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Effects of various phytase concentrations in diets with low-phytate corn on broiler chick performance and metabolism

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ABSTRACT

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Research indicates a reduction of phosphorus content in fecal excreta with the supplementation of phytase to corn-soybean based diets or with the use of low phytate corn (LPC) in broilers. This study examined how 0-to-3-wk broiler chicks are impacted by concomitant phytase supplementation with LPC (0.136% phytate P by analysis) in the diet. Various levels of phytase (250, 500, 750, 1000, 2000 FYT/kg) from either 2500 or 5000 FYT/g of dry *Peniophora lycii* phytase product were used as experimental treatments. Efficacy of treatments was determined using a response curve created with monocalcium phosphate providing 0.23%, 0.28%, 0.33%, and 0.38% levels of nonphytate phosphorus (nPP). All diets that included phytase contained 0.23% nPP, 0.8% calcium and LPC. Following a 6-day pre-test, 576 broiler chicks were randomly assigned to one of the 12 dietary treatments, with 8 replicates and 6 birds per cage. Measurements of live performance, tibia ash, mineral digestibility, and apparent metabolizable energy (AME) were obtained. Increasing phytase concentration led to a linear increase in live weight gain ($P=0.0309$) and a linear decrease in feed conversion ($P=0.0010$). At enzyme levels greater than 250 FYT/kg, digestible phosphorus, calcium, and AME increased ($P<0.05$). The two *Peniophora lycii* products, when used to make similar experimental treatments, did not differ regarding performance, mineral digestibility and AME ($P>0.05$). Thus, phytase supplementation in diets containing LPC had a positive impact on broiler chick growth.

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Background Information / Literature Review

Animal manure contains valuable nutrients for crops, such as nitrogen, phosphorus, and potassium. Manure is considered an amendment that improves the physical and chemical properties of soil, as well as the nutrient values of soil by adding organic material. Manure improves soil structure by aiding in its ability to hold water and retain nutrients. These nutrients are available for immediate use by plants when added to soil systems. Using animal waste to supplement soil improves soil quality and makes use of an otherwise excess waste product.

The broiler chicken produces the largest amount of manure, nitrogen, and phosphorus per pound animal unit compared to any farm animal (NRCS, 1995). Poultry diets may be formulated to crude protein specifications while the excess protein not utilized for growth or maintenance is excreted as nitrogen. On average, most protein contains 16% nitrogen, of this approximately 25% or less is retained by the birds (Leeson and Summers, 2001). Litter removed annually from a broiler house with 22,000 birds contains as much phosphorus as the sewage in a community of 6,000 people (NRCS, 1995).

The poultry industry and other livestock operations are facing growing concerns about the land application of litter contaminating surface waters (Sharpley, 1999). Surplus nutrients from fertilizers in crop run-off increase eutrophication. Eutrophication is the process by which a body of water becomes enriched in dissolved nutrients that stimulate growth of aquatic plant life, usually resulting in the depletion of oxygen. The overgrowth of this undesirable plant life, blocks out sunlight, causing these aquatic weeds to die. During the decomposition stage, soluble oxygen is utilized in the water leading to oxygen shortages for aquatic life. Thus, water quality is decreased by eutrophication. The addition of phosphorus to freshwater increases the eutrophication process, which restricts water use for fisheries, recreation, industry, and drinking due to increased growth of undesirable algae and aquatic weeds.

According to the Environmental Protection Agency, agriculture is the leading source of water quality impairments to rivers and lakes in the United States (NRC, 1993). Algal blooms of toxic cyanobacteria (blue-green algae) occur in drinking water supplies and may pose serious health hazards to humans and animals due to crop run-off (Sharpley, 1999). Cyanobacteria produce hepatoxins, neurotoxins, and non-specific toxins that may lead to illness in humans and death in animals due to ingestion of contaminated water (Morris, 2000). Primary contributors to outbreaks of dinoflagelate Pfisteria piscicida in the Chesapeake Bay are due to excess nutrients in waters, mainly phosphorus build-up (NRCS, 1995; Sharpley, 1999). Neurological damage in people exposed to toxic volatile chemicals produced by this dinoflagelate has dramatically increased the public awareness of eutrophication and the need for solutions (Grattan et al., 1998). Eutrophication has serious implications on health as well as the environment.

Scientists are exploring ways to increase the value of manure by altering biochemical characteristics to make it more suitable for crops. Nitrogen-based management of waste has been practiced for years; only since the early 1980s has the emphasis turned to phosphorus-based management of waste (Sharpley, 1999). Manure application rate recommendations are routinely based on nitrogen content and crop requirement for nitrogen to minimize the purchase of commercial fertilizer nitrogen (NRCS, 1995; Sharpley, 1999). The nitrogen-based management system often results in a build-up of phosphorus in soil and contributes to excess nutrient run-off. Phosphorus-based nutrient management regulations require that manure applications be limited to the phosphorus needs of the crop. Most livestock and poultry farmers produce more manure phosphorus than their crops require. Phosphorus-based management utilizes less manure than nitrogen-based management and the use of more supplemental nitrogen fertilizer (Knowlton, 2002). Waste from broiler chickens is a good source of manure because of its high nutritive quality in a concentrated form. The value of poultry fertilizer would be increased tremendously if the amount of phosphorus was reduced to more closely meet the needs for adequate fertilizer. Poultry nutritionists are working to maintain broiler performance and enhance the value of manure.

Phosphorus is an essential mineral to broiler chicken growth, especially skeletal tissue. Phosphorus acts as an integral part of many systems in the body and is required to adequately sustain life and promote growth in broilers. Phosphorus plays a key role in intermediary metabolism, contributes to cell membranes (phospholipids), functions in acid-base balance, and is an important component of nucleic acids (Angel et al., 2002).

Broiler chickens in the United States primarily consume corn-soybean based diets, which are naturally high in phosphorus. However, these plant phosphorus sources are phytate bound and not readily available to the bird. Inorganic phosphorus sources are more available and added to the diet usually as monocalcium, or dicalcium phosphate. The addition of inorganic phosphorus is costly and somewhat redundant because the bird is already receiving adequate phosphorus from organic ingredients in the diet.

Phosphorus is abundant in corn and soybean meal; but two-thirds of the total phosphorus contained in feed ingredients of plant origin is bound as phytic acid (Nelson, 1967; Sohail et al., 1999). Unavailable phosphorus is referred to as phytate phosphorus (PP) or phytic acid found in a cyclic complex. Phytate phosphorus is either unavailable or poorly utilized by monogastric animals due to insufficient quantities of endogenous phytase, which is used to break the phytic acid molecule (hexophosphoiniositol) (Waldroup et al., 2000; Angel et al., 2002). Phytates are associated with a number of anti-nutritional effects largely because they chelate divalent cations, such as calcium and zinc, and can reduce nutrient availability of protein and amino acids, as well as starch and other carbohydrates (Ravindran, 1995). Therefore, diminishing phytate phosphorus complexes may increase nutrient availability and dietary energy, in addition to liberating phosphorus (Ravindran et al., 1995; Sohail et al., 1999; Ravindran et al., 2000).

Phytate phosphorus may be defined as a ringed complex containing six phosphate molecules, as well as starch, protein, calcium, magnesium, zinc, iron, and other trace minerals (Angel et al., 2002; Yan et al., 2003). Chickens lack the endogenous enzyme phytase, and cannot digest or absorb phosphorus in the phytate form. Adding supplemental phytase to broiler diets increases the availability of phytate-bound phosphorus, and therefore may reduce the amount of phosphorus excreted into the environment (Waldroup et al., 2000; Yan et al., 2001; Angel et al., 2002; Yan et al., 2003) Phytase releases the phytate bound phosphorus, thus increasing phosphorus availability to the bird and therefore decreasing phosphorus in the excreta. The addition of dietary phytase can aid in reducing formulation cost by decreasing inorganic phosphorus inclusion in the feed (Waldroup et al., 2000).

A small amount of the phytase enzyme can produce a large benefit in the reduction of phosphorus waste. In broilers, the addition of phytase is reported to reduce phosphorus excretion by 25-40% and nitrogen excretion up to 10%, thus increasing the efficiency of nutrient utilization in the bird (NRC, 1993). Phytase can improve phosphorus and nitrogen utilization by broilers, as well as the value of chicken manure as a soil amendment.

Phytases are widely distributed in plants, animals, and microorganisms. Phytases are currently recognized in two classes: a 3-phytase and a 6-phytase, which initiates the dephosphorylation of phytic acid at different positions on the inositol ring (IUB, 1979; Angel et al. 2002). The 3-phytases do not always completely dephosphorylate the phytic acid complex, whereas the 6-phytases do. It has been stated that microorganisms normally produce the 3-

phytase and the 6-phytases are found in plants (Angel et al., 2002). There are exceptions to this general rule, for example, an enzyme with 3-phytase has been reported in soybeans, and an enzyme with 6-phytase activity has been reported in Escherichia coli (Angel et al., 2002; Radcliffe, 2000). Another exception, Peniophora lycii is a microbial, 6-phytase active over a pH range 4-4.5 (Linden, 2000). The efficacy of microbial phytase to improve dietary phosphorus bioavailability has been reported by several researchers (Nelson 1967, Waldroup et al. 2000, Yan et al. 2001).

