INFLUENCE OF PHYTASE ON PHOSPHORUS UTILIZATION IN BROILERS

By
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THESIS

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DECLARATION

I hereby declare that the thesis entitled "**INFLUENCE OF PHYTASE ON PHOSPHORUS UTILIZATION IN BROILERS**" 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, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that the thesis entitled "**INFLUENCE OF PHYTASE ON PHOSPHORUS UTILIZATION IN BROILERS**" is a record of research work done independently by **Shri. P. Kanagaraju**, 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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We, the undersigned members of the Advisory Committee of **Shri. P. Kanagaraju**, a candidate for the degree of Master of Veterinary Science in Poultry Science, agree that the thesis entitled "**INFLUENCE OF PHYTASE ON PHOSPHORUS UTILIZATION IN BROILERS**" may be submitted by Sri. P. Kanagaraju, in partial fulfilment of the requirement for the degree.

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Dedicated to
My Everloving
Parents & Teachers

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Introduction

1. INTRODUCTION

During the past few decades, the Indian poultry industry has registered remarkable progress. Based on the present growth rate, broiler production is expected to cross 1400 million by the turn of this century. Now India ranks 20th in the world in broiler production. It is estimated that the current per capita consumption of 520 g per annum could increase to 1500 g by 2000 AD (Anon, 1996). This stands testimony to the growth potential of the broiler segment of poultry industry.

It is expected that the broiler industry will expand two to three folds over the next few years. Considering this impressive growth potential, it is imperative to provide necessary inputs to cope with the enhanced production. This calls for increased feed availability in the coming years. In order to meet this heavy demand of feed, it becomes necessary to identify newer feed ingredients that will not compete with human food and also enhance the utilization of available alternate feed resources by the application of appropriate technology. Also any feedstuff which is being initially used as an ingredient for poultry feed rapidly becomes main input for some emerging industries thereby making it disadvantageous to poultry industry on cost front (Iqballuddin, 1996).

Among the inorganic elements, phosphorus is the most expensive mineral supplement to poultry rations. Cereals and plant materials which constitute major part of poultry feeds 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 feed ingredients. Availability of phytin phosphorus varies depending upon the cereals/plant materials and the species of animals that digest it. It is estimated that only about 30 per cent of plant phosphorus can be utilised by poultry and the remaining 70 per cent gets excreted.

In addition, the phytic acid may combine with starch and proteins and also with certain inorganic elements such as calcium and magnesium in the diets. Being insoluble, these complexes precipitate in the intestine and are excreted. On the other hand, almost all the phosphorus in inorganic form and that from animal sources can be fully utilized by poultry. Therefore, nutritionists routinely supplement diets with inorganic phosphorus usually beyond the level that birds actually require. This over supplementation leads to phosphorus excretion through droppings and leads to environmental pollution. These factors have forced the feed industry to investigate ways and means to increase the availability of phytin phosphorus to the animal. In order to make phytin phosphorus biologically available, it is necessary to hydrolyse the phytin by means of the phosphatase enzyme, phytase. Absence of phytase enzyme in the digestive tract of poultry necessitates the use of supplemental source of enzyme so that phytin phosphorus can be better utilized.

The potential of phytase as a feed additive was studied by Nelson *et al*. (1968, 1971). Production and use of this enzyme has generally been considered too costly to compete with feed phosphates such as dicalcium or deflourinated rock phosphate. Recently, interest in the use of phytase as a feed additive has been renewed because of advances in biotechnology and environmental concern over residual phosphate in animal wastes.

Phytase is naturally present in high concentrations in cultures of *Aspergillus ficum*, *Aspergillus niger* and in wheat bran. This discovery has now paved the way for the addition of it in poultry feeds. It is believed that addition of phytase enzyme in poultry diets hydrolyses the phytic acid to orthophosphate, inositol and other phospho-inositol intermediates in the intestine thereby increasing the bioavailability of phosphates and excretion in droppings.

In this context, the present study was planned to evaluate the influence of supplementation of phytase enzyme on phosphorus utilization and subsequent performance in commercial broilers.

Review of Literature

2. REVIEW OF LITERATURE

Enzymes play a vital role in the functioning of biological systems as catalysts for chemical reactions. As such, they can participate in a wide range of reactions under diverse environmental conditions. This is particularly true for enzymes derived from bacteria and fungi. Successful application of enzyme technology relies on knowledge of the chemical reaction to be affected and the conditions under which the reaction will occur. It is believed that phytase of fungal origin (*Aspergillus ficum*, *Aspergillus niger*) improves the phytate phosphorus availability in poultry diets based on feed ingredients of plant origin. Here, an attempt has been made to provide a review of the available literature related with supplementation of phytase enzyme on phosphorus utilization and subsequent performance in broilers.

2.1 Body weight and body weight gain

In a study to find out the effect of a mould phosphatase on the utilization of phytate phosphorus by chicks, Nelson *et al.* (1971) conducted three experiments with White Plymouth Rock cockerels. They were fed with basal diet containing 0.3, 0.47 and 0.5 per cent total phosphorus, supplemented with phytase at the rate of 0.4 per cent. The results showed that chicks fed the basal diet supplemented with phytase gained significantly ($P<0.01$) more weight than control birds.

Simons *et al.* (1990) reported that growth rate in broiler chickens fed low phosphorus diets supplemented with microbial phytase was comparable to or even better than that obtained on control diets.

Schoner *et al.* (1992a) studied the comparative effects of microbial phytase and inorganic phosphorus on the performance of broilers. In a factorial experiment day-old male broiler chicks were fed for two weeks on a basal maize-soyabean meal diet containing phytate phosphorus 2.3 g, phosphorus from monocalcium phosphate 1 g, total phosphorus 4.5 g and calcium 6 g/kg supplemented with microbial phytase at 800 units/kg. They observed a significant improvement in live weight gain by the addition of phytase.

Caged male Lohmann broilers fed maize-soyabean meal diet containing total phosphorus 7.1, 5.8 and 4.5 g/kg supplemented with phytase at levels of 500, 750 or 1000 units per kg of feed improved growth rate. Decreasing dietary phosphorus without phytase supplementation decreased the rate of growth (Vogt, 1992).

Edwards (1993) found that the addition of 1,25-dihydroxy cholecalciferol to broiler diet in the presence of dietary phytase 75, 150, 300 and 600 *u*/kg diet resulted in greater ninth day weight.

Schoner *et al.* (1993a) reported that supplementation of phytase (*A. niger*) at levels of 125, 250, 500 *u*/kg and phosphorus from monocalcium phosphate at the rate of 0.6, 1.2 or 1.8 g/kg to phosphorus deficient (P 3.5 g/kg) diet fed to broiler chickens for 40 days resulted in dose-dependent increase in live weight gain.

Farrell *et al.* (1993) conducted an experiment on broiler chickens for 18 days maintained on a sorghum-soyabean based diet supplemented with Natuphos^R-5000 (phytase) at 750 units per kg and observed significant improvement in growth rate throughout the experiment.

In a trial on broiler chickens fed with sorghum or wheat based diet low in phosphorus, supplemented with Natuphos^R-5000 (microbial phytase) at levels of 250, 500, 1000 *u*/kg of diet, Huyghebaert *et al.* (1993a) observed significant improvement in weight gain.

Phytase 750 *u*/kg or 1,25-dihydroxy cholecalciferal 5 μg/kg or both when added to maize-soyabean meal diets containing zinc 35 mg for broilers had no effect on body weight (Roberson and Edwards, 1994).

Three broiler diets containing three levels of inorganic phosphorus viz., 0.6, 1.1 and 1.6 g/kg were supplemented with two levels of phytase namely 500 and 1000 *u*/kg and were fed to broilers to study the effect of phytase on broiler performance (Richter *et al.*, 1994). The results showed that there were no negative effects on 14th and 35th day body weight when inorganic phosphorus in mineral mixture was decreased and phytase supplemented.

Richter and Cyriaci (1994) stated that addition of phytase enzyme at the levels of 300 and 750 *u*/kg of diet containing plant components only (trial 1) or plant and animal components (trial 2) with 0.4 to 0.5 per cent total phosphorus increased average daily weight gain of broilers.

In an experiment with male and female broilers fed starter and finisher diets containing phosphorus 0.6, 1.1 and 1.6 g/kg (as monocalcium phosphate) supplemented with phytase at the levels of 500 and 1000 *u*/kg gained 5 and 2.6 per cent more body weight respectively than control (Richter, 1994).

Kiiskinen *et al.* (1994) studied the effects of different intakes (250-1000 phytase units/g) of microbial phytase

(*A. niger*) on the performance of broiler chicks and observed an increase in body weight by 4-7 per cent.

Piva *et al*. (1994) conducted an experiment using broiler chickens and were fed with a control diet and diets with high activity phytase (500, 1000 or 2000 *u*/kg) and reported that final live weight was 8.5 per cent greater ($P<0.01$) for phytase supplemented diets than with the control diet.

Zyla and Koreleski (1994) stated that the substitution of calcium phosphate for a preparation of phytase in broiler feed containing rape seed oil meal did not cause 'significant differences in the body weight.

In order to evaluate the effectiveness of supplemental phytase for improving the availability of phytate phosphorus in soyabean meal, Denbow *et al*. (1995) conducted an experiment with day-old broiler chicks using a semipurified basal diet containing, 0.18 per cent phytate phosphorus. Seven levels of phytase (0, 200, 400, 600, 800, 1000 and 1200 *u*/kg of diet) were added to diets formulated to contain 0.20, 0.27 or 0.34 per cent non-phytate phosphorus or 0.38, 0.45 and 0.52 per cent total phosphorus respectively. They observed a significant improvement in body weight gain by phytase at all non-phytate phosphorus levels, but the magnitude of response was greatest at low non-phytate phosphorus level.

Edwards and Roberson (1995) evaluated the influence of commercial phytase enzyme on male broiler chicks fed a corn-soyabean meal basal diet containing 0.66 per cent calcium, 0.49 per cent phosphorus and 0.25 per cent phytate phosphorus and phytase was added at the levels of 150, 300 or 600 units/kg of diet. They reported that there were no treatment effects on 16th day body weight.

Sebastian *et al.* (1995) studied the influence of microbial phytase by supplementing at a level of 600 *u*/kg to low phosphorus corn-soyabean meal diets and were offered to 180 sexed day old broiler chicks for three weeks and observed significant (P<0.01) improvement in body weight in both male and female chicks by 13.2 and 5.8 per cent respectively.

Biehl *et al.* (1995) found that the combination of phytase (1200 *u*/kg) and 10 μg of 1,25 dihydroxy cholecalciferol per kg of soya protein diets deficient in zinc (13 mg Zn/kg) improved weight gain (P<0.05) than with either of these supplements alone in chicks.

In an experiment with broilers Kwon *et al.* (1996) offered diets containing 60, 80 or 100 per cent of the recommended phosphorus supplemented with phytase at the rate of 0 or 500 Feed Trend Units (FTU) for a period of 21 days. They found that broilers fed on diets containing 60 per cent of the required phosphorus had significantly lower live weight gains than those fed on 80 or 100 per cent phosphorus diets when no supplemental phytase was fed. However, when broilers were fed on phytase supplemented diets all groups had similar live weight gain.

Mitchell and Edwards (1996a) observed maximum body weight in male broilers fed with corn-soyabean meal diet containing 0.55 per cent total phosphorus supplemented either with phytase or 1,25-dihydroxy cholecalciferol and diet with 0.45 per cent total phosphorus supplemented with both phytase and 1,25-dihydroxy cholecalciferol for a period of 21 days.

Three experiments were conducted to determine the effect of vitamin D3 and a commercial phytase product on calcium and phosphorus requirements of 0 to 21 days old broiler males (Mitchell and Edwards, 1996b). The results showed that supplementation with 5 μg of vitamin D3 or 600 u of phytase/kg of feed can reduce total phosphorus requirements by upto 0.1 per cent for the criteria like body weight.

The usefulness of microbial phytase on the improvement of phytate phosphorus availability in corn and soyabean meal diet in broilers was studied by Kornegay *et al.* (1996). In a 21 day trial broilers were fed with corn-soyabean meal diet containing 2.0, 2.7 or 3.4 g/kg of non-phytate phosphorus or total phosphorus 4.0, 5.1 or 5.8 g/kg supplemented with phytase at the levels of 200, 400, 600, 800, 1000 or 1200 *u*/kg diet. They noted a linear increase ($P<0.01$) in body weight gain.

Huyghebaert (1996) opined that reduction in body weight could be alleviated by phytase supplementation at the rate of 500 FTU/kg of low phosphorus diets in broilers.

In a three week feeding trial 180 broiler chicks were maintained on maize-soyabean diets containing 0.46 (control) or 0.33 per cent (low) available phosphorus plus microbial phytase (Natuphos^{R}-5000) 600 *u*/kg of diet. Phytase supplementation increased ($P\leq 0.05$) body weight in male and female chickens by 13.2 and 5.8 per cent respectively (Sebastian *et al.*, 1996).

Munaro *et al.* (1996) evaluated the supplementation of phytase at levels of 250, 500, 750, 1000 or 1250 phytase units/g completely replacing dicalcium phosphate (DCP) as phosphorus source in a broiler diet and found that phytase supplementation improved body weight gain upto five weeks old.

Furuse *et al.* (1997) conducted an experiment in eight days old chicks for a period of two weeks. The chicks were fed with diets containing partially hydrolysed guar gum at different ratios supplemented with phytase at 500 *u*/kg. Although live weight gain improved with dietary phytase, it decreased in a dose-dependent manner as dietary guar gum increased.

2.2 Feed consumption and feed conversion efficiency

Simons *et al.* (1990) reported that the feed conversion ratio in broilers fed low phosphorus diets supplemented with phytase was comparable or even better than that of control diets.

In a factorial experiment, day-old male broiler chicks were fed for two weeks a diet containing phytate phosphorus 2.3, phosphorus from monocalcium phosphate 1, total phosphorus 4.5 and calcium 6 g/kg supplemented with microbial phytase at 800 *u*/kg and observed significant differences in feed intake but feed conversion efficiency was not affected (Schoner *et al.*, 1992a).

In a trial with broiler chickens fed low phosphorus diets (0.35 and 0.47 per cent non-phytate phosphorus) supplemented with Natuphos®-5000 (phytase) at the rate of 250, 500, 1000 *u*/kg for a period of six weeks, Huyghebaert *et al.* (1993a) observed significant improvement in feed intake index.

Schoner *et al.* (1993a) conducted an experiment in broiler chickens by supplementing phytase at levels of 125, 250 and 500 *u*/kg in phosphorus deficient (3.5 g/kg) maize-soyabean meal diets for a period of 40 days and recorded increased feed intake.

In another trial, Schoner *et al.* (1993b) observed increased feed conversion efficiency in Lohmann broilers fed diets containing total phosphorus 3.5, phytate phosphorus 2.3 and calcium 6 g/kg and supplemented with phytase 125, 250 and 1500 *u*/kg of feed as compared to control without supplementing phytase.

Improved feed conversion efficiency was observed in broiler chickens fed diets supplemented without or with phosphorus from calcium hydrogen phosphate and Natuphos[R]-5000 (phytase) from *A. niger* at a level of 750 *u*/kg for a period of 18 days (Farrell *et al.*, 1993).

Perney *et al.* (1993) conducted two experiments in broiler chicks to study the effect of dietary phytase on growth performance and phosphorus utilization. In the first experiment chicks were given diets containing 0.21, 0.29, 0.37 and 0.44 per cent available phosphorus plus 0.05, 0.1 or 0.3 per cent phytase and 0.29 per cent available phosphorus plus 0.1 per cent phytase. In the second experiment diets providing available phosphorus levels of 0.32, 0.38 and 0.44 per cent and phytase levels of 0.5, 1.0 and 1.5 per cent (250, 500 and 750 *u*/kg) fed to four groups of chicks. They observed increased feed intake and feed conversion efficiency by increasing dietary available phosphorus but not with phytase in both the experiments.

Richter and Cyriaci (1994) studied the effect of phytase supplementation on 720 broilers in two 35-day trials. The birds were fed with diets containing plant components only (trial 1) or plant and animal components (trial 2) with 0.4 to 0.5 per cent total phosphorus supplemented with phytase at the rate of 300 and 750 *u*/kg of diet. Addition of phytase increased feed intake when compared to control in both the trials.

Kiiskinen *et al.* (1994) studied the effects of different intakes (250-1000 phytase units/g) of microbial phytase (*A. niger*) on the performance of broiler chicks and observed increase in feed intake by 3-9 per cent.

Biehl *et al.* (1995) reported that supplementation of both phytase (1200 *u*/kg) and 1,25-dihydroxy cholecalciferol (10 μg/kg) in soya protein diets deficient in zinc (13 mg/kg) improved feed intake in chicks than with either of these supplements alone.

Denbow *et al.* (1995) conducted an experiment with broiler chicken by supplementing phytase at the rate of 200, 400, 600, 800, 1000 and 1200 *u*/kg to semipurified basal diet formulated to contain 0.20, 0.27 or 0.34 per cent non-phytate phosphorus and found that phytase supplementation improved feed intake significantly ($P<0.01$) but feed gain ratio was not affected.

The influence of phytase on male broiler chicks fed with corn-soyabean meal diet containing 0.66 per cent calcium, 0.49 per cent phosphorus and 0.25 per cent phytate phosphorus was studied by Edwards and Roberson (1995). Phytase was added at the rate of 150, 300 or 600 *u*/kg of diet. They reported that the feed gain ratio was not improved by the addition of phytase.

Sebastian *et al.* (1995) added phytase at the rate of 600 *u*/kg to low phosphorus corn-soyabean diets and were given to broiler chicks for three weeks. They found that phytase supplementation overcame ($P \leq 0.05$) the depression of feed intake observed on low phosphorus diet.

In an experiment, broiler chickens were given maize-soyabean meal diets supplemented with phytase at levels of 250, 500, 750, 1000 or 1250 phytase units/g completely replacing dicalcium phosphate as phosphorus source showed no differences in feed conversion efficiency (Munaro *et al.*, 1996).

Microbial phytase added to diets with low phosphorus levels improved feed conversion significantly in broilers

(Simons and Versteegh, 1996). The levels of phytase had a positive effect on this trait.

Kwon *et al.* (1996) conducted an experiment with broilers fed on diets containing 60, 80 or 100 per cent of the required phosphorus supplemented with phytase at the rate of 500 FTU for a period of 21 days and reported that feed intake and feed gain ratio decreased as the phosphorus content of the diet decreased when supplemental phytase was not fed.

In a study with day-old male broilers fed diets containing 2.0, 2.7 or 3.4 g/kg of non-phytate phosphorus (total phosphorus 4.0, 5.1 or 5.8 g/kg) supplemented with phytase enzyme at levels of 200, 400, 600, 800, 1000 or 1200 *u*/kg for a period of 21 days, Kornegay *et al.* (1996) observed linearly increased ($P<0.01$) feed intake in phytase supplemented groups.

Phytase supplementation in broiler chickens fed corn-soyabean diet had no effect on feed-gain ratio (Sebastian *et al.*, 1996). However supplementation of enzyme overcame ($P<0.05$) the reduction of feed intake observed on low phosphorus diet.

Carlos and Edwards (1997) reported that the supplementation of phytase enzyme at the levels of 150, 300 or 600 FTU/kg of corn-soya basal diet containing 1 per cent calcium and 0.5 per cent total phosphorus significantly ($P<0.05$) increased feed gain ratio in broiler chicks.

The results of an experiment in broilers fed wheat-canola based diets containing 0.25 per cent and 0.35 per cent of available phosphorus supplemented with phytase at the levels of 500 and 1000 *u*/kg of diet indicated that phytase supplementation improved feed intake and feed efficiency only in diets containing 0.25 per cent available phosphorus (Nernberg *et al.*, 1997).

In order to study the influence of supplemental phytase on the performance of broilers, Sohail and Roland (1997) formulated diets to contain two levels of available phosphorus (0.225 and 0.325 per cent) and three levels of phytase (0, 300, 600 FTU). They observed a significant reduction in feed consumption and feed efficiency when available phosphorus was reduced from 0.325 per cent to 0.225 per cent. Phytase at 300 FTU reduced feed intake in groups fed with diets containing 0.225 per cent available phosphorus compared to control.

2.3 Tibial ash

Nelson *et al.* (1971) reported an increase in the percentage of bone ash in chicks fed with corn and soyabean meal diet containing 0.18 to 0.24 per cent natural phytate phosphorus supplemented with phytase at the rate of 1 to 8 g/kg of diet.

Male broilers fed maize-soyabean meal based diets containing 2.3 g of phytate phosphorus, 4.5 g of total phosphorus and 6 g/kg of calcium supplemented with phytase at levels from 0 to 800 *u*/kg and observed improved crude ash content of toe (Schoner *et al.*, 1992a).

In an experiment, broiler chickens were given sorghum-soyabean diets supplemented with or without phosphorus from calcium hydrogen phosphate and Natuphos[R]-5000 (phytase) from *A. niger* at the rate of 750 *u*/kg for a period of 18 days resulted in an increase in tibial ash (Farrell *et al.*, 1993).

When Lohmann broilers were given a basal diet from plant (A) or plant and animal sources (B), both diets contained 0.4 to 0.5 per cent total phosphorus supplemented with phytase at the levels of 300 and 700 *u*/kg resulted in an increase in crude ash content of tibia (Richter *et al.*, 1993a).

Vogt (1993) found that decreasing dietary phosphorus decreased bone mineralization in broilers. However, bone mineralization was improved by phytase supplementation.
In an attempt to find out the effect of supplementation of microbial phytase on the mineralization of the skeleton, Huyghebaert *et al*. (1993a) reported that increasing amounts of

microbial phytase addition increased bone mineralization (per cent ash in tibia) in a curvilinear fashion.

The addition of phytase at levels of 500 and 1000 *u*/kg of starter and finisher diets containing phosphorus 0.6, 1.1 and 1.6 g/kg (as mono calcium phosphate) in broilers fed for a period of 35 days had no effect on tibia strength (Richter, 1994).

Broz *et al.* (1994) conducted three experiments on broilers to study the effect of supplemental phytase on performance and phosphorus utilization in broiler chicken fed with low phosphorus diets and concluded that phytase supplementation improved tibial ash percentages.

Zyla and Koreleski (1994) reported that substitution of calcium phosphate for a preparation of phytase in rape seed oil meal based diet fed to broilers did not cause significant differences in tibia mineralization.

In an investigation with male broiler chickens, given semipurified basal diet formulated to contain 0.2, 0.27 or 0.34 per cent non-phytate phosphorus supplemented with phytase at the rate of 200, 400, 600, 800, 1000 and 1200 *u*/kg, Denbow *et al.* (1995) observed that phytase addition increased tibia and toe ash percentages.

Edwards and Roberson (1995) reported increase in bone ash when commercial phytase was added at the rate of 600 *u*/kg of corn-soyabean meal diet containing 0.66 per cent calcium, 0.49 per cent phosphorus and 0.25 per cent phytate phosphorus.

Sebastian *et al.* (1995) reported that phytase addition at a level of 600 *u*/kg of corn-soyabean diets low in phosphorus fed to broilers resulted in an increase in the ash content of both head and shaft portions of dry, fat-free tibial bone.

Phytase addition at the rate of 1200 *u*/kg diet containing phosphorus 0.43 g/100 g (non-phytate phosphorus 0.1 g/100 g) increased bone ash by 65 per cent (Biehl *et al.*, 1995).

In a trial, broilers were given low phosphorus diets supplemented with phytase at the rate of 500 FTU/kg. The results indicated that reduction in bone mineralization was ameliorated by phytase supplementation (Huyghebaert, 1996).

