MODELING AND GENETICS OF PARAMETERS AFFECTING PHYTATE PHOSPHORUS BIOAVAILABILITY IN GROWING BIRDS

by

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ABSTRACT

Genetic methods may be used to improve phytate phosphorus utilization by modeling factors that affect PPB. A model was developed to determine the main factors affecting PPB. The effects of each trait were on PPB were determined and showed that body weight, feed consumption and feed conversion ratio, had a negative effect on phytate phosphorus utilization whereas body weight gain, relative growth, and the bioavailabilities of total phosphorus, calcium, nitrogen and energy had a positive effect. The results of this study also indicate that the major factors affecting phytate phosphorus utilization (PPB) in birds were body weight at 4 weeks, calcium bioavailability, nitrogen bioavailability and energy bioavailability. There were differences between the sexes with regard to the factors affecting PPB. The predictive model showed that nitrogen bioavailability had a positive effect on the PPB of females but had no influence on males. The fact that body weight and feed consumption showed a negative relationship while relative growth had a positive effect shows that feed intake traits have to be corrected for bodyweight to account for differences in efficiency of utilization. The heritabilities and genetic correlations between the parameters were also determined. The genetic correlations (r_g) between PPB and body weight at 4 weeks and nitrogen bioavailability were low whereas r_g between PPB and energy bioavailability and calcium bioavailability were moderate and positive implying that selection for improvement in calcium and energy bioavailability may improve PPB.

INDEX WORDS: phytate phosphorus, modeling

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B.Sc , University of Ghana, 1999

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial

Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2003

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December 2003

DEDICATION

To the Lord, who has been a source of great blessings, to my parents and sisters, and to all my friends.

ACKNOWLEDGEMENTS

First of all, I would like to thank God for giving me the grace to go through the masters program. I am indebted to my major professor, Dr. Aggrey, for all the assistance and advice he has given me for the past two years. I am grateful to the members of my advisory committee, Dr. Gene Pesti and Dr. Nicholas Dale for their advice and encouragement. I would like to thank Cheryl Pearson and Dr. Remzi Bakalli for their technical assistance and friendship. I would also like to thank all the graduate students of the poultry science department especially Tim Chendrimada and Wensheng Zhang, who were always willing to answer my questions and offer assistance in anyway that they could. Finally, I would like to thank my family and friends for their support and prayers.

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CHAPTER 1

INTRODUCTION

Phosphorus is an important mineral required in poultry diets for normal growth and development. The main sources of phosphorus in plant tissues however, are phosphates, phytic acid and other myo-inositol phosphates (Classen and Stevens, 1995; Sebastian et al., 1996). Phytates are largely unavailable to monogastrics because they lack significant sources of intestinal phytase (Kornegay, 1996). Phytase makes phosphorus available by removing phosphorus groups from the inositol molecule (Sandberg et al., 1993). Several studies have been conducted to increase phytate phosphorus utilization in poultry because phosphorus is essential for proper growth. Secondly, unutilized phosphorus excreted in manure causes environmental pollution when applied to land (Kies et al., 1983).

The addition of microbial phytase to the diet has been found to improve phytase phosphorus utilization. The efficacy of microbial phytase however, is influenced by several factors that affect phytate phosphorus utilization (Kornegay, 1996).These include: the amount of vitamin D present (Mohammed et al., 1991), calcium to total phosphorus (Ca: tP) ratio (Liu et al., 1998), bioavailability of calcium and phosphorus supplements, age of the animal (Kornegay, 1996), size of the bird (Punna and Roland, 1999) and the level of phytase enzyme in the diet.

Researchers are able to understand livestock production systems by using models which represent the existing knowledge of the system (Spedding, 1988). Models predict the outcomes of experiments and estimate certain parameters that cannot be estimated by other methods. (Korver and Van Arendonk, 1988). These parameters help us to make inferences from data, which we apply, to the population from which it was drawn. Models also help us to understand causal relationships and the interactions between and among variables (Burnham and Anderson, 1998).

Research Objectives

(1) To develop a model to predict factors that affect phytate phosphorus utilization in growing birds.

(2) To determine heritabilities and genetic correlations among model parameters.

CHAPTER 2

REVIEW OF LITERATURE

I. Structure and utilization of Phytic acid

A large portion of phosphorus of plant origin is in the form of phytic acid or its salts, which are collectively, called phytin or calcium magnesium phytate (Temperton and Cassidy, 1964a). Phytate or inositol hexaphosphate is found in many plant feedstuffs and is the main storage form of phosphorus in plant seeds. It accounts for about 60 to 80% of the total phosphorus found in plants (Cabahug et al., 1999). The amount of phytate varies with the type of plant. Oil seeds have about 1.5% phytate while whole grain cereals have about 1.0%. There is also some variation in the availability of phytates. Wheat bran is known to have a high phytase activity (Pointillart et al., 1984). Thus, phytates in this cereal grain are more available than the phytate found in other plant feedstuffs like corn. These workers found higher phytate phosphorus utilization in pigs fed a wheat diet than those fed a corn diet. Nelson (1976) found similar results in an earlier study with chicks and layers. Broilers at 4 and 9 weeks together with layers were fed diets containing either corn only or a combination of corn and wheat. The amount of phytate phosphorus utilized was 0, 2 and 8%, respectively for those fed a corn diet and 8, 13 and 13% respectively for birds fed a diet containing wheat. Incubating both corn and wheat resulted in an increase in phytate phosphorus utilization.

Anderson (1915) conducted one of the earliest studies on the structure of phytate. When wheat bran was combined with a dilute solution of phytin, about two-thirds of the total phosphorus was split off. Only a portion of the total phytin was completely hydrolyzed to form inosite and phosphoric acid. The rest were partially hydrolyzed to inorganic phosphoric acid and inosite tri, di and monophoshates.

Phytate phosphorus is not available for absorption unless phosphate groups are removed from the inositol molecule by the enzyme phytase or intestinal phosphatase (Harland and Morris, 1995). Temperton and Cassidy (1964b) reported the work of Singsen (1948) on chickens: Phytate phosphorus was adequate for the growth requirements of chickens but not for adequate bone calcification. Singsen (1948) stated that phytate phosphorus could be utilized in the metabolic processes required for growth without being hydrolyzed from the phytate molecule. Temperton and Cassidy (1964b) in their study found that even in the absence of feedstuffs of animal origin and inorganic phosphorus, birds were able to retain about 60% of phytate phosphorus and showed no sign of rickets, which implies effective bone mineralization. However there is sufficient evidence that monogastrics are not able to utilize phytate phosphorus efficiently because they lack sufficient phytase activity. They are only able to utilize phytates efficiently when microbial phytase is introduced into the feed or when the feedstuff has high phytase activity.

Gillis et al. (1957) found much higher levels of radioactive phosphorus in the blood and bones of chicks fed inorganic phosphorus than that of chicks fed organic phosphorus, indicating that chicks cannot make effective use of phytate phosphorus for growth and bone formation. The source of phytate under use however, was calcium phytate, which is less available than some other phytate forms. There is also some evidence that microbes in the intestinal tract of monogastrics produce enough phytase to improve phytate hydrolysis.

When fed a phosphorus deficient diet, rats increased digestion of total dietary phosphorus and phytate phosphorus (Moore and Veum, 1983). The levels of alkaline phosphatase and intestinal phytase did not differ between treatment groups. Thus, the increase in phytate hydrolysis was attributed to an increase in alkaline phosphatase or phytase activity by intestinal microflora.

The bird's ability to hydrolyze phytate phosphorus actually depends on several factors other than the phytate and phytase contents of the diet or the presence of microbial phytase. Among the factors that influence phytate phosphorus utilization are calcium levels, protein levels, calcium to phosphorus ratio (Qian et al.,1996) vitamin D (Edwards, 1993), phosphorus levels (Ballam et al., 1985) and age, species and sex of the birds (Kornegay, 1996; Punna and Roland, 1999).

Factors affecting phytate phosphorus hydrolysis

I. The effect of calcium on phytate phosphorus hydrolysis

Chickens have an effective homeostatic mechanism that regulates the levels of calcium but high levels still have a negative effect on phytate phosphorus utilization. The absorption of calcium is reduced when it precipitates and becomes insoluble. Edwards and Veltmann (1983) found that phosphorus retention depends on the level of calcium and phosphorus in the diet. Phosphorus retention is highest when the diet contains low levels of calcium and phosphorus. Ballam et al. (1985) observed that an increase in calcium levels decreased phytate phosphorus utilization irrespective of the phosphorus levels. These workers stated that excess calcium binds with phytate and reduces its hydrolysis since calcium has two ligands that have a high affinity for phosphate groups. In an earlier study, however these workers (Ballam et al., 1984) found that the decrease in phytate phosphorus utilization with an increase in calcium and inorganic phosphorus levels depended on the source of phytate. They found that high levels of these minerals decreased phytate phosphorus utilization for all feed sources except for rice bran. They attributed this result to the high bioavailability of phosphorus in rice bran.

Mohammed et al. (1991) also reported a marked increase in phytate phosphorus digestibility when the levels of both inorganic phosphorus and calcium in the diet were reduced. There was a further increase in phytate phosphorus utilization when cholecalciferol was added to the diet. The effect of cholecalciferol is also linked to a reduction in calcium since it increases calcium uptake. Excess calcium in the diet reduces phytate hydrolysis in two ways. It binds with phytate and makes it insoluble thus reducing its hydrolysis. Calcium also competes directly with phytate for the active sites of the phytase enzyme (Qian et al., 1996). The absorption of calcium can also be determined by the part of the intestine in which it is located. Thus, phytate degradation is also affected by the site of the small intestine in which it is found. Sandberg et al. (1993) reported that in pigs, phytate phosphorus degradation in the colon is minimal. They stated that this could be due to an increase in pH. The formation of insoluble complexes increases with an increase in pH. The reverse is true when pH is reduced. Thus, most minerals are absorbed in the duodenum or jejunum. The adverse effect of high levels of calcium is observed regardless of the type of diet that animals are fed (Skogland et al., 1997). These workers however noted that the decrease in degradation of phytate when high levels of calcium were fed occurred most significantly in the colon. There was no significant decrease in phytate hydrolysis in the small intestine and stomach.

Hydrolysis of phytate in the colon occurs mainly by the action of colon flora. High levels of calcium could decrease microbial activity leading to a decrease in phytate hydrolysis.

II. The effect of protein on phytate phosphorus hydrolysis

Proteins are positively charged and at low and neutral pH, they form insoluble complexes with the anionic phosphate groups of phytate (Yi et al., 1996). The source of protein and the level of protein in the diet usually determine the utilization of phosphorus, zinc, magnesium and other elements in the diet (Shafey and Mcdonald, 1991). Phytates form bonds with endogenous enzymes like trypsin and chymotrypsin and reduce their efficiency thus decreasing the availability of both protein and phosphates in the phytate compound (Biehl and Baker, 1997). These workers found that utilization of amino acids in chicks fed soybean meal can be improved when microbial phytase is added to the diet. This improvement however, depends on the amino acid content of the diet and source of protein. The utilization of amino acids in chicks fed a corn-peanut meal diet in the same experiment did not increase whether the diet was deficient in amino acids or not. These results were explained by the fact that substances that improve the availability of phytate bound nutrients vary in their efficiency depending on the ingredients that are fed. In another experiment in pigs where soybean meal was used as the sole source of protein, supplemental phytase had little or no effect on the apparent or true digestibility of crude protein or amino acids (Traylor et al., 2001). Yi et al. (1994) in a study on broilers noted that the addition of phytase to feed increases the apparent retention of phosphorus and nitrogen. Similar results were obtained with turkey poults (Yi et al., 1996) and pigs (Ketaren et al., 1993). These workers found that the addition of phytase to the diets of young pigs increased protein deposition and retention but did not affect the digestibility of crude protein.

Fammatre et al. (1977) found no protein by mineral interaction on performance criteria. There was however, a protein by mineral interaction for feed conversion. A high mineral level resulted in an improvement in feed conversion on a diet higher in protein. The availability of phosphorus in animals fed high levels of protein was low. They concluded that higher levels of calcium and phosphorus must be fed when protein levels are increased. Mahan et al. (1980) in a later study also obtained similar results. Their results were however attributed to the higher levels of phytate phosphorus in the higher protein diet. They observed that a dietary deficiency of phosphorus affects nitrogen retention more than adequate protein. Similar results have been found in humans (Hegsted et al., 1981). They explained that an increase in dietary protein requires a simultaneous increase in dietary phosphorus in order to maintain a bone mineral equilibrium as an increase in protein intake causes a simultaneous increase in urinary calcium.

III. Effect of Vitamin D on phytate phosphorus utilization

Vitamin D is the general name given to a group of compounds with antirachitic effects (Georgievski, 1982). It can be synthesized by the body but must also be supplied by the body to meet the animal's needs. Vitamin D is usually found in the liver where it is converted into hydroxyvitamin D. It is then further converted into dihydroxyvitamin D which responsible for the homeostatic regulation of both phosphorus and calcium (Littledike and Goff, 1987). It also plays an important role in the synthesis of enzymes like intestinal adenosine triphosphatase and alkaline phosphatase that aid in the absorption of calcium and phosphate ions. Some studies have shown that the serum levels of cholecalciferol increase with vitamin D deficient rats with low phosphorus levels (Moore and Veum, 1983; Pointillart et al. 1984). This increase results in a simultaneous increase in the levels of alkaline phosphatase and also the amount of phosphorus absorbed through the intestine.

Pointillart et al. (1984) reported an increase in serum levels of vitamin D in pigs fed low levels of phosphorus. They however, noted that this increase did not eliminate signs of phosphorus deficiency in pigs. In contrast, Mohammed et al. (1991) observed that increasing levels of cholecalciferol and decreasing the levels of calcium in the diet completely eliminated phosphorus deficiency symptoms. Edwards et al. (1993) found similar results and noted that an increase in the levels of cholecalciferol and phytase and a decrease in calcium levels resulted in an increase in phytate phosphorus utilization. Cholecalciferol enhances the utilization of phytate over a wide range of inorganic phosphorus levels. This may be due to an increase in the levels of intestinal phytase and increased hydrolysis of phytate to release phosphate ions. The efficiency with which phosphates are transported from mucosa to blood is also enhanced by 1, 25 dihydroxycholecalciferol (Edwards, 1993). Biehl and Baker (1997) however, reported that microbial phytase activity was not significantly affected by 1 alpha-cholecalciferol supplementation or by the level of phosphorus in the diet. The birds used in their experiment however, were fed cholcalciferol adequate diets. Weight gains in these birds however, were comparable to those birds fed phosphorus adequate diets.

It is generally believed that the improvement in phosphate absorption by Vitamin D occurs as a secondary result of improved absorption of calcium. Hurwitz and Bar (1972) observed that most phosphate absorption occurred in the jejunum and the degree of absorption depended on the phosphate concentration in the jejunum and also on the presence of calcium and potassium ions. Nicolaysen, (1937) showed that Vitamin D increases phosphate absorption in the rat only when the calcium levels in the diet are normal. They also noted that phosphate transport takes place by a pump that depends on calcium levels. The action of Vitamin D is also affected by the calcium to phosphorus ratio of the diet. Rao et al (1999) reported that at high Ca: P ratio, supplementation with Vitamin D failed to increase weight gains in broilers. The high calcium levels in a low phosphorus diet may increase intestinal pH and decrease the soluble fraction of mineral thus reducing their availability for absorption (Shafey, 1993).

IV. The effect of calcium to phosphorus ratio on phytate phosphorus utilization

The efficiency of phytate hydrolysis is reduced at sub- optimal calcium to available phosphorus ratios. At very low levels of calcium, absorbed phosphorus will be excreted in the urine alternatively, at very high levels of calcium degradation of calcium from the intestinal tract and phosphorus absorption is reduced (Van der Klis and Versteegh, 1996). There are varying opinions on the importance of calcium to phosphorus (Ca: P) ratio in the diet. The ideal calcium to available phosphorus ratio is generally estimated at between 1:1 and 2:1. This ratio could be higher if the levels of Vitamin D in the diet are adequate however, the adverse effects of a very wide calcium to phosphorus ratio will be seen regardless of the levels of Vitamin D in the diet (Bethke et al., 1929). Ca: P ratios also become important when the absolute levels of these elements in the diet are very low.

Bethke et al. (1929), observed that on adding between 1.5% and 2.5 % calcium carbonate to the diet of growing chicks, there was an increase in growth and ash content of bone. However, when the Ca: P ratio was greater than 3.5:1 there was a decrease in growth and bone ash. This reduction increased when the level of calcium was increased further. The deleterious effect of a high Ca: P ratio was observed regardless of the actual levels of calcium and phosphorus in the diet. Wilgus (1931) estimated the ideal calcium to phosphorus ratio to be 2.2: 1. When the calcium to phosphorus ratio was about 2.5: 1, growth and calcification were slightly reduced. Further increases in the calcium to phosphorus ratio resulted in a severe depression in growth and bone mineralization. Birds fed the ideal calcium to phosphorus ratio of 2.2:1 grew well because the phosphorus levels were high enough to buffer calcium levels. Wilgus (1931) explained the difference in his results from that of Bethke et al. (1929) as being due to the fact that birds in his study were fed a normal diet as opposed to a rachitic one. In a more recent study, Krieger et al., (1995) stated that a calcium to phosphorus ratio of 2:1 together with optimum levels of Vitamin D resulted in great improvements in the utilization of both organic and inorganic phosphorus. At a higher ratio of 4:1, vitamin D had only a slight positive effect on phytate phosphorus utilization. Vipperman et al. (1974) stated that an optimum Ca: P ratio is only of importance when the dietary levels of these minerals are inadequate. They observed that low calcium to available phosphorus ratios favored both calcium and phosphorus retention for any level of dietary phosphorus while higher ratios did not favor calcium and phosphorus retention for all levels of phosphorus. In a study of the effect of zinc zeolite in the diet, Edwards et al. (1992) found that the addition of synthetic zeolite to the diet causes a marked decrease in growth rate at all levels of calcium except at the lowest level. This was due to the Ca: total phosphorus (tp) ratio rather than the low levels of phosphorus that was fed. Qian et al. (1996) also reported that the effectiveness of phytase decreases at higher levels of total and inorganic phosphorus and at wide Ca: tp ratios. At 0.27% level of inorganic phosphorus, bodyweight gains were similar when Ca: tp ratios were between 1.1:1 and 1.4:1. As Ca: tp ratios became wider, bodyweight gains decreased. This effect increased with at 0.27% inorganic phosphorus and lower levels of phytase. The increase in Ca: tp ratio interferes with phytase activity in the digestive tract of pigs and broilers.

Rao et al. (1999) observed a decrease in weight gain when birds were fed diets with wide calcium to phosphorus ratios. Reduction of non-phytate phosphorus levels of the diet containing 10g/kg of calcium resulted in a 17.3% decrease in bodyweight gain. On the other hand, a decrease in non-phytate phosphorus levels at 7.5 g/kg led to a 6.8% decrease. From their study, the ideal Ca: P ratio was about 2:1 for optimal weight gain. In spite of the antagonistic relationship between calcium and phosphorus when they are fed together in the diet, birds do not utilize these minerals efficiently when they are fed alternately (Nelson et al., 1965). The alternate intake of these two elements results in a more rapid excretion of phosphorus thus there is less efficient utilization for deposition in the bone.

V. The effect of inorganic phosphorus on phytate phosphorus hydrolysis

High levels of inorganic phosphorus generally reduce organic phosphorus utilization. This is due to a number of factors including a negative feed back mechanism caused by excess phosphorus. The extra inorganic phosphorus might also inhibit phytase synthesis by mucosal cells in the small intestine of the chicken. (Wise, 1983).

Sanders et al. (1992) showed that phytate hydrolysis in birds was highest at low levels of non-phytate phosphorus. This increase in availability of phosphorus resulted in increased retention of phosphorus and a simultaneous decrease in its excretion.

Ballam et al. (1984) reported that chicks fed diets with 1% calcium and no inorganic phosphorus hydrolyzed more phytate phosphorus than those fed diets with non-phytate phosphorus. They also observed that increasing the levels of calcium and phosphorus further led to a greater reduction in phytate phosphorus utilization. A decrease in pH favors phytate phosphorus utilization because calcium absorption increases at low pH and the source of phosphorus used in this study caused an increase in pH thus reducing phytate phosphorus utilization. In a later study, Ballam et al. (1985) observed that there was an increase in phytate hydrolysis in the absence of supplemental calcium when inorganic phosphorus was added to the diet. This unusual result could have been due to the source of inorganic phosphorus that was used. Monobasic sodium orthophosphate dissociates to produce negatively charged ions. These anions combine with excess calcium and make them less available to bind with phytate. In rats, phosphorus deprivation leads to an increase in the digestion of total dietary and phytate phosphorus (Moore and Veum, 1983). These workers found that phytate phosphorus was more available to rats when there is a deficiency of phosphorus. Farm animals adapt to low intakes of minerals by adjusting their requirement for those minerals. They are able to do this by increasing the efficiency with which minerals are absorbed from the intestinal tract or reducing the rate at which they are excreted (Underwood, 1966).

