ABSTRACT

SANTOS JR., ANAEL ARAUJO. Improvement of Nutritional Value of Wheat by Dietary Enzyme Supplementation for Turkeys. (Under the direction of Dr. Peter R. Ferket and Jesse L. Grimes.)

The increasing world production of turkeys and broilers is increasing the demand for wheat as a major feedstuff. Wheat and wheat-by products vary in apparent metabolizable energy (AME) content due to the presence of non-starch polysaccharides (NSP). Enzymes have become an important dietary supplement to improve nutrient utilization and growth performance of poultry fed NSP-rich diets. This study explored the use of dietary enzyme supplementation to improve the nutritional value of inferior quality wheat for turkeys. The first research trial compared the nutritional value of frost-damaged and normal-mature wheat fed to turkeys and how a blend of NSP degrading enzymes with major endoxylanase activity influenced their nutritive value. Day-old toms were raised up to 17 days of age. Growth performance, nitrogen-corrected apparent metabolizable energy (AMEn), nitrogen retention (%NR), and viscosity were measured. The second trial evaluated the effect of three different supplemental enzymes on the growth performance and energy utilization on turkeys fed inferior-quality of wheat. The three enzyme preparations used included a natural enzyme blend of endoxylanase and other enzymes, an enzyme with predominantly endoxylanase activity, and an enzyme with predominantly phospholipase activity. Day-old toms were raised up to 18 weeks of

age. Growth performance, caked litter accumulation, AMEn, and viscosity were measured.

The supplementation of enzymes improved the nutritional value of wheat-based diets to a level similar to diets containing corn in place of wheat. The first study showed that frost damage during seed development significantly reduced the nutrient utilization of wheat, by presumably decreasing starch content and increasing the relative content of NSP. Supplementation of the enzyme improved the growth performance of turkey poults, regardless of the degree of frost damage of the wheat in the diet. In the second study, growth performance and energy utilization of turkeys fed diets containing a low AME wheat was significantly enhanced by phospholipase supplementation of starter feeds, while endoxylanase supplementation was most beneficial in growing and finishing feeds. Evidently, phospholipase alleviated the adverse effect of dietary NSP by improving fat digestion and absorption in young turkeys, whereas endoxylanase was more effective in older birds that have greater digestive capacity and more mature gut microbial ecosystem. Dietary supplementation of a natural blend of enzymes containing endoxylanase and other enzyme activities was beneficial for turkeys fed the wheat-based diet, regardless of the age of turkeys.

In conclusion, enzyme supplementation had positive effects on nutrient utilization of different wheat sources and cultivation conditions. Different sources of supplemental enzymes had variable effects according to the age of the birds. In general this research demonstrated that supplementation of appropriate enzymes is an effective way of dealing with grains with high NSP content in poultry diets.

IMPROVEMENT OF NUTRITIONAL VALUE OF WHEAT BY DIETARY ENZYME SUPPLEMENTATION FOR TURKEYS

by

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DEDICATION

To

God and my family

BIOGRAPHY

Anael Araujo Santos Jr. was born on November 17, 1975 in Belo-Horizonte, MG, Brazil. In 1979, Anael and his family moved to Uberlandia, MG, Brazil, where he received his elementary, secondary, and high school education. In 1993, he participated in the Youth for Understanding International Exchange Program at Bismarck, Missouri where Anael graduated his senior year at Bismarck High School.

After return to Brazil, in 1995, Anael began his undergraduate studies at the Federal University of Uberlandia (*Universidade Federal de Uberlandia*) with a major in Veterinary Medicine. During college, Anael participated in many internships, including poultry farm management and biosecurity, broiler and broiler breeder nutrition, biosecurity and management, commercial hatchery management and biosecurity, poultry and swine feed mill management, feed quality control, poultry slaughter house sanitary inspection, and work as a veterinary assistant in animal hospitals. During the fall of 1999, Anael worked as an international scholar at Poultry Science Department in North Carolina State University, Raleigh, NC.

After receiving his Bachelor of Science degree in December 1999, Anael entered North Carolina State University in August of 2000 to pursue a Master of Science degree in Poultry Science with a concentration in nutrition under the direction of Dr. P. R. Ferket. In 2002, Anael received award in recognition of the most outstanding graduate student research paper and presentation at the annual meeting of the Southern Poultry Science Society for his work examining dietary supplementation of endoxylanases and

phospholipase for turkeys fed wheat-based rations. After completion of his Master of Science, Anael will pursue Ph.D. in Nutrition at North Carolina State University.

The author is married to Fernanda Botaro O. Santos of Araguari, MG, Brazil. They have a daughter Amanda B. Santos.

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1. CHAPTER 1: LITERATURE REVIEW

1.1 INTRODUCTION

World poultry consumption has been on an upward trend for many years and it will continue as poultry becomes the world's primary choice meat product during the next decade or two (Roenigk, 1999). This growth is due in part to the rapid rise of the human population, which is expected to increase from 5,282 million in 1990 to 7,286 million in the year 2015 (Gueye, 2000), as well as the shift in national and global preferences towards quality animal protein products. Therefore, while making one of the best uses of available natural resources, the poultry industry is among the most efficient food commodity groups to supply the fast growing human population with quality protein.

Turkey production has increased significantly during the last decade due to new advances in management practices, increased exports, and increased consumer demand to low-fat food (Productschappen Vee and Vless en Eieren, 2001). Now the turkey industry is comprised of integrated turkey production operations with processing plants and distribution to retailers (Gascoyne, 1989). Turkey and broilers producers intend to produce a low-cost, high quality homogeneous meat product, in as short a period as possible, while maintaining good bird health and welfare. The rapid growth of turkeys and broilers require the use of high quality diets containing readily available nutrients.

The economics of turkey production is dependent upon feed ingredient availability and prices and feed formulation costs. The diet accounts for about 70-80% of the total cost of producing poultry (Acamovic, 2000). The formulation of poultry diets may vary among regions and seasons because it dependents upon the cost and availability of the ingredients. For example, the use of rye and barley in poultry feeds is relatively common in some parts of Eastern Europe, whereas it is unusual in the southeastern US (Bedford, 1995) due to the availability and cost of feedstuff. The poultry industry is occasionally challenged by high corn prices or short grain supply, causing many feed manufactures to consider wheat and wheat by-products as alternatives to corn for rations (Grimes and Crouch, 1997). Wheat production in the last several decades has increased world wide, and in conjunction with the North American Free Trade Agreement, General Agreement on Tariffs and Trade and other trade agreements, this grain may become competitive with corn on a routine basis (Ward, 1995).

Wheat is a cereal grain as are maize, rice, barley, oats, rye, millet, and sorghum. A respectable proportion of the world's cereal's and their by-products are used for animal feeds and the amount of cereals used to feed animals continues to increase as global animal production increases. Wheat is the most widely cultivated crop in the world, accounting for almost 30% (600.7 million metric tons in average of the years 1997 to 1999 – FAO, 2002a) of total cereal production, and it is one of the major energy contributors in diets for poultry (Steenfeldt et al., 1998). However, there is much concern about the high variability of AME among wheat used to feed poultry (Sibbald and Slinger, 1962; Wiseman and Inborr, 1990, Mollah et al., 1983; Scott et al, 1998). The

low-AME wheat in broilers, first described by Mollah et al. (1983) and Rogel et al. (1987), is caused by the presence of soluble non-starch polysaccharides (NSP), which are pentosans (mainly arabinoxylan and some β-glucan) in cell walls of the wheat kernels (Annison, 1993).

Non-starch polysaccharides have been shown to decrease nutrient absorption and increase variation in performance of poultry. Variation in performance is a consequence of different levels of NSP in the diet of poultry. The majority of NSP in poultry diets are of plant origin and there is a large variation in the amount and structure between ingredients and within the same ingredient (Smits and Annison, 1996). For example, the NSP content in the Australian wheat ranged from 5.4 to 7.2% and from 5.5 to 6.5% in the North American wheat samples harvested in 1992-1993 (Wootton et al., 1995). Also, agronomic conditions can change the NSP content of wheat. For example, wheat subjected to frost damage during seed maturation (immature wheat) contains increased levels of NSP (Ward, 1995). However, supplementation of diets with enzymes may help reduce the antinutritional properties of wheat. Feed ingredient technologists have developed supplemental enzymes that reduce the negative effects of NSP and improve the feeding value of wheat in poultry diets (Crouch et al., 1997). The dietary inclusion of exogenous glycanases (xylanase and β-gluconases) to improve the performance of poultry has become a prominent tool when diets contain a high concentration of wheat.

In conclusion, the increasing world demand for turkeys and broilers is expanding the need for wheat as an alternative for corn. Due to the antinutritive activity of NSP

present in wheat and wheat by-products, enzymes have become an important dietary supplement to improve growth performance.

The following review of the scientific literature will focus on the antinutritional effect of non-starch polysaccharides and the influence of enzyme supplementation in wheat-based diets. The primary objective of this thesis is to explore the use of dietary enzyme supplementation to improve the nutritional value of wheat for turkeys. Although, it is not the purpose of this thesis to review the considerably complex structure of wheat or to describe in detailed the digestive and absorptive processes in poultry, some simple appreciation of both is necessary to seek an explanation for the antinutritional effect of the non-starch polysaccharides and the influence of exogenous enzyme supplementation in wheat-based diets.

1.2 NUTRIENT DIGESTION IN THE GASTROINTESTINAL TRACT OF POULTRY

The major purpose of the digestive system is to assimilate nutrients required for energy, maintenance, growth, and reproduction. Digestion consists of a number of physical and chemical processes. Feed is ingested, broken down into smaller particles, macerated, mixed with digestive enzymes, and propelled through the digestive tract by the muscular activities of the tract. Salivary, gastric, pancreatic, biliary, and intestinal secretions collectively provide mucus for protection and lubrication of the tract, enzymes that aid in digestion, watery medium, and optimal pH required for digestion. Digestive enzymes aid in the hydrolysis of carbohydrates, protein, and lipids into a limited number of much smaller compounds suitable for absorption. Microorganisms, indigenous to the

digestive tract, can provide additional nutrients by breaking down structural carbohydrates that are not subject to digestion by endogenous enzymes and by synthesizing amino acids and vitamins essential to the host animal.

1.2.1 General characteristics of histomorphology and digestion in the intestinal tract

Histomorphology of the small intestine

Quantitatively, most of the digestion and absorption takes place in the small intestine (Turk, 1982). The small intestine is divided into duodenum, jejunum, and ileum. In birds, the duodenum is the section that extends from the gizzard to the pancreatic duct and biliary ducts, and encloses the pancreas to form structure also known as the duodenal loop. The jejunum is the segment extending from the pancreatic ducts to the Meckel's Diverticulum or yolk sac diverticulum. The ileum extends from the Meckel's Diverticulum to the ileo-caecal junction. The properties of the intestine grade continuously from the upper duodenum to the lower ileum (Turk, 1982). For instance, the intestinal mucosa decreases in thickness as the villi become shorter and the crypts decrease in depth from the duodenum to the ileum (Turk, 1982).

The avian intestinal tract is a multilayered tube containing a serosal layer, longitudinal muscular layer, circular muscle layer, submucosal layer and mucosal layer (Turk, 1982). Absorption takes place primarily through the mucosa of the small intestine. Most of the digestion of the bird intestinal tract occurs in the lumen of the intestine under the influence of the digestive enzymes secreted by the pancreas and intestinal wall and the bile secreted by the liver. Digestion of sugars and peptides, however, takes place

within the brush board by the enterocytes, facilitated by membrane-bound enzymes (Turk, 1982). The interior surface of the intestine is complexly folded into many structures called villi (Romanoff, 1960), which greatly increase its absorptive surface area. Between the villi are the crypts of Lieberkuhn, in which cells, called Crypt cells, proliferate and then migrate up the villi (Turk, 1982). These cells have a life cycle of 48 to 96 hours under normal conditions (Imondi and Bird, 1966; Cook and Bird, 1973; Fernando and McCraw, 1973; Turk, 1982; Moran, 1982; Uni et al., 1998). As the Crypt cells move up the villus, they differentiate into principal (absorptive), or goblet (secretory) cells. The absorptive epithelial cells are most abundant along the length of the villi, and goblet cells are intermittently dispersed.

The goblets cell produce mucopolysaccharides that are secreted into a layer covering the villar surface (Moran, 1985). This mucus layer serves as a protective barrier for the delicate absorptive surface from the epithelial lumenal contents. It has long been believed that mucin, which is the major component of the mucous layer, has a function that is largely associated with lubrication of bolus movement (Moran, 1985).

The absorptive cell is a very active columnar epithelial cell with a large basally located nucleus. The luminal surface of the absorptive cell is covered with extensive projections toward the lumen of the GI tract, called microvilli (Moran, 1985). The microvilli are cylindrical structures projecting from the cell surface and are bounded by a trilaminar membrane. The microvilli contain fibrous structures, which extend its length into the terminal web portion of the cell. Occasionally, these fibers may also be observed to extend into the mucus layer of glycocalyx, in which water is immobilized because of

the viscosity from accompanying mucin (Moran, 1985). Nimmerfall and Rosenthaler (1980) speculated that the rate at which molecules may move through this mucin water complex will depend on their charge, hydration, radius, ability to form hydrogen bonds, and molecular weight. Only the simplest saccharides, peptides, and fatty acids can readily transfer through this unstirred water layer (Moran, 1985). Borgstrom et al. (1985) described the unstirred water layer as an infinite number of water lamellae arranged in parallel with the enterocyte membrane. They, also, reported that the closer the water lamellae are situated to the enterocyte membrane, the lower the relative rate of stirring or movement is.

Disaccharidase enzymes are found on the luminal surface of the microvilli (Turk, 1982). Disaccharidases are enzymes catalyzing the reduction of sugars to glucose attached to the enterocyte membrane below the unstirred water layer (Moran, 1985). Mizuno (1982) showed that both maltase and the sucrase-isomaltase complexes are located on the chick's jejunal mucosa. By removing the glycocalyx from the small intestine, Kushak et al. (1981) were able to show that the digestive enzyme activity remained almost exclusively with the "denuded" surface. Thus, disaccharidases are present on surface of microvilli.

In the interior of the villi, beneath the epithelial cells, is the lamina propria, which contains connective tissue, capillaries, smooth muscle, and nerve fibers. The capillaries bring the blood stream to the base of the epithelial cells so that only one cell layer separates the lumen of the intestine from the blood (Romanoff, 1960). Thus, the

absorption of nutrient from the lumen of the intestine and its release into the blood stream is facilitated.

Relative to body weight, the digestive tract in avian species is shorter than in mammals. Most of this difference is in the intestinal region, suggesting that birds have less area for digestion and absorption than mammals (Moran, 1982). To compensate for this reduced absorptive surface area, birds achieve efficient digestion and absorption by maximizing digestive retention via periodic reverse peristalsis. Reverse peristalsis in poultry occur in three distinct regions of the gut: (a) the gastric reflux moves digesta from the gizzard back into the proventriculous once for each gastrointestinal contraction to optimize pectic digestion, (b) the small intestine reflux moves digesta from the jejunum up through the duodenum and sometimes into the gizzard to facilitate enzyme digestion, and (c) the cloaca-ceca reflux, which is a continuous, low amplitude colonic anti-peristalsis that conveys the urethral secretions along the epithelial surface of the rectum into the ceca where some uric acid nitrogen is converted into microbial biomass (Ferket and Veldkamp, 1999). The gizzard is the “pace-maker” of normal gut motility (Duke, 1992), and gizzard motility increases as particle size and integrity increases.

The cloaca-ceca reflux rate is dependent upon the activity of gut motility in the upper gut, particularly of the gizzard. Urine conveyed to the ceca facilitate water reabsorption and the absorption of volatile fatty acids (VFA) produced by bacterial fermentation. Of the total amount of water reabsorbed, 10-20% is absorbed in the ceca, 3-5% is reabsorbed in the rectum, and 85% is reabsorbed by the kidneys (Ferket and Veldkamp, 1999).

The rate of food passage is affected by many factors. Feed transit time or retention time through the small and large intestine increases with age (Shires et al., 1987). This may account for increases in metabolizable energy values of feedstuffs noted in older birds. Adding lipid (Sell et al., 1983), protein (Sibbald, 1979), or soluble non-starch polysaccharides (Fengler et al., 1988; Veldman and Vahl, 1994; Englyst and Hudson, 1996; Petersen et al, 1999; Preston et al., 2001) to the diet can increase retention time. Increases in environmental temperature (Denbow, 2000), or insoluble non-starch polysaccharides (Cummings and Englyst, 1992; Smits and Annison, 1996; Cao et al., 1998; Langhout, 1998; Knudsen, 2001) decreases retention time.

Insoluble and soluble NSP have different effects on digesta retention time or rate of passage. Soluble NSP alter the retention time through its physicochemical properties by forming a viscous digesta (Choct et al., 1999a). High gut viscosity decreases the rate of digesta passage (Gohl and Gohl, 1977; van der Klis and van Voorst, 1993). This effect of soluble-NSP is highlighted with more detail in section 1.4 of this thesis. In contrast, insoluble NSP decreases the retention time through its physical structure (Langhout, 1998). Although, soluble- and insoluble-NSP in wheat both consist mainly of arabinose and xylose residues, insoluble NSP decreases retention time because it has higher degree of branching (Medcalf and Gilles, 1968; D'Appolonia and MacArthur, 1975) and the polymers are highly linked to other macromolecules, such as lignin or protein or both (Lineback and Rasper, 1988).

Overview of digestion in the intestine tract

The principal organic components of plants and animals are carbohydrates, lipids, proteins, and nucleic acids (Stevens and Hume, 1995a). Although some are ingested in a form that can be readily absorbed, most of them require hydrolysis into a limited number of simpler compounds before their absorption. Digestion includes all physical and chemical processes by which the feed is broken down and made ready for absorption: swallowing, peristalsis, grinding in the gizzard, softening effect of water, action of enzymes and bacteria, etc.

Digestion starts when feed is picked up by the beak of the chicken, moistened with saliva, and swallowed without mastigation. It passes down to the crop, where is stored, softened by ingested water and liquid secretions. Feed moves from the crop, a small quantity at a time, through the proventriculus, where it is exposed to gastric juices, to the gizzard, where rhythmic contractions of the walls of the intestinal tract macerate, and force the feed along. Once the feed is ground to a fine particle size (< 1 mm in diameter), it is conveyed along the gizzard crevices through the pylorus sphincter, and duodenum. In the duodenum, the digesta is buffered and exposed to digestive enzymes from pancreatic secretions and bile salts. Then digestion proceeds in the small intestine and some of the undigested residue are assimilated by the microflora of the large intestine. The dietary fraction that was not digested and utilized by either the bird or its gut microflora is excreted into the feces.

Nutrient classification

The nutrients assimilated by the birds are chemically classified according to their nutritional properties. The macronutrients of the diet include protein, carbohydrate and fat, and the micronutrients are vitamins and minerals. Macro- and micro-nutrients are used according to the quantities required rather than their importance to the well-being of the organism (Stevens, 1996). The macronutrients provide protein and energy, and their digestion in the small intestine and their chemical classification will be considered in subsequent sections.

1.2.2 Protein classification and digestion

Proteins include the essential nitrogen-containing components of foods. Like carbohydrates and lipids, proteins contain carbon, hydrogen, and oxygen. Proteins contain about 16% nitrogen by weight (Spallholz et al., 1999a). “Proteins”, derived from Greek word *proteus*, means first. Proteins were the first substances to be recognized as a vital part of living tissue. About half of the dry weight of most animal cells consists of protein (Spallholz et al., 1999a).

Protein classification

Although an enormous range of proteins exists in nature, they are all composed of relatively the same simple units, the amino acids (Horton et al., 1996a; Evers et al., 1999; Spallholz et al., 1999a). About 20 to 22 amino acids are commonly found in most proteins. All the amino acids except proline and hydroxyproline (which are really imino acids) are α-amino carboxylic acids. They contain a basic amino group, a hydrogen atom

and an acidic carboxyl group attached to the α-carbon or C-2. In addition, a side chain, or "R" group, is attached to the α-carbon. The amino acids differ from each other as a result of differences in the side chains. With the exception of glycine, all amino acids assembled into protein in animals and humans are L-amino acids (Spallholz et al., 1999a).

Amino acids have been classified in several ways. They are frequently classified based on their structural similarities, which consist of aliphatic, acidic, basic, aromatic, sulfur-containing, selenium-containing, and secondary amino acids (Spallholz et al., 1999a). From a nutritional standpoint, however, amino acids can be classified as *essential* (indispensable) or *non-essential* (dispensable). *Essential* amino acids are those required in the diet to maintain a growing animal in positive nitrogen balance, or an adult in nitrogen equilibrium (Stevens, 1996). An animal requires all 20 amino acids to make its complement of body proteins, but some of these can be synthesized from the *essential* amino acids. Thus, the essential amino acids cannot be synthesized *in vivo* from nonprotein sources in sufficient quantity to meet the body's needs, and so must be supplied to the animal from either plant or animal protein (Spallholz et al., 1999a). The categorization into *essential* or *non-essential* amino acids is dependent upon the species and age of the animal in question. Arginine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine and histidine are essential for maintenance of nitrogen balance in adult domestic fowl and glycine is necessary for growing chicks to maintain maximum growth rate (Stevens, 1996). In broiler chickens and commercial

turkeys that have been selected for rapid growth rate, any deficiency in amino acid requirements is likely to be more pronounced than in a slower growing rate animal.

Despite the limited number of amino acid types, variations in the order in which amino acids are connected, in the numbers of amino acids per protein, cross-linkages, and tertiary structures allow an almost limitless variety of proteins (Horton et al., 1996a; Spallholz et al., 1999a). Plant and animal proteins consist of chains of L-amino (or imino) acids linked together with peptide (-NH•CO-) bonds. The terms dipeptide, tripeptide, oligopeptide, and polypeptide refer to chains of two, three, several (up to about 20), and many (usually more than 20) amino acid residues, respectively.

The animal body's protein needs are both qualitative and quantitative. The body needs each of the essential amino acids in certain proportions and also needs a sufficient quantity of protein (Stevens, 1996). Thus, functionally, one of the most significant means of classifying proteins is by the essential amino acid content of the protein, which determines the protein quality. Most protein feedstuffs contain many different proteins, so the quality of a protein feed-ingredient reflects a composite of the amino acid content of several different proteins. Complete protein contains all the essential amino acids needed by the body in proportions and amounts that are adequate. Incomplete proteins contain one or more essential amino acid(s) in insufficient quantity(ies) to meet the body needs.

The early studies on amino acid requirements focused primarily on the essential amino acids. The more recent studies on domestic fowl have focused on the quantitative aspects of efficiency of utilization of the protein source and the balance of amino acids.

The requirements for protein are actually based on the animal's requirements for individual amino acids (NRC, 1994). The amounts of proteins and amino acids required in avian diets depend on different conditions: (a) the physiological state of the bird, (b) the digestibility of dietary protein, and (c) the biological value of dietary protein. Dietary protein requirement can be influenced by several antinutritional factors. For example, excess dietary soluble-NSP increases the viscosity in the gastrointestinal tract (GI) and may interfere with protein digestion and diffusion of peptides and amino acids during absorption. Adequate protein digestibility is crucial to maintain the proper growth of poultry.

Protein digestion in the small intestine

Of the three macronutrients, protein may be considered the most important (Stevens, 1996). This is because dietary protein is able to act as a source of body protein, carbohydrate or fat, whereas neither fat nor carbohydrate alone can give rise to a net increase in body protein. Lack of dietary protein has the most serious consequences, since it is the source of synthesis of body protein. In the small intestine, protein is hydrolyzed by extracellular endopeptidases, which hydrolyze peptide bonds along the protein chain, and exopeptidases, which cleave terminal amino acids (Stevens and Hume, 1995a). The action of these enzymes releases oligopeptides, dipeptides, and amino acids. Oligopeptides and dipeptides are further hydrolyzed by enzymes in the brush border or contents of intestinal epithelial cells.

The principal endopeptidases found in birds are trypsin, chymotripsin, elastase, and collagenase from pancreatic secretion, and pepsin from gastric secretion. Among the

exopeptidases, the C-terminal peptidases (carboxypeptidases) hydrolyze peptide bonds with a free carboxyl group, and the N-terminal peptidases (aminopeptidases) hydrolyze substrates with a free amino group (Stevens and Hume, 1995a). Carboxypeptidase “A” has been observed in pancreatic tissues of birds (Stevens and Hume, 1995a). The enzymes in the brush border and cytosol of the enterocytes are believed to be principally responsible for the hydrolysis of peptides rather than of intact proteins (Kim and Erickson, 1985; Silk et al., 1985). They are exopeptidases involved in the final stages of peptide hydrolysis, which consist of aminopeptidases, tripeptidases, and dipeptidases. Brush border enzymes have greater activity against tetrapeptides and large oligopeptides. Much of the tripeptidases and dipeptidase activity resides within the cytosol of enterocytes (Stevens and Hume, 1995a).

The products of proteolysis are absorbed in the small intestine in the form of amino acids or small peptides, the majority being in the latter form (Matthews, 1975).

Evaluation of the digestion and enzyme activity is a good referential for the performance of the bird, and vice-versa. Stevens and Hume (1995a) reported that enzyme activity is modulated by the diet and time of the day. Francesch et al (1999) conducted an experiment to evaluate the effects of cereal non-starch polysaccharides and exogenous enzyme supplementation to determine the nutrient digestibility in broiler chickens. They found that dietary ileal nitrogen digestibility was highly affected by the grain species: nitrogen digestibility was higher for maize (4.3% NSP, DM), followed by wheat (6.5% NSP, DM), barley (10.9% NSP, DM) and rye (10.2% NSP, DM) diets. They also showed that supplementation of exogenous enzyme increases the apparent nitrogen digestibility

in the small intestine. Thus, as the level of water-soluble non-starch polysaccharides (mainly arabinoxylan and β-D-glucan) increases, the digestibility of nutrients decreases, and this effect was improved by the supplementation of exogenous enzymes in the feed.

1.2.3 Carbohydrates classification and digestion

The term carbohydrate originated in the belief that naturally occurring compounds of this class could be represented formally as hydrates of carbon, i.e. $C_x(H_2O)_y$. This definition is too rigid, however, as the important deoxy sugars like rhamnose, the uronic acids, and compounds such as ascorbic acid would be excluded and acetic acid would qualify for inclusion. Nevertheless, the term carbohydrate remains to describe those polyhydroxy compounds that reduce Fehlings solution either before or after hydrolysis with mineral acids (Percival, 1962).

Carbohydrates and fats form the main energy source in a typical avian diet. Although dietary protein can also be an energy source, it only occurs when it is in excess or when there is a shortage of dietary fat and/or carbohydrate relative to requirements. Dietary requirements for carbohydrates and fats are less exact than those for proteins (Stevens, 1996). Carbohydrates are essential components for all living organism, however carbohydrates can be completely eliminated from the diet as it can be synthesized from body protein (Horton et al., 1996b). The carbohydrates in plants and animals serve three main functions: structure, storage, and transport (Stevens and Hume, 1995a).

Carbohydrates classification

Carbohydrate can be classified according to their chemical, physiological and nutritional characteristics. The classification of carbohydrates has been reviewed in detail by several authors (Englyst, 1989; Cummings and Englyst, 1992; Southgate, 1995; Stevens and Hume, 1995a; Asp, 1996; Englyst and Hudson, 1996; Knudsen, 2001). It is customary to classify carbohydrates according to their degree of polymerization into monosaccharides (1 unit), oligosaccharides (2-20 units), and polysaccharides (>20 units) (Evers et al., 1999). Monosaccharides are the simplest carbohydrates and most of them are sugars. Monosaccharides may have 3-8 carbon atoms, but those with 5 carbons (pentoses) and 6 carbons (hexoses) are common (Evers et al., 1999). Both pentoses and hexoses exist in a number of isomeric forms; they may be polyhydroxyaldehydes or polyhydroxyketones (Evers et al., 1999). Structurally, they occur in a ring form, which may be 6-membered (pyranose form) or 5-membered (furanose form) (Evers et al., 1999). The oligosaccharides and polysaccharides are monosaccharides linked by glycosidic bonds to form a chain. Most of the carbohydrates in plants and animals are polysaccharides and they either provide structural support or serve as the intracellular storage of energy (Stevens and Hume, 1995a).

The quantitatively most import food carbohydrates are listed in Table 1 (Asp, 1996). The low-molecular weight carbohydrates consist of mono-, di- and oligo-saccharides (Asp, 1996). Nutritionally, sugars usually include mono- and disaccharides (2 units). The main food/feed monosaccharides are glucose, fructose, and galactose. Sucrose, lactose, and maltose are the main disaccharides. The predominant forms of

oligosaccharides are the raffinose series of α-galactosides, fructo-oligosaccharides, and malto-oligosaccharides (Asp, 1996).

TABLE 1: Quantitatively the main food carbohydrates[1].

CARBOHYDRATE	MONOMERS
Monosaccharides (LMW[2])	
Glucose	glucose
Fructose	fructose
Galactose	galactose
Disaccharides (LMW[2])	
Sucrose	glucose, fructose
Lactose	glucose, galactose
Maltose	glucose, glucose
Oligosaccharides (LMW[2])	
α- Galactosides (e.g. raffinose)	galactose, glucose, fructose
Fructooligosaccharides	fructose, glucose
Maltooligosaccharides	glucose
Polysaccharides	
Starch	
Amylose (α-1,4-D-glucan)	glucose
Amylopectin (α-1,4- and α-1,6- D-glucan)	glucose
Non-starch polysaccharides (NSP)	
Cellulose (β-1,4-D-glucan)	glucose
NCP[3]	
β-Glucans [β-(1,3)(1,4)-D-glucan]	glucose
Heteropolysaccharides	glucose, galactose, mannose, arabinose, xylose, rhamnose, uronic acids
e.g. Arabinoxylan	arabinose, xylose

[1]Asp, 1996.
[2]LMW = low molecular weight sugars.
[3]NCP = non-cellulosic polysaccharides .

In a review of the literature, Asp (1996) classified polysaccharides into starches, and non-starch polysaccharides (NSP). Starches are the major storage polysaccharides in plants. Starch are linear (amylose) or branched (amylopectin) homopolymers of glucose with α-glycosidic bonds (α-D-glucans). Amylose is α-1,4-D-glucans, while amylopectin

is α-1,4-D-glucans with α-1,6-D-glucan branch points. Glycogen, the principal storage carbohydrate of animals, has a structure similar to amylopectin, except it has shorter chains (10-14 units) between the α-1,6-D-glucan branches.

NSP are a very diverse group of molecules with varying degrees of water solubility, size, and structure (Cummings and Englyst, 1992). Plant-cell walls are the main source of dietary NSP. Non-starch polysaccharides consist of cellulose, which is a linear β-1,4-D-glucan, and a range of heteropolysaccharides non-α-D-glucans. The non-cellulosic NSP can be classified according to many different criteria, including neutral (containing mainly neutral sugar residues), acidic (containing mainly uronic acid residues, also referred to as pectic substances) and hemicellulose A, B, C (mixture of polysaccharides of varying composition, usually a common core of xylose) depending on solubility at various pH. The relative proportion of main monomeric residues (i.e. rhamnose, xylose, arabinose, galactose, glucose, mannose, and uronic acids) is another common way to characterize and name polysaccharides, including arabinoxylans, galactans, galactomonnans, rhamnogalacturonans, etc.

The main polysaccharides of plant cell walls are cellulose, arabinoxylans, mixed linked β-(1,3)(1,4)-D-glucan (β-glucan), xyloglucans, rhamnogalacturonans, and arbinogalactans (Bacic et al., 1988; Selvendran, 1984; Theander et al., 1989). Cellulose, and β-glucan are polymers of a single sugar species, but arabinoxylans, xyloglucans, rhamnogalacturonans, arbinogalactans are polymers that comprise two or more different species (heteropolymers), called pentosans (polymers of pentose sugars) (Evers et al., 1999). Arabinoxylans, found in endosperm walls of wheat and other cereals, have a

xylonopyranosyl backbone to which are attached single arabinofuranosyl residues. A better description of the structure and physiological effect of the non-starch polysaccharides is described in section 1.4 of this thesis.