Phytate content of corn is manipulated in another strategy to increase phosphorus utilization and decrease phosphorus waste; with the use of genetically engineered corn containing decreased phytate (Raboy and Gerbasi, 1996). This hybrid corn, known as low phytate corn (LPC) or high available phosphorus corn (HAPC) has enhanced phosphorus availability to animals due to low phytate levels and increased nonphytate phosphorus (nPP) (Huff, 1998; Li et al., 2000). Low phytate corn contains similar total phosphorus content to normal yellow dent corn, but PP and nPP distribution vary. Normal corn contains approximately 0.03% nPP where LPC contains about 0.17% nPP (Huff et al., 1998; Waldroup et al., 2000; Yan et al., 2000).

Li and coauthors (2000) clearly demonstrated that phosphorus in LPC is more available than phosphorus in normal corn, and that a reduction in phytate content with LPC does not compromise nutritional value. Results also indicated that phosphorus excretion could be substantially reduced by substituting LPC for normal corn in the diet (Li et al., 2000). The combined effects of LPC and phytase supplementation indicate marked reduction in phosphorus excretion without compromising broiler chick performance (Huff et al., 1998; Waldroup et al., 2000; Yan et al., 2000).

Energy as well as trace minerals can be liberated in varying amounts depending on phytate complexes (Angel, 2002). Inconsistencies in metabolizable energy may be due to variations in phytic acid content of feed. With the addition of phytase to broiler diets, variations in metabolizable energy from no change (Biehl and Baker, 1997) up to 5.5% increase in metabolizable energy have been reported (Camden et al. 2001).

Calcium is of particular interest because it is strongly chelated to the negatively charged phosphate groups of phytic acid. The calcium: total phosphorus ratio is recommended to be 2.0:1 ratio for maximum growth performance (NRC, 1994). Because calcium is liberated from the phytate complex with the addition of supplemental phytase, the addition of calcium can be reduced in the diet formulation (Qian, 1997). Calcium (Ca) and phosphorus (P) levels in the diet (Edwards and Veltmann, 1983; Qian, 1997) influence phytate phosphorus utilization of corn-soybean diets by broilers. Excess Ca binds with phytate to form an insoluble complex that is less accessible to phytase, and can have an effect on metabolizable energy (Kornegay, 1999).

Introduction

The poultry industry and other livestock operations are facing growing concerns about the land application of manure contaminating surface waters (Sharpley, 1999). A manure fertilizer for crops provides necessary, but often more, nutrients than required for crop growth. The surplus nutrients, phosphorus being of greatest concern, may leach into watersheds and contribute to eutrophication (Grattan et al., 1998; Sharpley, 1999).

Phosphorus is an essential mineral for broiler chicken metabolism and skeletal development. However, two-thirds of the phosphorus provided in typical broiler diet ingredients, corn and soybean, are bound to phytic acid (Nelson, 1967; Sohail et al., 1999). Phytate phosphorus (PP) is either unavailable or poorly utilized by monogastric animals, due to insufficient quantities of endogenous phytase enzyme that aids in digestion of the phytic acid complex (Waldroup et al., 2000; Angel et al. 2002). Phytic acid can act as an anti-nutrient, due to the ability of the complex to bind starch, proteins and trace minerals, such as phosphorus, zinc, iron, calcium, and magnesium (Kornegay, 1999; Camden et al., 2001; Radcliffe, 2002).

The addition of phytate-degrading enzymes can improve the nutritional value of plant-based foods by enhancing nutrient digestibility through phytate hydrolysis during digestion in the gut (Yi and Kornegay, 1996; Konietzny and Greiner, 2002). Research has shown that the supplementation of exogenous phytase to broiler diets is an effective means for increasing the availability of phosphorus to the bird as well as reducing phosphorus excretion, by liberating phytate bound phosphorus (Nelson, 1967; Jongbloed and Kemme, 1990; Kornegay et al., 1996; Waldroup, 2000; Angel, 2002). Results phytase dosage efficacy have been inconsistent, in part this may be due to variable PP content of the diet (Kornegay, 1999; Angel, 2002; Radcliffe, 2002) or uniformity of phytase distribution in the diet (Johnston and Southern, 2000; Angel, 2002).

The sparing ability of phytase on inorganic phosphorus is a common determination of phytase efficacy. The amount of inorganic phosphorus spared with the addition of phytase is termed the phosphorus sparing effect. Phytase supplementation is measured in phytase units (FYT). One phytase unit is defined as the quantity of phytase that generates one micromole of inorganic phosphorus from 5.1 mmol/L of sodium phytate at pH of 5.5 and 37 degrees Celsius (Johnston and Southern, 2000; McMullen et al., 2001). Typically, phytase manufacturers claim that 0.1% phosphorus sparing is obtainable with the addition of 300-500 phytase units/kg of diet. Past literature reports that 0.1% phosphorus sparing has been achieved with a range of 781-1413 phytase units/kg of diet (Angel et al., 2002). These large discrepancies in efficacy could be a result of variation in corn and soybean PP content or mix uniformity of the feed (Johnston and Southern, 2000).

Given that young chicks only consume a few grams of feed each day, it is necessary to provide all essential nutrients, in the proper quantity (Beumer, 1991). Utilizing different concentrations of phytase may alter growth and performance due to uniformity of mix. The addition of a more concentrated product leaves more space available in the diet for other ingredients, but it is more difficult to ensure that all ingredients are adequately dispersed. Commercial recommendations for mixer coefficient of variation (CV) is less than 10% but a mean CV of 30% on an average mixer has been reported (Johnston and Southern, 2000). Phytase efficacy and performance data may vary due to inadequate mixer uniformity.

Many studies analyze total phosphorus of the diet, but few include information on PP content and phytase analysis (Angel et al., 2002). In several studies that focused on similar corn-soybean meal diets, phytase supplementation, total phosphorus, and calculated PP, results of toe ash were largely varied (Kornegay et al., 1995; Qian et al., 1997, Camden et al., 2001). More specifically, nonphytate phosphorus was calculated to be 0.27-0.28% with 500-600 FYT/kg, and toe ash results ranged from 11.6-12.7%. Toe ash is comparable to tibia ash and both are highly sensitive indicators of phosphorus levels in the broiler chick, making these differences noteworthy (Angel et al., 2002). Inconsistencies in metabolizable energy may also be due to variations in phytic acid content of feed. With the addition of phytase to broiler diets, variations in metabolizable energy from no change (Biehl and Baker, 1997) up to 5.5% increase in metabolizable energy have been reported (Camden et al. 2001). Data are still limited as to the variability in PP content in feed ingredients (Applegate and Angel, 2003). Phytate phosphorus analysis of ingredients will allow for improved diet formulation (Angel et al., 2002).

Phytate content of corn can be manipulated to increase phosphorus utilization with the use of genetic engineering for a homozygous lpa1-1 gene (Raboy and Gerbasi, 1996). This hybrid corn, known as low phytate corn (LPC) or high available phosphorus corn (HAPC) has enhanced phosphorus availability to animals due to low phytate levels and increased nonphytate phosphorus (nPP) (Huff, 1998; Li et al., 2000). Low phytate corn contains similar total phosphorus content to normal yellow dent corn, but PP and nPP distribution vary. Normal corn has been reported to contain 0.03% nPP where LPC contains about 0.17% nPP (Huff et al., 1998; Waldroup et al., 2000; Yan et al., 2000).

Li and coauthors (2000) demonstrated that phosphorus in LPC is more available than phosphorus in normal corn, and that a reduction in phytate content with LPC does not compromise nutritional value. Results have also indicated that phosphorus excretion could be substantially reduced by substituting LPC for normal corn in the diet (Li et al., 2000). The combined effects of LPC and phytase supplementation indicate marked reduction in phosphorus excretion without compromising broiler chick performance (Huff et al., 1998; Waldroup et al., 2000; Yan et al., 2000).

In an attempt to determine appropriate levels of phytase supplementation in LPC-soybean meal diets, the current study explored the effects of varying phytase concentrations of two different commercial phytase products on feed uniformity, broiler performance and metabolism.

Materials and Methods

Diet Formulation

Feed Manufacture

Performance Study

Energy and Mineral Utilization

Diet Formulation

Experimental corn-soybean based mash diets were formulated to meet or exceed NRC (1994) recommendations for all nutrients except calcium and phosphorous. Formulations were adjusted to determine the efficacy of phytase in liberating phosphorus, calcium and energy from phytic acid. All dietary treatments used LPC, containing 0.23% total phosphorus, 0.14% PP, and by difference 0.09% nPP. Birds consumed a NRC (1994) based pre-test diet formulated with 1% calcium and 0.45% nPP from 1 to 5 d. A series of diets designated as the standard curve was created to determine phosphorus-sparing effect. Diets utilized for the standard curve contained 0.8% calcium and varying levels of nPP (0.23%, 0.28%, 0.33%, and 0.38%). Nonphytate phosphorus was adjusted with monocalcium phosphate, ground limestone, and cellulose. Concentrations of exogenous phytase varied by adding different levels of commercial phytase products. Experimental diets containing added phytase were formulated to contain 0.8% calcium and 0.23% nPP (Table 1).

