

## ABSTRACT

GODWIN, JENNIFER LEIGH, Effect of Dietary Phosphorus and Enzyme Levels on Reproduction in Turkey Breeder Hens (Under the direction of Dr. Jesse Grimes)

Previous work has demonstrated that the P requirement for turkey breeder hens has not been adequately determined. It is becoming apparent that there are distinctive P requirements of turkey breeder hens compared to poults and growing birds. Therefore, the objective of this study was to provide additional information regarding P requirement and use of phytase in turkey breeder hen diets. An experiment was conducted to determine the effect of dietary phosphorus (P) levels and phytase enzyme levels on turkey breeder hen productivity from 31 to 62 weeks of age (WOA). Four hundred and eighty turkey breeder hens were reared in a curtain-sided house with 48 pens (10 birds per pen; 8 pens per treatment) at 31 WOA. Hens were fed a breeder ration with treatments as follows: high P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E). The phytase enzyme product used (Allzyme<sup>TM</sup> Phytase, Alltech, Inc., Nicholasville, KY 40356) is derived from *Aspergillus niger* and contained 11.27 PTU/g. Feed and water were available *ad libitum* for a 28-week reproductive period. Diets were fed in mash form. All hens were photostimulated in January (31 WOA) with 15.5 h of light/day. Individual bird body weight and feed consumption, by pen, were determined at monthly intervals from 31 WOA to 62 WOA. Hens were inseminated weekly with pooled semen from same strain breeder males. Hens were observed for biweekly reproductive and hatchability performance for 28 weeks of lay (WOL) and recorded on a pen basis. The following parameters were measured: egg production, fertility, hatchability of all eggs, hatch of fertile, initial egg weight, % egg

weight loss, conductance, and embryonic mortality. Egg weight, shell thickness, albumen P and yolk P were measured monthly. At the end of the study P concentration was measured in plasma, tibia, fecal P and water-soluble fecal P.

There were no consistent differences for any of the reproductive parameters observed due to dietary treatments. Decreasing dietary P resulted in no major reproductive problems for turkey hens. However, the addition of enzyme did significantly increase the number of days hens remained in production. Lowering dietary P significantly decreased total fecal P and water-soluble fecal P suggesting that dietary P can be lowered in efforts to aid environmental concern without impairing reproduction.

The hatchability of eggs from turkey breeder hens fed dietary treatments was also measured. The few significant differences observed may be due to the hen's endogenous ability to efficiently breakdown the phytate molecule regardless of the inclusion level. Therefore, it was suggested that feeding 0.17% aP provided a sufficient source of P without impairing reproductive parameters. This suggests that eggs from hens fed 0.17% aP hatch as well or better than those fed industry or NRC levels with or without the addition of phytase.

In conclusion, the P requirement of turkey breeder hens may be lower than the current level recommended by the NRC (1994). This study has provided evidence that lowering dietary P levels does not affect the reproduction or hatchability of turkey breeder hens. Results suggest that poultry companies that feed turkey breeder hens lower levels of dietary P will maintain reproductive and hatchability status and lower the amounts of fecal P. This would reduce litter P which would lessen the impact of turkey production on the environment.

**Effect of Dietary Phosphorus and Enzyme Levels on Reproduction in Turkey  
Breeder Hens**

by

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## **BIOGRAPHY**

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## **Literature Review**

### **Introduction**

The food animal industry has advanced tremendously during the past forty years. The rapid rise in the human population and the shift in preferences towards quality animal protein products are two factors that have spurred the industry increase over the past four decades. As the demand for a healthier quality of meat has risen so has the need for larger and more complex animal production facilities. Over time, this demand has decreased the need for the small independent production and has resulted in a trend toward larger, more efficient, contract animal rearing complexes. Kerr (1995) identified these large facilities as those capable of producing up to 40,000 pigs, 400,000 chickens, 25,000 turkeys, or 2,000,000 layers per year. The poultry industry, the largest facet of the food animal industry, has seen a rapid increase in production due to consumers' perceptions that poultry products are a healthy source of low fat protein and less expensive.

The growth in production and the concentration of animals per complex that has occurred in the modern poultry industry has been concurrent with the need to allocate scarce and valuable resources to the efficient and cost-wise production of poultry (Aho, 1989). Farm consolidation, intensification, and concentration is sound from an economics and management perspective, but fails to adequately address the environmental impacts of the increased and intensified generation of animal wastes with in a confined area (Barker and Zublena, 1995). Poultry waste has the potential to become either a valuable resource or an environmental hazard (Parks, 1998). Agricultural output

trends of this scale result in significant demands on the surrounding geographical area in the form of waste disposal.

Historically, land application programs use nitrogen (N) as the target nutrient to achieve agronomic nutrient rates (Zublena, 1994). Recently, however, phosphorus (P) has received a great deal of attention as being the rate-limiting nutrient. In addition, future limits may be based on P levels as well as the possibility of heavy metals rather than the traditional use of N values (Cromwell *et al.*, 1970). Therefore all phases of poultry production including turkey breeder hen production should be concerned with limiting the amount of fecal nutrients excreted by the birds especially that of P.

The objective of this study was to examine the effect of decreased dietary P with and without phytase on the P digestibility and reproductive performance of turkey breeder hens. Egg production, fertility, tibia, plasma, total fecal P and water-soluble fecal P content, hatchability, and fecal P were analyzed in detail for significant differences. The reduction of dietary P could offer several advantages in poultry production such as optimal animal production and reduced P excretion. Decreasing the dietary P levels in breeder feed would have both immediate and long-term effects. The addition of phytase to livestock feed could increase the availability of P, thus making P more available for bone development and energy transfer. This is an important factor for egg production by breeder hens and for growth of market turkeys. Adding phytase could also result in fewer nutrients released into the environment while still meeting the nutritional requirements of the animal. This would require adjustments in P supplementation.

There are conflicting reports in the literature on this subject. A recent industry survey reports that the range for available P levels of turkey breeder hen diets is 0.45 to

0.55% (Kuhl, 1997; Kuhl, 1993). However, feeding turkey breeder hens closer to requirement for available P could aid in attempts to improve turkey breeder hen productivity and decrease the amount of land applied fecal P. The scope of the following review will be limited to the effects of dietary manipulation of P level in the diet and the addition of phytase on the reproductive performance and fecal P with a concentration on turkey breeder hens.

### **Poultry Nutrition**

Nutrition combines the knowledge of biochemistry and physiology into a working relationship between an organism and its food supply. Nutrition supplies the animal with the tools to function at an optimum level for processes such as growth, production, maintenance, and reproduction. Basic knowledge of the role nutrition plays in supplying animals and the interrelationship between various nutrients and other metabolites within the body is required before one can make practical scientific use of the knowledge of nutrition. Metabolism is a complexity of chemical reactions that continuously occur within animal cells. Therefore, it is necessary to feed nutrients in a form in which the animal is able to metabolize. Scott *et al.* (1969) described the avian as one of the most fastidious of all avian species. Through basic research scientists have achieved what is considered scientific nutrition of the poultry species. For the chicken alone it has been determined that at least 40 chemical compounds are essential nutrients, which must be present in the diet in adequate amounts to promote maximum growth, maintenance, and reproduction. Chicken has long since been economically important as a source of nutritious foods due to the amount of available amino acids, etc. the meat has to offer the consumer. The young chick is an excellent model on which to perform studies in order to

examine nutrition and its effects on the body of the bird. Much research has been generated on the nutritional needs of chicken compared to other species (Scott *et al.*, 1969). The amino acids, vitamins, and minerals must be present in the diet in a definite ratio to energy so that the animal will receive enough of all essential nutrients while satisfying its need for energy (Scott *et al.*, 1969).

The majority of information on the growth, reproduction, and dietary needs of poultry has been based on the chicken; therefore these data are often referred to in turkey research. Chickens vary greatly according to the purpose for which they have been developed; therefore the methods of feeding also differ. The nutrition of turkeys is divided into needs of birds used in meat production and needs of those used for reproduction, i.e. egg production. These two categories differ largely in the proportion of nutrients devoted to productive uses as opposed to those used for maintenance activity. Through the first 8-12 weeks of age (WOA) male and female turkeys grown for reproductive purposes are generally fed the same diet as birds intended for meat production (NRC, 1994). After 10-12 WOA efforts are made to avoid obesity. Historically, excess body weight of hens has been less of a problem than with males due to the loss of body weight during egg production. However, with increased selection in female lines for growth, turkey breeder hen obesity is becoming more of a concern.

The formulation of balanced diets is essential to economical poultry production. This process depends on knowing nutrient requirements of poultry and the nutritional characteristics of nutrient sources (NRC, 1994). Poultry diets are composed primarily of a mixture of several feedstuffs such as grains, soybean meal, animal by-products, fats, and vitamin and mineral premixes (NRC, 1994). Diets are formulated to provide the

energy and nutrients that are essential for the growth, maintenance, reproduction, and health (NRC, 1994). Minerals are the inorganic part of feeds and tissues. The body needs both macro (i.e. calcium, phosphorus) and micro (i.e. copper, zinc) amounts of minerals. Minerals are required for formation of the skeleton, as components of various compounds with certain functions within the body, cofactors of enzymes and for maintenance of osmotic balance within the body of the birds (NRC, 1994). Calcium (Ca) and phosphorus are two such minerals that are essential for the formation and maintenance of the skeletal system. In the growing bird Ca is used for bone formation versus its use for eggshell formation in a mature laying fowl. However, an excess of Ca can interfere with the availability of other minerals such as P (NRC, 1994).

Phosphorus plays a role in several functions such as bone formation, utilization of energy, and structural components of cells (NRC, 1994). Phosphorus can be introduced into the diet one of two methods organically (plant) and inorganically (ex. dicalcium phosphate; NRC, 1994). Plants contain two forms of P; available non-phytin P and unavailable phytin P (NRC, 1994). Animal by-products and P supplements are considered to be a more bioavailable source of P to the bird (NRC, 1994). Cromwell *et al.* (1970) defined bioavailability as the relative amount of mineral that is absorbed and utilized by an animal. Most of the minerals are partially supplied by the natural ingredients of the diet. However, the bioavailability of minerals such as Ca and P varies with the animal. Therefore, minerals must be provided to the animal as supplements. However, one must also take in to consideration that the bioavailability of mineral supplements also may vary. In order to accurately meet the requirement of the animal, information on the absolute bioavailability of the mineral in feedstuffs must be known.

## **Turkey Breeder Hen Requirements**

Formulating diets to precisely meet the P requirement of poultry should be a major economic and environmental concern. Any unused mineral becomes excreted into the environment for longer periods compared to other turkeys. There are a large number of interacting factors to consider, any one of which can influence the bird's requirement for P such as Ca, vitamin D<sub>3</sub>, age and type of birds, dietary ingredients and feed processing. Phytate P is poorly utilized by monogastrics such that plant based diets are routinely supplemented with inorganic phosphate to increase available phosphate in the diet. In areas of concentrated animal production, excess undigested phytate P in the manure poses an environmental concern. This leads to an accumulation of fecal excretion of unused plant phytate and supplemental P over 60 weeks in the case of a turkey breeder hen flock. Furthermore, the industry tends to feed turkey breeder hens large amounts of supplemental P for either what is believed to be better production or as a safety margin. The process of dietary phytate P digestion and absorption is poorly characterized and large discrepancies in the retention of phytate P are reported in the literature. Digestion and retention of dietary phytate P varies with the form of phytate in the diet and the mineral and vitamin D status of the animal. Therefore, the phosphorus requirement of laying hens has not been adequately defined. However, increasing concerns with regards to environmental P levels has caused the turkey industry to re-evaluate requirements of P in turkey diets. It has also made phytase a viable feed additive. The turkey industry may need to feed phytase as well as lower P levels in order to maintain productivity while reducing environmental P. These factors may account for the apparent discrepancies in the bioavailability of phytate P. One method in which to reduce the amount of P excreted

is to first identify the actual P requirements of the turkey breeder hen while maintaining egg and poult production. In 1950 and 1971, the National Research Council listed the non-phytate phosphorus (P) dietary requirement level for laying turkey hens at 0.75%. Over time the recommended requirement has been lowered to 0.35% (NRC, 1984; 1994). However, a recent industry survey reports that the range for available P levels of industry turkey breeder hen diets is 0.45% to 0.55% (Kuhl, 1997; 1993). Breeder recommendations range from 0.4% to 0.55% available P (B.U.T.A., Hybrid Turkeys, Nicholas Turkey Breeder Farms). This survey demonstrates that the turkey industry may be feeding higher than needed dietary P.

The metabolism of P derived from plant tissues is one of the least understood and most debated subjects in the field of mineral nutrition. Few studies have considered the needs during the reproductive period, during the time when feed consumption is the greatest. Variable results have been obtained in studies concerning the P requirement of turkey breeder hens. Atkinson *et al.* (1964) performed two experiments to study the effect of feeding various Ca and P levels to turkey breeder hens reared in cages and in floor pens. Atkinson *et al.* (1964) results suggested that the P requirement of turkey breeder hens was between 0.6 and 0.8% of the diet for rearing hens in both cages and floor pens. However, Wilcox *et al.* (1961) was unable to demonstrate a need for supplemental phosphate in practical breeder diet in studies conducted over a six-year period. Wilcox *et al.* (1961) performed these studies utilizing turkey breeder hens fed various levels of dietary P and reared on floor pens. Wilcox *et al.* (1957; 1961) reported that diets containing approximately 0.10% available P with no supplemental inorganic P versus a diet containing added P had no apparent detrimental effect on egg production

and fertility, and only a slight effect on hatchability. Thus suggesting that a very low requirement for P as well as the usual sources of unidentified factors that removal of a P supplement from a practical-type diet did not appear to have any influence reproductive parameters. Although, Waldroup *et al.* (1974) suggested inorganic P level of 0.30% was needed for maximum rate of egg production. Waldroup *et al.* (1974) observed the Ca and P requirements of 30-week-old turkey breeder hens kept in cages using 2 levels of Ca (2.25, 3.50%) and 4 levels of aP (0.10, 0.2, 0.3, and 0.4% aP). Results from this study suggested that the P content of the diet had no significant effect on the eggshell thickness or on the hatchability of fertile eggs. However, there was a significant effect of dietary P levels on fertility, thus indicating at least 0.20% aP is needed to maintain adequate fertility. Therefore, based on various findings, Wilcox *et al.* (1957; 1961), Atkinson *et al.* (1964), and Waldroup *et al.* (1974) placed the available P level needed by turkey breeder hens at approximately 0.1%, 0.6-0.8%, and 0.3% respectively. The lack of consensus among researchers as to the available P needs of the turkey breeder hen has warranted the need to further examine the dietary P requirement of turkey breeder hens.

Sewell *et al.* (1972) studied the reproductive performance of turkey breeder hens fed a basal diet (0.35% tP) and a diet supplemented with P in order to provide 1.01% tP. Sewell *et al.* (1972) obtained a significant increase in egg production and hatchability of fertile eggs when hens were fed a diet supplemented with inorganic P versus those fed diets with P from plant sources only. Other reproductive and physiological parameters did not show any differences, which could be related to the P levels of the diet. Sewell *et al.* (1972) found a significant increase in egg production when the diet contained 1.01% total P versus a basal diet with 0.35% P. Ferguson *et al.* (1974) also reported percent hen

day egg production increased significantly when inorganic P was added to the basal diet. Ferguson *et al.* (1974) fed caged turkey breeder hens basal diets supplemented with 0, 0.1, 0.2, 0.3, or 0.4% aP to provide diets with 0.35, 0.45, 0.55, 0.65, and 0.75% tP. Best production was with a diet containing 0.65 or 0.75% total P. Results indicated a higher requirement of P for egg production than the 0.4% (total), which Wilcox *et al.* (1957; 1961) found to be adequate. Ferguson *et al.* (1974) reported average egg weight to be greatest when the diet contained 0.55% total P. Ferguson *et al.* (1974) found hatchability of fertile eggs was significantly better in all groups receiving supplemental inorganic P versus basal diet with 0.35% P from plant sources. Agreeing with results by Wilcox *et al.* (1957; 1961) and Sewell *et al.* (1972). Thus, suggesting that the turkey breeder hen can utilize plant P sources but at least 0.65% total P would be required for optimum performance especially regarding egg production and hatchability of fertile eggs. Fertility did not appear to be related to dietary P.

In a later study, Slaugh *et al.* (1989) utilized 30-week-old turkey breeder hens kept in cages fed 4 treatment levels of aP in the diet (0.15, 0.30, 0.50, and 0.70% aP). Results agreed with those of Wilcox *et al.* (1957; 1961) that the egg production of turkey breeder hens was not impaired when the diet was not supplemented with inorganic P but conflicts with findings of Waldroup *et al.* (1974) and Ferguson *et al.* (1974). As observed by others, Slaugh *et al.* (1989) found that feeding a diet lacking supplemental inorganic P did not affect egg weight (Ferguson *et al.*, 1974) nor shell thickness (Waldroup *et al.*, 1974). Slaugh *et al.* (1989) reported all levels of P were equally as effective in maintaining body weight. However, both femur P and serum P were lower in birds fed 0.15% level. Slaugh *et al.* (1989) also reported hens fed low P diets (0.15 and 0.30%)

excreted and retained significantly less P than those fed the higher P diets (0.50 and 0.70%) and that fertility was significantly reduced when hens were fed diet with no supplemental P versus those fed diets with added P. Waldroup *et al.* (1974) made a similar observation. This disagrees with results by Wilcox *et al.* (1957; 1961) and Ferguson *et al.* (1974) who did not report a change in fertility. Slauch *et al.* (1989) did report no effect on hatchability of fertile eggs agreeing with Wilcox *et al.* (1957; 1961) and Waldroup *et al.*, (1974) but not with Ferguson *et al.* (1974).

The dietary requirements of P and its availability in plant origin feedstuffs are key issues in poultry nutrition. However, there are a large number of interacting factors that can influence the hen's requirement for P. Further research is needed to determine the P requirements of turkey breeder hens without impairing reproduction.

## **Phosphorus**

The inorganic part of plant and animal tissues consists of minerals. One such mineral is phosphorus, which like calcium, is essential for proper formation and maintenance of bones. Although the majority of the P needs are related to skeletal development (Axe, 1998), P is also needed for phosphorylation, high-energy phosphate bonds, and acid-base balance (Ewing, 1963), protein synthesis, and is part of the vitamin-mineral relationship (Jurgens, 1984). The need for a minimum level of inorganic P is indicated by the National Research Council (NRC) and based on the generally greater availability of inorganic P than of phytin P.

The dietary requirement of a nutrient for growth is the summation of the requirement for body maintenance and the total body tissue accretion. In the early stages of development when the tissues with high concentrations of P, such as bone and muscle,

are deposited rapidly, the need for P is higher (Axe, 1998). Conversely, during the later stages of development of mature poultry when skeletal structure is near completion and growth is directed toward fat deposition, the need for P decreases since the P content of fat is lower than muscle (Jongbloed, 1987) or skeleton. This is one reason turkey breeder hens requirements are less than that of growing poultry.

The requirement for dietary P is estimated using a variety of methods. Before determining the estimated dietary requirement for P, as for any nutrient, a response criteria must be identified, whether the criteria is body weight gain, feed efficiency, muscle growth, protein growth, P retention, plasma concentrations, and/or bone mineralization. The requirements for each of the various responses are different in some cases. For example, the dietary P intake required to maximize bone is greater than for that to maximize growth (Cromwell *et al.*, 1970; Kornegay *et al.*, 1981). Furthermore, the physiological status of the bird can affect the requirement for the different stages in life. For example, the relative amount of P deposited in bone decreases as the animal ages and the skeletal structure becomes fully developed (Mudd and Stranks, 1985).

Phosphorus is found in every cell within the body. Therefore, Ca and P deposition is essential for skeletal growth and a constant Ca to P ratio of slightly higher than 2:1 in bones is maintained throughout growth and development. Phosphorus is widely distributed throughout the body, which indicates its importance in several biological functions.

One of the most important functions of P is the mineralization of bone. Bone is a highly specialized form of connective tissue that serves as structural support for the body and as mobile storage for Ca and P. Bone development in the growing animal is

important in determining the Ca and P requirement for growth. During the preliminary stages of bone formation, osteoblasts are deposited to form an organic matrix. Once cartilage mineralization has been established, bone growth occurs via osteoblasts action (Loverbridge *et al.*, 1992). At this stage of bone development, dietary P and Ca intake is crucial. Adequate dietary P intake is a crucial contributor to bone integrity. Rats fed 33% of the P requirement decreased the rate of bone mineralization and protein matrix formation (Baylink *et al.*, 1971). Thus, adequate dietary intake is essential for body maintenance as well as maximum growth. Without adequate dietary intake, a P deficiency may develop.