Besides classification by their chemical structure, carbohydrate can be classified by their physiological and nutritional effects (Asp, 1996). The nutritional effects of carbohydrate can be described by: (a) the rate of digestion, which is the proportion of carbohydrates that provide substrate to enzymes and microflora; (b) the rate of absorption, which is the speed of absorption in the small intestine, and its effect on the blood glucose level (glycaemic index); (c) the proportion of monomer absorption, which is the relative amount of absorbed monomers, specially the fructose/glucose ratio; (d) the degree and rate of colonic fermentation associated to the profile of fermentation products. The main fermentation products are the volatile fatty acids (VFA) including acetate, propionate, and butyrate.

The nutritional value of carbohydrates is dependent upon their degree of digestibility. Soluble indigestible carbohydrates increase digesta viscosity, increase water-binding capacity, and are fermented by gut microflora to produce VFA (Asp, 1996). Carbohydrate classified by their chemical or physiological properties cannot always be characterized in similar categorical groups. For example, both oligosaccharides and polysaccharides may be indigestible in the small intestine. However, starch, a polysaccharide, is the quantitatively most important digestible carbohydrate, even though a fraction of the starch called resistant starch (RS) can be classified as undigestible along with non-starch polysaccharides and oligosaccharides.

Carbohydrates digestion in the small intestine

Dietary fiber can be defined as “the skeletal remains of plant cells that are resistant to digestion by enzyme of the digestive tract” (Trowell, 1972). This definition was later modified to “non-starch plant polysaccharides (NSP) and lignin that are resistant to hydrolysis by the digestive enzymes” (Trowell, 1976). Starch is the only plant polysaccharide that is hydrolyzed by the digestive enzymes (Englyst, 1989). In contrast, endogenous enzymes of vertebrates cannot hydrolyze the β-1,4 linkages of cellulose and hemicellulose (Stevens and Hume, 1995a).

Amylose, amylopectin, and glycogen are hydrolyzed by α-amylase to form maltose, isomaltose, maltotriose, and other α-1,4-linked and α-1,6-linked oligosaccharides. Amylase is secreted by pancreatic tissue of all vertebrates (Stevens and Hume, 1995a). In contrast to mammals, poultry do not secrete any salivary amylase, although some starch and glycogen digestion may occur in the crop as the result of microbial fermentation (Bolton, 1965). Therefore, pancreatic α-amylase is the major enzyme responsible for the digestion of starch and glycogen.

Oligosaccharides and disaccharides are hydrolyzed to monosaccharides by enzymes located in the brush border and microvilli membranes of enterocytes. Stevens and Hume (1995a) listed the brush border enzymes of birds as isomaltase, maltase, sucrase and cellobiase. Maltase and isomaltase hydrolyze maltose, isomaltose, and α-1,4-oligosaccharides to glucose. Sucrase hydrolyzes sucrose to glucose and fructose. Stevens and Hume (1995a) stated that the presence and function of cellobiase is difficult to understand, since cellobiase is a β-glucosidase that can hydrolyze the β-1,4-linkage of

cellobiose, the end product of cellulose hydrolysis by C_1 and C_x cellulolytic enzymes (Vonk and Western, 1984). However, cellulolytic enzymes are not endogenous enzymes of vertebrates; and microbial fermentation of cellulose leads primarily to the release of short-chain fatty acids, CO_2, and CH_4.

The products of the brush border carbohydrases are absorbed in the small intestine in the form of monosaccharides (Moran Jr., 1982; Moran, 1985; Fogarty, 1983). Some carbohydrates that are not degraded by pancreatic enzymes are utilized by gut microflora in the large intestine that produce volatile fatty acids as by-products of fermentation (Englyst, 1985; Englyst and Macfarlane, 1986). NSP are largely but not completely fermented in the large intestine (Cummings, 1981).

Like proteolytic enzymes, saccharidases are affected by a number of factors, including the age and physiological status of the animal, the diet being fed, and feedback or end product inhibition (Longland, 1991). For instance, high dietary inclusion of NSP increases the viscosity of digesta in the gastrointestinal tract, which impairs the diffusion and convective transport of enzymes within the gastrointestinal contents (Smits and Annison, 1996). Edwards et al. (1988) demonstrated in vitro that the convective transport of glucose and sodium was impaired in a viscous environment. Moreover, viscosity may reduce the degree of contact between dietary constituents (e.g. starch) and the digestive secretions, and it impairs the transport of nutrients to the epithelial surface (Smits and Annison, 1996).

1.2.4 Lipids, and fat-soluble vitamins classification and digestion

Lipids are water-insoluble substances that are soluble in organic solvents, such as chloroform, ether, or benzene. Lipids are long chains of hydrocarbon groups and are present in, or derived from living organisms (Kates, 1972). This definition covers a wide range of compounds, including long-chain hydrocarbons, alcohols, aldehydes and fatty acids, and derivatives such as glycerides, wax esters, phospholipids, glycolipids, and sulfolipids. Lipids also include fat-soluble vitamins A, D, E, and K, and their derivatives, as well as carotenoids and sterols and their fatty acid esters (Kates, 1972).

Although many fat-soluble compounds are generally classified as lipids, fats and oils are classified strictly as triglycerides (triacylglycerols) (Evers et al., 1999). Fats and oils are distinguished by their thermal melting point: fats are solid at room temperature, while oils are liquid. Although many fats and oils originate in living organisms (where they function as a means of storing energy), this is not a feature of their definition as it is for lipids (Evers et al., 1999).

Lipids, like proteins, and carbohydrates, are essential components for all living organism. Although the dietary requirement for fat is relatively much lower than for carbohydrates, it cannot be eliminated completely from an animals' diet (Stevens, 1996). The symptoms of dietary fat deficiency was first demonstrated in rats by Burr and Burr in 1929. They found that the deficiency symptoms could be overcome if certain polyunsaturated fatty acids were included in the diet, and they called them essential fatty acids.

Essential fatty acids include linoleic, linolenic, and arachidonic acids. Linoleic acid and arachidonic acid are required in poultry, but there is not convincing evidence for the requirement of α-linolenic acid (Scott et al., 1982). These acids are required in relatively small amounts: approximately 1% of the diet (Stevens, 1996). Most of the dietary lipids are used as a form of energy, although they may initially be stored in the fat depots, such as adipose tissue.

Lipids, and fat-soluble vitamins classification

Unlike the other types of biomolecules, lipids are widely varied structures. The extreme diversity of lipid compounds, both in structure and function, makes any classification of these molecules difficult (Spallholz et al., 1999b; Horton et al., 1996b). One of the lipid classifications frequently used in science is based on their polarity. Lipids are either hydrophobic (nonpolar) or amphipathic (containing both nonpolar and polar regions) (Horton et al., 1996b). The major types of lipids and their structural relationships are listed in Figure 1 (Horton et al., 1996b). Horton et al. (1996b) classified lipids in three groups, as follow: (a) phospholipids – lipid containing phosphate moieties, (b) glycosphingolipids - lipids containing both sphingosine and carbohydrate groups, and (c) isoprenoids - lipids containing the five-carbon molecule isoprene.

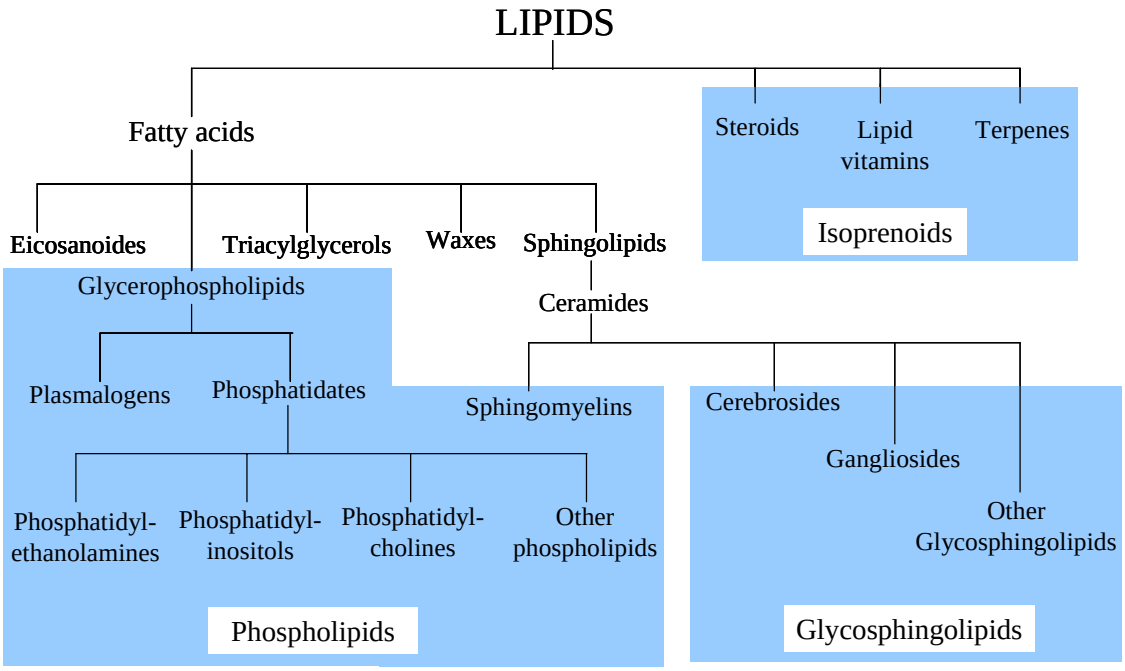

FIGURE 1: Major classes of lipids (Horton et al., 1996b).

The simplest lipids are fatty acids, which have the general formula R-COOH, where R represents a hydrocarbon chain (CH_3, CH_2, and/or CH). Fatty acids are components of many complex types of lipids, including triacylglycerols, glycerophospholipids, eicosanoids, waxes, and sphingolipids (Horton et al., 1996b). Fatty acids differ by the length of their hydrocarbon tails, the degree of unsaturation (the number of carbon-carbon double bonds), and the position of the double bonds in the chains (Spallholz et al., 1999b; Horton et al., 1996b). The number of carbon atoms in the most abundant fatty acids ranges from 12 to 20 and is almost always even, since fatty acids are synthesized by the sequential addition of two-carbon units (Horton et al., 1996b). Fatty acids without a carbon-carbon (C=C) double bond are classified as saturated, whereas those with at least one carbon-carbon double bond are classified

unsaturated. The physical properties of saturated and unsaturated fatty acids differ considerably (Horton et al., 1996b). Typically, saturated fatty acids are waxy solids at room temperature, whereas unsaturated are liquids.

Free fatty acids occur only in trace amounts in living cells. Most fatty acids are esterified to form more complex lipid (Horton et al., 1996b). The most common complex lipid, the neutral storage form of fatty acids, is the triacylglycerols. Triacylglycerols are composed of three fatty acyl residues esterified to glycerol (a three carbon sugar alcohol). Triacylglycerols are very hydrophobic (not soluble in water), which make them an efficient source of storage. Triacylglycerols (triglycerides) constitute the major dietary lipid component (Homan and Jain, 2001). However, monoacylglycerols, and diacylglycerols can be formed from the esterification of one or two acylglycerol of the glycerol molecule, respectively (Evers et al., 1999).

Glycerophospholipids, also called phosphoglycerides, are the second most abundant dietary lipid class (Homan and Jain, 2001), and the first most abundant lipids in membranes (Horton et al., 1996b). The simplest glycerophospholipids, phosphatidates, consist of two fatty acyl groups esterified to C-1 and C-2 of glycerol 3-phosphate (Horton et al., 1996b). In most glycerophospholipids, the phosphate group is esterified to both glycerol and another compound bearing an –OH group (e.g. water, choline, serine). Each type of glycerophospholipid is not a single compound but a family of molecules that have the same polar head group and different fatty acyl chains (Horton et al., 1996b).

Cholesterol is the most widely distributed sterol in animal and human tissues (Spallholz et al., 1999b). Its presence in the body comes from ingestion of only animal

foods, or actively synthesized from acetyl-CoA and isoprene by the liver, intestinal epithelium, adrenal glands, and skin (Spallholz et al., 1999b). Steroids contain four fused rings: three six-carbon rings designated A, B, and C, and a five-carbon D ring (Horton et al., 1996b).

Vitamins are compounds that are required in small quantities for growth and maintenance of normal cell and organ functions; they are nutrients required primarily by vertebrates and not by lower organisms (Meydani and Martin, 2001). Although vitamins may be grouped by their source or function, the most commonly used categorization relates to their chemical solubility, namely hydrophobicity (lipid-soluble) or hydrophilicity (water-soluble), which is directly associated with absorption processes. The water-soluble vitamins, which include the B-complex vitamins (thiamin, riboflavin, niacin, vitamin B_6, folacin, vitamin B_{12}, pantothenic acid, and biotin) and vitamin C, have known functions as precursors of coenzymes (Stevens, 1996; Meydani and Martin, 2001). The functions of the fat-soluble vitamins, which include the vitamin A, D, E, and K, are less clearly understood but include both regulatory roles and redox roles (Stevens, 1996; Meydani and Martin, 2001). The fat-soluble vitamins can usually be stored in appreciable amounts in the body, thus have low incidences of deficiency symptoms, however, excess in the dietary intake can be toxic. In contrast, the water-soluble vitamins cannot be stored in appreciable amounts and there excess are not generally harmful because they are readily excreted (Stevens, 1996; Meydani and Martin, 2001). Fat-soluble vitamins undergo distinct absorption processes and generally follow the requirements and pathways of lipid absorption and transport (Hollander, 1981).

For more information on lipid classification, can be found published by Spallholz et al. (1999b), Horton et al. (1996b), Evers et al. (1999), and Verkade and Tso (2001).

Lipids, and fat-soluble vitamins digestion in the small intestine

The first stage of lipid digestion is characterized by the formation of an emulsion-core from the action of amphipath molecules. Lipids enter the small intestine, where they are emulsified into the aqueous phase of the digesta to provide an oil-water interface required for digestion by lipase (Stevens and Hume, 1995a). The emulsification is accomplished principally by bile salts, phospholipids, cholesterol, and an apolipoprotein (Borgstrom and Patton, 1991), and facilitated by shear forces of the GI tract (Carey and Hernell, 1992). The lipid emulsion would not be stable unless it included amphiphilic lipids (high polar lipids), such as phospholipids from the diet. These molecules are strong detergents called amphipath molecules (Verkade and Tso, 2001) and they are required since lipids are not water-soluble (Horton et al., 1996b).

More polar lipids and bile salts are added to the emulsion-core to form the mixed micelles so that digestion and absorption of lipids can occur. The process of emulsification is aided by the secretion of bile into the intestine (Verkade and Tso, 2001). Bile acids, synthesized in the liver, contain significant amounts of phospholipids, mainly phosphatidylcholine (a potent amphipath molecule), and bile salts. Biliary salts and phosphatidylcholine play significant roles in micellarization (formation of micelles) (Verkade and Tso, 2001). Due to their physicochemical properties, phospholipids stabilize the emulsion, whereas bile salts destabilizes the emulsion, partially by the incorporation of polar lipids with less amphiphilic characteristics into mixed micelles

(Borgstrom et al., 1985). Destabilization of the emulsion by bile salts also increases the availability of triacylglycerols and diacylglycerols for lipolysis by the pancreatic lipase (Verkade and Tso, 2001). Micelles of bile salts solubilize fatty acids and monoacylglycerols so that they can diffuse through the brush border and be absorbed by the cells of the intestinal wall (Horton et al., 1996b).

Pancreatic lipase is responsible for most of the lipid digestion and its end products are either absorbed or they help with the stabilization, digestion, or transport of other lipids. Pancreatic lipase, the most abundant lipid digestion enzyme (Stevens and Hume, 1995a), catalyzes hydrolysis of the primary esters (at C-1 and C-3) of triacylglycerols, releasing fatty acids and monoacylglycerols. The rate of the pancreatic lipase is strongly related to the surface area of the oil-water interface, as well as to its physicochemical properties (Borel et al., 1994; Schmit et al., 1996; Dahim and Brockman, 1998). The products of lypolysis are initially present in small unilamellar vesicles (Verkade and Tso, 2001). When the concentration of fatty acids and monoacylglycerols significantly exceeds the bile salt concentration, a liquid crystalline phase is formed, characterized by multiple lipid layers covering the lipolyzed emulsion (Verkade and Tso, 2001). Multilamellar vesicles can develop from these multilayers, which thereafter can be dispersed into mixed micelles. The mixed micelles (composed of long-chain fatty acids, monoglycerides, phospholipids, and fat-soluble vitamins) are microdroplets of approximately 30-100 Aº as compared to the 5,000 Aº of the initial emulsion droplet, and 500 Aº of the distance in-between two microvilli (Odle, 2001). Thus, incorporation of

lipolytic end products into mixed micelles is a prominent and highly effective means of lipid transport through the aqueous phase of the intestinal lumen (Homan and Jain, 2001).

In summary, the predominant phase of the lipid digestion is an oil-water emulsion-core rich in triglyceride (Homan and Jain, 2001). A surface layer of more polar lipids including monoglycerides, fatty acids, phosphatidylcholine and bile acids are stabilized on the oil-water emulsion-core. Following the oil-water emulsion-core phase is the micellar, vesicular, and crystalline lipid phases. All phases are composed of the same lipids but enriched with more polar lipid (Homan and Jain, 2001).

The lipid digestion products are transported as micelles prior to their absorption. Micelles contain all the products of lipid digestion, with the exclusion of the short-chain free fatty acids and glycerol, which are water-soluble (Spallholz et al., 1999c). Free fatty acids of 10 or fewer carbons, and glycerol may pass directly through the microvilli membrane and into the capillary bed of the villus entering the general hepatic portal circulation (Spallholz et al., 1999c). However, the transport of absorbed monoacylglycerides and free fatty acids of more than 10 carbons through enterocytes requires reacylation of the absorbed lipid into triglycerides, followed by reacylation into chylomicron particles composed of a triglyceride-rich core, sufaced by apolipoproteins and a phospholipid monolayer (Spallholz et al., 1996c).

The digestion of fat-soluble vitamins mainly follows the lipid digestion with minor differences. Dietary fat-soluble vitamins are generally associated with proteins; thus, the vitamins are released as a result of gastric acidity and/or *via* the action of proteolytic enzymes (Meydani and Martin, 2001). The fat-soluble vitamins are

solubilized and aggregated into small fat globules formed from lipids of ingested feed through mechanical mixing of the chyme (Meydani and Martin, 2001). These fat droplets are then emulsified within the lumen of the small intestine by secretion and interaction of bile acids and pancreatic juice, which then, follow the lipid digestion.

For more information on the process of lipid digestion in monogastric animals refer to reviews published by Freeman, 1976; Davidson and Glickman, 1983; Freeman, 1984; Bezard and Buguat, 1986; Stremmel, 1987.

Like protein and carbohydrate digestion, lipid digestion is affected by a number of factors, including the age and physiological status of the animal, the diet being fed, and the physical and chemical characteristics of the fats. Wiseman et al. (1991) reported that the higher is the degree of saturation of fat, the lower is the metabolizable energy. In addition, the longer the fatty acid chain length, the poorer the digestibility of the fat (Ward and Marquardt, 1983). Antoniou et al. (1980), Fengler et al. (1988), and Preston et al. (2001) described that the gel forming properties of water-soluble non-starch polysaccharides have a detrimental effect on the digestibility of fat in poultry. Moreover, the effects on the fat digestibility by NSP are more pronounced in young birds. Due to the limited production of lipase enzyme and inefficient recycling of bile salts, poults (Sell et al., 1986; Krogdahl and Sell, 1989), chicks, and ducklings (Martin and Farrel, 1998) have lower lipid digestibility as compared to the adults. Noy and Sklan (1995) reported that lipase activity increased at a slower rate than for most other digestive enzymes; thus, a low level of natural lipase production in young birds likely limits fat digestion.

Endogenous phospholipase

Intestinal phospholipases A_2 (PLA_2) are a family of enzymes that catalyze hydrolysis of ester bond at sn-2 position of phospholipids producing fatty acids and lysophospholipids (Homan and Jain, 2001). Pancreatic phospholipase is one of several lipases released along the alimentary tract to facilitate lipid absorption by hydrolysis of the complex acylated lipids. The triglycerides, which constitute the major dietary lipid component, are hydrolyzed by the pancreatic lipases; however, glycerophospholipids, the second most abundant dietary lipid class, are hydrolyzed by phospholipase A_2 (Homan and Jain, 2001).

Besides hydrolyzing glycerophospholipids into absorbable products, phospholipase A_2 affect several stages in the lipid absorption process by the hydrolysis of phosphatidylcholine, which is the predominant glycerophospholipid in the lumenal contents. Consequently, phospholipase A_2 influence the absorptions of other lipid classes as well (Tso and Scobey, 1986; Homan and Hamelehle, 1998). The products of the hydrolysis of phosphatidylcholine by phospholipase are fatty acids and lyso-phosphatidylcholine (lyso-PC). Lyso-PC is a major amphiphile molecule, which acts to stabilize microdroplets of triglycerides, cholesterol, and other nonpolar dietary lipids that are otherwise insoluble in the aqueous environment of the intestinal contents (Carey et al., 1983). Therefore, phospholipase can enhance lipid absorption (Homan and Jain, 2001). Homan and Jain (2001) hypothesized that pancreatic phospholipase A_2 is the rate-limiting factor for the absorption of a range of lipid classes, in addition to the glycerophospholipids. Furthermore, they speculated that once an emulsion droplet is

attacked by phopholipase, the products of its hydrolysis might promote the binding of lipases.

Another mode in which lipid absorption may be linked to pancreatic phospholipase A_2 activity derives from the fact that the flux of absorbed lipid through the enterocytes of the intestinal epithelium depends directly on cellular phosphatidylcholine synthesis, which is limited by the supply of products available from hydrolysis of phosphatidylcholine in the lumenal contents (Mansbach, 1977). As discussed earlier, the transport of absorbed lipid through enterocytes requires reacylation of the absorbed lipid into triglycerides, followed by reacylation into chylomicron particles composed of a triglyceride-rich core surfaced by apolipoproteins and a phospholipid monolayer consisting primarily of phosphatidylcholine (Tso, 1994). In their review of the literature, Tso and Scobey (1986) concluded that the absorption of tryglycerides decreases significantly when the supply of phosphatidylcholine is limited for the production of intestinal chylomicron. Therefore, the capacity of enterocytes to transport absorbed lipids into the circulation depends on pancreatic phospholipase A_2 hydrolysis of phosphatidylcholine in the lumenal contents.

Additionally, phospholipase has shown to posses an intrinsic secretin-releasing activity. Chang et al. (1999) observed that secretin stimulated the release of pancreatic enzymes and bicarbonate in the duodenum, which influences the digestion of others macronutrients. Independent of its hydrolytic activity (active or inactivated by 4-bromophenacyl bromide), phospholipase A_2 causes the release of secretin by activation of a specific receptor in the small intestine. Chang et al. (1999) further suggested that

pancreatic phospholipase A_2 is released from the upper small intestinal mucosa to mediate the release of secretin in response to duodenal acidification.

1.2.5 Energy

Feedstuffs provide the energy and nutrients that are essential for the bird's growth, reproduction, and health (NRC, 1994). Genetic selection for rapid growth rate has made a large contribution to the improved performance of modern broilers, and turkeys. The outcome of this selection is a bird with high daily energy requirements. The gross energy yields for carbohydrates, protein, and fat, as determined by bomb calorimetric, are 4,099 kcal/kg, 5,399 kcal/kg, and 9,539 kcal/kg, respectively (Stevens, 1996). The actual amount of energy obtainable from these nutrients by birds is somewhat less, as this would require each of the nutrients to be absorbed by the gut with 100% efficiency (Stevens, 1996). Both fats and carbohydrate can be fully oxidized in the body to carbon dioxide and water, but the nitrogen of protein is not released in its fully oxidized state because most is excreted by the bird as uric acid, which reduces the energy available from protein to 4,299 kcal/kg (Stevens, 1996). Although protein can also be an energy source, it only occurs when an excess of protein or when dietary fat and/or carbohydrate is limited. Fatty acids are important metabolic fuels (Horton et al., 1996b) because the carbon atoms of fatty acids are more reduced than those in proteins or carbohydrates, and the oxidation of fatty acids yields more energy (~8,843 kcal/kg) than the oxidation of proteins or carbohydrates (~3,825 kcal/kg each) (Horton et al., 1996b). Therefore, fats and carbohydrates form the main energy source in a typical avian diet.

The energy system that has been adopted for poultry is apparent metabolizable energy (AME) (NRC, 1994). Metabolizable energy is the amount of energy assimilated from feed, and it is equal to the gross energy of the feed consumed minus the gross energy contained in the feces, urine, and gaseous products of digestion (NRC, 1994). For poultry the gaseous products are usually disregarded, so AME represents the gross energy, determined by bomb calorimeter, of the feed minus the gross energy of the excreta (NRC, 1994).

Apparent metabolizable energy data for ingredients usually apply a correction for nitrogen (N), yielding a nitrogen-corrected AME (AMEn). The correction for nitrogen removes the portion of food N that is deposited as protein tissue and not oxidized in the body to provide energy (NRC, 1994; Farrel, 1999). The N excreted as uric acid has an energy content of 8,222 kcal/kg, and this factor is used to make the necessary adjustment to zero N retained (Farrel, 1999). Consequently, the corrected metabolizable energy value is higher than the uncorrected value if birds are in negative N balance during the assay period, and lower if the birds are in positive N balance (Farrel, 1999). The AMEn measurement allows all feedstuffs to be compared on a similar basis, but AMEn underestimates the metabolizable energy of individual ingredients in practical dietary formulations (Farrel, 1999). Therefore, many researchers have used AMEn because it allows feedstuffs and/or diets to be compared (Ravindran et al., 1998; Santos Jr. et al., 2000; Scott and Pierce, 2001; Santos Jr. et al., 2002).

Considerations should be taken when determining the AME of feedstuff because it can vary according to the age of the animal. AME values for a particular ingredient or

diet have been shown to be low for young birds and increases linearly as the bird ages (Scott and Boldaji, 1997; Fuente et al., 1998; Smulikowska and Mieczkowska, 2000). Farrell (1999) indicated that the methods of AME for a feedstuff or diet should be determined using birds of an age at which the value will be applied. Also, Farrell (1999) reported that there is not a specific value for the difference in AME between young and old birds, because it depends on the diet composition.

A suitable inert digestibility marker (e.g. chromic oxide and Celite^{TM} - Celite Corp., Lompar, CA 93436) is used to estimate the digestibility of dietary nutrients, such as AME. The known concentration of the marker in the feed removes the need to determine quantitatively the feed intake and excreta output when calculating AME (Farrell, 1999). The ratio of the marker in the diet to the amount of marker in excreta or ileal digesta indicates the digestibility of the diet by the enzymes of the bird and its enteric microflora (Scott and Boldaji, 1997). Scott and Boldaji (1997) compared inert markers for determination of AME of wheat and barley broiler diets, with and without enzyme, and reported that Celite^{TM} (Celite Corp., Lompar, CA 93436) at level of 0.5 to 1.0% was the most suitable marker for determination of AME.

1.3 WHEAT PRODUCTION, STRUCTURE, CHEMISTRY, AND UTILIZATION

Cereals are cultivated grasses that are grown throughout the world. As well as providing food for man, cereals and their co-products are important components in the diets of farm stock (Evers et al., 1999). Cereals owe their English name to the Roman goddess Ceres, the giver of grain, indicative of the antiquity and importance of cereals

(Hill, 1937). Cereals are members of the large monocotyledonous grass family, the *Gramineae* (Morris and Bryce, 2000). Wheat, maize, and rice make up the bulk of world cereal production, but five other cereal crops also make important contribution to world nutrition, which in order of global production tonnage, these are barley, millet, oat, and rye (Morris and Bryce, 2000).

Wheat is among the oldest and most extensively grown of all crops (Orth and Shellenberger, 1988), accounts for over one quarter of the world's global cereal production (Morris and Bryce, 2000). Wheat production and its utilization have been intimately linked with the development of both agriculture and civilization over at least the last 12,000 years (Gooding and Davies, 1997). Some archaeological evidence suggests even earlier utilization, around 15,000 $_{BC}$ (Harlan, 1981). *Triticum aestivum* is the most commonly grown wheat species, today (Gooding and Davies, 1997; Morris and Bryce, 2000).

1.3.1 Wheat production

Wheat is extremely adaptable and it is grown from the Artic Circle to the equator, from sea level to 3000 m, and in areas with between 250 and 1800 mm of rainfall. However, wheat is best produced to areas between 30º and 50º North, and 25º and 40º South latitude (Stoskopf, 1992) partly because wheat is a C_3 plant and therefore photorespiration limits DM assimilation in conditions of high light intensity, high temperatures and/or water shortage (Hall and Rao, 1994). Nonetheless, significant

production extends through large areas of temperate, subtropical and tropical highland regions (Fischer, 1981).

Production season and hardness are the two main parameters that classify wheat. Classification into spring or winter wheat is common and traditionally refers to the seasons during which the crop is growing (Orth and Shellenberger, 1988). By the physical and chemical properties, commercial grades around the world can be compared according to their grain hardness and protein content, which has an influence on the end use in human diets (Orth and Shellenberger, 1988). Durum wheat is preferred for pasta; soft wheat for biscuits, cakes, and pastries; and hard wheats for breads, noodles, and others. Endosperm texture is not simply a function of hardness or softness, but that there are degrees from very hard to very soft (Wiseman et al., 2000).

The total cereal production in the year 2000 was 2,060 million tones and wheat accounted for over 28% (585.4 million tonnes) of this production (FAO, 2002b). The largest global producers of wheat are China, India, and the USA (Morris and Bryce, 2000).

1.3.2 Wheat structure and chemistry

Wheat structure

Wheat is rich in nutrients that vary in location according to the grain structure. Wheat and its products are recognized as an important source of essential nutrients. They provide energy, fiber, carbohydrates, proteins, B-vitamins, iron, calcium, phosphorus, zinc, potassium, and magnesium. These nutrients are located in various parts of the grain

(Orth and Shellenberger, 1988). The fruit of the cereal are economically and nutritionally the most important part of the plant. The fruit, also called caryopsis or kernel, contain a single seed, surrounded by the pericarp or fruit coat (Figure 2; Ferket, 2001). The seed comprises the germ, which is the plant of the next generation, whilst the testa is the outermost layer, which plays a role in regulating water and gaseous exchange (Evers et al., 1999).

