EFFECT OF CITRIC ACID AND MICROBIAL PHYTASE ON PHOSPHORUS UTILIZATION AND GROWTH IN BROILER CHICKEN

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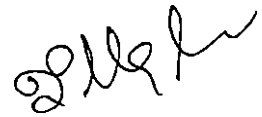Kerala Agricultural University, Thrissur

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DECLARATION

I hereby declare that this thesis, entitled "**EFFECT OF CITRIC ACID AND MICROBIAL PHYTASE ON PHOSPHORUS UTILIZATION AND GROWTH IN BROILER CHICKEN**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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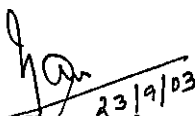Mannuthy.

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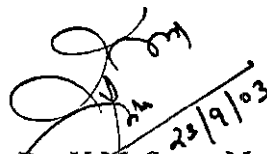
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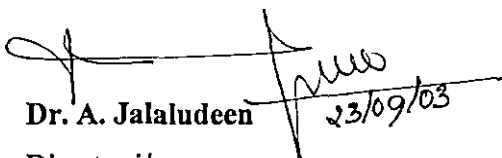College of Veterinary and Animal sciences
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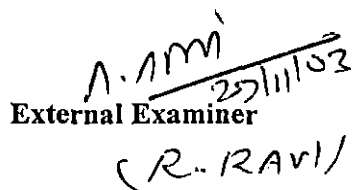Department of Animal Nutrition
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Dedicated to

My Beloved

Parents &

Teachers

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Introduction

1. INTRODUCTION

Poultry industry has attained remarkable growth in India with a phenomenal increase in broiler production over the past two decades. Among various livestock enterprises, poultry farming has metamorphosed into a modern and vibrant industry contributing substantially to the Gross Domestic Product (GDP). As our country faces an overwhelming demand for animal protein, the broiler chicken that provides the cheapest source of animal protein is being exploited to meet out the demand. However, the productivity level per bird in India on an average is still low compared to several other countries and a wide gap exists between availability and minimum requirement of egg and poultry meat.

Broiler production, which was only four million in1971, has gone up to 700 million in1998 (Anon, 2000). The present annual per capita availability of poultry meat is one kg in India compared to 15 kg in developed countries. There is immense scope for future development in poultry segment, since the ICMR recommendation is 10.8 kg meat per person per annum from all sources. The anticipated annual growth of commercial layers and broilers in the coming years is estimated at around 10 and 20 per cent,respectively.

One of the crucial inputs that would determine successful and sustainable development in poultry industry is the availability of quality feed in required quantity. As feed accounts for 70-75 per cent of total production cost of poultry, efficient utilization of feed is extremely important to poultry producers. But availability of feed ingredients is critical, due to the competition that exists between human beings and livestock for the same. A feedstuff found suitable as an ingredient for poultry feed rapidly becomes main input for some emerging industries making it unavailable for livestock and poultry production on cost front. This often necessitates the poultry farmer to look into ways and means to develop alternate strategies for maximum utilization of feed.

Major ingredients in poultry diets are of plant origin such as cereals, cereal by-products and oil seed cakes, which are blended together to provide necessary energy and protein for optimizing production. The plant-derived ingredients are rich in phosphorus (P), but only about one third of the P is present in inorganic form, which is easily digestible. The remaining two third is present as organic P especially in the form of salts of phytic acid – phytates (Myo-inositol hexakisphosphates) that cannot be utilized and excreted as such by poultry, due to insufficient quantities of enzyme phytase in the GI tract (Nelson, 1967).

Apart from its unavailability, phytates may combine with starch, protein and certain elements such as Ca, Mg, Zn, Mn, Cu, Fe, Co, Ni and K. Being insoluble these compounds precipitate in the gut, without getting absorbed and finally excreted. Over supplementation of P as well as other nutrients is also common in the feed industry because of the safety margin for the requirements. This excess supplementation leads to P excretion through droppings and is responsible for environmental pollution.

The need for inorganic P in the rations can be reduced considerably if phytate P can be utilized by poultry. To make phytate P biologically available, it is necessary to hydrolyse them by phytase. The enzyme phytase, a normal constituent of feed ingredients like soybean meal, rape seed meal, corn, wheat etc. help in degrading the phytate to a certain extent, but the activity of vegetable phytase is limited as they act only at a narrow pH range. This necessitates the use of extraneous source of this enzyme.

Phytase is more active at low (acidic) pH. Furthermore, low intestinal pH can increase the solubility of P and Ca and improve P and Ca absorption in the small intestine. So organic acids, which lower the gut pH, will potentiate the effects of phytase into the small intestine. Citric acid being an organic acid can improve phytate P utilization. Citric acid and phytase may have some additive or synergistic effects in poultry (Boling et al., 2000). A proper combination of citric

acid and phytase may represent a practical solution for improving phytate P utilization and decreasing P levels in poultry excreta, thus environmental pollution.

Apart from overcoming the ill effects of phytate P, citric acid and microbial phytase have been reported to enhance the growth performance of broiler chicken.

Since, no pioneering work has been done in India and only very few works have been done elsewhere to study the interaction effect of citric acid and microbial phytase in poultry, a study was undertaken to evaluate the effect of citric acid and microbial phytase on mineral utilization and growth performance in broiler chicken.

Review of Literature

2. REVIEW OF LITERATURE

Recently, supplementation of enzymes in animal feeds are getting popular to increase productivity, to use available resources more efficiently and to prevent environmental pollution. It is believed that phytase of fungal origin improves the utilization of phytate P, Ca and trace minerals along with protein in poultry and swine diets based on ingredients of plant origin. There is a general conception that organic acids like citric acid which lower the gut pH will potentiate the effects of phytase in the small intestine. In this chapter, a sincere attempt has been made to provide a review of the available literature allied with this topic as well as related topics.

2.1. MODE OF ACTION OF ORGANIC ACIDS

The mode of action of citric acid may be associated with its Ca-complexing property (Hamilton and Dewar, 1937; Day, 1940; Pileggi *et al.*, 1956).

Pileggi *et al.* (1956) suggested that the antirachitogenic effects of citric acid in rats were attributed to a reduction of the inhibitory effect of Ca on phytic acid hydrolysis.

Studies with chicks and pigs have indicated a positive response to dietary addition of various organic acids, including formic (Vogt *et al.*, 1981) and fumaric acids (Vogt *et al.*, 1981; Giesting and Easter, 1985). Alterations in gut pH, activation of protease enzyme and modifications of intestinal microflora were the possible modes of action for the organic acids.

Growth promotion action and improvement in the utilization of nutrients resulting from the addition of fumaric acid in the diet might be attributed not only to gastrointestinal effects, but also to improved energy and protein utilization in

intermediary metabolism and to changes in certain enzyme activity (Kirchgessner and Roth, 1982).

Falkowski and Aherne (1984) revealed that addition of 1 or 2 per cent fumaric or citric acid lowered the pH of pig diets from 5.6 (control) to 5.0, 4.5, 4.9 and 4.5 respectively and this reduction in pH might have decreased the pH value of stomach contents and increased pepsin activity.

Jongbloed et al. (1996) stated that supplemental citric acid might enhance the solubility of digesta P and the transit time of digesta in the small intestine by lowering digesta pH, thereby improving total P absorption and growth performance in pigs.

Qing et al. (1996) suggested that citric acid and phosphoric acid in the swine diet tended to decrease gastrointestinal pH values, increase activities of trypsin and amylase and reduce the number of coliform bacteria in the colon, thus improves the performance.

Organic acids are reported to cause a higher protein and energy digestibility and retention, an alteration of bacterial populations and metabolites in the gastro intestinal tract and possibly an effect on metabolism in young pigs (Roth and Kirchgessner, 1998).

Citric acid, a strong chelator of Ca, removes Ca from, or decreases Ca binding to, the phytate molecule, thus making it less stable and more susceptible to endogenous and exogenous phytase (Boling et al., 2000).

Broek (2000) opined that the organic acids could be divided into two groups based on the mode of action viz., 1. Acids with indirect effect on decreasing bacterial population by lowering the environmental pH (fumaric acid, malic acid, citric acid and lactic acid). Their action is mainly restricted to stomach and in intestine, the pH is brought back to 6.5 by high bicarbonate secretion. 2. Organic acids, which act directly by interfering in the bacterial cell with enzyme

complexes, destroy cell membranes and DNA-duplicating mechanism. Eg. Formic acid, acetic acid, propionic acid and sorbic acid. These acids also have the pH lowering action.

Phytase is more active at low (acidic) pH. Further more, low intestinal pH increases the solubility of P and Ca, and improves P and Ca absorption in the small intestine. So organic acids, which lower the gut pH, will potentiate the effects of phytase in the small intestine (Overland *et al.*, 2002).

Puyalto and Mesia (2002) reported that organic acids have a bactericidal action. They act by being diffused inside the cell without producing lysis in bacterial membranes. This causes a reduction in pH and increase in acidity in the cytoplasm and results in inhibition of synthesis of certain macromolecules as well as various membrane components, RNA, DNA, proteins and lipids.

2.2   BODY WEIGHT AND WEIGHT GAIN

2.2.1   Effect of Organic Acids

Sifri *et al.* (1977) from his studies in growing chicks for 28 days using 0.587 per cent P, 0.40 per cent and 0.85 per cent Ca diets with 0 and 0.71 per cent citric acid *observed significant increase in body weight gain due to increase in Ca,* but there was no effect of citric acid on body weight gain.

Falkowski and Aherne (1984) reported that on feeding crossbred pigs from *four to eight weeks of age with diets containing either 0, 1 or 2 per cent fumaric* acid or citric acid, average daily gain was four to seven per cent greater (P<0.05) for pigs fed diets containing fumaric or citric acid.

Patten and Waldroup (1987) *reported that addition of 0.5 or 1.0 per cent* fumaric acid significantly (P<0.05) improved body weights of broilers and addition of Ca formate at levels greater than 0.72 per cent significantly reduced body weight.

When corn-soybean meal (CS) or corn-soybean-whey (CSW) basal diets supplemented with 0 or 1.0 per cent and 0, 0.5 or 1.0 per cent of a commercial acid product consisting of citric acid and Na citrate (2:1), addition of one per cent acid significantly improved (P<0.01) growth rate of pigs fed the CS diet (Burnell *et al.*, 1988).

In a five week feeding trial, Risley *et al.* (1991) reported that supplementation with 1.5 per cent citric acid in diets for weanling pigs tended to improve body weight gain during the first four weeks with no effect during week five, while 1.5 per cent fumaric acid caused smaller and non significant improvement in weight gain.

Effect of acidification of starter diet was also studied by Maxwell *et al.* (1993) and stated that piglets given 0.5 per cent citric or fumaric acid (unprotected acid) diet had a greater body weight gain than those given 0.3 per cent Triacid 300® (a fatty acid coated protected acid containing 300 g acid/kg).

Krause *et al.* (1994) concluded that the 2.5 per cent citric acid or malic acid with sodium bicarbonate diets resulted in increased average daily gain in pigs compared with the 2.5 per cent fumaric acid with sodium bicarbonate and the diets without organic acids or sodium bicarbonate taken as the control.

Maheswari and Kadirvel (1996) reported that the inclusion of malic acid (0.5 and 1.0 per cent) in diets had resulted in a marginal but statistically insignificant improvement in weight gain in broiler chicken.

Roth and Kirchgessner (1998) observed a significant improvement in growth rate of weaning pigs by the dietary inclusion of formic, lactic, sorbic, fumaric, citric and malic acids as well as with different salts of formic acid.

In an experiment in broiler chicks from day old to 42 days of age, supplementation of citric acid, citric acid and sodium citrate (1:1 mixture) or citric acid, sodium citrate and potassium citrate (1:1:1 mixture) at levels of 0, 4.5 and

6.0 per cent to a P deficient diet containing 0.22 per cent available P and 0.91 per cent Ca, Metwally (2001) observed increased body weight gain by increased levels of dietary citrate to 4.5 per cent.

Boling-Frankenbach *et al.* (2001b) evaluated the effect of different levels of citric acid (0, 2, 4 or 6 per cent) on P utilization in chicks fed on low P (0.1 per cent available P) corn-soybean meal diets and found that 4 and 6 per cent citric acid produced the largest response in growth from eight to 21 days of age.

Diets supplemented with either 1.5 per cent citric acid, 1.6 per cent lactic acid or 50 ppm of enrofloxacin showed significantly better growth performance (P<0.05) in growing piglets than control group fed free of antimicrobials (Tsiloyiannis *et al.*, 2001).

Chinbin *et al.* (2002) showed that starter diets supplemented with 2.0 per cent citric acid or 0.8 per cent lactic acid did not improve the growth performance of weanling pigs.

2.2.2 Effect of Phytase

Phytase supplementation in low available P diets significantly increased growth rate in broiler chicks (Broz *et al.*, 1994).

Biehl *et al.* (1995) conducted a 12 day feeding trial (from eight to 20 days of post hatching) in chicks fed on a Zn deficient soya concentrate diet (Zn 13 mg/kg) and observed increased growth rate by 40 per cent when chicks were fed diet supplemented with 1, 25 $(OH)_2$ D_3 at 10 mg/kg or phytase at 1200 U/kg.

Growth promoting effect of phytase when supplemented to low P diets in chicks was also reported by several workers (Denbow *et al.*, 1995; Mitchell and Edwards, 1996a,b; Yi *et al.*, 1996b; Qian *et al.*, 1997) and in turkey poults by Qian *et al.* (1996).

Huff *et al.* (1998) reported that phytase supplementation at a level of 500 U/kg on control and high available P corn containing diets increased body weight (P<0.05) at 49 days of age in Cobb male broilers.

Kanagaraju (1998) observed that broilers maintained on low available P (0.40 per cent) diet supplemented with phytase at 750 U/kg feed recorded significantly higher body weight and weight gain both at sixth and eighth week of age and was statistically comparable to that of group maintained on standard broiler ration. The body weight recorded for control and phytase supplemented low available P diet groups was 1480.67 and 1450.33 g at sixth and eighth week of age, respectively. Likewise, the gain in weight was 1434.44 g and 1404.09 g at sixth week and 1996.54 g and 1985.75 g at eight week of age for control and phytase added low available P diet.

Zanini and Sazzad (1998) reported no influence of phytase on growth performance in broiler chicks.

Significantly higher body weight and weight gain on phytase supplementation were reported by Cabahug *et al.* (1999), Sohail and Roland (1999) and Balasubramanian (2000).

Waldroup *et al.* (2000) reported that phytase supplementation significantly increased body weights in birds, however the response to phytase was greater when it was added to diets containing normal corn, and the magnitude of the response was the greatest for the lower non-phytate P levels. Phytase supplementation (1000 U/kg) to low P diet (-0.15 per cent of NRC) significantly improved body weights in broiler chicks from day old to 56 days of age (Yan *et al.*, 2000)

Ravindran *et al.* (2001) conducted an experiment in broiler chicks from day seven to 28 post hatching with diets containing one per cent lysine and 0.45 per cent non-phytate P, supplemented with graded levels of phytase 125, 250, 375,

500, 750 or 1,000 U/kg diet and revealed that the response in weight gain to added phytase reached a plateau at 500 FTU/kg diet (quadratic effect, $P<0.001$).

Yan *et al.* (2001a) conducted a feeding trial in broiler chicks from three to six weeks of age fed diets with 0.10 to 0.45 per cent non-phytate P in increments of 0.05 per cent, with or without supplementation of phytase (800 U/kg) and concluded that body weight gain was linearly improved by phytase supplementation in diets containing non-phytate P up to 0.30 per cent.

Yan *et al.* (2001b) conducted a 21 day feeding trial in broiler chicks in a factorial arrangement of three Ca levels (0.5, 0.7 and 0.9 per cent) with eight levels of non-phytate P (0.15 to 0.50 per cent in increments of 0.05 per cent) supplemented with or without phytase (1000 U/kg) and concluded that addition of phytase to these diets improved body weight as a result of increased availability of P.

Aksakal and Bilal (2002) studied the effect of various Ca:total P ratios (1:1 and 2:1), the addition of microbial phytase (600 U/kg) or vitamin D_3 (5 µg/kg) on the performance of broilers and observed that live weight gain was significantly higher ($P<0.05$) in groups fed with Ca:total P ratio of 1:1 and added phytase.

Augspurger *et al.* (2003) found that an *E. coli* derived phytase (500 FTU/kg diet) resulted in superior ($P<0.01$) weight gain compared with *Aspergillus niger* (Natuphos®; 500 FTU/kg diet) or *Peniophora lycii* (Ronozyme®; 500 FTU/kg diet) phytase enzymes. They found no synergism when a 3-phytase (Natuphos®) was combined with a 6-phytase (Ronozyme®or *E. coli* phytase) in young chicks and pigs.

2.2.3 Combined Effect of Organic Acids and Phytase

Han *et al.* (1998) reported that average daily gain was similar between pigs fed with diets containing 15 or 10 per cent wheat middling, 300 U/kg microbial

phytase and 1.5 per cent citric acid with no inorganic P and control diets with added inorganic P (0.2 per cent).

Radcliffe *et al.* (1998) conducted a feeding trial in crossbred weanling pigs fed with corn-soybean meal based diet, low in Ca and P (0.33 per cent) supplemented with three levels of citric acid (0, 1.5 or 3.0 per cent) and four levels of phytase (0, 250, 500 or 750 U/kg) in a 3 x 4 factorial arrangement. They concluded that the addition of citric acid improved (P<0.05) average daily gain, but no synergistic effects were observed.

In chicks fed with diets containing 0.62 per cent Ca and 0.10 per cent available P, graded doses of citric acid + sodium citrate (1:1,wt:wt) mixture (1, 2, 4 or 6 per cent of diet) resulted in linear increases (P<0.01) in body weight gain (Boling *et al.*, 2000). Relative to chicks fed no citric acid, weight gain (g/day) was increased by 22 per cent in chicks, fed 6 per cent citrate diet. Addition of 1,450 U of phytase/kg of the diet with 6 per cent citrate caused further improvements (P<0.05).

Jongbloed *et al.* (2000) have also reported significant (P<0.05) increase in average daily gain in pigs on phytase and organic acid (lactic and formic) supplementation.

Angel *et al.* (2001a) indicated that phytase (300 and 600 FTU/kg) and citric acid (1, 2 and 3 per cent) added to a low non-phytate P (0.44 per cent) and 1.20 per cent Ca diet improved (P<0.05) body weight gain in broiler chicks from eight to 15 days of age, but no interactions were seen. Whereas, Angel *et al.* (2001b) reported that phytase (0, 200 and 500 FTU/kg), 25-hydroxy cholecalciferol (0 and 70 µg/kg) and citric acid (0 and 3 per cent) in a low non-phytate P (0.16 per cent) and 0.80 per cent Ca diet in a 3x2x2 factorial experiment did not affect (P>0.05) body weight gain in broiler chicks from 14 to 24 days of age.

2.3. FEED CONSUMPTION AND FEED CONVERSION RATIO

2.3.1 Effect of Organic Acids

Sifri *et al.* (1977) reported that using citric acid at 0 and 0.71 per cent levels in marginally low (0.40 per cent) and adequate (0.85 per cent) Ca diets resulted in no effect on gain: feed ratios in growing chicks up to 28 days of age.

Feeding trial conducted in cross bred pigs from four to eight weeks of age with diets containing either one or two per cent fumaric acid or citric acid resulted in improved feed conversion efficiency (P<0.05) approximately five to 10 per cent by addition of either acid to the diet (Falkowski and Aherne, 1984).

Edmonds *et al.* (1985) evaluated the efficacy of copper (Cu) sulfate (to provide 250 mg Cu/kg), antibiotic-sulfa combinations (ASP) and anhydrous citric or fumaric acid (0.75 to 1.50 per cent of the diet) in 32 day-old weaned piglets and found that citric or fumaric acid supplementation at 1.5 per cent of the diet increased feed utilization, with response occurring in both the presence and absence of Cu, ASP or the combination of Cu and ASP.

Burnell *et al.* (1988) recorded improved feed efficiency in pigs by addition of a commercial acid product consisting of citric acid and Na citrate (2:1) to both corn-soybean and corn-soybean-whey basal diets.

Radecki *et al.* (1988) reported that feed intake during first week was depressed (P<0.05) by adding citric acid (1.5 or 3.0 per cent), and gain: feed ratio during week one to two, in a four week trial in four week old weaning pigs.

Supplementation with 1.5 per cent citric acid in diets for weanling pigs tended to improve the efficiency of feed utilization during the first four weeks with no effect during week five, but 1.5 per cent fumaric acid caused smaller and non significant improvements in feed efficiency (Risley *et al.*, 1991).

Maxwell *et al.* (1993) reported that piglets given 0.5 per cent citric or fumaric acid (unprotected acid) diet had a greater feed intake than those given 0.3 per cent Triacid 300® (protected acid; a fatty acid coated product containing 300 g acid/kg).

Krause *et al.* (1994) reported increased feed intake in chicks, when fed with diets supplemented with 2.5 per cent citric acid or malic acid with sodium bicarbonate when compared to 2.5 per cent fumaric acid with sodium bicarbonate or the basal diets.

Campabadal *et al.* (1995) reported that citric acid (1.0 or 2.0 per cent) supplementation improved feed efficiency in weaned piglets.

Maheswari and Kadirvel (1996) reported that inclusion of malic acid at 0, 0.5 and 1.0 per cent levels in broiler diets up to four weeks of age did not produce any apparent difference either in feed consumption or feed/gain between the various groups.

When weaned piglets were fed diets without (control) or with 1.0 per cent citric acid, 3.0 per cent phosphoric acid and 3.0 or 0.5 per cent acid blends for 28 days, supplements of citric acid and acid blends resulted in increases of 11 to 15 per cent (P<0.05) and eight per cent, respectively, in feed conversion efficiency (Qing *et al.*, 1996).

Metwally (2001) reported that increasing levels of dietary citrate to 4.5 per cent increased feed consumption and FCR, when citric acid, citric acid and sodium citrate (1:1) mixture and citric acid, sodium citrate and potassium citrate (1:1:1) mixture were added at levels of 0, 4.5 and 6.0 per cent to a P deficient diet containing 0.22 per cent available P and 0.91 per cent Ca, fed to broiler chicks from one to 42 days of age.

In an experiment with weaned piglets, Tsiloyiannis *et al.* (2001) indicated that supplementation of either 1.5 per cent citric acid, 1.6 per cent lactic acid or 50 ppm of enrofloxacin improved (P<0.05) feed conversion ratio.

2.3.2   Effect of Phytase

Feeding trial conducted in broiler chicks with varying levels of available P viz., 0.32, 0.38 and 0.44 per cent and phytase at the levels of 0.5, 1.0 and 1.5 per cent (250, 500 and 750 U/kg) in a factorial arrangement by Perney *et al.* (1993) revealed increase in feed intake and feed conversion efficiency due to increased dietary available P, but not due to phytase, whereas Broz *et al.* (1994) reported that graded amounts of supplemental phytase (125, 250 or 500 PU/kg diet) significantly increased feed intake, but moderately improved feed conversion efficiency.

Biehl *et al.* (1995) conducted an experiment in broiler chicks from eight to 20 days of age to study the efficacy of 1200 U phytase/kg, 10 μg/kg 1,25 $(OH)_2$ D_3, and its combination in a low available P (0.1 per cent) diet on the performance of broilers and observed that both supplemental phytase and 1,25 $(OH)_2D_3$, alone or in combination, resulted in significantly higher (P<0.01) feed intake.

Denbow *et al.* (1995) stated that both added inorganic P and phytase *increased feed intake with the greatest response to added phytase at the lowest non-phytate P levels*, in male broiler chicken.

Qian *et al.* (1996) reported linear increase in feed intake in male turkey poults when fed with corn-soybean meal diet supplemented with 300, 600 or 900 U of phytase/kg in combination with four Ca:total P ratios of 1.1, 1.4, 1.7 and 2:1, and two levels of non-phytate P of 0.27 and 0.36 per cent in a 21 day trial.

Supplementation of defluorinated phosphate to provide 0.36, 0.45 or 0.54 per cent non-phytate P to a basal diet with 0.27 per cent non-phytate P and supplementation of phytase (350, 700 or 1050 U/kg) to the basal diet with 0.27 per

cent non-phytate P linearly increased feed consumption of broilers by six to 25 per cent (Yi *et al.*, 1996b).

In a 4 x 4 x 2 factorial experiment with 1.4, 1.7 and 2:1 Ca:total P ratio, 0, 300, 600 and 900 U phytase/kg diet and 66 and 660 μg of vitamin D_3/kg diet, Qian *et al.* (1997) reported that feed intake of broiler chicks was linearly increased (P<0.001) by added phytase, negatively influenced by widening the Ca:total P ratio and synergistically improved by addition of phytase and vitamin D_3.

Huff *et al.* (1998) could not observe any significant difference in feed conversion efficiency in broiler chicks by phytase supplementation (500 U/kg diet) in control and high available P corn diet.

Kanagaraju (1998) observed superior feed efficiency among birds fed diet containing 0.4 per cent available P with supplemental phytase (750 U/kg diet) compared to unsupplemented group.

Cabahug *et al.* (1999) conducted a 19 day feeding trial in seven day old male broilers fed on diets containing three concentrations of phytic acid (10.4, 13.2 and 15.7 g/kg; equivalent to 2.9, 3.7 and 4.4 g/kg phytate P) two of non-phytate P (2.3 and 4.5 g/kg) and three of microbial phytase (0, 400 and 800 FTU/kg diet) caused improvements in feed:gain of broilers, but the magnitude of the response was greater in low non-phytate P diets, resulting in significant non-phytate P and phytase interactions.

Sohail and Roland (1999) observed no effect of added phytase (300 and 600 FTU/kg) on FCR in broiler chicks. Balasubramanian (2000) reported higher cumulative feed intake from zero to eight weeks of age and significantly improved (P<0.01) feed efficiency on phytase supplementation at 750 and 1000 U/kg of low available P diets in broiler chicks.

Waldroup *et al.* (2000) conducted a study in young (zero to three weeks) broiler chicken fed with two sources of corn (normal or high available P corn) at

variable non-phytate P levels (0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50 per cent) with and without phytase supplementation (800 U/kg diet) and concluded that phytase supplementation significantly improved feed conversion and greater improvement was observed when phytase was added to the diets with high available P corn compared with diets containing normal corn.

Yan *et al.* (2000) also reported improved feed efficiency on phytase supplementation (1000 U/kg diet) to low P diets in broiler chicks.

Ravindran *et al.* (2001) observed no effect of phytase in broiler chicks on gain per feed up to 250 U/kg diet and increased (quadratic effect, $P<0.05$) by further additions (375, 500, 750 or 1000 U/kg diet) to a basal diet containing one per cent lysine and 0.45 per cent non-phytate P.