In an experiment on male broilers fed diets containing 2.0, 2.7 or 3.4 g/kg of non-phytate phosphorus (total phosphorus 4.0, 5.1 or 5.8 g/kg) supplemented with varying levels of phytase resulted in significant ($P<0.01$) increase in toe ash percentage (Kornegay *et al.*, 1996).

Carlos and Edwards (1997) reported that supplementation of phytase enzyme at the level of 150, 300 or 600 FTU/kg of corn-soya basal diet containing 1 per cent calcium and 0.5 per cent total phosphorus significantly ($P<0.05$) increased bone ash in male broiler chicks.

2.4 Serum calcium and phosphorus

Edwards (1993) reported that supplementation of either phytase or 1,25-dihydroxy cholecalciferol in broiler chickens did not have any effect on either plasma calcium or dialyzable phosphorus.

In three experiments broiler chickens were given low phosphorus diets (P 4.4, 4.5 and 5.2 g/kg) supplemented with fungal phytase (*A. niger*) at the rate of 0 or 500, 0, 125, 200 or 500 and 0, 125, 250 or 500 phytase units/kg of diet, Broz *et al.* (1994) found increased plasma inorganic phosphorus in groups fed low phosphorus diets supplemented with phytase compared to unsupplemented groups.

Edwards and Roberson (1995) observed that there were no treatment effects on plasma calcium and dialyzable phosphorus in male broiler chicks fed with soyabean meal basal diets containing 0.49 per cent phosphorus and 0.25 per cent phytate phosphorus supplemented with phytase at the levels of 150, 300 or 600 units/kg.

To assess the influence of microbial phytase supplementation on availability of minerals, Sebastian *et al.* (1995) conducted a trial in which broiler chicks were given low phosphorus corn-soyabean diet supplemented with 600 u of phytase/kg. They observed an increase in plasma phosphorus by 15.7 per cent, however calcium concentration was reduced ($P \leq 0.05$) by 34.5 per cent.

An experiment was conducted on male broilers fed corn-soyabean meal diets containing three levels of total dietary phosphorus 0.45, 0.55 and 0.65 per cent, supplemented with 5 µg/kg of 1,25-dihydroxy cholecalciferol, 600 *u*/kg of phytase or the combination of these. Both phytase and 1,25-dihydroxy cholecalciferol increased plasma phosphorus (Mitchell and Edwards, 1996a).

Munaro *et al.* (1996) in their experiment on broiler chickens fed a maize-soyabean meal basal diet supplemented with phytase at the rate of 250, 500, 750, 1000 or 1250 phytase units/g completely replacing dicalcium phosphate as phosphorus source showed increased plasma phosphorus level.

In a three week feeding trial with broiler chickens fed on maize-soyabean diets containing 0.46 (control) or 0.33 per cent (low) available phosphorus supplemented with Natuphos^{R}-5000 (phytase) at the level of 600 *u*/kg increased plasma phosphorus ($P \leq 0.03$) and copper ($P<0.02$) (Sebastian *et al.*, 1996).

2.5 Bio availability of calcium and phosphorus

Simons *et al.* (1990) reported that supplementation of microbial phytase to low phosphorus diets in broilers resulted in an increase in the availability of phosphorus to over 60 per cent. The amount of phosphorus in the droppings decreased by 50 per cent.

The results obtained from an experiment with male broilers fed a maize-soyabean meal based diet containing 2.3 g of phytate phosphorus, 6 g of calcium and 4.5 g/kg of total phosphorus supplemented with microbial phytase at levels of 0 to 800 *u*/kg indicated that phytase additions decreased phosphorus excretion by upto 50 per cent and improved ($P \leq 0.05$) retention of calcium and phosphorus (Schoner, 1992).

Schoner *et al.* (1992b) in their experiment on broilers fed diets supplemented with phytase resulted in 65 per cent utilization of total phosphorus and 50 per cent reduction of phosphorus excretion.

In a trial with broiler chickens fed low phosphorus diet (0.35 and 0.47 per cent non-phytate phosphorus) supplemented with Natuphos^{R}-5000 (commercial phytase), Huyghebaert *et al.*

(1993b) observed increased phosphorus metabolism by 12 to 14 per cent when phytase was supplemented at 500 *u*/kg of diet.

When Lohmann broilers were fed on a basal diet from plant (A) or plant and animal sources (B), both diets contained 0.4 to 0.5 per cent total phosphorus and supplemented with phytase at the levels of 300 and 700 *u*/kg, phosphorus excretion was decreased by 24 and 10 per cent respectively in diets A and B (Richter *et al.*, 1993a).

Phytase supplementation at levels of 125, 250 and 500 *u*/kg of maize-soyabean meal diet containing 3.5 g of total phosphorus, 2.3 g of phytate phosphorus and 6 g/kg of calcium resulted in an increase in the retention of calcium and phosphorus. Utilization of phosphorus and phytate phosphorus were also significantly improved (Schoner *et al.*, 1993a).

Farrell *et al.* (1993) reported 18 per cent improvement in phosphorus retention in broiler chickens fed sorghum-soyabean diets supplemented with phytase (Natuphos[R]-5000) at the level of 750 *u*/kg of diet for a period of 18 days.

Broilers offered low phosphorus diets supplemented with fungal phytase resulted in marked improvement in the availability of phosphorus (Broz *et al.*, 1994). They also observed significant reduction ($P<0.05$) of phosphorus concentration in the excreta.

Edwards and Roberson (1995) stated that phytase supplementation at 300 or 600 *u*/kg of diet for broilers increased the availability of phytate phosphorus in groups fed a diet containing 0.25 per cent phytate phosphorus.

Sebastian *et al.* (1995) studied the influence of phytase supplementation on the availability of minerals in broiler chickens and observed that phytase supplementation at 600 *u*/kg diet increased ($P \leq 0.05$) the relative retention of total phosphorus, calcium, copper and zinc by 24, 38, 78 and 225 per cent, respectively.

Different levels of phytase supplementation in broilers fed maize-soyabean meal diets containing 2.0, 2.7 or 3.4 g/kg of non-phytate phosphorus, 4.0, 5.1 or 5.8 g/kg of total phosphorus resulted in a linear increase ($P<0.01$) in the apparent retention (per cent of intake) or total amount (g) of retained calcium and phosphorus. However, there were a linear decrease ($P<0.01$) in the phosphorus excretion (g/kg DM intake) (Kornegay *et al*, 1996).

Huyghebaert (1996) observed decreased phosphorus excretion in broilers fed low phosphorus diets supplemented with phytase at the rate of 500 FTU/kg of diet.

In a trial with broilers fed maize, wheat and soyabean meal diets containing 0.62 or 0.79 per cent calcium and 0.59

per cent phosphorus supplemented with phytase at 600 units and above per kg of diet improved phosphorus utilization and retention (Windisch and Kirchgessner, 1996). They also found that calcium retention and utilization increased upto the highest level of phytase suplementation.

Carlos and Edwards (1997) in an experiment with broiler chicks fed corn-soya basal diet containing 1 per cent calcium and 0.5 per cent total phosphorus supplemented with phytase for a period of 16 days observed that the addition of increasing levels of phytase significantly ($P<0.05$) increased phosphorus retention.

Addition of phytase at a level of 1000 *u*/kg in broiler diet improved phytate digestibility to 62 per cent ($P<0.05$). Reduction of dietary available phosphorus from 0.45 to 0.35 per cent and to 0.25 per cent reduced total phosphorus excretion by 24 and 29 per cent respectively ($P>0.05$) (Nernberg *et al.*, 1997).

2.6 Processing yields and losses

In a 35-day feeding experiment 720 male and female Lohmann broilers were given plant-based feeds containing inorganic phosphorus at levels of 1350, 675, 338 and 0 mg/kg supplemented with phytase at the rate of 300 and 700 *u*/kg feed. When phosphorus was 0, slaughter weight decreased by upto 39 per cent. Medium phosphorus with phytase 300 or 700 *u*/kg increased slaughter weight by 17 and 23 per cent respectively (Richter *et al.*, 1993b).

2.7 Livability

Vogt (1992) conducted an experiment with caged male Lohmann broilers fed diets containing total phosphorus 7.1, 5.8 and 4.5 g/kg supplemented with phytase at the rate of 500, 750 or 1000 *u*/kg of feed. He observed that decreasing dietary phosphorus increased mortality.

In an experiment on 2880 day old broiler chicks fed maize-soyabean diets containing total phosphorus 3.5, phytate phosphorus 2.3 and calcium 6.0, 7.5 and 9 g/kg supplemented with 125, 250, 1500 *u*/kg of phytase. Control diets with calcium 6, 7.5 and 9 g/kg had 28, 56 and 58 per cent mortality respectively, which decreased significantly when phosphorus or phytase were supplemented (Schoner *et al.*, 1993b).

In a study, broilers were given diet with phosphorus content 5.2, 5.7 and 6.2 g/kg supplemented with phytase at levels of 200, 400, 800 and 1600 *u*/kg for 6 weeks, Vogt (1993) noted that decreasing dietary phosphorus increased mortality.

He also opined that increased mortality due to low phosphorus diet could be alleviated by phytase supplementation.

Richter and Cyriaci (1994) studied the influence of phytase supplementation in broilers. They were fed with two types of diet viz., plant based (trial 1) and plant and animal based (trial 2) with 0.4 to 0.5 per cent total phosphorus supplemented with phytase at levels of 300 and 750 *u*/kg. They reported that increased losses caused by phosphorus deficiency were lessened by phytase supplementation.

The effect of phytase supplementation in soyabean based broiler diet was studied by Denbow *et al.* (1995). They added phytase at levels of 200, 400, 600, 800, 1000 and 1200 *u*/kg of semipurified basal diet formulated to contain 0.20, 0.27 or 0.34 per cent non-phytate phosphorus. They found high mortality (35 to 45%) in 0.20 and 0.27 per cent non-phytate phosphorus diets without phytase, but this declined to normal levels with the addition of 200 to 400 u phytase/kg diet.

Kornegay *et al.* (1996) observed increased mortality in broiler chicks fed with diets containing 2.0 g/kg of non-phytate phosphorus and the condition was corrected by phytase addition.

In an experiment on broiler chicks fed diets formulated to contain 0.225 per cent and 0.325 per cent of available phosphorus supplemented with phytase at levels of 300 and 600 FTU showed that phytase 300 FTU had a much greater influence on livability in broilers fed 0.225 per cent available phosphorus than in broilers fed with 0.325 per cent available phosphorus (Sohail and Roland, 1997).

2.8 Economics

Newman (1993) stated that 1 kg of phytase enzyme per tonne of feed can potentially replace 6-7 kg of mono calcium phosphate, thus it reduces the supplementation cost of additional phosphorus.

In a trial with broilers fed diets containing inorganic phosphorus 0.6, 1.1 and 1.6 g/kg supplemented with phytase at levels of 500 and 1000 *u*/kg of diet Richter *et al.* (1994) concluded that phytase was more expensive than inorganic phosphorus.

Materials and Methods

3. MATERIALS AND METHODS

An experiment was conducted in the Department of Poultry Science, College of Veterinary and Animal Sciences, Mannuthy to evaluate the influence of phytase on the phosphorus utilization and subsequent performance in broilers.

3.1 Experiment materials

3.1.1 Experimental birds

Two hundred and sixteen, day-old straight-run broiler chicks (Varna) procured from the Revolving fund hatchery of the Centre for Advanced Studies in Poultry Science, Kerala Agricultural University, Mannuthy formed the experimental material.

3.1.2 Experimental rations

Two types of rations viz., standard broiler ration and broiler ration with low levels of available phosphorus were used in this study. The standard broiler ration (SBR) was formulated as per BIS (1992) specifications. Low available phosphorus broiler rations (LAPBR) were formulated keeping the level of available phosphorus at 0.3 and 0.4 per cent whereas, 0.5 per cent in the standard broiler ration. The level of all other nutrients were similar to that of SBR. Feed ingredients used for the formulation of the experimental diets were yellow maize, groundnut cake (expeller), gingelly oil cake, unsalted dried fish and rice polish. Initially, the ration with 0.3 per cent available phosphorus was formulated. By the addition of appropriate levels of dicalcium phosphate (DCP) to this ration, diets with 0.4 per cent and 0.5 per cent available phosphorus were formulated. Broiler starter diets were fed upto six weeks of age and then switched over to broiler finisher diets till the end of the experiment.

The ingredient composition and the chemical composition of the experimental rations 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. The enzyme was added at a level of 750 units per kg of diet.

Table 1. Percentage ingredient composition of experimental rations

Sl. No.	Ingredients	Standard broiler ration (SBR)		Low available phosphorus broiler ration (LAPBR)			
		Starter	Finisher	Starter		Finisher	
				0.3%	0.4%	0.3%	0.4%
1.	Yellow maize	54.10	53.65	54.35	54.35	53.40	53.65
2.	Groundnut cake (exp)	29.00	26.00	29.00	29.00	26.00	26.00
3.	Gingelly oil cake	8.00	0.00	8.00	8.00	0.00	0.00
4.	Unsalted dried fish	7.00	7.00	7.00	7.00	7.50	7.25
5.	Rice polish	0.00	11.00	0.00	0.00	11.00	11.00
6.	Shell grit	0.25	0.75	1.00	0.50	1.50	1.00
7.	Dicalcium phosphate	1.00	1.00	0.00	0.50	0.00	0.50
8.	Common salt	0.25	0.25	0.25	0.25	0.25	0.25
9.	Vitamin mixture	0.025	0.025	0.025	0.025	0.025	0.025
10.	Lysine Hydrochloride	0.195	0.145	0.195	0.195	0.145	0.145
11.	Coccidiostat	0.050	0.050	0.050	0.050	0.050	0.050
12.	Trace mineral mixture	0.130	0.130	0.130	0.130	0.130	0.130
	Total	100	100	100	100	100	100

Vitamin mixture composition:

Each gram contains : Vitamin A 40,000 IU, Vitamin B2 20 mg and Vitamin D3 5000 IU

Coccidiostat composition:

Each gram contains : Dinitro-ortho-toluamide 250 mg and Ethopabate 16 mg

Trace mineral mixture composition:

Composition/100 kg of feed : Manganese sulphate 30 gm, Copper sulphate 10 gm, Zinc oxide 20 gm, Ferrous sulphate 70 gm, and Potassium iodide 100 mg

Table 2. Percentage chemical composition of experimental rations (on dry matter basis)

Sl. No.	Nutrients	Standard broiler ration (SBR)		Low available phosphorus broiler ration (LAPBR)			
		Starter	Finisher	Starter		Finisher	
				0.3%	0.4%	0.3%	0.4%
	Analysed values*						
1.	Moisture	9.7	9.8	9.6	9.7	9.9	9.8
2.	Crude protein	23.5	20.2	23.4	23.5	20.2	20.3
3.	Ether extract	6.6	6.6	6.5	6.6	6.7	6.7
4.	Crude fibre	4.5	5.2	4.6	4.5	5.2	5.2
5.	NFE	53.8	56.3	53.8	53.8	56.2	56.1
6.	Total ash	11.6	11.7	11.7	11.6	11.7	11.7
7.	Acid insoluble ash	2.8	2.8	2.8	2.9	2.8	2.7
8.	Calcium	1.30	1.28	1.33	1.31	1.25	1.27
9.	Total phosphorus	0.83	0.85	0.62	0.73	0.64	0.75
10.	Available phosphorus	0.51	0.50	0.30	0.41	0.31	0.40
	Calculated values						
11.	ME (KCal/kg)	2835	2913	2843	2843	2917	2914
12.	Lysine	1.16	0.99	1.16	1.16	1.00	0.99
13.	Methionine	0.70	0.57	0.70	0.70	0.58	0.57
14.	Manganese (mg/kg)	96	97	96	96	97	97

* Average of seven samples

3.2 Experimental methods

3.2.1 Housing of birds

The experimental house, feeders, waterers and other equipments were cleaned thoroughly and disinfected prior to housing the chicks. The straight-run day-old-chicks were weighed, vaccinated and wing banded.

3.2.2 Experimental design

The chicks were randomly divided into eighteen groups of twelve chicks each. These groups were allotted randomly to six treatments viz., T1, T2, T3, T4, T5 and T6 with three replications in each treatment. The birds in each treatment were assigned to each of the six rations viz., SBR, LAPBR (0.3%), LAPBR (0.4%) and SBR with 750 units of phytase enzyme per kg of diet, LAPBR (0.3%) with 750 units enzyme per kg of diet and LAPBR (0.4%) with 750 units enzyme per kg of diet. The details of treatment particulars are presented in Table 3.

3.2.3 Management

Feed and water were provided *ad libitum* throughout the experiment and the birds were maintained under deep litter system of management. Standard managemental procedures were

Table 3. Distribution of the different dietary treatments

Treat-ment	Repli-cation	No. of birds	Diet	Enzyme treatment	Level of inclusion (u)
T1	R1	12	SBR(0.5%)	-	-
	R2	12	SBR(0.5%)	-	-
	R3	12	SBR(0.5%)	-	-
T2	R1	12	LAPBR(0.3%)	-	-
	R2	12	LAPBR(0.3%)	-	-
	R3	12	LAPBR(0.3%)	-	-
T3	R1	12	LAPBR(0.4%)	-	-
	R2	12	LAPBR(0.4%)	-	-
	R3	12	LAPBR(0.4%)	-	-
T4	R1	12	SBR(0.5%)	Phytase	750
	R2	12	SBR(0.5%)	Phytase	750
	R3	12	SBR(0.5%)	Phytase	750
T5	R1	12	LAPBR(0.3%)	Phytase	750
	R2	12	LAPBR(0.3%)	Phytase	750
	R3	12	LAPBR(0.3%)	Phytase	750
T6	R1	12	LAPBR(0.4%)	Phytase	750
	R2	12	LAPBR(0.4%)	Phytase	750
	R3	12	LAPBR(0.4%)	Phytase	750

adopted during the entire experimental period. The experiment was conducted for a period of eight weeks.

3.2.4 Climatic parameters

The maximum and minimum temperature were recorded at 8 A.M., on all days throughout the experimental period. The wet and dry bulb thermometer readings were taken at 8 A.M. and 2 P.M. daily. From these data, weekly mean maximum and minimum temperature and per cent relative humidity were arrived at.

3.2.5 Body weight

The body weight of individual birds was recorded at fortnightly intervals from day-old to study the pattern of body weight gain under different feeding regimes.

3.2.6 Feed consumption

Feed intake of the birds was recorded replicate-wise at the end of each week. From these data, the average feed intake per bird was calculated for various treatment groups.

3.2.7 Feed efficiency

Feed conversion efficiency (kg of feed consumed/kg body weight) was calculated based on the data on body weight and feed intake.

3.2.8 Metabolism trial

At the end of the experiment, a metabolic trial was undertaken using two birds, randomly selected from each replicate and housed in individual cages. Water was provided *ad libitum*. Excreta samples were collected over 24 hour period using total collection method as described by Summers *et al.* (1976). The droppings were weighed and samples were taken, oven dried at 70°C for two hours and ground prior to calcium and phosphorus estimation. The total amount of feed consumed and excreta voided were also recorded.

3.2.9 Chemical analysis

The chemical composition of the different experimental rations was determined as per the procedure described by AOAC (1990). Available phosphorus was estimated as per the procedure described by Bureau of Indian standards (BIS, 1992). The total phosphorus and calcium content of different rations and excreta were analysed as per the procedure of AOAC (1990).

The bioavailability of calcium and phosphorus was calculated utilizing the data on tibial ash content.

3.2.10 Tibial ash

At the end of sixth week, one male and one female bird from each replicate were randomly selected and sacrificed to collect tibia as per the method described by Kalango and Ademosun (1973). The birds were fasted overnight, 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 weighed and the fat-free bone ash content of the tibia were estimated as per the procedure of AOAC (1975). The weight of tibial ash was expressed as percentage of the weight of the dried tibia. Tibial ash was also determined at the end of eighth week from the tibia obtained from the birds utilized for slaughter studies.

3.2.11 Serum calcium and inorganic phosphorus

Blood samples of two birds from each replicate were collected at the end of the sixth and eighth week of age by severing the jugular vein for the estimation of serum calcium and inorganic phosphorus. The estimation of serum calcium was done using the kit (OCPC method), supplied by M/s AGAPPE Diagnostics, F-4, Shailesh Industrial Complex, Valiv post, Vasai (E), Thane, Maharashtra-401 208, India. The serum inorganic phosphorus was estimated using the kit (molybdate method) supplied by M/s Diagnostic and Biochemicals Innovations, Thrissur, Kerala, India.

3.2.12 Processing yields and losses

At the end of the experiment, one male and one female bird from each replicate were randomly selected and sacrificed to study the processing yields. Percentages of dressed yield, giblet yield and ready-to-cook yield were calculated from the data.

3.2.13 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.

3.2.14 Cost-benefit analysis

Cost of feed, cost of enzyme, live weight produced and quantity of feed consumed by birds in each treatment was calculated. From these data the cost-benefit analysis was worked out.

3.2.15 Statistical analysis

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

Results

4. RESULTS

The results of an experiment conducted to evaluate the effect of phytase supplementation on phosphorus utilization and subsequent performance in commercial broilers fed with diets containing low available phosphorus are presented in this chapter.

4.1 Climatic parameters

The mean maximum and minimum temperatures and per cent relative humidity during different weeks of this experiment (May to July 1998) are presented in Table 4. During the experimental period of eight weeks, the mean maximum temperature ranged from 27.20 to 34.42°C and the minimum temperature from 23.58 to 27.30°C. The per cent relative humidity in the morning varied from 86.43 to 96.14, while in the afternoon it ranged from 58.14 to 90.29. In general, the variation in the climatic profile was very small between different weeks of the experimental period.

4.2 Body weight

Data on mean body weight at fortnightly intervals as influenced by different treatments viz., standard broiler ration (T1), broiler ration with 0.3 per cent available

Table 4 Mean weekly meteorological data during the experimental period (12 May to 6 July 1998)

Period (weeks)	Temperature (°C)		Relative humidity	
	Maximum	Minimum	8 A.M.	2 P.M.
1	32.09	24.90	92.57	72.14
2	33.39	26.84	92.86	63.57
3	34.42	27.30	86.43	58.14
4	31.82	24.99	89.29	69.86
5	30.09	24.69	94.00	80.00
6	29.12	23.62	95.57	78.43
7	27.20	23.68	95.43	90.29
8	28.27	23.58	96.14	81.29
Mean	30.80	24.95	92.79	74.22
SE	±0.90	±0.51	±1.20	±3.67

phosphorus (AP) (T2), broiler ration with 0.4 per cent AP (T3), standard broiler ration with 750 units of phytase per kg of feed (T4), broiler ration with 0.3 per cent AP supplemented with 750 units of phytase per kg of feed (T5) and broiler ration with 0.4 per cent AP supplemented with 750 units of phytase per kg of feed are charted out in Table 5. It was evident from the table that day-old body weight of chicks among the different treatment groups was more or less uniform. Statistical analysis of the mean body weight data also confirmed this trend.

At second week of age higher body weight was noted among the birds fed with diets containing 0.3 per cent AP with 750 units of phytase (T5), whereas lower body weight was noted with groups fed a diet containing 0.3 per cent AP without enzyme (T2). Statistical analysis of the second week body weight data (Table 6) showed a significant ($P<0.01$) difference among various treatments. Statistically higher body weight was observed with all groups fed diets supplemented with enzyme. Significantly higher body weight was also observed with birds fed a standard diet without enzyme.

However, second week body weight was significantly lower for birds fed diets containing 0.3 and 0.4 per cent AP without phytase (T2 and T3).

