EFFECT OF PHYTASE SUPPLEMENTATION ON PHOSPHORUS UTILIZATION AND PERFORMANCE IN LAYER CHICKEN

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled "**EFFECT OF PHYTASE SUPPLEMENTATION ON PHOSPHORUS UTILIZATION AND PERFORMANCE IN LAYER CHICKEN**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title, of any other university or society.

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Certified that the thesis entitled **"EFFECT OF PHYTASE SUPPLEMENTATION ON PHOSPHORUS UTILIZATION AND PERFORMANCE IN LAYER CHICKEN"** is a record of research work done independently by **Sri. SUKUMAR D.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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Introduction

1. INTRODUCTION

Poultry farming in India has attained the status of an industry and has become a major contributor to the national economy. The value of poultry production has gone up from Rs. 800 crores in 1980 to Rs. 9500 crores in 1996 (Anon, 1994). India currently produces over 30,000 million eggs and 450 million kg of broiler meat annually. Though the growth in the poultry sector during the past few decades is remarkable, still it has to go a long way to meet its domestic requirements by enhancing the egg output five folds and chicken meat production by ten folds.

The estimated growth of layers in India is in the order of eight to ten per cent per annum. The feed requirement for the increasing poultry population in the country is estimated to be about eight million tonnes per year. It calls for adequate supply of feedstuffs. Spiralling feed cost is a major constraint in poultry production since it accounts for 65 to 70 per cent of the total production cost. In addition, availability of feed ingredients is also posing problems since there is competition between human beings and livestock for the same. This necessitates the poultry farmer to look into ways and means to reduce the cost of feed.

It has been known that many feed ingredients both conventional as well as non-conventional contains variable quantities of anti-nutritional factors which reduces opportunities to exploit their full nutritional value. For instance cereals, millets and other plant materials which constitute a major part of poultry feed contain phosphorus in the form of phytic acid (hexa phosphate inositol). It is the major form of plant phosphorus and assumes high levels in many of the commonly used ingredients. Phytin phosphorus varies in availability depending upon the cereals / plant materials and species of animals that digest it. Phytate bound phosphorus is poorly available for poultry because birds lack endogenous phytases which are able to degrade phytin effectively. It is estimated that only 30 per cent of plant phosphorus can be utilised by poultry and the remaining part cannot be utilised and is excreted in the faeces.

In addition, the phytic acid may combine with starch, protein and certain inorganic elements such as calcium, magnesium, zinc, copper, iron and potassium. Being insoluble, these complexes precipitate in the gut without getting absorbed and finally excreted. On the other hand, almost all the phosphorus in inorganic and from animal sources can be fully utilised by poultry. Therefore, farmers supplement diets with inorganic phosphorus usually beyond the level that birds actually require. This over supplementation leads to phosphorus excretion through waste and leads to environmental pollution. In order to make phytin phosphorus biologically available, it is necessary to hydrolyse phytin by means of phosphatase enzyme called phytase. This enzyme converts phytic acid into orthophosphate, inositol and other phosphoinositol intermediaries and thereby increasing the bio-availability of phosphorus.

In order to make the phosphorus from plant phosphorus source more available as well as to reduce the chances of chelation of other inorganic compounds especially minerals nutritionist's are currently trying to use extraneous source of enzyme.

Though the potential use of phytase as a feed supplement was identified long back (Nelson *et al.*, 1968), incorporation of this enzyme has generally been regarded not cost effective in comparison to dicalcium phosphate or defluorinated rock phosphate. The advance in biotechnology and environmental concern hastened the use of this enzyme as feed additive. Biotechnological innovation has had an important influence on the nutrition of poultry and its vital role in developing enzyme products (Jenson, 1996). Discovery of the natural presence of high concentrations of phytase in cultures of *Aspergillus ficuum, Aspergillus niger* and *Bacillus subtilis* paved the way for the addition of this enzyme in poultry feed.

Addition of phytase in poultry diets can effectively break down the phytin phosphorus and thereby increase the bio-availability of phosphates. It can also be used as a means of reducing the feed cost and the concern about phosphorus pollution. Comparatively little work has been carried out to evaluate the influence of phytase supplementation on laying house performance.

Therefore, a study on the supplementation of phytase enzyme on phosphorus utilization in practical layer ration and its impact on the performance in commercial layers was carried out.

Review of Literature

2. REVIEW OF LITERATURE

Enzymes are used in animal feeds either to balance the suboptimal synthesis of endogenous enzymes by animals or for breakdown of compounds which cannot be digested into absorbable nutrients by these enzymes. In general enzymes supplementation has been found to enhance the biological availability and utilization of nutrients. The phosphorus from plant based feed can be utilized only in small amounts by poultry. Approximately two third of the total phosphorus contained in plant feed is present as bound phytate phosphorus. Several experiments revealed that the release of phosphorus from phytic acid can be improved by addition of microbial phytase to the diets and therefore phosphorus in mineral form can be reduced. In this chapter, an attempt has been made to review the available literature related with the inclusion of phytase enzyme on phosphorus utilization and subsequent performance in layer chicken.

2.1 Egg production

In two experiments hybrid layers of white and brown varieties were offered a basal diet of maize-soyabean meal containing phosphorus1.2 g/kg and phytate-phosphorus 2.5 g/kg feed or basal diet plus monocalcium phosphate (total phosphorus 4.8 g/kg) and phytase 250 or 500 units/kg (Schoner *et al.*, 1993).

In both the trials phytase supplemented diets significantly improved laying performance.

Jeroch and Peter (1994) studied the effect of phytase on laying hens fed with maize based or wheat based diets and supplemented with phytase 500 units/kg or inorganic phosphorus 1 g/kg and observed that egg production was maintained at higher levels by supplementing maize-based diets with phytase or inorganic phosphorus, while egg production from wheat based diets was not improved by either supplement.

Budor *et al.* (1995) studied with laying hens from 22 to 64 weeks of age fed a diet containing 0.5 per cent phosphorus and 0.2 per cent available phosphorus supplemented with phytase at levels of 0 and 600 units/kg and noticed that phytase supplemented groups showed increased egg production. In another experiment also, with laying hens from 49 to 64 weeks old provided with diet containing the same phytase level but phosphorus at a level of 0.4 per cent (0.1 per cent available phosphorus), they could observe positive effect of phytase on egg production.

Thirty six weeks old laying hens were offered diets based on maize, wheat and soyabean oil meal with 100, 90 or 80 per cent available phosphorus or diets supplemented with 500 phytase units to the diets with 90 per cent and 80 per cent available phosphorus, Kwon *et al.* (1995) observed that egg production of hens fed on 90 per cent available phosphorus only, 90 per cent available phosphorus and phytase or 80 per cent available phosphorus and phytase was similar to that of hens fed on 100 per cent available phosphorus only, but that of hens fed on 80 per cent available phosphorus only was significantly lower.

Lettner *et al.* (1995) conducted an experiment with hybrid layers given diets containing 0.61 and 0.41 per cent phosphorus with 0.65 per cent monocalcium phosphate (control) or Natuphos 80 g (5000 units/g) plus feed grade calcium carbonate and observed that laying performance was not different between groups.

Usayran and Balnave (1995) studied the influence of supplementary phytase on the phosphorus requirements of laying hens fed on wheat based diets. Fifty nine weeks old laying hens were maintained on a diet containing wheat, sorghum and soyabean meals as the major ingredients. Dietary total phosphorus varied between 3.2 and 4.6 g/kg. Diets were given without or with supplemented phytase at 500 units/g. Results showed that inclusion of phytase in diets containing total phosphorus between 3.2 and 4.6 g/kg had an adverse effect on egg production at temperature 18 and 30°C. It was also observed that dietary total phosphorus at a level of 3.2 g/kg provided an available phosphorus at a level of 1.2 g/kg with a dietary phytase activity of < 200 units/kg satisfied phosphorus requirement of the hen.

In a trial with laying hens fed on wheat maize soybean meal diets containing 0.5,0.55 and 0.6 per cent total phosphorus, equivalent to 0.25,0.30 and 0.35 per cent available phosphorus supplemented with microbial phytase at levels of 0, 150, 300 or 450 FTU/kg, Kaminska *et al.* (1996) observed that hens fed on diet containing 0.50 per cent total phosphorus supplemented with 300 FTU/kg showed the highest laying rate (93 per cent)

Roland and Gordon (1996) carried out an experiment to study the efficacy of phytase with 21 week old chicken for 17 weeks. Birds were allocated 1 of 10 diets arranged factorially with 5 levels of available phosphorus from 0.1 to 0.5 per cent and phytase 0 and 300 FTU/kg and found that feeding 0.1 per cent available phosphorus diet without phytase decreased egg production and it was improved with phytase supplementation.

Effect of supplemental phytase to laying hens fed diets of different phosphorus content was studied by Kaminska (1997). Birds were fed on diet containing 0.45, 0.52 and 0.58 per cent total phosphorus (0.25,0.30 and 0.35 per cent available phosphorus) supplemented with 0, 150, 300 or 450 units of microbial phytase per kg of feed from 48 to 70 weeks of age. It was concluded that levels of phosphorus and phytase had no effect on egg production.

In a feed trial Klis *et al.* (1997) added phytase or monocalcium phosphate to a corn-soyabean meal layer diet containing calcium 40 g/kg and phosphorus 3.6 g/kg diet at levels of 0,100, 200 and 300 FTU/kg or levels of 0, 0.3, 0.6 and 0.9 g monocalcium phosphate per kg respectively and stated that production performances were significantly improved by dietary supplementation of the negative control diet with phytase or monocalcium phosphate.

Oloffs *et al.* (1997b) reported that performance of laying hens were not different when fed with phosphorus deficient maize soyabean oil meal diets supplemented with 0.1 per cent inorganic phosphorus or phytase (Natuphos) at a level of 500 FTU/kg

Gordon and Roland (1998) conducted an experiment in commercial laying hens for six weeks with diets containing inorganic phosphorus at two levels viz., 0.1 and 0.3 per cent supplemented with phytase at a level of 300 units/kg feed and opined that phytase supplementation had a positive effect on egg production.

To study the effect of phytase in laying hens, Hadorn and Wiedmer (1998) added 300 and 150 units/kg of commercial phytase (NOVO - L) in a phosphorus reduced diet and reported that during phase 1 (21 - 48 weeks) and phase 2 (49 - 60 weeks) no phytase related differences could be seen in egg production, but egg production was reduced (- 5 per cent) in phase 3 (61-68 weeks), among birds which receives low phosphorus diets inspite of phytase inclusion.

Rao *et al.* (1999)carried out an experiment with White Leghorn layers using a reference diet (36.0 g calcium and 2.0 g non phytate phosphorus/kg), basal diet A (36.0 g calcium and 1 g non phytate phosphorus/kg) and basal diet B (36 g calcium and 1.5 g non phytate phosphorus/kg). Both the basal diets were supplemented with phytase (250 units/kg), cholecalciferol (200 mcg/kg) or yeast culture (13.5 x 10^9 *Saccharomyces cerevisiae* cells/kg). All the diets were fed to layers for 57 days from 338 to 394 days of age. Feeding of basal diet A resulted in significantly ($p < 0.05$) lower egg production (62.8 eggs per 100 birds per day) compared to those fed the reference diet (90.8 eggs/100 birds/day). However, phytase supplementation to this diet improved ($p < 0.05$) egg production (91 eggs/100 birds/day). Feeding of basal diet B with or without enzyme supplementation resulted in layer performance that was similar to the reference diet fed layers.

2.2 Feed intake

Simons *et al.* (1992) reported that feed consumption by hens consuming a low phosphorus diet (0.33 per cent total phosphorus) with and without phytase was 108 and 89 g, respectively.

Budor *et al.* (1995) conducted three trials to study the effect of added phytase in laying hen diets on phosphorus excretion and assimilation and observed that phytase had no effect on feed consumption.

In a trial with laying hens fed diets containing 100, 90 or 80 per cent of the available phosphorus recommended by the NRC and supplemented with 500 units of phytase to 90 and 80 per cent of the recommended available phosphorus, (Kwon *et al.* 1995) reported that hens fed on 80 per cent available phosphorus and 80 per cent available phosphorus plus phytase had a higher feed intake than those on 100 per cent available phosphorus.

Zobac *et al.* (1995) carried out an experiment with pullets fed on diets containing 100, 50 or 0 per cent of recommended dicalcium phosphate supplemented with phytase (Natuphos). Feeding on the diet low or devoid of dicalcium phosphate with out phytase significantly reduced feed intake and addition of Natuphos considerably improved feed intake.

Roland and Gordon (1996) conducted a trial to study the efficacy of phytase with 21 week old chicken for 17 weeks. Birds were allocated 1 of 10 diets arranged factorially with 5 levels of available phosphorus from 0.1 to 0.5 per cent and phytase 0 and 300 FTU per kg and reported that feeding the 0.1 per cent available phosphorus diet without phytase resulted in decreased feed intake and supplementing with phytase corrected this effect.

Kaminska (1997) conducted a trial with brown hens from 48 - 70 weeks of age, fed on diets containing 0.45,0.52 and 0.58 per cent total phosphorus(0.25, 0.30 and 0.35 per cent available phosphorus respectively), supplemented with 0, 150, 300 or 450 units of microbial phytase per kg of feed. He opined that the different levels of phosphorus and phytase had no effect on feed intake.

Gordon and Roland (1998) conducted a 6 weeks study to determine the influence of supplemental phytase on calcium and phosphorus utilization in commercial laying hens. Diets were arranged factorially with three levels of dietary calcium (2.5, 3.8 and 3.1 per cent), fed at two levels of non - phytate phosphorus (0.1 and 0.3 per cent non - phytate phosphorus) with or without supplemental phytase (300 units/kg). They reported that phytase supplementation increased feed consumption at both levels of dietary phosphorus, but the magnitude of that increase was greatest when diets containing 0.1 per cent non-phytate phosphorus.

Hadorn and Wiedmer (1998) added 300 and 150 units/kg of commercial phytase (NOVO - L) in a phosphorus reduced diet of laying hens and opined that during phase 1 (21-48 weeks) and phase 2 (49 - 60 weeks) no phytase related differences could be seen in feed consumption. However, daily feed consumption was lower (- 6.2 per cent) during phase 3 (61 - 68 weeks).

2.3 Feed efficiency

Kwon *et al.* (1995) conducted an experiment with laying hens fed on diets based on maize, wheat and soyabean oil meal with 100 per cent available phosphorus, 90 per cent available phosphorus or 80 per cent available phosphorus recommended by the NRC and or supplemented with 500 phytase units to the diets with 90 per cent available phosphorus or 80 per cent available phosphorus and observed that feed gain ratio of hens fed on 90 per cent available phosphorus, 90 per cent available phosphorus plus phytase and 80 per cent available phosphorus plus phytase was similar to that of hens fed on 100 per cent available phosphorus. The feed gain ratio of hens fed on 80 per cent available phosphorus without enzyme was higher than that of hens maintained on 100 per cent available phosphorus.

In an experiment phytase or monocalcium phosphate was added to corn soyabean meal diet containing calcium 40 g/kg and phosphorus 3.6 g/kg diet at levels of 0, 100, 200 and 300 FTU/kg of Natuphos or 0, 0.3, 0.6 and 0.9 g monocalcium phosphate per kg and reported that feed conversion ratio of the hens from the phytase supplemented groups remained good throughout the experiment (Klis *et al.* 1997).

In an attempt to study the effect of phytase addition in a low phosphorus layer diet, Hadorn and Wiedmer (1998) conducted an experiment in which two levels of phytase, NOVA - L 300 and 150 units/kg were supplemented in low phosphorus layer diet. They found that feed conversion rate was not influenced by phytase during 21 - 48 and 61 - 68 weeks of age.

Commercial White Leghorn layers offered with a maize soya diet containing 3.6 g calcium and 1 g non phytate phosphorus/kg supplemented with phytase (250 units/kg), resulted in an improvement in feed conversion efficiency (Rao *et al.* 1999).

2.4 Body weight gain

Nahashon *et al.* (1994) opined that higher body weight gain was observed when Single Comb White Leghorn layers were fed with phytase and other microbials (lactobacillus) added diet.

Zobac *et al.* (1995) reported that phytase enzyme inclusion in the diet of pullets had positive effect on body growth and, feeding on the diet low or devoid of dicalcium phosphate without Natuphos significantly reduced growth.

Klis *et al.* (1997) studied the influence of phytase in laying hens provided with diets containing 0, 0.5 or 1 g phosphorus /kg feed and supplemented with 0, 250 or 500 units of phytase per kg feed respectively and opined that growth is significantly ($p < 0.01$) improved by dietary supplementation of phytase.

The efficiency of supplemental phytase in laying hens was studied by Carlos and Edwards (1998). The basal diet was formulated to contain 3 per cent calcium and 0.33 per cent total phosphorus. They concluded that phytase had a positive effect on body weight.

In a study to find out the efficacy of phytase to enhance the phytate phosphorus availability Rao *et al.* (1999) provided a reference diet (36 g calcium and 2 g non phytate phosphorus/kg), basal diet A (36 g calcium and 1 g non phytate phosphorus/kg), basal diet B (36 g calcium and 1.5 g non phytate phosphorus/kg). Both the basal diets were supplemented with phytase (250 units/kg), cholecalciferol (200 mcg/kg) or yeast culture (13.5 x 10^9 *Saccharomyces cerevisiae* cells/kg)to White Leghorn layers for 57 days from 338 to 394 days of age. Feeding of phytase supplementation resulted significant improvement in final body weight ($p < 0.05$) compared to reference diet.

2.5 Egg weight

In order to study the influence of phytase enzyme, Budor *et al.* (1995) conducted a trial in which laying hens were fed with diet containing 0.6 per cent total phosphorus (0.3

per cent available phosphorus) and supplemented with phytase at levels of 0 and 600 units/kg from 22 to 48 weeks of age. They reported that egg weight was decreased in phytase supplemented groups (62.45 Vs 61.62 g).

In an investigation, laying hens were fed diets containing 100, 90 or 80 per cent of the available phosphorus recommended by the NRC and supplemented with 500 units of phytase to 90 and 80 per cent of the recommended available phosphorus diets. (Kwon *et al.,* 1995). The results showed that there was no difference in egg weight or percentage of broken eggs among groups. However, daily egg weight of hens fed on 80 per cent of the recommended available phosphorus without enzyme was lower than that of hens in other groups.

Kaminska (1997) studied the effect of supplemental phytase to laying hen diets of different phosphorus level and reported that eggs were heaviest when no phytase was added or the diet contained the lowest phosphorus level.

Two experiments were conducted to investigate the effects of supplementing a corn - soyabean layer diet with either phytase, 1-25 - dihydroxy cholecalciferol or their combination (Carlos and Edwards, 1998). The basal diet was formulated to contain 3 per cent calcium and 0.33 per cent total phosphorus. They observed that phytase had no effect on egg weight.