Yan *et al.* (2001a) reported significant improvement in feed conversion efficiency in broiler chicks on phytase supplementation (800 U/kg) to diet with graded levels of non-phytate P (0.1 to 0.45 per cent in increments of 0.05 per cent).

On studying the effect of various Ca:total P ratios (1:1 and 1:2) with the addition of microbial phytase (600 U/kg) or vitamin D_3 (5 μg/kg) on the performance of broilers, Aksakal and Bilal (2002) revealed that phytase addition has increased the feed consumption and feed conversion in broiler diets having Ca:total P ratio of 1:1.

2.3.3 Combined Effect of Organic Acids and Phytase

Han *et al.* (1998) reported that gain:feed ratio was significantly higher ($P<0.02$) in pigs fed with diets containing 10 per cent wheat middling, 300 U/kg microbial phytase and 1.5 per cent citric acid with no added inorganic P when compared to control diets with added inorganic P (0.2 per cent) and without phytase, citric acid or wheat middling during week one to three.

In a feeding trial with crossbred weanling pigs fed with corn-soybean meal diet, low in Ca and P (0.33 per cent P) supplemented with three levels of citric acid (0, 1.5 or 3.0 per cent) and four levels of phytase (0, 250, 500 or 750 U/kg) in a 3 x 4 factorial arrangement, Radcliffe et al. (1998) reported that addition of citric acid resulted in improved (P<0.05) feed efficiency, but no synergistic effect was observed on supplementing citric acid along with phytase.

In an experiment with growing pigs fed with basal diet containing 0.6 per cent Ca and 0.1 per cent digestible P was either or not supplemented with phytase (410 U/kg) and with lactic acid (0, 1.6 and 3.2 per cent) or formic acid (0, 0.8 and 1.6 per cent), both microbial phytase and the organic acids had a positive effect (P<0.05) on growth rate and feed conversion ratio, but there was no synergistic effect (Jongbloed et al., 2000).

Supplementation of phytase, citric acid or both to a low P diet showed improvement (P<0.05) in feed consumption and feed efficiency from 8 to 15 days of age in broiler chicks, but no interactions were seen (Angel et al., 2001a).

Angel et al. (2001b) reported that feed consumption was lower (P<0.05) when citric acid (3.0 per cent) was added to low non-phytate P (0.16 per cent) diet, but feed to gain ratio was positively affected by addition of phytase (200 or 500 FTU/kg) and citric acid in broiler chicks from 14 to 24 days of age, but no interactions were seen.

2.4 PROTEIN EFFICIENCY RATIO

Maheswari and Kadirvel (1996) revealed that inclusion of malic acid at 0, 0.5 and 1.0 per cent in diets had nearly the same protein efficiency ratios in all the three experimental groups in broiler chicks up to four weeks of age.

Peter et al. (2000) conducted an experiment in chicks during the period of eight to 21 day post hatching fed with diets containing 10 per cent CP furnished by corn-gluten meal (CGM) or casein in the presence and absence of 1,200 U/kg

phytase. They observed a protein source x phytase interaction (P<0.05) for weight gain, gain:feed and protein efficiency ratio, indicating positive responses to phytase when casein was fed, but negative responses to phytase when CGM was fed.

Boling-Frankenbach et al. (2001a) studied the efficiency of phytase (1200 U/kg) for increasing protein efficiency ratio (PER) values for several feed ingredients fed to chicks from eight to 17 or 20 days of age and indicated that phytase addition had no significant effect (P>0.10) on PER values for any of the ingredients evaluated.

In growth trials with young chicks, Peter and Baker (2001) evaluated crude protein (CP) utilization in soybean meal as affected by dietary addition of 1200 U phytase/kg diet and concluded that phytase addition did not affect (P>0.10) measures of protein utilization namely, weight gain/protein intake (PER) and protein gain/protein intake at any of the CP levels that were fed.

2.5   NUTRIENT UTILIZATION

2.5.1 Dry Matter Retention

Campabadal et al. (1995) reported that citric acid supplementation (1.0 or 2.0 per cent) in the diet did not improve DM digestibility in weaned piglets.

Jongbloed et al. (1996) studied the main and interactive effects of microbial phytase Natuphos® (0 vs 550 FTU/kg) in combination with lactic acid (1.6 vs 3.6 per cent), formic acid (0.8 vs 1.6 per cent), and propionic acid (0.5 vs 1.0 per cent) in growing pigs and revealed that the organic acids and microbial phytase exerted a positive effect on the apparent digestibility of DM.

Yi et al. (1996b) reported that supplementation of defluorinated phosphate to provide 0.36, 0.45 or 0.54 per cent non-phytate P and phytase at 350, 700 or 1050 U/kg diet increased apparent digestibility of DM in broiler chicken when

compared to basal control diet with 0.27 per cent non-phytate P without added phytase.

Li *et al.* (1998) conducted an experiment in pigs with corn-soybean meal diet, containing 0.38 per cent total P and no added inorganic P, supplemented with phytase (750 U/kg) alone or in combination with either 1.5 per cent citric acid or 2000 IU/kg of added vitamin D_3 and concluded that apparent digestibility of DM was significantly improved (P<0.05) by the addition of phytase. They also stated that the addition of vitamin D_3 and/or citric acid tended to further increase (P>0.05) the DM digestibility.

Radcliffe *et al.* (1998) studied the effects of microbial phytase, citric acid and their interaction in a corn-soybean diet for weaning pigs and found that addition of phytase (250 or 500 U/kg) linearly improved DM digestibility (P<0.05), while citric acid had no influence on DM digestibility.

Um *et al.* (1999) studied the effects of microbial phytase supplementation to diets with low non-phytate P levels on the performance and bioavailability of nutrients in laying hens and reported apparent increase in DM retention by addition of phytase (250 U/kg) to low P diets, while Um and Paik (1999) observed significantly increased DM retention (P<0.05) in brown laying hens by phytase supplementation (500 U/kg) to low P diets.

Chung *et al.* (2000) reported that acidifier treatments with 1.5 per cent citric acid, 1.5 per cent fumaric acid, 1.5 per cent Ca formate, 0.2 per cent Acidlac® or 0.2 per cent Stacidem® (commercial preparations) tended to improve ileal digestibility of dry matter in early weaned piglets.

Jongbloed *et al.* (2000) conducted an experiment with growing pigs fed on basal diet containing 0.6 per cent Ca and 0.1 percent digestible P either or not supplemented with phytase (410 U/kg) and with lactic acid (0, 1.6 and 3.2 per cent) or formic acid (0, 0.8 and 1.6 per cent). They found that apparent total tract

digestibility of DM was significantly enhanced by organic acids (P<0.001) and microbial phytase (P = 0.015).

Mroz *et al.* (2000) randomly assigned eight treatments to pigs (2 x 4 factorial arrangement) differing in the contest of acidogenic Ca benzoate (0 vs 2.4 per cent) and organic acids (none vs formic acid vs fumaric acid vs n-butyric acid) in the concentration of 300 mEq acid/kg feed and revealed that addition of all organic acids (formic and n-butyric acid, in particular) exerted a positive effect (P<0.05) on DM digestibility.

Feeding trial conducted in weaned piglets with low Ca (0.92 per cent), low P (0.52 per cent) diets, supplied with potassium diformate (KDF; 0, 0.9 and 1.8 per cent) in the absence or presence of microbial phytase (1,450 FTU/kg) revealed that in the absence of phytase, KDF tended to improve the apparent digestibility of dry matter by 1.7 per cent. However, simultaneous and excessive addition of phytase reversed the tendency (Fevrier *et al.*, 2001)

2.5.2   Nitrogen Retention and Excretion

2.5.2.1 *Effect of Organic Acids*

Campabadal *et al.* (1995) reported that citric acid supplementation (one or two per cent of diet) improved CP digestibility in weaned piglets.

When the effect of additives (citric acid 10 g, olaquinodox 100 mg, Zn-bacitracin 40 mg or Zn (as Zn oxide) 3.0 g/kg feed) were compared with a control diet without additives on apparent precaecal digestibility of crude nutrients, Fasshauer and Kienzle (1995) observed that apparent precaecal protein digestibility tended to increase by additive supplementation.

Feeding of weaned piglets with diets supplemented without (control) or with 1.0 per cent citric acid, 3.0 per cent phosphoric acid and 3.0 or 0.5 per cent

acid blends for 28 days resulted in no significant differences in dietary protein digestibility among treatments (Qing *et al.*, 1996).

When early weaned piglets were subjected to six dietary treatments, namely 1.5 per cent citric acid, 1.5 per cent fumaric acid, 1.5 per cent Ca formate, 0.2 per cent Acidlac®, 0.2 per cent Stacidem® and control without any acidifier, citric acid treatment was highest in faecal digestibility of essential amino acids among treatments (Chung *et al.*, 2000).

2.5.2.2 Effect of Phytase

The beneficial effects of microbial phytase in diets of broilers was investigated by Farrell *et al.* (1993) and reported that N retention was significantly improved with phytase supplementation (750 U/kg diet).

In Cobb broilers, diet containing high activity phytase at a level of 1000 U/kg diet for 55 days showed a greater retention of protein than with control diet (Piva *et al.*, 1995).

Yi *et al.* (1996b) reported that phytase supplementation at levels of 350, 700 or 1050 U/kg in corn-soybean meal diets had significantly increased apparent retention of N ($P < 0.06$) in broiler chicken from day-old to 21 days of age, whereas Kwon *et al.* (1996) reported that addition of phytase at 500 FTU/kg diet had no influence on N excretion. Windisch and Kirchgessner (1996b) also could not observe any influence of phytase, supplemented at 0, 200, 400, 600 and 1000 U/kg feed on N utilization.

Biehl and Baker (1997) opined that true amino acid digestibility (TAAD) values of essential amino acids were increased by approximately two per cent when 1200 U/kg phytase was included with soybean meal diet and administered to cacectomized roosters.

In an attempt to reduce N and P waste production form broilers through diet manipulation, Ferguson *et al.* (1997) conducted a trial in which male broilers were given low crude protein and low P starter and grower diets and found that phytase supplementation at a level of 1.0 g/kg diet produced considerable savings on N wastage through droppings.

Piao *et al.* (1998) found considerable reduction in N excretion in Arbor acres broilers when fed with corn-soybean meal diets supplemented with 0.05 per cent Kemzyme® (commercial enzyme mixture) or 0.1 per cent phytase or 0.1 per cent yeast or combination of these three supplements.

To evaluate the effectiveness of supplemental Natuphos®-5000 phytase on the apparent digestibility, Zanini and Sazzad (1998) performed a 21-day trial with day-old broilers and observed that supplemental phytase at 500 U/kg diet increased apparent retention of N.

Phytase supplementation (250 U/kg diet) to low P diets had not significantly affected the retention of CP and excretion of N in brown laying hens (Um *et al.*, 1999).

Balasubramanian (2000) reported that graded levels of phytase (500, 750 and 1000 U/kg diet) addition to low available P (0.3 per cent) diet from zero to eight weeks of age linearly increased N retention in broiler chicken at eight weeks of age.

Ravindran *et al.* (2001) conducted an experiment in broiler chicks from seven to 28 day post hatching with diets containing one per cent lysine and 0.45 per cent non-phytate P which was supplemented with L-lysine monochloride to provide 1.06, 1.12 or 1.18 per cent lysine or with 125, 250, 375, 500, 750 or 1,000 U phytase/kg diet and concluded that addition of increasing levels of supplemental phytase to the lysine deficient diet improved (P<0.001) the digestibility of N and all amino acids.

2.5.2.3 Combined Effect of Organic Acids and Phytase

Li *et al.* (1998) stated that N digestibility was increased (P<0.05) by phytase (750 U/kg diet) in combination with vitamin D_3 (2000 IU/kg diet) and/or citric acid (1.5 per cent) but not when supplemented alone, compared to low (0.18 per cent) and high (0.36 per cent) available P control diets. They also reported that faecal N excretion was 8.2 per cent lower for pigs fed phytase compared to high available P control diets while further reductions were obtained when fed in combination with citric acid or vitamin D_3.

In a digestibility trial, Fevrier *et al.* (2001) studied the effect of adding potassium diformate (KDF; 0, 0.9 and 1.8 per cent) and phytase excess (1,450 FTU/kg) for weaned piglets and revealed that in the absence of phytase, KDF tended to improve the N retention by 2.1 per cent.

2.5.3   Calcium, Phosphorus and Magnesium Availability and Phosphorus Excretion

2.5.3.1 Effect of Organic acids

Metabolism trial conducted in pigs fed with liquid diets based on maize/soybean meal/tapioca meal having low, medium or high Ca and P supplemented with or without citric acid (2.0 per cent) revealed that citric acid increased apparent absorption of both Ca and P (Schenkel and Roser, 1991).

Effect of citric acid (1.5 per cent) added to a maize-soya diet with or without Zn supplementation (30 or 100 mg/kg) on the availability of minerals in pigs was studied by Hohler and Pallauf (1993) and indicated that availability of Ca and P was improved by citric acid supplementation.

Hohler and Pallauf (1994) also reported that on feeding diets supplemented with Zn 40, 60 or 80 mg/kg, Zn 40 mg/kg plus 1.5 per cent citric acid or Zn 60

mg/kg plus 1.5 per cent citric acid, the apparent absorption and retention of Ca, P and Mg were not affected by citric acid supplementation in piglets.

Roth *et al.* (1998) reported that addition of a complex of formic acid and potassium formate (Formi^TM LHS) at a dietary level of 1.8 per cent in pigs increased the retention by nine per cent for P, three per cent for Ca and eight per cent for Mg when compared to control diet. They also found that potassium diformate reduced P excretion through feces and urine by 8.1 per cent.

Boling *et al.* (2000) indicated that citric acid (1, 2, 3 or 4 per cent) did not improve the utilization of dietary P in laying hens from 20 to 40 weeks of age fed a corn-soybean meal diet containing 3.8 per cent Ca and 0.1 per cent available P.

A Ca-deficient basal diet (0.54 per cent Ca and 0.45 per cent available P) containing 0 to 0.9 per cent supplemental Ca in 0.1 per cent increments was fed with or without 6 per cent citric acid to chicks from eight to 21 days of age and the results indicated that citric acid did not significantly affect the Ca utilization. When a P deficient basal diet (0.20 per cent available P) supplemented with 0 to 0.25 per cent inorganic P with or without 4 or 6 per cent citric acid fed to chicks, citric acid increased the P utilization in corn-soybean meal diets (Boling-Frankenbach *et al.*, 2001b).

Metwally (2001) studied the influence of supplemental citric acid and sodium and potassium citrate on phytate P utilization in broiler chicks fed P deficient (0.22 per cent available P) diets from one to 42 days of age and indicated that the mixture of citric acid, sodium and potassium citrate (1:1:1 mixture) at a level of 4.5 per cent enhanced phytate P utilization in broiler diets.

2.5.3.2 *Effect of Phytase*

The effect of graded levels of dietary phytase (0, 250, 500 and 750 U/kg) and increasing levels of available P (0.32, 0.38 and 0.44 per cent) on P metabolism in broilers was evaluated by Perney *et al.* (1993) and could notice

significant linear increase in P excretion (P<0.01) by increasing dietary available P and significant decrease by supplemental phytase.

Broz *et al.* (1994) reported that graded levels of supplemental phytase (125, 250 or 500 PU/kg) improved apparent availability of P and reduced its concentration in excreta (P<0.05) in broiler chicken receiving low P diets (0.44 per cent) without additional inorganic P.

Denbow *et al.* (1995) found that the phytate bound P in soybean meal was made more available to broilers by microbial phytase.

Supplementation of phytase (600 U/kg), 1,25-$(OH)_2D_3$ (5 μg/kg) or both in broiler diets with varying P levels resulted in increased P retention in all the groups indicating significant phytase, Vitamin D_3 and dietary P interaction. The excreta P was also reduced by phytase (P<0.04) and lesser extent (P<0.10) by 1, 25-$(OH)_2 D_3$ (Mitchell and Edwards ,1996b).

Increased retention of Ca and P on phytase supplementation to low P diet was reported by Qian *et al.* (1996) in turkey poults and Sebastian *et al.* (1996a) and Yi *et al.* (1996b) in broiler chicken.

Qian *et al.* (1997) studied the utilization of phytate P and Ca as influenced by microbial phytase, cholecalciferol and the Ca:total P (tP) ratio in broiler diets and revealed that added phytase (0, 300, 600 and 900 U/kg) linearly increased (P<0.001) Ca and P retention, which was negatively influenced by widening of dietary Ca:total P ratio beyond 1.4:1 and synergistically improved by addition of vitamin D_3 (66 and 660 μg/kg).

Kanagaraju (1998) reported that in broilers bioavailability of Ca and P was significantly (P<0.01) more on phytase supplementation (750 U/kg feed) compared to corresponding unsupplemented groups. Enzyme supplementation also resulted in a significant reduction in P excretion.

Effectiveness of supplemental Natuphos®-5000 phytase on mineral utilization of broilers was also studied by Zanini and Sazzad (1998) and observed that phytase addition at the level of 500 U/kg diet had favouring effect on reducing P excretion and increasing apparent retention of Ca and P.

On studying the effects of phytase supplementation to diets with low non-phytate P levels (0.21, 0.16 and 0.11 per cent) in laying hens, Um *et al.* (1999) reported increased (P<0.05) retention of Ca, P and Mg on phytase supplementation (250 U/kg diet).

Um and Paik (1999) also reported that phytase supplementation (500 U/kg) significantly increased (P<0.05) the retention of Ca, P and Mg and reduced the excretion of P and Zn in laying hens.

Balasubramanian (2000) reported that the addition of phytase at levels of 500, 750 and 1000 U/kg feed in low available P (0.3 per cent) diets improved the Ca and P at all levels with significant (P<0.01) increase at levels of 750 and 1000 U/kg. P excretion was significantly (P<0.01) reduced on enzyme supplementation compared to control (0.5 per cent available P) and LAP (0.3 per cent available P) diet groups.

Waldroup *et al.* (2000) stated that faecal P output could be reduced in broilers by the use of reduced dietary non-phytate P, introduction of high available P corn and phytase supplementation.

Reducing dietary P levels in yellow dent corn (YDC) and high available P corn (HAPC) diets to 0.15 per cent below NRC (1994) recommendations along with phytase supplementation (1000 U/kg diet) resulted in 37.5 and 58.2 per cent reduction in faecal P levels respectively, as compared with broilers fed the YDC diet with NRC (1994) recommended P levels (Yan *et al.,* 2000).

Yan *et al.* (2001a) reported that phytase supplementation (800 U/kg) in broiler chicks fed diets with 0.10 to 0.45 non-phytate P in increments of 0.05 per cent reduced P excretion at the lower P levels.

2.5.3.3 *Combined Effect of Organic Acids and Phytase*

On studying the main and interactive effects of microbial phytase Natuphos® (0 vs 550 FTU/kg) in combination with lactic acid (1.6 vs 3.6 per cent), formic acid (0.8 vs 1.6 per cent), and propionic acid (0.5 vs 1.0 per cent) in growing pigs Jongbloed *et al.* (1996) concluded that phytase and organic acids exerted a positive effect on apparent digestibilities of Ca and total P with significant ($P<0.01$) interaction between organic acids and microbial phytase for P digestibility.

Li *et al.* (1998) reported that the apparent digestibility of Ca and P was significantly ($P<0.05$) improved by the addition of phytase (750 U/kg). They also stated that the addition of vitamin D_3 (2000 IU/kg) and/or citric acid (1.5 per cent) tended to further increase the digestibility of Ca and P ($P<0.05$) in corn-soybean meal diet in growing swine. P excretion from pigs fed low P (0.38 per cent) diet, phytase alone or in combination with citric acid or vitamin D_3 was 27 per cent less than pigs fed with control diets, containing 0.51 per cent P and no added phytase or vitamin D_3.

Radcliffe *et al.* (1998) studied the effects of microbial phytase, citric acid and their interaction in a corn-soybean meal-based diet for weanling pigs and found that addition of citric acid (1.5 or 3.0 per cent) improved ($P<0.05$) Ca digestibility and addition of phytase (250 or 500 U/kg) linearly increased ($P<0.05$) Ca and P digestibilities, but no synergistic effects were observed.

Jongbloed *et al.* (2000) reported that microbial phytase and organic acids significantly enhanced apparent total tract digestibility of Ca and P and a

synergistic effect of organic acids and microbial phytase was found for P and Mn in growing pigs.

Fevrier *et al.* (2001) conducted a digestibility trial in weaned piglets with low Ca (0.92 per cent) and low P (0.52 per cent) diets, supplied with potassium diformate (KDF; 0, 0.9 and 1.8 per cent) in the absence or presence of microbial phytase (1,450 FTU/kg) and concluded that the digestibility of P (P<0.001) and Ca (P<0.04) were significantly improved by the addition of phytase, however KDF at the highest level impaired the effect of phytase.

Addition of 500 U microbial phytase/kg and 0.35 per cent organic acid mixture (Nutri-acid®) composed mainly of citric acid to pig starter diets improved P digestion and utilization, thereby leading to a reduction in P excretion (Omogbenigun *et al.*, 2003).

2.5.4   Availability of Zinc and Manganese

2.5.4.1 Effect of Organic Acids

Hohler (1992) conducted three experiments to study the influence of supplementing diets with citric or fumaric acid (1.5 per cent) on utilization of Zn and other essential minerals in piglets and found improved utilization of Zn by citric acid supplementation, while fumaric acid had no effect. He also stated that Fe, Cu and Mn were not affected by acid supplementation.

Increased Fe and Zn availability was reported by Hohler and Pallauf (1993) in male pigs on dietary supplementation of citric acid at 1.5 per cent level, whereas according to Hohler and Pallauf (1994), apparent absorption and retention of Zn, Fe, Cu and Mn were not affected by citric acid supplementation.

Edwards and Baker (1999) observed a marked increase in Zn bioavailability of soybean meal in chicks on citric acid supplementation.

2.5.4.2 Effect of Phytase

Roberson and Edwards (1994) stated that supplementation of phytase at 600 U/kg feed along with 1,25-dihydroxy cholecalciferol at 5 µg/kg feed in corn-soybean diet increased Zn retention and Zn content of bone in broilers. They suggested that supplemental Zn might be decreased in a corn-soybean meal diet when phytate P utilization was improved.

When chicks were fed on a Zn deficient soya-concentrate diet (Zn-13 ppm) dietary supplementation of $1,25-(OH)_2 D_3$ or phytase increased growth rate by 40 per cent and tibial Zn content by more than 100 per cent. Adding $1,25-(OH)_2 D_3$ together with phytase increased tibial Zn content by 160 per cent. Utilization of Zn and Mn contained in the corn-soybean meal diet were also markedly enhanced by supplemental phytase (Biehl et al., 1995).

Sebastian et al. (1996a) reported that day-old broiler chicks fed on a corn-soybean diet containing 0.33 per cent available P plus phytase 600 U/kg had an increased relative retention of Zn by 62.3 per cent in comparison with control diet containing 0.46 per cent available P without phytase.

Windisch and Kirchgessner (1996a) conducted a trial in broilers to study the effect of phytase (0, 200, 400, 600 and 1000 U/kg diet) on apparent digestibility and gross utilization of Fe, Cu, Mn and Zn at different levels of Ca supply (0.62 or 0.79 per cent) and 0.59 per cent P and observed that increasing phytase increased gross utilization of Zn by 8.46 and Mn by 3.80 per cent. With high dietary Ca, gross utilization changed by –3.4 per cent of Zn and +1.4 per cent of Mn.

The effect of microbial phytase on retention and utilization of Zn in broilers was also studied by Yi et al. (1996a). A corn-soybean meal based diet containing Zn 20 mg/kg was fed alone or supplemented with Zn 5, 10 or 20 mg/kg as $ZnSO_4 7H_2O$ or with phytase 150, 300, 450 or 600 U/kg diet. The amount of

Zn retained by birds was linearly improved by adding Zn and phytase (P<0.01). Zn retained as a per cent of intakes was linearly decreased by adding Zn, but was linearly increased by adding phytase (P<0.01).

From a 21-day trial in day-old broilers with diets containing metabolizable energy of 2800 and 3000 kcal/kg and phytase at the levels of 0 and 500 U/kg, Zanini and Sazzad (1998) recorded improvement in apparent retention of Zn on phytase supplementation.

While Um *et al.* (1999) reported that phytase supplementation (250 U/kg) to low P diets did not significantly affect the retention of Zn in brown laying hens, Um and Paik (1999) recorded improved Zn and Fe retention (P<0.05) on phytase supplementation (500 U/kg) in laying hens.

Balasubramanian (2000) stated that Mn availability was more (P<0.01) with birds fed low available P diet supplemented with 750 and 1000 U of phytase/kg and lower (P<0.01) with birds fed standard diet. Phytase supplementation (500, 750 and 1000 U/kg diet) resulted in a significantly linear increase in the availability of Zn with higher levels of enzyme.

2.5.4.3 Combined Effect of Organic Acids and Phytase

Hohler *et al.* (1992) also observed improved Zn utilization in piglets on citric acid (1.5 per cent) and phytase (1000 U/kg feed) supplementation.

2.6   EFFECTS ON MINERAL REQUIREMENTS

2.6.1   Effect of Organic Acids

Boling-Frankenbach *et al.* (2001b) reported that citric acid (4 or 6 per cent) increased P utilization in corn-soybean meal diet containing 0.2 per cent available P and reduced the available P requirement in chicks by approximately 0.1 per cent of the diet.

2.6.2 Effect of Phytase

Broz *et al.* (1994) reported that phytase supplementation in practical broiler diets will allow the reduction or omission of additional dietary inorganic P.

Denbow *et al.* (1995) indicated that about 821 U of phytase were required to replace one g of inorganic P as defluorinated phosphate based on equally weighted BW gain and toe ash percentage for equivalent P averaged across 0.20 and 0.27 per cent non-phytate P, in male broilers.

Ravindran *et al.* (1995) conducted a three week feeding trial using poults to evaluate the addition of seven levels of phytase (0, 200, 400, 600, 800, 1000 and 1200 U/kg diet) and recorded that 625 U of microbial phytase is equivalent to one g of P from defluorinated phosphate in turkey starter diets using soybean meal as the only source of phytate P. The response per 100 U of phytase decreased as the total amount of phytase added was increased.

Mitchell and Edwards (1996a) have shown conclusively that either phytase (600 U/kg) or 1,25-(OH)$_2$ D$_3$ (5 μg/kg) will replace about 0.1 per cent dietary P in corn-soybean diets, whereas their combination will replace about 0.2 per cent dietary P in the diet of broilers.