Feed Manufacture

Two dry Peniophora lycii phytase products were used, each containing a different concentration of the enzyme (Product 1 and Product 2). Product 1 contained 2500 FYT/g and was added to diets in concentrations of 250, 500, 750, 1000, and 2000FYT/kg. Product 2, contained 5000FYT/g and was added to diets in concentrations of 250, 500, and 750FYT/kg.

Due to the small quantities of enzyme inclusion to feed, it was essential to ensure adequate homogeneity in the mixer by determining mix CV. All diets were mixed in a single screw vertical mixer[1]. Mixer coefficient of variation was determined by mixing four 454.5kg corn/salt batches for 40 minutes each. To mimic enzyme inclusion, salt was added at 0.01% of the test batch. Ten samples were analyzed from each batch with Quantab titrators[2] for chloride analysis. Testing procedure followed those of McCoy (1994).

Four 907kg basal diet batches were formulated (0.23%, 0.38%, Product 1 at 2000 FYT/kg, Product 2 at 750 FYT/kg) and assayed for phytase[3], total phosphorus[3], and PP[4] to ensure appropriate mix and formulation. High Performace Liquid Chromatography was used to determine phytate phosphorus with post column detection. The four basal diets were then blended in small rations, and in different proportions to create eight subsequent experimental diets (0.28% nPP, 0.33% nPP, Product 1 @ 250, 500, 750, and 1000 FYT/kg and Product 2 @ 250, 500 FYT/kg). Feed samples from all diets were analyzed for total phosphorus[3, 5], soluble phosphorus[3], calcium[5] and gross energy[6].

Performance Data

Following a 1 - 5 d adaptation period, 576 Ross 308 x Ross 344 straight run broilers[7] were randomly assigned to one of 12 dietary treatments. The experiment was conducted as a randomized complete block design, run from 6 – 21 d. Treatments were replicated 8 times using a pen of 6 birds as an experimental unit. Birds were housed in raised wire brooding cages in a cross-ventilated negative pressure room. Mash feed, supplied in external troughs, and water, supplied through nipple drinkers, was provided *ad libitum*. Nipple drinkers were adjusted by visual inspection to appropriate height for chicks (Lott et al. 2001). Live weight gain was determined by difference in chick weights at 6 and 21 days of age, total feed consumption was calculated and mortality weights were recorded throughout the experiment. Feed conversion was calculated as the feed intake to weight gain ratio (including mortality weight).

On day 21, after birds were euthanized, right tibias were extracted from all birds and pooled by pen for tibia ash determination of dry fat-free bone (AOAC, 1990). Tibias were dried for 48 hours at 110 degrees Celsius. After drying, bones were defatted with diethyl ether by the Soxhlet extraction method (AOAC, 1990). Dry defatted bones were ashed in an ashing oven at 550 degrees Celsius for 12 hours (AOAC, 1990). Tibia ash percentage was calculated by percent of the dry fat-free bone weight remaining as ash.

Energy and Mineral Utilization

Apparent metabolizable energy (AME) was estimated over 4 days (18 to 21 d). Total excreta was collected during re-feeding after an 18-hour fast. Feed intake and total excreta per pen were calculated. Excreta were dried for 48 hours at 65 degrees Celsius (Namkung and Leeson 1999), and ground using a Thomas-Wiley Mill, Model 4[8]. Gross energy via adiabatic bomb calorimetry[6] was determined on feed and excreta to calculate AME. Feed and excreta samples were analyzed for total phosphorus[3, 5], soluble phosphorus[3], and calcium[5] by Inductively Coupled Plasma Atomic Emissions Spectrophotometer[5]. Digestible P and digestible Ca were calculated by percentage differences between feed and excreta.

Statistical Analysis

Data analysis was performed with the general linear model program of SAS[9]. The analysis of variance probability values are presented for the overall 12-treatment comparison. Fischer's least significant difference test was utilized for multiple comparisons of the means. Four diets increasing in monocalcium phosphate, consisting of the standard curve, were evaluated for linearity. Diets containing each phytase product were evaluated across increasing levels for linear and quadratic effects. Experimental diets containing phytase were compared to the control diet (0.23 nPP %, without phytase). The three treatments containing Product 2 were compared with the three corresponding treatments of Product 1 based on enzyme activity level. Probability values are presented for the main effects of product type and enzyme activity level, as well as the interaction.

Results

Feed Manufacture

Performance Data

Mineral and Energy Utilization

Feed Manufacture

Mixer CV, with 0.01% salt inclusion, did not exceed 18%. Analysis of diets indicated total phytase, total phosphorus, and calcium within expected calculated values (Table 1).

Performance Data

Live weight gain increased in all experimental treatments compared to the control diet ($P<0.05$, Table 2). Increasing levels of Product 1 had a significant linear effect on LWG ($P=0.0309$). Increasing levels of Product 2 did not affect LWG ($P>0.05$); however, LWG produced from Product 2 did not vary from Product 1 ($P = 0.2796$). Feed intake (FI) was greater compared to the control diet for all treatments other than Product 2 at 250 FYT/kg ($P<0.05$, Table 2). Feed intake was similar in Product 1 and Product 2 ($P = 0.9132$). Feed conversion improved with the addition of enzyme in all treatments except Product 1 at 250 FYT/kg ($P<0.05$, Table 2). Increasing levels of Product 1 had a linear decrease in FC ($P=0.0010$). Product 1 at 1000 FYT/kg numerically had the lowest FC and highest phosphorus sparing effect (Table 2 and 3).

The percent of tibia ash increased from the control diet for Product 1 at 500, 1000, and 2000FYT/kg ($P<0.05$, Table 2). Tibia ash did not increase from basal control diet for Product 2. Product 1 and Product 2 were not different with respect to tibia ash ($P = 0.3343$). Treatments did not affect mortality (Table 2).

Mineral and Energy Utilization

The addition of phytase increased Ca digestibility from the control diet in treatments greater than 500FYT/kg for Product 1 and 250FYT/kg for Product 2 ($P<0.05$, Table 4).

Phosphorus digestibility increased with the addition of phytase from the control diet for both products at levels greater than 250FYT/kg ($P<0.05$, Table 4). Calcium and phosphorus digestibilities were similar at comparable levels of Product 1 and Product 2 ($P=0.2333$).

Water-soluble phosphorus content was variable (Table 4). Experimental diets were similar to the control diet in all treatments except Product 1 at 250 and 2000 FYT/kg ($P<0.05$, Table 4). Product 1 and Product 2 did not produce similar water-soluble phosphorus levels in the excreta ($P=0.0207$, Table 4).

Apparent metabolizable energy when adjusted to a constant feed and fecal dry matter of 88% indicated increased AME in all experimental treatments containing phytase compared to the control diet ($P<0.05$, Table 5). The effect on AME was similar for Product 1 and Product 2 ($P=0.1287$).

Discussion

No difference in the products could be detected for any measured variable, except water-soluble phosphorus levels in excreta. Variations in water-soluble phosphorus found due to variable results. Although products were not different, numerically reduced efficacy may have been a result of inadequate mix uniformity. A CV of less than 10% has become the accepted degree of variation that separates uniform from nonuniform feed mixes (McCoy et al., 1994; Johnston and Southern, 2000). Coefficient of variation up to 20% has been reported to be adequate for maximum growth performance in broiler chicks (fed diet with a 0.03- 0.04% tested inclusion) (McCoy et al., 1994). The current study utilized a more concentrated ingredient (Product 2 at 0.01% of the diet). A mixer CV of 18% may have been too high for optimal chick performance and reduction of phosphorus excretion.

The addition of phytase, in both products, at greater than 500 FYT/kg improved all measured variables, other than tibia ash. Tibia ash efficacy was not consistent, resulting in improvement in Product 1 at 500, 1000, and 2000 FYT/kg and no difference in all other treatments from the control diet. The addition of phytase increased ash percentage, therefore phosphorus stores in the bone were higher due to increased liberation of phosphorus.

To evaluate efficacy of phytase at improving phosphorus utilization, it was necessary to have all experimental treatments below NRC (1994) recommendations for both phosphorus and calcium. Minimum phosphorus and calcium requirements seem to have been met in treatments containing greater than 500 FYT/kg for both products because digestibilities improved from the control treatment.