Inadequate dietary intakes of P, Ca and/or vitamin D<sub>3</sub> can result in depressed growth, decreased efficiency of feed utilization, and impairment of bone mineralization (Cromwell *et al.*, 1970; Jongbloed, 1987). Rickets or osteomalacia will develop in young animals or adult animals, respectively, given insufficient dietary P intake or metabolism.

Scientists have recognized that there are portions of feedstuffs that are not easily digested and absorbed by poultry. The NRC (1994) lists the dietary non-phytate P requirement for turkey laying hens at 0.35%. Dietary P for growing birds is derived from mainly two sources, organic and inorganic sources. Organic P is incorporated in poultry diets by the selection of protein and energy sources such as grains and legumes. The phosphorus in plant feedstuffs can be separated into two groups; organically bound P present as salts of phytic acid (phytate P) and P present in other forms (nonphytate P) (Waldroup, 1999). Poultry diets largely consist of cereal grains and plant-derived products that contain high concentrations of phytic acid (Cromwell *et al.*, 1970), which in turn contains approximately 60-80% of the total P present in the diet (Simons *et al.*,

1990; Belanger, 2000). Organic P sources, such as corn and soybean meal, have a relatively low P bioavailability (approximately 14 to 50%) because the P is bound in the form of phytic acid (phytate). Endogenous phytase enzymes exist in plants that are capable of hydrolyzing phytate P. However, these enzymes are present in variable quantities depending on the plant source, and significant amounts of these enzymes are highly unlikely to survive the highly acidic conditions (pH 1.0-2.5) of the proventriculus and gizzard (Hill, 1971), where the solubility of phytate is also high (Von Sheuermann *et al.*, 1988). Endogenous phytase is also heat liable and is readily inactivated at 70-80 C, the temperatures commonly used in feed processing (Jongbloed and Kemme, 1990). This creates the need to supplement the diet with inorganic P.

However, inorganic P supplements have different degrees of availability; therefore, it is important that the minimum level of P be provided in a readily available form that meets the minimum requirement (Jurgens, 1984). Inorganic sources of P such as mono- and dicalcium phosphate have a relatively high P bioavailability (85 to 100%) and are typically used as a standard in the determination of P availability of other feedstuffs. Inorganic sources are often supplemented in the diet to meet the P requirement of the bird because grains and legumes, which constitute a majority of the diet, are low in dietary available P for poultry. This is true for cereals, legume seeds, and most plant sources containing phytic acid. Therefore, inorganic sources including mineral supplements and animal by-products are used in addition to that present in the plant to meet P requirements (Summers, 1997).

Phytic acid, commonly referred to as phytate, is a myo-inositol with 6 structural positions capable of containing phosphate groups (Sebastian *et al.*, 1998). Phytate is an

organic complex regarded as the primary storage form for both phosphate and inositol in plants (Cosgrove, 1966). Phytic acid containing the maximum 6 phosphates, myo-inositol hexakisphosphate (IP6), is a major source of P and myo-inositols available to the seed for development, comprising 60 to 80% of the P present in the plant. Phytate is a reactive anion that can form salts with dietary minerals (Oberleas, 1973; Erdman, 1979) and proteins (Cheryan, 1980; Cosgrove, 1980), thus reducing their solubility and digestibility when fed to animals. Most importantly, however, might be the low P availability from the phytate molecule and the subsequent increases in P excretion following increases in phytate in the diet of the animal. Historically, it has been documented that two-thirds of the total P in these materials is present in this form (Kornegay, 1998; Simons *et al.*, 1990). However, scientists now realize that the concentration of phytate P in feedstuffs depends largely on the source of plant from which it is derived (Sebastian *et al.*, 1998; Cromwell *et al.*, 1970). Ravindran *et al.* (1995) reported that oilseed and cereal by-products contain large amounts of phytate P compared to grain legumes. The concentration of phytate in plants may also depend on the stage of maturity, climate, method of processing, water availability, geographical location and the year during which they are grown (Reddy *et al.*, 1982).

There are many factors that influence the required non-phytate P level and utilization by the bird such as dietary calcium (Ca), inorganic P, vitamin D<sub>3</sub>, age and type of birds, dietary ingredients and feed processing (Summers, 1997; Sebastian *et al.*, 1998). Mohammed *et al.* (1991) demonstrated that both dietary Ca and P concentrations influenced the phytate P utilization by poultry. However, an important factor that determines the extent of phytate hydrolysis (Wise, 1983; Ballam *et al.*, 1984) is the use of

high concentrations of Ca, which may completely prevent the hydrolysis of phytate (Taylor, 1965). Bone formation is highly dependent on the dietary concentration of Ca and P (Hart *et al.*, 1922; Dunn, 1924). Under practical feeding conditions where the dietary Ca and P concentrations are formulated to result in maximum performance and bone calcification, phytate P is utilized very poorly by poultry (Sebastian *et al.*, 1998). This outcome has been confirmed in several studies where Ca was added at recommended rates (Nelson, 1976). The Ca to total P ratio also plays a role in phytate P utilization (Wise, 1983). The hydrogen bonds which occur between the phosphoric acid groups are broken in the presence of Ca, resulting in very stable Ca phytates which may precipitate over a wide range of pH (Graf, 1983). A high Ca or Ca:P (total) ratio of 2:1 impairs the digestion of phytate (Summers, 1997) because of the formation of an insoluble Ca phytate complex in the intestine (Nelson, 1967). As a result, an increase in phytate may lead to a deficiency in Ca attributed to the decrease in absorption of the Ca bound to the phytate molecule (Reddy *et al.*, 1982). The result of the action is that the phytate P as well as the Ca becomes largely unavailable for absorption as a result (Wise, 1983). Harms *et al.* (1961) demonstrated that widening the Ca:P ratio in the diets from 1:1 to 2:1 decreased the availability of the P from phytic acid, in laying hens, to a greater extent than that from inorganic supplements such as dicalcium phosphate. Thus, Ca plays an important role in phytate-P availability. Therefore, Ca levels should be factored into determining the P requirements of turkey breeder hens.

The hydrolysis and absorption of phytate P by monogastric animals is also complexed by vitamin D<sub>3</sub>. Poultry require vitamin D<sub>3</sub> to effectively use Ca (NRC, 1994). Bone formation is not only dependent upon the Ca and P concentrations; the adequate

intake of vitamin D<sub>3</sub> is also essential (McGowan and Emslie, 1934). After absorption, the vitamin is hydroxylated at the C-25 position in the liver and then transferred to the kidney where the 1, 25-dihydroxy metabolite is formed (Ameenuddin *et al.*, 1985). Vitamin D metabolites induce the synthesis of Ca-binding proteins in the intestine, kidney, and uterus (NRC, 1994). Calcium-binding proteins enhance Ca absorption from the intestine, recovery from the urine, and shell deposition (Coty, 1980, Jande *et al.*, 1981; Roth *et al.*, 1981; Clemens *et al.*, 1988).

Vitamin D<sub>3</sub> also mediates the intestinal absorption of P and activates bone mineralization by the osteoblasts (Schroder *et al.*, 1996). Therefore, dietary vitamin D<sub>3</sub> has the potential to increase P absorption and bone mineralization. Thus, high dietary vitamin D<sub>3</sub> intakes may increase P retention, especially in the early stages in growth when the rate of bone growth is high. However, any dietary condition that causes a decrease in muscle or bone growth would, theoretically, decrease P retention, because muscle and bone are high in P concentration. Phytate P utilization is depressed by feeding diets marginal or deficient in vitamin D<sub>3</sub> (Ewing, 1963). Several reports have indicated that the addition of vitamin D<sub>3</sub> enhanced the amount of phytate P retained by chickens (Edwards, 1993; Edwards *et al.*, 1989; Mohammed *et al.*, 1991). This improvement of phytate P utilization in response to vitamin D<sub>3</sub> supplementation may be due to increased synthesis or activity of intestinal phytase (Shafey *et al.*, 1991). This would increase phytate hydrolysis (Mohammed *et al.*, 1991) through stimulation of Ca absorption; therefore, rendering phytate more soluble and P more available for absorption (Wasserman and Taylor, 1973; Tanaka and De Luca, 1974).

Older birds hydrolyze phytate P to a greater extent than chicks (Peeler, 1972) because there is more phytase activity present in the gastrointestinal tract of older birds. This is consistent with a larger body size and small intestine mucosal surface area in the mature laying hen compared to the chick (Maenz and Classen, 1998). The recommended non-phytate P requirement for starting poults is approximately 0.6% (Almquist, 1954; Bailey *et al.*, 1986; Stevens *et al.*, 1986). This non-phytate P value has been shown to decrease with the age of the bird (Day and Dilworth, 1962; Sullivan, 1962). Edwards *et al.* (1989) reported that the ability of poultry to utilize phytate P increased with age. However, Nelson (1976) observed only a slight increase in P utilization by the older birds. Nelson (1976) did suggest that the failure to obtain a higher rate of hydrolysis could have been due to the variety of wheat tested on a more acid pH intestine, which could have reduced the phytase activity. Gillis *et al.* (1953) also reported that laying hens had considerably more ability to utilize the P of phytin than young chicks. Adult birds are not rapidly building skeletal or soft tissue, both which require large amounts of P. The requirements of a mature bird are mostly for replacement of P deposited in eggs and other normal catabolic losses. Adult birds are also able to draw upon their skeletal stores for a limited amount of P, thus probably delaying the effects of a dietary deficiency. Another theory to the lower P requirement as the bird ages may be due to increased consumption of feed. Studies have suggested that during the later stages of production, when a significant amount of feed is consumed, that there is little, if any, need for supplemental P in a typical corn-soybean meal broiler diet (Waldroup *et al.*, 1963, 1974; Skinner and Waldroup, 1992; Skinner *et al.*, 1992; Twining *et al.*, 1965; Tortuero and

Diez Tardon, 1983). There is limited evidence that there may be breed and strain differences in the utilization of phytate P within poultry (Sebastian *et al.*, 1998).

Hydrolysis of phytate could result from nonspecific phosphatase or specific phytase activities found in the intestinal brush border (IBB). Intestinal brush border vesicles prepared from mature laying hens had 35% higher total phytase activity in the small intestine than broiler chicks (Maenz and Classen, 1998). Thus indicating IBB phytase could contribute to phytate-P digestibility and may be subject to regulation in response to the dietary P and vitamin D<sub>3</sub> status of the chicken (Maenz and Classen, 1998). Maenz and Classen (1998) suggest that the larger surface area accompanied by a larger total activity of brush border phytase in the upper small intestine may improve the efficiency of endogenous phytate hydrolysis and is consistent with a more efficient utilization of phytate P in laying hens compared to chicks. The pH of the intestine has also been studied as a possible determining factor of P absorption efficiency. However, the results are often contradictory. Varying pH potentials may also cause a change in the intestinal absorption of P by changing P availability (Lee *et al.*, 1986). Maenz and Classen (1998) reported substantial phytate hydrolysis occurred over the range of 5 to 6.5 with maximum hydrolysis at pH of 6. However, the brush border membrane contains no acid phosphatase and no nonspecific phosphatase at pH 6. There is also the effect of intestinal microflora to consider. Some microbial organisms produce phytase; however, the majority of the microbes in poultry produce insufficient amounts of intrinsic phytase. Therefore, the net absorption of P in the large intestine, where possible microbial phytase is produced, is questionable and has not been studied conclusively. Nevertheless,

changes in intestinal microflora composition and concentrations have the potential to affect P availability.

While P is an essential and critical nutrient for animal production, both organic and inorganic phosphorus can serve as antinutritional factors for other nutrients and environmental pollutants when fed at rates considerably higher than required (BASF, 2000). Anti-nutritive components, like phytic acid, are common in many feed ingredients. However, these factors impair the animal's ability to digest and absorb nutrients (Stilborn, 1998). Special processing techniques must be used in order to maximize the nutritional value and minimize the antinutritional factor. The feeding value of plant-based ingredients such as cereal grains is influenced by these antinutritional factors (Stilborn, 1998). Inorganic phosphorus is also an antinutritional factor since it forms insoluble soaps with calcium and lipids (BASF, 2000). The formation of insoluble soaps reduces the digestibility of several nutrients including energy and amino acids (BASF, 2000). Increasing the level of dietary available P by the addition of inorganic phosphate consistently lowers apparent metabolizable energy (AME) and amino acid digestibility (BASF, 2000).

Nelson (1967) demonstrated that poultry poorly utilized the P in phytate. However, past studies have shown that poultry are capable of digesting and utilizing phytate P and that this capability ranges from zero (Nelson, 1976) to over 50% (Edwards, 1983; Mohammed *et al.*, 1991). This wide range concerning the ability of poultry to utilize phytate P appears to be caused by the complex nature of phytate hydrolysis (Sebastian *et al.*, 1998).

The dephosphorylation of phytic acid arises from the action of enzymes called phytase (Nayni and Markakis, 1986). Phytase is a naturally occurring enzyme, which is often present in vegetable sources. It has the specific role of a catalyst for breaking down the links between P and the phytate ring, therefore, liberating the phytin P (Oderkirk, 1998). The addition of phytase to the diet should decrease the amount of P needed from other sources as it works to make available the P in phytate (Oderkirk, 1998).

### **Phytase**

There are currently two methods to overcome poor nutritional availability of P in grains fed to swine and poultry. The most common method is to add inorganic phosphates and/or animal by-products to the feed. However, this approach does not address the phytate-P content of the diet. The consequences are two-fold a) supplementing P is an added expense to the producer and b) the undegraded phytate-P is excreted by the animal contributing to environmental risks. The other approach is to release the P from phytate with the addition of commercially produced phytate enzyme (phytase) to the feed. Twenty years ago, most feed manufacturers and nutritionists were uncertain about the value of feed-technology enzymes. Recent interest in dietary phytase has been stimulated by the realization that phytase provides a cost effective alternative to inorganic P supplementation and addresses the concern of pollution caused by excessive P in animal waste in areas of dense population and intensive livestock production (Campbell and Bedford, 1992). Previous research has shown that the use of phytase in poultry feed gives better utilization of phytate P (Almquist, 1954; Axe, 1998; Day and Dilworth, 1962). Some tests show P availability of total P to increase by 15-35% after the addition of phytase. This improvement in nutrient availability might allow

nutritionist to decrease concentrations of certain nutrients (i.e. phytate-P) in rations. This should allow the level of inorganic P supplementation to be reduced, therefore resulting in less P excreted in the manure.

Phytase supplementation is mainly incorporated into poultry diets to increase P bioavailability by liberating P from the phytate molecule. The amount of phytase activity is measured in phytase units (PU or FTU). One PU is the equivalent to 1 mmol of ortho-phosphates liberated from dietary phytic acid at 37 C/ 99 F and pH 5.5 within one minute. At least two different phytases are known to exist in nature: 3-phytase and 6-phytase referring to the site on the phytate molecule at which the enzyme begins to cleave the ortho-phosphates. The nonspecific phosphomonoesterase 3-phytase found in microorganisms such as *Aspergilli niger* (Ullah, 1988) and in mammalian small intestine (Cooper and Gowing, 1983), catalyzes the stepwise progression from the 3 position of the phosphate to the 6 position (Gibson and Ullah, 1990). The 6-phytase, found in higher plants such as wheat and barley, mimics the reaction of the 3-phytase with this exception: cleavage begins at C-6 of the phytate molecule and proceeds sequentially until hydrolysis is completed at C-1, provided conditions are optimal (Kemme, 1998).

The phytase enzyme breaks down the complex structure of phytate by its ability to hydrolyze phytate and increase P availability. This reaction begins in the crop and the proventriculus of the bird once moisture levels increase. Endogenous phytase is variable among both plant and animal species. The low intrinsic phytase activity located in the small intestine of the monogastric animal (Pointillart *et al.*, 1984; Pointillart, 1993) and the low phytase activity provided by common feedstuffs, such as corn and soybean meal, does not allow the animal to sufficiently utilize phytate bound P without the use of

exogenous phytase. Feed ingredients, versus supplemental P, usually provide approximately 50% of the total dietary P for most animal diets (Axe, 1998). Recent studies have examined the use of exogenous phytase. In one study aimed at determining the phytase activity of 51 feedstuffs (Eckhout and De Paepe, 1994), unprocessed grains such as rye, wheat, and barley were shown to possess high phytase activities consisting of 5130, 1193, and 582 FTU/kg, respectively. However, some widely used feedstuffs fed to poultry are extremely low in phytase activity such as corn, soybean meal, rapeseed meal, and lupins containing 15, 8, 16, and 0 FTU/kg respectively (Eckhout and De Paepe, 1994). Therefore, the total P of the diet is increased by the addition of supplemental inorganic P added to meet the bird's requirement. Early experiments showed the potential of increasing apparent total P digestibility by approximately 20% with phytase, thus decreasing required P intake and in turn decreasing P excretion as much as 30 to 40% (Nasi, 1990; Simons *et al.*, 1990). Phytase not only liberates P from the phytate molecule but also increase the utilization of other elements that might form a complex with phytate. One mole of phytic acid can bind 3 to 6 moles of calcium, to form insoluble phytates at the pH found in the poultry intestine. Furthermore, zinc, copper, cobalt, manganese, iron, magnesium, and protein can also form a complex with phytic acid, but zinc and copper have the strongest binding activity (Maddaiah *et al.*, 1964; Vohra *et al.*, 1979).

Due to the limited amount of research available on turkey breeder hens, it is useful to examine the effects of phytase supplementation in other monogastrics. Phytase enzyme added to feed increased P availability from 15% up to 45% in swine (Cromwell *et al.*, 1993; 1995). Coon and Leske (1998) reported that for broilers, retention of phytate

P varied from 29% for wheat midds to 35% for soybean meal. With phytase in the feed, the retention of phytate P varied from 72% for soybean meal to 47% for wheat (Coon and Leske, 1998). Microbial phytase was very effective for improving P availability in corn-soybean meal based diets (Qian *et al.*, 1996), and soybean meal-based semi-purified diets (Ravindran *et al.*, 1995) when fed to turkey poults from day of hatch to 21 days of age.

Young birds have very limited ability to hydrolyze phytate P; however, this ability increases with age up to maturity (Nelson, 1967). First cycle Single Comb White Leghorn (SCWL) laying hens responded well when low dietary P was supplemented with phytase (Gordon and Roland, 1997). The hens showed no changes in feed consumption or egg production until the 11th week of the lay cycle depending on which diet they were fed. At that time, feed consumption and egg production of the hens consuming the diet with 0.10% nonphytate P, without phytase, began to decline precipitously compared to the performance of the hens fed the other diets. Supplementation of phytase to the 0.10% nonphytate P diet completely corrected these effects (Gordon and Roland, 1997). In a similar study reported by Van Der Klis *et al.* (1997), SCWL laying hens which were fed a standard diet with 3.3 g/kg of total P with graded levels of phytase performed as well as hens fed the same standard diets with 3.6, 3.9, 4.2 g/kg total P (P was supplemented as monocalcium).

Phytase addition not only improves P availability and retention, it has also provided environmental benefits from the initiation of using supplemental phytase in poultry diets over the last few years. In a recent study, Bosch *et al.* (1998) estimated that the use of microbial phytase in feed reduced the land area required for waste lagoon liquid disposal from 98 acres to 71 acres on a low land-hog ratio and from 191 to 138

acres on a high land-hog ratio farm. Phytase enzyme decreased P excretion by broilers by as much as 24% (Kornegay *et al.*, 1996). However, there is concern that the addition of phytase to the diet may increase the amount of soluble fecal P that could potentially be released into the environment.

Phosphorus loss in runoff from agricultural land continues to pollute water resources and contributes to accelerated eutrophication. Although particulate P may constitute 75 to 95% of the P transported from conventionally tilled land and provide a variable but long-term source of P to aquatic biota, dissolved P accounts for most of the P loss in runoff from fields where soil erosion is minimal such as no-till fields or grassland (Sharpley *et al.*, 1994; Nash and Murdoch, 1997). Dissolved P, comprised mostly of orthophosphate, is immediately available for uptake by algae and aquatic plants and is therefore of concern for the receiving water bodies.

