

**OPTIMIZATION OF NON-PHYTATE PHOSPHORUS REQUIREMENT FOR
BROILER BREEDER FEMALES**

BY

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I. BALA SUNDARAM has satisfactorily prosecuted the course of research and that the thesis entitled **“OPTIMIZATION OF NON-PHYTATE PHOSPHORUS REQUIREMENT FOR BROILER BREEDER FEMALES”** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

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This is to certify that the thesis entitled” “**OPTIMIZATION OF NON-PHYTATE PHOSPHORUS REQUIREMENT FOR BROILER BREEDER FEMALES**” submitted in partial fulfillment of the requirements for the degree of **MASTER OF VETERINARY SCIENCE** in Animal Nutrition for P.V. Narsimha Rao Telangana Veterinary University, Hyderabad is a record of the bonafide research work carried out by **I. BALA SUNDARAM** under my guidance and supervision. Student's Advisory Committee has approved the subject of the thesis.

No part of the thesis has been submitted for any other degree or diploma. The author of the thesis has duly acknowledged all the assistance and help received during the course of investigation.

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I, **I. BALA SUNDARAM** hereby declare that the thesis entitled “**OPTIMIZATION OF NON-PHYTATE PHOSPHORUS REQUIREMENT FOR BROILER BREEDER FEMALES**” submitted to the **P.V.NARSIMHA RAO TELANGANA VETERINARY UNIVERSITY**, Hyderabad for the degree of **MASTER OF VETERINARY SCIENCE** in Animal Nutrition is the result of original research work done by me. I also declare that material contained in this thesis have not been published earlier.

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LIST OF ABBREVIATIONS

%	-	Per cent
@	-	At the rate of
<	-	Less than
>	-	Greater than
ALP	-	Alkaline Phosphatase
ANOVA	-	Analysis of Variance
Arg	-	Arginine
AV.P	-	Available Phosphorus
B.wt.	-	Body weight
BWG	-	Body weight gain
Cyst	-	Cysteine
Ca	-	Calcium
DM	-	Dry matter
DORB	-	Deoiled Rice bran
EM	-	Egg mass
EP	-	Egg production
<i>et al</i>	-	Co-workers
EW	-	Egg weight
FCR	-	Feed conversion ratio
FE	-	Feed efficiency
FI	-	Feed intake
g	-	Gram
g/d	-	Gram/day

IU	-	International Units
k cal	-	Kilo calorie
Kg	-	Kilo gram
Leu	-	Leucine
Lys	-	Lysine
ME	-	Metabolizable energy
Meth	-	Methionine
mg	-	milli gram
mm	-	millimeter
NRC	-	National research council
NPP	-	Non-phytate Phosphorus
P	-	Phosphorus
PA	-	Phytic acid
PP	-	Phytate Phosphorus
PTHrp	-	Parathyroid Hormone retention protein
SBM	-	Soybean meal
SEM	-	Standard error mean
Thr	-	Threonine
Trp	-	Tryptophan
Val	-	Valine

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ABSTRACT

An experiment was conducted for 112 days on Cobb (400Y) broiler breeders (51-66 week of age) to establish the non phytate phosphorus (NPP) requirement for broiler breeder females by feeding at different NPP (0.15, 0.2, 0.25, 0.30, 0.35 and 0.40%) levels. The experiment was conducted on 1200 broiler breeders (Cobb-400Y) of 51 week old having uniform body weight and egg production, which were randomly distributed in to six experimental groups with 10 replicates per group and each replicate contained 20 birds. Birds housed by placing 2 birds in a colony cages (18x15x18inch). The experiment was conducted in well ventilated raised platforms 2-bird cage breeder house. Six diets were formulated by altering NPP levels at 0.15, 0.20, 0.25, 0.30, 0.35, 0.40% and keeping all other nutrients constant. All replicate groups of birds were offered the respective diets from 51 to 66 weeks. Eggs produced by each replicate were collected on the daily basis. Feed restriction is being practiced and the birds were fed at the rate of 156g/bird/day for first 3 weeks and later birds were fed 154g/bird/day for remaining period. Egg weight was recorded weekly from each replicate and eggs laid during last day of every period were collected to assess the egg quality parameters. For hatching total eggs laid from all replicates were collected on daily basis and

stored at 15-17°C for one week. These eggs were kept for hatching on weekly interval basis for 16 weeks. Total chicks produced in each hatch were assessed for chick quality and at the end of experiment 60 birds were slaughtered for bone mineralization studies. The Broiler breeders fed different NPP (0.15, 0.2, 0.25, 0.30, 0.35 and 0.40%) levels in the diet did not show significance ($P>0.05$) on egg production, feed conversion ratio and hatching eggs %.

Feeding graded concentration of NPP (%) (0.15, 0.2, 0.25, 0.30, 0.35 and 0.40%) did not show any effect on egg weight between groups. However, NPP levels in the diet affected the egg density of broiler breeder females significantly ($P<0.05$) during period-2, period-3 and overall period. Egg density increased with increase in NPP levels from 0.15 to 0.4% in the diet in period-2, period-3 and overall period. The Haugh unit score was not significantly ($P>0.05$) affected by the NPP levels in diet during the periods 1, 2, 3 and overall except period 4. Shell weight was significantly different ($P<0.05$) during period-3 and period-4 in broiler breeder females fed with different levels of NPP. But the shell weight during the overall period was not differed significantly ($P>0.05$). Different NPP levels in the diet (0.15 to 0.4%) did not show significant ($P>0.05$) effect on shell thickness in broiler breeder females among all the four periods and treatment groups. The data on shell strength fed with different levels of NPP (%) shows that the graded concentration of NPP % levels significantly affected the shells strength at 2nd, 4th period. However in period 1, 3 and overall results were insignificant ($P>0.05$).

Different NPP levels in the diet (0.15 to 0.4%) did not show significant ($P>0.05$) effect on fertility% in broiler breeder females except in second period. Fertility% was low at 0.15% NPP level diet during the second period and the effect was not reflected in overall fertility % among the periods and levels of NPP. The level of NPP (%) the diets significantly ($P<0.05$) affected the per cent hatchability in broiler breeder during period 1 and

2 and overall. Hatchability percentage was highest at 0.25 % level. However results were insignificant ($P>0.05$) in period 3 and 4.

The per cent of saleable chicks was significantly ($P<0.05$) affected by levels NPP % in period 1, but this did not show any consistent trend related to NPP levels in diet. The difference in saleable chick's % was not affected in sub sequent periods including overall. The levels of NPP % significantly ($P<0.05$) affected the chick weight during period 3 and overall. Whereas the effect not seen in period 1, 2 and 4 with different levels NPP % in the diet. The highest chick weight was observed at 0.25 and 0.3% NPP level in the diet. Different NPP levels in the diet (0.15 to 0.4%) did not show significant ($P>0.05$) effect on bone weight, bone breaking strength, bone ash%, bone Calcium and Phosphorus content in broiler breeder females. Based on the results of the present study, it can be concluded that, different NPP levels in the diet did not significantly affect egg production performance, FCR, hatching eggs, egg weight, Haugh unit score, shell weight, shell thickness, shell strength, fertility, saleable chicks % and bone quality parameters. Different NPP levels in the diet significantly affected the egg density, hatchability % and chick weight. This study indicates that feeding 0.15% NPP level in the diet might be sufficient for broiler breeder for optimum production performance. But 0.25% NPP levels shows better hatchability %.

INTRODUCTION

CHAPTER I

INTRODUCTION

Phosphorous (P) is an essential, expensive, critical and is the second most abundant mineral in the body, not only for humans but for all domestic animals. It plays an important role in metabolism, being a part of many organic compounds involved in metabolic processes such as phosphorylation and synthesis of DNA and RNA (Scott *et al.*, 1999). Its abundance and ubiquitous presence in the entire body has become just one example of what the term “essential” could mean, especially as this mineral has been reported to influence metabolic, structural, and reproductive functions.

About two thirds of total P present in the plant are in the phytate form and is not available to the birds. The availability of P from vegetable sources is about one third (NRC, 1994)

The P needs of broiler breeder hens for production and progeny performance have not been well investigated. For decades scientist have worked to establish minimum dietary requirements for domestic animals in order to promote optimum health and growth that later will be converted to profits for animal producers. The conclusions drawn from the results continued to be controversial and elusive (Ronald, 1982).

The ninth edition (1994) of the Nutrient Requirements of Poultry from the NRC established that the minimum requirement of non-phytate phosphorus (NPP) was 0.45% and 0.35% for broilers from 0-3 week and 4-6 week, respectively. For broiler breeders, 0.45% NPP diets during the rearing phase was recommended. Intensive selection in domestic avian species during the last decade (Rauw *et al.*, 1998) has required advancements in nutrition and physiology to evolve as rapidly as those in genetics and genomics (Burt, 2002). This situation has made industry and academia constantly reevaluate the nutritional requirements necessary

to support genetically induced improvements in performance, while taking in consideration animal welfare and the environment. Nevertheless, P content of animal feeds in the India has remained high due to the predominant use of corn and soybean meal in animal diets. Furthermore, most of the P in cereals and legumes has been shown to be present in the phytate form, or inositol hexakisphosphate, where six phosphate groups were attached by phosphoester bonds to an inositol ring (phytate phosphorus) that has been reported to not be fully available to non-ruminants (O'Dell *et al.*, 1972; Maenz and Classen, 1998). Only 21% of the total P in corn and 35% in soybean meal (NRC, 1994) were reported to be available for absorption, while the remainder was excreted in the faeces due to the lack of sufficient endogenous intestinal phytase. As a result, nutritionists have been compelled to include significant amounts of expensive, but highly bioavailable, inorganic sources such as di calcium phosphate, mono calcium phosphate and de fluorinated phosphate in poultry diets. This has been a successful means to maintain performance of animals, but has had detrimental effects on the environment.

In plants, the majority of P is in the form of phytic acid (PA) and its salts with Ca, Mg, Zn and other divalent cations known as phytates (Pallauf and Rimbach, 1997). Phytate phosphorus (PP) is not available to the poultry because they do not contain sufficient amounts of intrinsic phytase required to hydrolyze the PP (Nelson, 1976; Schwarz, 1994). Therefore, poultry diets are supplemented with additional inorganic P to meet the bird's requirement. Formation of complexes of phytic acid with divalent cations and amino acids not only reduces the availability of P but also reduces the digestibility and availability of amino acids and cations to the birds (Sebastian *et al.*, 1998) Phosphorus is an essential and critical mineral in laying hen diet. Dietary levels of phosphorus that are too high or too low adversely affect not only egg production but also egg shell quality in White Leghorn layers (Owings *et al.*, 1977). Imbalanced phosphorus levels in poultry diet leads to increased excretion of

phosphorus and other minerals which leads to environmental pollution. Recently growing concern over environmental pollution from large scale poultry farming is primarily associated with excretion of phytate phosphorus and other minerals (Paik, 2000). The phytate phosphorus also chelates with Ca, Cu, Zn, Fe etc. (Kornegay *et al*, 1996) and made them not available to chicken and eventually excreted in the environment. The requirement of NPP well studied and being adapted under practical conditions in case of egg laying hens. Little data is available on the requirement of NPP for broiler breeder hens. (Bhanja *et al.*, 2005; Ekmay and Coon 2010). Since the data on NPP requirement for broiler breeder hens is scanty, under practical conditions highly variable levels of NPP is being adopted in India. Commercial broiler breeder companies have recommended NPP (Cobb-0.38-0.43%, Ross-0.32-0.35%, Hubbard-0.35-0.41%) which is considerably higher than the required levels suggested (0.18-0.2%) for broiler breeder hens (Bhanja *et al.*, 2005 and Ekmay and Coon 2010). Despite the research on P requirement of broiler breeder females, some uncertainty exists about dietary P levels that are nutritionally adequate and economical. Because of the high cost of P sources and the public concern over the contribution of poultry excreta to environmental pollution, the P requirement of broiler breeder females need to be reinvestigated. Keeping this in view the present study was conducted to optimize the NPP requirement for high yielding broiler breeder females (Cobb 400Y) being reared under cage system of management.

Therefore an attempt was made to optimize the NPP requirements of broiler breeder females (Cobb 400Y) with the following objectives

- 1) To establish the optimum requirement of NPP for broiler breeder hens for egg production, hatchability and fertility.
- 2) To study the effect of NPP supplemented at various levels in the diets of broiler breeder hens on shell quality, chick quality and bone mineralization.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Unlike laying hens, the eggs from broiler breeders must fulfill the requirements of the embryo and subsequent chick for optimal growth. The P requirements of the breeder hen cannot be completely accounted for by the amount deposited into the egg. Phosphorus loss due to bone mobilization also accounts for a portion of the overall hen P requirement. However, this endogenous P loss is difficult to quantify. The P needs of broiler breeder hens for production and progeny performance have not been well investigated; as such, it is common for industry to feed up to 600 mg NPP/ day.

Previous studies have shown evidence that increasing P intake above 0.4% available P confers no additional benefit to production but very little research has been conducted with lower NPP breeder diets. Chandramoni *et al.*, (1998) showed that egg production, shell weight and egg content did not improve with total P above 0.43% in caged layers; while Keshavrz (2000) found no differences in egg production in laying hens fed low levels of P.

Triyuwanta *et al.*, (1992) showed that increasing available P to amounts as high as 1% in the diet did not improve egg production, egg weight, or shell quality. Only marginal improvements were seen in fertility, hatchability, and hatching weight. In the same study, Triyuwanta *et al.*, (1992) showed that increasing dietary levels of available phosphorus did not improve two-week progeny weight or seven-week-progeny weight. The study by Triyuwanta *et al.*, (1992) shows the current practice of feeding 600 mg NPP/day to breeders may not be necessary but additional research is needed to show how much NPP is needed to maintain optimum performance for the breeder and her progeny.