Gordon and Roland (1998) conducted a 6 weeks study to determine the influence of supplemental phytase on calcium and phosphorus utilisation with commercial laying hens. Diets were arranged factorially with three levels of dietary calcium (2.5, 2.8 and 3.1 per cent), fed at two levels of non-phytate phosphorus (0.1 and 0.3 per cent non - phytate phosphorus) with or without supplemental phytase (300 units/kg). They reported that supplemental phytase significantly increased egg weights during weeks 4 and 6, when diets contain 0.1 per cent non - phytate phosphorus.

Hadorn and Wiedmer (1998) studied the effect of phytase supplementation in a low phosphorus layer diet and reported that the reduction of dietary total phosphorus content led to lower egg weight and a decrease of large eggs. This effect was lowered by supplementation of phytase enzyme.

Rao *et al.* (1999) stated that addition of phytase (250 units/kg) in layer diet containing 1 g non phytate phosphorus/kg) had a positive effect on egg weight.

2.6 Specific gravity

Nahashon *et al.* (1994) determined the presence of phytase activities in condensed cane molasses solubles (CCMS) and CCMS - Lactobacillus (Lacto) in White Leghorn layers. They found that Lacto supplemented to 0.25 per cent available phosphorus diet produced eggs with higher specific gravity than the unsupplemented 0.45 per cent available phosphorus diet, but not different from unsupplemented 0.25 per cent available phosphorus diet.

Roland and Gordon (1996) carried out an experiment to study the influence of phytase with 21 week old chicken for 17 weeks. Birds were allocated 1 of 10 diets arranged factorially with 5 levels of available phosphorus from 0.1 to 0.5 per cent and phytase 0 and 300 FTU/kg and opined that feeding 0.1 per cent available phosphorus diet without phytase resulted in decreased egg specific gravity and supplementing with phytase corrected this effect.

Kaminska (1997) held an experiment with laying hens in single cages offered diets containing 0.45, 0.52 and 0.58 per cent total phosphorus (0.25,0.30 and 0.35 per cent available phosphorus respectively), supplemented with 0, 150, 300 and 450 units of microbial phytase and observed that specific gravity of eggs significantly ($p < 0.05$) improved by supplementing diets with phytase.

The influence of supplemental phytase in laying hens was investigated by Carlos and Edwards (1998). The basal diet was formulated to contain 3 per cent calcium and 0.33 per cent total phosphorus. Phytase was supplemented at a level of 600

FTU/kg feed. They found that phytase had no effect on egg specific gravity.

Gordon and Roland (1998) conducted a trial for six weeks to study the influence of phytase on calcium and phosphorus utilisation in commercial laying hens. Birds were fed with diets containing two levels of non phytate phosphorus (0.1 and 0.3 per cent non - phytate phosphorus) with and without supplemental phytase and suggested that phytase showed a significant ($p < 0.01$) improvement in egg specific gravity.

2.7 Egg shell weight and thickness

Budor *et al.* (1995) conducted 3 experiments to study the influence of phytase in laying hens. In all the trials birds were given diets with phytase 0 and 600 units/kg. In trial 1, feed contained 0.6 per cent total phosphorus with 0.3 per cent available phosphorus and were given from 22 to 48 weeks of age, in trial 2, 0.5 per cent phosphorus and 0.2 per cent available phosphorus from 22 to 64 weeks of age and in trial 3, 0.4 per cent phosphorus with 0.1 per cent available phosphorus from 49 to 64 weeks of age. They concluded that egg quality was not influenced by phytase supplementation in those trials.

Lettner *et al.* (1995) studied the efficiency of phytase in hybrid layers offered diets containing 0.61 and 0.41 per cent phosphorus with 0.65 per cent monocalcium phosphate (control) or

Natuphos 80 g (5000 units/g) plus feed grade calcium carbonate. Number of cracked eggs decreased from 7.60 to 3.81 per cent with phytase supplementation. However, egg quality was not different between groups.

In a trial with laying hens fed on wheat – maize - soyabean meal diets containing 0.5, 0.55 and 0.6 per cent total phosphorus, equivalent to 0.25, 0.30 and 0.35 per cent available phosphorus respectively supplemented with microbial phytase at levels of 0, 150, 300 or 450 FTU/kg, Kaminska *et al.* (1996) reported that the hens fed on diets containing 0.5 per cent total phosphorus supplemented with 300 FTU/kg showed good egg shell quality. The thickest egg shells and the highest breaking strength were observed when diets containing 0.30 - 0.35 per cent available phosphorus were supplemented with 150 units of phytase.

In a study with laying hens fed on phosphorus deficient maize-soyabean meal diets supplemented with inorganic phosphorus 1.0 g/kg or phytase (Natuphos) at the levels of 0 or 500 FTU/kg, Oloffs *et al.* (1997a) observed that egg quality was improved with supplementation and declined after 5 weeks on the phosphorus deficient diet. There was no difference between the supplemented groups.

In an experiment with laying hens fed on diets containing 0.45, 0.52 and 0.58 per cent total phosphorus (0.25, 0.30, 0.35 per cent available phosphorus respectively) supplemented with 0, 150, 300 or 450 units of phytase enzyme per kg of feed Kaminska (1997) found that shell thickness was significantly ($p < 0.01$) improved in hens fed with phytase added diets.

Gordon and Roland (1998) conducted an experiment in commercial laying hens for 6 weeks with inorganic phosphorus at two levels viz., 0.1 and 0.3 per cent supplemented with phytase at a level of 300 units/kg feed and stated that the magnitude of shell quality improvement was greatest when the 0.1 per cent phosphorus diet was supplemented with phytase.

Rao *et al.* (1999) reported that supplementation of phytase to layer rations low in non phytate phosphorus resulted in an improvement in egg shell quality.

2.8 Serum calcium and phosphorus

Carlos and Edwards (1998) conducted two experiments to study the effects of supplementing a corn-soyabean layer diet with either phytase, 1-25 dihydroxy cholecalciferol or their combination. The basal diet was formulated to contain 3 per cent calcium and 0.33 per cent total phosphorus. In both these experiments, plasma dialyzable phosphorus increased by the supplementation of phytase at a level of 600 FTU/kg feed.

2.9 Tibial ash and phosphorus content

Schoner *et al.* (1993) conducted two experiments with hybrid layers of white and brown varieties to study the phosphorus balance of layers supplemented with phytase and reported that phytase supplemented diets significantly improved tibial crude ash in white varieties only.

In a trial conducted with pullets fed on diets containing 100, 50 or 0 per cent of the recommended levels of dicalcium phosphate supplemented with phytase, Zobac *et al.* (1995) reported that feeding on the diet low or devoid of dicalcium phosphate without Natuphos significantly reduced deposition of calcium and phosphorus in tibia and addition of phytase showed a considerable increase in the deposition.

In an experiment, phytase and monocalcium phosphate were added to a corn-soyabean meal layer diet at levels of 0, 100, 200 and 300 FTU/kg of phytase and 0, 0.3, 0.6 and 0.9 g of monocalcium phosphate per kg respectively. The basal diet contained 40 g calcium and 3.6 g phosphorus/kg diet. The tibial ash content was significantly improved by dietary supplementation of the negative control diet with phytase (Klis *et al.*, 1997).

In a study conducted with 24 week old laying hens for 8 weeks, offered with a corn soyabean diet added with either phytase (600 FTU/kg feed) or 1,25 - dihydroxy cholecalciferol or their combination, Carlos and Edwards (1998) reported that phytase had a positive effect on tibial bone ash.

Supplemental phytase in laying hens resulted in an increase in the bone mineral content and bone density (Gordon and Roland, 1998).

2.10 Bio availability

In an attempt to minimise phosphorus excretion in poultry Edwards (1992) conducted a study in broilers and laying hens and concluded that the amount of phosphorus in the excreta of birds can be accurately predicted. He also stated that supplementation of diets with phytase or 1, 25 – dihydroxy cholecalciferol increased phytate utilization.

Simons *et al.* (1992) reported that phytate phosphorus degradation increased from 20 per cent to 70 per cent when phytase at a level of 500 units/kg was added to laying hen diets.

Schoner *et al.* (1993) carried out two experiments with hybrid layers of white and brown varieties to study the phosphorus balance of layers supplied with phytase. The diet contained available phosphorus 1.2 and phytate phosphorus 2.5 g/kg feed. In both the trials phytase supplemented diet significantly improved phosphorus retention. Phosphorus utilization was 15, 24, 38 and 33 per cent for groups fed with basal diet, basal diet plus monocalcium phosphate, basal diet plus 250 phytase units and basal diet plus 500 phytase units/kg respectively for white laying birds and it was 9, 18 and 30 per cent for basal diet, basal diet plus monocalcium phosphate and basal diet plus 400 phytase units/kg respectively for brown layers.

Nahashon *et al.* (1994) reported that phytase activity was present in the condensed cane molasses solubles and Lactobacillus combination (Lacto) and that the presence of phytase and Lacto supplementation to a 0.25 per cent available phosphorus layer diet improved phosphorus retention in layers.

Budor *et al.* (1995) conducted three trials to study the effect of added phytase in laying hen diets on phosphorus excretion and assimilation. In all the trials birds were given diets with phytase 0 and 600 units/kg. In trial 1, feed contained 0.6 per cent total phosphorus with 0.3 per cent available phosphorus and were given from 22 to 48 weeks of age, in trial 2, 0.5 per cent phosphorus and 0.2 per cent available phosphorus from 22 to 64 weeks of age, and in trial 3, 0.4 per cent phosphorus with 0.1 per cent available phosphorus from 49 to 64 weeks of age. In trial 1, phytase had no effect on the retention of phosphorus. In trial 2, phosphorus absorption was 20 per cent more. However in trial 3, these trends were not confirmed.

Application of enzyme phytase in feed mixtures for reduction of the phosphorus content in poultry faeces was studied by Zobac *et al.* (1995). Pullets were fed on diets containing 100, 50, or 0 per cent of recommended dicalcium phosphate supplemented with phytase (Natuphos). Feeding the diet low or devoid of dicalcium phosphate without Natuphos significantly reduced digestibility of calcium and phosphorus but addition of Natuphos resulted considerable increase in the calcium and phosphorus digestibility.

In a study with laying hens fed rations on two basal diets containing calcium 30 or 40 g/kg diet supplemented with phytase (0,250 or 500 phytase units/kg diet), Klis *et al.* (1997) found that 250 phytase units/kg diet hydrolysed an amount of phytate phosphorus that was equivalent to 1.3 g phosphorus from monocalcium phosphate but at the highest phytase inclusion level (500 units/kg) a lower bioavailability was observed as phosphorus absorption was almost maximal at the lower level of phytase inclusion.

Gordon and Roland (1998) conducted a trial with commercial laying hens provided with corn soyabean based diet containing 0.1 and 0.3 per cent phosphorus and phytase at a level of 300 units/kg and stated that inclusion of phytase in layer diet improved calcium utilization.

Carlos and Edwards (1998) conducted two experiments to study the effects of phytase, 1-25 dihydroxy cholecalciferol or their combination. They reported that in both these experiments on laying hens with corn soya diet containing 3 per cent calcium or 0.33 per cent phosphorus, phytase enzyme had a positive effect on phytate phosphorus retention.

Maenz and Classen (1998) studied the phytase activity in the small intestinal brush border membrane of mature laying hens and suggested that the total activity of brush border phytase was 35 per cent higher in the small intestine ($p < 0.05$) and they contribute to higher phytate phosphorus digestibility.

2.11 Phosphorus excretion

Schoner *et al.* (1993) carried out two experiments with hybrid white and brown layers provided with diets containing 1.2 per cent available phosphorus and phytate phosphorus 2.5 g/kg feed. In white layers, diets were supplemented with phytase 250 or 500 units/kg and monocalcium phosphate (total phosphorus 4.8 g/kg). In brown layers diet were supplemented with phytase 400 units/kg. In both these experiments phosphorus excretion was decreased by 45 and 50 per cent respectively, when phytase was supplemented, as compared with positive control.

Three trials were conducted by Budor *et al.* (1995) to study the effect of added phytase in laying hen diets on phosphorus excretion and assimilation. In all the trials birds were given diets with phytase 0 and 600 units/kg. In trial 1, feeds contained 0.6 per cent total phosphorus with 0.3 per cent available phosphorus and were given from 22 to 48 weeks of age, in trial 2, 0.5 per cent phosphorus and 0.2 per cent available phosphorus from 22 to 64 weeks of age and in trial 3, 0.4 per cent phosphorus with 0.1 per cent available phosphorus from 49 to 64 weeks of age. In trial 1, phytase had no effect on excretion and retention of phosphorus. In trial 2 phosphorus absorption was 20 per cent more, while phosphorus excretion was decreased by 4 per cent. However in trial 3, these trends were not confirmed.

Kaminska *et al.* (1996) conducted an experiment with laying hens fed on wheat maize soyabean meal diets containing 0.5, 0.55 and 0.6 per cent total phosphorus equivalent to 0.25, 0.30 and 0.35 per cent available phosphorus respectively and supplemented with microbial phytase 0, 150, 300 or 450 FTU/kg. They observed that hens fed on 0.25 per cent available phosphorus plus 300 FTU/kg excreted 100 mg less phosphorus daily than those fed on 0.35 per cent available phosphorus (0.60 per cent total phosphorus) only.

In an experiment, laying hens were fed diets containing 100, 90 or 80 per cent of the available phosphorus recommended by the NRC and supplemented with 500 units of phytase to 90 and 80 per cent of the recommended available phosphorus diets (Kwon *et al.*, 1995). The results showed that phosphorus excretion by hens fed on diets containing 80 per cent available phosphorus and 500 phytase units was lower than that of hens fed on the other diets.

Lettner *et al.* (1995) conducted an experiment with hybrid layers given diets containing 0.61 and 0.41 per cent phosphorus with 0.65 per cent monocalcium phosphate (control) or Natuphos 80 g (5000 units/g) plus feed grade calcium carbonate, respectively. They observed that phosphorus excretion in faeces was reduced by 25 per cent in diets containing the enzyme preparation.

Zobac *et al.* (1995) carried out an experiment with pullets fed on diets containing 100, 50 or 0 per cent of recommended dicalcium phosphate supplemented with phytase (Natuphos) and reported that the addition of Natuphos decreased the phosphorus content of faeces.

Hadorn and Wiedmer (1998) added 300 and 150 units/kg of commercial phytase (NOVO - L) in phosphorus reduced diets of laying hens. They conducted the experiment from 21 to 80 weeks of age and opined that phosphorus excretion was reduced in phytase supplemented diets.

2.12 Livability

In a factorial experiment, 21 week old laying hens were fed with 5 levels of available phosphorus from 0.1 to 0.5

per cent and phytase 0 and 300 FTU/kg feed for 17 weeks and observed that feeding the 0.1 per cent available phosphorus diet without phytase resulted in increased mortality and supplementing with phytase corrected this adverse effect (Roland and Gordon, 1996).

2.13 Economics

Simons *et al.* (1992) reported that supplementation of phytase at 500 units/kg in laying hens resulted in an equivalency of monocalcium phosphate phosphorus 0.7 g / 250 units phytase.

After studying the influence of phytase during the whole laying period Vahl *et al.* (1993) opined that 300 units of Natuphos is equivalent to 1.8 g available phosphorus/kg diet (or 0.18 per cent available phosphorus) in the diet for layers.

After conducting a trial with laying hybrids provided with diets containing 0.61 and 0.41 per cent phosphorus with 0.65 per cent monocalcium phosphate or Natuphos 80 g plus feed grade calcium carbonate, Lettner *et al.* (1995) opined that supplementation of Natuphos could substantially contribute to reducing environmental phosphorus load, at the expense of some what higher feed costs.

In an attempt to enhance phytate phosphorus availability in commercial layers, Rao *et al.* (1999) supplemented phytase, cholecalciferol or yeast culture in the layer rations and opined that dicalcium phosphate can completely be replaced with phytase (250 units/kg) in layer diet.

Materials and Methods

3. MATERIALS AND METHODS

An experiment was conducted at the All India Co-ordinated Research Project on Poultry Improvement, Centre for Advanced Studies in Poultry Science, College of Veterinary and Animal Sciences, Mannuthy, to evaluate the effect of phytase supplementation on phosphorus utilization and production performance in layer chicken.

3.1 Experimental materials

3.1.1 Experimental birds

One hundred and fifty, 18 weeks old White Leghorn strain cross pullets of ILM-90 (ATHULYA) selected at random, formed the subjects of this experiment.

3.1.2 Experimental diets

Two types of rations viz., standard layer ration and layer ration with low available phosphorus, were used in this study. The standard layer ration (SLR) was formulated as per BIS (1992) specifications. Low available phosphorus layer ration (LAPLR) was formulated keeping the level of available phosphorus at 0.3 per cent (whereas 0.5 per cent in the SLR). The level of all other nutrients were similar to that of SLR. Initially, the ration with 0.3 per cent available phosphorus was formulated and then by the addition of appropriate levels of dicalcium phosphate to this ration, SLR was formulated. The ingredient composition and the chemical composition of the experimental diets are presented in Tables 1 and 2, respectively.

3.1.3 Enzyme

The enzyme used in this study was 'Natuphos® - 5000' a product manufactured and marketed by M/S BASF, D-67056. Ludwigshafen, Germany. It is a phosphatase enzyme of fungal origin (*Aspergillus* species) containing phytase as the only component.

3.2. Experimental methods

3.2.1 Housing of birds

The cage house, cages, feeders and waterers were cleaned thoroughly and disinfected. The birds were wing badged and housed in individual cages of identical dimensions of 25 x 37 x 37 cm at 18^{th} week of age.

3.2.2 Experimental design

The pullets were randomly divided into fifteen groups of ten birds each and allotted to five treatments viz., T_1,T_2,T_3,T_4 and T_5 with three replications in each treatment. The birds in each treatment were assigned to each of the five rations viz., SLR, LAPLR and three different levels of enzymes viz., 200,300 and 400 units per kilogram of feed in LAPLR. The details of treatment particulars are presented in Table 3.

3.2.3 Management

Feed and water were provided *ad libitum* throughout the experimental period. Uniform and standard managemental procedures were followed during the course of the experiment. The experiment was conducted over five 28-day periods covering 20 weeks from 21to 40 weeks of age.

3.2.4 Climatic parameters

The wet and dry bulb thermometer readings were taken at 8 am and 2 pm daily. The maximum and minimum temperatures were recorded at 8 am on all days. From these data, period-wise maximum and minimum temperatures and per cent relative humidity were arrived at.

3.2.5 Body weight

The body weight of individual birds was recorded at the commencement and at the end of the experimental period to study the pattern of body weight gain under the different feeding regimes.

3.2.6 Feed consumption

Feed intake was recorded replicate wise for each 28 -day period. From this data average feed intake per bird per day was calculated for various treatment groups.

3.2.7 Egg production

Individual egg production record of all the birds was maintained every day during the experimental period and from this data, per cent hen day egg production was calculated.

