# UTILIZATION OF LOW PHYTIC ACID CORN WITH PHYTASE TO REDUCE PHOSPHORUS EXCRETION FROM GROWING TURKEYS AND PIGS

By

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ABSTRACT

UTILIZATION OF LOW PHYTIC ACID CORN WITH PHYTASE TO REDUCE PHOSPHORUS EXCRETION FROM GROWING TURKEYS AND PIGS

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A series of experiments were conducted to determine if a low phytic acid, high protein corn (NutriDense Low Phytate™ (NDLP), Exseed Genetics L. L. C.) could replace yellow dent corn (YD) to reduce phosphorus excretion without affecting growth or bone parameters in growing turkeys and pigs. Phosphorus (P) bioavailability of NDLP was estimated to be 90%. Analyses of corns showed that 90% of total phosphorus (tP) in NDLP was in the form of non-phytate P (npP) (0.32% and 0.29%, respectively). Analyzed YD contained 33% of tP as npP (0.25% and 0.08%, respectively). Replacing YD with NDLP, with or without Natuphos® phytase, reduced P excretion by 30 to 45% (and 56% with phytase) in finishing toms. The NDLP can be safely formulated assuming a 90% P availability in growing-finishing pig diets. Also, NDLP can reduce P excretion by about 45% when fed instead of YD to grow-finish and weanling pigs.

I would like to devote this work to my family and friends who helped me get through it. They were all there to support me every step of the way, whether they realized it or not, especially after very long days and times of frustration. I especially thank my girlfriend, Raelene Charbeneau, whom I love dearly. I would also like to dedicate this work to Alpha Gamma Rho, who helped to shape me into the man I am today..."to make better men and through them a broader and better agriculture". I am very proud to continue the family Spartan tradition as a fourth generation graduate of Michigan State University.

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## KEY TO SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| A | cross-sectional area |
| ADF | acid detergent fiber |
| ADFD | average daily feed disappearance |
| ADG | average daily gain |
| ADP | adenosine diphosphate |
| AMP | adenosine monophosphate |
| ADPD | average daily phosphorus disappearance |
| ANOVA | Analysis of Variance |
| ASAE | American Society of Agricultural Engineers |
| ATP | adenosine triphosphate |
| B | major outside diameter of a bone cross section |
| BMD | bone mineral density |
| BUTA | British United Turkeys of America |
| BW | body weight |
| C | Celsius |
| Ca | calcium |
| $cm^3$ | cubic centimeter |
| CP | crude protein |
| CT | computed tomographic |
| Cu | copper |
| d | day |

| | |
|---|---|
| D | minor outside diameter of a bone cross section |
| dL | deciliter |
| DNA | deoxyribonucleic acid |
| et al. | "and others" |
| F | maximum force required to break a bone |
| Fe | iron |
| FIN | Finisher |
| FTU | BASF Natuphos® *Aspergillus niger* phytase units |
| g | gram |
| GF | gain:feed |
| GLM | General Linear Model |
| GR1 | Grower 1 |
| GR2 | Grower 2 |
| h, hr | hour |
| i.e. | "that is" |
| I | iodine |
| kcal | kilocalorie |
| kg | kilogram |
| kN | kiloNewton |
| L | distance between fulcra points |
| LP | Low Phytate |
| lsmeans | least square means |
| ME | metabolizable energy |

| | |
|---|---|
| Mg | magnesium |
| mg | milligram |
| min | minute |
| MJ | MegaJoule |
| mL | milliliter |
| mm | millimeter |
| Mn | manganese |
| MPa | MegaPascal |
| N | Newton |
| NCIUBMB | Nomenclature Committee of the International Union of Biochemistry and Molecular Biology |
| NDLP | NutriDense™ Low Phytate |
| ng | nanogram |
| npP | non-phytate phosphorus |
| NRC | National Research Council |
| P | phosphorus |
| Pa | Pascal |
| pP | phytate phosphorus |
| PTH | parathyroid hormone |
| PTU | Alko Ltd. Biotechnology Finase™ *Aspergillus niger* phytase activity units |
| PU | Alko Ltd. Biotechnology yeast phytase activity units |
| qCT | quantitative computed tomography |
| RNA | ribonucleic acid |

| | |
|---|---|
| s | second |
| Se | selenium |
| SEM | standard error of the mean |
| stderr | standard error |
| sP | soluble phosphorus |
| TME | true metabolizable energy |
| tonne | metric ton |
| tP | total phosphorus |
| Trt | treatment |
| US | United States |
| USA | United States of America |
| USDA | United States Department of Agriculture |
| USDA-ARS | United States Department of Agriculture-Agricultural Research Service |
| vs. | versus |
| w | average cortical wall thickness |
| wk | week |
| X | distance from neutral axis to outer fiber of a bone |
| YD | yellow dent |
| Zn | zinc |

# INTRODUCTION

As the human population has increased in the USA, so has the demand for food. The expansion in the size of farms has increased to partially meet that demand, with either crop or livestock production, and often both. The current trend is that the number of farms is decreasing while the average size of the farming operation is on the rise. In 1900 there were approximately 5.7 million farms in production with an average acreage of 147 acres. In 1997, there were about 1.9 million farms in production with an average size of about 487 acres. As examples of this trend, in 1978 the number of swine operations in existence was 635,000 and in 1992 it was 240,150. Similarly, the number of turkey operations in existence was 26,638 in 1978 and 13,766 in 1992. The increase in the number of animals raised for production from 1978 to 1992 was 12% for swine (from 88,512,000 to 99,142,000 pigs) and 109% for turkeys (from 138,939,000 to 289,880,000). From these numbers, clearly the number of animals raised and the number of animals raised per operation is increasing.

The use of domestic livestock wastes as sources of potential nutrients for crop production has been a long-standing tradition in the USA. Many years of manure application to crop lands on or around animal feeding operations has, in some instances, led to a saturation of soils with nutrients. If the nutrient saturation exceeds the soils' ability to retain nutrients, a potential for water pollution may exist. This may occur in one or more of several ways. Surface runoff is one major pathway. In this scenario, the loss of soil nutrients may occur as sediment bound or dissolved nutrients associated with organic material or soil particles erodes away from the soil and is washed away with surface water runoff. Rainfall or irrigation can then take this runoff into other bodies of

water including streams, rivers, ponds and lakes. Another pathway is by way of artificial drainage systems and subsurface water flows. Loss of nutrients by leaching down through the soil, especially in periods of drought followed by periods of rainfall, is another pathway. Nutrients in this situation could end up in groundwater and drinking water and become a concern for human health.

Eventually the manure-derived nutrients may end up in large bodies of water such as ponds and lakes. Coupled with the influx of nutrients from other sources, a potential for a nutrient overload of the aquatic system could lead to an increase in biological oxygen demand and a condition known as eutrophication. As organic material (manure particles or sediment-bound nutrients, for instance) enter bodies of water, plant growth will increase often preventing adequate oxygenation. Also, bacteria may act to decompose the organic matter, using dissolved water oxygen in the process. Dissolved oxygen concentrations can drop below sustainable concentrations for fish and other organisms in the aquatic system, leading to kills. Eutrophication can occur when an overabundance of phosphorus and other nutrients lead to a surge in aquatic plant growth, especially algae. Too much decaying algae can lead to depletion in dissolved oxygen in the water and may eventually cause acute fish kills. As the size of the animal feeding operation, proximity to watersheds, and soil nutrient saturation from applied manure increase so does the potential for water pollution. When managed properly, through a variety of different mechanisms, livestock feeding operations can pose only minimal risk for water pollution.

Phosphorus utilization from grain feedstuffs in poultry and swine diets is limited due to a difference in gastrointestinal metabolism. Most phosphorus in grains is part of

the phytic acid (phytate) complex. Turkeys and swine have limited phytase, an enzyme which splits phosphorus from the phytate complex, increasing its availability. Reducing manure nutrient concentrations allows for more manure application with a reduced risk of water pollution. Since corn is the leading cereal grain produced in the USA, and is the primary constituent of most USA turkey and swine diets, a reduced phytate phosphorus corn variety could be a potential feedstuff to reduce manure phosphorus. Addition of exogenous phytase enzyme could also reduce manure phosphorus by making more phytate-bound phosphorus available to turkeys and pigs. The focus in the following chapters will address how feeding a variety of low-phytic acid corn with phytase and reducing dietary sources of inorganic phosphorus (dicalcium phosphate) in turkey and swine diets could reduce manure concentrations of phosphorus. The impacts on growth performance and skeletal attributes will also be examined.

## Thesis Organization

The following thesis is organized as a literature review followed by two papers as thesis chapters. The first chapter is written in the style and format of the Journal of Applied Poultry Research. The second Chapter is written in the style and format of Animal Feed Science and Technology. Michael W. Klunzinger completed the research reported in the papers under the direction of Kevin D. Roberson, Gretchen M. Hill, Michael W. Orth, Allan P. Rahn, and Robert D. von Bernuth. Included in the appendices is an estimation of cost of dietary treatments used in the experiments.

# LITERATURE REVIEW

## Introduction

More than 95% of the corn that is harvested in the USA is marketed as mature commodity yellow dent corn, with 55% being used in livestock feeding (Hallauer, 2001). Corn is the leading cereal grain feedstuff fed in livestock diets in the USA. The goal to reduce livestock manure phosphorus, more specifically poultry and swine manure phosphorus, has led to the advent of corn varieties which have a higher available phosphorus content than conventional yellow dent corn. The use of the enzyme phytase to increase phosphorus availability of poultry and swine feeds, which results in the reduction of inorganic phosphorus supplementation in poultry and swine feeds, has also been proven to reduce poultry and swine manure phosphorus.

## Nutrient attributes of conventional yellow dent corn

Mature corn is used in livestock feeding because its nutrient content better matches the needs of livestock than immature corn. A mature kernel of corn commonly contains 70-75% starch, 8-10% protein, 4-5% oil, and 10-18% water, fiber, vitamins, and minerals (Boyer and Hannah, 2001). A kernel of corn includes four main parts: tip cap, germ, endosperm, and pericarp. Components of the kernel can be genetically modified to create a new corn nutrient profile.

### *Energy*

In livestock, energy can be separated into two categories: (1) heat energy to maintain body temperature; and (2) molecular energy available for work and biochemical processes in the body (but energy is stored as fat). When estimating the amount of

potential energy available in feed, the common unit is kilocalories (kcal), or the amount of heat energy required to raise the temperature of one gram of water by one degree centigrade, when the feed is completely oxidized in a bomb calorimeter. This value is then referred to as Gross Energy (GE). (Scott et al., 1982)

When formulating diets for swine and poultry, the energy requirements are generally based on Metabolizable Energy (ME), which is defined as the gross amount of potentially oxidizable energy (GE) in a consumed feed minus the oxidized Fecal Energy (FE) minus Gaseous Products of Digestion (GDP) minus Urinary Energy (UE). In poultry feeding, some nutritionists use True Metabolizable Energy (TME) in dietary formulation rather than apparent Metabolizable Energy (ME) (NRC, 1994). The difference is that TME is unaffected by variations in feed intake and accounts only for the gross energy of the feces, urine or urates, and gaseous products that are of feed origin, rather than endogenous. Because corn is rather high in digestible carbohydrates and fats, it is a good source of energy (Scott et al., 1982).

*Carbohydrate*

A majority of the carbohydrate in the corn kernel occurs as starch. Starch is the storage form of nutritionally available carbohydrate for the germinating corn seed, most of which (80-90%) is found in the endosperm. About 80% of the total mature corn kernel dry weight is comprised of starch. Starch has a GE value of approximately 4 kcal/g and is readily digested by poultry (Moran, 1985a). After pigs are two to three weeks old, they can utilize starch (Becker and Terrill, 1954; Cunningham, 1959; Sewell and Maxwell, 1966). Prior to that time, pigs lack the amounts of pancreatic amylase and intestinal disaccharides to digest cornstarch (Cunningham, 1959; Sewell and Maxwell, 1966).

Starch is made up of primarily α-1,4 linkages of glucose units arranged in a linear manner. A small amount of branching occurs as α-1,6 linkages of glucose units. Starches are made up of two distinct types of polymers: (1) amylose and (2) amylopectin. (Scott et al., 1982) Standard yellow dent corn starch is composed of about 25% amylose and 75% amylopectin. Amylose is a molecule made up of 100 to 1000 glucose subunits arranged by linear α-1,4 linkages and branched α-1,6 linkages about every 200 glucose subunits. Amylopectin molecules contain up to 200,000 glucose units. Approximately 8,000-10,000 of the total number of glucose subunits in amylopectin are arranged by α-1,6 linkages, with 19,000-192,000 arranged by α-1,4 linkages. The linear regions of amylopectin are either 10-20 or 30-50 glucose units long. (White, 2001) Amylases in swine and poultry hydrolyze starches in the saliva, crop, and small intestine, through a series of reactions to eventually break them down to glucose units. Monosaccharides can then be absorbed across the small intestine and converted to glucose in the intestinal cell. Glucose is then transported through the blood and can serve as an energy source to any tissue or organ in the body through a variety of different mechanisms. Glucose can be stored, to a certain extent, in the liver and muscle as glycogen. Glycogen is similar in structure to amylose. Glucose can also be used in the formation of adipose tissue. (Pond et al., 1995) Digestion and absorption of starch can be affected by many factors, including corn particle size, starch configuration (amylase and amylopectin proportions), interactions with protein and fa t, and the presence of antinutritional factors such as phytate (Pond et al., 1995).

*Protein*

Most of the protein in a corn kernel is found in the endosperm and germ. Standard yellow dent corn kernel protein is made of 7% albumins, 5% globulins, 52% prolamines (zeins), and 25% glutelins on a percent nitrogen basis. The endosperm may contain up to 80% of the total protein in a corn kernel, and accounts for 34% of kernel glutelins and 60% of kernel prolamines. The germ contains 60% albumins and 5-10% prolamines. (Boyer and Hannah, 2001) Albumins, globulins, and glutelins are more soluble than prolamines and provide a more desirable balance of essential amino acids for animals. The prolamine (zein) fraction of corn contains extremely low portions of lysine (an essential amino acid) and higher portions of glutamine, proline and alanine (non-essential amino acids), as compared to other protein fractions of corn (Vasal, 2001). Because standard yellow dent corn contains higher concentrations of prolamines and contributes more non-essential amino acids (e.g. glutamine, proline, and alanine) and less essential amino acids (e.g. lysine and tryptophan), it would be desirable to feed corn that would provide less zein protein and more albumin, globulin, and glutelin proteins. Antinutritional factors, such as phytate, can have a detrimental effect on protein digestion and absorption (de Rahm and Jost, 1979; Cosgrove, 1980; Caldwell, 1992).

*Lipids*

The majority (83-85%) of the lipid (oil) contained in a kernel of conventional yellow dent corn is found in the germ portion. The endosperm, tip cap, and pericarp contain 10-13% of the kernel oil. Corn oil is comprised of mainly triacylglycerides, which is a mixture of saturated and unsaturated fatty acids. Typical high quality yellow dent corn oil should contain approximately 50% linoleic acid, 40% oleic acid, 12%

palmitic acid, 2% stearic acid, and 1% linoleic acid. (Boyer and Hannah, 2001) Because there is a large percentage (50%) of linoleic acid and a low percentage (1%) of linolenic acid (an essential fatty acid) in corn oil, it is considered a good source of dietary fatty acid and, therefore, energy (Boyer and Hannah, 2001; Scott et al., 1982).

**Nutrient composition of yellow dent corn**

Table 1 illustrates a nutrient profile of US #2 Grade yellow dent corn grain used for poultry diet formulation (NRC, 1994). Table 2 illustrates a nutrient profile of US #2 Grade yellow dent corn grain used for swine diet formulation (NRC, 1998). Nutrient values listed are those of particular interest for this thesis. Grade US #2 is the standard for most animal feeds (Leeson and Summers, 1997).

**Table 1. Nutrient Composition of Yellow Dent Corn Grain, *Zea mays indentata*, for Poultry Diets (as-fed basis).[a]**

| Nutrient | Units | Values | Nutrient | Units | Values |
|---|---|---|---|---|---|
| Moisture | % | 12.00 | Histidine | % | 0.23 |
| TME | kcal/kg | 3,470 | Isoleucine | % | 0.29 |
| ME | kcal/kg | 3,350 | Leucine | % | 1.00 |
| Crude Protein | % | 8.50 | Lysine | % | 0.26 |
| Ether Extract | % | 3.80 | Methionine | % | 0.18 |
| Crude Fiber | % | 2.20 | Cystine | % | 0.18 |
| Calcium | % | 0.02 | Phenylalanine | % | 0.38 |
| Total Phosphorus | % | 0.28 | Tyrosine | % | 0.30 |
| Non-phytate phosphorus | % | 0.08 | Threonine | % | 0.29 |
| Arginine | % | 0.38 | Tryptophan | % | 0.06 |
| Glycine | % | 0.33 | Valine | % | 0.40 |
| Serine | % | 0.37 | | | |

[a]Based on NRC Nutrient Requirements of Poultry (1994) values.

**Table 2. Nutrient Composition of Yellow Dent Corn Grain, *Zea mays indentata*, for Swine Diets (as-fed basis).[a]**

| Nutrient | Units | Values | Nutrient | Units | Values |
|---|---|---|---|---|---|
| Moisture | % | 11.00 | Isoleucine | % | 0.28 |
| ME | kcal/kg | 3,420 | Leucine | % | 0.99 |
| Crude Protein | % | 8.30 | Lysine | % | 0.26 |
| Ether Extract | % | 3.90 | Methionine | % | 0.17 |
| ADF | % | 2.80 | Cystine | % | 0.19 |
| Calcium | % | 0.03 | Phenylalanine | % | 0.39 |
| Total Phosphorus | % | 0.28 | Tyrosine | % | 0.25 |
| Available phosphorus[b] | % | 0.04 | Threonine | % | 0.29 |
| Arginine | % | 0.37 | Tryptophan | % | 0.06 |
| Histidine | % | 0.23 | Valine | % | 0.39 |

[a]Based on NRC Nutrient Requirements of Swine (1998) values.
[b]Based on a phosphorus bioavailability estimate of 14%.

**Calcium and Phosphorus Nutrition in Swine and Poultry**

Although the focus of this thesis centers around phosphorus, the discussion of calcium is also essential. Calcium and phosphorus must be discussed in conjunction with one another because they have close associations in animal metabolism, especially in bone formation.

*Calcium*

Approximately 99% of the calcium found in animal stores is in the skeleton as part of bones and teeth (Pond et al., 1995). Calcium is found in roughly a 2:1 ratio with phosphorus in bone, mainly as crystals of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. Most of the blood calcium is in the extracellular plasma fraction as one of three forms (Lloyd et al., 1978): 60% as a free ion, 35% protein bound, and 5-7% as complexed with organic (e.g. citrate) or inorganic acids (e.g. phosphate). It is important that blood calcium is

maintained at a relatively constant concentration so that calcium is available for functions such as neurotransmission, muscle contraction, and blood clotting. The parathyroid gland is the calcium control point for calcium regulation in the body. A decrease in plasma calcium results in the stimulation of the parathyroid gland to release parathyroid hormone (PTH), which induces biochemical activation of vitamin $D_3$ eventually to 1,25-dihydroxycholecalciferol [1-25($OH_2$) D3] by the kidney, thereby causing increases in calcium absorption from the gastrointestinal tract (primarily in the duodenum of the small intestine) and resorption of calcium from bone (Lloyd et al., 1978; Groff and Gropper, 1998; Underwood and Suttle, 1999). Absorption of calcium across the small intestine is facilitated by the carrier protein calbindin. The kidney is also a very important organ in calcium reabsorption. An increase in plasma calcium stimulates the thyroid gland C-cells to release calcitonin, which is a hormone that represses calcium resorption from bone (Groff and Gropper, 2000).

Bone growth occurs by bone forming cells called osteoblasts. In general, bone formation occurs as a process in which mineral elements are deposited onto an organic matrix. Dicalcium phosphate is of great importance in the process. There are several events that take place during the formation of bone, outlined as follows: 1) dicalcium phosphate accumulates; 2) three molecules of dicalcium phosphate congregate to form one molecule of tricalcium phosphate, leaving one molecule of phosphoric acid; 3) ions of carbonate, fluorine, or hydroxyl attach to the unstable tricalcium phosphate to complete a characteristic crystalline structure of apatite; 4) further mineral complex additions contribute to structure and insolubility (Lloyd et al., 1978).

Bone resorbing cells called osteoclasts are responsible for bone surface resorption. Bone resorption also takes place deep within the bone by a process known as osteocytic resorption. Bone is in a constant state of flux, as more bone accretion occurs and changes in shape and density take place. Bone resorption also influences the structure and shape of bone.

Calcium is also necessary for normal blood coagulation and has functional roles in muscle and nervous tissue, regulation of cell membrane permeability, enzyme activation, and vitamin $B_{12}$ absorption (Lloyd et al., 1978). Dietary calcium is absorbed primarily in the duodenum and jejunum of the small intestine of swine and poultry. The efficiency of absorption is difficult to ascertain due to endogenous sources of calcium re-excreted into the intestine. Apparent digestibility values (those which does include endogenous losses) are therefore used, when the measurement of net absorption values (that which does not include endogenous losses) are not possible. Net absorption values involve very intensive balance studies. Absorption of calcium occurs by energy-dependent active transport and by passive diffusion transport across the intestinal epithelium. Acid conditions of the stomach or proventriculus and gizzard help to promote calcium absorption. (Pond et al., 1995) Active transport calcium absorption is controlled by the vitamin D dependent calcium binding protein calbindin (Hurwitz, 1996). About 50% of plasma calcium is filtered by the kidneys and 99% is reabsorbed. As a general rule, an increase in dietary calcium will result in a decrease in percentage calcium absorbed. Absorption of calcium can be adversely affected by the presence of phytic acid, which can form insoluble complexes with calcium in poultry and swine. (Wise, 1983; Pond et al., 1995) When high dietary concentrations of radio-labeled

calcium were fed to rats, insoluble calcium-phytate complexes were formed, which had a negative effect on phosphorus utilization (Nahapetian and Young, 1980).

Excretion of dietary calcium is by way of feces from the intestine and urine (pigs) or uric acid (poultry) from the kidneys. Fecal excretion of calcium includes both endogenous calcium and that which was not absorbed. Urinary or uric acid excretion of calcium is low due to filtration of plasma calcium and reabsorption by the kidneys. (Pond et al., 1995)

The calcium content of yellow dent corn grain is generally quite low not only in concentration, but also in bioavailability as calcium can be an integral part of the structure of phytin, a calcium-magnesium salt of phytic acid, which is mostly unavailable to swine and poultry. As a result, swine and poultry corn based diets must be supplemented with ingredients that will provide bioavailable calcium to prevent a deficiency. The bioavailability of calcium found in monocalcium phosphate, dicalcium phosphate, tricalcium phosphate, defluoronated phosphate, calcium gluconate, and calcium sulfate is very high, 90-100% (Baker, 1991; Soares, 1995), based on poultry research as compared to the calcium in calcium carbonate, which is assumed to be 100% bioavailable.

*Phosphorus*

Approximately 80% of the phosphorus in the body is present in the skeleton and teeth as phosphate phosphorus in the crystal hydroxyapatite and calcium phosphate essential to bone ossification. The remaining 20% of body phosphorus is found throughout the rest of the body tissues. In blood serum, phosphorus occurs in an organic form as part of blood lipids or as inorganic phosphorus (10% bound to protein, 50-60%

ionized, and about 35% complexed with Na, Ca, and/or Mg). (Lloyd et al., 1978; Pond et al., 1995; Underwood and Suttle, 1999)

The major role of dietary phosphorus in the animal is as a constituent of the skeleton and its involvement with bone metabolism, as outlined in the section on calcium. Phosphorus is also found in phospholipids, which have roles as cell membrane structure and lipid transport. Another very important function of phosphorus is its role in energy metabolism as a component of adenosine monophosphate (AMP), ADP, ATP, and creatine phosphate. Phosphorus has a functional role in protein synthesis as part of the structure of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Phosphorus also has an integral role in many enzyme systems including carbohydrate, amino acid, and lipid metabolism. (Scott et al., 1982; Pond et al., 1995; Groff and Gropper, 1998; Underwood and Suttle, 1999)

Phosphorus concentrations in the blood can fluctuate more than that of calcium because phosphorus is not as tightly regulated. The primary control point of phosphorus regulation in the body is the kidney. Low dietary phosphorus will increase phosphorus reabsorption in the proximal tubule of the kidney. Phosphorus reabsorption is upregulated by IGF-1, growth hormones, and thyroid hormones, particularly in growing animals that are increasing muscular mass. Parathyroid hormone downregulates phosphorus reabsorption in the kidney. The kidney is the key organ in keeping calcium and phosphorus under the saturation point in blood. Parathyroid hormone influences resorption of phosphate from bone. In contrast to calcium, PTH stimulates excretion of phosphorus in urine or uric acid sufficient to transcend bone resorption of phosphorus to effect a net decrease in plasma phosphorus. Calcitriol and the active for of vitamin $D_3$

(1,25-dihydroxycholecalciferol [1-25($OH_2$) D3]) enhances phosphate absorption across the gastrointestinal tract (primarily in the duodenum of the small intestine) and phosphate resorption from bone. (Groff and Gropper, 1998)

Although there are some endogenous losses of phosphorus, from secretion of phosphorus into the intestinal lumen, the losses are not as great as those seen with calcium. Apparent digestibility values for dietary phosphorus are, therefore, somewhat more reliable than apparent digestibility values for dietary calcium. Absorption of phosphorus occurs by energy-dependent active transport and by passive diffusion transport across the intestinal epithelium. (Pond et al., 1995) Acid conditions of the stomach or proventriculus and gizzard help to promote phosphorus absorption. In relation to calcium, an excess of dietary phosphorus can impair calcium absorption, presumably by the formation of insoluble calcium phosphate salts (Pond et al., 1995). If dietary phosphorus is present as phytic acid, absorbability will be very low (Pond et al., 1995).