Plumstead *et al.*, (2007) conducted three floor pen feeding studies with breeders housed on slats to determine the available P requirement while feeding corn/ soybean/ phytase diets. The researchers fed corn soy diets and added 0.10% available P from phytase

to the breeders and suggested 316 mg available P was needed for egg production. The researchers suggested additional available P (0.32% available P) was needed for maximum fertility if pullets were reared from 10 wk of age on only a corn /soybean and phytase diet. Common (1932, 1933, and 1936) working with laying hens reported a rise in excreta P corresponding to the period of shell formation, and attributed the excess phosphorus to phosphate set free when the calcium was removed from the bone for shell formation. Research has already shown that feeding commercial layers large particle size calcium improves both the eggshell quality and the bone ash during lay. Commercial layers are fed free choice whereas broiler breeders are control fed a restricted amount of feed throughout the rearing and production period and are generally fed one time early in the morning. Breeders are generally not fed large limestone particles for several reasons. It is thought that breeder hens do not produce as many eggs as commercial layers and thus the large particle limestone would not be as critical during a production period. Breeder feed may be fed as pellets by some integrators, which would require smaller calcium particles. The feeding of large Ca particles may result in slow release of Ca since larger limestone particles remain longer in the gizzard, leading to availability of Ca for intestinal absorption during the period of shell formation. Due to the continuous availability of Ca for gut absorption by feeding larger particle size limestone (calcium source), there may be a reduction in the bone mobilization of Ca along with its counter ion P, especially during shell calcification, thereby reducing P excretion.

Leske and Coon (1999) reported that commercial layers fed increasing levels of NPP along with large particle size limestone compared to small particle limestone excrete less total phosphorus per g P consumed. When marine shells were fed to broiler breeder hens and compared to ground limestone it was found that marine shells improved egg weight, 1-day progeny weight, yield stress and elastic deformation of tibiae from 1-day old progeny

(Guinotte and Nys, 1991). Feeding larger particle size limestone has been shown to increase the retention of limestone particles for a longer time period in the gizzard (Zhang and Coon, 1997) and increased the overall calcium retention for layers by improving calcium deposition in eggs.

2.1 FUNCTION OF PHOSPHORUS IN POULTRY

Phosphorous (P) has been one of the most widely studied minerals in animal nutrition due to its wide range of physiological functions in the animal body. The functions enumerated in the literature have included bone formation and maintenance. About 85% of the P in the body was located in the skeleton in the form of a calcium phosphate salt called hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ (Ternouth, 1990; Heaney, 2012). The remaining 15% was either located in cells or extracellular fluid or in blood as organic phosphate compounds and the common inorganic penta valent form of P (PO_4^{3-}). P was involved in muscle tissue and egg formation where phospholipids were a major component of the yolk and major structural component of cell membranes. Information coding and components of nucleic acid such as DNA and RNA required P (Karn, 2001). P had acted as a major intracellular buffer in the body and was involved in acid base balance as it acted as a buffer in its salt form (Cromwell *et al.*, 1995; Leeson and Summers, 2001). All energy production and storage have been shown to be dependent on phosphorylated compounds, such as adenosine triphosphate (ATP) and phosphocreatine (Karn, 2001). P was also involved as both a component and activator of enzyme systems. P regulated enzyme systems by phosphorylation of catalytic proteins. A number of enzymes, hormones, and cell-signalling molecules have been reported to depend upon phosphorylation for their activation.

2.2 SOURCE OF PHOSPHORUS AND BIOAVAILABILITY IN POULTRY

Typical P sources in animal feeds originate from plants that become animal feed ingredients and inorganic P that can be supplemented when the feed ingredient P level does not meet the requirement of the birds (Van der Klis and Versteegh, 1996; Waldroup, 1999). Plant source P in feed ingredients have been organized into two categories, the organically bounded P that were in the form of phytic acid (PA) and other forms (NPP) (Waldroup 1999). Phytic acid, also known as inositol hexakisphosphate (IP6), inositol polyphosphate, or myo-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate, and phytate when in its salt form. In the past, one-third of the P in plant source feed ingredients was assumed to be available to monogastric animals due to the fact that two thirds of the P in plant source feed ingredients was in the form of phytate. Phytate was the main storage form of P in most grain ingredients used in feed, possessing approximately 28.2% of bound P (Ravindran *et al.*, 1999). However, the bioavailability of P could be affected by various factors. For example, with feed ingredients the P bioavailability for poultry in cereals such as corn, barley, rye, wheat, rice, and sorghum ranged from 48 to 73%, 48 to 79% in rice and wheat, and 40 to 65% for oil-seed meals such as soybean meal, cottonseed meal, and rapeseed meal (Soares, 1995).

The presence of minerals and vitamins in excess may have affected the absorption of P. High calcium interacted with PO_4 forming insoluble complexes, and magnesium interacted with P to form complexes that reduced the absorption of both P and magnesium. The presence of vitamin D increased blood absorption and bone reabsorption of P (Edwards and Veltmann, 1983). In order to compensate for the low P level in plant source ingredients, inorganic P sources have been added to the diet to meet the nutritional requirement of poultry. The common supplemental P sources in animal feed have been dicalcium phosphate (22% Ca and 18.5% P), monocalcium phosphate (16% Ca and 21% P), and defluorinated phosphate or tricalcium phosphate [$\text{Ca}_3 (\text{PO}_4)_2$] (33% Ca and 18% P). Dicalcium and

monocalcium phosphate have been the two most frequently used sources of inorganic P in poultry feed (Newman and Leeson, 1997).

2.3 ISSUES OF PHYTATE AND EXCESS PHOSPHOROUS

Phytate, the major form of P stored in plants has acted like a chelating agent, where it interacted with other minerals such as calcium, magnesium, zinc, iron, copper, (Cheryan and Rackis, 1980; Maenz *et al.*, 1999; Maenz, 2001), amino acids (De Rham and Jost, 1979), and starch (Ravindran *et al.*, 1999). Phytate also constrained the activities of other digestive enzymes for example, protease, amylase, and trypsin (Liu 1998; Mate 2002; Lin, 2005; Isaksen, 2006). Numerous investigations have shown that phytate negatively affected growth and performance of animals. The anti-nutritional effect of phytate was demonstrated in broilers, where energy and amino acid digestibility declined with phytate inclusion level in the diet (Ravindran *et al.*, 2006). The lack of sufficient endogenous phytase combined with low absorption of P in animals has led to the excess P being excreted in the faeces.

Chicken manure normally contained faeces, urine, and bedding materials that were rich in nitrogen and P that can be used as fertilizer after decomposition. However, high density animal production has created a number of areas of high concentration of manure. Poultry manures on average supplied two to three times more P than plants require, which has raised concerns about the accumulation of P in soil, and pollution of surface water during runoff (Sharpley, 1996; Mikkelsen, 2000; Sims *et al.*, 2000). P has supported the growth of algae and aquatic plants that were the food source and habitat for a number of aquatic organisms. However, presence of excess P in water systems may cause over population of algae (toxic and non-toxic) and aquatic plants, which may result in water toxification and eutrophication that significantly reduced or eliminated oxygen in the water. Lack of oxygen in the water has caused illness or death of fish and aquatic organisms in large quantity. Due to these environmental concerns about excess P pollution, and the fact that poultry and other animal

production industries were considered to be one of the major P contributors to the environment, research has investigated methods to reduce the amount of P released into the environment. The fluctuation of price was affected by increased agriculture demand and tight supplies of phosphate rock. Due to the limited quantity of P resources, P has become one of the most expensive feed ingredients in animal feed (Summers, 1997). In poultry production, feed accounts for 60 to 75 % of the total production cost from hatching to the processing plant (Donohue and Cunningham, 2009). Thus, researchers had been focused on ways to increase the utilization of nutrients in the birds to minimize cost of production while finding a solution to prevent P pollution of the environment.

2.4 EFFECTS OF REDUCED NPP LEVELS ON BROILER BREEDER PRODUCTION, SKELETAL INTEGRITY, PROGENY QUALITY, AND P BALANCE.

Three experiments were conducted to investigate the impact of lowered NPP intake and changes in limestone particle size on production performance, progeny performance, and phosphorus retention for broiler breeders (Ekmay and Coon, 2008). In the first experiment, 700 Cobb 500 breeders were reared according to Cobb guidelines (Cobb-Vantress, 2005) and transferred to a production house at 147 days. At 168 days, 285 birds were switched over to one of five experimental diets (5 groups of 57 breeder hens) that differed only in the level of NPP. Phosphorus levels ranged from 0.2% to 0.4% NPP in 0.05% increments; and corresponded to a daily intake of 288, 360, 432, 504 and 576 mg NPP with 144g feed at peak. The breeders were fed 3.25% Ca primarily from large particle limestone ($X_{50} = 3489.73\mu\text{m}$). Results show that total egg production, egg number, age at sexual maturity, and egg weight were not negatively affected by lowering NPP levels to 0.20% in the breeder diet. Shell quality, though statistically impacted by NPP level, remained at high levels for all treatments (above 1.081). Reproductively, reduced dietary NPP did not negatively impact hatchability or

subsequent progeny performance. Day old and three-week progeny weights were comparable as was progeny bone quality. Breeder tibia ash and relative strength was impaired at 0.20% NPP. Breeders fed low levels of dietary NPP had lower total P excretion and greater overall P retention. The amount of P deposited into the egg was not different among the treatment groups.

In the second experiment, ninety-four 21 week-old broiler breeder hens were fed diets that consisted of the same five NPP levels as in the first experiment and one of two limestone particle sizes (large particle: X50 = 3489.73 μ m and small particle: X50 = 185.50 μ m). The breeders were peaked on 144g of feed. Production performance, egg quality, breeder skeletal quality, and P retention were monitored throughout the experimental period. No differences were seen in egg production, age at sexual maturity, specific gravity or tibia relative strength due to NPP intake. However, increasing dietary NPP led to the production of heavier eggs. Increasing dietary NPP also increased total P excretion but did not show an effect on overall Ca or P retention possibly being partially explained by increased bone ash in breeders fed larger intakes of NPP. Breeders fed large particle limestone, independent of dietary NPP intake, showed a tendency to reduce the amount of total P excreted ($p>0.08$). A rise in the amount of P deposited into the egg by hens fed large particle limestone may also partially account for these results. Feeding large particle limestone also improved shell quality and tibia relative strength. Results appear to indicate that lower levels of NPP intake do not impair a breeder hen's performance or progeny performance and that feeding large particle limestone may improve several aspects of a breeder hen's performance. Hens are able to maintain their performance by mobilizing bone reserves to meet the demands of egg formation and utilize dietary sources to replenish these reserves.

In the third experiment, Cobb 500 breeder pullets were reared to three different growth curves (20% under, Cobb standard, 20% over) and followed Cobb guidelines for all

other rearing parameters. At 21 wk., 648 breeders were transferred to a production house and individually fed one of two experimental diets (12 groups of 54 birds) that differed in the level of NPP; the other treatment groups included pullet rearing and feeding regimen for a 3x2x2 factorial study. Phosphorus levels were 0.15% or 0.4%NPP; and corresponded to a daily intake of 216 and 576 mg NPP at peak with 144g feed consumed, respectively. The breeders were fed 3.25% Ca with a 50% mix of large particle (X50 = 3159.41 μ m) and small particle (X50 =192.89 μ m) limestone. The normal regimen consisted of feed adjustments appropriate for the treatment group's performance. The altered regimen consisted of feed adjustments appropriate for the flock as a whole. Eggs per hen housed were diminished in hens fed the low dietary NPP, and by low pullet target weight. Hens fed low dietary NPP also had lower egg weights but better shell quality. Mortality was significantly higher in hens fed low dietary NPP and pullets with low BW were specifically affected, especially during weeks 26-28. Breeder tibia relative strength and ash was also significantly lower in hens fed low dietary NPP %, regardless of the quantitative amount. Hatch of fertile was not impacted by NPP intake or by rearing program, but was affected by breeder feeding regimen ($p>0.0031$). Progeny bone quality was not significantly affected by any treatment. However, an initial benefit in 1 day progeny weight was observed for hens fed 0.4% NPP. Circulating blood PTHrp was higher at 24 weeks of age than at 29 weeks of age (peak) and PTHrp levels were lower in hens reared 20% over target BW. Osteoclast activity and bone mobilization appears to peak 8-12 hours post feeding; followed by a period of bone re-deposition (total ALP). Deficiencies in dietary NPP do not appear to exert an effect until nearing peak production; pullet rearing appears to exert a greater effect during the transition into sexual maturity. Hens on 0.15% NPP will mobilize less bone than hens on 0.40% NPP, even at peak production. Plasma P readings support these conclusions showing breeders fed 0.14% NPP had less reactive P in plasma. Hens reared on a 20% under growth curve had reduced egg production.

“Under” hens had higher levels of bone deposition (total ALP) at 24 and 26 weeks, and lower levels of bone resorption at 24 weeks. In summary, 0.15% dietary NPP and the 20% under pullet growth curve negatively impact egg production as well as breeder skeletal integrity, but do not impair progeny skeletal integrity. Body composition appears to be a main contributor in bone remodeling mechanisms.

2.4.1 Effect of Dietary Non Phytate Phosphorus Levels on Performance

Panda *et al.*, (2008) investigated effect of calcium and NPP on performance of Vanaraja breeder (female parent) chicks during juvenile stage (0-6wks). Diets supplemented with Ca (0.8, 0.9 and 1%) and NPP (0.40, 0.45 and 0.5%) in factorial manner. Better FCR and optimum performance was found at 0.80% and 0.40% NPP.