3.2.8 Egg weight, egg shell weight, egg shell thickness and specific gravity of egg

The egg quality studies were carried out at the end of each 28 – day period with four eggs randomly selected from each replicate, for three consecutive days. Egg weight and egg shell weight were obtained from the weighment of eggs and shells respectively. Shell thickness was measured using shell thickness measure gauge. Specific gravity was obtained by brine floatation technique developed by Tyler and Geake (1961)

3.2.9 Feed efficiency

Feed conversion efficiency (feed per dozen eggs) was calculated based on the dozen of egg produced and kilogram of feed consumed.

3.2.10 Metabolism trial

At the end of the experiment, a metabolism trial was conducted for 3 days using four birds randomly selected from each treatment group. *Ad libitum* supply of feed and water was ensured. Feed intake, excreta voided and dry matter content of feed and excreta of individual birds were recorded for each day. The samples of droppings were taken and preserved for further analysis. Samples were pooled for each replicate and analysed for calcium and phosphorus content.

3.2.11 Chemical analysis

The chemical composition of the experimental rations and total phosphorus and calcium content of different rations and droppings were analysed as per the procedure described by AOAC (1990).

3.2.12 Tibial ash and phosphorus content

At the end of the trial four birds from each treatment were randomly selected and sacrificed to collect tibia as per the method described by Kalango and Ademosun (1973). The birds were fasted, slaughtered and dressed. The muscular layers covering the left tibia of each bird were removed as closely as possible with a scalpel. Adhering connective and soft muscular tissues were finally removed by boiling the bone in one per cent solution of sodium hydroxide for about ten minutes. Then the tibia were thoroughly washed, dried in the oven at 100°C overnight. The cooled tibia were used for ash and phosphorus estimation as per the procedure of AOAC(1990).

3.2.13 Serum calcium and inorganic phosphorus

Blood samples of four birds from each treatment group were collected at the end of the experiment by severing the jugular vein for the estimation of serum calcium and inorganic phosphorus. The estimation of serum calcium was done using Atomic Absorption Spectrophotometer (Parkin Elmer, Model 3110). The estimation of serum inorganic phosphorus was done by modified Metol method, phosphorus kit, supplied by Qualigeus Fine Chemicals, A division of GLAXO India Ltd., Dr. Annie Besant Road, Mumbai.

3.2.14 Livability

The mortality of birds from different treatment groups was recorded and post mortem examination was conducted in each case to find out the cause of death.

3.2.15 Cost-benefit analysis

Cost of feed and enzymes, number of eggs produced and quantum of feed consumed to produce an egg in each treatment group were calculated. From this data the cost-benefit analysis was worked out.

3.2.16 Statistical analysis

Data collected on various parameters were analysed as per the methods described by Snedecor and Cochran (1985).

Table 1 : Per cent ingredient composition of experimental diets

Ingredients	Standard layer ration (SLR)	Low available phosphorus layer ration (LAPLR)
Yellow maize	42.50	42.50
Ground nut cake	19.00	19.00
Gingelly oil cake	04.00	04.00
Rice polish	10.00	10.00
Bajra	09.89	09.89
Wheat bran	03.00	03.00
Unsalted dried fish	05.00	05.00
Shell grit	05.25	06.25
Dicalcium phosphate	01.00	00.00
Common salt	00.25	00.25
Trace mineral mixture	00.08	00.08
Vitamin mixture	00.03	00.03
Total	**100.00**	**100.00**

Vitamin mixture composition :-

Each gram contains : Vitamin A 40000 IU,
Vitamin B_2 20 mg and
Vitamin D_3 5000IU.

Trace mineral mixture composition:-

Each 100 kg contains : Manganese sulphate 17g,
Potassium iodide 0.15 g,
Copper sulphate 3.6 g,
Ferrous sulphate 39.5g,
Zinc sulphate 18.5 g.

Table 2 : Per cent chemical composition of experimental diets (on dry matter basis)

Nutrients	Standard layer ration (SLR)	Low available phosphorus layer ration (LAPLR)
Crude protein	18.300	18.200
Ether extract	4.130	4.180
Crude fibre	6.810	6.970
Total ash	16.430	16.630
Acid insoluble ash	3.810	3.740
Calcium	3.120	3.030
Total phosphorus	0.921	0.782
Available phosphorus*	0.500	0.320

* Calculated value

Table. 3. Treatment particulars

Treatment	Ration	Level of phytase inclusion (units/kg)
T_1	SLR	-
T_2	LAPLR	-
T_3	LAPLR	200
T_4	LAPLR	300
T_5	LAPLR	400

SLR – Standard Layer Ration

LAPLR – Low Available Phosphorus Layer Ration

Results

4. RESULTS

An experiment to study the influence of phytase supplementation in low available phosphorus diet on the production performance and nutrient availability of layer chicken was conducted for a period of 20 weeks. The results obtained are presented in this chapter.

4.1 Climatic parameters

The mean maximum and minimum temperatures and per cent relative humidity during the different periods of this experiment (From October 1998 to February 1999) are presented in Table 4. During the course of the experiment, the maximum temperature ranged from 28.0 to 34.5°C and minimum temperature from 21.5 to 23.3°C. The relative humidity in the morning varied from 76 to 94 per cent, while in the afternoon it ranged from 40 to 76 per cent. A gradual rise in mean maximum temperature was observed from the first period till the completion of the study, whereas not much variations could be seen in the mean minimum temperature among the different periods.

4.2 Egg Production

The data on per cent hen-day egg production among the dietary treatment groups viz., standard layer ration (T_1), low

Table 4 : Climatic parameters during experimental period

Periods	Temperature °C		Relative Humidity (%)	
	Maximum	Minimum	8A.M	2 PM
1 (21-24 weeks)	28.0	22.8	94	76
2 (25-28 weeks)	31.5	23.1	92	64
3 (29-32 weeks)	30.1	22.9	79	58
4 (33-36 weeks)	32.4	21.5	77	40
5 (37-40 weeks)	34.5	23.3	76	53

Table 5 : Influence of phytase supplementation on per cent hen day egg production

Periods	Treatments					Mean ± SE
	T_1	T_2	T_3	T_4	T_5	
1 (21-24 weeks)	68.57	65.35	73.21	69.76	75.71	70.52^{C} ± 1.80
2 (25-28 weeks)	87.95	91.90	92.97	80.28	91.01	90.55^{A} ± 0.90
3 (29-32 weeks)	90.71	87.74	91.54	90.95	92.22.	90.63^{A} ± 0.76
4 (33-36 weeks)	91.18	85.46	83.45	92.04	92.06	88.84^{AB} ± 1.82
5 (37-40 weeks)	87.61	80.18	84.88	88.14	84.20	85.00^{B} ± 1.42
Mean SE	85.20 ± 4.21	82.13 ± 4.60	85.21 ± 3.52	86.03 ± 4.12	87.04 ± 3.20	

CD = 3.896

Means bearing the same superscript do not differ significantly

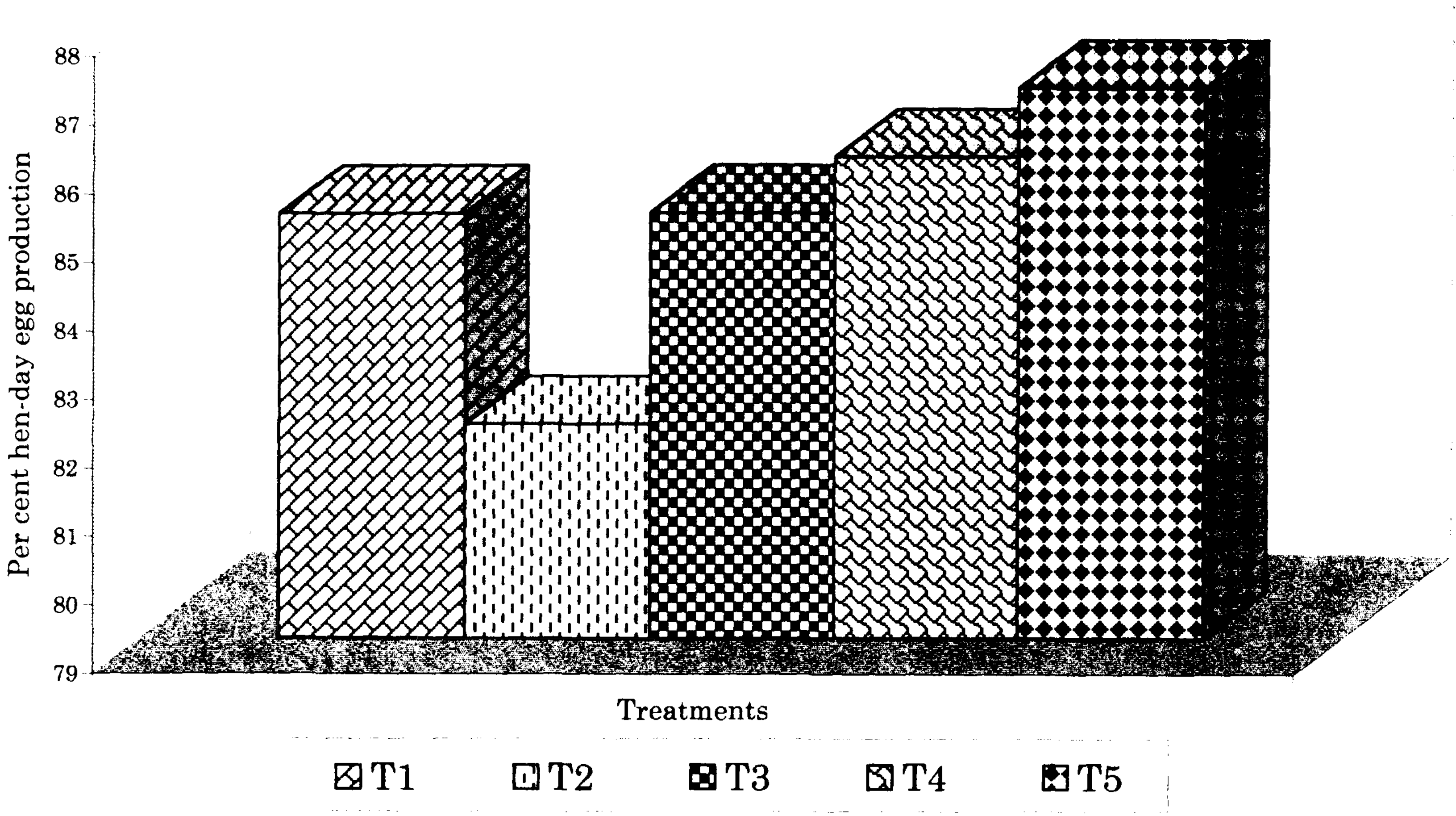
Table 6 : Influence of phytase supplementation on per cent hen day egg production- ANOVA

Source	DF	SS	MSS	F value
Treatment	4	67.466	16.867	1.997 NS
Period	4	1434.101	358.525	42.4495 **
Error	16	135.135	8.446	
Total	24	1636.703		

** Significant ($p < 0.01$)

NS - Not Significant

FIG. 1 : PER CENT HEN-DAY EGG PRODUCTION AS INFLUENCED BY PHYTASE SUPPLEMENTATION



available phosphorus layer ration without phytase (T_2), low available phosphorus layer ration with 200 units of phytase/kg (T_3), low available phosphorus layer ration with 300 units of phytase/kg (T_4) and low available phosphorus layer ration with 400 units of phytase/kg (T_5) was 85.20, 82.13, 85.21, 86.03 and 87.04, respectively (Table 5).

Highest per cent hen-day egg production was noticed in birds fed with low available phosphorus diet supplemented with 400 units of phytase (87.04) and lowest (82.13) in the group fed with low available phosphorus diet without added phytase. Other treatments were intermediary with respect to hen-day egg production. A perusal of the per cent hen-day egg production for the different periods revealed that it was lowest (70.52) in the first period (21-24weeks of age). The peak in egg production was attained as early as 25th week of age and was maintained till 32nd week. Thereafter, a gradual decline in egg production was noticed till 40th week of age.

Statistical analysis of data presented in Table 6 revealed that the variation in per cent hen-day egg production among different treatment groups was not statistically significant. Nonetheless there is apparent lower hen-day egg production in the group fed diet with 0.3 per cent available phosphorus without phytase supplementation (T_2) than either control or supplemental group. Likewise, there is apparent similarity among control and enzyme supplemented groups. The differences in hen day egg production among the different experimental periods were highly significant ($p < 0.01$). Egg production was significantly higher during the 2^{nd} and 3^{rd} periods.

Though the egg production was numerically lower during the 4^{th} period, it was comparable with 2^{nd} and 3^{rd} periods. Likewise, egg production between 4^{th} and 5^{th} periods was also statistically comparable. Significantly low egg production was observed during the first period (70.52 per cent).

The pattern of per cent hen-day egg production for the various dietary treatment groups is depicted in Fig 1.

4.3 Feed intake

Mean daily feed intake per bird in treatments T_1, T_2, T_3, T_4 and T_5 were 111.30, 107.88, 113.38, 112.88 and 115.13g respectively (Table 7). It could be seen from the table that birds offered a diet containing low available phosphorus layer ration supplemented with 400 units of phytase/kg consumed more feed (115.13g) and those fed low available phosphorus layer ration without phytase consumed less feed (107.88g). Birds in the other groups consumed feed in between these two values. It was also evident from the table that daily feed intake was lowest during the first period i.e., 21-24 weeks age and it gradually rose with subsequent periods.

Table 7 : Influence of phytase supplementation on mean daily feed in take per bird (g).

Periods	Treatments					Mean ± SE
	T_1	T_2	T_3	T_4	T_5	
1 (21-24 weeks)	98.69	100.14	105.48	104.83	105.20	102.87^{C} ±1.432
2 (25-28 weeks)	110.94	106.03	111.39	113.32	115.09	111.35^{B} ±1.522
3 (29-32 weeks)	117.18	109.61	114.75	115.06	117.54	114.83^{A} ±1.417
4 (33-36 weeks)	114.60	111.58	118.14	115.81	118.80	115.79^{A} ±1.298
5 (37-40 weeks)	115.08	112.03	117.13	115.37	119.02	115.73^{A} ±1.162
Mean SE	111.30^{B} ± 3.308	107.88^{C} ± 2.205	113.38^{AB} ± 2.291	112.88^{B} ± 2.056	115.13^{A} ± 2.579	

CD = 2.089

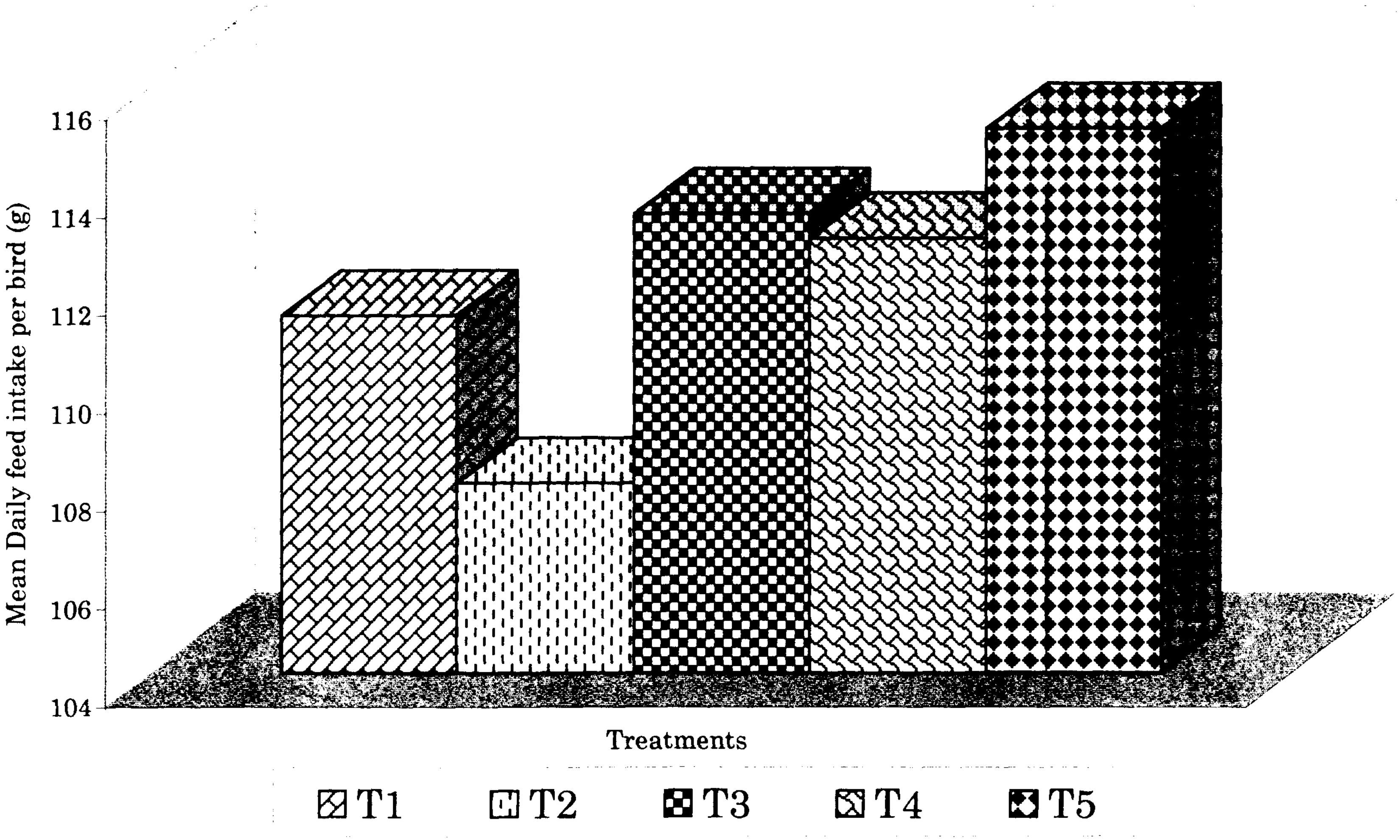
Means bearing the same superscript do not differ significantly

Table 8 : Influence of phytase supplementation on mean daily feed in take per bird (g) - ANOVA

Source	DF	SS	MSS	F value
Treatment	4	149.436	37.359	15.3915 **
Period	4	599.810	149.953	61.7788 **
Error	16	38.836	2.427	
Total	24	788.082		

** Significant ($p < 0.01$)

FIG.2 : MEAN DAILY FEED INTAKE PER BIRD AS INFLUENCED BY PHYTASE SUPPLEMENTATION.



The analysis of variance of the mean daily feed intake per bird presented in Table 8 indicated that this trait was significantly influenced by both treatments as well as periods. Significantly higher daily feed intake was observed with birds offered a low available phosphorus diet supplemented with 400 units of phytase/kg and it was statistically comparable with birds fed a low available phosphorus layer ration with 200 units of phytase. Mean daily feed intake among treatments T_1, T_3 and T_4 was also comparable. Significantly lower daily feed intake was noted with birds fed a low available phosphorus layer ration without enzyme.