Mitchell and Edwards (1996b) reported that the total dietary P requirements were estimated to be 0.55 and 0.60 per cent at the levels of 600 U/kg of phytase and 5 μg/kg of 1,25-(OH)$_2$ D$_3$, respectively or 0.45 per cent when the combination was added. The Ca requirements were estimated to be 0.77 per cent when 1,25-(OH)$_2$ D$_3$ was added to the diet and 0.9 to 0.95 per cent when phytase was added.

Li *et al.* (1998) indicated that the addition of phytase to a corn-soy diet containing no inorganic P liberated enough P from phytate to allow the pig performance similar to that achieved with a diet containing supplemental inorganic P.

When diets were prepared with high available P corn and supplemented with phytase (500 U/kg feed), the dietary addition of total P could be reduced by at least 25 per cent without affecting the broiler chicken performance (Huff *et al.*, 1998).

From studies on effects of microbial phytase in finisher diets of White Pekin ducks, Orban *et al.* (1999) suggested that on phytase supplementation (750 U/kg diet), finisher ducks (three to six weeks of age) can be fed on a diet without inorganic P, if they were fed on adequate P diet during the first phase of growth (zero to three weeks of age).

Addition of microbial phytase at 300 and 600 FTU/kg feed prevents P deficiency symptoms, in diets containing marginal (0.325 per cent) to deficient (0.225 per cent) levels of either non-phytate P or Ca (0.75 per cent) or both in broiler chicken from four to six weeks of age (Sohail and Roland, 1999).

Dietary P levels could be reduced by 0.15 per cent under NRC (1994) recommendations without affecting live performance of broilers in conjunction with phytase supplementation (Yan *et al.*, 2000).

Yan *et al.* (2001b) could not observe any Ca sparing effect on phytase supplementation in broilers.

2.6.3   Combined Effect of Organic Acids and Phytase

Jongbloed *et al.* (1996) studied the main and interactive effects of microbial phytase Natuphos® (0 vs 550 FTU/kg) in combination with lactic acid (1.6 vs 3.2 per cent) formic acid (0.8 vs 1.6 per cent) and propionic acid (0.5 vs 1.0 per cent) in growing pigs and concluded that the positive synergistic effect of the enzyme with formic acid were equal to 0.2 g of digestible P/kg feed.

Han *et al.* (1998) revealed that it is feasible to completely replace the Ca phosphate as P source to get a P concentration of 0.1 and 0.2 per cent, with 10 to

15 per cent wheat middling, 300 U/kg microbial phytase and 1.5 per cent citric acid in the corn soybean meal diets for growing pigs.

Angel *et al.* (2001a) studied the non-phytate P sparing effect of phytase and citric acid in broiler chicks and stated that the sparing effect, based on tibial ash, was 0.0280, 0.0898 and 0.0310 per cent non-phytate P (NPP) for 300, 600 FTU phytase/kg diet and 3 per cent citric acid, respectively. A similar sparing effect was found for 3 per cent citric acid and for 600 FTU phytase/kg diets, when toe ash was used (0.0310 and 0.0875 per cent NPP, respectively). When 600 FTU phytase/kg diet and 3 per cent citric acid were used together the sparing effect was 0.130 and 0.125 per cent NPP for tibia and toe ash, respectively.

In another experiment with broiler chicks, Angel *et al.* (2001b) suggested that the P sparing effects were 0.014, 0.048, 0.035 and 0.030 per cent non-phytate P (NPP) when 200 and 500 FTU phytase/kg diet, 3 per cent citric acid and 70μg 25-hydroxy chlolecalciferol/kg diet, respectively were added to diet and the sparing effect was 0.116 per cent, when the highest levels of all three feed additives were used together.

2.7   WEIGHT OF DRIED TIBIA AND TIBIAL ASH

2.7.1   Effect of Organic Acids

Sifri *et al.* (1977) reported that citric acid at levels 0 and 0.71 per cent in marginally low (0.40 per cent) and adequate (0.85 per cent) Ca diets did not exert any effect on tibial ash in growing chicks up to 28 days of age.

Boling-Frankenbach *et al.* (2001b) also evaluated the effect of different levels of citric acid in chicks fed 0.2 per cent P from eight to 21 days of age and the results indicated that 4 and 6 per cent citric acid produced the largest responses in growth and tibial ash content.

When citric acid, citric acid and sodium citrate (1:1 mixture, CS) and citric acid, sodium citrate and potassium citrate (1:1:1 mixture, CSP) were added at levels of 0, 4.5 and 6.0 per cent to P deficient diet (0.22 per cent available P) from one to 42 day of age improved tibial weight and ash for chicks fed 4.5 per cent of CSP. The bone ash response to the mixture of CSP was much greater than the bone ash response to the mixture of CS (Metwally, 2001).

2.7.2　Effect of Phytase

Supplementation of phytase at the levels of 1.0 to 8.0 g/kg in corn soybean meal diets with 0.18 to 0.24 per cent natural phytate P caused an increase in per cent bone ash (Nelson *et al.,* 1971).

Broiler chickens fed on grain sorghum/soybean meal diets supplemented with or without P from $CaHPO_4$ and phytase from *Aspergillus niger* (Natuphos[®]) at 750 U/kg showed an increase in tibial ash content (Farrell *et al.,* 1993).

Dietary phytase and increased levels of available P increased the toe and tibial ash content in broiler chicks (Perney *et al.,* 1993). They also reported that tibial bone breaking strength was improved by dietary phytase, but not by increased levels of available P.

Broz *et al.* (1994) obtained increased tibial ash on phytase supplementation in broiler chicken fed on a low available P diet without addition of inorganic P.

Biehl *et al.* (1995) conducted an experiment in broiler chicks from eight to 20 days of post hatching to study the efficacy of 1200 U phytase/kg, 10 µg/kg $1,25(OH)_2\ D_3$ and its combination in a low available P (0.1 per cent) diet. They observed that both supplemental phytase and $1,25(OH)_2\ D_3$ alone or in combination, resulted in higher values ($P<0.01$) for tibial ash. They also stated that additivity was observed for the combination of phytase and $1,25(OH)_2\ D_3$ relative to either supplement fed alone.

When the male broiler birds were given semi purified basal diet contained soybean meal as the only protein source with 0.20, 0.27 or 0.34 per cent non-phytate P and seven levels of phytase 0, 200, 400, 600, 800, 1000 and 1,200 U/kg diet for 21 days, Denbow *et al.* (1995) found that ash per cent of toes and tibia, shear force and shear stress of tibia increased with added phytase.

Mitchell and Edwards (1996b) conducted an experiment with three levels of total dietary P (0.45, 0.55 and 0.65 per cent) in corn-soybean meal diets supplemented with 56 µg/kg of 1,25(OH)$_2$ D$_3$, 600 U/kg of phytase or the combination of these supplements in male broiler chicks in factorial arrangement from day old to 21 days in battery brooders and also in floor pens for 35 days. They stated that the combination treatment improved bone ash over both phytase and 1,25(OH)$_2$ D$_3$ alone at all dietary P levels.

In corn-soybean meal diet, with 0.6, 1.0 and 1.25 per cent Ca supplemented with phytase at 600 U/kg diet increased the ash content in tibial head and shaft (Sebastian *et al.*, 1996b).

Yi *et al.* (1996b) revealed that supplementation of defluorinated phosphate to provide 0.36, 0.45 or 0.54 per cent non-phytate P and phytase at 350, 700 or 1050 U/kg diet linearly increased (P<0.01) per cent ash of dried toes in broiler chicks.

Kanagaraju (1998) reported that phytase supplementation at 750 U/kg diet had a positive effect on tibial ash in low available P rations (0.3 and 0.4 per cent available P).

Balasubramanian (2000) reported that tibial ash was significantly (P<0.01) increased by the addition of 750 and 1000 U of phytase/kg diet at both sixth and eighth week of age in broiler chicken when compared to low available P diet (0.3 per cent available P).

Phytase supplementation (800 U/kg) significantly improved tibial ash, when added to diets with low levels of non-phytate P and the improvement was greater in chicks fed with normal corn than high available P corn (Waldroup *et al.*, 2000).

Yan *et al.* (2001a) also reported that phytase supplementation (800 U/kg) in broiler chicks from three to six weeks of age fed diets with 0.10 to 0.45 per cent non-phytate P in increments of 0.05 per cent markedly improved tibial ash at lower levels of non-phytate P than their unsupplemented groups.

Yan *et al.* (2001b) reported that phytase (1000 U/kg diet) supplementation improved tibial ash in broiler chicks fed with low Ca (0.5, 0.7 or 0.9 per cent) and low non-phytate P (0.15 to 0.5 per cent in increments of 0.05 per cent) diets from one to 21 days of age.

Augspurger *et al.* (2003) found that an *E. coli* derived phytase (500 FTU/kg diet) resulted in superior (P<0.01) tibial ash (mg and per cent) values when compared with *Aspergillus niger* (Natuphos®; 500 FTU/kg diet) or *Peniophora lycii* (Ronozyme®; 500 FTU/kg diet) phytase enzymes in young chicks and pigs. They also found no synergism when a 3-phytase (Natuphos®) was combined with a 6-phytase (Ronozyme® or *E. coli* phytase).

2.7.3 Combined Effect of Organic Acids and Phytase

Radcliffe *et al.* (1998) studied the effects of microbial phytase, citric acid and their interaction in a corn soybean meal diet for weanling pigs and revealed that addition of phytase (250 or 500 U/kg diet) linearly increased (P<0.05) dry bone weight, ash weight and ash per cent.

Boling *et al.* (2000) reported that feeding of chicks with diets containing 0.62 per cent Ca, 0.10 per cent available P and graded doses of citric acid + Na citrate (1:1, wt:wt) mixture (1, 2, 4 or 6 per cent of diet) resulted in linear increase

(P<0.01) in tibial ash. They also stated that tibial ash was increased by 43 per cent in chicks fed 6 per cent citric acid than that of chicks fed no citric acid. They further stated that addition of 1,450 U of phytase/kg diet with 6 per cent citrate mixture caused further increase (P<0.05) in weight of bone ash.

Feeding trial conducted in broiler chicks fed low P diets supplemented with phytase (0, 300 and 600 FTU/kg) and citric acid (0, 1, 2 and 3 per cent) in 3 x 4 factorial experiment revealed that phytase and citric acid positively affected (P<0.05) toe and tibial ash content, but no interactions were seen (Angel *et al.*, 2001a).

In an experiment, Angel *et al.* (2001b) showed that addition of phytase (200 and 500 U/kg), 25-hydroxy cholecalciferol (70 μg/kg) or citric acid (3.0 per cent) in broiler chicks fed low non-phytate P (0.16 per cent) and 0.80 per cent Ca diet from 14 to 24 day of age increased the tibial ash content, but no interactions were seen.

2.8   TIBIAL MINERAL CONTENT

2.8.1   Effect of Organic Acids

Sifri *et al.* (1977) found that citric acid at the level of 0.71 per cent in diets with two levels of Ca (0.40 and 0.85 per cent) did not exert any effect on tibial Ca content in growing chicks up to 28 days of age. They also stated that there was no significant effect of ascorbic acid even at 0.65 per cent on Ca, Zn, or Fe deposition in the tibia.

Boling *et al.* (2000) studied the effect of graded levels of citric acid and sodium citrate (1:1) mixture (0, 1, 2, 4 or 6 per cent) in chick diets containing 0.62 per cent Ca and 0.10 per cent available P and found that tibia Zn was increased (P<0.05) with increasing levels of citrate between 0 and 6 per cent.

2.8.2 Effect of Phytase

Biehl $et\ al.$ (1995) evaluated the efficacy of 1200 U phytase/kg diet, 10 µg 1,25$(OH)_2$ D_3/kg diet and its combination in a low available P (0.1 per cent) diets of broiler chicks from eight to 20 days of post hatching. They concluded that both supplemental phytase and 1,25$(OH)_2$ D_3, alone or in combination, resulted in higher (P<0.01) values for tibia P and tibia Mn content than in basal diet fed chicks and the additivity was observed for the combination. They also reported that combination of phytase and 1,25$(OH)_2$ D_3 with non-supplemental P, Zn, or Mn resulted in increased tibia Zn (86 per cent) and tibia Mn (123 per cent) over the basal diets.

Piva $et\ al.$ (1995) conducted an experiment in broilers fed with control diets, diets with high activity phytase (500, 1000 or 2000 U/kg) and diets with low activity phytase (500, 1000 or 2000 U/kg) for 55 days and observed greater Mn content in bone of birds fed with low activity phytase diet (2000 U/kg) and greater Zn content in bone of birds fed with low activity phytase diet (500 U/kg) and high activity phytase diets than the control diet.

Thiel $et\ al.$ (1995) studied Zn retention in male broiler chicken given a corn soybean meal diet containing Zn 30 mg/kg with no supplement or with microbial phytase 700 U/kg and with no added Zn or with added Zn to provide totals of 34, 39 or 45 mg/kg. They reported that without phytase, femur Zn increased significantly with added Zn up to 39 mg/kg diet and with phytase, femur Zn increased with no added Zn by more than 20 per cent. On the basis of the increments in femur and whole body Zn, they concluded that the response to the phytase supplement was equivalent to that for a supplement of Zn 15 mg/kg diet and the per cent Zn retention decreased with each increment in dietary Zn.

Zanini and Sazzad (1998) found improvement in *tibial Zn concentration* with supplementation of phytase (500 U/kg diet) in a 21-day trial in day-old broilers with diets having metabolizable energy of 2800 and 3000 kcal/kg.

When broiler chicks fed diets with two levels of non-phytate P (NPP; 0.225 and 0.325 per cent) and three levels of phytase (0, 300 and 600 FTU/kg) with 0.75 per cent Ca, Sohail and Roland (1999) *revealed that phytase (300 FTU/kg diet) had greater influence on bone mineral content, bone density and bone breaking strength in broilers fed 0.225 per cent non-phytate P (NPP) than in broilers fed 0.325 per cent NPP.

Um *et al.* (1999) reported that phytase supplementation (250 U/kg diet) to low P diets increased the tibial Ca, P, Mg and Zn content in a eight week *feeding* trial with 48 week old brown laying hens.

2.9   SERUM MINERAL CONTENT AND ALKALINE PHOSPHATASE

2.9.1   Effect of Organic Acids

On feeding growing chicks with diet having 0.587 per cent P, 0.40 or 0.85 *per cent Ca and zero or three per cent citric acid,* Sifri *et al.* (1977) observed that there was a significant increase in plasma Ca due to increase in Ca in diet, but there was no effect of citric acid on plasma Ca.

Hohler (1992) reported that dietary supplementation of citric or fumaric acid (1.5 per cent) in low Zn diet in piglets resulted in increased plasma Zn concentration and decreased alkaline phosphatase activity with citric acid, while fumaric acid has no effect.

Increased plasma Zn concentration on citric acid supplementation (1.5 per cent) in pigs was also reported by Hohler and Pallauf (1993).

2.9.2 Effect of Phytase

Perney *et al.* (1993) reported that when broiler chicks were given corn-soybean diets contained 0.32, 0.38 and 0.44 per cent available P supplemented with 250, 500 and 750 U of phytase/kg feed, plasma inorganic P responded quadratically to increasing dietary phytase.

Phytase supplementation at varying levels (125, 250 or 500 U/kg) to low P diets (4.4, 4.5 and 5.2 g/kg) significantly (P<0.05) elevated plasma concentration of inorganic P in broiler chicks (Broz *et al.*, 1994).

Roberson and Edwards (1994) reported that corn-soybean meal diet supplemented with 1, 25-$(OH)_2$ D_3 at 5 µg /kg and phytase 600 U/kg had no effect on plasma alkaline phosphatase activity.

Mitchell and Edwards (1996a) observed that supplementation of 1,25-$(OH)_2$ D_3 at 5 µg/kg diet and phytase at 600 U/kg diet in broilers could replace up to 0.1 per cent of inorganic P for plasma P criteria in corn-soybean meal diets.

Mitchell and Edwards (1996b) conducted an experiment with three levels of total dietary P (0.45, 0.55 and 0.65 per cent) in corn-soybean meal diets supplemented with 5 µg/kg of 1, 25-$(OH)_2$ D_3, 600 U/kg of phytase, or the combination of these supplements in male broiler chicks in a factorial arrangement from day-old to 21 days in battery brooders and also in floor pens for 35 days. They concluded that plasma P content was increased by both phytase and 1, 25-$(OH)_2$ D_3, and further increased by its combination.

Sebastian *et al.* (1996a) reported that broiler chicks fed with corn soybean meal diets containing 0.46 per cent (control) or 0.33 per cent (low) available P supplemented with microbial phytase (Natuphos[®]) at 600 U/kg recorded increased plasma P by 15.7 per cent and reduced Ca concentration by 34 per cent.

Huff *et al.* (1998) observed that serum alkaline phosphatase activity was reduced in chicks fed with diets supplemented with phytase at the level of 500 U/kg.

Serum Ca and inorganic P were significantly higher in phytase supplemented groups (750 U/kg diet) than the control chicks at sixth week of age (Kanagaraju, 1998), but no difference could be detected at eighth week of age.

Balasubramanian (2000) reported that serum Ca and serum inorganic P were significantly ($P<0.01$) increased by the addition of 750 and 1000 U phytase/kg in low available P diets both at sixth and eighth week of age. The serum alkaline phosphatase level was significantly ($P<0.01$) decreased in the diets supplemented with phytase.

Kita *et al.* (2000) found that plasma concentrations of inorganic Ca and P were significantly increased in White Leghorn male chicks by barley soaked in water with the presence or absence of phytase (100 U/L or 250 U/L) from seven to 17 days of age indicating that soaking in water alone is sufficient for the hydrolysis of phytate by phytase present in barley.

2.9.3 Combined Effect of Organic Acids and Phytase

Han *et al.* (1998) found no significant difference in plasma inorganic P between pigs fed with diets containing wheat middling (15 or 10 per cent), microbial phytase (300 U/kg) and citric acid (1.5 per cent) with no added inorganic P and diets with added inorganic P (0.2 per cent) at week four and six.

2.10 PROCESSING YIELDS

2.10.1 Effect of Organic Acids

Studies with diets containing buffered propionic acid (BPA; 0, 0.2, 0.4, and 0.8 per cent) in broilers from day-old to 49 days of age, Izat *et al.* (1990b) stated that the BPA supplementation had significantly increased ($P<0.05$) dressing yield in female chicks. The BPA at 0.4 per cent fed continuously had significant reduction in the abdominal fat for males, while the BPA supplement at 0.8 per cent had no effect.

Roth *et al.* (1996) revealed that Formi™ LHS, a diformate based product in pig diets at 0.65, 1.30 and 1.95 per cent levels had no effects on carcass evaluation.

Addition of citric acid, citric acid and sodium citrate (1:1 mixture, CS) and citric acid, sodium citrate and potassium citrate (1:1:1 mixture, CSP) at levels of 0, 4.5 and 6.0 per cent to a P deficient diet containing 0.22 per cent available P and 0.91 per cent Ca, in broiler chicks from one to 42 days of age revealed that carcass quality was improved for chicks fed 4.5 per cent mixture of CSP (Metwally, 2001).

2.10.2 Effect of Phytase

Feeding of Lohmann broiler chicks with plant based seeds having varying levels of inorganic P (1350, 675, 338 and 0 m/kg diet) and phytase (0, 300 and 700 U/kg diet) for 35 days revealed that when inorganic P was 0, slaughter weight decreased by up to 39 per cent and medium P with phytase 300 or 700 U/kg diet increased slaughter weight by 17 and 23 per cent, respectively. Bending and breaking strength of tibia decreased with decreased P, but increased with phytase. Incidence of leg disorders was limited by phytase supplementation (Richter *et al.*, 1993).

Kanagaraju (1998) reported that the per cent dressed yield and ready-to-cook yield of broilers were significantly (P<0.01) higher in groups fed a diet having 0.4 per cent available P plus 750 U phytase/kg diet than other groups. He also noted that the per cent giblet yield was significantly (P<0.01) more in groups fed a standard diet supplemented with phytase than other groups.

Kornegay *et al.* (1998) stated that breast weight and breast weight as per cent of live weight and carcass weight were increased (P<0.05 to 0.01) as the level of crude protein/amino acid or phytase increased in the diet compared with birds fed the low protein diet.

Balasubramanian (2000) reported that supplementation of phytase (500, 750 or 1000 U/kg diet) had no effect on per cent dressed yield and giblet yield. The per cent ready-to-cook yield was significantly (P<0.05) higher in groups fed standard diet, LAP diet and 500 U phytase supplemented diet at sixth week, at eight week it was significantly higher in groups fed standard diet and 1000 U phytase added diet.

2.11   LIVABILITY

2.11.1 Effect of Organic Acids

Sifri *et al.* (1977) reported that feeding citric acid at 0 and 0.71 per cent levels in marginally low (0.4 per cent) and adequate (0.85 per cent) Ca diets did not reveal any pronounced effect on mortality, in growing chicks up to 28 days of age.

Inclusion of up to 1.0 per cent formic acid or 1.45 per cent Ca formate in broiler chicken from day old to 43 days had no adverse effect on livability (Izat *et al.*, 1990a).

From a 28-day feeding trial, Tsiloyiannis *et al.* (2001) observed that supplementation of either 1.5 per cent citric acid, 1.6 per cent lactic acid or 50 ppm of enrofloxacin reduced the mortality in weaned piglets.

2.11.2 Effect of Phytase

Feeding of Lohmann broilers with a maize/soybean meal diet having 5.2, 5.7 and 6.2 g P/kg plus phytase 0, 200, 400, 800 and 1000 U/kg for six weeks revealed that increased mortality with decreasing dietary P could be improved by supplemental phytase (Vogt, 1993).

Denbow *et al.* (1995) stated that mortality was 45 and 35 per cent in broilers fed the diet containing 0.20 and 0.27 per cent non-phytate P, respectively

without phytase and number of deaths declined markedly by the addition of phytase in diets.

High incidence of leg disorders and high mortality (40 per cent) observed in poults fed with the 0.27 per cent non-phytate P diet was declined with the addition of 200 to 400 U of phytase/kg of diet (Ravindran *et al.*, 1995).

Kanagaraju (1998) reported that the per cent livability of commercial broiler chicken was better with phytase supplemented groups than with unsupplemented groups.

When broiler chicks fed with two levels of non-phytate P (NPP; 0.225 and 0.325 per cent) and three levels of phytase (0, 300 and 600 FTU/kg) with 0.75 per cent Ca, Sohail and Roland (1999) found that phytase (300 FTU/kg) had greater influence on livability in broilers fed 0.225 per cent NPP than in broilers fed 0.325 per cent NPP.

Balasubramanian (2000) reported that the livability was not influenced by dietary phytase (500, 750 and 1000 U/kg) and available P (0.3 and 0.5 per cent) levels.

High mortality observed in broiler chicks (zero to three weeks) fed with lower level of non-phytate P diet could be considerably reduced on phytase (800 U/kg diet) supplementation (Waldroup *et al.*, 2000).

Yan *et al.* (2000) reported that birds supplemented with phytase (1000 U/kg diet) had significantly lower mortality than those fed unsupplemented diets, with a significant interaction between P level and phytase supplementation.

Yan *et al.* (2001a) conducted a feeding trial in broiler chickens from three to six weeks of age fed diets with 0.6 to 0.45 per cent non-phytate P in increments of 0.05 per cent, with or without phytase supplementation (800 U/kg) and observed that mortality was not influenced by any of the dietary treatments.

2.12   ECONOMICS

Newman (1993) stated that one kg of phytase enzyme per tonne of feed could potentially replace six to seven kg of monocalcium phosphate, thus allowed significant reduction in P supplementation cost.

After conducting an experiment with broilers fed diets containing inorganic P 0.6, 1.1 and 1.6 g/kg supplemented with phytase 0, 500 and 1000 U/kg diet, Richter *et al.* (1994) reported that phytase was more expensive than inorganic P.

The cost of production per kg live weight (when feed cost alone was considered) was cheaper in groups fed with 0.4 per cent available P supplemented with phytase at 750 U/kg diet. The net profit per kg live weight was 13 paise higher in groups maintained on 0.4 per cent available P diet supplemented with phytase 750 U/kg compared to standard broiler ration (Kanagaraju, 1998).

Balasubramanian (2000) reported that the net profit per kg live weight gain was highest in the group fed standard diet followed by 750, 1000 and 500 U of phytase/kg offered groups and the lowest in low available P diet group both at six and eight week of age.

Materials and Methods

3. MATERIALS AND METHODS

An experiment was conducted in the Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy for a period of eight weeks to study the effect of citric acid and microbial phytase on mineral utilization and subsequent growth performance in broiler chicken.

3.1   EXPERIMENTAL MATERIALS

3.1.1   Experimental Birds

One hundred and ninety two, day-old straight-run commercial broiler chicks (Ven cob) procured from Venkateshwara Hatcheries Ltd., Palakkad, Kerala formed the experimental subjects.

3.1.2   Experimental Rations

Four experimental rations were formulated, viz.,

1. T1 (Control) - Standard broiler ration (SBR) with 0.5 per cent available P as per BIS (1992) specifications.

2. T2 - Low available P broiler ration (LAPBR) with 0.3 per cent available P and 3.0 per cent citric acid.

3. T3 - LAPBR with 0.3 per cent available P + 700 U/kg microbial phytase.

4. T4 - LAPBR with 0.3 per cent available P and 1.5 per cent citric acid + 350 U/kg microbial phytase.

In low available P broiler rations (LAPBR), the level of available P was kept at 0.3 per cent as compared to 0.5 per cent in the standard broiler ration. The levels of all other nutrients were similar to that of SBR. Feed ingredients used in

the formulation of the experimental rations were yellow maize, rice polish, soybean meal and unsalted dried fish. These feed ingredients were procured from University Poultry Farm, Mannuthy.

Initially the ration with 0.3 per cent available P was formulated (LAPBR). By the addition of appropriate levels of dicalcium phosphate (DCP) to this ration, diet with 0.5 per cent available P was formulated (T1). LAPBR with 3.0 per cent citric acid formed the experimental ration T2. LAPBR was supplemented with microbial phytase at a level of 700 U/kg feed to form T3. LAPBR having both citric acid and microbial phytase at a level of 1.5 per cent and 350 U/kg feed, respectively formed the experimental ration T4.

Broiler starter diets were fed up to 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 four different starter and finisher rations are presented in Tables 1, 2, 3 and 4, respectively.

3.1.3 Enzyme

The enzyme used in this trial was 'Natuphos®-5000G' a product manufactured and marketed by M/s. BASF, D-67056, Ludwigshafen, Germany. It is a phosphatase enzyme of fungal origin *(Aspergillus niger)* containing phytase as the only component. The product contained 5000 U of phytase activity per gram, with one U of phytase activity defined as the quantity of enzyme required to produce one μmol of inorganic P per minute from 1.5 mmol/L of sodium phytate at a pH of 5.5 and temperature of 37°C (Biehl *et al.*, 1995). The enzyme was added in low available P broiler rations in two different levels viz., 700 (T3) and 350 (T4) U per kg of diet.