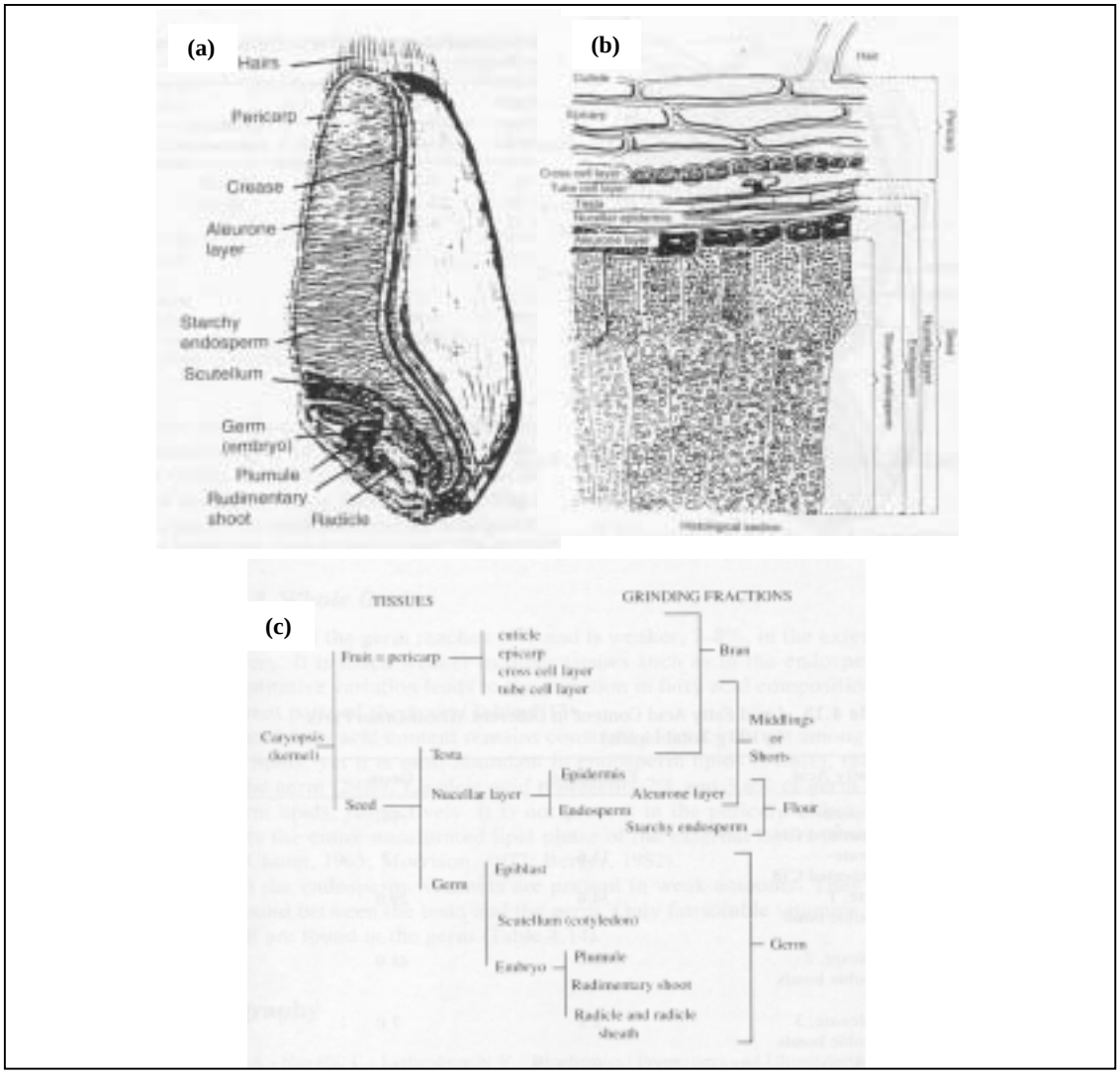

FIGURE 2: (a) Macroscopic longitudinal section of wheat kernel (b) Microscopic longitudinal section of wheat (c) Diagram of the tissues and grinding fractions of wheat (Ferket, 2001).

The nucellar layer is composed of the endosperm (starchy endosperm and aleurone layer) and nucellar epidermis. The endosperm is the largest tissue of the grain. The majority of the endosperm is called starchy endosperm that consists of cells packed with nutrients that can be mobilized to support growth of the embryonic axis at the onset of germination (Evers et al., 1999). Starch, in insoluble form or crystalline granules, is the most abundant nutrient in all cereal grains, constituting about 64% of the dry matter of the entire wheat grain (about 70% of the endosperm) (Evers et al., 1999). Wheat starch contains approximately 250-290g amylose/kg and 710-750g amylopectin/kg (Doublier, 1987; Tester and Morrison, 1990). The walls of the starchy endosperm of wheat are composed mainly of arabinoxylans, while in barley and oats β-(1,3)(1,4)-D-glucan predominates (Pettersson and Aman, 1988; Steenfeldt et al., 1995). Cellulose contributes little to cereal endosperm walls except in rice (Evers et al., 1999). The cell walls function as a structural framework and as a physical boundary to access by enzymes produced outside the cell (Evers et al., 1999). The second section of the endosperm tissue is the aleurone. Wheat aleurone layer surrounds the starchy endosperm, and consist of one layer of cubic cells that contains protein and lipid but no starch. The aleurone layer is important in grain development (aleurone cells differentiate into starchy endosperm cells), and germination (the site of synthesis of hydrolytic enzymes responsible for solubilising the reserves, for example endogenous arabinoxylanase) (Evers et al., 1999).

The pericarp or fruit coat is a multilayered structure consisting of several complete and incomplete layers. The pericarp, mainly consisting of empty cells, is dry in

all cereal grains at maturity. During development it serves to protect and support the growing endosperm and embryo (Evers et al., 1999).

Wheat chemistry

The carbohydrate fraction of wheat is mainly composed of starch in the endosperm and NSP in the cell walls (Evers et al., 1999). The carbohydrates in the wheat kernel make up 600-700 g/kg dry matters and are thus the major component (Steenfeldt et al., 1995; Pirgozliev et al., 2000). Starch is the major energy-yielding component of wheat (Choct et al., 1996). Wheat contains about 110g NSP/kg, which arabinoxylans and xylans account for 33 and 48 g/kg, respectively (Englyst, 1989; Steenfeldt et al., 1998). About 20% of the cereal proteins are enzymes that are important in many stages of the life cycle of the grain (Evers et al., 1999). Fretzdorf and Weipert (1990) reported that during maturation, enzymes in the grain act on the synthesis of storage products, while hydrolytic enzymes hydrolyze these storage products before and after the maturation. These enzymes hydrolyze the cell walls (e.g. endogenous arabinoxylanase) permitting greater access to storage products by enzymes that catalyze hydrolysis of starch and protein. In conjunction with the wide range of lipid compounds present in wheat, the principle phosphoglycerides are phosphatidylcholine, and phosphatidylethanolamine (Evers et al., 1999).

1.3.3 Wheat utilization

About 65% of the global wheat produced is utilized directly as food for humans, 21% as a feed for livestock, 8% as seed, and 6% for other such as industrial raw material

(Orth and Shellenberger, 1988). Because of the high total tonnage of wheat production, all of these sectors are of great importance in terms of production systems and utilization (Gooding and Davies, 1997).

Wheat is used for a vast variety of purposes. It is utilized and processed for a multitude of products for human consumption (Faridi and Faubion, 1995) such as breads, pasta, crackers, cookies, cereals, and others (Gooding and Davies, 1997). Wheat has the potential to be used as a non-food raw material in many industrial processes. Wheat starch can be used as a surface coating agent in the manufacture of paper and board (Jones, 1987). It serves as a fermentation substrate in the production of antibiotics, vitamins and hormones; emulsifier in paint; and other uses (Leygue, 1993).

Although wheat is a valuable energy source, its metabolizable energy is variable. The most important dietary significance of wheat is as an energy source for both human and livestock (Gooding and Davies, 1997). Wheat takes second place to maize on a worldwide basis as feed grain for livestock, even though wheat grain can equal maize in gross energy value and often better than maize with respect to protein concentration (Gooding and Davies, 1997), and it has better pelleting characteristics and lysine contents than corn (Crouch et al., 1997). The reason wheat is often not preferred over maize is because its metabolizable energy content is much more variable (Nicol et al., 1993; Veldman and Vahl, 1994; Grimes and Crouch, 1997; Dusel et al., 1998).

The livestock sector is often the recipient of wheat milling by-products or wheat that has been rejected for human consumption (Gooding and Davies, 1997). Because the quality of these feedstuffs is variable, dietary inclusion levels are limited to avoid growth

performance problems in poultry (Gooding and Davies, 1997; Crouch et al., 1997). Many different studies have evaluated how the chemical composition, and digestibility of wheat influence its variability as a feedstuff. Despite the small contribution to the total dry matter of wheat, the NSP content is the main cause of the low AME values (Choct and Annison, 1990, 1992a; Ward, 1995; Hughes and Choct, 1999; Wiseman et al., 2000). Among the NSP in wheat, the water-soluble arabinoxylans posses the greatest antinutritive activity because it increases intestinal digesta viscosity (Steenfeldt and Heindl, 2000). Saulnier et al. (1995) analyzed 22 samples of wheat grown at different locations and reported that the values for the water-soluble fraction ranged from 0.36% to 0.83% dry matter. They concluded that the proportion of water-soluble arabinoxylan present in wheat is dependent upon genetic and environmental influences. Many studies demonstrated differences in NSP content and composition among different wheat cultivars (Hong et al., 1989; Izydorczyk et al., 1991a,b; Saulnier et al., 1995).

The utilization of wheat in the diet of livestock depends upon its NSP content or ways to eliminate their adverse effects. Waters and Choct (1998), Choct et al. (1999b), and Scott and Pierce (2001) indicated that the cereal grains would be better utilized if the wheat was stored before it is fed to poultry. These authors attributed this change in nutritional value of wheat to the degradation of viscous non-starch polysaccharides by endogenous cell wall-degrading enzymes. Degradation of NSP by these endogenous enzymes would lower digesta viscosity and result in an improvement in performance, similar to that experienced when diets are supplemented with exogenous NSP degrading enzymes (Bedford, 1996). Saulnier et al. (1995) confirmed the presence of enzymes that

hydrolyze arabinoxalans in mature wheat grains, but these enzymes are deactivated by heat during the feed manufacturing process. In contrast, addition of whole wheat in the poultry diet seems to enhance metabolizable energy and performance. Geraert et al. (2000) observed that energy value of wheat was enhanced even when only half of the wheat was supplied as whole grains incorporated in the diet prior to pellet, compared to ground wheat.

The utilization of wheat in the diet of poultry has increased with the supplementation of NSP enzymes. The development and dietary supplementation of commercial enzyme products designed to target arabinoxylans has become a widespread practice in the animal feed manufacturing industry (Ravindran et al., 1999a). Supplementation of exogenous enzymes to wheat-based diet has been shown to improve performance (Choct et al., 1996; Hayat and Arif, 2000; Odetallah et al., 2002), nutrient digestion (Ouhida et al., 2000; Choct et al., 1999a; Preston et al., 2001), fecal moisture (Hayat and Arif, 2000), and feather condition (Odetallah et al., 2002). Therefore, the inclusion level of NSP-degrading enzyme in the diet of poultry improves the nutritional value of wheat that increases its utilization.

In conclusion, wheat is widely used as the major component of livestock diet, especially swine and poultry diets; but its nutritional value is variable both in terms of its chemical composition and the availability of energy. This variation can be attributed to a wide range of anti-nutritive factors, such as genetic, environmental factors, NSP, enzyme activity, and others factors. However, the NSP seems to be the predominant factor, especially the soluble fraction (Hughes and Choct, 1999). Fortunately, supplementation

of exogenous enzyme to wheat-based diets of poultry may improve the nutritional value of wheat and increase its utilization as an alternative feedstuff for poultry diets.

1.4 NUTRIENT LIMITATION BY NON-STARCH POLYSACCHARIDES IN THE GASTROINTESTINAL TRACT

Non-starch polysaccharides (NSP) are the main constituents of cell wall material in all parenchymatous and lignified tissues of the wheat plant (Lineback and Rasper, 1988) and it comprises about 70-90 % of the plant cell wall (Knudsen, 2001). NSP are the principal components of the plant that is not digested by endogenous secretions of the digestive tract (Lineback and Rasper, 1988).

Non-starch polysaccharides are components of the wheat kernel that adversely affects poultry nutrition. They are undesirable in poultry feeds because it reduces growth performance and nutrient digestibility (Hetland and Svihus, 2001). The NSP content is a major factor that limits the exploitation of many low-cost alternative ingredients in poultry nutrition (Iji et al., 2001). Although a typical poultry diet may contain ten times less NSP than starch, NSP has a much greater affect on lipid, carbohydrate and protein absorption because of its physical properties (Cummings and Englyst, 1992). The antinutritional effect of NSP depends on its dietary concentration and the ingredient composition, and animal related factors, such as age, endogenous enzyme activity, gastro-intestinal conditions, and gut ecosystem characteristics (Huyghebaert, 2000).

Although the effects produced by these carbohydrates may be considered to be undesirable in livestock nutrition, they are considered to be beneficial for humans. They

reduce fat and cholesterol absorption, alter the rate of glucose uptake, and enhance volatile fatty acid (VFA) production in the large intestine, which increase tolerance to insulin deficiency (diabetes mellitus), and reduce incidence of cancer and arteriosclerosis (Vahouny and Kritchvsky, 1982). Other investigators reported that NSP improve human health by preventing constipation (Cummings and Englyst, 1992), diverticulitis (Cummings and Englyst, 1992; Johnson, 1993), hemorrhoids (Johnson, 1993), and colorectal cancer (Stevens and Hume, 1995b).

1.4.1 Non-starch polysaccharides chemistry and determination

NSP chemistry

Non-starch polysaccharides are principally non-α-glucan polysaccharides of the plant cell wall. They are a heterogeneous group of polysaccharides with varying degrees of water solubility, size, and structure. They are classified into water-soluble and water-insoluble fractions (Sasaki et al., 2000), which delineate their functions and chemical structure (Izydorczyk et al., 1991a,b; Izydorczyk et al., 1998). The solubility of NSP is determined not only by their primary structure, but also by how they are bound to other cell wall components (protein and lignin). Water-soluble NSP have opposite effects on water binding capacity and viscosity than the insoluble fiber fraction (Sasaki et al., 2000). The water insoluble fiber fraction include cellulose, galactomannan, xylans, xyloglucans, and lignin, while the water-soluble fibers are the pectins, arabinogalactans, arabinoxylans, and β-(1,3)(1,4)-D-glucan (β-glucan) (Johnson, 1993).

Water-soluble β-glucans and arabinoxylans are the NSP of major concern when feeding poultry diets with high cereal grain content. β-Glucans are linear polymers of glucose with β-(1,3)(1,4) glycosidic links (figure 3) (Fincher and Stone, 1986). Arabinoxylans consist of long backbone chains of β-(1,4) anhydro-D-xylopyranosyl to which are attached single α-L-arabinofuranosyl residues at the 2 or 3-position (figure 4) (Lineback and Rasper, 1988). The great range of chemical structures of NSP results in a great diversity in physical properties.

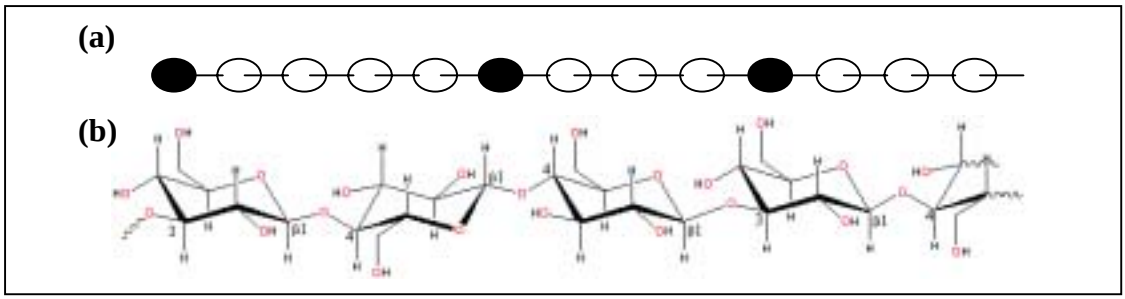

FIGURE 3: (a) Schematic representation of the β-glucan sugars (○– 1,4-glucose; ●– 1,3-glucose); (b) Structure of β-(1,3)(1,4)-D-glucan (Smits and Annison, 1996).

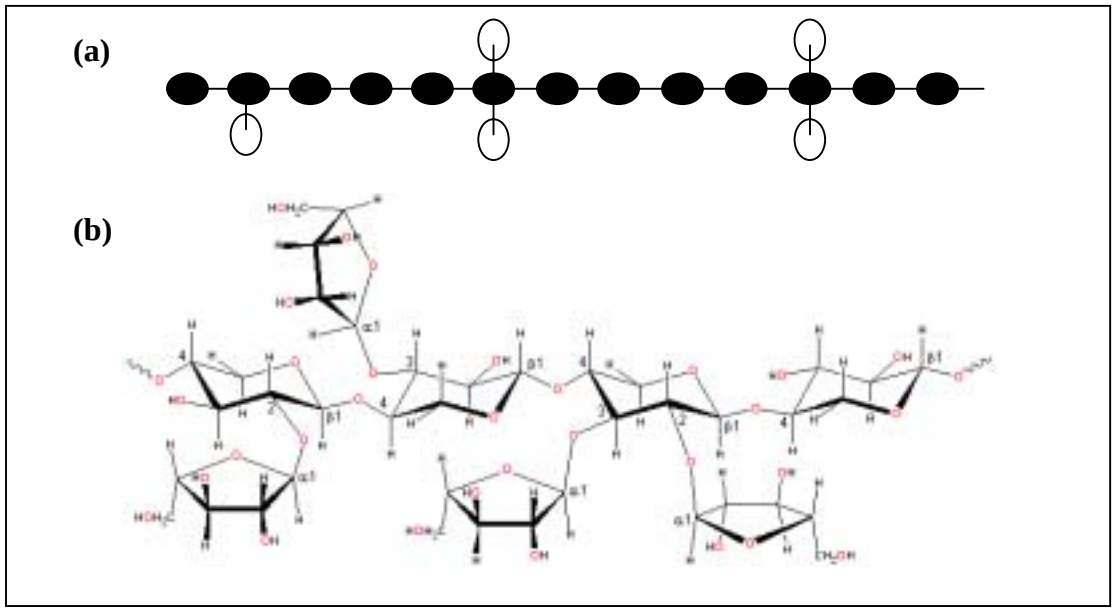

FIGURE 4: (a) Schematic representation of the arabinoxylan sugars (○– arabinose; ●–xylose); (b) Structure of arabinoxylan (Smits and Annison, 1996).

The concentrations and types of these fibrous polysaccharides vary between different parts of the plant. The cell wall polysaccharides of cereals are comprised mainly of arabinoxylans and β-glucan, with smaller quantities of cellulose (Lineback and Rasper, 1988). The main polysaccharides constituents of wheat endosperm cell walls are arabinoxylans (Mares and Stone, 1973; Henry, 1985), whereas arabinoxylans and β-glucans predominate in wheat aleurone layers, and arabinoxylan and cellulose predominate in cell walls of pericarp/testa (Bacic and Stone, 1981; Selvendran, 1984). Lineback and Rasper (1988) stated that arabinoxylan accounts for approximately 88% of wheat endosperm cell wall polysaccharides, of which one third to one half is soluble in water. Although arabinoxylan remains the principal constituent in aleurone, testa, and pericarp layers, the arabinoxylan in the testa and pericarp are quite different from endosperm cell walls. In the testa and pericarp they exist as glucoronoarabinoxylans that are linked to other macromolecules, such as lignin or protein or both, and this form of arabinoxylan is water-insoluble. This agrees with the findings of Delcour et al. (1999) who showed that the degree of water-soluble NSP decreases from the inner to the outer layer of the wheat kernel.

The NSP content and type can also differ among grains (Table 2). The NSP content relative to dry matter is lower in wheat kernel (11.4%) than in rye (13.2%) and barley (16.7%). Arabinoxylan is the predominant NSP in wheat (6-8%) and rye (8.9%), while β-glucan is the predominant NSP in barley (7.6%) (Smits and Annison, 1996). Arabinose side chain is the NSP-soluble portion of wheat. The long entangled polymers have a high water-holding capacity, resulting in an increase in intestinal viscosity.

Viscosity is directly proportional to the molecular weight of wheat arabinoxylans (Bedford and Classen, 1992a). Without arabinose, the xylan would precipitate from solution and virtually no change in viscosity would be expected (Ward, 1995). Thus, the antinutritive property of wheat is due to the polymeric characteristics of the NSP, in contrast to highly digestible monomeric sugars (Longstaff et al., 1988; Izydorczyk et al., 1991a). More information can be found on the literature on the NSP content and characteristics for non-cereal grains (Mohamed and Rayas-Duarte, 1995; Choct and Kocher, 2000), and other cereals besides wheat (Friesen et al., 1992; Gdala et al., 1997; Brien, 1999; Evers et al., 1999). In summary, NSP are a heterogeneous group of polysaccharides that the water-soluble arabinoxylan are the major concern when feeding wheat diets to poultry. Also the level and type of these polysaccharides can vary between plants and plant parts.

TABLE 2: Non-starch polysaccharides (NSP) content of some cereals[1].

GRAIN SOURCE	NSP FRACTIONS			MAIN NSP AND CONCENTRATION
	SOLUBLE	INSOLUBLE	TOTAL	
	(% of dry matter)			
Wheat	2.4	9.0	11.4	Arabinoxylan – 6.05 β-D-glucan – 0.5 Cellulose – 2.0
Rye	4.6	8.6	13.2	Arabinoxylan – 8.9 β-D-glucan – 1.2 Cellulose – 1.5
Barley	4.5	12.2	16.7	Arabinoxylan – 7.6 β-D-glucan – 3.3 Cellulose – 3.9
Maize	0.9	8.8	9.7	Arabinoxylan – 4.2 β -D-glucan – 0.1 Cellulose – 2.2

[1]Data from Smits and Annison (1996) and Knudsen (2001).

NSP determination

Water-soluble NSP content of grain is difficult to analyze, so it is not done on routine basis (Ward, 1996). The oldest and most commonly used method for analysis of fiber in feedstuff is the crude fiber method (Henneberg and Stohmann, 1859). However, this method only measures a small and variable fraction of the fiber components because it can solubilize some of the structural polysaccharides and lignin. The detergent method (Van Soest, 1963; Van Soest et al., 1991) provides a more descriptive measurement of the fiber that is insoluble in neutral detergent (NDF: neutral detergent fiber), and in acid detergent (ADF: acid detergent fiber). The NDF measures hemicellulose, cellulose and lignin, while ADF measures cellulose and lignin allowing calculation of hemicellulose by difference. However, this calculation does not give the exact measurement of NSP because water-soluble and water-insoluble NSP may be lost in the NDF procedure, starch and protein may contaminate the NDF residue, and hemicellulose may be left in the ADF fraction (Knudsen, 2001). More precise procedures of NSP analysis include enzymatic-chemical method or Englyst method (Englyst and Cummings, 1988; Englyst and Hudson, 1987) and the non-enzymatic gravimetric method of Prosky (Prosky et al., 1985) have shown to be the main approaches for the NSP measurement (Englyst, 1989; Cummings and Englyst, 1992; Englyst and Hudson, 1996; Knudsen, 2001). The enzymatic-chemical method has been shown to be easier and quicker to perform than the non-enzymatic gravimetric method of Prosky (Cummings and Englyst, 1992). Knudsen (2001) described that the enzymatic-chemical method yields information on the monomeric composition of the NSP divided into soluble and insoluble fractions. This method gives a general view of

the functional properties of the fiber, in particular when working with identifiable cell wall material.

1.4.2 Influence of grain variety, agronomic conditions, processing, and maturation on the NSP content

Influence of grain variety and agronomic conditions on NSP content

Generic and agronomic factors influences NSP content of grains. The NSP content of cereal grains can vary due to the genotype variance (Delcour et al., 1999; Hughes and Choct, 1999). Hong et al. (1989) reported that genotype variance affects the NSP variation more significantly than the environmental variance. However, high soluble-NSP content in grain has been associated with the amount of rainfall and environmental temperature patterns during the period of grain maturation (Aastrup, 1979; Hughes and Choct, 1999). Choct et al. (1999b) reported that the AME values of the Australian wheat differ significantly due to year of harvest, with AME ranging from 2,194 Kcal/kg to 3,580 Kcal/kg of dry matter basis. They proposed that the AME values depend upon the climatic condition (e.g. droughts or high rainfall) during the growing season of the wheat. Aastrup (1979) observed a similar response in barley. They observed that the variation in the nutritive value of barley was mainly attributed to the year of harvest, with little influence on the geographical locations. Hot and dry conditions increase the proportion of cell wall material high in soluble NSP content, whereas wet conditions during grain maturation decreases it (Longstaff and McNab, 1986). Consequently, the year of harvest influences the level of NSP. In contrast, Wiseman and

McNab (1995) found that variety, year of growth, location, and application of N fertilizer had no significant effect on true metabolizable energy values of wheat for young and adult poultry, even though they observed a difference due to age of birds.

Influence of grain processing on NSP content

Period of storage and feed-milling practices can affect the level of NSP in cereal-diets. The increase in nutritive value of the grain by post-harvest storage has been studied by several authors (Fuente et al., 1998; Choct et al., 1999b; Chesson, 2000; Scott, 2000). All cereal grains possess endogenous enzymes able to degrade the endosperm cell walls and make accessible starch granules, which appear constantly present during grain storage and may slowly degrade soluble NSP. Thus, AME of low quality wheat increases during storage (Choct and Hughes, 1997). However, thermal processing reduces or removes the endogenous enzymes in the grain (Vukic-Vranjes et al., 1994), and it can also form resistant-starch that are un-digestible (Blakeney, 1993) and solubilize NSP (Vukic-Vranjes et al., 1994).

Influence of grain maturation on NSP content

The composition of the plant cell wall is not only dependent on the plant species, variety, and processing, but also on the maturity of the plant at harvesting for feed. D'Appolonia and Mac Arthur (1975) examined the pentosans content in the bran (pericarp and testa) and endosperm layers of two immature and mature varieties of wheat. They reported that the pentosan content recovered from the bran and endosperm of immature wheat is higher than that recovered from the mature wheat samples. The ratio arabinose:xylose in the bran was similar for both immature and mature wheat samples,

whereas the ratio in the endosperm was higher for the immature than for the mature. In addition to containing a higher amount of pentosan, immature wheat has more water soluble pentosans because the arabinose side chain is the water-soluble portion of the NSP (Ward, 1995).

Immature wheat has lower starch content and its arabinoxylans has higher hydration capacity than mature wheat kernel. The hydration capacity of polymerized arabinoxylans is dependent on the molecular size and it varies between immature and mature wheat. Small molecules of NSP exhibit greater water absorption than larger ones (Izydorczyk et al., 1991a). Arabinoxylans in the endosperm of immature wheat has less branching (D'Appolonia and MacArthur, 1975), so immature wheat has a higher hydration capacity than mature wheat. The starch content in immature wheat is lower than mature ones. Jennings and Morton (1963) reported that starch synthesis starts early after pollination, and continues through the different stages of maturation. Consequently, mature wheat has more starch than immature wheat, and thereby higher AME value.

In conclusion, the inferior growth performance and low AME values of poultry fed diets containing immature wheat may be due to a several factors: increased pentosan content, higher water solubility of the arabinoxylans, or to the lower level of starch content in immature wheat.

1.4.3 Effects of water-soluble NSP

Physicochemical properties of water-soluble NSP

The physiological effects of NSP on the digestion and absorption of nutrients in human and monogastric animals have been attributed to its physicochemical properties. The main physicochemical properties of NSP that are of nutritional significance include: (a) hydration properties; (b) viscosity; (c) cation exchange capacity; and (d) organic compound absorptive properties (Knudsen, 2001). Each of these properties is outlined as follow.

The hydration properties of NSP are characterized by swelling. Water spread these macromolecules until they are fully extended and disperses it after they are solubilized (Thibault et al., 1992). The hydration properties of NSP influence its water holding capacity and water binding capacity (Knudsen, 2001). The water holding and water binding capacities depend on the physicochemical structure of the molecule and its ability to incorporate water within the molecular matrix.

The viscosity properties of the NSP depend on its molecular weight or molecule size (linear or branched), its ionically charged groups, the surrounding structures, and the concentration of NSP (Smits and Annison, 1996). The hydration properties of the NSP cause a direct interaction of water and polysaccharides. As the NSP concentration increases, the molecules of the NSP themselves interact forming a gel-like network (Morris and Ross-Murphy, 1981). The gel formations occur when the interaction of the NSP molecules *via* covalent-cross linkages become extensive (Izydorczyk et al., 1991a).

Soluble arabinoxylans are able to absorb up to 10 times their own weight of water, forming highly viscous solutions (Veldman and Vahl, 1994).

The cation exchange capacity is formed because the three-dimensional structure of the NSP molecule allows a chelation of ions to occur. Cations can form ionic bridges between NSP molecules, which profoundly influences their viscosities and gel-forming properties (Smits and Annison, 1996).

The organic compound absorptive properties of NSP are due to the capacity of the NSP to bind small molecules by both hydrophobic and hydrophilic bond interactions. NSP may also have a surface activity, which they present charge (mainly negative) as well as weakly hydrophobic and weakly hydrophilic surface, for example, the NSP may be on the surfaces of feed particles, on the surface of lipid micelles, or on the glycocalyx surface of the gut (Smits and Annison, 1996). Moreover, viscous NSP might physically restrict and/or interact with the digestive enzymes and reduce their activity (Ikeda and Kusano, 1983).

The viscous gel formed by soluble NSP interferes with the digestion and absorption of nutrients from wheat and other dietary components. Consequently the nutrients are less available to the bird (Choct and Annison, 1992a; Grimes and Crouch, 1997; Smits et al., 1997). This concept will be discussed further in the subsequent sections.

Physiological effects of the NSP on the nutrient entrapment

The detrimental effect of soluble NSP is mainly associated with the viscous nature of these polysaccharides and their physiological effects on the digestive medium.

However, because NSP represent a diversity of compounds possessing different physicochemical properties, their nutritional effects in poultry are also diverse. Early work identified the soluble β-glucans and arabinoxylans as being the fractions most responsible for impeding digestion by causing a viscous intestinal environment (Antoniou and Marquardt, 1982; White et al., 1981,1983; Claysen and Bedford, 1991; Teitge et al., 1991). This high gut viscosity is associated with the incapability of the animals to not digest cellulose, arabinoxylans, or β-glucans (Bedford, 1995; Steenfeldt et al., 1995). The rate of digestion of a feed and the absorption of the products of digestion relies on the formation of a complex between the digestive enzyme and its substrate and subsequent release of its product, and the diffusion of the product to the enterocyte for absorption to occur (Bedford, 1995). Unimpeded movement of enzymes, substrates and products by diffusion through the gut is essential for digestion. As the viscosity of the digesta increases by the NSP, the diffusion decreases. Moreover, the NSP gel may act as a physical barrier between substrates, enzymes, and digestion end-products (Petterson and Aman, 1989), thus limiting the mix of nutrients with pancreatic enzymes and bile acids (Edwards et al., 1988). NSP complex may reduce the brush border diffusion of nutrients, limiting their exposure to the brush border enzymes and absorption by the enterocytes (Edwards et al., 1988). Dietary NSP may also increase the thickness of the unstirred water layer of the mucosa *via* their interaction with the mucopolysaccharides (Johnson et al., 1981; Flourie et al., 1984), and NSP may bind the brush border digestive enzymes (Silva and Smithard, 2002) that limit fats, proteins, and carbohydrates digestion and

absorption. Therefore, NSP may reduce the digestion and absorption of nutrients by its physicochemical effect in the intestinal tract.

Physiological effects of NSP on the gut ecosystem characteristic

Wheat NSP inhibits nutrient absorption in chicken and turkeys not only by raising the viscosity of the digesta or their physicochemical effects, but also by altering the intestinal microflora. The influence of enteric microflora on nutrient digestion in birds fed high dietary NSP has been confirmed by observations of improved growth performance when birds fed barley or rye diets were supplemented with antibiotics (Wagner and Thomas, 1977; Cave et al, 1990). Also association of the microflora and NSP was studied by Jorgensen et al. (1996) who observed an increase in the levels of SCFA and H_2 (pH) as the dietary level of NSP increased. Bacteria colonize intestinal epithelium and lumen of the bird shortly after hatch (Yamauchi et al., 1989), and the development of microflora will depend on the composition of the diet. The combination of the viscous intestinal environment, slower rate of feed passage, and the presence of significant amounts of undigested materials derived from high levels of NSP in the feed, lead to the proliferation of microflora and its migration from the caeca to the small intestine where most of the nutrient absorption takes place (Jaroni et al., 1999; Preston et al., 2001). By fermenting and utilizing carbohydrates and protein, the microflora actively compete with the host for nutrients. This increased microflora-host competition contributes to the adverse effects of NSP. Thus, more nutrients are fermented instead of enzymatically hydrolyzed and digested (Bedford, 1995; Choct et al., 1996; Langhout et al., 2000).