During the first period of the experiment significantly lower daily feed intake was observed. However, the daily feed intake of birds increased during the 2^{nd} period (25-28 weeks of age) and was statistically different from other periods. The daily feed intake per bird during the periods 3,4 and 5 were statistically higher than other periods and were comparable.

The mean daily feed consumption per bird as influenced by phytase supplementation is presented in Fig. 2.

4.4 Feed efficiency

The data calculated for feed per dozen eggs for the different treatment groups are set out in Table 9. Feed per dozen eggs obtained from different treatments groups viz., T_1, T_2, T_3, T_4

Table 9 : Influence of phytase supplementation on feed efficiency (kg feed /dozen eggs)

Periods	Treatments					Mean ± SE
	T_1	T_2	T_3	T_4	T_5	
1 (21-24 weeks)	1.75	1.84	1.73	1.81	1.68	1.76^{A} ±0.028
2 (25-28 weeks)	1.51	1.38	1.44	1.52	1.52	1.47^{D} ±0.028
3 (29-32 weeks)	1.55	1.50	1.50	1.52	1.53	1.52^{CD} ±0.009
4 (33-36 weeks)	1.51	1.58	1.71	1.50	1.55	1.57^{BC} ±0.038
5 (37-40 weeks)	1.58	1.68	1.66	1.57	1.70	1.64^{B} ±0.026
Mean SE	1.58 ± 0.44	1.60 ± 0.78	1.61 ± 0.06	1.58 ± 0.06	1.60 ± 0.39	

CD = 0.09481

Means bearing the same superscript do not differ significantly

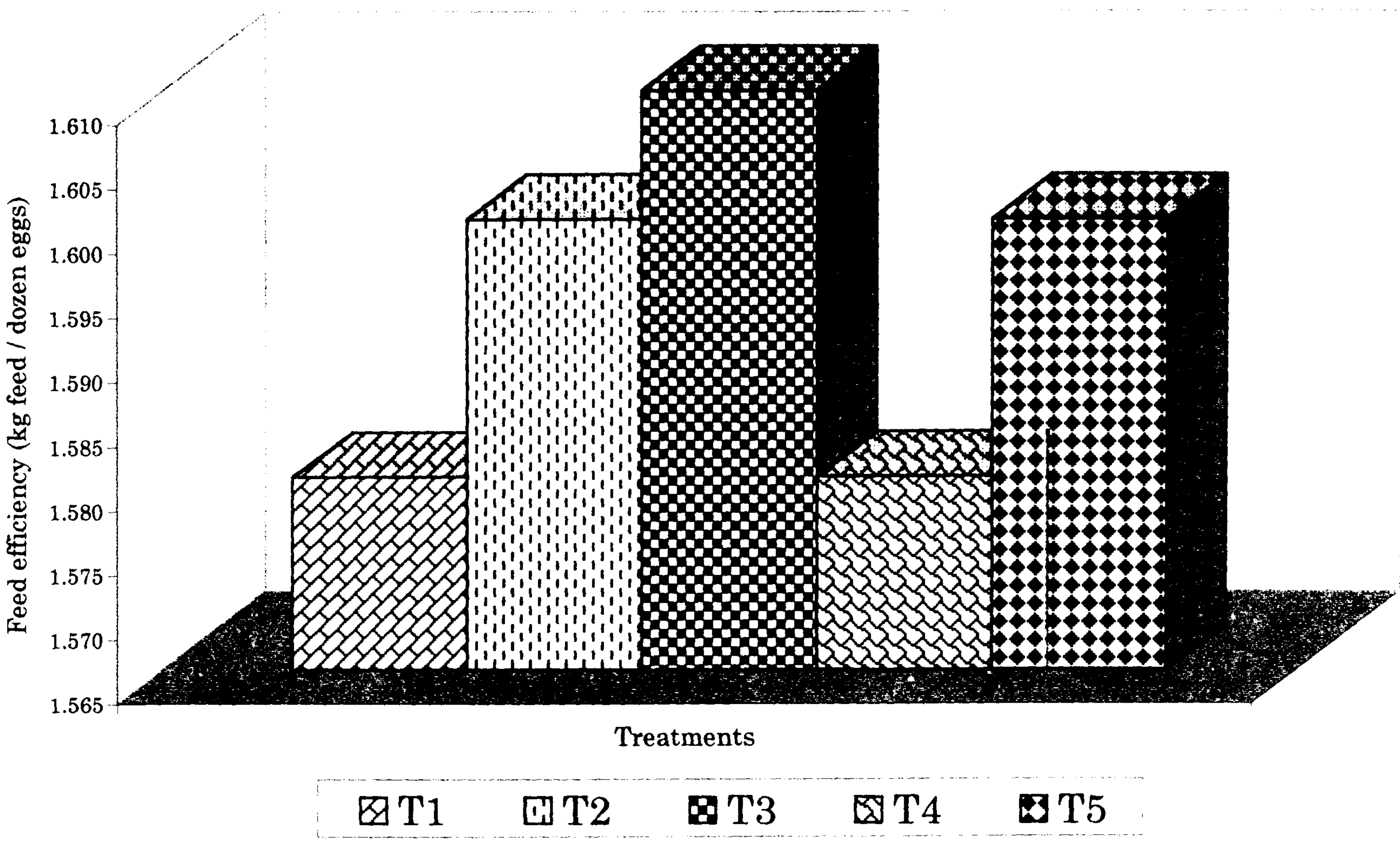
Table 10 : Influence of phytase supplementation on feed efficiency (kg feed /dozen egg) - ANOVA

Source	DF	SS	MSS	F value
Treatment	4	0.002	0.001	0.1335 NS
Period	4	0.253	0.063	13.7111 **
Error	16	0.074	0.005	
Total	24	1636.703		

** Significant ($p < 0.01$)

NS - Not Significant

FIG.3 : FEED EFFICIENCY (kg feed / dozen eggs) AS INFLUENCED BY PHYTASE SUPPLEMENTATION



and T_5 were 1.58, 1.60, 1.61, 1.58 and 1.60 respectively. In general much variations were not observed in feed per dozen eggs among the various dietary regimes employed in this study. On examining the feed per dozen eggs as influenced by different periods without looking into treatment effects it was observed that superior efficiency was noticed with 2^{nd} period (1.47), while it was inferior during 1^{st} period (1.76). Feed conversion efficiency of other periods were intermediary.

Statistical analysis of the feed per dozen eggs data presented in Table10 indicated that there was no significant difference among the treatment groups, whereas it differed significantly between periods. Superior feed efficiency was noticed in the second period and it was statistically comparable with third period. Similarly feed per dozen eggs between 3^{rd} and 4^{th} periods and that between 4^{th} and 5^{th} periods were statistically comparable. Lowest feed conversion efficiency was recorded for the first period.

The feed efficiency (feed per dozen eggs) for different dietary treatments is depicted in Fig.3.

4.5 Body Weight Gain

At the commencement and at the end of the experiment, individual body weight of birds in the different dietary treatment groups were recorded to assess the gain in body

Table 11 : Influence of phytase supplementation on body weight gain (kg)

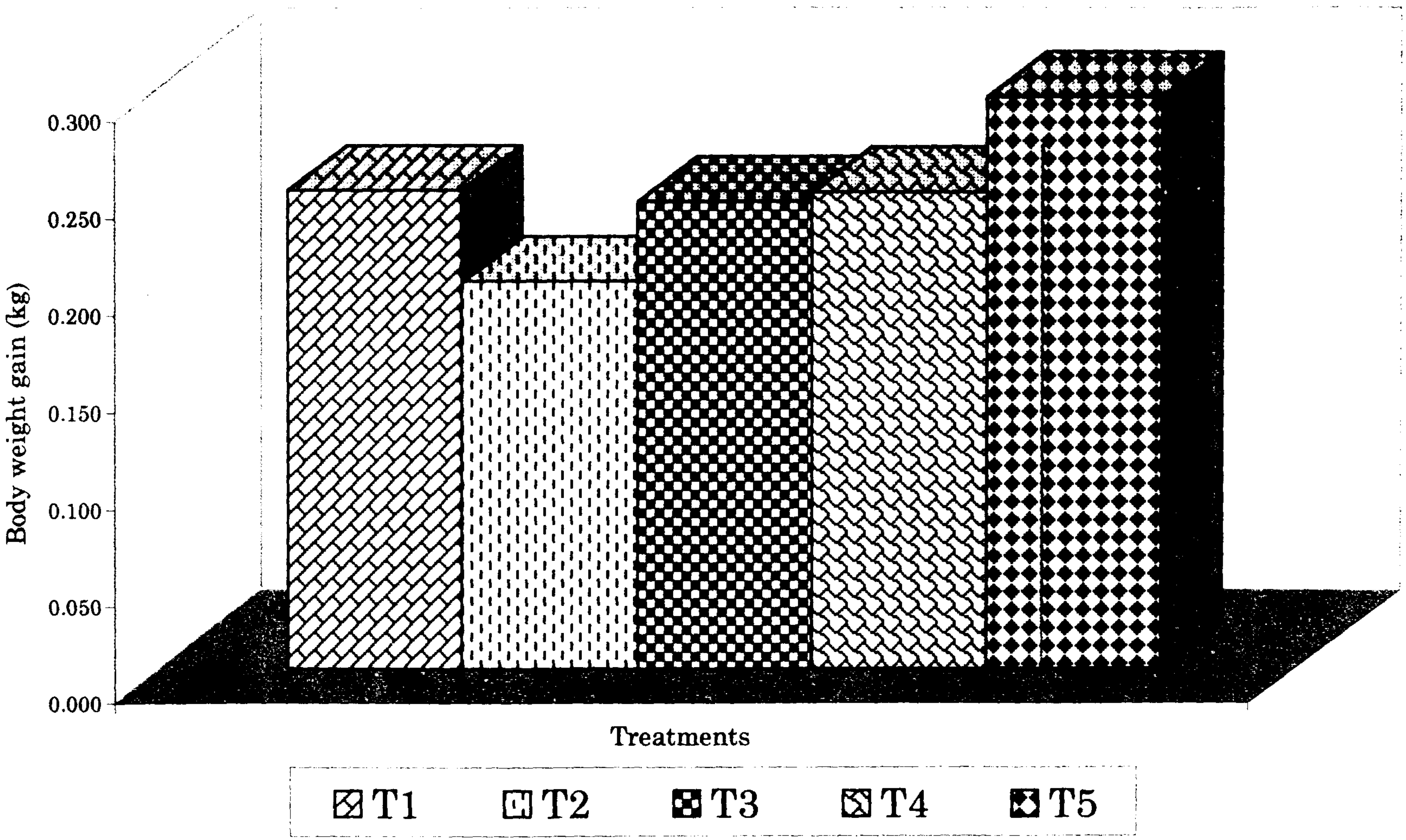
Replication	Treatments				
	T_1	T_2	T_3	T_4	T_5
1	0.228	0.167	0.164	0.348	0.585
2	0.254	0.207	0.298	0.170	0.309
3	0.259	0.227	0.260	0.221	0.241
Mean	0.247	0.200	0.241	0.246	0.295
SE	±0.011	± 0.017	± 0.040	± 0.052	± 0.029

Table 12 : Influence of phytase supplementation on hen day egg production- ANOVA

Source	DF	SS	MSS	F value
Treatment	4	0.014	0.003	1.012 NS
Error	10	0.033	0.003	
Total	14			

NS - Not Significant

FIG.4: BODY WEIGHT GAIN (kg) AS INFLUENCED BY PHYTASE SUPPLEMENTATION



weight due to enzyme supplementation. The mean values ranged from 0.200 to 0.295 kg (Table 11). The maximum body weight gain of 0.295kg was noticed in the group fed with low available phosphorus diet supplemented with 400 phytase units / kg (T5) and the lowest gain (0.200kg) in the group fed with low available phosphorus diet without supplementation of phytase (T_2).

Perusal of the body weight gain data presented in Table 11 revealed that enzyme supplementation resulted in numerical improvement of this trait. However, when the magnitude of difference in body weight gain between treatments were analysed statistically it failed to show any significant difference.

4.6 Egg weight

Data on mean egg weight for the different treatment groups as influenced by phytase supplementation are given in Table 13. Among the different feeding regimens, the highest mean egg weight of 51.16 g was noticed in birds fed low available phosphorus layer ration supplemented with 200 phytase units/kg and the lowest among the birds fed low available phosphorus layer ration without phytase supplementation (49.07g). Birds fed on a standard layer ration registered an egg weight of 50.17g which was higher than those fed a layer ration having 0.3 per cent available phosphorus. Similarly, eggs laid by birds offered a ration supplemented with phytase at 300 and 400

Table 13 : Influence of phytase supplementation on egg weight

Periods	Dietary Treatments					Mean ± SE
	T_1	T_2	T_3	T_4	T_5	
1 (21-24 weeks)	47.84	46.40	49.03	50.13	49.79	48.64^{C} ± 0.68
2 (25-28 weeks)	50.46	48.80	51.20	49.93	50.36	50.15^{B} ± 0.39
3 (29-32 weeks)	50.56	50.74	51.66	49.88	49.56	50.56^{AB} ± 0.38
4 (33-36 weeks)	51.58	50.64	52.54	51.74	51.25	51.55^{A} ± 0.31
5 (37-40 weeks)	50.01	48.79	51.37	49.60	50.01	49.96^{B} ± 0.42
Mean SE	50.17^{A} ± 0.64	49.07^{B} ± 0.79	51.16^{A} ± 0.58	50.26^{A} ± 0.38	50.19^{A} ± 0.30	

CD = 1.05

Means bearing the same superscript do not differ significantly

Table 14: Influence of phytase supplementation on egg weight - ANOVA

Source	DF	SS	MSS	F value
Treatment	4	10.946	2.737	4.4629 **
Period	4	22.249	5.562	9.0709 **
Error	16	9.811	0.613	
Total	24	1636.703		

** Significant ($p < 0.01$)

units/kg were also heavier than those fed a low available phosphorus ration. Mean egg weight for the periods 1,2,3,4 and 5 were 48.64, 50.15, 50.56, 51.55 and 49.96 g, respectively.

Statistical analysis of data presented in Table 14 showed that the difference in mean egg weight between the different dietary treatments as well as periods was statistically significant ($p < 0.01$). Eggs laid by birds fed with low available phosphorus layer ration supplemented with 200 phytase units/kg was significantly larger (51.16g) and birds fed a low available phosphorus layer ration without phytase supplementation was significantly smaller (49.07g). It was also revealed that egg weights of birds fed a standard layer ration as well as those fed low available phosphorus ration supplemented with varying levels of phytase were statistically comparable.

While considering the different periods, higher egg weight ($p < 0.01$) was recorded during the 4^{th} period (33-36 weeks of age), whereas eggs laid during 21-24 weeks of age (1^{st} period) were of lower weight ($p < 0.01$). Egg weights between 3^{rd} and 4^{th} periods were statistically comparable. Likewise, egg weights between 2^{nd} and 3^{rd} and that between 3^{rd} and 5^{th} were also comparable.

Fig.5 depicts the influence of phytase supplementation on egg weight.

4.7 Egg specific gravity

The influence of phytase supplementation on egg specific gravity was determined for all treatment groups period-wise and are presented in Table 15. Mean egg specific gravity was highest (1.094) for birds fed on low available phosphorus layer ration added with 200 phytase units/kg and it was lowest for birds fed on low available phosphorus layer ration without phytase inclusion. The egg specific gravity of other treatments were intermediary.

The mean egg specific gravity obtained for the periods 1,2,3,4 and 5 were 1.091, 1.089, 1.087, 1.089 and 1.089, respectively. It was highest during the 1^{st} 28 day period and lowest during the 3^{rd} period and the values for the periods 2,4 and 5 were medium.

The analysis of variance of the egg specific gravity presented in Table 16 revealed that this trait was influenced both by treatments as well as periods. Among the treatments, eggs laid by birds offered low available phosphorus diet with 200 units of phytase/kg registered significantly higher values (T_3). The egg specific gravity of birds fed low available phosphorus ration supplemented with 300 and 400 units of phytase/kg were higher ($p < 0.01$) than birds fed standard layer ration and those fed low available phosphorus diet without phytase, but lower than those fed low available phosphorus diet supplemented with 200 units of

Table 15 : Influence of phytase supplementation on egg specific gravity

Periods	Dietary Treatments					
	T_1	T_2	T_3	T_4	T_5	Mean ± SE
1 (21-24 weeks)	1.088	1.084	1.095	1.096	1.094	1.091^{A} ±.0002
2 (25–28 weeks)	1.087	1.083	1.095	1.090	1.091	1.089^{B} ±.0002
3 (29-32 weeks)	1.084	1.081	1.092	1.089	1.090	1.087^{C} ±.0002
4 (33-36 weeks)	1.086	1.084	1.093	1.091	1.090	1.089^{B} ±.0001
5 (37-40 weeks)	1.088	1.083	1.093	1.090	1.092	1.089^{B} ±.0002
Mean SE	1.087^{C} ± .001	1.083^{D} ± .001	1.094^{A} ± .001	1.091^{B} ± .001	1.091^{B} ± .001	

CD = 0.0015

Means bearing the same superscript do not differ significantly.

Table 16 : Influence of phytase supplementation on egg specific gravity - ANOVA

Source	DF	SS	MSS	F value
Treatment	4	.0003676	.0000919	68.4644 **
Period	4	.0000456	.0000114	8.3882 **
Error	16	.0000208	.0000013	
Total	24	.000434		

** Significant ($p < 0.01$)

phytase/kg. The egg specific gravity of birds fed a standard layer ration was higher ($p < 0.01$) than those fed a low available phosphorus ration without enzyme.

The egg specific gravity of the 1st period was significantly higher ($p < 0.01$) and was different from all other periods (1.091). Similarly, the egg specific gravity value of 3rd period was lower and was different from other periods (1.087). The egg specific gravity of periods 2,4 and 5 registered a medium value of 1.089.

The egg specific gravity for different dietary treatments is depicted in Fig. 5.

4.8 Egg shell weight

Data on mean egg shell weight among different treatment groups are given in Table 17. Mean egg shell weight for treatments T_1, T_2, T_3, T_4 and T_5 were 5.85, 5.77, 6.34, 6.05 and 6.18 g respectively. It was highest (6.34g) for the birds fed on low available phosphorus layer ration plus 200 phytase units/kg and lowest (5.77g) for the birds maintained as negative control (0.3 per cent available phosphorus without phytase). The shell weight values of other treatments were in between these two. The shell weight values determined for the periods 1,2,3,4 and 5 were 5.52, 6.18, 6.14, 6.19 and 6.18g respectively. An observation of the period-wise shell weight values presented in Table 17 revealed that barring 1st period i.e., 21-24 weeks of age, not much variation existed between the other periods.

