53	0.53	0.53	0.53	0.49	0.49	0.49	0.49	0.50	0.50	0.50	0.50
Met + Cys, %	0.97	0.97	0.97	0.97	0.85	0.85	0.85	0.85	0.78	0.78	0.78	0.78	0.77	0.77	0.77	0.77
Threonine, %	0.87	0.87	0.87	0.87	0.76	0.76	0.76	0.76	0.70	0.70	0.70	0.70	0.69	0.69	0.69	0.69
Analyzed value																
GE, kcal/kg $\times 10^{-2}$	41.58	41.80	41.51	41.93	41.14	41.33	41.35	41.56	39.57	40.93	40.38	40.90	39.64	40.38	39.70	40.42
Protein, %	19.62	20.02	20.79	20.28	19.31	18.83	18.94	18.20	14.88	16.98	15.32	16.63	15.12	15.63	14.58	14.91

[1] AR-/AN- was reduced/normal energy diet using corn A, while BR-/BN- with corn B; basal diets in experiment 3 were AN- and BN-. [2] Mineral premix provided the following in milligrams per kilogram of diet: Zn (ZnO), 210; Mn (MnO), 120; Fe ($FeSO_4.H_2O$), 40; Cu ($CuSO_4$), 20; I (CaI_2), 3; Co ($CoCO_3$), 0.05. [3]Vitamin premix provided the following per kilogram of diet: Vitamin A 8,818 IU, D3 2,646 ICU, E 22 IU, B12 26µg, riboflavin 8.8 mg, niacin 88 mg, d-pantothenic acid 22 mg, K 2.6mg, folic acid 2.2mg, B6 4.3mg, Thiamine 3.7mg, d-biotin 220µg. [4] 60g monensin sodium per lb.

with the EnzChek® Ultra Amylase Assay Kit [4]. Feed and water were provided *ad libitum*. Birds were on a step-lighting program of 24 h of light for d 0 to 10 d, 20 h light and 4 h dark for d 11 to 42, and 24 h of light for d 43 to 49. The research trial was conducted with the written approval of the Virginia Tech Institutional Animal Care and Use Committee.

Performance

On 7, 14, 28, 37, and 49 days of age, chicks and feed were weighed from each pen. Body weight gain (BWG) and feed intake (FI) were calculated. On d 49, birds were individually weighed to determine uniformity. Mortality and room temperature were recorded daily. Mortality-adjusted FCR was calculated as total feed per pen divided by total bird weight of live plus dead and culled .

Feed passage rate, AMEn, and nitrogen retention

On d 28, subsets of birds were randomly selected and transferred to Petersime batteries (n = 6 reps/trt) in a separate building. The birds from the same dietary treatment were combined, randomly assigned to one of six battery pens, and fed respective grower diets for 2 days. Feed was then removed for 12 h and then replaced with respective treatment mash diets mixed with 0.8% chromic oxide for determining feed passage rate. One hour later (time 0h), chromic oxide feed was replaced with respective normal grower feed and the remaining marked feed was weighed for FI calculation. Thirteen hours later (time 12h), the normal feed was removed and weighed, and FI of normal feed was calculated. Excreta were collected at 2, 4, 6, 8, 12, and 24 h after normal feed was supplied. Excreta were dried at 60 °C in an oven until samples were consistent in weight. Dry matter was calculated, while chromium was determined using a PerkinElmer AAnalyst 800 spectrometer (PerkinElmer Inc., Wellesley, MA). Samples were also

[4] Invitrogen Corporation, Carlsbad, CA

analyzed in duplicate for gross energy (cal/g) using a Parr 1271 automatic bomb calorimeter (Parr Instrument Company, Moline, IL) and nitrogen percentage using the combustion method according to AOAC (1990). Nitrogen retention was calculated as feed intake nitrogen minus excreta nitrogen. Apparent metabolizable energy with nitrogen correction (AMEn) was calculated as gross energy in feed minus excreta gross energy and nitrogen retention multiplied by 8.22 and then divided by dry matter feed (Lopez and Leeson, 2007).

Ileal digesta collection and sialic acids assay

On 7, 14, 28, and 49 days of age, one bird per pen was sacrificed (n = 8 birds/treatment) during EXP 3. Ileum tissue located between Meckel's diverticulum and the ileocecal junction was opened lengthwise. Digesta was gently scraped onto wax paper, weighed, flash frozen in liquid nitrogen, and stored at -80 °C.

Crude mucin in EXP 3 was extracted from ileal digesta according to previously reported methods (Lien *et al.,* 1996). Briefly, after 0.5 g digesta was homogenized in 0.9% sodium chloride solution and centrifuged at 20,000 × g for 15 min, the supernatant was mixed with 100% ice-cold ethanol to achieve a final concentration of 60% ethanol. The samples were held overnight at -20 °C. Crude mucin was obtained by centrifuging at 1,400 × g for 10 min and subsequently dissolving the pellet in 3 ml ddH_2O. Total and free sialic acids were determined by Jourdian's method modified for microplate assay (Jourdian *et al.,* 1971) with a FluoStar Optima microplate reader[5].

Carcass yield

On d 49, six birds per pen (n = 54 birds /treatment) were randomly selected and wing-banded. Birds in five blocks of each diet were fasted with only water access for 12 h. The birds

[5] BMG Labtech Inc. Durham, NC

were then transferred to the processing facility, weighed, stunned, euthanized and bled. After 3 min of bleeding, birds were scalded, de-feathered, and eviscerated. The necks and feet were removed. Resulting warm carcasses were weighed and then chilled in ice water for 4 h. Cold carcasses were weighed, and abdominal fat pad removed and weighed. Wings, thighs, drums and pectoral minor and major were dissected using stationary de-boning cones and individually weighed (Sun *et al.,* 2005). On d 50, the remaining four blocks were processed as previously described. Results of process products were reported as percentage of respective product weight to cold carcass weight.

Statistical Analysis

All data were analyzed using the MIXED procedures of SAS (SAS Institute Inc., Cary, NC, 2006) as a 2 ×2 factorial arrangement of dietary treatments in a randomized complete block design to determine overall significance of energy, enzyme, and the interaction of energy and enzyme in EXP 1 and 2 as well as corn quality and enzyme effects in EXP 3. Additional pair-wise contrasts between diets were used to evaluate the effect of diets when a significant interaction (P <0.05) was observed. When nitrogen retention data were evaluated, feed intake was used as a covariance.

Results

Amylase activity was determined for each diet following pelleting demonstrating the presence of the enzyme cocktail (Table 2).

Table 2. Amylase activity (U/kg feed) recovered from diets[1]

	Experiment 1				Experiment 2			
Diets[2]	AR-	AR+	AN-	AN+	BR-	BR+	BN-	BN+
Energy[3]	Reduced	Reduced	Normal	Normal	Reduced	Reduced	Normal	Normal
Enzyme[4]	-	+	-	+	-	+	-	+
Starter (d 1 to 14)	571	1059	61	793	502	1197	277	947
Grower (d 14 to 28)	136	810	255	1102	53	804	88	999
Finisher (d 28 to 37)	200	914	276	1110	455	701	515	1022
Withdrawal (d 37 to 49)	63	866	124	998	136	792	249	959

[1]Unit of amylase activity is standardized with *Bacillus* amylase (Sigma, A-6380). One unit of amylase activity will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C.
[2]AR-/AN- was reduced/normal energy diet using corn A, while BR-/BN- with corn B; basal diets in experiment 3 were AN- and BN-; plus sign meant diets with enzyme addition.
[3]In experiments 1 and 2, normal energy diet with 3042, 3141, 3196, 3218 kcal/kg ME at starter, grower, finisher, and withdrawal while reduced energy diet with reduction of 74, 84, 94, and 100 kcal/kg ME in respective period diets in experiment 1 of corn A and with reduction of 68, 78, 88, and 93 kcal/kg ME in respective diets in experiment 2 of corn B.
[4]Enzyme supplementation provided minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively

EXP 1

Cumulative BWG on d 14 and 37 had interactions ($P < 0.05$) between energy and enzyme (Table 3). On d 14, birds fed diet AR+ had higher ($P < 0.05$) cumulative BWG as compared to that of birds fed diet AR-. However, in the normal energy diet treatments, enzyme supplementation did not result in significant changes in BWG, which were also not different from AR- or AR+. On d 37, cumulative BWG in birds fed diet AR- were lower ($P < 0.05$) as compared to birds fed diet AR+ or AN-. After d 28, dietary energy level affected ($P < 0.05$) cumulative FCR with birds fed normal energy diets being more efficient (AN-/+) as compared to birds fed reduced energy diets (AR-/+), irrespective of enzyme addition.

Interactions of dietary energy and enzyme supplementation ($P < 0.05$) were also observed for period BWG and FI (Table 4). Between d 7 and 14 as well as d 28 and 37, birds fed diet AR+ had higher BWG ($P < 0.05$) as compared to birds fed diet AR-, while BWG of birds fed AN- and AN+ was performing similar. From d 28 to 37, birds fed diet AR+ or AN- consumed more ($P < 0.05$) feed as compared to birds fed diet AN+. During d 37 to 49, birds fed reduced energy diets (AR-/+) ate more ($P < 0.05$) feed as compared to birds fed normal energy diets (AN-/+). Between d 14 and 28 and from d 28 to 37, FCR in birds consuming normal energy diets was lower ($P < 0.05$) as compared to that of birds fed AR-/+.

Wing weight as a percentage of cold carcass weight (Table 5) in birds fed basal diets (AR- and AN-) was higher ($P < 0.05$) as compared to that of birds fed diets with enzyme (AR+ and AN+). Similarly, pectoral minor percent in birds fed diets with enzyme supplementation was higher ($P < 0.05$) as compared to that of birds fed basal diets. Fatpad percent in birds consuming AR-/+ was lower ($P < 0.05$) as compared to that of birds fed AN-/+.

Table 3. Effect of dietary energy and enzyme on cumulative body weight gain (BWG, kg/bird), feed intake (FI, kg/bird), and feed conversion ratio (FCR) in birds fed corn A diets[1]

Diet	AR-	AR+	AN-	AN+		Main Effects						
Energy[2]	Reduced	Reduced	Normal	Normal		Enz		Eng		Statistical effects		
Enzyme[3]	-	+	-	+	SEM[4]	-	+	Reduced	Normal	Eng×Enz	Enz	Eng
BWG												
d 7	0.130	0.136	0.134	0.132	0.0026	0.132	0.134	0.133	0.133	NS	NS	NS
d14	0.433[b]	0.448[a]	0.445[ab]	0.439[ab]	0.0050	0.307	0.309	0.308	0.309	0.03	NS	NS
d 28	1.573	1.591	1.602	1.581	0.0116	1.587	1.586	1.582	1.592	NS	NS	NS
d 37	2.443[b]	2.496[a]	2.502[a]	2.454[ab]	0.0178	2.472	2.475	2.470	2.478	0.009	NS	NS
d 49	3.652	3.693	3.675	3.678	0.0287	3.663	3.685	3.673	3.676	NS	NS	NS
FI												
d 7	0.169	0.173	0.170	0.170	0.0027	0.169	0.172	0.171	0.170	NS	NS	NS
d14	0.596	0.608	0.611	0.600	0.0063	0.603	0.604	0.602	0.605	NS	NS	NS
d 28	2.306	2.347	2.323	2.337	0.0222	2.315	2.342	2.327	2.330	NS	NS	NS
d 37	3.913	3.981	3.954	3.924	0.0317	3.934	3.952	3.947	3.939	NS	NS	NS
d 49	6.598	6.658	6.520	6.573	0.0537	6.559	6.616	6.628	6.547	NS	NS	NS
FCR												
d 7	1.296	1.273	1.268	1.292	0.0197	1.282	1.282	1.285	1.280	NS	NS	NS
d14	1.393	1.373	1.366	1.367	0.0169	1.379	1.370	1.383	1.366	NS	NS	NS
d 28	1.491	1.486	1.445	1.469	0.0116	1.468	1.477	1.488	1.457	NS	NS	0.01
d 37	1.765	1.732	1.648	1.670	0.0146	1.706	1.701	1.748	1.659	NS	NS	0.0001
d 49	1.911	1.890	1.816	1.829	0.0145	1.863	1.859	1.900	1.822	NS	NS	0.0001

[a,b] Different superscripts within row are significantly different (P <0.05)

[1] Values are means of 9 replicate pens; AR-/AN- was reduced/normal energy diet using corn A; plus sign meant diets with enzyme addition.

[2] Normal energy (Eng) diet with 3042, 3141, 3196, 3218 kcal ME/kg feed at starter, grower, finisher, and withdrawal while reduced energy diet with reduction of 74, 84, 94, and 100 kcal ME/kg feed in respective diets.

[3] Enzyme (Enz) supplementation provided minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively

[4] Standard error of the difference between means

Table 4. Effect of dietary energy and enzyme on period body weight gain (BWG, kg/bird), feed intake (FI, kg/bird), and feed conversion ratio (FCR) in birds fed corn A diets

Diet	AR-	AR+	AN-	AN+		Main Effects						
Energy[2]	Reduced	Reduced	Normal	Normal		Enz		Eng		Statistical effects		
Enzyme[3]	-	+	-	+	SEM[4]	-	+	Reduced	Normal	Eng×E	Enz	Eng
BWG												
d 7 to 14	0.303[b]	0.312[a]	0.311[ab]	0.307[ab]	0.0028	0.307	0.309	0.308	0.309	0.03	NS	NS
d 14 to 28	1.139	1.143	1.157	1.142	0.0084	1.148	1.143	1.141	1.150	NS	NS	NS
d 28 to 37	0.870[b]	0.905[a]	0.900[ab]	0.873[ab]	0.0115	0.885	0.889	0.887	0.886	0.01	NS	NS
d 37 to 49	1.210	1.197	1.173	1.223	0.0200	1.191	1.210	1.203	1.198	NS	NS	NS
FI												
d 7 to 14	0.427	0.435	0.441	0.430	0.0048	0.434	0.432	0.431	0.435	NS	NS	NS
d 14 to 28	1.711	1.739	1.713	1.737	0.0179	1.712	1.738	1.725	1.725	NS	NS	NS
d 28 to 37	1.607[ab]	1.633[a]	1.630[a]	1.587[b]	0.0134	1.619	1.610	1.620	1.609	0.02	NS	NS
d 37 to 49	2.685	2.678	2.566	2.649	0.0314	2.625	2.663	2.681	2.608	NS	NS	0.03
FCR												
d 7 to 14	1.438	1.420	1.411	1.404	0.0213	1.424	1.412	1.429	1.408	NS	NS	NS
d 14 to 28	1.532	1.534	1.479	1.513	0.0126	1.505	1.523	1.533	1.496	NS	NS	0.01
d 28 to 37	1.856	1.821	1.813	1.808	0.0111	1.834	1.814	1.838	1.811	NS	NS	0.02
d 37 to 49	2.233	2.244	2.232	2.200	0.0338	2.232	2.222	2.238	2.216	NS	NS	NS

[a,b] Different superscripts within row are significantly different (P <0.05)

[1] Values are means of 9 replicate pens; AR-/AN- was reduced/normal energy diet using corn A; plus sign meant diets with enzyme addition.

[2] Normal energy (Eng) diet with 3042, 3141, 3196, 3218 kcal ME/kg feed at starter, grower, finisher, and withdrawal while reduced energy diet with reduction of 74, 84, 94, and 100 kcal ME/kg feed in respective diets.

[3] With enzyme (Enz) supplementation to provide minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively

[4] Standard error of the difference between means

Table 5. Effect of dietary energy and enzyme on body weight coefficient of variation (CV, %) on d 49, total mortality (%), and process products (%) of birds fed corn A diets[1]

Diet	AR-	AR+	AN-	AN+		Main effects						
Energy	Reduced	Reduced	Normal	Normal		Enz		Eng		Statistical effects		
Enzyme	-	+	-	+	SEM	-	+	Reduced	High	Eng×Enz	Enz	Eng
CV	12.56	12.54	11.75	10.70	0.914	12.15	11.62	12.55	11.22	NS	NS	NS
Mortality	8.06	8.06	6.94	8.33	1.615	7.50	8.19	8.06	7.64	NS	NS	NS
Wing	10.65	10.51	10.62	10.48	0.067	10.64	10.50	10.58	10.55	NS	0.04	NS
Pectoral	25.84	25.57	25.19	25.51	0.247	25.52	25.54	25.71	25.35	NS	NS	NS
Pectoral	4.67	4.83	4.58	4.77	0.074	4.62	4.80	4.75	4.67	NS	0.02	NS
Drum	12.20	12.21	12.19	12.20	0.083	12.20	12.21	12.21	12.20	NS	NS	NS
Thigh	16.55	16.36	16.43	16.55	0.145	16.49	16.46	16.45	16.49	NS	NS	NS
Fatpad	1.87	1.99	2.15	2.19	0.074	2.01	2.09	1.93	2.17	NS	NS	0.004

[1]Values are means of 9 replicate pens; AR-/AN- was reduced/normal energy diet using corn A; plus sign meant diets with enzyme addition.
[2]Normal energy (Eng) diet with 3042, 3141, 3196, 3218 kcal ME/kg feed at starter, grower, finisher, and withdrawal while reduced energy diet with reduction of 74, 84, 94, and 100 kcal ME/kg feed in respective diets.
[3]With enzyme (Enz) supplementation to provide minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively
[4]Standard error of the difference between means

No difference in feed passage rate between dietary treatments was observed (Figure 1). There was an interaction ($P < 0.05$) between energy and enzyme on excreta chromium amount at 8 h post-feeding of normal feed. About half of the chromium was excreted 4 h after birds were fed normal diets. Nitrogen retention was higher ($P < 0.05$) in birds fed non-enzyme supplemented diets as compared to birds fed diets with supplemental enzyme (Table 6). Birds consuming low energy diets had higher ($P < 0.05$) nitrogen retention as compared to birds fed normal energy diets.