Although a reduction of total phosphorus is often emphasized in literature, water-soluble phosphorus has the greatest environmental implications on eutrophication (Miles et al., 2003). The combination of LPC and phytase in the diet has been reported to reduce water-soluble phosphorus in litter compared with normal corn (Miles et al., 2003). Water-soluble phosphorus did not change from control diet with greater than 250 FYT/kg (Table 4). Applegate and Angel (2003) reported that with correct phytase inclusion both total phosphorus and water-soluble phosphorus decrease. However, they also found that incorrect application of phytase and an insufficient decrease in dietary total phosphorus will result in no change in excreta phosphorus and an increase in water-soluble phosphorus. The lack of change in water-soluble phosphorus in the current study may have resulted from the addition of more phytase than necessary. Total and available phosphorus may have been too high with LPC and 0.23% nPP in control diet and resulted in liberation of excess phosphorus with the addition of phytase. More phosphorus than required for bird growth may have been liberated and therefore excess was excreted as water-soluble phosphorus.

Apparent metabolizable energy was markedly increased with phytase supplementation at all levels. Increases in AME up to 6% have been reported (Ravindran, 1999; Camden et al., 2001). The addition of phytase to diets liberated phosphorus as well as energy substrates bound to phytic acid. Increased energy utilization with added phytase is in part due to increased protein digestibility (Camden et al. 2001) and starch digestibility (Ravindran, 1999). In addition, past research has speculated that calcium-phytate complexes with fatty acids forming metallic soap in the gut lumen, therefore decreasing fat utilization (Leeson, 1993; Ravindran, 1999; Ravindran et al. 2000).

Efficacy of phosphorus increased with the addition of phytase to LPC, but not as much as reported with normal yellow dent corn (Waldroup et al. 2000). Low phytate corn contains less phytate bound phosphorus for the phytase to liberate; therefore, reduced efficacy is expected (Radcliffe, 1999; Kornegay, 1999; Angel et al. 2002).

Phosphorus sparing was not as effective as commercial recommendation of 0.1% with 300-500 FYT/kg in this experiment. At 500 FYT/kg in Product 1, a 0.052% phosphorus sparing effect was found based on tibia ash. These results were below observations reported by Applegate (2003) of 0.065% phosphorus sparing with 500FYT/kg based on tibia ash and normal corn. Feed conversion was the most effected variable for phosphorus sparing with 0.092% at 1000 FYT/kg in Product 1. Feed conversion and tibia ash are the most common indices and often the most sensitive for comparing phosphorus sparing (Applegate and Angel, 2003).

Analysis of phytate level of corn is important when utilizing phytase in research or practice to ensure appropriate use of enzyme. Inappropriate diet formulation is costly and results in either performance decrements or environmental burdens. Low phytate corn alters results of enzyme efficacy; inclusion of phytase to analyzed diets allows a more accurate diet formulation to achieve desired results. The addition of concentrated products, such as phytase, requires uniform mixing to ensure appropriate enzyme dispersion for maximal chick performance.

Tables

Table 1. Basal diets utilized for composition of experimental treatments in phytase study.

Ingredient	0.23% nPP Diet	0.38% nPP Diet	Product 1 @ 2000 FYT/Kg	Product 2 @ 750 FYT/Kg
Low phytate corn [AB]	56.21	56.21	56.21	56.21
Soybean meal (44%)	31.08	31.08	31.08	31.08
Corn Gluten Meal (60%)	5	5	5	5
Soybean oil	4.26	4.26	4.26	4.26
Limestone [B]	1.70	1.36	1.70	1.70
Cellulose [B]	0.46	0	0.40	0.44
Salt	0.44	0.44	0.44	0.44
L-Lysine HCl	0.09	0.09	0.09	0.09
DL-Methionine	0.18	0.18	0.18	0.18
Mono Calcium Phosphate [B]	0.34	1.13	0.34	0.34
NB 3000 Vitamin Premix[C]	0.25	0.25	0.25	0.25
Phytase	0	0	0.05	0.01
Calculated Composition				
ME (Kcal/Kg)	3200	3200	3200	3200
Crude protein (%)	21.95	21.95	21.95	21.95
Methionine + Cystine (%)	0.9	0.9	0.9	0.9
Lysine (%)	1.1	1.1	1.1	1.1
Crude fat (%)	6.86	6.85	6.86	6.86
Calcium (%)	0.8	0.8	0.8	0.8
Nonphytate P (%)	0.23	0.38	0.23	0.23
Analyzed Composition				
Phosphorus (%)	0.43	0.56	0.41	0.42
Phytate Phosphorus (%)	0.207	0.217	0.217	0.215
Calcium (%)	0.79	0.78	0.77	0.78
Phytase (FYT/kg)	50	58	1813	742

[A] Analyses of LPC: Total phosphorus, 0.23%; Phytate Phosphorus, 0.14%.
[B] Particle sizes of corn=697.5µ, limestone=169.5µ, cellulose=185.5µ, MonoCalPhos=789µ
[C] Supplied per kilogram of diet: manganese, 0.02%; zinc, 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69 mg; choline, 386 mg; riboflavin, 6.61 mg; biotin, 0.03 mg; vitamin B_6, 1.38 mg; niacin, 27.56 mg; panthothenic acid, 6.61 mg; thiamine, 2.20 mg; menadione, 0.83 mg; vitamin B_{12}, 0.01 mg; vitamine E, 16.53 IU; vitamin D_3, 2,133 ICU; vitamin A, 7,716 IU.

Table 2. Performance and Tibia Ash Data (day 6-to-day 21)

	Analyzed Total P (%)	Analyzed Ca (%)	Bird LWG[A] (kg)	Pen FI[B] (kg)	FC[C] (kg/kg)	Mortality[D] (%)	Tibia Ash[E] (%)
0.23 calc. nPP	0.43	0.79	0.3297[e]	3.393[e]	1.817[a]	8.33	28.02[c]
0.28 calc. nPP	0.46	0.75	0.4275[b]	4.117[b]	1.611[cde]	0	31.40[cd]
0.33 calc. nPP	0.50	0.77	0.4785[a]	4.427[a]	1.560[de]	2.08	34.16[ab]
0.38 calc. nPP	0.56	0.78	0.4927[a]	4.459[a]	1.509[de]	0	35.56[a]
Product 1[F] @ 250 FYT/kg	0.42	0.82	0.3764[d]	3.808[cd]	1.727[ab]	4.17	29.43[de]
Product 1 @ 500 FYT/kg	0.41	0.77	0.3853[cd]	3.750[cd]	1.625[bcd]	0	31.11[cd]
Product 1 @ 750 FYT/kg	0.42	0.78	0.3962[bcd]	3.822[cd]	1.629[bcd]	2.08	30.21[de]
Product 1 @ 1000 FYT/kg	0.42	0.78	0.4080[bcd]	3.802[cd]	1.591[cde]	4.17	30.83[cd]
Product 1 @ 2000 FYT/kg	0.41	0.77	0.4216[bc]	4.130[b]	1.634[bcd]	0	32.58[bc]
Product 2[G] @ 250 FYT/kg	0.43	0.79	0.3861[cd]	3.602[de]	1.681[bc]	8.33	29.32[de]
Product 2 @ 500 FYT/kg	0.43	0.78	0.3922[bcd]	3.873[c]	1.667[bcd]	2.08	29.80[de]
Product 2 @ 750 FYT/kg	0.42	0.78	0.4199[bc]	3.924[bc]	1.642[bcd]	6.25	30.06[de]
ANOVA P-value	--	--	0.0001	0.0001	0.0003	0.0653	0.0001
LSD[H]	--	--	0.038	0.226	0.115	--	2.32
Standard curve- linear effect P-value	--	--	0.0001	0.0001	0.0001	0.0158	0.0001
Product 1 linear effect P-value	--	--	0.0309	0.4255	0.0010	0.8549	0.5978
Product 1 quadratic effect P-value	--	--	0.1967	0.0925	0.0022	0.694	0.9648
Product 2 linear effect P-value	--	--	0.7840	0.1626	0.9907	0.2001	0.6074
Product 2 quadratic effect P-value	--	--	0.6500	0.2965	0.9340	0.217	0.7747
Product 1 vs. Product 2 (2 products x 3 levels- factorial arrangement)[I]							
Product P-value	--	--	0.2796	0.9132	0.9303	0.1192	0.3343
Level P-value	--	--	0.1953	0.0826	0.2472	0.1590	0.2502
Product x Level P-value	--	--	0.8348	0.0556	0.5942	0.9033	0.5886

[A] Live Weight Gain
[B] Feed Intake
[C] Feed Conversion
[D] Mortality
[E] Dry, Defatted right tibia
[F] Product 1 contains 2,500 FYT/g
[G] Product 2 contains 5,000 FYT/g
[H] Fischer’s least significant difference
[I] Levels include only 250, 500, and 750 FYT/kg