Secondly, low dietary phosphorus levels in chicks stimulate phosphate transport from the intestine. Serum levels of Vitamin D in animals that have low levels of phosphorus also increase, leading to a rise in intestinal levels of alkaline phosphatase. This enzyme ensures a more efficient uptake of phosphates. An increase in inorganic phosphorus levels decreases the ileal digestibility of phytate phosphorus since phosphorus levels in the body are already adequate. Thus, birds do not need to utilize phosphorus from phytate molecules. Though low levels of inorganic phosphorus have proved to have a favorable effect on phytate phosphorus utilization, poultry must still be given adequate sources of inorganic phosphorus even in diets that contain supplemental phytase. Pointillart et al. (1984) found that low levels of inorganic phosphorus caused hypophosphataemia in pigs even though there was an increase in serum levels of vitamin D. When levels of non-phytate phosphorus in the diet are too low, there is a decrease in mineral content of the tibia even with phytase supplementation (Lim et al., 2001). The addition of phytase to the diets of monogastrics reduces their requirement for inorganic phosphorus. Lei et al. (1993) fed added phytase to the diet of pigs without inorganic phosphorus supplementation. Pigs fed phytase retained 50% more phosphorus and excreted less phosphorus than the control. Serum and plasma concentrations of phosphorus were also higher in pigs fed a phytase supplemented diet. Alkaline phosphatase activity was, however, lower as expected. As dietary phytase increases, calcium and phosphorus utilizations also increase indirectly because dietary calcium can only be well utilized for skeletal growth when dietary phosphorus is simultaneously utilized.

V1. Effect of age, species and sex on phytate phosphorus utilization

There is a wide variation in the ability of various species to utilize phytate phosphorus. Generally monogastrics have low intestinal phytase activity though some phytase activity has been found in chickens (Davies et al., 1970). The ability of rats to utilize phytate phosphorus is influenced by age. Nelson and Kirby (1979) found that young rats hydrolyzed 71% of phytate while mature rats hydrolyzed only 39%. These results differ from the general theory that phytate phosphorus utilization increases with maturity (Nelson, 1967). Scheideler and Sell (1987) found similar results in layers. They realized from their study that phytate phosphorus utilization decreases with age. Phytate phosphorus retention was quite high at 34 weeks during early egg production but decreased at 50 and 72 weeks. These results imply that the effect of age on phytate phosphorus hydrolysis varies with different species and breeds of birds. Matyka et al. (1990) observed that at 16 to 21 days of age, phytate phosphorus utilization was between 2.8 and 7.2%. Mohammed et al. (1991) however realized that phytate digestibility in broilers fed normal amounts of inorganic phosphorus, calcium and cholecalciferol was 50%. The diets fed in these two studies were similar though the level of cholcaciferol in the later study was higher. The difference in phytate phosphorus hydrolysis could thus have been due to the difference in age (age of mohammed birds).

The degree to which phytate phosphorus is utilized is also influenced by the strain and breed of chicken. Single comb white leghorn chickens retain more phytate phosphorus than broilers (Edwards, 1983). Broilers within the same strain also showed differences in phytate phosphorus utilization (Punna and Roland, 1999). Shafey et al. (1990) compared the response of different broiler strains to variable dietary calcium, phosphorus and vitamin D. Leaner strains of birds were more tolerant to high levels of dietary phosphorus and calcium. Commercial broiler chickens vary in their growth rate and body composition, which may be an indication of variation in mineral requirement and metabolism. Male birds have a higher requirement for phosphorus than females. Lillie et al. (1964) showed a higher response to phosphorus levels in males than in females. Edwards et al. (1989) also found that male broilers retain more phytate phosphorus than females at high or low dietary calcium levels.

Models

I. Definition

A model is a set of mathematical relations between variables and parameters (Green, 1992). The relationship between the quantities in the model must correspond to the behavior of the real system in order for a model to be useful (Finkelstein and Carson, 1985). Models are used in science for understanding a system, predicting an unknown state or for controlling a system to produce a desirable condition. (Haefner, 1996). Models are also useful for determining the value of certain parameters and their influence on a particular system. Thirdly, a model that has been validated can be used to test a hypothesis. When a model is known to change according to a set of hypothesis, data from the real system can be used to test this hypothesis. (Bergman, 1988; Patterson et al., 1992). Mathematical models used in nutrition are usually of a descriptive nature (Green, 1992). They should not only be significant mathematically and biologically but should describe responses produced by many different types of nutrients and sources of nutrients in higher organisms (Mercer, 1980).

II. Types of models and their uses

Models used for descriptive purposes: These involve the expression of quantitative terms in equations. Models used for this purpose allow for easy description and analysis of data. Descriptive models are useful when a system is complex and has many interacting relations thus mathematical models used in nutrition could be of this nature. Models used for prediction: These determine how a system responds to stimuli or a change in the system (Finkelstein and Carson, 1985). Explanatory models: These kinds of models also show the relationship between variables but in addition to this, they explain the cause of the relationship (Gold, 1977).

III. Model formulation and validation.

Formulation of a model involves defining the purpose for which the model is being built and gathering information on the laws and theories that are relevant to the purpose of the model. It also involves collecting the appropriate data. (Finkelstein and Carson, 1985). After a model has been formulated, it must be tested to determine its usefulness. This depends on whether there is sufficient data to evaluate the model's validity and also on whether the model is consistent with the purpose for which it was formulated (Bergman, 1988).Testing of the model is done by comparing experimental data with the values that have been predicted by the model (Patterson et al., 1992).

Models that have been validated can be applied in many areas of nutrition research to explain systems which may otherwise have been too complex to explain.

CHAPTER 3

MODELING FACTORS AFFECTING PHYTATE PHOSPHORUS UTILIZATION IN GROWING BIRDS[1]

[1] Georgina Ankra-Badu, Samuel E. Aggrey, Gene Pesti, Remzi Bakalli and H.M Edwards. Submitted to *Poultry Science*, 9/21/2003.

ABSTRACT

The utilization of phytate phosphorus is affected by several factors including the genetic strain of the bird, bioavailability of calcium and phosphorus, level of Vitamin D, and the age of the bird. Though several studies have been conducted on the factors affecting phytate phosphorus utilization, no attempt has been made to model the factors affecting phytate phosphorus utilization. This study was conducted to develop a model that predicts the factors affecting phytate phosphorus utilization. A population of 1,004 Athens Canadian randombred birds from the mating of 40 males and 200 female chickens were used in the study. Chemical analysis was done to determine the amount of calcium, (gross) energy, nitrogen and phosphorus in feed and excreta. Phytate phosphorus, nitrogen and calcium contents were also determined by the NIRS method. Other traits measured were body weight (BW), BW gain, feed consumption, feed conversion ratio and inorganic phosphorus, organic phosphorus, calcium, nitrogen and energy intake. The PROC STEPWISE and PROC MAXIMUM R – SQUARED METHODS (SAS Institute, 1998) were used to determine traits that contributed significantly to phytate phosphorus bioavailability. The results showed that the main factors affecting phytate phosphorus bioavailability were energy, calcium and nitrogen bioavailability and body weight.

INTRODUCTION

Phosphorus is an important mineral required for growth and development because it is required for the metabolism of carbohydrates, amino acids, lipids and energy releasing compounds and also for the formation of bone. Phytate phosphorus is only available for absorption and utilization when the phosphate groups are removed from the inositol molecule by the enzyme phytase or intestinal phosphatase (Harland and Morris, 1995). The amount of available phosphorus required by the broiler chicken depends on age and ranges from 0.45 % to 0.3 % per 100g of the diet (NRC 1994). Poultry diets are mostly made up of ingredients like cereals and seeds. The main sources of phosphorus in these feeds is phytate or phytic acid. Phytates are largely unavailable to poultry because they lack intestinal phytase which is required to hydrolyze phosphate groups from the phytic acid molecule. The unutilized phosphorus is excreted in manure and sometimes causes environmental pollution when applied to land.

Several studies have been conducted to improve phytate phosphorus bioavailability in poultry. Phytate phosphorus utilization has been found to vary widely and ranges from zero (Nelson, 1976) to more than 50% (Mohammed et al. 1991). Research has shown that phytate phosphorus utilization depends on several factors, including the amount of Vitamin D in the diet (Mohammed et al., 1991), Calcium to Phosphorus ratio (Liu et al., 1998), type of ingredient (Pointillart et al., 1984), bioavailability of calcium and phosphorus and age of the bird (Kornegay, 1996), genetic strain of the bird (Edwards, 1983) sex of the bird and energy levels (Lillie et al. 1964).

The objective of this paper is to model factors affecting phytate phosphorus utilization in growing birds

MATERIALS AND METHODS

I. Experimental birds

A population of 1,004 birds was established from a mating of 40 males and 200 female chickens from an Athens Canadian random bred population. These birds were transferred into individual cages when they were 4 weeks old. After 3 days of acclimatization feed intake was measured and excreta produced in 3 consecutive days were collected. Chemical analysis of phytate phosphorus in feed and excreta was done by the method described by Latta and Eskim (1980), analysis of nitrogen was done by the method described by AOAC (1995) and calcium in the feed and excreta was determined by the method of Hill (1995). One hundred fecal samples and a representative sample of the feed were scanned in a Feed and Forage Analyzer Model 5000 with NIRS- 2 Software (Foss America, Eden Prairie, MN55344). The samples were used to create the calibration curves for the near infra red spectrophometer (NIRS). Based on these calibrations, phytate phosphorus, nitrogen and calcium contents were also determined by the NIRS method.

Body weights (BW) were measured before and after the feeding period. From 3-4 weeks of age, the birds were fed a practical diet containing 3.20 kcal/kg metabolizable energy, 20 % crude protein, 0.90% calcium, 0.675% total phosphorus and 0.45% available phosphorus. The supplemental source of phosphorus was reduced and the total phosphorus adjusted down to 0.35% during the acclimatization and excreta collection periods. Other traits that were measured were body weight gain (BWG)?, feed consumption (FC), feed conversion ratio (FCR), and inorganic phosphorus, organic phosphorus, calcium, energy and nitrogen intake. Feed conversion ratio was calculated as the ratio of FC per BWG. The bioavailabilities of total phosphorus, phytate phosphorus, inorganic phosphorus, calcium, nitrogen and (gross?)energy were also computed as follows:

$$NBA = (A-B)/A * 100 \%$$

where NBA= the bioavailability of the nutrient, A = content of the nutrient in the feed (%) * feed intake (g), B = content of the nutrient in dried excreta (%) * dried excreta weight (g). Data were obtained on 1,044 birds.

II. Data editing and Statistical Analysis

Editing of data was done by obtaining descriptive statistics using the PROC MEANS and PROC UNIVARIATE methods (SAS Institute, 1998). Data that was more than two standard deviations from the mean were identified as outliers and removed from the data. Body weight, BWG, FC FCR, relative growth (RG), total phosphorus bioavailability (TPB), phytate phosphorus bioavailability (PPB), calcium bioavailability (CaB), nitrogen bioavailability (NB), Energy bioavailability (EB), inorganic phosphorus intake (IPI) organic phosphorus intake (OPI), and calcium intake (CaI), energy intake (EI), nitrogen intake (NI), total phosphorus intake (TPI) and nitrogen to energy ratio (NE) were measured. The PROC GLM method was used to correct for hatch effects. The model used was:

$$Y_{ij} = \mu + H_i + \varepsilon_{ij}$$

where Y_{ij} is the individual observation for the trait, μ= overall mean, H_i = hatch group effect (i = 1, 2……6) and ε_{ij} = individual total random effect.

The PROC GLM (SAS Institute, 1998) was used to test the significance for the significance of sex for the traits. Body weight, BWG, FC, FCR, CaB, NB, IPI, OPI, CaI, EI, NI, TPI and NE were significantly different ($P \leq 0.05$) for different sexes. After editing, 919 individuals comprising of 476 males and 443 females were used for the analyses.

III. Modeling

The PROC STEPWISE and PROC MAXIMUM R-SQUARED (SAS Institute, 1998) were used to determine traits that significantly contributed towards phytate phosphorus bioavailability. The PROC STEPWISE method (SAS Institute, 1998) selects variables by adding variables that have a significant F statistic to the model. After all the variables have been added, the method deletes any variables that do not produce a significant F statistic at a given value specified in the model statement. The MAXR-SQUARED procedure (SAS Institute, 1998), finds the best one variable model to the best nth variable model. The variables that lead to the greatest increase in R^2 value are kept in the model.

RESULTS

Least square means and standard deviations for growth, feed utilization and bioavailability traits of the different sexes are presented in Table 1. There were significant differences ($P \leq 0.05$) between the means for the different sexes for BW, BWG, feed conversion, feed conversion ratio, and the bioavailabilities of calcium and nitrogen. The effect of each trait measured, on PPB is presented in Table 2. The R^2 values for BW4 weeks were 0.0037 and 0.04 for females and males respectively. Males with high body weight appeared to use phytate phosphorus less efficiently than birds with low BW4. Body weight gain had low R^2 values but had a significant effect on phytate phosphorus utilization. The R^2 values for relative growth were approximately 0.03 for both males and females. There was slightly positive linear relationship between relative growth and phytate phosphorus bioavailability for both males and females. The relationship between FC and PPB was slightly negative. High feed consumption resulted in a decrease in PPB. The R^2 values were 0.038 and 0.0004 for males and females, respectively. Feed conversion ratio also showed a negative relationship with PPB with R^2 values were 0.08 and 0.02 for males and females, respectively.

The relationship between PPB and nutrient intake and bioavailability are presented in Figures 1 to 30. From Figures 11 and 12, total phosphorus bioavailability had a highly positive linear relationship with phytate phosphorus utilization with R^2 values of 0.61 and 0.68 for males and females, respectively.

High calcium bioavailability for both sexes led to a corresponding increase in phytate phosphorus utilization with R^2 values of 0.40 and 0.39 for females and males, respectively.

When the Nitrogen to energy ratio in the feed was high, there was a decrease in phytate phosphorus utilization. R^2 values for males and females were 0.038 and 0.0023, respectively. Nitrogen bioavailability also had a positive linear relationship with efficiency of phytate phosphorus utilization with R^2 values of 0.31 and 0.52 for males and females, respectively. In both males and females, birds that utilized nitrogen effectively also had high phytate phosphorus bioavailability. The relationship between EB and PPB was positive. Energy bioavailability had high R^2 of 0.55 and 0.68for males and females, respectively and birds that utilized energy efficiently, also utilized phytate phosphorus well.

From Tables 3-5, the traits that significantly affected PPB were energy, calcium and nitrogen bioavailabilities as well as initial BW (BW at 4 wk). The predicting model for the females was:

$$PPB = 0.2061\ CaB + 0.2514\ NB + 2.4652\ EB - 0.0163\ BW4 - 186.7653. \quad R^2 = 0.74$$

and that for males was:

$$PPB = 0.2038\ CaB + 2.393\ EB - 0.0170\ BW4 - 170.9868. \quad R^2 = 0.64$$

From Tables 3 and 5 it is evident that calcium and energy bioavailability and body weight made highly significant contributions to the model for both males and females. Nitrogen bioavailability however, did not contribute significantly to the model for the males and therefore was not included in the model by the stepwise selection process. Nitrogen bioavailability was not significant for the males using the Maximum R- squared method (SAS Institute, 1998) (Table 5a) as well.

DISCUSSION

Table 1 shows that there was a significant difference between the sexes for body weight, body weight gain, feed conversion, feed conversion ratio, calcium and nitrogen bioavailability, and inorganic and phytate phosphorus levels .Results from previous studies indicate that male birds have a higher requirement for phosphorus (Lillie et al. 1964) and retain more phytate phosphorus than females at various dietary calcium levels (Edwards et al. 1989), however, there was no difference between the sexes from the present studies. This might be because the birds were four weeks of age and therefore sexually immature. The effect of age on PPB is well documented (Nelson, 1967; Kornegay, 1996). The mineral requirements of birds are also determined by sex (Georgievski, 1982). The differences in nutrient requirements are due to differences in body composition and growth rate (Fandrejewski and Rymark, 1986).

The effects of each trait on PPB are shown in Table 2. Though the R^2 values for body weight for both males and females were low, birds with high body weight at 4 weeks utilize phytate phosphorus less efficiently than birds with low body weight (Figures 1 and 2). Birds that consume more feed have a higher rate of passage than birds that consume less feed and this does not allow for efficient utilization of phytate phosphorus (Zhang et al. 2003). High feed consumption resulted in decreased phytate phosphorus utilization; whereas relative growth showed a positive relationship with PPB, therefore intake traits have to be corrected for body weight differences to account for differences in maintenance energy requirement and efficiency of utilization. Feed conversion ratio also showed a negative relationship with PPB and this may be related to the relationship with high feed consumption.

Bioavailability of calcium was positively related to PPB (Figures 13 and 14) whereas CaI is negatively related to PPB (Figures 21 and 22).There is an interaction between the metabolism of phosphorus and calcium. The homeostatic metabolism of both is controlled by similar mechanisms (Georgievski, 1982). An improvement in the retention of calcium in the skeleton is accompanied by improved retention of phosphorus because calcium is only well utilized for skeletal growth when phosphorus is available at the same time (Helander et al. 1996; Underwood and Suttle, 1999). Cholecalciferol, the active form of Vitamin D stimulates calcium uptake and improves calcium absorption. This would lead to an improvement in PPB since calcium is no longer available to form insoluble complexes (Georgievski, 1982; Underwood and Suttle, 1999). An increase in calcium absorption also leads to an increase in the levels of calcium and phosphorus in the serum and favors the deposition of these minerals in the bone. Though calcium and phosphorus are both required for the formation of bone, high levels of calcium intake would enable excess calcium to form insoluble complexes with phosphorus and make them unavailable. High levels of calcium also inhibit the action of intestinal phytase. Edwards and Veltmann (1983) showed that phosphorus retention depends on the levels of both calcium and phosphorus in the diet and is highest when the levels of these elements are low. The relationship between CaI and PPB is similar to observations made by Ballam et al. (1985) and Mohammed et al. (1991) on chickens. Thus, excess dietary calcium and phosphorus leads to a reduction in their bioavailability.

Birds that utilized nitrogen efficiently showed a corresponding increase in PPB (Figures 15 and 16). This may have been due to the fact that an increase in phytate phosphorus digestibility corresponds to an increase in protein availability (Ravindran et al., 2000). Shohl (1939) reported a slight relationship between nitrogen and mineral metabolism. A low nitrogen to phosphorus ratio causes an excess in phosphorus, which is excreted. Nitrogen retention is also limited when phosphorus is a limiting factor in growth and may be due to the fact that phosphorus plays a significant role in the synthesis of amino acids and proteins. Generally, phosphorus is more available on a low protein diet than a high protein diet. Hegsted et al. (1981) in a study on humans found that an increase in dietary protein requires an increase in dietary phosphorus in order to maintain bone equilibrium because a high protein intake causes an increase in urinary calcium. They also found that the effect of a change in phosphorus intake on calcium balance depended on the level of protein in the diet. In this study, phytate phosphorus retention is better with high nitrogen retention but decreases with high nitrogen intake. It is therefore evident that nutrient intake and retention have an opposite relationship with PPB. The effect of nitrogen by calcium interaction on phytate phosphorus was however, not significant. The nitrogen retention in this study did not take into account endogenous nitrogen sources in the excreta, and may be taken as a crude estimate of protein retention.

An increase in inorganic phosphorus intake caused a reduction in PPB (Figures 19 and 20) and has been confirmed in several studies (Sanders et al. 1992; Lei et al. 1993). High levels of inorganic phosphorus (IP) generally lead to a decrease in PPB due to their inhibitory effect on phytase activity (Ravindran, 2000). Wise (1983) also observed that extra inorganic phosphorus might inhibit phytate absorption and phytase production by the mucosa of the chicken. The decrease in PPB from high phosphorus intake can also attributed to a negative feedback mechanism caused by excess or adequate phosphorus. However, the effect of inorganic phosphorus utilization on phytate phosphorus bioavailability depends on the source of inorganic phosphorus (Ballam et al., 1984, 1985). Phosphorus compounds that tend to increase intestinal pH reduce PPB because high pH tends to decrease the absorption of calcium thus favoring the formation of calcium- phytate complexes.

From the predictive model, energy bioavailability had a great influence on PPB (Table 2) with high R^2 values of 0.55 and 0.68 for the males and females respectively, similar to the calcium, nitrogen and energy intake and bioavailability. Phosphorus plays an important role in the utilization and transfer of energy (Underwood and Suttle, 1999). Phosphorus is vital for the formation of adenosine triphosphate and adenosine diphospate, which are required for the release of energy. Increasing the energy level of the diet leads to a decrease in feed intake therefore increasing the energy levels of the diet may increase phosphorus requirement (Waibel et al. 1977). Some reports indicate that increased energy levels do not affect phosphorus requirement (Pepper et al. 1955; Lillie et al. 1964) while others show that changing dietary energy changes phosphorus requirement (Waldroup et al. 1974, 1975). However, from this study, birds that utilize PPB efficiently also utilize energy efficiently and vice versa.

SUMMARY

The major factors affecting phytate phosphorus bioavailability are body weight at 4 weeks, calcium bioavailability, nitrogen bioavailability and energy bioavailability. Nitrogen bioavailability did not affect PPB in males. High intake of calcium, energy and nitrogen decreased phytate phosphorus bioavailability but increased bioavailability of these nutrients had the opposite effect

Table 1. Least square means and standard deviation for growth and feed utilization traits in a random mating chicken population.

	Male			Female		
Traits	N = 476			N = 443		
Body weight (4wks) (g)	308.06	±	41.31[a]	273.73	±	34.58[b]
Body weight gain (g)	47.68	±	10.69[a]	41.22	±	41.22[b]
Relative growth (g/g/wk)	0.16	±	0.04	0.15	±	0.04
Feed consumption (g/3d)	108.02	±	14.34[a]	96.08	±	12.64[b]
Feed conversion ratio (g/g)	2.34	±	0.40[a]	2.42	±	0.43[b]
Phytate phosphorus bioavailability (%)	31.86	±	6.24	32.19	±	7.34
Calcium bioavailability (%)	23.59	±	9.86[a]	25.29	±	9.59[b]
Nitrogen bioavailability (%)	59.99	±	3.66[a]	58.68	±	4.75[b]
Energy bioavailability (%)	82.56	±	1.37	82.54	±	1.61
Inorganic phosphorus intake (g)	0.03	±	0.00[a]	0.02	±	0.00[b]
Phytate phosphorus intake (g)	0.35	±	0.05[a]	0.31	±	0.04[b]
Calcium intake (g/3d)	1.13	±	0.15[a]	1.01	±	0.13[b]
Energy intake (kcal/3d)	453.22	±	60.18[a]	403.11	±	53.05[b]
Nitrogen intake (g/3d)	3.44	±	0.45[a]	3.06	±	0.40[b]

[ab]Traits for males and females with no common superscript are significantly different ($P \leq 0.05$).