EXP 2

Similar to results with corn A diets, there were interactions between dietary energy levels and enzyme supplementation on performance of birds fed corn B diets. There were interactions ($P < 0.05$) of dietary energy level and enzyme addition noted for cumulative BWG through all feeding periods (Table 7) as indicated by higher BWG in birds fed reduced energy diets without enzyme (BR-) and normal energy with enzyme (BN+) as compared to their respective comparatives (BR+ and BN-, respectively). Birds fed diet BR- had higher ($P < 0.05$) cumulative BWG to d 37 as compared to birds fed diet BR+. Also on d 14, birds consuming diet BR- had greater ($P < 0.05$) cumulative BWG as compared to birds consuming diet BN-. Birds fed diet BN+ gained more ($P < 0.05$) as compared to birds consuming diet BN- on d 14 and 37. On d 37, birds fed reduced energy diets (BR-/+) had lower ($P < 0.05$) cumulative BWG as compared to birds consuming normal energy diets (BN-/+). Cumulative FI to d 37 had interactions ($P < 0.05$) of energy and enzyme, similar to the trend of cumulative BWG. Birds fed diet BR- consumed more ($P < 0.05$) feed to d 37 as compared to birds fed diet BR+. Interaction ($P < 0.05$) was also observed for cumulative FCR on d 37. Birds fed BR-/+ had higher ($P < 0.05$) cumulative FCR as compared to birds fed BN-/+ after d 14.

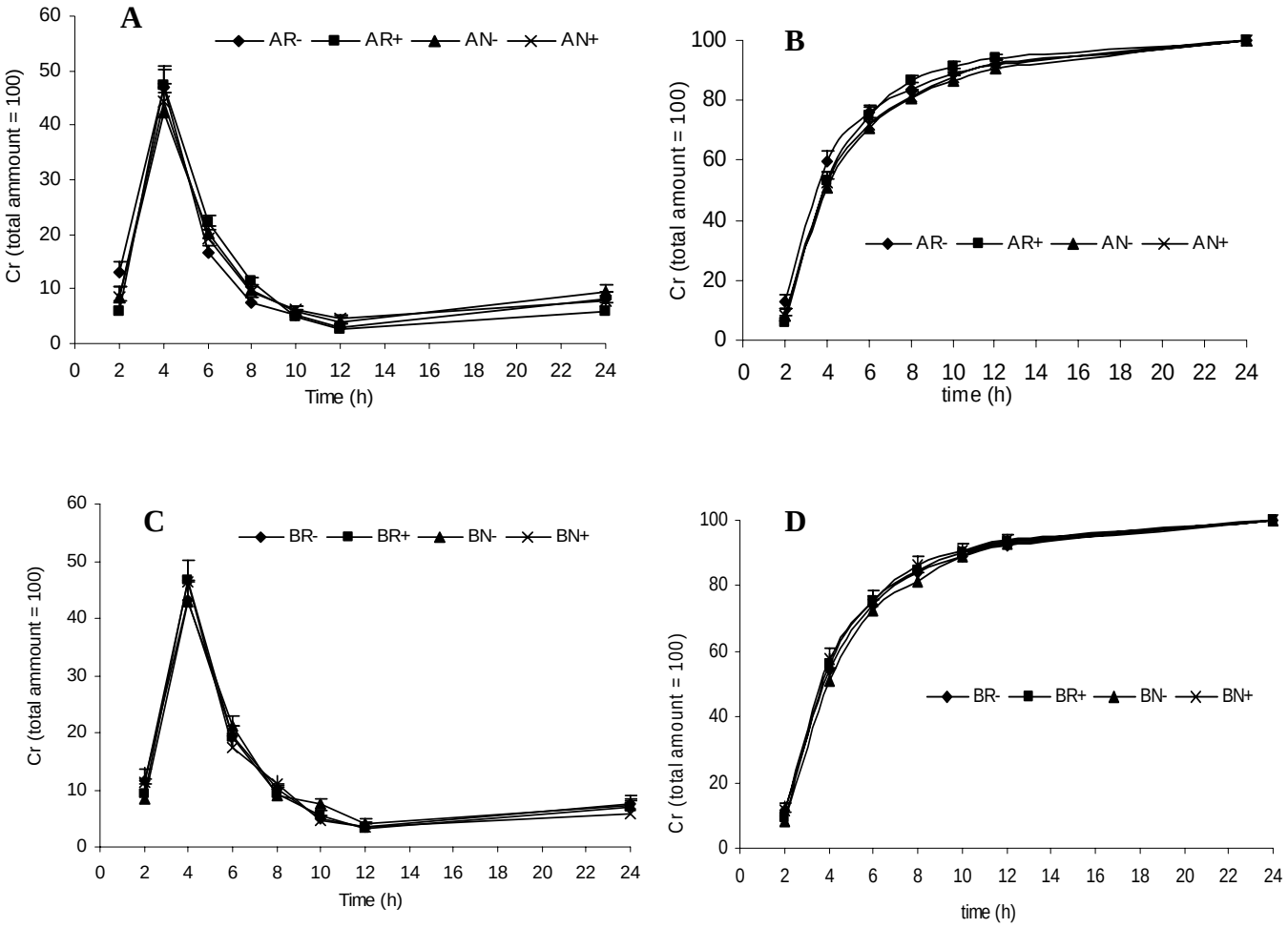

Figure 1. Period (Figure A and C) and cumulative (Figure B and D) feed passage rate in birds fed corn A or B on 30 days of age.

AR-/BR-: reduced energy basal diet; AR+/BR+: A/BR- plus enzyme; AN-/BN-: normal energy basal diet; AN+/BN+: AN-/BN- plus enzyme. Normal energy diet with 3042, 3141, 3196, 3218 kcal ME/kg feed at starter, grower, finisher, and withdrawal while reduced energy diet with reduction of 74, 84, 94, and 100 kcal ME/kg feed in respective corn A diets. Similar as corn A diet design and normal dietary energy levels with reduction of 68, 78, 88, and 93 kcal ME/kg feed instead in respective diets. With enzyme supplementation, there were minimal dietary 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively.

Table 6. Apparent metabolizable energy (AMEn) with nitrogen correction and nitrogen retention of birds on d 30 in experiment (EXP) 1 and 2[1]

EXP 1 Diet	AR-	AR+	AN-	AN+		Main effects						
Energy[2]	Reduced	Reduced	Normal	Normal		Enz		Eng		Statistical effects		
Enzyme[3]	-	+	-	+	SEM	-	+	Reduced	High	Eng×Enz	Enz	Eng
FI, g/bird	122	118	117	122	5.0	120	120	120	119	NS	NS	NS
AMEn, kcal/kg	3704	3647	3698	3689	21.1	3702	3669	3676	3694	NS	NS	NS
N retention, g/bird	2.08	1.88	1.87	1.64	0.085	1.98	1.76	1.98	1.75	NS	0.02	0.02

EXP 2 Diet	BR-	BR+	BN-	BN+								
Energy	Reduced	Reduced	Normal	Normal								
Enzyme	-	+	-	+								
FI, g/bird	119	122	121	128	9.0	120	125	120	124	NS	NS	NS
AMEn, kcal/kg	3579	3577	3566	3597	29.6	3573	3587	3579	3582	NS	NS	NS
N retention, g/bird	1.90	1.75	1.68	1.92	0.100	1.79	1.84	1.83	1.80	NS	NS	NS

[1]Values are means of 6 replicate cage pens; AR-/AN- was reduced/normal energy diet using corn A; plus sign meant diets with enzyme addition.
[2]Normal energy (Eng) diet with 3042, 3141, 3196, 3218 kcal ME/kg feed at starter, grower, finisher, and withdrawal while reduced energy diet with reduction of 68, 78, 88, and 93 kcal ME/kg feed in respective diets.
[3] With enzyme (Enz) supplementation to provide minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively.

Table 7. Effect of dietary energy and enzyme on cumulative body weight gain (BWG, kg/bird), feed intake (FI, kg/bird), and feed conversion ratio (FCR) in birds fed corn B diets[1]

Diet	BR-	BR+	BN-	BN+		Main Effects						
Energy[2]	Reduced	Reduced	Normal	Normal		Enz		Eng		Statistical effects		
Enzyme[3]	-	+	-	+	SEM[4]	-	+	Reduced	Normal	Eng×Enz	Enz	Eng
BWG												
d 7	0.141[a]	0.130[b]	0.137[a]	0.142[a]	0.0021	0.139	0.136	0.136	0.140	0.001	NS	NS
d14	0.457[a]	0.445[b]	0.446[b]	0.459[a]	0.0033	0.452	0.452	0.451	0.453	0.0008	NS	NS
d 28	1.595[ab]	1.551[c]	1.569[bc]	1.616[a]	0.0116	1.582	1.583	1.573	1.593	0.0006	NS	NS
d 37	2.448[ab]	2.364[c]	2.421[b]	2.497[a]	0.0176	2.435	2.431	2.406	2.459	0.0001	NS	0.006
d 49	3.614[ab]	3.543[b]	3.590[ab]	3.661[a]	0.0337	3.602	3.602	3.578	3.625	0.05	NS	NS
FI												
d 7	0.169[ab]	0.162[c]	0.164[bc]	0.172[a]	0.0019	0.166	0.167	0.165	0.168	0.0007	NS	NS
d14	0.608[a]	0.586[b]	0.596[ab]	0.604[a]	0.0050	0.602	0.595	0.597	0.600	0.007	NS	NS
d 28	2.338[a]	2.269[b]	2.299[ab]	2.325[a]	0.0167	2.318	2.297	2.304	2.312	0.009	NS	NS
d 37	3.940[a]	3.840[b]	3.874[ab]	3.926[a]	0.0253	3.907	3.883	3.890	3.900	0.006	NS	NS
d 49	6.546	6.423	6.438	6.489	0.0540	6.492	6.456	6.485	6.464	NS	NS	NS
FCR												
d 7	1.196	1.240	1.198	1.212	0.0207	1.197	1.226	1.218	1.205	NS	NS	NS
d14	1.347	1.341	1.334	1.308	0.0118	1.340	1.324	1.344	1.321	NS	NS	NS
d 28	1.491	1.486	1.456	1.434	0.0099	1.474	1.460	1.489	1.445	NS	NS	0.0002
d 37	1.775	1.776	1.677	1.646	0.0140	1.726	1.711	1.776	1.661	NS	NS	0.0001
d 49	1.906	1.922	1.840	1.815	0.0181	1.873	1.869	1.914	1.828	NS	NS	0.0001

[a,b,c] Different superscripts within row are significantly different (P <0.05)

[1] Values are means of 9 replicate pens; BR-/BN- was reduced/normal energy diet using corn B; plus sign meant diets with enzyme addition.

[2] Normal energy (Eng) diet with 3042, 3141, 3196, 3218 kcal ME/kg feed at starter, grower, finisher, and withdrawal while reduced energy diet with reduction of 68, 78, 88, and 93 kcal ME/kg feed in respective diets.

[3] With enzyme (Enz) supplementation to provide minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively

[4] Standard error of the difference between means

Interactions ($P < 0.05$) of dietary energy and enzyme supplementation were observed for period BWG and FI between d 14 to 28 and from d 28 to 37 (Table 8). Birds fed diet BR- had greater ($P < 0.05$) BWG as compared to BR+ during the two periods, while birds consuming BN+ diets had higher ($P < 0.05$) BWG as compared to BN- between d 14 to 28. Birds fed BN-/+ gained more ($P < 0.05$) as compared to birds fed BR-/+ during d 28 to 37. Birds fed diet BR- consumed more feed as compared to birds fed diet BR+ during d 14 to 28 and d 28 to 37. Birds fed diets supplemented with enzyme (BR+ and BN+) had more efficient ($P < 0.05$) period FCR as compared to birds fed diets without enzyme (BR- and BN-) from d 7 to 14. Birds consuming BR-/+ less efficient ($P < 0.05$) as compared to birds fed BN-/+ during d 14 to 28 and from d 28 to 37. Birds fed enzyme supplemented diets had less ($P < 0.05$) total mortality as compared to birds fed diets without enzyme (Table 9). Birds fed BN-/+ on d 49 showed a trend of more uniform weight ($P = 0.07$) as compared to birds consuming BR-/+ (CV, 11.52 vs. 12.95 %). Generally, feed passage rates between dietary treatments were not different (Figure 1). Half of the chromium intake was excreted after birds were fed normal diets for 4 h.

EXP 3

Cumulative BWG in birds fed low quality corn B diets was higher ($P <0.05$) as compared to birds fed high quality corn A diets on d 7 and 14 (Table 10). Interactions ($P <0.05$) of corn quality and enzyme supplementation on BWG were observed on d 14, 28, and 37. Birds fed BN+ gained more ($P <0.05$) as compared to birds fed BN- on d 14, 28, and 37. No difference of cumulative FI between dietary treatments was observed. Birds fed low quality corn B converted feed more efficiently ($P <0.05$) as compared to birds consuming high quality corn A on d 7 and 14. Interaction ($P < 0.05$) of corn quality and enzyme addition on FCR was observed on d 37.

Table 8. Effect of dietary energy and enzyme on period body weight gain (BWG, kg/bird), feed intake (FI, kg/bird), and feed conversion ratio (FCR) in birds fed corn B diets

Diet	BR-	BR+	BN-	BN+		Main Effects						
Energy[2]	Reduced	Reduced	Normal	Normal		Enz		Eng		Statistical effects		
Enzyme[3]	-	+	-	+	SEM[4]	-	+	Reduced	Normal	Eng×Enz	Enz	Eng
BWG												
d 7 to 14	0.316	0.315	0.309	0.317	0.0025	0.313	0.316	0.316	0.313	NS	NS	NS
d 14 to 28	1.138^{ab}	1.106^{c}	1.123^{bc}	1.157^{a}	0.0095	1.130	1.132	1.122	1.140	0.002	NS	NS
d 28 to 37	0.853^{a}	0.814^{b}	0.853^{a}	0.881^{a}	0.0118	0.853	0.847	0.834	0.867	0.008	NS	0.01
d 37 to 49	1.166	1.178	1.169	1.164	0.0261	1.167	1.171	1.172	1.166	NS	NS	NS
FI												
d 7 to 14	0.440	0.425	0.432	0.432	0.0045	0.436	0.429	0.432	0.432	NS	NS	NS
d 14 to 28	1.729^{a}	1.683^{b}	1.702^{ab}	1.721^{ab}	0.0139	1.716	1.702	1.706	1.712	0.03	NS	NS
d 28 to 37	1.602^{a}	1.571^{b}	1.575^{b}	1.602^{a}	0.0121	1.589	1.586	1.587	1.588	0.03	NS	NS
d 37 to 49	2.606	2.583	2.565	2.563	0.0368	2.585	2.573	2.595	2.564	NS	NS	NS
FCR												
d 7 to 14	1.420	1.387	1.399	1.353	0.0159	1.409	1.370	1.403	1.376	NS	0.02	NS
d 14 to 28	1.554	1.551	1.510	1.489	0.0121	1.532	1.512	1.552	1.499	NS	NS	0.002
d 28 to 37	1.862^{a}	1.915^{a}	1.842^{a}	1.835^{b}	0.0136	1.852	1.875	1.889	1.838	0.03	NS	0.001
d 37 to 49	2.208	2.230	2.245	2.224	0.0543	2.227	2.227	2.219	2.235	NS	NS	NS

[a,b] Different superscripts within row are significantly different (P <0.05)

[1] Values are means of 9 replicate pens; BR-/BN- was reduced/normal energy diet using corn B; plus sign meant diets with enzyme addition.

[2] Normal energy (Eng) diet with 3042, 3141, 3196, 3218 kcal ME/kg diet at starter, grower, finisher, and withdrawal while reduced energy diet with reduction of 68, 78, 88, and 93 kcal ME/kg diet in respective diets.