Table 17 : Influence of phytase supplementation on egg shell weight (g)

Periods	Treatments					Mean ± SE
	T_1	T_2	T_3	T_4	T_5	
1 (21-24 weeks)	5.43	4.33	5.66	5.64	5.53	5.52^{B} ± 0.6
2 (25-28 weeks)	5.95	5.86	6.52	6.17	6.38	6.18^{A} ± 0.12
3 (29-32 weeks)	5.97	5.87	6.48	6.11	6.26	6.14^{A} ± 0.11
4 (33-36 weeks)	5.95	5.90	6.54	6.19	6.37	6.19^{A} ± 0.12
5 (37-40 weeks)	5.95	4.89	6.51	6.15	6.38	6.18^{A} ± 0.12
Mean SE	5.85^{D} ±0.105	5.77^{D} ±0.110	6.34^{A} ±0.171	6.05^{C} ±0.104	6.18^{B} ±0.165	

CD = 0.1039

Means bearing the same superscript do not differ significantly

Table 18 : Influence of phytase supplementation on egg shell weight (g) – ANOVA

Source	DF	SS	MSS	F value
Treatment	4	1.105	0.276	44.3124 **
Period	4	1.708	0.427	68.4638 **
Error	16	0.100	0.006	
Total	24	2.913		

** Significant ($p < 0.01$)

When the mean egg shell weight values were subjected to statistical analysis, it was found that both treatment as well as periods did have a significant influence on this trait. The egg shell weight of T_3 i.e., birds offered a low available phosphorus ration with 200 units of phytase/kg was significantly higher and was different from all other treatments. Supplementation of 300 and 400 units of phytase/kg in low available phosphorus rations also caused significant increase in egg shell weight. The egg shell weight of enzyme supplemented groups were significantly different from one another. The egg shell weight values of birds fed standard layer ration as well as those maintained on low available phosphorus ration without phytase were significantly ($p < 0.01$) lower than enzyme supplemented groups and were statistically comparable.

Statistical analysis of the period-wise shell weight data indicated that the values were significantly lower for the 1st period. In the remaining four periods, the values were significantly higher and were statistically comparable.

The egg shell weight for different dietary treatments is depicted in Fig.5

4.9 Egg shell thickness

Mean egg shell thickness as influenced by supplementation of phytase in low available phosphorus layer ration is given in Table 19. Mean egg shell thickness was highest (0.354mm) for group fed a low available phosphorus layer ration supplemented with 200 units of phytase/kg (T_3) and lowest

Table 19 : Influence of phytase supplementation on egg shell thickness (mm)

Periods	Treatments					Mean ± SE
	T_1	T_2	T_3	T_4	T_5	
1 (21-24 weeks)	0.336	0.332	0.364	0.346	0.343	0.344^{A} ± 0.05
2 (25-28 weeks)	0.334	0.328	0.351	0.340	0.346	0.340^{AB} ±0.004
3 (29-32 weeks)	0.333	0.326	0.349	0.340	0.345	0.339^{B} ±0.004
4 (33-36 weeks)	0.335	0.330	0.352	0.341	0.347	0.341^{AB} ±0.003
5 (37-40 weeks)	0.334	0.329	0.351	0.339	0345	0.340^{AB} ±0.004
Mean SE	0.334^{C} ±0.001	0.329^{D} ±0.001	0.354^{A} ±0.003	0.342^{B} ±0.001	0.345^{B} ±0.001	

CD = 0.004

Means bearing the same superscript do not differ significantly

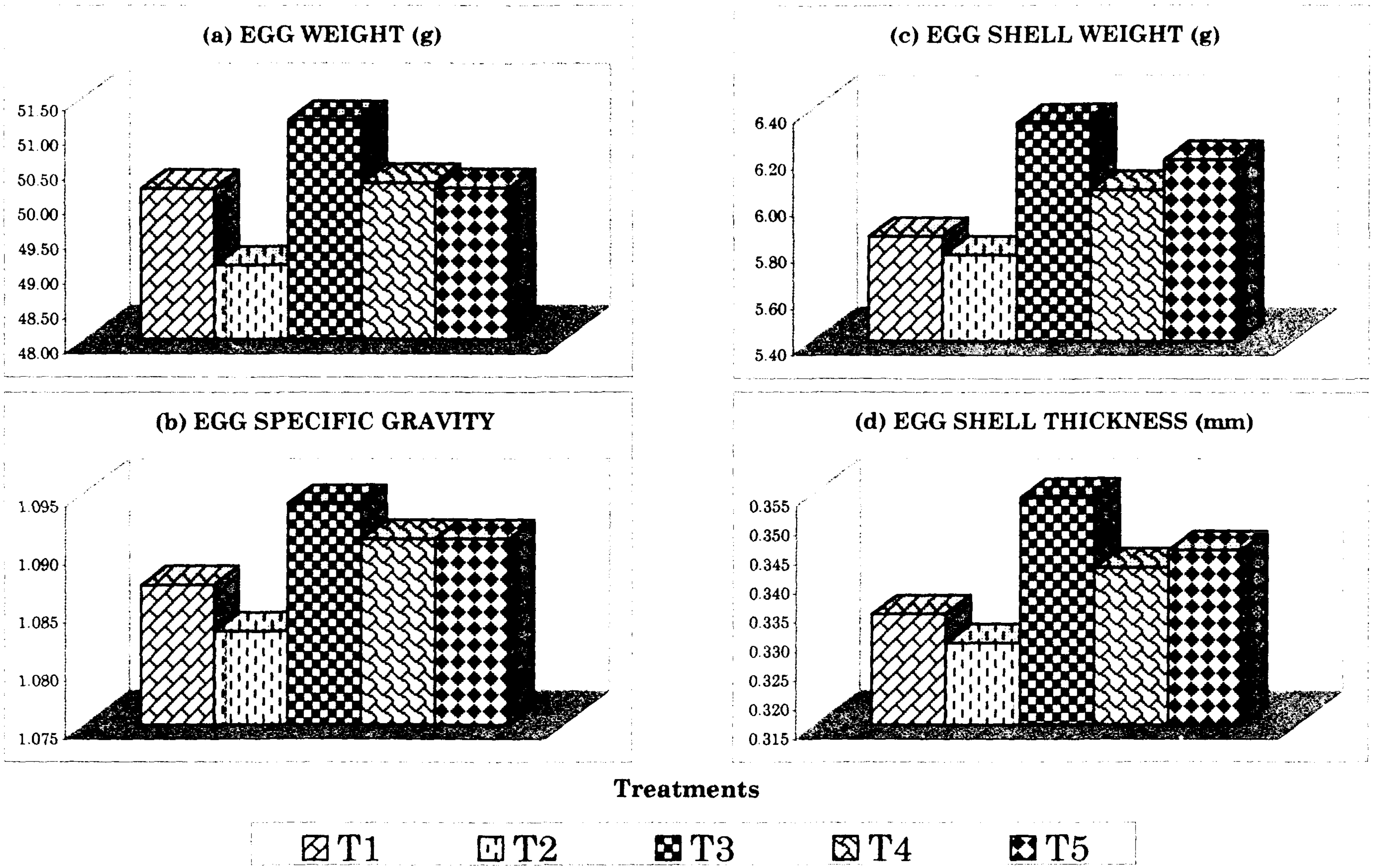
Table 20 : Influence of phytase supplementation on egg shell thickness (mm) - ANOVA

Source	DF	SS	MSS	F value
Treatment	4	0.00179176	0.00044794	61.65726 **
Period	4	0.00009376	0.00002344	3.22642 *
Error	16	0.00011624	0.000007265	
Total	24	0.00200176		

* Significant ($p < 0.05$)

** Significant ($p < 0.01$)

FIG. 5 : EGG QUALITY AS INFLUENCED BY PHYTASE SUPPLEMENTATION



(0.329 mm) for groups provided with low available phosphorus layer ration without phytase inclusion (T_2). Shell thickness of other treatments registered values in between these two.

The period-wise mean egg shell thickness was 0.344, 0.340, 0.339, 0.341 and 0.340mm for 1st, 2nd, 3rd, 4th and 5th periods respectively.

Statistical analysis of shell thickness data presented in Table 20 indicated that various dietary regimes employed in this study did have a significant influence. Mean shell thickness was significantly more among birds fed low available phosphorus layer ration supplemented with 200 units of phytase/kg (T_3), whereas the negative control (T_2) registered the lowest ($p < 0.01$) value. The shell thickness value of standard layer ration fed birds were significantly higher than those fed a low available phosphorus layer ration without phytase. Irrespective of levels of enzyme supplementation, all supplemented groups significantly improved shell thickness values. However, maximum improvement ($p < 0.01$) was noticed with the treatment offered 200 units of phytase/kg.

Analysis of variance of the period-wise shell thickness values also revealed that significant differences existed among periods. It was significantly higher in the first period and lower in the 3rd period. However, the shell thickness values during periods 1,2,4 and 5 were statistically comparable. Likewise, shell

Table 21: Influence of phytase supplementation on serum calcium (mg %)

Bird's No.	Treatments				
	T_1	T_2	T_3	T_4	T_5
1.	19.84	18.72	19.63	20.63	21.07
2.	21.37	17.29	22.47	21.47	19.64
3.	20.42	19.47	21.89	19.93	19.42
4.	18.93	17.07	20.43	22.40	23.84
Mean	20.14^{AB}	18.14^{B}	21.11^{A}	21.11^{A}	20.99^{A}
SE	± 0.51	± 0.57	± 0.65	± 0.53	± 1.01

CD = 2.062

Means bearing the same superscript do not differ significantly

Table 22 : Influence of phytase supplementation on serum calcium (mg %) - ANOVA

Source	DF	SS	MSS	F value
Treatment	4	25.926	6.482	3.465 *
Error	15	28.062	1.871	
Total	19	53.988		

* Significant ($p < 0.05$)

thickness during periods 2,3,4 and 5 were also found to be statistically similar. The egg shell thickness for different dietary treatments is depicted in Fig.5.

4.10 Serum calcium

The mean serum calcium levels as influenced by phytase supplementation estimated at the end of the experiment is presented in Table 21. Mean serum calcium levels for treatment groups T_1, T_2,T_3, T_4 and T_5 were 20.14, 18.14, 21.11, 21.11 and 20.99 mg per cent respectively.

The analysis of variance for serum calcium given in Table 22 revealed that there were significant ($p < 0.01$) differences among treatments. Significantly higher values were obtained for the treatments T_3, T_4 and T_5 i.e., enzyme supplemented groups. The serum calcium level of standard layer ration fed birds and enzyme supplemented groups were statistically comparable. Likewise, the serum calcium levels of standard layer ration and low available phosphorus layer ration without phytase were also comparable. Fig.6 depicts the influence of phytase supplementation on serum calcium.

4.11 Serum inorganic phosphorus

Estimated serum inorganic phosphorus levels for different treatment groups are provided in Table 23. Mean serum inorganic phosphorus for T_1, T_2, T_3, T_4 and T_5 were 4.38, 3.76, 4.33, 4.18 and 4.12mg per cent respectively. When the magnitude

Table 23 : Influence of phytase supplementation on serum inorganic phosphorus. (mg%)

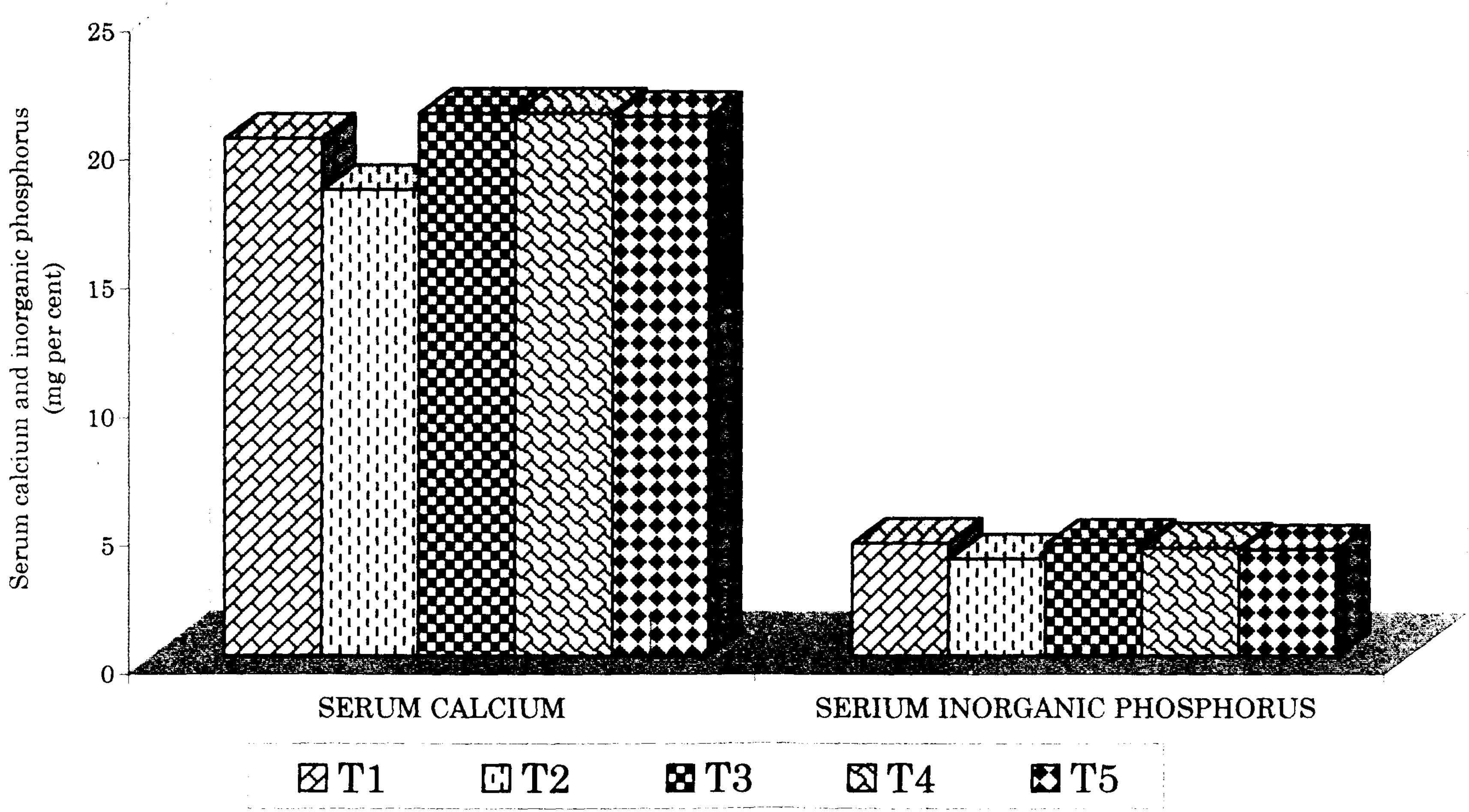
Bird's No.	Treatments				
	T_1	T_2	T_3	T_4	T_5
1.	4.17	4.09	4.25	3.82	4.22
2.	3.92	3.61	4.31	4.47	4.37
3.	4.45	3.01	3.83	4.03	3.92
4.	4.97	4.31	4.92	4.41	3.98
Mean	4.38	3.76	4.33	4.18	4.12
SE	$\pm$ 0.225	$\pm$ 0.29	$\pm$ 0.225	$\pm$ 0.155	$\pm$ 0.105

Table 24 : Influence of phytase supplementation in serum inorganic phosphorus (mg %)- ANOVA

Source	DF	SS	MSS	F value
Treatment	4	0.964	0.241	1.374 NS
Error	15	2.631	0.175	
Total	19	3.395		

NS - Not Significant

FIG.6: SER UM CALCIUM AND INORGANIC PHOSPHORUS AS INCLUENCED BY PHYTASE SUPPLEMENTATION



of difference in serum inorganic phosphorus among the treatments was tested statistically, it failed to show any difference between treatments. Fig.6. depicts the influence of phytase supplementation on serum inorganic phosphorus.

4.12 Tibial ash and phosphorus content

The influence of phytase supplementation on tibial ash and phosphorus content estimated at the end of the feed trial are given in Tables 25 and 27, respectively. Mean tibial ash content were 52.14, 48.70, 52.77, 51.89 and 52.05 per cent and mean tibial phosphorus content were 16.90, 15.06, 17.16, 17.16 and 17.18 per cent for treatment groups T_1, T_2, T_3, T_4 and T_5, respectively. Though, tibial ash and phosphorus levels were numerically higher in enzyme supplemented groups, statistical analysis revealed that no significant differences existed between the treatments with respect to these two traits (Tables 26 and 28). Tibial ash and phosphorus content for different dietary treatments is depicted in Fig.7.