Table 5. Influence of phytase supplementation on fortnightly body weight (g)

Treatments		Age in weeks				
		0	2	4	6	8
T1	R1	46.77	201.33	700.83	1472.00	2050.00
	R2	45.33	202.50	703.75	1490.00	2038.88
	R3	47.17	204.19	705.42	1480.00	2040.00
	Mean	46.42 a	202.67 a	703.33 b	1480.67 a	2042.97 a
	SE	±0.56	±0.83	±1.34	±5.21	±3.54
T2	R1	46.92	194.70	610.50	1311.60	1900.00
	R2	47.23	191.75	602.10	1308.00	1909.00
	R3	47.08	192.80	609.58	1303.30	1911.10
	Mean	47.08 a	193.08 c	607.39 e	1307.63 c	1906.70 c
	SE	±0.09	±0.87	±2.66	±2.40	±3.41
T3	R1	47.25	199.70	656.70	1403.00	1980.00
	R2	47.53	197.30	657.27	1375.50	1978.00
	R3	47.50	197.50	659.17	1390.00	1989.00
	Mean	47.43 a	198.17 b	657.71 c	1389.50 b	1982.33 b
	SE	±0.09	±0.77	±0.75	±7.95	±3.39
T4	R1	47.83	203.67	714.80	1440.00	2056.00
	R2	47.00	201.67	713.42	1450.00	2030.00
	R3	46.50	204.20	711.70	1472.00	2030.00
	Mean	47.11 a	203.18 a	713.31 a	1454.00 a	2038.64 a
	SE	±0.39	±0.77	±0.90	±9.46	±8.68
T5	R1	45.58	203.40	651.23	1370.00	1950.00
	R2	46.83	205.90	649.17	1441.00	1970.00
	R3	47.83	202.50	625.20	1405.00	1975.00
	Mean	46.75 a	203.93 a	650.87 d	1405.33 b	1965.00 b
	SE	±0.65	±1.02	±0.90	±0.50	±7.65
T6	R1	45.33	202.50	701.30	1430.00	2030.00
	R2	46.33	203.17	704.42	1451.00	2040.00
	R3	47.08	201.67	702.42	1470.00	2020.00
	Mean	46.25 a	202.45 a	702.71 b	1450.33 a	2030.00 a
	SE	±0.51	±0.43	±0.91	±11.57	±5.78
	C.D.	-	2.466	4.333	34.19	17.89

Means bearing the same superscript within the same column do not differ significantly (P<0.01)

Table 6. Influence of phytase supplementation on fortnightly body weight - ANOVA

Week	Source	d.f.	SS	MSS	F value
0	Treatment	5	5.023	1.005	1.680 NS
	Error	12	7.174	0.598	
	Total	17	12.197		
2	Treatment	5	263.686	52.737	27.456 **
	Error	12	23.050	1.921	
	Total	17	286.736		
4	Treatment	5	25362.690	5072.538	854.909 **
	Error	12	71.201	5.933	
	Total	17	25433.891		
6	Treatment	5	58055.339	11611.068	31.430 **
	Error	12	4433.146	369.429	
	Total	17	62488.485		
8	Treatment	5	42860.358	8572.072	84.755 **
	Error	12	1213.680	101.140	
	Total	17	44074.138		

** Significant (P<0.01)
NS Not significant

Fourth week body weight data (Table 5) indicated that it was higher (713.31 g) with birds fed a standard broiler diet supplemented with phytase (T4) whereas the group fed a diet containing 0.3 per cent AP without phytase (T2) registered a lower body weight (607.39 g). Statistical analysis of the fourth week body weight data (Table 6) showed significant (P<0.01) differences among different treatments. In general enzyme supplemented groups had significantly (P<0.01) higher body weight compared to their counterpart without enzyme.

Sixth week mean body weight (Table 5) showed that it was higher (1480.67 g) in groups fed a standard broiler diet without enzyme (T1) whereas it was lower (1307.63 g) with group received a diet with 0.3 per cent available phosphorus (T2). All other groups maintained body weights in between these two treatments. Statistical analysis of the sixth week mean body weight data (Table 6) indicated that there were significant (P<0.01) differences among different treatment groups. It was significantly (P<0.01) higher with birds fed a standard broiler diet without any added enzyme and was statistically (P<0.01) comparable with groups fed a standard broiler diet and a diet having 0.4 per cent available phosphorus both supplemented with 750 units of phytase.

Birds maintained on a diet having an AP level of 0.3 per cent without the addition of enzyme registered significantly

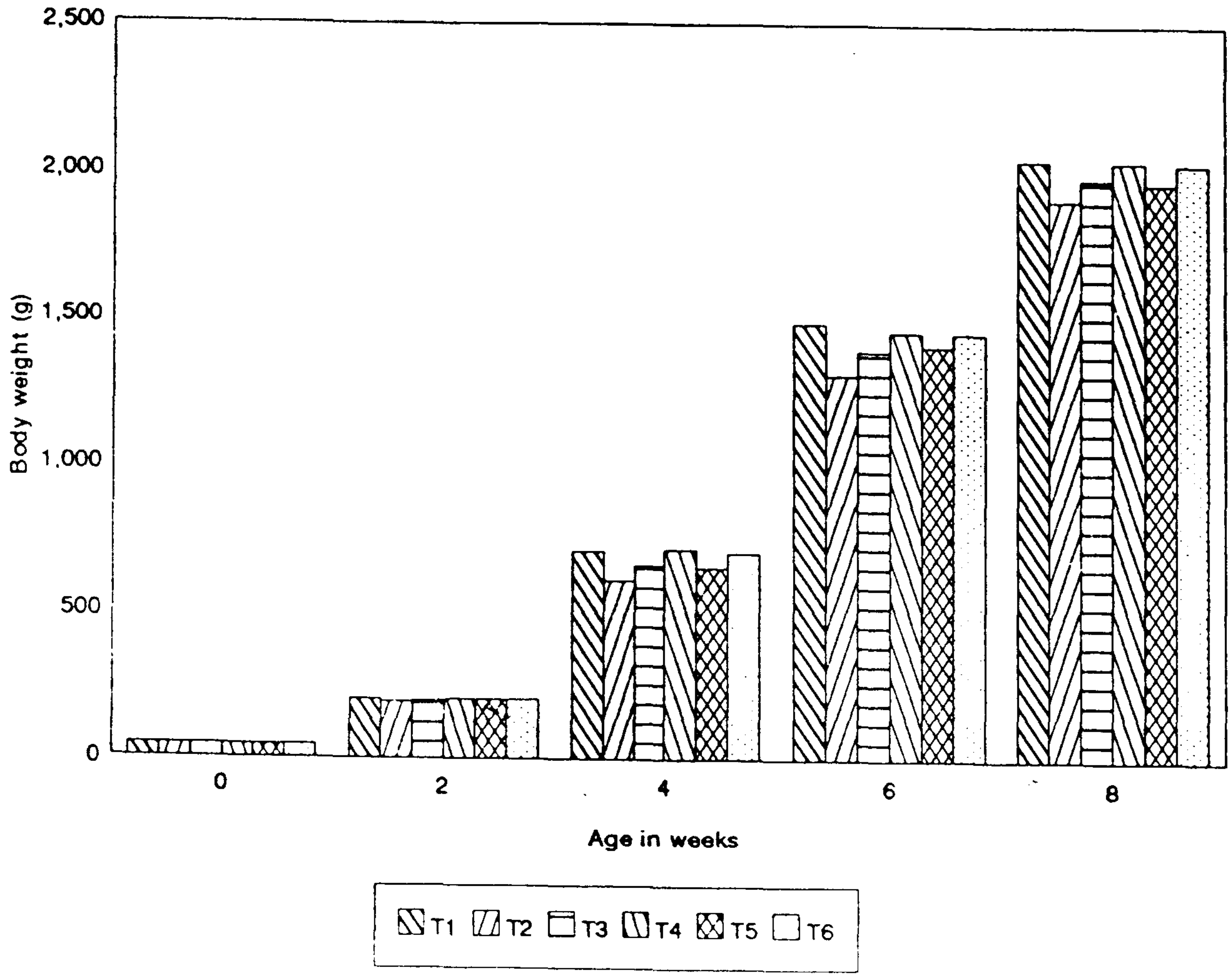
(P<0.01) lower body weight than all other groups. However, birds reared on a diet containing 0.4 per cent AP without enzyme and those on a diet with 0.3 per cent AP plus 750 units of phytase were statistically comparable and were significantly different from all other groups.

Eighth week mean body weight for the treatments T1, T2, T3, T4, T5 and T6 were 2042.97, 1906.70, 1982.33, 2038.64, 1965.00 and 2030.00 g respectively. Eighth week mean body weight data of different treatment groups given in Table 5 showed that the trend was very much similar to sixth week body weight of various treatments. Significantly (P<0.01) higher eighth week body weight was noted with those fed a standard broiler diet without enzyme (T1) and was statistically comparable with birds fed a standard broiler diet and those fed a diet having 0.4 per cent AP both supplemented with enzyme (T4 and T6).

Similarly eighth week body weight was significantly lower with birds offered a diet containing 0.3 per cent AP without enzyme. Statistically comparable body weights were obtained between groups fed a diet having an AP of 0.4 per cent without enzyme (T3) and those maintained on a diet with an AP of 0.3 per cent supplemented with enzyme (T5).

Mean fortnightly body weight of broiler chicks as influenced by phytase supplementation is depicted in Fig.1.

Fig.1 FORTNIGHTLY BODY WEIGHT AS INFLUENCED BY PHYTASE SUPPLEMENTATION



4.3 Body weight gain

The mean fortnightly body weight gain of chicks maintained on different dietary regimen during the experimental period is shown in Table 7.

The gain in weight when the birds attained two weeks of age was 156.25, 146.01, 150.31, 156.07, 157.19 and 156.20 g for the treatments T1, T2, T3, T4, T5 and T6 respectively.

Statistical analysis of the mean body weight gain data (Table 8) at two weeks revealed that it was significantly (P<0.01) more in birds offered a standard broiler diet (T1) and all other diets supplemented with phytase (T4, T5 and T6). However, it was significantly (P<0.01) lower with birds fed a diet containing 0.3 per cent AP without enzyme (T2). Though body weight gain was significantly better in group fed a diet having 0.4 per cent AP without phytase (T3), it was different from all other groups.

A perusal of the body weight gain data after four weeks of age (Table 7) revealed that it was numerically higher (510.13 g) in birds fed a standard broiler diet supplemented with phytase (T4) and was lower (414.31 g) in birds offered a diet having 0.3 per cent AP without phytase (T2). All other groups were intermediate with respect to gain in weight.

Table 7. Influence of phytase supplementation on fortnightly and cumulative body weight gain (g)

Treatments		Age in weeks				Cumulative body weight gain (g)	
		2	4	6	8	Sixth week	Eighth week
T1	R1	154.56	499.50	771.17	578.00	1425.23	2003.23
	R2	157.17	501.25	786.25	549.00	1444.67	1993.55
	R3	157.02	501.23	774.58	560.00	1433.43	1992.83
		a	b	a	a	a	a
	Mean	156.25	500.66	777.33	562.33	1434.44	1996.54
	SE	±0.85	±0.58	±4.57	±8.46	±5.64	±3.36
T2	R1	147.78	415.80	701.10	588.40	1264.68	1853.08
	R2	144.52	410.35	705.90	601.00	1260.77	1861.77
	R3	145.72	416.78	692.72	608.80	1256.22	1864.92
		c	e	c	a	c	d
	Mean	146.01	414.31	699.91	599.40	1260.56	1874.26
	SE	±0.95	±2.00	±3.86	±5.95	±2.45	±3.31
T3	R1	151.45	457.00	746.30	577.00	1354.75	1931.75
	R2	149.47	459.97	718.23	602.28	1327.67	1929.95
	R3	150.00	461.67	730.83	599.00	1342.50	1941.39
		b	c	bc	a	b	bc
	Mean	150.31	459.55	731.79	592.76	1341.64	1934.36
	SE	±0.59	±1.36	±8.13	±7.95	±7.84	±3.56
T4	R1	155.84	511.13	725.20	615.55	1392.17	2007.72
	R2	154.67	511.75	736.58	580.00	1403.00	1983.00
	R3	157.70	507.50	760.30	558.00	1425.50	1983.50
		a	a	b	a	a	ab
	Mean	156.07	510.13	740.69	584.52	1406.89	1991.41
	SE	±0.88	±1.33	±10.35	±16.79	±9.83	±8.17
T5	R1	157.82	447.83	718.77	580.00	1324.42	1904.42
	R2	159.07	443.27	791.83	529.00	1394.17	1923.17
	R3	154.67	449.70	752.80	570.00	1357.17	1927.17
		a	d	ab	a	b	cd
	Mean	157.19	446.93	754.47	559.67	1358.59	1918.25
	SE	±1.31	±1.91	±21.13	±15.62	±20.17	±7.02
T6	R1	157.17	498.80	728.70	600.00	1384.67	1984.67
	R2	156.84	501.25	746.58	589.00	1404.67	1993.67
	R3	154.59	500.75	767.58	550.00	1422.92	1972.92
		a	b	ab	a	a	ab
	Mean	156.20	500.27	747.62	579.67	1404.09	1983.75
	SE	±0.81	±0.75	±11.25	±15.18	±11.06	±6.01
	C.D.	2.846	4.387	35.14	-	31.5069	57.4358

Means bearing the same superscript within the same column do notdiffer significantly (P<0.01)

Table 8. Influence of phytase supplementation on fortnightly body weight gain - ANOVA

Week	Source	d.f.	SS	MSS	F value
2	Treatment	5	303.602	60.720	23.718 **
	Error	12	30.722	2.560	
	Total	17	334.324		
4	Treatment	5	21556.796	4311.359	709.047 **
	Error	12	72.966	6.081	
	Total	17	21629.762		
6	Treatment	5	9939.930	1987.986	5.096 **
	Error	12	4681.284	390.107	
	Total	17	14621.214		
8	Treatment	5	3854.362	770.872	1.668 NS
	Error	12	5546.466	462.206	
	Total	17	9400.828		

** Significant (P<0.01)
NS Not significant

Table 9. Influence of phytase supplementation on cumulative body weight gain - ANOVA

Source	d.f.	SS		MSS		F value	
		Cumulative body weight gain		Cumulative body weight gain		Cumulative body weight gain	
		6th wk	8th wk	6th wk	8th wk	6th wk	8th wk
Treatment	5	59247.31	36103.00	11849.46	7220.60	37.80**	83.13**
Error	12	3761.71	1042.33	313.48	86.86		
Total	17	63009.02	37145.33				

** Significant (P<0.01)

Statistical analysis of the fourth week mean body weight gain data also confirmed this trend. Weight gain was significantly (P<0.01) more in birds offered a standard broiler diet supplemented with enzyme (T4) and was significantly (P<0.01) lower in birds fed a diet having 0.3 per cent AP without enzyme (T2). Body weight gain was statistically comparable between the treatments T1 and T6 at fourth week of age.

. Fourth week body weight gain was significantly different among the treatments T2, T3 and T5. It was significantly better with T3, inferior with T2 and medium with T5.

Sixth week mean body weight gain data as influenced by phytase supplementation presented in Table 7 indicated that numerical differences existed between various treatments employed in this experiment. It was numerically higher (777.33 g) among birds offered a standard broiler diet without enzyme (T1) and was lower (699.91 g) in birds maintained on a diet having an AP level of 0.3 per cent without enzyme (T2). All other groups gained weight in between these two values. When the magnitude of difference in weight gain between the treatments were tested statistically, it was revealed that sixth week body weight gain was significantly (P<0.01) different among various treatments. It was significantly (P<0.01) higher in birds maintained on a standard broiler diet without added enzyme (T1) and was statistically comparable with groups fed a diet containing 0.3 and 0.4 per cent AP supplemented with 750 units of phytase per kg of diet (T5 and T6). Birds fed a diet containing 0.3 per cent AP without enzyme (T2) gained significantly ($P<0.01$) lower weight and was significantly on par with the group fed with a diet having 0.4 per cent AP without enzyme (T3).

Supplementation of enzyme to standard broiler diet failed to improve weight gain in comparison to that of standard diet without enzyme. However, the three treatment groups fed with diets supplemented with phytase (standard broiler diet and diets with 0.3 and 0.4 per cent AP supplemented with phytase) gained statistically comparable weights at sixth week of age.

Data on the influence of phytase supplementation on eighth week body weight gain, presented in Table 7 pointed out that a maximum gain of 599.40 g was noted with birds offered a diet having 0.3 per cent AP without enzyme (T2) and a minimum gain of 559.67 g with birds offered a diet having the same level of AP supplemented with enzyme (T5). The gain in weight of all other treatments were intermediary. Statistical analysis of the eighth week body weight gain data (Table 8) showed that no significant differences existed between treatments.

The mean body weight gain for the period from 0-6 weeks and 0-8 weeks as influenced by supplementation of phytase are presented in Table 7. The weight gain at sixth week of age was 1434.44, 1260.56, 1341.64, 1406.89, 1358.59 and 1404.09 g for the treatment groups T1, T2, T3, T4, T5 and T6 respectively. Highest gain in weight (1434.44 g) was noticed with birds offered a standard broiler diet without enzyme (T1) and lowest (1260.56 g) in the group fed a diet with 0.3 per cent AP without phytase (T2). Statistical analysis of the data showed that the difference in weight gain among the treatment groups was statistically significant (Table 9). Significantly ($P<0.01$) higher weight gain was noted with groups fed a standard diet without phytase (T1) and it was statistically comparable with groups fed a standard diet with added enzyme (T4) and those offered a diet having an AP content of 0.4 per cent plus phytase (T6). Significantly lower weight gain was observed in the group fed a diet containing 0.3 per cent AP without enzyme (T2) and was different from all other groups. Birds fed a diet containing 0.4 per cent AP without phytase (T3) and those fed a diet having 0.3 per cent AP with phytase gained (T5) statistically comparable weight and were different from other groups.

Cumulative weight gain from 0-8 weeks presented in Table 7 showed that the trend in gain among the different treatment groups were comparable with 0-6 weeks gain. Weight gain at 0-8 weeks for the treatments T1, T2, T3, T4, T5 and T6 were 1996.54, 1874.26, 1934.36, 1991.41, 1918.25 and 1983.75 g

Fig.2 FORTNIGHTLY BODY WEIGHT GAIN AS INFLUENCED BY PHYTASE SUPPLEMENTATION

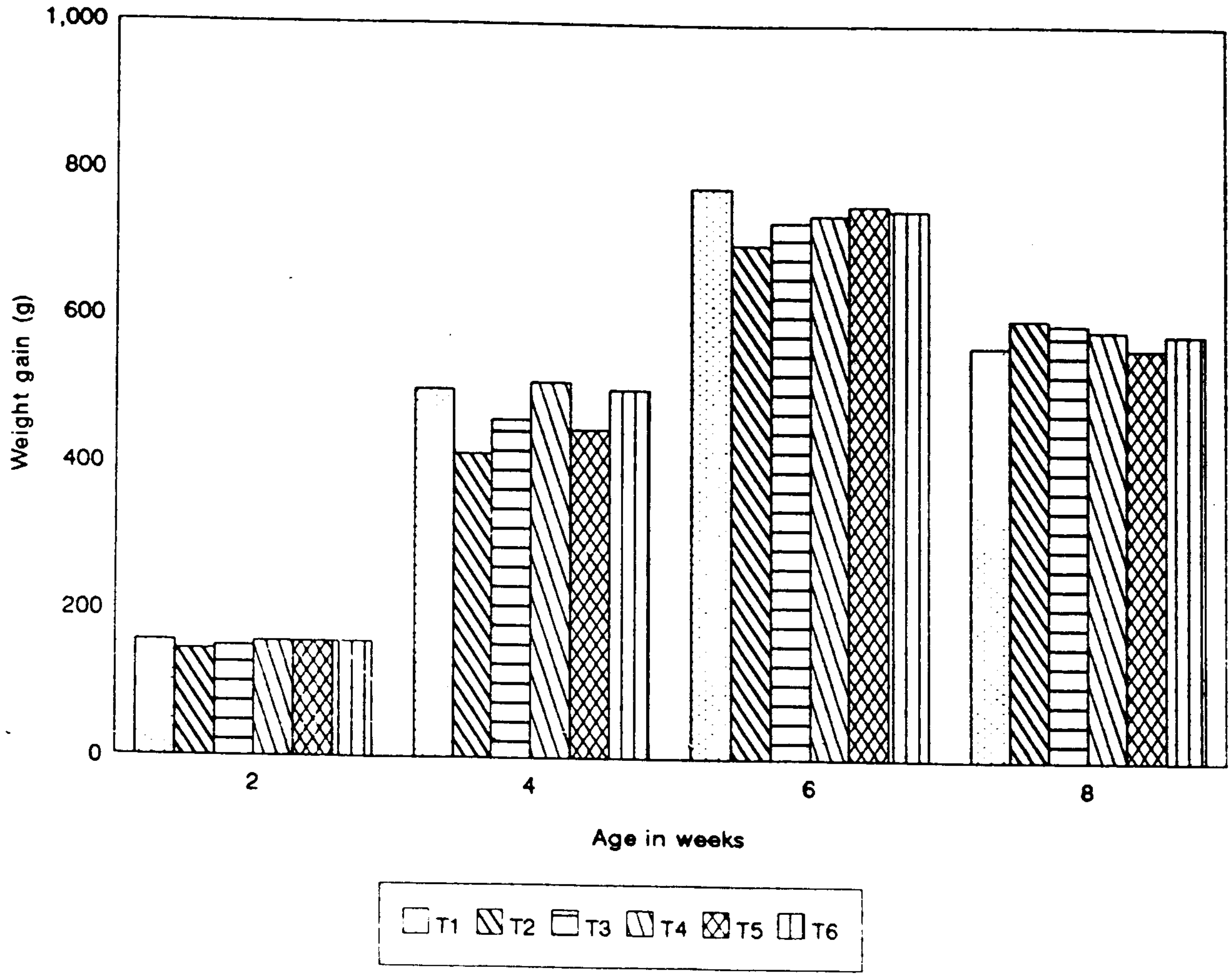
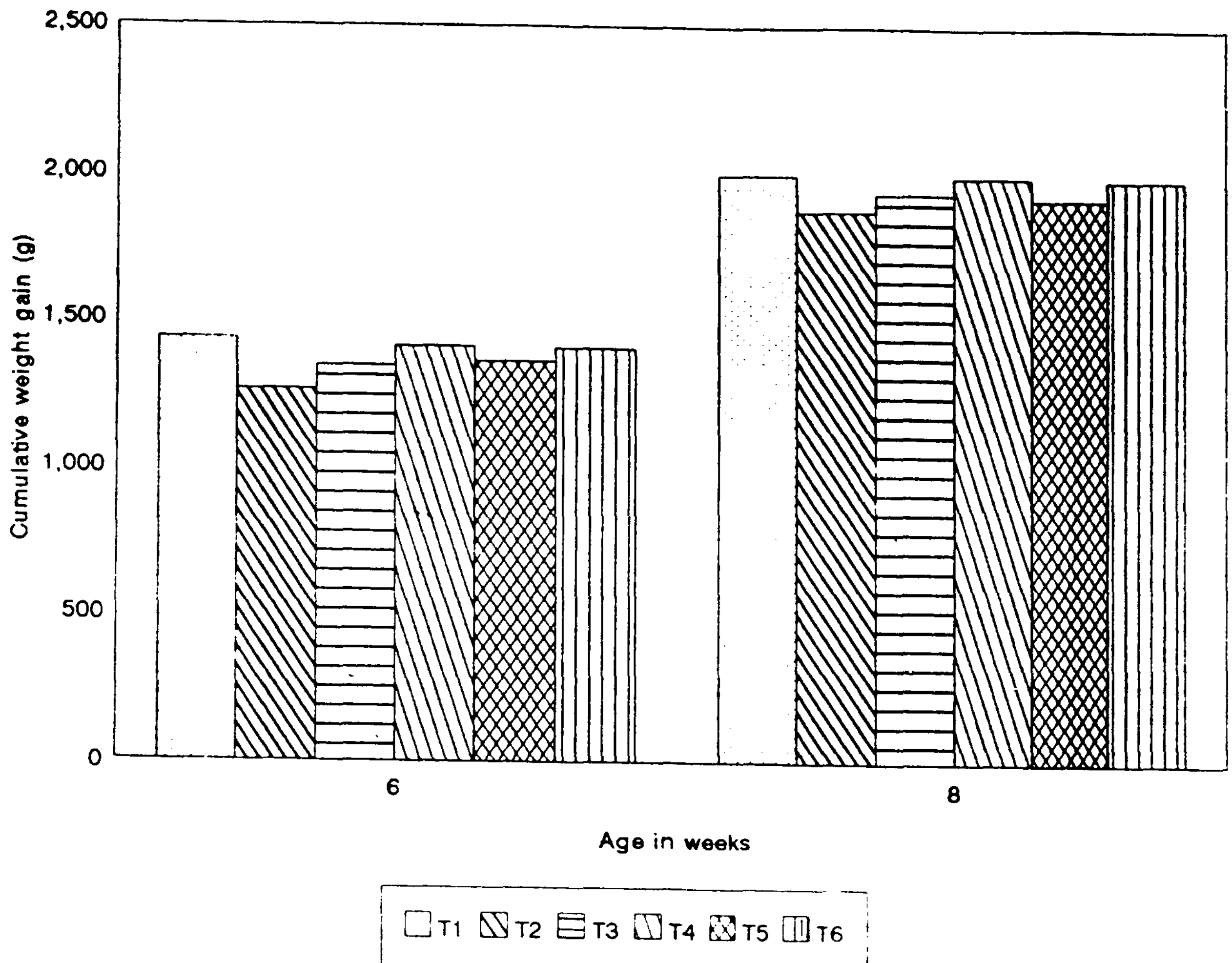


Fig.3 CUMULATIVE BODY WEIGHT GAIN AT SIXTH AND EIGHTH WEEKS OF AGE AS INFLUENCED BY PHYTASE SUPPLEMENTATION



respectively. Maximum gain (1996.54 g) was obtained for the birds fed a standard diet without enzyme (T1) and the lowest gain in the group fed a diet containing 0.3 per cent AP without phytase (T2). Statistical analysis of the 0-8 weeks weight gain data showed that the variations in gain among the different treatments were statistically significant (P<0.01). Significantly (P<0.01) higher gain (1996.54 g) was noticed with birds fed a standard broiler diet without phytase (T1) and were statistically comparable with T4 (1991.41 g) and T6 (1983.75 g). Significantly (P<0.01) lower 0-8 week weight gain was found with birds fed a diet containing 0.3 per cent AP without phytase (T2) and these values were comparable with those fed a diet having 0.3 per cent AP with phytase.