*Calcium and phosphorus deficiency*

If calcium and phosphate concentrations in fluids surrounding bone fall below a critical level necessary for calcium phosphate precipitation into the crystalline lattice structure of apatite, proper ossification (bone mineralization) fails to transpire. In growing animals, this results in a condition known as rickets. Rickets generally results from inadequate dietary calcium, vitamin $D_3$, phosphorus, or a very narrow or wide calcium: available phosphorus ratio. Symptoms often include growth failure, enlarged ends of long bones, "rubbery" or "springy" bones leading to bowed legs, beaded ribs, curved spine, curved sternum, with an increasing amount of loss of appetite, weakness,

and ataxia as the condition advances and could eventually result in death. (Lloyd et al., 1978; Scott et al., 1982; Pond et al., 1995)

*Phytic acid structure and function*

Phytic acid is the principal progenitor of inositol and inositol phosphates and the storage form of phosphorus in plant seeds (Cosgrove, 1980). Phytic acid is the common name for myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate ($C_6H_{18}O_{24}P_6$). The term "phytic acid" refers to the free acid; "phytate" refers to the free salt of phytic acid; and "phytin" refers to the calcium/magnesium salt of phytic acid. Anderson (1914) first proposed the most generally accepted chemical structure of phytic acid (Figure 1).

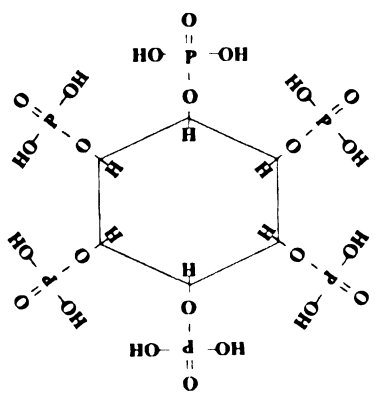

**Figure 1. Structure of phytic acid proposed by Anderson (1914) Adapted from Sebastian et al. (1998).**

This structure best explains the biochemical properties, interactions, and nutritional effects of phytic acid. At a neutral pH, the phosphate groups on the phytic acid molecule generally have one or two negatively charged oxygen atoms. For this reason, positively charged cations are able to strongly bond between two phosphate groups or weakly with an individual phosphate group on the phytic acid molecule to form insoluble salts (Figure 2) (Erdman, 1979).

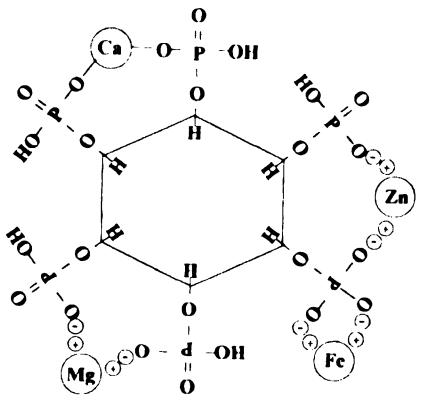


**Figure 2. Structure of phytic acid chelate at neutral pH (Erdman, 1979) Adapted from Sebastian et al. (1998).**

In the plant, phytic acid serves two major roles as the supply of inositol phosphates for signal transduction as well as storage of phosphorus in dormant seeds later used for ATP synthesis during germination. Concentrations of phytic acid (as a proportion of the total phosphorus) in various plants and plant seeds are somewhat variable, ranging between 60 and 80% (Simons et al., 1990). About two-thirds of the total phosphorus present in yellow dent corn (*Zea mays indentata*) grain is in the form of phytate phosphorus and about one-third is represented as non-phytate phosphorus (Nelson et al., 1968; NRC, 1994, 1998; Simons et al., 1990; Cromwell, 1992).

*Phytate as an antinutritional factor*

The phosphorus and other essential elements in phytate have lead to the recognition of phytate as a nutrient. Phytate has also been considered to be an antinutritional factor in poultry and swine diets because it binds other essential elements and reduces bioavailability of those elements (Erdman et al., 1979; Reddy et al., 1982). Nutritionally essential cations able to bond to the phytic acid molecule include calcium,

magnesium, manganese, iron, zinc, and potassium. Phytate can reduce the solubility of other essential dietary constituents of poultry and swine diets including proteins (Saio et al., 1967; Rojas and Scott, 1969; de Rahm and Jost, 1979; Cosgrove, 1980; Cheryan, 1980; Prattley and Stanley, 1982). Phytate can also cause a reduction in pepsin activity (Deshpande and Cheryan, 1984), a reduction in trypsin activity (Singh and Krikorian, 1982; Caldwell, 1992), and the inhibition of α-amylase activity by binding the calcium necessary for its stability and activation resulting in low starch digestibility and the reduction of available dietary energy (Deshpande and Cheryan, 1984; Knuckles and Betschart, 1987).

*Phytate digestibility and bioavailability*

For dietary phytate to be digested and utilized by poultry and swine, it must first be hydrolyzed to release inorganic phosphorus and other bound essential elements, such as calcium, magnesium, zinc, iron, manganese, and potassium. Although non-enzymatic hydrolysis of phytate has been suggested (Hegsted et al., 1954), the liberation of phytate-derived inorganic phosphorus and associated elements is strongly dependent on the presence of phosphatase enzymes, including phytase. Dietary phytate is poorly utilized by poultry and swine because they lack the quantities of phytase that would be sufficient to hydrolyze large amounts of dietary phytate and is, therefore, relatively poor in bioavailability of phosphorus from the phytate molecule; (Taylor, 1965; Nelson, 1967, 1976; Peeler, 1972; Cromwell, 1979).

*Phosphorus in grain feedstuffs*

Reported phytate phosphorus concentrations in yellow dent corn are somewhat variable ranging from 59-73%, when expressed as a percentage of total phosphorus

(Nelson et al., 1968; Cromwell, 1979; Reddy et al., 1989; Eeckhout and De Paepe, 1994; Ravindran et al., 1995a). The non-phytin, or non-phytate phosphorus content of dent corn is listed as 0.08%, with phytate phosphorus as 0.20% and total phosphorus as 0.28%, by the NRC (1994). Swine and poultry corn based diets must be supplemented with ingredients that will provide more bioavailable sources of phosphorus to avoid deficiency. The phosphorus from inorganic supplements, such as monocalcium phosphate, dicalcium phosphate, monosodium phosphate, defluoronated rock phosphates and animal byproduct ingredients is considered to be highly bioavailable, often approaching 100% bioavailability (Kornegay, 1972b; Hays, 1976; Clawson and Armstrong, 1982; Partridge, 1981; Tunmire et al., 1983; Cromwell et al., 1987; Cromwell, 1992). Steamed bone meal is often less bioavailable and more variable in phosphorus bioavailability (Cromwell, 1992). High-fluorine rock phosphates, soft phosphate, Curacao Island phosphate, and colloidal clay are relatively low in phosphorus bioavailability compared to other inorganic sources (Chapman et al., 1955; Plumlee et al., 1958; Hays, 1976).

*Dietary calcium and phosphorus requirements for turkeys and pigs*

The ratio of calcium:phosphorus normally found in bone is approximately 2:1 (Lloyd et al., 1978). Consequently, the recommendations for most starting, growing, and finishing diets for turkeys have a calcium:non-phytate phosphorus ratio of around 2:1 (NRC, 1994). For weanling pigs, the recommendations for nursery diets have a calcium:available phosphorus ratio of around 2:1 (NRC, 1998). For growing-finishing pigs, the recommended calcium:available phosphorus ratio increases from 2:1 to 3:1 as the pigs age and increase body weight (Jongbloed, 1987; Ketaren et al., 1989; Qian et al.,

1996; NRC, 1998). The reason why higher calcium:available phosphorus ratios are suggested (NRC, 1998) as pigs age and increase in body weight is not entirely clear, especially since some studies investigating the calcium:phosphorus ratio, growth, bone parameters, and serum measurements were not influenced by calcium:phosphorus ratio, even at a ratio as high as 3.1:1 (Koch et al., 1984). Other reports have stated that responses to higher (greater than 2:1) calcium:phosphorus ratios had adverse effects (Qian et al., 1996). Satisfactory calcium and phosphorus nutrition for swine and poultry is contingent upon an appropriate dietary calcium:available phosphorus ratio, adequate dietary vitamin D, and an adequate concentration of each macromineral in a bioavailable form in the diet. Other minerals, such as magnesium, zinc, and iron can bind calcium and phosphorus and must be fed in the right amounts (NRC, 1994; NRC, 1998) so as not to have a negative impact on calcium and phosphorus nutrition.

A limited amount of information is published on the dietary calcium and phosphorus recommendations for turkeys (Tables 3 and 4).

**Table 3. Suggested calcium requirements for growing turkeys as percentage of diet (90 percent dry matter)**

| Source | Recommendation (%) | Age of Turkey |
|---|---|---|
| Motzok and Slinger, 1948 | 1.70 | Starting poults (0-5 wks) |
| Wilcox et al., 1953 | 1.50 | Starting poults (0-4 wks) |
| Slinger et al., 1961 | 1.00 | Starting poults (0-8 wks) |
| Formica et al., 1962 | 0.81 | Starting poults (0-8 wks) |
| Neagle et al., 1968 | 1.20[a] | Starting poults (0-4 wks) |
| Nelson et al., 1961 | 1.20[a] | Growing turkeys |
| Sullivan et al., 1962 | | |
| Formica et al., 1962 | | |
| Nelson et al., 1984 | Can increase requirements when diets contain high levels of phytate P. | Growing turkeys |

[a] Requirement when dietary total P and vitamin D levels were 0.80% and 1,100 ICU/kg diet, respectively

| Table 4. Suggested non-phytate phosphorus requirements for growing turkeys as percentage of diet (90 percent dry matter) | | |
|---|---|---|
| Source | Recommendation (%) | Age of Turkey |
| Almquist, 1954 | 0.60 | Starting poults (0-6 wks) |
| Bailey et al., 1986 | 0.60 | Starting poults (0-3 wks) |
| Stevens et al., 1986 | 0.60 | Starting poults (0-3 wks) |
| Day and Dilworth, 1962 | non-phytate P requirement | Growing turkeys |
| Sullivan, 1962 | decreases with age from 0.60 to 0.45 | (9-16,17-24, and 8-20 wks, respectively) |

The National Research Council (NRC, 1994) has suggested recommendations for the dietary calcium and phosphorus requirements of growing turkeys based on research by Day, Dilworth, and Sullivan (1962) (Table 5). The NRC (1994) recommendations are based on male turkeys phase fed in four-week intervals from previous research. However, the NRC (1994) states that genetic improvements in growth performance and body weight gain would suggest that the dietary recommendations be implemented at an earlier age as 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, and 18-21 weeks, rather than the previously suggested four-week intervals. This is because as turkeys reach market weight faster, feed intake increases and physiological age increases at an earlier chronological age, and so the nutritional requirements must also increase.

| Table 5. Calcium and non-phytate phosphorus requirements of growing turkeys fed ad libitum as percentage of diet (90% dry matter)[a] | | | | | | |
|---|---|---|---|---|---|---|
| | Growing turkeys, males | | | | | |
| Nutrient | 0-4 wks[b] | 4-8 wks[b] | 8-12 wks[b] | 12-16 wks[b] | 16-20 wks[b] | 20-24 wks[b] |
| Calcium | 1.20 | 1.00 | 0.85 | 0.75 | 0.65 | 0.55 |
| Non-phytate phosphorus | 0.60 | 0.50 | 0.42 | 0.38 | 0.32 | 0.28 |

[a]Based on NRC recommendations (1994).

[b]Age intervals are based on previous research of actual chronology. Genetic improvements in body weight gain necessitate an earlier implementation of these levels, at 0-3, 3-6, 6-9, 9-12, 12-15, and 15-17 weeks, respectively.

[c]Organic phosphorus is considered to be of limited bioavailability as it is associated with phytin. Non-phytate phosphorus is, therefore, considered in the phosphorus requirements of growing turkeys.

A considerable amount of research has been published concerning the calcium and phosphorus requirements of weanling pigs (Rutledge et al., 1961; Combs and Wallace, 1962; Combs et al., 1962, 1966; Miller et al., 1962, 1964a,b, 1965b,c,d; Menehan et al., 1963; Zimmerman et al., 1963; Blair and Benzie, 1964; Mudd et al., 1969; Coalson et al., 1972, 1974; Mahan et al., 1980; Mahan, 1982) and growing-finishing pigs (Chapman et al., 1962; Libal et al., 1969; Cromwell et al., 1970, 1972b; Stockland and Blaylock, 1973; Doige et al., 1975; Pond et al., 1975, 1978; Fammatre et al., 1977; Kornegay and Thomas, 1981; Thomas and Kornegay, 1981; Maxson and Mahan, 1983; Combs et al., 1991a,b). The National Research Council (NRC) has suggested recommendations for the dietary calcium and phosphorus requirements of weanling pigs and growing-finishing pigs (Table 6). The NRC (1998) recommendations are based on pigs phase fed within particular body weight ranges (3-5, 5-10, 10-20, 20-50, 50-80, and 80-120 kg body weight).

**Table 6. Calcium, total phosphorus, and available phosphorus requirements of growing pigs fed ad libitum as percentage of diet (90% dry matter) or daily requirement[a]**

| | Growing pigs body weight (kg) | | | | | |
|---|---|---|---|---|---|---|
| Nutrient | 3-5 | 5-10 | 10-20 | 20-50 | 50-80 | 80-120 |
| Calcium[b] | 0.90 / 2.25 | 0.80 / 4.00 | 0.70 / 7.00 | 0.60 / 11.13 | 0.50 / 12.88 | 0.45 / 13.84 |
| Total phosphorus[b] | 0.70 / 1.75 | 0.65 / 3.25 | 0.60 / 6.00 | 0.50 / 9.28 | 0.45 / 11.59 | 0.40 / 12.30 |
| Available phosphorus[b] | 0.55 / 1.38 | 0.40 / 2.00 | 0.32 / 3.20 | 0.23 / 4.27 | 0.19 / 4.89 | 0.15 / 4.61 |

[a]Based on NRC recommendations (1998).
[b]Percentages of calcium, total phosphorus, and available phosphorus should increase by 0.05 to 0.10 percentage units for developing boars and replacement gilts in the 50 to 120 kg body weight growth period.

**Phytase**

The enzymatic dephosphorylation and hydrolysis of phytic acid occurs primarily by the action of a family of enzymes known as phytases (myo-inositol hexaphosphate phosphorohydrolases). Phytases have the ability to catalyze the removal of inorganic orthophosphates from the phytic acid molecule in a stepwise fashion (Nayni and Markalds, 1986). Phytases consequently also have the ability to release other elements or compounds that may be bound to phytate, such as the cations that may be bound (calcium, magnesium, manganese, etc.), phytate-associated proteins, and phytate-associated starch. There are two phytases that have been classified by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB). These two phytases fall under the recommended names of 3-phytase (myo-inositol hexakis phosphate 3-phosphorohydrolase; EC 3.1.3.8) and 6-phytase (myo-inositol hexakis phosphate 6-phosphorohydrolase; EC 3.1.3.26), where the numbers refer to the carbon positions of phytase removal of the phosphate groups. Eeckhaute and De Paepe (1994) reported that of the 50 feedstuffs they analyzed for phytase activity, only rye (5130 units/kg), triticale (1688 units/kg), wheat (1193 units/kg), and barley (582 units/kg) were phytase-rich. Maize, oats, sorghum, and oilseeds contained little or no phytase.

Phytases present in the gastrointestinal tract of poultry and swine emanate from dietary sources of plant matter, gut microflora, and endogenous secretions from the intestinal mucosa for the hydrolysis of the phytate molecule. Very low concentrations of phytase have been gleaned from the brush border region of the small intestine of mammals, such as pigs (Cooper and Gowing, 1983). The intestinal phytase activity in

poultry is rather controversial. Bitar and Reinhold (1972) and Maenz et al. (1995) showed that phytase activity exists in the small intestine of poultry. Davies and Motzok (1972) reported that a homogenate of chick intestinal mucosa was able to hydrolyze a sample of sodium phytate. In studies by Moore and Veum (1983), the presence of intestinal phytase was not confirmed. High concentrations of phytase are normally present in certain species of yeast, fungi including *Aspergillus ficuum, Aspergillus niger, Aspergillus oryzae, Aspergillus terreus, Aspergillus fumigatus, Emericella nidulans, Myceliophthora thermophila, and Talaromyces thermophilus*, as well as certain species of bacteria, including *Escherichia coli, Pseudomonas,* and *Bacillus subtilis* (Nelson et al., 1968; Sebastian et al., 1998). *Aspergillus niger* has been the most active fungal species isolated in terms of phytase activity (Sebastian et al., 1998). *Aspergillus ficuum*, a variant of *Aspergillus niger* has been shown to produce the largest concentrations of phytase and was found to be very thermostable (Nelson et al., 1967; Sebastian et al., 1998). Microbial phytases isolated from previously mentioned fungal species have a broader range in pH activity than plant phytases and have consequently been more effective in the lower pH gastrointestinal environments of swine and poultry (Simons et al., 1990; Sebastian et al., 1998).

Nelson et al. (1968, 1971) conducted extensive studies investigating fungal phytase supplementation in corn-soybean based poultry feeds. Cromwell and Stahly (1978) conducted an experiment in which a dried live yeast culture (*Saccharomyces cerevisiae*) was added to a corn-soybean based diet for pigs. The anticipation was that the live yeast culture would contain phytase enzyme to liberate phytate phosphorus from the feed. They concluded, however, that the yeast culture did not improve phytate

utilization because growth rate, feed conversion (as intake/gain), and bone strength were not impacted. Similar conclusions were drawn by Chapple et al. (1979) from a similar swine experiment. Shurson et al. (1984), however, did conclude that growing pigs had an improved growth rate due to yeast phytase supplementation. The researchers were not able to improve phytate phosphorus utilization by supplementing a yeast phytase in corn-soybean meal based diets in balance studies and feeding trials with piglets. In the past, production costs of fungal-derived phytase would have been high and the cost of inorganic sources of phosphate (such as dicalcium phosphate) would have been relatively low. Because of these reasons, the commercialization of phytase supplementation was not realized (Swick and Ivey, 1992).

Increased environmental awareness and advances in fermentation technology have renewed interest in feed phytase enzyme production and supplementation for poultry and swine diets. The use of phytase as a feed additive is enticing to poultry and swine producers in countries that have strict regulations on land application of animal waste phosphorus, such as in the Netherlands (Simell et al., 1989; Campbell and Bedford, 1992). In the U.S., concerns about surface runoff water contamination from poultry production in areas such as the Delaware-Maryland-Virginia area have spurred interest in phytase supplementation (Swick and Ivey, 1992). In Maryland, legislation has made it compulsory for poultry producers to feed phytase to reduce excreted phosphorus (Hansen, 2000). Recent developments in Michigan indicate that large animal feeding operations (greater than 1000 animal units [1,000 beef cattle = 3,000 pigs = 55,000 turkeys]) will need to develop a phosphorus-based comprehensive nutrient management plan (personal communication, Dr. Robert von Bernuth, Michigan State University).

Over the past decade or so, research involving the use of phytase supplementation in corn-soybean meal based diets for swine and poultry has intensified.

Microbial phytase supplementation improves phytate availability in broiler chickens fed corn-soybean meal based diets (Nelson et al., 1968; Denbow et al., 1995; others summarized in Table 7). On average, phytase supplementation of broiler diets improves phytate phosphorus availability by 20 to 40 percent. The amount of phytate phosphorus liberated from corn-soybean meal based broiler diets depends on the concentration and source of added phytase and phytate (Simons et al., 1990; Kornegay et al., 1996; Yi et al., 1996), calcium (Schoner et al., 1993; Sebastian et al., 1996), vitamin $D_3$ (Edwards, 1993; Roberson and Edwards, 1994; Ravindran et al., 1995b; Yi et al., 1995; Qian et al., 1995, 1997), and the calcium:phosphorus ratio (Schoner er al., 1993; Qian et al., 1993).

**Table 7. Impact of microbial phytase on bodyweight (BW) gain, feed conversion ratio (FCR), phosphorus retention (P ret.), phosphorus excretion (P excr), tibia ash, tibia breaking strength (BS), and toe ash in broiler chickens fed corn-soybean based diets**

| Source | Bird age (d) | Phytase source, Units/kg[b] | Diet npP[c] (%) | % Improvement[a] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | BW gain | FCR | P ret. | P excr | Tibia ash | Tibia BS | Toe ash |
| Simons et al., 1990 | 28 | *A. ficuum*, 700 | 0.16 | 38 | 0.63 | 20 | 50 | — | — | — |
| Perney et al., 1993 | 16 | *A. niger*, 0.15% | 0.32 | — | — | 11 | 10 | 25 | 14 | 39 |
| Aoyagi and Baker, 1995 | 20 | Natuphos 5000®, 600 | — | 77 | 3.7 | — | — | — | — | — |
| Sebastian et al., 1996a | 21 | Natuphos®, 600 | 0.30 | 13 | 0.67 | 24 | | — | — | — |
| Kornegay et al., 1996 | 21 | Natuphos®, 600 | 0.20 | 36 | 2.6 | 5.3 | | — | — | — |
| Yi et al., 1996a | 20 | Natuphos®, 600 | 0.45 | 11 | 3.5 | — | — | — | — | — |
| Yi et al., 1996 | 21 | Natuphos®, 1,050 | 0.27 | 22 | — | — | 19 | — | — | 15 |
| Korin et al., 1999 | 28d[d] | Natuphos®, 600 | 0.03 | — | — | 18 | — | — | — | — |
| Sohail et al., 1999 | 42 | Natuphos®, 600 | 0.23 | 1.5 | — | — | — | 13 | 8.6 | — |
| Zhang et al, 2000 | 35 | Natuphos®, 600 | 0.21 | 6.8 | 6.8 | 11 | 10 | — | — | 4.5 |
| Yan et al., 2001 | 42 | Natuphos®, 800 | 0.10 | 7.4 | 3.8 | — | 3.2 | 10 | — | — |

[a]Dashes in columns indicate that response data was not available form the literature.
[b]1 unit of phytase is the activity that releases 1 μmol of phosphorus from phytic acid in 1 minute.
[d]Broilers were fed using corn as the sole ingredient in a 5d bioassay from 25 to 30d of age.

Table 8 summarizes investigations of the supplementation of phytase to corn-soybean meal based dietary studies involving male growing turkeys.

**Table 8. Impact of microbial phytase on body weight (BW) gain, feed conversion ratio (FCR), phosphorus retention (Phos ret), and bone ash in growing turkeys fed corn-soybean meal based diets**

| Source | Bird age (d) | Phytase source, units/kg[b] | Diet npP[c] (%) | % Improvement[a] BW gain | FCR | Phos ret. | Bone ash |
|---|---|---|---|---|---|---|---|
| Yi et al., 1996 | 29 | Natuphos®, 750 | 0.45 | 11 | — | — | — |
| Qian et al., 1996 | 21 | Natuphos®, 600 | 0.27 | 83 | 32 | 24 | 49 |
| Atia et al., 1996 | 49 | Natuphos®, 500 | 52% of NRC (1994) | ... | — | — | 9.4 |
| | 112 | | | 12 | | | |

[a]Dashes in columns indicate that response data was not available from the literature.
[b]1 unit of phytase is the activity that releases 1 μmol of phosphorus from phytic acid in 1 minute.
[c]npP = non-phytate phosphorus

Additionally, Ravindran et al. (1995) reported that when turkey poults were fed 800 FTU/kg for 3 weeks, they had increased body weights, a 24% increase in bone ash, and a 35% increase in bone strength over poults fed no phytase with diets that contained 0.27% non-phytate phosphorus. Atia et al. (2000) reported that when turkeys were fed 500 FTU/kg phytase, from 4 to 16 weeks of age, there was a 9.5% increase in body weight, a 9% increase in bone ash, and a 22% increase in bone strength as compared to turkeys fed diets without phytase and 52% of NRC (1994) recommendations for dietary non-phytate phosphorus. Microbial phytase supplementation is beneficial in corn-soybean meal based swine diets (Table 9).

**Table 9. Impact of microbial phytase supplementation on body weight (BW) gain, feed conversion ratio (FCR), phosphorus retention (P ret.), phosphorus excretion (P excr.), bone ash, and bone breaking strength (BS) in pigs fed corn-soybean based diets**

| Source | Pig initial BW (kg), Trt[b] period (d) | Phytase source, units/kg[c] | Diet Phos. (%) | % Improvement[a] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | BW gain | FCR | P ret. | P excr. | Bone ash | Metacarpal/Metatarsal Bone Strength |
| Simons et al., 1990 | | *Aspergillus ficuum*, 700 | 0.33 | — | — | 24 | 35 | — | — |
| Jongbloed et al., 1992 | 37, 14 | *Aspergillus niger*, 1500 U/g[e] | 0.09 (aP) | — | — | 30 | 30 | — | — |
| Lei et al., 1993 | 7.4, 28 | *A. niger*, 1350 U/g[e] | — | 39 | — | 68 | — | — | — |
| | 7.6, 28 | *A. niger*, 750 U/g[e] | 0.29 | + | — | 61 | — | — | — |
| | 8.2, 14 | *A. niger*, 750 U/g[e] | 0.32 | — | — | 50 | 42 | — | — |
| Cromwell et al., 1993 | 20.7, 34 | FINASE™, 500 U/g | 0.32 (0.05) | 12 | — | — | — | — | 35 |
| Young et al., 1993 | 10.2, 21 | FINASE™, 500 U/g | 0.55 | 16 | 8.9 | 17 | — | 5.1 | — |
| Cromwell et al., 1995 | 23, 112 | Natuphos®, 500 | 0.35/ 0.30 | 9.4 | 3.2 | 82 | 11.8 | 4.9 | 32.3 |

[a]Dashes in columns indicate that information was not available from the literature; + indicates a positive effect.

The addition of phytase to corn-soybean meal based diets with low phytic acid corn as a corn source and lower than normal levels of dietary non-phytate phosphorus has also been shown to improve phosphorus availability without negatively affecting performance in growing swine, broilers, and turkeys (Table 10).