[3] With enzyme (Enz) supplementation to provide minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively

[4] Standard error of the difference between means

Table 9. Effect of dietary energy and enzyme on body weight coefficient of variance (CV, %) on d 49, total mortality (%), and process products (%) of birds fed corn B diets

Diet	BR-	BR+	BN-	BN+		Main effects						
Energy	Reduced	Reduced	Normal	Normal		Enz		Eng		Statistical effects		
Enzyme	-	+	-	+	SEM	-	+	Reduced	High	Eng×Enz	Enz	Eng
	---------- % ----------											
CV	12.54	13.35	12.16	10.88	0.765	12.35	12.12	12.95	11.52	NS	NS	NS
Mortality	6.94	4.44	7.78	5.56	1.060	7.36	5.00	5.69	6.67	NS	0.04	NS
Wing	10.49	10.70	10.57	10.65	0.100	10.53	10.67	10.59	10.61	NS	NS	NS
Pectoral	25.43	25.03	25.67	25.45	0.234	25.55	25.24	25.23	25.56	NS	NS	NS
Pectoral	4.76	4.83	4.58	4.67	0.096	4.67	4.75	4.80	4.63	NS	NS	NS
Drum	12.15	12.32	12.25	12.29	0.122	12.20	12.30	12.23	12.27	NS	NS	NS
Thigh	16.38	16.29	16.51	16.13	0.176	16.45	16.21	16.34	16.32	NS	NS	NS
Fatpad	2.02	2.12	1.98	2.21	0.110	2.00	2.17	2.07	2.10	NS	NS	NS

[a,b] Different superscripts within row are significantly different (P <0.05)

[1] Values are means of 9 replicate pens; BR-/BN- was reduced/normal energy diet using corn B; plus sign meant diets with enzyme addition.

[2] Normal energy (Eng) diet with 3042, 3141, 3196, 3218 kcal ME/kg feed at starter, grower, finisher, and withdrawal while reduced energy diet with reduction of 68, 78, 88, and 93 kcal ME/kg feed in respective diets.

[3] With enzyme (Enz) supplementation to provide minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively

[4] Standard error of the difference between means

Table 10. Effect of corn quality and enzyme supplementation on broiler cumulative body weight gain (BWG, kg/bird), feed intake (FI, kg/bird), and feed conversion ratio (FCR)[1]

Diet	AN-	AN+	BN-	BN+		Main Effects						
Corn quality[2]	High	High	Low	Low		Enz		Cn		Statistical effects		
Enzyme[3]	-	+	-	+	SEM[4]	-	+	High	Low	Cn×Enz	Enz	Cn
BWG												
d 7	0.134	0.132	0.137	0.142	0.0023	0.136	0.137	0.133	0.140	NS	NS	0.01
d14	0.445[b]	0.439[b]	0.446[b]	0.459[a]	0.0038	0.446	0.449	0.442	0.453	0.02	NS	0.01
d 28	1.602[ab]	1.581[ab]	1.569[b]	1.616[a]	0.0138	1.585	1.599	1.592	1.593	0.02	NS	NS
d 37	2.502[a]	2.454[ab]	2.421[b]	2.497[a]	0.0195	2.462	2.476	2.478	2.459	0.004	NS	NS
d 49	3.675	3.678	3.590	3.661	0.0335	3.632	3.669	3.676	3.625	NS	NS	NS
FI												
d 7	0.170	0.170	0.164	0.172	0.0023	0.167	0.171	0.170	0.168	NS	NS	NS
d14	0.611	0.600	0.596	0.604	0.0060	0.604	0.602	0.605	0.600	NS	NS	NS
d 28	2.323	2.337	2.299	2.325	0.0232	2.311	2.331	2.330	2.312	NS	NS	NS
d 37	3.954	3.924	3.874	3.926	0.0354	3.914	3.925	3.939	3.900	NS	NS	NS
d 49	6.520	6.573	6.438	6.489	0.0617	6.479	6.531	6.547	6.464	NS	NS	NS
FCR												
d 7	1.268	1.292	1.198	1.212	0.0202	1.233	1.252	1.280	1.205	NS	NS	0.001
d14	1.366	1.367	1.334	1.308	0.0156	1.350	1.338	1.366	1.321	NS	NS	0.008
d 28	1.445	1.469	1.456	1.434	0.0127	1.451	1.452	1.457	1.445	NS	NS	NS
d 37	1.648	1.670	1.677	1.646	0.0135	1.662	1.658	1.659	1.661	0.05	NS	NS
d 49	1.816	1.829	1.840	1.815	0.0179	1.828	1.822	1.822	1.828	NS	NS	NS

[a,b] Different superscripts within one row are significantly different (P <0.05)

[1] Values are means of 9 replicate pens; AN-/BN- was normal energy diet using corn A or B; plus sign meant diets with enzyme addition.

[2] High quality corn (Cn) A released 138 kcal ME/kg feed by enzyme supplementation, while corn B released 125 kcal ME/kg feed.

[3] Enzyme (Enz) supplementation provided minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively

[4] Standard error of the difference between means

Interaction (P <0.05) of corn quality and enzyme addition was observed for period BWG except during d 37 to 49 (Table 11). Birds fed BN+ had higher (P <0.05) period BWG as compared to birds fed BN- during d 7 to 14 and d 14 to 28. Birds consuming AN- gained more (P <0.05) as compared to birds fed BN- during d 14 to 28 and during d 28 to 37. There was an interaction (P <0.05) between corn quality and enzyme for period FI during d 28 to 37. Birds fed AN- consumed more (P <0.05) as compared to BN- birds during d 28 to 37. Compensatory gain and FI were noted in birds with previous lower BWG during d 37 and 49. Feed conversion was better (P <0.05) in birds fed high quality corn as compared to birds consuming low quality corn during d 28 and 37.

There were no significant differences in BW uniformity or carcass yield associated with dietary treatments (Table 12). Energy (ANEn) retention in birds fed corn A diets was 2.8 % greater (P <0.05) as compared to birds consuming corn B diets (Table 13) at 30 d of age. Interaction (P <0.05) of corn quality and enzyme addition on nitrogen retention was observed on d 30.

Intestinal mucin levels indicated by sialic acid concentration were higher (P <0.05) in birds fed corn A as compared to birds consuming corn B on d 7 (Table 14). Enzyme supplementation resulted in lower (P <0.05) amounts of sialic acids in ileal digesta on d 7. The ratio of total to free sialic acids was higher (P <0.05) in birds fed low quality corn as compared to high quality corn on d 49. Feed passage rate was not affected by dietary treatments (Figure 2).

Table 11 Effect of corn quality and enzyme supplementation on feeding period broiler body weight gain (BWG, kg/bird), feed intake (FI, kg/bird), and feed conversion ratio (FCR)[1]

Diet	AN-	AN+	BN-	BN+		Main Effects						
Corn quality[2]	High	High	Low	Low		Enz		Cn		Statistical effects		
Enzyme[3]	-	+	-	+	SEM[4]	-	+	High	Low	Cn×En	Enz	Cn
BWG												
d 7 to 14	0.311[ab]	0.307[b]	0.309[b]	0.317[a]	0.0026	0.310	0.312	0.309	0.313	0.02	NS	NS
d 14 to 28	1.157[a]	1.142[ab]	1.123[b]	1.157[a]	0.0111	1.140	1.150	1.150	1.140	0.04	NS	NS
d 28 to 37	0.900[a]	0.873[ab]	0.853[b]	0.881[ab]	0.0117	0.876	0.877	0.886	0.867	0.03	NS	NS
d 37 to 49	1.173	1.223	1.169	1.164	0.0208	1.171	1.194	1.198	1.166	NS	NS	NS
FI												
d 7 to 14	0.441	0.430	0.432	0.432	0.0053	0.437	0.431	0.435	0.432	NS	NS	NS
d 14 to 28	1.713	1.737	1.702	1.721	0.0193	1.708	1.729	1.725	1.712	NS	NS	NS
d 28 to 37	1.630[a]	1.587[ab]	1.575[b]	1.602[ab]	0.0150	1.603	1.594	1.609	1.588	0.03	NS	NS
d 37 to 49	2.566	2.649	2.565	2.563	0.0332	2.570	2.606	2.608	2.564	NS	NS	NS
FCR												
d 7 to 14	1.411	1.404	1.399	1.353	0.0214	1.405	1.379	1.408	1.376	NS	NS	NS
d 14 to 28	1.479	1.513	1.510	1.489	0.0147	1.494	1.501	1.496	1.499	NS	NS	NS
d 28 to 37	1.813	1.808	1.842	1.835	0.0106	1.828	1.821	1.811	1.838	NS	NS	0.01
d 37 to 49	2.232	2.200	2.245	2.224	0.0455	2.239	2.212	2.216	2.235	NS	NS	NS

[a,b] Different superscripts within one row are significantly different (P <0.05)

[1] Values are means of 9 replicate pens; AN-/BN- was normal energy diet using corn A or B; plus sign meant diets with enzyme addition.

[2] High quality corn (Cn) A released 138 kcal ME/kg feed by enzyme supplementation, while corn B released 125 kcal ME/kg feed.

[3] With enzyme (Enz) supplementation to provide minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively

[4] Standard error of the difference between means

Table 12. Effect of corn quality and enzyme supplementation on broiler body weight coefficient of variation (CV, %) on d 49, total mortality (%), and process products (%)[1]

Diet	AN-	AN+	BN-	BN+		Main effects						
Corn quality[2]	High	High	Low	Low		Enz		Cn		Statistical effects		
Enzyme[3]	-	+	-	+	SEM[4]	-	+	High	Low	Cn×Enz	Enz	Cn
	---------- % ----------											
CV	11.75	10.70	12.16	10.88	0.800	11.95	10.79	11.22	11.52	NS	NS	NS
Mortality	6.94	8.33	7.78	5.56	1.271	7.64	7.08	7.92	6.81	NS	NS	NS
Wing	10.62	10.48	10.57	10.65	0.080	10.61	10.55	10.56	10.60	NS	NS	NS
Pectoral major	25.19	25.51	25.67	25.45	0.267	25.43	25.48	25.35	25.56	NS	NS	NS
Pectoral minor	4.58	4.77	4.58	4.67	0.104	4.58	4.72	4.67	4.63	NS	NS	NS
Drum	12.19	12.20	12.25	12.29	0.115	12.22	12.24	12.20	12.27	NS	NS	NS
Thigh	16.43	16.55	16.51	16.13	0.145	16.47	16.34	16.49	16.32	NS	NS	NS
Fatpad	2.15	2.19	1.98	2.21	0.095	2.06	2.20	2.17	2.10	NS	NS	NS

[1] Values are means of 9 replicate pens; AN-/BN- was normal energy diet using corn A or B; plus sign meant diets with enzyme addition.
[2] High quality corn (Cn) A released 138 kcal ME/kg feed by enzyme supplementation, while corn B released 125 kcal ME/kg feed.
[3] With enzyme (Enz) supplementation to provide minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively
[4] Standard error of the difference between means

Table 13. Effect of corn quality and enzyme supplementation on apparent metabolizable energy (AMEn) with nitrogen correction and nitrogen retention of birds on d 30[1]

Diet	AN-	AN+	BN-	BN+		Main effects						
Corn quality[2]	High	High	Low	Low		Enz		Cn		Statistical effects		
Enzyme[3]	-	+	-	+	SEM	-	+	High	Low	Cn×Enz	Enz	Cn
FI, g/bird	117	122	121	128	5.9	119	125	119	124	NS	NS	NS
AMEn, kcal/kg	3698	3689	3566	3597	31.4	3644	3632	3694	3582	NS	NS	0.007
N retention, g/bird	1.93	1.70	1.67	1.88	0.094	1.80	1.79	1.81	1.78	0.03	NS	NS

[1] Values are means of 6 replicate cage pens; AN-/BN- was normal energy diet using corn A or B; plus sign meant diets with enzyme addition.
[2] High quality corn (Cn) A released 138 kcal ME/kg feed by enzyme supplementation, while corn B released 125 kcal ME/kg feed.
[3] With enzyme (Enz) supplementation to provide minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively.

Table 14. Effect of corn quality and enzyme supplementation on broiler intestinal mucin[1]

Diet	AN-	AN+	BN-	BN+		Main Effects						
Corn quality[2]	High	High	Low	Low		Enz		Cn		Statistical effects		
Enzyme[3]	-	+	-	+	SEM[4]	-	+	High	Low	Cn×En	Enz	Cn
Total												
d 7	13.3	11.2	11.7	6.2	1.54	12.5	8.7	12.3	9.0	NS	0.02	0.04
d 14	7.0	8.9	8.8	8.3	1.35	7.9	8.6	7.9	8.6	NS	NS	NS
d 28	5.7	6.0	6.5	8.9	1.01	6.1	7.4	5.9	7.7	NS	NS	NS
d 49	3.5	2.9	2.5	2.3	0.48	3.0	2.6	3.2	2.4	NS	NS	NS
Free : Total												
d 7	31.0	27.7	27.9	35.9	4.45	29.5	31.8	29.3	31.9	NS	NS	NS
d 14	29.6	23.1	36.0	23.8	7.14	32.8	23.4	26.3	29.9	NS	NS	NS
d 28	32.7	32.3	23.9	21.6	7.44	28.3	27.0	32.5	22.8	NS	NS	NS
d 49	23.6	20.6	43.1	37.5	7.48	31.8	30.5	22.1	40.3	NS	NS	0.02

[1]Intestinal mucin indicated by sialic acids as μmol/kg body weight for total and percentage of free to total sialic acids for ratio; AN-/BN- was normal energy diet using corn A or B; plus sign meant diets with enzyme addition; values are means ± SEM of 8 replicate pens.

[2]High quality corn (Cn) A released 138 kcal ME/kg feed by enzyme supplementation, while corn B released 125 kcal ME/kg feed.

[3]With enzyme (Enz) supplementation to provide minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively.

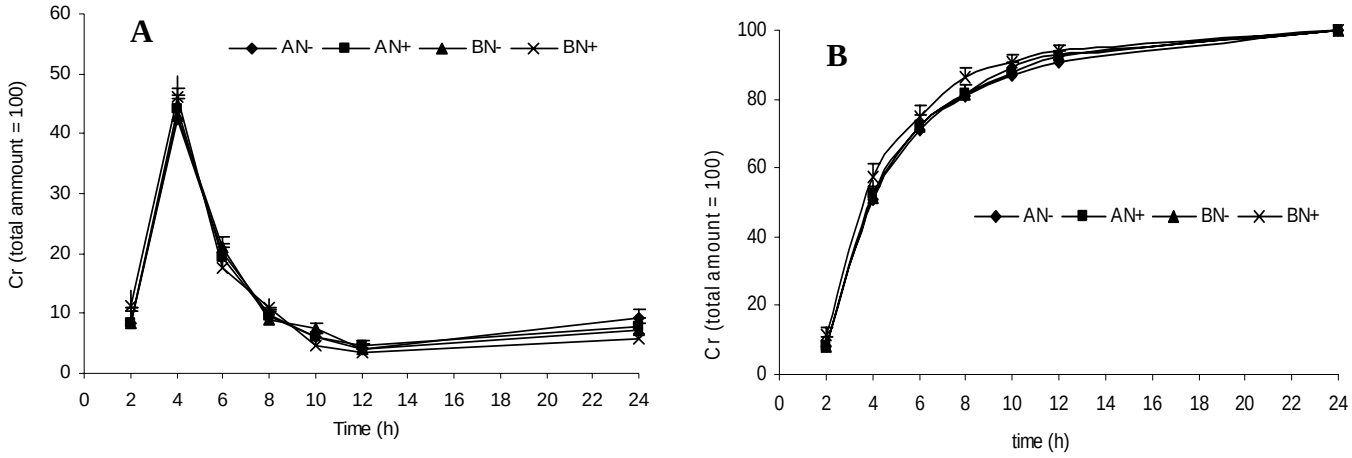

Figure 2. Period (Figure A) and cumulative (Figure B) feed passage rate in birds fed corn A and B on 30 days of age.

AN-: corn A normal energy basal diet; AN+; AN- plus enzyme; BN-: corn B normal energy basal diet; BN+: BN- plus enzyme. High quality corn (Cn) A released 138 kcal ME/kg feed by enzyme supplementation, while corn B released125 kcal ME/kg feed. With enzyme supplementation, there are minimal dietary 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively.

Discussion

The concept of corn quality or starch quality in corn has recently been applied more to poultry nutrition (Cowieson, 2005; Cowieson *et al.,* 2006a). Corn quality can be classified by its starch digestibility with low quality corn having low ME due to slowly digestible or resistant starch (Weurding *et al.,* 2001; Cowieson, 2005). In experiment 1 and 2, two formulation strategies were employed, reduced energy or normal energy ("over-the-top") diets supplemented with an enzyme cocktail of amylase, protease, and xylanase. The purpose of this design was to determine the best way to use enzymes with different quality corn. Birds fed high quality corn may digest appropriate starch with endogenous enzymes, while "over-the-top" exogenous enzyme addition may enhance the birds' ability to digest starch from low quality corn. Experiment 3 came from experiment 1 and 2 with birds fed normal energy diets. The purpose of experiment 3 was to investigate the feeding of two qualities of corn with or without exogenous enzyme on broiler performance, nutrient retention, and intestinal mucin.

Reduced BWG has been reported in birds fed diets with low (Reece *et al.,* 1984; Holsheimer and Veerkamp, 1992) and excessive (Cornejo *et al.,* 1991) energy. It is thus beneficial for birds to consume diets with proper energy levels to optimize performance. In EXP 1, birds fed AR+ and AN- were expected to have the proper dietary energy levels and indeed had greater BWG than their comparatives (AR- of low and AN+ of high energy), resulting in the expected interactions of dietary energy and enzyme supplementation. These results indicated that energy was released with enzyme supplementation. Similarly, Cowieson and colleagues (2006b) reported increased nutrient digestibility when birds were fed the enzyme cocktail and phytase. The numerically increased feed intake (FI) in birds fed reduced energy diets is in accordance with other research (Bartov and Plavnik, 1998) as a possible compensatory response to reduce energy deficit by consuming more feed (Yu and Robinson, 1992).