The use of phytase has allowed monogastric animals to maximize the use of the nutrients in their feed. Furthermore, the increase in P bioavailability from natural feedstuffs decreases the need for inorganic P and thus decreases the total P content of the diet. Therefore, more P is digestible and subsequently less P is excreted via fecal matter. Another benefit is the impact seen on the environment and the economics of production. Although phytase has the potential to reduce the amount of P excreted in the manure, some concerns do exist with this approach. These concerns include: application equipment cost, potential instability of the phytase enzyme, potential inconsistent distribution of the phytase enzyme throughout the ration, and the cost efficiency of supplementing the diet with phytase.

## Environmental

Phosphorus contamination of the aquatic environment from agriculture arises from point and non-point sources. Point sources such as sewage treatment outfalls, wastewater effluent, waste disposal sites, runoff and infiltration from animal feed lots (Issues in Ecology, 1998) were historically the most important sources. However, as treatment technologies improved and removed P more efficiently, and as land uses became more intense, nonpoint sources became more important (USEPA, 1990). Nonpoint sources are characterized by diffuse runoff from areas such as agriculture, pasture, range, urban and septic runoff, construction sites, and any activities on land that generate contaminants (Issues in Ecology, 1998). One of the major sources of P runoff from agricultural lands is animal waste (Moore and Miller, 1994). Several studies have addressed nutrient transport in runoff immediately following land application of poultry litter (Sauer *et al.*, 1999).

Runoff of P from fields receiving poultry has been speculated to be one of the primary factors affecting water quality in the US (Martin, 1997). Several investigators have characterized P runoff from land receiving poultry manure (Edwards and Daniel, 1993, McLeod and Hegg, 1984, Westerman *et al.*, 1983) also showing that P runoff increases as the manure or litter application rate increases and as rainfall intensity increases. Poultry litter contains approximately 20 g P kg<sup>-1</sup>, of which about 2 g P kg<sup>-1</sup> is water soluble (Moore and Miller, 1994). Recent studies have found that most of high concentrations of land applied P in runoff is dissolved inorganic P (Edwards and Daniel, 1993). Sonzogni *et al.* (1982) suggested that dissolved inorganic P is directly available to algae and concluded that the best management practices used to decrease P runoff should

consider the bioavailable P load, rather than the total-P load. Agricultural P losses in erosion and runoff are the most common area of concern; however, P leaching to ground waters or transport to surface waters via agricultural drainage systems is a serious environmental problem for areas of intense animal production (Mozaffari and Sims, 1996). The low intensity animal grazing lands release relatively low fluxes of nutrients, but higher more intense pastures are often among the most serious nutrient sources (Correll, 1999). In these operations, both exogenous and organics nutrients are applied at high rates, in addition to direct inputs of animal waste (Correll, 1999). Thus these lands become potential sources of nonpoint pollution.

Livestock manure has been spread on the land for centuries, at first, perhaps as a method to dispose of waste but later as fertilizer for crop production. Since World War II two things have changed the attitudes toward manure. First, livestock and poultry production has become concentrated in large scale, confinement-type operations. Such increases in the size of large-scale production facilities have greatly magnified the problems of handling wastes. Second, the convenience of commercial fertilizers has decreased the need to spread manure, thus causing manure to become a surplus by-product (Withers and Sharpley, 1995). The concentration of large numbers of animals into smaller areas through the use of confinement rearing has led to the perception there is increased environmental impact. This increased interest in the environmental management of poultry and other livestock manures has emphasized the need to evaluate manure management systems.

There are several strategies in which it is possible to reduce nutrient excretion through nutrient management and best management practices (BMP). Nutrient strategies

include more accurate nutrient requirement information for animals and feedstuffs, reduced feeding of excess nutrients through over formulation, feeding for optimal performance rather than maximum, and improving the availability of P with addition of feed enzyme phytase (Kornegay, 1998). Land application of poultry litter and manure is a BMP readily accepted by most growers and the general public if performed properly (Chapman and Synder, 1992). Best management practices may be used to achieve the greatest agronomic benefit and avoid potential water quality problems (Stilborn, 1998). A few of these strategies include the determination of appropriate P application rates based on environmental soil tests, balancing P inputs with P outputs in areas with confined animal operations, and basing manure application on soil P and crop removal of P, mitigating the excessive buildup of soil P (Eck and Stewart, 1995; Stilborn, 1998).

Determination of the biological availability of the various sources of P in the diet is one of the primary problems nutritionist face who want to reduce the P in the excreta. There are also many factors that contribute to the variability in nutrient requirements of animals such as species, age, and dietary ingredients. Therefore, in some cases nutritionists have utilized rather high margins of safety in order to avoid production problems due to the wide variation in the ability of the bird to utilize different sources of P (Waldroup, 1999). Diets containing P in excess of minimum dietary requirements are not proven to enhance animal performance but serve only to increase the amount of P excreted (Brodison *et al.*, 1989; Morse *et al.*, 1992).

The available P level, the uptake, and the utilization of P by animals from feed materials can influence the fate and transport of P in soils (Withers and Sharpley, 1995). The low P availability from feedstuffs can decrease utilization; therefore creating a need

to supplement with organic P. This results in excreted P from feedstuffs plus any unutilized supplemental P, which would increase the P load applied to the land. Due to the growing concerns regarding the potential contribution of P in poultry excreta on eutrophication of surface water, increasing pressure is being placed to limit the amount of excess P in diets and thus reduce fecal P output (Waldroup, 1999). Iserman (1990) demonstrated the poor retention of P by monogastric animals with P excretion rates of 70-80% for feeder pigs and 87% for poultry. Therefore, the use of phytase has been studied to provide better P retention without increasing dietary nutrients and resulting in lower P excretion. Lee (1992) reports that for non-ruminants, the level of phytase in the diet can have a considerable impact on the utilization and excretion of dietary P intake. The balancing of animal requirements for P and the amount of supplemental P added to rations (van Horn, 1991) has been shown to reduce excreted P and minimize many adverse environmental impacts such as eutrophication.

Eutrophication is the over enrichment of surface waters with mineral nutrients. Continual use of manure to supply the N needs of crops results in increased P content of the soil. Stewart (1970) reported that a significant amount of N is lost by volatilization in addition plants need less P than N. Therefore when manure is applied at rates sufficient to supply adequate N, excess amounts of P are added. Van Reimsdijk *et al.*, (1987) warned that land spreading of animal manure in quantities exceeding plant uptake of P would result in an accumulation of P. Phosphorus is not recycled in the atmosphere in any quantity; therefore a small but continuous loss of P by natural erosion and leaching, from the terrestrial environment to the oceans (Walker and Syers, 1976; Stevenson, 1986). The continual long-term application of P in manure at levels exceeding crop

requirements can raise soil test P levels above those required for optimum crop yields. Once P accumulation has exceeded the buffering capacity of the soil, the potential for P to leach into ground and surface water causing eutrophication increases (Van Reimsdijk *et al.*, 1987).

The relationship of total P concentration of lake water to eutrophication has been well documented (Vollenweider, 1975; Effler *et al.*, 1985). Over time several bodies of water have progressed from oligotrophic conditions (low mineral and high dissolved oxygen) to mesotrophic conditions and finally eutrophication conditions (high mineral and low dissolved oxygen). The adverse effect of P in aquatic system is that it, in combination with other plant nutrients, allows algae growth in freshwater environments. The result is the excessive production of algae and cyanobacteria and their high respiration rates leads to low dissolved oxygen, which leads to loss of aquatic life (Correll, 1999). High concentrations of P promote growth of algae and aquatic weeds and accelerate eutrophication of lakes and reservoirs. Algae proliferates in response to elevated nutrient levels, sunlight cannot penetrate surface waters; therefore the growth of submerged vegetation is inhibited. As algae die and decay, water is depleted of dissolved oxygen and fish kills may occur. Advanced eutrophication of surface water leads to problems with its use for fisheries, recreation, industry, or drinking, due to increased growth of undesirable algae and aquatic weeds and oxygen shortages caused by their senescence and decomposition (Withers and Sharpley, 1995). Phosphorus is the limiting nutrient for freshwater algae and aquatic plants. Therefore, phosphorus is the limiting nutrient for the eutrophication process (Schindler, 1977). Agriculture can make a significant contribution to decreasing eutrophication problems in inland and coastal waters

(Withers and Sharpley, 1995). There is clearly a need to minimize the adverse effects of P buildup in agricultural soils and the transport of soil and applied P to aquatic systems. Maybeck (1982) estimated that 20 million tons of manure was transported annually to the oceans by world rivers with consequent risks of eutrophication. In 1998, the USDA estimated that the USA poultry industry produced 300 million turkeys and 7.76 billion broiler chickens (National Statistics Service, 1998). This production generated in excess of 10 million tons of manure annually. The risk of P accumulation is greatest where there is a limited land area for recycling of livestock manures. This is one reason why restrictions on P inputs to land are in operation in the Netherlands. Western European countries have been the most progressive in investigating environmental concerns and declaring governing policies about P in animal waste and the subsequent accumulation in soils. In the Netherlands, there is a limited amount of land available for waste disposal. Therefore, in 1984, legislation set forth limitations in ground and surface water at 0.10 mg of ortho-P per liter and inhibited all poultry expansion. Specifically, the amount of animal manure application on land was limited by the concentration of P in the manure, the type of soil, and the amount of non-manure fertilizer applied to the land (Jongbloed *et al.*, 1999).

The phosphorus content and composition of fresh livestock manures is variable. This variability depends upon the type and age of animal, type of bedding material used (Azevedo and Stout, 1974), and composition and digestibility of the diet especially when mineral supplements are used (Withers and Sharpley, 1995). In stored manures, inorganic P represents 80-90% of total P content (Withers and Sharpley, 1995). Upon storage of litter, the inorganic P component in feces is further increased by microbial

mineralization of organic P fraction (Gerritse and Vriesema, 1974; Gerritse and Zugec, 1977). The accumulation of P in soil from unbalanced use of organic and inorganic P fertilizers raised concern over agriculture contribution to eutrophication of inland waters and marine environments (Vighi and Chiaudani, 1987; Uunk, 1991).

Historically, manure application rates have been mainly based on meeting nitrogen requirements of plants (Wallingford *et al.*, 1975) with little attention being paid to P accumulations (Shreve *et al.*, 1995). Although P does not have a direct negative impact on land to which it is applied, it can adversely impact surface waters if it is moved off-site by erosion or run-off (Sharpley and Menzel, 1987). Due to the growing environmental concern associated with P in surface water supplies, there are pressures mounting for limiting or even banning P additions to soils that exceed a certain level of plant-available P based on soil tests. The potential buildup from the use of manure poses a challenge for managing animal wastes. Recent research has lead to new proposed methods to dispose of waste such as burning litter for energy or pelleting litter as a fertilizer that can be used as a residential landscaping product. However, the market for these products still needs to be developed.

Environmentally safe management of nutrient excretion from poultry is essential for the sustainability of the industry. In order to successfully reduce P excretion by the bird while maintaining productivity, it is logical that one considers the contribution of the various sources of dietary P that occur in the feces and base their approach on minimizing these where feasible (Waldroup, 1999). The presence of dietary phytate in commonly used cereal grains and legumes decreases the digestibility of diet P resulting in high levels of P excretion from fecal matter. Approximately 50% of the P from a typical diet

is excreted in the feces. However, variation of fecal excretion is high as a result of varying digestibility of selected feedstuffs and antinutritional factors. Thus, fecal P is the primary contributors of P excretion from poultry. Therefore, nutritional interest is being focused on improving the efficiency of P digestion and thus recovery by poultry and other livestock. If this can be accomplished, less P would pass through the animal and P concentrations in manure and litter would be lower (Zublena, 1994).

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### **ABSTRACT**

An experiment was conducted to test the hypothesis that current supplementary levels of dietary P in turkey breeder diets are necessary and sufficient for reproductive performance. The objective was to determine the effect of levels lower than those recommended by the National Research Council (NRC) on turkey breeder hen productivity from 31 to 62 weeks of age (WOA). The effects of dietary phytase were studies also. Turkey breeder hens were placed in a curtain-sided house with 48 pens (10 birds per pen; 8 pens per treatment) at 31 WOA. Hens were fed a breeder ration with treatments as follows: high P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E). Feed and water were available *ad libitum* for a 28-week reproductive period. Diets were fed in mash form. All hens were photostimulated in January (31 WOA) with 15.5 h of light/day. Production data were recorded on a pen basis. Individual bird body weight and feed consumption, by pen, were determined at monthly intervals from 31 to 62 WOA. Hens were observed for biweekly reproductive performance for 28 weeks of lay (WOL). The following parameters were measured: hen housed (HH) egg production, hen day (HD) egg production, fertility, settable eggs, cumulative settable eggs, hatchability of all eggs, hatch of fertile, initial egg weight, % egg weight loss, conductance, conductance constant (K) and embryonic mortality hens out of lay, and hen mortality. At the end of the study tibia P, plasma P, total fecal P, and water-soluble fecal P were measured for P

concentration. Egg weight, shell thickness, albumen P and yolk P were measured monthly.

The data indicated that decreasing dietary P resulted in no major reproductive problems for turkey breeder hens over 28 WOL. Additionally, decreasing dietary P decreased total fecal P and water-soluble fecal P. Feeding phytase caused significantly fewer hens to go out of lay. There were no consistent differences for any of the other parameters observed due to dietary P, enzyme, or interactions. These results suggest that dietary P can be lowered in efforts to reduce environmental concerns over P without impairing reproduction. Further study is needed to fully develop the optimum P intake needed by turkey breeder hens to maintain reproductive performance and decrease excess fecal P.

## **INTRODUCTION**

Dietary requirements for P and its availability in plant origin feedstuffs are key issues in poultry nutrition. Less than one-third of the P in feedstuffs of plant origin is biologically available to monogastric animals (NRC, 1988, 1994; Cromwell *et al.*, 1993, 1995). This low availability is attributed to much of the plant's P being present as phytate. Phytate is present in the form of phytic acid as a complex of cations such as calcium, magnesium, zinc, potassium, and proteins (Jalal and Scheideler, 2001). Monogastric animals such as poultry and swine lack sufficient amounts of endogenous phosphophytase in the gastrointestinal tract to hydrolyze the phytate molecule and thus release plant P (Sebastian *et al.*, 1998). This results in the need to add inorganic sources of P to satisfy dietary requirements. However, addition of excess inorganic P and undigested phytate P results in excess P excretion in manure.

Increasing concerns about excess environmental P levels have caused the turkey industry to re-evaluate requirements of P in turkey breeder diets. It has also made phytase a viable feed additive. Turkey breeder hens are heavier birds compared to commercial layers and therefore consume more feed to maintain energy and nutrient requirements. Turkey breeder hens are also maintained for a relatively long period compared to other commercial turkeys. In combination, the longer rearing periods and higher feed consumption of turkey breeder hens versus other areas of the poultry industry may lead to an accumulation of fecal excretion of unused plant phytate and supplemental P over 60 weeks. Furthermore, the industry tends to feed turkey breeder hens large amounts of supplemental P for either what is believed to be better production or as a safety margin. Thus, targeting P levels of turkey breeder feeds may be a way to reduce environmental P. The turkey industry may need to feed phytase as well as lower P levels in order to maintain productivity while reducing environmental P.

One method to reduce the amount of P excreted is to first identify the actual P requirements of the turkey breeder hen while maintaining egg and poult production. The National Research Council (NRC; 1950, 1971) listed the non-phytate phosphorus (P) dietary requirement level for laying turkey hens at 0.75%. Over time the recommended requirement has been lowered to 0.35% (NRC, 1994). However, a recent industry survey reports that the range for available P levels of industry turkey breeder hen diets is 0.45% to 0.55% (Kuhl, 1997; 1993). Breeder recommendations range from 0.4% to 0.55% available P (B.U.T.A., Hybrid Turkeys, Nicholas Turkey Breeder Farms). The objective of this study was to provide additional information on the ability of the turkey hen to use supplemental P and the effect of adding phytase on turkey breeder hen performance.

## MATERIALS AND METHODS

Four hundred and eighty Hybrid EURO FP turkey breeder hen poults were brooded in 12 pens until 3 weeks of age (WOA). At 3 WOA, the hens were randomly distributed to 48 pens (10 poults per pen) in a curtain sided house. Hens were fed grower diets as reported by Crouch *et al.*, (2002). At 31 WOA, the hens were fed a typical corn-soybean meal based diet meeting all NRC (1994) nutrient recommendations except for P. The dietary treatments included three levels of P and two levels of phytase enzyme (0 and manufacture's level of 1 kg/ton of feed) (Table 1). The feed treatments were as follows: high P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E). The phytase enzyme product used (Allzyme™ Phytase, Alltech, Inc., Nicholasville, KY 40356) is derived from *Aspergillus niger* and contained 11.27 PTU/g. The enzyme contained trace activities of cellulase, protease, xylanase, and acid phosphatase according to the manufacturer. The feed was fed as mash to prevent possible degradation of the enzyme during pelleting. Diets were analyzed by the North Carolina Department of Agriculture to determine crude protein, calcium, and total P of the diets on an as fed basis. Hens received feed and water *ad libitum* and were photostimulated in January (31 WOA) with 15.5 h of light/24 h day. Hens were inseminated biweekly with pooled semen from same strain breeder males. Hens that could not be everted for weekly insemination were removed from the pen and the study and defined as out of lay. Significant, effects among treatments were not observed at 20 WOL; therefore, all supplemental P was removed from LP and LP+E to create basal diets where all available P was supplied only by the corn and soybean meal.

The facilities used in this experiment consisted of a curtain-sided house containing forty-eight pens each with 9 m<sup>2</sup> of floor space. Pen floors were covered with clean, soft pine shavings. Caked litter was removed as necessary throughout the trial. The curtains were lowered (opened) during the breeder period to promote natural ventilation.

Feed consumption by pen and individual BW were recorded at monthly intervals beginning at 31 WOA until 62 WOA. Hen housed (HH) and hen day (HD) egg production, settable eggs and cumulative settable eggs were measured biweekly for each pen. Hatchability was measured and recorded on a pen basis. This provided 14 hatches over the 28 weeks of lay. However, due to mechanical error, weeks 7-8 and 11-12 (hatches 4 and 6, respectively) were removed from both hatchability and hatch of fertile data sets.

Eggs were collected five times a day and classified as settable, double yolk, misshapened (crowded), cracked, or soft shell eggs. Settable eggs were sanitized and stored in an egg cooler at approximately 13°C and 70% relative humidity. All settable eggs from each WOL were set in trays and incubated by pen in a common incubator for each set date on a bi-weekly basis. Eggs were incubated for 24 days at 37.5°C and 53% relative humidity and then transferred into a hatcher operating at 37.2°C and 65% relative humidity. All eggs that failed to hatch after 28 days of incubation were broken open and examined macroscopically to determine true fertility and age at embryonic death. All unhatched eggs were categorized into one of the following groups; infertile, Week 1 dead, Week 2 dead, Week 3 dead, Week 4 dead, internal pip, external pip, crack, and rotten. There was also a category for cull poults. Percentages for each category were calculated

for each setting of eggs. Three eggs from each pen, preferably from the last day of production, were weighed to the nearest gram initially and at transfer (25d) to the hatcher; subsequent poults were also weighed. Three eggs were used to measure initial egg weight, weight loss (%), conductance (G), and conductance constant (K). Conductance and conductance constant were measured as reported by Christensen and Nestor (1994). Cumulative poults per hen were calculated by pen for each treatment.

At 3-4, 19-20, and 27-28 WOL, three eggs from each pen were numbered, weighed eggs were then and broken out to determine % shell thickness, albumen weight and yolk weight. The yolk and albumen were separately bagged and frozen. A P microtiter plate assay (Appendix I) was used to determine P content on both the yolk and albumen samples. Phosphorus concentration was measured in the albumen and yolk and presented on a relative basis at three intervals throughout the study (Table 2).

The shells from each egg were then washed to remove any residue and then dried. A dry weight of the shells was then recorded. The emptied shells were then used to measure shell thickness at the top, middle, and bottom of the egg using a micrometer.