3.1.4 Citric Acid

Commercial grade citric acid marketed by Premier Scientific Suppliers, Karur, Tamil Nadu was used in this trial. The citric acid is an organic acid, which is available as fine white powder. It was added in low available P broiler ration in two different levels viz., 3.0 per cent (T2) and 1.5 per cent (T4).

3.2 EXPERIMENTAL METHODS

3.2.1 Housing of Birds

The experimental pens, feeders, waterers and other equipment were properly cleaned and disinfected one week before the chicks were housed. The straight-run day-old chicks were wing banded, weighed individually and vaccinated against Ranikhet disease, before housing.

3.2.2 Experimental Design

The chicks were randomly divided into 16 groups of 12 chicks each. The groups were allotted randomly to four dietary treatments viz., T1, T2, T3 and T4 with four replicates in each treatment. The details of treatments are presented in Table 5.

3.2.3 Management

The birds were provided with feed and water *ad libitum* throughout the experimental period and were maintained under deep litter system of management. The birds were vaccinated against Infectious Bursal Disease at 14 and 28 days of age. Standard managemental procedures were adapted identically to all treatments during the entire experimental period of eight weeks.

3.2.4 Climatic Parameters

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

3.2.5 Body Weight

The body weight of individual birds was recorded at fortnightly intervals from day old to study the pattern of growth rate under different dietary treatments.

3.2.6 Feed Consumption

Feed intake of the birds was recorded replication wise at weekly intervals. From these data, the average feed intake per bird per day was calculated for various treatment groups.

3.2.7 Feed Conversion Ratio

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

3.2.8 Protein Efficiency Ratio

Protein intake (g) of the birds at replication wise was calculated at fortnightly intervals based on the data on feed intake and protein content of feed. Protein efficiency ratio (g body weight gain/g of protein consumed) was calculated based on the data on body weight gain and protein intake.

3.2.9 Metabolism Trial

Towards the end of the experiment, a three-day metabolism trial was conducted using one bird from each replicate selected randomly and housed in

individual metabolism cages with facilities for feeding, watering and excreta collection. Water was provided *ad libitum*. Excreta samples were collected over 24 hour period for three consecutive days using total collection method as described by Summers *et al.* (1976). The droppings were weighed and samples were taken and stored in airtight containers in a deep freezer for analysis. The total amount of feed consumed and excreta voided were also recorded.

3.2.10 Chemical Analysis

The chemical compositions of experimental rations were determined as per the standard procedures (AOAC, 1990). The content of N of the excreta samples were determined in fresh material as per the procedure described by AOAC (1990). Then the excreta samples were dried in the oven at 100°C overnight and ground prior to the estimation of minerals. For mineral analysis, the diet and excreta samples were subjected to wet digestion, using nitric acid and perchloric acid (2:1). Ca, Mg, Zn and Mn content of the digested sample were determined using atomic absorption spectrophotometer (Perkin-Elmer Model-AAS 3110) and inorganic P by colorimetric method (AOAC, 1990) using spectronic 1001_{plus} spectrophotometer (Milton Roy Co., USA).

From the data obtained on the total intake and outgo of nutrients during the metabolism trial, dry matter retention, N retention and availability of Ca, P, Mg, Zn and Mn were calculated.

3.2.11 Tibial Ash

At the end of sixth week, one bird from each replicate was 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 were removed as closely as possible. The adhering connective and muscular tissues were finally removed by boiling the bone in one per cent solution of sodium hydroxide for about ten minutes. Then the
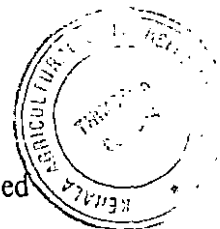
tibias were thoroughly washed, dried in the oven at 100°C overnight. The cooled tibias were weighed individually and the ash contents of the tibia were estimated as per the procedure of AOAC (1990). The weight of the tibial ash was expressed as percentage of the weight of the dried tibia. Tibial ash was also determined at the end of eighth week in birds utilized for slaughter studies.

3.2.12 Tibial Mineral Content

For mineral analysis, dried tibial bone samples were ground and subjected to wet digestion, using nitric acid and perchloric acid (2:1). Ca, Mg, Zn and Mn content of the digested sample were determined using atomic absorption spectrophotometer (Perkin-Elmer Model-AAS 3110) and inorganic P by colorimetric method (AOAC, 1990) using spectronic 1001_{plus} spectrophotometer (Milton Roy Co., USA).

3.2.13 Serum Mineral Content and Alkaline Phosphatase

At the end of sixth and eighth week of age, blood samples were collected from one bird in each replicate by severing the jugular vein for the estimation of serum Ca, P, Mg, Zn, Mn and alkaline phosphatase. The serum inorganic P was estimated colorimetrically using spectronic 1001_{plus} spectrophotometer (Milton Roy Co., USA) by phosphomolybdate method utilizing the kit supplied by M/s Agappe Diagnostics, F-4, Shailesh Industrial Complex, Valiv Post, Vasai (E), Thane, Maharashtra – 401 208, India. The serum alkaline phosphatase was estimated in blood analyzer (MISPA Plus) using the kit supplied by M/s Agappe Diagnostics. The serum Ca, Mg, Zn and Mn were determined using atomic absorption spectrophotometer (Perkin-Elmer Model- AAS 3110).

3.2.14 Processing Yields

At the end of sixth and eighth week, one bird from each replicate was randomly selected and sacrificed to study the processing yields as per the procedure described by BIS (1973). Percentages of dressed yield, giblet yield,

ready-to-cook yield and abdominal fat yield were calculated from the slaughter data.

3.2.15 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.

3.2.16 Cost-Benefit Analysis

Cost of feed for different dietary treatments was calculated from cost of ingredients including cost of citric acid and enzyme and from per cent ingredient composition. Cost of feed/kg live weight gain for different dietary treatments was calculated from cost of feed, live weight gain of birds and quantity of feed consumed by birds in each treatment groups.

3.2.17 Statistical Analysis

Data collected on various parameters were statistically analyzed by Completely Randomised Design (CRD) method as described by Snedecor and Cochran (1985). Means were compared by Least Significant Difference (LSD) test using MSTATC.

Table 1. Ingredient composition of starter rations, %

Ingredients	Treatments			
	T1	T2	T3	T4
Yellow maize	50.000	46.500	50.000	48.000
Rice polish	5.735	6.235	6.235	6.235
Soybean meal	36.000	36.500	36.000	36.500
Unsalted dried fish	5.200	5.200	5.200	5.200
Shell grit	0.800	1.500	1.500	1.500
Dicalcium phosphate	1.500	0.300	0.300	0.300
Citric acid	0.000	3.000	0.000	1.500
Common salt	0.250	0.250	0.250	0.250
Vitamin mixture[1]	0.025	0.025	0.025	0.025
Trace mineral mixture[2]	0.130	0.130	0.130	0.130
D.L. methionine	0.060	0.060	0.060	0.060
Toxin binder[3]	0.200	0.200	0.200	0.200
Coccidiostat[4]	0.050	0.050	0.050	0.050
Chloline chloride	0.050	0.050	0.050	0.050
Total	100.000	100.000	100.000	100.000
Phytase (U/kg)	0	0	700	350

[1]Vitamin mixture composition (INDOMIX + INDOMIX BE):
   Each gram contains: Vitamin A - 41250 IU, Vitamin D_3 - 6000 IU,
   Vitamin E - 20 mg, Vitamin K - 5 mg, Vitamin B_1 - 2 mg, Vitamin B_2 - 25
   mg, Vitamin B_6 - 4 mg, Vitamin B_{12} - 20 µg, Niacin - 30 mg, Ca
   pantothenate - 20 mg.

[2]Trace mineral composition (Ultra-TM)
   Each gram contains: Mn - 54 mg, Zn - 52 mg, Iron - 20 mg, Iodine - 2 mg,
   Copper - 2 mg, Cobalt - 1 mg.

[3]Toxin binder composition (Alusil Premix[TM]):
   Each gram contains: SIO_2: 400-500 mg, Al_2O_3: 320-400 mg, Fe_2O3: 3-10
   mg, MgO: 5-20 mg, CaO: 30-50 mg, Na_2O: 25-45 mg, K_2O: 5-10 mg.

[4]Coccidiostat composition (Anacox[TM] 1%):
   Each gram contains: Maduramycin ammonium 10 mg.

Table 2. Ingredient composition of finisher rations, %

Ingredients	Treatments			
	T1	T2	T3	T4
Yellow maize	61.750	58.250	61.750	59.750
Rice polish	2.125	2.525	2.525	2.525
Soybean meal	28.000	28.500	28.000	28.500
Unsalted dried fish	5.000	5.000	5.000	5.000
Shell grit	0.800	1.500	1.500	1.500
Dicalcium phosphate	1.600	0.500	0.500	0.500
Citric acid	0.000	3.000	0.000	1.500
Common salt	0.250	0.250	0.250	0.250
Vitamin mixture[1]	0.025	0.025	0.025	0.025
Trace mineral mixture[2]	0.130	0.130	0.130	0.130
D.L. methionine	0.020	0.020	0.020	0.020
Toxin binder[3]	0.200	0.200	0.200	0.200
Coccidiostat[4]	0.050	0.050	0.050	0.050
Chloline chloride	0.050	0.050	0.050	0.050
Total	100.000	100.000	100.000	100.000
Phytase (U/kg)	0	0	700	350

[1]Vitamin mixture composition (INDOMIX + INDOMIX BE):
Each gram contains: Vitamin A - 41250 IU, Vitamin D_3 - 6000 IU, Vitamin E - 20 mg, Vitamin K - 5 mg, Vitamin B_1 - 2 mg, Vitamin B_2 - 25 mg, Vitamin B_6 - 4 mg, Vitamin B_{12} - 20 μg, Niacin - 30 mg, Ca pantothenate - 20 mg.

[2]Trace mineral composition (Ultra-TM)
Each gram contains: Mn - 54 mg, Zn - 52 mg, Iron - 20 mg, Iodine - 2 mg, Copper - 2 mg, Cobalt - 1 mg.

[3]Toxin binder composition (Alusil Premix[TM]):
Each gram contains: SIO_2: 400-500 mg, Al_2O_3: 320-400 mg, Fe2O3: 3-10 mg, MgO: 5-20 mg, CaO: 30-50 mg, Na_2O: 25-45 mg, K_2O: 5-10 mg.

[4]Coccidiostat composition (Anacox[TM] 1%):
Each gram contains: Maduramycin ammonium 10 mg.

Table 3. Chemical composition of starter rations*, %

Nutrients	Treatments			
	T1	T2	T3	T4
Dry matter	91.14	91.59	91.25	91.62
Crude protein	23.59	23.14	23.62	23.36
Ether extract	4.62	4.44	4.70	4.45
Crude fibre	4.28	4.12	4.36	4.38
NFE	59.32	58.32	58.32	57.74
Total ash	8.19	9.98	9.00	10.07
Acid insoluble ash	2.45	2.44	2.46	2.63
N	3.77	3.70	3.78	3.74
Ca	1.21	1.20	1.21	1.21
Total P	0.82	0.59	0.59	0.60
Mg	0.21	0.20	0.20	0.20
Mn (mg/kg)	126.60	126.14	123.70	123.72
Zn (mg/kg)	81.63	84.50	88.36	82.99
Calculated values				
ME (kcal/kg)	2804.58	2814.08	2818.58	2814.08
Available P	0.50	0.30	0.30	0.30
Lysine	1.35	1.36	1.35	1.36
Methionine	0.45	0.45	0.45	0.45

*On dry matter basis

Table 4. Chemical composition of finisher rations*, %

Nutrients	Treatments			
	T1	T2	T3	T4
Dry matter	90.88	90.25	90.73	90.44
Crude protein	20.24	20.35	20.21	20.39
Ether extract	4.80	4.95	5.28	4.84
Crude fibre	3.48	3.47	3.86	3.48
NFE	64.71	64.11	63.43	64.75
Total ash	6.77	7.12	7.22	6.54
Acid insoluble ash	1.52	1.53	1.54	1.43
N	3.24	3.26	3.23	3.26
Ca	1.19	1.21	1.23	1.20
Total P	0.81	0.59	0.58	0.59
Mg	0.15	0.16	0.16	0.16
Mn (mg/kg)	123.31	124.73	128.07	129.50
Zn (mg/kg)	80.62	80.51	2.08	83.94
Calculated values				
ME (kcal/kg)	2894.25	2900.95	2905.45	2900.95
Available P	0.50	0.31	0.31	0.31
Lysine	1.12	1.13	1.12	1.13
Methionine	0.37	0.37	0.38	0.37

*On dry matter basis

Table 5. Distribution of the different dietary treatments

Treatment	Replication	No. of birds	Diet	Level of phytase inclusion (U/kg feed)	Level of citric acid inclusion (per cent diet)
T1	R1	12	SBR (0.5%)	0	0
	R2	12	SBR (0.5%)	0	0
	R3	12	SBR (0.5%)	0	0
	R4	12	SBR (0.5%)	0	0
T2	R1	12	LAPBR (0.3%)	0	3.0
	R2	12	LAPBR (0.3%)	0	3.0
	R3	12	LAPBR (0.3%)	0	3.0
	R4	12	LAPBR (0.3%)	0	3.0
T3	R1	12	LAPBR (0.3%)	700	0
	R2	12	LAPBR (0.3%)	700	0
	R3	12	LAPBR (0.3%)	700	0
	R4	12	LAPBR (0.3%)	700	0
T4	R1	12	LAPBR (0.3%)	350	1.5
	R2	12	LAPBR (0.3%)	350	1.5
	R3	12	LAPBR (0.3%)	350	1.5
	R4	12	LAPBR (0.3%)	350	1.5

Results

4. RESULTS

The results obtained in the present study are detailed here under the following headings.

4.1 CLIMATIC PARAMETERS

The data pertaining to climatic parameters viz., the mean maximum and minimum temperatures and per cent relative humidity during the feeding trial period from November 2002 to January 2003 are given in Table 6. The mean maximum and minimum temperatures were 32.59 and 23.76°C, respectively. The mean relative humidity was 61.86 per cent at 8 A.M and 44.55 per cent at 2 P.M.

4.2 BODY WEIGHT

The data on fortnightly mean body weight of birds as influenced by different dietary treatments are presented in Table 7. They are graphically represented in Fig. 1a and 1b. The mean body weight of birds belonging to the groups T1, T2, T3 and T4 were 1867.29, 1974.55, 1938.44 and 2071.56 g at sixth week and 2545.12, 2797.99, 2662.73 and 2806.59 g at eighth week, respectively.

4.3 BODY WEIGHT GAIN

The data on mean body weight gain of birds at fortnightly intervals and the cumulative mean weight gain up to six and eight weeks of age among different dietary treatments are presented in Table 8. The weight gains are graphically represented is Fig. 2 and 3. The cumulative mean body weight gain of birds belonging to the groups T1, T2, T3 and T4 were 1826.96, 1934.52, 1897.64 and 2031.44 g at sixth week and 2504.79, 2757.97, 2621.92 and 2766.47 g at eighth week, respectively.

4.4   FEED CONSUMPTION

The daily mean feed intake of birds maintained on different dietary treatments at weekly intervals is presented in Table 9.

Data on fortnightly and cumulative mean feed intake of the birds at sixth and eighth week of age as influenced by different dietary treatments are given in Table 10 and their graphical representation in Fig. 4 and 5. The cumulative mean feed intake of the birds for T1, T2, T3 and T4 were 3817.29, 3906.54, 3921.87 and 4075.79 g at sixth week and 5904.33, 6340.86, 6035.73 and 6337.33 g at eighth week, respectively.

4.5   FEED CONVERSION RATIO

The data pertaining to mean feed conversion ratio (FCR; kg of feed consumed/kg body weight gain) at fortnightly intervals and cumulative FCR at sixth and eighth week of age for different dietary treatments are set out in Table 11 and are graphically represented in Fig. 6. The mean cumulative FCR of experimental birds were 2.09, 2.02, 2.07 and 2.01 at sixth week and 2.36, 2.30, 2.30 and 2.29 at eighth week for the treatments T1, T2, T3 and T4, respectively.

4.6   PROTEIN EFFICIENCY RATIO

The data pertaining to mean protein efficiency ratio (PER; g body weight gain/g of protein intake) at fortnightly intervals and cumulative PER at sixth and eighth week of age for different dietary treatments are set out in Table 12 and are graphically represented in Fig. 7. The mean cumulative PER for T1, T2, T3 and T4 were 2.03, 2.14, 2.05 and 2.13 at sixth week and 1.89, 1.97, 1.94 and 1.96 at eight week, respectively.

4.7   METABOLISM TRIAL

The chemical compositions of droppings voided by the experimental birds during the metabolism trial are given in Table 13.

The data on average daily DM intake (g), DM outgo (g) and DM retention (per cent) of birds during the metabolism trial are set out in Table 14 and their graphical representation is in Fig. 8. The average DM retention was 66.07, 67.87, 70.06 and 67.19 per cent for T1, T2, T3 and T4, respectively.

4.8   N RETENTION AND EXCRETION

The data on N balance (g/day), retention (per cent) and excretion (g/kg DM intake) of the birds during the metabolism trial are given in Table 15. They are graphically represented in Fig. 8. The mean N retention for the experimental birds in T1, T2, T3 and T4 were 42.08, 46.38, 44.72 and 45.43 per cent, respectively.

4.9   AVAILABILITY OF MINERALS

The data pertaining to availability of Ca, P, Mg, Zn and Mn are set out in Tables 16 to 20. Their graphical representations are given in Fig 9.

The per cent availability of minerals in experimental birds of T1, T2, T3 and T4 were 38.05, 35.81, 46.57 and 28.23 for Ca; 26.56, 30.19, 36.38, and 32.82 for P; 7.52, 6.19, 19.99 and 6.39 for Mg; 8.32, 15.46, 26.25 and 14.66 for Zn and 9.06, 18.99, 31.71 and 19.25 for Mn, respectively.

4.10   P EXCRETION

The data obtained on P excretion (g/kg DM intake) of birds among different dietary treatments are presented in Table 17. They are graphically represented in Fig. 10. The mean P excretion in experimental birds was 5.92, 4.11, 3.71 and 3.94 g/kg DM intake for T1, T2, T3 and T4, respectively.

4.11   WEIGHT OF DRIED TIBIA AND TIBIAL ASH

The data on weight of dried tibia and per cent tibial ash content of experimental birds at sixth and eighth week of age as influenced by different dietary treatments are given in Table 21.   Their graphical representations are shown in Fig. 11 and 12, respectively.

The mean weight of dried tibia of experimental birds was varied from 5.62 (T1) to 7.08 (T4) g at sixth week and 10.04 (T1) to 11.98 (T2) g at eighth week. The per cent tibial ash content of experimental birds was ranged from 39.97 (T1) to 41.82 (T4) at sixth week and 40.51 (T1) to 42.67 at eighth week of age.

4.12   TIBIAL MINERAL CONTENT

Data on tibial mineral content of experimental birds at sixth and eighth week of age as influenced by different dietary treatments are given in Tables 22 and 23.

Tibial Ca ranged from 12.50 (T1) to 12.80 (T4) at sixth week and 13.90 (T3) to 15.61 (T2) per cent at eight week. Tibial P varied from 8.02 (T2) to 9.26 (T3) and 10.30 (T3) to 12.06 (T2) per cent at sixth and eighth week of age, respectively.   Tibial Mg content ranged from 0.36 to 0.44 per cent at sixth week and 0.43 to 0.56 per cent at eighth week.

Tibial Zn content varied from 84.73 to 147.55 ppm and 139.73 to 160.79 ppm at sixth and eighth week of age, respectively. Tibial Mn content varied between 61.23 ppm in control (T1) to 80.40 ppm in T2 and 77.88 ppm in T1 to 86.25 ppm in T4 at sixth and eighth week of age, respectively.

4.13   SERUM MINERALS AND ALKALINE PHOSPHATASE

The data on serum mineral concentration and alkaline phosphatase content of experimental birds as influenced by different dietary treatments at sixth and

eighth week of age are presented in Tables 24 and 25 and serum alkaline phosphatase (ALP) content of birds are graphically represented in Fig.13.

Serum Ca ranged from 8.63 (T2) to 10.44 (T3) and 8.38 (T1) to 10.44 (T4) mg per cent at sixth and eighth week of age, respectively. Serum iP varied between 5.12 (T1) to 6.56 (T2) and 6.89 (T3) to 7.64 (T2) mg per cent at sixth and eighth week of age, respectively. Serum Mg ranged from 2.10 (T4) to 2.60 (T3) mg per cent at sixth and 2.25 (T2) to 2.50 (T4) mg per cent at eighth week of age, respectively.

Serum Zn content ranged from 1.75 (T1) to 3.25 (T3) ppm at sixth week and 2.00 (T3) to 2.63 (T4) ppm at eighth week of age. Serum Mn content varied from 0.50 (T1) to 1.00 (T3) and 0.75 (T1) to 2.25 (T3) ppm at sixth and eighth week of age, respectively.

The mean serum ALP content of birds was 446.38, 425.31, 417.22 and 401.62 U/L at sixth week and 424.11, 411.64, 400.55 and 380.57 U/L at eighth week for T1, T2, T3 and T4, respectively.

4.14   PROCESSING YIELDS

Per cent dressed yield, ready-to-cook yield, giblet yield and abdominal fat yield of birds maintained on different dietary treatments at sixth and eighth week of age are given in Table 26.

The per cent dressed yield were 90.12, 90.44, 90.58 and 90.33 at sixth week and 91.91, 90.90, 91.56 and 90.67 at eighth week; the per cent ready-to-cook yield were 69.90, 70.17, 71.68 and 70.94 at sixth week and 72.54, 70.69, 72.38 and 71.61 at eighth week; the per cent giblet yield were 4.18, 4.33, 4.24 and 4.15 at sixth week and 3.77, 3.91, 3.87 and 3.90 at eighth week and the per cent abdominal fat yield were 1.67, 2.09, 1.46 and 1.29 at sixth week and 1.49, 2.09, 1.78 and 1.43 at eighth week for T1, T2, T3 and T4, respectively.

4.15 LIVABILITY

During the course of experiment only five birds died out of 192, in which one bird each from T1 and T4 and three birds from T2. The livability per cent was 97.92, 93.75, 100.00 and 97.92 for T1, T2, T3 and T4, respectively. They are graphically represented in Fig. 14.

4.16 ECONOMICS OF GAIN

Cost of experimental rations and cost of production (Rs.) per kg live weight gain of birds maintained on different dietary treatments are charted out in Tables 27 and 28. Their graphical representations are given in Fig. 15.

The cost/kg feed for T1, T2, T3 and T4 were Rs. 9.39, 11.72, 9.24 and 10.50 for starter ration and Rs. 8.89, 11.24, 8.76 and 10.02 for finisher ration, respectively. The cost feed/kg live weight gain were Rs. 19.63, 23.67, 19.12 and 21.07 at sixth week and Rs. 21.73, 26.53, 20.91 and 23.69 at eighth week for T1, T2, T3 and T4, respectively.

Table 6. Mean weekly meteorological data during the experimental period from November 29, 2002 to January 23, 2003.