Bhanja *et al.*, (2005) studied the effect of supplementing microbial phytase with reducing NPP from 0.30 to 0.18%. results revealed that no significant ($P>0.05$) difference was observed on egg production, feed intake, FCR and egg weight when fed with low NPP diet (0.18%) and concluded that optimum performance observed at 0.18% NPP in broiler breeder diet. Enhancing the NPP content or supplementation of phytase at 0.18% NPP had no advantage on performance.

Berry *et al.*, (2003) conducted experiment to determine the effect of phytase on performance in broiler breeders (27-60 wk. age) at low phosphorus diets (0.1 and 0.3% available Phosphorus) and results were concluded that phytase supplementation shown significant increase in egg production and no significant effect on egg weight, 0.1% available phosphorus diet shown determinant effect on hatchability.

Bhanja *et al.*, (2007) conducted experiment to optimize the NPP requirement (1.2, 1.8, 2.4, 3.0, 3.4 and 4.2g/kg diet) in synthetic broiler breeder diet on laying performance and hatchability of eggs and reported that NPP higher than 1.2g/kg diet had no significant effect on egg productivity and hatchability.

Triyuwanta and Nys (1992) conducted experiment on dwarf breeder hens to study the effect with different dietary concentration of phosphorus on the productive performance (0.2, 0.6, and 1.0%). Results have shown that there is no significant ($P>0.05$) difference in egg production fertility and hatchability.

Plumstead *et al.*, (2007) conducted experiment to study the effects of reduced dietary NPP levels (0.37, 0.27, 0.19 and 0.09%) and inclusion of phytase on broiler breeder performance, results showed that there was significant reduction in fertility at low NPP level (0.19%) and there was no significant difference in egg production, FCR and hatchability.

Brake *et al.*, (2003) conducted experiment to study the effect of dietary available phosphorus (0.1 and 0.4%) and phytase in broiler breeder diets on breeder performance, results showed that there is no significant difference on hen day production fertility and hatchability.

Panda *et al.*, (2006) conducted experiment to study six levels of NPP (0.15, 0.18, 0.21, 0.24, 0.27 and 0.30%) at a constant calcium level of 3.5% on egg production, egg weight and FCR. The results revealed that there is no significant effect on egg production, egg weight and FCR at all NPP levels and concluded that at 0.15% NPP in the diet of layers showed optimum production performances

Panda *et al.*, (2005) conducted experiment to examine the effect of microbial phytase supplementation of low NPP (1.2, 1.8, 2.4 and 3.02g/kg diet) on egg production and FCR. They reported that addition of 500 FTU microbial phytase per kg diet can allow the reduction of NPP content to 1.2g per kg in layer diet without affecting the production performance.

Rama rao *et al.*, (2007) conducted experiment on Vanaraja chicks to find out the optimum requirement of calcium and NPP during their juvenile stage (1-42 days). These chicks were fed with 0.5, 0.7 and 0.9% calcium and 0.25, 0.35, 0.45% NPP. Results revealed that feed intake and feed efficiency were not affected by the interaction between the calcium

and NPP levels in the diet. Increasing levels of calcium from 0.5 to 0.7% significantly increased weight gain and also suggested that interaction between calcium and NPP in the diet for optimum weight gain these birds need to feed 0.7% calcium and 0.35% NPP.

Rama rao *et al.*, (1997) conducted experiment to determine the CP, Ca, P requirement in force moulted WL layers. Three levels of calcium (2.75, 3.25 and 3.75%) were used with two levels of phosphorus (0.5 and 0.65%). Results revealed that dietary levels of 3.25% calcium and 0.65% phosphorus were found to be optimum better performance during post moult phase. Scott *et al.*, (1999) studied the effect of two levels of calcium (3.7 and 4.1%), available phosphorus (0.11 and 0.22%) and phytase levels added at the rate of 0, 250, 500 FTU per kg diet in layer rations. Results revealed that after 55 weeks phytase enzyme can compensate for low levels of available phosphorus in the diet and depends on calcium level.

Boling *et al.*, (2000) conducted experiment to determine the optimum phytase level (0, 100, 200, 250 and 300 FTU/kg diet) at low available phosphorus (0.1%) in commercial WL laying hens. Results were found that optimum performance observed at 0.1% Av. Phosphorus with 100 units of phytase per kg diet. These performances significantly not different with 0.45% Av. Phosphorus. Gordan and Roland (1998) conducted experiment to determine the effect of phytase on calcium (2.5, 2.8 and 3.1%) and NPP (0.1 and 0.3%) utilization in commercial layers. Results showed that addition of phytase to low NPP diets (0.1%) had no significant difference in performance when compared to 0.3% NPP with phytase.

Tuijl, (1998) reported that reductions in dietary phosphorus levels of the order of 20-25% had an adverse effect on the performance of broiler breeding stock (during the rearing and production stages), broilers and commercial layers. Among the effects were increased mortality, increased leg problems (including bone breakage in layers), poorer egg quality (in layers and breeders) and reductions in uniformity (in young broiler breeders and broilers).

Wilson *et al.*, (1980) reported that diets containing 0.41% total dietary P exhibited the best hatchability and egg production rate during 28 wk. of production, while 0.36% and 0.31% total P were evidenced by lower performance. Berry *et al.*, (2003) reported that egg production was maintained in a diet without any inorganic P with 0.1% Av. Phosphorous with supplementation of phytase at 300 FTU/kg. Plumstead *et al.*, (2007) also suggested that 0.1 % NPP with phytase to achieve 0.22% Av. Phosphorous maintained egg production. Ekmay and Coon (2009, 2010) reported that egg production and egg quality were not affected when dietary NPP was reduced to 0.2%. However, when NPP was reduced to 0.15%, production was impaired to 40 wk. of age. The research further suggested the NPP requirement for egg production was above 0.15% NPP. The same research group produced different data later when they compared breeder diet NPP levels of 0.15% versus 0.40%, and found that egg weight and egg production were decreased in conjunction with increased female breeder mortality (Ekmay *et al.*, 2012).

2.5 EFFECT OF DIETARY PHOSPHOROUS AND PHYTASE IN BROILER BREEDER AND LAYER DIETS ON PERFORMANCE

Harms *et al.*, (1964) suggested that maternal P supplementation had limited effect on progeny tibia ash. Ekmay and Coon (2010) found varying the NPP level from 0.4 % to 0.2% did not affect progeny BW or tibia ash. These results were repeated later, which no differences being observed for tibia ash when comparing breeder diet NPP levels of 0.15% versus 0.40% (Ekmay *et al.*, 2012). Triyuwanta *et al.*, (1992), on the other hand, reported that the progeny of dwarf broiler breeder hens fed 0.2, 0.6, or 1.0% Av. Phosphorous exhibited an initial tibia breaking strength that was strongly positive correlated with the Av. Phosphorous level of the maternal diet, but the effect did not persist as the progeny aged.

Egg quality can be easily affected by feed composition, in that dietary P and Ca have been found to have direct effects on eggshell quality. P and Ca were stored in the

hydroxyapatite crystal form in bones. When insufficient Ca was provided in the feed to meet eggshell formation needs, Ca was mobilized from the bone also released P into the blood. A high P intake has increased the P concentration of the blood, which inhibited bone Ca release and ultimately decreased eggshell quality (Miles *et al.*, 1983). However, Panda *et al.*, (2005) investigated the effect of dietary NPP levels of 0.15, 0.18, 0.21, 0.24, 0.27, and 0.30% on egg shell quality in white leghorn layers, and found no effect of NPP on egg shell weight, shell thickness, shell strength, or specific gravity.

Ekmay *et al.*, (2011) conducted an experiment on broiler breeder (cobb500, 21-40 weeks) to determine the effects of pullet body weight, dietary NPP on performance and bone remodeling. Breeder hens were fed with dietary NPP 0.15 and 0.4%. Results showed that hens fed with low NPP diets had low egg weights, high mortality, better shell quality and low tibial strength. Ahmadi and Rodehutsord (2012) conducted meta-analysis to quantify relationship between dietary NPP and phytase levels and performance of laying hens. Analysis of model shown that corn soya diets containing 0.22% NPP without phytase supplementation resulted in high egg production, egg mass and FCR in layers.

Scott *et al.*, (1999) conducted an experiment to determine low level of available phosphorus (0.2 and 0.4%) on performance in commercial layers. Results showed that there is no significant difference in production parameters with the decrease in available phosphorus (0.2%) when compared to 0.4% available phosphorus. Araujo *et al.*, (2013) conducted experiment in Cobb 500 broiler breeders with 3 levels (0.1, 0.2 and 0.3%) of available phosphorus to study the bone quality of progeny. Results showed that there was no significant difference in body weight and bone quality of progeny.

Sohail and Rolando. (2002) have conducted experiment to determine the effect of NPP on hen performance with 13 dietary treatments (0.1 to 0.7 NPP at graded increments of 0.05%). Results shown that feed consumption, egg production, body weight increased with

increase of NPP from 0.1 to 0.7%. Egg density, egg weight decreased with decrease of NPP. Mortality was higher in low NPP diets (0.1 and 0.15%). Bone breaking strength is higher in hens fed with 0.3 to 0.4% NPP.

Sohail and Rolando. (2002) have conducted experiment to determine the effect on commercial layer performance with two levels of calcium (3 and 4%), four levels of NPP (0.1, 0.2, 0.3 and 0.4%). Results indicated that egg production and feed consumption were adversely affected with low NPP diets (0.1%). Increased hen mortality, reduced bone density, bone breaking strength, bone mineral content and egg density was observed at 0.1% NPP level in the diet. Snow *et al.*, (2005) conducted an experiment to determine the performance of w-36 layers with 0.1, 0.14 and 0.45% NPP levels from 20 -50 weeks of age. Results shown that at 0.1% NPP experiment terminated at 35 weeks age due to low production. At 0.14% NPP there was no difference in egg production, FCR and tibial ash when compared to 0.45% NPP diets.

Jalal and Scheider, (2001) conducted experiment to observe the effect of different levels of NPP (0.35, 0.25, 0.15 and 0.1%) on production parameters in laying hens. Results indicated that there is no significant difference on feed intake, FCR, egg production and egg weight, egg quality parameters. High mortality, high bone ash% was observed in 0.1% NPP diets.

Boorman and Gunaratnae, (2001) conducted experiment to study the effect of dietary NPP on egg production parameters in layers (0.156, 0.213, 0.3 and 0.38%). Results showed that there is no significant difference between low, moderate and High NPP on egg production, shell quality and bone mineralization parameters. Punna and Roland, (1999) conducted an experiment in Pullets (19 wk. of age) previously fed varying levels of available Phosphorous on hen performance during Phase 1 (Week 21 to 36) and 2 (Week 37 to 48). The available phosphorous levels used in this portion of the study remained at 0.1, 0.2, 0.3,

and 0.4%. Feed consumption, egg production, egg weight, egg specific gravity, and mortality were the criteria used. Reduction of Av. Phosphorous from 0.4 to 0.2% had no effect on feed intake, egg production, egg weight, or eggshell quality ($P > 0.05$). However, hens fed 0.1% available phosphorous showed reduced feed intake, egg production, and bone mineral density ($P < 0.001$) and increased mortality ($P < 0.001$). Eggs from hens fed 0.2, 0.3 and 0.4% Av. Phosphorous diets were heavier than those fed 0.1% available phosphorous.

Liu *et al.*, (2007) conducted an experiment to study the effects of different levels (0.28 and 0.15% NPP) on the performance of layers (320 Hy-Line brown layers from 23 to 28 wk. age) fed with corn- soybean, and by-product meal based diets were assessed. The results revealed that the reduction of Ca and P from the positive control diet significantly depressed feed intake, egg mass, eggshell hardness ($P < 0.05$).

Pelicia *et al.*, (2009) conducted an experiment to study the effect of four available phosphorus levels (0.25, 0.30, 0.35, and 0.40%) in the diet of semi-heavy commercial layers after moulting on Hisex Brown® layers between 90 and 108 weeks of age on mortality, egg production, feed intake, egg mass, average egg weight, feed conversion ratio (per dozen eggs and per kg eggs), eggshell percentage and thickness, eggshell strength, albumen quality. The intake of Av. Phosphorous linearly increased ($P < 0.01$) with dietary Av. Phosphorous levels. The remaining parameters were not influenced ($P > 0.05$) by dietary Av. Phosphorous levels. The lowest Av. Phosphorous level fed (0.25%) is sufficient to maintain the performance and the egg quality of semi-heavy commercial layers after moulting.

Summers, (1995) conducted two experiments to study the response of White Leghorn hens to low dietary phosphorus levels from 18 to 64 wk. of age. A corn-soybean meal diet containing 0.2% available phosphorus gave similar performance, up to 32 wk. of age, as a similar control diet containing 0.4% available phosphorus. Beyond 32 wk., although shell quality and average egg weight were similar, egg production was significantly reduced with

the lower phosphorus diet. In a second experiment the test diet contained 0.3% rather than 0.2% available phosphorus. No differences were noted for any of the production variables measured between the 0.4 and 0.3% available phosphorus diets. Boling *et al.*, (2000) conducted experiments to study the effects of dietary available phosphorus levels and phytase on performance of young and older laying hens. In the first of two experiments (20 to 70 wk. of age), eight treatments consisted of corn-soybean meal diets (0.34% total P) containing 0.10, 0.15, 0.20, 0.25, or 0.45% Av. Phosphorous, with the three lowest Av. Phosphorous diets supplemented with 300 units of phytase/kg of diet. A second experiment evaluated the effect of feeding the 0.10% Av. Phosphorous diet with and without phytase on performance and time required for onset of P deficiency in older hens (70 to 76 wk. of age). In Experiment 1, the 0.10% Av. Phosphorous diet with no supplemental phytase depressed performance by 28 wk. of age. No other significant differences in performance were observed among treatments for the entire 20 to 70 wk. period except that the 0.15% Av. Phosphorous diet with no phytase resulted in body weights and tibia ash that were lower ($P < 0.05$) than those of hens fed the 0.45% Av. Phosphorous diet during the last 30 wk. In the second experiment, P deficiency signs occurred within 3 wk. of consuming the unsupplemented corn-soybean meal diet (0.10% Av. Phosphorous) compared with 8 wk. in Experiment 1.