On the final day of the study two birds per treatment were sacrificed to collect plasma and right tibias. The P microtiter assay was used to determine the P concentration of each plasma, tibia, and fecal (total and water-soluble P) samples. Blood was collected via the brachial vein of the wing. Samples were centrifuged for 10 minutes at 2000 rpm. The resulting plasma was used to determine P concentration. Tibias were stripped of all soft tissue and fat. The weight and length were recorded for each bone. The bone shafts were cut into uniform pieces using a band saw. Tibia samples were oven dried for 24 h at 100°C and then ashed in a muffle furnace at 600°C for 24 h. One gram samples were

then used to determine P concentration and were expressed as percent of dry weight. Fecal samples were collected over a five-day period from each treatment to measure total fecal P and water-soluble P. Fecal samples were dried at 100°C for 12 h, weighed, ground, and then used to determine P concentration. Water-soluble P samples were prepared as reported by Self-Davis and Moore (2000) and P content was measured using the P assay.

The design of this study was a randomized complete block design with a 2X3 factorial arrangement of treatments (2 levels of phytase and 3 levels of P). The experimental period was 28 WOL. The house is designed in quad formation with each quad representing a block of 12 pens. Dietary treatments were randomly assigned to 2 pens in each block. The pen served as the experimental unit. Each parameter was regressed on enzyme, dietary P, and enzyme X dietary P. Percentage data were divided by 100 and subjected to arc sin transformation of the square root before analysis; however, actual percentage means are presented. Differences among treatment means were partitioned using the least square means procedure of SAS (SAS Institute, Inc., 1992). Statements of significance are based on  $P \leq 0.05$  unless otherwise stated.

## **RESULTS**

Birds consumed approximately 265 g/bird/day of feed at the beginning of the study (Figure 1). Daily feed consumption continued to increase up to an average of 308g/bird/day at weeks 21-22. There was an enzyme by P interaction for feed consumption at 8 WOL. Hens fed MP+E and LP consumed significantly more feed than the other treatments at 8 WOL. There were no other differences in feed consumption

during the experiment. Body weight was not affected by treatment regardless of dietary P or enzyme level (Figure 2).

Average HH egg production rose up to a peak of 64-70% at 2 WOL (Figure 3). Subsequently, there was a steady decline in HH egg production for hens of all treatments to the end of the egg production period. Hen day egg production is presented in Figure 4. By weeks 2 and 3, all treatments had reached a maximum of 67-72% with a steady decline thereafter. The cumulative total eggs set over 28 WOL did not differ among dietary treatments. The total settable eggs per hen per week were also not affected by treatment (Figure 5).

Fertility was 90% or higher for weeks 1 thru 18 and then began to decline (Figure 6) with no consistent differences among dietary treatments. Fertility was significantly increased by enzyme only at 13-14 WOL. At the beginning of the study hatchability treatment means were highly variable (Figure 7). However, by the second sampling variability was decreased. There were no consistent differences due to treatments.

Hatchability of fertile eggs (HOF) responded in a similar way as hatchability of all eggs (Figure 8). However, the means of HOF for all treatments began to become more uniform during the last 10 weeks of the study with no one diet giving superior performance to the others. Embryonic mortality resulted in few significant differences that were inconsistent by treatment over the 28 WOL (Table 3).

Initial egg weight, recorded before eggs were incubated, increased from 80-84 g up to 92-96 g (Figure 9). There was a significant ( $p \leq 0.006$ ) difference at 7-8 WOL due to treatment. Hens fed LP and HP+E produced heavier eggs than hens fed the other four diets. At 11-12 WOL there was a significant enzyme effect ( $p \leq 0.02$ ) indicating that birds

fed diets without the enzyme produced heavier eggs than those with enzyme. At 27-28 WOL initial egg weight was significantly affected by an enzyme by P interaction. Hens fed HP, MP+E, and LP+E diets had heavier eggs than hens fed the other three diets.

Egg weight loss was not consistently different over 28 WOL (Figure 10). At 3-4 WOL, hens fed the LP diet had significantly ( $p \leq 0.001$ ) higher egg weight loss. At 23-24 and 27-28 WOL, hens fed HP+E, MP, and LP+E had significantly ( $p \leq 0.05$ ) higher egg weight loss. However, there was not a consistent trend for egg weight loss over 28 WOL due to treatment.

There were few differences for the conductance and the conductance constant ( $K^2$ ) of the eggshell due to treatment. At 3-4 WOL, hens fed 0.17% aP diets had a higher conductance than those fed 0.3 and 0.5% aP (Figure 11). Hens fed LP diets at 3-4 WOL produced eggs with significantly ( $p \leq 0.01$ ) higher conductance  $K^2$  (Figure 12). Conductance  $K^2$  was then consistent until 23-24 WOL hens fed HP+E, MP+E, MP, and LP+E produced eggs that had significantly ( $p \leq 0.05$ ) higher conductance  $K^2$ . At 27-28 WOL hens fed HP+E, MP and LP+E diets had significantly higher conductance  $K^2$ .

The weight of the eggs (g) sampled for P content increased from 80 g at the beginning of the lay period to 93 g after 14 WOL (Figure 13). Egg weight was steady at 92-96 g for the remainder of the study. At 27-28 WOL hens fed MP+E and HP produced significantly heavier eggs than those fed other treatments. Shell thickness as expected made a steady decline as the weight of the egg increased (Figure 14). Hens fed LP+E, HP+E, MP and MP produced eggs with significantly thicker shell during 2 WOL.

Albumen P concentrations decreased linearly over the 28 WOL (Figure 15). Eggs produced during 3-4 WOL had significantly higher P concentration than those produced

during 19-20 or 27-28 WOL. Eggs laid during 19-20 WOL also had significantly higher P levels than those laid during 27-28 WOL. Hens that were fed diets with enzyme produced eggs with significantly higher P in the albumen during 27-28 WOL. Calculated total P albumen levels did not decrease over the 28 WOL (Table 2).

Yolk P concentrations decreased linearly over the 28 WOL (Figure 16). Hens produced eggs laid during 3-4 WOL had significantly higher P concentrations in yolk than those produced during 19-20 or 27-28 WOL. Eggs laid during 19-20 WOL also had significantly higher P than those laid during 27-28 WOL. At 27-28 WOL, hens fed MP had significantly higher yolk P concentration than those fed LP or HP treatments. Calculated total P yolk levels increased over the 28 WOL (Table 2).

At the end of the study plasma P (Figure 17) and tibia P (Figure 18) were not significantly affected by dietary P, enzyme levels, or an interaction of the two. However, fecal P (Figure 19) and water-soluble P (Figure 20) decreased as dietary P was reduced in the diet. Hens fed diets without enzyme also produced a significantly lower amount of P in the feces.

Hens that could no longer be everted were considered out of lay and were removed from the study (Figure 21). Hens fed dietary phytase enzyme were significantly less likely to go out of production than hens fed diets without enzyme. Mortality was less than 1% during the 28 WOL, and was not significantly different among treatments.

## **DISCUSSION**

Limited information is available on P requirements for turkey breeder hens. Wilcox *et al.* (1957, 1961) found that the removal of supplemental P from a practical-type diet did not appear to have any influence on egg production. Pepper *et al.* (1958)

reported similar results for laying hens. In the present study, there were no differences in HH and HD egg production among the dietary treatments. Turkey breeder hens fed low levels of supplemental P (0.17% aP) performed as well as those fed NRC (0.35% aP) or industry level (0.55% aP) diets with or without enzyme even with the withdrawal of all supplemental P at 20 WOL. This study also agrees with results reported by Slaugh *et al.* (1989) who reported that the reproductive parameters such as hen body weight and egg production were not affected by decreasing the available P from 0.7, 0.5, and 0.3 to 0.15%. Feed consumption was relatively consistent throughout the lay cycle.

The maintenance of egg production despite low P intakes may represent a depletion of body stores of P to meet physiological needs. Alternately, the naturally occurring forms of P may be more available to the turkey hen than previously thought. Hens consistently produced fertile eggs at industry standards regardless of treatment. No differences of treatments were observed for embryonic mortality over 28 WOL, which suggests that level of dietary P or enzyme did not influence embryonic death. This is more evidence that feeding lower P levels does not impair reproductive performance. Even at 20 WOL when supplemental P was removed from the LP and LP+E diets, fertility was comparable to eggs from hens fed diets that contained higher levels of supplemental P with and without enzyme. Wilcox *et al.* (1957; 1961) reported that diets with approximately 0.1% aP and no supplemental inorganic P had no apparent effect on fertility. Ferguson *et al.* (1974) also observed when feeding similar diets a reduction in egg production and hatchability of fertile eggs but no effect on fertility. However, Slaugh *et al.* (1989) reported that fertility was reduced when available P was reduced from 0.3 to 0.15% for Medium White turkey hens. Current results agree with findings of Wilcox *et*

*al.* (1957, 1961), Ferguson *et al.* (1974) and Sewell *et al.* (1972) who reported that fertility did not appear to be related to dietary P.

Wilcox *et al.* (1961) reported that a low level of P has no apparent effect on hatchability of fertile eggs thus indicating a low requirement for phosphorus. However, this contradicts findings made by Ferguson *et al.* (1974) who reported significantly higher hatchability of fertile eggs from hens fed higher levels of P or Sewell *et al.* (1972) who found that P supplementation of a diet containing 0.35% P from plant sources resulted in a significant increase in the hatch of fertile.

Reinhart and Moran (1979) and Shanawany (1987) reported that hen age is positively correlated with egg size and egg size is positively correlated with poult weight. Initial egg weights were similar regardless of treatment throughout the 28 WOL even with the removal of all supplemental P from the LP+E and LP diets. Results by Ferguson *et al.* (1974) were contradictory to this with average egg weights being greatest when the diet contained 0.55% total P and significantly lower at 0.35 and 0.45% total P. However the increase in egg weight are not necessarily accompanied by proportional increases in egg components (Applegate and Lilburn, 1996). Current results agree with Reidy *et al.* (1994) who reported that the weight of eggs from commercial turkey breeders increased 11% between the onset of lay and 24 weeks of production. During that time, yolk weight increased 21% but albumen weight only increased 7%.

Percent egg weight loss, conductance, and conductance constant (K) express functional egg shell qualities (Rahn *et al.*, 1981). Differences were observed in the percentage egg weight loss; however, the results were few and inconsistent. Conductance measures the ease with which gases diffuse across the pores of the shell (Christensen and

Nestor, 1994). This parameter differed very little between treatments over the 28 WOL. Conductance K exhibited a similar pattern to data from egg weight loss. While, the differences were significant; however, there was no trend. Results from egg weight loss, conductance, and conductance K suggests that all dietary treatments can be fed without impaired hatchability. Egg weights of eggs sampled each hatch for conductance and egg weight loss consistently increased as shell thickness declined over the 28 WOL. Results suggest that all dietary treatments were adequate in providing P for expected increases in egg weight.

Albumen weights declined slightly as yolk weight increased over the 28 WOL. This agrees with findings made by Applegate and Lilburn (1996) who reported that as hens aged, egg weight increased along with relative yolk weight at the expense of albumen. Both albumen and yolk P concentrations were significantly affected by dietary treatments at 27-28 WOL. During this time phytase increased P concentration in albumen whereas dietary P levels increased yolk P concentrations. Results suggest that towards the end of the lay cycle eggs produced are more susceptible to dietary treatment.

In this study, the range for plasma P was 5.50 to 9.52 mg % P which agreed with findings made by Ledoux, *et al.* (1995) who reported plasma P levels for turkey hens to range from 7.55 to 8.24 mg % P. In the current study this wider variation between plasma P ranges may be attributed to the time of day in which the blood was drawn. Past studies have shown that plasma P levels fluctuate due to daily egg production status (Miller *et al.*, 1977; Reichmann and Conner; 1977). Woodard and Mather (1967) also noted that in turkey plasma, P level remains higher for a longer than period of time than

chickens and that this may be due to the longer shell formation. This suggests that the variation may be due to the time of day samples were collected.

Tibia P content ranged from 14.6 to 15.3 mg % P agreeing with reports made by Slauch *et al.* (1989) that femur P increased from 15.8 to 16.4% as the level of P increased from 0.15 to 0.70% aP. This could be an indication that hens could be fed as little as 0.17% aP and efficiently utilized available P in the diet to maintain production which would result in a decrease the amount of excreted fecal P.

In the current study, dietary P levels and enzyme addition significantly affected fecal P. This agrees with findings made by Slauch *et al.* (1989) who reported fecal levels to increase from 1.93 to 3.10% as the level of P increased from 0.15 to 0.70% aP. As expected, hens fed 0.7% total P (0.55% available P) excreted significantly more fecal P than those fed lower dietary P levels. Gordon and Roland (1997) observed that hens fed 0.1% aP with phytase supplementation excreted 25% less P than those consuming the same diet without phytase. Another explanation for reductions in fecal P could be associated with rates in feed consumption. It is known that consumption of larger amounts of feed with increased dietary P will increase fecal P regardless of inclusion of phytase (Waldroup, 1999). Current results do not support a reduction in fecal P with the addition of phytase to the diet. The amount of fecal P was lower regardless of enzyme addition as the dietary P decreased. There was no explanation of why hens fed diets without enzyme produced lower fecal P concentrations than those fed diets with enzyme addition. The fact that hens fed both LP+E and LP diets excreted less total fecal P than all other treatments strongly suggests that formulating diets with low levels of dietary P with or without the addition of enzyme will decrease the amount of fecal P excreted by

the hen; therefore, lowering the environmental impact of highly concentrated areas of animal production.

Overall, soluble P content of the poultry manure was 5.1 to 19.3 mg %. Hens fed either LP+E or LP excreted lower water-soluble P levels than all other treatments. When hens were fed low dietary P plus enzyme, the water-soluble P level was still lower than those fed diets at industry or NRC levels (0.55 and 0.35% aP) with or without the addition of phytase. These findings clearly suggest higher levels of dietary P increased water-soluble P, which indicated that feeding lower P would have less impact on the environment.

In each parameter observed, hens fed phytase demonstrated similar results to those fed diets without phytase over the 28 WOL except for the number of hens that went out of lay. At 28 WOL on average 28% more hens remained in production when fed phytase. It has been proposed that the improvements in growth rate and feed conversion with the addition of phytase to the diet are due to the phytase's ability to release and make available for absorption minerals and trace elements complexed with phytic acid (Simons *et al.*, 1990). The increased availability of nutrients may be a determining factor for keeping hens in production longer.

Hens fed phytase did not respond with significant or increase performance in production parameters as expected. This agrees with observations made by Van der Klis *et al.* (1997) that production performance in turkey breeder hens fed diets supplemented with phytase was comparable to hens receiving supplemental inorganic P. This also agrees with findings made by Simons *et al.*, (1990) that the addition of phytase to feed containing 4.5 g/kg of total P resulted in levels of feed conversion and performance

which were as good or better than the levels achieved using rations which were supplemented with inorganic P to a total P level of 7.5 g/kg. According to Sebastian *et al.* (1998) it is well documented that the content of intestinal phytase is very low in young birds but increases with age. Sebastian *et al.* (1998) noted that according to published work only a 1/3 of the total P in plant-derived ingredients is available to young chicks and perhaps up to 1/2 is available to adult birds. The hens used in this study were over 31 WOA, therefore, corresponding with past theory that older birds are better suited to utilize plant-derived P. Peeler *et al.* (1972) reported that older birds hydrolyze phytate P to a greater extent than chicks, because there is more phytase activity present in the gastrointestinal tract of older birds. This suggests that older hens have more endogenous phytase capacity, which reduces the efficacy of exogenous phytase. This leads to the idea that P may be over supplemented to turkey breeder hens. If hens are able to more fully utilized plant P then dietary phytase might not be needed.

From the results of this study, one can conclude that feeding low dietary P (0.17% aP) results in turkey breeder hen reproduction performance comparable to hens fed dietary P levels fed by the turkey industry (0.55% aP) or those fed NRC recommended levels (0.35% aP). Dietary phytase results in little of no effect since hens seem to be able to perform adequately with no supplemental P. Complete removal of all supplemental P may be possible as seen from results after 20 WOL when corn and soybean meal provided the only source of available P. Therefore, the turkey industry might be able to lower dietary P without impairing reproductive performance while reducing fecal P. This reduction in fecal P in effect lowers the amount of P being applied to the land and its impact on the environment. However, further research, such as P balance studies, to

ascertain the precise levels of dietary P for turkey breeder hens is needed for the varied conditions under which they are expected to perform.

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**Table 1. Feed ingredients for six dietary treatments<sup>1</sup>.**

| Ingredients (%)                  | Dietary Treatments |        |        |        |        |        |
|----------------------------------|--------------------|--------|--------|--------|--------|--------|
|                                  | HP+E               | HP     | MP+E   | MP     | LP+E   | LP     |
| Corn                             | 64                 | 64     | 64     | 64     | 64     | 64     |
| Soybean meal (45% protein)       | 22                 | 22     | 22     | 22     | 22     | 22     |
| Limestone                        | 6.5                | 6.5    | 7.0    | 7.0    | 7.5    | 7.5    |
| Dicalcium phosphate              | 2.2                | 2.2    | 1.3    | 1.3    | 0.3    | 0.3    |
| Poultry Fat                      | 4.0                | 4.0    | 4.0    | 4.0    | 4.0    | 4.0    |
| DL-Methionine                    | 0.1                | 0.1    | 0.1    | 0.1    | 0.1    | 0.1    |
| Salt                             | 0.4                | 0.4    | 0.4    | 0.4    | 0.4    | 0.4    |
| Minerals (TM-90) <sup>2</sup>    | 0.1                | 0.1    | 0.1    | 0.1    | 0.1    | 0.1    |
| Vitamins (NCSU-90) <sup>3</sup>  | 0.1                | 0.1    | 0.1    | 0.1    | 0.1    | 0.1    |
| Selenium Supplement <sup>4</sup> | 0.1                | 0.1    | 0.1    | 0.1    | 0.1    | 0.1    |
| Rendox (45g)                     | 0.01               | 0.01   | 0.01   | 0.01   | 0.01   | 0.01   |
| Sand                             |                    |        | 0.4    | 0.4    | 0.9    | 0.9    |
| Phytase                          | 0.1                |        | 0.1    |        | 0.1    |        |
| Total                            | 100                | 100    | 100    | 100    | 100    | 100    |
| Calculated analysis              |                    |        |        |        |        |        |
| Protein, %                       | 16.14              | 16.14  | 16.14  | 16.14  | 16.14  | 16.14  |
| ME, kcal/kg                      | 2970.7             | 2970.7 | 2970.7 | 2970.7 | 2970.7 | 2970.7 |
| Calcium, %                       | 2.9                | 2.9    | 2.9    | 2.9    | 2.9    | 2.9    |
| Phosphorus (available, %)        | 0.55               | 0.55   | 0.35   | 0.35   | 0.17   | 0.17   |
| Lysine, %                        | 0.89               | 0.89   | 0.89   | 0.89   | 0.89   | 0.89   |
| DL-Methionine, %                 | 0.39               | 0.39   | 0.39   | 0.39   | 0.39   | 0.39   |
| Analyzed                         |                    |        |        |        |        |        |
| Crude Protein, %                 | 16.9               | 16.4   | 16.2   | 16.4   | 16.3   | 16.3   |
| Ca, %                            | 3.5                | 4.1    | 3.9    | 4.0    | 3.8    | 3.5    |
| P (total), %                     | 0.7                | 0.8    | 0.6    | 0.5    | 0.4    | 0.3    |

<sup>1</sup>High P (HP), available P=0.5%; high P + enzyme (HP+E), available P=0.5%; medium P (MP), available P=0.3%; medium P + enzyme (MP+E), available P=0.3%; low P (LP), available P=0.17%; and low P + enzyme (LP+E), available P=0.17%.

<sup>2</sup>Mineral composition (in g/kg of diet): zinc sulfate, 120; manganous sulfate, 120; copper sulfate, 10; calcium iodate, 2.5; cobalt sulfate, 1.0.

<sup>3</sup>Vitamins in amounts per kilogram of diet: vitamin A, 26,400 IU; vitamin D<sub>3</sub>, 8,000 IU; vitamin E 132 IU; vitamin B<sub>12</sub>, 79.2 µg; riboflavin 26.4.

<sup>4</sup>Provided 0.3 mg Se per kilogram of diet.