Period (Weeks)	Temperature (°C)		Relative humidity (per cent)	
	Maximum	Minimum	8 A.M.	2 P.M.
1	32.50	23.93	61.29	41.36
2	31.57	25.43	60.57	40.14
3	32.21	24.14	60.00	46.57
4	32.07	21.86	57.43	39.29
5	32.00	22.21	72.43	50.00
6	32.43	23.93	63.29	51.00
7	33.43	24.21	61.47	46.21
8	34.50	24.36	58.43	41.86
Mean ±SE	**32.59** 0.33	**23.76** 0.41	**61.86** 1.64	**44.55** 1.60

Table 7. Fortnightly mean body weight of birds maintained on different dietary treatments, g

Treatments		0	2**	4**	6**	8*
				Age in weeks		
T1	R1	40.34	368.82	1105.83	1885.00	2545.45
	R2	40.27	380.50	1137.08	1909.17	2601.86
	R3	40.47	369.68	1093.75	1863.33	2566.36
	R4	40.25	353.63	1044.58	1811.67	2466.82
	Mean	**40.33**	**368.16**^{b}	**1095.31**^{bc}	**1867.29**^{b}	**2545.12**^{b}
	±SE	0.05	5.52	19.22	20.77	28.58
T2	R1	39.95	306.78	1020.91	1950.45	2693.33
	R2	40.85	304.76	1039.17	1944.58	2798.18
	R3	39.47	336.07	1074.17	2045.42	2830.45
	R4	39.82	312.93	976.25	1957.73	2870.00
	Mean	**40.02**	**315.14**^{c}	**1027.63**^{c}	**1974.55**^{ab}	**2797.99**^{a}
	±SE	0.29	7.19	20.38	23.78	37.85
T3	R1	40.27	378.21	1128.75	1994.58	2767.27
	R2	40.54	377.47	1095.00	1845.42	2540.45
	R3	40.91	389.46	1180.83	2012.08	2776.36
	R4	41.49	387.43	1108.33	1901.67	2566.82
	Mean	**40.80**	**383.14**^{ab}	**1128.23**^{ab}	**1938.44**^{b}	**2662.73**^{ab}
	±SE	0.26	3.09	18.86	39.35	63.24
T4	R1	40.16	394.64	1217.08	2064.58	2736.82
	R2	39.96	393.10	1140.83	2110.42	2988.64
	R3	39.63	393.53	1211.25	2093.75	2835.45
	R4	40.73	395.54	1155.83	2017.50	2665.45
	Mean	**40.12**	**394.20**^{a}	**1181.25**^{a}	**2071.56**^{a}	**2806.59**^{a}
	±SE	0.23	0.55	19.29	20.36	69.98
CD		-	20.73	83.99	117.50	228.00

a, b, c - Means bearing the different superscript within the same column differed significantly
* Significant (P<0.05); ** Significant (P<0.01)
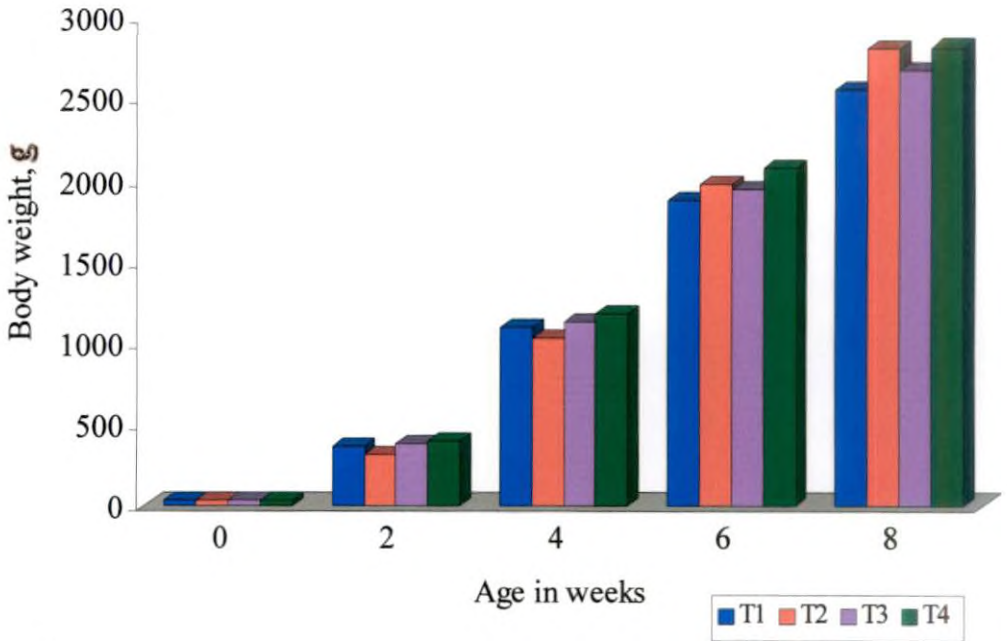
Fig. 1a. Fortnigtly mean body weight of birds maintained on different
dietary treatments
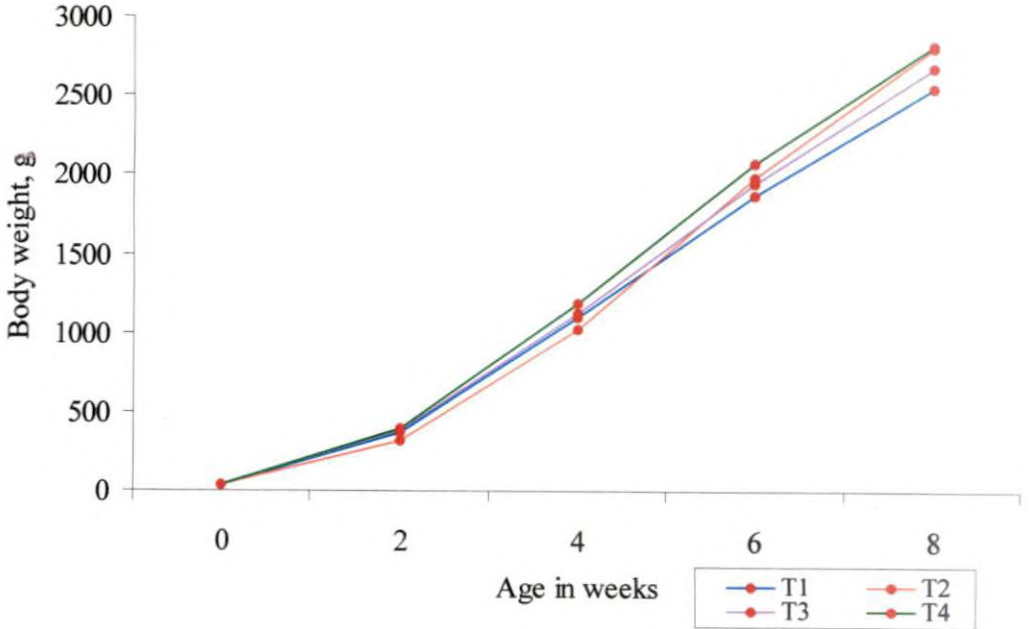
Fig. 1b. Fortnightly mean body weight of birds maintained on different
dietary treatments

Table 8. Fortnightly and cumulative mean body weight gain of birds maintained on different dietary treatments, g

Treatments		Fortnightly mean body weight gain (g)				Cumulative mean body weight gain (g)	
		Age in weeks				Age in weeks	
		2**	4*	6**	8*	0-6**	0-8*
T1	R1	328.48	737.01	779.17	660.45	1844.66	2505.11
	R2	340.23	756.58	772.09	692.69	1868.90	2561.59
	R3	329.21	724.07	769.58	703.03	1822.86	2525.89
	R4	313.38	690.95	767.09	655.15	1771.42	2426.57
	Mean	327.83[b]	727.15[b]	771.98[c]	677.83[b]	1826.96[b]	2504.79[b]
	±SE	5.51	13.79	2.60	11.81	20.76	28.56
T2	R1	266.83	714.13	929.54	742.88	1910.50	2653.38
	R2	263.91	734.41	905.41	853.60	1903.73	2757.33
	R3	296.60	738.10	971.25	785.03	2005.95	2790.98
	R4	273.11	663.32	981.48	912.27	1917.91	2830.18
	Mean	275.11[c]	712.49[b]	946.92[a]	823.45[a]	1934.52[ab]	2757.97[a]
	±SE	7.42	17.22	17.82	37.38	23.98	37.91
T3	R1	337.94	750.54	865.83	772.69	1954.31	2727.00
	R2	336.93	717.53	750.42	695.03	1804.88	2499.91
	R3	348.55	791.37	831.25	764.28	1971.17	2735.45
	R4	345.94	720.90	793.34	665.15	1860.18	2525.33
	Mean	342.34[ab]	745.09[ab]	810.21[bc]	724.29[ab]	1897.64[b]	2621.92[ab]
	±SE	2.89	17.12	24.83	26.29	39.40	63.34
T4	R1	354.48	822.44	847.50	672.24	2024.42	2696.66
	R2	353.14	747.73	969.59	878.22	2070.46	2948.68
	R3	353.90	817.72	882.50	741.70	2054.12	2795.82
	R4	354.81	760.29	861.67	647.95	1976.77	2624.72
	Mean	354.08[a]	787.05[a]	890.32[ab]	735.03[ab]	2031.44[a]	2766.47[a]
	±SE	0.37	19.27	27.39	51.70	20.57	70.14
CD		20.93	52.27	88.81	107.90	117.90	162.90

a, b, c - Means bearing the different superscript within the same column differed significantly
* Significant (P<0.05)
** Significant (P<0.01)
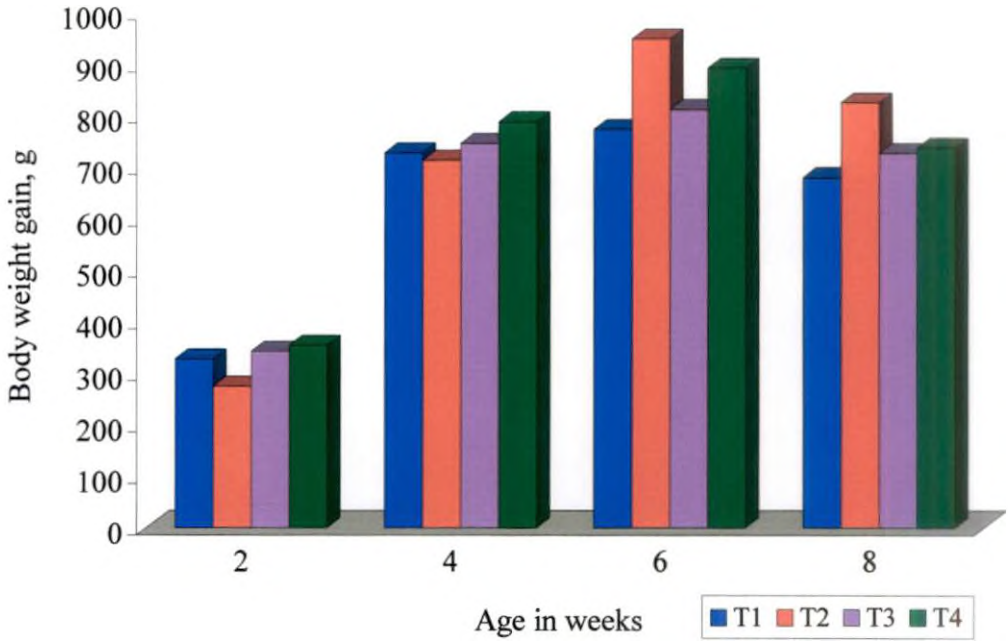
Fig. 2. Fortnighly mean body weight gain of birds maintained on different dietary treatments
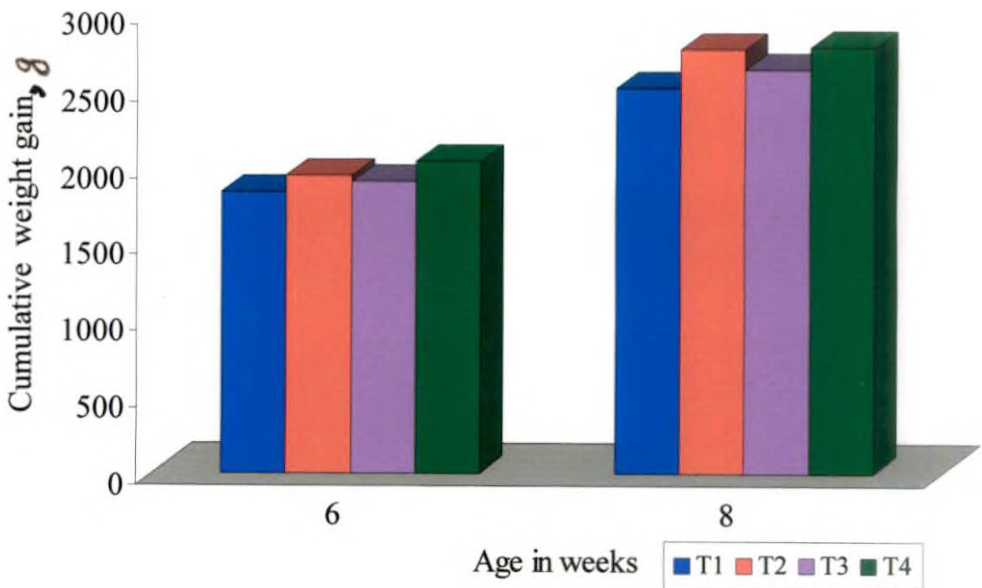
Fig. 3. Cumulative mean body weight gain of birds maintained on different dietary treatments

Table 9. Mean daily feed intake of birds maintained on different dietary treatments at weekly intervals, g

Treatments		1	2**	3*	4**	5**	6**	7*	8**
					Age in weeks				
T1	R1	16.67	45.60	88.57	126.55	129.88	147.38	148.70	152.08
	R2	17.50	46.79	88.14	127.74	122.02	145.24	152.21	145.84
	R3	16.19	46.55	88.14	116.90	125.12	137.98	152.86	143.64
	R4	18.57	46.79	88.45	127.14	126.67	140.60	156.88	140.39
	Mean	17.23	46.43^a	88.33^a	124.58^a	125.92^c	142.80^{bc}	142.80^c	152.66^{bc}
	±SE	0.52	0.28	0.11	2.57	1.64	2.14	2.14	1.68
T2	R1	14.50	39.40	80.54	113.75	146.23	155.71	161.43	183.90
	R2	15.80	39.05	79.64	115.36	137.80	166.37	153.77	178.31
	R3	17.14	44.76	81.13	120.06	145.83	162.38	163.77	187.14
	R4	17.02	41.90	82.02	114.64	136.13	165.06	172.34	190.39
	Mean	16.12	41.28^b	80.83^b	115.95^b	141.50^{ab}	162.38^a	162.83^a	184.94^a
	±SE	0.62	1.32	0.50	1.41	2.64	2.37	3.82	2.58
T3	R1	15.71	45.36	83.21	125.36	140.48	142.98	155.19	164.03
	R2	17.26	52.14	83.69	124.40	137.86	135.12	147.40	145.06
	R3	18.10	53.33	87.86	127.38	143.57	143.57	156.49	146.10
	R4	16.31	53.10	96.07	123.81	139.64	134.76	144.42	149.22
	Mean	16.85	50.98^a	87.71^a	125.24^a	140.39^b	139.11^c	150.88^{bc}	151.10^c
	±SE	0.53	1.89	2.98	0.78	1.19	2.41	2.94	4.40
T4	R1	18.45	49.40	89.17	127.24	148.30	147.50	157.40	158.44
	R2	17.98	49.52	86.19	125.48	148.24	155.12	159.74	170.91
	R3	18.63	47.32	90.71	131.79	158.69	152.26	158.05	173.76
	R4	17.50	48.69	85.49	122.75	146.48	145.60	152.72	162.34
	Mean	18.14	48.73^a	87.89^a	126.82^a	150.43^a	150.12^b	156.98^{ab}	166.36^b
	±SE	0.25	0.51	1.23	1.90	2.79	2.18	1.50	3.59
CD		-	5.14	5.03	7.73	9.37	9.84	8.20	14.48

a, b, c - Means bearing the different superscript within the same column differed significantly
* Significant (P<0.05)
** Significant (P<0.01)

Table 10. Fortnightly and cumulative mean feed intake of birds maintained
on different dietary treatments, g

Treatments		Fortnightly mean feed intake				Cumulative mean feed intake	
		Age in weeks				Age in weeks	
		2	4	6	8	0-6	0-8
T1	R1	435.84	1505.83	1940.84	2105.45	3882.51	5987.96
	R2	450.00	1511.67	1870.84	2086.36	3832.51	5918.87
	R3	439.13	1435.83	1841.66	2075.45	3716.62	5792.07
	R4	457.50	1509.17	1870.84	2080.91	3837.51	5918.42
	Mean	455.62^{ab}	1490.63^a	1881.05^b	2087.04^c	3817.29^b	5904.33^b
	±SE	4.98	18.30	21.08	6.53	35.39	40.83
T2	R1	377.50	1360.00	2113.64	2417.27	3851.14	6268.41
	R2	384.16	1365.00	2129.16	2324.54	3878.32	6202.86
	R3	433.33	1408.34	2157.50	2456.36	3999.17	6455.53
	R4	412.50	1376.67	2108.37	2539.09	3897.54	6436.63
	Mean	401.87^b	1377.50^b	2127.17^a	2434.32^a	3906.54^b	6340.86^a
	±SE	12.94	10.86	11.03	44.54	32.31	62.33
T3	R1	427.50	1460.00	1984.16	2234.54	3871.66	6106.20
	R2	485.83	1456.66	1910.83	2047.27	3853.32	5900.59
	R3	500.00	1506.66	2010.00	2118.18	4016.66	6134.84
	R4	485.84	1539.17	1920.83	2055.46	3945.84	6001.30
	Mean	474.79^a	1490.62^a	1956.46^b	2113.86^c	3921.87^{ab}	6035.73^b
	±SE	16.11	19.80	24.13	43.23	37.39	53.41
T4	R1	475.00	1518.34	2070.80	2210.91	4064.14	6275.05
	R2	472.50	1481.66	2123.52	2314.55	4077.68	6392.23
	R3	461.67	1557.50	2176.66	2285.46	4195.83	6481.29
	R4	463.33	1457.69	2044.50	2235.23	3965.52	6200.75
	Mean	468.13^a	1503.80^a	2103.87^a	2261.54^b	4075.79^a	6337.33^a
	±SE	3.31	21.82	29.30	23.52	47.18	62.10
CD		46.47	78.50	96.76	141.10	166.20	239.20

a, b, c - Means bearing the different superscript within the same column differed
significantly (P<0.01)
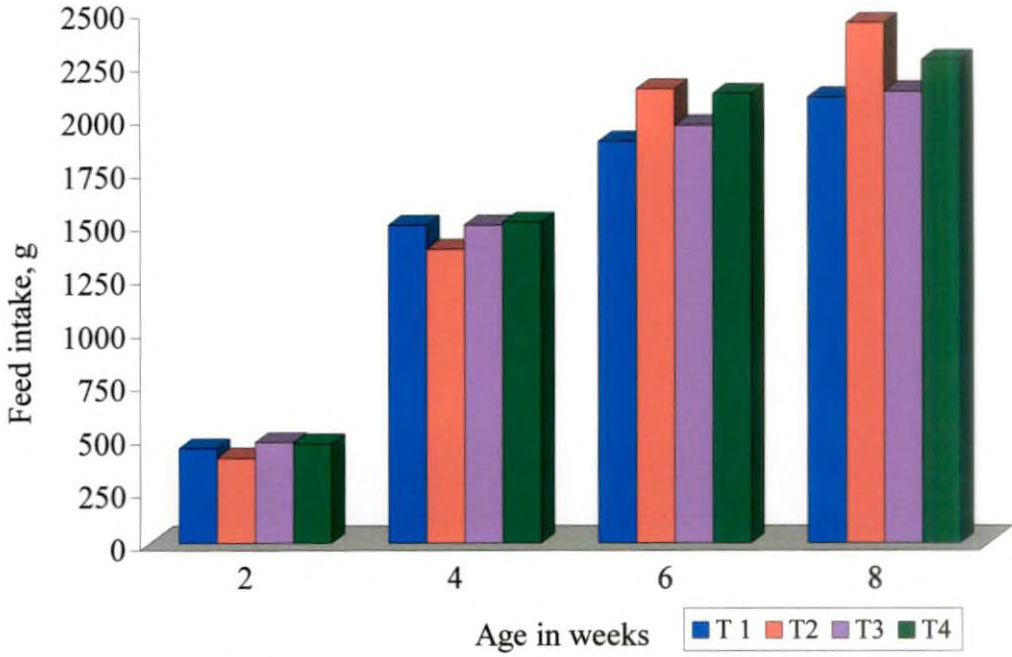
Fig. 4. Fortnightly mean feed intake of birds maintained on
different dietary treatments
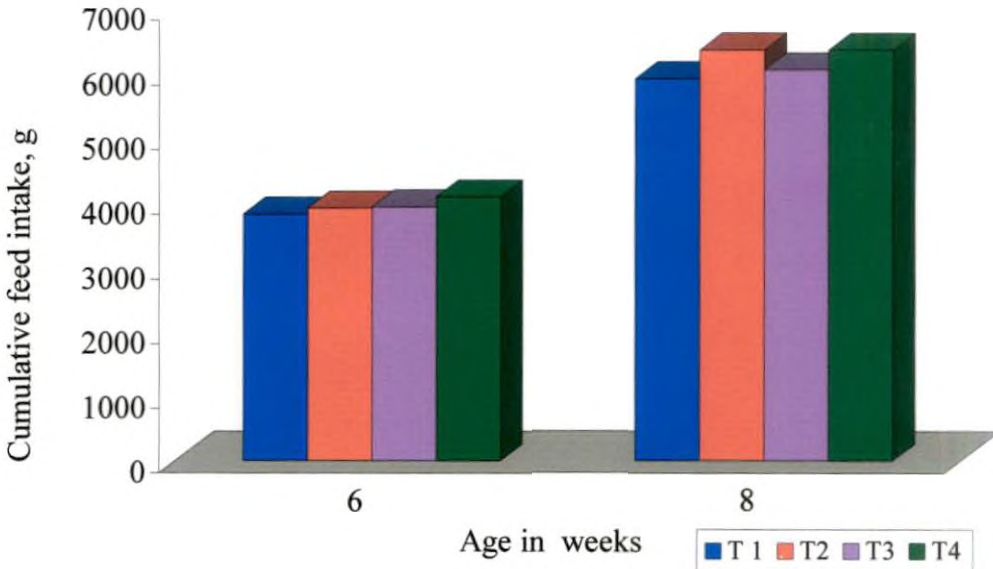
Fig. 5. Cumulative mean feed intake of birds maintained on different
dietary treatments

Table 11. Fortnightly and cumulative mean feed conversion ratio of birds
maintained on different dietary treatments

Treatments		Fortnightly mean feed conversion ratio				Cumulative mean feed conversion ratio	
		Age in weeks				Age in weeks	
		2	4	6	8	0-6	0-8
T1	R1	1.33	2.04	2.49	3.19	2.10	2.39
	R2	1.32	2.00	2.42	3.01	2.05	2.31
	R3	1.33	1.98	2.39	2.95	2.04	2.29
	R4	1.46	2.18	2.44	3.18	2.17	2.44
	Mean	**1.36^b**	**2.05**	**2.44**	**3.08**	**2.09**	**2.36**
	±SE	0.03	0.05	0.02	0.06	0.03	0.03
T2	R1	1.41	1.90	2.27	3.25	2.02	2.36
	R2	1.46	1.86	2.35	2.72	2.04	2.25
	R3	1.46	1.91	2.22	3.13	1.99	2.31
	R4	1.51	2.08	2.15	2.78	2.03	2.27
	Mean	**1.46^a**	**1.94**	**2.25**	**2.97**	**2.02**	**2.30**
	±SE	0.02	0.05	0.04	0.13	0.01	0.02
T3	R1	1.27	1.95	2.29	2.89	1.98	2.24
	R2	1.44	2.03	2.55	2.95	2.13	2.36
	R3	1.43	1.90	2.42	2.77	2.04	2.24
	R4	1.40	2.14	2.42	3.09	2.12	2.38
	Mean	**1.39^{ab}**	**2.00**	**2.42**	**2.92**	**2.07**	**2.30**
	±SE	0.04	0.05	0.05	0.07	0.04	0.04
T4	R1	1.34	1.85	2.44	3.29	2.01	2.33
	R2	1.34	1.98	2.19	2.64	1.97	2.17
	R3	1.30	1.90	2.47	3.08	2.04	2.32
	R4	1.31	1.92	2.37	3.45	2.01	2.36
	Mean	**1.32^b**	**1.90**	**2.37**	**3.11**	**2.01**	**2.29**
	±SE	0.01	0.03	0.06	0.18	0.01	0.04
CD		0.09	-	-	-	-	-

a, b, c - Means bearing the different superscript within the same column differed
significantly (P<0.05)

Table 12. Fortnightly and cumulative mean protein efficiency ratio of birds maintained on different dietary treatments

Treatments		Fortnightly mean protein efficiency ratio				Cumulative mean protein efficiency ratio	
		Age in weeks				Age in weeks	
		2	4	6	8	0-6	0-8
T1	R1	3.19	2.07	1.70	1.55	2.01	1.87
	R2	3.21	2.12	1.75	1.64	2.07	1.93
	R3	3.18	2.14	1.77	1.67	2.08	1.95
	R4	2.90	1.94	1.74	1.56	1.96	1.83
	Mean	**3.12**	**2.07**	**1.74**[b]	**1.60**	**2.03**[b]	**1.89**
	±SE	0.07	0.04	0.01	0.03	0.03	0.03
T2	R1	3.05	2.27	1.90	1.51	2.14	1.92
	R2	2.97	2.33	1.84	1.80	2.12	2.01
	R3	2.96	2.26	1.95	1.57	2.17	1.96
	R4	2.86	2.08	2.01	1.77	2.13	2.00
	Mean	**2.96**	**2.24**	**1.92**[a]	**1.66**	**2.14**[a]	**1.97**
	±SE	0.04	0.05	0.04	0.07	0.01	0.02
T3	R1	3.35	2.18	1.85	1.71	2.14	2.00
	R2	2.94	2.09	1.66	1.68	1.98	1.89
	R3	2.95	2.22	1.75	1.79	2.08	1.99
	R4	3.01	1.98	1.75	1.60	2.00	1.87
	Mean	**3.06**	**2.12**	**1.75**[b]	**1.69**	**2.05**[b]	**1.94**
	±SE	0.10	0.05	0.04	0.04	0.04	0.03
T4	R1	3.19	2.32	1.75	1.49	2.13	1.93
	R2	3.20	2.16	1.95	1.86	2.17	2.07
	R3	3.28	2.25	1.74	1.59	2.10	1.93
	R4	3.28	2.23	1.80	1.42	2.13	1.90
	Mean	**3.24**	**2.24**	**1.81**[ab]	**1.59**	**2.13**[a]	**1.96**
	±SE	0.02	0.03	0.05	0.10	0.02	0.04
CD		-	-	0.109	-	0.077	-

a, b, c - Means bearing the superscript within the same column differed significantly (P<0.05)
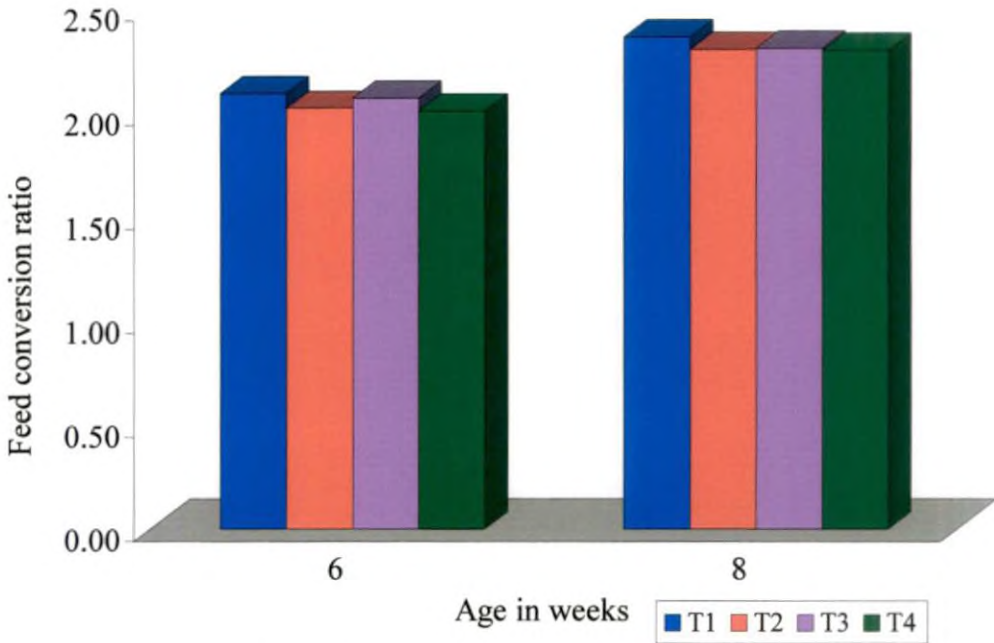
Fig. 6. Cumulative mean feed conversion ratio of birds
maintained on different dietary treatments
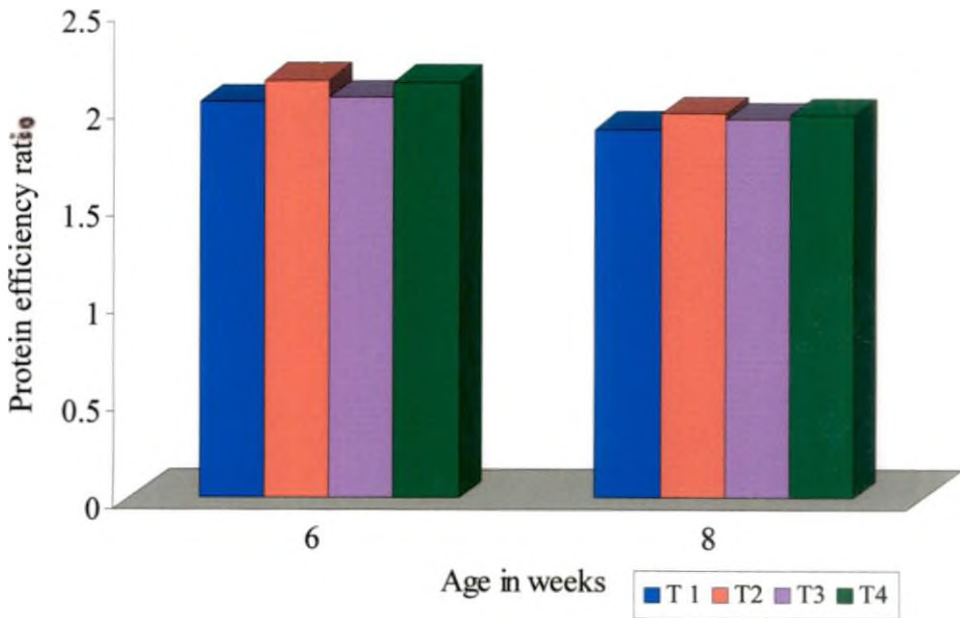
Fig. 7. Cumulative mean protein efficiency ratio of birds maintained on
different dietary treatments