Table 2. The effect of single traits on Phytate phosphorus bioavailability in a random mating chicken population

	Male		Female	
Traits	R^2	P ≤ | t	R^2	P ≤ | t
Body weight (4weeks) (g)	0.04	<0.0001	0.0037	0.1999
Body weight gain (g)	0.01	0.0122	0.01	0.0279
Relative growth (g/g/wk)	0.03	0.0002	0.03	0.0003
Feed Consumption (g)	0.038	<0.0001	0.0004	0.6863
Feed Conversion Ratio (g/g)	0.07	<0.0001	0.02	0.0036
Calcium bioavailability (%)	0.39	<0.0001	0.40	< 0.0001
Nitrogen bioavailability (%)	0.31	<0.0001	0.52	< 0.0001
Energy bioavailability (%)	0.55	<0.0001	0.68	< 0.0001

Table 3a. Summary of Stepwise Selection for an equation describing Phytate phosphorus bioavailability (males).

Step	Trait	Number of traits	Partial R^2	Model R^2	F value	P> F
1	Energy bioavailability	1	0.5220	0.5220	583.99	<0.0001
2	Calcium bioavailability	2	0.0789	0.6309	101.15	<0.0001
3	Body weight at 4 weeks	3	0.0121	0.6430	15.99	<0.0001

Table 3b. Summary of Stepwise selection for an equation describing Phytate phosphorus bioavailability (females).

Step	Trait	Number of traits	Partial R^2	Model R^2	F value	P> F
1	Energy bioavailability	1	0.6802	0.6802	938.17	<0.0001
2	Calcium bioavailability	2	0.0452	0.7254	72.40	<0.0001
3	Nitrogen bioavailability	3	0.0086	0.7340	14.16	0.0002
4	Body weight at 4 weeks	4	0.0058	0.7398	9.84	0.0018

Table 4a. The best fit equation of factors affecting Phytate phosphorus bioavailability using Stepwise Selection (Males)

Variable	Parameter estimate	Standard error	F value	Pr > F
Intercept	- 182.39502	11.90373	234.78	< 0.0001
Body weight at 4 weeks	-0.01673	0.00419	15.99	< 0.0001
Calcium bioavailability	0.20009	0.02045	95.77	< 0.0001
Energy bioavailability	2.60032	0.14604	317.03	< 0.0001

Table 4b. The best fit equation of factors affecting Phytate phosphorus bioavailability using Stepwise Selection (Females)

Variable	Parameter Estimate	Standard error	F value	Pr > F
Intercept	-186.76526	15.54630	144.32	< 0.0001
Body weight at 4 weeks	-0.01632	0.00520	9.84	0.0018
Calcium bioavailability	0.20608	0.02286	81.27	< 0.0001
Nitrogen bioavailability	0.25139	0.07071	12.64	0.0004
Energy bioavailability	2.46516	0.23156	113.33	< 0.0001

Table 5a. The best fit equation describing factors affecting Phytate phosphorus bioavailability using Maximum R squared Improvement procedure (Males).

Variable	Parameter Estimate	Standard Error	F value	Pr > F
Intercept	-164.01797	15.86159	106.93	< 0.0001
Feed conversion ratio	0.68461	0.68461	1.28262	0.5938
Body weight gain	0.09363	0.06819	1.89	0.1704
Body weight 4 wk	-0.02040	0.00777	6.89	0.0089
Calcium bioavailability	0.20566	0.02130	93.21	< 0.0001
Nitrogen bioavailability	0.10123	0.07461	1.84	0.1755
Energy bioavailability	2.32044	0.22616	105.27	<0.0001
Relative growth	-14.54058	9.27640	2.46	0.1177

Table 5b. The best fit equation describing the factors affecting Phytate Phosphorus bioavailability using Maximum R squared Improvement procedure (Females)

Variable	Parameter Estimate	Standard Error	F Value	Pr > F
Intercept	-190.53779	16.16589	138.92	< 0.0001
Feed conversion ratio	1.72213	1.11581	2.38	0.1235
Body weight gain	0.03080	0.06330	0.24	0.6268
Body weight 4 wk	-0.01115	0.00863	1.67	0.1968
Calcium bioavailability	0.20841	0.02318	80.82	<0.0001
Nitrogen bioavailability	0.24934	0.07264	11.78	0.0007
Energy bioavailability	2.42893	0.23806	104.10	<0.0001
Relative growth	16.36754	9.52872	2.95	0.0866

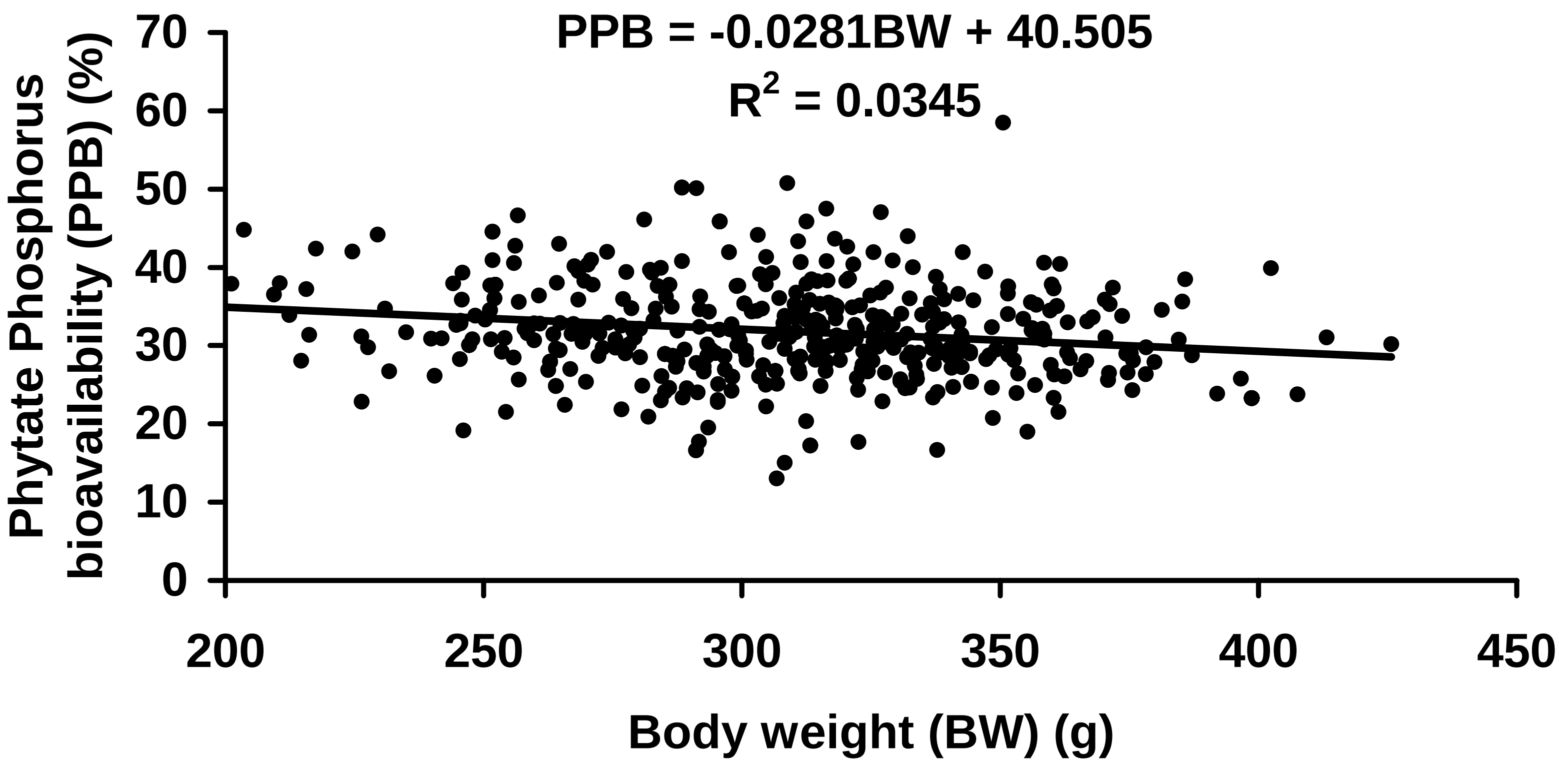

Figure 1. Effect of body weight on Phytate phosphorus bioavailability (Males)

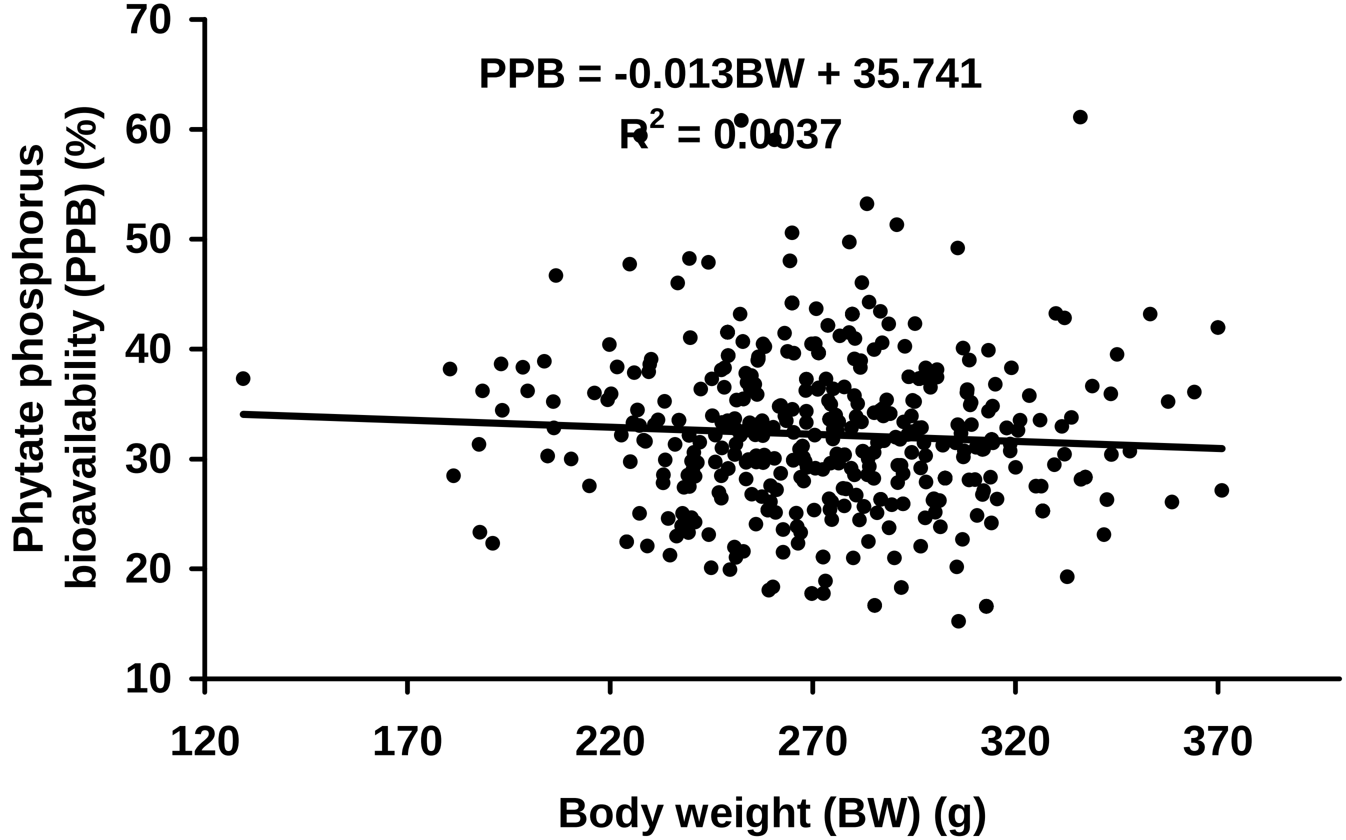

Figure 2. Effect of body weight on Phytate phosphorus bioavailability (Females)

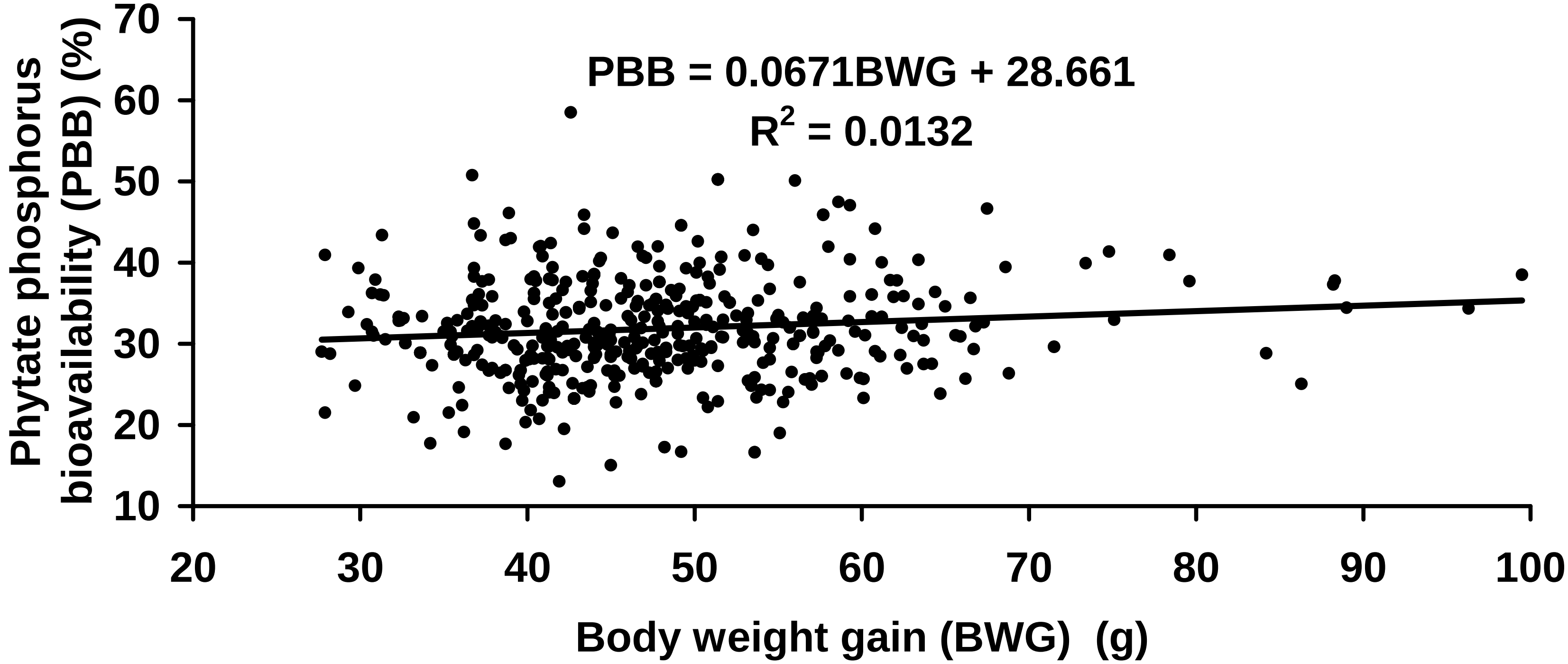

Figure 3. Effect of body weight gain on Phytate phosphorus bioavailability (Male)

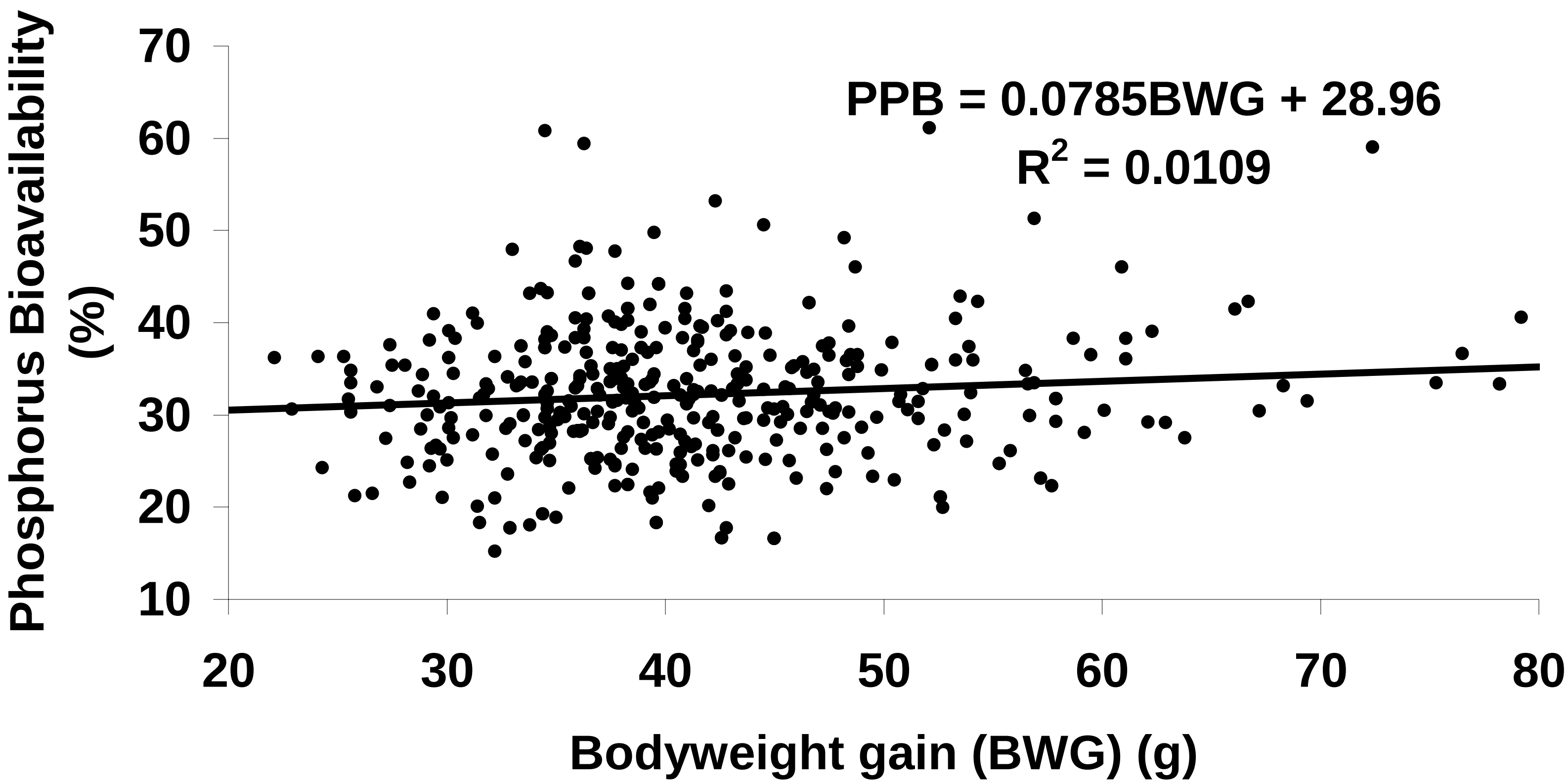

Figure 4. Effect of body weight gain on phytate phosphorus bioavailability (females)

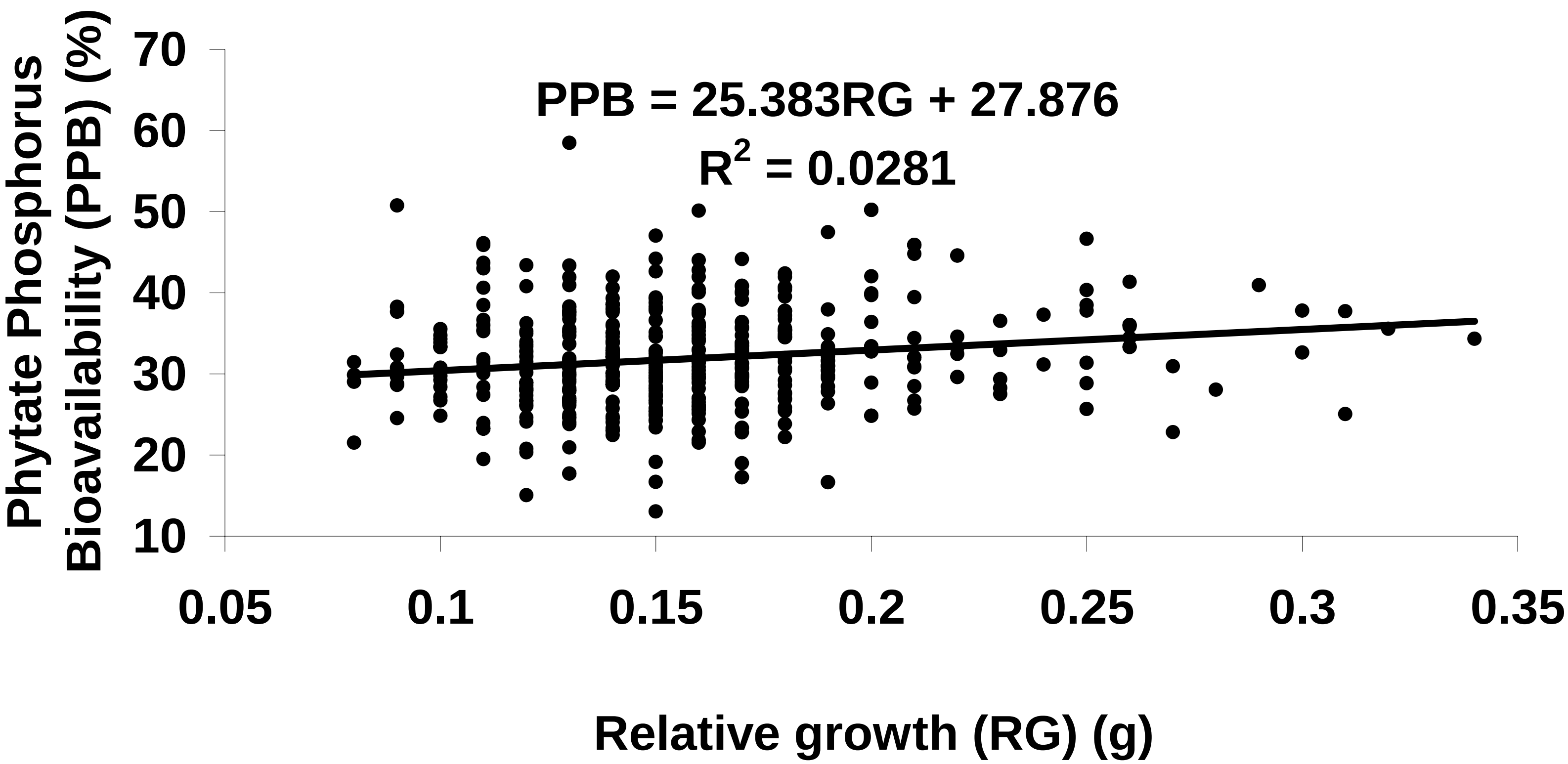

Figure 5. Effect of relative growth on phytate phosphorus bioavailability (males)