The mean fortnightly and cumulative body weight gain of birds as influenced by phytase supplementation are shown in Figs.2 and 3, respectively.

4.4 Feed consumption

The mean daily feed intake per bird during the eight weeks period among different treatment groups and the cumulative feed intake upto six weeks and eight weeks are given in Tables 10 and 12, respectively.

The mean daily feed intake per bird among the treatment groups viz., T1, T2, T3, T4, T5 and T6 were 122.23, 117.42,

Table 10. Influence of phytase supplementation on mean daily feed intake per bird (g)

Treatments		Age in weeks							
		1	2	3	4	5	6	7	8
T1	R1	9.97	20.64	50.42	65.16	92.09	121.68	140.00	150.00
	R2	10.01	20.54	50.30	65.04	91.93	122.40	140.52	150.26
	R3	10.01	20.61	51.04	64.98	92.04	122.60	139.92	150.39
	Mean	10.00^{e}	20.60^{f}	50.59^{b}	65.06^{e}	92.02^{b}	122.23^{c}	140.15^{b}	150.22^{b}
	SE	±0.01	±0.03	±0.23	±0.05	±0.05	±0.28	±0.19	±0.12
T2	R1	10.54	21.13	41.82	65.01	79.43	118.76	130.00	138.00
	R2	10.56	21.05	41.79	64.95	79.19	117.89	130.21	138.79
	R3	10.49	21.02	41.76	64.80	78.02	115.61	129.70	135.30
	Mean	10.53^{d}	21.07^{e}	41.79^{f}	64.92^{e}	78.88^{f}	117.42^{d}	129.97^{e}	137.36^{d}
	SE	±0.02	±0.03	±0.02	±0.06	±0.43	±0.94	±0.15	±1.06
T3	R1	10.68	21.57	46.56	67.54	86.88	130.86	135.00	143.00
	R2	10.70	21.65	46.70	68.00	87.20	132.00	133.40	142.00
	R3	10.69	21.61	46.83	67.60	86.23	131.81	136.20	144.00
	Mean	10.69^{c}	21.61^{d}	46.71^{d}	67.71^{c}	86.94^{d}	131.56^{a}	134.87^{c}	143.00^{c}
	SE	±0.01	±0.02	±0.08	±0.14	±0.14	±0.35	±0.81	±0.58
T4	R1	10.99	23.04	56.80	73.10	99.80	133.10	145.20	156.70
	R2	10.94	22.96	56.37	72.63	101.10	132.65	146.10	157.20
	R3	10.90	23.10	56.90	72.74	101.16	132.99	145.70	155.50
	Mean	10.94^{a}	23.03^{a}	56.69^{a}	72.82^{a}	100.69^{a}	132.91^{a}	145.67^{a}	156.47^{a}
	SE	±0.03	±0.04	±0.16	±0.14	±0.45	±0.31	±0.26	±0.50
T5	R1	10.69	22.37	43.92	66.43	81.41	127.98	135.10	144.80
	R2	10.63	22.42	43.75	66.25	81.45	127.12	134.80	145.10
	R3	10.72	22.50	46.73	66.41	81.25	127.66	134.70	143.20
	Mean	10.68^{c}	22.43^{c}	43.80^{e}	66.36^{d}	81.37^{e}	127.59^{b}	134.87^{c}	144.37^{c}
	SE	±0.03	±0.04	±0.06	±0.06	±0.06	±0.25	±0.12	±0.59
T6	R1	10.84	22.80	47.54	68.13	89.58	126.78	132.80	140.67
	R2	10.88	22.86	47.49	68.19	91.04	127.83	135.96	143.23
	R3	10.93	22.73	47.42	68.23	88.19	125.16	128.21	130.87
	Mean	10.88^{b}	22.80^{b}	47.48^{c}	68.18^{b}	89.60^{c}	126.59^{b}	132.32^{d}	138.26^{d}
	SE	±0.03	±0.04	±0.03	±0.03	±0.83	±0.78	±2.25	±3.77
	C.D.	0.05626	0.0974	0.3774	0.2869	0.8074	1.365	1.145	1.817

Means bearing the same superscript within the same column do not differ significantly (P<0.01)

Table 11. Influence of phytase supplementation on daily feed intake - ANOVA

Week	Source	d.f.	SS	MSS	F value
1	Treatment	5	1.737	0.347	257.346 **
	Error	12	0.016	0.001	
	Total	17	1.753		
2	Treatment	5	14.526	2.905	831.564 **
	Error	12	0.042	0.003	
	Total	17	14.568		
3	Treatment	5	420.607	84.121	1861.769 **
	Error	12	0.542	0.045	
	Total	17	421.149		
4	Treatment	5	128.255	25.651	990.806 **
	Error	12	0.311	0.026	
	Total	17	128.566		
5	Treatment	5	939.138	187.828	910.164 **
	Error	12	2.476	0.206	
	Total	17	941.614		
6	Treatment	5	594.370	118.874	201.848 **
	Error	12	7.067	0.589	
	Total	17	601.437		
7	Treatment	5	511.577	102.315	247.353 **
	Error	12	4.964	0.414	
	Total	17	516.541		
8	Treatment	5	723.212	144.642	138.669 **
	Error	12	12.517	1.043	
	Total	17	735.729		

** Significant (P<0.01)

Table 12. Influence of phytase supplementation on cumulative feed intake per bird (g)

Treatments		Cumulative feed consumption (g)	
		Sixth week	Eight week
T1	R1	2519.71	4549.71
	R2	2521.52	4556.98
	R3	2528.95	4561.12
	Mean	2523.39^{d}	4555.94^{b}
	SE	±2.52	±3.34
T2	R1	2456.83	4332.83
	R2	2348.01	4331.01
	R3	2373.16	4278.16
	Mean	2392.67^{f}	4314.00^{e}
	SE	±32.93	±17.95
T3	R1	2548.71	4494.71
	R2	2564.00	4491.80
	R3	2556.89	4518.29
	Mean	2556.53^{c}	4501.00^{c}
	SE	±4.42	±8.40
T4	R1	2778.00	4891.30
	R2	2776.55	4899.65
	R3	2783.04	4891.44
	Mean	2779.20^{a}	4894.13^{a}
	SE	±1.97	±2.76
T5	R1	2532.64	4491.94
	R2	2461.47	4420.77
	R3	2465.93	4411.23
	Mean	2486.68^{e}	4441.31^{d}
	SE	±23.04	±25.49
T6	R1	2609.70	4474.00
	R2	2608.08	4532.38
	R3	2608.60	4352.20
	Mean	2608.79^{b}	4452.86^{d}
	SE	±0.48	±0.57
	Grand mean	2549.54	4546.64
	SE	±32.09	±50.75
	C.D.	32.39	40.94

Means bearing the same superscript within the same column do not differ significantly ($P<0.01$)

Table 13. Influence of phytase supplementation on cumulative feed intake - ANOVA

Source	d.f.	SS		MSS		F value	
		Cumulative feed intake		Cumulative feed intake		Cumulative feed intake	
		6th wk	8th wk	6th wk	8th wk	6th wk	8th wk
Treatment	5	255388.57	781616.49	51077.71	156323.29	61.85 **	295.18 **
Error	12	9910.03	6354.97	825.84	529.581		
Total	17	265298.60	637971.46				

** Significant (P<0.01)

131.56, 132.91, 127.59 and 126.59 g during the sixth week and 150.22, 137.36, 143.00, 156.47, 144.37 and 138.26 g during the eighth week respectively.

The analysis of variance of the data on daily feed intake presented in Table 11 indicated that this trait was significantly (P<0.01) influenced by different treatment groups throughout the experimental period. The mean daily feed intake data did not reveal any definite trend based on different feeding regimen employed in this study. Meaningful conclusions may possibly be drawn if cumulative feed intake for 0-6 weeks and 0-8 weeks are analysed critically.

The mean cumulative feed consumption per bird were 2523.39, 2392.67, 2556.53, 2779.20, 2486.68 and 2608.79 g during 0-6 weeks and 4555.94, 4314.00, 4501.00, 4894.13, 4441.31 and 4452.86 g during 0-8 weeks period, for the treatment groups T1, T2, T3, T4, T5 and T6 respectively.

Six weeks total feed consumption was more with T4 (2779.20 g) and less with T2 (2392.67 g) and the values for other treatments were intermediary. T4 continued to consume more feed (4894.13 g) for the whole eight weeks period and T2 less feed (4314.00).

The analysis of variance of the total feed consumed for six weeks and eight weeks presented in Table 13 revealed that

Fig.4 DAILY FEED INTAKE AS INFLUENCED BY PHYTASE SUPPLEMENTATION

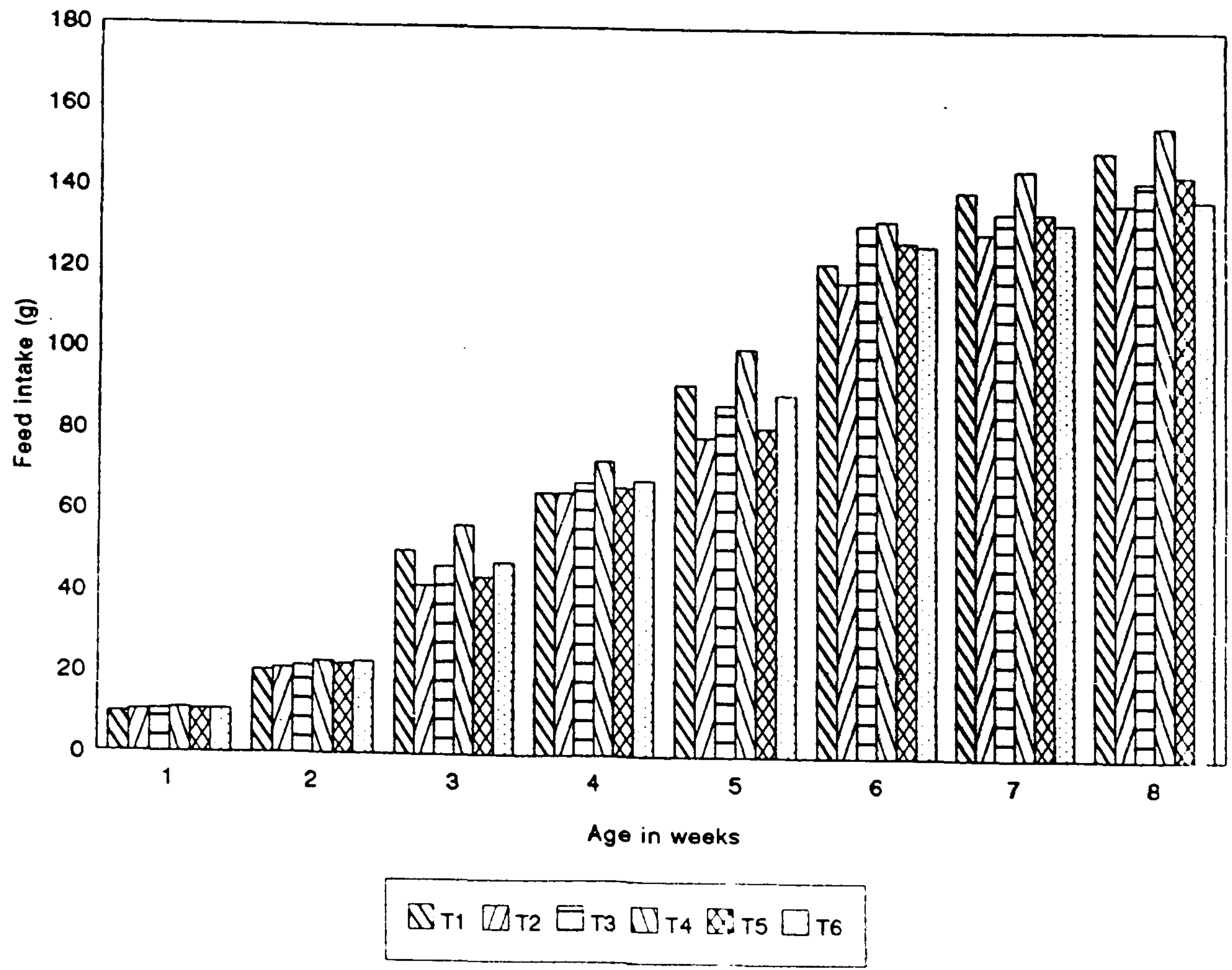
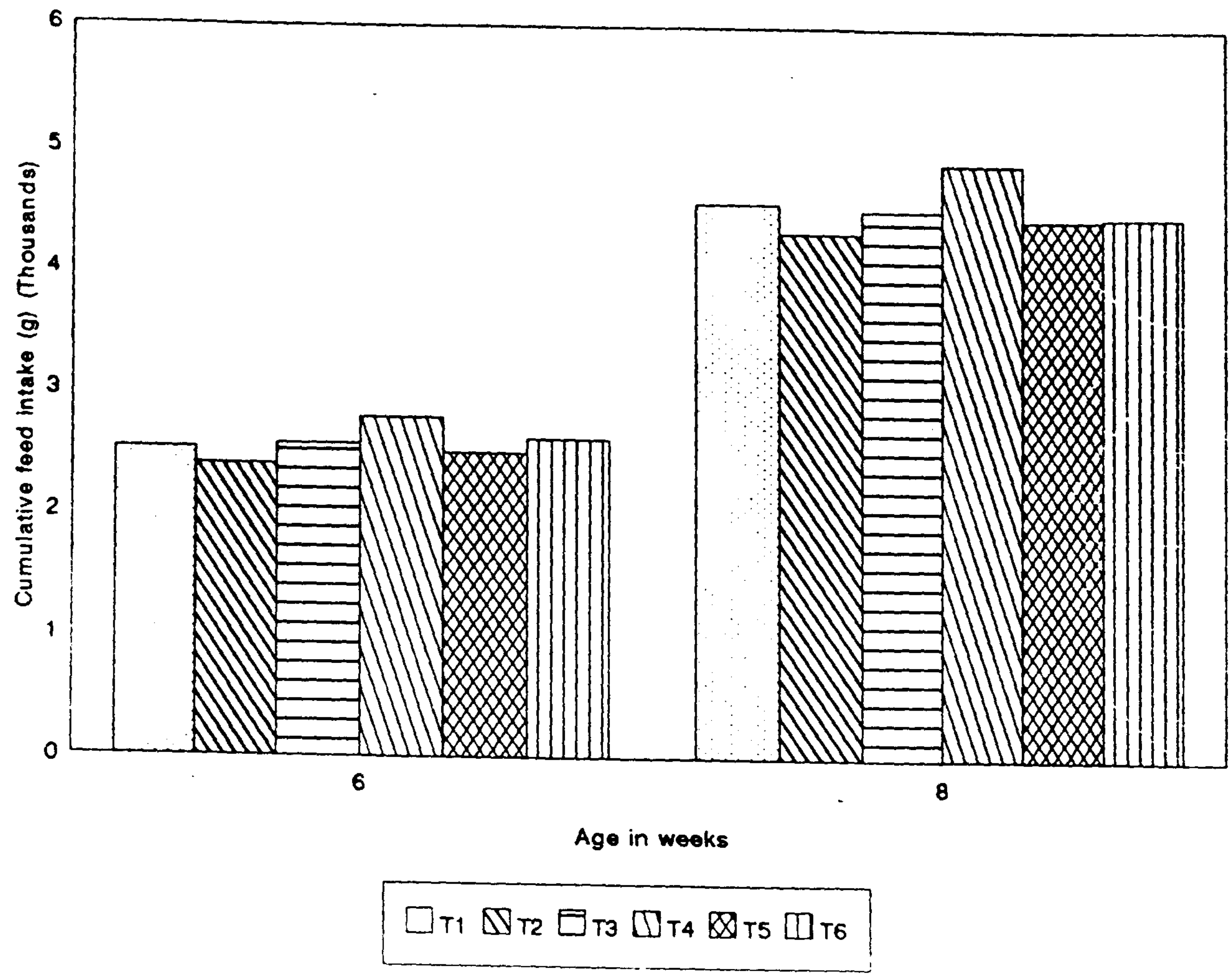


Fig.5 CUMULATIVE FEED INTAKE AT SIXTH AND EIGHTH WEEKS OF AGE AS INFLUENCED BY PHYTASE SUPPLEMENTATION



this trait was statistically different among various treatment groups. Six weeks feed consumption was significantly ($P<0.01$) more in the group fed a standard diet supplemented with phytase (T4) and was significantly less in the group fed a diet containing 0.3 per cent available phosphorus without phytase (T2). All the treatments differed significantly ($P<0.01$) between each other with respect to six and eight weeks total feed consumption. Eight week feed consumption was also significantly ($P<0.01$) more with T4 and less with T2 and all other groups being intermediary.

The mean daily feed intake (weekly basis) and cumulative feed intake per bird as influenced by phytase supplementation are presented in Figs.4 and 5, respectively.

4.5 Feed conversion efficiency

The data on fortnightly feed conversion efficiency (FCE) and cumulative feed conversion efficiency at sixth and eighth weeks of age among different treatment groups are set out in Table 14.

The cumulative feed conversion efficiency obtained for different treatment groups viz., T1, T2, T3, T4, T5 and T6 were 1.70, 1.83, 1.84, 1.91, 1.77 and 1.80 at sixth week and 2.23, 2.26, 2.27, 2.40, 2.26 and 2.19 at the end of the eighth week.

Table 14. Influence of phytase supplementation on mean fortnightly and cumulative feed conversion efficiency

Treatments		Age in weeks 2	4	6	8	Cumulative feed efficiency Sixth week	Eighth week
T1	R1	1.39	1.62	1.94	3.51	1.71	2.22
	R2	1.36	1.61	1.91	3.71	1.69	2.24
	R3	1.36	1.62	1.94	3.63	1.71	2.24
	Mean	1.37^{c}	1.62^{c}	1.93^{d}	3.63^{a}	1.70^{d}	2.23^{c}
	SE	±0.01	±0.01	±0.01	±0.06	±0.006	±0.006
T2	R1	1.50	1.80	1.98	3.19	1.87	2.23
	R2	1.53	1.82	1.95	3.13	1.80	2.27
	R3	1.52	1.79	1.96	3.05	1.82	2.24
	Mean	1.52^{a}	1.80^{a}	1.96^{c}	3.12^{c}	1.83^{b}	2.26^{bc}
	SE	±0.01	±0.01	±0.01	±0.04	±0.02	±0.006
T3	R1	1.49	1.75	2.04	3.37	1.82	2.27
	R2	1.52	1.75	2.14	3.20	1.86	2.27
	R3	1.51	1.73	2.09	3.27	1.84	2.27
	Mean	1.51^{a}	1.74^{ab}	2.09^{b}	3.28^{bc}	1.84^{b}	2.27^{b}
	SE	±0.01	±0.01	±0.03	±0.05	±0.01	±0.00
T4	R1	1.53	1.78	2.25	3.43	1.93	2.38
	R2	1.53	1.76	2.22	3.66	1.91	2.41
	R3	1.51	1.79	2.15	3.78	1.89	2.41
	Mean	1.52^{a}	1.78^{ab}	2.21^{a}	3.62^{a}	1.91^{a}	2.40^{a}
	SE	±0.01	±0.01	±0.03	±0.10	±0.01	±0.01
T5	R1	1.49	1.72	2.04	3.38	1.85	2.30
	R2	1.45	1.74	1.84	3.70	1.71	2.24
	R3	1.50	1.71	1.94	3.41	1.76	2.23
	Mean	1.48^{b}	1.72^{b}	1.94^{cd}	3.50^{ab}	1.77^{c}	2.26^{bc}
	SE	±0.02	±0.01	±0.06	±0.10	±0.04	±0.02
T6	R1	1.50	1.62	2.08	3.19	1.82	2.20
	R2	1.51	1.62	2.05	3.32	1.80	2.22
	R3	1.42	1.62	1.95	3.30	1.77	2.15
	Mean	1.48^{b}	1.62^{c}	2.03^{bc}	3.27^{bc}	1.80^{bc}	2.19^{d}
	SE	±0.01	±0.00	±0.04	±0.04	±0.02	±0.006
	C.D.	0.02813	0.0725	0.0974	0.2452	0.05626	0.03602

Means bearing the same superscript within the same column do not differ significantly ($P<0.01$)

Statistical analysis of the fortnightly feed conversion efficiency (Table 15) and cumulative feed conversion efficiency at sixth and eighth week of age (Table 16) revealed that significant ($P<0.01$) differences existed among various treatments at different periods. The mean fortnightly feed conversion efficiency presented in Table 14 did not reveal any definite trend due to supplementation of phytase.