Table 13. Chemical composition of droppings voided by the birds during the metabolism trial*, %

Treatments		Moisture	Dry matter	Crude protein	N	Ca	P	Mg	Zn (mg/kg)	Mn (mg/kg)
T1	R1	78.87	21.13	27.36	4.38	2.33	1.45	0.43	218.43	331.83
	R2	82.42	17.58	34.44	5.51	2.43	1.93	0.44	237.80	380.27
	R3	80.49	19.51	31.40	5.02	2.14	1.83	0.48	251.50	367.57
	R4	77.46	22.54	42.24	6.76	1.91	1.80	0.34	181.68	272.52
	Mean	79.81	20.19	33.86	5.42	2.20	1.75	0.42	222.35	338.05
	± SE	1.07	1.07	3.15	0.50	0.11	0.11	0.03	15.16	24.13
T2	R1	80.12	19.88	29.02	4.64	2.22	1.18	0.44	206.35	304.70
	R2	85.68	14.32	36.33	5.81	2.59	1.41	0.49	222.51	333.77
	R3	78.17	21.83	32.69	5.23	2.12	1.18	0.41	195.60	297.95
	R4	79.68	20.32	38.08	6.09	2.79	1.36	0.50	225.53	323.76
	Mean	80.91	19.09	34.03	5.44	2.43	1.28	0.46	212.50	315.04
	± SE	1.64	1.64	2.01	0.32	0.16	0.06	0.02	7.03	8.30
T3	R1	84.06	15.94	33.74	5.40	2.10	1.23	0.39	180.63	280.03
	R2	86.11	13.89	40.85	6.54	2.12	1.09	0.47	190.03	234.73
	R3	83.91	16.09	30.17	4.83	1.98	1.13	0.45	197.84	301.21
	R4	85.59	14.41	43.51	6.96	2.52	1.46	0.42	232.92	340.03
	Mean	84.92	15.08	37.07	5.93	2.18	1.23	0.43	200.36	289.00
	± SE	0.55	0.55	3.09	0.49	0.12	0.08	0.02	11.41	21.94
T4	R1	86.37	13.63	38.98	6.24	2.27	1.22	0.47	217.61	306.29
	R2	82.55	17.45	31.90	5.10	2.72	1.17	0.43	211.85	326.16
	R3	82.40	17.60	31.40	5.02	2.62	1.30	0.48	225.10	337.65
	R4	81.87	18.13	33.51	5.36	2.86	1.13	0.45	219.64	305.91
	Mean	83.30	16.70	33.95	5.43	2.62	1.21	0.46	218.55	319.00
	± SE	1.03	1.03	1.74	0.28	0.12	0.04	0.01	2.74	7.81

*On dry matter basis

Table 14. Average daily dry matter intake, outgo and retention in birds recorded during the metabolism trial

Treatments		DM intake (g/day)	DM outgo (g/day)	DM retention (per cent)[NS]
T1	R1	151.68	51.00	66.38
	R2	103.74	28.94	72.11
	R3	123.36	37.16	69.88
	R4	36.37	16.04	55.90
	Mean	**103.79**	**33.28**	**66.07**
	± SE	24.53	7.33	3.59
T2	R1	152.43	47.37	68.92
	R2	129.50	41.30	68.11
	R3	155.49	55.51	64.30
	R4	131.01	39.11	70.14
	Mean	**142.11**	**45.82**	**67.87**
	± SE	6.88	3.67	1.26
T3	R1	158.80	44.09	72.24
	R2	100.19	27.78	72.28
	R3	132.19	39.36	70.22
	R4	104.61	36.10	65.49
	Mean	**123.95**	**36.83**	**70.06**
	± SE	13.60	3.44	1.59
T4	R1	128.99	40.74	68.42
	R2	159.29	55.49	65.17
	R3	173.55	52.76	69.60
	R4	144.10	49.59	65.58
	Mean	**151.48**	**49.64**	**67.19**
	± SE	9.61	3.20	1.08

NS – Not significant

Table 15. Data on N retention and excretion in birds maintained on different dietary treatments

Treatments		N intake (g/day)	N outgo (g/day)	N balance (g/day) [NS]	N retention (per cent) [NS]	N excretion (g/kg DM intake) [NS]
T1	R1	4.91	2.23	2.68	54.55	14.72
	R2	3.36	1.59	1.76	52.53	15.37
	R3	4.00	1.87	2.13	53.26	15.14
	R4	1.18	1.08	0.09	7.97	29.80
	Mean	3.36	1.69	1.67	42.08	18.76
	±SE	0.79	0.26	0.56	11.38	3.68
T2	R1	4.96	2.20	2.76	55.67	14.43
	R2	4.22	2.40	1.82	43.06	18.54
	R3	5.06	2.90	2.16	42.66	18.67
	R4	4.27	2.38	1.88	44.14	18.19
	Mean	4.63	2.47	2.18	46.38	17.46
	±SE	0.22	0.15	0.22	3.11	1.01
T3	R1	5.14	2.38	2.76	53.65	14.99
	R2	3.24	1.82	1.42	43.96	18.12
	R3	4.27	1.90	2.37	55.54	14.38
	R4	3.38	2.51	0.87	25.71	24.02
	Mean	4.01	2.15	1.86	44.72	17.88
	±SE	0.44	0.17	0.43	6.82	2.21
T4	R1	4.21	2.54	1.67	39.61	19.70
	R2	5.20	2.83	2.36	45.50	17.78
	R3	5.66	2.65	3.01	53.19	15.27
	R4	4.70	2.66	2.04	43.44	18.45
	Mean	4.94	2.67	2.27	45.43	17.80
	±SE	0.31	0.06	0.28	2.86	0.93

NS – Not significant

Table 16. Data on Ca balance and per cent availability in birds maintained on different dietary treatments

Treatments		Ca intake (g/day)	Ca outgo (g/day)	Ca balance (g/day) NS	Ca availability (per cent) NS
T1	R1	1.80	1.19	0.62	34.29
	R2	1.23	0.70	0.53	42.99
	R3	1.47	0.79	0.67	45.88
	R4	0.43	0.31	0.13	29.04
	Mean	**1.24**	**0.75**	**0.49**	**38.05**
	±SE	0.29	0.18	0.12	3.88
T2	R1	1.84	1.05	0.79	43.06
	R2	1.57	1.07	0.50	31.72
	R3	1.88	1.18	0.70	37.36
	R4	1.59	1.09	0.49	31.10
	Mean	**1.72**	**1.10**	**0.62**	**35.81**
	±SE	0.08	0.03	0.08	2.80
T3	R1	1.95	0.92	1.03	52.69
	R2	1.23	0.59	0.64	52.12
	R3	1.63	0,78	0.85	52.17
	R4	1.29	0.91	0.38	29.30
	Mean	**1.52**	**0.80**	**0.72**	**46.57**
	±SE	0.17	0.08	0.14	5.76
T4	R1	1.55	0.93	0.62	40.18
	R2	1.91	1.51	0.40	21.07
	R3	2.08	1.38	0.70	33.62
	R4	1.73	1.42	0.31	18.07
	Mean	**1.82**	**1.31**	**0.51**	**28.23**
	±SE	0.12	0.13	0.09	5.21

NS – Not significant

Table 17. Data on P balance and excretion in birds maintained on different dietary treatments

Treatments		P intake (g/day)	P outgo (g/day)	P balance (g/day) [NS]	P availability (per cent) [NS]	P excretion (g/kg DM intake)
T1	R1	1.223	0.737	0.486	39.71	4.86
	R2	0.836	0.558	0.279	33.32	5.38
	R3	0.994	0.679	0.315	31.68	5.51
	R4	0.293	0.289	0.005	1.54	7.94
	Mean	**0.837**	**0.566**	**0.271**	**26.56**	**5.92[a]**
	±SE	0.198	0.100	0.100	8.52	0.69
T2	R1	0.896	0.557	0.340	37.89	3.65
	R2	0.762	0.581	0.181	23.76	4.48
	R3	0.914	0.658	0.257	28.09	4.23
	R4	0.770	0.532	0.239	31.00	4.06
	Mean	**0.836**	**0.582**	**0.254**	**30.19**	**4.11[b]**
	±SE	0.040	0.027	0.033	2.97	0.17
T3	R1	0.927	0.543	0.384	41.40	3.42
	R2	0.585	0.303	0.282	48.21	3.02
	R3	0.771	0.447	0.325	42.11	3.38
	R4	0.610	0.526	0.084	13.80	5.03
	Mean	**0.723**	**0.455**	**0.269**	**36.38**	**3.71[b]**
	±SE	0.079	0.055	0.065	7.68	0.45
T4	R1	0.757	0.496	0.261	34.51	3.85
	R2	0.935	0.651	0.285	30.44	4.08
	R3	1.019	0.685	0.334	32.77	3.95
	R4	0.846	0.562	0.284	33.56	3.90
	Mean	**0.889**	**0.598**	**0.291**	**32.82**	**3.94[b]**
	±SE	0.056	0.043	0.015	0.87	0.05
CD				-	-	1.294

a, b, c - Means bearing the different superscript within the same column differed significantly (P<0.05)

NS - Not significant

Table 18. Data on Mg balance and per cent availability in birds maintained on different dietary treatments

Treatments		Mg intake (g/day)	Mg outgo. (g/day)	Mg balance (g/day)[NS]	Mg availability (per cent)
T1	R1	0.23	0.22	0.01	4.87
	R2	0.16	0.13	0.03	19.71
	R3	0.19	0.18	0.01	5.51
	R4	0.06	0.06	0.00	0.00
	Mean	**0.16**	**0.15**	**0.01**	**7.52**[b]
	±SE	0.04	0.04	0.01	4.24
T2	R1	0.24	0.21	0.03	12.32
	R2	0.20	0.20	0.00	0.00
	R3	0.24	0.23	0.02	6.68
	R4	0.21	0.19	0.01	5.74
	Mean	**0.22**	**0.21**	**0.01**	**6.19**[b]
	±SE	0.01	0.01	0.01	2.52
T3	R1	0.26	0.17	0.08	33.20
	R2	0.16	0.13	0.03	19.54
	R3	0.21	0.18	0.04	17.69
	R4	0.17	0.15	0.02	9.54
	Mean	**0.20**	**0.16**	**0.04**	**19.99**[a]
	±SE	0.02	0.01	0.01	4.91
T4	R1	0.21	0.19	0.02	7.87
	R2	0.26	0.24	0.02	5.93
	R3	0.28	0.26	0.02	8.02
	R4	0.23	0.22	0.01	3.74
	Mean	**0.24**	**0.23**	**0.02**	**6.39**[b]
	±SE	0.02	0.01	0.00	1.00
CD		-	-	-	10.84

a, b, c - Means bearing the different superscript within the same column differed significantly (P<0.05)

NS – Not significant

Table 19. Data on Zn balance and per cent availability in birds maintained on different dietary treatments

Treatments		Zn intake (mg/day)	Zn outgo (mg/day)	Zn balance (mg/day) [NS]	Zn availability (per cent) [NS]
T1	R1	12.23	11.14	1.09	8.90
	R2	8.36	6.88	1.48	17.73
	R3	9.95	9.35	0.60	6.03
	R4	2.93	2.91	0.02	0.62
	Mean	**8.37**	**7.64**	**0.86**	**8.32**
	±SE	1.98	1.72	0.27	3.58
T2	R1	12.27	9.78	2.50	20.34
	R2	10.43	9.19	1.24	11.86
	R3	12.52	10.86	1.66	13.27
	R4	10.55	8.82	1.73	16.36
	Mean	**11.44**	**9.66**	**1.78**	**15.46**
	±SE	0.55	0.44	0.26	1.88
T3	R1	13.03	7.96	5.07	38.90
	R2	8.22	5.28	2.94	35.81
	R3	10.85	7.79	3.06	28.22
	R4	8.59	8.41	0.18	2.07
	Mean	**10.17**	**7.36**	**2.81**	**26.25**
	±SE	1.12	0.71	1.00	8.37
T4	R1	10.83	8.87	1.96	18.12
	R2	13.37	11.75	1.62	12.09
	R3	14.57	11.88	2.69	18.48
	R4	12.10	10.89	1.20	9.95
	Mean	**12.72**	**10.85**	**1.87**	**14.66**
	±SE	0.81	0.70	0.32	2.15

NS – Not significant

Table 20. Data on Mn balance and per cent availability in birds maintained on different dietary treatments

Treatments		Mn intake (mg/day)	Mn outgo (mg/day)	Mn balance (mg/day)	Mn availability (per cent)
T1	R1	18.70	16.92	1.78	9.52
	R2	12.79	11.00	1.79	13.99
	R3	15.21	13.66	1.55	10.21
	R4	4.48	4.37	0.11	2.54
	Mean	12.80	11.49	1.31[b]	9.06[b]
	±SE	3.03	2.66	0.40	2.39
T2	R1	19.01	14.43	4.58	24.08
	R2	16.15	13.78	2.37	14.67
	R3	19.39	16.54	2.86	14.72
	R4	16.34	12.66	3.68	22.51
	Mean	17.73	14.35	3.37[b]	18.99[ab]
	±SE	0.86	0.81	0.48	2.50
T3	R1	20.34	12.35	7.99	39.29
	R2	12.83	6.52	6.31	49.18
	R3	16.93	11.86	5.07	29.97
	R4	13.40	12.27	1.12	8.38
	Mean	15.87	10.75	5.12[a]	31.71[a]
	±SE	1.74	1.41	1.46	8.71
T4	R1	16.71	12.48	4.23	25.30
	R2	20.63	18.10	2.53	12.27
	R3	22.48	17.81	4.66	20.74
	R4	18.66	15.17	3.49	18.71
	Mean	19.62	15.89	3.73[b]	19.25[ab]
	±SE	1.24	1.31	0.47	2.71
CD				2.56	15.03

a, b, c - Means bearing the different superscript within the same column differed significantly (P<0.05)

Fig. 8. DM retention, N retention and excretion in birds
maintained on different dietary treatments

Fig. 9. Availability of Ca, P, Mg, Zn and Mn in birds maintained on
different dietary treatments

Table 21. Weight of dried tibia and tibial ash content of birds maintained on different dietary treatments at sixth and eighth week of age

Treatments		Weight of dried tibia (g)		Tibial ash (per cent)	
		Age in weeks		Age in weeks	
		6	8	6	8
T1	R1	7.28	8.29	38.07	39.14
	R2	4.74	11.90	40.86	40.94
	R3	5.33	8.30	40.10	39.18
	R4	4.94	11.66	40.85	42.78
	Mean	5.62^b	10.04	39.97	40.51
	±SE	0.63	1.01	0.66	0.86
T2	R1	6.74	9.85	42.49	43.22
	R2	7.30	14.58	37.40	42.70
	R3	6.64	9.75	43.49	41.90
	R4	7.26	13.73	38.33	41.66
	Mean	6.98^a	11.98	40.43	42.37
	±SE	0.17	1.27	1.51	0.36
T3	R1	5.52	8.85	42.32	40.68
	R2	6.09	13.54	41.55	42.81
	R3	7.34	10.93	38.11	40.48
	R4	6.38	13.43	41.72	42.02
	Mean	6.33^{ab}	11.69	40.92	41.50
	±SE	0.38	1.12	0.95	0.55
T4	R1	6.77	9.86	40.38	44.42
	R2	7.32	14.64	43.69	41.87
	R3	7.14	10.22	40.34	43.30
	R4	7.08	12.85	42.87	41.07
	Mean	7.08^a	11.89	41.82	42.67
	±SE	0.11	1.13	0.86	0.75
CD		1.117	-	-	-

a, b, c - Means bearing the different superscript within the same column differed significantly (P<0.05)

Fig. 10. P excretion in birds maintained on different dietary
treatments
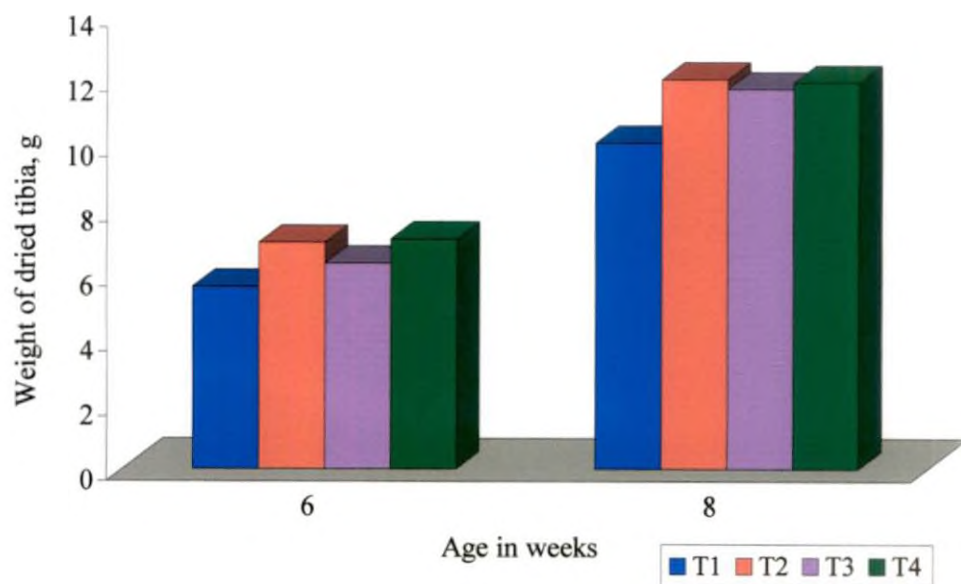
Fig. 11. Weight of dried tibia of birds maintained on different
dietary treatments at sixth and eighth week of age

Table 22. Tibial Ca, inorganic P and Mg content of birds maintained on different dietary treatments at sixth and eighth week of age, mg %

Treatments		Tibial Ca		Tibial iP		Tibial Mg	
		Age in weeks		Age in weeks		Age in weeks	
		6	8	6	8	6	8
T1	R1	11.98	13.74	8.81	9.87	0.35	0.39
	R2	12.83	15.86	9.86	12.38	0.40	0.43
	R3	11.95	15.39	9.05	10.18	0.33	0.43
	R4	13.25	12.22	9.28	14.77	0.34	0.47
	Mean	12.50	14.30	9.25	11.80	0.36^c	0.43
	±SE	0.32	0.83	0.22	1.14	0.01	0.02
T2	R1	11.64	14.72	8.57	10.88	0.38	0.47
	R2	14.41	14.68	7.44	10.54	0.38	0.46
	R3	12.95	12.74	7.93	10.20	0.42	0.52
	R4	11.92	20.31	8.13	16.62	0.46	0.44
	Mean	12.73	15.61	8.02	12.06	0.41^{ab}	0.47
	±SE	0.63	1.63	0.23	1.53	0.02	0.02
T3	R1	12.63	13.77	9.16	9.89	0.35	0.39
	R2	12.59	13.77	8.51	10.74	0.35	0.43
	R3	12.53	13.44	10.20	9.96	0.39	0.43
	R4	13.09	14.63	9.17	10.63	0.38	0.47
	Mean	12.71	13.90	9.26	10.30	0.37^{bc}	0.43
	±SE	0.13	0.25	0.35	0.22	0.01	0.01
T4	R1	11.46	18.28	8.66	12.19	0.44	0.42
	R2	14.31	16.09	9.19	12.68	0.49	0.38
	R3	11.77	13.94	8.06	10.83	0.41	1.04
	R4	13.66	14.08	11.10	10.42	0.43	0.40
	Mean	12.80	15.60	9.25	11.53	0.44^a	0.56
	±SE	0.70	1.02	0.66	0.54	0.02	0.16
CD		-	-	-	-	0.049	-

a, b, c - Means bearing the different superscript within the same column differed significantly ($P<0.05$)

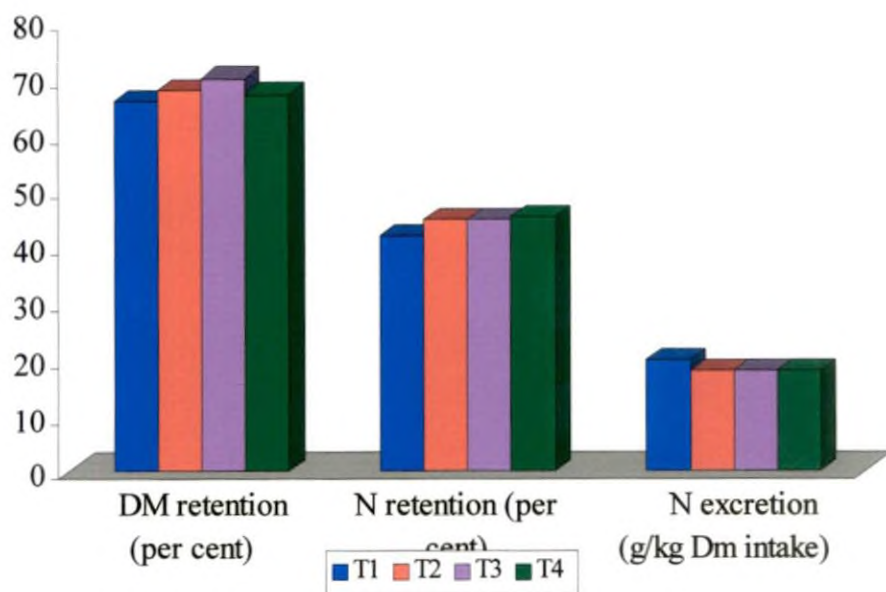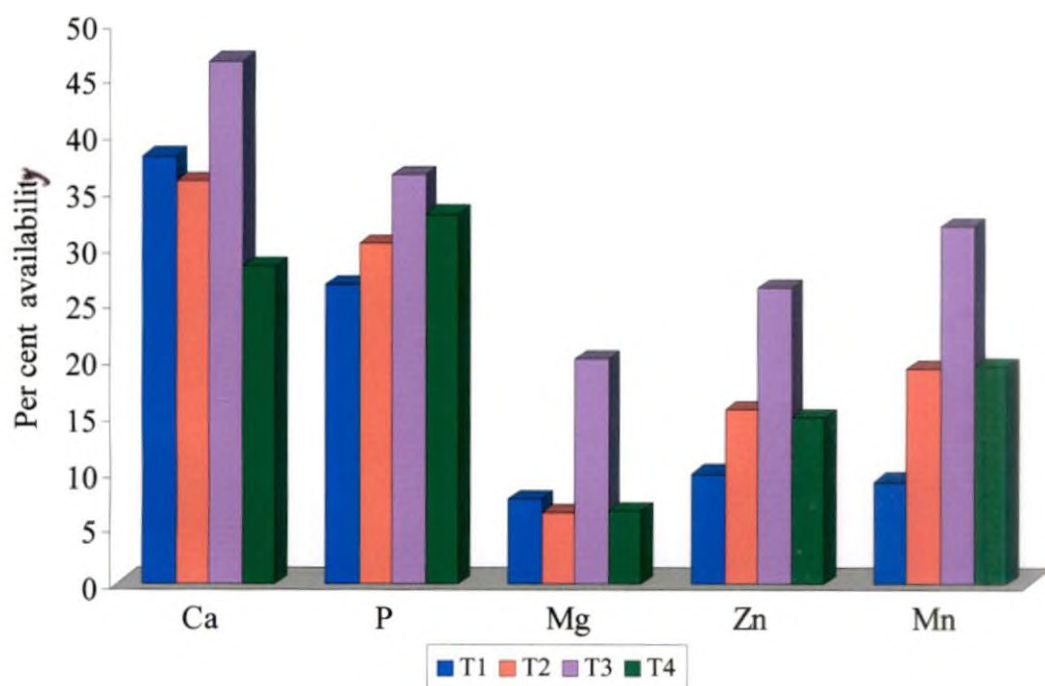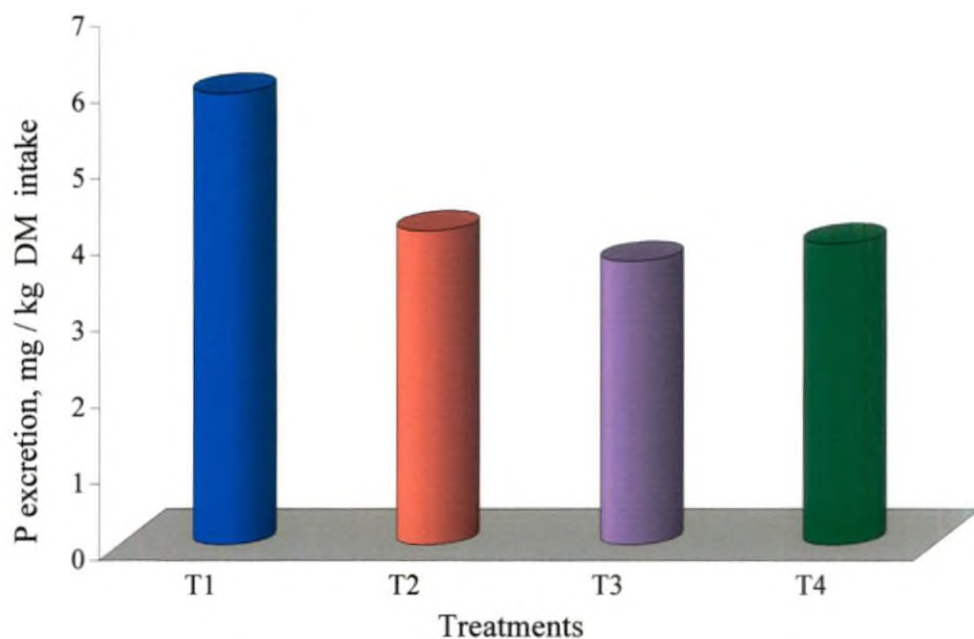Table 23. Tibial Zn and Mn content of birds maintained on different dietary treatments at sixth and eighth week of age, ppm