**Table 10. Impact of microbial phytase supplementation on body weight (BW) gain, feed conversion ratio (FCR), phosphorus retention (P ret.), phosphorus excretion (P excr.), bone ash, and bone breaking strength (BS) in growing broilers, turkeys, and pigs fed low phytic acid corn-soybean meal based diets**

| Source | Species age (d) | Phytase source, units/kg[b] | Diet npP[c] (%) | % Improvement[a] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | BW gain | FCR | P ret. | P excr. | Bone ash | Bone BS |
| Yan et al., 1998, 2000 | Broilers 56 | Natuphos®, 1000 | NRC-0.15% NpP | — | — | — | 54 | — | — |
| Huff et al., 1998 | Broilers 49 | Natuphos®, 500 | NRC-0.10% NpP | + | — | — | — | — | — |
| Saylor et al., 1999 | Broilers 53 | Natuphos®, 600 | NRC-0.20% NpP | + | + | — | 13 | — | — |
| Waldroup et al., 2000 | Broilers 21 | Natuphos®, 800 | 0.17 | 2.3 | 3.5 | — | 18 | 2.8 | — |
| Saylor et al., 2000 | Broilers 21 | Natuphos®, 600 | Control-0.24% NpP | + | — | — | 54 | + | + |
| Yan et al., 2000 | Turkey 18 | Natuphos®, 1000 | 0.17 | — | — | — | + | + | — |
| Cromwell et al., 1999 | Pigs 63 | Natuphos®, 600 | 0.19 | — | — | — | 11 | — | 22 |

[a]Dashes in columns indicate that response data was not available from the literature; + indicates a positive effect.
[b]1 unit of phytase is the activity that releases 1 μmol of phosphate from phytic acid in 1 minute.
[c]npP = non-phytate phosphorus

Evidence suggests that there may be breed and strain differences in the ability to utilize dietary phytate in poultry. In a study by Edwards (1983), differences in phytate utilization between three strains of broilers was noted. More recently, a heritability estimate of 0.33 was recognized for phytate utilization in a pedigreed population of chickens (Zhang et al., 2002), indicating that phytate phosphorus utilization is heritable and that selection for phytate utilization is a possibility. Transgenic pigs have been developed that secrete phytase enzyme in their saliva from the introduced transgene composed of mouse parotid secretory protein promoter and the *Escherichia coli appA* phytase gene (Golovan et al., 2001).

Several companies have developed feed grade phytase products, such as Natuphos™ (BASF, Inc.), Ronozyme™ (Roche Novo Nordisk, Inc.), Allzyme™ (Alltech, Inc.), and Finase™ (Alko, Ltd.). In recent years, the goal to improve phytase heat stability for addition to pelleted diets has become a major selling point (Ward, 2002). The Ronozyme™ phytase product is advertised as being more heat stable than other forms of commercially available phytases. In this project, we will be using Natuphos™ (BASF, Inc.) as a source of phytase. Phytase activity, or phytase units per kilogram of feed, for this product is defined as the amount of phytase enzyme required to release 1 μmol of inorganic phosphorus from sodium phytate at a temperature of 37° C and pH of 5.5 in one minute.

**History of Low-Phytic Acid Corn**

Low-phytic acid corn was discovered in 1992 by Dr. Victor Raboy, a geneticist for the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) National Small Grains Germplasm Research Facility, Aberdeen, Idaho. The goal of the USDA-ARS research team was to find non-lethal mutants of mature corn that would contain reduced amounts of phytic acid phosphorus and have increased inorganic phosphorus, but still contain the same amount of total phosphorus. Two non-lethal mutant alleles were found; *low phytic acid 1-1* (*lpa1-1*) and *low phytic acid 2-1* (*lpa2-1*). Under a cooperative research agreement, the low phytic acid genes were brought into commercial corn lines of Pioneer Hi-Bred International, Johnston, Iowa. The Pioneer low-phytic corn became known as High Available Phosphorus (HAP) corn. The USDA-ARS holds a patent on this variety of low-phytic acid corn.

Other grain companies brought in the mutations for low phytic acid and have produced similar varieties of low phytic acid corn. Optimum Quality Grains, L.L.C. (now DuPont Specialty Grains, DesMoines, Iowa) is associated with Pioneer Hi-Bred International. Exseed Genetics, L.L.C., Decatur, Illinois (owned by BASF, Corp., Mount Olive, New Jersey) developed varieties of low phytic acid corn known as NutriDense™ LP (NDLP) corn and Yellow Dent LP (LP) corn, which became commercially available in 1999.

Several poultry studies and swine studies have been conducted using varieties of low-phytic acid corns in place of yellow dent corn. Results of these studies are summarized in Tables 11 and 12.

**Table 11. Research highlights of studies utilizing low phytic acid corn varieties as an alternative to conventional yellow dent corn in growing meat-type poultry diets**

| Source | Species | Treatment Period | Phosphorus availability | Corn variety | Research highlights |
|---|---|---|---|---|---|
| Ertl et al., 1998 | Broiler chicks | 18d | 75% | Lpa1-1 | Reduction in fecal P ranging from 9 to 40% |
| Huff et al., 1998 | Broilers | 49d | 63% (0.17%npP, 0.27% tP) | HAP | -Decrease in serum cholesterol by feeding HAP corn<br>-Can decrease total P in diet by 11% by using HAP corn. |
| Kersey et al., 1998 | Broiler chicks | 21d | 63% (0.17%npP, 0.27% tP) | HAP | -Peak tibia ash with 0.35%npP HAP corn diet<br>-41.4% decrease in fecal P from a 0.45% npP YD corn diet to a 0.35% npP HAP corn diet |
| Li et al., 1998, 2000 | Broiler chicks | 21d | 69% (0.18%npP, 0.26% tP) 65% from in vitro assay | HAP | Chicks on HAP corn diets retained more Ca and P than chicks on YD corn diets |
| Moore et al., 1998 | Broiler litter | | | HAP | -Litter from broilers fed HAP corn diets decreased P runoff by 22% (P>0.05) as compared to litter from broilers fed YD corn diets when applied to fescue plots and using a rainfall simulator |
| Yan et al., 1998, 2000 | Broilers | 56d | 63% (0.17%npP, 0.27% tP) | HAP | Reduction in fecal P ranging from 8 to 55% |
| Douglas et al., 1999, 2000 | Chicks | 8-20d | | LP | -Average phosphorus availability of 86.2% for LP corn<br>-Phosphorus availability for LP corn ranged from 59 to 95% based on tibia response curves |
| Kornegay et al., 1999 | Young turkeys | 8-28d | 51.2% | HAP | Linear increase in BW, toe ash, tibia ash, and P retention as HAP corn replaced YD corn and as Pi was added into YD corn P-reduced diets |
| Malone et al., 1999 | broilers | 54d | N/A | HAP | No consistent diet effect on skeletal parameters |

| Table 11 (cont'd). Research highlights of studies utilizing low phytic acid corn varieties as an alternative to conventional yellow dent corn in growing meat-type poultry diets | | | | | |
|---|---|---|---|---|---|
| Saylor et al., 1999 | broilers | 53d | 63% (0.17%npP, 0.27% tP) | HAP | -A 1.7% increase in BW in broilers fed HAP corn<br>-A 2.2% increase in feed efficiency in broilers fed HAP corn |
| Pierce et al., 1999 | Broiler chicks | 14d | 50% | HAP | -Weight gain and tibia strength were similar for chicks fed HAP corn and YD corn with the same levels of dietary npP<br>-Excreted P was reduced by feeding HAP corn in place of YD corn |
| Sims et al., 1999 | broilers | 53d | 63% (0.17%npP, 0.27% tP) | HAP | Litter from broilers fed HAP corn decreased by 3% as compared to litter from broilers fed YD corn (tP = 1.43 vs. 1.38%, respectively) |
| Saylor et al., 2000 | broilers | 21d | N/A | NDLP | -A 24% decrease in excreta tP from broilers fed NDLP corn diets compared to excreta from broilers fed YD corn<br>-A 26% decrease in excreta sP from broilers fed NDLP corn diets compared to excreta from broilers fed YD corn diets |
| Waldroup et al., 2000 | Broiler chicks | 21d | 63% (0.17%npP, 0.27% tP) | HAP | -Greatest need for npP was for maximum tibia ash (0.39% for YD and 0.37% for HAP)<br>-A 28% decrease in fecal P from broilers fed HAP corn as compared to fecal P from broilers fed YD corn (YD = 1.09%, HAP = 0.78%) |
| Yan et al., 2000 | Young turkeys, Broilers | 18d<br>56d | 63% (0.17%npP, 0.27% tP) | HAP | Decrease in fecal P |
| Klunzinger and Roberson, 2001 | Turkeys | 115d | 90% (0.29% npP, 0.32% tP) | NDLP | -Increase in BW for turkeys fed NDLP corn as compared to turkeys fed YD corn diets<br>-Decrease in ulna strength in NDLP corn diets formulated with 75% P availability assumption |

**Table 12. Research highlights of studies utilizing low phytic acid corn varieties as an alternative to conventional yellow dent corn in growing swine diets**

| Source | Pig Class | Trt Period | P availability | Corn | Research highlights |
|---|---|---|---|---|---|
| Cromwell et al., 1998 | Growing pigs | 15.6 kg initial BW for 40d | 77% | *lpa1-1* | P in *lpa1-1* corn is 3 times more available than P in YD corn |
| Pierce et al., 1998 | Growing pigs | 52-105 kg BW | 75% (0.21% aP, 0.28% tP) | *lpa1-1* | Less supplemental P is required when *lpa1-1*corn is fed in place of YD corn |
| Pierce et al., 1998 | Growing pigs | 60kg initial BW for 5d, 22.7-51 kg BW for 37d | 67%<br><br><br>75% | *lpa1-1* | -Pigs fed *lpa1-1* corn excreted less P than pigs fed YD corn<br><br><br>-With *lpa1-1* corn, 0.09% less dietary P was needed to maximize performance and bone traits |
| Spencer et al., 1998, 2000 | Growing pigs | 9 kg initial BW for 35d and 20 kg initial BW for 5d, 72 and 112 kg initial BW | 62-64<br><br><br><br><br>57% from in vitro assay | *lpa1-1* | -Pigs fed *lpa1-1* corn with no added P had increased P digestibility and retention and reduced P excretion<br>-*lpa1-1* corn has 5 times as much aP as YD corn<br><br><br><br>-Pigs fed *lpa1-1* corn had increased P digestibility<br>-*lpa1-1* corn greatly decreased excreted P and increased the N:P ratio in manure |
| Veum et al., 1998 | Growing barrows | 14.5 kg initial BW for 35d | 69% (0.18% aP, 0.26% tP) | *lpa1-1* | Pigs fed *lpa1-1* corn had increased Ca and P retention and decreased excreted Ca and P |
| Pierce et al., 1999 | Growing pigs | 18 kg initial BW for 42d | 75% | *lpa1-1* | P excretion can be reduced by 25-35% by adding *lpa1-1* corn in place of YD corn |

Additionally, Spencer et al.(1998, 2000) found that *lpa1-1* corn increased loin eye area in finished swine; availability of phosphorus in *lpa1-1* corn diets improved over yellow dent corn diets and may be sufficient for grow-finish swine with no added phosphorus. Pigs fed *lpa1-1* corn diets had carcasses with less back fat and higher percentage lean. Diets containing *lpa1-1* corn with reduced phosphorus supplementation could be fed to growing-finishing swine (27-122 kg body weight) under experimental and commercial conditions with no deleterious effects on pig performance, bone strength, or carcass characteristics.

In general, feeding low-phytic acid corn varieties, in place of conventional yellow dent corn, to poultry and swine on corn-soybean meal based diets can reduce phosphorus excretion. When diets contain lower than required phosphorus levels, using low-phytic acid corn in place of dent corn, can have an effect of increased phosphorus retention in the animal and could possibly improve bone strength relative to low phosphorus diets.

**Nutrient Composition of NutriDense™ Low Phytate Corn**

NutriDense™ Low Phytate (NDLP) Corn is a variety of corn that was developed by Exseed Genetics, L.L.C. (owned by BASF, Inc.) is lower in phytic acid and higher in total phosphorus and non-phytate phosphorus, amino acids, energy, crude protein, and oil content than conventional yellow dent corn. The marketing strategy behind NDLP corn, with respect to poutlry and swine nutrition, was to be able to provide an alternative variety of corn that would reduce excreted phosphorus and other minerals that might otherwise be chelated by the phytic acid complex, as well as providing a better amino acid balance than conventional dent corn. In doing so, the need for supplementing diets

with inorganic sources of phosphate, such as dicalcium phosphate, would decrease. The need for phytase supplementation might also decrease because of the lower levels of phytic acid in NDLP corn based diets. Other nutritional benefits from NDLP corn include the possibility of improvements in protein digestibility, weight gain, and feed efficiency. Studies involving feeding NDLP corn to meat type poultry were outlined in the previous section. The specific nutrient profile of NDLP corn, as compared to YD corn is given in Table 12. Corn analysis was obtained from the University of Missouri-Columbia nutrition laboratory. Estimated ME value of NDLP for pigs (3,498 kcal/kg) and poultry (3,490 kcal/kg) came from Exseed Genetics, L.L.C. Estimated ME value for YD (3,480 kcal/kg) came by personal communication from Vernon Felts, Goldsboro Milling, Raleigh, NC. Amino acid composition of the two corn types was analyzed at the University of Missouri-Columbia. Phytate phosphorus composition of the two corn types was analyzed at the University of Georgia.

| Table 13. Analyzed nutrient content of NutriDense™ Low Phytate (NDLP) and Yellow Dent (YD) corn | | |
|---|---|---|
| Nutrient[A] | NDLP | YD |
| ME,[B] kcal/kg | 3,480 | 3,410 |
| Crude Protein | 10.00 | 8.20 |
| Crude Fat | 2.60 | 2.20 |
| Arginine | 0.45 | 0.36 |
| Cystine | 0.22 | 0.17 |
| Glycine | 0.37 | 0.30 |
| Histidine | 0.29 | 0.23 |
| Isoleucine | 0.34 | 0.25 |
| Leucine | 1.31 | 0.97 |
| Lysine | 0.32 | 0.28 |
| Methionine | 0.21 | 0.16 |
| Phenylalanine | 0.51 | 0.39 |
| Proline | 0.87 | 0.68 |
| Serine | 0.43 | 0.33 |
| Threonine | 0.33 | 0.27 |
| Tryptophan | 0.07 | 0.05 |
| Tyrosine | 0.29 | 0.24 |
| Valine | 0.47 | 0.37 |
| Ca | 0.03 | 0.01 |
| pP | 0.03 | 0.17 |
| npP | 0.29 | 0.08 |
| tP | 0.32 | 0.25 |
| [A]Nutrient values are expressed as % unless otherwise specified.<br>[B]Estimated values. | | |

**Bone Parameters in Poultry and Swine Nutrition**

Because phosphorus is a major constituent of bone tissue and has a prominent role in its structure, several techniques to surmise the nutritional contribution of phosphorus to bone have been devised. These include bone breaking strength, bone ash, and computed tomography.

*Bone breaking strength*

Animal nutritionists have used bone-breaking strength as a measure of response to nutritional regimens for many years, including dietary phosphorus concentrations. A very good review of bone strength as a measure of bone mineralization of swine was

reported by Crenshaw et al. (1981). The authors define "bone breaking strength" as the force per unit area required for breaking a bone. Most publications, which include "bone breaking strength" only involve a measure of force, or mass, with no consideration for the area of bone over which the force is applied. Many types of tests have been devised to determine the strength of materials.

Modern devices used to measure bone strength have a better capability to reduce variations in bone strength than those in the past. An Instron Universal Testing Machine, for instance, has the capability to sustain a constant rate of deformation, electronically. A deformation rate of 5 mm/min is reported to be optimal for plotting a force deformation curve with an Instron Universal Testing Machine (Crenshaw et al., 1981).

Variation also exists in the procedures used for bone preparation. Freezing bones before testing will not negatively impact the mechanical properties of the bones. Changes in temperature at the time of bone testing may slightly affect bone strength (Seldin, 1965). Wet bones bend more than dry fat extracted bones when comparing bone strain to the point of ultimate stress and consequent breakage (Crenshaw et al., 1981). As wet bones are exposed to air at room temperature, the bones begin to dry and begin to show an increase in strength (Seldin and Hirsch, 1966; Crenshaw et al., 1981). Wet bones are preferable during bone testing as they more closely resemble the state of the bones as they exist inside the living animal.

Consideration must also be given to the inside hollow cross section of the bone as well as the bone shape (Crenshaw et al., 1981). Altered dietary calcium and phosphorus concentrations can result in changes in bone wall thickness without changing outside bone diameters (Cromwell et al., 1972; Tanksley et al., 1976; and Crenshaw et al., 1981).

In recent years, an attempt to standardize procedures for bone strength testing has been reported by the American National Standards Institute (March, 1998) for the shear and three-point bending test of animal bone. The outlined standards were developed by the American Society of Agricultural Engineers (ASAE) Physical Properties of Agricultural Products Committee approved by the ASAE Food and Process Engineering Institute Standards Committee and published in 1999. The standards outlined include ultimate shear stress (strength) and ultimate bending stress (strength). Standards for testing instrumentation are outlined.

*Bone ash*

Bone ash has been used as a response variable in countless studies investigating dietary phosphorus regimens for many years. Bone ash is the inorganic mineral portion of the bone and is most often reported as a percentage of fat-free dry bone. Fat-free bone is obtained by using solvents, such as ether, to extract fat and marrow out of bones and allowing to dry by removing the moisture in an oven set to greater than 100°C. Bone ash is obtained by burning bone in an oven at high temperature (600°C) for several hours (usually greater than 4, depending on the size of the bone) until every component of bone is combusted except "ash", which is made up of bone minerals.

*Computed Tomography*

Computed tomography has been used as a radiological tool for the medical community since the 1970's. Computed tomography (CT) scans employ the use of 360-degree x-ray beams accompanied by the computer production of images. Computed tomography scans allow for cross-sectional views of body tissues and organs, including bones. The images from a CT scan are much more pronounced, sharp, focused, than

standard x-ray equipment. This allows for a better view of body tissues (Hathcock and Stickle, 1993).

The use of CT scans for studies involving the effects of dietary calcium and phosphorus on bone development in growing turkeys has been reported (Rosenstein et al., 2000). Computed tomography has also been utilized in studies involving vitamin and mineral supplement withdrawal and wheat middling inclusion in growing pig diets (Shaw, 2001).

Bone mineral density (BMD) can be estimated using CT scanning equipment and have been found to correlate with turkey bones from turkeys fed different concentrations of dietary calcium and phosphorus (Rosenstein et al., 2000). Hydroxyapatite crystal standards can be scanned with bone samples simultaneously and analyzed using a bone mineral density software package to compare the x-ray linear attenuation coefficient of bone to the hydroxyapatite crystal standards.

**Conclusion**

From the review of literature, it is apparent that nutritional as well as environmental benefits exist for phytase supplementation and low phytic acid corn inclusion in swine and turkey diets. Although the amount of swine and turkey research in the area of phytase supplementation and low phytic acid corn usage is limited, the potential benefits they may offer are worthy of investigation. The objective of this thesis is to investigate the impact of low phytic acid corn (NutriDense™ Low Phytate) substitution for yellow dent (YD) corn with and without microbial-derived phytase supplementation on turkey and swine growth performance, bone parameters, and reduce phosphorus excretion.

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# CHAPTER 1: IMPACT OF FEEDING LOW PHYTATE CORN TO GROWING-FINISHING LARGE WHITE MALE TURKEYS


## Summary

Two studies were conducted to verify the availability of P in a high amino acid, low-phytic acid corn (NDLP) variety as compared to conventional yellow dent corn (YD) fed to large white (British United Turkeys of America 'Big 6' breed) male turkeys. The NDLP contained 0.29% non-phytate P (npP), which is assumed to be available, and 0.32% total P (tP). The YD contained 0.08% npP and 0.25% tP. Results of these studies verify that P in NDLP is more available than P in YD. The NDLP can be safely formulated with an assumption of a 90% P availability without compromising growth or bone attributes, and feeding NDLP in place of YD can reduce excreted P by 30 to 44% without phytase and by up to 56% with 600 FTU/kg Natuphos® 600 phytase supplementation.


## Description of Problem

Approximately two-thirds of the P in cereal grains and oilseed meals is bound to phytate, which is generally considered to have limited availability to poultry [1]. Because poultry diets in the US are based primarily on corn and soybean meal, they need to be supplemented with inorganic P in order to meet the P requirements of the bird. The limited ability of poultry to utilize phytate from corn and soybean meal based diets can result in a large amount of P in the manure [2]. Manure is often disposed of by applying it to cropland as a nutrient source for the crops. In areas of highly concentrated poultry numbers, manure application can exceed the nutritional needs of the planted crop. In this case, manure derived nutrients such as P can pose an environmental risk. Runoff of P

into water bodies can contribute to the process of eutrophication, which can be perceived as environmental pollution.

Scientists have recently developed corn mutants that contain less phytate P (pP) and more npP, without reducing the concentration of tP [3]. If these corn mutants are fed in place of YD, less supplemental dietary inorganic P (i.e., dicalcium phosphate) is necessary to achieve the same dietary concentrations of npP. Ertl et al. [4] first reported that a variety of corn with the *lpa1-1* mutation for low-phytic acid had a P availability of 75% when fed to broiler chicks and the chicks showed a reduction in excreted P ranging from 9 to 40%. Huff et al. [5] reported that total P can be reduced by 11% in the diet when using a high available P (HAP) [6] corn variety over YD in broiler diets. Kersey et al.[7] showed that excreted P could be reduced by 41.4% when a HAP corn diet was fed to broiler chicks with npP reduced by 0.10% as compared to a YD corn diet. Li et al. [8] reported that broiler chicks fed HAP corn diets retained more calcium and phosphorus than chicks fed a YD corn diet. Yan et al. [9] showed that broilers fed HAP corn diets had reductions in excreted P ranging from 8 to 55% as compared to broilers fed YD corn diets. Kornegay et al. [10] showed that young turkeys fed HAP corn in place of YD corn in P reduced diets had a linear increase in body weight, toe ash, tibia ash, and P retention. Waldroup et al. [11] reported that the greatest need for npP was for maximum tibia ash (0.39% for YD and 0.37% for HAP) and excreted P was reduced by 28% when broilers were fed HAP corn in place of YD corn. Yan et al. [12] showed that when young turkeys and broilers were fed HAP corn in place of YD corn, there was a decrease in excreted P.

Douglas et al. [13] showed that a different variety of low-phytic acid (LP) corn [14] had an average phosphorus availability of 86.2% with a range of 59 to 95%, based

on chick tibia response curves. A variety of low-phytic acid, high amino acid corn has been developed under the trade name NutriDense™ LP corn (NDLP) [14]. This low-phytic acid corn has more npP and tP and less pP concentration as compared to YD. Saylor et al. [15] reported that broilers fed NDLP corn based diets had a 24% decrease in excreted total P (tP) and a 26% decrease in soluble P (sP) as compared to broilers fed YD corn diets.

Phytase enzyme addition is also a way to make dietary P more available to poultry [16]. Atia et al. [17] reported an increase ($P<0.05$) in body weight (in 4, 8, 12, and 16 wk old male turkeys), bone ash (of 7 wk old turkeys), and bone density (of 7, 11, and 15 wk old male turkeys) when 500 FTU/kg Natuphos® phytase was added to diets containing npP at 52% of NRC [1]. Ravindran et al. [18] reported that when poults were fed 800 FTU/kg Natuphos® phytase added to diets containing 0.27% npP for 3 wk, linear increases in body weight, bone ash, and bone strength were observed. Yan et al. [19] reported that when 1000 FTU/kg Natuphos® was supplemented to young turkey diets (0-18 d of age) with npP reduced by 0.15 percentage units from NRC [1], excreted P was reduced and bone ash was increased.

The objectives of the two experiments were to evaluate what P availability of NDLP for toms grown to market age for consumer toms (ca. 16 kg) and whether phytase could be used in conjunction with NDLP to further reduce P excretion without reducing growth performance and bone strength.

**Materials and Methods**

The Michigan State University All University Committee for the Use and Care of Animals approved all animal experimentation procedures. Both experiments were

conducted from April to August using large white male turkeys [20]. All turkeys were procured from a commercial hatchery [21].

**Experiment 1**

BIRDS AND HUSBANDRY

Four hundred twenty one 3-wk old poults were randomly assigned to four treatments. A completely randomized design was used with four dietary treatments and four replicate pens of 25 to 27 poults allotted randomly to each dietary treatment from 3 to 17 wk of age. Prior to allotment, 428 day-old poults were group brooded with the same four dietary treatments and two replicate pens of 53 to 55 poults placed randomly to each dietary treatment from day-old to 3 wk of age. Supplemental heat was provided for the first 8 d of the experiment with heat lamps, hung 45.72 cm above the pen floor in the center of the pen. For the first 6 d, turkeys were maintained on 24 h of light. On Days 7 and 8, turkeys were given 1 h of darkness. On Day 8, heat lamps were removed. Turkeys were given 1 more h of light and 1 less h of darkness each day, until 16 h of light and 8 h of darkness were achieved on Day 13. Turkeys were maintained on a 16-h light 8-h dark lighting schedule for the rest of the experiment. One 40-watt incandescent bulb at the center of each pen provided light for the entire study. Light intensity was approximately 60 lux. Environmental temperature was set to provide temperature targets (Table 1) using computerized propane gas powered heating and electronic ventilation.

**Table 1: Environmental temperature target guidelines.**

| Days of age | Room Temperature (°C) |
|---|---|
| 21-28 | 26.67 ± 2.22 |
| 29-35 | 23.88 ± 2.22 |
| 36-42 | 21.11 ± 2.78 |
| 43-49 | 18.33 ± 2.78 |
| 49-119 | 15.56 ± 2.78 |

Temperature settings were gradually decreased each day by approximately 0.56°C until the final temperature target setting was reached. Turkeys were housed in 2.46m X 3.08m floor pens with fresh pine shavings for bedding. Water was provided ad libitum using automatic red plastic bell type waterers. Feed was provided ad libitum using trough type feeders (day-old to 1 wk of age) and plastic tube round type feeders from day old-9 wk of age and galvanized steel tube round type self feeders from 9 wk until 17 wk of age.