Table 3. Phosphorus sparing effect (%) comparison

Treatment	Analyzed Total P (%)	Analyzed Ca (%)	Derived Calc P (%) LWG[A]	LWG % P sparing effect[B]	Derived Calc P (%) FC[C]	FC % P sparing effect	Derived Calc P (%) Ash[D]	Ash % P sparing effect[B]
0.23 calc. nPP	0.43	0.79	---	---	---	---	---	---
0.28 calc. nPP	0.46	0.75	---	---	---	---	---	---
0.33 calc. nPP	0.50	0.77	---	---	---	---	---	---
0.38 calc. nPP	0.56	0.78	---	---	---	---	---	---
Product 1[E] @ 250 FYT/kg	0.42	0.82	0.253	0.023	0.252	0.022	0.249	0.019
Product 1 @ 500 FYT/kg	0.41	0.77	0.262	0.032	0.305	0.075	0.282	0.052
Product 1 @ 750 FYT/kg	0.42	0.78	0.271	0.042	0.303	0.073	0.264	0.034
Product 1 @ 1000 FYT/kg	0.42	0.78	0.282	0.053	0.322	0.092	0.276	0.046
Product 1 @ 2000 FYT/kg	0.41	0.77	0.295	0.065	0.300	0.070	0.311	0.081
Product 2[F] @ 250 FYT/kg	0.43	0.79	0.262	0.032	0.276	0.046	0.247	0.017
Product 2 @ 500 FYT/kg	0.43	0.78	0.268	0.038	0.283	0.053	0.256	0.026
Product 2 @ 750 FYT/kg	0.42	0.78	0.293	0.064	0.296	0.066	0.261	0.031

[A] Calculated P values derived from the linear Regression of LWG for the standard curve (LWG-0.1028)/1.07951=calc. P, r^2=0.6825
[B] Sparing effect based on Monocalcium P
[C] Calculated P values derived from the linear Regression of FC for the standard curve (FC-2.2192)/-1.95051=calc. P, r^2=0.4534

[D] Calculated P values derived from the linear Regression of Tibia Ash for the standard curve (Ash%-16.7975)/50.77848=calc. P, r^2=0.4935
[E] Product 1 contains 2,500 FYT/g
[F] Product 2 contains 5,000 FYT/g

Table 4. Mineral Digestibility Data (Day 18-to-Day 21)

	Analyzed Total P (%)	Analyzed Ca (%)	Digestible Ca (%)	Digestible P (%)	Water Soluble P (g/kg)
0.23 calc. nPP	0.43	0.79	67.1^{c}	71.3^{c}	1.94^{e}
0.28 calc. nPP	0.46	0.75	75.5^{ab}	75.7^{bc}	2.80^{c}
0.33 calc. nPP	0.50	0.77	77.5^{ab}	76.7^{abc}	3.44^{b}
0.38 calc. nPP	0.56	0.78	81.2^{a}	77.4^{ab}	4.16^{a}
Product 1[A] 250 FYT/kg	0.42	0.82	71.7^{bc}	74.8^{bc}	2.58^{cd}
Product 1 500 FYT/kg	0.41	0.77	72.2^{bc}	77.2^{ab}	1.86^{e}
Product 1 750 FYT/kg	0.42	0.78	77.1^{ab}	81.5^{a}	1.93^{e}
Product 1 1000 FYT/kg	0.42	0.78	76.0^{ab}	80.5^{ab}	2.14^{de}
Product 1 2000 FYT/kg	0.41	0.77	75.1^{b}	79.3^{ab}	2.34^{cde}
Product 2[B] 250 FYT/kg	0.43	0.79	71.6^{bc}	75.8^{abc}	2.11^{de}
Product 2 500 FYT/kg	0.43	0.78	73.4^{b}	79.2^{ab}	2.24^{de}
Product 2 750 FYT/kg	0.42	0.78	73.9^{b}	77.8^{ab}	1.95^{e}
ANOVA P-value	--	--	0.0001	0.0001	0.0001
LSD[C]	--	--	6.03	5.76	0.49
Standard curve-linear effect P-value	--	--	0.0092	0.2177	0.0001
Product 1 linear effect P-value	--	--	0.2349	0.0843	0.0585
Product 1 quadratic effect P-value	--	--	0.2879	0.1173	0.0466
Product 2 linear effect P-value	--	--	0.7863	0.3394	0.4184
Product 2 quadratic effect P-value	--	--	0.8488	0.3801	0.3654
Product 1 vs. Product 2 (2 products x 3 levels- factorial arrangement)[D]					
Product P-value	--	--	0.6534	0.8444	0.8724
Level P-value	--	--	0.1214	0.0579	0.0399
Product x Level P-value	--	--	0.4759	0.2333	0.0393

[A] Product 1 at 2,500 FYT/g
[B] Product 2 at 5,000 FYT/g
[C] Fischer's least significant difference value
[D] Levels include only 250, 500, and 750 FYT/k

Table 5. Apparent Metabolizable Energy Data (Day 18-to-Day 21)

	Analyzed Total P (%)	Analyzed Ca (%)	Apparent Metabolizable Energy (kcal/kg) adjusted to a constant DM (88%) [standard deviation]
0.23 calc. nPP	0.43	0.79	3452[c] [183]
0.28 calc. nPP	0.46	0.75	3680[a] [94]
0.33 calc. nPP	0.50	0.77	3588[ab] [247]
0.38 calc. nPP	0.56	0.78	3643[ab] [241]
Product 1[A] 250 FYT/kg	0.42	0.82	3573[b] [269]
Product 1 500 FYT/kg	0.41	0.77	3595[ab] [140]
Product 1 750 FYT/kg	0.42	0.78	3691[a] [126]
Product 1 1000 FYT/kg	0.42	0.78	3644[ab] [96]
Product 1 2000 FYT/kg	0.41	0.77	3639[ab] [176]
Product 2[B] 250 FYT/kg	0.43	0.79	3607[ab] [103]
Product 2 500 FYT/kg	0.43	0.78	3665[ab] [137]
Product 2 750 FYT/kg	0.42	0.78	3610[ab] [150]
ANOVA P-value	--	--	0.0147
LSD[C]	--	--	116
Standard curve-linear effect P-value	--	--	0.1502
Product 1 linear effect P-value	--	--	0.2474
Product 1 quadratic effect P-value	--	--	0.2969
Product 2 linear effect P-value	--	--	0.3350
Product 2 quadratic effect P-value	--	--	0.3330
Product 1 vs. Product 2 (2 products x 3 levels- factorial arrangement)[D]			
Product P-value	--	--	0.7966
Level P-value	--	--	0.2811
Product x Level P-value	--	--	0.1287

[A] Product 1 at 2,500 FYT/g
[B] Product 2 at 5,000 FYT/g
[C] Fischer's least significant difference value
[D] Levels include only 250, 500, and 750 FYT/kg

Footnotes

Notations for footnotes in article:

[1] Weigh-Tronix vertical mixer at West Virginia University
[2] Hach Company, Loveland, Colorado, 80539
[3] Roche Vitamins Inc., Parsippany, New Jersey 07054
[4] University of Maryland, Roselina Angel
[5] New Jersey Feed Laboratory Inc., Trenton, New Jersey 08650
[6] Parr Instrument Co., Moline, Illinois 61265
[7] Pilgrim's Pride, Moorefield, West Virginia 26836
[8] Thomas Scientific Co., Swedesboro, New Jersey 08085
[9] SAS Institute. 1991. SAS User's Guide: Statistics. Version 6.03 Edition. SAS Institute, Inc., Cary, North Carolina.

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Appendix
West Virginia University Animal Care and Use Committee
Protocol Statement

Pre-11/02 Protocol Forms will not be reviewed

Research Compliance Use Only
Protocol # ____________ OSP# ____________
Reviewers ____________

Protocol Statement

West Virginia University Animal Care and Use Committee

Research Compliance Office, 886 Chestnut Ridge Road
PO Box 6845 phone (304) 293-7073 fax (304) 293-7435

1. **Protocol Title** Impact of phytase supplementation on phosphorous and calcium digestibility and metabolizable energy in broilers

2. **Principal Investigator (Instructor) PI*** Joe Moritz, PhD. Position Assistant Professor
Department Animal and Veterinary Sciences PO Box 6108 Phone 293-2631 x 4435 Fax 293-2232
HA* [x] ES* 15 PP* [x] EP* 15 AW* [x] * Explanation for Codes

3. **Other Personnel (See panel above for instructions)**

Name	Department	PO Box	Signature	CO*	HA*	ES*	PP*	EP*	AW*
Nicole Baker	A&VS			x	x	1	x	1	x

4. **For Academic Courses** Course Number N/A Estimated # of Students ____
When Offered ____

5. **Classification of Protocol** New [x] Renewal [ ] (Current Protocol Number)
If the protocol is a Renewal of a **Research** Project, submit a progress report of 500 words or less to the ACUC.

6. **Years Requested** 1 year [ ] 2 years [ ] 3 years [x]

7. **Signatures**
As the Principal Investigator (Instructor), I am responsible for assuring that the conduct of all procedures and any animal care provided outside of centralized animal holding facilities or areas comply with all applicable standards and regulations. I have read and am familiar with the appropriate federal regulations and standards for animal care and use. I certify that all proposed animal use is necessary for this project and will be carried out within the provisions of the Animal Welfare Act, Public Health Service Policy on university regulations, Policies or guidelines. All other personnel involved in animal use under this protocol will be educated in their responsibilities prior to the use of animals. I also understand that any changes in to this project must be reviewed and approved by the WVU Animal Care and Use Committee before implementation.