Liebert *et al.*, (2005) conducted experiment with laying hens (22-62 wk.) to evaluate the effect of low phosphorus corn soya diets on performance with diets 0.12%, 0.15% NPP. Results indicated that there is no difference in egg production however increased egg weight, FCR, tibial ash and tibial P content were better in 0.15% NPP diets. Snow *et al.*, (2004) conducted experiment (one cycle) in hens to study the effect of difference levels of Av. Phosphorous (0.10, 0.115, 0.125, 0.135, 0.15, or 0.45%) from 40 to 56 wk. of age, with the last diet being a positive control. Egg production, egg mass, and BW were reduced ($P < 0.05$) by all lower Av. Phosphorous levels except 0.15% Av. Phosphorous when compared with the

0.45% Av. Phosphorous treatment. In the second experiment (two cycles with a moult), hens were initially fed diets containing 0.10, 0.12, 0.14, 0.16, 0.18, 0.20, or 0.45% Av. Phosphorous from 21 to 63 wk. of age. Diets containing 0.10, 0.12, and 0.14% AP were terminated at 35, 39, and 50 wk., respectively, due to low egg production and increased mortality. Hens fed 0.16% Av. Phosphorous also had significantly lower production performance than hens fed 0.45% Av. Phosphorous during the first cycle. Hens on the 0.16 to 0.45% Av. Phosphorous treatments were induced moulted at 64 wk. of age by 10 d of feed removal. The hens were then returned to the same Av. Phosphorous layer diet they had been fed from 21 to 63 wk. For the 68 to 108 wk. post-moult second-cycle period, hens fed the 0.16 to 0.20% Av. Phosphorous diets (166 to 209 mg/d) had significantly lower egg production, egg mass, and feed efficiency than hens fed 0.45% Av. Phosphorous. The results of our study indicated that first-cycle hens required approximately 0.18% Av. Phosphorous or 198 mg Av. Phosphorous /hen per day, and moulted hens in their second cycle had a requirement that was greater than 0.20% Av. Phosphorous or 209 mg Av. Phosphorous /hen per day.

Keshavarz, (2000) conducted an experiment to re-evaluate the nonphytate P (NPP) requirement of laying hens with and without phytase. The experiment involved 12 treatments. The hens of the control group (T1) were fed a sequence of 0.40-0.35-0.30% NPP during 30 to 42, 42 to 54, and 54 to 66 wk., respectively. The NPP was reduced in increments of 0.05% in T2 to T6. The hens of T7 to T12 were fed NPP regimens similar to T1 to T6 but with 300 units phytase/kg diet. Results show that at 0.1 and 0.15% NPP levels there is decrease in egg production and egg mass and no change in egg weight and feed intake. Production and specific gravity were not influenced by NPP regimens used during the growing period. There is increased FCR and decrease in tibial ash content. In the absence of phytase, production traits were not different for hens fed a NPP regimen of 0.25-0.20-0.15% than for the

unsupplemented phytase control group for the entire experiment ($P > 0.05$). However, production traits were inferior for hens fed the lower NPP regimens ($P < 0.05$). In the presence of phytase, production traits were not different for hens fed the lowest NPP regimen (0.15-0.10-0.10%) than for the unsupplemented phytase control for the entire experiment ($P > 0.05$).

Skrivan *et al.*, (2010) conducted an experiment to study the effects of concentration of available phosphorus (AP; about 4, 3 and 2 g/kg) on the parameters of hen performance and egg quality as well as shell calcium (Ca) and phosphorus (P) contents. Two experiments were carried with corn and wheat based diets in which (0.12, 0.24, 0.27, 0.3, and 0.41 %,.) respectively with older ISA Brown hens housed in enriched cages. The interaction of diet type and Av. Phosphorous concentration was ascertained for all evaluated characteristics except the amount of Ca and P deposited in shells in the first experiment. In the second experiment, the interaction of diet type and Av. Phosphorous concentration was found for feed intake, egg weight, shell thickness and weight as well as the albumen quality parameters. Furthermore, Ca deposition in shells increased ($P < 0.001$) with the wheat diet. Hens fed a maize-based diet ($P < 0.001$) laid heavier eggs. The highest level of Av. Phosphorous (4.1 g/kg) in the wheat-based diet significantly ($P < 0.001$) decreased albumen height, albumen index and Haugh units (HU). These trends were the same in both experiments. The results indicate that 0.27% Av. Phosphorous in wheat-based diet and 0.30% Av. Phosphorous in maize-based diet are adequate for hens with the intake of 115g of feed with 3.5% of Ca without a negative impact on performance or egg quality. The results indicated that there is no difference in hen day egg production but reduced egg weight, increased FCR per kg eggs. There is no difference in shell strength, shell thickness and shell weight in different Av. Phosphorous diets.

Ekmay and Coon, (2010) conducted a 40-wk study to assess the effects of reduced dietary Non-Phytate Phosphorus (NPP) on broiler breeder performance. Seven hundred Cobb 500 chicks were reared according to Cobb guidelines (Cobb-Vantress, 2005) and transferred to a production house at 21 wk. At 24 wk., 285 birds were switched over to one of five experimental diets (5 groups of 57) that differed only in NPP. Phosphorus levels ranged from 0.2% to 0.4% NPP in 0.05% increments; and corresponded to a daily intake of 288, 360, 432, 504 and 576 mg at peak. Production performance, egg quality, breeder and progeny skeletal quality, hatchability, progeny weight and P retention were monitored throughout the experimental period. Results show that total egg production, egg number, age at sexual maturity and egg weight were not negatively affected by lowering NPP levels to 0.20% (288 mg/day).

Wei Nie *et al.*, (2013) conducted experiment to study the effects of various levels of dietary nonphytate phosphorus on laying performance and the expression patterns of phosphorus metabolism related genes in Dwarf pink-shell laying hens. A total of 405, 28-week-old Dwarf pink-shell laying hens were fed the same corn-soybean basal meals but containing 0.20%, 0.25%, 0.30%, 0.35% or 0.40% non-phytate phosphorus. The results showed that feed intake, egg production, and average egg weights were quadratically correlated with dietary nonphytate phosphorus content ($P < 0.05$), and the highest egg production, feed intake and average egg weights were achieved when dietary non phytate phosphorus was at 0.3% ($P < 0.05$). mRNA expression of intestinal sodium phosphorus co-transporter linearly decreased when dietary nonphytate phosphorus increased. mRNA and protein expression of intestinal calcium binding and vitamin D receptor correlated quadratically with dietary nonphytate phosphorus, and the highest expression was found when dietary available phosphorus was at 0.25% to 0.3%. In conclusion, the ideal phosphorus requirement for Dwarf pink-shell layer hens is estimated to be 0.3% in a corn-soybean diet.

With this level of phosphorus supplementation, calcium binding and vitamin D receptor reached their highest expression.

2.5.1 Effect of Dietary Non Phytate Phosphorus Levels on Egg Quality

Raju *et al.*, (1995) conducted experiment to study the effect of egg weight on hatchability and chick weight. Hatching eggs up to 60 gm (A), 60.1-65gm (B), 65.1-70gm (C) and above 70gm (D). Results showed that fertility is unaffected by egg size, hatchability decreased with egg weight. Day old chick weight increased significantly with increase in egg weight.

Triyuwanta and Nys (1992) conducted experiment on dwarf breeder hens to study the effect with different dietary concentration of phosphorus on the productive performance (0.2, 0.6, and 1.0%). Results have shown that there is no significant ($P>0.05$) difference in egg production fertility and hatchability. Plumstead *et al.*, (2007) conducted experiment to study the effects of reduced dietary NPP levels (0.37, 0.27, 0.19 and 0.09%) and inclusion of phytase on broiler breeder performance, results showed that there was significant reduction in fertility at low NPP level (0.19%) and there was no significant difference in egg production and hatchability.

Brake *et al.*, (2003) conducted experiment to study the effect of dietary available phosphorus (0.1 and 0.4%) and phytase in broiler breeder diets on breeder performance, results showed that there is no significant difference on hen day production fertility and hatchability. Tuijl (1998) reported that reductions in dietary phosphorus levels of the order of 20-25% had an adverse effect on the performance of broiler breeding stock (during the rearing and production stages), broilers and commercial layers. Among the effects were poorer egg quality (in layers and breeders) and reductions in uniformity (in young broiler breeders and broilers).

Wilson *et al.*, (1980) reported that diets containing 0.41% total dietary P exhibited the best hatchability and egg production rate during 28 wk. of production, while 0.36% and 0.31% total P were evidenced by lower performance. Ekmay *et al.*, (2011) conducted an experiment on broiler breeder (cobb500, 21-40 weeks) to determine the effects of pullet body weight, dietary NPP on performance and bone remodeling. Breeder hens were fed with dietary NPP 0.15 and 0.4%. Results showed that hens fed with low NPP diets had low egg weights, high mortality and better shell quality.

Godwin *et al.*, (2005) conducted an experiment to determine the effect of dietary P levels and dietary phytase enzyme inclusion on Large White turkey breeder hen reproductive performance (Available P 0.55, 0.35 and 0.17). Results shown that there was no difference in hens out of lay%, egg hatchability and hatchability of fertile eggs, poult weight, egg weight, egg shell thickness and egg quality parameters in turkey breeder hens fed with low phosphorus diets.

Arguelles-Ramos (2011) conducted experiment to determine the effect of low levels of NPP in broiler breeder diets. Results have shown that there is no significant difference in production, egg quality, fertility and hatchability parameters.

Jalal and Scheider (2001) conducted experiment to observe the effect of different levels of NPP (0.35, 0.25, 0.15 and 0.1%) on production parameters in laying hens. Results indicated that there is no significant difference on egg production and egg weight, egg quality parameters. Boorman and Gunaratnae (2001) conducted experiment to study the effect of dietary NPP on egg production parameters in layers (0.156, 0.213, 0.3 and 0.38%). Results showed that there is no significant difference between low, moderate and high NPP on egg production, and shell quality.

Punna and Roland (1999) reported that reduction of Av. Phosphorous from 0.4 to 0.2% had no effect on egg weight, or eggshell quality ($P > 0.05$). Liu *et al.*, (2007) conducted an

experiment to study the effects of different levels of NPP (0.28 and 0.15%) on the performance of layers. The results revealed that the reduction of Ca and P from the positive control diet significantly depressed egg mass, eggshell hardness ($P < 0.05$).

Pelicia *et al.*, (2009) reported that egg mass, average egg weight, feed conversion ratio (per dozen eggs and per kg eggs), eggshell percentage and thickness, eggshell strength, albumen quality were not influenced ($P > 0.05$) by dietary Av. Phosphorous levels (0.25, 0.30, 0.35, and 0.40%).

Ekmay and Coon (2010) revealed that Shell quality, though statistically impacted by NPP level, remained at high levels for all treatments (above 1.081). Reproductively, reduced dietary NPP did not negatively impact hatchability or subsequent progeny performance. Day old progeny weight and progeny bone quality were not significantly different from the breeders fed different NPP levels. Breeder tibia ash and relative strength was impaired at 0.20% NPP. The % total P (TP) retention showed a negative linear response with increasing dietary NPP, although absolute P retained increased with increasing dietary NPP. The amount of P deposited into the egg was not different among the treatment groups. Results appear to indicate that 360 mg NPP/day at peak (0.25% NPP) is sufficient for breeder hen performance, progeny 1 d weight and progeny skeletal quality. Breeder hens are able to maintain their performance by mobilizing bone reserves to meet the demands of egg formation and utilize dietary sources to replenish these reserves.

2.5.2 Effect of Dietary Non Phytate Phosphorus Levels on Bone Quality

Bootwalla and Harms (1988) conducted experiment to determine the effect of dietary phosphorus on reproductive capacity and bone integrity of broiler breeder males at 0, 0.05 and 0.13%, results have showed that significant difference on bone integrity and body weight change. Tuijl (1998) reported that reductions in dietary phosphorus levels of the order of 20-25% had increased leg problems (including bone breakage in layers). Ekmay *et al.*, (2011)

conducted an experiment on broiler breeder (cobb500, 21-40 weeks) to determine the effects of pullet body weight, dietary NPP on performance and bone remodeling. Breeder hens were fed with dietary NPP 0.15 and 0.4%. Results showed that hens fed with low NPP diets had low egg weights, high mortality, better shell quality and low tibial strength.

Sohail and Rolando (2002) have conducted experiment to determine the effect of NPP on hen performance with 13 dietary treatments (0.1 to 0.7 NPP at graded increments of 0.05%) and reported that bone breaking strength is higher in hens fed with 0.3 to 0.4% NPP.

Sohail and Rolando (2002) have conducted experiment to determine the effect on commercial layer performance with two levels of calcium (3 and 4%), four levels of NPP (0.1, 0.2, 0.3 and 0.4%). Results indicated that reduced bone density, bone breaking strength and bone mineral content was observed at 0.1% NPP level in the diet.

Jalal and Scheider (2001) conducted experiment to observe the effect of different levels of NPP (0.35, 0.25, 0.15 and 0.1%) on production parameters in laying hens. Results indicated high bone ash% in 0.1% NPP diets.

Boorman and Gunaratnae (2001) conducted experiment to study the effect of dietary NPP in layers (0.156, 0.213, 0.3 and 0.38%). Results showed that there is no significant difference between low, moderate and high NPP on bone mineralization parameters. Punna and Roland (1999) conducted an experiment in Pullets (19 wk. of age) previously fed varying levels of Av. Phosphorous on hen performance during Phase I (Week 21 to 36) and II (Week 37 to 48). The Av. Phosphorous levels used in this portion of the study remained at 0.1, 0.2, 0.3, and 0.4%. The study revealed that hens fed 0.1% Av. Phosphorous showed reduced bone mineral density ($P < 0.001$).