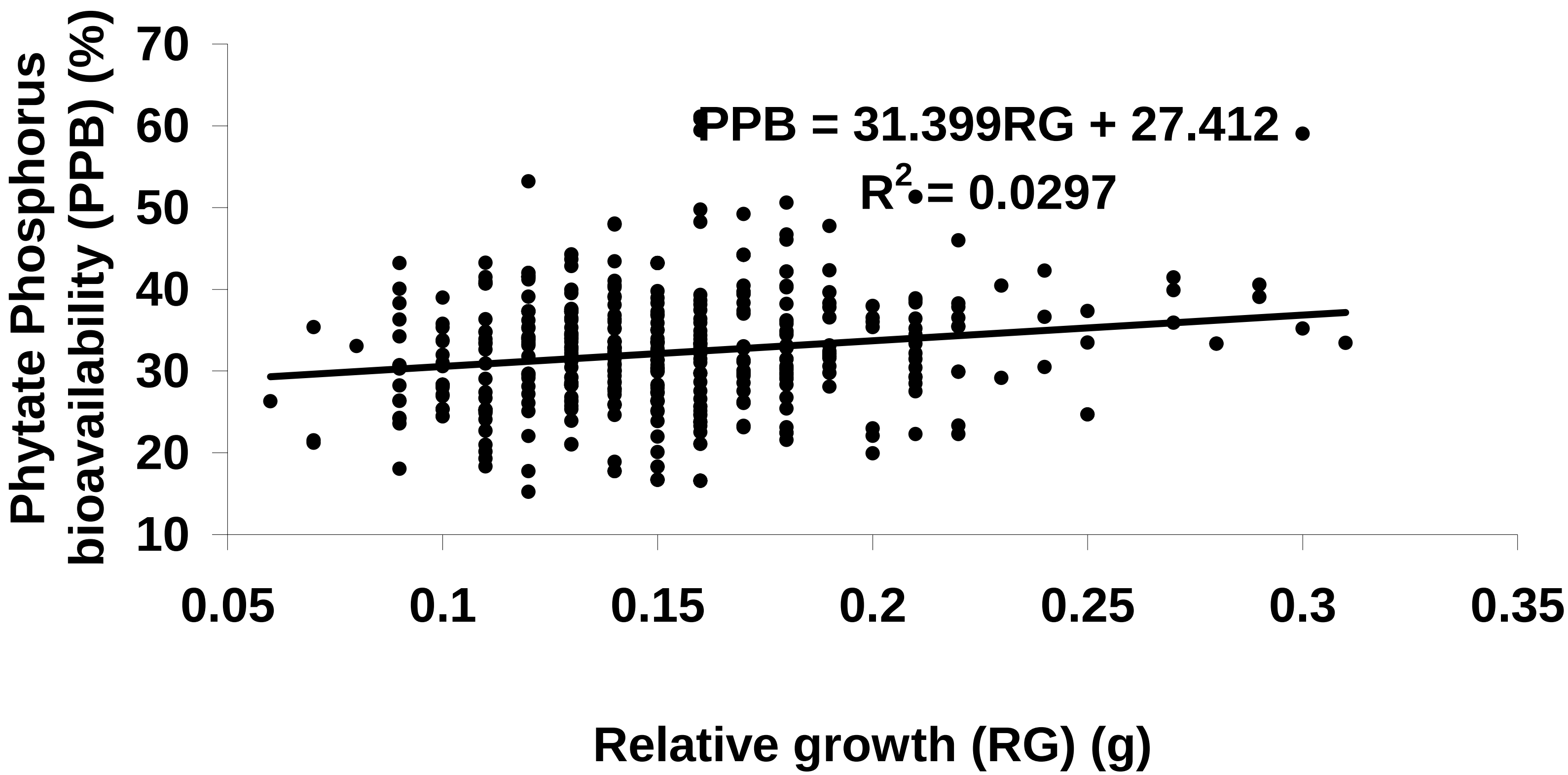

Figure 6. Effect of relative growth on phytate phosphorus bioavailability (females)

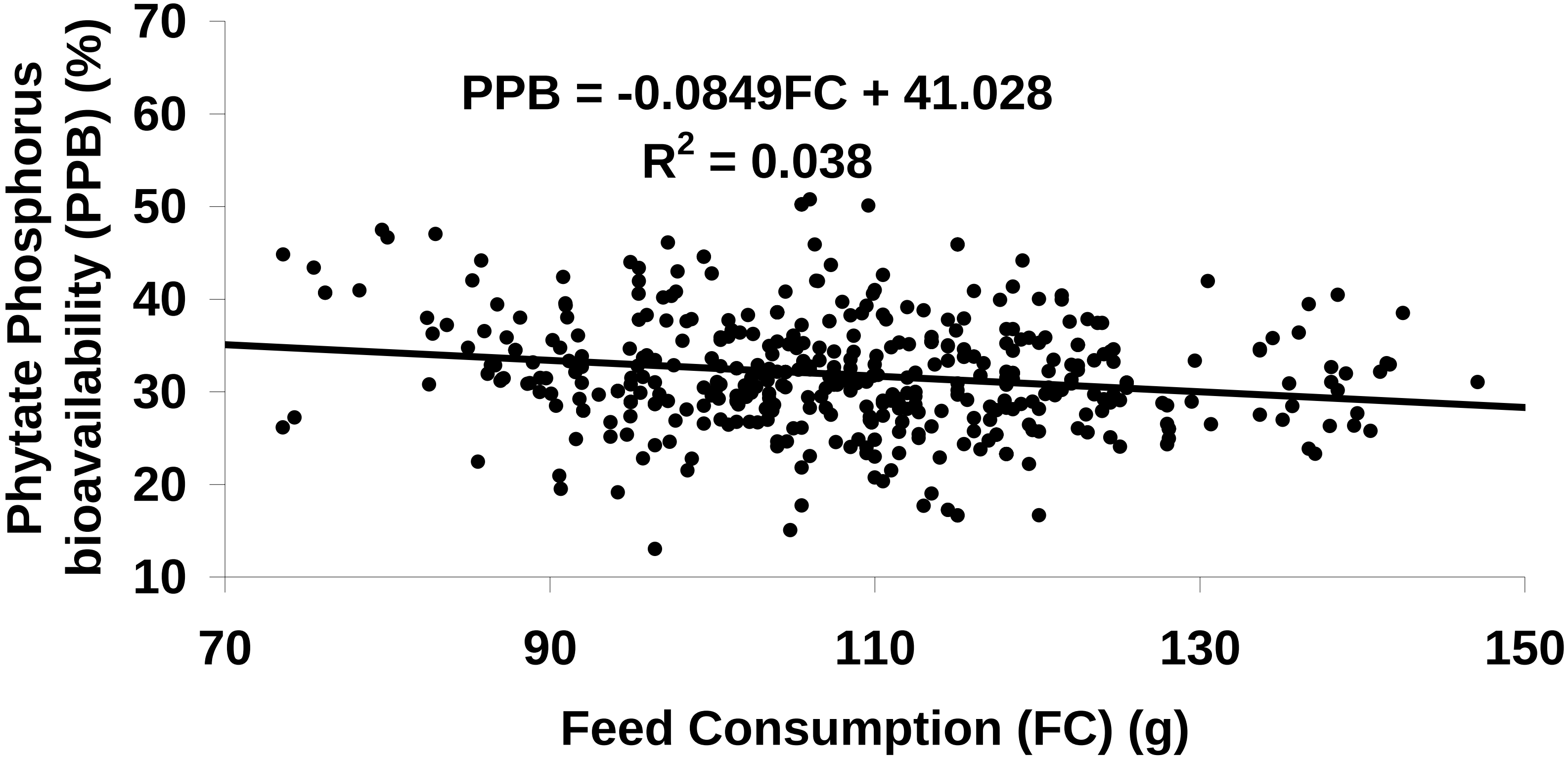

Figure 7. Effect of feed consumption on phytate phosphorus bioavailability (males)

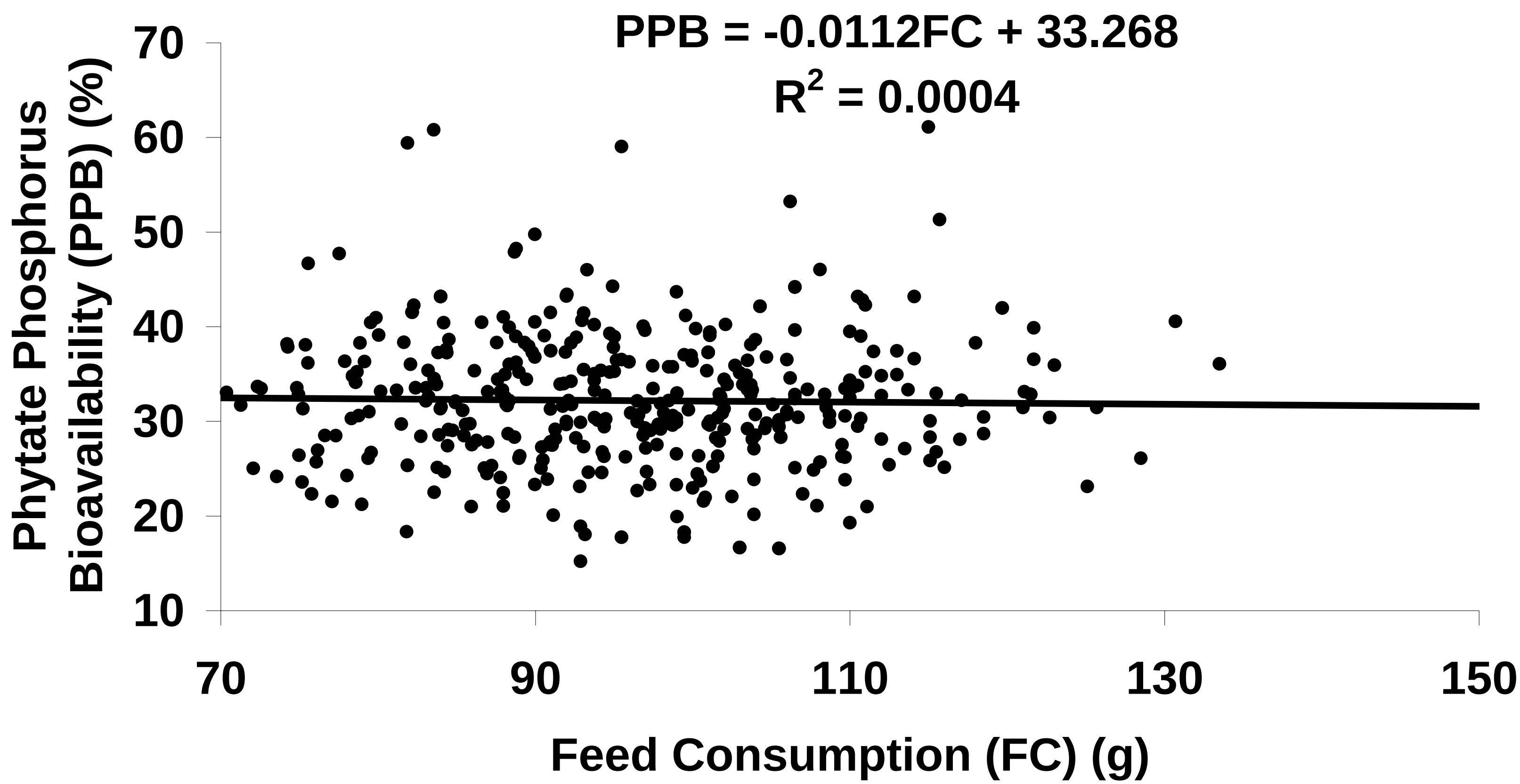

Figure 8. Effect of feed consumption on phytate phosphorus bioavailability (females)

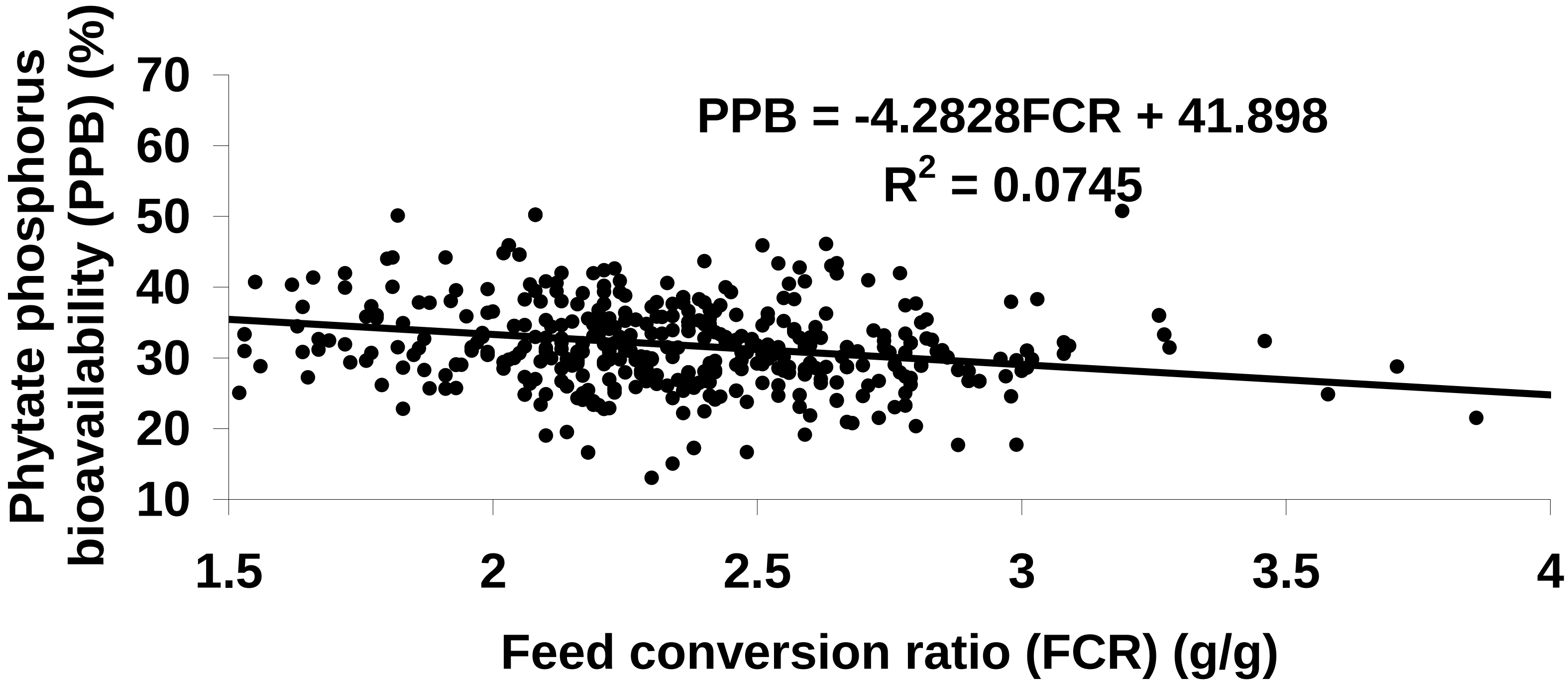

Figure 9. Effect of feed conversion ratio on phytate phosphorus bioavailability (males)

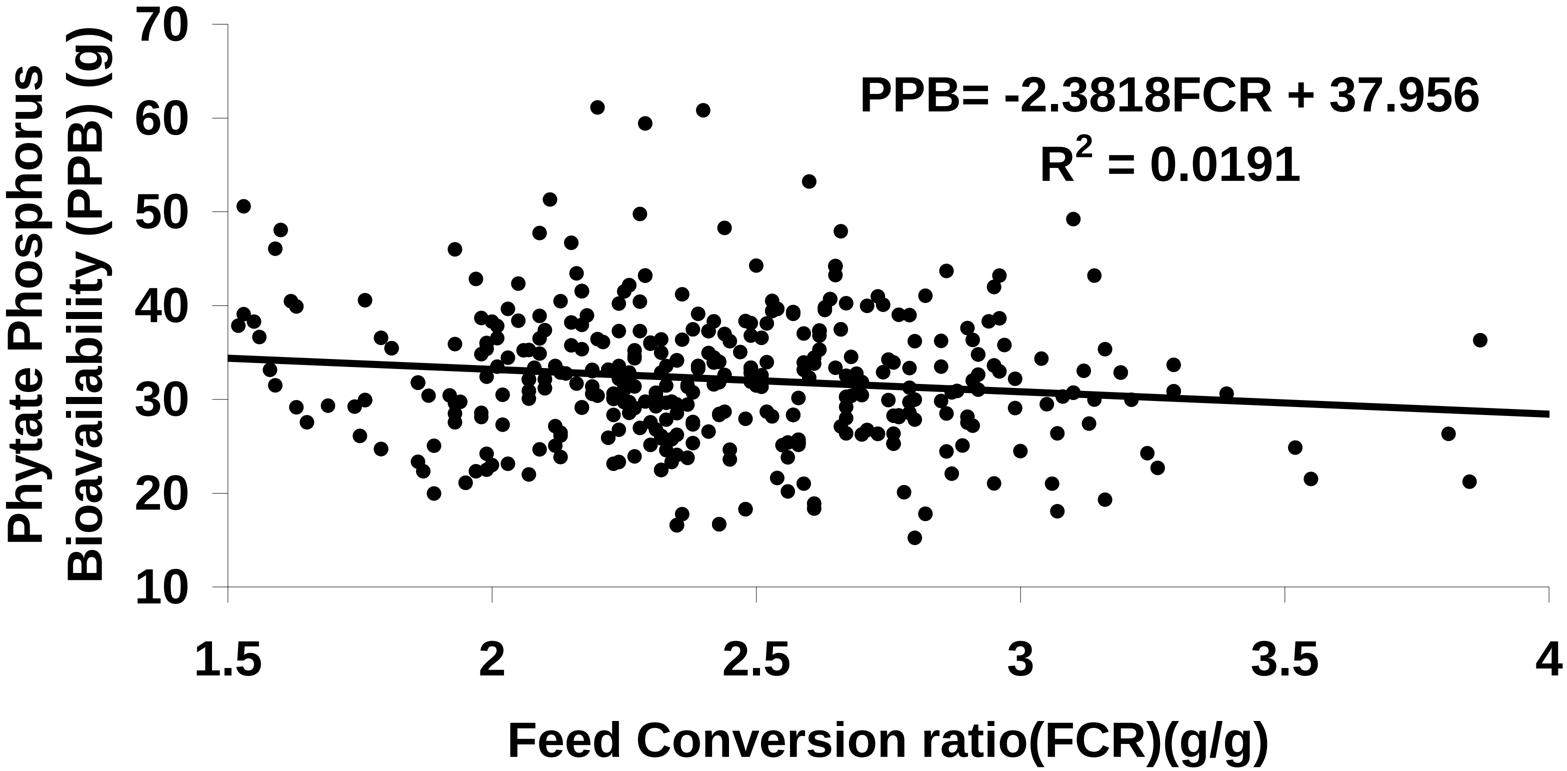

Figure 10. Effect of feed conversion ratio on phytate phosphorus bioavailability (females)