4.13 Bio-availability of calcium

Bio-availability of calcium as influenced by different feeding regimes employed in this investigation was determined and is given in Table 29. It was numerically higher with both standard layer mash fed birds and birds fed low available phosphorus layer ration supplemented with 200 phytase units/kg (60.85 per cent) and lowest with those fed on low available phosphorus layer ration without enzymes addition (57.68per cent)

Table 25 : Influence of phytase on tibial ash (per cent)

Bird's No.	Treatments				
	T_1	T_2	T_3	T_4	T_5
1.	52.39	47.29	51.73	52.37	51.52
2.	51.47	48.91	53.91	50.91	48.77
3.	54.70	51.17	54.72	54.97	53.87
4.	49.98	47.43	50.73	49.33	54.03
Mean	52.14	48.70	52.77	51.89	52.05
SE	± 0.99	± 0.90	± 0.93	± 01.20	± 1.23

Table 26 : Influence of phytase on tibial ash (per cent) - ANOVA

Source	DF	SS	MSS	F value
Treatment	4	41.271	10.318	2.299 NS
Error	15	67.325	4.488	
Total	19	108.596		

NS - Not significant

Table 27: Influence of phytase supplementation on tibial phosphorus content (per cent)

Bird's No.	Treatments				
	T_1	T_2	T_3	T_4	T_5
1.	15.21	15.20	17.73	16.12	15.98
2.	18.97	15.09	16.67	18.97	17.87
3.	16.44	14.21	18.21	16.31	16.48
4.	16.97	15.72	16.03	17.23	18.37
Mean	16.90	15.06	17.16	17.16	17.18
SE	± 0.78	± 0.31	± 0.49	± 0.65	± 0.56

Table 28: Influence of phytase supplementation on tibial phosphorus content - ANOVA

Source	DF	SS	MSS	F value
Treatment	4	13.564	3.391	2.495 NS
Error	15	20.388	1.359	
Total	19	33.951		

NS - Not significant

FIG.7 : TIBIAL ASH AND PHOSPHORUS CONTENT AS INFLUENCED BY PHYTASE SUPPLEMENTATION

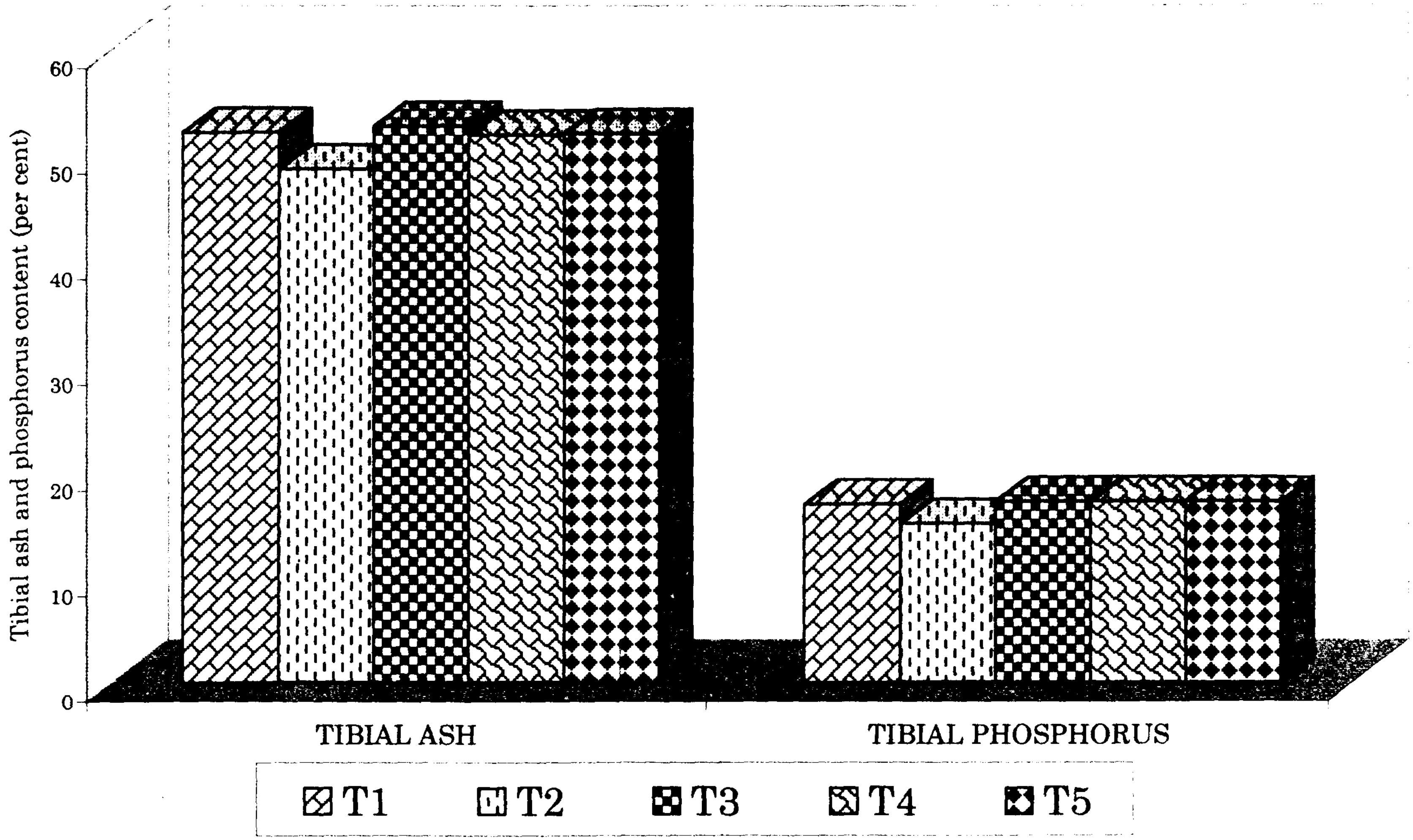


Table 29 : Influence of phytase supplementation on bio availability of calcium (per cent)

Bird's No.	Treatments				
	T_1	T_2	T_3	T_4	T_5
1.	58.93	56.06	62.87	60.80	58.87
2.	62.74	58.23	59.56	61.17	62.13
3.	60.02	58.92	60.30	58.13	57.53
4.	61.71	57.50	60.66	61.93	58.96
Mean	60.85	57.68	60.85	60.51	59.37
SE	± 0.85	± 0.61	± 0.71	± 0.82	± 0.97

Table 30 : Influence of phytase supplementation on bio availability of calcium (per cent) - ANOVA

Source	DF	SS	MSS	F value
Treatment	4	29.500	7.375	2.845 NS
Error	15	38.889	2.593	
Total	19	68.389		

NS - Not significant

The data on bio-availability of calcium was subjected to statistical analysis and is presented in Table 30. It showed that there were no significant differences among treatments. Bio-availability of calcium for different dietary treatments is depicted in Fig. 8.

4.14 Bio-availability of phosphorus

Bio-availability of phosphorus estimated at the end of the experiment presented in Table 31, showed that it was highest (54.85 per cent) for the birds fed low available phosphorus layer ration supplemented with 400 phytase units/kg (T_5) and lowest (46.85 per cent) with T_2, i.e., birds fed low available phosphorus layer ration without phytase. In general the bio-availability of phosphorus was more with enzyme supplemented groups. Similarly, it was more with standard layer mash fed birds than the negative control. The analysis of variance for bio-availability of phosphorus presented in Table 32 showed that significant ($p < 0.01$) differences existed among treatments. Significantly higher values were obtained for all enzyme supplemented groups as well as standard layer mash fed birds, whereas bio-availability of phosphorus was significantly inferior with low available phosphorus layer ration fed birds without phytase. It was also revealed that T_1, T_3, T_4 and T_5 were statistically comparable. Fig.8. depicts the influence of phytase supplementation on the bio-availability of phosphorus.

4.15 Phosphorus excretion

Phosphorus excretion calculated as gram per kilogram dry matter intake is shown in Table 33. Mean phosphorus

Table 31 : Influence of phytase supplementation on bio-availability of phosphorus (per cent)

Bird's No.	Treatments				
	T_1	T_2	T_3	T_4	T_5
1.	52.14	49.25	52.19	52.15	57.35
2.	52.14	46.79	55.55	57.21	61.08
3.	50.71	45.68	54.32	50.38	49.76
4.	53.57	45.67	56.58	52.37	51.20
Mean	52.14^{A}	46.85^{B}	54.66^{A}	53.03^{A}	54.85^{A}
SE	± 0.58	± 0.84	± 0.94	± 1.46	± 2.65

CD= 4.493

Table 32 : Influence of phytase supplementation on bio-availability of phosphorus (per cent) - ANOVA

Source	DF	SS	MSS	F value
Treatment	4	169.375	42.344	4.765 **
Error	15	133.308	8.887	
Total	19	302.684		

** Significant ($p < 0.01$)

FIG.8: BIO AVAILABILITY OF CALCIUM AND PHOSPHORUS AS INFLUENCED BY PHYTASE SUPPLEMENTATION

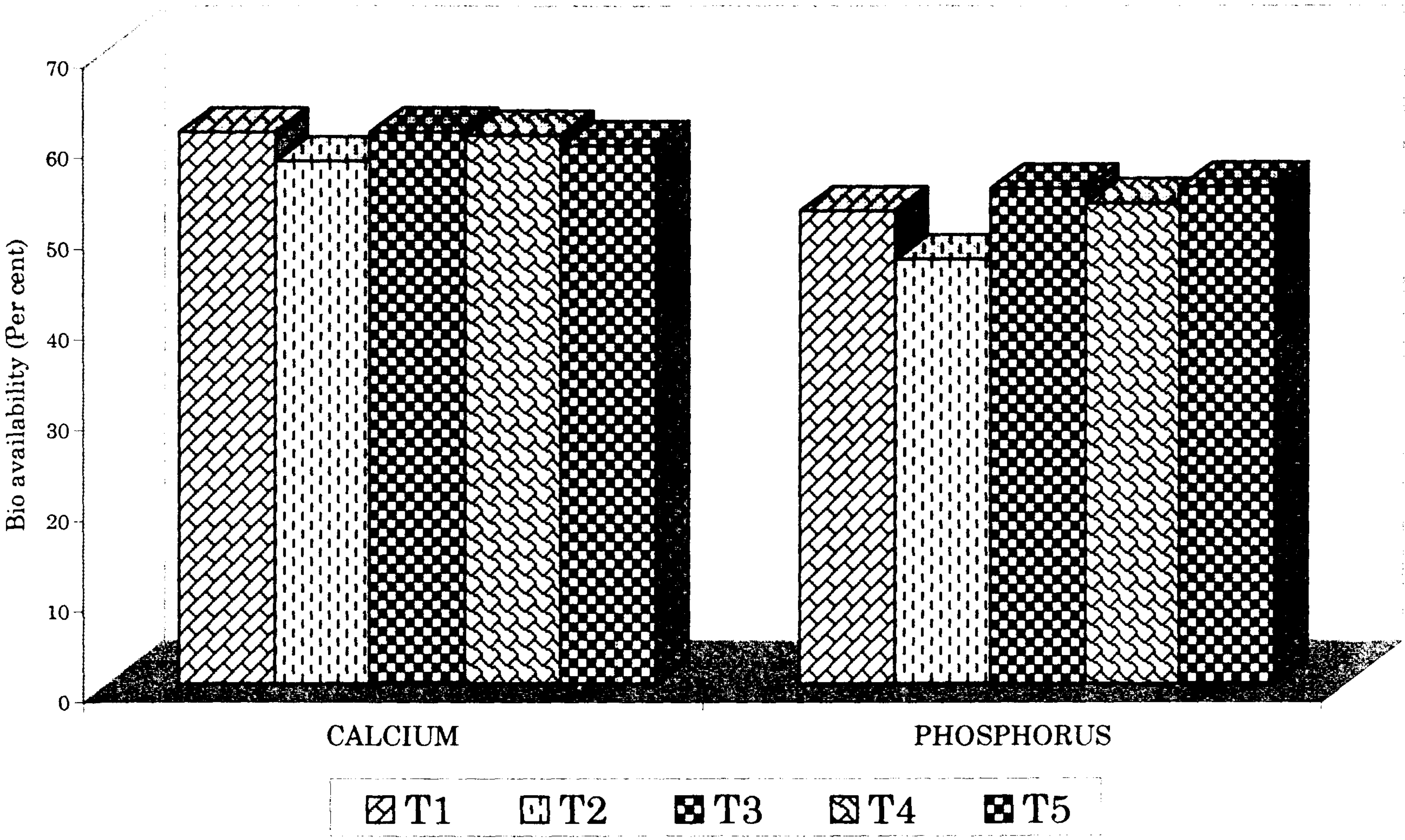


Table 33 : Influence of phytase supplementation on phosphorus excretion (g/kg DM intake)

Bird's No.	Treatments				
	T_1	T_2	T_3	T_4	T_5
1.	6.10	5.25	3.23	2.78	2.95
2.	6.70	4.52	2.92	3.30	2.84
3.	6.76	5.41	3.68	2.46	2.69
4.	6.58	6.00	2.82	3.30	3.08
Mean	6.54^{A}	5.30^{B}	3.16^{C}	2.96^{C}	2.89^{C}
SE	± 0.15	± 0.30	± 0.19	± 0.20	± 0.08

CD= 0.6066

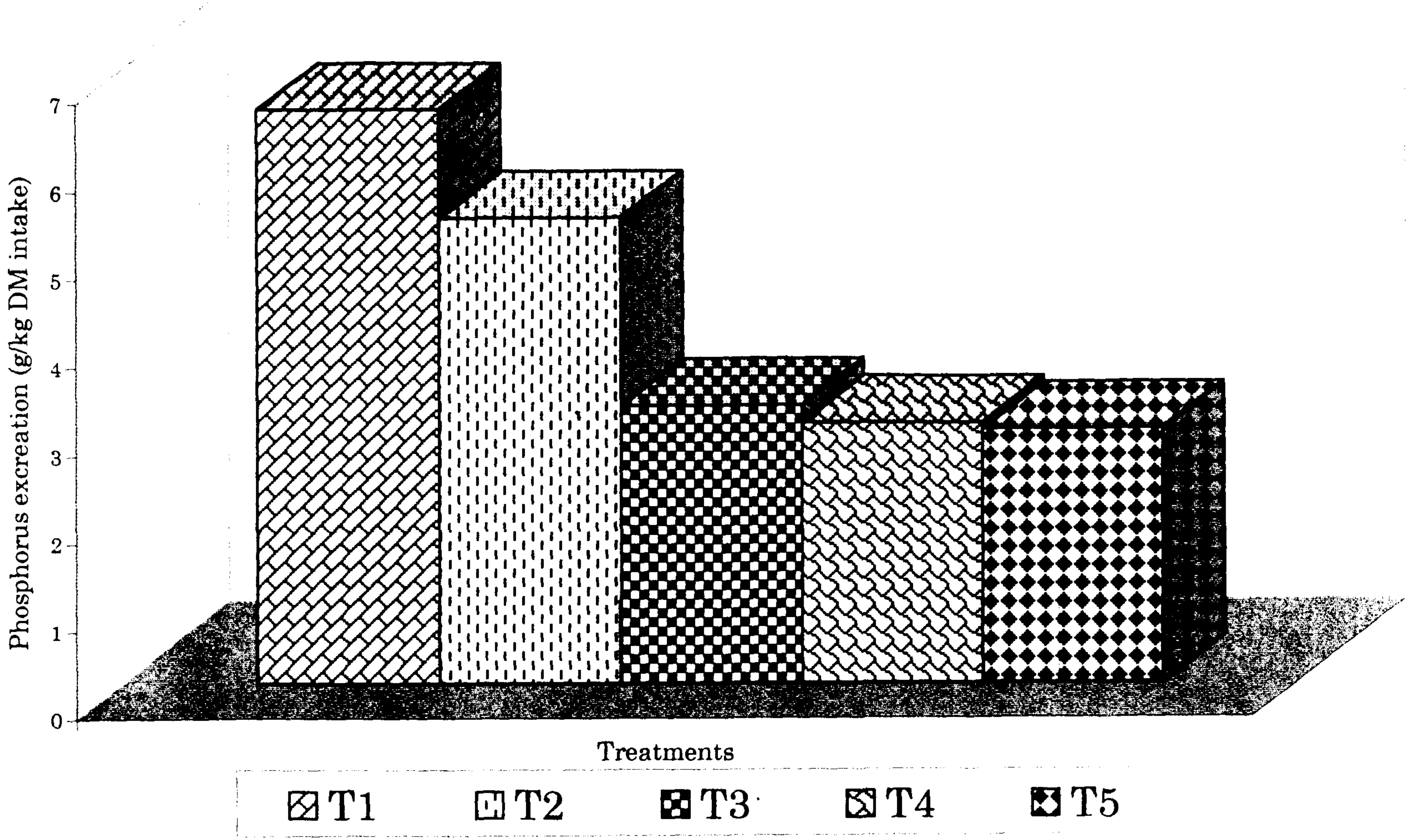
Means bearing the same superscript do not differ significantly

Table 34 : Influence of phytase supplementation on phosphorus excretion (g/kg DM intake) - ANOVA

Source	DF	SS	MSS	F value
Treatment	4	43.906	10.976	67.860 **
Error	15	2.426	0.162	
Total	19	46.332		

** Significant ($p < 0.01$)

FIG.9 : PHOSPHORUS EXCRETION (g/kg DM intake) AS INFLUENCED BY PHYTASE SUPPLEMENTATION



excretion was 6.54, 5.30, 3.16, 2.96 and 2.89 g/kg dry matter intake for T_1, T_2, T_3, T_4 and T_5 respectively. Perusal of the mean phosphorus excretion data presented in Table 33 indicated that it was more among birds fed a ration containing 0.5 per cent available phosphorus (T_1) and that reduction of available phosphorus content of diet resulted in a simultaneous reduction of phosphorus excretion. It was also revealed that by the incremental addition of phytase to low available phosphorus rations resulted in a linear decrease in phosphorus excretion.

Statistical analysis of the phosphorus excretion data given in Table 34 showed that this trait was significantly influenced by various treatments. Phosphorus excretion was significantly more with birds fed standard layer ration. Feeding of low available phosphorus ration resulted in a significant reduction in the phosphorus excretion. When phytase was supplemented to low available phosphorus ration phosphorus excretion was still reduced and was significantly different from other treatments. Phosphorus excretion of enzyme supplemented groups were statistically comparable. The phosphorus excretion for different dietary treatments is depicted in Fig.9.

4.16 Livability

The effect of phytase supplementation on survivability of hens was studied based on the mortality pattern observed in the course of the experiment (Table 35). Altogether three birds died during the entire period of study. The per cent

Table 35 : Percentage livability among different treatments

Period	Mortality				
	T_1	T_2	T_3	T_4	T_5
1 (21-24 weeks)					1
2 (25-28 weeks)					
3 (29-32 weeks)					
4 (33-36 weeks)		1			
5 (37-40 weeks)				1	
Total mortality	0	1	0	1	1
Percent Livability	100	96.7	100	96.7	96.7

livability for T_1 and T_3 were 100 per cent. It was 96.7 per cent for T_2, T_4 and T_5. Necropsy of dead birds did not reveal any pathogenic signs that are attributable to treatment effect. The overall mortality in the experiment was within the standards prescribed for laying house mortality.

4.17 Economics

In order to assess the cost benefit particulars of supplementation of phytase in low available phosphorus layer ration, the cost of different rations used in the study was calculated based on the actual price of feed ingredients which prevailed at the time of experiment and are presented in Table 36. Cost of rations computed for the different treatments viz., T_1, T_2, T_3, T_4 and T_5 were 6.58, 6.45, 6.46, 6.47 and 6.48 rupees per kg respectively. Among the rations, cost of standard layer ration was the highest (Rs. 6.58) and low available phosphorus layer ration without phytase supplementation was the cheapest (Rs. 6.45). Addition of dicalcium phosphate to the standard layer ration, for obtaining an available phosphorus of 0.5 per cent resulted this ration to cost more.

When graded levels of enzyme were added to the low available phosphorus layer ration, their costs gradually increased. Even after the addition of different levels of enzyme to the low available phosphorus diets their costs were well below that of standard layer ration.

Table 36 : Cost of experimental diets

Ingredients	Cost/kg (Rs)	Cost of diets per 100kg (Rs.)				
		T_1	T_2	T_3	T_4	T_5
Yellow maize	5.08	215.90	215.90	215.90	215.90	215.90
Ground nut cake	9.45	179.50	179.50	179.50	179.50	179.50
Gingelly oil cake	9.45	037.80	037.80	037.80	037.80	037.80
Bajra	5.90	058.35	058.35	058.35	058.35	058.35
Rice Polish	4.74	047.40	047.40	047.40	047.40	047.40
Wheat bran	5.57	016.71	016.71	016.71	016.71	016.71
Fish meal	10.50	052.50	052.50	052.50	052.50	052.50
Shell grit	3.40	017.85	021.25	021.25	021.25	021.25
Common salt	1.74	000.43	000.43	000.43	000.43	000.43
Dicalcium phosphate	16.50	016.50	000.00	000.00	000.00	000.00
Natuphos®	400.00	000.00	000.00	01.60	02.40	03.20
Vit. AB_2D_3	430.00	010.75	010.75	010.75	010.75	010.75
Trace min-mix	51.10	004.09	004.09	004.09	004.09	004.09
Total		657.78	644.68	646.28	647.00	647.88
Cost./Kg.		6.58	6.45	6.46	6.47	6.48

Table 37 : Economics of production

Sl.No	Particulars	Treatments				
		T_1	T_2	T_3	T_4	T_5
1.	Total feed intake in kg (21-40 weeks)	467.45	453.03	476.19	474.09	483.54
2.	Total no. of eggs produced (21 - 40 weeks)	3579.00	3477.00	3579.00	3614.00	3557.00
3.	Feed consumed per egg (g) (21 - 40 weeks)	130.61	130.29	133.05	131.18	135.94
4.	Cost of feed /kg (Rs)	6.58	6.45	6.46	6.47	6.48
5.	Cost of feed /egg (paise)	85.94	84.04	85.95	84.87	88.08

The economics of production (Table 37) indicated that cost of production of an egg varied from 84.04 to 88.08 paise for different treatments. The cost of production of eggs was lowest for birds fed with low available phosphorus layer ration without phytase and was highest for birds fed with low available phosphorus layer ration supplemented with 400 units of phytase/kg.