Boling *et al.*, (2000) reported that the 0.15% Av. Phosphorous diet with no phytase resulted in body weights and tibia ash that were lower ($P < 0.05$) than those of hens fed the 0.45% Av. Phosphorous diet during the last 30 wk. in layers. Liebert *et al.*, (2005) conducted

experiment with laying hens (22-62 weeks) to evaluate the effect of low phosphorus corn soya diets on performance with diets 0.12% and 0.15% NPP. Results indicated that there is no difference in tibial ash and tibial P content was better in 0.15% NPP diets. Keshavarz, (2000) conducted an experiment to re-evaluate the nonphytate P (NPP) requirement of laying hens with and without phytase. Results show that there is decrease in tibial ash content at low NPP levels.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

An experiment was carried out on broiler breeders (Cobb-400Y) at Kalinga breeding farms Pvt. Ltd, Gajwel, Siddipet district to study the “Optimum requirement of non phytate phosphorus for broiler breeder females”.

The objective of the experiment was to establish the non phytate phosphorus (NPP) requirement for broiler breeder females by feeding graded concentrations of NPP (0.15, 0.2, 0.25, 0.30, 0.35 and 0.40%).

3.1 EXPERIMENTAL DESIGN

The experiment was conducted on 1200 broiler breeders (Cobb-400Y) of 51 week old having uniform body weight and egg production, which were randomly distributed in to six experimental groups with 10 replicates per group and each replicate contained 20 birds following randomized block design. Experiment was conducted during the period of July 2016 to November 2016. The birds were fed respective diets for four laying periods, each period consisting of 28 days. Eggs produced by each replicate were collected on daily basis and the mean egg production was calculated by dividing the total number of eggs produced by number of hen days. Feed restriction is being practiced and the birds were fed at the rate of 156g/bird/day for first 3 weeks and later birds were fed 154g/bird/day for remaining experimental period as per the breeder recommendation. Egg weight was recorded weekly on all eggs produced on seventh day of the week from each replicate and one egg from each replicate was collected on the last day of every period were collected to assess the egg quality parameters. For hatching variables, total eggs laid from all replicates were collected on daily basis and stored at 15-17°C for one week. These eggs were kept for hatching on weekly interval basis for 16 weeks considering the replicate as the unit for statistical analysis.

Total chicks produced in each replicate and all the replicates for each treatment were assessed for chick quality hatch were assessed for chick quality and at the end of experiment (66 weeks) one bird from each replicate was slaughtered for assessing bone mineral variables.

3.2 PREPARATION OF EXPERIMENTAL DIETS

Six Maize-Soya-Deoiled rice bran diets were formulated and ingredient and nutrient composition is shown in the Table 1. Analysed composition of Ca and P of individual feed ingredients were considered while formulating the diets. MCP was supplemented as inorganic phosphorus source to attain graded concentration of NPP at (0.15, 0.20, 0.25, 0.30, 0.35 and 0.40%) in the respective diets. Levels of maize, soya bean meal and shell grit were altered to get uniform concentration of calcium (3%) in the diet. The composition of other nutrients were kept similar to the breeder recommendation.

3.3 HOUSING AND MANAGEMENT

The birds of all groups were kept under uniform management and standard hygienic conditions throughout the experiment. A total of 1,200 birds were equally distributed to 60 replicate groups consists of 20 birds in each. Birds housed by placing 2 birds in a colony cages (18x15x18inch). The experiment was conducted in well ventilated raised platforms 2-bird cage breeder house. All replicate groups of birds were offered the respective diets. Clean and fresh drinking water provided *ad libitum* throughout the experimental period.

Table1. Ingredient and nutrient composition (%) of broiler breeder experimental diets

Ingredients	NPP%					
	0.15	0.20	0.25	0.30	0.35	0.40
Maize	65.37	65.50	65.64	65.77	65.90	66.03
Soya DOC 45%	17.37	17.46	17.56	17.66	17.76	17.86
De-Oiled Rice Bran	6.30	5.97	5.64	5.31	4.97	4.64
Mono-calcium phosphate	0.155	0.395	0.634	0.874	1.114	1.353
Lime stone powder	2.00	2.00	2.00	2.00	2.00	2.00
Shell Grit	7.67	7.54	7.40	7.27	7.13	6.99
Salt	0.276	0.276	0.277	0.278	0.278	0.279
DL- Methionine 98%	0.06	0.06	0.06	0.06	0.06	0.06
L- Lysine Hcl 98%	0.075	0.075	0.074	0.073	0.073	0.072
Trace Mineral mix ¹	0.12	0.12	0.12	0.12	0.12	0.12
Vitamin Premix ²	0.12	0.12	0.12	0.12	0.12	0.12
Choline Chloride 60%	0.15	0.15	0.15	0.15	0.15	0.15
Toxin Binder	0.10	0.10	0.10	0.10	0.10	0.10
Liver Tonic	0.05	0.05	0.05	0.05	0.05	0.05
Sodium bi-carbonate	0.15	0.15	0.15	0.15	0.15	0.15
Total	100	100	100	100	100	100
Nutrient composition*						
Metabolizable Energy	2700	2700	2700	2700	2700	2700
Protein (%)	14.50	14.50	14.50	14.50	14.50	14.50
Calcium (%)	3.00	3.00	3.00	3.00	3.00	3.00
NPP (%)	0.15	0.20	0.25	0.30	0.35	0.40
Lysine (%)	0.750	0.750	0.750	0.750	0.750	0.750
Methionine (%)	0.290	0.290	0.290	0.290	0.290	0.290
Sodium (%)	0.160	0.160	0.160	0.160	0.160	0.160

Trace mineral mix¹ provided per kg diet: manganese, 60mg; Zinc, 60mg; Iron, 48mg; Copper,12mg; Iodine, 0.6mg; Chromium, 0.2mg and Selenium, 0.3mg. Vitamin premix² provided per kg diet: Vitamin A, 22500IU; Vitamin D₃, 4500IU; Vitamin E, 60mg; Vitamin K, 8mg; Riboflavin, 20mg; Vitamin B₁, 4mg; Vitamin B₆, 6mg; Vitamin B₁₂, 300mcg; Niacin, 60mg; Vitamin C, 100mg; Folic acid, 4mg; Pantothenic acid, 30mg and Biotin, 0.2mg.

*Calculated values

Photo plate 1. Feeding of experimental broiler breeder females



Photo plate 2. Collection of eggs replicate wise



Photo plate 3. Hatch pull out



Photo plate 4. Counting of chicks replicate wise



Photo plate 5. Weighing of chicks



Photo plate 6. Feed mill



3.4 PERFORMANCE PARAMETERS

3.4.1 Egg Production

Data on egg production (EP) was recorded from 51 to 66 weeks for four laying periods consisting of 28 days each and data were subjected to statistical analysis for every period. Per cent hen day egg production was calculated and number of hen days was considered while calculating the egg production for each replicate.

3.4.2 Feed Intake and Feed Conversion Ratio

Measured quantity of the feed was offered daily as per the recommendations for the breed. No residue was left at the end of period, during all the four periods of the study. Feed consumption of each replicate was recorded daily during each period and feed conversion ratio was calculated at end of each period and also on cumulative basis on entire study period. Feed conversion ratio (feed intake/egg production) was calculated considering mortality.

3.4.3 Hatching Eggs

Total eggs from all replicates were collected daily. Small eggs (below 52g), extra-large eggs (above 72g), hair line cracked eggs, broken eggs and misshapen eggs were discarded. The remaining eggs were considered as hatching eggs.

Hatching eggs% = $100 \times (\text{Total eggs} - \text{discarded eggs} / \text{total eggs})$.

3.4.4 Egg Weight

Egg weight was recorded on total eggs produced on seventh day of each week using electronic balance (Shimadzu – ATX-224) to the nearest 0.1 g accuracy.

3.5 EGG QUALITY PARAMETERS

Internal egg quality parameters were evaluated by utilizing one egg from each replicate during the end of each period. In total, 10 eggs per treatment per period were utilized for measurement of egg quality traits viz., shell weight (g), shell thickness (mm),

Haugh unit score, egg density and breaking strength. Egg quality variables were recorded on last day of each period for all the four laying periods

3.5.1 Haugh Unit Score

Haugh unit score of all eggs was measured by using Ames Haugh unit meter.

3.5.2 Shell Weight

The shell of each egg was cleaned with tissue paper and air dried for two days at room temperature before weighing on a precision balance (Shimadzu – ATX-224) nearest to 0.1mg accuracy.

3.5.3 Shell Thickness

From the dried shell, the thickness of air dried egg shell was measured using Mitutoya dial gauge meter (Model no.7301, Japan), to an accuracy of 0.01 mm. The values were measured at three places viz., narrow, middle, and broad ends of the egg and expressed in millimetres.

3.5.4 Egg Density

The egg density was measured by using digital density balance fitted with density kit (LCGC – AS220/X) by dipping the egg in clean distilled water.

3.5.5 Shell strength

The shell strength of egg was measured by using (Kalpak; KIC-1-100-C)

3.6 HATCHING PARAMETERS

3.6.1 Fertility

On 12th day of incubation eggs were candled to remove the infertile eggs from the incubator. The percentage of fertility is the percentage of fertile eggs of all the eggs set. Fertility was determined using the formula:

Fertility % = 100 (number of fertile eggs/number of total eggs set)

3.6.2 Hatchability

Total eggs produced after grading were used to determine hatchability traits.

The hatchability traits considered were, per cent live germ (fertility) at 12th day and per cent hatchability of total eggs set.

Incubation was done using an electrically heated incubator. All the hatching eggs (settable eggs) produced were cleaned and stored at 15-17⁰C before transferring them in an egg incubator. Regular/standard temperature/ humidity were maintained in both incubator and hatcher. The incubation temperature and relative humidity were 99.5-99.9⁰F and 86.5 to 87.0 per cent, respectively. Eggs were turned hourly basis. Candling was done on the 12th day to determine infertile eggs and dead embryo. Eggs with living embryo were then transferred to the hatcher unit. Hatched chicks were collected, counted and weighed. Hatchability was determined using the formula:

$$\text{Hatchability} = \left[\frac{\text{Number of chicks hatched}}{\text{Number of total eggs set}} \right] \times 100$$

3.7 CHICK QUALITY PARAMETERS

At the end of incubation, the numbers of hatched chicks were recorded. All chicks from each replicate were examined to determine the chick quality parameters. A chick of good quality (saleable) was defined as being clean, dry, and free from deformities (no skin lesions, well-formed beak, normal conformation of legs), completely sealed navel, and no yolk sac or residual membrane protruding from the navel area (Tona et al., 2003). The chicks with deformities were treated as weaklings and rejected. Within each replicate, all the saleable chicks were weighed and average chick weight was calculated.

3.8 TIBIAL QUALITY VARIABLES/BONE MINERALIZATION PARAMETERS

At the end of experiment (66 week of age), one bird from each replicate was selected and killed by cervical dislocation. Left tibia from each bird was excised, weighed pooled group-wise and preserved for analysis for ash, tibial strength and Ca and P contents. The tibia collected from birds was pressure

cooked for 1 h, cleaned manually to clear off the attached muscle and cartilage. The bones were dried at 100°C for 3h. The fat from dried bone samples were extracted by soaking them in the petroleum ether for 48h and weight of dried bone was recorded. The breaking strength of the bone was measured using Universal testing machine (Kalpak Tech services). Tibial ash content was determined by ashing the bones in a muffle furnace for 4h at 600°C.

3.9 STATISTICAL ANALYSIS

The results obtained were subjected to analysis with software (version 16.0; SPSS, 2007) by applying one way analysis of variance (Snedecor and Cochran 1980) through generalized linear model and the treatment means were compared using Duncan's multiple range test (Duncan, 1955) at $P < 0.05$. All the statistical analysis was followed as per the procedures of Snedecor and Cochran (1980).

RESULTS

CHAPTER IV

RESULTS

An experiment was conducted for 112 days on Cobb (400Y) broiler breeders (51-66 week of age) to establish the non phytate phosphorus (NPP) requirement for broiler breeder females by feeding graded concentrations of NPP (0.15, 0.2, 0.25, 0.30, 0.35 and 0.40%). The results of various parameters are presented here under.

4.1 PRODUCTION PARAMETERS

4.1.1 Egg Production (%)

Broiler breeders fed different NPP (0.15, 0.2, 0.25, 0.30, 0.35 and 0.40%) levels in the diet did not show significant ($P>0.05$) influence on egg production (Table -2) during different periods and overall experimental period. The Fourth period egg production percentage were lower compare to 1, 2 and 3rd period in all the treatment groups overall the per cent egg production was comparable among the treatments.

4.1.2 Feed Conversion Ratio (g/egg)

The results of the broiler breeder females fed with different NPP (0.15, 0.2, 0.25, 0.30, 0.35 and 0.40%) levels in the diet on FCR were presented in (Table-3). The data of feed conversion ratio is non-significant ($P>0.05$) among all the graded concentration of NPP (%).