In EXP 2, higher BWG and FI in birds fed diets with either the lowest energy (BR-) or the highest energy (BN+) diets of low quality corn B was observed as compared to that of birds fed diets BR+ or BN-. Cowieson and coworkers (2006a) reported a similar result in which higher BWG was observed in birds fed normal nutrients with enzyme supplementation. The increased BWG in birds fed BN+ seems to fit previous assumptions that a normal energy diet of low quality corn plus enzyme ("over-the-top") will help the birds release proper nutrients from the corn and achieve greater BWG. The 2.8% lower AMEn in corn B as compared to corn A indicated that corn B was lower quality, and corn B diets were at lower levels of actual energy. The interesting observation in EXP 2 was the increased FI and BWG in birds fed BR-. This might be a result of an incidental optimal dietary ratio of protein (or amino acid) to energy which stimulated enhanced FI and BWG (Bartov and Plavnik, 1998), while enzyme supplementation may have released additional energy and altered the ratio which limited FI in birds fed BR+.

Interestingly, birds fed lower quality corn B diets gained more BW as compared to birds consuming high quality corn A diets until d 14 in EXP 3, while the reverse response of BWG was observed after d 14. The changing response in BWG to corn quality during aging may come from varied intestinal nutrient digestion and absorption between young and older birds. Young birds fed slowly digestible starch (low quality) have been shown to have better BWG and feed efficiency between d 9 and 30 (Weurding *et al.,* 2003). These researchers concluded that the improved BWG was a result of more efficient energy utilization with slowly digested starch. Birds gain mature and more extensive intestinal microflora after d 28 (Lu *et al.,* 2003). Animals fed a higher concentration of slowly digestible or resistant starch often have more active fermentation as a result of the microflora in the colon (Kleessen *et al.,* 1997). Feeding of low quality corn may result in reduced nutrients available to the bird because of more extensive competition from microflora for nutrients. The reduced availability of nutrients as a result of utilization by microflora may exceed the increased nutrient availability from slow digestion after

d 14 in the present experiment, resulting in the lower BWG in birds fed low quality corn. The reduced performance in birds fed low quality corn diets may also come from increased maintenance energy due to increased body weight.

There were interactions of enzyme supplementation and corn quality on BWG between d 14 and 37 in EXP 3 due to greater BWG in birds fed BN+ and AN- as compared to birds consuming BN- and AN+, respectively. This could suggest that birds fed high quality corn with supplemental enzymes had excess dietary energy which reduced growth and performance while birds fed low quality corn B with enzymes had optimal available dietary energy resulting in better BWG. Similar results have been indicated, in which performance was improved in birds fed diets supplemented with enzymes (Cowieson *et al.,* 2006a), while decreased BWG is observed in birds fed excessive dietary energy (Cornejo *et al.,* 1991).

Compensatory growth in birds with previously lower BWG was noted during the last feeding period (withdrawal diets) in both experiments. The energy that supports growth compensation may come from the reduced requirement for maintenance energy related to a lower BW and metabolic adaptation (Yu and Robinson, 1992), as well as greater FI relative to BW and its associated digestive adaptations (Zubair and Leeson, 1994).

Birds fed low energy diets are reported to have inferior feed efficiency (Holsheimer and Veerkamp, 1992; Leeson *et al.,* 1996). Similar results were observed in the present research with inferior feed efficiency in birds fed reduced energy diets after 28 days of age. The best FCR (1.816) in EXP 1 was in birds fed AN-, while the best FCR (1.815) in EXP 2 was in birds fed BN+. This result may come from similar optimal energy levels or energy to protein ratio in the two diets (Bartov and Plavnik, 1998), and nutrients should be released by enzyme supplementation in the latter diets to obtain required nutrient levels. The relative higher protein retention may also have contributed to a better FCR due to reduced loss of endogenous secretion (Cowieson *et al.,* 2003). The worst FCR (1.911) in birds consuming AR- in EXP 1 was caused

by lower nutrient density (Leeson *et al.,* 1996). The highest FCR (1.922) in birds fed BR+ in EXP 2 may have been caused by improper energy to protein ratio and increased loss of endogenous secretion (Cowieson *et al.,* 2004). In other reports, percent fatpad was greater in birds fed high energy diets as compared to birds fed reduced energy diets (Leeson and Zubair, 1997; Bartov and Plavnik, 1998). The increased fatpad percentage with linear trend in EXP 1 indicated that the deposit of fat was associated with either increased dietary energy levels or energy released by enzyme supplementation. The increased pectoral minor percentage in birds fed enzyme supplemented diets in EXP 1 indicated a positive effect of enzyme supplementation on protein turnover in breast muscle. An increased percent pectoral minor was also observed in a previous experiment (data not published) by our laboratory. The reduced mortality in birds fed enzymes in EXP 2 may indicate a positive protection effect of enzyme on bird's health when birds were fed low quality corn diets. A reduction of pathogenic bacteria of *Clostridium perfringen* and an increase of beneficial bacteria of *Bifidobacterium* spp. in birds fed enzyme supplementated diets was observed in a previous trial (data not published).

Although FI was only affected ($P < 0.05$) by dietary treatments from d 28 to 37 during EXP 3 with highest FI in birds fed AN-, numerically increased FI is often associated with superior BWG (Vandegrift *et al.,* 2003). However, birds fed low quality corn B had better feed conversion as compared to birds fed high quality corn A during d 1 to 14 as a result of greater BWG and lower FI. This result indicates more efficient nutrient utilization in birds fed more slowly digestible or resistant starch of low quality corn at an early age (Weurding *et al.,* 2003). With age, better feed utilization was evident in birds fed high quality corn A, and especially from d 28 and 37. Birds fed high quality corn had significantly improved feed conversion as compared to birds fed low quality corn. This changed response in feed efficiency due to age may come from increased nutrient expense by intestinal microflora exceeding the nutrient availability provided by slow digestion. It is also possible that superior feed efficiency in birds fed AN- and

BN+ was the result of providing optimal dietary energy level or energy to protein ratio (Holsheimer and Veerkamp, 1992) by diet formulation (AN-) or enzyme release (BN+).

The AMEn of high quality corn was 3.1% or 112 kcal/kg more ME as compared to corn B diets at d 30. This result showed that the previous determination of quality by Avicheck® (Cowieson *et al.,* 2006b) matches *in vivo* nutrient utilization. The interaction of dietary corn quality and enzyme addition on nitrogen retention is mainly caused by the higher nitrogen retention in birds fed AN- and BN+ as compared to birds fed AN+ and BN-. The nitrogen retention result is also in accordance with the higher BWG and better feed efficiency (Shafey and McDonald, 1991) in birds fed the former two diets. Corn is low in soluble non-starch polysaccharides, and therefore does not present problems of viscosity (Gracia *et al.,* 2003). The similar passage rate between dietary treatments in the present experiment was likely a result of similar digesta viscosity.

Sialic acid, one of the components of intestinal mucin, can be evaluated as mucoprotein production and loss of intestinal endogenous secretion (Cowieson *et al.,* 2004). Higher mucin secretion comes with more loss of endogenous secretion (saccharides, amino acids, etc.), poor nutrient retention, and reduced performance (Cowieson *et al.,* 2003). In EXP 3, lower sialic acid in birds fed BN+ on d 7 was associated with more BWG and improved feed conversion as compared to birds fed AN-. This result suggests that performance may be partially affected by endogenous secretion loss such as mucin. Mucin present in the intestinal lumen is degraded by microflora into free sialic acid for a nutrient source. Sialylated mucins from germ-free rats are rapidly degraded (90%) after 1 h incubation in the presence of whole caecal flora from normal rats (Fontaine *et al.,* 1998). A higher ratio of free to total sialic acid is thought to be a result of higher microflora activity, and contribute to an adverse effect on performance. Less efficient feed utilization in birds fed low quality corn B during the withdrawal period was in accordance with higher sialic acid ratio on d 49.

In conclusion, reducing dietary energy level was a better strategy for improving broiler BWG when supplementing the enzyme cocktail in high quality corn diets, while adding enzyme "over-the-top" was beneficial for improving broiler BWG and feed efficiency when supplementing the enzyme in low quality corn diets. Birds fed low energy diets resulted in inferior feed efficiency regardless of the quality of the corn. Quality of low quality corn was indicated as low AMEn as compared to high quality corn.

Chapter 4. Response of broilers fed two varieties of corn with or without an enzyme cocktail of amylase, protease, and xylanase on gastrointestinal enzyme activity and jejunal gene expression

Response of broilers fed two varieties of corn with or without an enzyme cocktail of amylase, protease, and xylanase on gastrointestinal enzyme activity and jejunal gene expression

Abstract A 2×2 factorial experiment was conducted to study the effect of corn quality and exogenous enzyme supplementation on broiler gastrointestinal enzyme activity and jejunal gene expression from 0 to 49 days of age. Four dietary treatments were comprised of: (1) diet AN-, corn A basal diet; (2) diet AN+, AN- plus Avizyme 1502® (AZ); (3) diet BN-, corn B basal diet; (4) BN+, BN- plus AZ. A total of 1,440 Ross 708 male chicks were randomly assigned with one of the four dietary treatments (9 reps/trt and 40 chicks/ rep) on d 1. Digesta samples from gizzard, jejunum, and ileum as well as pancreatic tissue samples were collected for evaluating amylase, protease, and xylanase activities. Intestinal tissue samples were collected for determining maltase, sucrase, and aminopeptidase N activities. On d 28, jejunal tissue was collected for total RNA isolation and subsequent genome-wide microarray assay. Corn A had higher endogenous amylase and xylanase activities as compared to corn B in the native cereal Xylanase activity in jejunal and ileal digesta of birds fed corn A diets were higher ($P < 0.05$) as compared to that of corn B fed birds on d 7 and 49. There were interactions of corn quality and enzyme addition ($P < 0.05$) with activities of ileal maltase, sucrase and aminopeptidase N on d 7. Sucrase-isomaltase contributed 63, 80, and 74 % of total maltase activity in duodenum, jejunum, and ileum, respectively. Most maltase and sucrase activities were correlated ($P < 0.05$) between intestinal segments. Pancreatic amylase activity was negatively correlated ($P < 0.05$) with ileal maltase, sucrase, and aminopeptidase N activities. Sucrase activities in duodenum and jejunum were correlated ($P < 0.05$) with performance, whereas duodenal aminopeptidase N was negatively correlated ($P < 0.05$) with performance except for period body weight gain. Pancreatic amylase and protease activities and gizzard activities of protease and xylanase were correlated ($P < 0.05$) with performance. The number of genes differentially regulated ($P < 0.05$) by corn quality was 77 as compared to those by enzyme supplementation in corn A diets (30 genes) and corn B diets

(23 genes). Immune response and metabolism related genes were the most frequent among those changed. In conclusion, differences in response to corn quality may come from active components, such as amylase and xylanase, and improved performance may relate to increased digestive enzyme activity, optimal microflora with minimal disease challenge, immune response, and metabolic demand.

Key words: broiler, enzyme, corn, gene regulation

Introduction

While exogenous microbial enzymes are often used in wheat/barley based diets to reduce the adverse effect of anti-nutritive factors, corn-soybean based diets have been supplemented with exogenous enzymes to eliminate nutritive variation of ingredient (Cowieson, 2005; Cowieson and Adeola, 2005). Improved performance was noted in birds fed corn-soybean diets supplemented with exogenous enzymes (Ghazi *et al.,* 1997b; Zanella *et al.,* 1999; Francesch *et al.,* 2005) as a result of increased nutrient digestibility (Cowieson *et al.,* 2006b). In contrast, no improvement of performance by enzyme supplementation has also been observed (Iji *et al.,* 2003), even with an increase in nutrient digestibility (Troche *et al.,* 2007).

In most reported studies, enzyme supplementation improves nutrient digestibility. The mechanism by which enzymes provide this improvement are not fully elucidated but may include altered gastrointestinal activities of absorption, secretion, and immune response or increased total digestive enzyme activity. Evidence of each of these mechanisms exists in the literature. In birds, amylase activity in crop, pancreas, and small intestine was not consistently changed by dietary supplementation of amylase and xylanase (Ritz *et al.,* 1995), whereas in humans, depressed pancreas secretion was observed when trypsin was administrated into the duodenum (Owyang *et al.,* 1986). Birds with early feed restriction and exogenous protease and amylase supplementation have increased sucrase, maltase, and lipase activities as compared to birds fed control diets (Pinheiro *et al.,* 2004). Components of dietary ingredients may also affect pancreatic secretion and subsequent intestinal enzyme activity. When phenylalanine was injected into chick wing veins, increased trypsinogen and chymotrypsinogen secretion was observed (Yang *et al.,* 1989). Zinc oxide supplementation increased amylase, lipase, trypsin and total protease activity in rat pancreatic homogenates and small intestinal digesta (Szabo *et al.,* 2004).

Most balanced diet formulations are currently based on proximate nutrient values. Increasing evidence suggests that nutrient values of dietary ingredients are also affected by active components such as enzyme inhibitors. Trypsin inhibitor in soybean has been recognized

for a long time. Reduced performance was reported when birds were fed a normal raw soybean diet as compared to birds fed a diet with soybean genetically altered to remove trypsin inhibitor (Palacios *et al.,* 2004). Increased secretion of pancreatic enzymes was observed when the synthetic protease inhibitor camostat was applied in duodenum of men (Adler *et al.,* 1988). Currently, soybean meal is processed to eliminate inhibitor activity and other anti-nutritional factors prior to being utilized in animal diets. Although corn accounts for more than sixty percent of complete diets, little attention has been focused on possible active components in raw corn, such as enzymes and their inhibitors. Indeed, a bi-functional Hageman factor inhibitor from corn is reported to inhibit animal protease and amylase activities (Behnke *et al.,* 1998).

In the present experiment, birds were fed two varieties (qualities) of corn combined with supplementation of an enzyme cocktail of amylase, protease, and xylanase. The objective of this research was to investigate the alteration of gastrointestinal enzyme activities and genome-wide jejunal gene expression by dietary enzymes and corn quality. The effect of dietary enzymes and corn quality on performance, carcass yield, nutrient retention, and intestinal mucin has been presented in chapter 3.

Materials and methods

Animals and diets

Two varieties of corn (A and B) were analyzed for proximate composition and amino acids and were evaluated for metabolizable energy (ME) released by Avizyme 1502®[6] addition with Avicheck™ Corn program as described previously (Cowieson *et al.,* 2006a). Mycotoxin was also analyzed in both corn samples. Dry matter, crude protein, crude fat, crude fiber, crude ash, calcium, phosphorus, and sodium in corn gluten and soybean meal were also analyzed. Amino acid composition in soybean meal and corn gluten meal was evaluated by AminoDat®

[6] Danisco Animal Nutrition, IL

3.0[7]. Based on analyzed proximate results, ME in corn, soybean meal, and corn gluten was determined by ME estimating equations (WPSA, 1989).

A total of 1,440 male Ross 708 broiler chicks were placed on fresh pine shaving in 36 pens with 9 replicates (pens) per treatment and 40 chicks per replicate (12.8 chicks /m^2 at day of age). The four feeding periods used were starter (d 0 to 14), grower (d 14 to 28), finisher (d 28 to 37), and withdrawal (d 37 to 49). Chicks were assigned to one of four dietary treatments from 0 to 49 days of age. The four diets consisted of diet AN-: corn A with normal matrix energy; diet AN+: AN- plus 0.05 % Avizyme 1502®; diet BN-: corn B with normal matrix energy; diet BN+: BN- plus 0.05% Avizyme 1502®;

Corn-soybean meal based diets were formulated as recommended in the nutrient requirement guide of the Ross 708 male broiler (Ross broiler nutrition specification, 2006). Mash diets were mixed at the Virginia Tech Research facility and transported to a local commercial feed mill to be either pelleted or crumbled. To estimate recovery of amylase, protease, and xylanase in corn, corn A and B samples with or without heat treatments (Dried 24 h at 100 °C or autoclaved 30 min at 121 °C then dried 24 h at 100 °C) received a combination of amylase, protease, and xylanase. Recovery of the enzyme activities was conducted to evaluate the endogenous enzyme and inhibitor activities in the corn. Feed and water were provided *ad libitum*. Chicks were on a step lighting program with 24 h of light for the first 10 d of age, 20 h of light and 4 h of dark during d 11 to 42, and 24 h of light during d 43 to 49. The research trial was conducted with the written approval of the Virginia Tech Institutional Animal Care and Use Committee.

Tissue and digesta sample collections

One bird per pen was euthanized (n = 8 birds/treatment) on 7, 14, 28 and 49 d of age. The pancreas was isolated from the duodenal loop and homogenized with glass slides. The

[7] Degussa GmbH Bennigsenplatz 1 40474 Düsseldorf, Germany

duodenum, jejunum, and ileum were located and opened lengthwise. The digesta of these tissues was gently scraped onto wax paper, and intestinal tissues were briefly rinsed in ice-cold PBS buffer, scraped and homogenized with glass slides. The homogenized tissue was weighed, flash frozen in liquid nitrogen, and then stored at -50 °C until analysis. On d 28, an additional jejunal tissue sample was collected without association of added RNase inhibitor and stored at -80 °C for a microarray assay. The digesta from gizzard, jejunum, and ileum were collected using the same birds for tissue sampling. The digesta was homogenized and weighed, flash frozen in liquid nitrogen, and stored at -50 °C until analysis.