Treatments		Zn		Mn	
		Age in weeks		Age in weeks	
		6 ^{NS}	8 ^{NS}	6 ^{NS}	8 ^{NS}
T1	R1	151.52	138.12	34.53	75.76
	R2	169.49	155.52	77.76	59.32
	R3	83.68	131.23	65.62	83.68
	R4	185.53	134.05	67.02	92.76
	Mean	**147.55**	**139.73**	**61.23**	**77.88**
	±SE	22.40	5.45	9.30	7.10
T2	R1	91.32	148.04	74.02	91.32
	R2	91.16	147.60	73.80	91.16
	R3	149.37	166.53	83.26	74.68
	R4	86.58	181.00	90.50	86.58
	Mean	**104.61**	**160.79**	**80.40**	**85.94**
	±SE	14.96	8.05	4.03	3.91
T3	R1	77.40	138.50	69.25	77.40
	R2	85.11	138.50	69.25	85.11
	R3	84.75	152.44	76.22	84.75
	R4	91.66	150.94	75.47	91.66
	Mean	**84.73**	**145.10**	**72.55**	**84.73**
	±SE	2.91	3.82	1.91	2.91
T4	R1	166.39	85.32	83.19	85.32
	R2	73.86	191.20	73.86	95.60
	R3	68.12	160.77	68.12	80.39
	R4	157.60	167.36	78.80	83.68
	Mean	**116.49**	**151.17**	**75.99**	**86.25**
	±SE	26.36	22.90	3.24	3.28

NS - Not significant

Table 24. Serum Ca, inorganic P and Mg content of birds maintained on different dietary treatments at sixth and eighth week of age, mg %

Treatments		Serum Ca		Serum iP		Serum Mg	
		Age in weeks		Age in weeks		Age in weeks	
		6^{NS}	8^{NS}	6^{NS}	8^{NS}	6^{NS}	8^{NS}
T1	R1	8.75	8.00	4.43	7.70	2.20	2.40
	R3	8.50	8.25	5.30	5.65	1.80	2.40
	R3	9.25	8.50	4.08	8.21	1.80	2.40
	R4	11.00	8.75	6.67	6.78	3.00	2.40
	Mean	**9.38**	**8.38**	**5.12**	**7.09**	**2.20**	**2.40**
	±SE	0.56	0.16	0.58	0.56	0.58	0.00
T2	R1	9.5	8.50	7.90	7.62	2.40	2.40
	R3	8.25	9.25	6.00	5.68	3.00	2.20
	R3	8.0	7.25	4.40	6.18	1.80	2.20
	R4	8.75	11.0	7.95	11.06	1.80	2.20
	Mean	**8.63**	**9.00**	**6.56**	**7.64**	**2.25**	**2.25**
	±SE	0.73	0.78	0.85	1.21	0.29	0.05
T3	R1	12.5	9.00	6.80	8.04	4.00	2.20
	R3	9.75	11.75	5.18	6.27	2.20	3.00
	R3	9.25	8.75	6.90	6.02	2.40	2.00
	R4	10.25	9.75	4.78	7.22	1.80	2.40
	Mean	**10.44**	**9.81**	**5.92**	**6.89**	**2.60**	**2.40**
	±SE	0.72	0.62	0.55	0.46	0.48	0.22
T4	R1	10.25	8.50	5.22	6.69	2.20	1.80
	R3	7.75	13.00	7.61	6.43	2.00	3.60
	R3	8.0	12.75	6.63	8.08	2.00	2.60
	R4	8.75	7.5	6.50	7.47	2.20	2.00
	Mean	**8.69**	**10.44**	**6.49**	**7.17**	**2.10**	**2.50**
	±SE	0.56	1.42	0.49	0.38	0.49	0.40

NS - Not significant

Table 25. Serum Zn, Mn and alkaline phosphatase (ALP) content of birds maintained on different dietary treatments at sixth and eighth week of age

Treatments		Serum Zn (ppm)		Serum Mn (ppm)		Serum ALP (U/L)	
		Age in weeks		Age in weeks		Age in weeks	
		6*	8	6	8	6**	8**
T1	R1	2.00	3.00	1.00	0.00	457.9	419.69
	R3	2.00	2.00	0.00	0.00	430.19	434.30
	R3	2.00	2.00	1.00	1.00	458.25	414.24
	R4	1.00	3.00	0.00	2.00	439.16	428.20
	Mean	1.75^b	2.50	0.50	0.75	446.38^a	424.11^a
	±SE	0.25	0.29	0.29	0.48	7.03	4.45
T2	R1	2.00	2.00	2.00	1.00	431.23	402.90
	R3	2.00	3.00	0.00	2.00	423.7	419.60
	R3	3.00	2.00	1.00	3.00	434.89	407.10
	R4	2.00	3.00	0.00	1.00	411.4	416.96
	Mean	2.25^{ab}	2.50	0.75	1.75	425.31^{ab}	411.64^a
	±SE	0.25	0.29	0.48	0.48	5.19	3.97
T3	R1	3.00	2.00	1.00	0.00	426.27	397.23
	R2	4.00	2.00	1.00	3.00	408.16	403.60
	R3	2.00	2.00	2.00	5.00	420.59	392.20
	R4	4.00	2.00	0.00	1.00	413.85	409.18
	Mean	3.25^a	2.00	1.00	2.25	417.22^{bc}	400.55^{ab}
	±SE	0.48	0.00	0.41	1.11	3.94	3.70
T4	R1	3.00	0.20	0.10	3.00	411.1	357.50
	R3	2.00	0.30	0.20	1.00	392.13	394.19
	R3	3.00	0.30	0.00	1.00	407.85	363.00
	R4	2.00	0.25	0.00	1.00	395.41	407.58
	Mean	2.50^{ab}	2.63	0.75	1.50	401.62^c	380.57^b
	±SE	0.29	0.02	0.05	0.50	4.63	12.10
CD		1.02	-	-	-	22.99	30.20

a, b, c - Means bearing the different superscript within the same column differed significantly
* Significant (P<0.05)
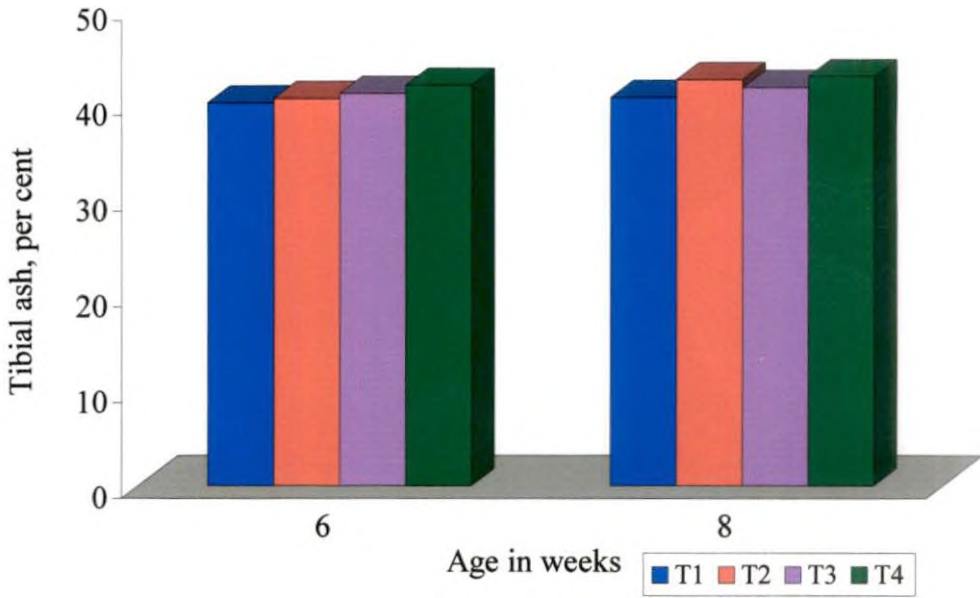** Significant (P<0.01)

Fig. 12. Tibial ash content of birds maintained on different dietary treatments at sixth and eighth week of age
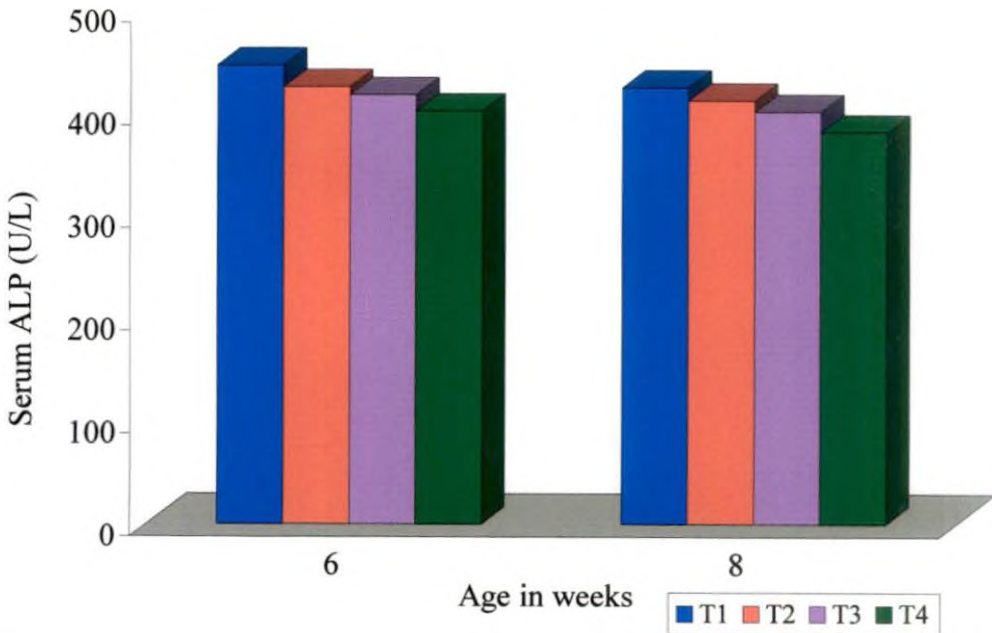

Fig. 13. Serum alkaline phosphatase content of birds maintained on different dietary treatments at sixth and eighth week of age

Table 26. Processing yields of birds maintained on different dietary treatments at sixth and eighth week of age, %

| Treatments | | Dressed yield | | Ready-to-cook yield | | Giblet yield | | Abdominal fat yield | |
| | | Age in weeks | | Age in weeks | | Age in weeks | | Age in weeks | |
		6^{NS}	8^{NS}	6^{NS}	8^{NS}	6^{NS}	8^{NS}	6^{NS}	8^{NS}
T1	R1	91.04	94.05	69.48	73.42	3.92	4.10	1.44	1.78
	R2	90.60	91.16	72.88	73.33	4.06	3.48	1.67	0.74
	R3	90.44	90.22	71.67	71.14	4.07	3.85	1.53	1.96
	R4	88.39	92.22	65.57	72.25	4.69	3.63	2.04	1.50
	Mean	**90.12**	**91.91**	**69.90**	**72.54**	**4.18**	**3.77**	**1.67**	**1.49**
	±SE	0.59	0.82	1.60	0.54	0.17	0.13	0.13	0.27
T2	R1	93.25	90.67	72.38	69.82	4.40	3.90	1.78	2.47
	R2	90.08	91.06	69.40	71.63	4.75	3.90	2.91	1.14
	R3	88.97	92.29	69.21	71.35	3.90	4.02	2.00	3.33
	R4	89.46	89.57	69.68	69.97	4.26	3.81	1.66	1.42
	Mean	**90.44**	**90.90**	**70.17**	**70.69**	**4.33**	**3.91**	**2.09**	**2.09**
	±SE	0.96	0.56	0.74	0.46	0.18	0.04	0.28	0.50
T3	R1	91.94	90.23	72.80	71.35	4.06	3.70	1.69	2.75
	R2	91.46	90.92	72.51	72.71	4.09	3.30	1.76	0.57
	R3	91.25	92.37	72.37	72.54	4.22	4.16	1.30	2.28
	R4	87.67	92.71	69.02	72.92	4.59	4.33	1.10	1.54
	Mean	**90.58**	**91.56**	**71.68**	**72.38**	**4.24**	**3.87**	**1.46**	**1.78**
	±SE	0.98	0.59	0.89	0.35	0.12	0.23	0.16	0.48
T4	R1	91.93	91.22	71.94	71.96	4.38	4.00	0.84	1.56
	R2	90.28	91.63	70.71	72.79	4.04	3.66	1.55	1.16
	R3	89.28	88.43	70.81	70.58	3.82	3.57	1.37	1.69
	R4	89.84	91.39	70.29	71.10	4.38	4.35	1.41	1.32
	Mean	**90.33**	**90.67**	**70.94**	**71.61**	**4.15**	**3.90**	**1.29**	**1.43**
	±SE	0.57	0.75	0.35	0.49	0.14	0.18	0.15	0.12

NS - Not significant

Table 27. Cost of ingredients in the experimental rations

Ingredients	Cost/kg (Rs)*	Broiler starter ration				Broiler finisher ration			
		T1	T2	T3	T4	T1	T2	T3	T4
Yellow maize	6.38	319.00	296.67	319.00	306.24	393.97	371.64	393.97	381.21
Rice polish	4.41	25.29	27.50	27.50	27.50	9.37	11.14	11.14	11.14
Soybean meal	11.97	430.92	436.91	430.92	436.91	335.16	341.15	335.16	341.15
Unsalted dried fish	12.00	62.40	62.40	62.40	62.40	60.00	60.00	60.00	60.00
Shell grit	2.76	2.21	4.14	4.14	4.14	2.21	4.14	4.14	4.14
Dicalcium phosphate	20.00	30.00	6.00	6.00	6.00	32.00	10.00	10.00	10.00
Citric acid	90.00	0.00	270.00	0.00	135.00	0.00	270.00	0.00	135.00
Common salt	2.38	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Vitamin mixure	521.36	13.03	13.03	13.03	13.03	13.03	13.03	13.03	13.03
Trace minerl mixure	34.60	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50
D.L.methionine	314.00	18.84	18.84	18.84	18.84	6.28	6.28	6.28	6.28
Toxin binder	45.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Coccidiostat	357.60	17.88	17.88	17.88	17.88	17.88	17.88	17.88	17.88
Choline chloride	98.00	4.90	4.90	4.90	4.90	4.90	4.90	4.90	4.90
Phytase	400.00	0.00	0.00	5.60	2.80	0.00	0.00	5.60	2.80
Total cost/100 kg feed		938.57	1172.36	924.30	1049.73	888.89	1124.24	876.19	1001.61
Cost/kg feed		9.39	11.72	9.24	10.50	8.89	11.24	8.76	10.02

* Cost calculated using the rate contract values fixed for feed ingredients by College of Veterinary and Animal Sciences, Mannuthy (2002-03)

Table 28. Cost of production of birds maintained on different dietary treatments

| Sl. No	Parameters	Treatments			
		T1	T2	T3	T4
1.	Cost of feed/kg (Rs)				
	Starter	9.39	11.72	9.24	10.50
	Finisher	8.89	11.24	8.76	10.02
2.	Total feed intake (kg)				
	Starter	3.817	3.907	3.922	4.076
	Finisher	2.087	2.434	2.114	2.261
3.	Live weight gain of birds (kg)				
	Zero-sixth week	1.827	1.935	1.898	2.031
	Zero-eighth week	2.505	2.758	2.622	2.767
4.	Cost of production/kg live weight gain (Rs.)				
	Sixth week	19.63	23.67	19.12	21.07
		±0.27	±0.12	±0.34	±0.16
	Eighth week	21.73	26.53	20.91	23.69
		±0.32	±0.28	±0.34	±0.45
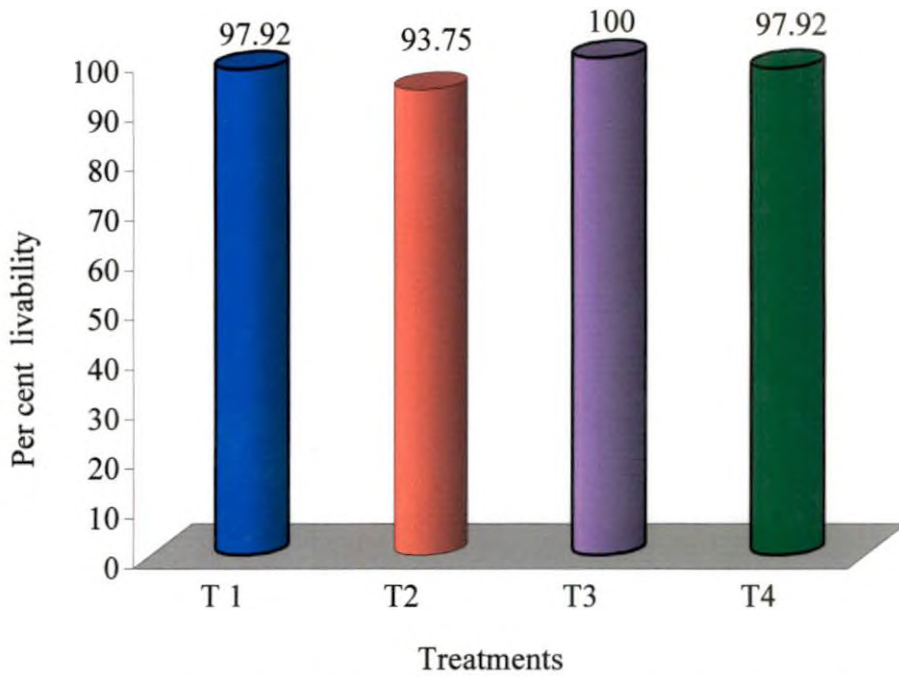
Fig. 14. Per cent livability of birds maintained on different dietary treatments
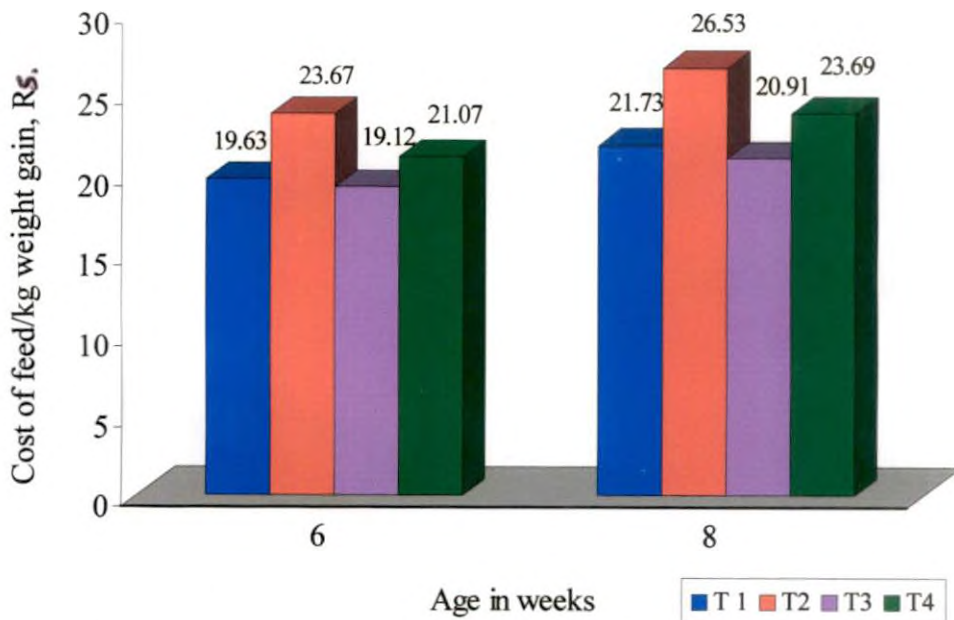

Fig. 15 Cost of production of birds maintained different dietary treatments

Discussion

5. DISCUSSION

The results obtained during the course of present study on effect of citric acid and microbial phytase on nutrient utilization and subsequent performance in broiler chicken are discussed below under separate heads.

5.1   CLIMATIC PARAMETERS

The climatic observations presented in Table 6 revealed that the weekly maximum temperature was lowest at the second week (31.57°C) and highest during eighth week (34.50°C). Although the magnitude of variations in the maximum temperature between weeks was minimal, it was comparatively higher at the finishing period. The minimum temperature during the study ranged from 21.86°C to 25.43°C and was within the comfort zone for broilers. The range in the relative humidity in the forenoon hours during the experimental period was from 57.43 to 72.43 per cent and in the afternoon hours it was from 40.14 to 51.00 per cent. In general, the forenoon and afternoon relative humidity was on the higher side during fifth and sixth week period. These findings indicated that the birds were reared under a hot-humid environment throughout the experiment.

5.2   BODY WEIGHT

The data on fortnightly mean body weight presented in Table 7 with their graphical representation in Fig. 1a and 1b showed that the initial mean body weight of day-old chicks varied from 40.02 to 40.80 g.

In the control group (T_1) the fortnightly mean body weight at second, fourth, sixth and eighth week of age were 368.16, 1095.31, 1867.29 and 2545.12 g, respectively observing the normal pattern of growth in broilers.

Inclusion of citric acid at 3.0 per cent level in low available P diet (T_2) resulted in lower fortnightly body weights at second (P<0.01) and fourth week of

age than any of the treatments and the difference could be due to the reduced feed intake. Sifri *et al.* (1977) and Angel *et al.* (2001b) also observed no effect of citric acid on body weight of broiler chicks during first four weeks of age and are in close agreement with the present study.

After four weeks of age T2 birds showed appreciable increase in body weight and attained statistically similar body weight at sixth week and significantly higher (P<0.01) body weight at eighth week compared to control birds on standard broiler ration (T_1). Boling *et al.* (2000), Angel *et al.* (2001a), Boling-Frankenbach *et al.* (2001b) and Metwally (2001) also could observe significant increase in body weight on citric acid supplementation.

Supplementation of phytase at a level of 700 U/kg in low available P diet (T_3) tended to result in higher fortnightly body weight right from second week onwards when compared to control group (T1).

This finding is in agreement with Yi *et al.* (1996b) who could observe linear increase in body weight in broilers on supplementation of phytase at levels of 350, 700 or 1050 U/kg in a basal diet containing 0.27 per cent non-phytate P. Significant improvement in body weight among broiler chicken fed low available P diet supplemented with phytase was also reported by Broz *et al.* (1994), Biehl *et al.* (1995), Qian *et al.* (1997), Huff *et al.* (1998), Kanagaraju (1998), Sohail and Roland (1999), Balasubramanian (2000), Waldroup *et al.* (2000), Yan *et al.* (2000, 2001a,b) and Ravindran *et al.* (2001). On the contrary, Zanini and Sazzad (1998) observed that supplemented phytase at 500 U/kg diet had no effect on growth performance in broilers when fed with different metabolizable energy levels of 2800 and 3000 kcal ME/kg diet.

Significantly superior body weights at all fortnights were noticed in the combination group (T_4) containing 0.3 per cent available P with 1.5 per cent citric acid and 350 U phytase/kg diet, when compared to control group and substantial increase when compared to all other treatments.

This results are in close agreement with Boling *et al.* (2000) who stated that addition of 1,450 U of phytase/kg of the diet with 6.0 per cent citrate and 0.1 per cent available P caused further improvements (P<0.05) in body weight. On the contrary, Angel *et al.* (2001a,b) observed no interactions between citric acid and phytase, when added together.

The results in the present study revealed that a reduction of 0.2 per cent available P in T2, T3 and T4 was compensated and even resulted in better performance of commercial broilers by inclusion of citric acid and/or phytase. The citric acid and/or phytase in the feed might have acted upon the bound phytate P in the feed resulting in release of more inorganic P and other nutrients for utilization by the bird and subsequently better performance in comparison with standard broiler diet.

Moreover, the effect of phytase on growth performance can be synergistically improved by inclusion of citric acid, which enhances the phytase activity.

5.3 BODY WEIGHT GAIN

From the results on body weight gain presented in Table 8, it can be seen that the weight gain recorded at second week of age were 327.83, 275.11, 342,34 and 354.08 g for T1, T2, T3 and T4, respectively. The combination group (T4) recorded the highest weight gain (P<0.01) followed by T3, T1 and T2 in which T2 being the lowest (P<0.01).

In the second fortnight also a similar trend was observed in weight gain, the values being 787.05 for T4 (P<0.05) followed by 745.09 (T3), 727.15 (T1) and 712.49 g (T2).

The weight gain for T1, T2, T3 and T4 at third fortnightly interval was 771.98, 946.92, 810.21 and 890.32 g and at fourth fortnightly interval, it was 677.83, 823.45, 724.29 and 735.03 g, respectively.

The highest weight gain at sixth (P<0.01) and eighth (P<0.05) week of age was observed in T2 group which was comparable with T4 at sixth week and with T3 and T4 at eighth week. T1 attained the lowest and was comparable with T3 at sixth week and T3 and T4 at eight week of age (Fig. 2).

The cumulative body weight gain recorded were 1826.96, 1934.52, 1897.64 and 2031.44 g at sixth week and 2504.79, 2757.97, 2621.92 and 2766.47 g, at eighth week for T1, T2, T3 and T4, respectively.

Similar to the data on sixth week body weight, the cumulative body weight gain of sixth week showed that the supplementation of phytase (700 U/kg diet) to low available P diet (T3) had a trend for improvement in body weight gain and was statistically comparable with gain of birds offered standard broiler diet (T1). Similar to sixth week body weight, body weight gain was also highest in the combination group (T4), which was statistically comparable with citric acid group (T2). Body weight gain of citric acid group (T2) at sixth week was also comparable with control (T1) and phytase group (T3).

The trend in the cumulative body weight gain from zero to eight weeks of age was also similar to body weight at eighth week of age (Fig. 3).

The results of the present study are in close agreement with Boling *et al.* (2000) who observed linear increase (P<0.01) in body weight gain by inclusion at 1, 2, 4 or 6 per cent citric acid + sodium citrate (1:1, wt: wt) mixture to low available P (0.1 per cent) diet. Improvement in weight gain by inclusion of citric acid in broiler diets was also recorded by Angel *et al.* (2001a), Boling-Frankenbach *et al.* (2001b) and Metwally (2001).

Qian *et al.* (1997) informed that phytase supplementation at the level of 300, 600 and 900 U/kg of low P diet linearly increased body weight gain in broilers. Kanagaraju (1998) and Balasubramanian (2000) also reported improvements in weight gain due to phytase supplementation.

The results of the present study are in agreement with Boling *et al.* (2000) who observed further improvements (P<0.05) in weight gain when phytase (1,450 U/kg diet) and citric acid (6 per cent) were added together to low P (0.1 per cent available P) diet, while the findings of present study are inconsistent with the results of Angel *et al.* (2001a,b) who could not observe any interaction effects of citric acid and phytase on body weight gain of broilers which might be due to application of shorter feeding trial (one week).