The increased microflora fermentation associated with high dietary NSP also decreases the digestion and absorption of lipids and fat-soluble vitamins by adversely affect the function of bile acids in micelle formation. Increased in microbial activity in the small intestine increases the rate of bile acid deconjugation (Coates et al., 1981; Feigner and Dashkevicz, 1988; Langhout et al., 2000). This reduction in conjugated bile acids reduces the formation of micelles of dietary fat and fat-soluble vitamins (Hoffman and Small, 1967). Digestion and absorption of dietary fats containing long-chain saturated fatty acids are particularly hindered by bile acid decongugation (Garrett and Young, 1975, Choct et al., 1996), whereas short-chain and unsaturated fatty acids are more easily absorbed in the absence of bile acids (Garrett and Young, 1975). Campbell et al. (1983) and Fengler et al. (1988) showed that the addition of conjugated bile acids improved digestibility of fat for birds fed rye-based diet. Because bile acids are also thought to stabilize pancreatic proteases in the intestinal lumen, protein digestion could also be compromised (Bedford, 1995). Moreover, bile acids recycling may be reduced when diets contain high levels of NSP because the gut microflora may produce bile acid degrading enzymes (Silva and Smighard, 2002). Bile acids can also bind to the NSP, causing increased bile excretion in the feces (Gestel et al., 1994; Overton et al., 1994; Kishimoto et al., 1995). High microbial activity may also compromise the absorption of fat-soluble vitamins by reducing the emulsification of lipids (Bedford, 1995).

Gut microflora have a significant role on the antinutritional effects of NSP. Bacteria are able to incorporate amino acids into microbial protein (Salter and Coates, 1974), which may explain the low nitrogen retention by conventional birds fed diets rich

in NSP (Furuse and Yokota, 1985; Langhout et al., 2000). Langhout et al. (2000) studied the role of the microflora on the antinutritive effects of NSP in broiler chicks. They observed that germ-free chicks were less affected by dietary NSP than conventional chicks. They hypothesized that the NSP were partly fermented into smaller fractions by the microflora, which resulted in increased ileal viscosity, decreased nutrient diffusion in the intestinal tract, and reduced hydrolysis of dietary nutrients. They also observed that the increased fermentation of starch by gut microflora in birds fed NSP-containing diets led to decreased ileal digestibility of macronutrients. These authors also observed a significant change in ileal villi morphology by dietary NSP (from predominantly zigzag pattern villi orientation to a more disorderly pattern of ridge-shaped or tongue-shaped villi). This altered villi morphology was associated with a reduction in the effective absorptive surface area, reducing nutrient absorption. This change in villi morphology was observed more in conventional chicks than in germ-free ones. The morphological results led Langhout and co-workers to hypothesize that the change observed in gut wall morphology was due to the increased amount of microbial fermentation products that are toxic to enteric tissues. These fermentation toxic products include amines, NH_3 and toxins. Similar findings have been reported by other authors (Wagner and Thomas, 1978; Campbell et al., 1983, Choct et al., 1996). Therefore, the gastrointestinal microflora plays an important role in the magnitude of the antinutritive effects of water-soluble NSP in birds.

Morphological changes in the gut villi by the microflora due to high levels of NSP in the diet have also been seem by other authors. Visek (1978) observed that high

bacterial counts could irritate and inflame the gut lining, thus damaging the microvilli and reducing nutrient absorption further. Viveros et al. (1994) observed that the jejunum epithelium of barley-fed birds was histologically different than birds fed the corn-soy diet. The barley-fed birds had shorter and thicker villi with occasional villi atrophy as compared to elongated villi observed in birds fed the corn-soybean meal type diet.

Effect of NSP on malabsorption syndrome

High non-starch polysaccharides in the diet may predispose turkeys to have malabsorption syndrome (MAS) that cause poor performance and intestinal health problems. Viscous NSP may alter mucin secretion and villus morphology when ingested at high levels because of their effect on the microbial ecosystem (Smits, 1996; Langhout, 1998). The effect of NSP on the luminal ecosystem and its effect on the integrity of the mucus and mucosa may reduce the bird's resistance to enteric disease. The mucus layer acts as a highly selective physicochemical and microbial barrier at the surface of the mucosa (Ter Huurne and Smits, 1999). The bacterial enteric disease caused by NSP make the intestinal tract less resistant to entero-viruses, which can cause signs of malabsorption syndrome (MAS) (Ter Huurne and Smits, 1999). Malabsorption syndrome is characterized by poor growth, retarded feathering, diarrhea with undigested food, pigment loss, bone abnormalities, and increased mortality (McNulty and McFerran, 1993). It is still unknown whether the underlying pathophysiology is based on either maldigestion (Griffiths and Williams, 1985) or malabsorption or both (Ter Huurne and Smits, 1999). Exogenous NSP degrading enzymes has been noted to prevent MAS by

reducing the viscosity in the intestinal tract, improving the gut environment and the digestibility of nutrients (Ter Huurne and Smits, 1999).

Effect of NSP on apparent metabolizable energy

As stated previously, determining dietary apparent metabolizable energy (AME) is one way to measure the antinutritive effect of NSP in feedstuffs. The AME is a major determinant of the nutritive value of poultry feed as it is a variable indicating not only the nutritive composition of the feed but also how the animal responds to that feed (Choct et al., 1999b). The evidence for the contribution of NSP on the low-AME wheat comes from studies demonstrating the following: (a) AME and soluble NSP are negatively correlated (Choct and Annison, 1990; Annison, 1991; Choct et al., 1999b); (b) various sources of purified NSP depress AME (Choct and Annison, 1990); (c) degradation of cell wall NSP by glycanases increases AME (Annison, 1992); and (d) NSP isolated from wheat depress AME in a dosage dependent manner (Choct and Annison, 1990; Hughes and Choct, 1999).

NSP content have a great effect on the low and variable values of AME among wheat cultivars. The AME of wheat is influenced by the antinutritive effect of NSP rather than the starch content (Choct et al., 1999b), protein content (Mollah et al., 1983), or grain hardness (Mollah et al., 1983; Rogel et al., 1987). Choct et al. (1999b) observed that wheat with low AME have a higher content of NSP, which they described it as low-AME wheat. The low-AME wheat had an AME value of less than 3,107 kcal/kg DM and caused sticky and watery droppings accompanied by poor growth and feed efficiency in broilers when included above 50% of the diet (Choct et al., 1999b). The wheat produced

in the U.S. and many other countries have a variable value of AME and significant amounts of this wheat are considered low AME-wheat. Metabolizable energy values for wheat used in the U.S. tend to be within a range of 2,866-3,000 kcal/kg, but broader ranges of 2,491-3,975 kcal/kg exist (Ward, 1995). In Australia, two major surveys in which a total of 60 wheats were assayed revealed substantial variation, with AME values ranging from 2,474 to 3,800 kcal/kg DM (Mollah et al., 1983; Rogel et al., 1987).

Relationship between NSP, apparent metabolizable energy and bird's age

There is evidence that older birds utilize cereal-based diets better than younger birds. Apparent metabolizable energy of birds fed cereal diets has been shown to be higher for older birds than for younger ones (Salih et al., 1991; Scott and Boldaji, 1997; Fuente et al., 1998; Smulikowska and Mieczkowska, 2000). Fuente et al. (1998) reported that the AME of 30 day-old chickens was 4.6% higher than that of 10 day-old chicks. Salih et al. (1991) also showed that negative effects of diets containing high levels of barley decreased as broilers grew. Brenes (1992) explained that young birds are more sensitive to the negative effects of antinutritional factors in cereals and other raw materials, than older birds because their digestive tract is less mature.

The major cause for the high sensitivity of young birds to NSP is due to their low lipid digestibility as compared to older birds. Poults and chicks have limited synthesis of lipase enzyme and inefficient recycling of bile salts (Sell et al., 1986; Martin and Farrel, 1998; Krogdahl and Sell, 1989). Further, young birds are not able to replace lost bile as efficiently as older birds (Serafin and Nesheim, 1970). The decrease in lipid digestibility caused by the NSP has been shown to be the major effect for the low-AME in wheat

(Smits et al, 1997). Ward and Marquardt (1983) showed that older birds utilize 55% more fat than younger birds when fed rye diets.

The ability of adult birds to tolerate higher levels of NSP in the diet and to maintain their normal productive capacity may be due to a variety of factors. Classen and Campbell (1990) reported that as birds age the effect of NSP on the growth rate decrease, even though the digesta viscosity remained the same. They also observed that the performance was reduced only when the transit time was reduced by dietary NSP. This observation indicates that older birds are capable of transporting viscous materials more easily than younger birds. This is supported by the study of Shires et al. (1987), who concluded that the feed transit time or retention time through the small and large intestine increases with age. In addition, the tolerance of older birds to NSP may reflect a more stable gut microflora (Choct and Annison, 1992b; Veldman and Vahl, 1994). The stability of the microflora may come from acclimatization of the digestive system to the diet (Petersen et al., 1999) through changes on the type and/or number of microorganism. Young birds have a higher variability in the numbers and types of microorganisms among birds than among older birds (Annison, 1989). As discussed by Ferket (1991), the larger the number of species of microorganisms, the greater is the stability of the overall population and its ability to cope with minor changes in gut environment.

In conclusion, NSP influence the AME values of wheat. The correlation of AME and NSP values is affected by the birds' age attributed to the gut maturity, and ecosystem characteristics. Thus, the effect of NSP is more pronounced in young birds due to their immature gut and poor ecosystem characteristics.

Effect of NSP on performance and nutrient digestion

It is generally considered that increased digesta viscosity and proliferation of the microflora are ways whereby antinutritional factors, such as arabinoxylans in wheat and β-glucans in barley, can reduce the digestion and absorption of nutrients in diets fed to broilers and turkeys. The antinutritive effect is manifested by poor growth of the chickens accompanied by depressed nutrient utilization. Depression in bird performance results from inhibition of starch, lipid, and protein digestion (Choct and Annison, 1990, 1992a). Choct and Annison (1992b) reported that the inclusion of 30g of wheat pentosans per kg of broiler diet caused significant depression in the AME, weight gain, and feed conversion ratio (FCR). They observed that the pentosans decreased the digestibility of starch, nitrogen, and fatty acids, especially the digestion of saturated fatty acids. Similarly, Pawlik et al. (1990) observed that water-soluble NSP in rye decreased the growth performance of chickens by reducing dietary nutrient utilization, particularly on fat absorption. Choct et al. (1996) reported that starch, protein, and lipid digestibility was depressed by 37.4%, 33.9%, and 59.0%, respectively, in a diet containing 188g/kg (DM) of arabinoxylan isolated from wheat. Using a lower amount of arabinoxylans/kg of diet (40g/kg diet DM) than Choct et al. (1996), Choct and Annison (1992a) observed significant ($P<0.001$) depression of growth performance and nutrient digestibility of the broilers, and correlated these responses to the level of pentosans in the diet. In agreement, Bedford and Classen (1992a) observed that growth performance of broilers fed wheat- and rye-based diets was linearly and negatively correlated to the intestinal viscosity.

Dusel et al. (1998), also found a good correlation ($r= 0.98$, $p<0.001$) between soluble pentosans and extract viscosity.

Arabinoxylans and β-glucans are different polysaccharides that act similarly by increasing the digesta viscosity, but they differ by their level and/or physicochemical properties, which can determine different degrees of antinutritive activity between grains. Friesen et al. (1992) stated that fat digestibility decreased 43, 67, and 77% for chicks fed diets containing 70% barley, rye, and oats respectively, in comparison to those fed 70% wheat diet. Steenfeldt et al. (1995) observed that apparent protein digestibility decreased from 90.6% to 75.2% in poultry fed diets with 60 and 385g/kg of NSP, respectively. Therefore, the influence on the performance and nutrient digestibility depends on the level and type of polysaccharides in the cereal in question.

Many researches have shown the negative effect of NSP on nutrient digestion and performance. Addition of soluble NSP decreases performance (Ward and Marquardt, 1987; Choct et al., 1992; Alvarez et al., 2000; Heindl and Steenfeldt, 2000; Iji et al., 2001) and decreases carbohydrate, protein and fat digestion (Choct and Annison, 1992a; Steenfeldt and Heindl, 2000; Langhout et al., 2000). Besides the depression in the utilization of macronutrients, the utilization of micronutrients is also adversely affected by dietary NSP via compromised absorption of fat-soluble vitamins (Ward, 1995). Calcium and Phosphorus digestibility has also been shown to decrease with high levels of wheat NSP in Leghorn hens (Jaroni et al., 1999).

Accompanying the dramatic effect of NSP on the performance and nutrient digestibility, birds often exhibit symptoms of considerable gastrointestinal stress. Choct

and Annison (1992a) reported that birds fed high NSP diets excreted excessive amounts of fluid in their feces. Ward (1995) stated that wheat is often associated with wet litter for poultry, especially when fed at levels exceeding about 20% of the diet. Osmotic diarrhea caused by high levels of NSP in the diet of poultry is associated with higher concentration of dietary components that are not completely digested and/or absorbed in the small intestine, giving rise to an increased amount of osmotically active compounds in the gut (Choct and Annison, 1992a). Moisture content of the excreta has been shown to be 10% higher in birds fed NSP-rich diets (Choct et al., 1996). This increased diarrhea can adversely affect the health of birds. It can increase the susceptibility of birds to enteric disease and leg problems (Grimes and Crouch, 1997), and increase carcass downgrading (Hughes et al., 2000). Moreover, excessive wet litter causes litter handling and disposal problems of litter (Pawlik et al., 1990).

Even though wheat NSP has detrimental effects on nutrient utilization and growth performance, these problems have been shown to be significantly alleviated by the supplementation of exogenous enzymes. Recent estimates indicate that more than 95% of the wheat- and barley-based broiler diets in the United Kingdom contain supplemental enzymes, as do about two-thirds of such diets worldwide (Ward, 1995). The use of enzymes to improve the nutrient value of poultry feed is the focus of the following section.

1.5 APPLICATION OF SUPPLEMENTAL ENZYMES TO IMPROVE NUTRIENT DIGESTIBILITY AND UTILIZATION

Enzymes are organic catalysts that by their mere presence in trace amounts can initiate or accelerate the speed of reactions occurring in organic matter that would not otherwise proceed at an appreciable rate (Schaible, 1970). Even the simplest living organisms contain multiple copies of nearly a thousand different enzymes. The first enzyme to be isolated was urease, crystallized by James B. Sumner in 1926 (Horton et al., 1996c). Most enzymes are named by adding the suffix "-ase" to the name of the substrate they act on or to a descriptive term for the reactions they catalyze. For example, urease has urea as a substrate; alcohol dehydrogenase catalyzes the removal of hydrogen from alcohols. However, a few enzymes, such as trypsin and chymotripsin, are known by their historic names (Horton et al., 1996c). Enzymes are categorized according to the general class of the organic reactions they catalyze. Oxidoreductases catalyze oxidation-reduction reactions. Transferases catalyze group-transfer reactions. Hydrolases, a special class of transferases that water serves as the acceptor of the group transferred, catalyze hydrolysis reactions. Lyases catalyze the lysis of a substrate, generating a double bond. Isomerases catalyze a structural change within one molecule. Ligases catalyze the ligation or joining of two substrates (Horton et al., 1996c). All digestive enzymes belong to the class of hydrolases (Odetallah, 2000). Today, isolated enzymes are used for a great variety of commercial purposes, including supplementation of enzymes to improve nutritional value of feed.

With the development of enzyme products targeting specific substrates, the use of enzymes to improve the nutritional value of feed has received increased attention. Since the 1920s, researchers have observed beneficial effects from enzyme supplementation in poultry feeds, particularly feeds that contain grains with a high fiber component (Hastings, 1946; Moran Jr. and McGinnis, 1968; Pettersson and Aman, 1989; Ritz et al., 1995). Supplemental enzymes in the feed are used to achieve one or all the following objectives: (a) increase the animal's own supply (Schaible, 1970); (b) alleviate the adverse effects of antinutritional factors, such as arabinoxylans, β-glucans, etc; and (c) render certain nutrients more available for absorption and enhance the energy value of feed ingredients (Classen and Bedford, 1991; Lyons, 1993; Lyons and Walsh, 1993).

In order for an enzyme to be applicable in animal feeds several criteria must be met. First, adequate substrate (target substance) must be available in the feed for a specific enzyme being used (Dale, 2002). The animal should be able to utilize the end product of enzyme action (e.g. fatty acid absorption from lipase supplementation) (Dale, 2002), or benefit from the breakdown of specific substrates in the feed (e.g. fat digestion improvement from NSP breakdown by arabinoxylanase). The enzyme must be stable in the animal's gut where it should have its greatest effect (Odetallah, 2000). The enzyme should be stable in the feed until it is consumed by the bird (Odetallah, 2000). The enzyme should be stable during and after feed processing (Odetallah, 2000). Finally, the enzyme should interact efficiently with its target substrate (Odetallah, 2000). For example, xylanase should be used for wheat-based diets because it contains substantial

amounts of xylan. In contrast, xylanase would be of little benefit in corn/soy diets because it contains a negligible amount of xylan.

Dietary enzyme supplementation may also serve to modify enteric microflora and promote growth as an alternative to antibiotic growth promoters. Antibiotics are effective modulators of enteric microflora and enteric health and they have been commonly used to promote growth in the poultry industry (Cook, 2002). However, due to the increasing public concern about the development of antibiotic-resistant pathogens, there is great interest in feasible non-pharmaceutical alternatives such as enzymes (McCracken et al., 2000). Lundeen (2002) stated that enzyme supplementation might be a tool available for modification of the microbial ecology of the turkey gut in the absence of antibiotics.

1.5.1 Common enzymes used in poultry feed

Table 3 represents the major enzymes used in animal feeds and their substrate specificity (Odetallah, 2000). All enzyme preparations recommended for use in animal husbandry to improve dietary nutrient utilization are hydrolases (Modyanov and Zel'ner, 1983). Commercial enzymes products are typically a blend of several different enzymes that are effective on a wide variety of substrates. The enzymes with proven efficacies for animal husbandry include xylanase, arabinoxylanase, β-glucanase, cellulase, and phytase (Choct and Kocher, 2000).

TABLE 3: Commercial enzymes used in poultry feed: general enzyme classifications relative to substrate specificity and its effect[1].

SUBSTRATE	ENZYME	EFFECTS
Protein	Protease, Peptidase	Supplementation of endogenous enzymes
Starch	Amylase	
Lipids	Lipase	
Phytate (phytin complex w/ P, etc.)	Phytase	Enhance plant phosphate use
Hemicellulose (grains)	Hemicellulase	Reduction of intestinal viscosity – enhance nutrient digestibility.
Pentosans (xylose, arabinose)	Pentosanase	
β-glucans	β-glucanase	
Pectins (plant protein sources)	Pectinase	
Oligosaccharides (mannans, galactans, etc.)	α-galactosidase	
Cellulose (plant cell wall)	Cellulase, Cellobiase	Cellulose digestion - release nutrient

[1]Odetallah, 2000. Adapted from Campbell and Bedford, 1992; Amado, 1993; Lyons and Walsh, 1993.

Besides NSP-enzymes, phytase and cellulase are important enzymes used in commercial poultry practice. Phytate is a universally antinutrient present in all plant material that irreversibly chelates divalent cations and interferes with amino acid absorption in the gastrointestinal tract of birds as well as other monogastrics. Moreover, the fecal excretion of phytate phosphorus and chelated minerals is a major source of soil and water pollution when wastes are applied to farmland. Dietary phytase supplementation is used to improve utilization of phosphorus and other ionically active nutrients (i.e. amino acids, minerals), ultimately reducing mineral emissions (Odetallah, 2000). The action of cellulase is complex because cellulose is generally associated with other polymers, such as lignin and pentosans. Lignin encrustation renders access to cellulose by the enzyme difficult if not impossible (Sears and Walsh, 1993).

1.5.2 Non-starch polysaccharides and phospholipase enzymes

The enzymes used in the research work reported in this thesis were phospholipase and NSP degrading enzymes, especially endoxylanase. The following is a brief description of each of these enzymes, and their mode of action in turkey feed.

NSP enzymes

The use of enzymes active against NSP is now an established part of the feed industry and its effect on the nutrient utilization and improvement in performance has been widely studied. The use of fungal and microbial enzymes to avoid the adverse effects of non-starch polysaccharides in cereal-based poultry diets has been studied for decades (Jensen et al., 1957), and interest in the effects of the enzymes and their modes of action has been sustained up to the present (Odetallah et al., 2002; Silva and Smithard, 2002). Chickens and turkeys do not produce enzymes that are capable of digesting xylans and β-glucans, which is why exogenous NSP-enzymes are added to the feed (Silversides and Bedford, 1999). The mechanism of action of the exogenous xylanases was long believed to be by the release of nutrients through the destruction of cell wall (Silversides and Bedford, 1999). Even though this idea is partially true, there is considerable evidence that enzymes elicit benefits other than simply releasing nutrients from cellular constituents (Silversides and Bedford, 1999).

Supplementing cereal-based diets with microbial enzyme preparations capable of hydrolyzing endosperm cell walls, may improve dietary nutrient availability by several means. Endoxylanase for example, degrades the xylan backbone of arabinoxylan into smaller units, which has several beneficial consequences. It renders the xylose units more

available to monogastrics (Odetallah, 2000). It disrupts the water holding capacity of the NSP (Scott and Boldaji, 1997) and reduces the viscosity of the digesta in the small intestine (Bedford and Schulze, 1998; Choct et al., 1999a). Reduced digesta viscosity increases the diffusion rates of nutrients and endogenous enzymes enabling the bird to digest and absorb more nutrients (Pawlik et al., 1990). Endoxylanase releases entrapped nutrients for the digestion by the endogenous enzymes of the bird (Chesson, 2000). Endoxylanase inhibits the proliferation of the fermentative microorganisms in the small intestine by increasing the digesta passage rate and nutrient digestion (Choct et al., 1999a). Thus, nutrient utilization is improved by reducing the competition between the host and its enteric microflora.

Many authors have observed improved nutrient utilization by dietary enzyme supplementation. Pettersson and Aman (1989) examined the effect of enzyme supplementation on the digestibility of nutrients in broilers fed wheat-based diets. Enzyme supplementation increased the digestibility of organic matter, crude protein and starch in the ileum, and increased the digestibility of organic matter and crude fat in the excreta. Silva and Smithard (2002) studied the effect of xylanase (Avizyme 1300, Finnfeeds International of Marlborough Ltd [EC Registration ATCC 2105]) on rye-based diets and observed that the reduction of small intestinal viscosity by the enzyme improved nutrient digestion and consequently performance. Several researchers have shown increased performance of broiler chickens fed cereal-based diets with enzyme addition (Jensen et al., 1957; Burnett, 1966; White et al., 1981; Hesselman and Aman, 1986; Annison, 1992; Van Paridon et al., 1992; Frigard et al., 1994; Silva and Smithard,

2002). Likewise, Ritz et al. (1995) observed improvement in body weight gain, and feed consumption of male turkeys fed xylanase supplementation.

Many authors have shown the interaction between pentosans, microflora, and enzyme supplementation. Fischer and Classen (2000) reported that bacterial count from the small intestine of broilers fed wheat-based diets was lower in xylanase-supplemented birds than the unsupplemented ones. Because enzymes supplementation reduces the microbial population in the small intestine (Choct et al., 1995b; Dunn, 1996), the entire gut ecosystem changes. These conditions in the gut alter the composition and activity of intestinal microflora (Vukic-Vranjes and Wenk, 1996). As the microflora changes by enzyme supplementation, there is a decrease in adverse effects of microbial fermentation, including the following: deconjugation of bile salts reducing fat digestion (Langhout, 1999); competition between the host and the microflora for nutrients (Bedford, 1995; Choct et al., 1996; Langhout et al., 2000); atrophy of the intestinal villi and enlargement of digestive organs (Brenes et al., 1993a,b; Viveiros et al., 1994).

Although microflora fermentation in the small intestine decreases when xylanase is supplemented in the diet, microbial fermentation increases in the large intestine and ceca. Steenfeldt et al. (1998) observed a decrease in the pH in the caecal content of chickens as a result of enzyme supplementation. The pH decreased as indicated by higher production of SCFA caused by an increased microbial fermentation. They also observed a significant negative correlation between pH and the apparent digestibility of total-NSP ($r= -0.73$, $P<0.0006$). They also found a negative correlation between pH and apparent digestibility of xylose ($r= -0.72$, $P<0.001$) and arabinose residues ($r= -0.69$, $P<0.001$).

Therefore, the results observed by Steenfeldt et al. (1998) indicate that degradation of cell wall arabinoxylan in the enzyme-supplemented diets increases the amount of material available for microbial fermentation in the caeca. This increased fermentation in the large intestine and caeca lead to a higher production of short-chain fatty acids that is utilized by the bird as energy, thus enhancing the benefits of the enzyme supplementation (Choct et al., 1996). Likewise, Choct et al. (1996) reported that volatile fatty acids (VFA) concentration in the caeca was not influenced by elevated amounts of soluble NSP, but it was significantly increased by enzyme supplementation. In contrast, they reported that ileal VFA was significantly higher in birds fed diets containing soluble NSP as compared to those fed the control (sorghum/soybean meal) or the enzyme-supplemented diets.

Enzyme supplementation has been shown to have different effect depending on the age of the bird. Ward (1996) reported in a review of the literature that the effect of enzyme supplementation on the performance of broilers fed wheat diets varied on the age of the bird. He reported that dietary enzyme supplementation improved performance in older birds (22-42 days), but not in younger birds (1-21 days). Similarly, Odetallah et al. (2002) observed higher live weight of male turkeys fed enzyme supplementation at 84-112 days but not at 7-49 days as compared to the unsupplemented control birds. Older birds have more mature gut ecosystem with a higher fermentation capacity than younger birds when diets contain a high amount of NSP. Therefore, the effect of the enzyme on the microflora, as a consequence of the NSP breakdown, is higher in older birds. Vukic Vranjes and Wenk, (1996) reported that the enzyme effect was completely eliminated by antibiotic supplementation on the performance of broilers fed diet supplemented with

both enzyme and antibiotic. This significant enzyme by antibiotic interaction demonstrates that the positive enzyme effect in older birds is, to a great extent, mediated by the activity of intestinal microbes. In contrast to Ward (1996), Brenes et al. (1993a) observed improved performance of broilers fed enzyme supplementation, regardless of age (0-42 days). Mcknight (1997), however, observed a benefit of enzyme supplementation (Natugrain 33%, BASF) only when fed to young turkeys (up to 70 d of age). He attributed the diminishing effects of enzyme supplementation in his trial to gradual adaptation of turkeys to the wheat-based diets. Likewise, Leeson et al. (1996) reported that NSP enzyme supplementation to turkey diets had a positive effect on growth rate of turkeys up to 72 d of age but not subsequently.

Several other researches have studied the effects of enzyme supplementation on NSP-rich diets of poultry feed. Dietary enzyme supplementation has been shown to decrease gut viscosity and improve performance (Friesen et al., 1992; Preston et al., 2001), increase AME (Annison and Choct, 1991; Auclair and Larbier, 2000), and enhance the digestibility of dietary calcium and phosphorus (Jaroni et al., 1999), starch (Hetland and Svihus, 2001), nitrogen (Bedford, 1995; Huyghebaert, 2000), and fat (Friesen et al., 1992). Many of these studies have shown that AME was positively correlated to fat digestion (Friesen et al., 1992; Steenfeldt et al., 1998; Steenfeldt and Heindl, 2000). Smulikowska and Mieczkowska (2000) reported that 62% of the increase of the AMEn values was due to better fat digestibility when broilers are fed wheat-based diet supplemented with enzymes containing xylanase and β-glucanase activities. Hence, appropriate dietary enzyme supplementation can restore the digestion of starch, protein,

and lipid, leading to more consistent and uniform growth performance (Choct et al., 1999a). Similar results have been observed with the supplementation of carbohydrases in diets containing legume and non-cereal grains (Bedford and Morgan, 1995; Annison et al., 1996). Furthermore, turkeys fed wheat-based diets supplemented with enzyme (Natugrain Blend and Lyxasan Forte, BASF - Germany) have been shown to have better feather scores than unsupplemented control birds because of a decrease in feather picking and aggression (Odetallah et al., 2002). Odetallah et al. (2002) suggested that this reduced aggressive behavior was due to improved amino acid availability and better intestinal comfort. Poor amino acid balance and perturbed gut microflora can stress turkeys (Ferket and Veldkamp, 1999). Moreover, Odetallah et al. (2002) reported that supplemental xylanase to turkeys decreased mortality rate. They stated that mortalities among the control-fed toms were primarily due to enteric infections, whereas mortalities among the enzyme treatment groups were due to cardio-pulmonary disorders often associated with the most rapidly growing birds.

There is some controversy among opinions on the effect of enzyme supplementation on feed intake. Some researchers have reported increases in feed intake after enzyme supplementation and attributed this effect to reduced intestinal viscosity and increased in passage rate (Bedford and Classen, 1992a; Antoniou et al., 1981). Other researchers reported a decrease in feed intake and attributed the enzymes effect on enhanced digestibility of carbohydrates, protein, and lipid (Annison, 1992; Osei and Oduro, 2000; Hayat and Arif, 2000; Scott, 2000). Still other researchers have observed no

significant effect of dietary enzymes supplementation on feed intake (Ravindran et al., 1999a; McCracken and Quintin, 2000; Preston et al., 2001).

Enzyme has been shown to reduce the differences between different batches of wheat. Many authors have observed that the addition of NSP enzymes significantly reduces the variation among different wheat cultivars (Scott, 2000; Geraert et al., 2000). Scott and Pierce (2001) measured the feeding value of western Canadian wheat by bioassays and reported that variation in AME among wheat- or barley-based diets was significantly reduced with enzyme supplementation. Diets containing low AME cereal grains generally benefit more from enzyme supplementation than diets containing high AME cereal grains. Choct et al. (1995a) reported that enzyme supplementation significantly improved the nutritive value of a low-AME wheat diet (< 3,107 kcal AME/kg DM; Mollah et al., 1983; Rogel et al., 1987). Therefore, use of appropriate enzymes is an effective way of using grains with high NSP contents in poultry diets (Choct et al., 1999a).

The effect of enzyme supplementation on the performance and nutrient digestion has been reported to increase as the enzyme level increased. Odetallah (2000) reported that the level of dietary enzyme addition is crucial for improving the performance of turkey. Several researchers have shown dose-dependent responses for dietary supplementation of NSP-enzymes (Hesselman et al., 1982; Petterson and Aman, 1989; Bedford and Classen, 1992a). Other workers, however, have not been able to demonstrate the increase in AME and performance by increasing the level of dietary enzyme supplementation (Annison, 1992). Furthermore, dietary supplementation of enzyme has

been shown to alleviate the osmotic diarrhea and improve litter quality in poultry by reducing the amount of osmotically materials in the gut because it disrupted the water holding capacity of the NSP (Pettersson and Aman, 1988; Veldman and Vahl, 1994; Fischer and Classen, 2000).

Dietary enzyme supplementation occasionally has been shown to have little influence on productive performance of poultry. Preston et al. (2000) studied the effect of xylanase supplementation on the performance and energy utilization of broilers fed diets containing 67% of wheat. They reported no significant effects of enzyme supplementation on performance and energy utilization. Odetallah (2000) attributed this poor enzyme efficacy to the low level of enzyme dosing (Odetallah, 2000). In agreement, Crouch et al. (1997) stated that one possible reason for the general lack of response with some enzymes might be due to high NSP content in some cultivars of wheat to the low dosage of enzyme used. Other authors attributed poor enzyme efficacy to the inappropriateness of the enzyme for the type of grain (Friesen et al., 1992). Friesen et al. (1992) stated that an enzyme with xylanase or pentosanase activity is required for rye and wheat, and a β-glucanase or cellulase for oats and barley. Furthermore, a blended preparation of enzymes has been shown to be more effective on improving performance than single enzyme preparations (White et al., 1981; Rotter et al., 1989; Bedford and Classen, 1992b; Gdala et al., 1997; Li et al., 2000; Odetallah et al., 2002). When diets are supplemented with a blend of enzymes, the activity of one type of feed enzyme is facilitated by the activity of another (Ravindran et al., 1999b).

The effects of enzyme supplementation are dependant upon the source and type of wheat. Veldman and Vahl (1994) studied the effect of four wheat varieties supplemented with xylanase on the growth performance of broilers. The enzyme had a different effect on broiler performance for each variety of wheat. Saulnier et al. (1995) attribute these variations in enzyme effect among wheat cultivars due to their high heterogeneity in water-soluble arabinoxylan content. Wheat contains higher levels of pentosans when it is subjected to frost-damaged during seed maturation (immature wheat) than when it is grown under more ideal conditions (Ward, 1995). However, the effect of enzyme(s) on immature frost-damaged wheat has not been reported.