The cumulative feed conversion efficiency at sixth week (Table 14) indicated that superior efficiency ($P<0.01$) was noted with T1 (1.70) whereas it was inferior with T4 (1.91). The feed conversion efficiency values of all other treatments were between these two.

When the feed conversion efficiency was considered for the whole experimental period of 0-8 weeks of age, significantly ($P<0.01$) superior efficiency was noted with the group offered a diet containing 0.4 per cent AP supplemented with phytase (T6), whereas the group fed a standard diet supplemented with phytase (T4) exhibited significantly ($P<0.01$) inferior efficiency (T4). The feed conversion efficiency values observed with all other treatments were intermediary.

The mean fortnightly feed efficiency for different dietary treatment groups during the eight weeks period and

Table 15. Influence of phytase supplementation on mean fortnightly feed conversion efficiency - ANOVA

Week	Source	d.f.	SS	MSS	F value
2	Treatment	5	0.050	0.010	35.848 **
	Error	12	0.004	0.00025	
	Total	17	0.054		
4	Treatment	5	0.094	0.019	133.278 **
	Error	12	0.002	0.00017	
	Total	17	0.096		
6	Treatment	5	0.183	0.037	11.754 **
	Error	12	0.037	0.003	
	Total	17	0.221		
8	Treatment	5	0.643	0.129	6.779 **
	Error	12	0.228	0.019	
	Total	17	0.870		

** Significant (P<0.01)

Table 16. Influence of phytase supplementation on cumulative feed conversion efficiency - ANOVA

Source	d.f.	SS		MSS		F value	
		Cumulative feed efficiency		Cumulative feed efficiency		Cumulative feed efficiency	
		6th wk	8th wk	6th wk	8th wk	6th wk	8th wk
Treatment	5	0.0733	0.066	0.01466	0.013	91.625 **	32.758 **
Error	12	0.0187	0.005	0.00016	0.00041		
Total	17	0.0920	0.071				

** Significant (P<0.01)

Fig.6 FORTNIGHTLY FEED EFFICIENCY AS INFLUENCED BY PHYTASE SUPPLEMENTATION

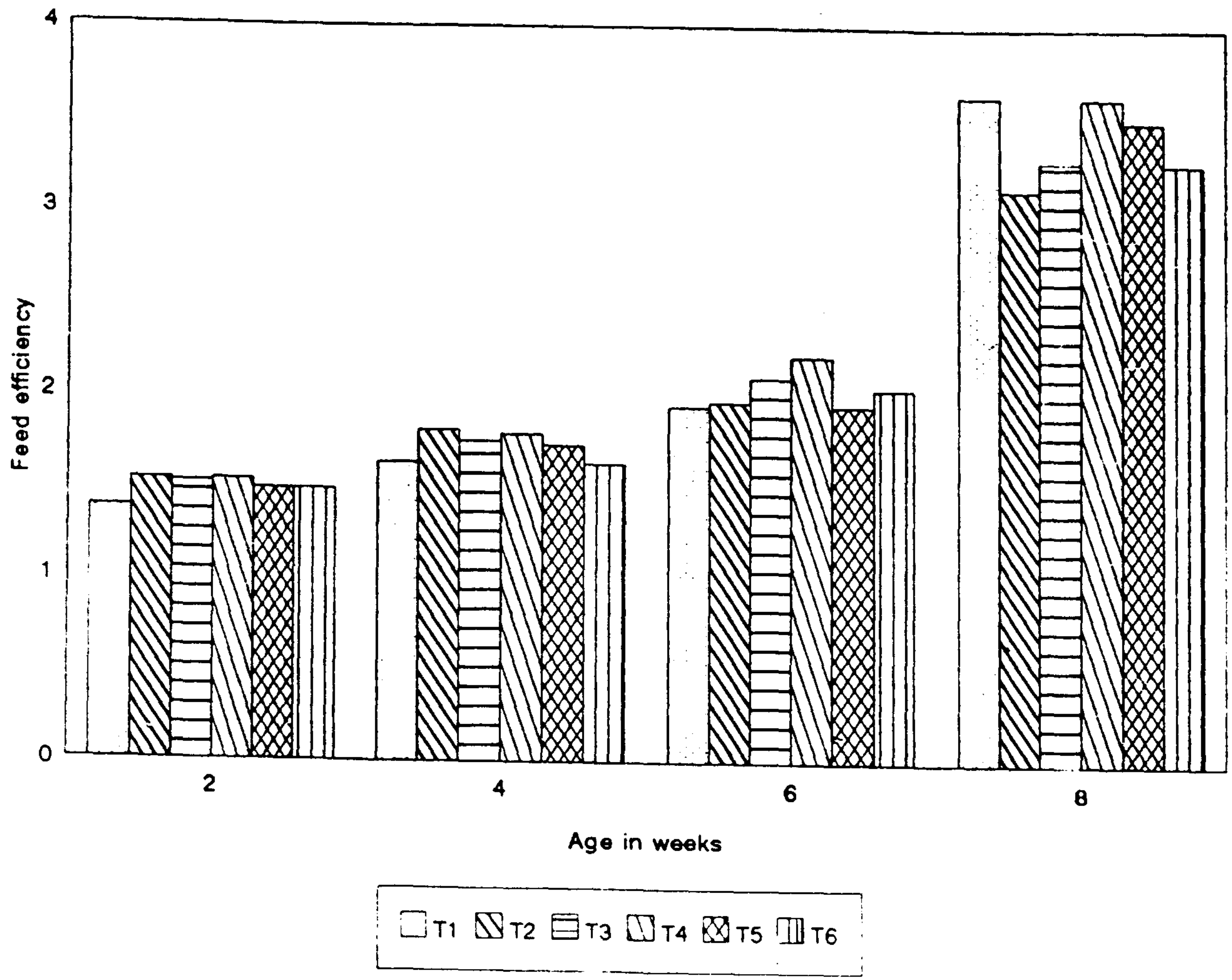
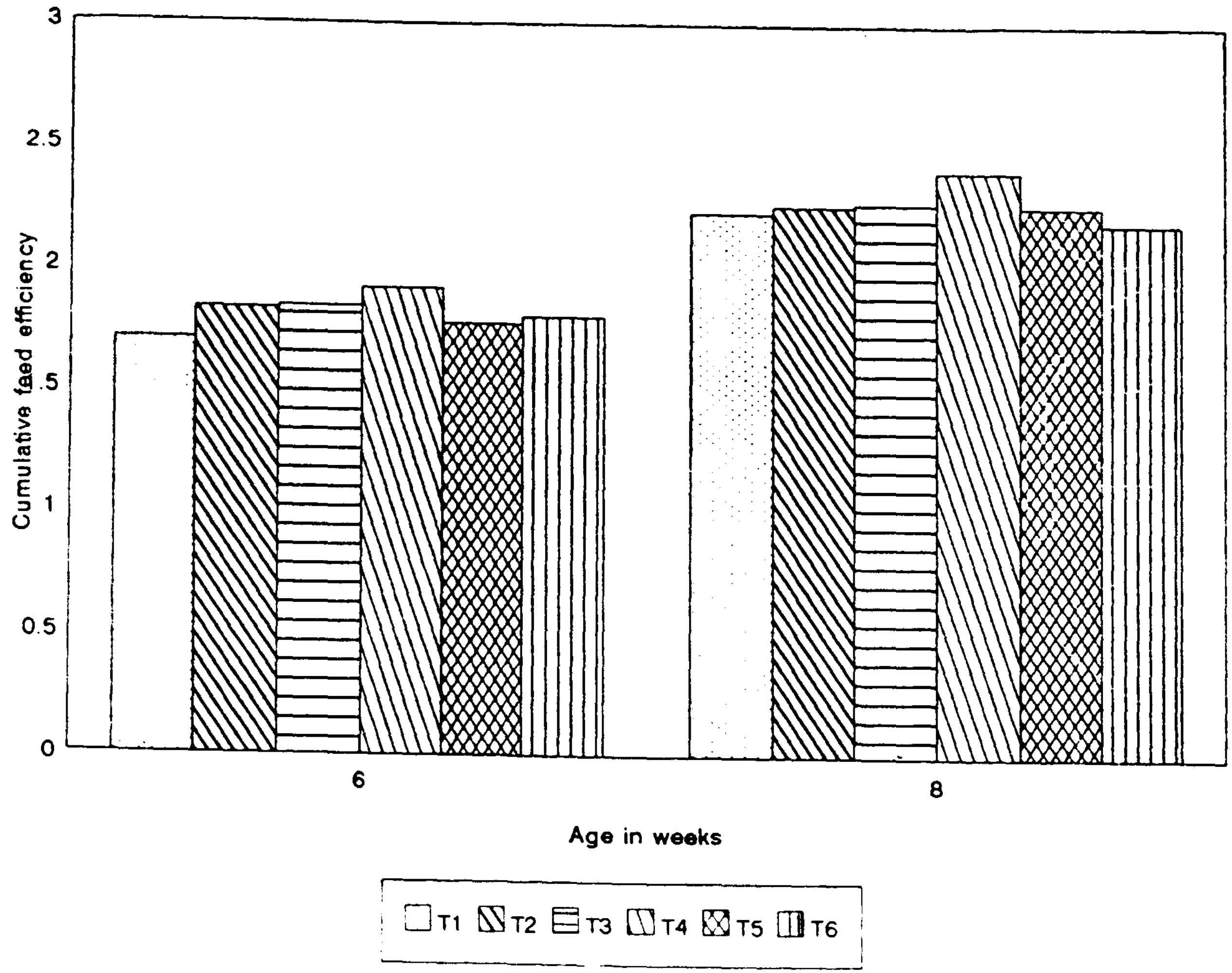


Fig.7 CUMULATIVE FEED EFFICIENCY AT SIXTH AND EIGHTH WEEKS OF AGE AS INFLUENCED BY PHYTASE SUPPLEMENTATION



cumulative feed efficiency at sixth and eighth week of age are depicted in Figs.6 and 7, respectively.

4.6 Tibial ash

The data pertaining to the per cent ash content of tibia estimated at sixth and eighth week of age among different treatment groups are given in Table 17 and 19 respectively.

The per cent ash content of tibia obtained for different treatment groups viz., T1, T2, T3, T4, T5 and T6 were 44.37, 38.70, 40.55, 44.47, 39.90 and 42.38 at sixth week of age and 46.06, 39.25, 41.87, 46.94, 44.22 and 45.69 at the end of the experiment (eight week) respectively.

The analysis of variance of the data presented in Tables 18 and 20 showed that tibial ash content was significantly ($P<0.01$) influenced by different treatments at sixth and eighth week of age. At sixth week tibial ash content was significantly ($P<0.01$) higher in T4 (44.47%) and T1 (44.37%) ie., standard diets with and without supplementation of phytase when compared to all other groups. It also showed that addition of enzyme to low available phosphorus diets resulted in significant ($P<0.01$) increase in the tibial ash content. Further, it indicated that the group fed a diet containing 0.4 per cent AP without phytase supplementation

Table 17. Influence of phytase supplementation on sixth week tibial ash (per cent)

Bird No.	Tibial ash (per cent)					
	T1	T2	T3	T4	T5	T6
1	44.63	39.61	41.57	45.01	40.21	42.83
2	44.75	38.97	41.62	44.82	40.31	42.94
3	45.01	38.73	41.82	45.21	40.35	42.79
4	43.83	38.78	39.78	43.79	39.52	41.72
5	44.02	39.01	40.01	44.12	39.83	42.02
6	43.97	37.12	39.52	43.87	39.29	41.97
Mean	44.37^{a}	38.70^{d}	40.55^{c}	44.47^{a}	39.90^{c}	42.38^{b}
SE	±0.20	±0.34	±0.54	±0.25	±0.18	±0.22

C.D. 0.9065

Means bearing the same superscript do not differ significantly (P<0.01)

Table 18. Influence of phytase supplementation on sixth week tibial ash - ANOVA

Source	d.f.	SS	MSS	F value
Treatment	5	172.661	34.532	58.397 **
Error	30	17.740	0.591	
Total	35	190.401		

** Significant (P<0.01)

Table 19. Influence of phytase supplementation on eighth week tibial ash (per cent)

Bird No.	Tibial ash (per cent)					
	T1	T2	T3	T4	T5	T6
1	47.00	39.91	42.98	47.21	44.53	46.12
2	45.92	39.64	42.14	47.58	44.68	46.93
3	46.21	38.93	41.75	47.10	44.41	45.12
4	46.31	39.21	42.31	46.87	44.11	45.72
5	45.00	39.54	41.83	46.92	44.38	45.32
6	45.92	38.25	40.23	45.97	43.20	44.90
Mean	46.06^{b}	39.25^{e}	41.87^{d}	46.94^{a}	44.22^{c}	45.69^{b}
SE	0.27	0.24	0.38	0.22	0.22	0.31

C.D. - 0.7997

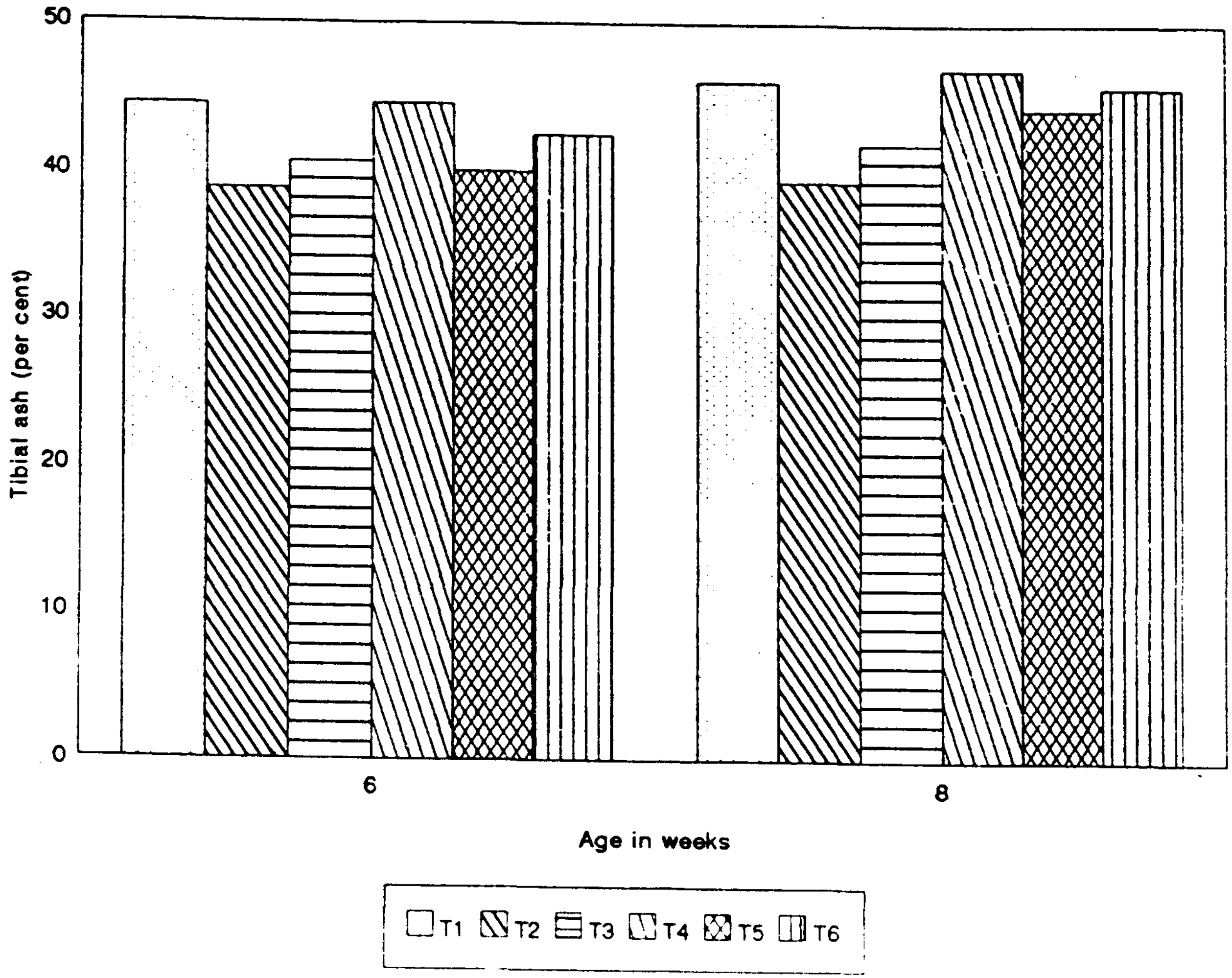
Means bearing the same superscript do not differ significantly (P<0.01)

Table 20. Influence of phytase supplementation on eighth week tibial ash - ANOVA

Source	d.f.	SS	MSS	F value
Treatment	5	257.421	51.484	111.933 **
Error	30	13.799	0.460	
Total	35	271.220		

** Significant (P<0.01)

Fig.8 TIBIAL BONE ASH AT SIXTH AND EIGHTH WEEKS OF AGE AS INFLUENCED BY PHYTASE SUPPLEMENTATION



(T3) was statistically comparable with group fed a diet having 0.3 per cent AP supplemented with phytase (T5).

Tibial ash content at the end of eight weeks of age was significantly (P<0.01) higher in group offered a standard broiler diet supplemented with phytase (T4) when compared to all other groups. It was significantly (P<0.01) lower in birds given a diet having 0.3 per cent AP without enzyme (T2). All other groups were intermediary with respect to tibial ash content. The tibial ash content of group fed a standard broiler diet without enzyme (T1) was statistically comparable with those fed a ration having an AP content of 0.4 per cent with phytase (T6). Tibial ash content of low available phosphorus fed groups without enzyme was significantly (P<0.01) lower than those supplemented with enzyme.

The percentage tibial ash content at six and eight weeks of age as influenced by phytase supplementation is shown in Fig.8.

4.7 Serum calcium and inorganic phosphorus

4.7.1 Serum calcium

The mean serum calcium levels of birds during sixth and eighth weeks of age among different treatment groups are given in Table 21 and 23 respectively.

Table 21. Influence of phytase supplementation on sixth week serum calcium (mg per cent)

Bird No.	Serum calcium (mg per cent)					
	T1	T2	T3	T4	T5	T6
1	10.89	12.20	10.00	12.22	12.22	11.11
2	11.02	12.20	11.10	12.22	11.11	12.22
3	11.10	12.20	11.10	12.22	11.11	12.22
4	10.67	11.10	11.10	13.10	12.22	13.33
5	11.21	11.10	10.89	12.67	12.22	12.22
6	11.30	11.10	11.10	12.44	13.33	11.11
Mean	11.03^{cd}	11.65^{bc}	10.88^{d}	12.48^{a}	12.04^{ab}	12.04^{ab}
SE	±0.09	±0.25	±0.18	±0.14	±0.34	±0.34

C.D. - 0.6228

Means bearing the same superscript do not differ significantly (P<0.01)

Table 22. Influence of phytase supplementation on sixth week serum calcium - ANOVA

Source	d.f.	SS	MSS	F value
Treatment	5	9.303	1.861	6.667 **
Error	30	8.372	0.279	
Total	35	17.675		

** Significant (P<0.01)

Table 23. Influence of phytase supplementation on eighth week serum calcium (mg per cent)

Bird No.	Serum calcium (mg per cent)					
	T1	T2	T3	T4	T5	T6
1	11.20	12.20	11.10	12.20	11.10	11.10
2	11.10	11.10	10.00	12.20	11.10	10.00
3	12.20	10.00	12.20	13.33	11.10	13.33
4	11.10	10.00	11.10	13.33	12.20	11.10
5	10.00	11.10	10.00	12.20	11.10	8.89
6	11.10	10.00	11.10	11.10	10.00	11.10
Mean SE	11.12^{a} ±0.28	10.73^{a} ±0.37	10.92^{a} ±0.34	12.39^{a} ±0.34	11.10^{a} ±0.28	10.92^{a} ±0.60

Means bearing the same superscript do not differ significantly (P<0.01)

Table 24. Influence of phytase supplementation on eighth week serum calcium - ANOVA

Source	d.f.	SS	MSS	F value
Treatment	5	8.821	1.764	2.391 NS
Error	30	22.135	0.738	
Total	35	30.956		

NS - Not significant

The mean serum calcium levels among the six treatment groups viz., T1, T2, T3, T4, T5 and T6 were 11.03, 11.65, 10.88, 12.48, 12.04 and 12.04 mg per cent at sixth week and 11.12, 10.73, 10.92, 12.39, 11.10 and 10.92 mg per cent at the end of experiment (eighth week) respectively.

The statistical analysis of the data presented in Tables 22 and 24 indicated that differences in mean serum calcium levels between the different treatments were statistically significant ($P<0.01$) at sixth week of age, whereas the magnitude of difference between treatments at eighth week was not significant. At sixth week of age serum calcium was significantly ($P<0.01$) higher in birds fed a standard diet supplemented with phytase (T4) and it was significantly ($P<0.01$) lower among the birds fed a diet having an AP content of 0.4 per cent without enzyme (T3). The serum calcium level of enzyme supplemented groups were statistically higher than other groups. However, the serum calcium values obtained for T2 (11.65), T5 (12.04) and T6 (12.04) were comparable.

4.7.2 Serum inorganic phosphorus

The mean serum inorganic phosphorus levels of birds during sixth and eighth week of age among different treatment groups are given in Tables 25 and 27, respectively.

Table 25. Influence of phytase supplementation on sixth week serum inorganic phosphorus (mg per cent)

Bird No.	Serum inorganic phosphorus (mg per cent)					
	T1	T2	T3	T4	T5	T6
1	5.49	4.25	4.98	5.98	4.78	5.23
2	5.50	4.49	5.12	5.98	4.92	5.37
3	5.37	4.51	5.02	5.78	4.92	5.61
4	5.78	4.78	5.52	6.12	5.12	5.76
5	5.92	4.92	5.61	6.12	5.23	5.92
6	5.89	4.63	5.51	6.23	5.12	5.92
Mean	5.66^{b}	4.60^{d}	5.29^{c}	6.04^{a}	5.02^{c}	5.64^{b}
SE	±0.10	±0.09	±0.12	±0.06	±0.07	±0.12

C.D. - 0.3518

Means bearing the same superscript do not differ significantly (P<0.01)

Table 26. Influence of phytase supplementation on sixth week serum inorganic phosphorus - ANOVA

Source	d.f.	SS	MSS	F value
Treatment	5	13.186	2.637	29.794 **
Error	30	2.655	0.089	
Total	35	15.841		

** Significant (P<0.01)

Table 27. Influence of phytase supplementation on eighthweek serum inorganic phosphorus (mg per cent)

Bird No.	Serum inorganic phosphorus (mg per cent)					
	T1	T2	T3	T4	T5	T6
1	5.82	4.97	5.40	6.21	5.87	6.30
2	6.10	6.83	5.37	6.10	7.85	8.40
3	5.92	5.10	5.40	5.98	5.91	6.17
4	7.84	6.75	7.43	8.10	7.87	8.10
5	7.92	5.20	7.52	7.98	5.85	6.10
6	8.10	6.52	7.63	8.03	7.83	8.30
Mean SE	6.95^{a} ±0.45	5.90^{a} ±0.36	6.46^{a} ±0.48	7.07^{a} ±0.44	6.86^{a} ±0.44	7.23^{a} ±0.47

Means bearing the same superscript do not differ significantly (P<0.01)

Table 28. Influence of phytase supplementation on eighth week serum inorganic phosphorus - ANOVA

Source	d.f.	SS	MSS	F value
Treatment	5	9.658	1.932	1.264 NS
Error	30	45.832	1.528	
Total	35	55.490		

NS - Not significant

The mean serum inorganic phosphorus among the six treatment groups viz., T1, T2, T3, T4, T5 and T6 were 5.66, 4.60, 5.29, 6.04, 5.02 and 5.64 mg per cent at sixth week and 6.95, 5.90, 6.46, 7.07, 6.86 and 7.23 mg per cent at the end of experiment (eight week) respectively.