Table 2. Relative yolk, albumen, and shell thickness of eggs from turkey breeders fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).

| Treatment            |      |      |       |         |         |      |         |       |      |
|----------------------|------|------|-------|---------|---------|------|---------|-------|------|
| 3-4 WOL <sup>2</sup> |      |      |       |         |         |      |         |       |      |
|                      | Egg  | Yolk |       |         | Albumen |      |         | Shell |      |
|                      | g    | g    | %     | Total P | g       | %    | Total P | g     | %    |
| HP+E                 | 81.3 | 23.6 | 29.0  | 29.5    | 49.9    | 61.3 | 6.94    | 7.9   | 9.7  |
| HP                   | 79.2 | 24.2 | 30.5  | 30.8    | 47.6    | 60.0 | 9.63    | 7.5   | 9.4  |
| MP+E                 | 80.0 | 24.2 | 30.2  | 28.4    | 48.3    | 60.3 | 6.52    | 7.7   | 9.6  |
| MP                   | 78.5 | 23.9 | 30.4  | 28.6    | 47.1    | 60.0 | 6.35    | 7.6   | 9.7  |
| LP+E                 | 81.6 | 24.0 | 29.4  | 26.8    | 49.7    | 60.9 | 9.98    | 7.9   | 9.7  |
| LP                   | 79.8 | 25.3 | 31.7  | 30.1    | 46.9    | 58.8 | 5.49    | 7.6   | 9.6  |
| $\bar{x}$            | 80.1 | 24.2 | 30.2  | 29.0    | 48.3    | 60.2 | 7.49    | 7.7   | 9.6  |
| SEM                  | 1.46 | 0.81 | 10.72 | 2.82    | 1.01    | 4.20 | 4.20    | 0.16  | 0.70 |

| 19-20 WOL |      |      |      |         |         |      |         |       |      |
|-----------|------|------|------|---------|---------|------|---------|-------|------|
|           | Egg  | Yolk |      |         | Albumen |      |         | Shell |      |
|           | g    | g    | %    | Total P | g       | %    | Total P | g     | %    |
| HP+E      | 94.1 | 30.2 | 32.2 | 33.7    | 55.1    | 58.5 | 9.88    | 8.4   | 8.9  |
| HP        | 91.3 | 29.1 | 31.9 | 37.6    | 55.3    | 60.6 | 12.18   | 7.8   | 8.6  |
| MP+E      | 94.2 | 28.4 | 31.2 | 37.2    | 58.1    | 61.7 | 13.06   | 8.2   | 8.7  |
| MP        | 92.8 | 30.0 | 32.3 | 31.3    | 53.9    | 58.0 | 9.64    | 8.0   | 8.6  |
| LP+E      | 94.1 | 29.7 | 32.6 | 35.3    | 55.4    | 58.9 | 5.85    | 8.1   | 8.6  |
| LP        | 93.0 | 28.6 | 30.7 | 33.5    | 56.0    | 60.2 | 13.14   | 8.4   | 9.0  |
| $\bar{x}$ | 93.3 | 29.3 | 31.8 | 34.8    | 55.6    | 59.7 | 10.6    | 8.2   | 8.7  |
| SEM       | 1.14 | 0.56 | 8.97 | 2.61    | 1.18    | 2.77 | 4.80    | 0.16  | 1.13 |

| 27-28 WOL |      |      |      |         |         |      |         |       |      |
|-----------|------|------|------|---------|---------|------|---------|-------|------|
|           | Egg  | Yolk |      |         | Albumen |      |         | Shell |      |
|           | g    | g    | %    | Total P | g       | %    | Total P | g     | %    |
| HP+E      | 91.9 | 31.0 | 33.7 | 40.5    | 54.0    | 58.7 | 11.01   | 7.7   | 8.3  |
| HP        | 94.2 | 32.5 | 34.4 | 42.5    | 53.4    | 56.6 | 9.86    | 7.9   | 8.4  |
| MP+E      | 96.2 | 33.1 | 34.4 | 43.4    | 55.2    | 57.3 | 9.03    | 8.2   | 8.6  |
| MP        | 92.3 | 32.1 | 34.8 | 43.0    | 51.4    | 55.7 | 7.85    | 7.7   | 8.3  |
| LP+E      | 93.0 | 32.0 | 34.4 | 42.1    | 53.4    | 57.4 | 12.09   | 7.7   | 8.3  |
| LP        | 91.3 | 31.5 | 34.5 | 43.7    | 52.5    | 57.6 | 13.13   | 7.7   | 8.5  |
| $\bar{x}$ | 93.2 | 32.0 | 34.4 | 42.5    | 53.3    | 57.2 | 10.5    | 7.8   | 8.4  |
| SEM       | 1.16 | 0.80 | 4.95 | 1.82    | 1.31    | 2.97 | 5.42    | 0.16  | 0.61 |

<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup> The relative amount of yolk at 27-28 WOL was significantly ( $P \leq 0.05$ ) higher than at 3-4 or 19-20 WOL. There was a linear increase in total yolk P from 3-4 WOL to 27-28 WOL ( $P \leq 0.05$ ).

Table 3. Weekly embryonic mortality for eggs produced by turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).

| Hatch     | Effect | Treatment         |                   |                   |                   |                   |                   |
|-----------|--------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|           |        | HP+E              | HP                | MP+E              | MP                | LP+E              | LP                |
| 1 Week 1  | Px E   | 1.4               | 1.9               | 0.6               | 1.4               | 0.9               | 0.0               |
| Week 2    |        | 0 <sup>c</sup>    | 4.2 <sup>ab</sup> | 0.8 <sup>c</sup>  | 1.4 <sup>bc</sup> | 5.2 <sup>a</sup>  | 0.0 <sup>c</sup>  |
| Week 3    |        | 0.7               | 0.0               | 0.0               | 0.0               | 0.7               | 0.0               |
| Week 4    |        | 2.9               | 0.6               | 0.8               | 3.5               | 2.3               | 1.4               |
| Pips      |        | 22.1              | 15.5              | 14.4              | 16.5              | 13.6              | 18.6              |
| 2 Week 1  | Px E   | 4.0               | 7.4               | 6.6               | 2.0               | 7.0               | 4.5               |
| Week 2    |        | 0.0               | 1.0               | 0.0               | 0.0               | 0.0               | 0.0               |
| Week 3    |        | 0.5               | 0.0               | 0.0               | 0.7               | 0.0               | 0.0               |
| Week 4    |        | 0.9               | 2.6               | 2.9               | 0.9               | 1.6               | 1.1               |
| Pips      |        | 10.6 <sup>a</sup> | 7.8 <sup>b</sup>  | 8.6 <sup>ab</sup> | 15.7 <sup>a</sup> | 10.9 <sup>a</sup> | 10.3 <sup>a</sup> |
| 3 Week 1  |        | 6.0               | 3.8               | 1.7               | 3.3               | 3.9               | 6.3               |
| Week 2    |        | 0.0               | 0.0               | 0.8               | 0.6               | 0.0               | 0.0               |
| Week 3    |        | 0.6               | 0.0               | 0.0               | 0.4               | 0.7               | 0.0               |
| Week 4    |        | 2.5               | 4.3               | 0.8               | 2.5               | 1.1               | 3.6               |
| Pips      |        | 15.3              | 12.9              | 12.5              | 9.6               | 11.5              | 15.5              |
| 4 Week 1  | E      | 9.7               | 5.5               | 5.9               | 10.2              | 6.3               | 6.2               |
| Week 2    |        | 0.0 <sup>b</sup>  | 2.7 <sup>a</sup>  | 0.0 <sup>b</sup>  | 0.0 <sup>b</sup>  | 2.6 <sup>a</sup>  | 0.7 <sup>ab</sup> |
| Week 3    |        | 2.6               | 0.0               | 0.0               | 0.9               | 0.6               | 0.0               |
| Week 4    |        | 15.1              | 22.6              | 13.8              | 10.0              | 18.2              | 12.8              |
| Pips      |        | 16.6              | 11.9              | 10.3              | 8.4               | 13.4              | 9.9               |
| 5 Week 1  |        | 1.3               | 2.4               | 1.1               | 2.5               | 1.4               | 1.5               |
| Week 2    |        | 0.8               | 2.0               | 1.2               | 1.1               | 2.0               | 0.7               |
| Week 3    |        | 0.0               | 1.5               | 1.5               | 0.6               | 0.0               | 1.0               |
| Week 4    |        | 1.9               | 3.8               | 2.4               | 1.3               | 0.0               | 0.9               |
| Pips      |        | 14.6              | 16.5              | 16.1              | 24.2              | 19.0              | 25.9              |
| 6 Week 1  | E      | 3.4               | 2.8               | 5.1               | 6.5               | 2.9               | 1.1               |
| Week 2    |        | 0.0               | 2.3               | 1.3               | 0.0               | 0.0               | 1.3               |
| Week 3    |        | 1.0               | 1.4               | 2.3               | 1.7               | 0.6               | 0.0               |
| Week 4    |        | 3.1 <sup>b</sup>  | 3.1 <sup>a</sup>  | 0.7 <sup>b</sup>  | 6.7 <sup>a</sup>  | 0.7 <sup>b</sup>  | 5.1 <sup>a</sup>  |
| Pips      |        | 29.3              | 36.7              | 23.6              | 37.5              | 26.4              | 33.4              |
| 7 Week 1  |        | 2.7               | 1.4               | 2.9               | 3.8               | 5.2               | 4.7               |
| Week 2    |        | 0.7               | 0.6               | 0.0               | 0.0               | 0.8               | 0.6               |
| Week 3    |        | 0.0               | 0.0               | 3.1               | 1.7               | 2.2               | 0.0               |
| Week 4    |        | 0.0               | 1.3               | 0.0               | 0.0               | 1.5               | 2.5               |
| Pips      |        | 22.9              | 15.7              | 20.9              | 24.9              | 17.4              | 23.6              |
| 8 Week 1  | E      | 1.6               | 1.8               | 4.2               | 1.7               | 3.7               | 3.0               |
| Week 2    |        | 0.0               | 0.0               | 0.0               | 2.6               | 0.0               | 2.6               |
| Week 3    |        | 3.2               | 2.0               | 5.8               | 3.0               | 4.0               | 3.2               |
| Week 4    |        | 0.9 <sup>b</sup>  | 0.8 <sup>a</sup>  | 0.0 <sup>b</sup>  | 3.4 <sup>a</sup>  | 0.7 <sup>b</sup>  | 3.2 <sup>a</sup>  |
| Pips      |        | 15.2              | 14.4              | 6.5               | 7.9               | 10.2              | 13.3              |
| 9 Week 1  | E      | 3.0 <sup>b</sup>  | 3.6 <sup>a</sup>  | 0.6 <sup>b</sup>  | 8.7 <sup>a</sup>  | 2.5 <sup>b</sup>  | 6.3 <sup>a</sup>  |
| Week 2    |        | 1.1               | 1.6               | 0.0               | 1.9               | 0.8               | 0.0               |
| Week 3    |        | 4.7               | 3.7               | 1.0               | 3.3               | 2.9               | 0.0               |
| Week 4    |        | 7.7 <sup>ab</sup> | 5.3 <sup>b</sup>  | 2.0 <sup>b</sup>  | 13.5 <sup>b</sup> | 14.2 <sup>a</sup> | 9.6 <sup>a</sup>  |
| Pips      |        | 6.8               | 10.5              | 8.2               | 11.0              | 13.4              | 10.3              |
| 10 Week 1 |        | 8.0               | 3.3               | 6.9               | 5.6               | 9.0               | 7.4               |
| Week 2    |        | 1.0               | 0.0               | 0.0               | 0.6               | 0.0               | 0.0               |
| Week 3    |        | 0.0               | 0.0               | 0.0               | 0.8               | 1.7               | 0.0               |
| Week 4    |        | 6.4               | 5.6               | 5.2               | 6.8               | 3.9               | 3.7               |
| Pips      |        | 10.7              | 12.2              | 8.3               | 12.0              | 14.1              | 13.3              |
| 11 Week 1 | E      | 5.0               | 4.3               | 4.1               | 4.8               | 3.7               | 4.4               |
| Week 2    |        | 0.6 <sup>a</sup>  | 0.0 <sup>b</sup>  | 0.8 <sup>a</sup>  | 0.0 <sup>b</sup>  | 1.8 <sup>a</sup>  | 0.0 <sup>b</sup>  |
| Week 3    |        | 3.3               | 4.3               | 4.0               | 3.2               | 2.6               | 1.7               |

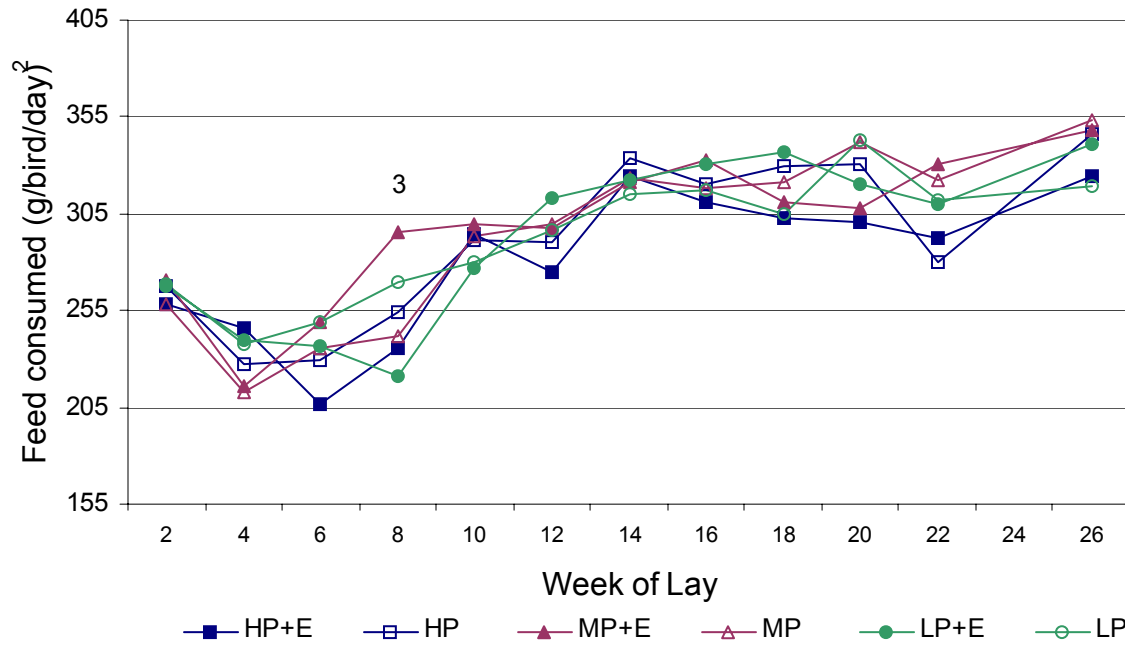
Table 3. Continued.

|    |        |      |      |      |      |      |      |
|----|--------|------|------|------|------|------|------|
|    | Week 4 | 3.3  | 7.0  | 4.8  | 5.2  | 7.2  | 3.6  |
|    | Pips   | 7.6  | 10.3 | 6.9  | 10.1 | 10.6 | 10.0 |
| 12 | Week 1 | 4.9  | 3.5  | 3.7  | 4.2  | 7.7  | 2.5  |
|    | Week 2 | 1.0  | 0.5  | 0.3  | 0.4  | 2.3  | 0.9  |
|    | Week 3 | 1.9  | 2.2  | 0.8  | 4.1  | 2.1  | 4.5  |
|    | Week 4 | 5.7  | 7.8  | 3.4  | 8.0  | 6.0  | 5.8  |
|    | Pips   | 12.3 | 10.7 | 13.1 | 15.3 | 9.6  | 20.2 |
| 13 | Week 1 | 6.4  | 2.3  | 7.0  | 3.8  | 3.8  | 4.8  |
|    | Week 2 | 0.0  | 1.2  | 0.0  | 0.0  | 1.0  | 2.1  |
|    | Week 3 | 2.9  | 2.2  | 1.8  | 2.9  | 2.0  | 1.7  |
|    | Week 4 | 2.1  | 7.0  | 6.2  | 4.6  | 5.1  | 3.0  |
|    | Pips   | 9.0  | 9.1  | 10.3 | 17.3 | 12.2 | 13.8 |
| 14 | Week 1 | 5.8  | 6.3  | 5.9  | 7.3  | 6.4  | 6.9  |
|    | Week 2 | 0.0  | 0.4  | 0.0  | 0.0  | 1.6  | 0.0  |
|    | Week 3 | 1.6  | 3.7  | 2.7  | 1.9  | 4.3  | 1.9  |
|    | Week 4 | 6.1  | 3.6  | 2.0  | 7.4  | 4.6  | 3.0  |
|    | Pips   | 11.7 | 10.6 | 10.1 | 16.1 | 13.0 | 14.5 |

<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>a,b,c</sup> Means with no common superscripts within a row are significantly different ( $P \leq 0.05$ ).

Figure 1. Feed consumption (g/bird/day) of turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).

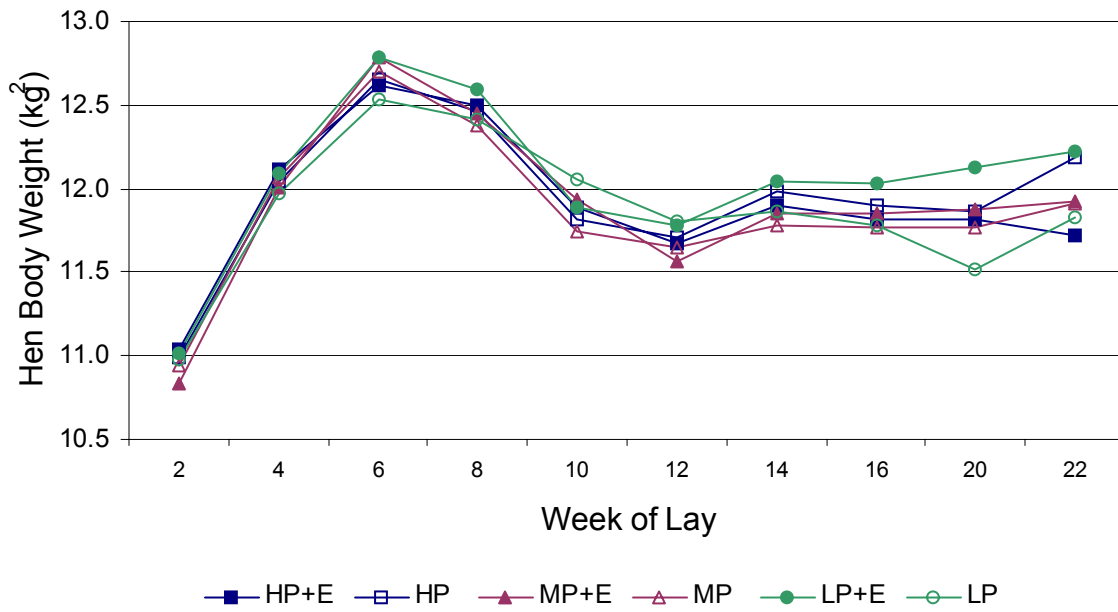


<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup> SEM=5.62

<sup>3</sup> At week of lay 7-8 there was a treatment effect ( $p \leq 0.02$ ). Hens fed MP+E, LP, and HP had significantly higher feed consumption. Hens fed HP also had similar feed consumption as those fed MP, HP+E, and LP+E.

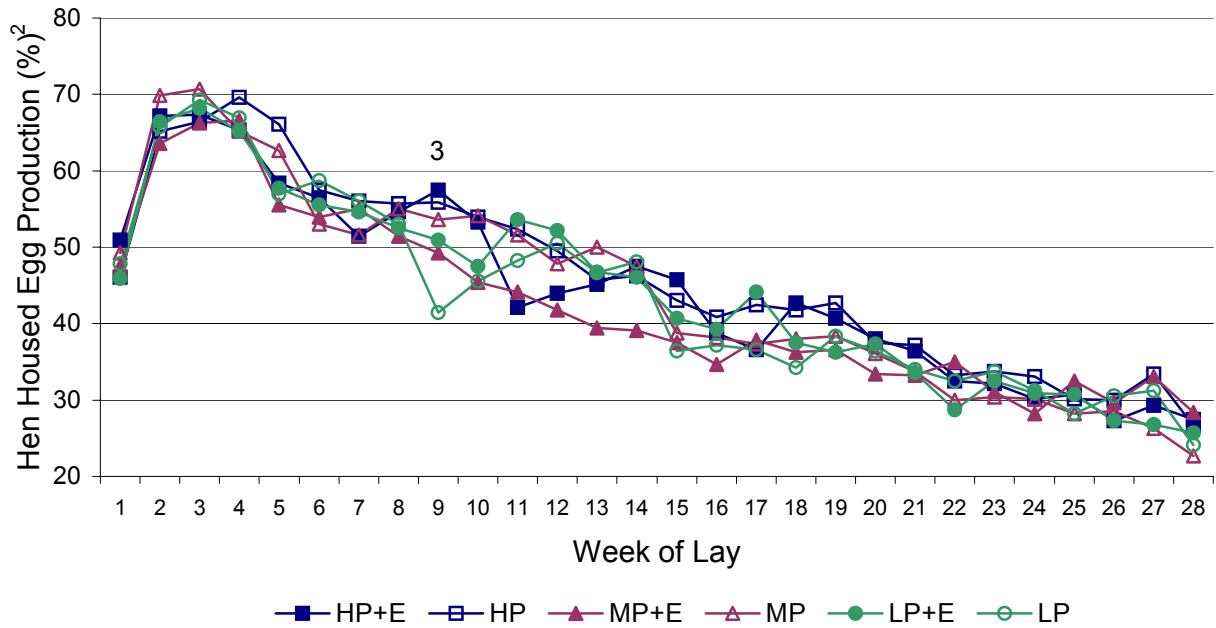
Figure 2. Body weights (kg) of turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).