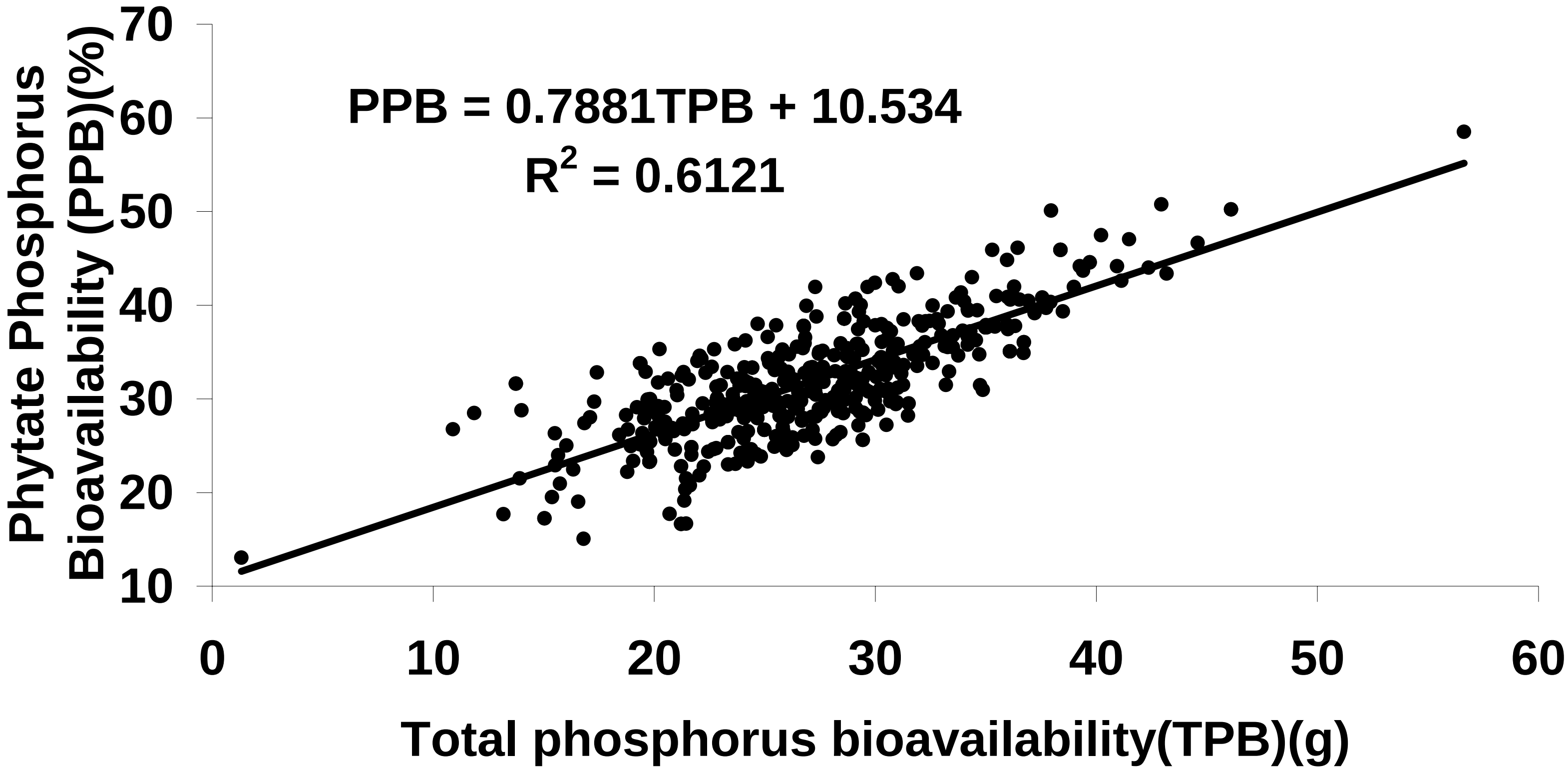

Figure 11. Effect of total phosphorus bioavailability on phytate phosphorus (males)

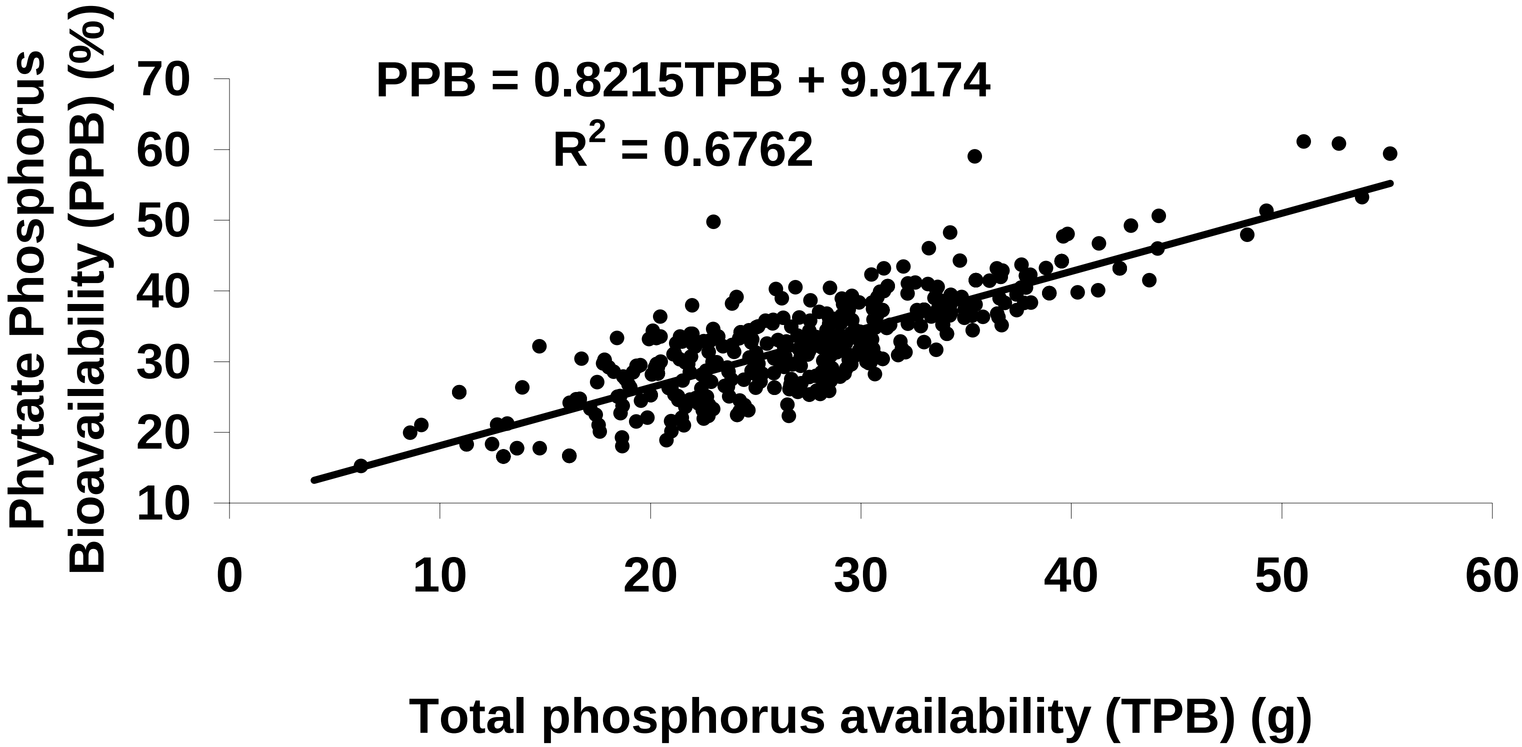

Figure 12. Effect of total phosphorus bioavailability on phytate phosphorus (females)

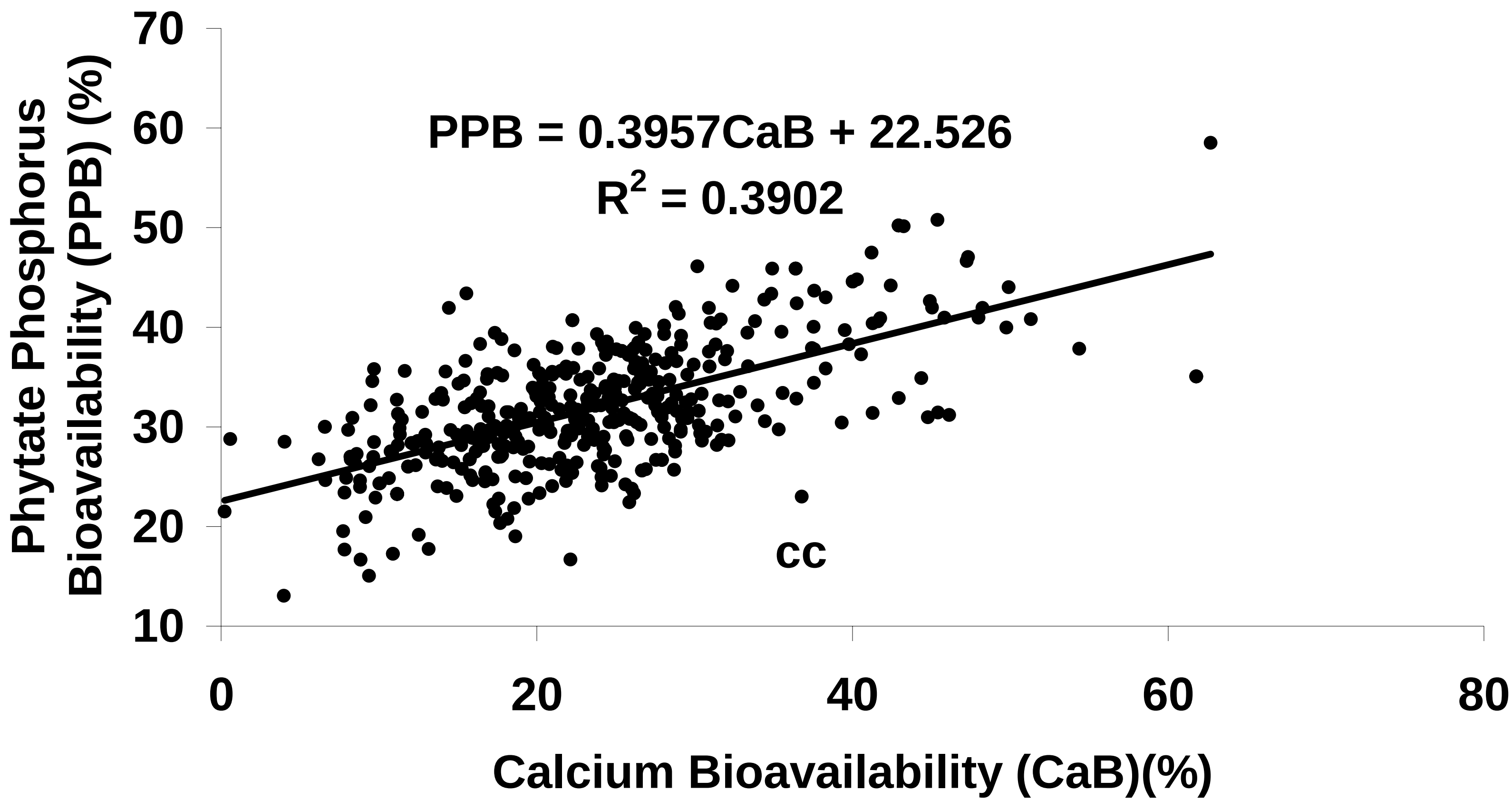

Figure 13. Effect of calcium bioavailability on phytate phosphorus (males)

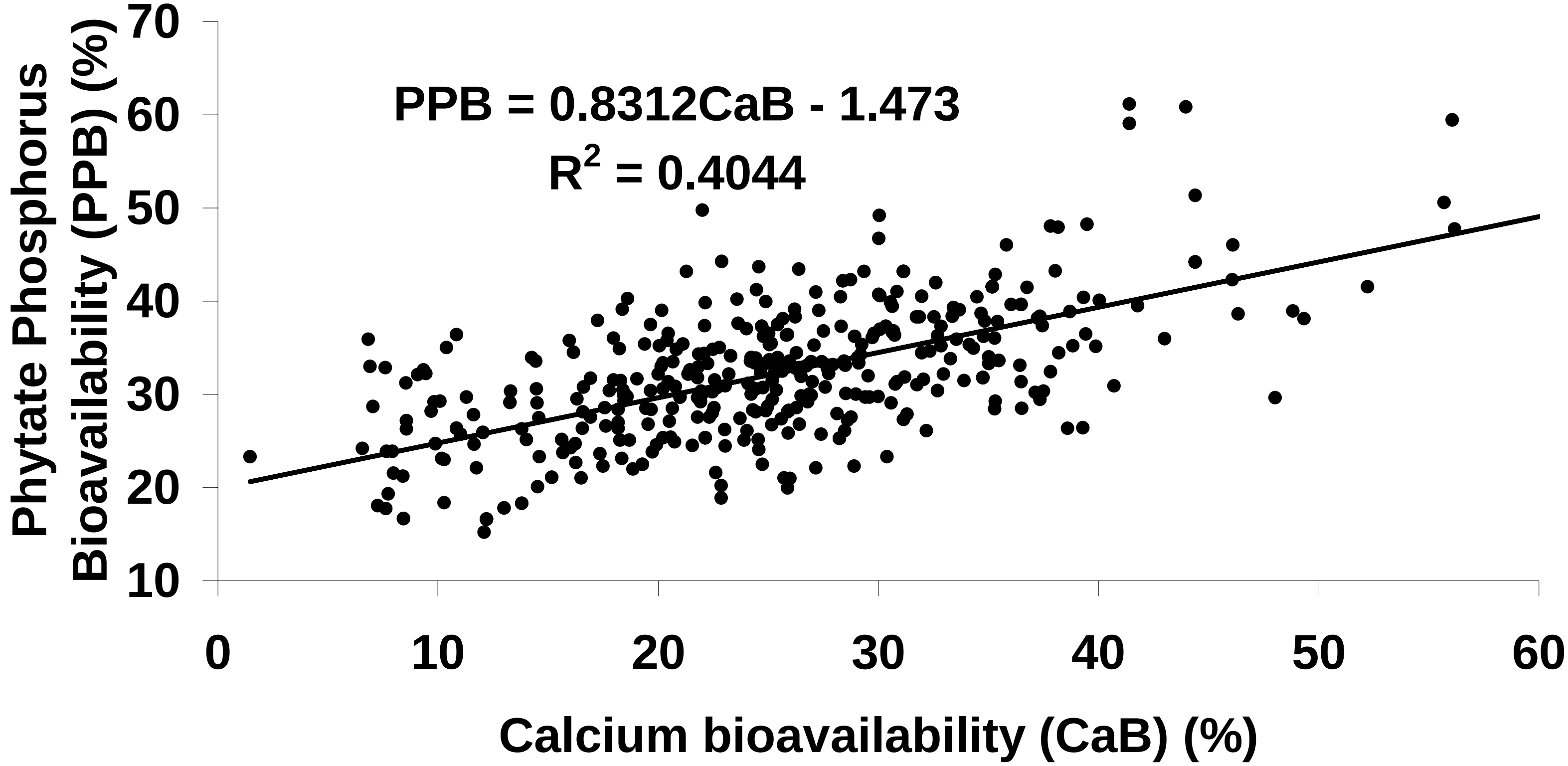

Figure 14. Effect of calcium bioavailability on Phytate Phosphorus (females)

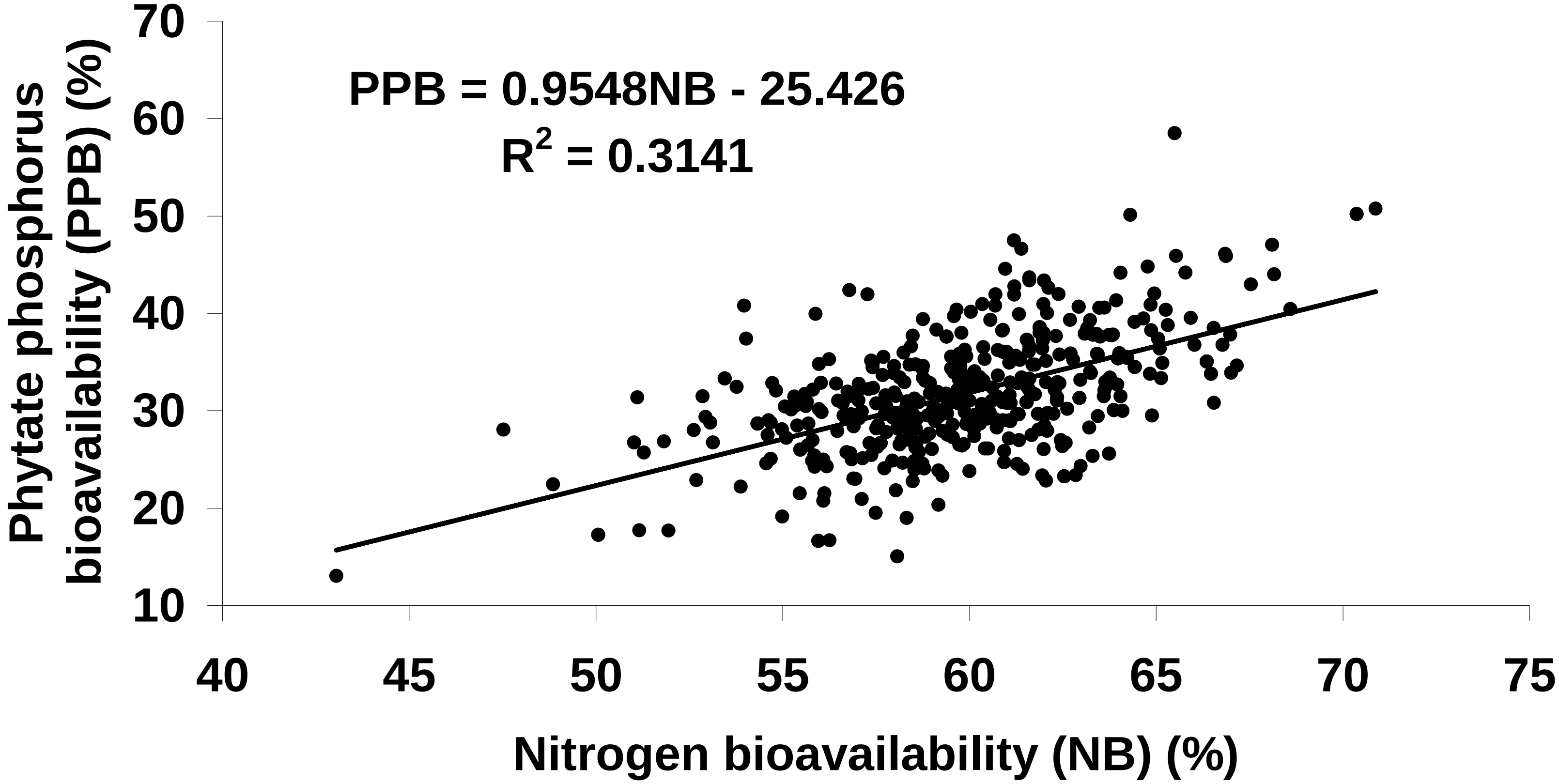

Figure 15. Effect of nitrogen bioavailability on phytate phosphorus (males)

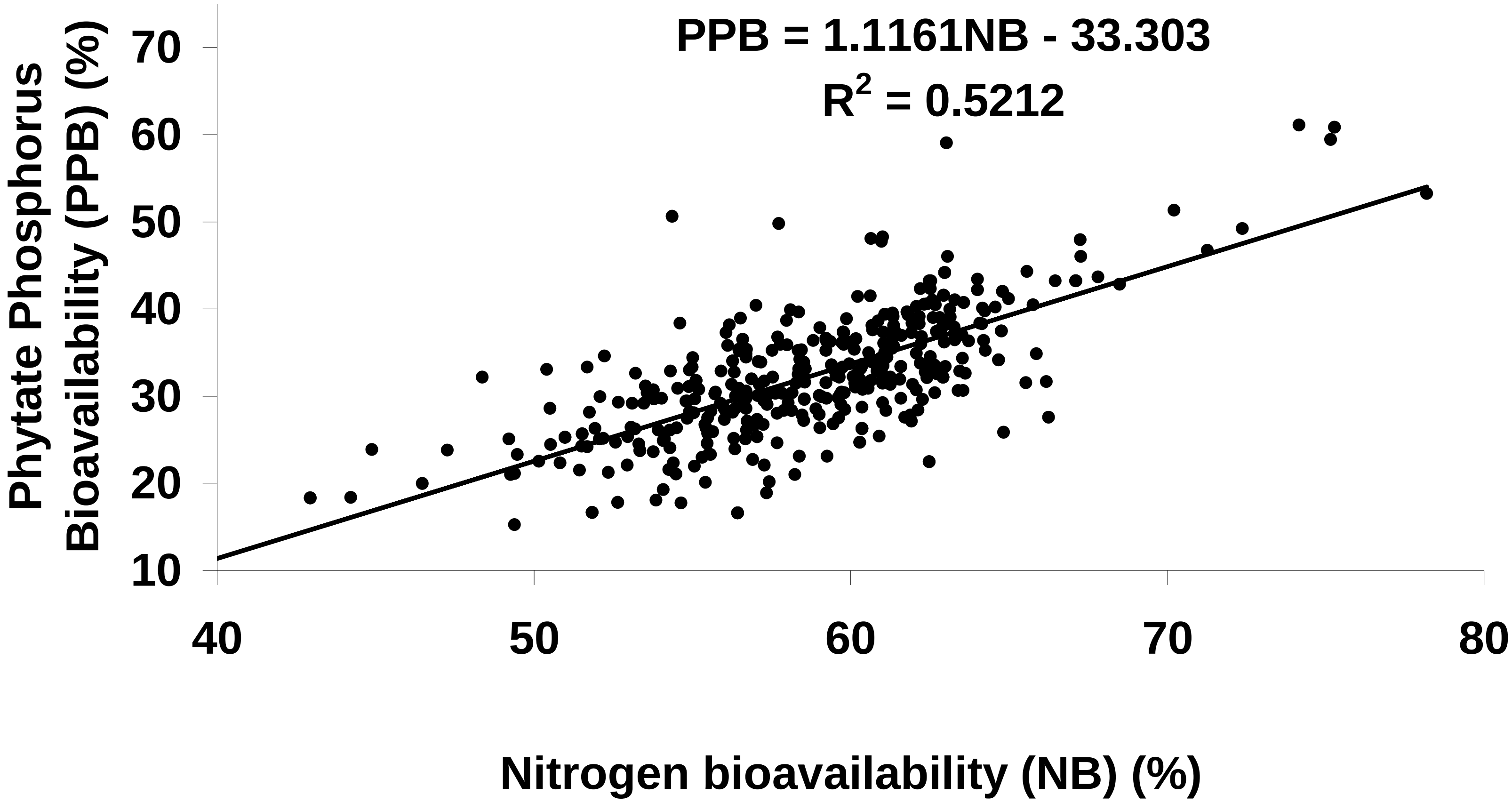

Figure 16. Effect of Nitrogen Bioavailability on Phytate Phosphorus (females)