The analysis of variance of data on serum inorganic phosphorus set out in Tables 26 and 28 showed that serum inorganic phosphorus was significantly (P<0.01) influenced by different treatments at sixth week of age, whereas the magnitude of difference between the values at eighth week of age was statistically comparable. Higher serum inorganic phosphorus value was obtained for T4 (6.04 mg per cent) which was statistically higher than all other groups at sixth week of age. Among the treatments, T2 registered significantly lower value (4.60 mg per cent).

Apparently higher serum inorganic phosphorus was noted with T6 (7.23 mg per cent) while T2 registered a lower value (5.90 mg per cent) at eighth week of age. However, the difference in serum inorganic phosphorus among the treatments failed to show any significant difference at this age.

The mean serum calcium and inorganic phosphorus levels obtained at sixth and eighth week of age as influenced by phytase supplementation are depicted in Figs.9 and 10, respectively.

Fig.9 SERUM CALCIUM AT SIXTH AND EIGHTH WEEKS OF AGE AS INFLUENCED BY PHYTASE SUPPLEMENTATION

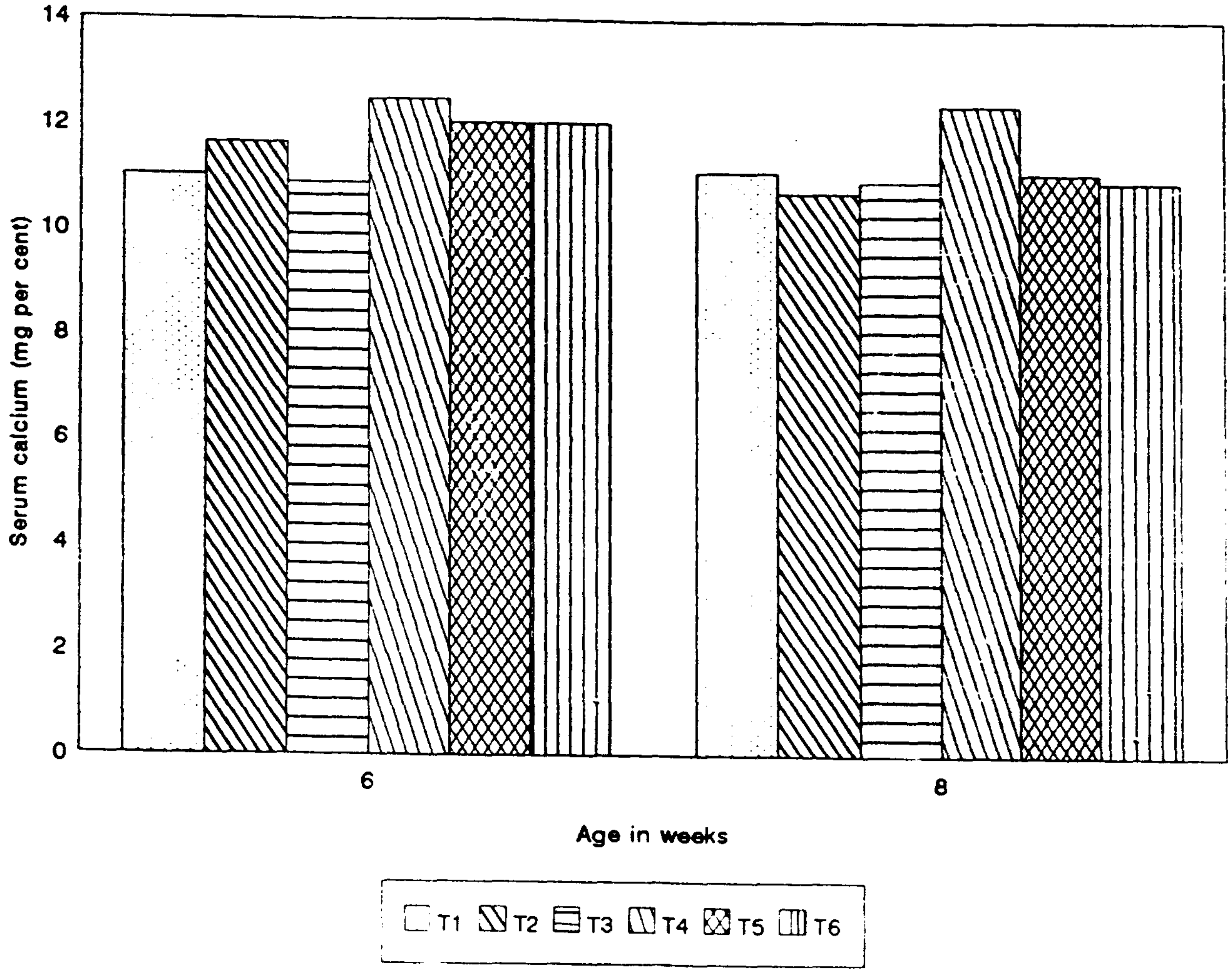
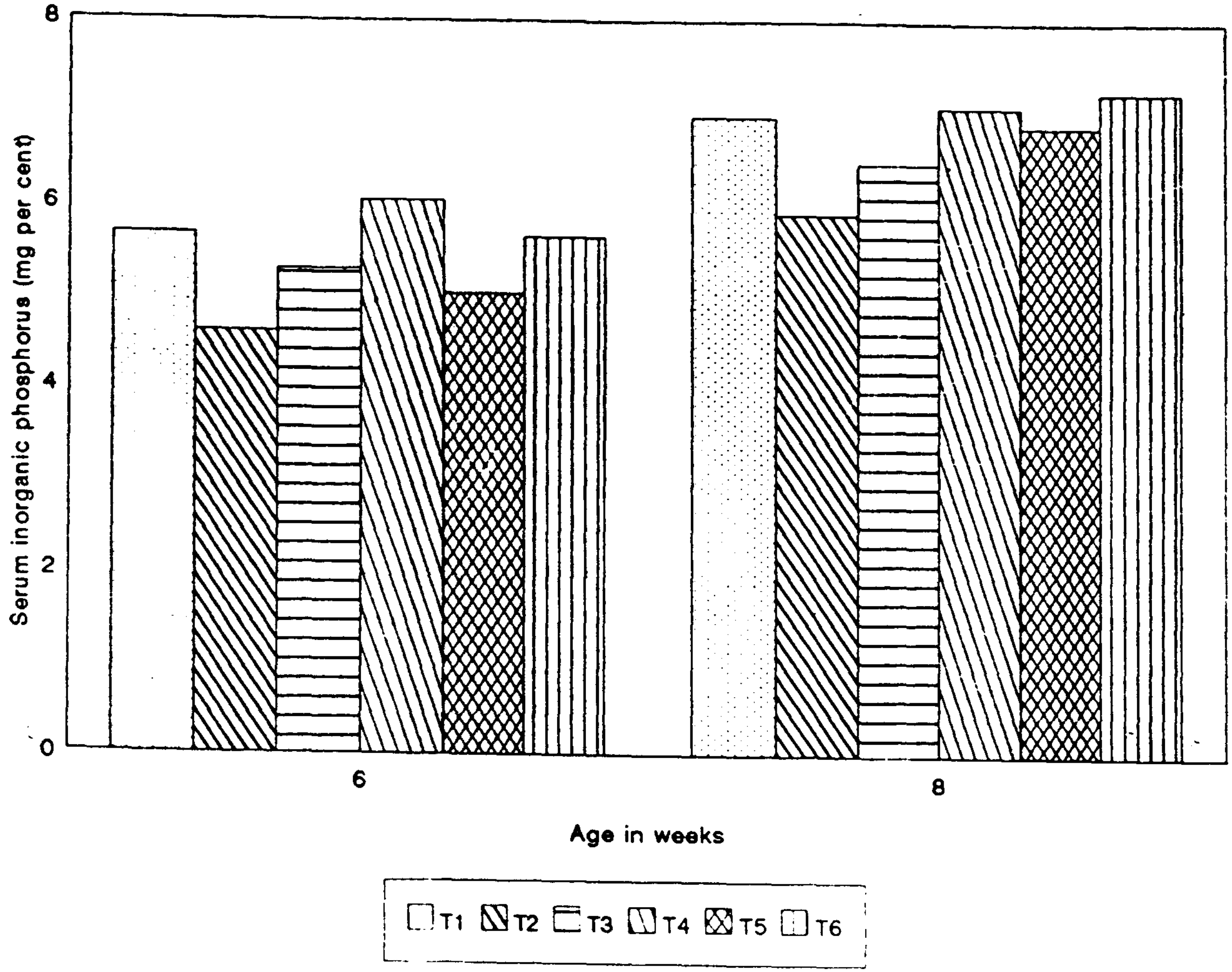


Fig.10 SERUM INORGANIC PHOSPHORUS AT SIXTH AND EIGHTH WEEKS OF AGE AS INFLUENCED BY PHYTASE SUPPLEMENTATION



4.8 Bioavailability of calcium and phosphorus

4.8.1 Calcium bioavailability

The data pertaining to bioavailability of calcium of birds among different treatment groups are given in Table 29.

Bioavailability of calcium obtained from different treatment groups viz., T1, T2, T3, T4, T5 and T6 were 62.13, 53.09, 58.37, 64.19, 57.28 and 61.28 per cent respectively.

The statistical interpretation showed that bio availability of calcium was significantly ($P<0.01$) influenced by supplementation of phytase (Table 30). Irrespective of the level of AP in the diet, it was significantly ($P<0.01$) higher in enzyme supplemented groups than their respective counter parts. Significantly ($P<0.01$) higher value was observed in T4 (64.19 per cent) and lower value in T2 (53.09 per cent).

4.8.2 Phosphorus bioavailability

The data on phosphorus bioavailability among different treatment groups as influenced by phytase supplementation is presented in Table 31.

The per cent bioavailability of phosphorus for the treatments T1, T2, T3, T4, T5 and T6 were 51.28, 38.08, 46.08, 55.85, 45.60 and 52.31, respectively. Statistical analysis of

Table 29. Influence of phytase supplementation on bioavailability of calcium (per cent)

Bird No.	Bioavailability of calcium (per cent)					
	T1	T2	T3	T4	T5	T6
1	62.13	52.13	58.19	64.13	57.18	61.28
2	62.18	53.01	58.23	64.28	57.23	61.34
3	62.23	54.17	59.14	64.14	57.48	61.41
4	62.31	53.18	59.10	64.22	57.31	61.18
5	61.98	54.12	58.31	64.13	57.15	60.93
6	61.96	51.93	57.23	64.25	57.30	61.52
Mean	62.13^{b}	53.09^{f}	58.37^{d}	64.19^{a}	57.28^{e}	61.28^{c}
SE	±0.06	±0.39	±0.29	±0.03	±0.05	±0.09

C.D. - 0.0305

Means bearing the same superscript do not differ significantly ($P<0.01$)

Table 30. Influence of phytase supplementation on bioavailability of calcium - ANOVA

Source	d.f.	SS	MSS	F value
Treatment	5	476.056	95.211	386.536 **
Error	30	7.390	0.246	
Total	35	483.446		

** Significant ($P<0.01$)

Table 31. Influence of phytase supplementation on bioavailability of phosphorus (per cent)

Bird No.	Bioavailability of phosphorus (per cent)					
	T1	T2	T3	T4	T5	T6
1	51.45	38.39	46.16	55.72	45.71	52.13
2	51.62	37.92	45.93	54.98	44.98	51.98
3	51.21	38.13	46.19	56.32	46.21	53.15
4	49.93	37.97	45.97	56.13	45.87	52.42
5	52.14	37.65	46.31	55.85	45.12	51.83
6	51.32	38.43	45.92	56.12	45.71	52.32
Mean	51.28 [c]	38.08 [e]	46.08 [d]	55.85 [a]	45.60 [d]	52.31 [b]
SE	±0.30	±0.12	±0.07	±0.20	±0.19	±0.19

C.D. 0.5530

Means bearing the same superscript do not differ significantly (P<0.01)

Table 32. Influence of phytase supplementation on bioavailability of phosphorus - ANOVA

Source	d.f.	SS	MSS	F value
Treatment	5	1191.214	238.243	1081.669 **
Error	30	6.608	0.220	
Total	35	1197.822		

** Significant (P<0.01)

the data on bioavailability of phosphorus presented in Table 32 indicated that significant (P<0.01) differences existed between different treatments. It was significantly (P<0.01) more in birds fed a standard diet supplemented with phytase (T4). Significantly (P<0.01) lower value was observed in birds fed a diet having 0.3 per cent AP without enzyme (T2).

Bioavailability of phosphorus was significantly (P<0.01) more among groups fed diet supplemented with phytase than their respective counterparts in the unsupplemented groups.

4.8.3 Phosphorus excretion

Data on phosphorus excretion of birds as influenced by different dietary treatments are charted out in Table 33.

The amount of phosphorus excreted by birds of different treatment groups viz., T1, T2, T3, T4, T5 and T6 were 5.38, 3.64, 4.76, 4.20, 2.35 and 3.85 g per kg of dry matter intake respectively.

The analysis of variance of the data on phosphorus excretion is set out in Table 34. It showed that there is significant (P<0.01) difference between different treatments. Significantly (P<0.01) lower phosphorus excretion was observed in enzyme supplemented groups when compared to their respective unsupplemented groups. Significantly (P<0.01)

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

Bird No.	Phosphorus excretion (g/kg DM intake)					
	T1	T2	T3	T4	T5	T6
1	5.36	3.62	4.82	4.21	2.35	3.86
2	5.35	3.65	4.76	4.28	2.40	3.81
3	5.39	3.64	4.74	4.15	2.31	3.94
4	5.51	3.65	4.76	4.17	2.33	3.85
5	5.30	3.67	4.73	4.19	2.39	3.89
6	5.38	3.61	4.74	4.17	2.34	3.78
Mean	5.38[a]	3.64[e]	4.76[b]	4.20[c]	2.35[f]	3.85[d]
SE	±0.03	±0.008	±0.01	±0.02	±0.02	±0.02

C.D. 0.05273

Means bearing the same superscript do not differ significantly (P<0.01)

Table 34. Influence of phytase supplementation on phosphorus excretion - ANOVA

Source	d.f.	SS	MSS	F value
Treatment	5	32.293	6.459	2857.812 **
Error	30	0.068	0.002	
Total	35	32.361		

** Significant (P<0.01)

Fig.11 BIOAVAILABILITY OF CALCIUM AND PHOSPHORUS AS INFLUENCED BY PHYTASE SUPPLEMENTATION

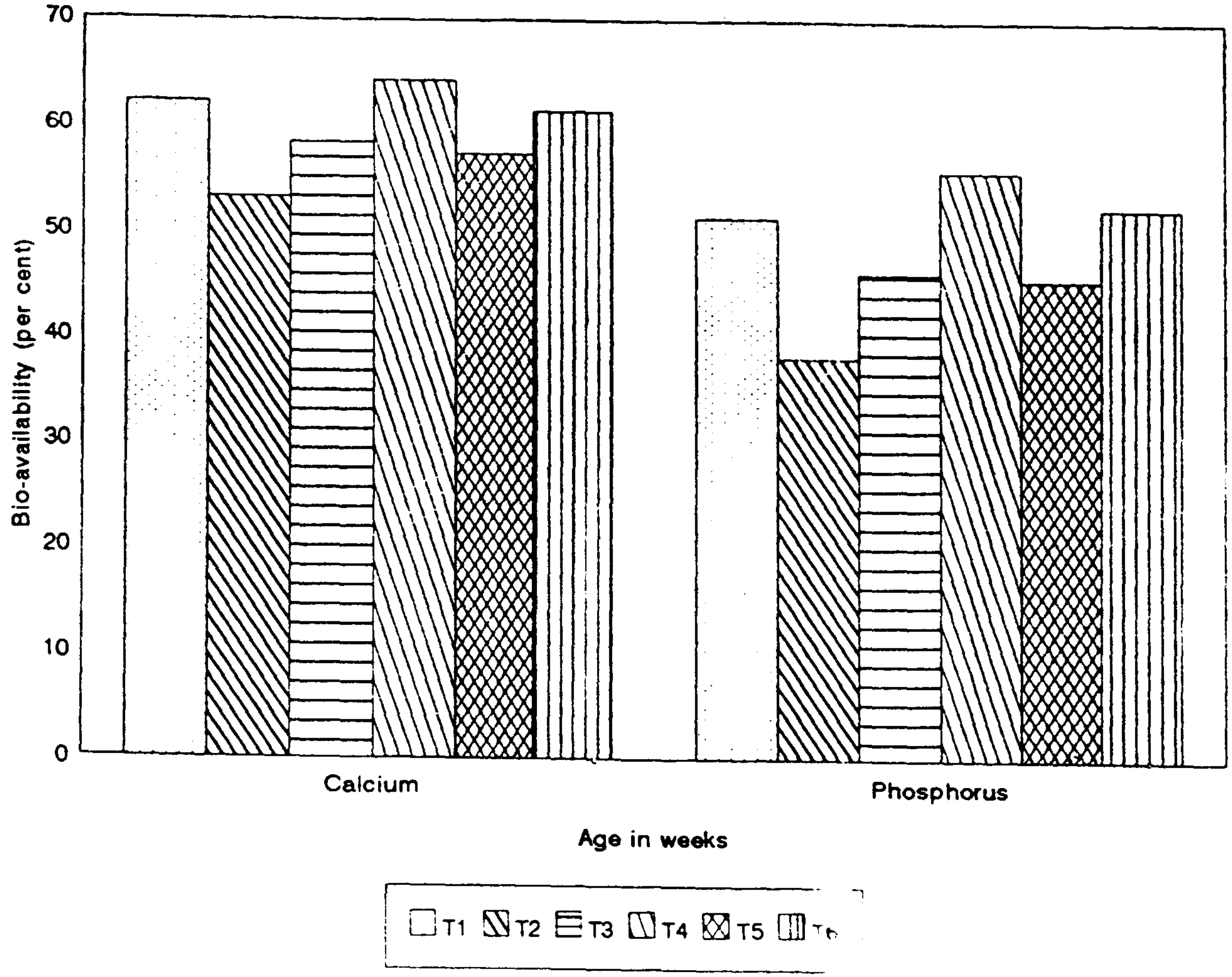
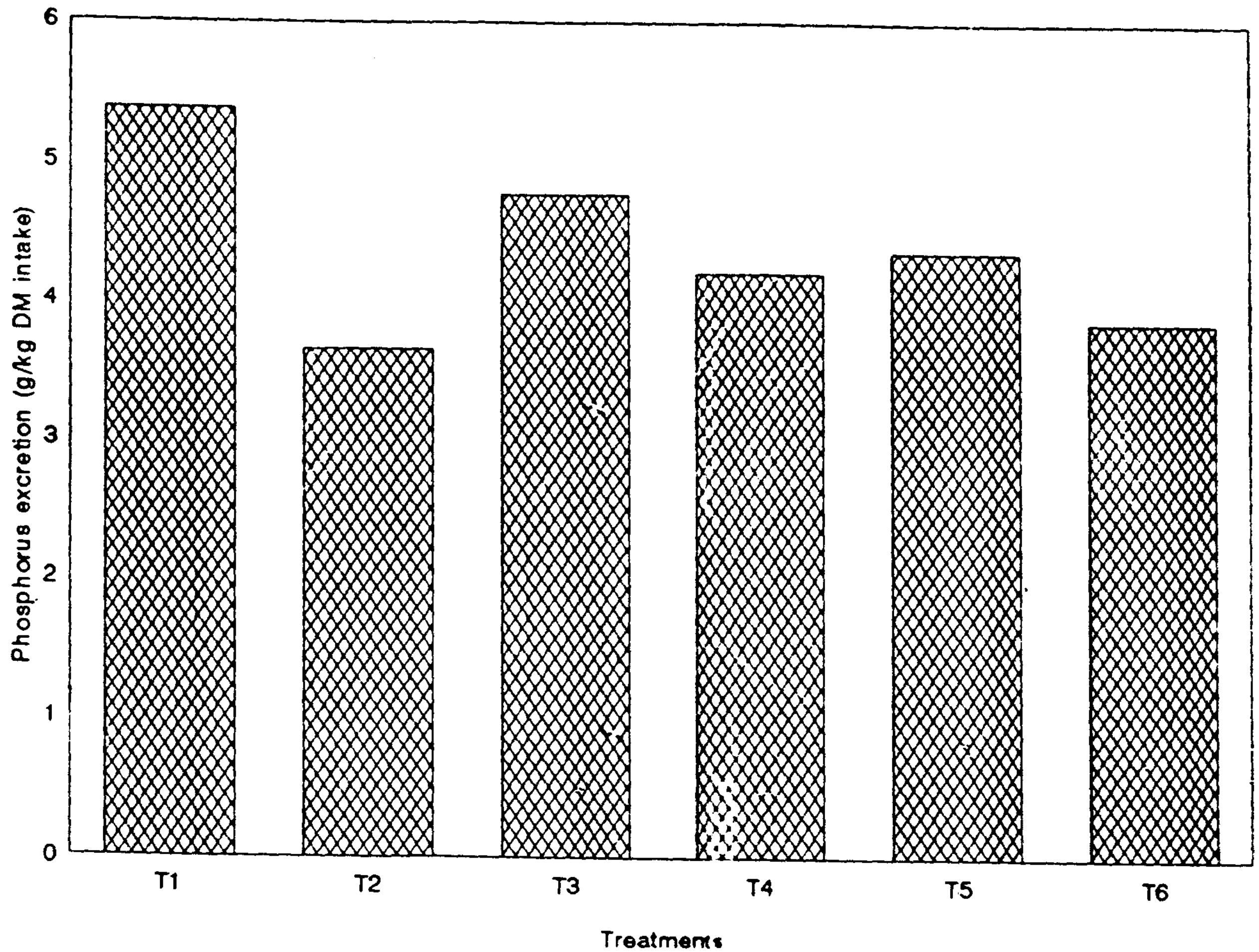


Fig.12 PHOSPHORUS EXCRETION IN DROPPINGS AS INFLUENCED BY PHYTASE SUPPLEMENTATION



higher value was obtained in T1 (5.38 g/kg DM intake) and lower value in T5 (2.35 g/kg DM intake).

The influence of phytase supplementation in broilers with respect to bioavailability of calcium, phosphorus and phosphorus excretion are shown in Figs.11 and 12, respectively.

4.9 Processing yields and losses

4.9.1 Dressed yield

Data on percentage dressed yield of birds among different treatment groups are given in Table 35.

The percentage dressed yield of birds of treatments T1, T2, T3, T4, T5 and T6 were 85.80, 85.78, 85.07, 85.67, 86.61 and 87.55 respectively.

The statistical analysis of the data on per cent dressed yield presented in Table 36 showed significant ($P<0.01$) differences between treatments. It was significantly ($P<0.01$) more in the group fed a diet containing 0.4 per cent AP and supplemented with phytase (T6) than all other groups. The per cent dressed yield was statistically comparable between treatments T5 (86.61 per cent) and T6 (87.55 per cent).