EXPERIMENTAL DIETS

Diets were phase fed in mash form on a 3-wk interval from day old-3wk, 3-6, 6-9, 9-12, 12-15, and 15-17 wk of age. Dietary treatments included

Treatment 1: YD-soybean meal based diet with a control level of tP

Treatment 2: YD-soybean meal based diet with a negative control level of tP (-0.10% of treatment 1 tP)

Treatment 3: NDLP-soybean meal based diet with the same tP as treatment 2, and a 75% availability assumption for npP in NDLP

Treatment 4: NDLP-soybean meal based diet with the same tP as treatment 2 and a 90% availability assumption for npP in NDLP

All treatments maintained a Ca:npP ratio at approximately 2:1. Nutrient composition of YD and NDLP are listed in Table 2 as analyzed [22, 23]. The ME values listed for YD and NDLP were estimated [24,25].

| Table 2. Analyzed nutrient content of NutriDense™ Low Phytate and Yellow Dent corn | | |
|---|---|---|
| Nutrient[A] | NDLP | YD |
| ME,[B] kcal/kg | 3,480 | 3,410 |
| Crude Protein | 10.00 | 8.20 |
| Ether Extract | 2.60 | 2.20 |
| Arginine | 0.45 | 0.36 |
| Cysteine | 0.22 | 0.17 |
| Glycine | 0.37 | 0.30 |
| Histidine | 0.29 | 0.23 |
| Isoleucine | 0.34 | 0.25 |
| Leucine | 1.31 | 0.97 |
| Lysine | 0.32 | 0.28 |
| Methionine | 0.21 | 0.16 |
| Phenylalanine | 0.51 | 0.39 |
| Proline | 0.87 | 0.68 |
| Serine | 0.43 | 0.33 |
| Threonine | 0.33 | 0.27 |
| Tryptophan | 0.07 | 0.05 |
| Tyrosine | 0.29 | 0.24 |
| Valine | 0.47 | 0.37 |
| Ca | 0.03 | 0.01 |
| pP[C] | 0.03 | 0.17 |
| npP[C] | 0.29 | 0.08 |
| tP[C] | 0.32 | 0.25 |

[A]Nutrient values are expressed as % unless otherwise specified.
[B]Estimated values; YD estimate by personal communication with V. Felts, Goldsboro Milling, Goldsboro, NC; NDLP estimate from equation for ME from N. Dale (2000 Midwest Poultry Federation Conference, St. Paul, MN)
[C]Values analyzed from the University of Georgia Poultry Nutrition Laboratory (Athens, GA 30602)

The ingredient concentration and selected nutrient composition of Experiment 1 diets are given in Table 3 for Prestarter (day-old to 3 wk) Table 4 for Starter 1 (3-6 wk) diets, Table 5 for Starter 2 (6-9 wk), Table 6 for Grower 1 diets (9-12 wk), Table 7 for Grower 2 diets (12-15 wk), and Table 8 for finisher diets (15-17 wk). Treatment 1 dietary calcium and phosphorus are considered to be the levels required for sufficient growth and

adequate bone strength, as determined in a previous experiment [26]. Washed fine sand was added to NDLP diets to maintain similar fat additions and ME values for the diets.

**Table 3. Composition and selected nutrient content of Prestarter diets (Experiment 1)**

| | Prestarter diets (day old-3 wk) | | | |
|---|---|---|---|---|
| Ingredient, % | 1 | 2 | 3 | 4 |
| YD corn[A] | 42.29 | 42.94 | - | - |
| NDLP corn[B] | - | - | 44.49 | 44.38 |
| Soybean meal (48% CP) | 48.44 | 48.33 | 46.26 | 46.28 |
| Choice White Grease | 1.81 | 1.58 | 1.19 | 1.23 |
| Limestone | 1.47 | 1.50 | 1.78 | 1.71 |
| Menhaden Meal | 1.00 | 1.00 | 1.00 | 1.00 |
| Blood Meal | 1.00 | 1.00 | 1.00 | 1.00 |
| Sodium Bicarbonate | 0.30 | 0.30 | 0.30 | 0.30 |
| Dicalcium phosphate | 2.89 | 2.54 | 2.41 | 2.52 |
| Salt | 0.14 | 0.14 | 0.14 | 0.14 |
| DL-Methionine | 0.27 | 0.27 | 0.25 | 0.25 |
| L-Lysine-HCl | 0.09 | 0.09 | 0.14 | 0.14 |
| Trace mineral mix[C] | 0.12 | 0.12 | 0.12 | 0.12 |
| Vitamin mix[D] | 0.13 | 0.13 | 0.13 | 0.13 |
| Choline | 0.05 | 0.05 | 0.05 | 0.05 |
| Washed sand | - | - | 0.75 | 0.75 |
| Nutrients | | | | |
| ME, kcal/kg | 2,850 | 2,850 | 2,850 | 2,850 |
| CP, % | 28.50 | 28.50 | 28.50 | 28.50 |
| Ca, % | 1.44 | 1.38 | 1.44 | 1.44 |
| Calculated tP, % | 0.98 | 0.91 | 0.91 | 0.94 |
| Analyzed tP, % | 1.04 | 0.94 | 0.92 | 0.92 |
| Calculated npP, % | 0.72 | 0.66 | 0.72 | 0.72 |

AYellow Dent corn

BNutriDense™ Low Phytate corn

[C]Mineral mix provided (mg/kg diet): 100 Mn, 100 Zn, 50 Fe, 10 Cu, 1 I.

[D]Vitamin mix provided (/kg diet): vitamin A, 11,000 IU; vitamin $D_3$, 5,000 IU; vitamin E, 35 IU; menadione, 2.75 mg; pantothenic acid, 20 mg; riboflavin, 10 mg; niacin, 80 mg; thiamine, 2.9 mg; pyridoxine, 4.3 mg; folic acid, 2.2 mg; biotin, 0.20 mg; vitamin $B_{12}$, 0.025 mg; vitamin C, 0.10 mg; selenium, 0.275 mg; ethoxyquin, 125 mg.

**Table 4. Composition and selected nutrient content of Starter 1 diets (Experiment 1)**

| | Starter 1 diets (3-6 wk) | | | |
|---|---|---|---|---|
| Ingredient, % | 1 | 2 | 3 | 4 |
| YD corn[A] | 46.49 | 47.24 | - | - |
| NDLP corn[B] | - | - | 48.66 | 48.54 |
| Soybean meal (48% CP) | 45.56 | 45.43 | 43.21 | 43.23 |
| Choice White Grease | 2.59 | 2.32 | 1.99 | 2.03 |
| Limestone | 1.44 | 1.47 | 1.75 | 1.67 |
| Dicalcium phosphate | 2.85 | 2.46 | 2.29 | 2.42 |
| Salt | 0.38 | 0.38 | 0.38 | 0.38 |
| DL-Methionine | 0.29 | 0.29 | 0.27 | 0.27 |
| L-Lysine-HCl | 0.12 | 0.12 | 0.18 | 0.18 |
| Trace mineral mix[C] | 0.12 | 0.12 | 0.12 | 0.12 |
| Vitamin mix[D] | 0.13 | 0.13 | 0.13 | 0.13 |
| Choline | 0.03 | 0.03 | 0.03 | 0.03 |
| Washed sand | - | - | 1.00 | 1.00 |
| Nutrients | | | | |
| ME, kcal/kg | 2,933 | 2,933 | 2,933 | 2,933 |
| CP, % | 26.00 | 26.00 | 26.00 | 26.00 |
| Ca, % | 1.34 | 1.27 | 1.34 | 1.34 |
| Calculated tP, % | 0.92 | 0.85 | 0.85 | 0.88 |
| Analyzed tP, % | 0.91 | 1.05 | 0.98 | 1.00 |
| Calculated npP, % | 0.67 | 0.60 | 0.67 | 0.67 |

AYellow Dent corn

BNutriDense™ Low Phytate corn

[C]Mineral mix provided (mg/kg diet): 100 Mn, 100 Zn, 50 Fe, 10 Cu, 1 I.

[D]Vitamin mix provided (/kg diet): vitamin A, 11,000 IU; vitamin $D_3$, 5,000 IU; vitamin E, 35 IU; menadione, 2.75 mg; pantothenic acid, 20 mg; riboflavin, 10 mg; niacin, 80 mg; thiamine, 2.9 mg; pyridoxine, 4.3 mg; folic acid, 2.2 mg; biotin, 0.20 mg; vitamin $B_{12}$, 0.025 mg; vitamin C, 0.10 mg; selenium, 0.275 mg; ethoxyquin, 125 mg.

**Table 5. Composition and selected nutrient content of Starter 2 diets (Experiment 1)**

| | Starter 2 Diets (6-9 wk) | | | |
|---|---|---|---|---|
| Ingredient, % | 1 | 2 | 3 | 4 |
| YD corn[A] | 52.92 | 54.12 | - | - |
| NDLP corn[B] | - | - | 55.53 | 55.39 |
| Soybean meal (48% CP) | 38.23 | 38.04 | 35.53 | 35.56 |
| Choice White Grease | 3.81 | 3.39 | 3.08 | 3.13 |
| Limestone | 1.32 | 1.16 | 1.71 | 1.63 |
| Dicalcium phosphate | 2.62 | 2.20 | 2.01 | 2.16 |
| Salt | 0.38 | 0.38 | 0.38 | 0.38 |
| DL-Methionine | 0.30 | 0.30 | 0.30 | 0.30 |
| L-Lysine-HCl | 0.17 | 0.17 | 0.24 | 0.24 |
| Trace mineral mix[C] | 0.12 | 0.12 | 0.12 | 0.12 |
| Vitamin mix[D] | 0.13 | 0.13 | 0.13 | 0.13 |
| Choline | - | - | - | - |
| Washed sand | - | - | 1.00 | 1.00 |
| Nutrients | | | | |
| ME, kcal/kg | 3,080 | 3,080 | 3,080 | 3,080 |
| CP, % | 23.00 | 23.00 | 23.00 | 23.00 |
| Ca, % | 1.24 | 1.09 | 1.24 | 1.24 |
| Calculated tP, % | 0.85 | 0.78 | 0.78 | 0.80 |
| Analyzed tP, % | 0.79 | 0.80 | 0.89 | 0.89 |
| Calculated npP, % | 0.62 | 0.54 | 0.62 | 0.62 |

AYellow Dent corn

BNutriDense™ Low Phytate corn

[C]Mineral mix provided (mg/kg diet): 100 Mn, 100 Zn, 50 Fe, 10 Cu, 1 I.

[D]Vitamin mix provided (/kg diet): vitamin A, 11,000 IU; vitamin $D_3$, 5,000 IU; vitamin E, 35 IU; menadione, 2.75 mg; pantothenic acid, 20 mg; riboflavin, 10 mg; niacin, 80 mg; thiamine, 2.9 mg; pyridoxine, 4.3 mg; folic acid, 2.2 mg; biotin, 0.20 mg; vitamin $B_{12}$, 0.025 mg; vitamin C, 0.10 mg; selenium, 0.275 mg; ethoxyquin, 125 mg.

**Table 6. Composition and selected nutrient content of Grower 1 diets (Experiment 1)**

| | Grower 1 diets (9-12 wk) | | | |
|---|---|---|---|---|
| Ingredient, % | 1 | 2 | 3 | 4 |
| YD corn[A] | 57.50 | 58.79 | - | - |
| NDLP corn[B] | - | - | 60.59 | 60.44 |
| Soybean meal (48% CP) | 33.46 | 33.25 | 30.47 | 30.50 |
| Choice White Grease | 5.36 | 4.91 | 4.48 | 4.53 |
| Limestone | 0.95 | 0.77 | 1.37 | 1.28 |
| Dicalcium phosphate | 1.70 | 1.25 | 1.03 | 1.19 |
| Salt | 0.38 | 0.38 | 0.36 | 0.36 |
| DL-Methionine | 0.26 | 0.26 | 0.23 | 0.23 |
| L-Lysine-HCl | 0.15 | 0.15 | 0.23 | 0.22 |
| Trace mineral mix[C] | 0.12 | 0.12 | 0.12 | 0.12 |
| Vitamin mix[D] | 0.13 | 0.13 | 0.13 | 0.13 |
| Washed sand | - | - | 1.00 | 1.00 |
| Nutrients | | | | |
| ME, kcal/kg | 3,250 | 3,250 | 3,250 | 3,250 |
| CP, % | 21.00 | 21.00 | 21.00 | 21.00 |
| Ca, % | 0.88 | 0.71 | 0.88 | 0.88 |
| Calculated tP, % | 0.66 | 0.58 | 0.58 | 0.61 |
| Analyzed tP, % | 0.70 | 0.60 | 0.48 | 0.56 |
| Calculated npP, % | 0.44 | 0.36 | 0.44 | 0.44 |

AYellow Dent corn

BNutriDense™ Low Phytate corn

[C]Mineral mix provided (mg/kg diet): 100 Mn, 100 Zn, 50 Fe, 10 Cu, 1 I.

[D]Vitamin mix provided (/kg diet): vitamin A, 9,000 IU; vitamin $D_3$, 3,500 IU; vitamin E, 25 IU; menadione, 1.5 mg; pantothenic acid, 15 mg; riboflavin, 6 mg; niacin, 70 mg; thiamine, 1.4 mg; pyridoxine, 3.0 mg; folic acid, 2.0 mg; biotin, 0.10 mg; vitamin $B_{12}$, 0.014 mg; selenium, 0.25 mg; ethoxyquin, 125 mg.

**Table 7. Composition and selected nutrient content of Grower 2 diets (Experiment 1)**

| | Grower 2 diets (12-15 wk) | | | |
|---|---|---|---|---|
| Ingredient, % | 1 | 2 | 3 | 4 |
| YD corn[A] | 62.40 | 63.80 | - | - |
| NDLP corn[B] | - | - | 65.94 | 65.76 |
| Soybean meal (48% CP) | 28.72 | 28.49 | 25.44 | 25.47 |
| Poultry Fat | 5.38 | 4.89 | 4.36 | 4.41 |
| Limestone | 0.92 | 0.73 | 1.39 | 1.29 |
| Dicalcium phosphate | 1.63 | 1.14 | 0.90 | 1.07 |
| Salt | 0.38 | 0.38 | 0.36 | 0.36 |
| DL-Methionine | 0.26 | 0.26 | 0.23 | 0.23 |
| L-Lysine-HCl | 0.05 | 0.05 | 0.14 | 0.14 |
| Trace mineral mix[C] | 0.12 | 0.12 | 0.12 | 0.12 |
| Vitamin mix[D] | 0.13 | 0.13 | 0.13 | 0.13 |
| Washed sand | - | - | 1.00 | 1.00 |
| Nutrients | | | | |
| ME, kcal/kg | 3,300 | 3,300 | 3,300 | 3,300 |
| CP, % | 19.00 | 19.00 | 19.00 | 19.00 |
| Ca, % | 0.84 | 0.66 | 0.84 | 0.84 |
| Calculated tP, % | 0.63 | 0.54 | 0.54 | 0.57 |
| Analyzed tP, % | 0.60 | 0.62 | 0.51 | 0.52 |
| Calculated npP, % | 0.42 | 0.33 | 0.42 | 0.42 |

AYellow Dent corn

BNutriDense™ Low Phytate corn

[C]Mineral mix provided (mg/kg diet): 100 Mn, 100 Zn, 50 Fe, 10 Cu, 1 I.

[D]Vitamin mix provided (/kg diet): vitamin A, 9,000 IU; vitamin $D_3$, 3,500 IU; vitamin E, 25 IU; menadione, 1.5 mg; pantothenic acid, 15 mg; riboflavin, 6 mg; niacin, 70 mg; thiamine, 1.4 mg; pyridoxine, 3.0 mg; folic acid, 2.0 mg; biotin, 0.10 mg; vitamin $B_{12}$, 0.014 mg; selenium, 0.25 mg; ethoxyquin, 125 mg.

**Table 8. Composition and selected nutrient content of Finisher diets (Experiment 1)**

| Ingredient, % | Finisher diets (15-17 wk) 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| YD corn[A] | 62.49 | 63.50 | - | - |
| NDLP corn[B] | - | - | 65.97 | 65.81 |
| Soybean meal (48% CP) | 26.82 | 26.65 | 23.54 | 23.57 |
| Choice White Grease | 7.45 | 7.10 | 6.45 | 6.51 |
| Limestone | 0.88 | 0.93 | 1.35 | 1.25 |
| Dicalcium phosphate | 1.54 | 1.00 | 0.82 | 0.99 |
| Salt | 0.36 | 0.36 | 0.36 | 0.36 |
| DL-Methionine | 0.21 | 0.21 | 0.18 | 0.18 |
| L-Lysine-HCl | - | 0.002 | 0.09 | 0.09 |
| Trace mineral mix[C] | 0.12 | 0.12 | 0.12 | 0.12 |
| Vitamin mix[D] | 0.13 | 0.13 | 0.13 | 0.13 |
| Washed sand | - | - | 1.00 | 1.00 |
| Nutrients | | | | |
| ME, kcal/kg | 3,430 | 3,430 | 3,430 | 3,430 |
| CP, % | 18.00 | 18.00 | 18.00 | 18.00 |
| Ca, % | 0.80 | 0.70 | 0.80 | 0.80 |
| Calculated tP, % | 0.60 | 0.50 | 0.51 | 0.55 |
| Analyzed tP, % | 0.63 | 0.46 | 0.45 | 0.42 |
| Calculated npP, % | 0.40 | 0.30 | 0.40 | 0.40 |

AYellow Dent corn

BNutriDense™ Low Phytate corn

[C]Mineral mix provided (mg/kg diet): 100 Mn, 100 Zn, 50 Fe, 10 Cu, 1 I.

[D]Vitamin mix provided (/kg diet): vitamin A, 9,000 IU; vitamin $D_3$, 3,500 IU; vitamin E, 25 IU; menadione, 1.5 mg; pantothenic acid, 15 mg; riboflavin, 6 mg; niacin, 70 mg; thiamine, 1.4 mg; pyridoxine, 3.0 mg; folic acid, 2.0 mg; biotin, 0.10 mg; vitamin $B_{12}$, 0.014 mg; selenium, 0.25 mg; ethoxyquin, 125 mg.

Turkeys were weighed at the end of each dietary phase and feed disappearance was recorded. Feed conversion (feed:gain) was calculated after correcting for mortality.

**Experiment 2**

BIRDS AND HUSBANDRY

Four hundred 6-wk old turkeys were randomly assigned to four treatments. A completely randomized design was used with four dietary treatments and four replicate pens of 25 turkeys allotted randomly to each dietary treatment from 6 to 18 wk of age. Dietary treatmens started at 6 wk of age due to a low availability of NDLP for this experiment. Hence, the turkeys were fed a common control diet adequate in P during the brooding period (day old-6 wk of age). The same husbandry practices used for Experiment 1 were used for Experiment 2.

EXPERIMENTAL DIETS

Diets were phase fed on a 3-wk interval from 6-9, 9-12, 12-15, and 15-18 wk of age. Dietary treatments included:

Treatment 1: YD-soybean meal based diet with a control level of tP,

Treatment 2: YD-soybean meal based diet with a negative control level of tP (-0.20% of treatment 1 tP)

Treatment 3: NDLP-soybean meal based diet with the same tP as treatment 2

Treatment 4: NDLP based diet with the same tP as treatment 2 plus Natuphos®600 phytase (600 FTU/kg).

A 90% P availability assumption (90% of tP as npP) for NDLP was included in the dietary ingredient formulation matrix. Treatment 1 and treatment 3 diets maintained a Ca:npP ratio of 2:1. The same nutrient compositions for NDLP and YD were used as in Experiment 1 (refer to Table 2), with the exception of ME values. The ME values used in Experiment 2 for NDLP and YD were 3,490 (TME by analysis [23]) and 3,390 kcal/kg (ME poultry industry average [25]), respectively. The ingredient composition and selected nutrient concentrations of Experiment 2 diets are given in Table 9 for Starter 2 diets (6-9 wk), Table 10 for Grower 1 diets (9-12 wk), Table 11 for Grower 2 diets (12-15 wk), and Table 12 for finisher diets (15-16.5 wk).

**Table 9. Composition and selected nutrient content of Starter 2 diets (Experiment 2)**

| | Starter 2 diets (6-9 wk) | | | |
|---|---|---|---|---|
| Ingredient, % | 1 | 2 | 3 | 4 |
| YD corn[A] | 54.14 | 55.69 | - | - |
| NDLP corn[B] | - | - | 60.96 | 60.96 |
| Soybean meal (48% CP) | 37.86 | 37.58 | 34.32 | 34.32 |
| Choice White Grease | 3.61 | 3.06 | 1.13 | 1.13 |
| Limestone | 1.25 | 1.38 | 1.47 | 1.47 |
| Dicalcium phosphate | 2.02 | 1.15 | 0.97 | 0.97 |
| Salt | 0.35 | 0.35 | 0.33 | 0.33 |
| DL-Methionine | 0.29 | 0.29 | 0.28 | 0.28 |
| L-Lysine-HCl | 0.23 | 0.24 | 0.29 | 0.29 |
| Trace mineral mix[C] | 0.10 | 0.10 | 0.10 | 0.10 |
| Vitamin mix[D] | 0.15 | 0.15 | 0.15 | 0.15 |
| Natuphos® 600[E] | - | - | - | + |
| Nutrients | | | | |
| ME, kcal/kg | 3,080 | 3,080 | 3,080 | 3,080 |
| CP, % | 23.00 | 23.00 | 23.00 | 23.00 |
| Ca, % | 1.00 | 0.86 | 0.86 | 0.86 |
| Calculated tP, % | 0.76 | 0.60 | 0.60 | 0.60 |
| Analyzed tP, % | 0.59 | 0.59 | 0.59 | 0.52 |
| Calculated npP, % | 0.50 | 0.34 | 0.43 | 0.53 |

AYellow Dent corn

BNutriDense™ Low Phytate corn

[C]Mineral mix provided (mg/kg diet): 100 Mn, 100 Zn, 50 Fe, 10 Cu, 1 I.

[D]Vitamin mix provided (/kg diet): vitamin A, 11,000 IU; vitamin $D_3$, 5,000 IU; vitamin E, 35 IU; menadione, 2.75 mg; pantothenic acid, 20 mg; riboflavin, 10 mg; niacin, 80 mg; thiamine, 2.9 mg; pyridoxine, 4.3 mg; folic acid, 2.2 mg; biotin, 0.20 mg; vitamin $B_{12}$, 0.025 mg; vitamin C, 0.10 mg; selenium, 0.275 mg; ethoxyquin, 125 mg.

[E]2 lb/ton phytase was added = 600 FTU/kg to release 0.10% npP, according to the manufacturer (BASF Corp., Mount Olive, NJ)

**Table 10. Composition and selected nutrient content of Grower 1 diets (Experiment 2)**

| | Grower 1 diets (9-12 wk) | | | |
|---|---|---|---|---|
| Ingredient, % | 1 | 2 | 3 | 4 |
| YD corn[A] | 57.26 | 58.83 | - | - |
| NDLP corn[B] | - | - | 64.40 | 64.30 |
| Soybean meal (48% CP) | 33.25 | 32.97 | 29.52 | 29.52 |
| Choice White Grease | 5.75 | 5.19 | 3.16 | 3.16 |
| Limestone | 1.08 | 1.22 | 1.32 | 1.32 |
| Dicalcium phosphate | 1.63 | 0.74 | 0.54 | 0.54 |
| Salt | 0.35 | 0.35 | 0.34 | 0.34 |
| DL-Methionine | 0.26 | 0.26 | 0.24 | 0.24 |
| L-Lysine-HCl | 0.21 | 0.21 | 0.27 | 0.27 |
| Trace mineral mix[C] | 0.10 | 0.10 | 0.10 | 0.10 |
| Vitamin mix[D] | 0.12 | 0.12 | 0.12 | 0.12 |
| Natuphos® 600[E] | - | - | - | + |
| Nutrients | | | | |
| ME, kcal/kg | 3,250 | 3,250 | 3,250 | 3,250 |
| CP, % | 21.00 | 21.00 | 21.00 | 21.00 |
| Ca, % | 0.84 | 0.70 | 0.70 | 0.70 |
| Calculated tP, % | 0.66 | 0.50 | 0.50 | 0.50 |
| Analyzed tP, % | 0.54 | 0.42 | 0.50 | 0.46 |
| Calculated npP, % | 0.42 | 0.25 | 0.35 | 0.45 |

AYellow Dent corn

BNutriDense™ Low Phytate corn

[C]Mineral mix provided (mg/kg diet): 100 Mn, 100 Zn, 50 Fe, 10 Cu, 1 I.

[D]Vitamin mix provided (/kg diet): vitamin A, 9,000 IU; vitamin $D_3$, 3,500 IU; vitamin E, 25 IU; menadione, 1.5 mg; pantothenic acid, 15 mg; riboflavin, 6 mg; niacin, 70 mg; thiamine, 1.4 mg; pyridoxine, 3.0 mg; folic acid, 2.0 mg; biotin, 0.10 mg; vitamin $B_{12}$, 0.014 mg; selenium, 0.25 mg; ethoxyquin, 125 mg.

[E]2 lb/ton phytase was added = 600 FTU/kg to release 0.10% npP, according to the manufacturer (BASF Corp., Mount Olive, NJ)

**Table 11. Composition and selected nutrient content of Grower 2 diets (Experiment 2)**

| Ingredient, % | Grower 2 diets (12-15 wk) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| YD corn[A] | 62.63 | 64.25 | - | - |
| NDLP corn[B] | - | - | 70.32 | 70.32 |
| Soybean meal (48% CP) | 28.33 | 28.05 | 24.28 | 24.28 |
| Choice White Grease | 5.64 | 5.07 | 2.85 | 2.85 |
| Limestone | 0.99 | 1.17 | 1.28 | 1.28 |
| Dicalcium phosphate | 1.45 | 0.51 | 0.29 | 0.29 |
| Salt | 0.35 | 0.35 | 0.34 | 0.34 |
| DL-Methionine | 0.26 | 0.26 | 0.24 | 0.24 |
| L-Lysine-HCl | 0.12 | 0.13 | 0.19 | 0.19 |
| Trace mineral mix[C] | 0.10 | 0.10 | 0.10 | 0.10 |
| Vitamin mix[D] | 0.12 | 0.12 | 0.12 | 0.12 |
| Natuphos® 600[E] | - | - | - | + |
| Nutrients | | | | |
| ME, kcal/kg | 3,300 | 3,300 | 3,300 | 3,300 |
| CP, % | 19.00 | 19.00 | 19.00 | 19.00 |
| Ca, % | 0.76 | 0.62 | 0.62 | 0.62 |
| Calculated tP, % | 0.61 | 0.44 | 0.44 | 0.44 |
| Analyzed tP, % | 0.48 | 0.39 | 0.40 | 0.43 |
| Calculated npP, % | 0.38 | 0.21 | 0.31 | 0.41 |

AYellow Dent corn

BNutriDense™ Low Phytate corn

[C]Mineral mix provided (mg/kg diet): 100 Mn, 100 Zn, 50 Fe, 10 Cu, 1 I.

[D]Vitamin mix provided (/kg diet): vitamin A, 9,000 IU; vitamin $D_3$, 3,500 IU; vitamin E, 25 IU; menadione, 1.5 mg; pantothenic acid, 15 mg; riboflavin, 6 mg; niacin, 70 mg; thiamine, 1.4 mg; pyridoxine, 3.0 mg; folic acid, 2.0 mg; biotin, 0.10 mg; vitamin $B_{12}$, 0.014 mg; selenium, 0.25 mg; ethoxyquin, 125 mg.