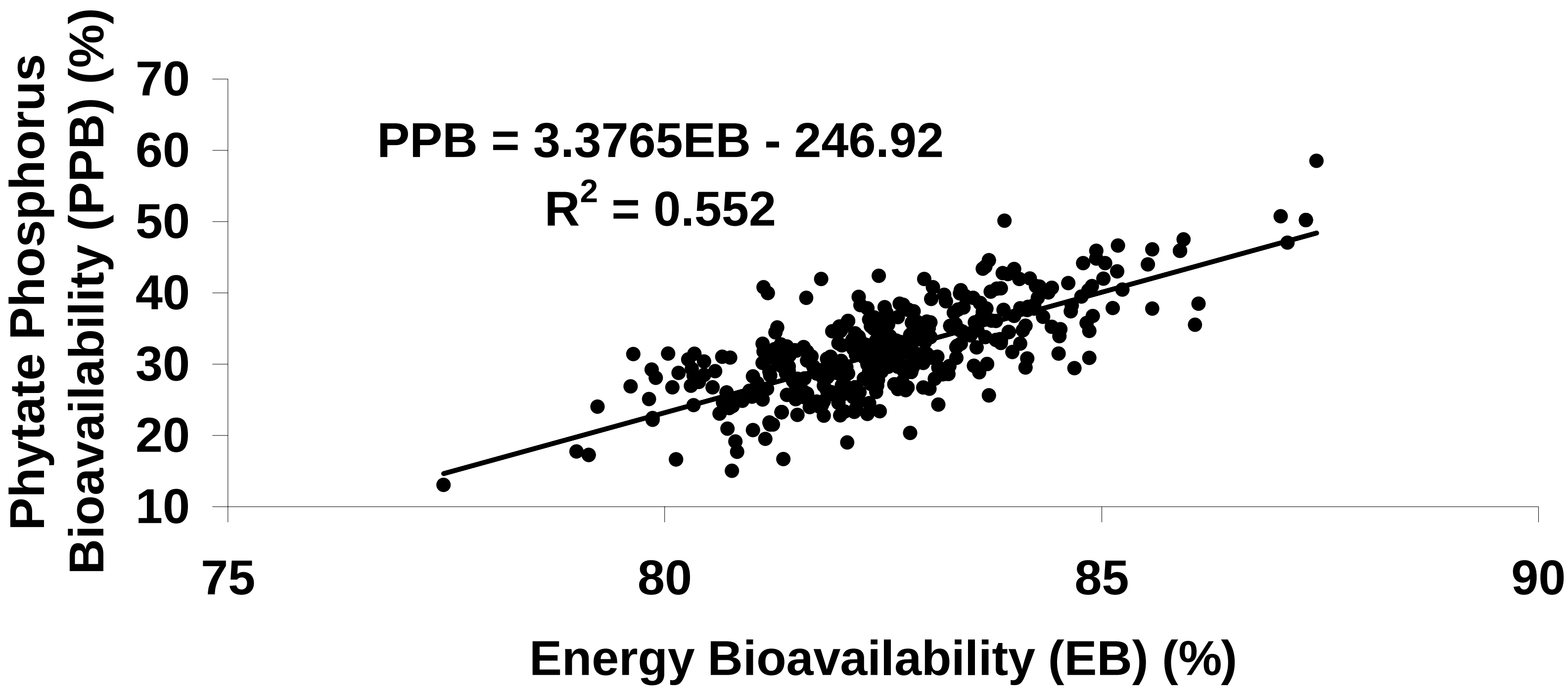

Figure 17. The effect of energy bioavailability on phytate phosphorus (males)

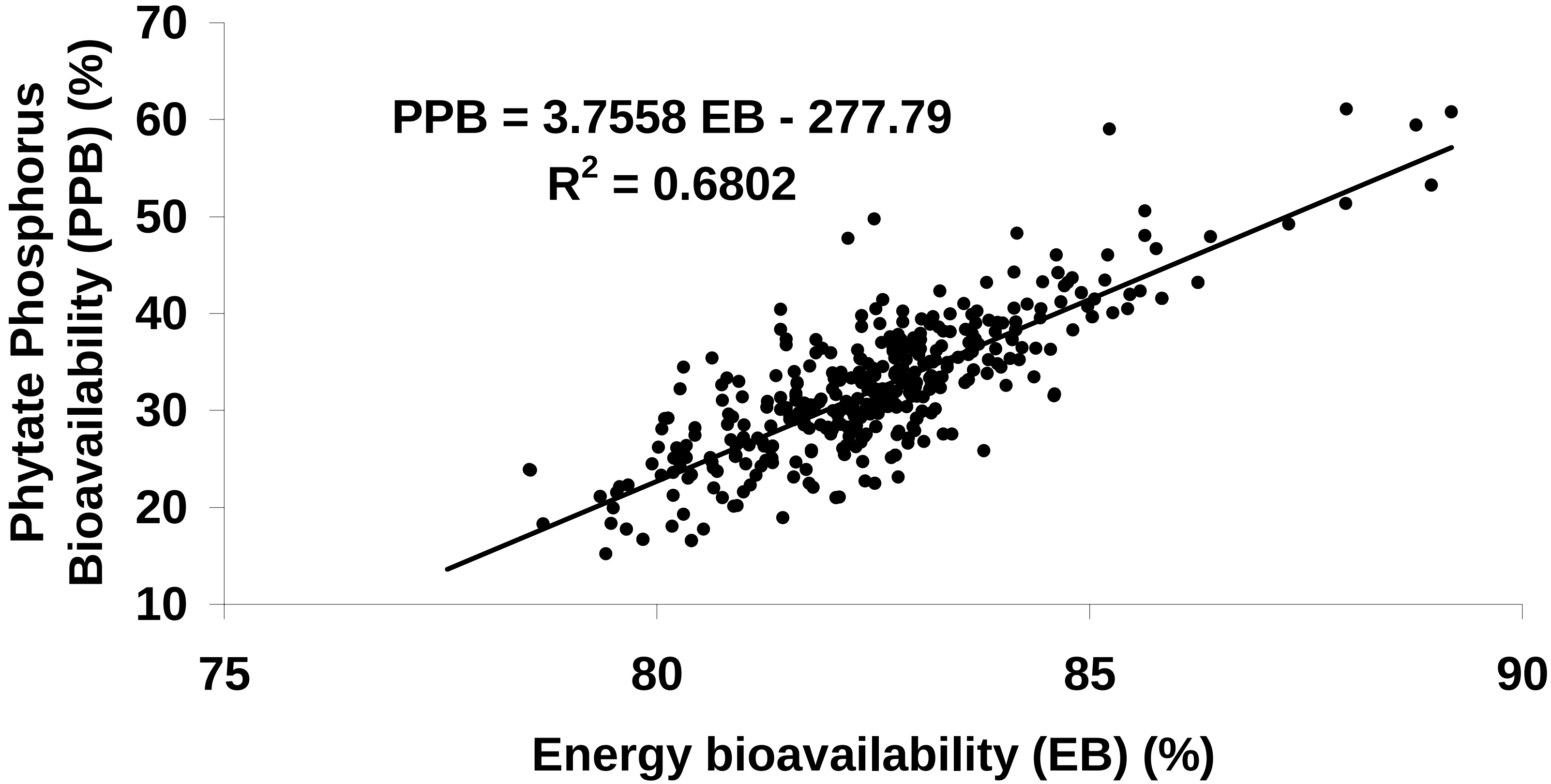

Figure 18. The Effect of Energy Bioavailability on Phytate Phosphorus (females)

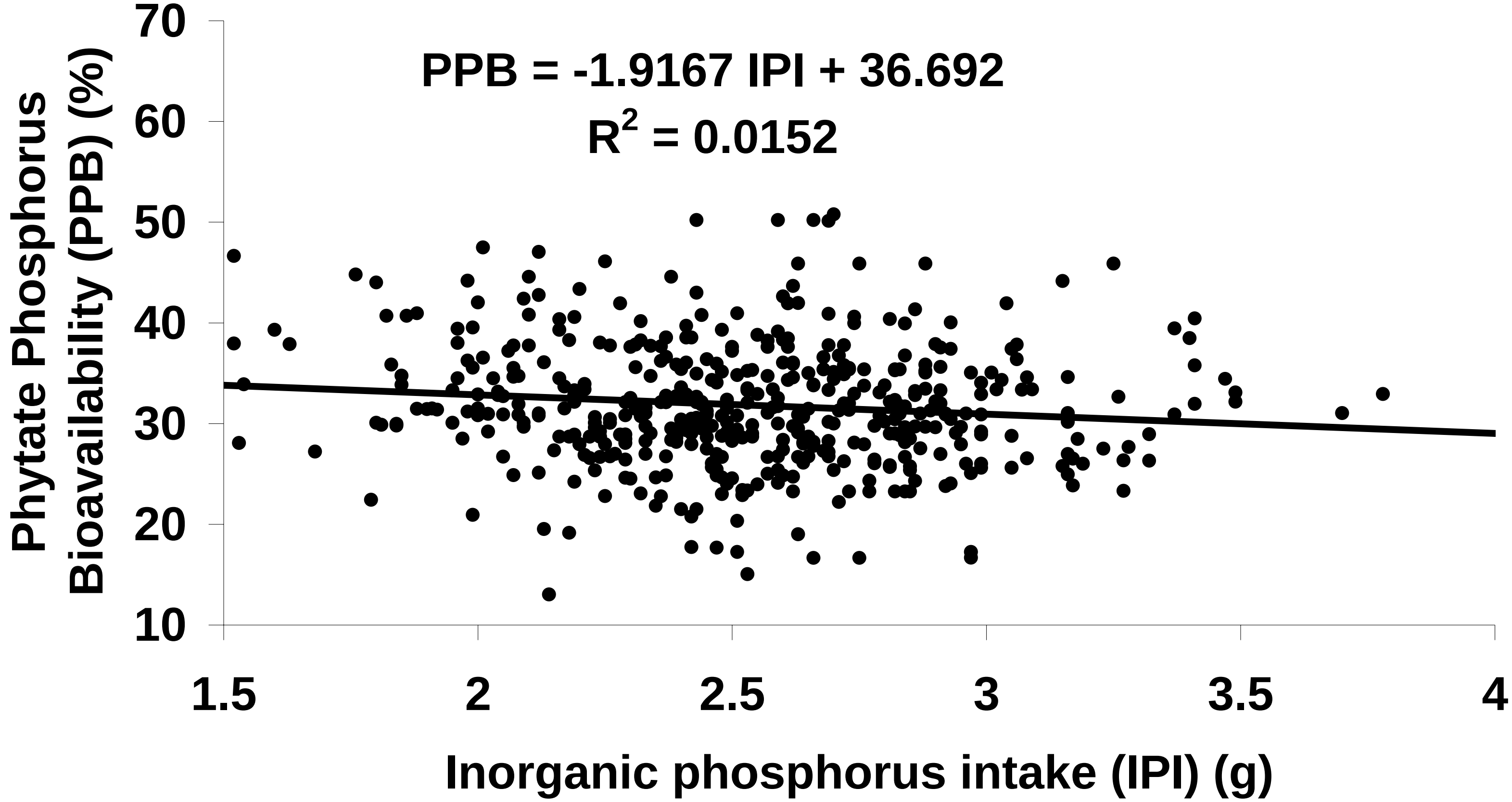

Figure 19. Effect of inorganic phosphorus intake on phytate phosphorus (males)

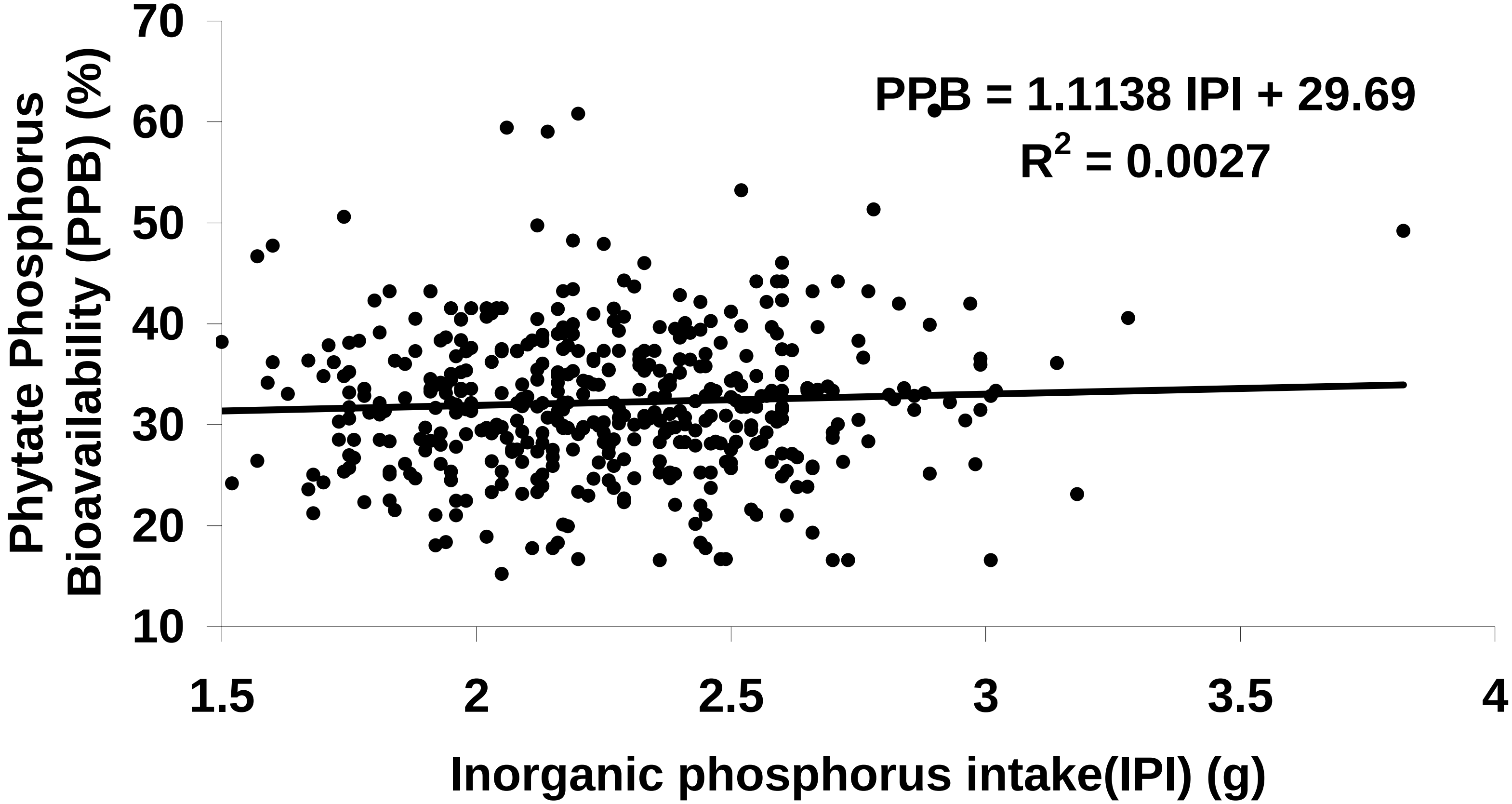

Figure 20. The effect of inorganic phosphorus intake on phytate phosphorus (females)

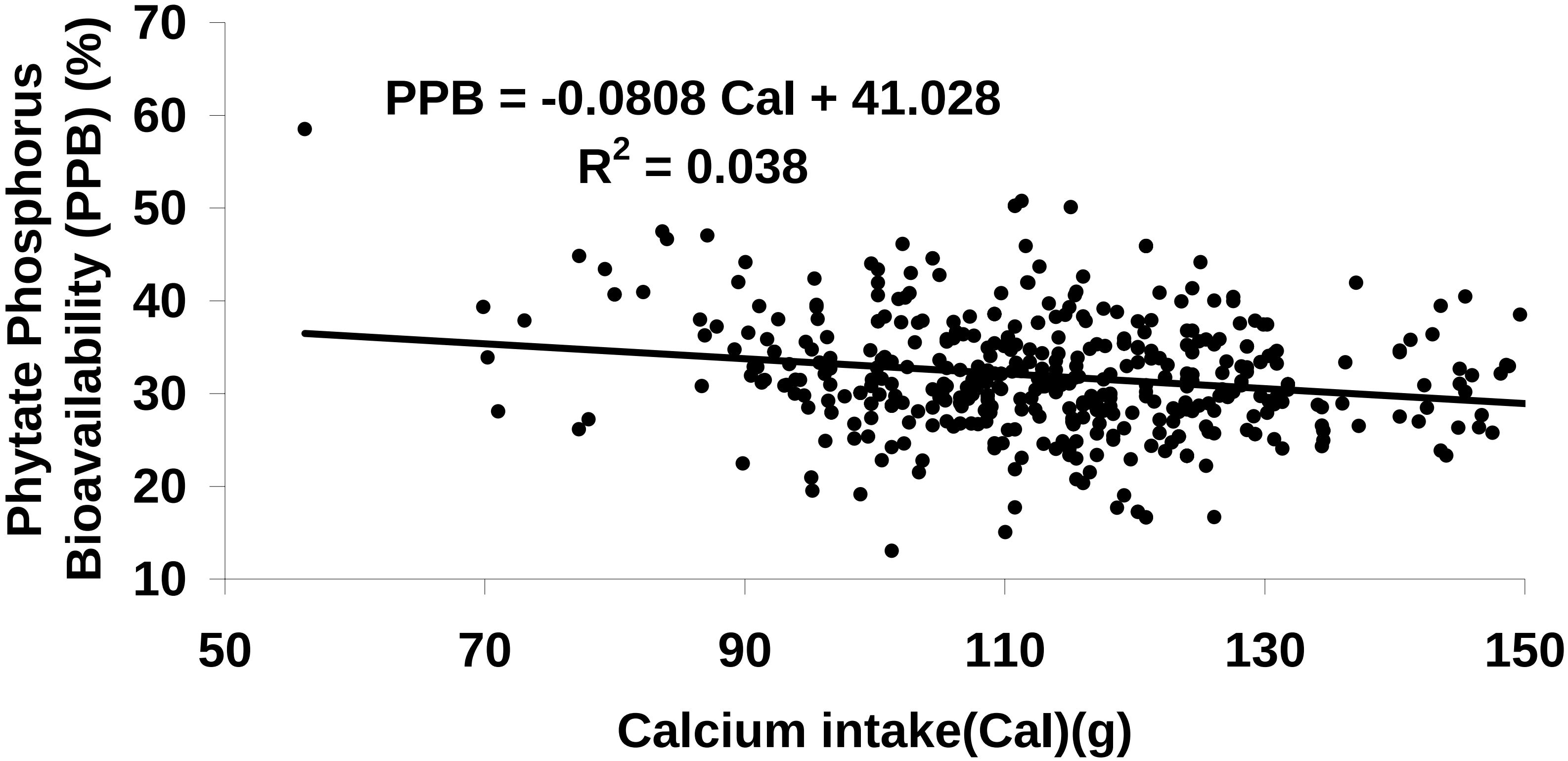

Figure 21. Effect of calcium intake on phytate phosphorus (males)

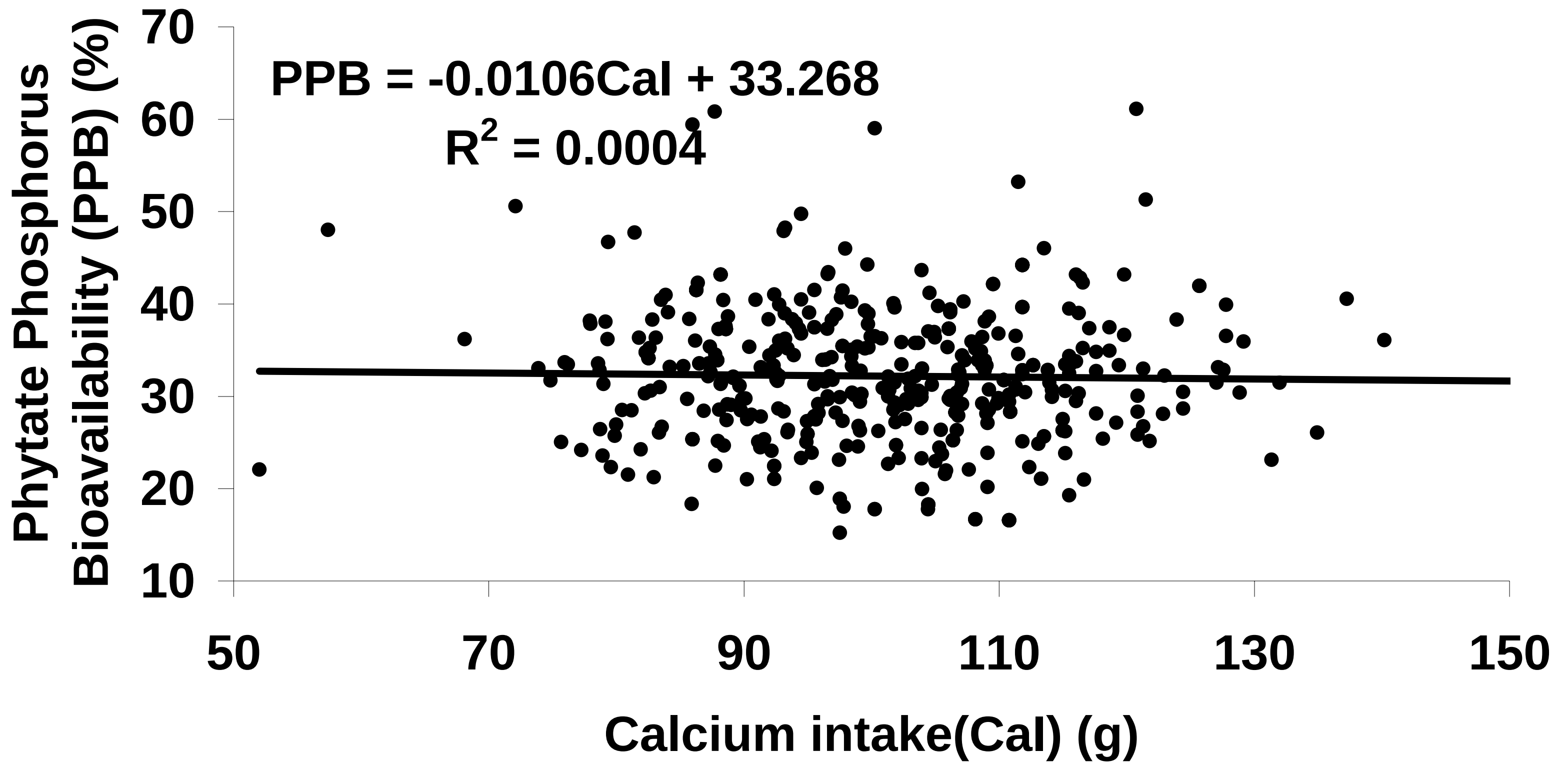

Figure 22. Effect of calcium intake on phytate phosphorus (females)