Discussion

5. DISCUSSION

5.1 Climatic parameter

Climatic observations set out in Table 4 revealed that the overall mean maximum and minimum temperature recorded inside the experimental house during the trial period was 31.3°C and 22.7°C, respectively. The mean relative humidity was 83.6 per cent in the morning and 54.6 per cent in the afternoon. The first two experimental periods (October and November, 1998) fall within the rainy season and the last three experimental periods (December, 1998, January and February, 1999) fall in the dry season of Kerala (Somanathan, 1980).

5.2 Egg production

On perusal of the mean hen-day egg production as influenced by phytase supplementation presented in Table 5, it was revealed that addition of phytase in low available phosphorus layer diets numerically improved egg production and that the quantum of improvement was in tune with the amount of enzyme added. The improvement in egg production from that of low available phosphorus diet without phytase was 3.08, 3.90 and 4.91 per cent for the groups fed with low available phosphorus diets supplemented with 200, 300 and 400 units of phytase per kg, respectively. On the other hand not much variation in egg production could be observed numerically in enzyme supplemented groups from that of standard layer mash fed birds. The groups fed low available phosphorus diets supplemented with 200, 300 and 400 units of phytase/kg laid only 0.01, 0.83 and 1.84 per cent more eggs, respectively than the standard layer mash fed group. The hen-day egg production was lowest numerically in group fed diet with low available phosphorus (0.3 per cent) indicating the possibility of inadequacy of this level for satisfactory egg production. When the data on hen-day egg production was subjected to statistical analysis, it was observed that phytase supplementation in low available phosphorus diet did not have a significant influence (Table 6). Therefore, the numerical improvement noted with the enzyme supplemented groups has to be adjudged along with other production traits viz., feed intake, feed conversion efficiency, egg weight, etc.

The period-wise egg production without consideration of the treatment effects given in Table 5 indicated that it was statistically lower (70.52 per cent) during the 1st period (21-24 weeks of age) than the subsequent periods. Hen-day egg production was 70.52, 90.55, 90.63, 88.84, 85.00 for the periods 1,2,3,4 and 5 respectively. On observation it could be seen that the period-wise egg production shows the normal laying curve for commercial egg type pullets.

The numerical improvement in the hen-day egg production points towards the beneficial responses of enzyme addition in layer ration. The improvement in egg production observed in this trial is in agreement with the result of the study carried out by Budor *et al.* (1995) who conducted an investigation with laying hens fed a diet containing 0.27 per cent available phosphorus supplemented with phytase at 600 units/kg and noted increased egg production among the enzyme supplemented birds. Similarly, in a trial with laying hens fed diets containing 0.5, 0.55 and 0.6 per cent total phosphorus, equivalent to 0.25, 0.30 and 0.35 per cent available phosphorus supplemented with microbial phytase at different levels, Kaminska *et al.* (1996) observed higher laying rate over the birds fed on diets containing 0.5 per cent total phosphorus with enzyme.

Significant improvement in egg production due to phytase inclusion in layer diets was also reported by Schoner *et al.* (1993), Roland and Gordon (1996), Klis *et al.* (1997), Gordon and Roland (1998) and Rao *et al.* (1999).

On the contrary, Kaminska (1997) and Oloffs *et al.* (1997 b) reported that supplementing phytase to laying hen diets containing low phosphorus levels did not have any effect on egg production.

By considering the findings in this study and those reported by other workers it can be seen that phytase supplementation in low available phosphorus layer ration can improve egg production. The beneficial effects in egg production could be due to the effect of added enzyme, which may be break up the organic phytate present in the feed and augment the utilization of phosphorus as well as other minerals which subsequently leads to better performance though the birds are provided with low available phosphorus ration. Absence of beneficial effects in egg production due to phytase addition, as reported by some workers could be due to variation in the available phytase units in the enzyme preparation.

5.3 Feed intake

The mean daily feed intake per bird in the different treatments ranged from 107.88 to 115.13 g. The data on mean daily feed intake per bird as influenced by phytase supplementation presented in Table 7 indicated that it was lowest among the birds fed low available phosphorus diet without enzyme and that phytase supplementation enhanced daily feed intake to a tune of 5.00 to 7.25 g/bird. Birds fed standard layer mash recorded a mean feed intake of 111.30 g/bird/day. While comparing the daily feed intake of birds fed standard ration and that between enzyme supplemented groups, it could be seen that addition of graded levels of enzymes did cause only an increase of 1.08 to 3.83 g feed/bird.

Statistical analysis of the mean daily feed intake data confirmed that phytase supplementation to low available phosphorus diets caused a significant increase in feed intake. It was significantly higher among birds offered a low available phosphorus diet with 400 units of enzyme per kg. Daily feed intake per bird was statistically comparable between groups supplemented with 200 and 400 units of phytase per kg and that between standard layer ration fed birds and those fed a ration supplemented with 300 phytase units/kg.

Mean daily feed intake for the periods 1,2,3,4 and 5 were 102.87, 111.35, 114.83, 115.79 and 115.73 g respectively per bird. It showed a linear increase in feed intake with each period. Statistical analysis of the period wise feed intake data revealed that it was significant between periods. Daily feed intake was significantly more in the last three periods than the other two.

On scanning the literature, not much work could be seen on the possible effects of phytase supplementation on feed in take of egg type layers. Budor *et al.* (1995) conducted three trials to study the effect of added phytase in laying hen diets on phosphorus excretion and assimilation and reported that phytase had no effect on feed consumption. On the other hand Simons *et al.* (1992), reported that feed consumption by hens consuming low phosphorus diets (0.33 per cent total phosphorus) with and without phytase was 108 and 89 g , respectively. Zobac *et al.* (1995) also opined that feeding on the diet low or devoid of dicalcium phosphate without phytase significantly reduced feed intake and addition of Natuphos considerably improved feed intake.

Increase in feed intake consequent to phytase supplementation in low available phosphorus diet was also reported by Roland and Gordon (1996)and Gordon and Roland (1998).

Significantly higher feed intake observed in enzyme supplemented groups might be due to increased phytate phosphorus digestibility, since phytic acid may be imposing a restraint on voluntary feed intake. Feed intake was significantly lower during the first period and improved statically in the subsequent periods. Lowered body weight of pullets during the initial period might have resulted in a lowered feed intake at that time. Moreover lowered egg production during the initial periods could also have contributed to the lesser feed intake. Significantly high feed intake in the subsequent periods must be due to high rate of production during that time and the increment in body weight.

5.4 Feed efficiency

The effect of phytase inclusion in low available phosphorus diets on feed efficiency is given in Table 9. It did not reveal much variation among treatments. The difference between various treatments was only 0.03 . Statistical analysis of the data on feed per dozen egg (Table 10) showed that feed required to produce one dozen of eggs was not different between treatments irrespective of the level of available phosphorus in the diet or inclusion of graded levels of phytase to low available phosphorus diets. Though daily feed intake was significantly less with the group fed a low available phosphorus diet without enzyme, numerically higher egg production recorded in the enzyme supplemented groups might have contributed to the similar feed conversion efficiency noted among various treatments.

The above finding is in agreement with Hadron and Wiedmer (1998) who studied the effect of phytase addition in a low phosphorus layer diet and reported that feed conversion rate was not influenced by phytase when added at the rate of 300 and 150 units/kg during 21-48 and 61-68 weeks of age. On the other hand, Klis *et al.* (1997) reported that feed conversion ratio of the hens from the phytase supplemented groups remained good throughout the experiment. Kwon *et al.* (1995) could not observe any improvement in feed gain ratio of hens fed on 90 per cent available phosphorus, 90 per cent available phosphorus plus phytase and 80 per cent available phosphorus plus phytase. However, it was higher among hens fed 80 per cent available phosphorus without phytase than birds maintained on 100 per cent available phosphorus. Rao *et al.* (1999) found an improvement in feed conversion efficiency when White Leghorn layers were fed with a diet containing 3.6 g calcium and 1 g non-phytate phosphorus (250 units/kg). Since the enzyme activity is essentially dependent on temperature, pH value, type of substrate and substrate concentration, the absence of improvement in feed conversion efficiency in the present trial might be due to reduced activity of phytase.

The feed per dozen egg for the period 1,2,3,4 and 5 were 1.76, 1.47, 1.52, 1.57, and 1.64, respectively. Statistical analysis of the period-wise feed conversion efficiency showed that significant difference existed between different periods. Superior efficiency was noted in the 2^{nd} period. The feed per dozen eggs was statistically similar between periods 2 and 3, 3 and 4 and 4 and 5. In all the treatments, most of the birds came to production only during the later part of 1^{st} period. This could have contributed for poor feed efficiency observed in the 1^{st} period.

5.5 Body weight gain

The mean gain in body weight was 0.247, 0.200, 0.241, 0.246 and 0.295 kg for the treatment T_1, T_2, T_3, T_4 and T_5, respectively (Table 11). Perusal of the body weight gain data revealed that birds offered a low available phosphorus diet supplemented with 400 units of phytase/kg gained more weight than all other treatments. Body weight gain was less with the group fed a low available phosphorus diet without enzyme. When the body weight gain data was subjected to statistical analysis, it was revealed that no significant difference existed between treatments.

Nahashon *et al.* (1994) found higher body weight gain when egg type layers were fed with phytase and other microbial added diet. Improvement in body weight gain subsequent to supplementation of phytase in layer diets was also reported by Zobac *et al.*(1995), Klis *et al.*(1997), Carlos and Edwards (1998) and Rao *et al.* (1999). In the present trial also numerical improvement in body weight gain was observed among the phytase supplemented groups. However, the magnitude of difference in weight gain between treatments were not enough to show any statistical separation. Absence of significant difference in the body weight gain among the enzyme supplemented groups might be due to the comparatively higher levels of available phosphorus (0.3 per cent) present in the treatment diets.

5.6 Egg weight

Egg number and egg weight are the two principal traits in layer stocks which determine profitability. While, the former is directly related to economic return, the latter is related to marketability of eggs. Body weight, age at sexual maturity, season, clutch size and management practices may bring about significant variations in egg weight. Therefore, these factors should also be taken into consideration while discussing this trait.

A perusal of the data on mean egg weight presented in Table 13 revealed that the average egg weight recorded in this study is fairly similar to those recorded for this genetic group at the farm (Anon 1997). Among the treatments, the groups maintained on a low available phosphorus diet supplemented with 200 units of phytase/kg attained comparatively higher egg weights (51.16 g), whereas, the group fed with a low available phosphorus diet without phytase recorded a mean egg weight of 49.07 g. Thus it could be seen that variation of 2.09 g in egg weight existed among the various treatments.

Statistical analysis of the mean egg weight data indicated that birds fed with a low available phosphorus diet without enzyme laid egg with significantly lower ($p < 0.01$) weights. Whereas, birds among all other treatments, i.e., those maintained on standard layer diet as well as those fed low available phosphorus diets supplemented with varying levels of phytase laid heavier ($p < 0.01$) eggs.

It was evident from the data presented (Table 13) that egg weight increases as age advances. Statistical analysis also indicated a significant difference in egg weight with respect to periods. It was significantly lower in the first period and was different from all other periods. Maximum egg weight was recorded during the 4^{th} period i.e., 33-36 weeks of age. Egg weight recorded between 3^{rd} and 4^{th} period and the between 2^{nd}, 3^{rd} and 5^{th} periods were statistically comparable.

The results of the present study closely agree with Gordon and Roland (1997), who opined that when hens were given lower non – phytate phosphorus diets, egg weights decreased and that supplementing the non-phytate phosphorus diet with phytase, eliminated that effect of low available phosphorus diet. Gordon and Roland (1998), Hadron and Wiedmer (1998) and Rao *et al.* (1999) also reported that phytase supplementation in low available phosphorus diet had a positive effect on egg weight. On the other hand, Carlos and Edwards (1998) could not observe any improvement in egg size with supplementation of phytase. Addition of phytase enzyme in the experimental ration might have not only helped the liberation of phosphorus from the phytase salts but also other minerals which are bound to phosphate. Microbial phytase could have also helped protein and amino acid digestibility which in turn lead to improved egg weight in enzyme added rations.

5.7 Egg specific gravity

Since eggs are easily broken during gathering, transportation and handling, it is important that such losses are kept minimum. Both egg specific gravity and egg shell weight are positively correlated t o egg shell strength.

The mean egg specific gravity as affected by phytase supplementation given in Table 15 revealed that it was lowest with birds fed a low available phosphorus diet without phytase supplementation (1.083). It was also evident from the table that addition of phytase to low available phosphorus diets markedly improved egg specific gravity. Statistical analysis of the mean egg specific gravity values presented in Table 16 confirmed the above trend. Mean egg specific gravity was highest ($p < 0.01$) with birds fed a low available phosphorus diet supplemented with 200 units of phytase/kg and was different from all other treatments. Though the groups offered low available phosphorus diets supplemented with phytase at 300 and 400 units/kg registered a mean egg specific gravity value significantly lower than the group fed a low available phosphorus diet containing 200 units/kg, it was significantly higher than the standard layer mash fed birds as well negative control. The egg specific gravity value of standard layer diet fed birds were statistically higher than birds fed a low available phosphorus diet without phytase but was significantly lower than phytase supplemented groups. Thus it could be seen that phytase supplementation has a definite role in improving egg specific gravity.

While considering the egg specific gravity as influenced by the different periods, it could be noted that significantly higher value was registered in the 1st period (21-24 weeks of age) and later slight decline was seen.

After conducting experiments in commercial laying hens, Roland and Gordon (1996) and Gordon and Roland (1998) suggested that phytase supplementation significantly improved egg specific gravity. The results of the present study also confirm the observation of Kaminska (1997) who conducted an experiment with laying hens fed diets containing 0.25, 0.30 and 0.35 per cent available phosphorus and supplemented with 0, 150, 300 and 450 units of microbial phytase and observed that specific gravity of eggs significantly improved by supplementing diets with phytase. On the contrary, Carlos and Edwards (1998) could not observe any improvement in egg specific gravity with phytase supplementation. The improvement in specific gravity of eggs as a result of phytase supplementation noted in this experiment could be due to the improved phosphorus availability consequent to the increased phytase breakdown.

5.8 Egg shell weight and egg shell thickness

A perusal of the mean egg shell weight as influenced by different feeding regimens employed in this study presented in Table 17 indicated that it was significantly more with the group offered a low available phosphorus diet added with 200 units of phytase per kg (6.34 g). It was also observed that both standard layer mash fed birds as well as low available phosphorus diet without phytase fed birds recorded significantly lower shell weights. All the enzyme supplemented groups had significantly higher egg shell weight than other treatments but differed ($p < 0.01$) between them.

The mean egg shell weight as influenced by different periods indicated that it was significantly lower with the 1st period and it was improved ($p < 0.01$) in the subsequent periods.

The data on mean egg shell thickness as influenced by supplementation of phytase enzyme given in Table 19 also showed a similar trend as that of egg shell weight. The egg shell thickness recorded for the period T_1, T_2, T_3, T_4, and T_5 were 0.334, 0.329, 0.354, 0.342, and 0.345 mm, respectively. Statistical analysis of the mean egg shell thickness presented in Table 20 revealed that it was more ($p < 0.01$) with birds offered a low available phosphorus diet supplemented with 200 phytase units/kg. The overall trend showed that all enzyme supplemented groups registered higher ($p < 0.01$) egg shell thickness than other treatments. The egg shell thickness of low available phosphorus diet fed birds were significantly lower than all other treatments. Though standard layer diet fed birds recorded significantly higher egg shell thickness than the low available phosphorus diet given birds, it was lower ($p < 0.01$) than phytase supplemented groups. Irrespective of the treatment effects, the egg shell thickness values of all birds were within the normal range. Though the egg shell thickness was influenced by different periods no definite conclusion could be made since the trend was erratic.

The beneficial effects of phytase supplementation on egg shell quality were well documented. Lettner *et al.* (1995) studied the efficiency of phytase in hybrid layers offered diets containing 0.61 and 0.41 per cent phosphorus with 0.65 per cent mono calcium phosphate or Natuphos plus calcium carbonate and opined that number of cracked eggs decreased from 7.60 to 3.81 per cent with phytase supplementation. In another trial with laying hens fed diets containing 0.5, 0.55 and 0.6 per cent total phosphorus equivalent to 0.25, 0.30 and 0.35 per cent available phosphorus, respectively supplied with microbial phytase at levels of 0, 150, 300 or 450 units/kg, Kaminska *et al.*(1996) reported that birds fed diets containing 0.5 per cent total phosphorus supplemented with 300 units/kg showed good egg shell quality. Thickest egg shell and the highest breakage strength were observed when diets containing 0.30 to 0.35 per cent available phosphorus were supplemented with 150 units of phytase. In an experiment with laying hens fed diets containing 0.25, 0.30 and 0.35 per cent available phosphorus supplemented with 0, 150, 300 or 450 units of phytase enzyme/kg feed, Kaminska *et al.*(1996) observed significant improvement in shell thickness in hens fed with phytase added diets. Improvement in egg shell quality with supplementation of phytase to layer rations low in non phytate phosphorus was also reported by Gordan and Roland (1998) and Rao *et al.* (1999).

The result of the present study and those reported by other workers indicated that phytase supplementation has a definite role in improving egg shell quality. Addition of phytase to the diet could have lead to the release of phosphorus from phytic acid which in turn causes improvement in shell quality.

The effect of phytase in breaking down phytate appear to be largely independent of the level of enzyme in the diet.

5.9 Serum calcium and phosphorus levels

Influence of phytase supplementation on serum calcium levels are given in Table 21. It was evident from the table that enzyme addition did have a significant influence on this trait. Irrespective of the levels of phytase addition, all supplemented groups had significantly higher serum calcium levels. Birds offered a low available phosphorus diet, without phytase had significantly lower serum calcium levels. Birds fed standard layer mash had serum calcium levels that were statistically comparable with other treatments.