<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup>SEM=0.14

Figure 3. Hen housed egg production (%) of turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).

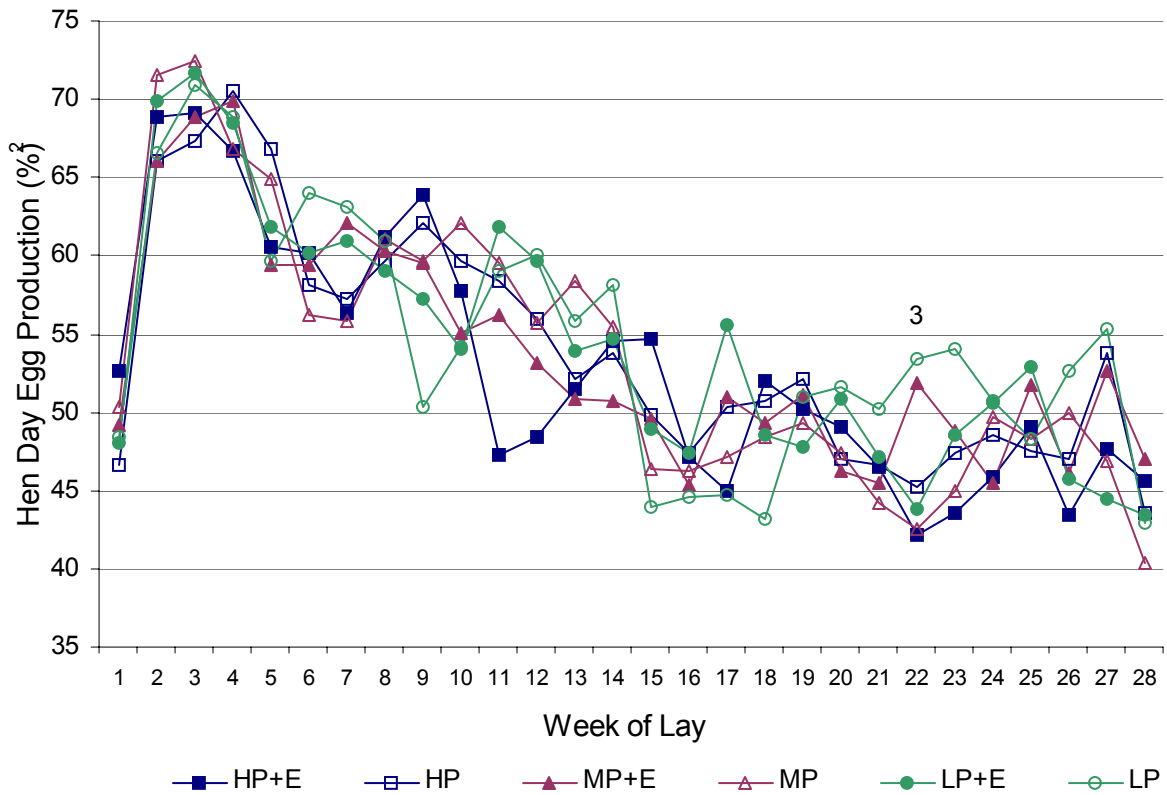


<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup> SEM=0.81

<sup>3</sup> At week of lay 9 there was a P effect ( $p \leq 0.05$ ). Hens fed the HP dietary treatment had higher hen house egg production than hens fed LP. Hens fed MP dietary treatment performed as well as those fed HP and LP treatments.

Figure 4. Hen day egg production (%) of turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).

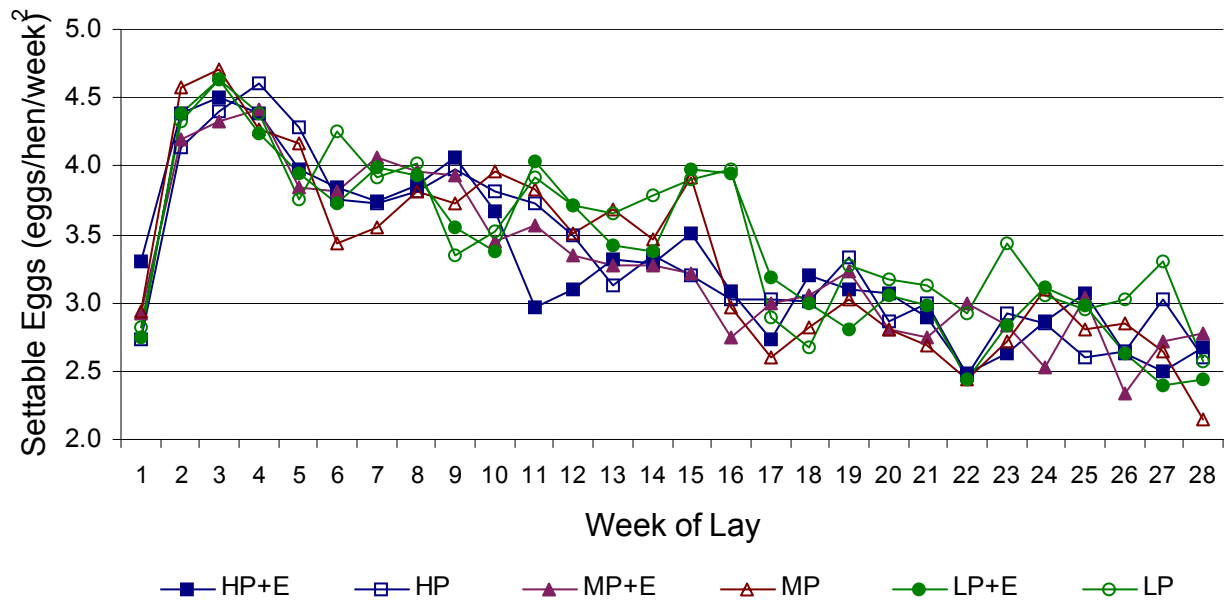


<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup> SEM=0.99

<sup>3</sup> At week of lay 22 there was a treatment effect ( $p \leq 0.04$ ) hens fed LP, MP+E, HP, and LP+E had significantly higher hen day egg production than hens fed HP+E and MP. Hens fed LP+E had similar hen day egg production as those fed HP+E and MP.

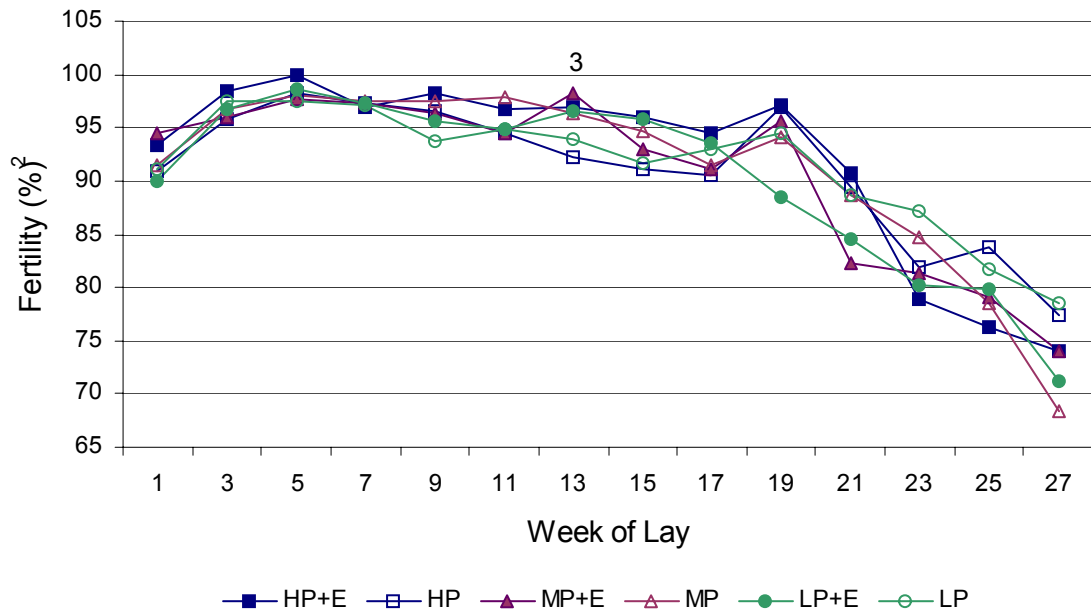
Figure 5. Total settable eggs per week from turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).



<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup>SEM=0.60

Figure 6. Fertility (%) of eggs from turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).

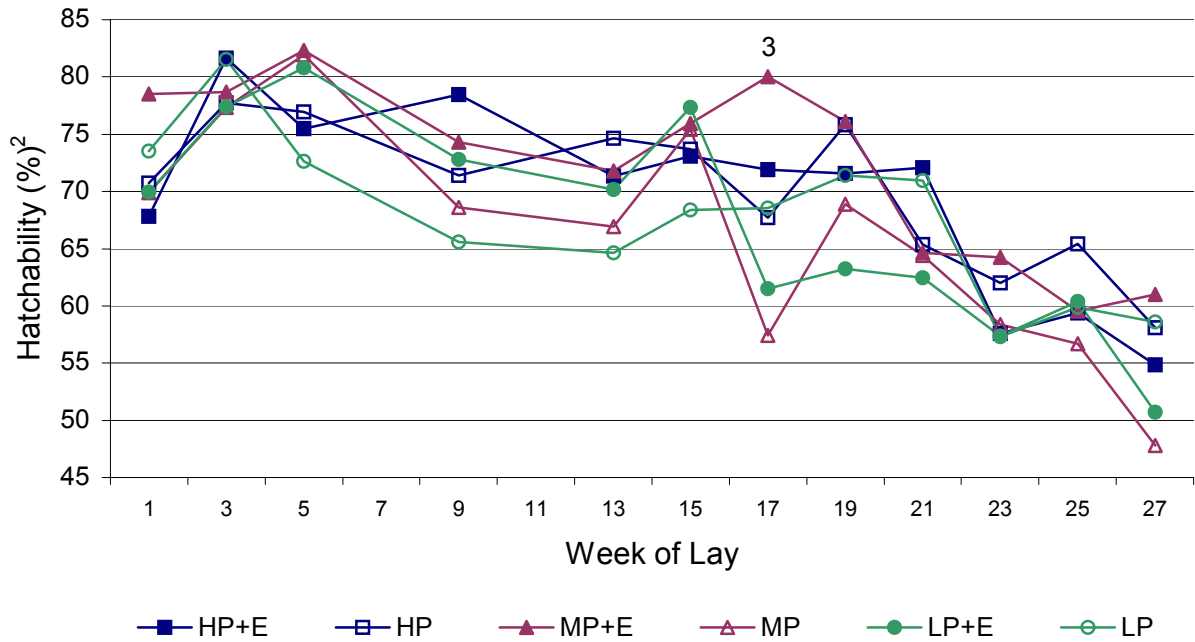


<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup> SEM=1.01

<sup>3</sup> At week of lay 13-14 there was a E effect ( $p \leq 0.03$ ) hens fed HP+E, MP+E, and LP+E had significantly higher fertility than hens fed HP, MP, and LP

Figure 7. Hatchability (%) of eggs from turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).

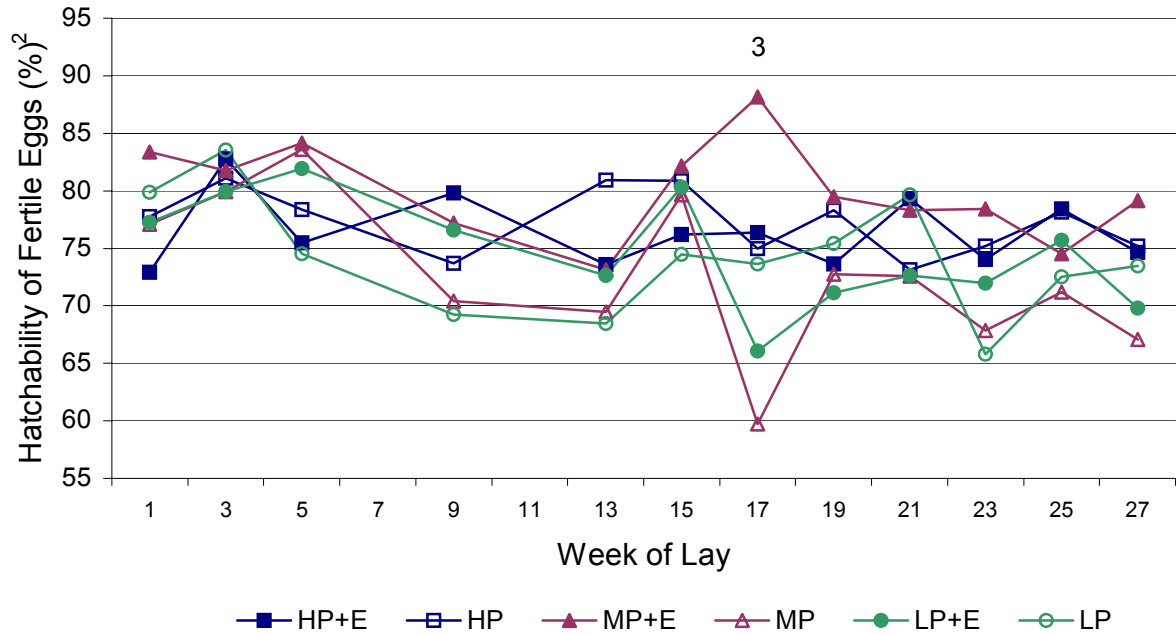


<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup> SEM=1.66

<sup>3</sup> At 17-18 WOL there was a treatment effect ( $p \leq 0.02$ ). Hens fed MP+E, HP+E, and LP had significantly better hatchability than those fed LP+E and MP. Hens fed HP performed equal to all treatments.

Figure 8. Hatchability of fertile eggs (%) from turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).

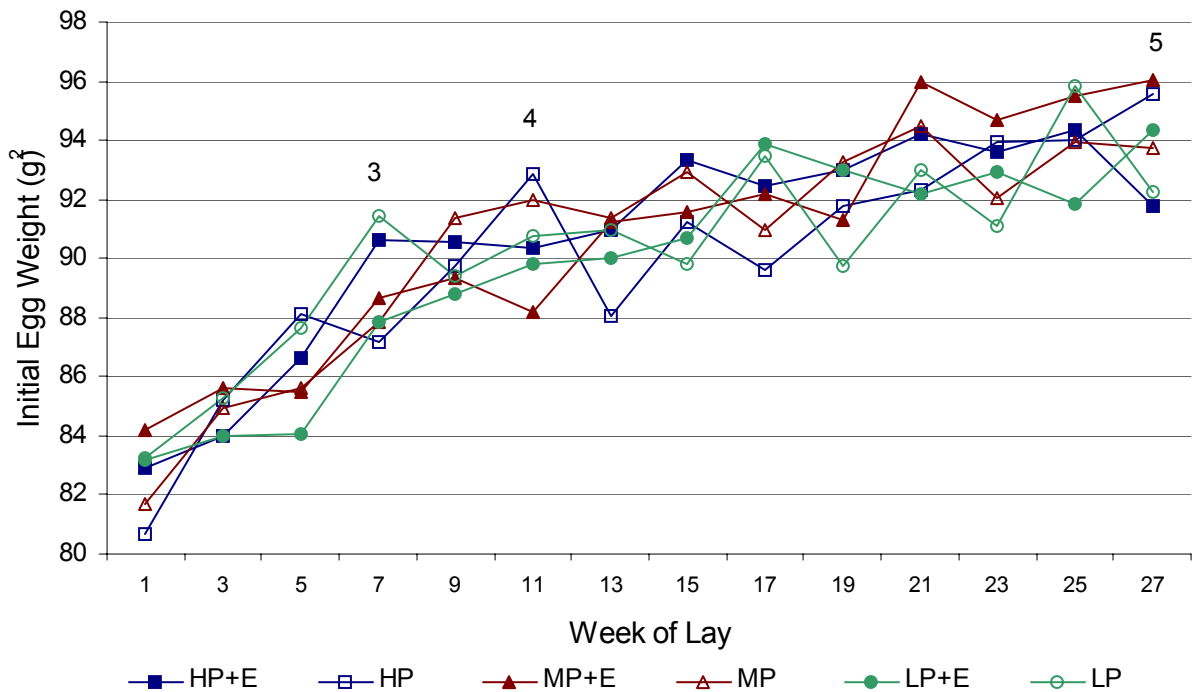


<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup> SEM=1.57

<sup>3</sup> At 17-18 WOL there was a treatment effect ( $p \leq 0.01$ ). Hens fed MP+E, HP+E and HP had significantly better hatchability of fertile eggs than all other diets. Hens fed HP also performed as well as those fed LP diets. Hens fed LP+E performed as well as those fed LP and MP. Hens fed MP diets performed only as well as those fed LP+E.

Figure 9. Initial egg weight (g) of eggs from turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).



<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

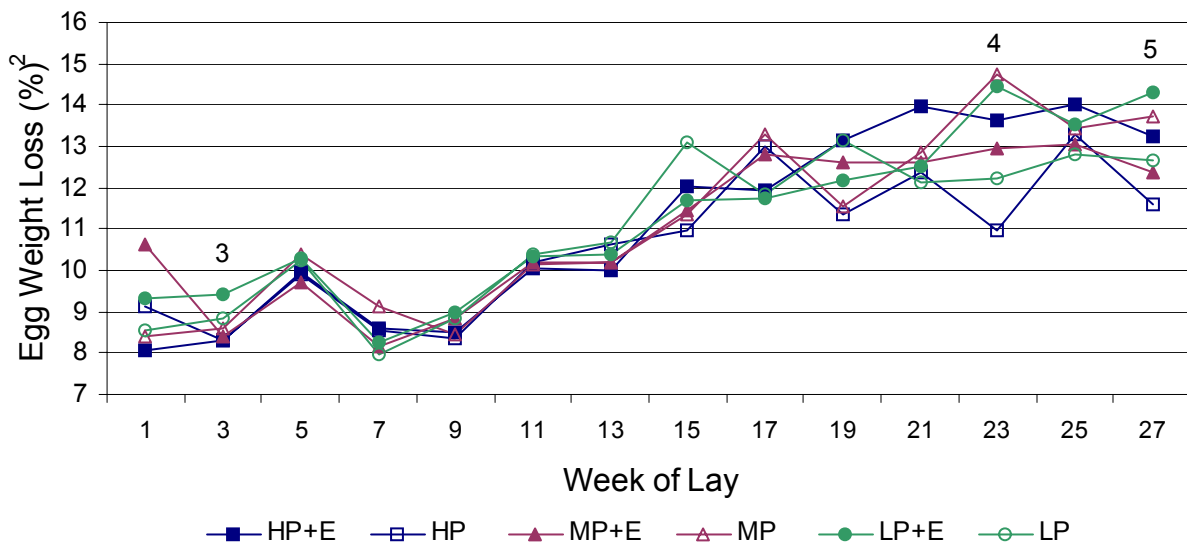
<sup>2</sup> SEM=0.38

<sup>3</sup> At 7-8 WOL there was a treatment effect ( $p \leq 0.01$ ). Hens fed LP, HP+E, and MP+E had significantly higher initial egg weight. Hens fed MP+E performed as well as those fed MP, LP+E, and HP.

<sup>4</sup> At 11-12 WOL there was an E effect ( $p \leq 0.02$ ). Hens fed HP, MP, and LP had significantly higher initial egg weights than those fed HP+E, LP+E, and MP+E.