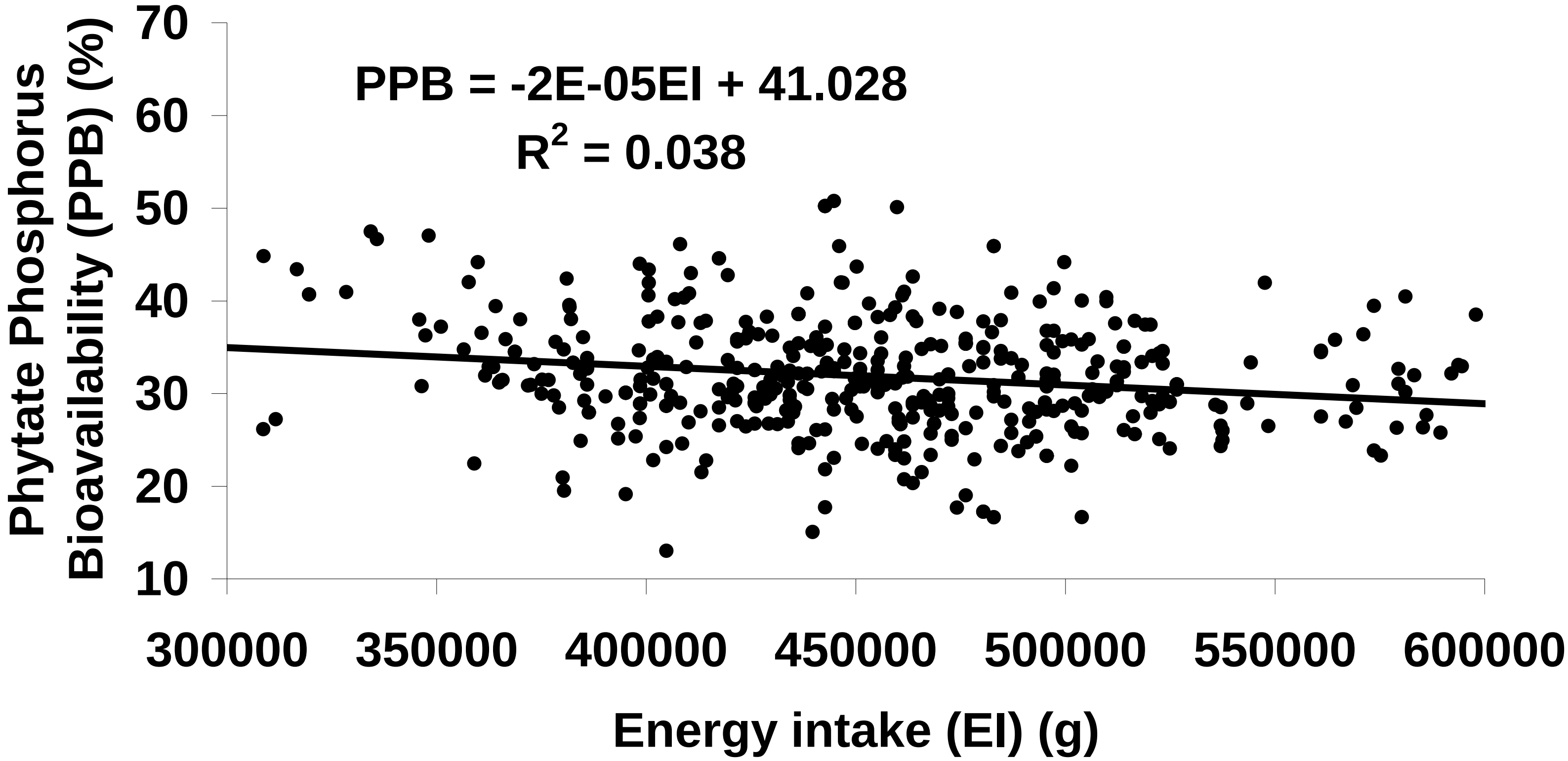

Figure 23. Effect of energy intake on phytate phosphorus (males)

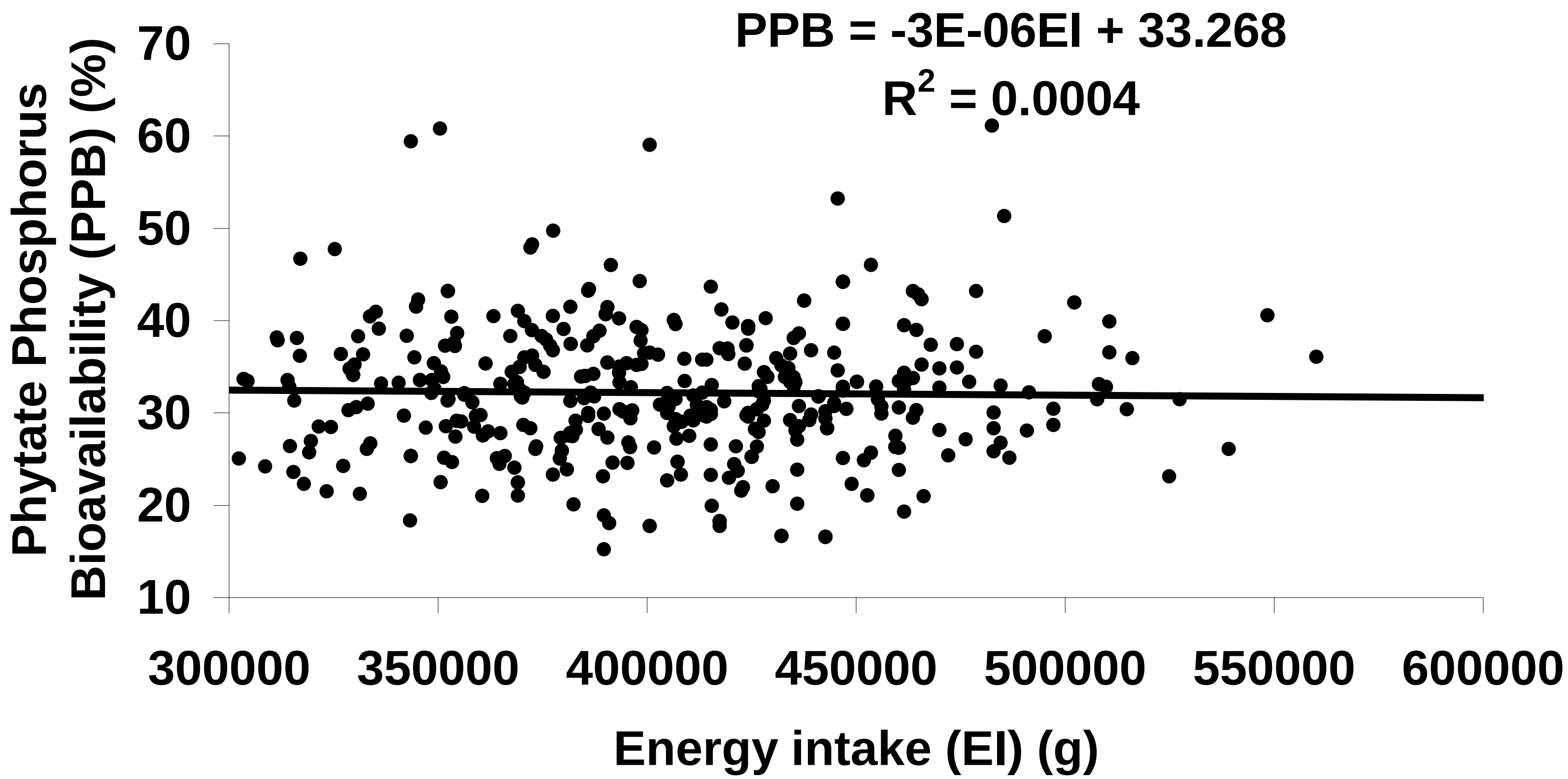

Figure 24. Effect of energy intake on phytate phosphorus (females)

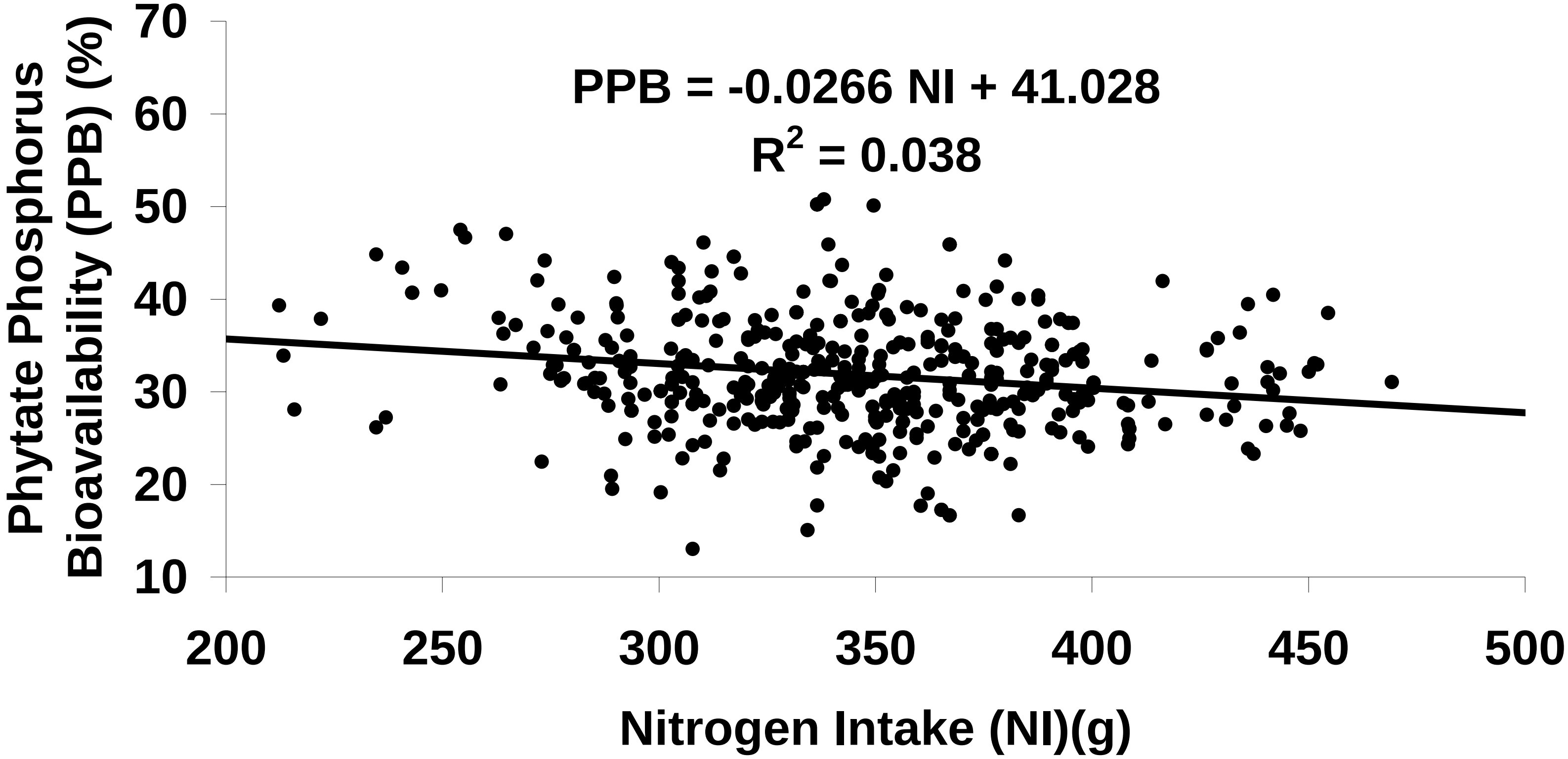

Figure 25. Effect of nitrogen intake on phytate phosphorus (males)

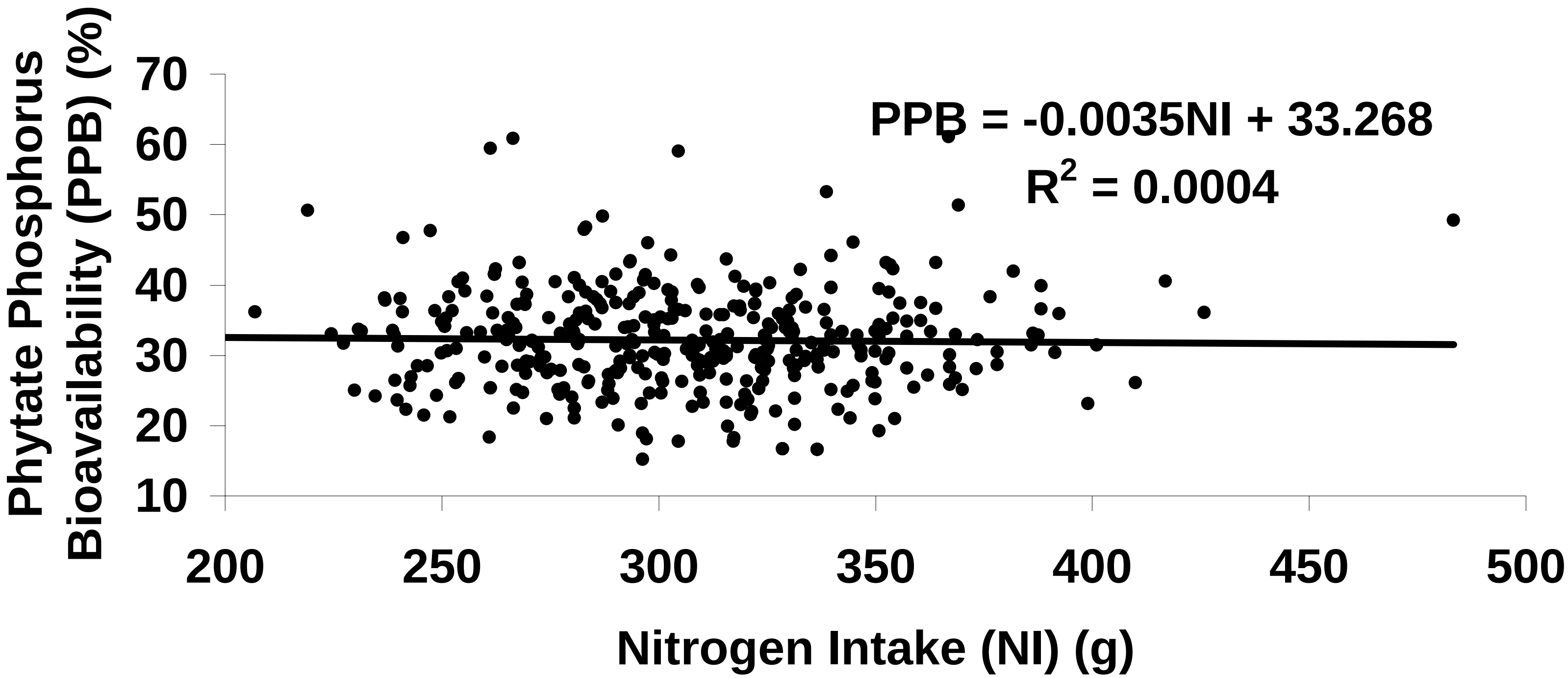

Figure 26. Effect of nitrogen intake on phytate phosphorus (females)

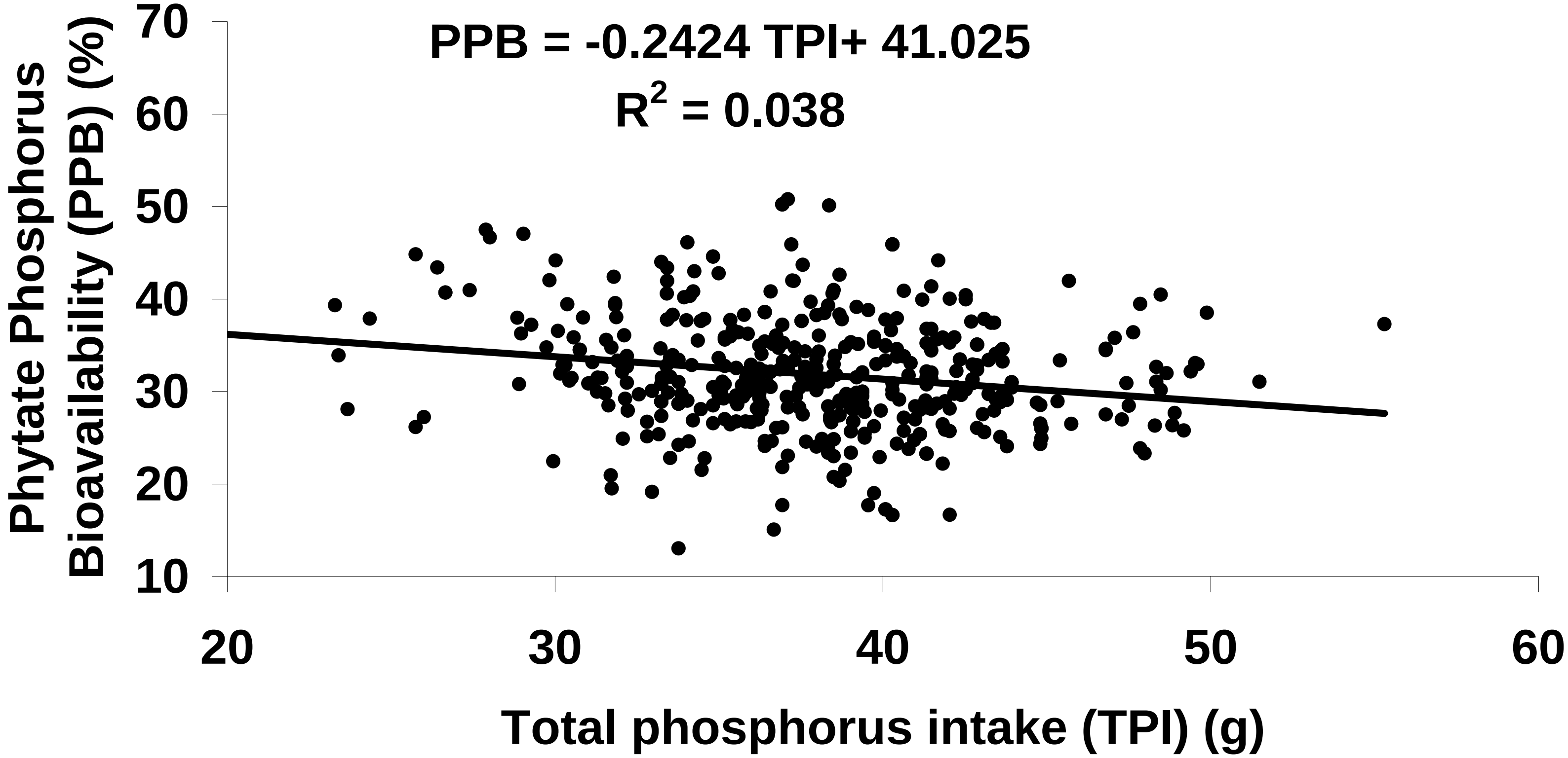

Figure 27. Effect on total phosphorus intake on phytate phosphorus (males)