A close look on the mean serum inorganic phosphorus presented in Table 23 indicated that similar to serum calcium levels, birds fed a low available phosphorus diet without phytase had lower value than other treatments. However, statistical analysis of the mean serum inorganic phosphorus did not show any significant difference between treatments due to phytase supplementation. An overall assessment of the biochemical parameters clearly indicated that phytase supplementation to low available phosphorus diets did cause an increase in their values over the negative control.

On reviewing the literature no work could be traced on the influence of phytase supplementation on serum calcium and inorganic phosphorus levels. However, the influence of phytase supplementation on plasma dialyzable phosphorus of layers was studied by Carlos and Edwards (1998). They conducted 12 experiments to study the effect of supplementation of layer diet with either phytase, 1-25 dihydroxy cholecalciferol or their combination. In both the experiments they could observe an increase in plasma dialyzable phosphorus by the supplementation of phytase at a level of 600 FTU/kg feed. In the present study, significant increase in serum calcium levels and a numerical increase in serum inorganic phosphorus was observed in birds when supplemented with phytase at different level. This indicated that phytase supplementation causes liberation of phosphorus from bound phytate phosphorus thereby, improving the bio-availability of phosphorus which in turn results in increased serum calcium and inorganic phosphorus. Absence of significant increase in serum inorganic phosphorus observed in the study might possibly be due to smaller sample size.

5.10 Tibial ash and phosphorus content

The per cent tibial ash and its phosphorus content of representative birds fed the experimental rations estimated at the end of the experiment are given in Table 25 and 27, respectively. A perusal of the data revealed that numerical differences existed between different treatments with regard to these traits.

Reduction of the available phosphorus content from 0.5 to 0.3 per cent in the layer ration resulted in a reduction of 3.44 per cent in tibial ash. When the low available phosphorus layer ration were supplemented with 200,300 and 400 units of phytase/kg caused an increase in tibial ash content to the extent of 4.07, 3.19 and 3.35 per cent respectively.

Similarly, a reduction of 1.84 percent in the tibial phosphorus content was observed when available phosphorus in the diet was reduced from 0.5 to 0.3 per cent. However an increase of 2.10, 2.10 and 2.12 per cent in the tibial phosphorus content could be found when low available phosphorus diets were supplemented with 200, 300 and 400 units of phytase/kg, respectively. When the data on tibial ash and phosphorus content were subjected to statistical analysis it was revealed that no significant difference existed between the various feeding regimens employed. The numerical improvement in these traits points towards the beneficial effects of phytase supplementation.

The present results confirm the observations of Schoner *et al.* (1993), Zobac *et al.* (1995) and Klis *et al.*(1997) who reported that addition of phytase enzymes in low available phosphorus layer diets considerably improved the tibial ash content. Like wise, Carlos and Edwards (1998) also reported that phytase had a positive effect on tibial bone ash.

Gordon and Roland (1998) also observed an increase in the bone mineral content and bone density with phytase supplementation in laying hens. Thus it could be concluded that phytase will act upon the bound phytase phosphorus of the plant portion of the feed thereby liberating phosphorus. This enhances phosphorus bio-availability and which in turn result in increased tibial ash and phosphorus content.

5.11. Bio-availability of calcium and phosphorus

Per cent bio-availability of calcium and phosphorus presented in Tables 29 and 31, respectively revealed that numerical difference existed between treatments. A reduction of 3.17 per cent in the bio-availability of calcium was found when the available phosphorus content of layer feed was reduced to 0.3 per cent. When the low available phosphorus rations were supplemented with 200, 300 and 400 units of phytase/kg an increase of 3.17, 2.83 and 1.69 per cent respectively of bio-availability of calcium resulted. However, the statistical analysis of bio-availability of calcium among the treatments as influenced by phytase supplementation failed to show significant difference.

Per cent bio-availability of phosphorus was significantly influenced by phytase supplementation ($p < 0.01$). Bio-availability of phosphorus was more in enzyme supplemented groups as well as in standard layer mash fed birds. Feeding layer with an available phosphorus content of 0.3 per cent resulted in a significant reduction in the bio-availability of phosphorus.

Schoner *et al.*(1993) and Nahashon *et al.*(1994) reported significant improvement in the retention of phosphorus when phytase was supplemented in layer diets. Zobac *et al.*(1995) opined that feeding the diet low or devoid of dicalcium phosphate without Natuphos significantly reduced digestibility of calcium and phosphorus but addition of Natuphos resulted in considerable increase in the calcium and phosphorus digestibility. Like wise, Gordon and Roland (1998) stated that inclusion of phytase in layer diet improved calcium utilization. Carlos and Edwards (1998) also found that phytase enzyme had a positive effect on phytate phosphorus retention in laying hens.

Based on the results of the present study and the findings of other workers it can be inferred that phytase supplementation in layers has a positive effect in improving the bio-availability of calcium and phosphorus. Addition of phytase in the diet acts upon the bound phytate phosphorus and liberated inorganic phosphorus molecules. It results in the enhancement of bio-availability of calcium and phosphorus.

5.12 Phosphorus excretion

Data on phosphorus excretion (g/kg dry matter intake) as influenced by phytase supplementation given in

Table 33 indicated that significant difference existed between treatments. Phosphorus excretion was significantly more (6.54 per cent) with the birds offered a diet containing an available phosphorus of 0.5 per cent. When the available phosphorus content of the diet was reduced to 0.3 per cent it resulted in a significant reduction in the phosphorus excretion (5.30 per cent). Supplementation of phytase to the low available phosphorus diets also caused a significant reduction in the phosphorus excretion. It was also revealed that phosphorus excretion of phytase supplemented groups were statistically comparable. It shows that when the birds are offered a standard layer feed containing an available phosphorus of 0.5 per cent it results in more excretion of phosphorus since most of the plant phosphorus are in the from of phytate phosphorus and they are not utilized and hence excreted. It also shows that phosphorus excretion is less in diet containing an available phosphorus of 0.3 per cent. It confirms that when phytase was added to diets it facilitates in enhancing the availability of phosphorus from phytate phosphorus for utilization and hence less amount is excreted in the droppings.

Reduction of phosphorus excretion in the droppings by phytase supplementation was reported by Schoner *et al.*(1993), Kaminska *et al.*(1996), Kwon *et al.*(1995), Lettner *et al.*(1995), Zobac *et al.*(1995) and Hadron and Wiedmer (1998). Thus supplementation of phytase not only is of important in enhancing the availability of phosphorus to bird but also is important in reducing soil pollution by reduced phosphorus excretion.

5.13 Livability

During the 20 weeks of experimental period covering 20-40 weeks of age only three birds died. The data on per cent livability of birds (Table 35) revealed that it ranged from 96.7 to 100. Necropsy findings indicated non specific reasons.

No mortality was observed among the birds offered with standard layer ration and low available phosphorus ration with 200 phytase units. From the pattern of livability it is evident that phytase supplementation did not have any detrimental effects on the physiological well being of laying hens. Roland and Gordon (1996) observed increased mortality among birds fed with diets containing 0.1 per cent available phosphorus diet without phytase and further supplementation of the enzyme corrected it.

5.14 Economics

The cost of different rations employed in the experiment (Table 36) revealed that standard layer ration formulated as per BIS specification was costlier than others. Reduction of phosphorus level below the BIS recommendation (0.3 per cent) reduced the cost of ration by 13 paise per kg.

however, supplementation of phytase enzyme to low available phosphorus layer ration enhanced the cost of ration in proportion to the level of enzyme addition. But even with higher level of enzyme incorporation (400 units/kg) the cost was 10 paise per kg lesser than standard layer ration indicating economic viability of phytase supplementation.

When the cost of egg production based on feed cost alone was calculated (Table 37), it was observed that in T_4 (birds offered with low available phosphorus layer ration plus 300 units phytase) the cost was lower than standard layer ration. Although low cost of feed/kg for production of an egg was noticed in T_2, considering the lower egg size and egg shell quality compared to other treatments T_3 and T_4 were found to be better.

The economic analysis point out the fact that incorporation of phytase at a level of 200 units/kg in layer diets having an available phosphorus level of 0.3 per cent could bring down the feed cost as well as production cost.

Summary

6. SUMMARY

An investigation was carried out at the Mannuthy Centre of All India Coordinated Research Project on Poultry Improvement using one hundred and fifty White Leghorn strain cross pullets (ATHULYA) at 21 weeks of age to assess the influence of phytase supplementation in low available phosphorus layer ration on production performance and nutrient availability. The pullets were randomly distributed to five dietary treatments with each treatment having three replicates of ten birds each. The dietary treatments consisted of standard layer ration (T_1), low available phosphorus layer ration (T_2), low available phosphorus layer ration with 200, 300 and 400 units of phytase per kg feed (T_3,T_4 and T_5), respectively. All the diets were formulated as per BIS specification except the level of available phosphorus.

Feed ingredients used in the formulation were yellow maize, groundnut cake, gingelly oil cake, bajra, rice polish, wheat bran, unsalted dried fish and dicalcium phosphate. The birds were housed in individual cages. Standard managemental procedures were adopted throughout the experimental period. The duration of the experiment was five 28-day periods covering 21-40 weeks of age. The body weight of individual birds was recorded at the commencement and at the end of the experiment. Daily feed intake and egg production were recorded. All the eggs collected during the last three consecutive days of each 28-day period were weighed individually to arrive at mean egg weight. These eggs were also used for measuring egg specific gravity, egg shell weight and shell thickness. From these data per cent hen-day production, daily feed intake per bird, feed conversion efficiency (feed per dozen eggs), body weight gain, mean egg weight, egg specific gravity, egg shell weight and shell thickness were worked out.

At the end of the experimental period a metabolism trial was conducted using four birds from each treatment. Based on the data obtained form the metabolic trial, bio-availability of calcium and phosphorus and phosphorus excretion were calculated. After the completion of metabolism trial, twenty birds were sacrificed to collect blood samples and tibia for the estimation of serum calcium, serum inorganic phosphorus, tibial ash and phosphorus content. Mortality of the birds were recorded. Cost benefit analysis was also worked out.

The overall performance of the birds fed different dietary treatment are presented in Table 38.

Based on the results obtained in this study, the following conclusions were made.

1) Enzyme supplementation caused a numerical increase in egg production. The improvement in hen-day egg production from that of low available phosphorus diet with out phytase

was 3.08, 3.90 and 4.91 per cent for the groups fed with low available phosphorus diets supplemented with 200, 300 and 400 units of phytase per kg, respectively. However, phytase supplementation did not have any significant influence on hen-day egg production. The difference in hen-day egg production among the different experimental periods were statistically significant.

2) The mean daily feed intake per bird in the different treatments ranged from 107.88 to 115.13g. Daily feed intake per bird was significantly higher ($p < 0.01$) among birds fed different levels of enzyme in low available phosphorus layer ration. Significantly lower feed intake was noticed in the treatment provided ration without enzyme. Daily feed intake showed significant differences among periods.

3) There was no significant difference among treatment groups with respect to feed per dozen eggs, whereas it differed significantly between periods.

4) The mean body weight gain of different treatments varied from 0.200 to 0.295 kg. Enzyme supplementation did not have any significant influence upon body weight gain.

5) The mean egg weight in the different dietary treatments varied from 49.07 to 51.16 g. Significantly higher egg weight was registered for the groups fed low available

phosphorus diets supplemented with varying levels of phytase. Birds fed with diet containing 0.3 per cent available phosphorus without enzyme laid eggs with lower ($p > 0.01$) weights. The egg weight of standard layer mash fed birds was statistically comparable with enzyme supplemented groups.

6) Mean egg specific gravity was highest ($p < 0.01$) with birds fed a low available phosphorus diet supplemented with 200 units of phytase per kg and those maintained on diet without phytase recorded significantly lower values. The egg specific gravity of standard layer diet fed birds were statistically higher than birds fed a low available phosphorus diet without phytase but was significantly lower than phytase supplemented groups.

7) The egg shell weight of birds offered low available phosphorus diet with 200 units of phytase/kg was significantly higher and was different from all other treatments. Supplementation of 300 and 400 units of phytase /kg in low available phosphorus ration also resulted in increase ($p < 0.01$) of egg shell weight. The egg shell weight values of birds fed standard layer ration as well as those maintained on low available phosphorus ration without phytase were significantly lower.

8) Irrespective of the levels of enzyme addition, all supplemented groups significantly improved shell thickness values; maximum improvement ($p < 0.01$) was noticed with the treatment offered 200 units of phytase /kg.

9) Mean serum calcium levels were significantly higher for the enzyme supplemented groups. Though phytase supplementation resulted in numerical improvement in the serum inorganic phosphorus no significant difference was noted between treatments.

10) Per cent tibial ash and phosphorus levels were numerically higher in enzyme supplemented groups. However, no statistical difference existed between the treatments with respect to these traits.

11) Bio-availability of calcium was not significantly different between treatments. It was numerically higher in both standard layer mash fed birds and those fed low available phosphorus ration supplemented with phytase. Bio-availability of phosphorus was significantly more in enzyme supplemented groups as well as in standard layer mash fed birds.

12) Phytase supplementation resulted in a significant reduction in the phosphorus excretion in the droppings (g/kg DM intake).

13) The survivability of laying hens was not affected by phytase inclusion in the diet.

14) Cost of rations computed for the treatments viz., T_1, T_2, T_3, T_4 and T_5 were 6.58, 6.45, 6.46, 6.47 and 6.48 rupees/kg respectively. The feed cost for producing one egg varied from 84.04 to 88.08 paise for the different treatments. Cost of production of eggs was lowest for birds fed with low available phosphorus diet without phytase and was highest for birds fed with low available phosphorus layer ration supplemented with 400 units of phytase per kg.

Although, cost of production of one egg is comparatively less with birds fed a diet having an available phosphorus content of 0.3 per cent, significantly lower egg weight and inferior egg shell qualities observed in this treatment deter in recommending low available phosphorus diets for layers. When graded levels of phytase were added to low available phosphorus layer diets a marked improvement in egg shell thickness, egg shell weight, egg specific gravity and egg size were observed. As compared to standard layer mash fed birds, the feed cost for production of an egg was 0.01 and 2.14 paise more in the groups fed low available phosphorus diets supplemented with 200 and 400 units of phytase per kg, respectively and 1.07 paise less in the group fed with low available phosphorus diet added with 300 phytase units /kg. The superior egg shell qualities as well as lower phosphorus excretion in the dropping noted among the enzyme supplemented groups in comparison to standard layer mash fed birds gives a definite advantage for phytase supplementation.

Among the different levels of phytase tested, incorporation at the rate of 200 units per kg feed was found to be optimum. Based on the results of this study it could be inferred that by the addition of 200 units of phytase enzyme per kg of diet, the available phosphorus level in layer rations using common feed ingredients can be reduced to 0.3 per cent without affecting the overall performance.

Table 38 Influence of phytase supplementation on overall performance of layer chicken

Parameters	Dietary treatments				
	T_1	T_2	T_3	T^4	T_5
Mean hen day egg production (%)	85.20	82.13	85.21	86.03	87.04
Mean feed intake per bird per day (g)	111.30	107.88	113.38	112.88	115.13
Mean feed efficiency (kg feed/dozen eggs)	1.58	1.60	1.61	1.58	1.60
Mean body weight gain (kg)	0.247	0.200	0.241	0.246	0.295
Mean egg weight (g)	50.17	49.07	51.16	50.26	50.19
Mean egg specific gravity	1.087	1.083	1.094	1.091	1.091
Mean egg shell weight (g)	5.85	5.77	6.34	6.05	6.18
Mean egg shell thickness (mm)	0.334	0.329	0.354	0.342	0.345
Serum calcium (mg %)	20.14	18.14	21.11	21.11	20.99
Serum inorganic phosphorus (mg %)	4.38	3.76	4.33	4.18	4.12
Tibial ash (%)	52.14	48.70	52.77	51.89	52.05
Tibial phosphorus (%)	16.90	15.06	17.16	17.16	17.18
Bio-availability of calcium (%)	60.85	57.68	60.85	60.51	59.37
Bio-availability of phosphorus (%)	52.14	46.85	54.66	53.03	54.85
Phosphorus excretion (g/kg intake)	6.54	5.3	3.16	2.96	2.89
Cost of feed (Rs/kg)	6.58	6.45	6.46	6.47	6.48
Feed cost per egg (paise)	85.94	84.04	85.95	84.87	88.08

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EFFECT OF PHYTASE SUPPLEMENTATION ON PHOSPHORUS UTILIZATION AND PERFORMANCE IN LAYER CHICKEN

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ABSTRACT OF A THESIS

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ABSTRACT

The effect of three levels of phytase enzyme viz., 200, 300 and 400 units per kg in the low available phosphorus layer ration on phosphorus utilization and performance in egg type chicken was evaluated using one hundred and fifty White Leghorn strain cross pullets (ATHULYA) of 21 weeks of age for a period of 20 weeks. The birds were divided into five dietary treatment groups viz., standard layer ration (T_1), low available phosphorus layer ration (T_2), low available phosphorus layer ration with 200, 300 and 400 units of phytase per kg feed (T_3, T_4 and T_5), respectively. Standard layer ration was formulated as per BIS specification for layer chicken. Initially, the ration with 0.3 per cent available phosphorus was formulated. By the addition of appropriate levels of dicalcium phosphate to this ration, diets with 0.5 per cent available phosphorus was formulated. A numerical improvement in per cent hen-day egg production was noticed among enzyme supplemented groups. However, the increase was not statistically significant. Daily feed intake per bird was significantly higher among birds fed with different levels of enzyme in low available phosphorus layer ration. Phytase supplementation did not have significant influence upon feed per dozen eggs. Body weight gain was not influenced by enzyme supplementation. Significantly higher egg weight was obtained for the groups fed low available phosphorus diets supplemented with varying levels of phytase. Phytase addition to low available phosphorus diets significantly improved egg specific gravity.

Supplementation of phytase resulted in significant increase in egg shell weight. Mean shell thickness was significantly more among enzyme supplemented groups. Serum calcium levels of the enzyme supplemented groups registered significantly ($p < 0.01$) higher values whereas only numerical improvement could be noticed in serum inorganic phosphorus levels. Per cent tibial ash and phosphorus content were not influenced by phytase supplementation. Enzyme supplementation resulted in a numerical improvement in the bio-availability of calcium. Bio-availability of phosphorus was significantly more among enzyme supplemented groups as well as standard layer mash fed birds. Phosphorus excretion in the droppings (g/kg DM intake) was significantly ($p < 0.01$) reduced in the enzyme supplemented groups. The per cent survivability of laying hens was not affected by phytase inclusion. Cost of the feed per kg was lower in all the enzyme supplemented groups than the standard layer ration of this study.

Based on the findings, it can be concluded that by the inclusion of 200 units per kg of phytase enzyme in layer diet, the available phosphorus level in the feed can be reduced to 0.3 per cent without affecting the over all performance. This also facilitates to reduce phosphorus excretion which is an eco-friendly factor in commercial layer operations.