In conclusion, supplementation of wheat-based diets with NSP-enzymes has been shown to improve the performance of poultry by improving the digestion and absorption of nutrients. However, much of the information is based on research with chickens rather than turkeys. Thus, the research work reported in this thesis studied the effect of NSP-enzymes fed to turkeys.

Phospholipase enzymes

Lipase is another enzyme that has been studied in wheat, since the high inclusion of wheat in the diet has considerable effect on the fat digestion. Martin and Farrell (1998) studied the supplementation of lipase in wheat-based diets, while Al-Marzooqi and Lesson (1999, 2000) studied its effect in corn/soy diets. Both research groups hypothesized that supplemental lipase would improve the digestibility of fat when wheat or corn-based diets were fed to young birds because they exhibit a lower level of natural lipase production than older birds. In wheat-based diets, supplemental lipase was

anticipated to be especially effective in increasing the digestibility of the fat that may be impeded by highly viscous water soluble NSP. However, dietary supplementation of lipase did not improve the performance of the chicken and ducklings, regardless of the type of cereal base diet. Al-Marzooqi and Lesson attributed the lack of lipase response to the contamination of the enzyme product with cholecystokinin by the microorganism responsible to produce the lipase. Cholecystokinin is a hormone that reduces feed intake.

Dietary fat digestibility may be improved by dietary supplementation of exogenous phospholipase. Endogenous intestinal phospholipase A_2 (PLA) catalyzes the hydrolysis of ester bond at *sn*-2 position of glycerophospholipids (GPL) producing fatty acids and lysophospholipids (e.g. Lyso-phosphatidylcholine or Lyso-PC). The fatty acids are then absorbed from the lumen as part of the fat micelle. Lyso-PC, the predominant GPL product in the luminal content, is essential for the emulsification (lipid emulsification is the first stage of lipid digestibility) of water-insoluble lipids (Homan and Jain, 2001). Lyso-PC is a major amphiphile molecule, which acts to stabilize microdroplets of triglycerides, cholesterol, and other nonpolar dietary lipids that are otherwise insoluble in the aqueous environment of the intestinal contents (Carey et al., 1983). Also, PLA influences the capacity of the enterocyte to transport absorbed lipids into the circulation, which depends on cellular phosphatidylcholine synthesis controlled by the hydrolysis of phosphatidylcholine in the lumenal contents. Additionally, PLA posses an intrinsic secretin-releasing activity (Chang et al., 1999) that stimulates the release of pancreatic secretion and bicarbonate in the duodenum, which influences the digestion of others macronutrients. Thus, dietary supplementation of phospholipase could

alleviate the adverse effects of NSP by (a) facilitating the formation of micelles of triglyceride, cholesterol, and other nonpolar dietary lipids; (b) enhancing the capacity of the enterocytes to absorb lipids; and (c) increasing the digestion of the others macronutrients. However, supplementation of exogenous phospholipase in wheat-based diets for poultry has not been investigated.

1.6 CURRENT STUDY

From the preceding literature survey, it is apparent that the soluble non-starch polysaccharides, especially arabinoxylan, are responsible for the antinutritive activity present in wheat. Moreover, the level of NSP can vary significantly among grain sources, and agronomic practices (e.g. immature frost-damage wheat has higher level of NSP). However, enzyme technology has become a prominent tool to overcome the adverse effects of NSP. This thesis addresses the application of dietary enzyme supplementation to improve the nutritional value of wheat for turkeys. The first part of this study compared the nutritional value of frost-damaged and normal-mature wheat for turkeys, and how a blend of NSP degrading enzymes influenced their nutritive value. The second part of this research studied the effect of different sources of supplemental enzymes on the growth performance and health of turkeys fed inferior-quality of wheat. The enzymes tested were (a) Natugrain Blend (blend with high endoxylanase activity), (b) Lyxasan (exclusive endoxylanase) at two application rates, and (c) exclusive phospholipase. Natugrain Blend® had the same level of endoxylanase activity in the feed as Lyxasan Forte® at 100ml/tonne feed, therefore we could test the effect of a blended product

compared to a single endoxylanase preparation. Lyxasan Forte® was applied at two-application rate (100 and 50 ml/tonne) so we could investigate the dose-dependent responses for dietary supplementation of NSP-enzymes. Phospholipase were used to test our hypothesis that dietary supplementation could alleviate the adverse effects of NSP by facilitating lipid digestibility.

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CHAPTER 2

DIETARY PENTOSANASE SUPPLEMENTATION OF DIETS CONTAINING DIFFERENT QUALITIES OF WHEAT ON GROWTH PERFORMANCE AND METABOLIZABLE ENERGY OF TURKEY POULTS[1]

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2.1 ABSTRACT

Wheat varies in apparent metabolizable energy (AME) due to the presence of non-starch polysaccharides (NSP), which can be improved by dietary enzyme supplementation. Poults from 0-17 d of age were fed diets containing various wheat sources (WS) with or without Natugrain Blend® (NB, BASF Corporation, Ludwigshafen, Germany) and then growth performance, AMEn, nitrogen retention (%NR), and viscosity were measured. Five replicate cages of 10 poults were assigned to each 8 SBM/wheat treatment diets and a control SBM/corn diet. The treatments were in a factorial arrangement with 4 WS (A, B, C, and D) and 2 enzyme levels (0 and 200 mg NB/kg). The WS differed by the degree of frost damage during seed development. Regardless of the source of wheat, NB increased 17 d BW (351 *vs.* 381 g, $P<0.001$), decreased 1-17 d FCR (1.55 *vs.* 1.49, $P<0.05$), increased AMEn (2,204 *vs* 2,455 kcal/kg, $P<0.001$), and increased %NR (35.0 *vs.* 41.4 %, $P<0.05$). No effects of WS were seen on growth performance, but WS A and B had higher ($P<0.05$) AMEn than sources C and D (2,396 and 2,460 *vs*. 2,246 and 2,216 kcal/kg, respectively). Viscosity was higher ($P<0.05$) in poults fed wheat-based diets than the control diet. Addition of NB to the wheat-based diets decreased gut viscosity (5.57 *vs.* 3.98 cP, $P<0.05$) to a level similar to the corn-based control diet, and it resulted in equivalent growth performance. Gut viscosities were negatively correlated with AMEn and ANR. The results demonstrated a positive effect of enzyme supplementation on nutrient utilization and performance of turkeys.

(*Key words*: wheat, enzymes, growth performance, metabolizable energy, turkey)

2.2 INTRODUCTION

Wheat is an important feed ingredient for poultry in many parts of the world. Although it can supply up to 70% of the metabolizable energy in the feed, the dietary inclusion rate of wheat is often limited because of the variability of apparent metabolizable energy (AME). Wheat may contribute up to 35% of the protein and 25% of the lysine in a broiler diet when included at high concentrations. However, the greatest nutritional significance comes from starch, which is the major energy-yielding component (Wiseman et al., 2000).

Water-soluble β-glucans and arabinoxylans are the non-starch polysaccharides (NSP) of major concern when feeding to poultry diets with high cereal grain content. β-Glucans are linear polymers of glucose with β-(1,3)(1,4) glycosidic links (Fincher and Stone, 1986). Arabinoxylans consist of long backbone chains of β-(1,4) anhydro-D-xylopyranosyl to which are attached single α-L-arabinofuranosyl residues at the 2- or 3-position. The predominant NSP in wheat are mainly arabinoxylan and some β-glucans. Recent studies (Mollah et al., 1983; Choct et al., 1995; Hughes and Choct, 1997) reported the occurrence of low AME of wheat. The low-AME wheat is caused by the presence of soluble NSP in cell walls of the wheat kernels (Annison, 1993). Water-soluble pentosans of wheat have the ability to bind large quantities of water (Bushuk, 1966) and to form viscous gels in the digesta of poultry *via* covalent cross-linking (Geissmann and Neukom, 1973). The viscous gel lowers the rate of diffusion of nutrients in the digesta and it acts as a physical barrier that impedes the interactions between substrates, enzymes, and digestion end-products (Pettersson and Aman, 1989). Consequently, NSP compromises

enteric digestion and nutrient absorption. By increasing digesta viscosity, NSP reduces digesta mixing, feed passage rate, and luminal oxygenation; thus increasing in the proliferation of the microflora and their fermentation in the small intestine (Preston et al., 2001). Some enteric microflora compete with the host for nutrients, reduce fat absorption by deconjugating bile salts (Coates et al., 1981; Feigner and Dashkevicz, 1988), and cause morphological changes in the gut villi by irritating the gut lining and damaging microvilli (Visek, 1978). Thus, some microflora adversely affect digestive processes by limiting the digestibility and absorption of nutrients.

Dietary pentosanase (e.g. arabinoxylanase, β-glucanase) supplementation is commonly used as a means to improve the feeding value of wheat for poultry. The appropriate enzyme supplementation to wheat-based diets improves the digestion of dietary starch, protein, and lipid in the small intestine, and it results in more consistent and uniform poultry performance (Choct et al., 1999). Supplementing cereal-based diets with microbial enzyme preparations capable of hydrolyzing endosperm cell walls may improve dietary nutrient availability by degrading the xylan backbone of arabinoxylan into smaller units that reduces the viscosity of the digesta in the small intestine (Bedford and Schulze, 1998; Choct et al., 1999). Reduced digesta viscosity increases the diffusion rates of nutrients and endogenous enzymes and decreases the proliferation of the microflora enabling the bird to digest and absorb more nutrients (Pawlik et al., 1990; Choct et al., 1999). The beneficial effects of enzymes are more apparent if diets are supplemented with a blend of enzymes (enzyme cocktail) than single-activity enzymes (Bedford and Classen, 1992a, Odetallah et al., 2002).

The effects of enzyme supplementation in wheat-based diets are dependant upon the source and type of wheat. Veldman and Vahl (1994) studied the effect of four wheat varieties supplemented with xylanase on the growth performance of broilers. The enzyme had a different effect on broiler performance for each different variety of wheat. Saulnier et al. (1995) attribute variations in enzyme effects among wheat cultivars to the high heterogeneity in water-soluble arabinoxylan content. Wootton et al. (1995) reported that the pentosan content ranged from 5.4 to 7.2% in Australian wheat and from 5.5 to 6.5% in North American wheat samples harvested in 1992-1993. Choct et al. (1999) reported that the AME values of the Australian wheat differ significantly due to year of harvest, with the values ranging from 2,194 kcal/kg to 3,580 kcal/kg (dry matter basis - DM). They proposed that the AME values depend on the climatic condition during the growing season of the wheat: wet conditions during grain maturation dramatically decrease the extract viscosity of the grain, whereas it is elevated when conditions during grain maturation are dry. Aastrup (1979) observed a similar response in barley. Similarly, wheat contains higher levels of pentosans when frost-damaged during seed maturation (immature wheat) (Ward, 1995). However, the effect of enzyme(s) on immature frost-damaged wheat fed to turkeys has not been reported. Therefore, the NSP level varies widely depending on environmental factors and variety.

The purpose of this research was to compare the nutritional value of immature frost-damaged wheat with mature wheat for turkeys, and how a blend of NSP degrading enzymes influences their nutritive value.

2.3 MATERIALS AND METHODS

Enzymes

The enzyme used in this study was Natugrain Blend®[1] (NB), a liquid enzyme preparation obtained from fungal fermentation of *Trichoderma longibrachiatum*. It contains standardized activities of β-glucanase and endoxylanase of at least 9,000 β-glucanase units (BGU) per gram of product and at least 36,600 endoxylanase units (EXU) per gram of product (BASF, 2001). One BGU is defined as the activity required to liberate 0.278 μmol of reducing sugar (measured as glucose equivalents) per minute at pH 3.5 and 40°C at a substrate concentration of 0.5% β-glucan from barley. One EXU is defined as the enzyme activity required to liberate 1 μmol of reducing sugar (measured as glucose equivalents) per minute from a 1% xylan solution at pH 3.5 and 40°C. Natugrain Blend® also contains some hemicellulase (e.g. pectinase), cellulase and protease activities (BASF, 1997). The enzyme dosage was 200 g NB/tonne of feed, as recommended by the manufacture. Thus the enzyme concentration in the feed was at least 7,300 EXU/kg and 1,800 BGU/kg.

Diets

The experimental diets are presented in Table 1. The chemical composition of the wheat used in the experimental diets is shown on Table 2. A basal diet containing all ingredients except corn or wheat was prepared as a single batch. Then the basal diet was split into 9 equal portions. One portion was used for the corn-based diet and 8 for the

[1]BASF AG, 67059 Ludwigshafen, Germany

wheat diets. The 8 wheat-based dietary treatments were a factorial arrangement of 4 wheat sources (A, B, C, and D) and 2 enzyme supplementation levels (0 and 200 mg NB/kg). The wheat sources differed by the degree of frost damage during seed development. The wheat were all of the same variety grown on the same farm in Saskatchewan, Canada, but planted at different times (approximately one week apart). An early fall frost event occurred when wheat A and B were near full maturity (mid- to end of ripening), and wheat C and D were in the milk to the soft-dough stage of seed development. The four wheat groups were harvested within the same week during the fall of 1999. After harvest, all wheat sources were stored for about 8 months before being used in the experiment.

All feeds were formulated using least-cost linear programming software, such that the diets were about 95% of the NRC (1994) recommendations for amino acids and energy. The diets were formulated slightly below requirements so that any improvement in nutrient availability due to enzyme supplementation could be observed as an improvement in growth performance. The corn-based control diet was prepared by mixing 50% corn, 0.1% Solka-Floc[2] (cellulose), and 49.9% basal diet. The wheat-based diets were prepared by mixing 50% wheat (sources A, B, C, or D), 0.1% Solka-Floc or enzyme, and 49.9% basal diet. Half of the wheat diets were supplemented with NB (200 mg/kg diet) and the other half of the diet had no supplementation of NB. The enzyme was applied as a fine spray onto the feed using a plant mister. Celite[3], a source of acid-

[2]Solka-Floc 40, Powdered cellulose, FS&D Fiber Sales and Development Corp, Urbana, OH, 43078.
[3]Celite™, A diatomite product, Food Chemicals Codex Grade. Celite Corp., Lompar, CA 93436.

insoluble ash (AIA), was used as an indigestible marker at 0.7% of the total diet. All feeds were prepared and fed as a mash. Composite feed samples from each diet were taken immediately after manufacture and analyzed for crude protein, fat, ash, Ca, and P.

Bird husbandry and nutrient digestibility (AMEn and ANR) bioassay

One-day-old commercial Large White BUTA[4] male poults were obtained from a commercial hatchery[5] and randomly assigned to the cages. Wing bands were applied to the birds at the time of placement to identify their treatment assignment. All the birds were housed in four Petersime battery cage units within a climate-controlled animal room. Each battery unit containing 12 cages was considered as an experimental block to account for error due to differences in temperature, light, or position. All cages contained individual heating units that were monitored throughout the trial for consistency. The Petersime battery brooder temperature was set at 35ºC and altered as needed to suit bird comfort. Room temperature was set at 28ºC on the day of placement and then reduced 2ºC per week. The room was illuminated with incandescent lights at about 50 lux on a continuous basis. Feed and water were provided *ad libitum* throughout the duration of the study. Visual heath inspection of all birds within the study was performed daily and weights of culled birds and reasons they were removed were recorded. Crippled or dead birds were removed and replaced up through day 3 at which time any further mortality was removed and recorded but not replaced. All mortality was weighed soon after death and recorded so that an appropriate adjustment to feed conversion could be made.

[4]British United Turkeys of America, Lewisburg, WV.
[5]Goldsboro Milling Company, Goldsboro, NC, USA.

Nine dietary treatments were randomly assigned to cages within each of the four blocks using the Proc-Plan procedure of SAS® (SAS, 1996). Each cage of 10 poults, the experimental unit, were subjected to one of 9 dietary treatments from 1 to 17 days of age, as follow: (1) corn/SBM unsupplemented control, (2) wheat A no enzyme, (3) wheat A with enzyme, (4) wheat B no enzyme, (5) wheat B with enzyme, (6) wheat C no enzyme, (7) wheat C with enzyme, (8) wheat D no enzyme, and (9) wheat D with enzyme. Each treatment combination was replicated by 5 cages.

Data collection

Group feed consumption and body weights (BW) were recorded at 1, 7, 13, and 17 days of age. Fecal collections were done daily at 9:00-11:00 am on days 11 to 14. The samples were put into plastic bags and stored frozen at -20ºC until chemical analysis associated with the determination of apparent metabolizable energy nitrogen-corrected (AMEn) and apparent nitrogen retention (ANR).

Dietary viscosity measurement

At 17 days of age, the birds were fasted over night (8 hours) and then given *ad libitum* access to feed for 3 hours before sampling. Two birds from each cage were weighed and euthanized by cervical dislocation. Then about 2 grams of digesta was gently expressed from the terminal part of the jejunum (midway between the duodenum and the Mechel's diverticulum to 0.5 cm above the Mechel's diverticulum), placed into micro-centrifuge tubes, centrifuged at 3,000 rpm for 2 minutes, and the supernatant

collected. The viscosity of the supernatant was determined using Brookfield Digital Viscometer LVDVII+CP[6].

Chemical analysis

The frozen fecal samples collected daily from days 11-14 were thawed overnight at room temperature in the Poultry Science Department in North Carolina State University. Daily fecal samples from each cage were pooled (approximately total of 300g excreta) and mixed in a blender[7] to slurry after the addition of approximately 100 ml distilled water. The pH of the fecal slurry was then adjusted to 5.4 by the addition of sulfuric acid (0.1 N) to minimize the volatilization of nitrogen during overnight drying in a forced-air convection oven[8] at 70ºC. The dried samples were ground in a blender[7], and then stored at -20ºC before analysis to determine AMEn and ANR. Dry matter content was obtained by drying 3 to 5 grams of the materials for 6 hours in a forced-air convection oven[8] at 105ºC. Feed and fecal energy values were obtained by combustion in an adiabatic oxygen bomb calorimeter[9]. Celite recovery was performed using the method described by Vogtmann et al. (1975). The nitrogen content of all samples was determined using a Kjeldahl automatic nitrogen analyzer[10]. The AMEn and ANR values were calculated relative to the acid-insoluble ash marker as shown in the footnote of Table 8. The AMEn was corrected to zero N-retention by using a value of 8.22 kcal/g nitrogen retained (Hill and Anderson, 1958).

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[6]Brookfield Engineering Laboratories Inc., Stoughton, MA.
[7]Waring Commercial Laboratory Blender, Model # 31BL91-7010, Torrington, CT.
[8]Blue-M, Model # DC-326F, Serial # DC-509, Blue M, Atlanta, GA.
[9]IKA Calorimeter System C5000 control, IKA® Werke Labortechnik, Staufen, Germany.
[10]KJELTEC Auto 1030 Analyzer, Tecator, Sweden.

Statistical analysis

All data were analyzed using the general linear models procedure for analysis of variance (ANOVA) (SAS, 1996). Cage means served as the experimental units for statistical analysis. Variables having a significant F-test were compared using the least-squares-means function of SAS (SAS, 1996), and the treatment effects were considered to be significant at $P<0.05$. All percentage data were transformed to arc sine of the square root to the data distribution before statistical analysis. Correlation analysis of digesta viscosity and AMEn and ANR were performed using PROC CORR procedure (SAS, 1996).

Animal ethics

The experiments reported herein were conducted according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) at North Carolina State University. All husbandry practices and euthanasia were done with full consideration of animal welfare.

2.4 RESULTS

Performance

Dietary supplementation of NB enzyme significantly ($P<0.05$) increased body weight (BW) of poults fed the wheat-based diets, but there were only marginal response differences due to wheat source (Table 3). Poults fed the wheat-based diets supplemented with the enzyme had equal BW to those fed the corn-based control diet. Enzyme supplementation also significantly ($P<0.05$) increased feed consumption (FC) among

poults fed the wheat-based diets (Table 4), although the birds fed the corn-based control diet had higher FC than those fed the wheat-based diets during the 1-17 days period (509.4 vs. 467.9, $P<0.05$). The enzyme supplementation improved feed conversion ratio (FCR) by about 4% ($P<0.05$) for poults fed the wheat-based diets (Table 5). Poults fed the wheat-based diets had a significantly lower cumulative FCR (1-17 days) than poults fed corn-based diets (1.524 vs. 1.716, $P<0.05$). There were no significant influences ($P<0.05$) of wheat source or enzyme X wheat source interactions on body weight, feed consumption, and feed conversion ratio. Cumulative livability averaged 99% for the entire experiment and was not significantly influenced by grain type (corn or wheat), wheat source, or enzyme supplementation (Table 6). In general, enzyme supplementation increased the growth performance of birds consuming the wheat-based diets to a level similar to those consuming the corn-based diets, regardless of source of wheat.

Ileum digesta viscosity

There were no significant differences on the jejunum digesta viscosity among the treatments (Table 7). However, jejunum digesta viscosity of birds fed the wheat-based diets without enzyme supplementation was significantly higher than those fed the corn-based diets (Figure 1). Enzyme supplementation significantly reduced gut viscosity regardless of source of wheat, such that it was statistically equivalent to the corn-based diets. Jejunum digesta viscosity was negatively correlated with AMEn, although the correlation coefficient was very low ($r= -0.22$, $P<0.05$) (Table 7). The negative correlation between jejunum digesta viscosity and ANR approached significance ($r= -0.20$, $P=0.056$).

Energy and protein utilization (AMEn, ANR)

AMEn and ANR of birds fed the wheat-based diets were significantly lower than those fed the corn-based diets; however, enzyme supplementation significantly increased nutrient utilization, such that it was statistically equivalent to the corn-based diets (Table 8). The addition of NB to the wheat-based diets significantly increased the AMEn of wheat from 2,204 kcal/kg DM to 2,455 kcal/kg DM ($P<0.001$). There was no wheat X enzyme interaction. However, there was a wheat source effect on nutrient utilization: wheat A and B had significantly higher AMEn and ANR than wheat C and D (frost damaged during grain filling).

2.5 DISCUSSION

This research compared the nutritional value of wheat that was frost-damaged during different stages of seed development, and how they are influenced by a blend of NSP-degrading enzymes. The commercial enzyme product used in this experiment, Natugrain Blend®, predominantly contained endoxylanase (36,600 EXU/g of product) along with β-glucanase (9,000 BGU/g of product), some hemicellulase (e.g. pectinase), cellulase, and protease activities (BASF, 1997). A blended preparation of enzymes was chosen because enzyme blends usually improves the nutritional value of wheat-based diets for monogastric animals more effectively than single enzyme preparations (White et al., 1981; Rotter et al., 1989; Bedford and Classen, 1992a; Gdala et al., 1997; Ravindran et al., 1999; Li et al., 2000; Odetallah et al., 2002). When diets are supplemented with a

blend of enzymes, the activity of one type of feed enzyme is facilitated by the activity of another (Ravindran et al., 1999).

In the present experiment, enzyme supplementation significantly improved body weight (BW), feed consumption (FC), feed conversion ratio (FCR), dietary energy (AMEn) and protein (ANR) utilization of poults fed wheat-based diets. These positive responses were attributed to the enzyme's ability to alleviate the adverse effects of excess dietary NSP. The poor performance of birds fed wheat-based diets that have not been supplemented with exogenous enzymes was attributed to NSP, mainly arabinoxylans, in the endosperm cell walls of wheat kernel. As these NSP increase in the diet, nutrient digestion and absorption and growth performance decreases (Petterson and Aman, 1989; Choct and Annison, 1990; Langhout et al., 2000). However, supplementation of NSP-degrading enzyme preparations to cereal-based diets improves growth performance of monogastric animals, and this response has been associated with the reduction of viscosity in the intestinal tract (Choct et al., 1996).

Our results confirm the hypothesis that the improvement in growth performance by the enzyme was mediated through a reduction in gut viscosity. Digesta viscosity was significantly higher in poults fed the wheat-based diet than those fed the corn-diet. However, enzyme supplementation to the wheat-based diets reduced jejunum viscosity to a level similar to the birds fed the corn-based diets. The reduction in digesta viscosity is associated with the improvement of digestion of starch, protein, and lipid in the small intestine (Choct et al., 1999). Similar results have been observed by other researchers that

reported that endoxylanase improved dietary nutrient availability and increased performance (Bedford, 1995; Preston et al., 2001; Hetland and Svihus, 2001).

Dietary endoxylanase supplementation elicits its beneficial effects on poultry by several means. Endoxylanase renders the xylose units more available to monogastrics (Odetallah, 2000). It disrupts the water holding capacity of the NSP (Scott and Boldaji, 1997) and reduces the viscosity of the digesta in the small intestine (Bedford and Schulze, 1998; Choct et al., 1999). Reduced digesta viscosity increases the diffusion rates of nutrients and endogenous enzymes, enabling the bird to digest and absorb more nutrients (Pawlik et al., 1990). Endoxylanase releases entrapped nutrients for the digestion by the endogenous enzymes of the bird (Chesson, 2000). Therefore, dietary endoxylanase supplementation increases the performance and nutrient utilization of poultry by disrupting the gel-forming capacity of the NSP, which in turn enables better digestion and nutrient absorption in the intestinal tract.

Besides improving nutrient utilization by decreasing the viscosity, endoxylanase also may improved AME and ANR by reducing the enteric microflora fermentation. Langhout et al. (2000) reported that excess dietary NSP increased digesta viscosity, which caused changes in gut microflora and decreased nutrient digestion and absorption. In agreement, we observed a significant decrease in AMEn as jejunum viscosity increased, although the negative correlation coefficient between jejunum viscosity and AMEn was very low ($r = -0.22$, $P<0.05$). Therefore, there are other factors that influence AMEn more than gut viscosity, such as microflora interaction on the antinutritive effect of wheat.

The endo-β (1,4)-D-xylanase is capable of hydrolyzing the xylan backbone of arabinoxylans to smaller fragments (Veldman and Vahl, 1994). A partial hydrolysis of β-glucan and arabinoxylan could considerably reduce their water holding capacity and viscosity. A reduction in gut viscosity could increase the feed passage rate and digestion, which decrease the amount of indigestible material in the intestinal tract and decreases the proliferation of microflora in the small intestine (Bedford et al., 1991; Van Paridon et al., 1992). As the microflora changes by enzyme supplementation, there is a decrease in adverse effects of microbial fermentation. Adverse effects of microbial fermentation in the small intestine include: deconjugation of bile salts reducing fat digestion (Langhout, 1999), competition between the host and the microflora for nutrients (Bedford, 1995; Choct et al., 1996; Langhout et al., 2000), atrophy of the intestinal villi, and enlargement of digestive organs (Brenes et al., 1993; Viveiros et al., 1994). Therefore, xylanase addition could have increased nutrient digestion and performance of poults fed wheat-based diets by improving digesta viscosity and gut ecosystem characteristics.

The beneficial effect of low gut viscosity could be supported by our observations in the corn-diet group. This effect on the corn group could be associated with the influence of enzyme on reducing digesta viscosity. Poults fed the corn-based diets had significantly higher FCR, although body weight did not differ significantly from those fed the wheat-based diets. One reason for this observation could be that the birds in corn-based diet had a lower gut viscosity, which allowed increased feed passage rate and thus increased feed intake. This observation was supported by the significantly higher feed consumption among the birds fed corn-based diets. Similarly, Bedford and Classen

(1992b) and Antoniou et al. (1981) reported increased feed intake by enzyme supplementation of wheat-based diets and attributed this response to the enzyme's effect on reducing gut viscosity, and consequently increased feed passage rate.

There was no wheat source effect on BW, FC, FCR, viscosity, and mortality, although wheat source effects were observed on the indicators of nutrient digestion (AMEn, and ANR). Wheat A and B (frost-damaged near full maturity) had significantly higher nutrient digestibility than wheat C and D (frost-damaged wheat during grain filling). The four wheat sources used in this study were similar in every aspect except planting time and the degree of maturation when they were frost damaged. All four wheat sources were from the same variety, grown on the same farm near Saskatoon in Saskatchewan, Canada. Therefore, the variation in response measurement among wheat sources due to the variety, geographical location and agronomic practices was minimized relative to the variation due to the degree of frost damage. The 1999 wheat-growing season in Saskatchewan, which usually starts in the second half of April, was delayed by heavier than normal snow cover until the first week of May and excessive soil moisture levels due to high precipitation until the first half of June (Morgan, 1999). Temperatures in May, June and July also were cooler than normal, with reporting deviations of 0.5ºC to 3ºC below normal. This cooler condition prolonged crop development. In August of 1999 in the Saskatchewan region, warm temperatures and a decrease in rain helped the crop to develop, although most of them were 10 to 15 days behind normal development (Morgan, 1999). Wheat A, B, C, and D utilized in this research were planted in the second, third, and fourth week of May and first week of June, respectively (Figure 2). In Saskatchewan,

the harvest period usually starts early September and finishes at the end of October (Morgan, 1999). In Saskatchewan region temperatures usually averaging 5-17ºC in September helps the crop to reach maturation before harvest. Although an early fall frost event in the first week of September occurred when wheat A and B were near full maturity (mid- to end of ripening), wheat C and D were in the milk to the soft-dough stage of seed development (mid-milk to the mid-dough stage). Reports of the 1999 spring-wheat crop in Saskatchewan and many other regions in Canada showed predominant grade of frost damage and green immature kernels due to frost (Morgan, 1999).

The milk stage is defined when the color and consistency of the kernel resembles that of milk. The endosperm thickens as the grain progresses into the dough stage. The dough stage is often further divided into soft and hard dough stages, as the moisture content of the grain decreases and it becomes more difficult to split with a fingernail. The grain reaches maturity as the grain hardens further and loses moisture to 30-35%. It typically takes 30 days for wheat to reach physiological maturity from flowering (Simmons et al., 1995), and a total of 103 days from planting (Fowler and Hermenean, 1996). However, variation in this growth period can occur because temperature determines the rate of wheat development.

Frost damage is critical depending on the stage of maturation of wheat kernel. Wheat is tolerant to frost damage before the initiation of flowering, usually 8-10 weeks after germination when it can become dormant, as is the case of winter wheat (Bendigo, 2000). However, wheat is very susceptible to frost damage during the period from the

formation of flowering parts to grain filling (milk and dough stage) (Bendigo, 2000). The grain fill period usually takes 12-21 days. The adverse effect of frost during the growth of the reproductive tissue and flowering is floret death and reduced yield. Frost damage during the grain filling, as occurred in the wheat C and D used in this experiment, can result in shriveled or shrunken grain that result at harvest in low test weight, low falling number and high screenings (Arnott and Richardson, 2001). Wheat kernels that reach full size and nearly full weight after mid-dough stage, like wheat A and B used in this experiment, are more resistant to freezing temperatures, and usually the only visible sign of frost damage may be unsightly wrinkled appearance of the kernels and a slightly reduced test weight (Warrick and Miller, 1999).

Frost damage during grain fill can change the chemical composition of the grain and nutrient availability. Frost damage during grain fill may prevent a significant proportion of the sugars being converted to starch (Arnott and Richardson, 2001). The fiber and ash content may be higher in frost damage grain during grain fill, because a higher proportion of seed coat and a lower proportion of endosperm in the frost damage grain. Thus, frost damaged wheat may contain less gross energy and lower gross energy digestibility than normal wheat at full maturity. Frost-damaged grain often contains higher levels of crude protein than normal grain because frost tends to reduce or prevent grain fill (less starch), thereby increasing the proportion of protein-to-starch (Arnott and Richardson, 2001).

There was a marginal variation in chemical composition of wheat due to the degree of frost damage (Table 2). Although gross energy content did not differ

significantly among wheat sources, the frost-damaged wheat during seed development had slightly higher crude protein, fiber and ash than the wheat exposed to frost during the ripening stage. The decrease in energy digestibility observed for the wheat C and D support our hypothesis that frost damaged during the grain filling stage of maturation adversely affects total starch content in favor of more fiber. Moreover, the degree of maturity at harvest may have a significant influence on the nutritional value of wheat.