[E]2 lb/ton phytase was added = 600 FTU/kg to release 0.10% npP, according to the manufacturer (BASF Corp., Mount Olive, NJ)

**Table 12. Composition and selected nutrient content of Finisher diets (Experiment 2)**

| | Finisher diets (15-18 wk) | | | |
|---|---|---|---|---|
| Ingredient, % | 1 | 2 | 3 | 4 |
| YD corn[A] | 63.25 | 64.86 | - | - |
| NDLP corn[B] | - | - | 70.99 | 70.99 |
| Soybean meal (48% CP) | 26.31 | 26.03 | 22.23 | 22.23 |
| Choice White Grease | 7.58 | 7.01 | 4.77 | 4.47 |
| Limestone | 0.86 | 1.04 | 1.14 | 1.14 |
| Dicalcium phosphate | 1.15 | 0.21 | - | - |
| Salt | 0.35 | 0.36 | 0.34 | 0.34 |
| DL-Methionine | 0.20 | 0.20 | 0.18 | 0.18 |
| L-Lysine-HCl | 0.07 | 0.08 | 0.13 | 0.13 |
| Trace mineral mix[C] | 0.10 | 0.10 | 0.10 | 0.10 |
| Vitamin mix[D] | 0.12 | 0.12 | 0.12 | 0.12 |
| Natuphos® 600[E] | - | - | - | + |
| Nutrients | | | | |
| ME, kcal/kg | 3,430 | 3,430 | 3,430 | 3,430 |
| CP, % | 18.00 | 18.00 | 18.00 | 18.00 |
| Ca, % | 0.64 | 0.50 | 0.50 | 0.50 |
| Calculated tP, % | 0.54 | 0.37 | 0.37 | 0.37 |
| Analyzed tP, % | 0.53 | 0.42 | 0.44 | 0.36 |
| Calculated npP, % | 0.32 | 0.15 | 0.25 | 0.35 |

AYellow Dent corn

BNutriDense™ Low Phytate corn

[C]Mineral mix provided (mg/kg diet): 100 Mn, 100 Zn, 50 Fe, 10 Cu, 1 I.

[D]Vitamin mix provided (/kg diet): vitamin A, 9,000 IU; vitamin $D_3$, 3,500 IU; vitamin E, 25 IU; menadione, 1.5 mg; pantothenic acid, 15 mg; riboflavin, 6 mg; niacin, 70 mg; thiamine, 1.4 mg; pyridoxine, 3.0 mg; folic acid, 2.0 mg; biotin, 0.10 mg; vitamin $B_{12}$, 0.014 mg; selenium, 0.25 mg; ethoxyquin, 125 mg.

[E]2 lb/ton phytase was added = 600 FTU/kg to release 0.10% npP, according to the manufacturer (BASF Corp., Mount Olive, NJ)

Turkeys were weighed every 3 wk and at 16.5 wk and again at 18 wk. Feed disappearance was recorded every 3 wk. All performance measurements were calculated in the same way for Experiment 2 as for Experiment 1.

BONE ANALYSES

At the end of both experiments (17 wk for Experiment 1 and 16.5 wk for Experiment 2, three and six birds, respectively, per pen were chosen for slaughter at the Michigan State University USDA inspected Meat Laboratory to obtain bone samples. Birds were chosen to match target body weight from breeder guidelines [20].

*Bone Breaking Strength*

Procedures for bone breaking strength were followed using shear and three-point bending tests of animal bone guidelines from the ASAE Standards guide [27]. The standards guide does not give equations for cross-sectional area. Ulna, tibia, and femur cross sectional shape most closely resembled a hollow quadrant of an ellipse. The cross sectional area of a hollow ellipse was determined by the following equation:

$$\text{A (m}^2\text{)} = \frac{1}{4} \times \pi \times \left[ (BD - bv) + \left( v \times \frac{B-b}{2} \right) + \left( b \times \frac{D-v}{2} \right) \right]$$, where A = cross sectional area, $m^2$; $D$ = minor outside diameter of the bone cross section (m); $B$ = major outside diameter of the bone cross section (m); $w$ = average cortical bone wall thickness (m); $v = D - 2w$ (m); and $b = B - 2w$ (m). Bone wall thickness and cross-sectional diameters were measured at the point of breakage with a digital caliper [28] in Experiment 1 and with computed tomography [29] in Experiment 2. All breaking tests were performed using an Instron Universal Testing Machine [30] equipped with a 10 kN load cell that had

a crosshead speed of 5 mm/min. Bone fracture force values, measured in Newtons (N) of force, were recorded. Ultimate shear stress (strength) was calculated for ulna and femur bone samples according to the ASAE Standards guide [27] equation: Ultimate shear stress (Pa) = $\tau = \frac{F}{2A}$, where F = maximum force required to break a bone (N); and A = cross sectional area of the bone ($m^2$). Ultimate bending stress (strength) was calculated for tibia bone samples according to the ASAE Standards guide [27] equation: Ultimate bending stress (MPa) = $\sigma_u = \frac{FLC}{4I}$, where $F$ = maximum force required to break a bone (N); L = distance between fulcra supports (m), which was set to 0.10 m; C = distance from neutral axis to outer fiber = 0.57559D (m); and I = moment of inertia ($m^4$), which was determined by the equation $0.0549 \times \left(BD^3 - bv^3\right)$.

*Computed Tomography*

Computed tomographic (CT) scanning was performed on the ulna, humerus, tibia, and femur bones from Experiment 2 when machinery became available at MSU Veterinary Radiology to acquire cross-sectional images in the transverse plane. Individual bone samples, of the same type, were positioned five at a time on a bone density phantom pad with built-in hydroxyapatite standards for bone mineral density (BMD) analysis. One slice of 10 mm thickness was acquired at the mid-diaphysis using the bone algorithm at a 512 x 512 matrix. Table height was 142 cm to position the specimens in the center of the imaging field. Measurements of cortical bone thickness, total bone diameter (major and minor) and cross-sectional area (cortical and medullary) were performed on the CT computer. Image data was transferred to a remote CT console with BMD analysis software. The CT density values of four selected regions of cortical

bone were compared to the CT density of the hydroxyapatite standards within each image to estimate average cortical BMD. This technique is known as quantitative CT (qCT) [31].

*Bone Ash*

Broken bones (all pieces) were soaked in ethanol for a minimum of 1 wk, dried, extracted with ether [32], oven dried at 106°C for 24 h, and ashed in a muffle furnace at 600°C for 24 h.

TOTAL PHOSPHORUS ANALYSIS

Feed samples were collected during the mixing of each dietary phase for tP analysis. During the last week of each study, raw excreta samples (four pens per treatment) were collected for 3 consecutive days for tP analysis. Litter samples (four pens per treatment) were collected at the end of each study according to the method described by Smith and Lacy [33]. Excreta and litter samples were dried in an oven at 50°C for 24 h or until no moisture accumulated when warm samples were placed in plastic storage bags. Feed, excreta, and litter samples were ground first to pass through a 1.0-mm sieve [34] then through a 0.5-mm sieve [35]. Duplicate samples of feed, excreta, and litter were digested by a nitric acid microwave wet digestion system [36]. Phosphorus concentrations in feed, excreta, and litter samples were analyzed colorimetrically [37] by a molybdo-sulfuric method [38].

STATISTICAL ANALYSIS

All data were analyzed using the General Linear Models (GLM) procedure of SAS® software [39]. Pen was defined as the experimental unit. Significantly different dietary treatment pen means were separated using the least square means option of GLM.

Standard errors for treatment pen means were determined using the standard error option of GLM. Treatment means were considered significantly different at $P < 0.05$.

**Results and Discussion**

*Growth Performance*

Results of Experiment 1 diets on growth performance are reported in Table 13.

**Table 13. Growth performance of turkeys fed diets containing yellow dent (YD) or NutriDense™ Low Phytate (NDLP) corn for Starter 1 (ST1), Starter 2(ST2), Grower 1 (GR1), Grower 2 (GR2), and Finisher (FIN) phases (Experiment 1)**

| TREATMENT | | | | PHASE | | | | |
|---|---|---|---|---|---|---|---|---|
| Diet | Corn Source | npP[A] | P availability of corn (%) | ST1 (3-6 wk) | ST2 (6-9 wk) | GR1 (9-12 wk) | GR2 (12-15 wk) | FIN (15-17 wk) |
| | | | | BODY WEIGHT (kg)[B] | | | | |
| 1 | YD | Control | 32 | 2.20 | 5.30 | 8.93 | 12.29 | 14.47 |
| 2 | YD | Control-0.10% | 32 | 2.21 | 5.27 | 8.82 | 12.34 | 14.35 |
| 3 | NDLP | as Diet 2 | 75 | 2.29 | 5.46 | 9.13 | 12.56 | 14.71 |
| 4 | NDLP | as Diet 2 | 90 | 2.27 | 5.44 | 9.17 | 12.72 | 14.77 |
| SEM | | | | 0.03 | 0.06 | 0.09 | 0.13 | 0.13 |
| Probability | | | | 0.07 | 0.09 | 0.05 | 0.14 | 0.12 |
| | | | | FEED INTAKE (kg)[B] | | | | |
| 1 | YD | Control | 32 | 2.75 | 5.90 | 8.59 | 10.45[a] | 6.85 |
| 2 | YD | Control-0.10% | 32 | 2.70 | 5.68 | 8.50 | 11.14[b] | 6.53 |
| 3 | NDLP | as Diet 2 | 75 | 2.80 | 5.76 | 8.64 | 11.04[ab] | 6.85 |
| 4 | NDLP | as Diet 2 | 90 | 2.65 | 5.85 | 8.60 | 11.41[b] | 6.41 |
| SEM | | | | 0.05 | 0.09 | 0.11 | 0.15 | 0.22 |
| Probability | | | | 0.30 | 0.38 | 0.85 | 0.01 | 0.39 |
| | | | | FEED CONVERSION (kg:kg)[BC] | | | | |
| 1 | YD | Control | 32 | 1.76 | 1.93 | 2.41 | 3.12 | 3.22 |
| 2 | YD | Control-0.10% | 32 | 1.69 | 1.86 | 2.47 | 3.22 | 3.25 |
| 3 | NDLP | as Diet 2 | 75 | 1.70 | 1.86 | 2.47 | 3.22 | 3.25 |
| 4 | NDLP | as Diet 2 | 90 | 1.62 | 1.85 | 2.30 | 3.26 | 3.24 |
| SEM | | | | 0.04 | 0.04 | 0.05 | 0.06 | 0.12 |
| Probability | | | | 0.15 | 0.42 | 0.15 | 0.49 | 0.95 |

[A]npP = non-phytate P

[B]Data are means of four replicate pens

[C]Feed conversion (feed:gain) was calculated by $\frac{FI}{G}$, where $FI$ = feed intake for the phase and $G$ = mortality adjusted pen gain for the phase

[a,b]For the same varible, mean values in the same column with unlike superscripts are significantly different ($P<0.05$)

Results of Experiment 2 diets on growth performance are presented in Table 14.

**Table 14. Growth performance of turkeys fed diets containing yellow dent (YD) or NutriDense™ Low Phytate (NDLP) corn for Starter 2(ST2), Grower 1 (GR1), Grower 2 (GR2), and Finisher (FIN) phases (Experiment 2)**

| TREATMENT | | | | PHASE | | | |
|---|---|---|---|---|---|---|---|
| Diet | Corn Source | npP[A] | Phytase (FTU/kg[B]) | ST2 (6-9 wk) | GR1 (9-12 wk) | GR2 (12-15 wk) | FIN (15-18 wk) |
| | | | | BODY WEIGHT (kg)[C] | | | |
| 1 | YD | Control | 0 | 5.72 | 9.37 | 12.74 | 16.78 |
| 2 | YD | Control-0.20% | 0 | 5.60 | 9.21 | 12.59 | 16.46 |
| 3 | NDLP | as Diet 2 | 0 | 5.54 | 9.15 | 12.45 | 16.28 |
| 4 | NDLP | as Diet 2 | 600 | 5.53 | 9.33 | 12.58 | 16.49 |
| SEM | | | | 0.06 | 0.07 | 0.12 | 0.20 |
| Probability | | | | 0.12 | 0.17 | 0.44 | 0.40 |
| | | | | FEED INTAKE (kg)[C] | | | |
| 1 | YD | Control | 0 | 5.99 | 9.30 | 8.24 | 11.98 |
| 2 | YD | Control-0.20% | 0 | 5.91 | 9.03 | 8.15 | 12.17 |
| 3 | NDLP | as Diet 2 | 0 | 5.97 | 9.24 | 8.23 | 11.93 |
| 4 | NDLP | as Diet 2 | 600 | 5.92 | 9.09 | 8.47 | 11.72 |
| SEM | | | | 0.17 | 0.17 | 0.12 | 0.61 |
| Probability | | | | 0.98 | 0.66 | 0.28 | 0.96 |
| | | | | FEED CONVERSION (kg:kg)[CD] | | | |
| 1 | YD | Control | 0 | 1.88 | 2.55 | 2.49 | 3.50 |
| 2 | YD | Control-0.20% | 0 | 1.94 | 2.51 | 2.54 | 3.28 |
| 3 | NDLP | as Diet 2 | 0 | 1.98 | 2.55 | 2.54 | 3.69 |
| 4 | NDLP | as Diet 2 | 600 | 1.98 | 2.43 | 2.68 | 3.30 |
| SEM | | | | 0.05 | 0.06 | 0.07 | 0.21 |
| Probability | | | | 0.45 | 0.45 | 0.33 | 0.49 |

[A]npP = non-phytate P

[B]Natuphos® phytase (BASF Corp., Mount Olive, NJ); 1 FTU is defined as the amount necessary to liberate 1 µmole of inorganic P per minute from 0.0015 mole of sodium phytate at 37°C and pH 5.5

[C]Data are means of four replicate pens

[D]Feed conversion (feed:gain) was calculated by $\frac{FI}{G}$, where $FI$ = feed intake for the phase and $G$ = mortality adjusted pen gain for the phase

Diet 1 in both experiments was designed to insure that control diets were meeting nutritional needs to support growth parameters (body weight, feed intake, and feed conversion). The standard expected growth responses used in our studies were breeder [ ] performance goals. Across dietary phases, in Experiment 1, turkeys fed control diets had body weights that were numerically lower than breeder [20] performance goals (2.20 vs. 2.65 kg for Starter 1; 5.30 vs. 5.63 kg for Starter 2; 8.93 vs. 9.14 kg for Grower 1; 12.29 vs. 12.77 kg for Grower 2; 14.47 vs. 15.12 kg for Finisher). In Experiment 2, turkeys fed control diets had body weights that were numerically higher for all phases except the Grower 2 phase as compared to breeder [20] recommendations. Although numerical differences in body weight exist between turkeys from our experiments and breeder [20] performance goals, the differences may not be statistically significant, indicating that our control diets were adequate to support similar body weight as breeder [20] performance goals. Compared to growing-finishing turkey studies at other universities, such as those reported by Atia et al. [17] or Waldroup et al. [40], turkeys grown in our facilities generally had higher body weight, probably due to differences in facilities. Turkeys in Experiment 1 did not grow as well as in other studies [26,41] at Michigan State University. However, turkeys from Experiment 2 were comparable in body weight to what we have seen in previous studies [26,41].

The calculated npP concentrations in our control diets were less than typical npP concentrations fed in the Midwestern USA [42] or Southeastern USA [43] commercial tom diets. Due to warmer temperatures in the Southeastern USA compared to the Midwestern USA, turkeys would be eating less feed and would therefore require a greater concentration of npP in the diet to meet their npP requirements. Even though our diets

contained less than typical concentrations of npP, growth performance was still adequate, based on breeder performance goals [20] for body weight.

In Experiment 1, turkeys fed control diets consumed more feed for Starter 2, Grower 1, and Grower 2 as compared to breeder [20] estimates for feed consumption and numerically less feed for Starter 1 and Finisher phases. In Experiment 2, turkeys fed control diets consumed more feed for Starter 2 and Grower 1 phases and numerically less feed for Grower 2 and Finisher phases, as compared to breeder [20] estimates for feed consumption. Numerical differences in feed consumption between turkeys in our studies as compared to breeder [20] estimates for feed consumption might fluctuate due to environmental temperature or the form of feed that was fed, since the turkeys were fed a mash form of diet in this study.

In Experiment 1, turkeys fed Diets 2 and 4 consumed more ($P<0.02$) feed than turkeys fed Diet 1 (11.14 and 11.41 vs. 10.45 kg) for the Grower 2 phase. Such differences in feed intake were unexpected and cannot be explained. Turkeys fed in Experiment 2 appeared to have eaten slightly more feed than turkeys fed in Experiment 1 for the Starter 2 and Grower 1 periods. Turkeys fed in Experiment 1, however, ate more ($P<0.05$) feed than turkeys fed in Experiment 2 for the Grower 2 phase. This may have been compensatory feed consumption if poults were of lower quality and matured later as compared to poults from Experiment 2. Also, in Experiment 2, feed consumption did not increase for the Grower 2 phase right after a period of higher growth (Grower 1) possibly because those turkeys matured earlier than those in Experiment 1.

Turkeys fed the NDLP diets tended ($P<0.10$) to be heavier at the end of Starter 1, Starter 2, and Grower 1 phases (Table 13) in Experiment 1. The increase in body weight

during these phases was most likely due to incorrect estimates of ME for YD and NDLP. The higher BW may have been due to higher energy consumption if ME was underestimated for NDLP and/or overestimated for YD. The estimated ME value for NDLP in Experiment 1 was 3,480 kcal/kg and 3,410 kcal/kg for YD. The estimate of ME for YD is one used in the commercial turkey industry [24]. The estimated ME value for NDLP was determined using the equation described by Dale et al. [25] for oil content and added energy content in consideration of the increased CP compared to YD. Starch was not accounted for by this equation in Experiment 1, which likely resulted in an underestimation of ME. Prior to the start of Experiment 2, a sample of NDLP was sent to the University of Georgia for a cecectomized rooster assay to obtain an analyzed TME value, which was 3,490 kcal/kg [23]. From these analyzed values, our original estimate for ME (Experiment 1) of NDLP may have been slightly underestimated, which could have resulted in heavier body weights, keeping in mind that dietary treatments were designed to be isonitrogenous and isocaloric. Estimated ME for YD in Experiment 2 was decreased to the value reported by Dale et al. [25]. There was no differences in body weight due to corn type in Experiment 2, suggesting that ME values in Experiment 2 were correct and dietary treatments were isocaloric.

In both experiments, Diet 2 was designed to be a negative control diet for P and turkeys fed this diet were expected to be lighter than turkeys fed other diets, if analyzed dietary P had met calculated dietary P. Atia et al.[17], Yan et al. [19], and Ledoux et al.[44] showed that when turkeys are fed lower concentrations of dietary P (30 and 52% < NRC [1]; a low P concentration; and NRC [1] – 0.15% for Atia et al. [17], Yan et al. [19], and Ledoux et al. [44], respectively), decreased body weight can result. Our experiments

did not yield lower turkey body weights for Diet 2 fed birds, although turkeys in Experiment 1 fed Diet 2 tended ($P<0.10$) to be lighter than turkeys fed all other diets at 17 wk of age.

Estimates for tP intake were determined for each phase by multiplying estimated feed intakes by the analyzed concentrations of dietary tP. From these estimates, turkeys fed Diet 2 did not necessarily consume the least amount of dietary tP, as compared to other diets. In Experiment 1, turkeys fed Diet 2 consumed less ($P<0.0001$) dietary tP than turkeys fed Diet 1 (223.98 vs. 237.51 g), but more ($P<0.0001$) dietary tP than Diets 3 and 4 (223.98 vs. 207.25 and 212.94 g). In Experiment 2, turkeys fed Diet 2 consumed less ($P<0.0001$) dietary tP than turkeys fed Diet 1 (155.71 vs. 188.60 g), but consumed the same amount of dietary tP as Diets 3 and 4 (155.71 vs. 166.80, and 151.22 g). Because turkeys fed Diet 2 in both experiments did not necessarily consume the least amount of dietary tP, body weights were not necessarily lighter, as would have been expected. Some variation in the P concentration of YD used for diets may have contributed to deviation from calculated dietary P concentrations. Mixing error may have also contributed to variations in dietary P concentrations as compared to calculated values.

Growth performance of turkeys fed Diets 3 and 4 were similar ($P>0.05$) to turkeys fed Diet 1 in both experiments. These results matched expectations for growth performance, which was that replacing YD with NDLP with or without phytase should not negatively impact growth performance.

*Bone Parameters*

Bone parameters (fracture force, strength, ash, and mineral density) were investigated as indirect measures of P status. Because approximately 80% of the P found in the body exists as part of the skeleton, bone characteristics should be indicators of P status [45].

The femur was selected as a bone for testing because it has been observed as the bone that is often broken in the commercial turkey industry [46]. Lilburn [47] reported that the femur and tibia are sensitive bones in investigating leg development in growing-finishing turkeys (8 to 16 wk of age). Crenshaw et al.[48] reported that the femur was a sensitive bone for measuring responses to dietary P in pigs. Observations from previous experiments [48] at Michigan State University indicated that the femur, tibia, and ulna are sensitive bones for measuring responses to dietary P. Studies involving the investigation of dietary P on performance [17,44] of growing-finishing turkeys have reported the tibia as a bone useful as an indication of dietary P utilization.

The effects of dietary treatments on bone fracture force and ultimate stress (strength) for Experiment 1 are presented in Table 15. No treatment differences in bone fracture force were observed in Experiment 1. When an equation to determine ultimate shear stress (strength) was applied, using bone fracture force values, treatment differences ($P<0.02$) in ulna ultimate shear stress were observed. Ulnas from turkeys that were fed Diet 2 withstood less pressure than ulnas from turkeys that were fed Diet 1 (19.10 vs. 16.82 MPa). Ulna ultimate shear stress values from turkeys that were fed Diets 3 and 4 (17.30 and 17.99 MPa) did not differ from Diets 1 or 2. No treatment differences in bone ultimate stress were observed for other bones in Experiment 1.

**Table 15. Bone fracture force and bone strength (stress) of turkeys fed diets containing yellow dent (YD) or NutriDense™ Low Phytate (NDLP) corn (Experiment 1)**

| TREATMENT | | | | BONE TYPE | | |
|---|---|---|---|---|---|---|
| Diet | Corn Source | npP[A] | P avail. of corn (%) | Ulna (shear) | Femur (shear) | Tibia (bend) |
| | | | | BONE FRACTURE FORCE (N)[BC] | | |
| | | | | $F_s$ | $F_s$ | $F_b$ |
| 1 | YD | Control | 32 | 2578.97 | 1968.46 | 938.50 |
| 2 | YD | Control-0.10% | 32 | 2415.71 | 2170.63 | 878.33 |
| 3 | NDLP | as Diet 2 | 75 | 2290.07 | 1894.99 | 913.89 |
| 4 | NDLP | as Diet 2 | 90 | 2366.83 | 2012.69 | 837.61 |
| SEM | | | | 76.78 | 130.85 | 48.32 |
| Probability | | | | 0.11 | 0.52 | 0.50 |
| | | | | BONE STRENGTH (MPa)[BD] | | |
| | | | | $\tau$ | $\tau$ | $\sigma_u$ |
| 1 | YD | Control | 32 | 19.10[a] | 7.97 | 105.52 |
| 2 | YD | Control-0.10% | 32 | 16.82[b] | 8.65 | 104.01 |
| 3 | NDLP | as Diet 2 | 75 | 17.30[ab] | 8.15 | 106.53 |
| 4 | NDLP | as Diet 2 | 90 | 17.99[ab] | 8.40 | 95.39 |
| SEM | | | | 0.43 | 0.27 | 8.08 |
| Probability | | | | 0.02 | 0.36 | 0.76 |

[A]npP = non-phytate P

[B]Data are means of four replicate pens of three turkeys each

[C]Fracture force is the force required to break a bone, measured in Newtons (N); 1 N = 0.102 kgf (kilograms force) = 9.8 kg (kilograms mass)

[D]Bone strength (ultimate stress, $\sigma_u$), measured in Pascals (Pa) is determined by the equation $\sigma_u = \frac{F_b LC}{4I}$ for ultimate bending stress and $\tau = \frac{F_s}{2A}$, where $F_b$= bone bending fracture force (N), $F_s$ = bone shear fracture force (N), A = cross-sectional area of the bone ($m^2$), L = distance between fulcra supports (tibia = 0.10 m), $C$ = distance from neutral axis to outer fiber (m), $I$ = moment of inertia ($m^4$); 1 MegaPascal (MPa) = $Pa \times (1 \times 10^{-6})$; 1 Pa = $1 \frac{N}{m^2}$

[a,b]For the same variable, mean values in the same column with unlike superscripts are significantly different ($P<0.05$)

Results from Experiment 2 for the effects of dietary treatments on bone fracture force and ultimate stress (strength) are presented in Table 16. Turkeys fed Diet 3, in Experiment 2 had femurs that withstood less breaking force than femurs from turkeys fed Diet 2 (1511.35 vs. 1685.40 N, respectively). When ultimate stress values were calculated from bone cross-sectional measurements using a digital micrometer, no treatment differences were observed. When qCT was used to measure bone cross-sectional area, however, treatment differences for femur samples were observed ($P<0.02$). This would indicate that perhaps using qCT technology is a more accurate way to determine cross-sectional area than by hand measurements with a digital caliper. The advantage of qCT is that bones do not need to be broken to take cross-sectional measurements. Femurs from turkeys fed Diet 3 withstood less pressure than femurs from turkeys fed Diet 2 (18.36 vs. 20.96 MPa). Ultimate stress values for femurs from turkeys fed Diets 1 and 4 (18.21 and 20.12 MPa) did not differ from ultimate stress values for femurs from turkeys fed Diet 3 or Diet 2. These results were not consistent with the results from Experiment 1 ulna stress, which may be partially explained by inconsistencies in dietary tP concentrations, particularly in Starter 2 and Grower 1. Diet 1 contained 0.15% less tP than expected (0.76%), which may partially explain why femurs from turkeys fed Diet 1 were not stronger than femurs from turkeys fed the negative control diet. Similarly, in Grower 2, Diet 1 contained 0.12% less tP than expected. Why Diet 2 femurs were stronger than Diet 3 femurs is questionable since they were formulated to contain the same concentration of tP, and Diet 3 was calculated to contain a greater concentration of npP than Diet 2. This should have yielded stronger femurs in Diet 3. No other bone strength differences were observed in Experiment 2.