Principal Investigator (Instructor) ____ Date ____ Faculty Advisor ____ Date ____
I approve the submission of this Animal Care and Use Protocol to the WVU Animal Care and Use Committee.

Department Chair ____ Date ____ Dean ____ Date ____
If the Principal Investigator (Instructor) is the Dept. Chair, the Associate Chair or a senior faculty member must sign in the Chair's place.

8. Animal Information (complete this page for each species used)

Scientific Name		Common Name	Strain/ Stock/ Breed	Sex	Age or Weight
Gallus *Domesticus*		Chicken	Ross 308 x Ross 344	F and M	day old
Protocol Total	**Yearly Number**	**Expected Avg. Daily Consensus**	**Source of Animals**		
1815 x 3	1815	605	Broilers, Commercial Source		

Maintenance of a Breeding Colony No [x] Yes [ ]

9. Sites / rooms where Procedures will be Conducted

	Facility*	Bldg/Room
Nonsurgical procedures or conditions	Poultry Farm (Morgantown)	Barn B
Nonsurvival surgery	N/A	
Survival surgery	N/A	
Postsurgical / Postanesthesia / Postprocedural Recovery	N/A	

*** If procedures will be conducted at one of the University facilities, specify above which site will be used for each type of procedure:**

Facilities include: OLAR, Stewartstown Farm, Life Sciences Bldg., Wardensville (Reymann Memorial) Farm, Willow Bend Farm, Potomac State College, Reedsville Farm.

Bldg/Rooms include: Food Animal Research Facility (FARF), Pole Barn, Dairy Barn, Sheep Barn, Beef Handling Area, Beef Holding Area, Poultry Barn, and Labs or Rooms within OLAR or the Life Sciences Building.

10. Animal Housing and Care

Attach a completed Animal Housing and Care Request Form for each species (see last page). The Animal Housing and Care Request Form will be forwarded to either the Office of Laboratory Animal Resources (OLAR) or the College of Agriculture, Forestry and Consumer Sciences. Approval of the protocol does not guarantee accommodations requested.

11. Special Animal Housing

Animals will be housed outside of the centralized animal holding facilities or areas, such as in laboratories, for:

Special Housing	No		Yes		If yes	Building / Site	Room	Duration
12 to 24 hours (Study Area)	x							
More than 24 hours (Satellite Facility)	x							

If yes provide

A. **Scientific justification for housing animals outside of the centralized animal holding facilities for more than 12 hours or more than 24 hours**

N/A

B. **Describe housing for animals and who will provide daily care for animals maintained outside the centralized animal holding facilities for more than 12 hours or more than 24 hours**

N/A

C. **Describe provisions for weekend, holiday, after-hours and emergency care for animals maintained outside the centralized animal holding facilities or areas for more than 12 hours or more than 24 hours.**

N/A

12. Fund Source

Federal Name: ______________________ Funding Status: Pending [ ] Active [ ] OSP# ________

Anticipated Funding Period: ________ Application deadline ________

State Name: ______________________ Funding Status: Pending [ ] Active [ ] OSP# ________

Anticipated Funding Period: 4/03-4/04 Application deadline ________

Private Name: Roche Vitamins Inc. Parsippany, New Jersey Funding Status: Pending [x] Active [ ] OSP# ________

Anticipated Funding Period: ________ Application deadline ________

Internal Name: ______________________ Funding Status: Pending [ ] Active [ ] OSP# ________

Anticipated Funding Period: ________ Application deadline ________

13. Lay Description

Briefly describe in nontechnical (lay) terms the goal of the project and the role of living vertebrate animals in the work. Include the benefits to be derived from the project. This description should be written so that it could be disseminated to the public through the media and understood by a nonscientist. It is not intended for peer review purposes. Do not use a grant abstract or exceed the space provided. For an example of a lay description go to http://www.wvu.edu/~rc/acuc/lay_desc.htm

Eutrophication of water sources from animal waste is a growing environmental concern. Eutrophication may be defined as the process by which a body of water becomes enriched in dissolved nutrients (as phosphates) that stimulate the growth of aquatic plant life usually resulting in the depletion of dissolved oxygen. Broiler chickens produce substantial amounts of phosphorous waste in comparison to other livestock. Litter removed annually from a broiler house with 22,000 birds contains as much phosphorous as the sewage community of 6,000 people, according to the USDA's Natural Resources Conservation Service (NRCS 1995). Broiler chickens consume corn-soybean based diets, which are high in phosphorous, however the phosphorous is phytate bound and not available to the bird. Phytate phosphorus may be defined as a ringed complex containing six phosphorus molecules as well as protein, calcium, zinc, iron and other minerals. Chickens are lacking or are limited in the enzyme phytase. Adding phytase from microbial sources to broiler feed increases the availability of phytate-bound phosphorus, reducing the amount of phosphorus released into the environment. In addition, diminishing phytate phosphorus complexes may increase calcium availability and dietary energy.

14. Experimental (or Teaching) Design

A. Provide a flow diagram (or verbal description if a diagram is not possible) showing how experimental groups (or groups used for teaching) and experimental (or teaching) procedures or conditions are integrated in the project. Indicate clearly which groups will undergo which procedures or experimental conditions over what periods of time. Details of the experimental (or teaching) procedures or conditions should be described in the appropriate sections on the following pages.

605 birds- 4 day pretest
National Research council (NRC) based diet

48 birds 48 birds 48 birds 48 birds 48 birds 48 birds 48 birds 48 birds 48 birds 48 birds 48 birds 48 birds

Each group of 48 birds will be fed one of 12 experimental diets formulated to NRC specifications with the exception of a 0.8% calcium level and various phosphorus levels, which include 0.2, 0.25, 0.3 and 0.35%. In addition eight treatments will contain a commercial phytase enzyme at various concentrations.

Throughout the experiment, feed and water will be provided for ad libitum consumption and temperature will be regulated to maximize bird comfort.

The experimental period will proceed from day 5 to day 21. Measurements from this period will include broiler performance, fecal phosphorus, digestible phosphorus, digestible calcium and metabolizable energy. On day twenty-one, all birds will be sacrificed and the left middle toe and right tibia will be excised to determine bone mineralization.

B. What (estimated) percentage of animals will be unusable due to unintended mortality or morbidity?

Moritz et. al. (2001), Nir et. al. (1995), and Moritz et. al. (2002) each calculated a mortality rate of 5% in feed performance studies. Therefore approximately 5% more chicks from this same hatch will be maintained on a control diet to replace mortality through the five day pretest period.

C. Describe any clinical problems that may arise from experimental manipulation.

With the exception of phosphorous and calcium, all nutrients meet or exceed NRC recommendations. In order to test phytase efficacy, it is necessary to utilize phosphorous and calcium at less than optimum levels. The NRC recommends a minimum level of 0.45% nonphytate phosphorous; however, research with 0-3 week chicks illustrates satisfactory feed conversion at 0.20% nonphytate phosphorous (Waldroup et. al. 2000). Although, weight gain may be reduced the birds will not experience pain or discomfort.

If the protocol involves only the observation of animals, stop here.

15. Nonsurgical Experimental (or Teaching) Procedures or Conditions

A. Details of experimental (or teaching) procedures, including frequency of treatments per animal.

Dietary ingredients will include corn, soybean meal (44%), corn gluten meal (60%), fat (soy oil), limestone, monocalcium phosphate, salt, vitamin premixture, methionine, lysine, and cellulose. All nutrients will meet or exceed NRC recommendations with the exception of phosphorous and calcium, which will be included in the diet at levels no lower than 0.2% and 0.8%, respectively. The experiment will have twelve treatments and eight replications per treatment. Six birds will be randomly allocated to each pen. Four treatments will comprise the standard curve control (without phytase supplementation) and eight treatments will include phytase. There will be two commercial types of enzyme used; Ronozyme 2500 FYT/g and Ronozyme 5000 FYT/g, utilized at different concentrations. Birds will provided with trough feeders and nipple drinkers to allow ad libitum consumption of feed and water.

B. If anesthetics, analgesics, tranquilizers, and /or experimental materials will be used during nonsurgical procedures, provide the following:

	Agent	Dose (mg/kg)	Route	Frequency
Anesthetics				
Analgesics				
Tranquilizers				
Experimental materials	Ronozyme 2500 FYT/g	250-2000 FYT/kg	Mixed with feed	Ad libitum daily
	Ronozyme 5000 FYT/g	250-700 FYT/kg	Mixed with feed	Ad libitum daily

C. Methods that will be used to detect and evaluate pain and distress in animals and any steps planned to avoid or minimize pain or distress to animals, including the criteria used to determine when animals should be euthanized, if appropriate:

Chickens will be observed daily. No pain or clinical illness is expected.