Table 2. Effect of graded concentration of NPP (%) on egg production (%) of Broiler Breeder females (51-66 weeks)

NPP (%)	Period-1	Period-2	Period-3	Period-4	Overall
	(51-54wks)	(55-58wks)	(59-62wks)	(63-66wks)	(51-66wks)
0.15	64.83	64.01	61.49	57.84	62.04
0.20	66.23	64.40	62.07	56.85	62.39
0.25	65.57	63.99	60.97	57.52	62.02
0.30	66.49	66.19	62.20	58.02	63.22
0.35	65.73	65.22	62.72	60.28	63.49
0.40	65.86	65.55	62.34	59.36	63.28
SEM	0.36	0.39	0.434	0.431	0.320
n	10	10	10	10	10
P Value	0.834	0.485	0.891	0.203	0.623

Table 3. Effect of graded concentration of NPP on Feed Conversion Ratio (g/egg) of Broiler Breeders (51-66 weeks)

NPP (%)	Period-1	Period-2	Period-3	Period-4	Overall
	(51-54wks)	(55-58wks)	(59-62wks)	(63-66wks)	(51-66wks)
0.15	241.1	243.4	251.4	266.9	249.9
0.20	235.8	242.4	249.1	271.8	248.8
0.25	237.8	243.3	253.1	268.0	249.8
0.30	235.4	235.4	248.3	266.2	245.3
0.35	237.3	238.8	246.1	255.9	244.1
0.40	237.4	237.5	247.6	260.9	245.1
SEM	1.333	1.438	1.794	1.971	1.282
n	10	10	10	10	10
P Value	0.867	0.495	0.900	0.235	0.650

4.1.3 Hatching eggs %

The total eggs collected were graded and hatching eggs % was determined. The data obtained on hatching eggs % was presented in (Table-4).

Among all the graded concentration of NPP (%), there was no significant difference ($P>0.05$) found in all the four periods and overall experimental period.

Table 4. Effect of graded concentration of NPP (%) on hatching eggs (%) of Broiler Breeders (51-66 weeks)

NPP (%)	Period-1	Period-2	Period-3	Period-4	Overall
	(51-54wks)	(55-58wks)	(59-62wks)	(63-66wks)	(51-66wks)
0.15	94.25	94.25	89.61	89.94	91.34
0.20	93.69	93.69	88.53	91.59	91.27
0.25	91.84	91.84	89.65	88.92	90.02
0.30	93.14	93.14	89.52	89.86	90.62
0.35	92.68	92.68	89.85	89.93	90.73
0.40	93.56	93.56	91.49	91.06	91.96
SEM	0.35	0.35	0.418	0.426	0.344
n	10	10	10	10	10
P Value	0.433	0.433	0.507	0.538	0.684

4.2 EGG QUALITY PARAMETERS

4.2.1 Egg Weight (g)

Feeding graded concentration of NPP (%) (0.15, 0.2, 0.25, 0.30, 0.35 and 0.40%) did not show any effect on egg weight (Table -5).

Table 5. Effect of graded concentration of NPP (%) on Egg Weight (g) of Broiler Breeders (51-66 weeks)

NPP (%)	Period-1	Period-2	Period-3	Period-4	Overall
	(51-54wks)	(55-58wks)	(59-62wks)	(63-66wks)	(51-66wks)
0.15	70.03	70.14	70.81	71.38	70.59
0.20	69.71	70.41	70.71	70.67	70.37
0.25	68.94	70.36	70.94	70.77	70.25
0.30	68.82	70.25	69.85	70.43	69.84
0.35	69.23	70.15	70.45	70.45	70.07
0.40	68.32	69.90	69.96	70.91	69.77
SEM	0.18	0.17	0.216	0.180	0.153
N	10	10	10	10	10
P Value	0.085	0.967	0.607	0.682	0.628

4.2.2 Egg Density

Egg density was not affected by ($P>0.05$) by increasing dietary NPP concentration from 0.15 to 0.40% for broiler breeders, during period one and four (Table-6). However, the egg density was significantly influenced with dietary NPP concentrations during period two, three and overall experimental period. In general during period two the egg density increased progressively with the concentration of NPP in the diet. The egg density was significantly higher in breeders fed with highest concentration of NPP (0.40%) compared to those fed with lower concentration (0.15-0.25%). Similarly, egg density in period three and overall experimental period, the egg density in breeders fed with 0.40% NPP was significantly higher than those fed with lower concentration of NPP (0.15-0.35%).

Table 6. Effect of graded concentration of NPP (%) on Egg density of Broiler Breeders (51-66 weeks)

NPP (%)	Period-1	Period-2	Period-3	Period-4	Overall
	(51-54wks)	(55-58wks)	(59-62wks)	(63-66wks)	(51-66wks)
0.15	1.076	1.085 ^c	1.081 ^b	1.058	1.075 ^b
0.20	1.080	1.094 ^{bc}	1.087 ^b	1.056	1.079 ^b
0.25	1.085	1.091 ^{cb}	1.088 ^b	1.056	1.080 ^b
0.30	1.073	1.119 ^{ab}	1.096 ^b	1.069	1.089 ^{ab}
0.35	1.069	1.124 ^{ab}	1.096 ^b	1.055	1.086 ^b
0.40	1.090	1.145 ^a	1.117 ^a	1.060	1.103 ^a
SEM	0.003	0.005	0.003	0.002	0.002
N	10	10	10	10	10
P Value	0.312	0.001	0.004	0.178	0.004

a, b, c means with different superscripts in a column differ significantly (P<0.05)

4.2.3 Haugh Unit Score

The Haugh unit score was not significantly (P>0.05) affected by the NPP levels in diet (Table-7) during the experimental period except period four where the level of NPP was significantly affected the HU score and was highest in breeders fed with 0.30 and 0.40 % NPP compared to the 0.20% of the NPP group. The HU score in other groups was intermediate.

4.2.4 Shell Weight (g)

The egg shell weight was not significantly influenced by the concentration of NPP in breeder diet during period one, two and overall experimental period (Table -8). The shell weight was significantly affected due to variation in NPP concentration in diets. Though the shell weight was influenced by the dietary NPP concentration, no specific trend was observed.

Table 7. Effect of graded concentration of NPP (%) on Haugh unit score of Broiler Breeders (51-66 weeks)

NPP (%)	Period-1	Period-2	Period-3	Period-4	Overall
	(51-54wks)	(55-58wks)	(59-62wks)	(63-66wks)	(51-66wks)
0.15	71.25	67.87	69.56	70.92 ^a	69.90
0.20	69.22	70.01	69.62	67.18 ^b	69.01
0.25	69.46	68.46	68.96	69.40 ^{ab}	69.07
0.30	71.47	67.46	69.46	70.49 ^a	69.72
0.35	70.35	66.70	68.53	69.56 ^{ab}	68.78
0.40	71.11	67.54	69.33	70.05 ^a	69.51
SEM	0.331	0.406	0.278	0.371	0.238
N	10	10	10	10	10
P Value	0.220	0.256	0.870	0.054	0.736

^{a, b} means with different superscripts in a column differ significantly (P<0.05)

Table 8. Effect of graded concentration of NPP (%) on shell weight (g) of Broiler Breeders (51-66 weeks)

NPP (%)	Period-1	Period-2	Period-3	Period-4	Overall
	(51-54wks)	(55-58wks)	(59-62wks)	(63-66wks)	(51-66wks)
0.15	7.160	6.825	6.991 ^a	7.005 ^{ab}	6.994
0.20	7.124	6.966	7.045 ^a	6.539 ^c	6.917
0.25	6.756	6.561	6.658 ^b	7.071 ^{ab}	6.762
0.30	6.980	6.604	6.793 ^{ab}	6.884 ^b	6.815
0.35	7.360	6.755	7.057 ^a	6.899 ^b	7.019
0.40	7.032	6.483	6.758 ^{ab}	7.268 ^a	6.887
SEM	0.065	0.056	0.044	0.054	0.034
N	10	10	10	10	10
P Value	0.144	0.116	0.021	0.003	0.197

^{a, b} means with different superscripts in a column differ significantly (P<0.05)

4.2.5 Shell Thickness (mm)

Different NPP levels in breeder diet (0.15 to 0.4%) did not show significant ($P>0.05$) effect on shell thickness in broiler breeder females during the experimental period (Table-9).

Table 9. Effect of graded concentration of NPP (%) on shell thickness (mm) of Broiler Breeders (51-66 weeks)

NPP (%)	Period-1	Period-2	Period-3	Period-4	Overall
	(51-54wks)	(55-58wks)	(59-62wks)	(63-66wks)	(51-66wks)
0.15	0.408	0.385	0.397	0.426	0.405
0.20	0.421	0.373	0.397	0.426	0.404
0.25	0.409	0.372	0.390	0.433	0.404
0.30	0.412	0.368	0.390	0.426	0.400
0.35	0.407	0.366	0.386	0.436	0.399
0.40	0.410	0.378	0.394	0.439	0.407
SEM	0.002	0.003	0.002	0.003	0.001
N	10	10	10	10	10
P Value	0.350	0.421	0.313	0.665	0.546

4.2.6. Shell strength (N)

Egg shell strength was significantly affected due to variation in dietary NPP concentration in breeder diet during period two and four. During period two and four data showed a trend of higher shell strength in groups fed with higher concentration of NPP (0.30-0.40%) compared to the lower levels particularly 0.25% NPP fed group. The trend of shell strength during period four was not clear. The egg shell strength during period one, three and overall experimental period was not affected ($P>0.05$) with increase in dietary NPP concentration (Table-10).

Table 10. Effect of graded concentration of NPP (%) on shell strength (N) of Broiler Breeders (51-66 weeks)

NPP (%)	Period-1	Period-2	Period-3	Period-4	Overall
	(51-54wks)	(55-58wks)	(59-62wks)	(63-66wks)	(51-66wks)
0.15	20.53	19.29 ^{bc}	10.27	28.49 ^{ab}	22.05
0.20	21.78	23.08 ^{abc}	10.89	25.95 ^b	23.31
0.25	19.66	16.87 ^c	9.83	29.44 ^{ab}	21.06
0.30	16.98	29.23 ^a	8.49	25.21 ^b	23.63
0.35	17.90	29.10 ^a	8.95	32.37 ^a	25.72
0.40	21.06	23.78 ^{ab}	10.53	26.72 ^b	23.50
SEM	0.878	1.053	0.439	0.746	0.518
N	10	10	10	10	10
P Value	0.598	0.001	0.598	0.054	0.165

a, b, c means with different superscripts in a column differ significantly ($P < 0.05$)

Load cell capacity (Kgf)-20, Load cell speed (mm/min)-50 (for period 1,2 and 4) and Load cell speed (mm/min)-100 (period 3).

4.3. Hatchability parameters

4.3.1. Fertility

Variations in NPP levels in the diet (0.15 to 0.4%) did not show significant ($P > 0.05$) effect on fertility in broiler breeder females during different periods and overall experimental period except in second period. During second period the fertility in groups fed 0.20% NPP was significantly higher than those fed with lowest concentration of NPP (0.15%) in diet. However, no further improvement in fertility was observed with additional increase NPP content in the diet (Table-11).

4.3.2. Hatchability %

The level of NPP (%) in the diets significantly ($P < 0.05$) affected the per cent hatchability in broiler breeder during period one, two and overall experimental period. Hatchability percentage was highest at 0.25 % level and no improvement in the hatchability with further increase in NPP levels in diet. However, difference in hatchability was not significant ($P > 0.05$) during in period 3 and 4 (Table-12).

Table 11. Effect of graded concentration of NPP (%) on fertility (%) of Broiler Breeders (51-66 weeks)

NPP (%)	Period-1	Period-2	Period-3	Period-4	Overall
	(51-54wks)	(55-58wks)	(59-62wks)	(63-66wks)	(51-66wks)
0.15	93.58	92.26 ^c	92.14	90.81	92.20
0.20	94.15	93.25 ^a	92.44	90.52	92.59
0.25	93.88	93.07 ^{ab}	92.46	90.80	92.55
0.30	93.26	92.93 ^{ab}	92.57	90.60	92.34
0.35	93.25	92.78 ^{ab}	92.71	90.97	92.42
0.40	93.88	92.71 ^{bc}	92.33	90.79	92.43
SEM	0.127	0.075	0.057	0.058	0.043
N	10	10	10	10	10
P Value	0.224	0.002	0.065	0.248	0.085

a, b, c means with different superscripts in a column differ significantly (P<0.05)

Table 12. Effect of graded concentration of NPP (%) on hatchability (%) of Broiler Breeders (51-66 weeks)

NPP (%)	Period-1	Period-2	Period-3	Period-4	Overall
	(51-54wks)	(55-58wks)	(59-62wks)	(63-66wks)	(51-66wks)
0.15	88.06 ^{bc}	87.13 ^{ab}	84.23	81.95	85.34 ^b
0.20	88.39 ^{ab}	87.26 ^{ab}	84.81	82.64	85.77 ^{ab}
0.25	88.67 ^a	87.64 ^a	85.18	82.46	85.99 ^a
0.30	87.81 ^c	87.57 ^a	84.98	82.58	85.74 ^{ab}
0.35	88.07 ^{bc}	86.86 ^b	84.91	82.21	85.51 ^b
0.40	88.24 ^{bc}	87.15 ^{ab}	84.45	82.75	85.64 ^{ab}
SEM	0.065	0.077	0.146	0.132	0.061
N	10	10	10	10	10
P Value	0.002	0.028	0.429	0.516	0.041

a, b, c means with different superscripts in a column differ significantly (P<0.05)

4. 4. Chick quality parameters

4.4.1. Saleable chicks

The data pertaining to the per cent of saleable chicks is presented in Table-13. The % of saleable chicks was significantly (P<0.05) affected by levels NPP % during period one, but

data did not show any consistent trend related to NPP levels in diet. The difference in saleable chick was not affected during the sub sequent periods including overall experimental period (Table -13).