**Table 16. Bone fracture force and bone strength (stress) of turkeys fed diets containing yellow dent (YD) or NutriDense™ Low Phytate (NDLP) corn (Experiment 2)**

| TREATMENT | | | | BONE TYPE | | |
|---|---|---|---|---|---|---|
| Diet | Corn Source | npP[A] | Phytase (FTU/kg[B]) | Ulna (shear) | Femur (shear) | Tibia (bend) |
| | | | | BONE FRACTURE FORCE (N)[CD] | | |
| | | | | $F_s$ | $F_s$ | $F_b$ |
| 1 | YD | Control | 0 | 2542.90 | 2919.54[ab] | 702.65 |
| 2 | YD | Control-0.20% | 0 | 2414.48 | 2929.51[a] | 654.60 |
| 3 | NDLP | as Diet 2 | 0 | 2439.05 | 2863.38[b] | 688.28 |
| 4 | NDLP | as Diet 2 | 600 | 2326.81 | 2810.20[ab] | 683.18 |
| SEM | | | | 71.45 | 160.05 | 21.30 |
| Probability | | | | 0.25 | 0.95 | 0.47 |
| | | | | BONE STRENGTH (MPa)[CE], USING qCT[F] | | |
| | | | | $\tau$ | $\tau$ | $\sigma_u$ |
| 1 | YD | Control | 0 | 21.26 | 18.21[ab] | 71.46 |
| 2 | YD | Control-0.20% | 0 | 20.72 | 20.96[a] | 63.56 |
| 3 | NDLP | as Diet 2 | 0 | 20.87 | 18.36[b] | 69.30 |
| 4 | NDLP | as Diet 2 | 600 | 20.26 | 20.12[ab] | 64.62 |
| SEM | | | | 0.69 | 1.15 | 2.34 |
| Probability | | | | 0.79 | 0.30 | 0.10 |
| | | | | BONE STRENGTH (MPa)[CE] | | |
| | | | | $\tau$ | $\tau$ | $\sigma_u$ |
| 1 | YD | Control | 0 | 32.79 | 15.66 | 74.57 |
| 2 | YD | Control-0.20% | 0 | 31.42 | 15.83 | 66.06 |
| 3 | NDLP | as Diet 2 | 0 | 31.52 | 16.69 | 73.38 |
| 4 | NDLP | as Diet 2 | 600 | 28.79 | 15.72 | 67.85 |
| SEM | | | | 1.40 | 0.57 | 2.97 |
| Probability | | | | 0.28 | 0.56 | 0.18 |

[A]npP = non-phytate P

[B]Natuphos® phytase (BASF Corp., Mount Olive, NJ); 1 FTU is defined as the amount necessary to liberate 1 µmole of inorganic P per minute from 0.0015 mole of sodium phytate at 37°C and pH 5.5

[C]Data are means of four replicate pens of six turkeys each

[D]Fracture force is the force required to break a bone, measured in Newtons (N);
1 N = 0.102 kgf (kilogram force) = 9.8 kg (kilograms mass)

[E]Bone strength (ultimate stress, $\sigma_u$), measured in Pascals (Pa) is determined by the equation $\sigma_u = \frac{F_b LC}{4I}$ for ultimate bending stress and $\tau = \frac{F_s}{2A}$, where $F_b$= bone bending fracture force (N), $F_s$ = bone shear fracture force (N), A = cross-sectional area of the bone ($m^2$), L = distance between fulcra supports (tibia = 0.10 m), $C$ = distance from neutral axis to outer fiber (m), $I$ = moment of inertia ($m^4$); 1 MegaPascal (MPa) = $Pa \times \left(1 \times 10^{-6}\right)$; 1 Pa = $1 \frac{N}{m^2}$

[F]qCT = quantitative computed tomography for cross-sectional x-ray scan of a bone to determine cross-sectional area and wall thickness prior to bone breakage

[a,b]For the same variable, mean values in the same column with unlike superscripts are significantly different ($P<0.05$)

The ulna was the most consistent bone for breaking tests. The ulna appeared to be more uniform in shape and did not shift noticeably in position during Instron testing. The femurs and tibias were more likely to slide on the fulcra points or shift in position while testing. However, attempts were made to minimize this from occurring in Experiment 2. The breaking pattern of the femurs were much different than the other bones. Femurs would shatter into many different pieces whereas ulnas would have a clean shear through the midsection of the bone and tibias would begin to break along the length of the bone. The breaking pattern of the femur may indicate that it might be a more sensitive bone for phosphorus utilization, but a lack of expected responses to dietary treatments in the current study does not lend support to this theory. Bones from turkeys fed Diet 2 would have been expected to be weaker than bones from turkeys fed other diets. Failure to obtain expected bone responses in Experiment 2 may have resulted because analyzed dietary P did not match calculated dietary P in the Starter 2 through Grower 2 periods. Ulna responses in Experiment 1 may have been partly attributed to proper dietary implementation of treatments during the 3-week Prestarter group brooding period. Previous studies have shown that differences in cortical thickness of turkey bones were only significant in younger turkeys [49]. Why there were no responses from other bones, however, is puzzling. Other evidence indicates that dietary treatment differences should have been observed. Roberson et al. [50] observed linear increases in femur, tibia, and ulna bone strength when 300-600 FTU/kg Natuphos® phytase was added to low npP diets (0.45, 0.40, or 0.35% dietary npP in Grower 1, Grower 2, and Finisher diets, respectively) and fed to male turkeys up to 17 wk of age. Since Roberson et al. [50] observed

treatment differences in bone strength when dietary treatments were started when turkeys were 9 wk of age, their findings do not lend support to the theory that dietary treatments should be implemented in the Starter periods. Ledoux et al. [44] reported that when turkey hens were fed either 0 or 1000 FTU of Natuphos® phytase to diets containing either NRC [1] or NRC [1] –0.15% concentrations of dietary npP, no dietary treatment differences ($P$=0.082) were observed in tibia breaking strength taken from 15 wk old hens. Mean tibia-breaking strength ranged from 70.6 to 76.6 kg force across treatment groups, which would equate to a range of 692.3 to 751.2 N. Tibia fracture force values appeared to be greater (in Experiment 1) than values reported by Ledoux et al. [44] with mean tibia bending strength values ranging from 837.6 to 938.5 N (or 85.4 to 95.7 kg force). In Experiment 2, our mean tibia strength values ranged from 654.6 to 701.65 (or 66.8 to 71.5 kg force), which appeared to be less than what Ledoux et al. [44] had reported. Our calculated diets should have contained more npP than NRC [1] recommendations. Previous research has indicated that the npP requirement for growth may not necessarily be the optimum for bone ash and bone strength [11]. If that is the case, then that may be why Ledoux et al. [44] did not see responses for bone strength. Atia et al. [17] observed that when 500 FTU/kg Natuphos® phytase was added to diets containing 30% of the NRC [1] recommendation of dietary npP, tibia strength of 7 wk old male turkeys was improved. Because our dietary npP concentrations were formulated to contain more npP than NRC [1] recommendations and NDLP with or without phytase should have improved P utilization, we should have seen more responses in bone strength in both experiments. Other research investigating turkey bone responses to dietary P [12,18,19] lends support that the current studies should have shown more responses in

bone fracture force and strength. Analysis of dietary pP was not performed in the current study and we cannot determine exactly what npP concentrations were fed, so it is not known precisely where the discrepancies exist in the analyzed vs. calculated dietary P concentrations.

Most values of bone "strength" are reported as kg. This is not the preferred way to report bone "strength", according to the ASAE standards guide [27]. The preferred units for reporting bone fracture force is as Newtons (*N*). One Newton is the force required to accelerate a mass of one kilogram 1 $m/s^2$; force equals mass times acceleration. The acceleration due to gravity on Earth is 9.80665 $m/s^2$, so 1 N is associated with 0.10141 kg mass. Another unit of force is kgf, or kilogram force. One kgf is equivalent to 1/9.80665 of a Newton, so 1 N = 0.10197 kgf. Also, what has often been reported as "strength" may actually be "fracture force". The correct measure of bone "strength", according to the ASAE Standards guide [27] is as "ultimate stress", which is the fracture force applied per unit area, measured in Pascals (Pa), which is equivalent to 1 $\frac{N}{m^2}$. This value can be converted to MegaPascals (MPa) for the ease of looking at numbers. One MPa is equivalent to $Pa \times \left(1 \times 10^{-6}\right)$.

No treatment differences in bone average cortical wall thickness or percentage bone ash (Table 17 for Experiment 1 and Table 18 for Experiment 2) were observed.

**Table 17. Bone cortical wall thickness and bone ash of turkeys fed diets containing yellow dent (YD) or NutriDense™ Low Phytate (NDLP) corn (Experiment 1)**

| TREATMENT | | | | BONE TYPE | | |
|---|---|---|---|---|---|---|
| Diet | Corn Source | npP[A] | P availability of corn (%) | Ulna | Femur | Tibia |
| | | | | BONE WALL THICKNESS (mm)[B] | | |
| 1 | YD | Control | 32 | 1.22 | 1.50 | 1.55 |
| 2 | YD | Control-0.10% | 32 | 1.32 | 1.51 | 1.45 |
| 3 | NDLP | as Diet 2 | 75 | 1.18 | 1.39 | 1.50 |
| 4 | NDLP | as Diet 2 | 90 | 1.19 | 1.45 | 1.46 |
| SEM | | | | 0.05 | 0.06 | 0.03 |
| Probability | | | | 0.20 | 0.52 | 0.15 |
| | | | | BONE ASH (%)[B] | | |
| 1 | YD | Control | 32 | 45.85 | 41.20 | 45.71 |
| 2 | YD | Control-0.10% | 32 | 45.92 | 41.48 | 45.39 |
| 3 | NDLP | as Diet 2 | 75 | 44.74 | 40.98 | 46.74 |
| 4 | NDLP | as Diet 2 | 90 | 45.01 | 43.07 | 47.23 |
| SEM | | | | 1.05 | 1.10 | 1.36 |
| Probability | | | | 0.81 | 0.55 | 0.76 |

[A]npP = non-phytate P
[B]Data are means of four replicate pens of three turkeys each

Also, no treatment differences in average bone mineral density for Experiment 2 (Table 18) were observed.

**Table 18. Bone cortical wall thickness, bone ash, and bone mineral density of turkeys fed diets containing yellow dent (YD) or NutriDense™ Low Phytate (NDLP) corn (Experiment 2)**

| TREATMENT | | | | BONE TYPE | | |
|---|---|---|---|---|---|---|
| Diet | Corn Source | npP[A] | Phytase (FTU/kg[B]) | Ulna | Femur | Tibia |
| | | | | BONE WALL THICKNESS (mm)[C] | | |
| 1 | YD | Control | 0 | 1.24 | 2.06 | 2.12 |
| 2 | YD | Control-0.20% | 0 | 1.16 | 2.01 | 2.04 |
| 3 | NDLP | as Diet 2 | 0 | 1.18 | 1.88 | 2.07 |
| 4 | NDLP | as Diet 2 | 600 | 1.17 | 1.98 | 2.13 |
| SEM | | | | 0.03 | 0.08 | 0.04 |
| Probability | | | | 0.29 | 0.53 | 0.30 |
| | | | | BONE ASH (%)[C] | | |
| 1 | YD | Control | 0 | 43.59 | 49.04 | 49.24 |
| 2 | YD | Control-0.20% | 0 | 42.79 | 58.62 | 50.40 |
| 3 | NDLP | as Diet 2 | 0 | 46.33 | 50.22 | 49.43 |
| 4 | NDLP | as Diet 2 | 600 | 44.11 | 47.57 | 49.22 |
| SEM | | | | 1.51 | 1.79 | 0.99 |
| Probability | | | | 0.42 | 0.77 | 0.81 |
| | | | | BONE MINERAL DENSITY ($mg/cm^3$)[CD] | | |
| 1 | YD | Control | 0 | 617.44 | 538.12 | 765.64 |
| 2 | YD | Control-0.20% | 0 | 596.64 | 537.61 | 741.90 |
| 3 | NDLP | as Diet 2 | 0 | 616.45 | 519.08 | 722.36 |
| 4 | NDLP | as Diet 2 | 600 | 588.88 | 527.66 | 731.17 |
| SEM | | | | 37.54 | 35.19 | 26.41 |
| Probability | | | | 0.93 | 0.98 | 0.69 |

[A]npP = non-phytate P

[B]Natuphos® phytase (BASF Corp., Mount Olive, NJ); 1 FTU is defined as the amount necessary to liberate 1 µmole of inorganic P per minute from 0.0015 mole of sodium phytate at 37°C and pH 5.5

[C]Data are means of four replicate pens of six turkeys each

[D]Bone mineral density determined by computed tomography technology

[a,b]For the same variable, mean values in the same column with unlike superscripts are significantly different ($P<0.05$)

The lack of response to dietary treatments for cortical wall thickness, percentage bone ash, or bone mineral density was surprising, since bones from turkeys fed Diet 2 in both experiments should have had lower bone ash and bone mineral density values as wall as thinner wall thickness compared to turkeys fed control diets. Crenshaw et al. [50] reported that when pigs were fed higher concentrations of Ca and P (0.8 and 0.8 vs. 0.4 and 0.4 %, respectively), bone wall thickness and percentage bone ash increased ($P<0.01$). From this data, the current study may not have yielded differences in bone wall thickness because the differences in dietary P between treatments were not as wide as those reported by Crenshaw et al. [50]. Atia et al. [17], Ledoux et al. [44], Roberson et al. [48] and others [11,12,18,19] reported that feeding higher concentrations of dietary P to turkeys and broilers, as compared to diets containing lower concentrations of dietary P, would have higher ($P<0.05$) bone ash values. Atia et al. [17] also reported that feeding higher concentrations of dietary P could increase bone mineral density ($P<0.05$). Tibias ashed in the current study appear to contain a higher percentage of ash than values reported by Atia et al. [17]. Atia et al. [17] reported tibia mean ash values ranging from 31.3 to 49.0% from 7 wk old male turkeys. In the current study, tibia mean ash values ranged from 45.71 to 50.40% from 17 wk old male turkeys. Tibia ash values in the current study are higher than those observed by Atia et al. [17] because bones were taken 10 wk later than those collected in the study reported by Atia et al. [17], and that our diets contained higher concentrations of P. Why no treatment differences were observed in our study might be again due to analyzed dietary P concentrations not matching calculated dietary P concentrations.

*Excreted Phosphorus*

The effects of dietary treatments on litter P and excreta P for Experiment 1 are shown in Table 19.

**Table 19. Litter phosphorus and excreta phosphorus of turkeys fed diets containing yellow dent (YD) or NutriDense™ Low Phytate (NDLP) corn (Experiment 1)**

| TREATMENT | | | | LITTER P (%)[B] | EXCRETA P (%)[B] |
|---|---|---|---|---|---|
| Diet | Corn Source | npP[A] | P availability of corn (%) | 17 wk | 17 wk |
| 1 | YD | Control | 32 | 1.75[a] | 1.87[a] |
| 2 | YD | Control-0.10% | 32 | 1.42[b] | 1.43[b] |
| 3 | NDLP | As Diet 2 | 75 | 1.42[b] | 1.27[b] |
| 4 | NDLP | As Diet 2 | 90 | 1.42[b] | 1.30[b] |
| SEM | | | | 0.05 | 0.07 |
| Probability | | | | 0.001 | 0.0002 |

[A]npP = non-phytate P
[B]Data are means of four replicate pens, samples were collected as random grab samples during 15 and 17 wk of age
[a,b]For the same variable, mean values in the same column with unlike superscripts are significantly different ($P<0.05$)

Turkeys fed Diets 2, 3, and 4 in Experiment 1 had litter (collected at 116 d of age) that contained 19% less ($P<0.002$) tP than litter from turkeys that were fed Diet 1 (1.42 vs. 1.75%). Compared with turkeys fed Diet 1, turkeys fed Diets 2, 3, and 4 excreted 24, 32, and 30% less ($P<0.0002$) tP, respectively (1.87 vs. 1.43, 1.27, and 1.30%). By multiplying feed intake by the percentage of analyzed dietary tP, turkeys fed Diet 1 ate more ($P<0.0001$) tP than turkeys fed Diets 2, 3, and 4 in the Finisher phase (43.17 vs. 30.03, 30.81, and 26.91 g/bird) which matches results of excreta tP.

The effects of dietary treatments on litter P and excreta P for Experiment 2 are shown in Table 20.

**Table 20. Litter phosphorus and excreta phosphorus of turkeys fed diets containing yellow dent (YD) or NutriDense™ Low Phytate (NDLP) corn (Experiment 2)**

| TREATMENT | | | | LITTER P (%)[C] | EXCRETA P (%)[C] |
|---|---|---|---|---|---|
| Diet | Corn Source | npP[A] | Phytase (FTU/kg[B]) | 18 wk | 18 wk |
| 1 | YD | Control | 0 | 1.48[a] | 1.30[a] |
| 2 | YD | Control-0.20% | 0 | 1.14[b] | 0.89[b] |
| 3 | NDLP | As Diet 2 | 0 | 1.11[b] | 0.72[bc] |
| 4 | NDLP | As Diet 2 | 600 | 0.89[c] | 0.57[c] |
| SEM | | | | 0.40 | 0.07 |
| Probability | | | | <0.0001 | <0.0001 |

[A]npP = non-phytate P
[B]Natuphos® phytase (BASF Corp., Mount Olive, NJ); 1 FTU is defined as the amount necessary to liberate 1 μmole of inorganic P per minute from 0.0015 mole of sodium phytate at 37°C and pH 5.5
[C]Data are means of four replicate pens, samples were collected as random grab samples during the last wk of the experiment (18wk of age)
[a,b,c]For the same variable, mean values in the same column with unlike superscripts are significantly different ($P<0.05$)

Turkeys fed Diet 4 had litter that contained 40% less ($P<0.0001$) than litter from turkeys fed Diet 1 (0.89 vs. 1.48%). Litter from turkeys fed Diets 2 and 3 had 23 and 25% less ($P<0.0001$) P than litter from turkeys fed Diet 1, respectively (1.14 and 1.11 vs. 1.48%). Excreta from 18 wk-old turkeys fed Diet 4 had 58% less ($P<0.0001$) tP than excreta from turkeys fed Diet 1 (0.57 vs. 1.30%). Also, excreta from turkeys fed Diets 2 and 3 had 32 and 45% less ($P<0.0001$) tP than excreta from turkeys fed Diet 1, respectively (0.89 and 0.72 vs. 1.30%). Turkeys fed Diet 1 consumed more dietary tP than Diets 2 and 3 or 4 (188.60 vs. 155.71 and 166.80 or 151.22 g/bird). This relationship closely matches results for litter and excreta tP. A further decrease in excreted tP in Diet 4 can be

attributed to an increase in available (np) dietary P by the addition of 600 FTU/kg Natuphos® phytase.

Ledoux et al. [44] reported higher litter P values from birds fed diets that contained NRC [1] concentrations of npP than we observed (mean reported values ranged from 1.62 to 2.25%). Why we saw lower tP concentrations in our turkey litter may be due to differences in the type of shavings used or procedures for analysis employed. We used pine shavings whereas Ledoux et al. [44] used cedar shavings. We used a nitric acid microwave wet digestion procedure whereas Ledoux et al. [44] used a nitric perchloric digestion procedure. Ledoux et al. [44] reported that the P content of the cedar shavings was subtracted from the litter P value. This may have resulted in a lower than actual value of litter P. The P content of the cedar shavings was not reported in the publication. Furthermore, percentage of litter as shavings vs. feathers vs. wasted feed and excreta is not reported. These values would be nearly impossible to ascertain. Because the percentage of litter as wasted feed, feathers, and excreta is difficult, if not impossible to determine, we collected raw excreta in addition to litter. The tP concentration of pine shavings was found to be 0.03% in our studies. An average book value for turkey litter phosphorus [51] is 1.67%. Our control level turkey litter values were 1.75 and 1.48% at the end of Experiments 1 and 2, respectively, which agrees with normal expected values. Similar results were also observed in studies where broilers were fed NDLP [15].

One of the objectives of these studies was to evaluate phosphorus availability of NDLP for toms grown to market age. Diets 3 and 4 of Experiment 1 were designed to investigate this objective, with Diet 3 assuming 75% P availability for NDLP and Diet 4 assuming 90% P availability for NDLP. Because dietary analyzed P values did not

always match calculated dietary P values, claims about P availability are inconclusive. However, results from ulna stress data in Experiment 1 did provide some support to the 90% P availability assumption from NDLP analysis.

Deviation from dietary treatment regimen may have been attributed to fluctuations in ingredient P concentrations or errors in adding the right amount of each ingredient during mixing. Concentrations of P in YD are known to vary by as much as 0.10 percentage units [52]. Deviations from prescribed dietary treatment regimen have been reported in other studies [17,53].

Another objective of these studies was to investigate whether dietary phytase supplementation could further reduce excreted P when added to the NDLP diet. Diet 4 in Experiment 2 was designed to address this objective. According to the manufacturer's [54] recommendation, 2 lb/ton Natuphos® was added to the Diet 4 treatments, in which dietary P was the same as Diet 3 (except for phytase addition), to give 600 FTU/kg phytase activity, which should be adequate to replace 0.10% dietary npP. This objective was apparently met with a 20% decrease in litter P as compared to litter from turkeys fed Diet 3. Other researchers [18,55,56] have found that feeding 600-800 FTU/kg Natuphos® to poults fed diets containing low concentrations of dietary P was adequate (compared to 0 FTU/kg) to improve body weight gain and toe ash, which demonstrates that phytase is an effective way to improve dietary P utilization. Atia et al. [17] demonstrated that 500 FTU/kg Natuphos® fed to male growing-finishing turkeys on low P diets was able to improve tibia ash and tibia strength as well as body weight. Roberson et al. [48] observed that 300 FTU/kg Natuphos® phytase was adequate to maintain growth and bone strength of commercial toms gaining 178 g/d fed from 9-17 wk of age.

In summary, results from these studies suggested that P from NDLP was more available than YD, when comparing ulna data from Experiment 1. Other experimental results indicate that good growth and bone strength can be obtained with low P diets. Results showed that turkeys fed NDLP and phytase excreted 56% less excreta P and had 40% less litter P than turkeys fed YD with an adequate level of dietary P for growth.

Cost of the Finisher diet (Experiment 1, Diet 1 vs. 4) was reduced by about $6.00/ton, just as an example of how replacing YD with NDLP might result in feed cost savings. Costs of ingredients are based on current sources and our assumptions [57, 58].

**Conclusions and Applications**

1. Laboratory analyses showed that npP made up 90% of the tP in NDLP, whereas YD had 33% of the tP as npP. Ulna ultimate stress results imply that an estimate of 90% P availability for NDLP in Experiment 1 may be appropriate. Further conclusions about P availability of NDLP are limited in this study because analyzed P values in feed at various phases did not always agree with calculated values.
2. Feed cost could be reduced by approximately 5% if NDLP were fed in place of YD corn, based on our assumptions for ingredient costs and that the market price of NDLP is assumed to be the same as YD. Reduction in feed cost would arise due to the higher energy, crude protein and available P added value of NDLP, which would replace some ingredient supplementation, such as fat, soybean meal, and dicalcium phosphate, respectively.
3. Replacing YD with NDLP with or without phytase can reduce excreted P by approximately 56 (with phytase) or 40% (without phytase) in finishing toms, based on results from Experiment 2.

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## CHAPTER 2: IMPACT OF FEEDING LOW PHYTATE CORN TO WEANLING AND GROWING-FINISHING PIGS


### Abstract

Two studies were completed to determine if phosphorus in NutriDense™ Low Phytate maize (NDLP) can be formulated assuming a 90% phosphorus availability without affecting growth or bone parameters (for growing-finishing pigs only) when fed to crossbred weanling and growing-finishing pigs as compared to yellow dent maize (YD). The NDLP contained 2.9 g $kg^{-1}$ non-phytate phosphorus (npP) and 3.2 g $kg^{-1}$ total phosphorus (tP), by analysis. The YD contained 0.8 g $kg^{-1}$ npP and 2.5 g $kg^{-1}$ tP, by analysis. In Experiment 1, Treatment 1 diets contained YD with 5.7 g $kg^{-1}$ calculated tP for Grower 1 (0-33 d), 5.0 g $kg^{-1}$ calculated tP for Grower 2 (33-58 d), and 4.7 g $kg^{-1}$ calculated tP for Finisher (58-91 d). Treatment 2 diets contained NDLP with 4.7 g $kg^{-1}$ calculated tP for Grower 1, 4.0 g $kg^{-1}$ calculated tP for Grower 2, and 3.6 g $kg^{-1}$ calculated tP for Finisher. No treatment differences ($p>0.05$) in bone or growth parameters were observed. Faecal phosphorus decreased ($p<0.01$) by 41, 47, and 49% in the three phases, respectively, when NDLP was fed. In Experiment 2, Treatment 1 diets contained YD with 6.1 g $kg^{-1}$ calculated tP for Phase I (1-14 d) and 5.3 g $kg^{-1}$ calculated tP for Phase II (14-28 d). Treatment 2 diets contained YD with 4.2 g $kg^{-1}$ calculated tP for Phase I and 3.5 g $kg^{-1}$ calculated tP for Phase II. Treatment 3 and 4 diets contained NDLP with 4.5 g $kg^{-1}$ calculated tP for Phase I and 3.9 g $kg^{-1}$ calculated tP for Phase II. Treatment 4 diets also contained 500 units (FTU) $kg^{-1}$ of phytase (Natuphos™) for Phase I and 250 FTU $kg^{-1}$ for Phase II. Growth parameters were not different ($p>0.05$) were observed. Serum P was decreased in Treatment 2 as compared to Treatments 1, 3, and 4

(39.18 vs. 53.25, 48.65, and 51.60 g $kg^{-1}$). Faecal phosphorus decreased ($p<0.006$) by up to 55% when NDLP (with phytase) was fed, as compared to YD control. Faecal phosphorus was reduced by 35% when NDLP was fed in place of YD.

**Introduction**

Phytate phosphorus is of limited availability to weanling and growing-finishing pigs. Diets fed to pigs containing maize and soyabean meal have two-thirds of the phosphorus in the form of phytate phosphorus. To assure adequate growth, bone mineralization, and the prevention of phosphorus deficiency inorganic phosphorus must be supplemented. Faecal phosphorus is a challenge in swine waste management when manure phosphorus content exceeds the plants' phosphorus requirement. Water runoff can carry excess phosphorus into streams, so phosphorus could contribute to eutrophication. This phosphorus excess can be partially alleviated by increasing the bioavailability of dietary phosphorus to swine by reducing dietary phytate phosphorus or using exogenous phytase.

Raboy first described maize with a non-lethal mutation on the *lpa1-1* allele, known as low-phytate maize (Raboy et al., 2000). This mutation contained reduced phytate phosphorus without affecting the total amount of phosphorus. Phosphorus availability of low phytate maize ranges from 57 to 75% (Cromwell et al., 1998; Pierce et al., 1998; Spencer et al., 2000a) and reduced phosphorus excretion and increase phosphorus retention (Pierce et al., 1998; Veum et al., 1998; and Spencer et al., 2000a).