<sup>5</sup> At 27-28 WOL there was a treatment effect ( $p \leq 0.01$ ). Hens fed MP+E, HP, LP+E, and MP had significantly higher initial egg weights. Hens fed MP performed as well as those fed LP and HP+E.

Figure 10. Percent egg weight loss (%) of eggs from turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).



<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

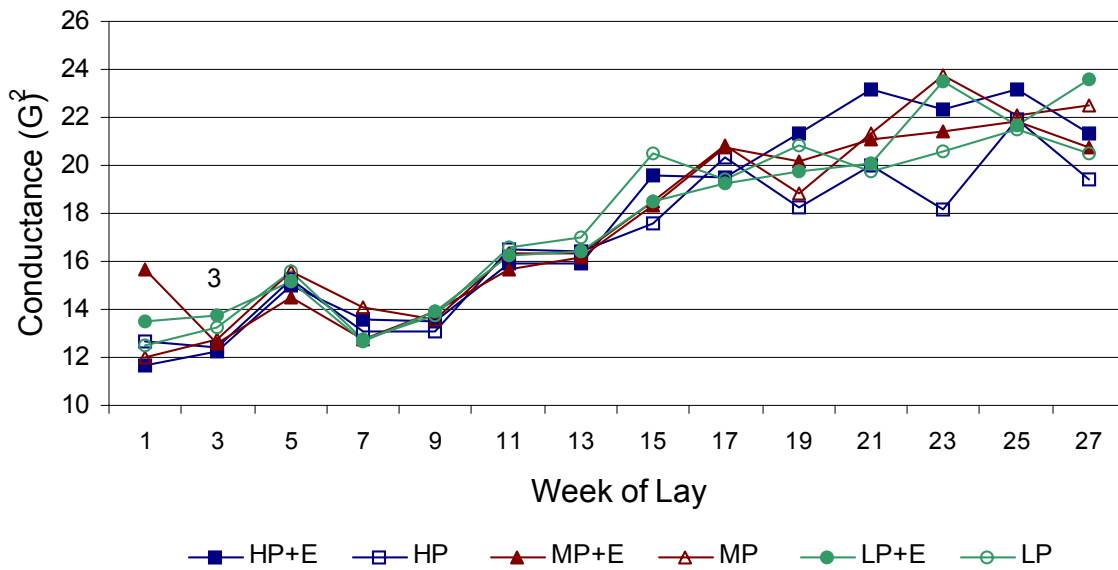
<sup>2</sup> SEM=0.20

<sup>3</sup> At 3-4 WOL there was a P effect ( $p \leq 0.01$ ). Hens fed LP had significantly higher percent egg weight loss than those fed HP and MP.

<sup>4</sup> At 23-24 WOL there was a treatment effect ( $p \leq 0.05$ ). Hens fed MP, LP+E, HP+E, MP+E and LP had significantly higher percent egg weight loss. Hens fed LP had percent egg weight losses similar to hens fed HP.

<sup>5</sup> At 27-28 WOL there was a treatment effect ( $p \leq 0.05$ ). Hens fed LP+E, MP, HP+E, and LP had significantly higher percent egg weight loss. Hens fed LP had percent egg weight losses similar to hens fed MP+E and HP.

Figure 11. Egg shell conductance (G) of eggs from turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).

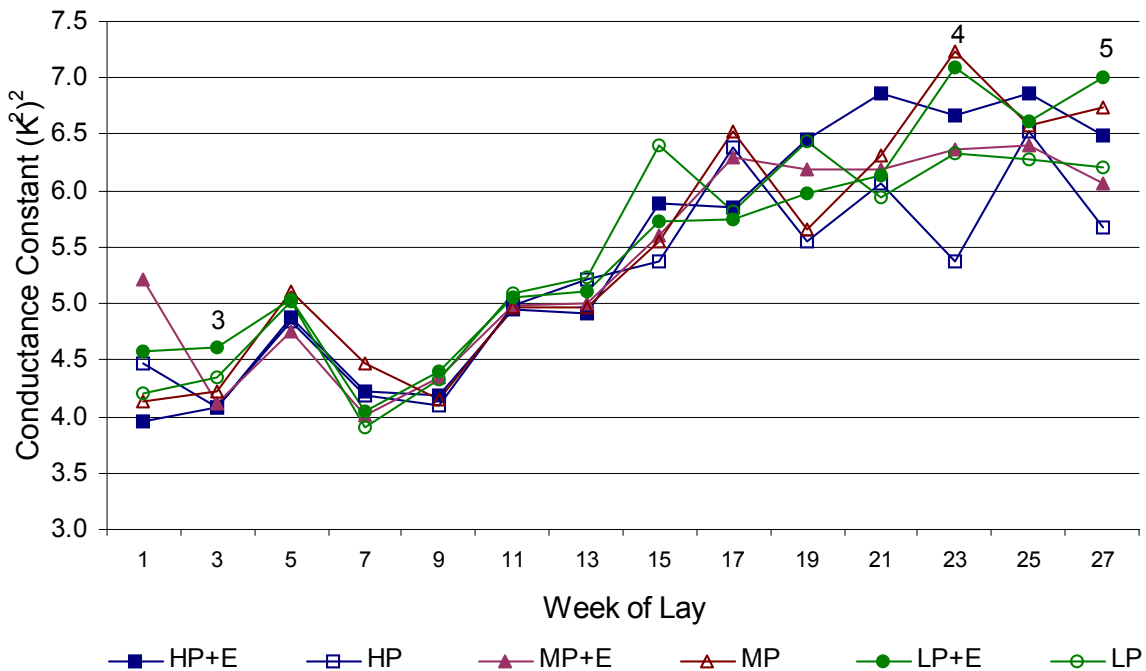


<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup> SEM=0.33

<sup>3</sup> At 3-4 WOL there was a P effect ( $p \leq 0.01$ ). Hens fed LP had significantly higher conductance than those fed MP and HP.

Figure 12. Egg shell conductance constant ( $K^2$ ) of eggs from turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).



<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

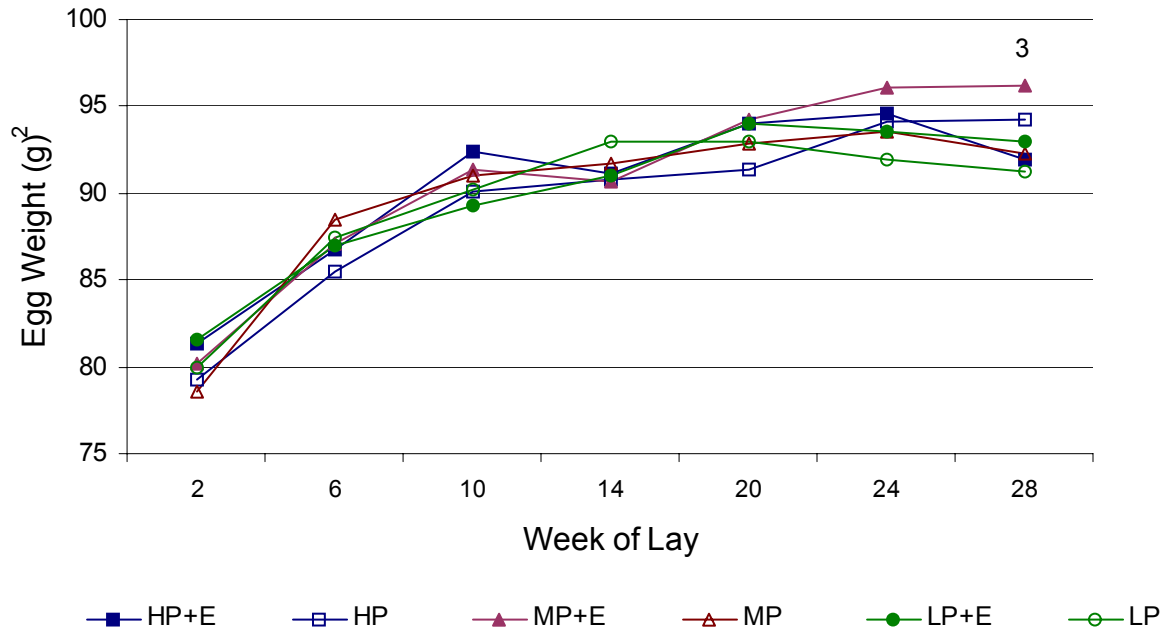
<sup>2</sup>SEM=0.10

<sup>3</sup> At 3-4 WOL there was a P effect ( $p \leq 0.01$ ). Hens fed LP had significantly higher conductance constant than those fed MP and HP.

<sup>4</sup> At 23-24 WOL there was a treatment effect ( $p \leq 0.05$ ). Hens fed MP, LP+E, HP+E, MP+E, and LP had significantly higher conductance constant. Hens fed LP diets had similar conductance constant values as hens fed HP.

<sup>5</sup> At 27-28 WOL there was a treatment effect ( $p \leq 0.05$ ). Hens fed LP+E, MP, HP+E, and LP had significantly higher conductance constant. Hens fed LP diets had similar conductance constant values as hens fed MP+E and HP.

Figure 13. Egg weight (g) of turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).

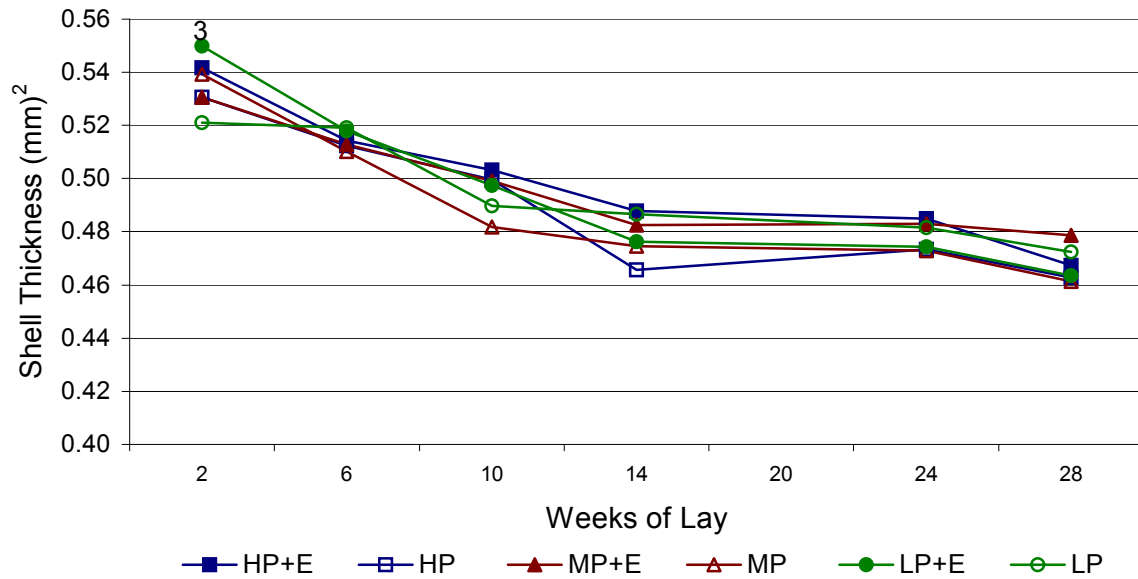


<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup>SEM=0.60

<sup>3</sup> At 27-28 WOL there was significant ( $p \leq 0.03$ ) due to a treatment effect. Hens fed MP+E and HP had significantly higher egg weights. Hens fed HP diets had egg weights similar to hens fed LP+E, MP, HP+E, and LP.

Figure 14. Shell thickness (mm) of eggs from turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).

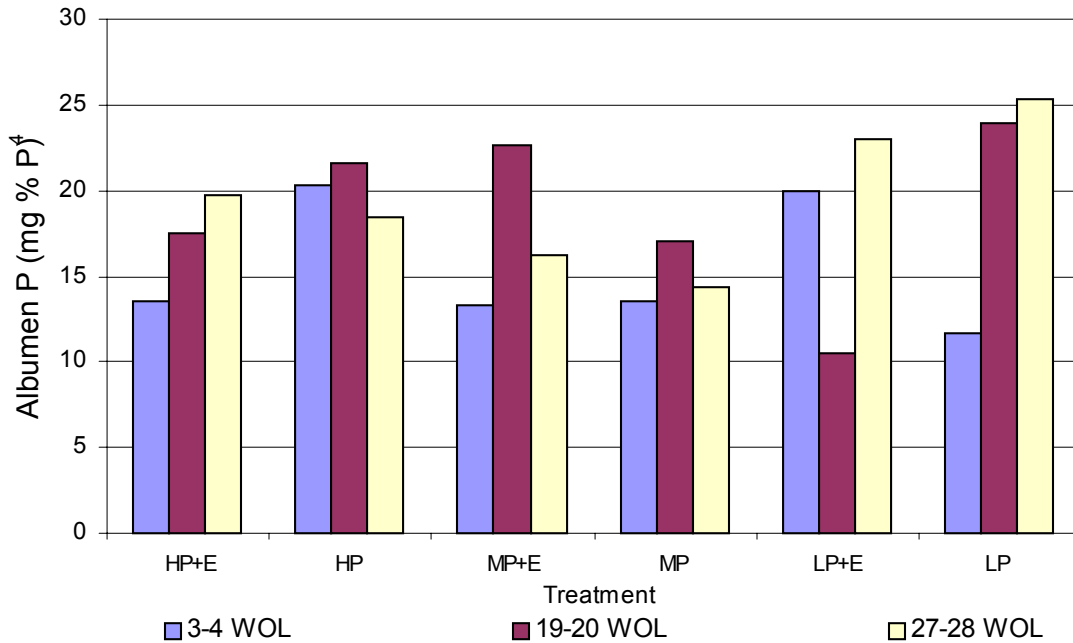


<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup> SEM=0.33

<sup>3</sup> At 1-2 WOL there was a treatment effect ( $p \leq 0.03$ ). Hens fed LP+E, HP+E, MP and MP had significantly thicker shells. Hens fed MP diets had shells as thick as those fed MP+E, HP, and LP.

Figure 15. Egg albumen P (mg % P) levels due to dietary treatments<sup>1</sup> fed to turkey breeder hens over 28 weeks of lay (WOL).<sup>2,3</sup>



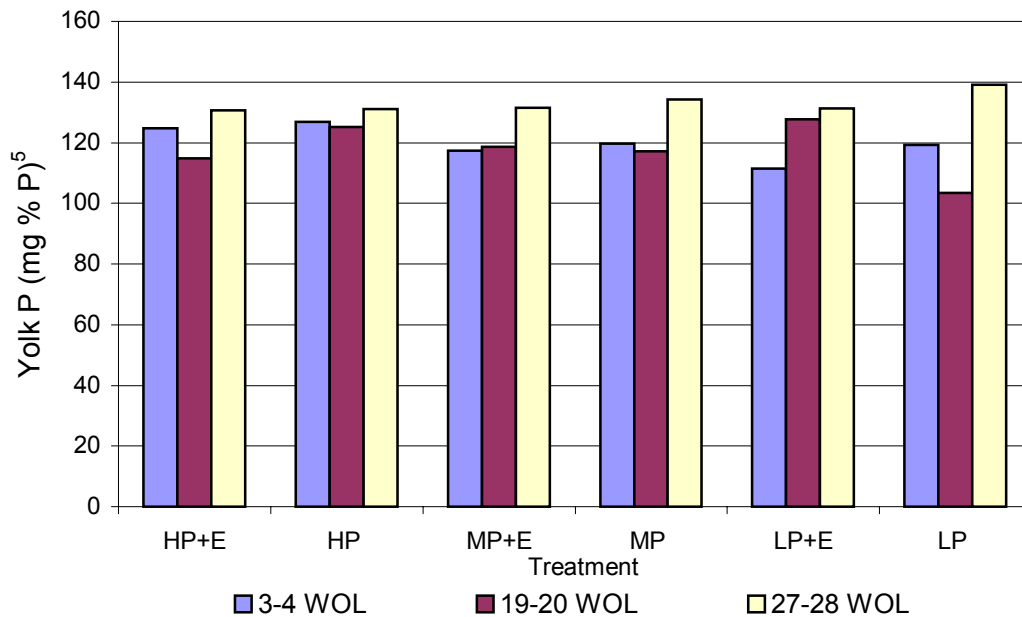
<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup> There was a significant ( $p \leq 0.0001$ ) difference due to month. Eggs produced at 3-4 WOL (February) had significantly higher concentration in albumen than those produced at 19-20 WOL (June) or at 27-28 WOL (August). Eggs produced at 19-20 WOL had significantly higher P albumen concentrations than those at 27-28 WOL.

<sup>3</sup>Eggs produced at 27-28 WOL were significantly ( $p \leq 0.01$ ) affected by enzyme addition. At that time hens fed diets containing phytase, produced eggs with significantly higher P albumen concentration than those fed diets without phytase supplementation.

<sup>4</sup>SEM=1.6

Figure 16. Egg yolk P (mg % P) levels due to dietary treatments<sup>1</sup> fed to turkey breeder hens over 28 weeks of lay (WOL).<sup>2,3,4</sup>



<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

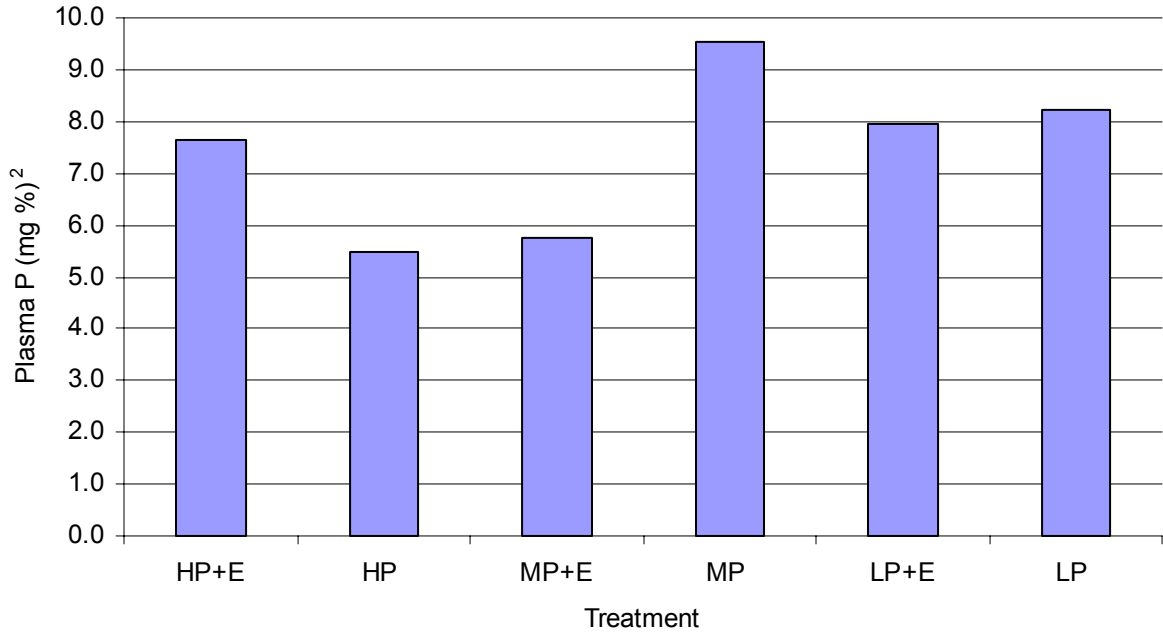
<sup>2</sup> There was a significant ( $p \leq 0.0001$ ) difference due to month. Eggs produced at 3-4 WOL (February) had significantly higher P concentration in yolk than those produced in 19-20 WOL (June) or 27-28 WOL (August). Eggs produced at 19-20 WOL had significantly higher P yolk concentrations than those from 27-28 WOL.

<sup>3</sup> Eggs produced at 19-20 WOL were significantly ( $p \leq 0.05$ ) affected by dietary treatment. Hens fed diets HP, MP+E, LP, and LP+E had significantly higher P concentration. Hens fed LP+E diets performed as well as HP+E and MP.

<sup>4</sup> Eggs produced at 27-28 WOL were significantly ( $p < 0.03$ ) affected by dietary P level. Hens fed MP had higher yolk P concentrations than those fed LP or HP.