16. Nonsurvival Surgery (Note: Nonsurvival surgery does not include euthanasia followed by procedures conducted postmortem)

A. Provide a description of the nonsurvival surgery

N/A

B. If anesthetics, analgesics, tranquilizers, and /or experimental materials will be used during nonsurvival surgery, provide the following:

	Agent	Dose (mg/kg)	Route	Frequency
Anesthetics				
Analgesics				
Tranquilizers				
Experimental materials				

C. Methods (such as intraoperative monitoring techniques) that will be used to detect and evaluate pain and distress in animals and any steps planned to avoid or minimize pain or distress to animals, including the criteria used to determine when animals should be euthanized, if appropriate:

17. Survival surgery

A. Provide a description of the surgery, including aseptic techniques.

N/A

B. If anesthetics, analgesics, tranquilizers, and /or experimental materials will be used during survival surgery, provide the following:

	Agent	Dose (mg/kg)	Route	Frequency
Anesthetics				
Analgesics				
Tranquilizers				
Experimental materials				

C. Methods (such as intraoperative monitoring techniques) that will be used to detect and evaluate pain and distress in animals and any steps planned to avoid or minimize pain or distress to animals, including the criteria used to determine when animals should be euthanized, if appropriate:

18. Multiple Major Survival Surgery

Provide scientific justification and the time proposed between procedures on the same animal:

N/A

19. Description of procedures using death as a measured end-point * (indicate why morbidity cannot be used instead of mortality):

***Death as an end point refers to projects in which the animals' non-experimentally induced death is required as a measured data point. It does not refer to projects in which the animals will be euthanized prior to non-experimentally induced death for tissue / sample collection or project termination.**

N/A

20. Euthanasia

Describe the methods used to euthanize animals.

Electrical stunning and exsanguinations, acceptable methods of euthanasia according to the 2000 Report of the AVMA Panel on Euthanasia (JAVMA, Vol. 218, No. 5, 2000), are common amongst poultry research settings. Electrical stunning, if done properly, leads to rapid unconsciousness, but may not cause death. Therefore, exsanguination is used to insure death. Thus excluding any possible tissue contamination caused by chemical agents used for euthanization. All personnel will be adequately trained in proper technique.

List the agent, dose in mg/kg body weight and route of administration if applicable.

Agent	Dose (mg/kg)	Route

Provide Scientific Justification for methods that are not described as "Acceptable" by the American Veterinary Medical Association (AVMA) in '2000 Report of the AVMA Panel on Euthanasia', JAVMA Vol. 218, No. 5, 2001, p1.

The method stated above is accepted by the AVMA for euthanasia of poultry.

21. Disposition of Animals Other than by Euthanasia

If animals will not be euthanized as a part of the project, describe exactly what will be done with them (e.g., transfer to another project).

N/A

22. Shared Biological Samples
If tissues, cells or other products derived from animals will be shared with other investigators during or after the project, describe this material and how it will be transferred. Provide reasons for the transfer, and give the name and address of the person receiving the material.

N/A

23. Transportation of Animals by Investigators
If you (instead of the animal care staff or a commercial vendor / transporter) plan to pick up or deliver a shipment of animals, or transport animals out of doors, describe how you plan to conduct this move.

At 1 day-old, chicks will be placed in hatchery crates and transported to the Poultry Barn on the Stewartstown Farm at West Virginia University in Morgantown, West Virginia.

24. Experimental Materials and Safety Considerations

Materials and Agents Used in Animals	No	Yes	Specify
Flammable or explosive materials (e.g. ether)	x		
Biological samples of human origin	x		
Biological materials (e.g. transplantable tumors) that might contain adventitious infectious agents	x		

Attach Radiation Safety Committee approval letters if appropriate. For Each Radioactive material, Infectious Agent including Oncogenic Viruses, Toxic Chemical or Carcinogen used in animals, complete the following:

Material or Agent	Concentration Used in Animals	Route of Administration	Duration of Exposure	Length of Time that Animals are Maintained After Exposure

25. Category of Animal Use (Circle the corresponding letter(s) for all that apply)

*** If Category B or C is chosen, complete the Refinement, Reduction, and Replacement Sections Below below.**

A. X Animals will <u>not</u> undergo procedures or experience conditions that would normally cause more than momentary or slight pain or distress in the absence of anesthetics, analgesics or tranquilizers, which will not be administered.

B. * Animals may potentially experience more than momentary or slight pain or distress and will receive anesthetics, analgesics or tranquilizers during or after the procedure or conditions listed below. Alternatively, animals may be euthanized to alleviate pain or distress. (Check all that apply)

- [ ] Nonsurgical experimental or teaching procedures or conditions
- [ ] Nonsurvival surgery
- [ ] Survival surgery
- [ ] Postsurgical / Postanesthesia / Postprocedural recovery period

C. * Animals may potentially experience more than momentary or slight pain or distress and will <u>not</u> receive anesthetics, analgesics or tranquilizers to alleviate pain or distress (except for euthanasia when appropriate).

Category C Scientific Justification for withholding anesthetics, analgesics or tranquilizers:

*** If Category A is chosen, sections 26 and 27 are not required.**

Refinement

Refinement refers to efforts made to improve procedures and methods to:

1. Use fewer animals
2. Reduce trauma to the animals (either physical or stressful). This could include improvement in procedures that provide for better outcomes, shorter recovery times, lower morbidity and mortality
3. Achieve more definitive results

26. Describe the methods and sources employed to determine the availability of alternatives to procedures that could potentially cause more than momentary or slight pain or distress.

The information requested in this section is required even if you plan to alleviate pain or distress by the use of anesthetics, etc.

A. **Database literature search:** See AWIC Tips for Searching for Alternatives or at http://www.nal.usda.gov/awic/alternatives/tips.htm

If a database / literature search was used to determine the unavailability of alternatives to potentially painful or distressful procedures, complete the following:

1] Name of search engine used: ______

2] Database searched: [ ] Medline [ ] Other --- Specify ______

3] Keywords used in the search and / or key references used to document the unavailability of alternatives:

4] Date of the search: ______

5] Years covered by the search: ______

B. If methods other than database / literature searches were used, describe them below:

N/A

- **Please note that while consultation with experts is allowed for the purpose of obtaining Reduction, Refinement and Replacement ("3 R's") information, it is generally discouraged by the USDA.**
- If a consultation with an expert is used to obtain information on the "3 R's," the following information about the consultation is required.
- Each component of the "3 R's" components, Reduction, Refinement and Replacement, must be addressed in the information from the expert
- The following information must also be provided:
  - Name of the expert
  - Expert's academic degree(s) and title of current position
  - Date of the consultation. If the consultation occurred at a meeting, provide the name, date and location of the meeting.
  - The expert not only must have knowledge in the field of interest, he/she must also have current knowledge of the "3 R's" information related to the field of interest. This special knowledge must be verified and described for each of the "3 R's" components, Reduction, Refinement and Replacement.

C. Results of the search

- Do relevant alternatives exist?
- If alternatives exist, why are they not adequate for this study?

N/A

27. Justification of the use of procedures iuvolving prolonged restraint (i.e., longer than one hour) of unanesthetized animals. Provide details of the restrain procedure and care of restrained animals.

N/A

Replacement

Replacement refers to consideration of non-animal model alternatives or the use of animals lower on the phylogenetic scale. Some issues include:

1. **Has computer modeling been employed to assess this subject? Why is computer modeling not relevant or sufficient for the goals of this study?**
2. **Can cell culture systems be substituted for the "animals"?**
3. **Can an animal lower on the phylogenetic scale be used as a model? For example, can a mouse or rat be used instead of a cat or a dog?**

28. Rationale for using animals rather than nonanimal alternatives
(in vitro systems, human clinical trials, computer models, etc.)

Eutrophication is a problem which stems from animal manure derived from the inability of animals to metabolize plant phosphorus. This study would not be possible without the use of animals.

29. Justification of the choice of animal species
(literature, previous studies, unique anatomic or physiologic characteristics, etc.)

Waste management challenges are not the same for all livestock. Broiler chickens excrete high amounts of phosphorous waste because they lack the enzyme phytase, which metabolizes phytate bound phosphorous from plant sources. Phosphorus associated with broiler waste continues to be a problem due to increased broiler production. In West Virginia alone, 2,000,000 broiler chickens are processed each week.

Reduction

Reduction refers to efforts to minimize the numbers of animals utilized in animal studies. Some common issues might include:

1. **Are the numbers of animals requested for each experimental group, the appropriate number to achieve statistically significant results?**
2. **Has a power test been performed to estimate necessary numbers?**

30. Assurance that proposed project does not necessarily duplicate previous work: (Check one)

[X] This project does not duplicate previous experiments

[ ] Duplication is necessary. Justification:

31. Justification for use of the number of animals requested

Describe: a] the experimental (or teaching) groups and animals needed per group, and/or b] the quantity of biological samples (e.g. tissue etc.) needed from animals relative to the number of animals requested (e.g. quantity of sample that can be obtained from each animal), and/or c] the statistical analysis and results used to determine the number of animals requested.