Exogenous phytase addition to maize-soyabean meal based pig diets has been shown to improve phosphorus availability (Cromwell et al., 1993; Lei et al., 1993a,

1993b; Young et al., 1993; Mroz et al., 1994; Adeola, 1995; Cromwell et al., 1995; Kornegay et al., 1995; Roberson, 1999).

In 1999, a variety of maize, known as NutriDense™ LP (NDLP) that was 82% lower in phytate phosphorus, 72% higher in non-phytate phosphorus, 18% higher in crude protein, and 15% higher in crude fat than conventional maize became commercially available. Table 1 compares the analyzed nutrient compositions of NDLP and YD maize.

**Table 1. Analyzed nutrient content of NutriDense™ Low Phytate (NDLP) and Yellow Dent (YD) maize**

| *Nutrient*[A] | NDLP[C] | YD[D] |
|---|---|---|
| ME,[B] (MJ $kg^{-1}$) | 14.74 | 14.31 |
| Crude Protein | 100.0 | 82.0 |
| Ether Extract | 26.0 | 22.0 |
| Arginine | 4.5 | 3.6 |
| Cysteine | 2.2 | 1.7 |
| Glycine | 3.7 | 3.0 |
| Histidine | 2.9 | 2.3 |
| Isoleucine | 3.4 | 2.5 |
| Leucine | 13.1 | 9.7 |
| Lysine | 3.2 | 2.8 |
| Methionine | 2.1 | 1.6 |
| Phenylalanine | 5.1 | 3.9 |
| Proline | 8.7 | 6.8 |
| Serine | 4.3 | 3.3 |
| Threonine | 3.3 | 2.7 |
| Tryptophan | 0.7 | 0.5 |
| Tyrosine | 2.9 | 2.4 |
| Valine | 4.7 | 3.7 |
| Ca | 0.3 | 0.1 |
| Phytate P | 0.3 | 1.7 |
| Available (non-phytate) P | 2.9 | 0.8 |
| total P | 3.2 | 2.5 |

[A]Nutrient values are expressed as g $kg^{-1}$ unless otherwise specified.
[B]Estimated values.

The objectives of these studies were to 1) determine if NDLP phosphorus was 90% available in growing-finishing and weanling pigs, 2) to determine if exogenous

phytase fed with NDLP would further improve dietary phosphorus utilization, and 3) to determine if substitution of NDLP for YD maize would alter growth parameters.

**Materials and Methods**

Two experiments were conducted. In Experiment 1, 64 castrates and female crossbred (Musclor™ ;Multigene USA, L.L.C., Clearfield, Iowa, USA 50840 x [Yorkshire-Landrace]) pigs (mean of 59 d of age; mean body weight of 20.48 kg). Pigs were allotted based on weight and sex. to eight pigs per pen, four pens per treatment and fed for 106 d. Treatment 1 diets with YD met phosphorus requirements of NRC (1998). Treatment 2 diets with NDLP were formulated to contain 1.0 g $kg^{-1}$ less total phosphorus than in Treatment 1, and the same concentration of available phosphorus. Three dietary phases were fed with calculated total phosphorus at 5.7 and 4.7 for Grower 1, 5.0 and 4.0 for Grower 2, and 4.7 and 3.6 g $kg^{-1}$ for Finisher for Treatments 1 and 2, respectively. Diet compositions are given in Table 2.

**Table 2. Composition and nutrient content of Grower 1 (GR1), Grower 2 (GR2), and Finisher (FIN) diets[A] (Experiment 1)**

| | GR1 diets (1-42 d) | | GR2 diets (42-77 d) | | FIN diets (77-106 d) | |
|---|---|---|---|---|---|---|
| *Ingredient, g kg$^{-1}$* | 1 | 2 | 1 | 2 | 1 | 2 |
| Yellow Dent maize | 689.8 | - | 737.3 | - | 774.5 | - |
| NutriDense™ Low Phytate maize | - | 756.4 | - | 808.5 | - | 849.7 |
| Soyabean meal (440 g kg$^{-1}$ Crude Protein) | 250.4 | 206.0 | 210.2 | 162.7 | 174.1 | 124.0 |
| Choice White Grease | 22.4 | 3.3 | 21.1 | 0.7 | 22.7 | 1.1 |
| Dicalcium phosphate | 11.8 | 4.5 | 9.1 | 1.3 | 8.5 | - |
| Limestone | 10.6 | 14.1 | 8.6 | 12.4 | 7.2 | 11.3 |
| Salt | 4.0 | 4.0 | 3.5 | 3.5 | 3.0 | 3.0 |
| L-Lysine HCl | - | 0.8 | 0.1 | 0.9 | - | 0.9 |
| Vitamin premix[B] | 6.0 | 6.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Trace mineral premix[C] | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| *Nutrient* | | | | | | |
| Crude Protein | 164.0 | 164.0 | 150.0 | 150.0 | 137.0 | 137.0 |
| ME (MJ kg$^{-1}$) | 13.8 | 13.8 | 14.0 | 14.0 | 14.1 | 14.1 |
| Lysine | 9.0 | 9.0 | 8.0 | 8.0 | 7.0 | 7.0 |
| Calcium | 7.5 | 7.5 | 6.3 | 6.3 | 5.5 | 5.5 |
| Calculated tP[D] | 5.7 | 4.7 | 5.0 | 4.0 | 4.7 | 3.6 |
| Analyzed tP[D] | 4.7 | 4.2 | 5.0 | 3.2 | 4.9 | 3.1 |
| Calculated aP[E] | 3.6 | 3.6 | 3.0 | 3.0 | 2.8 | 2.8 |

[A]Dietary phases were fed based on NRC (1998) body weight intervals and counted as days from the start of the experiment (GR1: 10-20 kg from Days 1 to 33), (GR2: 20-50 kg from Days 33 to 58), (FIN: 50-80 kg from Days 58 to 91)

[B]Vitamin premix provided (kg$^{-1}$ diet): Vitamin A 5511 IU (GR1), 4592 IU (GR2 and FIN); Vitamin $D_3$ 551 IU (GR1), 459 IU (GR2 and FIN); Vitamin E 66 IU (GR1), 55 IU (GR2 and FIN); Vitamin K activity 13 mg (GR1), 11mg (GR2 and FIN); menadione 4.4 mg (GR1), 3.7 mg (GR2 and FIN); Vitamin $B_{12}$ 0.03 mg; riboflavin 4.4 mg (GR1), 3.7 mg (GR2 and FIN); d-pantothenic acid 18 mg (GR1), 15 mg (GR2 and FIN); niacin 26 mg (GR1), 22 mg (GR2 and FIN); thiamine 1.1 mg (GR1), 0.9 mg (GR2 and FIN); pyridoxine 1.0 mg (GR1), 0.8 mg (GR2 and FIN).

[C]Mineral premix provided (mg kg$^{-1}$ diet); 670 Ca; 10 Cu; 0.2 I; 100 Fe; 10 Mn; 0.3 Se; 100 Zn.

[D]tP = total phosphorus

[E]aP = available (non-phytate) phosphorus

Pigs were housed in an environmentally controlled facility with concrete slatted flooring, nipple waterers, and a stainless steel feeders. Feed and water were supplied ad libitum. Pigs and feed were weighed every 14 d.

During the last two days of each dietary phase, faecal samples were collected randomly from pigs within in a pen and dried in an oven at 50°C for 24 hours or until dry. Feed and faecal samples were ground through a 1.0-mm sieve (Thomas Wiley Mill Model AH2151X, Arthrup H. Thomas Co., Philadelphia, Pennsylvania, USA 19019) and then through a 0.5-mm sieve (Cyclotec Model 1093 Sample Mill, Foss North America, Eden Prairie, Minnesota, USA 55344). At termination of the study, blood was collected into 8.5 mL collection tubes with no anticoagulant from the vena cava of one castrate and one gilt, chosen at random per pen. Samples were maintained on ice until centrifuged at 4°C at 3,000 revolutions per minute for 5 minutes, using a Beckman 6S-6KR centrifuge (Beckman Instruments, Inc., Fullerton, California, USA 92634). Serum samples were harvested and frozen at -18°C until analyzed. At the termination of the study, one gilt per pen was selected, based on mean pen body weight (92.93 kg), for slaughter at the Michigan State University USDA-inspected Meat Laboratory. All four feet and both femurs from each pig were collected for bone analyses.

In Experiment 2, 96 castrate and gilt crossbred Duroc x (Yorkshire-Landrace) pigs were weaned (mean of 22 d of age; 6.56 kg mean body weight) and assigned to one of four treatment based on sex and weight. There were six pigs per pen and four pens per treatment. Treatment 1 diets with YD and calculated total phosphorus of 1.0 g $kg^{-1}$ less than the NRC (1998) recommendations werved as a negative control. Treatment 2 diets with YD had 2.0 g $kg^{-1}$ and 1.5 g $kg^{-1}$ less total phosphorus (calculated) in Phase I and

Phase II, respectively than diets in Treatment 1. Treatment 3 diets contained NDLP with the same calculated total phosphorus concentration as Treatment 2 diets. Treatment 4 diets were the same as Treatment 3 diets with the addition of 500 FTU $kg^{-1}$ Natuphos® 600 phytase (BASF, Mount Olive NJ 07828). Dietary compositions are given in Tables 3 and 4 for Phase I diets (1-14 d) and phase II diets (14-28 d), respectively.

**Table 3. Composition of Phase I diets[A] (Experiment 2)**

| | Phase I diets (1-14 d) | | | |
|---|---|---|---|---|
| *Ingredient (g kg$^{-1}$)* | 1 | 2 | 3 | 4 |
| Yellow Dent maize | 474.8 | 482.4 | - | - |
| NutriDense™ Low Phytate maize | - | - | 500.7 | 500.07 |
| Soyabean meal (440 g kg$^{-1}$ CP) | 283.7 | 282.9 | 274.0 | 274.0 |
| Dried whey | 100.0 | 100.0 | 100.0 | 100.0 |
| Spray dried plasma | 30.0 | 30.0 | 30.0 | 30.0 |
| Spray dried blood cells | - | - | - | - |
| Lactose | 50.0 | 50.0 | 50.0 | 50.0 |
| Corn oil | 18.5 | 15.6 | 6.3 | 6.3 |
| L-Lysine-HCl | 1.5 | 1.5 | 1.5 | 1.5 |
| DL-Methionine | 1.1 | 1.1 | 1.1 | 1.1 |
| Salt | 3.5 | 3.5 | 3.5 | 3.5 |
| Copper sulfate | 0.5 | 0.5 | 0.5 | 0.5 |
| Dicalcium phosphate | 10.3 | - | - | - |
| Limestone | 8.8 | 15.2 | 15.1 | 15.1 |
| Trace mineral premix[B] | 5.0 | 5.0 | 5.0 | 5.0 |
| Vitamin premix[C] | 6.0 | 6.0 | 6.0 | 6.0 |
| Zinc oxide | 2.8 | 2.8 | 2.8 | 2.8 |
| Antibiotic[D] | 2.5 | 2.5 | 2.5 | 2.5 |
| Natuphos™ phytase[E] | - | - | - | 0.63 |
| Chromic oxide | 1.0 | 1.0 | 1.0 | 1.0 |
| *Nutrient (g kg$^{-1}$)* | | | | |
| Crude Protein | 198.2 | 198.4 | 204.0 | 204.0 |
| ME (MJ kg$^{-1}$) | 13.66 | 13.66 | 13.66 | 13.66 |
| Lysine | 13.5 | 13.5 | 13.5 | 13.5 |
| Calcium | 8.0 | 8.0 | 8.1 | 8.0 |
| Calculated tP[F] | 6.1 | 4.2 | 4.5 | 4.5 |
| Analyzed tP[F] | 5.9 | 3.9 | 4.5 | 4.6 |
| Calculated aP[G] | 4.0 | 2.1 | 3.1 | 4.1 |

[A]Dietary phase was based on NRC (1998) body weight intervals and counted as days from the start of the experiment (Phase I: 5-10 kg from Day 1 to 14)

[B]Mineral premix provided (mg kg$^{-1}$ diet): 670 Ca; 10 Cu; 0.2 I; 100 Fe; 10 Mn; 0.3 Se; 100 Zn.

[C]Vitamin premix provided (kg$^{-1}$ diet): Vitamin A 5511 IU; Vitamin $D_3$ 551 IU; Vitamin E 66 IU; Vitamin K activity 13 mg; menadione 4.4 mg; Vitamin $B_{12}$ 0.03 mg; riboflavin 3.7 mg; d-pantothenic acid 18 mg; niacin 26 mg; thiamine 1.1 mg; pyridoxine 0.9.

[D]Mecadox™ (Carbadox) (Pfizer, Inc., Exton, Pennsylvania, USA 19341) is commonly used in the US to maintain healthy status.

[E]1.0 kg tonne$^{-1}$ will provide 500 FTU kg$^{-1}$ phytase activity according to the manufacturer (BASF Corp., Mount Olive, New Jersey 07828) to replace 1.0 g kg$^{-1}$ available dietary phosphorus.

[F]tP = total phosphorus

[G]aP = available (non-phytate) phosphorus

**Table 4. Composition of Phase II diets[A] (Experiment 2)**

| | Phase II diets (14-28 d) | | | |
|---|---|---|---|---|
| *Ingredient (g kg$^{-1}$)* | 1 | 2 | 3 | 4 |
| Yellow Dent maize | 652.1 | 659.5 | - | - |
| NutriDense™ Low Phytate maize | - | - | 665.9 | 665.2 |
| Soyabean meal (440 g kg$^{-1}$ CP) | 223.8 | 223.1 | 213.0 | 213.0 |
| Dried whey | 50.0 | 50.0 | 50.0 | 50.0 |
| Spray dried plasma | - | - | - | - |
| Spray dried blood cells | 20.0 | 20.0 | 20.0 | 20.0 |
| Lactose | - | - | - | - |
| Corn oil | 12.5 | 9.8 | 4.0 | 4.0 |
| L-Lysine-HCl | 1.5 | 1.5 | 1.5 | 1.5 |
| DL-Methionine | 1.1 | 1.1 | 1.1 | 5.0 |
| Salt | 3.5 | 3.5 | 3.5 | 5.0 |
| Copper sulfate | 0.5 | 0.5 | 0.5 | 0.5 |
| Dicalcium phosphate | 9.6 | - | - | - |
| Limestone | 8.1 | 14.1 | 13.9 | 13.9 |
| Trace mineral mix[B] | 5.0 | 5.0 | 5.0 | 5.0 |
| Vitamin mix[C] | 6.0 | 6.0 | 6.0 | 6.0 |
| Zinc oxide | 2.8 | 2.8 | 2.8 | 2.8 |
| Antibiotic[D] | 2.5 | 2.5 | 2.5 | 2.5 |
| Natuphos™ phytase[E] | - | - | - | 0.7 |
| Chromic oxide | 1.0 | 1.0 | 1.0 | 1.0 |
| *Nutrient* | | | | |
| Crude Protein | 174.7 | 175.0 | 181.7 | 181.7 |
| ME (MJ kg$^{-1}$) | 13.66 | 13.66 | 13.66 | 13.66 |
| Lysine | 11.5 | 11.5 | 11.5 | 11.5 |
| Calcium | 7.0 | 7.0 | 7.0 | 7.0 |
| Calculated tP[F] | 5.3 | 3.5 | 3.9 | 3.9 |
| Analyzed tP[F] | 4.2 | 3.2 | 3.3 | 3.6 |
| Calculated aP[G] | 3.2 | 1.4 | 2.7 | 3.7 |

[A]Dietary phase was based on NRC (1998) body weight intervals and counted as days from the start of the experiment (Phase II: 10-20 kg from Day 14 to 28)

[B]Mineral premix provided (mg kg$^{-1}$ diet): 670 Ca; 10 Cu; 0.2 I; 100 Fe; 10 Mn; 0.3 Se; 100 Zn.

[C]Vitamin premix provided (kg$^{-1}$ diet): Vitamin A 5511 IU; Vitamin $D_3$ 551 IU; Vitamin E 66 IU; Vitamin K activity 13 mg; menadione 4.4 mg; Vitamin $B_{12}$ 0.03 mg; riboflavin 3.7 mg; d-pantothenic acid 18 mg; niacin 26 mg; thiamine 1.1 mg; pyridoxine 0.9.

[D]Mecadox™ (Carbadox) (Pfizer, Inc., Exton, Pennsylvania, USA 19341) is commonly used in the US to maintain healthy status.

[E]1.0 kg tonne$^{-1}$ will provide 500 FTU kg$^{-1}$ phytase activity according to the manufacturer (BASF Corp., Mount Olive, New Jersey 07828) to replace 1.0 g kg$^{-1}$ available dietary phosphorus.

[F]tP = total phosphorus

[G]aP = available (non-phytate) phosphorus

Pigs were housed in an environmentally controlled facility with steel slatted flooring, nipple waterers, and stainless steel feeders. Feed and water were supplied ad libitum. Pigs and feed were weighed every 14 d. Chromic oxide was added (1.0 g $kg^{-1}$) to estimate apparent phosphorus digestibility. At the end of each phase, faecal samples were collected randomly as in Experiment 1. Laboratory analyses were the same as in Experiment 1, plus chromium concentration was determined by atomic absorption spectrometry.

*Analyses*

Feed and faecal samples were wet-ashed in duplicate using a nitric acid microwave digestion procedure (Model 907055 Microwave Accelerated Reaction System 5 with HP-500 Plus Vessel Accessory sets (CEM Corp., Matthews, North Carolina, USA 28105)). Feed, faecal, and serum phosphorus was measured colorimetrically (Gomori, 1942), using a Beckman DU-7400 spectrophotometer (Beckman Instruments, Inc., Fullerton, California, USA 92634). Feed and faecal samples were digested in duplicate for chromic oxide determination by the phosphoric acid method (Williams et al., 1962). Digested samples were read on a Unicam 989 atomic absorption spectrometer (Unicam Atomic Absorption, Cambridge CB1YF, UK).

Third and fourth metacarpal and metatarsal bone strength was measured using the shear method (ASAE, 1999) with an Instron Universal Testing Machine (Model 4202, Instron, Canton, Massachussetts, USA ) fitted with a 10 kN load cell with a crosshead speed of 5 mm $min^{-1}$. Femur bone strength was measured using the bend method (ASAE, 1999) with a Texture Expert (Model TA-Hdi, Texture Technologies Corp., Scarsdale, New York, USA 10583). Bone ash was determined with third and fourth metacarpal and

metatarsal bones and 20-mm cross sections of both femurs were soaked in ethanol for a minimum of 7 d, dried, extracted with ether in a Soxhlet extraction apparatus (Pyrex® Model 3885, Corning Glass Works, Corning, New York, USA 14831), oven dried (Model DN-81 Constant Temperature Oven (American Scientific Products Co., Erie, Pennsylvania, USA 16506)) at 106°C for 24 hr, and ashed (Thermolyne Type 30400 muffle furnace (Barnsted/Thermolyne, Dubuque, Iowa, USA 52044)). Osteocalcin in serum was measured in duplicate using a commercially available ELISA testing kit (Novocalcin®; Metra Biosystems, Mountain View, California, USA 94043).

*Calculations and Statistics*

Apparent phosphorus digestibility was calculated by a method described by Kersey et al. (1995) using analyzed chromic oxide and total phosphorus values from faecal and feed samples. Ultimate bone stress (strength) was calculated according to the ASAE Standards guidelines (ASAE, 1999). The cross-sectional area of a quadrant of a hollow ellipse, which most closely matched the shape of the cross-section of bones tested, was estimated by the following equation:

$$A = \frac{1}{4} \times \pi \times [(B \times D) - (b \times v)] = \left[ v \times \left( \frac{B-b}{2} \right) \right] + \left[ b \times \left( \frac{D-v}{2} \right) \right]$$, where A = cross-sectional area of the bone ($m^2$), D = minor outside diameter of the bone cross section (m), B = major outside diameter of the bone cross section (m), $v = D - (2 \times w)$, $b = B - (2 \times w)$, and w = average bone wall thickness (m).

Experiment 1 data were analyzed by the T-TEST procedure of SAS® software (1999) for a randomized complete block design. Experiment 2 data were analyzed by the least squares (lsmeans) Analysis of Variance (ANOVA) with the General Linear Model

(GLM) procedure of SAS® software (1999) for a randomized complete block design. Pen was defined as the experimental unit. Significantly different dietary treatment pen means were adjusted with a Tukey multiple pairwise comparison method for Experiment 2 data. Standard errors for pen means were ascertained using the standard error (stderr) option of GLM. Differences in treatment means were considered significant at $P<0.05$.

**Results**

The effects of dietary treatments on growth performance for Experiment 1 are presented in Table 5. No dietary treatment differences were observed for body weight, average daily gain, average daily feed intake, or feed efficiency. For overall estimated average daily feed intake, pigs fed Treatment 2 consumed more ($P$=0.04) feed than pigs in Treatment 1.

**Table 5. Effects of dietary treatments on body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency[A] (G:F) (Experiment 1)**

| | Treatment | | Probability | |
|---|---|---|---|---|
| | 1 | 2 | SEM | Trt |
| Source of maize | YD[A] | NDLP[B] | | |
| *ADG (kg day$^{-1}$)* | | | | |
| Grower 1 | 0.478 | 0.490 | 0.01 | 0.52 |
| Grower 2 | 0.933 | 1.01 | 0.06 | 0.30 |
| Finisher | 0.797 | 0.837 | 0.07 | 0.60 |
| Overall | 0.736 | 0.798 | 0.04 | 0.32 |
| *ADFI (kg day$^{-1}$)* | | | | |
| Grower 1 | 1.05 | 1.13 | 0.03 | 0.14 |
| Grower 2 | 2.14 | 2.38 | 0.11 | 0.17 |
| Finisher | 2.83 | 2.93 | 0.08 | 0.30 |
| Overall | 1.98 | 2.14 | 0.09 | 0.04 |
| *G:F* | | | | |
| Grower 1 | 0.454 | 0.434 | 0.01 | 0.06 |
| Grower 2 | 0.438 | 0.423 | 0.01 | 0.43 |
| Finisher | 0.281 | 0.286 | 0.02 | 0.80 |
| Overall | 0.366 | 0.363 | 0.02 | 0.73 |

[A]Feed efficiency as gain:feed (G:F)
[B]Yellow Dent maize
[C]NutriDense™ Low Phytate maize

The effects of dietary treatments on bone measurements for Experiment 1 are shown in Table 6. No differences in third and fourth metacarpal and metatarsal or femur fracture force or ultimate stress (strength), average cortical bone wall thickness, percentage bone ash, or serum osteocalcin were observed.

**Table 6. Effects of dietary treatments on bone fracture force, bone ultimate stress (strength), average cortical wall thickness, serum osteocalcin, and bone ash (Experiment 1)**

| | Treatment | | Probability | |
|---|---|---|---|---|
| | 1 | 2 | SEM | Trt |
| Source of maize | YD[A] | NDLP[B] | | |
| *Bone fracture force (N)*[C] | | | | |
| 3rd and 4th metacarpal and metatarsals-shear ($F_s$)[C] | 4077.72 | 3810.20 | 138.74 | 0.13 |
| Femur-bend ($F_b$)[C] | 4654.24 | 4793.61 | 308.70 | 0.67 |
| *Ultimate stress (MPa)*[D] | | | | |
| 3rd and 4th metacarpal and metatarsals-shear ($\tau$)[D] | 15.04 | 13.42 | 0.61 | 0.60 |
| Femur-bend ($\sigma_u$)[D] | 5.26 | 5.28 | 0.93 | 0.98 |
| *Average wall thickness (mm)* | | | | |
| 3rd and 4th metacarpal and metatarsals | 1.38 | 1.43 | 0.04 | 0.44 |
| Femur | 5.01 | 5.63 | 0.53 | 0.83 |
| *Bone ash (g $kg^{-1}$)* | | | | |
| 3rd and 4th metacarpal and metatarsals | 573.08 | 574.7 | 9.85 | 0.22 |
| Femur cross section | 707.13 | 704.48 | 3.18 | 0.24 |
| *Serum osteocalcin (ng $mL^{-1}$)* | 296.99 | 275.90 | 28.46 | 0.49 |

[A]Yellow Dent maize
[B]NutriDense™ Low Phytate maize
[C]Fracture force is the force required to break a bone, measured in Newtons (N); 1 N = 0.102 kgf (kilograms force) = 9.8 kg (kilograms mass)
[D]Bone strength (ultimate stress, $\sigma_u$), measured in Pascals (Pa) is determined by the equation $\sigma_u = \frac{F_b LC}{4I}$ for ultimate bending stress and $\tau = \frac{F_s}{2A}$, where $F_b$= bone bending fracture force (N), $F_s$ = bone shear fracture force (N), A = cross-sectional area of the bone ($m^2$), L = distance between fulcra supports (tibia = 0.10 m), $C$ = distance from neutral axis to outer fiber (m), $I$ = moment of inertia ($m^4$); 1 MegaPascal (MPa) = $Pa \times \left(1 \times 10^{-6}\right)$; 1 Pa = $1 \frac{N}{m^2}$

The effects of dietary treatments on faecal phosphorus excretion for Experiment 1 are included in Table 7. Pigs excreted less ($P<0.01$) phosphorus when fed Treatment 2 for all phases, as compared to pigs fed Treatment 1 diets.

**Table 7. Effects of dietary treatments on faecal phosphorus (Experiment 1)**

| | Treatment | | Probability | |
|---|---|---|---|---|
| | 1 | 2 | SEM | Trt |
| Source of maize | YD[A] | NDLP[B] | | |
| *Faecal phosphorus (g kg$^{-1}$)* | | | | |
| Grower 1 | 15.9[a] | 9.4[b] | 5.25 | 0.008 |
| Grower 2 | 15.0[a] | 7.9[b] | 1.44 | 0.009 |
| Finisher | 18.9[a] | 9.6[b] | 3.37 | 0.0001 |

[A]Yellow Dent maize
[B]NutriDense™ Low Phytate maize
[a,b]For the same variable, mean values in the same row with unlike superscripts are significantly different ($P < 0.05$)

The effects of dietary treatments on growth performance for Experiment 2 are summarized in Table 8. Pigs fed Treatment 1 diets during Phase II gained more ($P<0.01$) weight per day than pigs fed Treatments 2 and 4. No differences in average daily gain were observed for Phase I. For the entire study, pigs fed Treatment 1 had greater average daily gain ($P<0.03$) than pigs fed Treatment 2. No treatment differences were observed for feed intake in either dietary phase. During Phase II, pigs fed Treatment 1 were more efficient ($P=0.01$) than pigs fed Treatments 3 and 4.