Assay activities of amylase, protease, and xylanase

Soluble protein in digesta and feed samples was extracted with 0.05 M Tris buffer pH 7.0 twice. The supernatants were pooled, and an aliquot of the supernatant was stored at -20 °C. Pancreatic tissues were homogenized in a solution of 100 mM mannitol 2 mM HEPES/KOH (pH 6.5), centrifuged at 2200 ×g for 10 min, and aliquots of the supernatant were stored at -20 °C. Protein concentration of pancreas was determined in assay microplates (Costar® 3631)[8] with the Bio-Rad protein assay kit[9]. Amylase activity in these aliquots was determined in assay microplates (Costar® 3915) by the EnzChek®[10] Ultra Amylase Assay Kit (E33651). *Bacillus* sp. amylase (Sigma A-6380)[11] was used as a standard for each plate assay. One unit of amylase activity will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C. Protease activity in these samples was analyzed in assay microplates (Costar® 3915) by the EnzChek® Protease Assay Kit (E6638). Protease from *Aspergillus saitoi* (Sigma P-2143) was used as a standard for each plate assay. One unit of protease activity will hydrolyze hemoglobin to produce color equivalent to 1.0 μmole (181 μg) of tyrosine per min at pH 2.8 at 37 °C (color by Folin-Ciocalteu

[8] Corning Incorporated, Corning, NY.
[9] Bio-Rad Laboratories, Hercules, CA.
[10] Invitrogen Corporation, Carlsbad, CA.
[11] Sigma-Aldrich, St. Louis, MO.

reagent). Xylanase activity in digesta samples was determined in assay microplate (Costar® 3915) by the EnzChek® Ultra Xylanase Assay Kit (E33650). Xylanase from *Trichoderma viride* (Sigma 95595) was used as a standard for each plate assay. One unit of xylanase corresponds to the amount of enzyme which liberates 1 μmole remazol brilliant blue R per min at pH 6.0 and 40°C. Fluorescence levels in the assay plates were detected using a FluoStar Optima microplate reader[12].

Assay of maltase, sucrase, and aminopeptidase N

Tissues of duodenum, jejunum, and ileum were homogenized in 100 mM mannitol 2 mM HEPES/KOH (pH 6.5), then centrifuged at 2200 ×g for 10 min. Aliquots of the supernatant were stored at -20 °C. Protein concentration was determined in assay microplates (Costar® 3631) with the Bio-Rad protein assay kit. Maltase and sucrase activities in the tissues were analyzed according to Dahlqvist methods (Dahlqvist, 1964) with modification for microplate (Costar® 3631) assay at 42 °C for 20 min incubation. Aminopeptidase N activity was assayed with microplate (Costar® 3631). Briefly, 8μl of undiluted homogenized tissue was incubated with 100 μl 1 mM L-leucine-p-nitroanilide 2 mM $MgCl_2$ at 42 °C for 16 min. 4-nitroaniline (Fisher AC18069-1000)[13] was used as a standard. Change of absorbance was detected on a FluoStar Optima microplate reader at 405 mM. One unit of maltase, sucrase, or aminopeptidase N is defined as the hydrolysis of 1 μmol of substrate in one minute at 42 °C, pH 7.0.

Jejunal gene expression

Total RNA in jejunum tissue was extracted with Qiagen RNeasy® mini kit[14]. RNA integrity was evaluated with gel electrophoresis, and the concentration was determined by a

[12] BMG Labtech Inc. Durham, NC.
[13] Fisher Scientific Co., Pittsburgh PA
[14] Qiagen Inc. Valencia, CA.

Genesys™ 5 spectrophotometer[15]. RNA from four replicate samples per treatment was pooled (n = 2 pools/trt) and sent to the Virginia Bioinformatics Institute for microarray gene expression assay using GeneChip® Chicken Genome Array[16] (n = 8 chips). Data were processed with ArrayAssist® Expression software[17] to generate gene expression values using GC Robust Multi-array Average (GCRMA) procedure. The gene expression data were further analyzed with GeneSpring software[18].

Statistical Analysis

All data were analyzed using the MIXED procedures of SAS (SAS Institute In., 2006) by a 2 ×2 factorial arrangement of dietary treatments in a randomized complete design to determine the overall significance of enzyme and corn variety as well as the interaction between enzyme and corn variety. Additional pair-wise contrast between diets was carried out to evaluate the effect of diets when an interaction significance (P <0.05) was determined. Correlations between enzyme activity and performance were analyzed using the CORR procedure of SAS. Greater than 1.5-fold changes of normalized gene expression values between dietary treatments were analyzed with t-test of two-sample equal variance, and results were reported at $P < 0.05$. False discovery rate (FDR) was not used due to limited replicates.

Results

Proximate analysis values were similar between the two varieties of corn (Table 15). Corn B had 1.1, 0.4, and 0.1 ppm Fumonisin B1, B2, and B3, respectively. Higher amylase and xylanase

[15] Thermo Spectronic, Rochester, NY.
[16] Affymetrix, Santa Clara, CA.
[17] Stratagene, Cedar Creek, TX
[18] Agilent Technologies, Inc. Santa Clara, CA

Table 15 Proximate analysis (%) and mycotoxin (ppm) values of corn A and B[1]

	Corn A	Corn B
Proximate, %		
Dry matter	84.9	86.6
Protein	9.4	10.0
Fat	4.0	4.4
Fiber	1.5	1.9
ash	1.2	1.6
Nitrogen-free extract	85.3	82.9
Starch	70.4	70.7
Phytate	1.2	1.0
Mycotoxin, ppm		
Fumonisin B1	ND	1.1
Fumonisin B2	ND	0.4
Fumonisin B3	ND	0.1

[1]Proximate analysis values were based on dry matter

activities in corn A were noted as compared to corn B in untreated condition (Table 16). When the enzyme cocktail of amylase, protease, and xylanase was added into the corn, recovery of xylanase and protease activities was inaccurate as a result of endogenous enzyme and inhibitor activities in the corn, whereas amylase activity was recovered to some degree. After corn was treated with heat, activities of amylase and protease were recovered when corn was dried for 24 h at 100 °C, while activity of xylanase was recovered only when corn was autoclaved for 30 min at 121 °C and then dried for 24 h at 100 °C.

Dietary corn and enzyme supplementation interaction was observed with amylase activity in pancreas on d 7 (Table 17). Pancreatic amylase activity in Birds fed BN- (corn B basal diet) was lower as compared to birds fed BN+ (corn B with enzyme supplementation diet) and AN- (corn A basal diet). Interaction of corn quality and enzyme addition on jejunal amylase activity was observed on d 14. Birds fed BN- had greater jejunal amylase activity as compared to birds consuming BN+.

Birds fed corn A diets had higher protease activity as compared to birds fed low quality corn B diets on d 28 (Table 18) and there was interaction of corn quality and enzyme addition on jejunal protease activity on d 49. Birds fed BN- had greater jejunal protease activity as compared to birds fed BN+ on d 49. Birds fed high quality corn A diets had greater ileal protease activity as compared to birds consuming low quality corn diets on d 14. No differences in protease activity were observed for gizzard or pancreas.

Xylanase activity in gizzard digesta of birds fed high quality corn was greater as compared to birds consuming low quality corn on d 14 (Table 19). On d 7 and 49, birds fed high quality corn had higher jejunal xylanase activity and ileal xylanase activity as compared to birds fed low quality corn. No differences in xylanase activity were observed from enzyme supplementation or the interaction of corn quality and enzyme addition.

Table 16 Activities of amylase (mU/g corn), protease (U/g), and xylanase (mU/g) in corn A and B[1]

Corn	Enzyme activity	A-	A+	B-	B+
Untreated corn	amylase	282	644	40	609
	protease	17	92	14	227
	xylanase	46	48	17	19
Corn dried for 24h at 100 °C	amylase	113	4715	37	4483
	protease	18	821	8	985
	xylanase	30	43	2	9
Corn autoclaved 30 min and dried for 24h at 100 °C	amylase	51	4579	33	4759
	protease	105	1573	38	1434
	xylanase	< 0.1	148	3	156

[1]A- and B- are corn A and B mash without enzyme supplementation, A+ and B+ mash are corn A and Corn B with supplemented an enzyme cocktail of 2,000 mU amylase, 43 mU xylanase, and protease 700 U per gram corn. One unit of amylase activity will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C. One unit of protease activity will hydrolyze hemoglobin to produce color equivalent to 1.0 μmole (181 μg) of tyrosine per min at pH 2.8 at 37 °C (color by Folin-Ciocalteu reagent). One unit of xylanase corresponds to the amount of enzyme which liberates 1 μmole remazol brilliant blue R per min at pH 6.0 and 40°C.

Table 17. Effect of corn quality and enzyme supplementation on broiler amylase activity in the gastrointestinal tract[1]

Diet	AN-	AN+	BN-	BN+		Main Effects						
Corn quality[2]	High	High	Low	Low		Enz		Cn		Statistical effects		
Enzyme[3]	-	+	-	+	SEM[4]	-	+	High	Low	Cn×En	Enz	Cn
Gizzard, mU												
d 7	63	197	39	19	62.9	51	108	130	29	NS	NS	NS
d 14	348	60	44	258	126.2	196	159	204	151	NS	NS	NS
d 28	82	102	37	73	30.7	60	87	92	55	NS	NS	NS
d 49	422	394	220	250	208.1	321	322	408	235	NS	NS	NS
Pancreas												
d 7	7.6[a]	5.6[ab]	3.4[b]	6.9[a]	1.17	5.5	6.2	6.6	5.1	0.02	NS	NS
d 14	33.7	33.8	26.7	31.3	3.40	33.8	29.0	32.5	30.2	NS	NS	NS
d 28	39.0	34.8	32.6	45.1	8.20	35.8	40.0	36.9	38.9	NS	NS	NS
d 49	25.9	28.2	25.4	25.7	2.80	25.6	27.0	27.0	25.6	NS	NS	NS
Jejunum												
d 7	739	1137	772	1061	216.2	756	1099	938	917	NS	NS	NS
d 14	871[ab]	1023[ab]	1235[a]	779[b]	130.8	1053	901	947	1007	0.03	NS	NS
d 28	1242	1413	1071	1019	236.2	1157	1216	1328	1045	NS	NS	NS
d 49	1190	1557	1228	922	260.6	1209	1240	1374	1075	NS	NS	NS
Ileum												
d 7	892	1031	1098	809	184.7	995	920	962	953	NS	NS	NS
d 14	514	377	627	709	116.6	571	543	445	668	NS	NS	NS
d 28	759	855	962	698	170.8	861	777	807	830	NS	NS	NS
d 49	701	793	820	678	148.8	760	735	747	749	NS	NS	NS

[a,b] Different superscripts within one row are significant different (P <0.05). One unit of amylase activity will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C.

[1] Values are means of 8 replicate pens; pancreas U/mg protein, jejunum and ileum U/g dry digesta; AN-/BN- was normal energy diet using corn A or B; plus sign meant diets with enzyme addition.

[2] High quality corn (Cn) A released 138 kcal ME/kg feed by enzyme supplementation, while corn B released 125 kcal ME/kg feed.

[3] With enzyme (Enz) supplementation to provide minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively.

[4] Standard error of the difference between means

Table 18. Effect of corn quality and enzyme supplementation on broiler protease activity in gastrointestinal tract[1]

Diet	AN-	AN+	BN-	BN+		Main Effects						
Corn quality[2]	High	High	Low	Low		Enz		Cn		Statistical effects		
Enzyme[3]	-	+	-	+	SEM[4]	-	+	High	Low	Cn×En	Enz	Cn
Gizzard												
d 7	2.01	1.96	1.52	0.89	0.451	1.76	1.42	1.98	1.20	NS	NS	NS
d 14	1.73	2.82	0.63	0.74	0.863	1.18	1.78	2.27	0.68	NS	NS	NS
d 28	3.09	3.64	2.40	1.93	0.667	2.75	2.79	3.36	2.17	NS	NS	NS
d 49	5.61	5.97	3.80	6.33	1.814	4.70	6.15	5.79	5.07	NS	NS	NS
Pancreas												
d 7	0.16	0.23	0.27	0.08	0.081	0.16	0.21	0.19	0.17	NS	NS	NS
d 14	2.65	3.22	2.47	2.39	0.520	2.56	2.80	2.94	2.43	NS	NS	NS
d 28	5.24	4.05	4.38	4.73	0.869	4.81	4.39	4.64	4.55	NS	NS	NS
d 49	5.13	5.61	5.57	5.71	0.440	5.35	5.66	5.37	5.64	NS	NS	NS
Jejunum												
d 7	2.77	1.92	1.22	2.24	0.502	2.00	2.08	2.34	1.73	NS	NS	NS
d 14	2.25	2.16	2.48	1.97	0.210	2.36	2.07	2.21	2.22	NS	NS	NS
d 28	3.63	3.16	3.04	2.78	0.414	3.33	2.97	3.40	2.91	NS	NS	0.01
d 49	2.59[ab]	3.13[ab]	3.35[a]	2.14[b]	0.395	2.97	2.64	2.86	2.75	0.04	NS	NS
Ileum												
d 7	1.12	0.99	1.07	1.28	0.275	1.09	1.14	1.06	1.18	NS	NS	NS
d 14	1.29	1.11	1.75	1.68	0.233	1.52	1.39	1.20	1.72	NS	NS	0.03
d 28	1.43	1.55	1.34	1.24	0.316	1.38	1.40	1.49	1.29	NS	NS	NS
d 49	1.06	0.78	1.11	0.94	0.249	1.08	0.86	0.92	1.03	NS	NS	NS

[a,b] Different superscripts within one row are significant different (P <0.05). One unit of protease activity will hydrolyze hemoglobin to produce color equivalent to 1.0 μmole (181 μg) of tyrosine per min at pH 2.8 at 37 °C (color by Folin-Ciocalteu reagent).

[1] Values are means of 8 replicate pens, pancreas 1,000U/mg protein and gizzard 1,000U/dry digesta, jejunum and ileum 1,000,000 U/dry digesta; AN-/BN- was normal energy diet using corn A or B; plus sign meant diets with enzyme addition.

[2] High quality corn (Cn) A released 138 kcal ME/kg feed by enzyme supplementation, while corn B released 125 kcal ME/kg ME feed.

[3] With enzyme (Enz) supplementation to provide minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively.

[4] Standard error of the difference between means.

Table 19. Effect of corn quality and enzyme supplementation on broiler xylanase activity (mU/g dry digesta) in gastrointestinal tract[1]

Diet	AN-	AN+	BN-	BN+		Main Effects						
Corn quality[2]	High	High	Low	Low		Enz		Cn		Statistical effects		
Enzyme[3]	-	+	-	+	SEM[4]	-	+	High	Low	Cn×En	Enz	Cn
Gizzard												
d 7	44	51	39	22	11.0	41	36	47	30	NS	NS	NS
d 14	64	59	18	50	11.6	41	54	62	34	NS	NS	0.03
d 28	55	188	33	102	52.1	44	145	122	67	NS	NS	NS
d 49	157	106	65	160	36.8	111	133	132	113	NS	NS	NS
Jejunum												
d 7	323	315	212	244	33.6	267	280	319	228	NS	NS	0.01
d 14	447	512	435	394	35.6	441	453	480	415	NS	NS	NS
d 28	463	460	381	440	83.8	422	450	461	410	NS	NS	NS
d 49	491	627	269	279	116.5	380	453	559	274	NS	NS	0.02
Ileum												
d 7	1217	1177	803	1090	97.3	1010	1133	1197	946	NS	NS	0.02
d 14	883	856	775	879	43.0	829	867	870	827	NS	NS	NS
d 28	865	966	678	906	105.7	771	936	916	792	NS	NS	NS
d 49	1124	1108	843	733	87.4	983	920	1116	788	NS	NS	0.001

[1] Values are means of 8 replicate pens. One unit of xylanase corresponds to the amount of enzyme which liberates 1 μmole remazol brilliant blue R per min at pH 6.0 and 40°C; AN-/BN- was normal energy diets using corn A or B; plus sign meant diets with enzyme addition.
[2] High quality corn (Cn) A released 138 kcal ME/kg feed by enzyme supplementation, while corn B released 125 kcal ME/kg feed.
[3] With enzyme (Enz) supplementation to provide minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively.
[4] Standard error of the difference between means

Interaction of corn quality and enzyme addition on jejunal maltase activity was observed on d 7 (Table 20). Birds fed AN- had higher jejunal maltase activity as compared to birds fed BN- on d 7. Similarly, dietary treatments of corn quality and enzyme addition had interaction on ileal maltase activity on d 7. Maltase activity in ileum of birds fed AN- was greater as compared to that of birds fed AN+ and BN-. The only differences observed in sucrase (Table 21) or aminopeptidase N (Table 22) activities were from interaction of corn quality and enzyme supplementation on d 7. Birds fed AN+ and BN- on d 7. No differences in maltase activity were observed from corn quality alone or enzyme supplementation. AN- had higher ileal sucrase activity as compared to birds consuming AN+ and BN-. Birds fed high quality corn diets had higher aminopeptidase N as compared to birds fed low quality corn diets.