<sup>5</sup> SEM=25.55

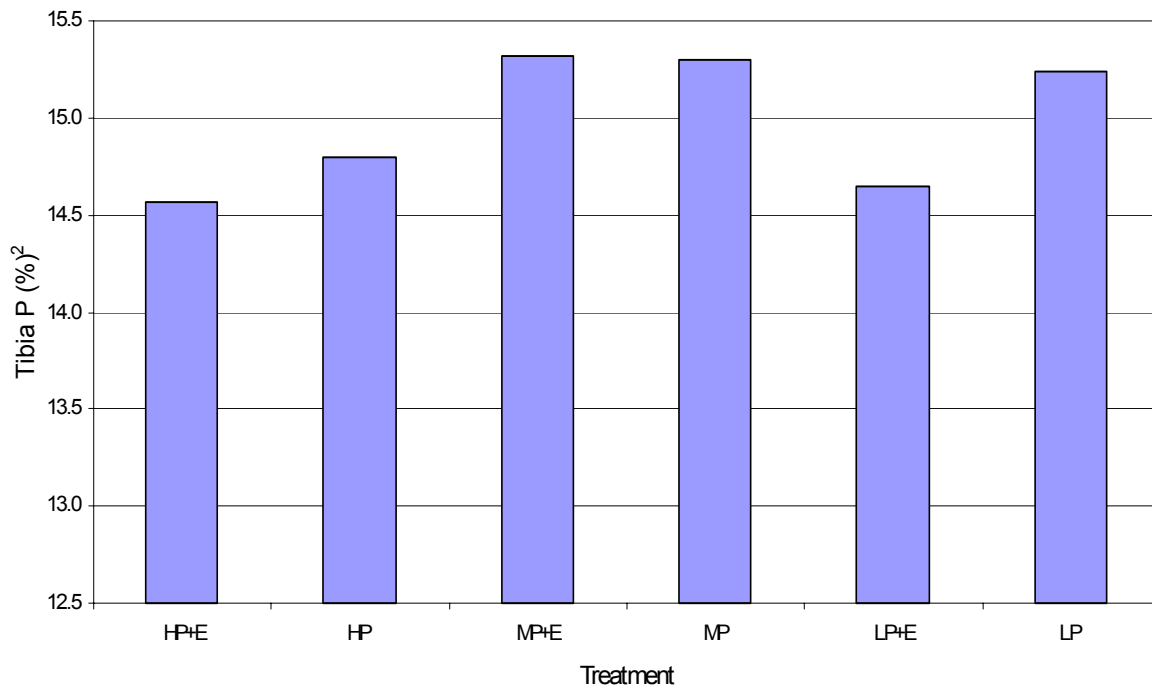
Figure 17. Plasma P (mg %) of turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).



<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup>SEM=0.60

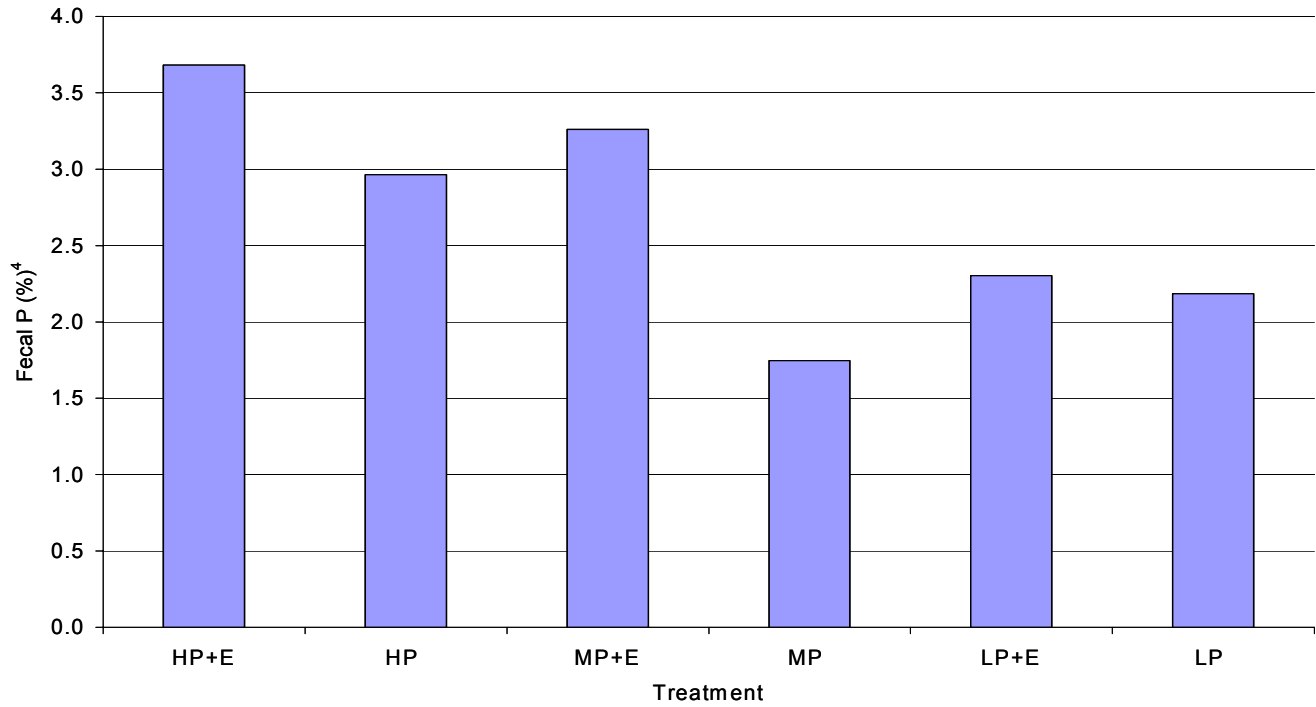
Figure 18. Tibia P (%) taken at the end of the study from turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).



<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup> SEM=0.20

Figure 19. Fecal P (%) analyzed at the end of the study from turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL)<sup>2,3</sup>.



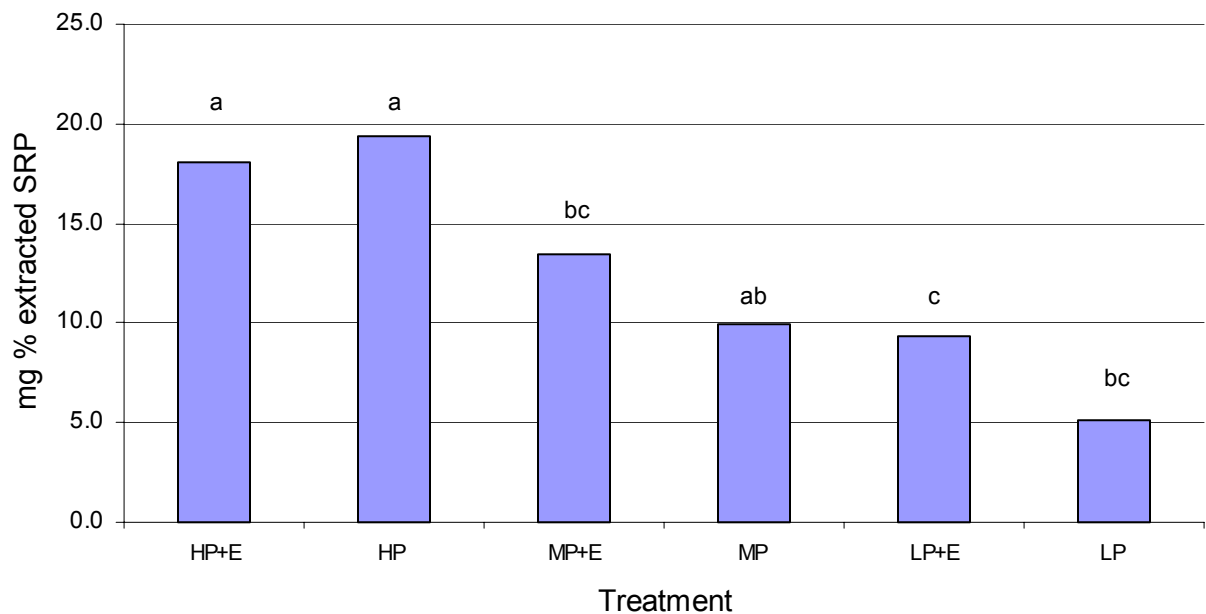
<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup> Fecal P was significantly reduced ( $P \leq 0.001$ ) in hens fed MP and LP treatments compared with hens fed HP.

<sup>3</sup> Fecal P was significantly ( $P \leq 0.006$ ) increased by E at all levels of dietary P.

<sup>4</sup> SEM=0.15

Figure 20. Fecal soluble reactive P (mg %) from turkey breeder hens fed dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).

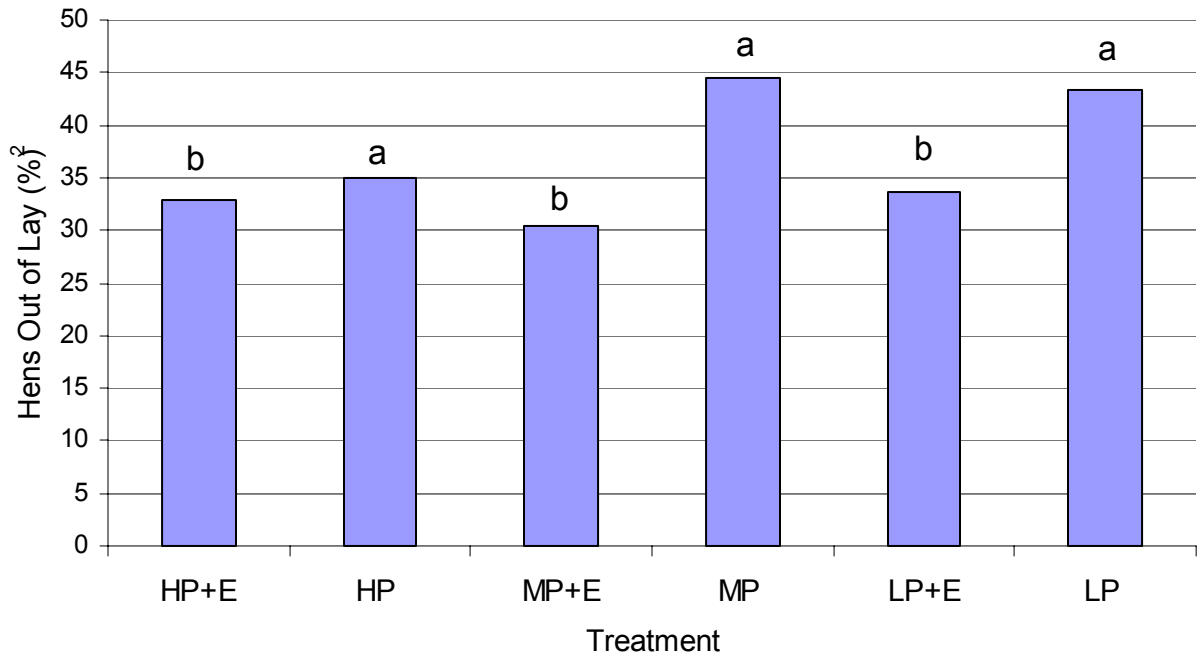


<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

<sup>2</sup> SEM=2.52

<sup>a,b,c</sup> Means with no common superscripts are significantly different ( $P < 0.05$ ). N=4 samples per treatment. There was a linear decrease in fecal soluble P as dietary P was reduced ( $P \leq 0.05$ ).

Figure 21. The proportion (%) of turkey hens out of lay due to dietary treatments<sup>1</sup> over 28 weeks of lay (WOL).



<sup>1</sup> High P (HP), available P=0.55%; high P + enzyme (HP+E), medium P (MP), available P=0.35%; medium P + enzyme (MP+E), low P (LP), available P=0.17%; and low P + enzyme (LP+E).

\  
<sup>2</sup>SEM=0.52

<sup>a,b</sup> There was an E effect ( $p \leq 0.04$ ) in that hens fed HP+E, MP+E, and LP+E had significantly less number of hens that went out of lay compared to those fed HP, MP, and LP.

## Appendix I: Phosphorus Assay

### Microtiter Plate Protocol

(tibia, fecal, water-soluble fecal P, yolk, albumen, serum/plasma)

This assay is used to measure P content using a microtiter plate reader. Past methods of measuring P were time consuming and required materials such as chemicals and lab equipment on a larger scale. This assay was developed in order decrease the time and the amount materials needed to perform the assay.

A. All samples (except blood) must be ashed in the muffle furnace. The crucibles used must first be acid washed to avoid phosphorus contamination. (See section D. to run blood (serum/plasma samples.)

B. Reagents:

1. Molybdate reagent:

[chemicals: ammonium molybdate and sulfuric acid ( $\text{H}_2\text{SO}_4$ )]

1. Into a clean, acid washed 500ml vol. flask, add about 200ml distilled  $\text{H}_2\text{O}$ ; set in an ice bath.
2. Slowly, with cooling add 45ml conc.  $\text{H}_2\text{SO}_4$
3. Dissolve 22g Ammonium Molybdate in an acid washed beaker containing 200ml distilled  $\text{H}_2\text{O}$
4. Pour Ammonium Molybdate solution into  $\text{H}_2\text{SO}_4$  solution; mix; let stand until completely cooled-bring to the 500ml mark; mix
5. Store in a brown bottle (stable for several years)

2. Iron-TCA Reagent:

[chemicals: TCA, ferrous ammonium sulfate, and thiourea]

- a. Into a clean, acid washed, 1000ml flask dissolve the following:
- b. 125g TCA (Trichloroacetic Acid)
- c. 37.5g Ferrous Ammonium Sulfate
- d. 12.5g Thiourea
- e. Store in Brown Bottle (stable for 6-12 months)

NOTE: A precipitate of sulfur will form after several days.

FILTER before using

## 1. Phosphorus Standard

(use same standards for all samples)

Use standards in 0-20% range for accuracy

Dry 0.0878g of  $\text{KH}_2\text{PO}_4$  (1hour)

Place in 100 ml of  $\text{dH}_2\text{O}$

To make:

| %  | Stock (ml) | $\text{dH}_2\text{O}$ (ml) |
|----|------------|----------------------------|
| 20 | 10         | 0                          |
| 18 | 9          | 1                          |
| 16 | 8          | 2                          |
| 14 | 7          | 3                          |
| 12 | 6          | 4                          |
| 10 | 5          | 5                          |
| 8  | 4          | 6                          |
| 6  | 3          | 7                          |
| 4  | 2          | 8                          |
| 2  | 1          | 9                          |
| 0  | 0          | 10                         |

### C. Parameters:

All samples except serum/plasma, water-soluble fecal P

1. Weigh
2. Dry (oven at 65-100 °C)
3. Weigh (fecal and tibia must be ground before next step)
4. Ash in muffle furnace at 600 °C (1 gram sample)
5. Weigh
6. Digest with HCl
  - A. Albumen 1.5  $\text{H}_2\text{O}$  and 0.5 HCl
  - B. Yolk\* 1.5  $\text{H}_2\text{O}$  and 0.5 HCl
  - C. Bone\* 2.5  $\text{H}_2\text{O}$  and 1.5 HCl
  - D. Fecal\* 1.5  $\text{H}_2\text{O}$  and 0.5 HCl

\* Samples are too concentrated and need to be serial diluted in order to use 0-20% standards

1. Yolk is a 10 fold dilution
2. Tibia is a 100 fold dilution
3. Fecal is a 100 fold dilution

Water-soluble fecal P:

1. Following steps 1-3 (section C.)
2. In a centrifuge tube place 1 gram sample
3. Add 20 ml dH<sub>2</sub>O
4. Shake for 1 hour
5. Centrifuge at 2200rpm for 30 minutes
6. Filter through 0.45 millapore filter paper
7. This solution will be used in the assay as follows in Section D.

Parameter Units:

1. Albumen P (mg %)
2. Yolk P (mg %)
3. Tibia P (%)
4. Fecal P (%)
5. Plasma P (mg %)
6. Water-soluble fecal P (mg % extracted Soluble Reactive Phosphorus)

D. Running the Assay:

1. Run samples in triplicate.
2. Place blank into the first three wells in column 1:  
240µl TCA  
10µl distilled H<sub>2</sub>O  
25µl molybdate
3. Place standards\* into the first three wells of columns 2-6 respectively:  
240µl TCA  
10µl standards (values)  
25µl molybdate
4. Place samples into the first three wells of the columns remaining:  
240µl TCA  
10µl sample  
25µl molybdate
5. Follow the procedure for the Softmax program and setting up the Microplate Reader.
6. Then read plate.

\*The use of the standards should produce a straight line. First test all standards to identify if concentration is correct. Next use at least five points to perform assay.

#### E. Serum/Plasma Phosphorus

[A protein-free filtrate must be prepared from the serum or plasma:]

1. 0.2ml serum/plasma in small plastic tube (bullet tube)
2. add 0.8ml 5% TCA
3. mix on vortex – let stand for 15-20 min.
4. spin
5. pour off the clear supernatant into another bullet tube
6. use 0.1ml of this filtrate

#### Chemicals

| <u>Chemical</u>            | <u>Company*</u> | <u>Chem. No.</u> | <u>Amt.</u> |
|----------------------------|-----------------|------------------|-------------|
| Ammonium Molybdate         | S               | A-7302           | (100g)      |
| Sulfuric Acid              | F               | A-300-500        | (500ml)     |
| TCA (Trichloroacetic Acid) | F               | A-322-500        | (500g)      |
| Ferrous Ammonium Sulfate   | S               | F-1543           | (500g)      |
| Thiourea                   | F               | T-101-100        | (100g)      |

\* S=Scientific; F=Fisher

#### Bones:

| <u>Chemical</u> | <u>Company*</u> | <u>Chem. No.</u> | <u>Amt.</u> |
|-----------------|-----------------|------------------|-------------|
| Chloroform      | F               | C-295-4          | 4L          |

\* S=Scientific; F=Fisher

## SUMMARY

There is a lack of agreement in the literature concerning the P requirement for turkey breeder hens. The different dietary needs that turkey breeder hens have during reproductive period compared to their needs as poults and growing birds is becoming more apparent. A turkey breeder hen P requirement in conjunction with the environmental impact and overload of fecal P from land application of manure has also become an increasing concern. The objective of the current study was to determine if the reproductive performance of turkey breeder hens is affected by feeding reduced levels of dietary P, compared to current industry practices and NRC recommendations, with and without the addition of phytase enzyme.

Reproductive performance was observed from turkey breeder hens fed six dietary treatments over 28 WOL. The diets contained 0.55, 0.35, or 0.17% aP with and without dietary phytase. Bird performance as measured by feed consumption, body weight, reproductive performance, hatchability, fertility, shell qualities, and mortality was unaffected by dietary treatments. Indicators of hatchability, such as conductance and egg weight, were also unaffected, further establishing that lower dietary P can be successfully fed to turkey breeder hens. However, dietary enzyme supplementation reduced the number of hens that went out of production. No effect of treatment was observed in plasma or tibia P. Although, fecal P and water-soluble fecal P were significantly decreased as dietary P was lowered.

## CONCLUSION

In conclusion, lowering dietary P did not impair turkey breeder hen reproductive performance or hatchability. Data measured from reproductive performance and hatchability suggests that hens fed 0.17% aP with or without the addition of phytase will reproduce at a similar rate to those fed industry (0.55% aP) and NRC (0.35% aP) levels with or without the addition of enzyme. These findings suggest that NRC turkey breeder hen requirements may need to be re-evaluated. However, the addition of enzyme does make a significant impact on the number of hens that go out of production, which could be beneficial to the industry for improving productivity of turkey breeder hens and should be further explored.

Lowering the P level fed to turkey breeder hens would enable poultry companies to lower P levels closer to the hen's requirement without impairing reproduction or hatchability. The lowering of dietary P reduces fecal P. The reduction in fecal P in effect lowers the amount of P being applied to the land helping to prevent P saturation of the land. Decreasing P saturation decreases its potential to contribute as an environmental pollutant. Therefore, implications for industry not only include reduced feed costs but more importantly reduction in fecal P over a 30 week period of lay which has serious costs implications for environment and industry.

It was observed in this study, that lowered dietary P does not affect performance of turkey breeder hens or their egg hatchability during the lay cycle. It was also observed that the addition of phytase increases the number of days of turkey hens remain in production. Evaluation of other factors such as levels of dietary calcium (Ca), inorganic P, vitamin D<sub>3</sub>, age of hen, dietary ingredient interaction and/or feed processing may offer

a greater understanding of turkey breeder hen dietary P requirements rather than studying P level alone.