**Table 8. Effects of dietary treatments on body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency[A] (G:F) (Experiment 2)**

| | Treatment | | | | Probability | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | SEM | Trt |
| Source of maize | YD[B] | YD[B] | NDLP[C] | NDLP[C] | | |
| Dietary P | Control | control – 2.0 g $kg^{-1}$ | control – 2.0 g $kg^{-1}$ | control – 2.0 g $kg^{-1}$ | | |
| Phytase (FTU $kg^{-1}$)[D] | 0 | 0 | 0 | 500 | | |
| *ADG (kg $day^{-1}$)* | | | | | | |
| Phase I | 0.232 | 0.170 | 0.227 | 0.209 | 0.02 | 0.29 |
| Phase II | 0.396[a] | 0.259[b] | 0.346[ab] | 0.293[b] | 0.02 | 0.004 |
| Overall | 0.314[a] | 0.214[b] | 0.287[ab] | 0.251[ab] | 0.02 | 0.02 |
| *ADFI (kg $day^{-1}$)* | | | | | | |
| Phase I | 0.416 | 0.402 | 0.498 | 0.467 | 0.03 | 0.17 |
| Phase II | 0.908 | 0.742 | 1.08 | 1.09 | 0.09 | 0.05 |
| Overall | 0.662 | 0.572 | 0.790 | 0.777 | 0.05 | 0.05 |
| *G:F* | | | | | | |
| Phase I | 0.561 | 0.429 | 0.455 | 0.449 | 0.05 | 0.35 |
| Phase II | 0.439[a] | 0.350[ab] | 0.324[b] | 0.279[b] | 0.03 | 0.01 |
| Overall | 0.477[a] | 0.376[ab] | 0.364[ab] | 0.332[b] | 0.03 | 0.04 |

[A]Feed efficiency as gain:feed (G:F)
[B]Yellow Dent maize
[C]NutriDense™ Low Phytate maize
[D]Natuphos® phytase (BASF Corp., Mount Olive, New Jersey, USA 07828); 1 FTU is defined as the amount necessary to liberate 1 µmole of inorganic P per minute from 0.0015 mole of sodium phytate at 37°C and pH 5.5
[a,b]For the same variable, mean values in the same row with unlike superscripts are significantly different ($P < 0.05$)

The effects of dietary treatments on serum phosphorus, faecal phosphorus, and apparent phosphorus digestibility for Experiment 2 are given in Table 9. Pigs fed Treatment 2 had lower ($P$=0.03) plasma concentrations of phosphorus compared to pigs fed Treatments 1, 3, and 4 while pigs fed Treatment 2 diets excreted less ($P$<0.0001) phosphorus than pigs fed Treatment 1 diets during Phase I period. However, pigs fed Treatments 3 and 4 excreted less ($P$<0.0001) phosphorus than pigs fed Treatments 1 or 2 during Phase I. Pigs fed Treatments 3 and 4 had greater ($P$<0.0001) apparent phosphorus digestibilities than pigs fed Treatments 1 and 2 for Phase I .

**Table 9. Effects of dietary treatments on plasma phosphorus, faecal phosphorus, and apparent phosphorus digestibility (Experiment 2)**

| | Treatment | | | | Probability | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | SEM | Trt |
| Source of maize | YD[A] | YD[A] | NDLP[B] | NDLP[B] | | |
| Dietary P | Control | control – 2.0 g $kg^{-1}$ | control – 2.0 g $kg^{-1}$ | control – 2.0 g $kg^{-1}$ | | |
| Phytase (FTU $kg^{-1}$)[C] | 0 | 0 | 0 | 500 | | |
| *Plasma P (mg $dL^{-1}$)* | 5.33[a] | 3.92[b] | 4.87[a] | 5.16[a] | 0.22 | 0.003 |
| *Faecal P[D] (g $kg^{-1}$)* | | | | | | |
| Phase I | 14.1[a] | 11.8[b] | 7.6[c] | 6.3[c] | 0.45 | <0.0001 |
| *Apparent P digestibility[D] (%)* | | | | | | |
| Phase I | 33.90[a] | 38.30[a] | 60.29[b] | 62.30[b] | 2.89 | <0.0001 |

[A]Yellow Dent maize

[B]NutriDense™ Low Phytate maize

[C]Natuphos® phytase (BASF Corp., Mount Olive, NJ); 1 FTU is defined as the amount necessary to liberate 1 µmole of inorganic P per minute from 0.0015 mole of sodium phytate at 37°C and pH 5.5

[D]Faecal P and apparent P digestibility values for Phase II were not obtained due to technical difficulties

[a,b,c]For the same variable, mean values in the same row with unlike superscripts are significantly different ($P < 0.05$)

**Discussion**

*Growth performance*

Treatment 1 diets in Experiment 1 met all nutritional needs. Growing-finishing pigs in Experiment 1 had comparable growth performance to previous studies where growing-finishing pigs were fed low phytic acid maize (Spencer et al., 2000).

Weanling pigs fed Treatment 1 control diets in Experiment 2 had comparable feed efficiencies to a similar study by Roberson et al. (1999). Findings by Spencer et al. (2000b) are in agreement with our study where high available phosphorus (HAP) maize can be fed in place of YD maize without having deleterious impacts on growth parameters. Although HAP maize is a different variety than NDLP and has a lower (62%) phosphorus availability than NDLP (90%), it was still useful in improving maize phosphorus availability.

Pierce et al. (1998) reported that 0.9 g $kg^{-1}$ less dietary phosphorus was needed to maximize performance. Treatment 2 in Experiment 2 was designed as a negative control diet for phosphorus and resulted in decreased gain. Treatments 3 and 4 with NDLP improved growth similar to our positive control diets. However, this positive improvement when phytase was fed was not observed. A decrease in Phase II gain:feed for Treatments 3 and 4 as compared to Treatment 1 was unexpected. Data for the overall study indicates that phosphorus availability limited feed efficiency. This observation necessitates validation with further experimentation.

*Bone Parameters*

Bone breaking load (fracture force), stress-testing of bone, and percent bone ash are sensitive indicators of dietary P availability (Crenshaw et al., 1981; Cromwell, 1992).

The percent ash of 3rd and 4th metacarpal and metatarsal bones of finishing swine have been reported to be between 570.0 to 600.0 g $kg^{-1}$ ash (Doige et al., 1975; Crenshaw et al., 1981; Spencer et al., 2000a, 2000b), which is similar to ours.

Bone strength has been reported as kg. However, the 1999 ASAE Standards suggests that bone strength is ultimate stress, or the force per unit area required to break a bone. Therefore, Pascals (Pa), which is equivalent to 1 $\frac{N}{m^2}$, are the appropriate unit. Bone fracture force or breaking load is reported in Newtons (*N*). One Newton is the force required to accelerate a 1 kilogram mass by 1 $m/s^2$. Combs et al. (1991) reported a 4th metacarpal and metatarsal ultimate shear stress value of 13.42 MPa for pigs fed to 113 kg fed NRC (1979) recommendations for dietary calcium and phosphorus. This is in agreement with our findings. Values for bone strength reported by Crenshaw et al. (1981), Carter and Cromwell (1998), and Spencer et al. (2000a, 2000b) ranging from 490 to 1863 N (converted from kgf). These values were smaller than values reported in our study.

Carter et al. (1996) reported that serum concentration of osteocalcin was a good indicator of bone turnover in pigs. Osteocalcin was inversely correlated with growth rate, bone strength, metacarpal ash, femur ash (Carter et al., 1996). As osteocalcin concentrations in serum increase, bone strength, growth rate, and bone ash decrease (Carter et al., 1996). If osteocalcin concentrations are elevated, that would indicate that there is a greater degree of bone turnover and the strength and ash of bones are decreasing indicating a greater need for phosphorus than supplied by diet. Osteocalcin concentrations reported by Carter et al. (1996) are lower than the values from our study (4.74-6.07 ng $mL^{-1}$ vs. 275.9-296.99 ng $mL^{-1}$). The Differences may be due to differences

in assay, genetics, diets, or age of animals. Because serum osteocalcin concentrations in Experiment 1 were found to be the same for both treatments, it would appear that there was not a change in bone turnover in pigs on the four diets. Likewise, no differences in bone strength or bone ash indicate that dietary available phosphorus was probably adequate in the NDLP diets.

*Plasma phosphorus*

Plasma concentration of phosphorus has been reported as an indicator of dietary phosphorus utilization (Lei et al., 1993). Our plasma inorganic phosphorus concentrations were similar for controls. When dietary phosphorus was reduced by 2.0 g $kg^{-1}$ (Diet 2), plasma phosphorus concentration was reduced. Also, because pigs fed Diets 3 and 4 had similar plasma concentrations of phosphorus as pigs fed Diet 1, NDLP with or without phytase appear to be effective in increasing dietary phosphorus utilization.

*Faecal phosphorus and phosphorus digestibility*

Several researchers have shown that maize with low phytic acid concentration fed with or without phtase reduced faecal phosphorus and increased apparent phosphorus digestibility (Pierce et al., 1998; Veum et al., 1998; Spencer et al., 2000). In these studies, low phytic acid maize reduced faecal phosphorus excretion by 13 to 50%, which is in agreement with our studies having a 46% reduction in faecal phosphorus. Although several researchers have used genetically different pigs of different ages and body weight, low phytic acid maize varieties (HAP or NDLP) can reduce faecal phosphorus excretion significantly.

*Phytase*

The NDLP maize in Treatment 3 replaced YD maize was designed to increase available phosphorus by 1.0 g $kg^{-1}$ in Experiment 2. Natuphos® phytase was added (500 FTU $kg^{-1}$ in Phase I and 250 FTU $kg^{-1}$ in Phase II so available phosphorus concentrations should be similar to control (Treatment 1) diets. The NDLP addition in Treatment 3 diets may have been adequate enough to increase available phosphorus to control levels (Treatment 1) but phytase addition may not have had an effect. Roberson (1999) reported that 500 FTU $kg^{-1}$ was adequate to replace dietary available phosphorus by 1.0 g $kg^{-1}$. Manufacturer analysis for phytase activity of Phase I and II Treatment 4 diets yielded 640 and 400 FTU $kg^{-1}$. This confirmed that phytase addition to Treatment 4 diets yielded a minimum 500 FTU $kg^{-1}$ for Phase I and 250 FTU $kg^{-1}$ for Phase II. The lack of response by feeding phytase was unexpected and the interaction between NDLP and phytase warrants further investigation.

Qian et al. (1996) suggested that a decrease in the calcium:total phosphorus ratio from 2.0 to 1.2:1 increases phytase efficiency. In our analyzed diets, calcium:total phosphorus for Treatment 4 diets was 1.76 and 1.94:1 for Phases I and II, respectively. Qian et al. (1996) recommended that a calcium:available phosphorus ratio of between 1.5:1 and 2.5:1 should be efficacious for the use of 500 FTU $kg^{-1}$ Natuphos® phytase. Our calculated calcium:phosphorus ratios for Phases I and II Treatment 4 diets were 1.6:1 and 2.6:1, which falls into the range suggested by Qian et al. (1996).

## Conclusion

The results of the two experiments clearly show that NDLP maize can safely replace YD maize using a 90% phosphorus availability assumption for NDLP, without affecting growth performance and serum phosphorus of weanling pigs or growth performance and bone traits of growing-finishing pigs. NDLP maize can reduce phosphorus excretion by about 45% when fed in place of YD corn to weanling and growing-finishing crossbred pigs. Cost of finisher diets was reduced by US$3.71 tonne$^{-1}$ due to replacement of soyabean meal, choice white grease and dicalcium phosphate by the addition of NDLP maize in place of YD maize, assuming the cost of the two maize varieties to be the same.

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## SUMMARY AND GENERAL CONCLUSION

The objective of this thesis was to verify the phosphorus bioavailability of NutriDense Low Phytate™ corn as 90% bioavailable when fed to growing-finishing commercial tom turkeys and growing-finishing and weanling crossbred pigs. Another goal was to investigate the degree to which phosphorus excretion could be reduced without negatively impacting growth performance or bone characteristics.

Although there were inconsistencies in dietary treatments between analyzed and calculated phosphorus concentrations, the increased nutrient profile of NutriDense Low Phytate™ corn was adequate to replace dietary phosphorus to reduce excreted phosphorus by 30 to 45% and up to 56% (with phytase addition) in commercial finishing toms when fed in place of conventional yellow dent corn. Feeding NutriDense Low Phytate™ corn reduced fecal phosphorus excretion by approximately 45% in both weanling and growing-finishing crossbred pigs when substituted for conventional yellow dent corn.

In conclusion, this work suggests that NutriDense Low Phytate™ has the potential to reduce phosphorus excretion from turkeys and swine. Economics will be dictated by market prices of ingredients and dietary percentage composition of those nutrients. As Michigan animal feeding operations move towards more comprehensive nutrient management plans, lowered dietary phosphorus, NutriDense Low Phytate™ corn, and/or phytase enzyme may become useful as tools in the planning process.

# VITA

Michael W. Klunzinger is originally from Grosse Pointe, Michigan where he was raised part of his life in the country and part of his life in the city. His mother's side hails from Almont, Michigan and has been actively involved in farming for centuries in fruit production, crop production, livestock, and poultry production. His father's side hails from East Lansing, Michigan, home of the Spartans. His interest in animals probably began as a small child when he was exposed to sheep, cattle, and poultry. In third grade, he moved to the city. His interest in animals never waned as he became employed at a veterinary hospital throughout high school. He came to Michigan State University in the fall of 1995 as a fifth generation Michigan State University student. He was actively involved as a member of the Alpha Gamma Rho fraternity and took interest in the Animal Science department as a sophomore and decided to pursue a Bachelor's degree in Animal Science. He worked at the MSU sheep farm, where he gained the skill of sheep shearing and has run a successful business travelling throughout the state of Michigan. He worked at the MSU poultry and mink farm as well. Summer experiences included working at Bennett Farms Dairy in Prescott, Michigan; study abroad through the College of Agriculture and Natural Resources in Australia and New Zealand; and attending the Midwest Poultry Consortium program at the University of Wisconsin-Madison with an invaluable internship at Jennie-O Foods, Inc. in Willmar, Minnesota. Upon graduation with a B.S. in Animal Science in 1999, he continued on for his Masters degree in turkey and swine nutrition at Michigan State University under the direction of Dr. Kevin D. Roberson. While in graduate school, he met the love of his life, Raelene A. Charbeneau.

# APPENDICES

## Appendix A

Calculations for ultimate bending stress of bone:
Adapted from ASAE Standards, 1999

*Ultimate bending stress (strength):*

$$\sigma_u = \frac{F \times L \times C}{4 \times I},\ C = 0.57559 \times D, \text{ and } I = 0.0549 \times \left[\left(B \times D^3\right) - \left(b \times v^3\right)\right]$$

Where:
$\sigma_u$ = ultimate bending stress, Pa
$F$ = bone fracture force, N
$L$ = distance between fulcra supports
(0.1000 mm for turkey tibia, 0.0596 mm for swine femur)
$C$ = distance from neutral axis to outer fiber of bone, m
$I$ = moment of inertia, $m^4$
$B$ = outside major diameter of the bone, m
$D$ = outside minor diameter of the bone, m
$b$ = inside major diameter of the bone, m
$= B \times (2 \times w)$
$v$ = outside major diameter of the bone, m
$= D \times (2 \times w)$
$w$ = average cortical bone wall thickness, m

*Ultimate shear stress (strength):*

$$\tau = \frac{F}{2 \times A},\ A = \frac{1}{4} \times \pi \times \left[\left((B \times D) - (b \times v)\right) + \left(v \times \frac{B-b}{2}\right) + \left(b \times \frac{D-v}{2}\right)\right]$$

Where:
$\tau$ = ultimate shear stress, Pa
$A$ = cross-sectional area of a quadrant of a hollow ellipse (bone), $m^2$
$B$ = outside major diameter of the bone, m
$D$ = outside minor diameter of the bone, m
$b$ = inside major diameter of the bone, m
$= B \times (2 \times w)$
$v$ = outside major diameter of the bone, m
$= D \times (2 \times w)$
$w$ = average cortical bone wall thickness, m

| Table 1. Ultimate stress equation variables from swine study (Experiment 1) | | | | |
|---|---|---|---|---|
| Variable | Femur | | $3^{rd}$ and $4^{th}$ Metacarpals and Metatarsals | |
| | Diet 1 | Diet 2 | Diet 1 | Diet 2 |
| F | 4654.24 | 4793.61 | 4077.72 | 3810.20 |
| L | 0.0596 | 0.0596 | | |
| C | 0.012362 | 0.012615 | | |
| I | $1.73818 \times 10^{-7}$ | $1.74738 \times 10^{-7}$ | | |
| A | | | $1.376 \times 10^{-4}$ | $1.429 \times 10^{-4}$ |
| B | | | 0.0183188 | 0.0185650 |
| D | | | 0.0149250 | 0.0152469 |
| w | | | 0.0019156 | 0.0019521 |
| b | | | 0.0144880 | 0.1474600 |
| v | | | 0.0110940 | 0.0113430 |

| Table 2. Ultimate stress equation variables from turkey study (Experiment 1) | | | | |
|---|---|---|---|---|
| | Tibia | | | |
| Variable | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
| F | 938.50 | 878.33 | 913.89 | 837.61 |
| L | 0.1 | 0.1 | 0.1 | 0.1 |
| C | 0.008302886 | 0.008312479 | 0.008192564 | 0.008322072 |
| I | $1.87137 \times 10^{-9}$ | $1.79150 \times 10^{-9}$ | $1.78699 \times 10^{-9}$ | $1.84714 \times 10^{-9}$ |
| | Ulna | | | |
| F | 2578.97 | 2415.713 | 2290.06 | 2366.83 |
| B | 0.014433 | 0.014533 | 0.014567 | 0.014625 |
| D | 0.010708 | 0.015000 | 0.010800 | 0.010483 |
| w | 0.001219 | 0.001317 | 0.001178 | 0.001186 |
| b | 0.011994 | 0.011900 | 0.122110 | 0.012253 |
| v | 0.008269 | 0.007867 | 0.008444 | 0.008111 |
| | Femur | | | |
| F | 1968.46 | 2170.63 | 1894.97 | 2012.69 |
| B | 0.018967 | 0.018958 | 0.01900 | 0.018842 |
| D | 0.017458 | 0.017275 | 0.01755 | 0.017075 |
| w | 0.001503 | 0.001511 | 0.001389 | 0.001453 |
| b | 0.015961 | 0.015936 | 0.016222 | 0.015936 |
| v | 0.014453 | 0.014253 | 0.014772 | 0.014169 |

| **Table 3. Ultimate stress equation (measurements taken using computed tomography (CT) technology) with variables from turkey study (Experiment 2)** | | | | |
|---|---|---|---|---|
| Variable | Tibia | | | |
| | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
| F | 702.66 | 654.60 | 688.28 | 683.17 |
| L | 0.1 | 0.1 | 0.1 | 0.1 |
| C | 0.007965 | 0.008389 | 0.008133 | 0.008329 |
| I | $1.97055 \times 10^{-9}$ | $2.2623 \times 10^{-9}$ | $2.05658 \times 10^{-9}$ | $2.23946 \times 10^{-9}$ |
| | Ulna | | | |
| F | 2548.78 | 2411.52 | 2408.82 | 2372.20 |
| A, by CT scan | $3.90952 \times 10^{-5}$ | $3.90909 \times 10^{-5}$ | $4.00952 \times 10^{-5}$ | $4.06818 \times 10^{-5}$ |
| A, using CT measurements then equation | $8.2187 \times 10^{-5}$ | $8.4576 \times 10^{-5}$ | $8.1046 \times 10^{-5}$ | $8.9272 \times 10^{-5}$ |
| B | 0.014329 | 0.014450 | 0.014514 | 0.014555 |
| D | 0.010519 | 0.010614 | 0.010614 | 0.010682 |
| w | 0.001727 | 0.001771 | 0.001671 | 0.001877 |
| b | 0.010875 | 0.010908 | 0.011171 | 0.010800 |
| v | 0.007065 | 0.007071 | 0.007271 | 0.006927 |
| | Femur | | | |
| F | 1628.54 | 1685.40 | 1511.35 | 1549.12 |
| A, by CT scan | $7.3250 \times 10^{-5}$ | $7.2750 \times 10^{-5}$ | $7.2375 \times 10^{-5}$ | $7.0083 \times 10^{-5}$ |
| A, using CT measurements then equation | 0.00016252 | 0.00016805 | 0.00016082 | 0.00016221 |
| B | 0.018796 | 0.019058 | 0.018721 | 0.018792 |
| D | 0.017363 | 0.017792 | 0.017375 | 0.017325 |
| w | 0.002307 | 0.002331 | 0.002290 | 0.002311 |
| b | 0.014182 | 0.014397 | 0.014140 | 0.014169 |
| v | 0.012749 | 0.013131 | 0.012794 | 0.012703 |

| **Table 4. Ultimate stress equation (measurements taken using a digital caliper) with variables from turkey study (Experiment 2)** | | | | |
|---|---|---|---|---|
| Variable | Tibia | | | |
| | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
| F | 702.66 | 654.60 | 688.28 | 683.17 |
| L | 0.1 | 0.1 | 0.1 | 0.1 |
| C | 0.007787 | 0.008128 | 0.007895 | 0.008116 |
| I | $1.85849 \times 10^{-9}$ | $2.08919 \times 10^{-9}$ | $1.88643 \times 10^{-9}$ | $2.091437 \times 10^{-9}$ |
| | Ulna | | | |
| F | 2548.78 | 2411.52 | 2408.82 | 2372.20 |
| A | $7.8487 \times 10^{-5}$ | $8.2439 \times 10^{-5}$ | $7.7273 \times 10^{-5}$ | $8.4718 \times 10^{-5}$ |
| B | 0.013919 | 0.014186 | 0.014019 | 0.013977 |
| D | 0.010157 | 0.010405 | 0.010243 | 0.010277 |
| w | 0.001727 | 0.001767 | 0.001671 | 0.001877 |
| b | 0.010465 | 0.010652 | 0.010676 | 0.010223 |
| v | 0.006703 | 0.006871 | 0.006900 | 0.006523 |
| | Femur | | | |
| F | 1628.54 | 1685.40 | 1511.35 | 1549.12 |
| A | 0.00015914 | 0.00015958 | 0.00015952 | 0.00015051 |
| B | 0.0184 | 0.018375 | 0.0184375 | 0.0187667 |
| D | 0.016525 | 0.017033 | 0.0164875 | 0.0165208 |
| w | 0.002367 | 0.002297 | 0.0023780 | 0.0021970 |
| b | 0.013667 | 0.014143 | 0.0136820 | 0.0140720 |
| v | 0.011792 | 0.012439 | 0.0117320 | 0.0121260 |

## Appendix B

Example of feed cost savings from substituting NutriDense Low Phytate™ corn for conventional Yellow Dent corn in turkey and swine finishing diets:

*Ingredient cost assumptions (priced on a per ton basis):*

| | |
|---|---|
| $91.43 | corn |
| $181.00 | soybean meal (48% protein) |
| $230.00 | choice white grease |
| $2200.00 | DL-Methionine |
| $1000.00 | L-Lysine HCl |
| $250.00 | dicalcium phosphate |
| $30.00 | limestone |

*other ingredients not considered because they remained the same between treatments*

**Table 5. Feed cost savings by replacing yellow dent (YD) corn with NutriDense Low Phytate™ (NDLP) corn in turkey Finisher diets (Experiment 1)**

| | | | $/ton | | | | $/ton |
|---|---|---|---|---|---|---|---|
| Item[a] | % in Diet | Item price | Cost in YD control diet | % in Diet | Item price ($/ton) | Cost in NDLP diet (90% P bioavailability) | |
| Corn | 62.49 x | $91.43 | = $57.13 | 65.81 x | $91.43 | = $60.17 | |
| SBM | 26.82 x | $181.00 | = $48.54 | 23.57 x | $181.00 | = $42.66 | |
| CWG | 7.45 x | $230.00 | = $17.14 | 6.51 x | $230.00 | = $14.97 | |
| DL-Met | 0.21 x | $2200.00 | = $4.62 | 0.18 x | $2200.00 | = $3.96 | |
| L-Lys | 0.00 x | $1000.00 | = $0.00 | 0.09 x | $1000.00 | = $0.90 | |
| DP | 1.54 x | $250.00 | = $3.85 | 0.99 x | $250.00 | = $2.48 | |
| Lime | 0.88 x | $30.00 | = $0.26 | 1.25 x | $30.00 | = $0.38 | |
| Total | | | = $131.55 | | | = $125.51 | |
| | | | | | Total cost savings = $131.55/ton - $125.51/ton = $6.03/ton | | |

[a]Item abbreviations:
SBM= soybean meal (48% protein); CWG = choice white grease; DL-Met = DL-Methionine; L-Lys = L-Lysine; DP = dicalcium phosphate; lime = limestone

**Table 6. Feed cost savings by replacing yellow dent (YD) corn with NutriDense Low Phytate™ (NDLP) corn in turkey Finisher diets (Experiment 1)**

| | | | $/ton | | | | $/ton |
|---|---|---|---|---|---|---|---|
| Item[a] | % in Diet | | Item price | Cost in YD control diet | % in Diet | Item price ($/ton) | Cost in NDLP diet (90% P bioavailability) |
| Corn | 77.45 | x | $91.43 | = $70.81 | 84.97 x | $91.43 | = $77.69 |
| SBM | 17.41 | x | $181.00 | = $31.51 | 12.40 x | $181.00 | = $22.44 |
| CWG | 2.27 | x | $230.00 | = $5.22 | 0.11 x | $230.00 | = $0.25 |
| L-Lys | 0.00 | x | $1000.00 | = $0.00 | 0.09 x | $1000.00 | = $0.90 |
| DP | 0.85 | x | $250.00 | = $2.13 | 0.00 x | $250.00 | = $0.00 |
| Lime | 0.72 | x | $30.00 | = $0.22 | 1.13 x | $30.00 | = $0.34 |
| Total | | | | = $109.89 | | | = $104.68 |
| | | | | Total cost savings = $109.89/ton - $101.63/ton = $8.26/ton | | | |

[a]Item abbreviations:
SBM= soybean meal (48% protein); CWG = choice white grease; L-Lys = L-Lysine; DP = dicalcium phosphate; lime = limestone