Strong correlations between intestinal maltase and sucrase activities in the duodenum, jejunum, and ileum were observed (Figure 3). The slope of this relationship estimated the contribution of sucrase-isomaltase to maltose hydrolysis and the intercept evaluated the activity of maltase-glucoamylase (Table 23). The contribution of sucrase-isomaltase to total maltase activity was estimated by multiplying the slope of the sucrase-maltase regression line by the mean sucrase activity. Sucrase contributed 63, 80, and 74 % of total maltase activity in the duodenum, jejunum, and ileum, respectively, whereas similar maltase-glucoamylase activity existed across the three segments.

Most maltase and sucrase activities itself or with each other were correlated between the intestinal segments of duodenum, jejunum, and ileum (Table 24). Similarly, aminopeptidase N activity was correlated with maltase and sucrase activities in segments of duodenum, jejunum, or ileum. Pancreatic amylase activity was negatively correlated with ileal maltase and sucrase activities as well as aminopeptidase N activities in duodenum and ileum, whereas positively correlated with jejunal sucrase and aminopeptidase N activities. Protease activity in jejunum was correlated with intestinal maltase, duodenal and jejunal sucrase, and jejunal aminopeptidase N

Table 20. Effect of corn quality and enzyme supplementation on broiler maltase activity in the intestine[1]

Diet	AN-	AN+	BN-	BN+		Main Effects						
Corn quality[2]	High	High	Low	Low		Enz		Cn		Statistical effects		
Enzyme[3]	-	+	-	+	SEM[4]	-	+	High	Low	Cn×En	Enz	Cn
Duodenum												
d 7	2.34	1.66	1.40	1.73	0.341	2.00	1.57	1.70	1.87	NS	NS	NS
d 14	1.45	1.04	1.41	1.26	0.141	1.43	1.15	1.25	1.34	NS	NS	NS
d 28	1.97	2.59	2.33	2.13	0.536	2.15	2.36	2.28	2.23	NS	NS	NS
d 49	2.06	2.10	2.18	1.91	0.158	2.12	2.01	2.08	2.05	NS	NS	NS
Jejunum												
d 7	4.39[a]	2.78[ab]	2.11[b]	3.24[ab]	0.587	3.25	3.01	3.59	2.67	0.03	NS	NS
d 14	3.09	3.12	3.70	3.40	0.387	3.39	3.26	3.11	3.55	NS	NS	NS
d 28	3.31	3.43	3.83	3.08	0.333	3.57	3.25	3.37	3.45	NS	NS	NS
d 49	5.01	3.99	4.79	3.73	0.721	4.90	3.86	4.50	4.26	NS	NS	NS
Ileum												
d 7	3.74[a]	2.44[bc]	1.62[c]	3.22[ab]	0.375	2.68	2.83	3.09	2.42	0.001	NS	NS
d 14	1.90	2.42	2.61	2.65	0.380	2.26	2.53	2.16	2.63	NS	NS	NS
d 28	2.04	2.38	2.51	2.28	0.278	2.27	2.33	2.21	2.39	NS	NS	NS
d 49	2.66	2.97	2.86	2.82	0.265	2.76	2.90	2.82	2.84	NS	NS	NS

[a,b,c] Different superscripts within one row are significant different (P <0.05). One unit of maltase is defined as the hydrolysis of 1 μmol of substrate in one minute at 42 °C, pH 7.0.

[1] Values are means of 8 replicate pens; AN-/BN- was normal energy diet using corn A or B; plus sign meant diets with enzyme addition.

[2] High quality corn (Cn) A released 138 kcal ME/kg feed by enzyme supplementation, while corn B released 125 kcal ME/kg feed.

[3] With enzyme (Enz) supplementation to provide minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively.

[4] Standard error of the difference between means

Table 21. Effect of corn quality and enzyme supplementation on broiler sucrase activity in the intestine[1]

Diet	AN-	AN+	BN-	BN+		Main Effects						
Corn quality[2]	High	High	Low	Low		Enz		Cn		Statistical effects		
Enzyme[3]	-	+	-	+	SEM[4]	-	+	High	Low	Cn×En	Enz	Cn
Duodenum												
d 7	0.23	0.14	0.12	0.15	0.036	0.17	0.14	0.19	0.13	NS	NS	NS
d 14	0.13	0.11	0.14	0.10	0.016	0.14	0.11	0.12	0.12	NS	NS	NS
d 28	0.35	0.44	0.39	0.35	0.080	0.37	0.39	0.39	0.37	NS	NS	NS
d 49	0.41	0.45	0.46	0.35	0.041	0.43	0.40	0.43	0.41	NS	NS	NS
Jejunum												
d 7	0.64	0.48	0.46	0.49	0.082	0.55	0.48	0.56	0.48	NS	NS	NS
d 14	0.65	0.76	0.74	0.66	0.096	0.70	0.71	0.70	0.70	NS	NS	NS
d 28	0.68	0.82	0.87	0.72	0.091	0.78	0.77	0.75	0.79	NS	NS	NS
d 49	1.09	0.92	1.05	0.73	0.143	1.07	0.82	1.00	0.89	NS	NS	NS
Ileum												
d 7	0.54[a]	0.36[bc]	0.27[c]	0.47[ab]	0.055	0.41	0.42	0.45	0.37	0.002	NS	NS
d 14	0.29	0.30	0.32	0.32	0.046	0.31	0.31	0.29	0.32	NS	NS	NS
d 28	0.30	0.36	0.36	0.29	0.045	0.33	0.33	0.33	0.33	NS	NS	NS
d 49	0.32	0.39	0.29	0.32	0.039	0.31	0.36	0.36	0.31	NS	NS	NS

[a,b,c] Different superscripts within one row are significant different (P <0.05). One unit of sucrase is defined as the hydrolysis of 1 μmol of substrate in one minute at 42 °C, pH 7.0.

[1] Values are means of 8 replicate pens; AN-/BN- was normal energy diet using corn A or B; plus sign meant diets with enzyme addition.

[2] High quality corn (Cn) A released 138 kcal ME/kg feed by enzyme supplementation, while corn B released 125 kcal ME/kg feed.

[3] With enzyme (Enz) supplementation to provide minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively.

[4] Standard error of the difference between means.

Table 22. Effect of corn quality and enzyme supplementation on broiler aminopeptidase activity in the intestine[1]

Diet	AN-	AN+	BN-	BN+		Main Effects						
Corn quality[2]	High	High	Low	Low		Enz		Cn		Statistical effects		
Enzyme[3]	-	+	-	+	SEM[4]	-	+	High	Low	Cn×En	Enz	Cn
Duodenum												
d 7	41.5	30.1	28.8	30.1	5.28	35.1	30.1	35.8	29.4	NS	NS	NS
d 14	26.1	24.6	28.2	26.2	1.67	27.1	25.4	25.3	27.2	NS	NS	NS
d 28	24.8	33.0	28.4	28.0	4.04	26.6	30.5	28.9	28.2	NS	NS	NS
d 49	22.7	23.2	24.3	20.4	1.34	23.5	21.8	22.9	22.3	NS	NS	NS
Jejunum												
d 7	35.5	35.4	29.9	36.2	3.10	32.7	35.8	35.5	33.1	NS	NS	NS
d 14	44.6	40.9	40.2	41.0	3.57	42.4	41.0	42.8	40.6	NS	NS	NS
d 28	38.5	39.8	38.3	38.4	3.24	38.4	39.1	39.1	38.3	NS	NS	NS
d 49	46.7	41.9	36.2	35.1	4.82	41.5	38.5	44.3	35.7	NS	NS	NS
Ileum												
d 7	28.0[a]	23.3[ab]	18.7[b]	25.3[a]	1.79	23.3	24.3	25.6	22.0	0.004	NS	0.05
d 14	19.0	20.1	21.0	20.7	2.66	20.0	20.4	19.5	20.9	NS	NS	NS
d 28	22.5	21.6	22.8	19.2	2.61	22.7	20.4	22.1	21.0	NS	NS	NS
d 49	18.9	20.7	19.8	18.9	1.17	19.3	19.8	19.8	19.3	NS	NS	NS

[a,b] Different superscripts within one row are significant different (P <0.05). One unit of aminopeptidase N is defined as the hydrolysis of 1 μmol of substrate in one minute at 42 °C, pH 7.0.

[1] Values are means of 8 replicate pens; AN-/BN- was normal energy diet using corn A or B; plus sign meant diets with enzyme addition.

[2] High quality corn (Cn) A released 138 kcal ME/kg feed by enzyme supplementation, while corn B released 125 kcal ME/kg feed.

[3] With enzyme (Enz) supplementation to provide minimum of 300, 400, and 4000 U/kg of xylanase, amylase, and protease, respectively.

[4] Standard error of the difference between means

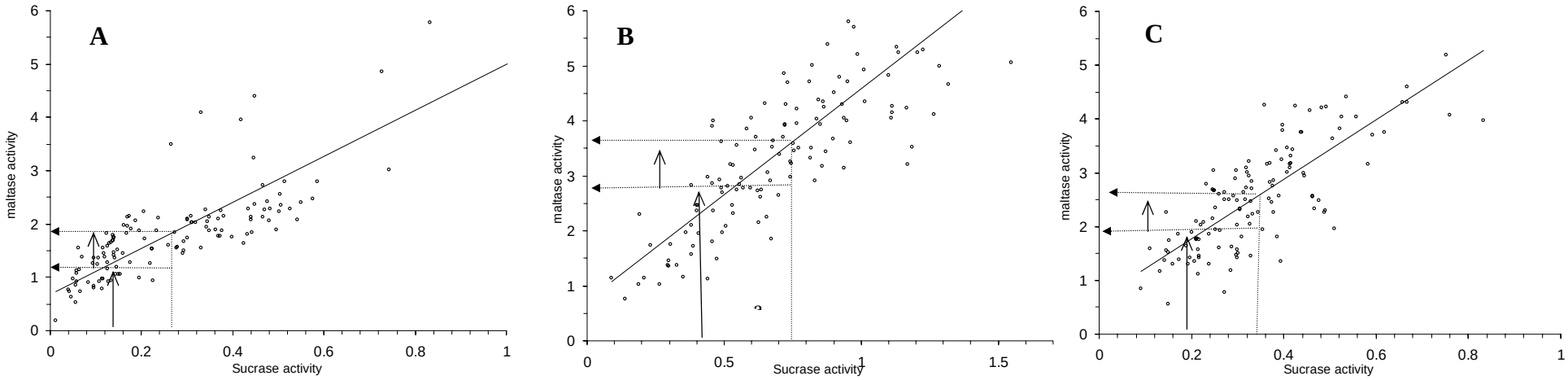

Figure 3. Contribution of sucrase-isomaltase to total maltase activity.

Enzyme activity is expressed as substrate hydrolyzed at μmole · min^{-1} · mg $protein^{-1}$. The relationships between maltase (Y) and sucrase (X) are Y = 4.30X + 0.68 at the duodenum (A), Y = 3.86X + 0.73 at the jejunum(B), and Y = 5.52X + 0.66 at the ileum (C).

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Table 23. Contribution of sucrase and sucrase-independent maltase to maltase activity[1].

	Duodenum	Jejunum	Ileum
Sucrase activity	0.27	0.73	0.35
Maltase activity	1.85	3.56	2.57
Slope	4.30	3.86	5.52
R^2	0.67	0.66	0.61
Sucrase contribution to maltase activity (%)	1.16 (63%)	2.84 (80%)	1.90 (74%)
Glucoamylase contribution to maltase activity (%)	0.69 (37%)	0.72 (20%)	0.67 (26%)

[1]Mean activity was expressed as substrate hydrolyzed at μmole · min^{-1} · mg $protein^{-1}$. The contribution of sucrase activity to maltase activity was estimated by multiplying mean sucrase activity by the slope of the regression line (Figure 3). The contribution of glucoamylase was the value of the intercept of the regression line.

Table 24. Correlation of maltase, sucrase, and aminopeptidase activities with amylase, protease, and xylanase activities as well as performance[1]

		Maltase			Sucrase			Aminopeptidase		
		D	J	I	D	J	I	D	J	I
Maltase	D									
	J	0.28								
	I	0.38	0.28							
Sucrase	D	0.82	0.34	0.30						
	J	0.18	0.81	0.19	0.38					
	I	0.38	--	0.78	0.21	--				
Aminopeptidase	D	0.71	--	0.30	0.35	--	0.44			
	J	--	0.50	--	0.19	0.65	--	--		
	I	0.33	--	0.74	--	--	0.66	0.39	--	
Amylase	J	--	--	--	0.22	--	--	--	--	--
	I	--	--	--	--	--	--	--	--	--
	P	--	--	-0.23	--	0.29	-0.26	-0.23	0.27	-0.28
	G	--	--	--	--	--	--	--	--	--
Protease	J	0.25	0.28	0.21	0.35	0.31	--	--	0.23	--
	I	--	--	--	--	--	--	--	0.20	--
	P	--	0.18	--	0.37	0.42	--	-0.30	0.20	-0.33
	G	0.23	--	--	0.37	--	--	--	--	--
xylanase	J	--	0.26	--	0.20	0.29	--	--	0.42	--
	I	--	0.26	0.21	--	--	--	--	0.20	--
	G	0.35	--	--	0.40	--	--	--	0.18	--
cBWG[3]		0.25	0.27	--	0.66	0.44	--	-0.27	--	-0.18
pBWG		0.23	--	--	0.53	0.31	-0.19	--	--	--
cFI		0.22	0.28	--	0.63	0.43	--	-0.29	--	-0.18
pFI		0.24	0.21	--	0.63	0.41	--	-0.24	--	--
cFCR		--	0.26	--	0.53	0.40	--	-0.28	--	-0.17
pFCR		--	0.27	--	0.49	0.38	--	-0.29	--	--

[1]Significant correlation ($P < 0.05$) indicated by the presence of value, otherwise with "--"
[2]P = pancreas, G = gizzard, D = duodenum, J = jejunum, I = ileum
[3]cBWG = cumulative body weight gain, pBWG = period body weight gain, cFI = cumulative feed intake, pFI = period feed intake, cFCR = cumulative feed conversion ratio, pFCR = period feed conversion ratio

activities. Protease activity in pancreas was correlated with jejunal sucrase, maltase, and aminopeptidase N activities, while negatively correlated with aminopeptidase N activities in duodenum and ileum. Protease and xylanase activities in gizzard were correlated with maltase and sucrase activities in the duodenum. Xylanase activity in jejunum was correlated with jejunal maltase, sucrase, and aminopeptidase N activities as well as duodenal sucrase activity. Most maltase and sucrase activities in the duodenum and jejunum were correlated with bird performance parameters, whereas duodenal and ileal aminopeptidase N activities were negatively correlated with cumulative performance.

Amylase activity in jejunum was correlated with ileal amylase activity, while pancreatic amylase activity was negatively correlated with ileal amylase activity (Table 25). Protease and amylase activities were correlated between and within jejunum and ileum. Protease and amylase activities were correlated in the pancreas. Pancreatic protease activity was correlated with protease activity in the gizzard, while negatively correlated with ileal protease activity. Jejunal xylanase activity was correlated with amylase and protease activities in the jejunum and pancreas. Jejunal and pancreatic amylase and protease activities were correlated with performance except for no correlation between period feed conversion ratio and amylase in the jejunum and pancreas. Protease and xylanase activities in the gizzard were correlated with bird performance parameters.

The numbers of up- (> 1.5 fold) or down- (< 0.67 fold) regulated genes in the jejunal mucosa of birds on d 28. The number of regulated genes between birds fed AN- and AN+ diets was greater than that of BN+/BN- diets (209 vs. 153 genes). The highest number of regulated genes (449 genes) was between birds fed BN- and AN- diets. Birds fed the BN- diet had a total of 77 genes significantly changed as compared to birds fed AN- diet. Fifteen genes were down-regulated, while 62 genes were up-regulated (Table 26 and Table 27). Four of fifteen down-regulated genes related to immune responses consisted of sialyltransferase-4C, N-acyl-

Table 25. Correlation of amylase, protease, and xylanase activities with performance[1].