Table 13. Effect of graded concentration of NPP (%) on saleable chicks (%) of Broiler Breeders (51-66 weeks)

NPP (%)	Period-1	Period-2	Period-3	Period-4	Overall
	(51-54wks)	(55-58wks)	(59-62wks)	(63-66wks)	(51-66wks)
0.15	87.18 ^{abc}	86.14	83.01	80.67	84.25
0.20	87.55 ^{ab}	86.38	83.66	81.30	84.72
0.25	87.74 ^a	86.65	84.03	81.06	84.87
0.30	86.70 ^c	86.48	83.88	81.37	84.61
0.35	87.11 ^{bc}	85.84	83.85	81.06	84.46
0.40	87.36 ^{ab}	86.25	83.32	81.64	84.64
SEM	0.087	0.092	0.176	0.161	0.073
N	10	10	10	10	10
P Value	0.008	0.153	0.558	0.640	0.198

^{a, b, c} means with different superscripts in a column differ significantly (P<0.05)

4.4.2. Chick weight (g)

The levels of NPP significantly (P<0.05) affected the chick weight during period 3 and overall where the lowest chick weight was observed in breeders fed with 0.20% NPP. Whereas such difference was not seen during period one, two and four with variations in NPP levels in the diet (Table -14).

Table 14. Effect of graded concentration of NPP (%) on chick weight (g) of Broiler Breeders (51-66 weeks)

NPP (%)	Period-1	Period-2	Period-3	Period-4	Overall
	(51-54wks)	(55-58wks)	(59-62wks)	(63-66wks)	(51-66wks)
0.15	47.42	47.19	46.93 ^a	47.26	47.20 ^a
0.20	47.28	47.28	45.66 ^b	47.29	46.88 ^b
0.25	47.41	47.26	46.77 ^a	47.47	47.23 ^a
0.30	47.27	47.42	47.01 ^a	47.29	47.25 ^a
0.35	47.04	47.30	47.07 ^a	47.37	47.20 ^a
0.40	46.90	47.31	46.93 ^a	47.31	47.11 ^{ab}
SEM	0.063	0.051	0.106	0.047	0.038
N	10	10	10	10	10
P Value	0.102	0.887	0.000	0.829	0.042

^{a, b} means with different superscripts in a column differ significantly (P<0.05)

4.5. Bone quality parameters:

Different NPP levels in the diet (0.15 to 0.4%) did not show significant (P>0.05) effect on tibia weight, breaking strength, total ash%, calcium and phosphorus content in tibia ash broiler breeder females (Table-15).

Table 14. Effect of graded concentration of NPP (%) on chick weight (g) of Broiler Breeders (51-66 weeks)

NPP (%)	Bone wt.	Strength	Ash (%)	Ca (%)	Phosphorus
0.15	11.76	87.37	54.97	38.98	15.59
0.20	11.45	86.24	55.41	38.46	16.52
0.25	12.55	89.10	57.66	38.95	15.71
0.30	11.15	92.14	56.01	39.09	15.70
0.35	12.18	89.59	57.25	39.59	16.09
0.40	12.58	87.47	56.50	39.53	16.21
SEM	0.197	1.379	0.326	0.163	0.129
N	10	10	10	10	10
P Value	0.191	0.869	0.126	0.376	0.250

DISCUSSION

CHAPTER V

DISCUSSION

Phosphorous plays an important role in metabolism, integral component of many organic compounds involved in metabolic processes such as phosphorylation and synthesis of nucleic acids (Scott and Baynes 1982). Its abundance and ubiquitous presence in the entire body has become just one example of what the term “essential” could mean, especially as this mineral has been reported to influence metabolic, structural, and reproductive functions. Therefore an attempt was made to establish the non phytate phosphorus (NPP) requirement for broiler breeder females (Cobb 400Y) by feeding graded concentrations of NPP (0.15, 0.2, 0.25, 0.30, 0.35 and 0.40%). The results obtained in terms of egg production, feed conversion ratio, egg quality, hatchability, and bone mineral variables were discussed in this chapter.

4.1 PRODUCTION PARAMETERS

4.1.1 Egg Production

Different NPP (0.15, 0.2, 0.25, 0.30, 0.35 and 0.40%) levels in the diet did not influence the egg production (EP) in broiler breeders during the 4 periods of experiment (51-66 weeks of age).

The EP data, indicates that broiler breeders fed 0.15% NPP yielded similar production to those fed 0.40% NPP diet. Therefore, a concentration of 0.15% NPP is appears to be adequate for broiler breeders during 51-66 weeks of age. The calculated daily intake of NPP in breeders fed 0.15% NPP is 234 mg/b/d considering the daily feed intake of 156 g/b/d. Based on the result, it can be concluded that an intake of 234 mg NPP/b/d is adequate for broiler breeders (51-66 weeks of age) for optimum EP.

Similar to our findings several authors (Wilson *et al.*, 1980, Triyuwanta and Nys, 1992; Rama Rao *et al.*, 1999; Keshavarz, 2000; Boling *et al.*, 2000; Bhanja *et al.*, 2005;

2007; Panda *et al.*, 2006; Pelicia *et al.*, 2009; Ekmay and Coon, 2010) have found no difference in EP of broiler breeder hens or egg laying hens fed with a wide range of 0.15 to 0.4% NPP in diet.

Similar to the findings of our study several authors (Harms *et al.*, 1964; Wilson *et al.*, 1980; Waldroup *et al.*, 1967; Wilson and Harms, 1984; Bhanja *et al.*, 2005; 2007; Ekmay *et al.*, 2009; 2010) have recommended lower concentrations of NPP (0.12 to 0.22%) for broiler breeder than the levels recommended by NRC (1994) or Cobb Broiler Breeder Manual (**0.45%**)

In contrast to the above findings, Ekmay *et al.* (2012) reported reduced EP in broiler breeders (21 to 40 weeks of age) fed 0.15% NPP compared to those fed 0.40% in control group. The reduced egg production observed by the above authors might be due to the age at which the experiment was conducted and the amount of feed offered to the hens during the experiment. The birds were at peak egg production (21 to 40 weeks of age) when the trial was conducted which need more nutrient intake (including P) to sustain egg production and body weight gain. Further the quantity of feed offered to the birds was 144 g/b/d, therefore the calculated daily NPP intake was considerably lower in Ekmay *et al.* (2012) (216 mg/day) compared to the intake of current experiment (234 mg/b/d).

Another probable reason for variations observed in NPP levels recommended by different authors might be due to the level of Ca used in their test diets. Few authors (Wilson *et al.*, 1980; Wilson and Harms, 1984) used relatively lower concentrations of Ca (2.25 – 3.1%) in diet and accordingly reported lower levels of NPP for optimum breeder performance. The depression in breeder performance observed by Ekmay *et al.* (2012) when fed 0.15% contained relatively higher levels of Ca (3.25%) compared to the levels used by the former group of researchers, which is known to increase the dietary P requirements.

Higher levels of Ca in diet was reported to reduce the utilization of P (Evans and Carver, 1942)

4.1.2 Feed Conversion Ratio

In the present study, feed conversion ratio (g/egg) in all the four periods (51-66 weeks) was not affected ($p>0.05$) with increase in NPP from 0.15 to 0.40% in broiler breeder diet.

Similarly to the current findings few authors (Bhanja *et al.*, 2005; Pelicia *et al.*, 2009; Panda *et al.*, 2006) reported that feed intake per egg mass was not affected by reducing the NPP levels from 0.4 to 0.15% in broiler breeders / layer diets.

In contrast to our findings, Snow *et al.* (2004) reported that hens fed 0.16 to 0.20% available P diets (166 to 209 mg/d) had significantly lower feed efficiency than hens fed 0.45% available P during 68 to 108 weeks post moult second egg laying cycle.

As the feed intake is restricted and constant among all the treatment groups and there is no difference in egg production during 51-66 weeks in broiler breeders fed with different NPP (0.15, 0.2, 0.25, 0.30, 0.35 and 0.40%) levels in the diet, hence there was no significant difference on the feed efficiency.

4.2 HATCHING EGGS

Dietary variations in NPP concentration (0.15, 0.2, 0.25, 0.30, 0.35 and 0.40%) did not influence the hatching eggs per cent during 51-66 weeks). The results thus suggest that the lower levels of NPP (0.15%) tested is adequate for obtaining optimum number of hatching eggs.

4.2 EGG QUALITY PARAMETERS

4.2.1 Egg Weight

Egg weight was not affected by variation in NPP concentration (0.15, 0.2, 0.25, 0.30, 0.35 and 0.40%) in broiler breeder diet during the post peak egg production phase (51-66 weeks). Similar to the present findings, Berry *et al.* (2003); Bhanja *et al.* (2005) and Ekmay and Coon, (2010) in broiler breeders, Pelicia *et al.* (2009) in commercial layers, Panda *et al.* (2006) in Vanaraja breeders and Godwin *et al.* (2005) in turkey breeders did not find any variation in egg size when fed varied concentration of P in the diets. Contrarily, Ekmay *et al.* (2012) reported significant depression in egg weight of broiler breeders fed low NPP (0.15%) diet compared to 0.40% NPP fed group. Though the levels of NPP tested in the above study and the current experiment was the same (0.15%), the net intake of P in Ekmay et a. (2012) was considerably lower than the intake of P in our experiment (216 vs. 234 mg/b/d).

4.2.2 Haugh Unit Score

Haugh unit score indicates the quality of egg albumin and egg storage conditions and indicate the shelf life of the egg. The Haugh unit score was not affected by the NPP levels tested in the current study.

In contrast to our results, Skrivan *et al.* (2010) reported decreased albumen height, albumen index and Haugh units (HU) score at the highest level of available phosphorous (4.1 g/kg) compared to lower available phosphorous levels (3 and 2 g/kg). The probable reduction in egg shell quality (shell weight) with higher NPP in diet (Bhanja *et al.*, 2005) than the optimum concentration might have increased the water evaporation and in turn the albumin quality in layer fed higher concentration of P in diet.

4.2.3 Egg Density

Egg density of broiler breeders increased with increase in NPP from 0.15 to 0.40% during period 2, period 3 and overall experiment. Similar to the present findings Sohail and Rolando, (2002) found reduced egg density and egg weight with reduction of NPP from 0.7 to 0.1 % in laying chicken diet. Contrarily, Punna and Roland (1999), Keshavarz (2000) and Panda *et al.* (2005) reported no significant difference in egg specific gravity when fed different levels of NPP in layer diet.

4.2.4 Shell Weight (g)

Shell weight was not affected during 51-66 weeks of age in broiler breeder females fed with different levels of NPP. Similarly, Panda *et al.* (2005), reported no difference in shell weight in layers fed different levels of available phosphorous in diets. In contrast, Bhanja *et al.* (2005) reported that egg shell weight increased by reducing the NPP levels from 0.30 to 0.18% in broiler breeder diets.

4.2.5 Shell Thickness

Variation in dietary NPP levels (0.15 to 0.4%) did not effect the shell thickness in broiler breeder females. Similarly Bhanja *et al.* (2005) reported that shell thickness was not affected by phosphorus levels in diet. Godwin *et al.* (2005) reported that shell thickness was not affected by reducing the available P levels (0.55, 0.35 and 0.17) in turkey breeder's diets.

4.2.6. Shell strength

Breaking strength of egg shell was not affected due to variations in concentration of NPP in diet during 51-66 weeks of age in broiler breeder females. Similarly, Panda *et al.* (2005) reported no effect of dietary NPP levels (0.15, 0.18, 0.21, 0.24, 0.27, and 0.30%) on shell strength in White Leghorn layers Pelicia *et al.* (2009) also concluded that egg shell strength was not influenced by dietary Av. P levels (0.25, 0.30, 0.35, and 0.40%) in diet of semi-heavy commercial layers. Skrivan *et al.* (2010) and Liu *et al.* (2007) reported that there

is no difference in shell strength in commercial layers fed with different Av.P levels in the diets.

Considering the above egg shell quality variables (shell weight, shell thickness and shell breaking strength) data, it can be concluded that a concentration of 0.15% NPP appears to be adequate for broiler breeders. Similar to our current study Bhanja *et al.* (2007) also did not find different in shell breaking strength in broiler breeders fed diet with no supplemental P (0.12%). Contrarily, Triyuwanta *et al.* (1992) found significant reduction in egg breaking strength with increase in dietary Av.P, the higher P tested in their study (0.60 and 1.0%) might have interfered with Ca deposition in egg shell and therefore resulted in reduced egg shell strength in breeders fed higher concentrations of Av.P in diet.

4.5.1. Fertility

Gradual increase in NPP levels in diet (0.15 to 0.4%) did not effect on fertility in broiler breeder females except during second period. Fertility was low in group fed 0.15% NPP diet during the second period and such reduction in fertility was not observed during the entire experiment (51 to 66 weeks of age).

Similarly, Triyuwanta and Nys (1992) and Brake *et al.* (2003) observed no significant difference in fertility in broiler breeder hens fed with different dietary concentration of phosphorus (0.1 to 1.0%). The fertility is usually affected by quality of semen and the nutrition profile of male diet, which in turn influence this parameter. Bootwalla and Harms (1988) found no significant affect in semen quality variables (semen volume, sperm per ejaculate and intact sperm cells) when breeder males (32 to 38 weeks of age) were diet without any supplemental phosphorus (0.09% NPP).

Contrary to our results, Plumstead *et al.* (2007) observed that there was significant reduction in fertility fed 0.19% compared to those fed control diet containing 0.37% NPP in diet in broiler breeders reared on slat floor and 2.7% Ca. The reduction in fertility observed in Plumstead *et al.* (2007) study could be due to long duration of experiment (22 to 64 weeks of

age), feeding of the same low NPP to cockerels, feeding low NPP (0.27%) for both male and hens during growing period (10 to 21 weeks of age). In our current study the breeders were on the recommended NPP during growing and pre experimental phase (0.43 and 0.36% NPP, respectively) and males were reared on high P (0.35% NPP) than the levels fed by Plumstead *et al.* (2007).