Table 35. Influence of phytase supplementation on dressed yield (per cent)

Bird No.	Dressed yield (per cent)					
	T1	T2	T3	T4	T5	T6
1	85.70	87.03	85.00	86.67	85.71	86.24
2	84.26	86.05	84.78	88.10	88.00	88.00
3	86.67	86.15	85.22	84.80	86.30	88.10
4	85.71	84.56	84.46	85.26	86.65	86.27
5	85.64	85.67	87.89	84.72	87.02	89.23
6	86.84	85.22	83.08	84.44	86.00	87.47
Mean	85.80^{b}	85.78^{b}	85.07^{b}	85.67^{b}	86.61^{ab}	87.55^{a}
SE	±0.38	±0.35	±0.64	±0.58	±0.34	±0.47

C.D. 1.217

Means bearing the same superscript do not differ significantly (P<0.01)

Table 36. Influence of phytase supplementation on dressed yield - ANOVA

Source	d.f.	SS	MSS	F value
Treatment	5	58.478	11.696	10.982 **
Error	30	31.948	1.065	
Total	35	90.426		

** Significant (P<0.01)

Similarly, the differences in per cent dressed yield among the treatments T1, T2, T3, T4 and T5 were not enough to make them statistically significant ($P<0.01$).

4.9.2 Ready-to-cook yield

The data pertaining to ready-to-cook yield of birds as influenced by phytase supplementation is presented in Table 37. Higher ready-to-cook yield was obtained for the treatment T6 (71.57 per cent), while T3 recorded a lower ready-to-cook yield of 63.45 per cent. When the differences in ready-to-cook yield among the treatments were analysed (Table 38), it showed that significant ($P<0.01$) differences existed between treatments. It was significantly ($P<0.01$) more with T6 (71.57 per cent), while treatments T2 (64.72 per cent) and T3 (63.45 per cent) recorded significantly ($P<0.01$) lower ready-to-cook yield. The ready-to-cook yield of treatments T1, T4 and T5 were statistically comparable. Similarly there was no significant difference between treatments T1 and T6.

4.9.3 Giblet yield

The mean giblet yield of birds fed different dietary regimen is shown in Table 39. The group fed a standard diet supplemented with phytase (T4) had a greater per cent giblet yield (9.29 per ent), while those maintained on a standard

Table 37. Influence of phytase supplementation on ready-to-cook yield (per cent)

Bird No.	Ready-to-cook yield (per cent)					
	T1	T2	T3	T4	T5	T6
1	68.98	65.71	65.00	66.15	65.31	73.17
2	70.28	64.72	63.04	71.40	68.00	71.11
3	71.49	66.67	62.61	66.01	69.77	71.43
4	72.83	64.57	62.70	68.95	68.11	71.35
5	64.62	62.86	65.79	66.32	67.03	71.28
6	68.43	63.78	61.54	67.22	70.00	71.05
Mean	69.44[ab]	64.72[c]	63.45[c]	67.68[b]	68.04[b]	71.57[a]
SE	±1.17	±0.55	±0.66	±0.87	±0.71	±0.33

C.D. - 1.35

Means bearing the same superscript do not differ significantly ($P<0.01$)

Table 38. Influence of phytase supplementation on ready-to-cook yield - ANOVA

Source	d.f.	SS	MSS	F value
Treatment	5	100.842	20.168	15.397 **
Error	30	39.296	1.310	
Total	35	140.138		

** Significant ($P<0.01$)

Table 39. Influence of phytase supplementation on giblet yield (per cent)

Bird No.	Giblet yield (per cent)					
	T1	T2	T3	T4	T5	T6
1	5.71	5.71	7.00	9.74	7.76	8.05
2	6.29	7.43	6.76	8.95	8.75	8.00
3	6.81	6.05	6.09	9.05	8.37	8.33
4	6.15	6.29	6.32	10.53	8.17	8.11
5	5.90	5.64	6.09	8.00	8.65	8.21
6	5.26	6.96	6.92	9.44	9.00	8.42
Mean	6.02^{c}	6.35^{c}	6.53^{c}	9.29^{a}	8.45^{b}	8.19^{b}
SE	±0.22	±0.29	±0.17	±0.35	±0.18	±0.07

C.D. - 0.7269

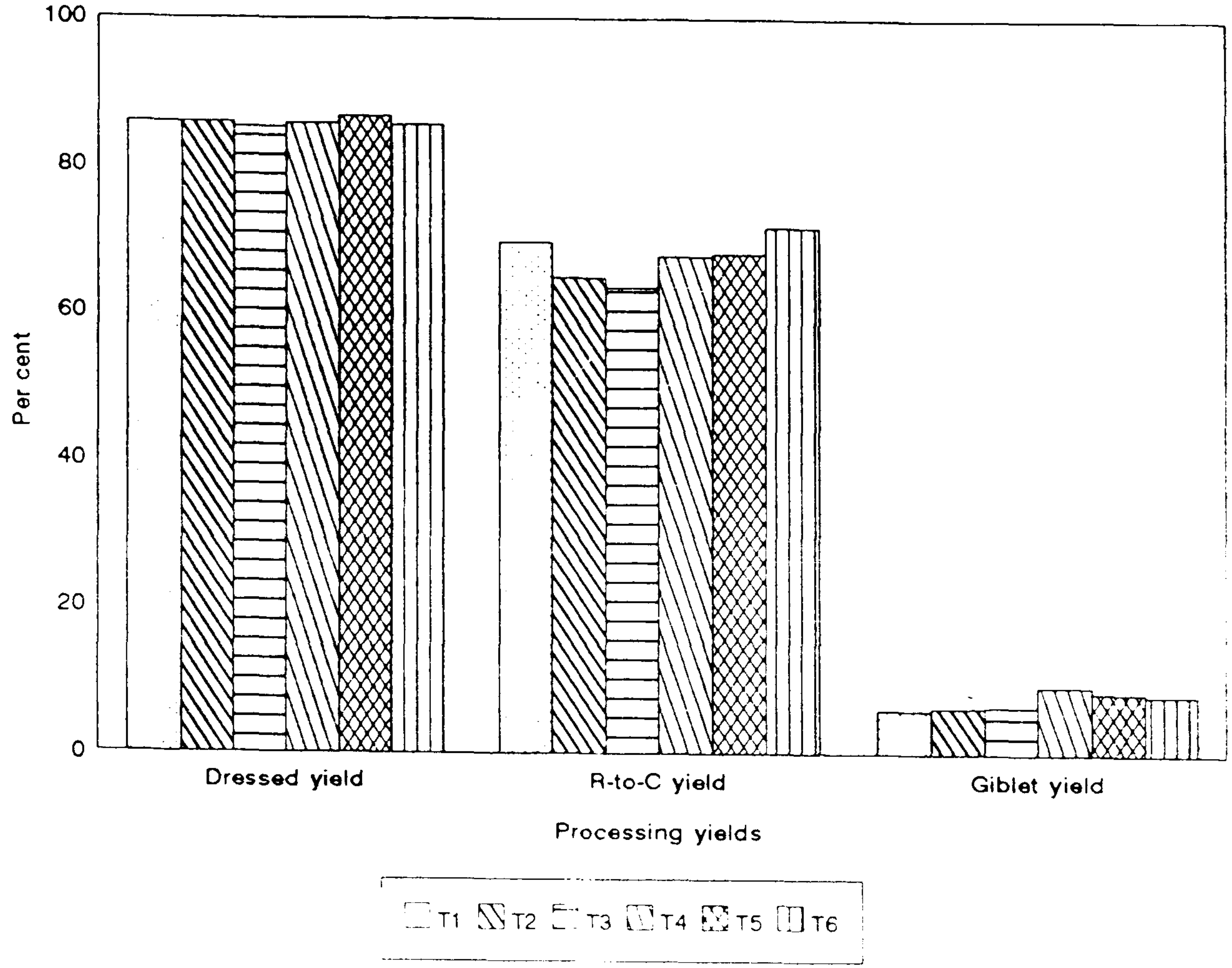
Means bearing the same superscript do not differ significantly (P<0.01)

Table 40. Influence of phytase supplementation on giblet yield - ANOVA

Source	d.f.	SS	MSS	F value
Treatment	5	64.152	12.830	33.803 **
Error	30	11.387	0.380	
Total	35	75.540		

** Significant (P<0.01)

Fig.13 DRESSED YIELD, READY-TO-COOK YIELD AND GIBLET YIELD AS INFLUENCED BY PHYTASE SUPPLEMENTATION



diet without phytase (T1) had numerically less giblet yield (6.02 per cent). When the differences in giblet yield among the various treatments were analysed statistically (Table 40), it was observed that phytase supplementation had a significant ($P<0.01$) influence on this trait. Significantly ($P<0.01$) more yield was obtained. From the birds offered a standard diet supplemented with phytase (T4) and was different from all other groups. It was also revealed that all the three phytase unsupplemented groups (T1, T2 and T3) had significantly ($P<0.01$) lower per cent giblet yield. Similarly, the treatments T5 and T6 had significantly higher giblet yield (8.45, 8.19 per cent respectively) than phytase unsupplemented groups but significantly lower yield than T4 (Standard diet + phytase).

The per cent dressed yield, ready-to-cook yield and giblet yield as influenced by phytase supplementation among different dietary treatments are depicted in Fig.13.

4.10 Livability

Mortality pattern of birds among the different treatment groups is presented in Table 41. Altogether eight birds died during the entire period of study. The percentage livability ranged from a minimum of 94.4 to a maximum of 97.8. There was numerically less mortality in groups fed diet supplemented

Table 41. Percentage livability among different dietary treatments

Period weeks	Mortality					
	T1	T2	T3	T4	T5	T6
1	-	-	-	-	-	1
2	-	1	-	-	1	-
3	1	-	-	1	-	-
4	-	1	1	-	-	-
5	-	-	1	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-
8	-	-	-	-	-	-
Total mortality	1	2	2	1	1	1
Per cent livability	97.2	94.4	94.4	97.2	97.2	97.2

with phytase and control group. Necropsy of dead birds did not show any signs that are attributable to treatment effect. The over all mortality in the experiment was within the standards prescribed for commercial broilers.

4.11 Economics

In order to assess the cost-benefit particulars of supplementation of phytase enzyme in low AP diet, 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 42. Cost of rations computed for different treatments viz., T1, T2, T3, T4, T5 and T6 were 9.30, 9.17, 9.24, 9.36, 9.23 and 9.30 Rupees per kg starter and 8.47, 8.32, 8.42, 8.53, 8.38 and 8.48 Rupees per kg finisher feed, respectively.

The cost-benefit analysis for different dietary treatments is set out in Table 43. Feed cost for production of one kilogramme live weight was worked out to Rs.19.92, 19.90, 20.19, 21.62, 20.02 and 19.74 for the treatments viz., T1, T2, T3, T4, T5 and T6 respectively. This revealed that the cost was lower in T6 (Rs.19.74) and higher in T4 (Rs.21.62). Total cost incurred for the production of one kilogramme of live weight chicken was found to be Rs.28.24, 28.82, 28.76, 29.96, 28.67 and 28.11 for the treatments T1,

Table 42. Cost of experimental rations

Ingredients	Cost/ kg* (Rs.)	Broiler starter ration						Broiler finisher ration					
		T1	T2	T3	T4	T5	T6	T1	T2	T3	T4	T5	T6
Yellow maize	6.09	331.90	333.42	333.42	331.90	333.42	333.42	328.86	327.33	328.86	328.86	327.33	328.86
Groundnut cake (exp)	12.34	357.86	357.86	357.86	357.86	357.86	357.86	320.84	320.84	320.84	320.84	320.84	320.84
Gingelly oilcake	10.34	82.72	82.72	82.72	82.72	82.72	82.72	-	-	-	-	-	-
Unsalted dried fish	10.25	71.75	71.75	71.75	71.75	71.75	71.75	71.75	76.88	74.31	71.75	76.88	74.31
Rice polish	4.89	-	-	-	-	-	-	53.79	53.79	53.79	53.79	53.79	53.79
Shell grit	3.49	0.87	3.49	1.75	0.87	3.49	1.75	2.62	5.25	3.49	2.62	5.25	3.49
Common salt	1.89	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Dicalcium phosphate	16.50	16.50	-	8.25	16.50	-	8.25	16.50	-	8.25	16.50	-	8.25
Trace mineral mixture	73.28	4.60	4.60	4.60	4.60	4.60	4.60	4.60	4.60	4.60	4.60	4.60	4.60
Vitamin mixture	441.00	11.03	11.03	11.03	11.03	11.03	11.03	11.03	11.03	11.03	11.03	11.03	11.03
Lysine Hydrochloride	170.00	42.50	42.50	42.50	42.50	42.50	42.50	27.20	27.20	27.20	27.20	27.20	27.20
Coccidiostat	181.00	9.05	9.05	9.05	9.05	9.05	9.05	9.05	9.05	9.05	9.05	9.05	9.05
Enzyme phytase	400.00	-	-	-	6.00	6.00	6.00	-	-	-	6.00	6.00	6.00
Total cost (100 kg)		929.25	916.89	923.40	935.25	922.89	929.40	846.71	831.19	841.89	852.71	837.19	847.89
Cost (kg)		9.30	9.17	9.24	9.36	9.23	9.30	8.47	8.32	8.42	8.53	8.38	8.48

* The rate contract approved by the university was taken as cost of feed ingredients

Table 43. Cost benefit analysis per bird for the different treatment groups

Sl. No.	Particulars	T1	T2	T3	T4	T5	T6
1.	Live weight (g)	2043	1906	1982	2038	1965	2030
2.	Total feed consumption (g)	4556	4314	4502	4894	4441	4453
	a) Starter ration (g)	2523	2392	2557	2779	2487	2609
	b) Finisher ration (g)	2033	1922	1945	2115	1954	1864
3.	Feed cost (Rs.)	40.69	37.93	40.01	44.06	39.33	40.07
4.	Feed + chick cost (Rs.)	52.69	49.93	52.01	56.06	51.33	52.07
5.	Total cost (Rs.)*	57.69	54.93	57.01	61.06	56.33	57.07
6.	Returns from sale of broilers (Rs.)	73.55	68.62	71.35	73.37	70.74	73.08
7.	Profit over feed cost (Rs.)	32.86	30.69	31.34	29.31	31.41	33.01
8.	Profit over feed + chick cost (Rs.)	20.86	18.69	19.34	17.31	19.41	21.01
9.	Net profit per bird (Rs.)	15.86	13.69	14.34	12.31	14.41	16.01
10.	Feed cost per kg body weight (Rs.)	19.92	19.90	20.19	21.62	20.02	19.74
11.	Total cost per kg body weight (Rs.)	28.24	28.82	28.76	29.96	28.67	28.11
12.	Profit over feed cost per kg body weight (Rs.)	16.08	16.10	15.81	14.38	15.98	16.26
13.	Net profit per kg body weight (Rs.)	7.76	7.18	7.24	6.04	7.33	7.89

* Rs.5/- per bird was accounted as miscellaneous cost for vaccination, medicines etc.

T2, T3, T4, T5 and T6 respectively. It was higher in the group fed a standard diet supplemented with phytase (T4) and lower in the treatment offered a diet with an AP level of 0.4 per cent plus phytase. The net profit per kilogramme live weight was higher in the group fed a diet containing 0.4 per cent AP supplemented with phytase than all other treatments.

Discussion

5. DISCUSSION

The results obtained from the study of phytase supplementation in low available phosphorus diet on the performance of broilers and other related parameters are discussed in this chapter.

5.1 Climatic parameters

The overall mean maximum and minimum temperatures recorded inside the experimental house during the trial period of eight weeks was 30.80°C and 24.95°C, respectively. The mean relative humidity was 92.79 per cent in the morning and 74.22 per cent in afternoon. These climatic observations revealed that the experimental period fell within the rainy season of Kerala.

5.2 Body weight

The mean fortnightly body weight data, as influenced by phytase supplementation, presented in Table 5 indicated that among the different treatments, the group fed standard broiler starter and finisher rations registered maximum body weight during sixth and eighth week of age. However, when the magnitude of difference in sixth and eighth week body weight was tested statistically, it was evident that the groups fed standard broiler starter and finisher rations with and without phytase and those fed ration containing an AP of 0.4 per cent supplemented with phytase had comparable body weights. All other groups obtained statistically lower body weights. The body weights of groups fed a diet having an AP of 0.4 per cent without enzyme and those maintained on a diet with an AP of 0.3 per cent supplemented with enzyme were statistically comparable.

The above finding is in agreement with Farrell *et al.* (1993) who studied the influence of phytase supplementation at a level of 750 units/kg diet and observed significant improvement in growth rate of broilers.

Significant improvement in body weight among broiler chicken fed low phosphorus diets supplemented with phytase was also reported by Huyghebaert *et al.* (1993a), Kiiskinen *et al.* (1994), Piva *et al.* (1994), Denbow *et al.* (1995), Sebastian *et al.* (1995) and Huyghebaert (1996). On the other hand, Zyla and Koreleski (1994) and Edwards and Roberson (1995) could observe neither significant improvement in growth nor impairment of growth when phytase was supplemented in broiler diets.

The results of this study revealed that a reduction of 0.1 per cent AP was compensated by the addition of 750 units of phytase per kg diet in as much as body weight of commercial broilers is concerned.

The microbial phytase supplemented in the diet might have acted upon the bound phytate phosphorus in the feed which resulted in the release of more inorganic phosphorus and other nutrients for effective utilization by the bird and subsequent better body weight. Increased feed intake in phytase supplemented groups might also have been responsible for better body weight.

5.3 Body weight gain

The mean fortnightly body weight gain data given in Table 7 revealed that except in the last fortnight there were significant differences in weight gain among the different treatments. In the first fortnight, birds fed a standard broiler diet and those fed diets supplemented with phytase irrespective of its AP content gained more weight than unsupplemented groups. However, in the second fortnight the group fed a standard diet supplemented with phytase gained 9.47 and 9.86 g more weight than groups offered a standard broiler diet and diet containing 0.4% AP supplemented with phytase respectively and the difference was statistically significant. All other groups gained significantly less weight.

In the third fortnight birds fed a standard broiler diet gained significantly more weight than all other groups except those fed diets containing 0.3 and 0.4 per cent AP both supplemented with phytase. It was also noticed that weight gain of all the enzyme supplemented groups were statistically comparable. The weight gain of birds fed diets having low AP was significantly lower. In the last fortnight, all birds irrespective of the treatment effects gained statistically comparable weights.

Since the fortnightly weight gain data did not show any definite trend due to treatment effects a critical assessment of cumulative weight gain data becomes essential in order to spell out meaningful conclusions.

A perusal of the mean cumulative body weight gain data presented in Table 7 indicated that 0-6 weeks gain was more (1434.44 g) with birds fed a standard diet without added enzyme (T1). Compared to this, T2, T3, T4, T5 and T6 gained 173.88, 92.80, 27.55, 75.85 and 30.35 g less weight, respectively. When the magnitude of difference in 0-6 weeks weight gain was analysed statistically, it was revealed that groups offered standard broiler diets with and without phytase and those fed diet containing 0.4% AP with phytase gained significantly more weight than other groups. It was also revealed that birds fed diet containing 0.4% AP without phytase gained comparable weight with those offered a 0.3% AP diet supplemented with phytase.

Cumulative weight gain data upto eight weeks showed that the control group i.e., birds offered a standard ration gained more weight (1996.54 g) than all other groups. Compared to control, the gain in weight was less to a tune of 5.13 and 12.79 g for the treatments T4 and T6 respectively. The cumulative weight gain from 0-8 weeks was statistically comparable among these three groups. Similar to six weeks weight gain trend eight weeks weight gain was statistically comparable between T3 and T5.

It implies that 750 units of phytase is capable of making available 0.1 per cent more AP without impairing the weight gain performance of broilers.

In a trial on broiler chickens fed diet low in phosphorus, supplemented with different levels of microbial phytase, Huyghebaert *et al.* (1993a) observed significant improvement in weight gain. Schoner *et al.* (1993a) also reported that supplementation of different levels of phytase to phosphorus deficient diets when fed to broiler chickens resulted in dose-dependent increase in live weight gain. The usefulness of different levels of microbial phytase on the improvement of phytate phosphorus availability in broiler diets was studied by Kornegay *et al.* (1996) and they observed a linear increase in body weight gain. The results of the present study is also in agreement with those reported by Sebastian *et al.* (1996) and Munaro *et al.* (1996). Improvement in body weight gain due to phytase supplementation observed in the present study was mainly related to the increased feed intake and in the light of the results of similar studies can be surmised that phytase supplementation can reduce available phosphorus requirements for the criteria like body weight/body weight gain.

5.4 Feed intake

The feed consumption (g/bird/day) of the birds in different dietary treatments (Table 10) revealed that it was significantly influenced by phytase supplementation in all the weekly periods of the experiment. The birds offered a standard diet supplemented with phytase continued to consume significantly more feed from the first week till the end of the experiment i.e., eight weeks of age than all other groups. Barring this trend, no specific pattern in daily feed consumption could be noticed due to treatment effects.

Irrespective of the treatments, the mean cumulative feed intake per bird was 2549.54 and 4546.64 g for six weeks and eight weeks respectively. The mean cumulative feed consumption per bird from 0 to 6 weeks and 0 to 8 weeks presented in

Table 12 indicated that this trait was significantly influenced by phytase supplementation. The mean cumulative feed intake upto six weeks was significantly more in T4 i.e., birds fed a standard diet supplemented with phytase and less in T2 i.e., birds fed 0.3% AP diet without phytase. It was also revealed that all enzyme supplemented groups consumed significantly more feed than their respective counterparts in the unsupplemented groups except in the group offered 0.4 per cent AP diet.

When the cumulative feed intake for the whole experiment was considered, birds in T4 continued to consume significantly more feed (4894.13 g) than all other treatments. Cumulative feed intake per bird was significantly less with birds under T2. Compared to T4, the treatments T1, T2, T3, T5 and T6 consumed 338.19, 580.13, 393.13, 452.82 and 441.27 g less feed respectively.

A critical evaluation of the total feed intake data also revealed that enzyme supplemented groups consumed significantly more feed than their respective unsupplemented groups except in the 0.4% AP diet group.

Denbow *et al.* (1995) conducted an experiment with broiler chicken by supplementing phytase at different levels to diets formulated to contain 0.2, 0.27 or 0.34% nonphytate phosphorus and reported that phytase supplementation significantly improved feed intake. In the present study also feed intake was significantly more with enzyme supplemented group except in the 0.4% AP diet group when 0-8 weeks total feed consumption was considered. However, this has to be viewed in conjunction with the general performance of this group. Significant increase in feed intake consequent to phytase supplementation in low phosphorus diet was also reported by Huyghebaert *et al.* (1993a), Schoner *et al.* (1993a), Richter and Cyriaci (1994), Kiiskinen *et al.* (1994) and Kornegay *et al.* (1996). However, Nernberg *et al.* (1997) made a study in which broiler chickens were fed diets containing 0.25% and 0.35% of available phosphorus supplemented with phytase and reported that improved feed intake was noticed only in diets containing 0.25% available phosphorus. Significantly higher feed intake observed in enzyme supplemented groups could be due to increased phytate phosphorus digestibility, since phytic acid may be imposing a restraint on voluntary feed intake.

5.5 Feed efficiency

Mean fortnightly feed conversion efficiency given in Table 14 revealed that significant differences existed among different treatments at different periods. Except in the last fortnight, birds fed standard broiler starter and finisher diets exhibited superior feed efficiency than other groups.

Similarly, the birds offered standard diet supplemented with phytase was inferior in feed efficiency throughout the experimental period. There was no specific pattern in feed conversion efficiency in other treatments during the entire course of this study.

The data on cumulative feed efficiency for six weeks (Table 14) indicated that enzyme supplemented groups were significantly inferior in converting feed to meat than the respective unsupplemented groups except in 0.4% AP diet fed groups in which the feed efficiency was comparable between phytase supplemented and unsupplemented birds. When the cumulative feed efficiency was considered for the whole experimental period, a deviation in the trend could be seen. The cumulative feed efficiency was 2.23, 2.26, 2.27, 2.40, 2.26 and 2.19 for the groups T1, T2, T3, T4, T5 and T6 respectively. The superior feed efficiency among the six treatments was recorded in birds fed with low available phosphorus (0.4%) diet with 750 units per kg diet of phytase enzyme (T6) followed by T1, T2, T5, T3 and T4.