One pen of six birds will designate an experimental unit. Each dietary treatment will be replicated eight times. Moritz et. al. (2001), Nir et. al. (1995), and Moritz et. al. (2002) each calculated a mortality rate of 5% in feed performance studies. Therefore approximately 5% more chicks from this same hatch will be maintained on a control diet to replace mortality through the five day pretest. Six-hundred-five chicks will be required to properly conduct the experiment. To effectively solve industry type problems it is necessary to conduct highly replicated experimentation. In replicating the twelve treatments eight times, the growing rooms will be fully utilized, thus mimicking industry conditions. A past protocol from the Moritz laboratory justified the use of thirteen replications for detecting appreciable broiler performance differences. This replication number was determined by a statistical power analysis. While thirteen replications would be ideal for the current study, facility pen numbers dictate a maximum of eight replications.

Animal Housing and Care Request Form

Pre-8/02 Protocol Forms will not be reviewed

Research Compliance Use Only
Protocol # ______ OSP# ______
Date Submitted ______

- **Complete One Request Form for Each Species**
- **Return this form to the Research Compliance Office with the Protocol Statement**
- **Submission of this request form does not guarantee the arrangements requested**
- **If you have any questions about animal housing and care arrangement, contact the appropriate office: the Office of Laboratory Animal Resources (OLAR) or the College of Agriculture, Forestry and Consumer Sciences**

Principal Investigator (Instructor) Joe S. Moritz **Species to be used** Broilers

1. Sites where animals will be maintained

[ ] Health Sciences Center
[X] University Farm Specify Site: ______
[ ] Eberly College of Arts and Sciences
[ ] Other Specify Site: ______

2. Rodents

A Room:
___ Standard Room
___ Exclusive use of an animal room (subject to availability – Check with OLAR)

B Caging:
___ Conventional plastic bottom cage
___ Conventional wire bottom cage
___ Microisolator cage without sterile food, water or bedding
___ Microisolator cage with sterile food, bedding and water (acidified/autoclaved)
___ Other (explain): ______

C Housing:
___ Standard (typically the maximum number of animals per cage allowed by federal standards)
___ One animal per cage
___ Other (explain): ______

D Care:
___ Standard
___ Other (special light cycle, diet or water, technical assistance, etc): Contact OLAR

3. Non-Rodents

A Room or Animal Holding Area:
_X_ Standard
___ Other (explain): ______

B Primary Enclosure (if applicable):
_X_ Standard
___ One animal per primary enclosure
___ Other (explain):

C Housing or Animal Holding Arrangements:
_X_ Standard
___ One animal per primary enclosure
___ Other (explain):

D Care:
_X_ Standard
___ Other (special light cycle, diet or water, technical assistance, etc): Contact the college of Agriculture, Forestry and Consumer Sciences

* Explanation for codes

Back

PI	If the Principal Investigator (Instructor) is a post-doctoral fellow, the faculty advisor must be listed as a co-investigator (co-instructor) and sign as the faculty advisor. Students may not serve as principal investigators.
CO	Are you a co-investigator? (X)
HA	Will you handle animals? (X)
ES	Length of experience with species (in years)
PP	Will you perform procedures? (X)
EP	Length of experience with procedures (in years)
AW	Have you passed the test for Animal Welfare Core Training at WVU? (X)

For all applicable regulations, policies and guidelines

Section	Section Heading
2, 3	Personnel performing procedures on animals
8	Animal Information
9	Sites / Rooms where Procedures will be Conducted
10	Animal Housing and Care
11	Special Animal Housing
13	Lay Description
14	Experimental (or Teaching) Design
15	Nonsurgical Experimental (or Teaching) Procedures or Conditions
16	Nonsurvival Surgery
17	Survival Surgery
18	Multiple Major Survival Surgery
19	Description of Procedures using Death as a Measured Endpoint
20	Euthanasia
21	Disposition of Animals Other than by Euthanasia
22	Shared Biological Samples
23	Transportation of Animals by Investigators
24	Experimental Materials and Safety Considerations
25	Category of Animal Use
26	Alternatives to Potentially Painful or Distressful Procedures
27	Justification for the Use of Procedures Involving Prolonged Restraint
28	Rationale for Using Animals Rather Than Nonanimal Alternatives
29	Justification of the Choice of Animal Species
30	Assurance that the Proposed Project Does Not Unnecessarily Duplicate Previous Work
31	Justification for the Use of the Number of Animals Requested

Vita

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OBJECTIVE
A position in nutrition-related research and development or technical service.

EDUCATION

Masters of Science Degree in Nutrition
West Virginia University, Morgantown, WV
Expected Graduation Date- August 2004
Concurrent Dietetic Internship, eligible for Registered Dietetics exam August 2004
Masters degree advisors Dr. Joseph Moritz and Dr. Cindy Fitch

Bachelor of Science Degree in Nutrition and Food Sciences and Dietetics
The University of Vermont, Burlington, VT
Graduation Date- May 2002

RELATED EXPERIENCE

Animal Nutrition
- First author of abstract and poster presentation at Poultry Science Association meeting in Madison, Wisconsin, July 6-9, 2003
- First author of abstract and oral presentation at Joint Federal Animal Science Society meeting in St. Louis, Missouri, July 25-29, 2004
- Practical experience and experimentation of conventional and organically reared poultry
- Researched, implemented, and performed Phytase supplementation experiment in poultry
- Experienced in conducting dry matter, ash, bomb calorimetry, ether extractions, Kjeldahl protein, and HPLC amino acid analysis
- Experienced in diet formulation, experimental design, and statistical analysis

Human Nutrition
- Research assistantship providing nutrition services to children with special needs at West Virginia University Center of Excellence and Disabilities, Morgantown, West Virginia
- Active participant in development and creation of curriculum and course work for class at West Virginia University entitled Rural Education for Appalachian Community Health at Home, in conjunction with WVU Center for Excellence and Disabilities
- Completed internship with dietitian at Health South Rehabilitation Hospital in Morgantown, West Virginia
- Coordinated and implemented nutrition participation with “organic farming field day”
- Oversee and advocate sanitation and proper food handling at food service facility

Animal Nutrition Publications

Peer Reviewed Journal Articles

- Baker, N. J., A. S. Parsons, N. P. Buchanan, and J. S. Moritz, 2004. Effects of various phytase concentrations in diets with low phytate corn on broiler chick performance and metabolizable energy. (In preparation for International Journal of Poultry Science).
- Moritz, J. S., A. S. Parsons, N. J. Baker, and J. Jaczynski, 2004. Synthetic methionine and feed restriction effects on performance and meat quality of organically reared broiler chickens. (In preparation for Journal of Applied Poultry Research).

Abstracts

- Baker, N. J., A. S. Parsons, N. P. Buchanan, and J. S. Moritz, 2004. Effects of various phytase concentrations in diets with low phytate corn on broiler chick performance and metabolizable energy. (To be presented at the annual meeting of Federation of Animal Science Society in St. Louis, Missouri; July 25-29, 2004).
- Buchanan, N. P., A. S. Parsons, N. J. Baker, and J. S. Moritz, 2004. Synthetic methionine and feed restriction effects on performance and meat quality of organically reared broiler chickens in fall months. (To be presented at the annual meeting of Federation of Animal Science Society in St. Louis, Missouri; July 25-29, 2004).
- Parsons, A. S., N. J. Baker, and J. S. Moritz, 2004. Synthetic methionine and feed restriction effects on performance and meat quality of organically reared broiler chickens in summer months. (To be presented at the annual meeting of Federation of Animal Science Society in St. Louis, Missouri; July 25-29, 2004).
- Kiess, A. S., N. J. Baker, A. S. Parsons, J. S. Moritz, and K. P. Blemings, 2003. Effect of feed form on lysine and nitrogen retention in broilers. (Presented at the annual meeting of the Poultry Science Association, Madison, Wisconsin, July 6-9, 2003).

Human Nutrition Publications and Media Experience

- Brown, G. and N. Baker, 2004. Get the low down on low carb diets. West Virginia University Extension Services educational packet (In preparation).
- Baker, N. J., 2003. Watching Winter Weight, The Dominion Post, November 23, 2003.
- Baker, N. J., 2001. The 4 C's of Food Safety. 96.1 FM Vermont Public Radio, presented and aired December 10, 2001.
- Baker, N. J. and S. A. Burczy, 2001. Enjoy Holiday Meals in Moderation, SERVE New England, Vol. 10, No. 11, November/December 2001.
- Baker, N. J., S. A. Burczy, and J. Harvey-Berino, 2001. Food & Fitness Choices for a Healthy Weight. University of Vermont Extension and WCAX-TV viewer handout, April 4, 2001.
- Baker, N. J. and S. A. Burczy, 2001. Feed your bones. Words to the Wise. University of Vermont Extension, Spring 2001 pp. 1-4.

Professional Profile

- Diverse experience and interest in animal and human nutrition
- Thrive on working in a challenging environment and excel at multi-tasking
- Have excellent writing, oral, and interpersonal skills
- Proficient with Microsoft Word, Excel, Power Point, WordPerfect, SAS, and Internet use