		Amylase				Protease				xylanase			
		J[2]	I	P	G	J	I	P	G	J	I	G	
Amylase	J												
	I	0.20											
	P	--	-0.26										
	G	--	--	--									
Protease	J	0.47	0.21	--	--								
	I	0.21	0.29	--	--	0.40							
	P	--	--	0.72	--	0.21	--						
	G	--	--	--	--	--	-0.19	0.27					
xylanase	J	0.29	--	0.28	--	0.18	--	0.23	--				
	I	--	0.21	-0.22	--	--	--	--	--	0.29			
	G	--	--	--	0.18	--	--	0.20	0.57	0.26	--		
cBWG[3]		0.21	--	0.40	0.20	0.31	--	0.78	0.44	0.19	--	0.35	
pBWG		0.19	--	0.62	--	0.37	0.09	0.69	0.20	0.25	-0.23	0.25	
cFI		0.20		0.33	0.23	0.28	--	0.75	0.47	--	--	0.35	
pFI		0.21	--	0.53	--	0.35	--	0.79	0.37	0.23	-0.18	0.33	
cFCR		0.18	--	0.23	0.25	0.22	--	0.66	0.48	--	--	0.33	
pFCR		--	--	--	0.25	0.19	--	0.62	0.48	--	--	0.32	

[1]Significant correlation ($P < 0.05$) indicated by the presence of value, otherwise with "--"
[2]P = pancreas, G = gizzard, J = jejunum, I = ileum
[3]cBWG = cumulative body weight gain, pBWG = period body weight gain, cFI = cumulative feed intake, pFI = period feed intake, cFCR = cumulative feed conversion ratio, pFCR = period feed conversion ratio

Table 26. Regulated jejunal gene expression ≥1.5-fold for BN- vs. AN- fed birds on d 28[1]

Gene description (gene symbol or Unigene)	Fold change of gene expression level (BN- /AN-)[2]
Down-regulated	
Immune response genes	
Gal-beta, Sialyltransferase-4C (gga.19476)	0.35
Interleukin-1 receptor-associated kinase 4 (Irak4)	0.62
CD3Z antigen (Cd3z)	0.65
Other genes	
Importin 13 (Ip013)	0.34
N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (LOC417719)[#]	0.60
Unknown (gga.7740)	0.50
Unkown1 (gga.17335)[#]	0.53
SEC24 related gene family, member B (Sec24b)	0.55
Calpastatin (Cast)[#]	0.56
Unkown2 (LOC422745)[#]	0.56
Unknown (LOC415786)	0.60
Apical early endosomal glycoprotein precursor (LOC420512)[#]	0.62
Fetal Alz-50 reactive clone 1 (LOC417422)[#]	0.62
Unknown (gga.19065)	0.62
Homologous Alu RNA binding protein (Srp14)	0.65
Up-regulated	
Immune response genes	
Lymphocyte antigen 6 complex, locus E (Ly6e)	7.78
Interferon stimulated gene 12-2 protein (Isg12-2)	5.08
Interferon-induced transmembrane protein 1 (gga.4832)	3.19
TIR domain containing adaptor inducing interferon-beta (TRIF)	1.61
Membrane-associated prostaglandin E synthase 2 (mPGES-2)[#]	2.30
Epilepsy holoprosencephaly candidate-1(EHOC-1)	1.85
DnaJ (Hsp40) homolog, subfamily A, member 1 (Dnaja1)	1.84
B6.1, an immunoglobulin M (LOC396098)	1.67
Pinin (Pnn)	1.57
Periostin, osteoblast specific factor (Osf-2)	1.55
Liver expressed antimicrobial peptide 2 (cLEAP-2)	1.49
Metabolism genes	
Fatty acid binding protein 6 (Fabp6)*	3.36
Pyruvate dehydrogenase phosphatase, catalytic subunit 2 (PDPC 2)	1.80
Adiponectin receptor 1 (Adipor1)*	1.71
Alkyl hydroperoxide reductase (gga.7797)	1.67
Thioesterase B (LOC415786)	1.65
Aspartyl aminopeptidase (Dnpep)*	1.55
Alcohol dehydrogenase 1A, alpha polypeptide (Adh1a)	1.55
Alcohol dehydrogenase 5 (Adh5)	1.52
MAWD binding protein (LOC423685)	1.71
Mitochondria 12S ribosomal RNA (no)*	2.09
Mitochondria 16S ribosomal RNA (no)	1.99

[1]Normalized gene expression values were significantly different ($P < 0.05$) between dietary treatments by t-test; BN -: corn B basal diet; AN-: corn A basal diet.
[2]Fold of change was calculated as normalized gene expression values of BN- divided by that of AN-; *genes were also regulated in BN+/BN-; [#]genes were also regulated in AN+/AN-.

Table 27. Regulated gene expression ≥ 1.5-fold for BN- vs. AN- fed birds on d 28[1]

Gene description (gene symbol or Unigene)	Fold change of gene expression level (BN-/AN-)[2]
Up-regulated	
Metabolism genes	
Cardiolipin synthase 1 (Crls1)*	1.98
Mitochondrial ribosomal protein S18A (Mrps18a)	1.59
Mitochondrial substrate carrier (LOC429678)	1.59
Mitochondrial ribosomal protein L15 (Mrpl15)	1.58
Peptidylprolyl isomerase F (Ppif)	1.56
cytochrome P-450, CYP2A6 (LOC423753)	2.73
Other genes	
ATPase, Ca++ transporting, plasma membrane 1 (Atp2b1)	2.03
Solute carrier family 25, member 17 (Slc25a17)	1.76
Endothelin receptor type A (Ednra)	1.58
Hypothetical protein DKFZp762D096.1 (LOC415756)	2.87
Microtubule-associated protein homolog (LOC395081)	2.45
Unkown6 (gga.20445)#	2.44
Ubiquitin specific peptidase 10 (Usp10)	2.16
Retinoblastoma-associated protein (gga.1927)*	2.10
Unknown (gga.20821)	2.01
Clathrin adaptor complex (LOC41558)*	2.06
Sulfate transporter/antisigma-factor antagonist STAS (Slc26a6)	1.53
Similar to chromosome 20 open reading frame 7 isoform 1 (LOC416742)	1.83
Minichromosome maintenance deficient 6 (MCM6)	1.80
DEAD/DEAH box helicase (LOC422427)	1.73
Zinc finger protein 64 homolog (Zfp64)	1.51
TSC22 domain family 1 (Tsc22d1)	1.47
Dynactin 6 (gga.6970)	1.71
NESH protein (gga.6972)	1.68
N-ethylmaleimide-sensitive factor (Nsf)	1.51
Ring finger protein 103 (Rnf103)	1.53
Cerebellar degeneration-related protein 2 (CDR2)	1.53
Inositol monophosphatase (Bpnt1)	1.53
Unknown (gga.16200)	1.93
Hypothetical protein FLJ13081 (LOC423933)	1.82
Unknown (gga.1530)	1.73
Unknown (gga.3211)	1.65
Unkown5 (Gga.8013)#	1.58
Unkown4 (gga.14364)#	1.56
Similar to X96994 BR-1 protein (gga.1706)	1.56
Unknown (gga.20156)	1.54
Unknown (gga.5869)	1.52
Unknown (LOC422001)	1.51
Unknown (gga.18157)	1.51
Unkown3 (ens:ENSGALT00000021614.1)#	1.49

[1]Normalized gene expression values were significantly different ($P < 0.05$) between dietary treatments by t-test; BN -: corn B basal diet; AN-: corn A basal diet.
[2]Fold of change was calculated as normalized gene expression values of BN- divided by that of AN-; *genes were also regulated in BN+/BN-; #genes were also regulated in AN+/AN-.

phosphatidylethanolamine-hydrolyzing phospholipase D, interleukin-1 receptor-associated kinase 4 (*Irak4*), and CD3Z antigen (*Cd3z*). Eleven of 62 up-regulated genes were related to the immune response. These genes were lymphocyte antigen 6 complex, locus E (*Ly6e*), interferon stimulated gene 12-2 protein (*Isg12-2*), interferon-induced transmembrane protein 1, TIR domain containing adaptor inducing interferon-beta (*TRIF*), membrane-associated prostaglandin E synthase 2 (*mPGES-2*), epilepsy holoprosencephaly candidate-1(*EHOC-1*), DnaJ (Hsp40) homolog, subfamily A, member 1 (*Dnaja1*), an immunoglobulin M B6.1, pinin (*Pnn*), osteoblast specific factor (*Osf-2*), and liver expressed antimicrobial peptide 2 (*cLEAP-2*). Nine up-regulated genes related with metabolism were fatty acid binding protein 6 (*Fabp6*), pyruvate dehydrogenase phosphatase, catalytic subunit 2 (*PDPC 2*), adiponectin receptor 1 (*Adipor1*), alkyl hydroperoxide reductase, thioesterase B, aspartyl aminopeptidase (*Dnpep*), alcohol dehydrogenase 1A, alpha polypeptide (*Adh1a*), alcohol dehydrogenase 5 (*Adh5*), and MAWD binding protein (*Mawbp*). Eight up-regulated genes related with mitochondria activity consisted of mitochondria 12S ribosomal RNA, mitochondria 16S ribosomal RNA, cardiolipin synthase 1 (*Crls1*), mitochondrial ribosomal protein S18A (*Mrps18a*), mitochondrial substrate carrier, mitochondrial ribosomal protein L15 (*Mrpl15*), and peptidylprolyl isomerase F (*Ppif*), cytochrome P-450, CYP2A6. Four genes are similar between NB- and NB+ birds while differently regulated ($P < 0.05$) as compared to AN- birds. These genes were: *Ip013*, fetal Alz-50 reactive clone 1, minichromosome maintenance complex component 6 (*MCM6*), and B 6.1. Expression nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha (*NFKBIA*) was up-regulated in BN+ birds as compared to AN- birds.

Among those differently regulated genes, 23 genes were significantly ($P < 0.05$) changed for birds fed BN+ diet as compared to birds fed BN-. Down-regulated genes totaled 18, while 5 genes were up-regulated (Table 28). Seven of the 18 down-regulated genes were metabolism related: fatty acid binding protein 6 (*Fabp6*), adiponectin receptor 1 (*Adipor1*), zinc binding

Table 28. Regulated jejunal gene expression ≥ 1.5-fold for BN+ vs. BN- fed birds on d 28[1]

Gene description (gene symbol or Unigene)	Fold change of gene expression level (BN+/BN-)[2]
Down-regulated	
Metabolism genes	
Fatty acid binding protein 6 (Fabp6)*	0.55
Adiponectin receptor 1(Adipor1)*	0.58
Zinc binding alcohol dehydrogenase (Zadh1)	0.58
Short-chain dehydrogenase/reductase SDR (Dhrs7)	0.66
Aspartyl aminopeptidase (Dnpep)*	0.66
Cytochrome oxidase c (Coc)	0.67
Mitochondria 12S ribosomal RNA (gb:AP003580-1)*	0.58
Other genes	
unknown (LOC416262)	0.47
unknown (gga.14273)	0.48
PDZ domain-containing guanine nucleotide exchange factor I (LOC422417)	0.52
Clathrin adaptor complex (LOC415581)*	0.55
Cardiolipin synthase 1 (Crls1)*	0.60
Retinoblastoma-associated protein (Rbl1)*	0.60
GRIP domain (ens:ENSGALT00000020020.1)	0.64
Unknown (LOC421341)	0.64
Survival motor neuron protein domain containing 1(Smndc1)	0.67
Epithelial V-like antigen 1 (Eva1)	0.67
Unknown (LOC416262)	0.67
Up-regulated	
Zinc finger protein 236 (Znf236)	1.61
Phosphoprotein phosphatase 3, catalytic subunit, alpha isoform (Ppp3ca)	1.56
Seb4D protein (LOC768866)	1.75
Unknown (gga.19802)	1.51
Unknown (gga.22567)	1.51

[1]Normalized gene expression values were significant different ($P < 0.05$) between dietary treatments by t-test; BN -: corn B basal diet; BN+: BN- plus an enzyme cocktail of amylase, protease, and xylanase.
[2]Fold of change was calculated as normalized gene expression values of BN+ divided by that of BN-; *genes were also regulated in BN-/AN-

alcohol dehydrogenase, domain containing 1(*Zadh1*), short-chain dehydrogenase/reductase SDR (*Dhrs7*), aspartyl aminopeptidase (*Dnpep*), cytochrome oxidase c (*Coc*), and mitochondria 12S rRNA. BN+ birds had the same seven down-regulated genes as those in AN- birds. Two of five up-regulated genes were metabolism related: zinc finger protein 236 (*Zfp236*) and phosphoprotein phosphatase 3, catalytic subunit, alpha isoform (Ppp3ca).

Birds fed AN+ diet had 30 genes regulated ($P < 0.05$) as compared to birds fed AN- (Table 29). One half of the genes were down-regulated in birds fed corn A with supplementation of the enzyme cocktail. Three genes related to immune response were Zn-finger, PIAS1 (*Pias1*), haloacid dehalogenase-like hydrolase, and N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D. Similar as the BN- group, down-regulated calpastatin (*cast*) gene in AN+ may result in increased mirovilli of epithelial cell. There were also ten other genes differentially expressed in AN- group as compared to those in AN+, while similar between AN+ and BN-. Among fifteen up-regulated genes, three genes related to immune response and consisted of membrane-associated prostaglandin E synthase 2, major histocompatibility complex protein, class I (*B-FIV*), and hematopoietic lineage cell-specific protein HS1. Three up-regulated genes related to metabolism were cytochrome P450 CYP2c18 (*Cyp2c18*), fibroblast growth factor 19 (*FGF19*), and protein phosphatase 2c.

Discussion

Similar proximate analysis values were observed for corn A and B, whereas corn A had 100 kcal/kg feed more AMEn as compared to corn B as reported in a previous chapter of this dissertation. This discrepancy showed the limitation of proximate analysis as an evaluation approach for corn nutritive value. Starch classification based on *in vitro* digestion is applied to solve this problem. In this experiment, corn quality was evaluated based on energy released with

Table 29. Regulated gene expression by over 1.5-fold for AN+ vs. AN- fed birds on d 28[1]

Gene Description (gene symbol or Unigene)	Fold change of gene expression level (AN+/AN-)[2]
Down-regulated	
Zn-finger, PIAS1 (Pias1)	0.61
Haloacid dehalogenase-like hydrolase (LOC416385)	0.64
N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (LOC417719)[#]	0.66
Calpastatin (gga.11782)[#]	0.58
Unknown (gga.8900)	0.32
Fetal Alz-50 reactive clone 1 (LOC417422)[#]	0.55
Coiled-coil domain containing 4 (LOC422777)	0.55
Unknown1 (gga.17335)[#]	0.56
Apical early endosomal glycoprotein precursor (LOC420512)[#]	0.57
Unknown (gga.15079)	0.57
Inner nuclear membrane protein Man1 (LOC419907)	0.60
Formin isoform IV (gga.5846)	0.61
Unknown2 (LOC422745)[#]	0.61
Inositol 1,3,4-triphosphate 5/6 kinase (Itpk1)	0.65
Unknown (gga.20024)	0.66
Up-regulated	
Immune response genes	
Membrane-associated prostaglandin E synthase 2 (mPGES-2)[#]	1.78
Major histocompatibility complex protein, class I (B-FIV)	1.59
Other genes	
Hematopoietic lineage cell-specific protein HS1 (LOC417719)	1.58
Cytochrome P450 CYP2c18 (Cyp2c18)	15.85
Fibroblast growth factor 19 (FGF19)	1.57
Protein phosphatase 2c (LOC424850)	1.49
Regenerating islet-derived family, member 4 (REG4)	3.06
Unknown6 (gga.20445)[#]	2.43
Unknown3 (ens:ENSGALT00000021614.1)[#]	1.84
Gastrin-releasing peptide precursor (GRP)	1.68
Unknown5 (BU449047)[#]	1.66
Unknown (gga.6241)	1.60
Unknown (gga.7779)	1.53
Unknown4 (gga.14364)[#]	1.51
splA/ryanodine receptor domain and SOCS box containing 3 (Spsb3)	1.49

[1]Normalized gene expression values were significantly different ($P < 0.05$) between dietary treatments by t-test; AN-: corn B basal diet; AN+: AN- plus an enzyme cocktail of amylase, protease, and xylanase.
[2]Fold of change was calculated as normalized gene expression values of AN+ divided by that of AN-; [#]genes were also regulated in BN-/AN-

supplementation of an enzyme cocktail of amylase, protease, and xylanase (Cowieson *et al.,* 2006a). The estimation procedure is based on analysis of corn starch digestibility *in vitro* with pancreatic enzymes (Englyst *et al.,* 1992). This analysis method is used for evaluating food starch in human nutrition, in which most food will be cooked before analysis. The cooking may inactivate most of the active components in food or feed.

When corn is used in animal diets, it is in raw form. Most active components in corn should remain intact when corn is mixed into feed, pelleted, and subsequently consumed by birds. The detected activities of amylase, protease, and xylanase in untreated corn without enzyme supplementation (A- and B-) indicate the existence of endogenous enzymes. Additionally, the incomplete recovery of enzyme activities in untreated corn with enzyme supplementation (A+ and B+) suggests enzyme inhibitors were present in the corn. Protease activity was recovered less in A+ while xylanase activity was recovered less in B+. Therefore, corn A likely had more protease inhibitor, while corn B had more xylanase inhibitor. A bi-functional corn Hageman factor inhibitor is reported to inhibit protease and amylase activities (Behnke *et al.,* 1998). After corn was heated for 24h at 100 °C, amylase inhibitor and most protease inhibitor activities were removed as indicated by the levels of detected enzyme activities. Xylanase inhibitor was removed only after the corn was autoclaved for 30 min at 121 °C and heated for 24h at 100 °C. The differential existence of endogenous enzyme and inhibitor activities for amylase, protease, and xylanase in corn A and B may contribute to the difference of AMEn between the two varieties of corn.