The feed efficiency of T4 continued to be inferior when it was considered for the whole period. However, it was statistically comparable among the treatments T2, T3 and T5. Significantly better feed efficiency in group fed with 0.4% available phosphorus supplemented with phytase than other groups indicated the increased availability of nutrients for utilization. The release of phosphorus from phytic acid could be improved by the addition of microbial phytase to the diet. Phytase addition might also have contributed to the release of other nutrients too since in addition to the binding of phosphorus there are chances of interaction between phytic acid and other nutrients because of its molecular structure.

The present results confirm the observations of Simons *et al.* (1990), Schoner *et al.* (1993b), Simons and Versteegh (1996) and Carlos and Edwards (1997) who reported that addition of phytase enzymes in low phosphorus broiler diets had significantly improved feed efficiency. However, Denbow *et al.* (1995) could not observe any change in feed gain ratio due to phytase supplementation in broiler chicken. Similarly Nernberg *et al.* (1997) reported that phytase supplementation improved feed efficiency only in diets containing 0.25% AP whereas it was not affected in diets with 0.35% AP. Absence of any significant improvement in feed efficiency in these trials might be due to less activity of phytase since the enzyme activity is essentially dependent on temperature, pH value, type of substrate and substrate concentration.

5.6 Tibial ash

The per cent ash content of tibia of representative birds fed the experimental rations estimated at the end of sixth and eighth week of age is given in Tables 17 and 19 respectively. A perusal of the data revealed that tibial ash content was significantly (P<0.01) influenced by different treatments at both the periods.

At sixth week of age per cent tibial ash was significantly (P<0.01) more in groups fed standard broiler rations with and without supplementation of phytase. The results also indicated that supplementation of phytase to 0.3 and 0.4% AP diets caused an increase in tibial ash content of 1.20 and 1.83% respectively. The margin of difference was statistically significant between supplemented and unsupplemented groups.

An assessment of eighth week tibial ash (Table 19) revealed that birds offered a standard diet supplemented with phytase had the highest tibial ash content which was significantly (P<0.01) higher than all other treatments. The tibial ash content of T1 and T6 was statistically comparable. Birds maintained on diets having an AP content of 0.3 and 0.4 per cent had lower tibial ash values. The results indicated that phytase supplementation in diets have a positive effect on tibial ash. Therefore, it is reasonable to conclude that phytase will act upon the bound phytate phosphorus of the plant portion of the feed thereby liberating phosphorus. This enhanced phosphorus bioavailability causes increase in tibial ash per cent. Farrell *et al.* (1993), Richter *et al.* (1993a) and Broz *et al.* (1994) also recorded increased tibial ash content in broilers by the addition of phytase. A similar trend has also been reported by Huyghebaert *et al.* (1993a), Denbow *et al.* (1995), Edwards and Roberson (1995), Sebastian *et al.* (1995) and Biehl *et al.* (1995).

5.7 Serum calcium and inorganic phosphorus

The data on mean serum calcium (mg per cent) levels of birds of different treatments at sixth and eighth week of age presented in Tables 21 and 23 respectively, indicated that phytase supplementation did have a significant influence at sixth week of age only. It was significantly higher ($P<0.01$) in birds fed a standard diet supplemented with phytase. It was also revealed that sixth week serum calcium levels were statistically comparable among enzyme supplemented groups. Similarly, serum calcium levels of unsupplemented groups were significantly lower than their respective counterparts.

Mean serum inorganic phosphorus (mg per cent) measured at sixth and eighth week of age as influenced by different feeding regimen set out in Tables 25 and 27 respectively showed that this trait was significantly influenced only at sixth week of age. T4 registered significantly higher serum inorganic phosphorus while T2 the lowest. Statistically comparable values were obtained for the treatments T1 and T6. Similar to serum calcium levels, serum inorganic phosphorus at eight weeks of age was not significantly altered by phytase supplementation. However, enzyme supplemented birds had numerically higher values.

On screening the literature no work could be traced throwing light on the possible influence of phytase supplementation on serum calcium and inorganic phosphorus levels. However, the effects of phytase addition on plasma calcium and inorganic phosphorus were investigated by a few researchers. Edwards (1993) reported that supplementation of either phytase or 1,25-dihydroxy cholecalciferol in broiler chickens did not have any effect on plasma calcium or dialyzable phosphorus. Likewise, Edwards and Roberson (1995) could not observe any effect on plasma calcium and dialyzable phosphorus in broiler chicks fed diets supplemented with phytase. However, Sebastian *et al.* (1995) observed a significant reduction in calcium and increase in plasma phosphorus concentrations and increase in plasma phosphorus when broiler chicks were given low phosphorus diets supplemented with phytase.

Broz *et al.* (1994) found increased plasma inorganic phosphorus in birds fed low phosphorus diets supplemented with phytase compared to unsupplemented birds. Similarly, Munaro *et al.* (1996) and Sebastian *et al.* (1996) reported increased plasma phosphorus in broiler chickens fed diets supplemented with phytase. In the present study, a significant increase in serum calcium and inorganic phosphorus was observed in birds supplemented with phytase at sixth week of age. This indicated that phytase supplementation causes liberation of phosphorus from bound phytin phosphorus thereby improving the bioavailability of phosphorus which inturn results in increased serum calcium and inorganic phosphorus. Absence of significant increase in serum calcium and inorganic phosphorus at eight weeks of age observed in this study might possibly be due to smaller sample size. It is suggested to take up elaborate study with more sample size so that definite conclusion can be drawn.

5.8 Bioavailability of calcium and phosphorus

Per cent bioavailability of calcium and phosphorus was significantly ($P<0.01$) influenced by phytase supplementation (Tables 29 and 31). Bioavailability of both these nutrients was significantly more in enzyme supplemented groups. In each of the phytase supplemented group, bioavailability of calcium and phosphorus was significantly more than their corresponding unsupplemented treatment.

Schoner *et al.* (1993a), Sebastian *et al.* (1995), Kornegay *et al.* (1996) and Windisch and Kirchgessner (1996) reported significant increase in the retention of calcium when phytase was supplemented in broiler diets.

Likewise, Simons *et al.* (1990), Schoner (1992), Schoner *et al.* (1992b), Huyghebaert *et al.* (1993b), Schoner *et al.* (1993a), Farell *et al.* (1993), Broz *et al.* (1994), Edwards and Roberson (1995), Sebastian *et al.* (1995), Kornegay *et al.* (1996), Windisch and Kirchgessner (1996) and Carlos and Edwards (1997) also reported increased bioavailability of phosphorus with phytase supplementation.

From the results of the present study and the findings of other workers it can be inferred that phytase supplementation in broilers has a positive effect in improving the bioavailability of calcium and phosphorus. Addition of phytase in the diet acts upon the bound phytate phosphorus, thereby liberating inorganic phosphorus molecules and ultimately enhancing the bioavailability of nutrients like calcium and phosphorus.

5.8.1 Phosphorus excretion

Data on phosphorus excretion as influenced by phytase supplementation presented in Table 33 indicated that phosphorus content of the droppings (g/kg DM intake) was significantly reduced in enzyme supplemented groups compared to the respective unsupplemented group. It shows that phytase when added to diets facilitates in enhancing the availability of phosphorus from phytate phosphorus for utilization and hence less amount is excreted in droppings. Reduction of phosphorus excretion in the droppings by phytase supplementation was reported by Schoner (1992), Schoner *et al.* (1992b), Richter *et al.* (1993a), Broz *et al.* (1994), Huyghebaert (1996), Kornegay *et al.* (1996) and Nernberg *et al.* (1997).

5.9 Processing yields and losses

The per cent dressed yield was significantly (P<0.01) higher in birds fed diet having 0.4 per cent AP supplemented with phytase (T6) than other groups. The reduction in per cent dressed yield for the treatments T1, T2, T3, T4 and T5 as compared to T6 was 1.75, 1.77, 2.48, 1.88 and 0.94, respectively. Dressed yield percentage was statistically comparable between treatments T5 and T6. Likewise, it was comparable among the treatments T1, T2, T3, T4 and T5.

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5.9 Processing yields and losses

The per cent dressed yield was significantly ($P<0.01$) higher in birds fed diet having 0.4 per cent AP supplemented with phytase (T6) than other groups. The reduction in per cent dressed yield for the treatments T1, T2, T3, T4 and T5 as compared to T6 was 1.75, 1.77, 2.48, 1.88 and 0.94, respectively. Dressed yield percentage was statistically comparable between treatments T5 and T6. Likewise, it was comparable among the treatments T1, T2, T3, T4 and T5.

Per cent ready-to-cook yield data of birds belonging to different treatments (Table 37) showed that significantly ($P<0.01$) more yield was obtained for the treatment T6 i.e., 0.4 per cent AP diet supplemented with phytase. There was no significant difference between T6 and T1. Likewise, the per cent ready-to-cook yield of treatments T1, T4 and T5 was statistically comparable. The per cent ready-to-cook yield was less to the tune of 2.13, 6.85, 8.12, 3.89 and 3.53 for the treatments T1, T2, T3, T4 and T5 respectively in comparison to T6.

The per cent giblet yield data given in Table 39 revealed that it was significantly more in the group fed a standard diet supplemented with phytase and was different from other treatments. The giblet yield was statistically comparable between treatments T5 and T6. Birds fed diet without phytase (T1, T2 and T3) had significantly lower per cent giblet yield.

On reviewing the literature it was felt that information on the influence of phytase supplementation on the processing yields and losses is meagre. Therefore, a comparison between the findings of this study and other similar investigations becomes difficult. Richter *et al.* (1993b) conducted two separate trials to find out the effects of supplementing phytase in broiler diets and reported that phytase supplementation significantly increased slaughter weight. In the present study also per cent dressed yield, ready-to-cook yield and giblet yield improved consequent to phytase addition. However, more studies are warranted before making a final conclusion.

5.10 Livability

The data on the per cent livability of birds under different dietary treatments revealed that it ranged from 94.4 to 97.8 per cent. During the entire course of the experiment covering eight weeks only eight birds died. Low mortality was observed in control and groups fed low available phosphorus diets supplemented with phytase. Higher mortality was recorded among birds fed low available phosphorus diet without enzyme. Necropsy findings revealed that the birds died due to non-specific reasons. Thus it is evident that phytase supplementation did not have any detrimental effects on the physiological well being of broiler chicken.

In the same line, Vogt (1992), Schoner *et al.* (1993b), Richter and Cyriaci (1994), Denbow *et al.* (1995), Kornegay *et al.* (1996) and Sohail and Roland (1997) reported improvement in survivability of birds fed low phosphorus diet supplemented with phosphatase enzyme (phytase). The better percentage of livability might be due to enhanced availability of nutrients with phytase supplementation.

5.11 Economics

An analysis of the cost of different rations employed in this experiment revealed that the standard broiler diets for both starter and finisher periods, formulated as per BIS specifications were costlier than low phosphorus diets with or without enzyme but cheaper than standard rations with enzyme. In order to prepare rations with 0.3 and 0.4 per cent AP instead of standard mineral mixture only required minerals of feed grade were incorporated. This resulted in the reduction of the cost of ration with low AP. However, supplementation of phytase to low phosphorus diets enhanced the cost of rations. But even with phytase supplementation (750 units/kg diet) the cost of low phosphorus diet was six paise less for starter diet with 0.3 per cent AP than the standard and the cost of diet with 0.4 per cent AP was comparable with that of the standard, whereas in finisher diet it was nine paise less and one paise higher for diets with 0.3 per cent and 0.4 per cent AP supplemented with enzyme than the standard, respectively.

When the cost of production per kg live weight on feed cost alone was calculated, it was observed that compared to low phosphorus diets all other rations were costlier except the diet with 0.4 per cent AP supplemented with phytase (T6) which is being the cheapest compared to all other rations.

The net profit per bird was 15 paise more for diets with 0.4 per cent AP supplemented with phytase compared to standard control. The economic analysis indicated the desirability of incorporation of phytase at a level of 750 units/kg in broiler diets having an AP level of 0.4 per cent in as much as it could bring down the feed cost as well as production cost per kilogramme of live weight.

Summary

6. SUMMARY

An investigation was carried out in the Department of Poultry Science, College of Veterinary and Animal Sciences, Mannuthy, using two hundred and sixteen day-old commercial broiler chicks (Varna) to assess the influence of phytase supplementation on phosphorus utilization and subsequent performance. The chicks were randomly distributed into six dietary treatments each having three replicates of 12 birds each. The dietary treatments consisted of standard broiler ration (T1), broiler ration with 0.3 per cent available phosphorus (AP) (T2), broiler ration with 0.4 per cent AP (T3), standard broiler ration with 750 units of phytase (Natuphos[R]-5000) per kg of feed (T4), broiler ration with 0.3 per cent AP supplemented with 750 units of phytase per kg of feed (T5) and broiler ration with 0.4 per cent AP supplemented with 750 units of phytase per kg of feed (T6). All the diets were formulated as per BIS specifications except in the level of AP.

Feed ingredients viz., yellow maize, groundnut cake (exp), gingelly oil cake, unsalted dried fish and rice polish were used for the formulation of experimental diets. Each replicate was housed at random in individual pens and reared under deep litter system of management. Scientific managemental procedures were adopted throughout the

experimental period. The duration of the experiment was eight weeks. Feed and water were provided *ad libitum*. Broiler starter diets were fed upto six weeks of age and then switched over to broiler finisher diets till the end of the experiment. The body weight of individual birds were recorded at the beginning of the experiment followed by fortnightly intervals. Replicatewise weekly feed consumption was recorded. From the above data, the feed conversion efficiency and the body weight gain for different treatments were worked out.

At the end of the sixth week, two birds from each replicate were randomly selected and sacrificed to collect blood samples and tibia for the estimation of serum calcium, serum inorganic phosphorus and tibial ash. Serum calcium, serum inorganic phosphorus and tibial ash were also determined at the end of eighth week. Two birds from each replicate were utilized for slaughter studies at eight weeks of age to assess dressed yeild, ready-to-cook yield and giblet yield. At the end of the experiment, metabolism trial was also undertaken using six birds from each treatment. Total collection method was employed. Based on the data obtained from the metabolism trial, bioavailability of calcium, phosphorus and phosphorus excretion were calculated. Mortality of the birds was recorded. Cost-benefit analysis was also worked out.

The overall performance of the birds fed different dietary regimens are presented in Table 44.

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

1. The mean fortnightly body weight of the birds were significantly ($P<0.01$) influenced by different dietary regimens. The groups fed standard broiler starter and finisher rations with and without phytase and those fed rations containing an AP of 0.4 per cent supplemented with phytase had significantly higher body weights at sixth and eighth week of age. All other groups obtained statistically lower body weights. The body weights of groups fed a diet having an AP of 0.4 per cent without enzyme and those maintained on a diet with an AP of 0.3 per cent supplemented with enzyme were statistically comparable. The mean body weight of the birds for different treatment groups, ranged from 1308 g to 1481 g at six weeks and 1907 g to 2043 g at eight weeks of age.

2. The mean fortnightly body weight gain data revealed that except in the last fortnight there were significant ($P<0.01$) differences in weight gain among the different treatments. It was also revealed that birds fed diet containing 0.4 per cent AP without phytase gained comparable weight with those offered a 0.3 per cent AP

diet supplemented with phytase. It implies that 0.1 per cent AP in broiler diets could be substituted with 750 units of phytase without impairing the weight gain performance of broilers.

3. The mean daily feed consumption (g/bird/day) of the birds in different dietary treatments was significantly influenced by phytase supplementation in all the weekly periods of the experiment. Cumulative feed intake per bird was significantly less with birds fed diet having 0.3 per cent AP without enzyme. All the enzyme supplemented groups consumed significantly ($P<0.01$) more feed than their respective counterparts in the unsupplemented groups except in the group offered 0.4 per ent AP. The mean cumulative feed consumption per bird under different dietary regimens ranged from 2.393 kg to 2.779 kg upto six weeks and 4.314 kg to 4.894 kg upto eight weeks.

4. The cumulative feed efficiency for different dietary treatment groups ranged from 1.70 to 1.91 upto six weeks and 2.19 to 2.27 upto eight weeks. Significantly superior feed efficiency was recorded in birds fed a diet having 0.4 per cent AP supplemented with 750 units of phytase per kg diet.

5. The per cent tibial ash content was significantly (P<0.01) influenced by different treatments at sixth and eighth week of age. Phytase supplementation had a positive effect on tibial ash content.

6. Serum calcium and inorganic phosphorus (mg per cent) were significantly higher in enzyme supplemented groups than their respective counterparts at sixth week of age. However, eighth week serum calcium and inorganic phosphorus were not influenced by enzyme supplementation.

7. Per cent bioavailability of calcium and phosphorus was significantly (P<0.01) influenced by phytase supplementation. In each of the phytase supplemented groups, bioavailability of calcium and phosphorus was significantly more than their corresponding unsupplemented treatments. Phosphorus excretion in the droppings (g/kg DM intake) was significantly reduced in enzyme supplemented groups compared to the respective unsupplemented group.

8. The per cent dressed yield and ready-to-cook yield were significantly (P<0.01) higher in birds fed diet having 0.4 per cent AP supplemented with phytase (T6) than other groups. The per cent giblet yield was significantly (P<0.01) more in the group fed a standard diet supplemented with phytase (T4) than other treatment.

9. Per cent livability was better with enzyme supplemented groups.

10. The feed cost per kg of weight varied from Rs.19.74 to Rs.21.62 for the different treatments. The cost of production per kg live weight when feed cost alone was considered was less in groups fed with 0.4 per cent AP supplemented with phytase (T6). The net profit per kg body weight was 13 paise higher in groups maintained on 0.4 per cent AP supplemented with phytase compared to standard broiler ration.

Among the different treatments the performance of birds fed with 0.4 per cent available phosphorus diet with 750 *u*/kg diet of phytase was found to be the best and economical. Based on the results of this study it could be inferred that by the addition of 750 units of phytase enzyme per kg of diet, the available phosphorus level in broiler rations using common feed ingredients can be reduced upto 0.1 per cent without affecting the overall performance. The supplementation of phytase will not only enhance the utilization of phosphorus and other nutrients but also reduces the phosphorus excretion in the dropping that are normally encountered with normal poultry diets.

Table 44. Influence of phytase supplementation on the phosphorus utilization and subsequent performance in broilers

Sl. No.	Particulars	Dietary treatments					
		T1	T2	T3	T4	T5	T6
1.	Live weight (g)	2043	1906	1982	2038	1965	2030
2.	Body weight gain (g)	1997	1874	1934	1991	1918	1984
3.	Total feed consumption (g)	4556	4314	4502	4894	4441	4453
	a) Starter ration (g)	2523	2392	2557	2779	2487	2609
	b) Finisher ration (g)	2033	1922	1945	2115	1954	1864
4.	Cumulative feed efficiency	2.23	2.26	2.27	2.40	2.26	2.19
5.	Tibial bone ash (per cent)						
	a) At sixth week of age	44.37	38.70	40.55	44.47	39.90	42.38
	b) At eighth week of age	46.06	39.25	41.87	46.94	44.22	45.69
6.	Biochemical parameters						
	i) Serum calcium (mg per cent)						
	a) At sixth week of age	11.03	11.65	10.88	12.48	12.04	12.04
	b) At eighth week of age	11.12	10.73	10.92	12.39	11.10	10.92
	ii) Serum inorganic phosphorus (mg per cent)						
	a) At sixth week of age	5.66	4.60	5.29	6.04	5.02	5.64
	b) At eighth week of age	6.95	5.90	6.46	7.07	6.86	7.23
7.	Bioavailability studies						
	a) Calcium (per cent)	62.13	53.09	58.37	64.19	57.28	61.28
	b) Phosphorus (per cent)	51.28	38.08	46.08	55.85	45.60	52.31
	c) Phosphorus excretion (g/kg DM intake)	5.38	3.64	4.76	4.20	2.35	3.85
8.	Slaughter studies						
	a) Dressed yield (%)	85.80	85.78	85.04	85.67	86.61	87.55
	b) Ready-to-cook yield (%)	89.44	64.72	63.45	67.68	68.04	71.57
	c) Giblet yield (%)	6.02	6.35	6.53	9.29	8.45	8.19
9.	Livability (per cent)	97.2	94.4	94.4	97.2	97.2	97.2
10.	Cost per kg of feed (Rs.)						
	a) Starter ration	9.30	9.17	9.24	9.36	9.23	9.30
	b) Finisher ration	8.47	8.32	8.42	8.53	8.38	8.48
11.	Net profit per kg body weight (Rs.)	7.76	7.18	7.24	6.04	7.33	7.89

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INFLUENCE OF PHYTASE ON PHOSPHORUS UTILIZATION IN BROILERS

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ABSTRACT OF A THESIS

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ABSTRACT

An investigation was carried out utilizing 216 commercial day-old broiler chicks to evaluate the influence of supplementation of phytase enzyme on phosphorus utilization and subsequent performance. The birds were divided into six dietary treatment groups viz., standard broiler ration (T1), broiler ration with 0.3 per cent available phosphorus (AP) (T2), broiler ration with 0.4 per cent AP (T3), standard broiler ration with 750 units of phytase per kg of feed (T4), broiler ration with 0.3 per cent AP supplemented with 750 units of phytase per kg of feed (T5) and broiler ration with 0.4 per cent AP supplemented with 750 units of phytase per kg of feed (T6). Standard broiler ration was formulated as per BIS (1992) specifications. Initially, the ration with 0.3 per cent AP was formulated. By the addition of appropriate levels of dicalcium phosphate to this ration, diets with 0.4 and 0.5 per cent AP were formulated. The groups fed standard broiler starter and finisher rations with and without phytase and those fed rations containing an AP of 0.4 per cent supplemented with phytase had significantly ($P<0.01$) higher body weights and body weight gain than other treatments. All enzyme supplemented treatments consumed significantly ($P<0.01$) more feed (g/bird/day) than their respective unsupplemented groups except in the group offered 0.4 per cent AP. Significantly ($P<0.01$) superior feed efficiency was recorded in groups fed a diet having 0.4 per cent AP supplemented with 750 units of phytase per kg of feed.

Phytase supplementation had a positive effect on tibial ash. Serum calcium and inorganic phosphorus (mg per cent) were significantly ($P<0.01$) higher in enzyme supplemented groups than their respective counterparts at sixth week of age but were not influenced at eighth week of age. Bioavailability of calcium and phosphorus was significantly ($P<0.01$) more in enzyme supplemented groups than their corresponding unsupplemented groups. Phosphorus excretion in the droppings (g/kg DM intake) was significantly ($P<0.01$) reduced in enzyme supplemented groups. The per cent dressed yield and ready-to-cook yield were significantly ($P<0.01$) higher in groups fed a diet having 0.4 per cent AP supplemented with phytase than other groups. The per cent giblet yield was significantly ($P<0.01$) more in group fed a standard diet supplemented with phytase than other groups. The per cent livability was better with enzyme supplemented groups. The cost of production per kg live weight (when feed cost alone was considered) was cheaper in groups fed with 0.4 per cent AP supplemented with phytase. The net profit per kg live weight was 13 paise higher in groups maintained on 0.4 per cent AP supplemented with phytase compared to standard broiler ration.

Based on the above findings it can be concluded that by the addition of 750 units of phytase per kg of diet, the available phosphorus level in broiler rations can be reduced by 0.1 per cent without affecting the overall performance.