Frost damage during the milk-stage of seed development, as occurred in wheat C and D, arrests the synthesis and accumulation of starch into the wheat kernel and thus prevents the grain to fully mature. Jennings and Morton (1963) and D'Appolonia and Mac Arthur (1975) reported that the pentosan/kernel in the endosperm increases throughout kernel development, as it is attributed to the synthesis of new cell walls to accommodate the newly synthesized starch. Starch synthesis starts early after pollination and continues until the grain kernel matures. Because immature wheat kernels contain less starch than more mature wheat, the NSP content is proportionally greater. Immature or frost-damaged wheat has a higher proportion of seed coat relative to endosperm than mature wheat. D'Appolonia and Mac Arthur (1975) reported that immature wheat has higher percentage levels of NSP, primarily arising from the bran (pericarp and testa) and endosperm layers. They observed that the ratio arabinose:xylose in the bran fraction was similar for both immature and mature wheat samples, but this ratio for the endosperm was higher in the immature than in the mature wheat. Therefore, immature wheat not only contains a higher amount of pentosan, but its pentosan is more water soluble than in mature wheat because the arabinose side chain is the water-soluble portion of the NSP

(Ward, 1995). In addition, the NSP in immature wheat has a lower degree of branching than in mature wheat (D'Appolonia and MacArthur, 1975), which increases the hydration capacity and gel viscosity. The hydration capacity of polymerized arabinoxylans is dependent on the size of the molecules: water absorption increases as molecule size decreases (Izydorczyk et al., 1991). The results obtained in the present study further support the findings of D'Appolonia and MacArthur (1975) as we observed lower AMEn and ANR for the wheat frost damaged at immature stage of seed development. The low AME found for the immature frost-damaged wheat is in agreement with findings of other researchers who reported different enzyme responses due to the high heterogeneity in water-soluble arabinoxylan content among wheat cultivars (Saulnier et al., 1995; Crouch et al., 1997).

Many research have shown that enzyme supplementation reduces the variability in nutrient utilization among different sources of wheat. Choct et al. (1995) reported that enzyme supplementation significantly improved the nutritive value of a diet containing a particular a low AME wheat. Using broiler chickens, Scott and Pierce (2001) measured the feeding value of western Canadian wheat. They reported that variations in AME of wheat- or barley-based diets was significantly reduced by enzyme supplementation, indicating that low AME cereal grains generally benefit more from enzyme supplementation than high AME cereal grains. This is of great interest for the poultry industry because not only does enzyme supplementation significantly improve nutrient utilization, but it also leads to a more consistent uniform performance (Choct et al., 1999). However, in our study, enzyme supplementation was equally effective for all the

wheat groups as indicated by growth performance, gut viscosity, and nutrient utilization. An insignificant enzyme X wheat effect may have been due to the fact that the wheat used in this study only differed by the degree of frost damage and there were no other genetic or agronomic differences.

From the results obtained, it can be concluded that supplementation of enzymes, as contained in Natugrain Blend®, improved the nutritional value of wheat-based diets to a level similar to diets containing corn in place of wheat. Frost damage during seed development significantly reduces the nutritional value of wheat, presumably by increasing the relative content of NSP to starch. This study demonstrated the positive effects of dietary enzyme supplementation on nutrient utilization of different wheat sources and cultivation conditions.

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2.6 TABLES AND FIGURES

TABLE 1: Composition and calculated nutrient content of the experimental diets fed to turkeys from 1 to 17 days of age

Ingredient	Basal diets	
	Corn	Wheat
	(%)	
Corn	50.0	0
Wheat A, B, C, or D[1]	0	50.0
Soybean meal (48%)	39.0	39.0
Poultry meal (60%)	5.0	5.0
Dical (18.5% P)	2.05	2.05
Calcium Carbonate	1.44	1.44
Salt	0.29	0.29
DL-Methionine	0.19	0.19
L-Lysine HCl	0.15	0.15
Minerals[2]	0.20	0.20
Vitamins[3]	0.10	0.10
Choline Cl (60%)	0.20	0.20
Selenium Premix[4]	0.075	0.075
Celite[5]	0.70	0.70
Enzyme or Solka-Floc[6]	0.10	0.10
Titanium Oxide	0.50	0.50
Calculated Analysis		
ME, Kcal/kg	2,766	2,676
Crude Protein, %	26.7	27.4
Methionine + Cystine, %	1.05	1.02
L-Lysine, %	1.60	1.60
Calcium, %	1.25	1.27
Non-Phytate Phosphorus, %	0.60	0.64
Sodium, %	0.17	0.18

[1]The wheat sources differed by the degree of frost damage during seed development. Wheat A and B were frost damaged near full maturity, and wheat C and D were damaged during grain filling stage (mid-milk to soft-dough stage).

[2]Supplied the following per kilogram of feed: 120 mg Zn as $ZnSO_4 \cdot H_2O$; 120 mg MN as $MnSO_4 \cdot H_2O$; 80 mg Fe as $FeSO_4 \cdot H_2O$; 10 mg Cu as $CuSO_4$; 2.5 mg I as $Ca(IO_3)_2$; 1.0 mg Co as $CoSO_4$.

[3]Supplied the following per kilogram of feed: vitamin A, 13,200 IU; cholecalciferol, 4,000 IU; niacin, 110 mg; pantothenic acid, 22 mg; riboflavin, 13.2 mg; pyridoxine, 7.9 mg; menadione, 4 mg; folic acid, 2.2 mg; thiamin, 4 mg; biotin, 0.253 mg; vitamin B_{12}, 0.04 mg; ethoxyquin, 100 mg; selenium, 0.30 mg. The vitamin E premix provided the necessary amount of vitamin E as DL-α-tocopheryl acetate.

[4]Selenium premix supplied 3 ppm Se as sodium selenate.

[5]Celite (Celite™, Celite Corp., Lompar, CA 93436), a source of acid-insoluble ash, were used as an indigestible marker.

[6]Enzyme treatments were supplemented with enzyme products that accounted as a dry ingredient (0.1%) and an equivalent amount of Solka-Floc (cellulose) was applied to the unsuplemented treatments.

TABLE 2: Chemical composition of wheat used in the experimental diets fed to turkeys from 1 to 17 days of age

Analysis	Wheat Type[1]			
	A	B	C	D
Gross Energy, Kcal/kg	4,240	4,430	4,430	4,430
Crude Protein, %	13.7	16.0	15.9	16.5
Crude Fat, %	1.50	1.60	1.70	1.70
L-Lysine, %	0.34	0.38	0.38	0.40
Fiber (ADF), %	3.30	3.70	3.80	4.20
Fiber (NDF), %	19.8	17.9	20.7	20.6
Ash, %	2.17	1.93	2.10	2.20
Calcium, %	0.05	0.05	0.05	0.06
Phosphorus, %	0.49	0.47	0.33	0.40
Sodium, %	0.02	0.02	0.02	0.02

[1]The wheat sources differed by the degree of frost damage during seed development. Wheat A and B were frost damaged near full maturity, and wheat C and D were damaged during grain filling stage (mid-milk to soft-dough stage).

TABLE 3: Effects of wheat source[1] with (+) and without (-) enzyme (Natugrain Blend®[2]) supplementation on body weight of poults raised from 0 to 17 days of age

Dietary Treatments		Days of Age		
Cereal Base	Enzyme	7	13	17
		(g)[3]		
Corn	-	143.3[a]	248.7[d]	353.7[bcd]
Wheat A	-	129.8[c]	253.7[cd]	364.3[abcd]
Wheat A	+	139.9[ab]	271.7[ab]	376.4[abc]
Wheat B	-	128.3[c]	257.1[bcd]	349.4[bcd]
Wheat B	+	142.6[a]	272.7[ab]	380.5[ab]
Wheat C	-	132.9[bc]	255.5[cd]	348.0[cd]
Wheat C	+	135.3[abc]	265.7[bc]	370.9[abcd]
Wheat D	-	131.7[bc]	248.1[d]	341.1[d]
Wheat D	+	144.8[a]	283.6[a]	394.2[a]
P-Value		0.0043	0.0005	0.0227
SEM(36)[4]		3.35	5.57	10.89
Main effect of enzyme supplementation among wheat treatments		(g)[5]		
Enzyme -		130.7[b]	253.6[b]	350.7[b]
Enzyme +		140.7[a]	273.4[a]	380.5[a]
Source of variation among wheat treatments		(P-Value)		
Enzyme		0.0002	0.0001	0.0007
Wheat		0.6360	0.7840	0.7957
Wheat X enzyme		0.2955	0.1471	0.3272
SEM(32)[6]		3.37	5.60	11.25

[a-d]Means with different superscripts within a column differ significantly ($P < 0.05$). There were no significant differences in poults starting weights at 1 d of age (60g).

[1]The wheat sources differed by the degree of frost damage during seed development. Wheat A and B were frost damaged near full maturity, and wheat C and D were damaged during grain filling stage (mid-milk to soft-dough stage).

[2]Natugrain Blend® contains 36,600 EXU/g of xylanase activity; 9,000 BGU/g of β-glucanase activity; and some hemicellulase, cellulase and protease activities (BASF, 1997). The dosage used was 200 mg/kg of feed, as recommended by the manufacture.

[3]Values represent means of 5 cages containing 10 poults each. Data from 9 treatments (1 corn, 8 wheat).

[4]SEM(36)= Standard Error of the mean with 36 degrees of freedom.

[5]Values represent means of 20 cages containing 10 poults each. Data from 8 wheat treatments (4 wheat sources and 2 level enzyme).

[6]SEM(32)= Standard Error of the mean with 32 degrees of freedom.

TABLE 4: Effects of wheat source[1] with (+) and without (-) enzyme (Natugrain Blend®[2]) supplementation on periodic feed consumption of poults raised from 0 to 17 days of age

Dietary Treatments		Days of Age			
Cereal Base	Enzyme	1-7	8-13	14-17	1-17
		(g)[3]			
Corn	-	103.1^{ab}	198.6	207.7^{a}	509.4^{a}
Wheat A	-	91.5^{cd}	203.1	185.1^{bcd}	479.7^{abc}
Wheat A	+	95.9^{bcd}	194.8	188.1^{bcd}	478.8^{abc}
Wheat B	-	88.8^{d}	179.0	174.7^{d}	442.5^{c}
Wheat B	+	98.0^{abc}	186.6	192.5^{abc}	477.1^{abc}
Wheat C	-	94.0^{cd}	180.7	176.5^{cd}	451.2^{c}
Wheat C	+	93.3^{cd}	184.4	192.9^{abc}	470.6^{bc}
Wheat D	-	90.6^{cd}	176.1	175.4^{d}	442.1^{c}
Wheat D	+	103.9^{a}	198.6	195.2^{ab}	497.7^{ab}
P-Value		0.0014	0.1251	0.0040	0.0094
SEM(36)[4]		2.62	7.50	5.82	13.35
Main effect of enzyme supplementation among wheat treatments		(g)[5]			
Enzyme -		91.2^{b}	184.7	177.9^{b}	453.9^{b}
Enzyme +		97.8^{a}	191.1	192.2^{a}	481.1^{a}
Source of variation among wheat treatments		(P-Value)			
Enzyme		0.0020	0.2266	0.0021	0.0085
Wheat		0.4488	0.1066	0.9670	0.4642
Wheat X enzyme		0.0828	0.2321	0.5009	0.2266
SEM(32)[6]		2.75	7.31	6.03	13.69

[a-d]Means with different superscripts within a column differ significantly (P< 0.05).

[1]The wheat sources differed by the degree of frost damage during seed development. Wheat A and B were frost damaged near full maturity, and wheat C and D were damaged during grain filling stage (mid-milk to soft-dough stage).

[2]Natugrain Blend® contains 36,600 EXU/g of xylanase activity; 9,000 BGU/g of β-glucanase activity; and some hemicellulase, cellulase and protease activities (BASF, 1997). The dosage used was 200 mg/kg of feed, as recommended by the manufacture.

[3]Values represent means of 5 cages containing 10 poults each. Data from 9 treatments (1 corn, 8 wheat).

[4]SEM(36)= Standard Error of the mean with 36 degrees of freedom.

[5]Values represent means of 20 cages containing 10 poults each. Data from 8 wheat treatments (4 wheat sources and 2 level enzyme).

[6]SEM(32)= Standard Error of the mean with 32 degrees of freedom.

TABLE 5: Effects of wheat source[1] with (+) and without (-) enzyme (Natugrain Blend®[2]) supplementation on cumulative feed conversion ratio[4] of poults raised from 0 to 17 days of age

Dietary Treatments		Days of Age		
Cereal Base	Enzyme	1-7	1-13	1-17
			(g/g)[3]	
Corn	-	1.200	1.578[a]	1.716[a]
Wheat A	-	1.312	1.522[a]	1.590[b]
Wheat A	+	1.172	1.362[b]	1.504[b]
Wheat B	-	1.258	1.342[b]	1.518[b]
Wheat B	+	1.166	1.348[b]	1.494[b]
Wheat C	-	1.246	1.384[b]	1.552[b]
Wheat C	+	1.182	1.326[b]	1.496[b]
Wheat D	-	1.232	1.398[b]	1.560[b]
Wheat D	+	1.184	1.338[b]	1.480[b]
P-Value		0.0912	0.0001	0.0047
SEM(36)[4]		0.035	0.036	0.039
Main effect of enzyme supplementation among wheat treatments			(g/g)[5]	
Enzyme -		1.262[a]	1.411[a]	1.555[a]
Enzyme +		1.176[b]	1.343[b]	1.493[b]
Source of variation among wheat treatments			(P-Value)	
Enzyme		0.0017	0.0133	0.0431
Wheat		0.7647	0.0512	0.7967
Wheat X enzyme		0.5899	0.1772	0.8730
SEM(32)[6]		0.035	0.037	0.041

[a,b]Means with different superscripts within a column differ significantly (P< 0.05).

[1]The wheat sources differed by the degree of frost damage during seed development. Wheat A and B were frost damaged near full maturity, and wheat C and D were damaged during grain filling stage (mid-milk to soft-dough stage).

[2]Natugrain Blend® contains 36,600 EXU/g of xylanase activity; 9,000 BGU/g of β-glucanase activity; and some hemicellulase, cellulase and protease activities (BASF, 1997). The dosage used was 200 mg/kg of feed, as recommended by the manufacture.

[3]Values represent means of 5 cages containing 10 poults each. Data from 9 treatments (1 corn, 8 wheat).

[4]SEM(36)= Standard Error of the mean with 36 degrees of freedom.

[5]Values represent means of 20 cages containing 10 poults each. Data from 8 wheat treatments (4 wheat sources and 2 level enzyme).

[6]SEM(32)= Standard Error of the mean with 32 degrees of freedom.

TABLE 6: Effects of wheat source[1] with (+) and without (-) enzyme (Natugrain Blend®[2]) supplementation on cumulative livability rate of poults raised from 0 to 17 days of age

Dietary Treatments		Days of Age		
Cereal Base	Enzyme	1-7	1-13	1-17
			(%)[3]	
Corn	-	100	100	100
Wheat A	-	94	94	94
Wheat A	+	100	100	100
Wheat B	-	100	100	100
Wheat B	+	100	98	98
Wheat C	-	98	98	98
Wheat C	+	98	98	98
Wheat D	-	100	100	100
Wheat D	+	98	98	98
P-Value		0.2816	0.4102	0.4102
SEM(36)[4]		0.018	0.019	0.019
Main effect of enzyme supplementation among wheat treatments			(%)[5]	
Enzyme -		98	98	98
Enzyme +		99	99	99
Source of variation among wheat treatments			(P-Value)	
Enzyme		0.4552	0.7260	0.7260
Wheat		0.4270	0.7133	0.7133
Wheat X enzyme		0.1838	0.1686	0.1686
SEM(32)[6]		0.019	0.020	0.020

[a,b]Means with different superscripts within a column differ significantly (P< 0.05).

[1]The wheat sources differed by the degree of frost damage during seed development. Wheat A and B were frost damaged near full maturity, and wheat C and D were damaged during grain filling stage (mid-milk to soft-dough stage).

[2]Natugrain Blend® contains 36,600 EXU/g of xylanase activity; 9,000 BGU/g of β-glucanase activity; and some hemicellulase, cellulase and protease activities (BASF, 1997). The dosage used was 200 mg/kg of feed, as recommended by the manufacture.

[3]Values represent means of 5 cages containing 10 poults each. Data from 9 treatments (1 corn, 8 wheat).

[4]SEM(36)= Standard Error of the mean with 36 degrees of freedom.

[5]Values represent means of 20 cages containing 10 poults each. Data from 8 wheat treatments (4 wheat sources and 2 level enzyme).

[6]SEM(32)= Standard Error of the mean with 32 degrees of freedom.

TABLE 7: Effects of wheat source[1] with (+) and without (-) enzyme (Natugrain Blend®[2]) supplementation on the jejunum digesta viscosity of 17 days-old poults. And Pearson correlation coefficients (r) of the jejunum viscosity versus AMEn and apparent nitrogen retention (ANR)

Dietary Treatments		Jejunum Digesta Viscosity
Cereal Base	Enzyme	
		(Centipoise, cP)[3]
Corn	-	3.46
Wheat A	-	5.56
Wheat A	+	2.87
Wheat B	-	5.65
Wheat B	+	4.99
Wheat C	-	6.38
Wheat C	+	4.20
Wheat D	-	4.88
Wheat D	+	3.55
P-Value		0.0548
SEM (80)[4]		0.83
Main effect of enzyme supplementation among wheat treatments		(cP)[5]
Enzyme -		5.57[a]
Enzyme +		3.98[b]
Source of variation among wheat treatments		(P-Value)
Enzyme		0.0132
Wheat		0.4737
Wheat X enzyme		0.6719
SEM(72)[6]		0.88
Correlation variable	*Correlation coefficient (r)*	(P-Value)
Viscosity vs. AMEn	-0.2216	0.0358
Viscosity vs. ANR	-0.2020	0.0562

[a,b]Means with different superscripts within a column differ significantly (P< 0.05).

[1]The wheat sources differed by the degree of frost damage during seed development. Wheat A and B were frost damaged near full maturity, and wheat C and D were damaged during grain filling stage (mid-milk to soft-dough stage).

[2]Natugrain Blend® contains 36,600 EXU/g of xylanase activity; 9,000 BGU/g of β-glucanase activity; and some hemicellulase, cellulase and protease activities (BASF, 1997). The dosage used were 200 mg/kg of feed, as recommended by the manufacture.

[3]Centipoise (cP). A centimeter-gram-second unit of dynamic viscosity equal to one dyne-second per square centimeter. Values represent means of 5 cages of 2 poults sampled per cage. Data from 9 treatments (1 corn, 8 wheat)

[4]SEM(80)= Standard Error of the mean with 80 degrees of freedom.

[5]Values represent means of 20 cages of 2 poults sampled per cage. Data from 8 wheat treatments (4 wheat sources and 2 level enzyme).

[6]SEM(72)= Standard Error of the mean with 72 degrees of freedom.

TABLE 8: Effects of wheat source[1] with (+) and without (-) enzyme (Natugrain Blend®[2]) supplementation on apparent metabolizable energy nitrogen-corrected[8] (AMEn) and apparent nitrogen retention[9] (ANR) of poults[3]

Dietary Treatments			
Cereal Base	Natugrain Blend®	AMEn	ANR
		(kcal/kg)[3]	(%)[3]
Corn	-	2,582[a]	46.1[a]
Wheat A	-	2,291[bc]	37.4[cd]
Wheat A	+	2,501[ab]	45.3[ab]
Wheat B	-	2,297[bc]	39.1[bcd]
Wheat B	+	2,624[a]	46.0[a]
Wheat C	-	2,223[cd]	33.1[de]
Wheat C	+	2,270[c]	33.7[de]
Wheat D	-	2,005[d]	30.3[e]
Wheat D	+	2,428[abc]	40.8[abc]
P-Value		0.0001	0.0001
SEM (36)[4]		77.09	2.20
Main effect of enzyme supplementation among wheat treatments		(kcal/kg)[5]	(%)[5]
Enzyme -		2,204[b]	35.0[b]
Enzyme +		2,455[a]	41.4[a]
Main effect of wheat source among wheat treatments		(kcal/kg)[6]	(%)[6]
Wheat A		2,396[ab]	41.4[a]
Wheat B		2,460[a]	42.6[a]
Wheat C		2,246[bc]	33.4[b]
Wheat D		2,216[c]	35.6[b]
Source of variation among wheat treatments		(P-Value)	
Enzyme		0.0001	0.0002
Wheat		0.0098	0.0004
Wheat X enzyme		0.1158	0.1695
SEM(32)[7]		78.36	2.21

[a-e]Means with different superscripts within a column differ significantly (P< 0.05).

[1]The wheat sources differed by the degree of frost damage during seed development. Wheat A and B were frost damaged near full maturity, and wheat C and D were damaged during grain filling stage (mid-milk to soft-dough stage).

[2]Natugrain Blend® contains 36,600 EXU/g of xylanase activity; 9,000 BGU/g of β-glucanase activity; and some hemicellulase, cellulase and protease activities (BASF, 1997). The dosage used were 200 mg/kg of feed, as recommended by the manufacture.

[3]Values represent means of 5-pooled samples of excreta per treatment collected from 11 to 14 days of age.

[4]SEM(36)= Standard Error of the mean with 36 degrees of freedom.

[5]Values represent means of 20 cages containing 10 poults each.

[6]Values represent means of 10 cages containing 10 poults each.

[7]SEM(32)= Standard Error of the mean with 32 degrees of freedom.

[8]Equation to determine AMEn, kcal/g diet on dry matter basis.

Ediet = kilocalories combustible energy per gram of diet dry matter (determined directly by bomb calorimeter)

Eexcreta = kilocalories combustible energy in excreta per gram of diet dry matter =

= kilocalories per gram excreta x (g celite per gram diet/g celite per gram excreta)

N = Nitrogen retention per gram of diet dry matter =

= N per gram diet – N per gram excreta x (g celite per gram diet/g celite per gram excreta)

AMEn = Metabolizable energy per gram diet dry matter nitrogen corrected =

= Ediet – Eexcreta – 8.22 N

[9]Equation to determine apparent nitrogen retention (ANR), %.

Nretained = Nitrogen retained per gram of diet dry matter =

= N per gram diet – N per gram excreta x (g Celite per gram diet/g celite per gram excreta)

ANR = Percentage of Apparent Nitrogen retention per gram of diet, percentage =

= (Nretained/N per gram diet) x 100

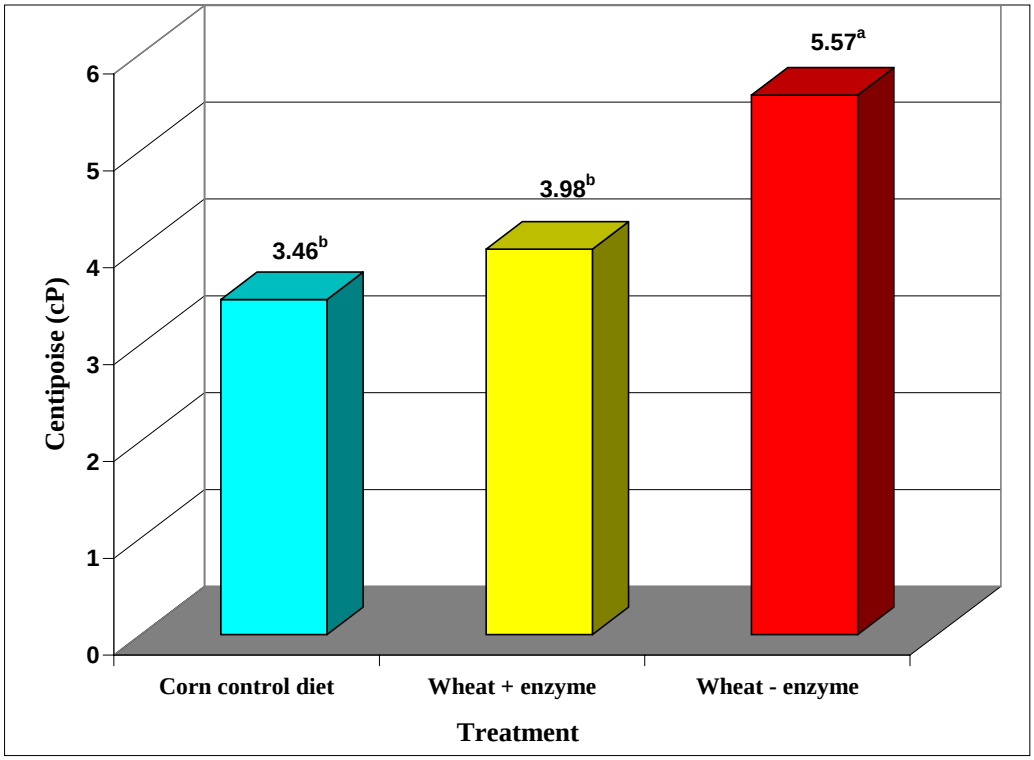

FIGURE 1: Effects of wheat with (+) and without (-) enzyme (Natugrain Blend®) supplementation on the jejunum digesta viscosity of 17 days-old poults. Different letters on each bar signify a significant ($P<0.05$) difference between mean values of jejunum digesta viscosity. Mean values represent average of two samples from twenty cages (corn had 5 cages) of 10 poults each. Natugrain Blend® contained 36,600 EXU/g of xylanase activity; 9,000 BGU/g of β-glucanase activity; and some hemicellulase, cellulase and protease activities (BASF, 1997). The dosage used were 200 mg/kg of feed, as recommended by the manufacture.

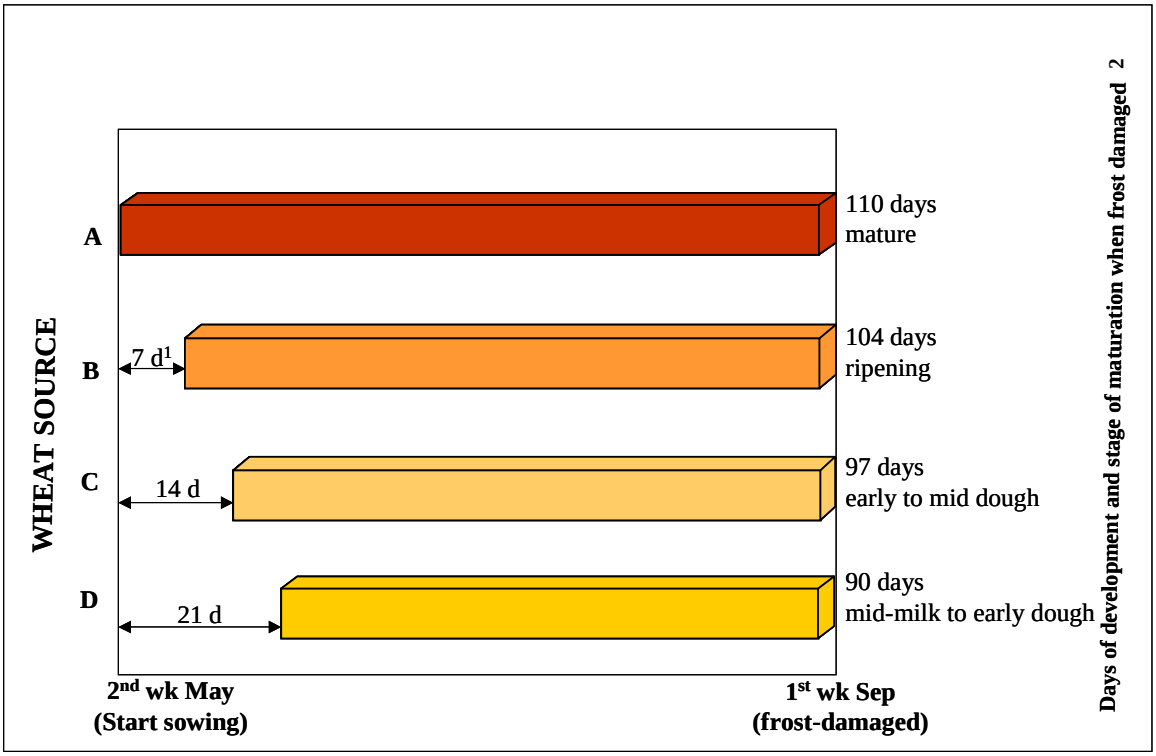

FIGURE 2: Schematic representation of days of development and degree of maturation of four different sources of wheat used in the experimental feed. The wheats were all of the same variety, grown on the same farm in Saskatchewan, Canada. [1]Wheat A was planted on the second week of May, and wheat B, C, and D were planted on the following weeks, approximately one week apart. An early fall frost event occurred on the first week of September when wheat A and B were near full maturity (mid- to end of ripening), and wheat C and D were in the milk to the soft-dough stage of seed development (mid-milk to the mid-dough stage). [2]The days of development and stage of maturation are estimates according to the information received from BASF AG, Ludwigshafen, Germany.

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CHAPTER 3

DIETARY SUPPLEMENTATION OF ENDOXYLANASES AND PHOSPHOLIPASE FOR TURKEYS FED WHEAT-BASED RATIONS[1]

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[1]The use of trade names in this publication does not imply endorsement by the North Carolina Agriculture Research Service or the North Carolina Cooperative Extension Service of the products mentioned nor criticism of similar products not mentioned.

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3.1 ABSTRACT

The adverse effects of non-starch polysaccharides (NSP) on turkeys fed wheat-based diets may be alleviated by dietary supplementation of endoxylanase (to reduce the adverse effects of digesta viscosity) or phospholipase (to improve the digestibility of fat). Nicholas toms were fed wheat-based diets containing one of 5 enzyme treatments: unsupplemented control (C), Natugrain Blend® (≥5,500 EXU/kg diet; NB), Lyxasan®-50 (≥2,250 EXU/kg diet; LX50), Lyxasan®-100 (≥5,500 EXU/kg diet; LX100), and Phospholipase (≥500 PLU/kg diet; PL) (BASF, Germany). Each treatment group was assigned to 8 pens containing 12 birds to evaluate growth performance in experiment 1 (1-128 d), and 2 pens of 12 birds (excluding LX50) for the AMEn and gut viscosity determination in experiment 2 (56-128 d). All enzyme treatments improved growth performance. In comparison to control, dietary enzyme increased ($P<0.05$) BW and decreased 1-128 d feed/gain (2.45 vs. 2.37, $P<0.005$). PL was most effective in reducing feed/gain during the starting phase (1 to 14 d), LX100 was most effective during the finishing phase, while NB had intermediate benefits throughout the experiment. Dietary AMEn was increased by PL from 9-12 wk, while NB and LX-100 resulted in the highest dietary energy utilization during the later finishing period. Ileum digesta viscosity was significantly higher for PL than the other treatments (13.5 vs. 7.07 cP, $P<0.001$). Growth performance and energy utilization of turkeys fed wheat-based diets can be significantly enhanced by phospholipase (PL) supplementation of starter feeds and endoxylanase (LX100) supplementation of growing and finishing feeds. However, a natural blend of enzymes (NB) may provide a positive response regardless of turkey age.

(*Key words*: non-starch polysaccharides, wheat, enzymes, turkey, growth performance)

3.2 INTRODUCTION

Cereals are important foodstuffs for man and livestock, but some contain high levels of antinutrients that limit their use. Cereals and their co-products comprise a significant proportion of livestock diets (Evers et al., 1999). Wheat is the most widely cultivated crop in the world, accounting for almost 30% (600.7 million metric tons in average of the years 1997 to 1999 – FAO, 2002) of total cereal production, and it is one of the major energy contributors to the diets of turkeys and broilers (Steenfeldt et al., 1998). Wheat contributes up to 80% of finishing poultry diets in the United Kingdom (Longstaff and McNab, 1986; Wiseman and Inborr, 1990). However, the use of wheat in commercial turkey and broiler diets has traditionally been limited by their low and variable energy values.

The apparent metabolizable energy (AME) of wheat can be lower and more variable than expected among different cultivars because of the amount of non-starch polysaccharides (NSP) contained in the kernel. The AME and soluble NSP are negatively correlated in wheat (Choct and Annison, 1990; Choct et al., 1999a) and various sources of purified NSP depress AME (Choct and Annison, 1990). Moreover, NSP isolated from wheat depress AME in a dosage dependent manner (Choct and Annison, 1990; Hughes and Choct, 1999). In contrast, degradation of cell wall NSP by glycanases increases AME (Annison, 1992).