5.2. Hatchability

Hatchability during the initial 2 periods (1 and 2) and overall experiment was significantly lower at 0.15% NPP in diet and maximum hatchability was observed in groups fed 0.25% NPP. Though the hatchability showed significant difference due to dietary P concentrations in our study, the actual difference in hatchability percent between the lowest NPP (0.15%) and 0.25% NPP (where significantly higher hatchability was observed during the overall experiment) was less than 0.50% (0.35%) which may be of less importance under practical conditions. In contrast, several authors reported that reduced dietary Av.P / NPP from 1.00 to 0.12% did not negatively impact hatchability (Triyuwanta and Nys, 1992; Bhanja *et al.*, 2007; Plumstead *et al.*, 2007; Ekmay and Coon, 2010; Arguelles-Ramos, 2011) in broiler breeders, Godwin *et al.* (2005) in turkey breeders. Similarly, Triyuwanta *et al.* (1992) reported no significant effect of increase in dietary Av.P from 0.20 to 1.0% on fertility and hatchability in dwarf broiler breeders. Increased dietary P levels was reported to progressively increase excreta phosphorus content (Bhanja *et al.*, 2007; Plumstead *et al.*, 2007; Ekmay and Coon, 2010) and the net accretion of P in egg yolk / egg content was not affected by dietary variation in NPP content. Though the P content in egg was not estimated in the current study, many authors (Bhanja *et al.*, 2005; 2007; Ekmay and Coon, 2010) reported uniform quantity of P deposition in egg irrespective of NPP levels in breeder diet, which might be responsible for lack of influence of dietary NPP on egg hatchability. On the contrary, improved hatchability was observed by Harms *et al.* (1964) and Waldroup *et al.* (1967) when the NPP levels (0.48 and 0.19%, respectively) was increased above the

requirements the mineral for EP (0.13 and 0.16%, respectively) for egg laying chicken. The discrepancy could be due to use of egg laying chicken and the supplemental P source. The latter group of authors used defluorinated rock phosphate as a source of supplemental phosphorus, whose availability is known to be less and therefore, higher dietary levels to support hatchability.

4.6.1. Saleable chicks

The per cent of saleable chicks was significantly higher in groups fed 0.25% NPP compared to the those fed further higher levels of NPP (0.30 / 0.35%), but there was no difference in the number of saleable chicks in groups fed 0.15 and 0.25% NPP. The improved number of saleable chicks could be due to significant increase in hatchability of eggs in breeders fed 0.25% NPP compared to those fed higher concentrations (0.30% or higher) of NPP in diet. The difference in saleable chicks during other periods and overall experimental was not affected due to increase in NPP level from 0.15 to 0.40% in breeder diet. Though Thus the data suggest that the lowest concentration of NPP tested (0.15%) appears to be adequate for broiler breeders (51 to 66weeks of age) to get optimum number of saleable chicks per hen.

4.6.2. Chick weight

The levels of NPP significantly ($P < 0.05$) affected the chick weight during period 3 and overall, but this did not show any consistent trend related to NPP levels in diet. Whereas the effect not seen in period 1, 2 and 4 with different levels NPP % in the diet.

Chick weight was significantly lower in breeders fed 0.20% NPP compared to other levels during period 3 and overall experimental period. Whereas, such reduction in chick weight was not found during other periods (period 1, 2 and 4). Though the chick weight in groups fed 0.20% NPP during period 3 and overall experiment was significantly lower (45.66 and 46.88 g, respectively), the difference in chick weight in 0.20% NPP fed group and the highest weight (0.35 and 0.30% NPP fed groups respectively) were 1.41 and 0.35 g, respectively.

The precise reason for lower body weights in 0.20% group during period 3 and overall experiment could not be identified as there was no significant difference in egg weight during the above mentioned periods. Also the chick weight in the lowest NPP (0.15%) fed group was higher than those fed 0.20% NPP add to the confusion exist over the low body weight of chicks in 0.20% NPP group. Significant reduction in body weight of day old chick from breeder hens fed 0.15% NPP was reported by Ekmay *et al.* (2012). The reduced egg weight and probably reduced body weight of breeder hens fed the 0.15% NPP might be responsible for the reduced body weight of day old chicks as observed by the above authors.

4.7 BONE QUALITY PARAMETERS

Tibia mineralization variables (bone weight, bone breaking strength, bone ash, bone calcium and phosphorus contents) in broiler breeder females (51 to 66 weeks of age) were not affected due to the increase in NPP concentrations from 0.15 to 0.40% in diet. Thus data of bone mineral variables suggest that a level of 0.15% NPP is adequate for optimum bone mineralization and skeletal strength for broiler breeders during the post peak egg production phase (51 to 66 weeks of age).

The data of our study indicate that the requirement of NPP is the same for both egg production and bone mineral variables. Contrarily, increased bone mineralization was observed by several authors (Waldroup *et al.*, 1975; Triyuwanta *et al.*, 1992; Bhanja *et al.*, 2007; Ekmay and Coon, 2009, 2010) at higher dietary P levels (0.225 to 1.0, 0.225) than the levels required for optimum EP.

Bone is the main storage organ for Ca and P, which serves as a dynamic source of both the minerals for egg production and several metabolic functions. As this trial was conducted for considerable long duration (16 weeks), any sub-optimal or deficiency of NPP could have influenced EP, shell quality and showed symptoms of lameness in breeder during the study period, which was not observed. Further, the concentrations of Ca and P in tibia ash

were also not affected in breeders fed with the lowest concentration of NPP in diet also suggest that 0.15% NPP was adequate to support the bone mineral content and strength, which is one of the major metabolic functions of P in chicken diet.

Similarly, Boorman and Gunaratnae (2001) also did not find any difference in bone mineral variables when layers were fed diet with 0.156% NPP compared to those fed 0.38% NPP in diet. Similar to our findings, Bootwalla and Harms (1988) also did not find variation in tibia ash content when broiler breeder males were fed a calculated level of 0.09% NPP in diet (no supplemental P). The previous results from the authors laboratory also confirms that the tibia ash and breaking strength were not affected when the broiler breeder diets contained as low as 0.12 to 0.18% NPP (Bhanja *et al.*, 2005; 2007). It is worth to mention that the above levels were the lowest concentrations of NPP tested in their experimental diets. Similarly, higher bone ash content was observed in laying hens fed 0.1% NPP compared to those fed higher levels of the mineral in diet (Jalal and Scheider, 2001). This decrease in bone strength at the higher NPP levels might be attributed to the reduced bone accretion because of increased excretion of P and Ca due to high blood P levels (Gordon and Roland 1998).

Contrarily to our findings, reduced tibia ash and tibia breaking strength were observed by Ekmay *et al.* (2012) in broiler breeders (21 to 40 weeks of age) fed 0.15% compared to those fed 0.4% NPP in diet. These findings suggest that the 0.15% NPP is not adequate for the broiler breeders to support optimum skeletal development. The discrepancy between the current findings and Ekmay *et al.* (2012) might be due to age of the birds. Ekmay *et al.* (2012) used young flock (21 to 40 weeks of age) where egg production is relatively higher (63.1 to 69.2%) and the birds were in growing phase which demand high P than the birds for P requirement after peak production (51 to 66 weeks of age) which were used in our current experiment.

In the current study, the minimum NPP level tested (0.15%) appears to be adequate for EP, shell quality, hatchability and tibia mineral variables in broiler breeders with an average intake of 234 mg NPP/b/d. Contrarily Ekmay and Coon, (2010) suggested 0.20% NPP (288 mg/kg) for EP and further higher levels of 0.25% NPP (360 mg/b/d) for bone mineral variables in broiler breeder diet. The higher recommendations made by Ekmay and Coon (2010) might be due to the use of 0.20% NPP as the lowest levels tested in their experiment, as they did not found difference in the breeder performance (EP) the authors recommended that minimum level used as the requirement. The higher NPP requirements suggested by Ekmay and Coon (2010) contradict our observations. The probable reason for higher P recommendation for bone ash might be due to higher mineral requirement for young birds which produce more number of eggs and grow constantly till up to peak egg production phase.

By observing the literature it is evident that majority of authors have recommended the lowest levels of P tested as the optimum requirement for different production variables (like EP, EW, shell quality, bone mineralization, hatchability, etc.), when they could not find statistical difference among the tested levels of P. The data of current study suggest 0.15% NPP as optimum, while Ekmay and Coon (2010) data suggest 0.20% NPP for EP, while Bhanja *et al.* (2007) tested the lowest NPP (0.12%) for coloured synthetic broiler breeders without supplementing inorganic P and yet got similar production compared to the levels recommended by the breeder production manual (0.40%). One of the major limitations in adoption of very low NPP (without inorganic P supplementation) as observed by Bhanja *et al.* (2007) is the low egg production and egg size compared to the broiler breeds being used by the poultry industry. In our current study also we have not tested the real low NPP levels that contain no additional inorganic P which might have given the optimum performance similar to the current findings. Considering the current scenario of literature on P requirement

for broiler breeders, it is quite convincing that NPP requirements are much lower than the recommendations suggested by the breeding companies. However, a comprehensive detailed study involving the high yielding broiler breeders from the point of lay to culling phase with wide range of NPP concentration in diet will suggest the actual NPP requirements which may find more practical application by the poultry industry.

Sohail and Rolando, 2002 and Gordon and Roland (1998) reported that bone breaking strength is higher in hens fed with 0.3 to 0.4% NPP. Punna and Roland, 1999 revealed that hens fed 0.1% available phosphorous showed reduced bone mineral density ($P < 0.001$).

SUMMARY AND CONCLUSIONS

Chapter VI

SUMMARY

An experiment was conducted for 112 days on Cobb (400Y) broiler breeders (51-66 week of age) to establish the non phytate phosphorus (NPP) requirement for broiler breeder females by feeding varied concentrations of NPP (0.15, 0.2, 0.25, 0.30, 0.35 and 0.40%) in diet. The experiment was conducted on 1200 broiler breeders (Cobb-400Y) of 51 week old having uniform body weight and egg production, which were randomly distributed in to six experimental groups with 10 replicates per group and each replicate contained 20 birds. Birds housed by placing 2 birds in a colony cages (18x15x18 inch) and successive 10 cages were considered as a replicate. A linear feed was provided for each replicate and therefore, the replicate mean was considered as a unit for stat analysis. The experiment was conducted in well ventilated raised platforms poultry house. Six diets were formulated having 6 graded concentrations of NPP (0.15, 0.20, 0.25, 0.30, 0.35, and 0.40%) and keeping all other nutrients constant among diets. Birds were offered the respective diets from 51 to 66 weeks. Eggs produced by each replicate were collected on the daily basis. Feed restriction is being practiced and the birds were fed at the rate of 156 g/bird/day for first 3 weeks (51 to 53 weeks of age) and 154 g/bird/day for the rest of experimental period. Egg weight was recorded weekly on total eggs produced by each replicate. All the eggs laid during last day of every period were collected to assess the egg quality parameters (egg weight, egg density, Haugh unit score, shell weight, shell thickness and shell strength). For hatching total eggs laid from all replicates were collected on daily basis and stored at 15-17 °C for one week. These eggs were kept for hatching on weekly intervals all through the experiment (16 weeks). Total chicks produced in each hatch were examined for chick quality and at the end of experiment, one bird from each replicate (60 birds) were slaughtered by cervical dislocation to study bone

mineralization variables (Tibia weight, breaking strength total ash, calcium and phosphorus content in tibial ash). The summary of the experiment results with regard to egg production, FCR, hatching eggs %, egg quality, hatchability, chick quality and bone quality variables were presented below.

The broiler breeders fed different NPP (0.15, 0.2, 0.25, 0.30, 0.35 and 0.40%) levels in the diet did not show significance ($P>0.05$) influence on egg production, feed conversion ratio and hatching egg %.

Feeding graded concentration of NPP (0.15, 0.2, 0.25, 0.30, 0.35 and 0.40%) did not show any effect on egg weight. Egg density increased ($P<0.05$) with increase in NPP levels from 0.15 to 0.4% in the diet during period 2, period 3 and overall period. The Haugh unit score was not significantly ($P>0.05$) affected by the NPP levels in diet during experimental period except period 4. Shell weight was significantly different ($P<0.05$) during period 3 and period 4 due to different levels of NPP. But the shell weight during the overall period was not differed significantly ($P>0.05$). Different NPP levels in the diet (0.15 to 0.4%) did not show significant ($P>0.05$) effect on shell thickness. The data on shell strength during overall experiment was not affected ($P>0.05$) by the dietary NPP concentration.

Variation in NPP levels in diet (0.15 to 0.4%) did not show significant ($P>0.05$) effect on fertility in broiler breeders during the experiment except during period 2. Fertility was low at 0.15% NPP during the second period and the effect was not reflected in overall experiment. The level of NPP in the diets significantly ($P<0.05$) affected the per cent hatchability in broiler breeder during period 1 and 2 and overall experiment, where highest hatchability percentage was observed at 0.25% NPP, such difference in hatchability was not noticed during period 3 and 4.

The difference in saleable chick per cent was not affected due to NPP concentration in diet during period 2, 3 and 4, and overall experiment. The levels of NPP significantly

($P < 0.05$) affected the chick weight during period 3 and overall. Whereas the effect was not seen during period 1, 2 and 4 due to NPP levels in diet. The highest chick weight was observed at 0.25 and 0.3% NPP in diet. Bone mineral variables (bone weight, bone breaking strength, bone ash, calcium and phosphorus in bone ash) were not affected with increase in NPP from 0.15 to 0.40% in broiler breeder diet.

Based on the results of the present study it is concluded that,

1. This study indicates that feeding 0.15% NPP level in the diet might be sufficient for broiler breeder for production parameters. But 0.25% NPP levels shows better hatchability %.
2. Variation in concentration of NPP in diet did not influence egg production performance, FCR, hatching eggs, egg weight, Haugh unit score, shell weight, shell thickness, shell strength, fertility, saleable chicks per cent and bone quality parameters in broiler breeders (51 to 66 weeks of age).
3. Different NPP levels in the diet significantly affected the egg density, hatchability % and chick weight.

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