Amylase activity in the gizzard, pancreas, jejunum, and ileum were sporadically different between dietary treatments, therefore, corn variety and dietary enzyme supplementation had little effect on amylase activity in the broiler gastrointestinal tract (GIT). Similarly, minor alteration of amylase activity in GIT is observed in turkeys fed diet supplemented with amylase and xylanase (Ritz *et al.,* 1995). Amylase activity in feed was about 1 U/g, and amylase activity in jejunal

digesta was more than 700 U/g. This means that most of the measured intestinal amylase activity came from endogenous pancreatic secretion. The exogenous feed amylase may have had a limited effect on digestion of the feed after passage through the duodenum. A trend of higher protease activity in the gizzard of birds fed corn A diets may come from the higher protease inhibitor in corn A. Increased pancreatic secretion has been observed when humans were administered trypsin inhibitor in the duodenum (Adler *et al.,* 1988). In the present experiment, the higher protease inhibitor might stimulate proventriculus secretion of pepsin and result in higher protease activity in the gizzard of corn A fed birds. Also noted is numerically higher gizzard protease activity in birds fed diets supplemented with enzymes, indicating a possible additive effect of enzyme supplementation. Protease activity in feed was about 140 U/g, while protease activity was more than 1000 U/g in gizzard digesta and 1 million U/g in jejunal and ileal digesta. The feed protease might have a limited effect on nutrient digestion after passage through the duodenum except that the feed protease can degrade feed inhibitors which host protease can not break down. Higher xylanase activity on d 14 and numerically higher xylanase activity on d 7, 28, and 49 in the gizzard digesta of birds fed corn A diets agrees with higher endogenous xylanase activity in corn A. Higher endogenous xylanase activity in corn A was further indicated by elevated xylanase activity in the jejunal and ileal digesta as compared to that of corn B fed birds. Starch digestibility and energy retention were improved when birds were fed wheat diet supplemented with xylanase (Choct *et al.,* 1999). Xylanase activity in corn A may have contributed to starch digestibility and subsequent higher energy retention in birds fed corn A diets.

Birds fed carbohydrate-containing diets had 2-fold higher maltase and sucrase activity as compared to birds fed carbohydrate-free diets (Biviano *et al.,* 1993). Under early feed restriction, birds fed diets supplemented with amylase and protease showed higher sucrase and maltase activities as compared to control birds (Pinheiro *et al.,* 2004). Birds with feed restriction had

higher aminopeptidase N activity as compared to birds fed *ad libitum* (Susbilla *et al.,* 2003). In the present experiment, dietary treatments had sporadic effects on intestinal maltase, sucrase, and aminopeptidase N activities. This result may come from similar dietary starch and protein levels in the diets provided, whereas other results suggests that the intestinal enzymes are altered more with substantial dietary change (Biviano *et al.,* 1993; Pinheiro *et al.,* 2004). The contribution of sucrase-isomaltase on total maltase activity was changed between different intestinal segments, while the contribution of glucoamylase on total maltase activity was similar. Sucrase-isomaltase has high alpha-glucogenesis under higher concentration of lumen oligomer substrates (maltose and maltotriose), whereas glucoamylase has high alpha-glucogenic activity under low concentration of oligomer substrates (Quezada-Calvillo *et al.,* 2007). In this experiment, birds were fed high starch diets, resulting in varied high concentration of oligomer substrates along the intestinal lumen. To digest these substrates and respond to varied substrate concentration, it would be more efficient to change sucrase-isomaltase instead of glucoamylase.

Correlations between aminopeptidase N and maltase/sucrase activities in the same intestinal segment may indicate simultaneous response of these three intestinal enzymes to lumen nutrients. In other words, abruptly changing dietary composition, such as ratio of starch to protein, may result in a delayed response of the enzymes. The negative correlation between pancreatic amylase and the three mucosal enzymes in the ileum may come from reduced substrate present in the lower small intestine as a result of increased digestion in the upper small intestine by pancreatic amylase, or vice versa. The correlation between the three mucosal enzymes in the jejunum and protease in the jejunum and pancreas show a possible chain reaction of digestion in which protease digests protein into peptides and may release attached starch, and the three intestinal enzymes of maltase, sucrase, and aminopeptidase N further degrade the products of protease degradation. The correlation between xylanase and maltase/sucrase in jejunum shows a similar digestion chain, and the same happens between gizzard xylanase and

duodenal maltase/sucrase. The correlation between bird performance and sucrase/maltase activity in the duodenum and jejunum supports the importance of energy release in the upper small intestine. The negative correlation between bird performance and duodenal aminopeptidase may originate from the negative correlation between duodenum aminopeptidase N and pancreatic amylase and protease, resulting in reduced digestion of starch and protein.

The pancreas may change its activity to respond to intestinal luminal nutrients. Pancreatic protein synthesis in mice increased in response to refeeding a diet through activation of the translational machinery downstream of mammalian Target Of Rapamycin (mTOR) (Sans *et al.,* 2004a). Amino acids, particularly branched chain amino acids, can activate mTOR and its downstream effector S6 Kinase (S6K), resulting in elevated translation of pancreatic enzymes (Sans *et al.,* 2006). An increase in trypsin synthesis in humans has been observed following enteral but not parenteral (intravenous) feeding (O'Keefe S *et al.,* 2006). Amylase, lipase, trypsin and total protease activity in rat pancreas and small intestinal digesta are increased with dietary ZnO (Szabo *et al.,* 2004). The correlation between amylase and protease activities in the pancreas, jejunum, and ileum indicates possibly simultaneous synthesis, storage, and discharge of the two enzymes in the present experiment. This may relate to the capacity by which birds can digest certain compositions of feed ingredients or in other words optimal ratio of dietary energy to protein. Jejunal xylanase activity correlated with amylase and protease activities in the pancreas and jejunum shows a cooperative relationship between xylanase and the other two enzymes. The same cooperation happened in the gizzard. The correlations between bird performance and amylase and protease in the jejunum and pancreas indicate the importance of digesting starch and protein in the upper intestine.

Genes regulated by corn variety (quality) were higher as compared to those by enzyme supplementation in either corn diets. This result agrees with bird performance parameters and enzyme activity patterns in which more differences of performance and enzyme activity were

caused by corn variety. The four innate immune response genes in birds fed BN- as compared to AN- fed birds were down-regulated, which consisted of sialyltransferase-4C, *Irak4, Cd3z*, and *cast*. These genes are responsible for mucin synthesis (Yamauchi *et al.,* 2006), Toll-like receptor (TLR) signaling (Kim *et al.,* 2007), natural killer (NK) cell activation (Moretta *et al.,* 2001), and microvilli formation (Potter *et al.,* 2003), respectively. However, eleven innate and adaptive immune response genes were up-regulated, which were *Ly6e*, *Isg12-2*, interferon-induced transmembrane protein 1, *TRIF*, *mPGES-2*, *EHOC-1*, *cLEAP*-2, immunoglobulin M B6.1, *Dnaja1*, *Pnn*, and *Osf-2*. These genes have been reported to be implicated to play a role in virus infection (Zhang *et al.,* 2007), inflammation (Kawai and Akira, 2005; Murakami and Kudo, 2006), autoimmune disorder (Yamakawa *et al.,* 1995), disease challenge (Townes *et al.,* 2004), *Candida albicans* infection (Han and Cutler, 1995), stress response (Qiu *et al.,* 2006), and cell junction (Zimowska *et al.,* 2003; Kudo *et al.,* 2007). Overall, birds fed BN- diets seemed to have experienced disease challenge and higher immune response as compared to birds consuming AN- diets. This challenge might not come from the presence of Fumonisin B1 to B3 in corn diets. Performance of broiler chicks was not affected by dietary Fumonisin B1 up to 50 ppm until d 49 (Broomhead *et al.,* 2002) or up to 80 ppm until d 21 (Henry *et al.,* 2000).

Nine metabolism related genes were expressed at higher levels in birds fed BN- diets as compared to birds fed AN- diets. These genes have been involved in metabolizing cholesterol (Ohmachi *et al.,* 2006), carbohydrate (Huang *et al.,* 1998), organic hyperoxides (Jacobson *et al.,* 1989), amino acids (Yokoyama *et al.,* 2006), alcohol (Crabb *et al.,* 2004), glucose (Yamauchi *et al.,* 2001), fatty-acid (Kadowaki and Yamauchi, 2005), and folate (Herde and Blankenfeldt, 2006). Over 90% of cellular energy generation takes place in the mitochondria, and mitochondria are primarily responsible for meeting the enormous energy demands of the immune response by oxidizing large amounts of substrates (Manoli *et al.,* 2007). Increased mitochondrial activity in BN- birds was indicated by elevated expression of mitochondria 12S ribosomal RNA,

mitochondria 16S ribosomal RNA, mitochondrial ribosomal protein S18A (*Mrps18a*), *Mrpl15*, and mitochondrial substrate carrier, *Crls1* (Chen *et al.,* 2006), *Ppif* (Tsujimoto *et al.,* 2006), and Cytochrome P-450 (Ding and Kaminsky, 2003; Matsuda *et al.,* 2007). These 17 up-regulated genes might relate with increased energy demand in association with the elevated immune response in BN- birds.

The immunoglobulin M B6.1 is specific for mannan epitope in the adhesin fraction of *Candida albicans* (Han and Cutler, 1995). *C. albicans* is the most common cause of opportunistic fungal diseases in humans (Schaberg *et al.,* 1991). Birds fed BN+ and BN- diets had increased B6.1 gene expression as compared to AN- birds, suggesting a disease challenge in birds fed corn B diets as compared to birds consuming AN- diets. Importin 13 (*Ip013*), a member of the importin β family of nuclear import proteins, transports glucocorticoid receptor and is involved in glucocorticoid effect on metabolism and antiinflammation (Tao *et al.,* 2006). Down-regulated *Ip013* in corn B fed birds may result in reduced blood glucose level and inflammation as compared to birds fed AN- diets.

Most of the up-regulated genes in birds fed BN- as compared to AN- fed birds were similar expression levels between birds fed AN- diets and birds fed BN+ diets, which were formulated by supplementing the enzyme cocktail into BN- diets. In addition, up-regulated *NFKB1A* expression in birds fed BN+ diets as compared to birds fed AN- suggests the presence of anti-inflammation pathway in birds fed BN+, resulting in a reduced immune response. Seven metabolism related genes were down-regulated in BN+/BN- birds, suggesting a reduction of energy demand stress by the enzyme supplementation. Expression levels of seven genes were similar between birds fed BN+ and AN- diets but different from birds fed BN- diets. Performance was similar in birds fed BN+ and AN-, while inferior in BN- birds. The result of the genes suppressed by enzyme supplementation in corn B diet suggests a possible relationship between bird performance and gene expression.

Interestingly, with enzyme supplemented corn A diets, six immune related genes were regulated and three metabolism related genes were up-regulated. Expression levels of eleven genes were similar between birds fed AN+ and BN- diets, whereas different from birds fed AN- diets. Therefore enzyme supplementation in high quality corn diets seemed to result in an adverse effect similar to BN-, maybe relating with poor performance in AN+ fed birds.

Based on the results of gene expression data, performance alteration by supplementing the enzyme cocktail was not through digestion and absorption of starch and protein but through alteration of immune response and the resulting metabolic demand. When nutrients are at low levels, microflora has to compete for nutrients with the host and may even invade the host to acquire nutrients to survive. Subsequently, this can result in increased disease challenge, immune response, and metabolic demand for energy. Enzyme supplementation increases the nutrient supply and decreases the stress induced by nutrient shortage. However, when enzyme supplementation is in higher quality corn and nutrients are over-supplied, microflora will proliferate, invade intestinal tissue, produce detrimental metabolites from the microflora, and reduce performance.

In conclusion, corn quality may come from active components of corn such as endogenous enzymes and enzyme inhibitors. Enzyme supplementation affected gastrointestinal enzyme activities, which may be related with performance. Birds fed high quality corn diets without enzyme supplementation had advantages in higher protease and xylanase activities, minimal immune response, and minimal energy demand. Enzyme supplementation in low quality corn eliminates most detrimental effects.

Chapter 5. Epilogue

Quality of feed ingredients has been determined based on proximate analysis (PS) values for a long time. This method is essential in distinguishing the difference in quality between various feed ingredients. For example, based on proximate analysis, corn has high gross energy and low non-soluble polysaccharides (NSP), while wheat has low gross energy and high NSP. Therefore, it is easy to conclude that corn is a high quality energy source as compared to wheat. However, when PS is used to determine the quality within same feed ingredient, problems arise. For example, in the experiments presented in this dissertation, the PS values of the two varieties of corn were highly similar, and it was impossible to differentiate which one had better quality strictly based on PS values.

To solve the discrepancies between real nutritive values and PS values of a feed ingredient, other analysis methods have been developed, such as *in vitro* digestion. Different starch digestibility between same the food or feed ingredient has been successfully determined by *in vitro* digestion with pancreatic enzymes. Based on this method, the ingredients can be classified into high quality (easily digested) or low quality (difficult to digest) for energy release. In these experiments, the two qualities of corn were represented as high quality corn A compared to low quality corn B.

Feeding experiments were conducted to evaluate the method of corn quality determination. Low energy retention in birds fed low quality corn B as compared to corn A validated the *in vitro* digestion method for determination of corn quality. Performance was changed with birds fed different corn diets. Furthermore, enzyme activity analysis in corn revealed that the quality might partially be attributed to active components of native corn, such as protease inhibitor and xylanase activity. The active components of the corn may have affected gastrointestinal enzyme activities, which might relate to observed differences in performance.

We have known that there are quality differences between feed ingredients such as corn. Therefore, the best way to formulate poultry diets is to perform *in vitro* digestion of corn every time before dietary formulation. However, it is impossible to do so in practice with hundreds or thousand batches of feed ingredients in industry operations. The feasible approach has been to use exogenous enzymes in diets. Enzyme supplementation can reduce or eliminate the nutritive difference by increasing nutrient digestibility. In the present experiments, energy released by supplementation of Avizyme 1502 improved overall broiler performance. There were some variations as indicated:

1) When supplementing the enzyme in high quality corn diets, reducing dietary energy level ("reformulation") resulted in better BWG, while similar BWG was observed in birds fed normal energy diets ("over-the-top"). Birds fed the enzyme supplemented diets had increased fatpad and pectoral minor percentage.
2) When adding the enzyme in low quality corn diets, birds with "over-the-top" supplementation had improved BWG, while "reformulation" resulted in lower BWG. Birds fed the enzyme supplemented diets had reduced mortality.
3) Feed efficiency was inferior in birds fed reduced energy diets regardless of the quality of the corn or enzyme supplementation.

These improvements and variation in broiler performance in these experiments indicated the feasibility of reducing nutrient variation by the "over-the-top" supplementation of exogenous enzymes. The approach of "reformulation" of diets seems to be a second choice if improving feed efficiency is the primary aim. If the aim is to grow birds faster and to earn more profit, "reformulation" may be a feasible choice after considering the reduced feed cost and time to processing.

To elucidate the underlying mechanism(s) for differences in bird performance by dietary factors of energy levels, enzyme supplementation, or corn quality in present experiments,

additional data was collected and analyzed. Based on enzyme activities in the gastrointestinal tract and correlations between the enzyme activities and performance, protease activity in gizzard and xylanase activity seemed to play a role in performance. Other enzyme activities, such as amylase, maltase, sucrase, and aminopeptidase-N activities, may have effects on performance for a transient time. This finding indicates that enzyme cocktail should include protease and xylanase for corn soybean diets.

Besides enzyme activities, other physiological parameters in jejunal tissue were suggestive of an effect on performance as indicated by genome-wide gene expression data using microarrays. Some genes involved in immune reactions and energy metabolism were changed in expression between birds fed different quality of corn. Supplementing the enzyme in the low quality corn diets resulted in similar gene expression levels of most genes as compared to the high quality corn basal diets. This finding suggests that collecting and analyzing data of immune response and energy metabolism activity are also important when we try to investigate underlying mechanisms in nutrition research.

Although there are many findings in these experiments, more research with improved design are necessary to further increase our understanding on the effect of energy levels, enzyme supplementation, and ingredient quality. Explained below are some suggestions for further research.

First, experimental dietary design needs to be improved. 1) Supplementing enzymes individually and in combination instead of the enzyme cocktail will facilitate analysis of the dietary effects on performance and physiological parameters. 2) The difference between reduced and normal energy diets should be increased so as to result in more clearly differentiated performance. 3) It is necessary to determine apparent metabolizable energy (AME) of corn, soybean meal, and corn gluten by tube-feeding and to analyze amino acid profiles in soybean

meal and corn gluten before diet formulation. 4) Include phytase in an enzyme cocktail because phytase may inactivate some enzyme inhibitors as well as release enzyme activators.

Second, when a nutrient retention trial is conducted, it would be better to have birds eat allotted amounts of feed instead of *ad libitum* to eliminate the effect of feed intake. The retention of most essential amino acids should also be analyzed to estimate their balance.

Third, it would be better to determine the microbial population in the intestine either by traditional culture method or molecular real time PCR. Based on these experiments, the tested microbes should include not only bacteria but also other microbes such as protozoa and viruses.

Fourth, real time PCR should be performed to validate the gene expression data by microarray.

In conclusion, the present experiments have provided evidence of broiler performance alteration by dietary energy level, enzyme supplementation, and corn quality. Underlying mechanisms for these alterations were explored, and data suggested that differences in enzyme activity and gene regulation may contribute to the responses. Considerations on further researches have been listed.

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