Arabinoxylan is the major NSP in wheat that produce the antinutritive effects of wheat. Arabinoxylan is a linear polymer of variable length and consists of a long backbone chain of β-(1,4) anhydro-D-xylopyranosyl to which are attached single α-L-

arabinofuranosyl residues at the 2 or 3-position (Lineback and Rasper, 1988). The principal antinutritive factor of arabinoxylans is their capacity to bind water and increase digesta viscosity (Knudsen, 2001). In the digestive tract, viscous solutions interfere with the digestion and/or absorption of nutrients (Marquardt et al., 1979) directly by affecting the rate of diffusion and indirectly by stimulating the growth of anaerobic microflora (Vukic Vranjes and Wenk, 1996). The viscous intestinal environment slows digestion and digesta passage rate, thus increasing the amount of undigested materials available for microflora proliferation and microbial fermentation in the small intestine (Preston et al., 2001). The microflora compete with the host for nutrients by converting nutrients into microbial protein and impeding digestion and absorption. An increase in microbial activity in the small intestine increases deconjugation of bile acids (Langhout et al., 2000). This reduction in conjugated bile acids reduces the formation of micelles, limiting digestion of fat and fat-soluble vitamins that decrease AME of wheat (Hoffman and Small, 1967).

Accompanying the dramatic effect of NSP on the performance and nutrient digestibility, birds often exhibit symptoms of considerable gastrointestinal stress. Choct and Annison (1992a) reported that birds fed high NSP diets excreted excessive amounts of fluid in their feces. Ward (1995) stated that wheat is often associated with wet litter for poultry, especially when fed at levels exceeding about 20% of the diet. Osmotic diarrhea caused by high levels of NSP in the diet of poultry is associated with a high concentration of dietary components that are not completely digested and/or absorbed in the small intestine, giving rise to an increased amount of osmotically active compounds in the gut (Choct and Annison, 1992a). Moisture content of the excreta has been shown to be 10%

higher in birds fed NSP-rich diets than in birds fed a corn-soy diet that contains little NSP (Choct et al., 1996). This increased diarrhea can adversely affect the health of birds by increasing the susceptibility of poultry to enteric disease and leg problems (Grimes and Crouch, 1997), and increase carcass downgrading (Hughes et al., 2000). Moreover, excessive wet litter causes problems with litter handling and disposal (Pawlik et al., 1990) and nuisance complaints from neighbors about flies and odor.

Dietary supplementation of fungal and microbial enzymes has been studied as a means to avoid the adverse effects of wheat NSP. Dietary enzyme supplementation has been shown to improve the feeding value of wheat by disrupting the water holding capacity of the NSP, improving nutrient digestion, and reducing microflora fermentation in the small intestine (Choct et al., 1999b). Supplementing wheat-based diets with enzyme preparation capable of hydrolyze the long complex of xylan into smaller units has increased the performance and nutrient digestibility (Annison and Choct, 1991; Steenfeldt et al., 1998; Preston et al., 2001). Furthermore, dietary enzyme supplementation has been shown to alleviate the osmotic diarrhea by improving nutrient digestion via reduction in the concentration of osmotically active compounds in the gut (Fischer and Classen, 2000). Thus, appropriate enzyme supplementation to wheat-based diets not only improves growth performance and AME, but it also reduces disease and management problems associated with poor and wet litter conditions.

The benefit of NSP-enzymes in wheat-based diets is mainly attributed to endoxylanase, but a blend including other enzymes may be more effective than a single endoxylanase preparation. Endoxylanase degrades the xylan backbone of arabinoxylan into smaller units, which decreases gut viscosity, and increases productive performance

and nutrient digestability (Odetallah, 2000). A blend of different enzymes has been shown to improve performance to a greater extent than single enzyme preparations because the activity of one type of feed enzyme is synergistically facilitated by another (Ravindran et al., 1999; Odetallah et al., 2002). Odetallah et al. (2002) studied the effect of three different enzyme products. One of the products was exclusively endoxylanase produced by a genetically modified organism, while the other enzyme preparation had major activities of β-glucanase and endoxylanase and minor activities of hemicellulase, cellulase and protease. The third enzyme preparation evaluated was a blend of the first two products. They reported that a blend of enzyme that contained high endoxylanase activities resulted in the best growth performance in turkeys fed wheat-based diets. Furthermore, the effect of enzyme supplementation on the performance and nutrient digestion increased as the enzyme level increased. Odetallah (2000) reported that the total amount of enzyme activity supplemented to the diet is crucial to its effect on the performance of turkey. Several other researchers have shown dose-dependent responses for dietary supplementation of NSP-enzymes (Hesselman et al., 1982; Petterson and Aman, 1989; Bedford and Classen, 1992).

Endoxylanase and enzyme complex products decrease gut viscosity and improve fat digestability by preserving the integrity and function of bile salts and allowing the formation of fat micelles. Another approach to enhance fat digestion in diets with high inclusion levels of wheat was studied using lipase (Martin and Farrell, 1998). However, dietary supplementation of lipase did not improve the performance of the birds. Al-Marzooqi and Lesson (1999) attributed the lack of lipase response to the contamination

of the enzyme product with cholecystokinin by the microorganism responsible to produce the lipase. Cholecystokinin is a hormone that reduces feed intake. In contrast, dietary supplementation of exogenous phospholipase may improve significantly fat digestibility (Carey et al., 1983). Endogenous intestinal phospholipase A_2 catalyzes the hydrolysis of glycerophospholipids (GPL) producing fatty acids and lysophospholipids (e.g. Lyso-phosphatidylcholine or Lyso-PC). The fatty acids are then absorbed from the lumen as part of the fat micelle. Lyso-PC, the predominant GPL product in the luminal content, is essential for the emulsification of water-insoluble lipids (Homan and Jain, 2001). Thus, dietary supplementation of phospholipase could alleviate the adverse effects of NSP by facilitating the formation of micelles of triglyceride, cholesterol, and other nonpolar dietary lipids. However, supplementation of exogenous phospholipase in wheat-based diets for poultry has not been investigated.

The general objective of this study was to evaluate the efficacy of supplemental enzymes with different enzyme activities on growth performance and energy utilization of turkeys fed an inferior-quality of wheat. The enzymes used in this study included (a) Natugrain Blend (blend with high endoxylanase activity), (b) Lyxasan (exclusively endoxylanase) at two application rate, and (c) exclusive phospholipase. The specific objectives of this study were (1) to determine the effect of dietary endoxylanase supplementation level on growth performance; (2) to compare the efficacy of endoxylanase from an organism that produces high endoxylanase activity and one that produces endoxylanase along with several other enzymes; and (3) to evaluate the effect of endoxylanase and phospholipase on growth performance and dietary energy utilization.

3.3 MATERIALS AND METHODS

The study was divided into two experiments to achieve the desired objectives. Experiment 1 included performance and caked litter analysis. Experiment 2 included intestinal digesta viscosity and energy digestibility (AMEn).

Enzymes

The same enzymes were used in experiment 1 and 2. The enzyme activity in the product and feed, and application rate used in the experimental diets are shown in Table 1. Natugrain Blend® 66% is a commercial liquid enzyme preparation obtained from fungal fermentation of *Trichoderma longibrachiatum*. It contained standardized activities of at least 9,000 β-glucanase activity (BGU) per gram of product and at least 36,600 endoxylanase unit (EXU) per gram of product (BASF, 2001). One BGU is defined as the activity required to liberate 0.278 μmol reducing sugar (measured as glucose equivalents) per minute at pH 3.5 and 40°C, at a substrate concentration of 0.5% β-glucan from barley. One EXU is defined as the enzyme activity required to liberate 1 μmol reducing sugar (measured as glucose equivalents) per minute from a 1% xylan solution at pH 3.5 and 40°C. Natugrain Blend® also contained some hemicellulase (e.g. pectinase), cellulase and protease activities (BASF, 1997). Lyxasan Forte® is a commercial liquid preparation obtained from a genetically modified *Aspergillus niger* that produces endoxylanase exclusively. Lyxasan Forte® had an endoxylanase activity of at least 56,000 EXU/g of product. Phospholipase is an experimental liquid enzyme preparation obtained from a microbial source and it contained activities of at least 5,000 units of phospholipase A_2 per

gram of product (BASF, 2001). All the enzyme preparations were kindly supplied by BASF[1].

The Natugrain Blend® was supplemented to the diet to achieve the same level of endoxylanase activity in the feed as supplemented with Lyxasan Forte® at 100g/tonne feed. This treatment design allowed us to evaluate the effect of endoxylanase as a single enzyme preparation or along with several other enzymes. Lyxasan Forte® was applied at two-application rates (100 and 50 ml/tonne) so we could investigate the dose-dependent responses for dietary endoxylanase supplementation. Phospholipase was used to test our hypothesis that its dietary supplementation could alleviate the adverse effects of NSP by facilitating lipid digestibility.

Experiment 1: performance trial

The facility used in this study was an industry-standard curtain-sided house containing ninety-six 9.3 square meter pens. Each pen was top-dressed with 4 cm of soft pine shavings at the start of the experiment. Ventilation was provided by natural air movement through appropriately adjusted curtain sides and air mixing fans located on the ceiling throughout the house. High and low ambient temperatures within the house were recorded at two places twice daily throughout the duration of the trial. The recorded temperatures are reported in figure 1. The house temperatures was kept at 29-31ºC during the first week (wk), and then gradually stepped down to the ambient outside temperature, which ranged from 6ºC (43ºF) to 34ºC (94ºF). The house was illuminated with incandescent lights for 23 hours per day on the first week and subsequently by natural

[1]BASF AG, 67059 Ludwigshafen, Germany.

daylight thereafter. Heat lamp unit[2] with 125-watt bulb[3] provided supplemental heat for each pen. Feed and water were provided *ad libitum* throughout the duration of the study. Visual heath inspection of all birds within the study was performed daily and weights of culled birds and reasons they were removed were recorded. Crippled or dead birds were removed and replaced up through day 7 at which time any further mortality were removed and recorded but not replaced. All mortality were weighed soon after death and recorded so that an appropriate adjustment to feed conversion could be made.

One-day-old commercial Large White BUTA[4] male turkeys were obtained from a commercial hatchery[5] and randomly assigned to the pens. They were used for both the growth performance trial (Experiment 1) and the digestability trial (Experiment 2).

The experimental diets are presented in Table 2. Five feed phases were used during the coarse of the experiment. All feeds were formulated using least-cost linear programming software, such that the diets contained about 95% of the NRC (1994) recommendations for amino acids and energy. The diets were formulated slightly below requirements so that any improvement in nutrient availability due to enzyme supplementation could be observed as an improvement in growth performance. All experimental diets consisted of the same wheat-SBM basal diet with different supplemental enzyme treatment using inclusion level of 0.1%. The unsupplemented control diet was supplemented with 0.1% washed builders sand. All feed was pellet-processed and fed in crumble form up to 4 weeks of age, and subsequently as a whole

[2]Heat Lamp, Model # 54411-Heave Gauge Aluminium Base, Hog Slats, Inc., Newton Grove, NC, 28366.
[3]125-watt bulb, SLI, China; Distributor: Hog Slats, Inc., Newton Grove, NC, 28366.
[4]British United Turkeys of America.
[5]Goldsboro Milling Co., Goldsboro, NC, USA.

5/16-pellet form. Composite feed samples from each diet were taken immediately after manufacture and subjected for analysis for crude protein, fat, ash, Ca, and P.

The enzymes were applied to the feed in a 500 kg capacity horizontal double ribbon mixer and then bagged into 20kg bags. The three enzymes (Natugrain Blend®, Lyxasan Forte®, and Phospholipase) were applied as a fine spray onto the pelleted feed during mixing using a plant mister. The enzymes were added to the diet in amounts recommended by the supplier. The enzymes were diluted in water to a volume of 1 litter, such that the dosage per tonne of feed were 150g of Natugrain Blend®, 100g and 50g of Lyxasan Forte® (Lyxasan-100 and Lyxasan-50, respectively), and 100g of Phospholipase. The wheat sample used in this experiment was previously determined to be a low-AME wheat (2,216 kcal/kg) (Santos Jr. et al., 2000) because wheat samples assaying less than 2,850 kcal/kg are arbitrarily classified as low-AME wheat (Mollah et al., 1983). This wheat, from Western Canada, had been exposed to a damaging frost event during grain filling stage (mid-milk to early-dough stage).

The purpose of experiment was to evaluate the influence of dietary enzyme supplementation on growth performance (body weight, feed consumption, feed conversion ratio, mortality) and litter condition of turkeys fed wheat-based diet. To achieve this objective forty pens of the experimental house were assigned to this trial, such that the inside 20 pens were contained in blocks two and three and the outside 20 pens were contained in blocks one and four. Five dietary treatments were randomly assigned to pens within each of the four blocks so that position effects within the turkey house were removed statistically. The dietary treatments were randomly assigned to pens

using the Proc-Plan procedure of SAS® (SAS, 1996). Each pen of 12 turkeys, the experimental unit, were subjected to one of 5 dietary enzyme treatments from 1 to 128 (0-18 wk) days of age, as follows: (1) unsuplemented control, (2) Natugrain Blend, (3) Lyxasan-50, (4) Lyxasan-100, and (5) Phospholipase. Each treatment combination was replicated in 8 pens (2 pens per block).

Feed consumption and body weights (by pen and by individual bird, respectively) were recorded at 0, 2, 4, 8, 12, 16, and 18 weeks of age. Caked litter from each pen was removed (11, 14, 16, and 18 wk of age) when required to maintain acceptable litter conditions, and the weight of the caked litter was recorded.

Experiment 2: digestibility trial

Experiment 2 used a similar facility, husbandry practice, and animals as in experiment 1 except there were a few modifications to the experimental diets and pen facilities to accommodate the objectives of determining dietary energy utilization. The experimental diets of the digestibility trial was formulated to contain 0.8% Celite[6] (w/w), a source of acid-insoluble ash (AIA), as an indigestible marker (Table 3).

The objective of the digestability trial was to evaluate the influence of enzyme addition on energy utilization (AMEn) and digesta viscosity of turkeys fed wheat-based diets. Twenty-five turkeys were maintained in each of 8 conventional floor-pens and they were randomly assigned to one of 4 dietary treatments: (1) unsupplemented control; (2) Natugrain Blend; (3) Lyxasan-100; and (4) Phospholipase. At 56 days of age (8 wk), 8 birds per pen were banded and sampled for digesta viscosity. The remaining twelve birds per pen were weighed and then transferred to 8 digestibility pens for AMEn assay. The

[6] Celite™, A diatomite product, Food Chemicals Codex Grade. Celite Corp., Lompar, CA 93436.

digestibility pens were modified floor-pens with plastic slats secured upon 2x4 lumber frames (Figure 2). The 2.5cm slats were spaced 2.5cm apart, which allowed the excreta of the birds to fall onto a plastic sheet under the pen. The plastic sheet could be pulled from under the slatted floor to facilitate excreta sampling. New plastic sheets were replaced after every excreta collection time.

The birds used for the digesta viscosity measurement were fasted over night (8 h) and then given *ad libitum* access to feed for 3 h before sampling. Eight birds from each pen were weighed and euthanized with carbon dioxide. Then about 2 grams of digesta was gently expressed from the terminal part of ileum (midway between the Meckel's diverticulum and the ileo-caecal junction to 1 cm above the ileo-caecal junction), placed into micro-centrifuge tubes, centrifuged at 3,000 rpm for 2 min, and approximately 500 µl of the supernatant collected. The viscosity of the supernatant was determined using Brookfield Digital Viscometer LVDVII+CP[7] at 15ºC according to the method described by the Brookfield Digital Viscometer Operating Instructions Manual[8]. Each bird was considered an experimental unit for the statistical analysis of the viscosity.

The digestibility experiment was done with toms from 9 to 18 wk of age. Excreta were collected twice per week from several locations on the plastic sheets from each pen, and care was taken to avoid contamination of feathers, scales and other debris. After each collection, the excreta samples were sealed in moisture-impermeable sample bags and stored at -20ºC until they were processed for nutrient analysis. Before analysis, the frozen-excreta were placed on the laboratory table to thaw overnight at room

[7]Brookfield Engineering Laboratories Inc., Stoughton, MA.
[8]Brookfield Digital Viscosimeter, Model DV-II+ Version 2.0, Operating Instructions Manual No. M/92-161-F1193, Brookfield Engineering Laboratories Inc., Stoughton, MA.

temperature. Then the two-200 g samples of excreta from each pen collected during each week were blended[9] together to slurry after the addition of approximately 100 ml of distilled water to form a pool sample. The pH of the fecal slurry was then adjusted to 5.4 by the addition of sulfuric acid (0.1 N) to minimize microbial fermentation of the fecal nitrogen during overnight drying in a forced-air convection oven[10] at 70ºC. The dried samples were ground in a blender[9], and then stored at -20ºC before analysis to determine AMEn. Gross energy content of feed and dried-excreta samples were determined by combustion in an adiabatic oxygen bomb calorimeter[11]. Celite recovery was performed using the method described by Vogtmann et al. (1975). Moisture analysis was obtained by drying 3 to 5 g of the materials for 6 h in a forced-air convection oven[10] at 105ºC. Nitrogen content of all feed and dried-excreta samples was determined using a Kjeldahl automatic analyzer[12]. The AMEn values were determined using the equations shown on the footnotes of the Table 10. The values of AMEn were calculated relative to the acid-insoluble ash marker, and corrected to zero N-retention, by using a value of 8.22 kcal/g nitrogen retained (Hill and Anderson, 1958).

Statistical analysis

All data were analyzed using the general linear models procedure for analysis of variance (ANOVA) (SAS, 1996). Pen means served as the experimental units for statistical analysis, unless otherwise stated. Variables having a significant F-test were compared using the least-squares-means (lsmeans) function of SAS (SAS, 1996), and

[9]Waring Commercial Laboratory Blender, Model # 31BL91-7010, Torrington, CT.
[10]Blue-M, Model # DC-326F, Serial # DC-509, Blue M, Atlanta, GA.
[11]IKA Calorimeter System C5000 control, IKA® Werke Labortechnik, Staufen, Germany.
[12]KJELTEC Auto 1030 Analyzer, Tecator, Sweden.

were considered to be significant at P<0.05. All percentage data was transformed to arc sine of the square root before analysis.

Animal ethics

The experiments reported herein were conducted according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) at North Carolina State University. All husbandry practices and euthanasia were done with full consideration of animal welfare.

3.4 RESULTS

3.4.1 Experiment 1: performance trial

Performance

All the enzyme supplementation treatments significantly (P<0.05) increased body weight (BW) of turkeys throughout the experiment, although their effect on 18-weeks body weight was marginal (15.8 vs. 15.3 kg, P=0.062) (Table 4). In comparison to the unsupplemented control treatments, enzyme supplementation improved BW from 3.1 to 28.1%. Although there was no significant treatment effect observed in the feed consumption (Table 5), the enzyme treatments clearly improved feed conversion ratio (FCR) in comparison to the unsuplemented control treatment (Table 6). The phospholipase supplementation had a greater effect on feed conversion ratio during the starting phase (0-2 wk) than during the growing-finishing phase (3 to 18 wk). In contrast, Lyxasan-100 was most effective during the growing-finishing period. Natugrain Blend had an intermediate improvement on FCR throughout the entire period studied. The

effect of endoxylanase supplementation on the FCR was dose-dependent, as indicated by the difference between the Lyxasan-50 and Lyxasan-100 treatments.

The average final cumulative mortality rate (0 to18 wk) was 12% (Table 7) with statistical significance ($P<0.05$) observed only during the period from 16 to 18 weeks of age. During this period (16-18 wk), treatments supplemented with Natugrain Blend and Lyxasan-50 had the highest mortality rate (8.3 vs. 3.13 %, $P<0.05$), and treatments supplemented with Lyxasan-100 had the lowest mortality rate (1.0 vs. 5.6 %, $P<0.05$). In general, enzyme supplementation increased the growth performance of turkeys consuming wheat-based diets.

Caked litter

The results on the effect of different exogenous enzyme supplementation on cumulative caked litter are presented in Table 8. During the period of 0 to 11 weeks of age, the highest amount of cumulative caked litter was found among the control treatment and the lowest value was among treatments supplemented with Lyxasan-100 ($P<0.05$). Dietary enzyme supplementation reduced the amount of caked litter by 40% in comparison to the control group ($P<0.10$). Generally, treatments supplemented with Lyxasan-100 presented the lowest values for cumulative caked litter.

3.4.2 Experiment 2: digestibility trial

Ileum digesta viscosity

The ileum digesta viscosities of turkeys fed the wheat-based diets supplemented with phospholipase were 48% higher ($P< 0.001$) than those fed the other dietary treatments (Table 9). There was no difference between the control birds and turkeys fed

diets supplemented with enzyme preparations containing endoxylanase (Lyxasan-100 and Natugrain Blend treatments) on the ileum digesta viscosity at 56 days of age.

Apparent metabolizable energy nitrogen-corrected (AMEn)

Dietary AMEn was increased by phospholipase from 9 to 11 wk, whereas treatments supplemented with Natugrain Blend and Lyxasan-100 resulted in the highest dietary energy utilization after 12 wk of age (Table 10 and Figure 3). There was no statistical difference between treatments from 15 to 17 weeks, but the treatment group receiving the dietary phospholipase supplementation had lower AMEn than the other treatments at 18 weeks. Although not amenable to statistical analysis, there was a clear increasing trend in AMEn throughout the trial. Diets supplemented with Natugrain Blend and Lyxasan-100 produced a more consistent increase in AMEn than the control diet or the diet supplemented with Phospholipase. A great fluctuation in the AMEn values was observed throughout the trial in the phospholipase and unsupplemented control treatment groups.

3.5 DISCUSSION

This research evaluated the effect of different sources of supplemental enzymes on growth performance and energy utilization of turkeys fed diets containing an inferior-quality of wheat. The wheat source used in this experiment was previously determined in our laboratory to be a low-AME wheat (2,216 kg/kg) when fed to turkeys (Santos Jr. et al., 2000). This wheat was frost-damaged during the grain filling (mid-milk to early-dough) stage of seed development. Frost damaged during grain filling reduced nutrient

utilization of wheat presumably by increased relative content of NSP to starch (Santos Jr. et al., 2000). We chose this wheat because there is evidence that responses to enzymes are greatest with low-AME wheat because of its higher NSP content (Choct et al., 1994).

As hypothesized, all the enzyme treatments improved growth performance and increased energy utilization throughout the experiment, but this effect was age dependent. Enzyme supplementation increased BW and decreased feed conversion ratio throughout the trial. No treatment effects were observed on feed consumption, although differences were noted in AMEn. Phospholipase treatment was most effective in reducing FCR and increasing AMEn during the early phase of growth, while Lyxasan-100 was most effective towards the later phases of growth, and Natugrain Blend had intermediate to best results throughout the study.

Endoxylanase activity contributes the main effect of Natugrain Blend and Lyxasan Forte. Evidently, degradation of the NSP in wheat by the added endoxylanase is one of the main reasons for the better performance of endoxylanase treated birds. Endoxylanase degrades the xylan backbone of arabinoxylan into smaller units, which disrupts the water holding capacity of the NSP (Scott and Boldaji, 1997) and reduces the viscosity of the digesta in the small intestine (Bedford and Schulze, 1998; Choct et al., 1999b). Reduced digesta viscosity increases the diffusion rates of nutrients and endogenous enzymes, enabling the bird to digest and absorb more nutrients that lead to increased growth performance (Pawlik et al., 1990). Also, endoxylanase inhibits the proliferation of the fermentative microorganisms in the small intestine by increasing the digesta passage rate and nutrient digestion (Choct et al., 1999b). As the microflora characteristics are changed by enzyme supplementation, there is a decrease in the adverse

effects of microbial fermentation, including the reduction in fat digestion by the deconjugation of bile salts (Langhout, 1999) and an increased competition between the host and the microflora for available nutrients (Bedford, 1995; Choct et al., 1996; Langhout et al., 2000). Therefore, endoxylanase improved performance and energy utilization by improving digestion and absorption of nutrients, and decreasing the fermentation of microorganisms in the intestinal tract.

The effect of dietary enzyme supplementation on the growth performance of turkeys appears to be dependent upon the dose of endoxylanase It is possible that the level of endoxylanase in the Lyxasan-50 treatment ($\geq$ 2,250 EXU/kg feed) was not enough to completely breakdown the high level of xylan backbone present in the high inclusion level of wheat, since the double dosage of endoxylanase used in Lyxasan-100 treatment ($\geq$ 5,500 EXU/kg feed) was able to produce a superior effect. Several researchers have shown dose-dependent responses for dietary supplementation of NSP enzymes (Hesselman et al., 1982; Petterson and Aman, 1989; Bedford and Classen, 1992). Crouch et al. (1997) stated that one possible reason for the general lack of response with some enzymes might be the presence of a higher content of water-soluble pentosans (arabinoxylan) in some cultivars of wheat. Other authors, attributes it to the low level of enzyme (Odetallah, 2000), and/or not an appropriate enzyme for the type of grain (Friesen et al., 1992).

The beneficial effect of endoxylanase may be enhanced by synergy with other enzymes in a blended enzyme preparation. In comparison to Lyxasan Forte and Phospholipase treatment, dietary supplementation of Natugrain Blend, which contained endoxylanase and other enzymes, improved performance and AMEn regardless of turkey

age. Even though the level of endoxylanase activity in the feed (at least 5,500 EXU/kg feed) was the same for both the Lyxasan-100 and Natugrain Blend treatment groups, the presence of hemicellulase (e.g. pectinase), cellulase, and protease (BASF, 1997) in the Natugrain Blend mixture may have afforded this enzyme product the greatest degree of versatility for use in wheat-rich turkey diets. Similar results have been reported by Odetallah et al. (2002), who observed that enzyme mixtures with high endoxylanase activity, like Natugrain Blend, resulted in best growth performance in turkeys fed wheat-based diets. Ravindran et al. (1999) stated that there is considerable synergy in activities among enzymes when they are supplemented as blended preparation.

It is widely accepted that endoxylanase improves fat digestability of birds fed wheat-based diets by decreasing viscosity and microbial fermentation in the gut. In this study we investigated the direct improvement of fat digestion by supplementation of phospholipase. To our knowledge, this study is the first to report on the dietary supplementation of phospholipase to poultry diets, considering that fat digestion is compromised in wheat-based diets (Choct and Annison, 1992a; Friesen et al., 1992). Endogenous phospholipase A_2 (PLA) catalyzes the hydrolysis of ester bond at *sn*-2 position of glycerophospholipids (GPL), producing fatty acids and lysophospholipids (e.g. Lyso-phosphatidylcholine or Lyso-PC). The fatty acids are then absorbed from the lumen as part of the fat micelle. Lyso-PC, the predominant GPL product in the luminal content, is essential for the emulsification of water-insoluble lipids (Homan and Jain, 2001). Lipid emulsification is the first stage of lipid digestibility. Lyso-PC is an important amphiphile molecule, which acts to stabilize microdroplets of triglycerides, cholesterol, and other nonpolar dietary lipids that are otherwise insoluble in the aqueous

environment of the intestinal contents (Carey et al., 1983). Also, PLA influences the capacity of the enterocyte to transport absorbed lipids into the circulation, since its capacity depends on cellular phosphatidylcholine synthesis controlled by the hydrolysis of phosphatidylcholine in the lumenal contents (Carey et al., 1983). Additionally, PLA may possess an intrinsic secretin-releasing activity (Chang et al., 1999) that stimulates the release of pancreatic secretion and bicarbonate in the duodenum, which enhances the digestion and absorption of others macronutrients.

We hypothesized that the exogenous source of phospholipase would act on a similar matter as endogenous PLA. Therefore, dietary phospholipase supplementation may alleviate the effects of dietary NSP by facilitating the formation of micelles of triglyceride, cholesterol, and other nonpolar dietary lipids, enhancing the capacity of the enterocytes to absorb lipids, and increasing the digestion of the other macronutrients. This hypothesis is consistent with the positive response observed in the growth performance and AMEn in turkeys up to 12 weeks of age. However, the beneficial effect of dietary phospholipase supplementation on growth performance diminished during the growing-finishing phases, which may be associated with the increase ability of the birds to digest lipid as the birds get older.

These data demonstrate that the effect of enzyme treatment is age-dependent. Phospholipase had a significantly better affect than the pentosanase enzyme treatment during the beginning of the trial, whereas the endoxylanase-containing enzyme products were more effective during later half end of the trial. These differences could be attributed to differences in gut maturity among young and older birds. The sensitivity of young birds to high dietary NSP is associated with their limited synthesis of lipase

enzyme and inefficient recycling of bile salts (Sell et al., 1986; Martin and Farrel, 1998; Krogdahl and Sell, 1989), and their incapability to replace lost bile as efficiently as older birds (Serafin and Nesheim, 1970). Thus, the antinutritive effects of dietary NSP from wheat in young birds were ameliorated more effectively by the phospholipase supplementation *via* enhancement of lipid digestion than by the xylanase supplementation. In contrast, older birds have a more mature and stable gut ecosystem with greater fermentation capacity than younger birds and they are more tolerant to the effects of NSP (Choct and Annison, 1992b; Veldman and Vahl, 1994). The stability of the microflora in older birds may come from acclimatization of the digestive system to the diet through changes in the type and number of microorganism (Petersen et al., 1999). A greater variability between birds is found in the numbers and types of microorganisms in young birds than in older birds (Annison, 1989). As discussed by Ferket (1991), the gut ecosystem becomes more resistant to change as the number of microbes increase. Thus, endoxylanase had a superior effect on older birds compared to phospholipase, because older birds have a more mature gut with a greater capacity for lipid digestion than younger birds.

Another possible reason for the lower performance and AMEn shown by the birds fed phospholipase as compared to the ones supplemented with endoxylanase during the growing-finishing phase could be due to the effect of phospholipase on increasing gut viscosity. Phospholipase increases the stability of the emulsion, which is positively correlated to the viscosity of the oil (Jumaa and Muller, 1998; Jumaa et al., 1998, Chung et al., 2001). In our study, we observed significantly higher ileum digesta viscosity of toms fed wheat-based diets supplemented with phospholipase than the other treatments at

8 weeks of age. However, no differences in the ileum digesta viscosity were observed between control and endoxylanase treatments. Similar results have been seen by other investigators who observed no decrease in digesta viscosity but an increase in performance and nutrient utilization from endoxylanase supplementation as compared to birds fed a NSP-rich diet without enzyme supplementation (Silva and Smithard, 2002; Veldman and Vahl, 1994).

Regardless of the dietary treatment, AMEn increased as the birds aged. These findings are in substantial agreement with many other authors that have observed higher dietary energy digestability among older birds than younger ones (Salih et al., 1991; Scott and Boldaji, 1997; Fuente et al., 1998; Smulikowska and Mieczkowska, 2000), indicating that older birds utilize cereal-based diets better. Fuente et al. (1998) reported that the AME of 30 days old chickens was 4.6% higher than that of 10 days old chicks. Salih et al. (1991) also showed that negative effects of high barley levels in broiler diets decreased as the birds grew older. Jaroni et al. (1999) observed better fat utilization at 60 wk of age than at 50 wk of age in laying hens supplemented with wheat middlings. Brenes (1992) explained that younger birds are more sensitive to the negative effects of antinutritional factors in cereal and other raw material due to the immaturity of their digestive tract.

The increasing trend in AMEn as birds aged was most consistent among birds fed diets supplemented with Natugrain Blend and Lyxasan-100. The phospholipase-supplemented and control diets had highly variable AMEn values throughout the trial (figure 3). The AMEn observed in the phospholipase and control treatments deviated significantly from the other treatments at 12 weeks of age, which coincided with the

change from diet 3 (65.66% of wheat) to diet 4 (73.54% of wheat). This observation demonstrated that dietary endoxylanase supplementation increased the bird's tolerance to high levels of wheat by hydrolyzing the xylan backbone of arabinoxylan. Similarly, Choct et al. (1995) reported that enzyme supplementation reduced the variability in nutrient utilization in diets with different levels of NSP. They observed that endoxylanase improved significantly the nutritive value of a diet containing a particular a low-AME wheat with a high level of NSP. Thus, endoxylanase addition not only improved energy utilization, but it also led to a more consistent and uniform nutrient utilization.