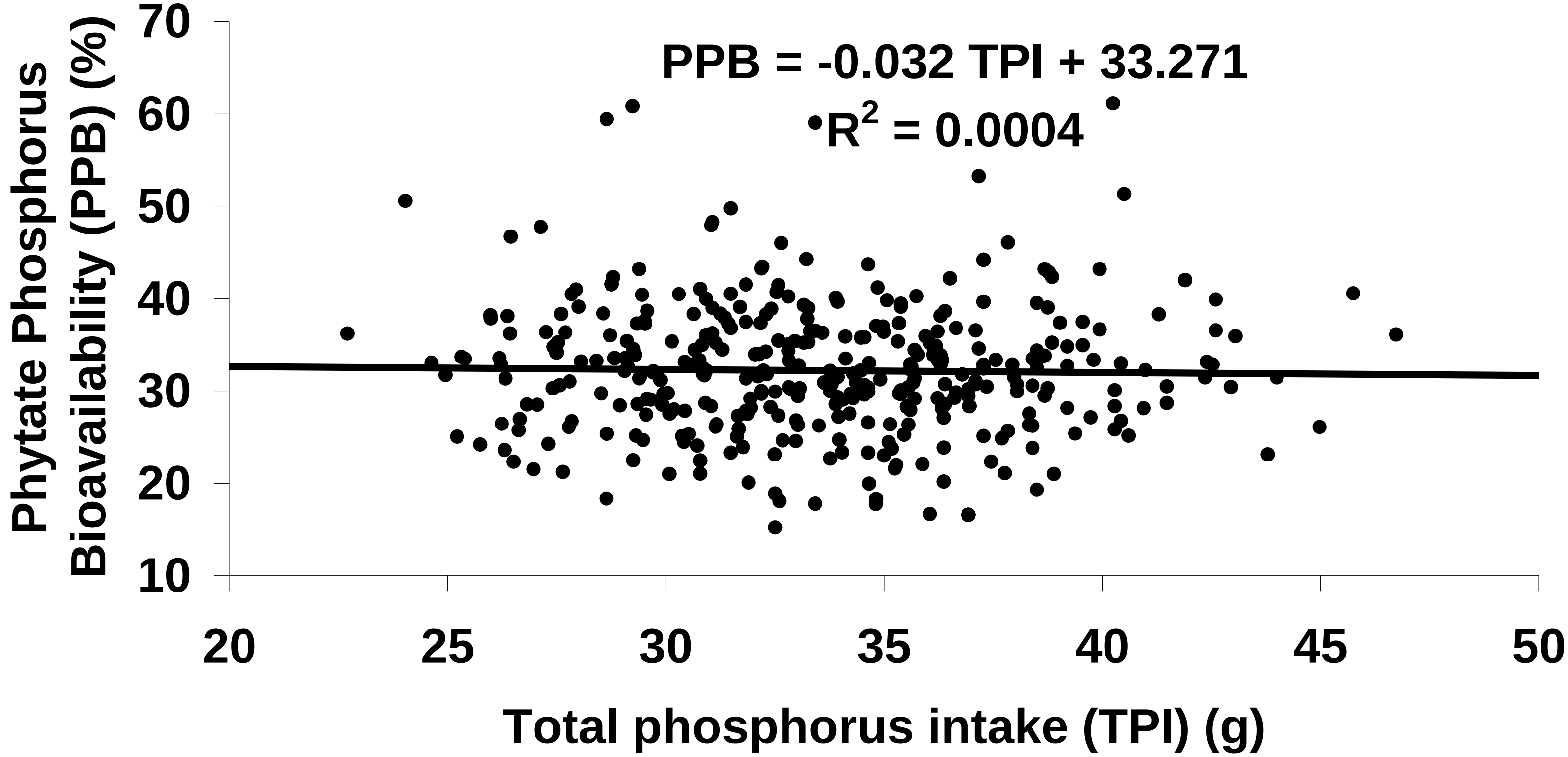

Figure 28. Effect of total phosphorus intake on phytate phosphorus (females)

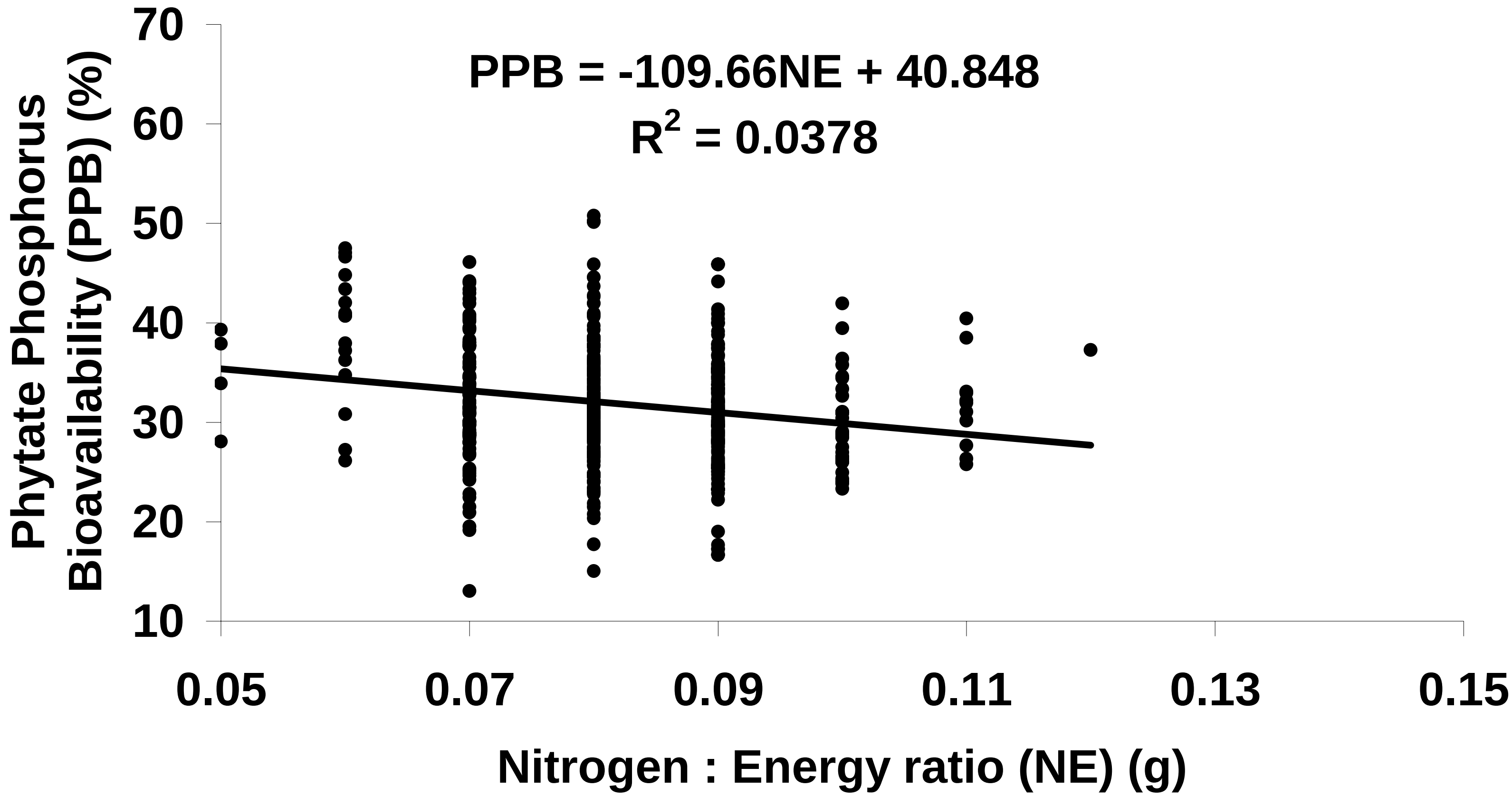

Figure 29. Effect of Nitrogen : Energy ratio on phytate phosphorus (males)

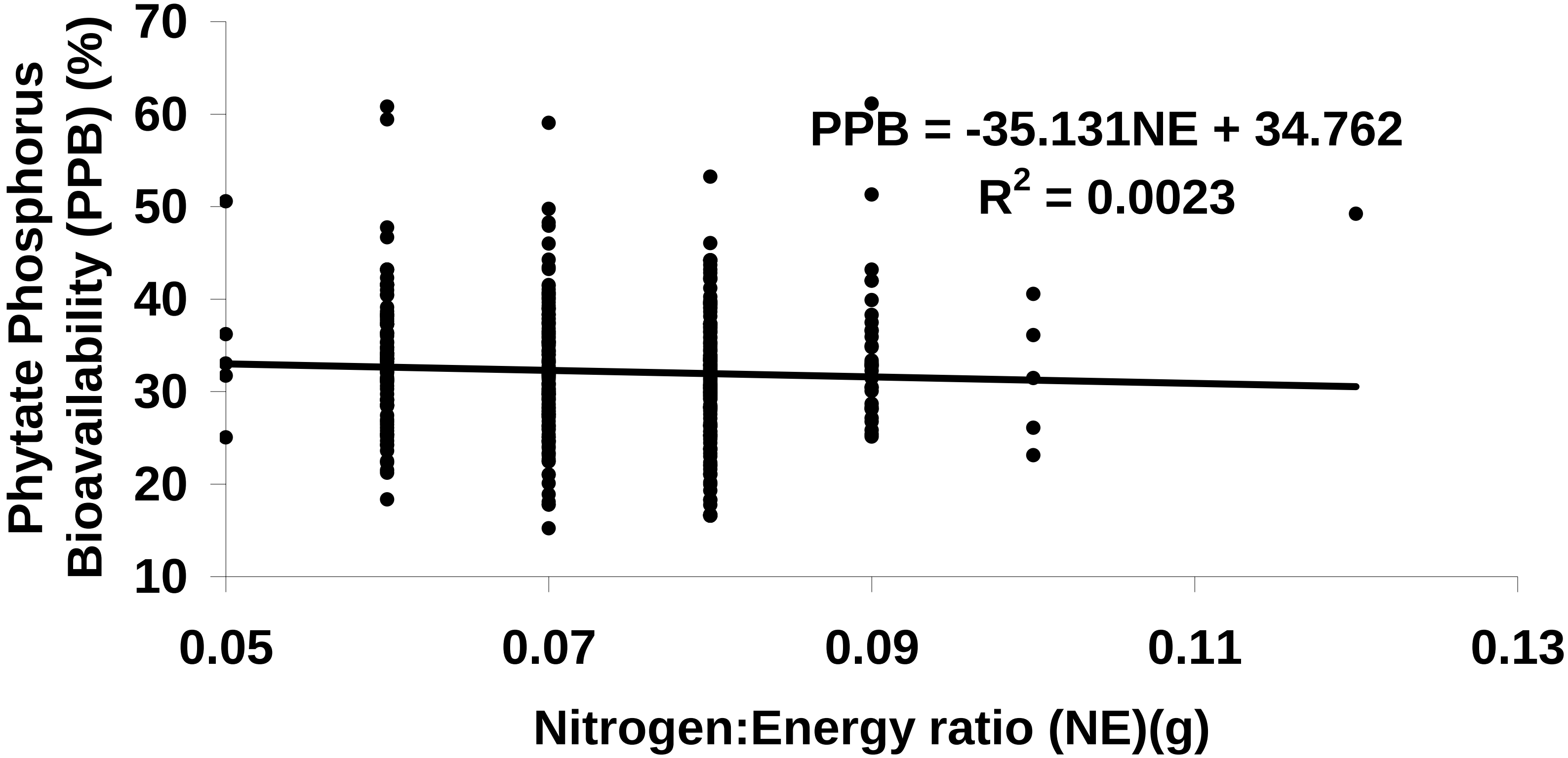

Figure 30. Effect of Nitrogen : Energy ratio on phytate phosphorus (females)

CHAPTER 4

THE GENETICS OF FACTORS AFFECTING PHYTATE PHOSPHORUS UTILIZATION[2]

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ABSTRACT

This study was conducted to estimate the heritabilities of the traits that affect phytate phosphorus utilization and the genetic and phenotypic relationships among these traits. The original data consisted of 1,004 Athens Canadian random bred birds. After editing, 919 birds were used in the analysis. The data was corrected for hatch effects using The PROC GLM (SAS Institute, 1998) method. The sire, dam and error components of the variance were estimated using the Restricted Maximum Likelihood (REML) in the PROC VARCOMP (SAS Institute, 1998). Heritability estimates and genotypic and phenotypic correlations were estimated for feed conversion ratio (FCR), body weight at 4 weeks (BW4), phytate phosphorus bioavailability (PPB), nitrogen bioavailability (NB), calcium bioavailability (CaB), energy bioavailability (EB) and relative growth (RG). The results of this study showed that the heritability of phytate phosphorus was 0.09. Those of feed conversion ratio, bodyweight at 4 weeks, calcium, nitrogen, and energy bioavailability and relative growth were 0.10, 0.66, 0.13, 0.16, 0.1 and 0.15, respectively. The phenotypic correlation between PPB and FCR, and BW4 were low and negative whereas that between PPB and RG was low and positive. There was a high positive phenotypic relationship between PPB and CaB, NB and EB. Genetic correlation between PPB and NB was low and positive. The genetic correlation between PPB and BW4, and FCR was moderate and negative thus an improvement in PPB will have an adverse effect on growth. Calcium bioavailability, EB, and RG had moderate and positive genetic correlations with PPB indicating that an increase in PPB will result in an increase in the bioavailabilities of calcium, energy and relative growth. It is thought that

improvement in energy utilization would be beneficial in improving PPB and efficiency of growth.

INTRODUCTION

Phosphorus is an important mineral required in poultry diets for normal growth and development. The main sources of phosphorus in plant tissues are phosphates, phytic acid and other myo-inositol phosphates (Classen and Stevens, 1999; Sebastian et al., 1996). Phytates are largely unavailable to monogastrics because they lack significant sources of intestinal phytase (Kornegay, 1996). Phytase makes phosphorus available by removing phosphorus groups from the inositol molecule (Sandberg et al., 1993). Several studies have been conducted to increase phytate phosphorus utilization in poultry because phosphorus is essential for proper growth. Secondly, unutilized phosphorus excreted in manure causes environmental pollution when applied to land (Kies et al., 1983).The addition of microbial phytase to the diet has been found to improve phytase phosphorus utilization (Kornegay, 1996).

Another way of improving phytate phosphorus utilization could be through genetic methods. Genetic improvement of a trait may be based on mathematical models predicting the trait from various inputs. Genetic parameters of the mathematical model parameters would provide the geneticist with the necessary information needed for improvement. A predictive model developed for phytate phosphorus utilization indicated that feed conversion ratio, bodyweight at 4 weeks, phytate phosphorus bioavailability, calcium bioavailability, nitrogen bioavailability, energy bioavailability and relative growth are major factors affecting phytate phosphorus bioavailability (PPB). The objective of this study was to determine the level of inheritance and the relationship among factors affecting PPB.

MATERIALS AND METHODS

Initial data collected for the experiment was edited after which 919 individuals comprising of 476 males and 443 females were used in the analysis. The sire ($\delta^2{}_s$), dam ($\delta^2{}_d$) and error ($\delta^2{}_{e)}$ components of the variance were estimated by using the Restricted Maximum Likelihood (REML) in PROC VARCOMP (SAS Institute, 1998). The restricted maximum likelihood method (REML) (Henderson, 1985) was used to estimate variance components of the traits. The animal model used was

$$y \quad = XB + Zu + e$$

where, $y = (y_1, y_2, \ldots\ldots y_t)$ and y_t is the vector of phenotypic observations for trait t; X = matrix that relates fixed effects to the phenotypic record; Z = matrix that relates animals to the records; $B = (B_1, B_2 \ldots B_t)$, and B_t = vector of fixed traits for trait t; $u = (u_1, u_2 \ldots u_t)$ and u_t = vector of random animal effects for trait t; $e = (e_1, e_2 \ldots\ldots e_t)$, and e_t = vector of residual effects for trait t. The variances of random animal effects were var(u) = A x G and var(e) = I x R, where A is the additive relationship matrix, G is the co(variance) matrix for genetic effects of the traits, I is the identity matrix and R is the co(variance) matrix for the corresponding residual effects. Sex and hatch groups were considered as fixed effects. Heritability estimates and genetic and phenotypic correlations were estimated for feed conversion ratio, body weight at 4 weeks, phytate phosphorus bioavailability-, calcium-, nitrogen- and energy bioavailabilities and relative growth. The estimates of genetic parameters were calculated according to the definitions (Falconer and Mackay, 1996) and obtained from the estimated co (variance) matrices for genetic and residual effects.

RESULTS

The data used in determining heritabilities and correlations have already been described in the previous chapter. The traits used for the estimation of variance component are described in Table 6. Heritability estimates of the traits are presented in table 7. The heritability of phytate phosphorus was 0.09. Those of feed conversion ratio (FCR), bodyweight at 4 weeks (BW4), calcium bioavailability (CaB), nitrogen bioavailability (NB), energy bioavailability (EB) and relative growth (RG) were 0.10, 0.66, 0.13, 0.16, 0.1 and 0.15 respectively. Phenotypic and genetic correlations among traits were estimated with a multivariate REML method, and the results are presented in Table 8. The phenotypic correlation between PPB and FCR, and PPB and BW4 were low and negative, and that between PPB and RG was low and positive. There was however, a high phenotypic relationship between PPB and CaB, NB and EB. Genetic correlation between PPB and NB was low and positive whereas that between PPB and BW4 was low and negative. Genetic correlation between PPB and FCR was moderate and negative, but those between PPB and CaB, PPB and EB, and PPB and RG were moderate and positive.

DISCUSSION

The heritabilities of the traits measured are shown in Table 7. The heritability of PPB was 0.09 showing that selection to improve this trait would be difficult with mass selection. As shown in the previous chapter, the utilization of phytate phosphorus is quite complex and depends on factors like the bioavailability of calcium, energy and nitrogen, and inorganic phosphorus intakes. This implies that the genetic parameters used here are only applicable under similar nutritional environments.

The genetic correlation between PPB and NB was low and positive, implying that improving PPB through selection will not lead to a corresponding improvement in NB. That between PPB and BW4 was negative but also low thus improving PPB will not have a negative impact on body weight. The moderate negative genetic correlation between PPB and FCR suggest that selection for improved PPB would have a moderately adverse effect on FCR. The negative correlation between PPB and FCR may be related to the negative correlation between PPB and FC. Zhang et al. (2003) found a negative correlation between PPB and FC and growth. They stated that this might be due to the fact that secretion of endogenous phytase may not be enough to compensate for high intake. Fast growing birds also have a high rate of passage due to high feed consumption thus reducing the efficiency of PPB. Phyate phosphorus bioavailability had a moderate genetic correlation with CaB, EB, and RG thus selection for improvement in these traits will have a moderately positive effect on PPB. With the exception of CaB, NB and EB, phenotypic correlations between PPB and the other traits were low and may be indicative of a stable environment.

SUMMARY

The heritability estimate of phytate phosphorus was 0.09. The estimates for FCR, BW4, CaB, NB, EB and RG were 0.10, 0.66, 0.13, 0.16, 0.1 and 0.15, respectively. The

genetic correlation between PPB and BW4, and PPB and NB were low but genetic correlation between PPB and EB, and PPB and CaB were moderate and positive therefore selection for calcium and energy bioavailability may improve phytate phosphorus bioavailability.

Table 6. Least square means (LSMEAN) and standard deviation for growth and feed utilization traits in a random mating chicken population.

Trait	Mean	Standard Deviation	Minimum	Maximum
Feed conversion ratio (g/g)	2.38	0.41	1.06	4.15
Body weight, 4 wk (g)	291.52	41.87	129.5	425.7
Phytate phosphorus bioavailability (%)	32.02	6.79	5.05	61.11
Calcium bioavailability (%)	24.41	9.76	0.24	77.16
Nitrogen bioavailability (%)	59.36	4.27	38.2	78.2
Energy bioavailability (%)	82.55	1.49	77.47	89.18
Relative growth (g/g/d)	0.15	0.04	0.06	0.34

Table 7. Estimates of heritability (h^2) for phytate P bioavailability (PPB), Feed conversion ratio (FCR), bodyweight at 4 weeks (BW4), calcium bioavailability (CaB), nitrogen availability (NB) energy bioavailability (EB), relative growth (RG).

Trait	$h^2 \pm SE$
Feed Conversion ratio	0.10 ± 0.03
Body weight at 4 weeks	0.66 ± 0.07
Phytate phosphorus bioavailability	0.09 ± 0.03
Calcium bioavailability	0.13 ± 0.01
Nitrogen bioavailability	0.16 ± 0.01
Energy bioavailability	0.10 ± 0.04
Relative growth	0.15 ± 0.01

Table 8.Genetic correlation (above diagonal) and phenotypic correlations of Body weight (BW), Relative growth (RG), Feed conversion ratio (FCR), Phytate phosphorus bioavailability (PPB), Calcium bioavailability (CaB), Nitrogen bioavailability (NB) and Energy bioavailability (EB).

	BW	RG	FCR	PPB	CaB	NB	EB
BW		**-0.39**	**-0.10**	**-0.25**	**-0.13**	**0.50**	**0.37**
RG	-0.25		**-0.71**	**0.55**	**0.37**	**-0.23**	**-0.23**
FCR	0.11	-0.73		**-0.55**	**-0.58**	**0.03**	**-0.25**
PPB	-0.12	0.17	-0.21		**0.55**	**0.19**	**0.51**
CaB	-0.10	0.21	-0.26	0.63		**-0.37**	**0.29**
NB	0.06	0.08	-0.12	0.65	0.33		**0.61**
EB	-0.01	0.11	-0.18	0.79	0.53	0.80	

CHAPTER 5

GENERAL CONCLUSIONS

Genetic methods may be used to improve phytate phosphorus utilization by modeling factors that affect PPB. A model was developed to determine the main factors affecting PPB. The effects of each trait were on PPB were determined and showed that body weight, feed consumption and feed conversion ratio, had a negative effect on phytate phosphorus utilization whereas body weight gain, relative growth, and the bioavailabilities of total phosphorus, calcium, nitrogen and energy had a positive effect. The results of this study also indicate that the major factors affecting phytate phosphorus utilization (PPB) in birds were body weight at 4 weeks, calcium bioavailability, nitrogen bioavailability and energy bioavailability. There were differences between the sexes with regard to the factors affecting PPB. The predictive model showed that nitrogen bioavailability had a positive effect on the PPB of females but had no influence on males. The fact that body weight and feed consumption showed a negative relationship while relative growth had a positive effect shows that feed intake traits have to be corrected for bodyweight to account for differences in efficiency of utilization. The heritabilities and genetic correlations between the parameters were also determined. The genetic correlations (r_g) between PPB and body weight at 4 weeks and nitrogen bioavailability were low whereas r_g between PPB and energy bioavailability and calcium bioavailability were moderate and positive implying that selection for improvement in calcium and energy bioavailability may improve PPB.

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