The dietary enzyme supplementation treatments used in this study reduced the amount of caked litter accumulation in the pens in comparison to the control treatment. This observation could be attributed to a reduction in osmotically active compounds in the gut because of improved digestion (Choct and Annison, 1992a; Choct et al., 1996). This result is in accordance with the results of other investigators who reported that the use of exogenous enzyme in poultry feed improved litter quality (Pettersson and Aman, 1988; Veldman and Vahl, 1994; Fischer and Classen, 2000), and reduced the incidence of health problems associated with poor litter quality. Such problems include pododermatitis, leg mobility abnormalities, and respiratory problems, which could lead to increased mortality rate. Even though no significant difference were observed in the cumulative mortality (0-18 wk), significant treatment effects on mortality rate were observed during the period of 16 to 18 weeks of age. Natugrain Blend and Lyxasan-50 treatments had the highest mortality attributed to cardiopulmonary disorder. In agreement, Odetallah et al. (2002) reported that mortalities that occurred among toms fed wheat-based diets without enzyme supplementation were primarily due to enteric

infections, whereas mortalities among the enzyme treatments groups were due to cardiopulmonary disorders often associated with the most rapidly growing birds.

In conclusion, the use of appropriate enzymes is an effective way to deal with grains with high NSP content in poultry diets. Growth performance and energy utilization of turkeys fed wheat-based diets can be significantly enhanced by phospholipase supplementation of starter feeds and endoxylanase (Lyxasan-100) supplementation of growing and finishing feeds; however, a natural blend of enzymes (Natugrain Blend) may provide a positive response regardless of turkey age. Evidently, phospholipase alleviated the adverse effect of dietary NSP by improving fat digestion and absorption in young turkeys, whereas endoxylanase is more effective in older birds that have greater digestive capacity and more mature gut microbial ecosystem. However, further studies are required to better elucidate the phospholipase mechanism of action. Future investigations with phospholipase in NSP-rich diets fed to broiler are also warranted.

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3.6 TABLES AND FIGURES

TABLE 1: Enzyme activity in the products[1] and in the feed, and rate of application used in the experimental diets[2]

Enzymes	Activity (units/g DM)[3]	Application rate (g/tonne feed)	Enzyme activity (units/kg feed)
Natugrain Blend® (66%)	≥ 36,600 EXU/g ≥ 9,000 BGU/g	150	≥ 5,500 EXU/kg ≥ 1,350 BGU/kg
Phospholipase	5,000 PLU/g	100	500 PLU/kg
Lyxasan Forte®	≥ 56,000 EXU/g	100 and 50[4]	≥ 5,500 EXU/kg[5] ≥ 2,250 EXU/kg[6]

[1]Products supplied by BASF (BASF AG, 67059 Ludwigshafen, Germany).
[2]Data from BASF, 2001.
[3]EXU= endoxylanase units, BGU= β-glucanase units, PLU= phospholipase units.
[4]Lyxasan Forte® was used at 2 application rate (Lyxasan-100 and Lyxasan-50 treatments).
[5]Concentration in feed from Lyxasan Forte® at 100 g/tonne feed.
[6]Concentration in feed from Lyxasan Forte® at 50 g/tonne feed.

TABLE 2: Composition and calculated nutrient content of the experimental diets fed to turkey toms from 1 to 128 days of age (Experiment 1)

Ingredients	Feed 1 (1 to 28 d)	Feed 2 (29 to 56 d)	Feed 3 (57 to 84 d)	Feed 4 (85 to 112 d)	Feed 5 (113 to 128 d)
	(%)				
Wheat	46.82	61.18	66.90	75.09	76.45
Soybean meal (48% CP)	42.83	23.25	19.92	10.35	11.44
Poultry meal (60% CP)	0.00	5.00	1.77	2.62	0.00
Poultry Fat	3.85	4.62	6.21	7.45	8.00
Dicalcium phosphate (18.5% P)	2.42	3.13	1.22	0.89	0.88
Limestone	1.66	0.17	1.28	1.00	1.01
Crude soy oil	1.00	1.00	1.00	1.00	1.00
Salt	0.35	0.19	0.36	0.28	0.31
Mineral premix[1]	0.20	0.20	0.20	0.20	0.20
Choline Cl (60%)	0.18	0.11	0.07	0.03	0.00
DL-Methionine	0.16	0.16	0.11	0.09	0.05
L-Threonine	0.00	0.14	0.11	0.19	0.00
L-Lysine HCL	0.08	0.41	0.41	0.36	0.20
Vitamin premix[2]	0.20	0.20	0.20	0.20	0.20
Sand or enzyme[3]	0.10	0.10	0.10	0.10	0.10
Selenium premix[4]	0.15	0.15	0.15	0.15	0.15
Calculated analysis					
ME, kcal/kg	2,700	2,800	2,900	3,000	3,050
Crude protein, %	27.0	23.0	20.0	17.0	15.8
Lysine, %	1.55	1.40	1.23	0.95	0.80
Methionine + Cysteine, %	1.00	0.90	0.76	0.67	0.61
Threonine, %	0.97	0.90	0.76	0.71	0.50
Calcium, %	1.25	1.00	0.90	0.75	0.65
Non-phytate phosphorus, %	0.60	0.85	0.42	0.38	0.32
Sodium, %	0.18	0.15	0.18	0.15	0.15

[1]Supplied the following per kilogram of feed: 120 mg Zn as $ZnSO_4 \cdot H_2O$; 120 mg MN as $MnSO_4 \cdot H_2O$; 80 mg Fe as $FeSO_4 \cdot H_2O$; 10 mg Cu as $CuSO_4$; 2.5 mg I as $Ca(IO_3)_2$; 1.0 mg Co as $CoSO_4$.

[2]Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B_{12}, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL-α-tocopheryl acetate.

[3]Enzyme treatments were supplemented with enzyme products that accounted as a dry ingredient (0.1%) and an equivalent amount of sand was applied to the unsuplemented control treatment.

[4]Selenium premix provided 0.3 ppm Se from sodium selenate.

TABLE 3: Composition and calculated nutrient content of the diets fed to turkey toms from 56 to 128 days of age on the AMEn assay (Experiment 2)[6]

Ingredient	Feed 3 (56 to 84 d)	Feed 4 (85 to 112 d)	Feed 5 (113 to 128 d)
	(%)		
Wheat	65.66	73.54	76.45
Soybean meal (48% CP)	19.58	10.63	11.04
Poultry meal (60% CP)	2.30	2.75	0.00
Dicalcium phosphate (18.5% P)	6.54	7.83	7.60
Poultry Fat	1.16	0.89	0.88
Limestone	1.25	0.99	1.01
Crude soy oil	1.00	1.00	1.00
Salt	0.35	0.28	0.31
Mineral premix[1]	0.20	0.20	0.20
Choline Cl (60%)	0.07	0.01	0.00
DL-Methionine	0.11	0.09	0.05
L-Threonine	0.11	0.19	0.00
L-Lysine HCL	0.41	0.35	0.20
Vitamin premix[2]	0.20	0.20	0.20
Celite[3]	0.80	0.80	0.80
Sand or enzyme[4]	0.10	0.10	0.10
Selenium premix[5]	0.15	0.15	0.15
Calculated analysis			
ME, kcal/ kg	2900	3000	3050
Crude protein, %	20.0	17.0	15.8
Lysine, %	1.23	0.95	0.80
Methionine + Cysteine, %	0.76	0.67	0.61
Threonine, %	0.76	0.71	0.50
Calcium, %	0.90	0.75	0.65
Available phosphorus, %	0.42	0.38	0.32
Sodium, %	0.18	0.15	0.15

[1]Supplied the following per kilogram of feed: 120 mg Zn as $ZnSO_4 \cdot H_2O$; 120 mg MN as $MnSO_4 \cdot H_2O$; 80 mg Fe as $FeSO_4 \cdot H_2O$; 10 mg Cu as $CuSO_4$; 2.5 mg I as $Ca(IO_3)_2$; 1.0 mg Co as $CoSO_4$.

[2]Supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B_{12}, 0.08 mg; ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL-α-tocopheryl acetate.

[3]Celite (Celite™, Celite Corp., Lompar, CA 93436), a source of acid-insoluble ash, were used as an indigestible marker.

[4]Enzyme treatments were supplemented with enzyme products that accounted as a dry ingredient (0.1%) and an equivalent amount of sand was applied to the unsuplemented control treatment.

[5]Selenium premix provided 0.3 ppm Se from sodium selenate.

[6]Prior to 56 d of age, the birds were fed Feed 1 (1-28 d) and Feed 2 (29-56) as shown in table 2.

TABLE 4: Effects of different exogenous enzyme supplementation on body weight of turkey toms fed wheat-based diets from 0 to 18 weeks of age

Treatment	2 wk	4 wk	8 wk	12 wk	16 wk	18 wk
			(kg)			
Control[1]	0.288[b]	0.922[c]	4.051[b]	7.958[b]	12.565[b]	15.290
Natugrain Blend[2]	0.327[a]	1.007[ab]	4.294[a]	8.420[a]	13.305[a]	15.953
Lyxasan-50[3]	0.336[a]	1.020[a]	4.280[a]	8.386[a]	13.131[a]	15.528
Lyxasan-100[4]	0.322[a]	0.981[b]	4.186[ab]	8.403[a]	13.291[a]	15.953
Phospholipase[5]	0.321[a]	1.004[ab]	4.204[a]	8.282[a]	13.107[a]	15.643
Statistical analysis						
SEM(32)[6]	0.0056	0.0128	0.0518	0.1096	0.1603	0.1801
P-value	0.0001	0.0001	0.0174	0.0286	0.0168	0.0617

[a-c]Means with different superscripts within a column differ significantly ($P < 0.05$). There were no significant differences in poults starting weights at 1 d of age (60g).
[1]Unsupplemented wheat/SBM basal diet.
[2]150g of Natugrain Blend/tonne of basal diet provided at least 5,500 EXU/kg feed and 1,350 BGU/kg feed.
[3]50g of Lyxasan Forte/tonne of basal diet provided at least 2,250 EXU/kg feed.
[4]100g of Lyxasan Forte/tonne of basal diet provided at least 5,500 EXU/kg feed.
[5]100g of Phospholipase/tonne of basal diet provided 500 PLU/kg feed.
[6]SEM(32)= Standard Error of the mean with 32 degrees of freedom.

TABLE 5: Effects of different exogenous enzyme supplementation on cumulative feed consumption of turkey toms fed wheat-based diets from 0 to 18 weeks of age

Treatment	0 to 2 wk	0 to 4 wk	0 to 8 wk	0 to 12 wk	0 to 16 wk	0 to 18 wk
			(kg)			
Control[1]	0.725	1.766	7.012	16.254	28.630	36.775
Natugrain Blend[2]	0.716	1.774	7.198	16.712	29.680	37.657
Lyxasan-50[3]	0.815	1.873	7.138	16.444	29.015	36.822
Lyxasan-100[4]	0.749	1.798	7.027	16.343	29.052	36.706
Phospholipase[5]	0.678	1.731	7.278	16.628	29.496	37.220
Statistical analysis						
SEM(32)[6]	0.0319	0.0403	0.0756	0.2012	0.3663	0.4147
P-value	0.0601	0.1639	0.0867	0.4718	0.2923	0.4562

[a,b]Means with different superscripts within a column differ significantly (P< 0.05).
[1]Unsupplemented wheat/SBM basal diet.
[2]150g of Natugrain Blend/tonne of basal diet provided at least 5,500 EXU/kg feed and 1,350 BGU/kg feed.
[3]50g of Lyxasan Forte/tonne of basal diet provided at least 2,250 EXU/kg feed.
[4]100g of Lyxasan Forte/tonne of basal diet provided at least 5,500 EXU/kg feed.
[5]100g of Phospholipase/tonne of basal diet provided 500 PLU/kg feed.
[6]SEM(32)= Standard Error of the mean with 32 degrees of freedom.

TABLE 6: Effects of different exogenous enzyme supplementation on cumulative feed conversion ratio[7] of turkey toms fed wheat-based diets from 0 to 18 weeks of age

Treatment	0 to 2 wk	0 to 4 wk	0 to 8 wk	0 to 12 wk	0 to 16 wk	0 to 18 wk
	(g/g)					
Control[1]	3.201[a]	2.051	1.775[a]	2.079[a]	2.327[a]	2.451[a]
Natugrain Blend[2]	2.695[b]	1.904	1.706[c]	2.003[bc]	2.255[b]	2.384[b]
Lyxasan-50[3]	2.968[ab]	1.954	1.724[ab]	1.992[bc]	2.232[b]	2.399[ab]
Lyxasan-100[4]	2.883[ab]	1.956	1.713[c]	1.970[c]	2.212[b]	2.322[c]
Phospholipase[5]	2.603[b]	1.847	1.761[ab]	2.025[b]	2.261[b]	2.400[ab]
Statistical analysis						
SEM(32)[6]	0.1425	0.0477	0.0139	0.0155	0.0172	0.0183
P-value	0.0466	0.0624	0.0039	0.0003	0.0008	0.0006

[a-c]Means with different superscripts within a column differ significantly (P< 0.05).
[1]Unsupplemented wheat/SBM basal diet.
[2]150g of Natugrain Blend/tonne of basal diet provided at least 5,500 EXU/kg feed and 1,350 BGU/kg feed.
[3]50g of Lyxasan Forte/tonne of basal diet provided at least 2,250 EXU/kg feed.
[4]100g of Lyxasan Forte/tonne of basal diet provided at least 5,500 EXU/kg feed.
[5]100g of Phospholipase/tonne of basal diet provided 500 PLU/kg feed.
[6]SEM(32)= Standard Error of the mean with 32 degrees of freedom.
[7]Equation to determine feed conversion ratio (FCR), g/g.
FCR = total pen feed consumed / total weight gained including mortality

TABLE 7: Effects of different exogenous enzyme supplementation on mortality rate of turkey toms fed wheat-based diets from 0 to 18 weeks of age

Treatment	0 to 4 wk	4 to 12 wk	12 to 16 wk	16 to 18 wk	0 to 18 wk
			(%)		
Control[1]	0.000	6.250	5.208	5.208[ab]	16.667
Natugrain Blend[2]	2.083	3.125	4.167	9.375[a]	16.667
Lyxasan-50[3]	0.000	5.208	2.083	7.292[a]	14.583
Lyxasan-100[4]	0.000	2.083	2.083	1.042[b]	5.208
Phospholipase[5]	1.042	4.167	1.042	3.125[ab]	8.333
Statistical analysis					
SEM(32)[6]	0.0265	0.0588	0.0543	0.0549	0.0712
P-value	0.2187	0.7108	0.4550	0.0378	0.1824

[a,b]Means with different superscripts within a column differ significantly (P< 0.05). The data was transformed to arc sine of the square root before statistical analysis.
[1]Unsupplemented wheat/SBM basal diet.
[2]150g of Natugrain Blend/tonne of basal diet provided at least 5,500 EXU/kg feed and 1,350 BGU/kg feed.
[3]50g of Lyxasan Forte/tonne of basal diet provided at least 2,250 EXU/kg feed.
[4]100g of Lyxasan Forte/tonne of basal diet provided at least 5,500 EXU/kg feed.
[5]100g of Phospholipase/tonne of basal diet provided 500 PLU/kg feed.
[6]SEM(32)= Standard Error of the mean with 32 degrees of freedom.

TABLE 8: Effects of different exogenous enzyme supplementation on cumulative caked litter[7] per turkey toms fed wheat-based diets from 0 to 18 weeks of age

Treatment	0 to 11 wk	0 to 14 wk	0 to 16 wk	0 to 18 wk
	(Kg)			
Control[1]	1.823[a]	5.157	7.524	9.666
Natugrain Blend[2]	1.205[ab]	3.248	4.516	5.576
Lyxasan-50[3]	1.381[ab]	3.979	5.495	6.881
Lyxasan-100[4]	0.847[b]	2.549	3.700	5.016
Phospholipase[5]	1.440[ab]	2.984	4.153	5.513
Statistical analysis				
SEM(32)[6]	0.2156	0.6786	1.0074	1.2224
P-value	0.0467	0.0839	0.0830	0.0729

[a,b]Means with different superscripts within a column differ significantly (P< 0.05).
[1]Unsupplemented wheat/SBM basal diet.
[2]150g of Natugrain Blend/tonne of basal diet provided at least 5,500 EXU/kg feed and 1,350 BGU/kg feed.
[3]50g of Lyxasan Forte/tonne of basal diet provided at least 2,250 EXU/kg feed.
[4]100g of Lyxasan Forte/tonne of basal diet provided at least 5,500 EXU/kg feed.
[5]100g of Phospholipase/tonne of basal diet provided 500 PLU/kg feed.
[6]SEM(32)= Standard Error of the mean with 32 degrees of freedom.
[7]Equation to determine cumulative caked litter per bird (CCL), kg.
CCL = kilogram of caked litter removed from a pen / number of birds in the pen, adjusted for mortality

TABLE 9: Influence of different sources of exogenous enzymes on the ileum viscosity of 56 days-old (8 wk) turkey toms fed wheat-based diets

Treatment	Mean viscosity (Centipoise, cP)[6]
Control[1]	7.118[b]
Natugrain Blend[2]	6.705[b]
Lyxasan-100[3]	7.389[b]
Phospholipase[4]	13.551[a]
Statistical analysis	
SEM(58)[5]	1.2892
P-value	0.0009

[a,b]Means with different superscripts within a column differ significantly (P< 0.05).
[1]Unsupplemented wheat/SBM basal diet.
[2]150g of Natugrain Blend/tonne of basal diet provided at least 5,500 EXU/kg feed and 1,350 BGU/kg feed.
[3]100g of Lyxasan Forte/tonne of basal diet provided at least 5,500 EXU/kg feed.
[4]100g of Phospholipase/tonne of basal diet provided 500 PLU/kg feed.
[5]SEM(58)= Standard Error of the mean with 58 degrees of freedom.
[6]Centipoise (cP). A centimeter-gram-second unit of dynamic viscosity equal to one dyne-second per square centimeter.

TABLE 10: Effects of different exogenous enzyme supplementation on apparent metabolizable energy nitrogen-corrected[6] (AMEn) of turkey toms fed wheat-based diets from 9 to 18 weeks of age

Treatment	9 wk	10 wk	11 wk	12 wk	13 wk	14 wk	15 wk	16 wk	17 wk	18 wk
					(kcal / kg)					
Control[1]	3,260	3,029^{b}	3,117^{b}	2,789^{b}	3,158^{c}	3,458^{b}	3,475	3,458	3,442	3,615^{a}
Natugrain blend[2]	3,310	3,114^{b}	3,217^{b}	3,287^{a}	3,457^{a}	3,543^{a}	3,612	3,583	3,577	3,647^{a}
Lyxasan-100[3]	3,249	2,997^{b}	3,137^{b}	3,247^{a}	3,396^{ab}	3,588^{a}	3,629	3,594	3,616	3,585^{a}
Phospholipase[4]	3,462	3,290^{a}	3,377^{a}	3,098^{a}	3,266^{bc}	3,449^{b}	3,439	3,449	3,423	3,389^{b}
Statistical analysis										
SEM(4)[5]	0.0464	0.0421	0.0310	0.0758	0.0391	0.0160	0.0522	0.0371	0.0632	0.0343
P-value	0.0905	0.0263	0.0128	0.0305	0.0189	0.0090	0.1361	0.0915	0.2164	0.0196

[a-c]Means with different superscripts within a column differ significantly (P< 0.05).
[1]Unsupplemented wheat/SBM basal diet.
[2]150g of Natugrain Blend/tonne of basal diet provided at least 5,500 EXU/kg feed and 1,350 BGU/kg feed.
[3]100g of Lyxasan Forte/tonne of basal diet provided at least 5,500 EXU/kg feed.
[4]100g of Phospholipase/tonne of basal diet provided 500 PLU/kg feed.
[5]SEM(4)= Standard Error of the mean with 4 degrees of freedom.
[6]Equation to determine AMEn, kcal/kg diet on dry matter basis.

Ediet = kilocalories combustible energy per gram of diet dry matter (determined directly by bomb calorimeter)

Eexcreta = kilocalories combustible energy in excreta per gram of diet dry matter =
= kilocalories per gram excreta x (g celite per gram diet/g celite per gram excreta)

N = Nitrogen retention per gram of diet dry matter =
= N per gram diet – N per gram excreta x (g celite per gram diet/g celite per gram excreta)

AMEn = Metabolizable energy per gram diet dry matter nitrogen corrected =
= Ediet – Eexcreta – 8.22 N

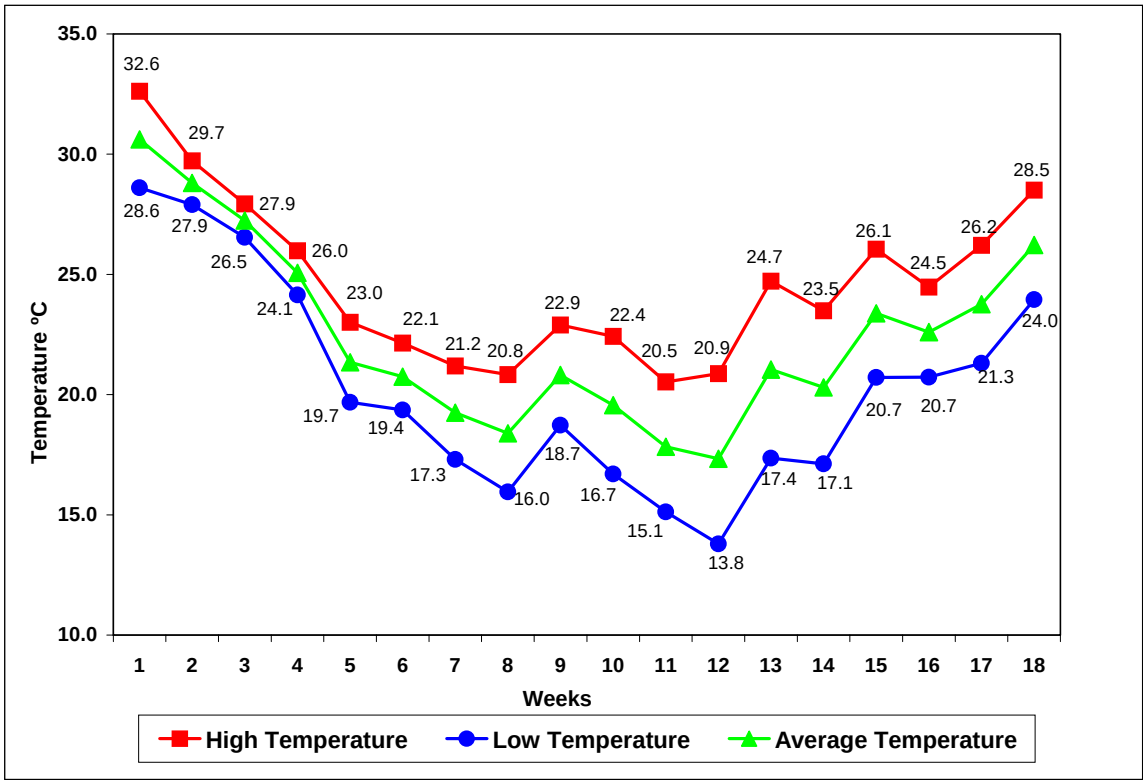

FIGURE 1: Mean weekly high, low and average ambient temperature throughout the trial. The ambient temperature was recorded twice daily from two different points within the experimental house.

FIGURE 2: Digestibility pens used for AMEn assay. (a) The digestibility pens were modified floor-pens with plastic slats secured upon 2x4 lamber frames. The 2.5cm slats were spaced 2.5cm apart, which allowed the excreta of the birds to fall onto a plastic sheet under the pen. (b) The plastic sheet could be pulled from under the slatted floor to facilitate excreta sampling.

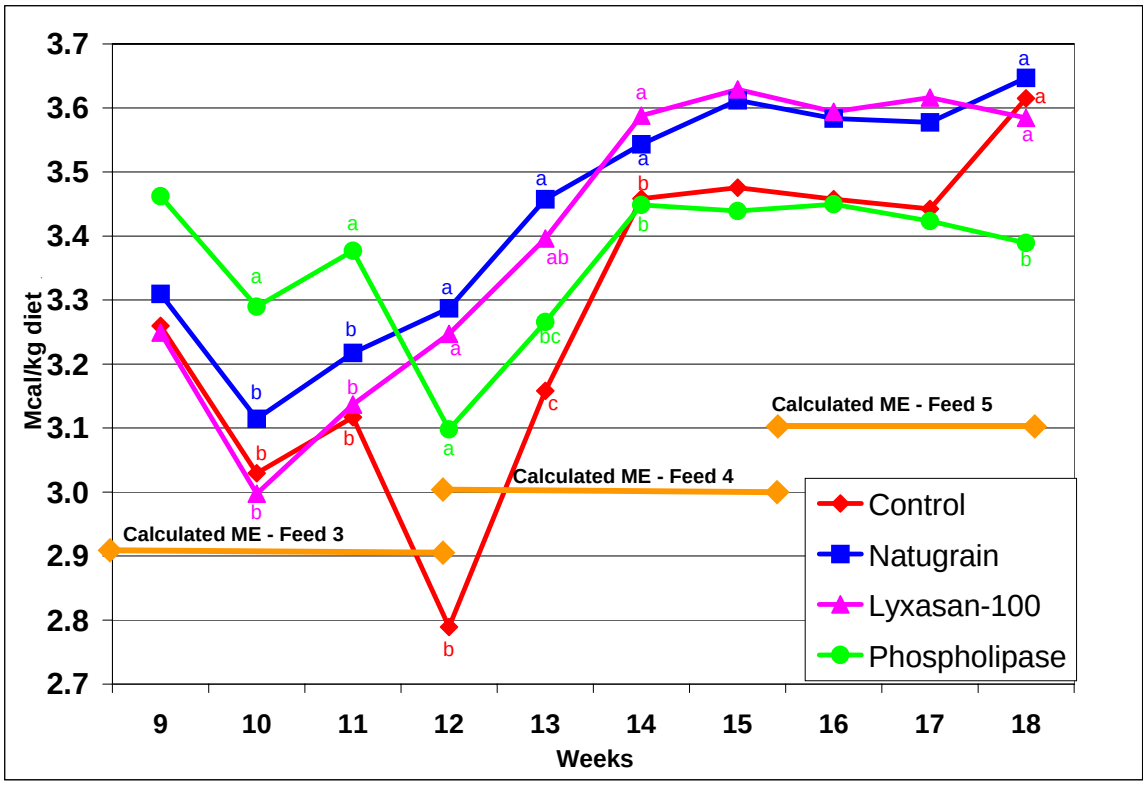

FIGURE 3: Effects of different exogenous enzyme supplementation on apparent metabolizable energy nitrogen-corrected (AMEn) of turkeys fed wheat based-diets from 9 to 18 weeks of age. Different letters on each line-point within each week, signify a significant (P< 0.05) difference between mean values of AME (Mcal per kilogram diet). Mean values represent values from two pooled samples of excreta. Pooled samples represent two samples of excreta of each pen (approximately 200g from each) collected from each week mixed together. The treatments were: unsupplemented wheat/SBM basal diet (control); 150g of Natugrain Blend/tonne of basal diet that provided at least 5,500 EXU/kg feed and 1,350 BGU/kg feed (Natugrain); 100g of Lyxasan Forte/tonne of basal diet that provided at least 5,500 EXU/kg feed (Lyxasan-100); and 100g of Phospholipase/tonne of basal diet that provided 500 PLU/kg feed (Phospholipase).

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4. SUMMARY

World poultry consumption has been on an upward trend for many years and it will continue as poultry becomes the world's primary choice meat product during the next decade or two. Turkey production has increased significantly during the last decade due to new advances in management practices, increased exports, and increased consumer demand to low-fat food. The economics of turkey production is dependent upon feed ingredient availability and prices, and feed formulation costs. This increase in the poultry industry is causing many feed manufactures to consider wheat and wheat by-products as alternatives to corn for rations. However, there is much concern about the high variability of apparent metabolizable energy (AME) among wheat used to feed poultry.

The low-AME wheat is caused by the presence of soluble non-starch polysaccharides (NSP), which are pentosans (mainly arabinoxylan and some β-glucan) in cell walls of the wheat kernels. Agronomic conditions can change the NSP content of wheat. For example, wheat subjected to frost damage during seed maturation (immature wheat) contains increased levels of NSP. However, supplementation of diets with enzymes may help reduce the antinutritional properties of wheat and improve the feeding value of wheat in poultry diets.

Experiments described in this thesis were designed to evaluate the effect of dietary enzyme supplementation on the nutritional value of wheat for turkeys. The general objectives of this research was to 1) evaluate the effect of a natural enzyme blend preparation on the improvement of nutritional value of low-AME wheat (immature-frost

damaged) on growth performance, energy and protein utilization and gut viscosity of turkey toms; and 2) to study the efficacy of endoxylanases and phospholipase on growth performance, energy utilization, digesta viscosity and litter quality of turkey toms fed low-AME wheat.

In the first experiment (reported in Chapter 2), growth performance, jejunum viscosity, energy (AMEn) and protein (ANR) utilization of poults (1-17 d of age) were studied to determine if the reduction in the nutritional value of wheat frost damaged during seed development can be alleviated by dietary supplementation of a blend of NSP enzymes. The enzyme concentration in the feed, containing 50% wheat in place of corn, was at least 7,300 endoxylanase units (EXU) per kg and 1,800 β-glucanase (BGU) per kg. In general, enzyme supplementation improved body weight, feed conversion, AME, ANR, and jejunum digesta viscosity. Frost damage during seed development reduced nutritional value of wheat (AMEn and ANR), presumably by preventing the conversion of sugars to starch thus increasing the relative content of NSP to starch.

The second experiment (reported in Chapter 3) determined if the adverse effects of non-starch polysaccharides (NSP) on turkeys fed wheat-based diets might be alleviated by dietary supplementation of endoxylanase (to reduce the adverse effects of digesta viscosity) or phospholipase (to improve the digestibility of fat). A blend of enzyme was supplemented to the diet to achieve the same level of endoxylanase activity in the feed (5,500 EXU/kg) as supplemented from an enzyme product produced by a genetically modified organism (GMO) with exclusive endoxylanase production. This treatment design allowed us to evaluate the effect of endoxylanase as a single enzyme preparation

or along with several other enzymes. Also, the enzyme produced from the GMO organism was applied at two-application rates (5,500 and 2,250 EXU/kg) so we could investigate the dose-dependent responses for dietary endoxylanase supplementation. Phospholipase (500 phospholipase units per kg feed) was used to test our hypothesis that its dietary supplementation could alleviate the adverse effects of NSP by facilitating lipid digestibility. In this experiment, performance, caked litter, AMEn, and ileum viscosity were determined.

The results in this experiment demonstrated that all enzyme treatments improved growth performance and energy utilization. Growth performance and energy utilization of turkeys fed wheat-based diets was significantly enhanced by phospholipase supplementation of starter feeds and endoxylanase supplementation of growing and finishing feeds; however, a natural blend of enzymes provided a positive response, regardless of the age of the turkeys. Evidently, phospholipase alleviated the adverse effects of dietary NSP on growth performance in young turkeys by improving fat digestion and absorption, whereas endoxylanase was more effective in older birds having a greater digestive capacity and a more mature gut microbial ecosystem. Our results also showed that the mode of action of an exclusively endoxylanase supplementation is more limited than from a blend of enzyme that contains several enzymes.

In summary, the results presented and discussed in this thesis demonstrated several points as follows:

- ✓ Enzyme supplementation to wheat-based turkey diets can enhance growth performance and nutrient utilization.

- ✓ Enzyme supplementation to wheat-based diets of turkey poults result in performance equivalent to corn-based diets.
- ✓ Frost damage during seed development significantly reduces nutrient utilization, presumably by reducing the amount of starch per kernel and increasing the relative content of NSP to starch.
- ✓ The adverse effect of NSP on turkeys fed wheat-based diets can be alleviated by dietary supplementation of endoxylanase by reducing digesta viscosity.
- ✓ Phospholipase improves the digestibility of fat when it is supplemented to diets containing NSP from wheat.
- ✓ Young birds have lower metabolizable energy primarily due to their limited lipase synthesis as compared to older birds that have a more mature digestive tract.
- ✓ Phospholipase was most effective in the starting feeds, likely because of the limited capacity for fat digestion in young turkeys, whereas endoxylanase was most effective during the growing and finishing phases. However a natural blend of enzymes provided positive response regardless of turkey age, presumable because of the synergistic activity among the different enzymes.