Beneficial effects in body weight gain by inclusion of citric acid and/or phytase in feed for broilers were mainly due to increase in feed intake (Table 9 and 10). Moreover, citric acid and/or phytase supplementation might have acted upon phytate molecule and resulted in the better utilization of P and other nutrients. In addition to its function in bone formation, P is also required in the utilization of energy and the resultant body weight gain.

Moreover, the cumulative body weight gain (Table 8) of the present study indicated 5.89, 3.83 and 11.19 per cent higher weight gain (P<0.01) at sixth week and 10.10, 4.6 and 10.45 per cent higher weight gain (P<0.05) at eighth week for T2, T3 and T4, respectively than control group (T1). This finding shows the synergistic effect (P<0.01) of citric acid and phytase combination (1.5 per cent citric acid and 350 U phytase/kg) on body weight gain when included in the starter diets.

5.4   FEED CONSUMPTION

The data on average daily feed intake of birds maintained on different dietary treatments at weekly intervals presented in Table 9 indicate that it differed significantly between groups in all weeks except first week. The fortnightly feed intake of birds also differed significantly (P<0.01) in all fortnights (Table 10 and Fig. 4).

The mean cumulative feed intake (Table 10) of birds were 3817.29, 3906.54, 3921.87 and 4075.79 g from zero to sixth week and 5904.33, 6340.86, 6035.73 and 6337.33 g from zero to eighth week for T1, T2, T3 and T4, respectively. When the cumulative feed intake was considered, it can be seen that the group offered standard broiler diet (T1) consumed less feed (P<0.01) and a statistically similar feed intake was observed by T2 and T3 at sixth week and T3 at eighth week. The combination group (T4) consumed more feed (P<0.01) than other groups and was statistically comparable with T3 at sixth week and with T2 at eighth week (Fig. 5).

The findings of the present study are in agreement with Angel et al. (2001b) who observed improvement (P<0.05) in feed consumption in broilers by supplementation of citric acid (1, 2 and 3 per cent) to low P diets. Metwally (2001) also observed increased feed consumption in broilers by increasing levels of citrate to 4.5 per cent in low P diets. Similar results were also reported by Krause et al. (1994). On the contrary, Angel et al. (2001a) observed lower (P<0.05) feed consumption, when citric acid (3 per cent) was added to low non-phytate P (0.16 per cent) diet.

The results of the present study are in close agreement with Balasubramanian (2000) who also observed increasing trend for feed intake form zero to eight weeks of age in groups fed phytase supplemented diets. Increasing trend in feed intake by supplementation of phytase in low available P diet obtained in the present trial also agrees with the findings of Perney et al. (1993), Broz et al. (1994), Denbow et al. (1995), Yi et al. (1996b), Qian et al. (1997), Kanagaraju (1998) and Aksakal and Bilal (2002).

Combination of citric acid and phytase (T4) or citric acid alone (T2) consumed almost equal amount of feed from zero to eighth week of age. But highest (P<0.01) feed intake in T4 from zero to sixth week of age showed the interaction effects. This finding is inconsistent with the results of Angel et al.

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(2001a,b) who observed no interaction effect. This might be due to application of shorter feeding trial of one week in their study.

Higher feed intake observed in T2, T3 and T4 groups could be due to increased phytate P digestibility, since phytic acid may be imposing restraint on voluntary feed intake.

Moreover, the cumulative mean feed intake (Table 10) showed 2.34, 2.74 and 6.77 per cent higher feed intake at sixth week and 7.39, 2.22 and 7.33 per cent higher feed intake at eight week of age for T2, T3 and T4, respectively than control group (T1). The results of the present study indicate that inclusion of citric acid alone or phytase alone had a favouring effect on feed intake, whereas when both added together in low P diets had a synergistic effect ($P<0.01$) on feed intake, when compared to standard diet.

5.5 FEED CONVERSION RATIO

The mean fortnightly feed conversion ratio (FCR) as influenced by different dietary treatments (Table 11) does not reveal any definite trend among the treatments. It ranged from 1.32 (T4) to 1.46 (T2) at the first fortnight, 1.90 (T4) to 2.05 (T1) in the second fortnight, 2.25 (T2) to 2.44 (T1) in the third fortnight and 2.92 (T3) to 3.11 (T4) in the fourth fortnight.

The analysis of variance of the data on mean feed conversion ratio showed that it was not significantly influenced in all fortnights except in the first fortnight, in which superior ($P<0.05$) feed conversion was recorded in combination group (T4) which was statistically comparable with phytase (T3) group and the feed efficiency was lowest with the group fed citric acid alone (T2), which was also statistically comparable with phytase (T3) group.

The cumulative feed conversion efficiency recorded were 2.09, 2.02, 2.07 and 2.01 for zero to sixth week and 2.36, 2.30, 2.30 and 2.29 for zero to eighth week of age in T1, T2, T3 and T4, respectively. On statistical analysis, no

significant difference could be observed. However, the supplemented groups are showing an improving trend in cumulative FCR from zero to six week. (Fig. 6).

Metwally (2001) also reported improvement in feed conversion efficiency by increasing levels of dietary citrate to 4.5 per cent. Whereas, Sifri *et al.* (1977) stated that the feed:gain ratio was not significantly affected, after conducting studies for 28 days on broilers fed on diets containing marginally low (0.4 per cent) and adequate (0.85 per cent) Ca along with citric acid (0.71 per cent). This findings correlate well with the present study.

The favouring effect of phytase supplementation in low available P diet on feed efficiency, in broilers observed in the present study is in agreement with Perney *et al.* (1993), Huff *et al.* (1998) and Sohail and Roland (1999).

Whereas, Broz *et al.* (1994), Kanagaraju (1998), Balasubramanian (2000), Waldroup *et al.* (2000), Yan *et al.* (2000, 2001a), Ravindran *et al.* (2001), and Aksakal and Bilal (2002) observed significantly improved feed conversion efficiency by dietary supplementation of phytase, when compared to unsupplemented low available P diet.

The results of the present study confirm the findings of Angel *et al.* (2001a,b) who observed no significant interactions of citric acid and phytase for FCR in broilers.

The inclusion of citric acid and/or phytase might have acted upon the phytate P; with the result, P could have been released for utilization by the bird, which indicates the apparent improvement in feed conversion efficiency than control (T1) group. Increased feed intake in relation to increased body weight gain resulted in no significant difference between treatments for FCR.

The cumulative feed conversion efficiency showed 3.5, 0.96 and 3.83 per cent higher feed conversion efficiency at sixth week and 2.54, 2.54 and 2.97 per cent higher feed conversion efficiency at eighth week of age for T2, T3 and T4,

respectively than control group (T1). These results indicate that addition of phytase and citric acid together improves feed utilization than when fed alone.

5.6 PROTEIN EFFICIENCY RATIO

From the results presented in Table 12, it can be seen that birds of all the four groups recorded almost similar PER during the various periods of the experiment, except at sixth week.

The second week PER values showed that T4 has registered the highest value of 3.24 and T2 has the lowest value of 2.96 and no significant difference exits between treatments. The fourth week PER values for T1, T2, T3 and T4 were 2.07, 2.24, 2.12 and 2.24 respectively (P>0.05).

In the sixth week, the PER values differed significantly (P<0.05) and values were 1.74, 1.92, 1.75 and 1.81 for T1, T2, T3 and T4, respectively. There is no significant difference in eighth week PER values and the values were 1.60, 1.66, 1.69 and 1.59 for T1, T2, T3 and T4, respectively.

The trend in cumulative PER at sixth and eighth week of age was also similar to fortnightly PER at sixth and eighth week of age (Fig. 7). The values were 2.03, 2.14, 2.05 and 2.13 at sixth week and 1.89, 1.97, 1.94 and 1.96 at eighth week for T1, T2, T3 and T4, respectively.

Maheswari and Kadirvel (1996) obtained nearly same PER values at fourth week ranging from 2.43 to 2.44 by supplementation of malic acid, when compared to unsupplemented groups and are in agreement with the present study. However, at sixth week, significantly higher (P<0.05) PER value for T2 and T4 in the present study might be due to increased nutrient utilization on citric acid supplementation in diet and it correlate well with increase in body weight gain.

Boling-Frankenbach *et al.* (2001a) and Peter and Baker (2001) also observed no significant effect of phytase (1200 U/kg diet) on PER values and agree well with the present study.

A close look at the protein efficiency values and the weight gain of birds in the various treatments groups reveal that they correlate well with the body weight gain.

5.7   DRY MATTER RETENTION

From the results given in Table 14 and Fig. 8, it can be seen that the average dry matter retention of birds maintained on different dietary treatments did not differ significantly. The mean values were 66.07, 67.87, 70.06 and 67.19 for T1, T2, T3 and T4, respectively with an apparent increase of DM retention by 2.7, 6.04 and 1.70 per cent, respectively in T2, T3 and T4 on citric acid and/or phytase supplementation compared to control group (T1).

These results confirm the findings of Campabadal *et al.* (1995) who stated that citric acid (1 or 2 per cent) supplementation did not significantly improve DM digestibility. Yi *et al.* (1996b) observed 72.8, 76.0, 76.4 and 75.9 per cent DM retention by supplementation of 0, 350, 700 or 1050 U of phytase/kg diets. The over all higher values when compared to present study might be due to application of treatments to younger birds (21 days old) than the present study (eight weeks old). The increasing trend for DM retention by phytase supplementation correlates well with the present study.

Radcliffe *et al.* (1998) also found that addition of phytase (250 or 500 U/kg) linearly improved DM digestibility, while citric acid had no influence on DM digestibility.

Apparent increase in DM retention of birds reveals that increase in nutrient utilization by inclusion of citric acid and/or phytase in broiler diets.

5.8   NITROGEN RETENTION AND EXCRETION

The effect of different dietary treatments on N balance, per cent retention and excretion were determined and the values with their statistical analysis are given in Table 15. Even though there is no significant difference between treatments, for all these parameters, T2, T3 and T4 registered 30.54, 11.38 and 35.93 per cent higher N balance and 10.22, 6.27 and 7.96 per cent higher N retention, respectively than control group (T1). N excretion was reduced by 6.93, 4.70 and 5.12 per cent, respectively for T2, T3 and T4 when compared to control (T1) group (Fig. 8).

Increased availability of protein could be the reason for the apparent improvement in N balance among citric acid and/or phytase-supplemented groups.

Several workers have reported the increase in N retention in broiler chicken on phytase supplementation (Farrell et al., 1993; Piva et al., 1995; Yi et al., 1996b; Zanini and Sazzad, 1998 and Balasubramanian, 2000). Their findings confirm the results of the present study. However, Windisch and Kirchgessner (1996b) could not observe any change in N retention by supplementing different levels of microbial phytase in broiler chicks fed diets containing 0.62 or 0.79 per cent Ca and 0.59 per cent total P. It could be due to the variation in the source of P contained in the diet. Reduced N excretion by phytase supplementation was also reported by Ferguson et al. (1997) and Piao et al. (1998). On the other hand, Kwon et al. (1996) reported no influence of phytase on N excretion. Li et al. (1998) stated that fecal N excretion was lower for pigs fed phytase (750 U/kg) supplemented diet, while further reductions were obtained when fed in combination with citric acid (1.5 per cent) and it supports the present study.

Phytate forms bonds with the amino terminus of protein, thereby affecting the digestibility of protein. Therefore inclusion of citric acid and/or phytase in low available P diet might have acted upon the bound phytates and resulted in an

increase in the availability of nutrients and resulted improvement in N retention and reduced excretion.

5.9   AVAILABILITY OF MINERALS

5.9.1   Availability of Calcium, Phosphorus and Magnesium

The balance and per cent availability of Ca, P and Mg as influenced by different dietary treatments is presented in Tables 16, 17, 18 and Fig. 9. It indicated that Ca balance ranged from 0.49 to 0.72 g/day, highest in phytase group (T3), while lowest in control (T1) group. The Ca availability ranged from 28.23 to 46.59 per cent, highest in phytase (T3) group and lowest in combination (T4) group. Even though there is no significant difference between treatments in these parameters, phytase (T3) group attained 23.79 per cent higher Ca availability than control (T1) group.

The P balance ranged from 0.254 to 0.291 g/day, highest in combination (T4) group and lowest in citric acid supplemented group (T2). The P availability ranged from 26.56 to 36.38 per cent. Phytase (T3) group attained the highest availability, whereas lowest availability in control (T1) group. However, there is no significant difference between treatments. These findings indicate that even though lowering of available P by 0.2 per cent in T2, T3 and T4 diets resulted in 13.67, 36.97 and 23.57 per cent higher availability of P, respectively than control (T1) group fed with standard broiler diet with 0.5 per cent available P by inclusion of citric acid and/or phytase, which could be due to the enhanced solubility and availability of P in the gut.

The Mg balance ranged from 0.01 to 0.04 g/day, highest in phytase (T3) group and lowest in T1 as well as T2 groups. But there was no significant difference between treatments. Moreover, Mg availability was significantly ($P<0.05$) higher in phytase (T3) group. Supplementation of 700 U of phytase/kg low available P (0.3 per cent) diet (T3) resulted in nearly three times more

availability of Mg, when compared to birds fed standard broiler diet (T1). This could be due to enhanced solubility and availability of phytate bound Mg in the gut of birds fed diets supplemented with phytase (700 U/kg).

The effectiveness of phytase supplementation on the availability and utilization of minerals was also studied by Denbow *et al.* (1995), Sebastian *et al.* (1996a), Yi *et al.* (1996b), Qian *et al.* (1997), Kanagaraju (1998), Zanini and Sazzad (1998) and Balasubramanian (2000) and reported marked improvement in the retention and bioavailability of Ca, P and Mg.

On the other hand, Boling-Frankenbach *et al.* (2001b) reported that inclusion of citric acid in broiler diets improved P utilization, but not Ca utilization. This finding confirms the results of the present study. Metwally (2001) also reported that dietary citrate mixture at level of 4.5 per cent enhanced P utilization in broilers.

Radcliffe *et al.* (1998) observed no synergistic effects of phytase and citric acid on Ca and P digestibilities in weanling pigs and agrees well with the present study.

5.9.2 Phosphorus Excretion

Influence of various dietary treatments on P excretion is set out in Table 17 and Fig. 10. The excretion of P ranged from 3.71 to 5.92 g/kg DM intake. Significantly ($P < 0.05$) higher P excretion was noticed in the control group (T1). Inclusions of citric acid and/or phytase in low available P diets caused significant ($P < 0.05$) reduction in P excretion and are statistically comparable with each other. Inclusion of citric acid or phytase and both (T2; T3 and T4) resulted in a reduction of P excretion to the tune of 30.57, 37.33 and 33.45 per cent, respectively than the control group (T1). The phytase and/or citric acid acting on the organically bound phytic acid might have liberated more P for utilization and ultimately less quantities of P is excreted in the droppings.

Li *et al.* (1998) and Roth *et al.* (1998) also reported reduction in excretion of P by organic acid supplementation. Perney *et al.* (1993) studied the effect of dietary phytase and increasing levels of available P on P metabolism. They noticed increased P excretion by increasing dietary available P and that it could be decreased by supplemented phytase. Reduction of P excretion in the droppings by phytase supplementation was also reported by Broz *et al.* (1994), Mitchell and Edwards (1996b), Yi *et al.* (1996b), Kanagaraju (1998), Zanini and Sazzad (1998), Balasubramanian (2000), Waldroup *et al.* (2000) and Yan *et al.* (2000, 2001a).

Phytase alone or in combination with citric acid or vitamin D$_3$ lowered the P excretion by 27 per cent in pigs was observed by Li *et al.* (1998). Omogbenigun *et al.* (2003) also observed similar results and are in close agreement with the present study.

5.9.3 Availability of Zinc and Manganese

From the results set out in the Table 19, it could be seen that the Zn balance and availability ranged from 0.86 to 2.81 g/day and 8.32 to 26.25 per cent, respectively and there is no significant difference between treatments. However, T2 and T4 registered nearly two times higher and T3 three times higher availability of Zn when compared to control (T1) group (Fig. 9).

The Mn balance and availability differed significantly (P<0.05) between treatments, and ranged from 1.31 to 5.12 g/day and 9.06 to 31.71 per cent, respectively (Table 20). Phytase supplemented group (T3) registered significantly higher (P<0.05) Mn balance. Control group (T1) attained significantly lower (P<0.05) Mn balance and was also statistically comparable with T2 and T4.

Percentage availability of Mn was also significantly higher (P<0.05) with phytase (T3) group and was statistically comparable with T2 and T4. T1 registered significantly lower value and was also statistically comparable with T2

and T4 (Fig.9). Mn availability was nearly two times higher for T2 and T4 and three times higher for T3 when compared to control (T1).

Increased retention of Zn and Mn by organic acid supplementation was also reported by Hohler and Pallauf (1993). Whereas, Hohler and Pallauf (1994) observed no effect of citric acid on apparent absorption and retention of Zn and Mn.

Improvement in the availability and utilization of Mn and Zn by phytase supplementation was reported by Biehl *et al.* (1995), Piva *et al.* (1995), Windisch and Kirchgessner (1996a) and Balasubramanian (2000). The effect of microbial phytase on retention and utilization of Zn also studied by Thiel *et al.* (1995), Sebastian *et al.* (1996a), Yi *et al.* (1996a), Zanini and Sazzad (1998) and Balasubramanian (2000) and observed increased Zn availability by phytase supplementation in broilers.

Hohler *et al.* (1992) observed improved Zn utilization on citric acid and phytase supplementation and confirm the results of the present study. The increased availability of Mn and Zn could be due to the effect of phytase and/or citric acid on bound complexes and release of bound minerals.

5.10   WEIGHT OF DRIED TIBIA AND TIBIAL ASH

Perusal of the weight of dried tibia presented in Table 21 indicated that this trait was significantly (P<0.05) influenced by dietary treatments at sixth week. At sixth week, it was significantly higher (P<0.05) for T4 and statistically comparable with T2 and T3. At eight week, the values ranging from 10.04 to 11.89 g and no significant difference exist between treatments (Fig. 11).

From the data on tibial ash presented in Tables 21 and Fig.12, it can be seen that there is no significant difference between treatments both at sixth and eighth week of age. At both these ages, it was higher in T4 and lower in T1. The

increase in tibial ash content for T2, T3 and T4 were 1.15, 2.38 and 4.62 per cent at sixth week and 4.59, 2.44 and 5.33 per cent at eighth week, respectively.

The results of the present study revealed that a reduction of 0.2 per cent available P in the standard broiler diet was compensated and even resulted in comparatively better bone development and mineralisation of commercial broilers by inclusion of citric acid and/or phytase. The citric acid and/or phytase in the feed might have acted upon the bound phytate P in the feed resulting in release of more inorganic P and other minerals for utilization by the birds.

Boling *et al.* (2000), Boling-Frankenbach *et al.* (2001b) and Metwally (2001) observed increased tibial ash content by inclusion of citric acid or citrate mixture and confirm the results of present study.

A positive relationship between phytase supplementation and tibial weight and ash content was also established by Farrell *et al.* (1993), Perney *et al.* (1993), Broz *et al.* (1994), Sebastian *et al.* (1996b), Kanagaraju (1998), Waldroup *et al.* (2000) and Yan *et al.* (2000).

Angel *et al.* (2001a,b) observed that phytase and citric acid supplementation improved tibial ash content, but no interactions were seen and are in partial agreement with the present study.

5.11   TIBIAL MINERAL CONTENT

The data on tibial mineral content presented in Tables 22 and 23, indicated that there is no significant difference between treatments for tibial Ca, P, Mg, Zn and Mn. Tibial Ca ranged from 12.50 (T1) to 12.80 (T4) at sixth week and 13.90 (T3) to 15.61 (T2) per cent at eighth week. Tibial P varied from 8.02 (T2) to 9.26 (T3) and 10.30 (T3) to 12.06 (T2) per cent at sixth and eighth week of age, respectively. It indicates that reducing available P level by 0.2 per cent in T2, T3 and T4 did not affect the bone mineralisation and P content due to the effect of citric acid and/or phytase on mineral availability. Tibial Mg content at sixth week

was significantly higher (P<0.05) in T4 and comparable with T2 and lower (P<0.05) in T1. T2 and T3 attained statistically comparable values at sixth week. At eighth week also T4 recorded relatively higher value. T1 and T3 had the same value (0.43 per cent) for tibial Mg content at eighth week. Tibial Zn content was high in T1 at sixth week, but the trend has reversed in eighth week in which T2, T3 and T4 registered 15.07, 3.80 and 8.14 per cent increasing trend for tibial Zn content than control (T1) group. Tibial Mn content varies between 61.23 ppm in control (T1) to 80.40 ppm in T2 and 77.88 in T1 to 86.25 ppm in T4 at sixth and eighth week of age, respectively. It indicates that inclusion of citric acid and/or phytase relatively increased tibial Mn content.

The results on tibial Ca correlate well with the studies of Sifri *et al.* (1977) who observed no significant effect of citric acid on tibial Ca. Increased bone mineral content on phytase supplementation was also observed by Piva *et al.* (1995), Thiel *et al.* (1995), Zanini and Sazzad (1998) and Sohail and Roland (1999) and are in partial agreement with the present study.

Biehl *et al.* (1995) observed significant increase in tibial P and Mn on phytase supplementation. Boling *et al.* (2000) also observed significantly increased tibial Zn content by increasing levels of citric acid. This finding correlates well with the results of the present study.

5.12 SERUM MINERAL CONTENT AND ALKALINE PHOSPHATASE

5.12.1 Serum Calcium, Inorganic Phosphorus and Magnesium

From the results presented in Tables 24 and 25, it can be seen that there was no significant difference between treatments for serum Ca, P and Mg at sixth and eighth week of age. However, serum Ca ranges from 8.63 (T2) to 10.44 (T3) mg per cent and 8.38 (T1) to 10.44 (T4) mg per cent in sixth and eighth week of age, respectively. An increasing trend for serum iP in T2 group both at sixth and eighth week, which indicate that citric acid, improves P utilization. Serum iP

varies between 5.12 (T1) to 6.56 (T2) and 6.89 (T3) to 7.64 (T2) mg per cent in sixth and eighth week of age, respectively. Serum Mg was relatively high in T3 at sixth and in T4 at eight week of age. It ranges from 2.10 (T4) to 2.60 (T3) mg per cent at sixth and 2.25 (T2) to 2.50 (T4) mg per cent at eighth week of age, respectively.

Sifri *et al.* (1977) also observed no effect of citric acid on plasma Ca. Han *et al.* (1998) found no significant difference in plasma iP by supplementation of phytase and citric acid. These findings are in close agreement with the present study. Whereas significantly increased serum Ca and iP (Kanagaraju, 1998 and Balasubramanian, 2000) or iP alone (Perney *et al.*, 1993; Broz *et al.*, 1994; Mitchell and Edwards, 1996b and Sebastian *et al.*, 1996a) on phytase supplementation was reported by several other workers, since they compared phytase supplemented groups with negative control group fed with low available P diet.

5.12.2 Serum Zinc, Manganese and Alkaline Phosphatase

The results given in Table 25 indicate that serum Zn varied significantly (P<0.05) at sixth week. Phytase supplemented group (T3) registered significantly higher (P<0.05) serum Zn content at sixth week and was statistically comparable with T2 and T4. However there is no significant difference between groups at eight week and values varied from 2.00 to 2.63 ppm.

The data on serum Mn levels of birds under different treatments at sixth and eighth week of age (Table 25) indicate that serum Mn content varied from 0.50 (T1) to 1.00 (T3) ppm and 0.75 (T1) to 2.25 (T3) ppm at sixth and eighth week of age, respectively. However there is no significant difference between treatments both at sixth and eighth week of age.

Serum alkaline phosphatase (U/L) measured at sixth and eighth week of age as influenced by different feeding regimes set out in Table 25 and Fig. 13

reveal that serum ALP was differed significantly (P<0.01) both at sixth and eighth week of age. T4 recorded significantly lower value and was statistically comparable with T3 both at sixth and eighth week of age.

While screening the literature on the influence of citric acid and/or phytase addition in feeds on serum Zn, Mn and alkaline phosphatase, much work could not be traced. However, increased plasma Zn concentration and decreased alkaline phosphatase activity on citric acid supplementation in pigs was also reported by Hohler (1992) and Hohler and Pallauf (1993). Reduced alkaline phosphatase activity on phytase supplementation was also observed by Huff et al. (1998), whereas Roberson and Edwards (1994) reported dietary phytase and vitamin D had no effect on plasma alkaline phosphatase activity.

In this trial, a relative increase in serum Zn and Mn levels were observed when birds were fed with low available P diets supplemented with phytase and/or citric acid, which indicates the release of bound Zn and Mn by the enzyme or citric acid and the both. On the other hand, lowering of serum alkaline phosphatase on the influence of citric acid and/or phytase might reflect the down regulation of this enzyme resulting from the increased availability of P.

5.13   PROCESSING YIELDS

The per cent dressed yield did not differ significantly among the groups both at six and eight weeks of age (Table 26). The range of mean values was 90.12 to 90.58 per cent at sixth week and 90.90 to 91.91 per cent at eighth week.

The per cent ready-to-cook yield ranged from 69.90 to 71.68 per cent at sixth week and 70.69 to 72.54 per cent at eighth week and did not differ significantly among the four treatments.

The mean giblet yield at sixth week ranged from 4.15 to 4.33 and from 3.77 to 3.91 per cent at eighth week and it did not differ significantly between

treatments. The difference between the lowest and highest value for all these parameters was almost same and narrow at both these ages.

The mean abdominal fat content did not differ significantly among groups both at sixth and eighth week of age. The range of mean values were 1.29 (T4) to 2.09 (T2) and 1.43 (T1) to 2.09 (T2) per cent at sixth and eighth week of age.

These findings correlate with the results of Roth *et al.* (1996) who observed organic acid had no effects on carcass evaluation. Whereas, Izat *et al.* (1990b) observed significantly increased dressed yield on buffered propionic acid supplementation. It might be due to source of organic acid used. They also observed no effect of organic acid on abdominal fat yield and are in agreement with the present study.

Balasubramanian (2000) also observed no effect of dietary phytase supplementation on dressed yield and giblet yield, whereas he observed significantly higher ready-to-cook yield in groups fed standard diet and phytase (500 U/kg) supplemented low P diet. Kanagaraju (1998) also observed significantly improved per cent dressed yield, ready-to-cook yield and giblet yield in phytase supplemented groups, is not in agreement with the present study. It might be due to difference in P levels in the diets.

5.14 LIVABILITY

Per cent livability of birds under different dietary treatments employed in this trial is presented in Fig. 14 and not influenced by different dietary treatments. During the course of experiment only five birds died out of 192, in which one bird each from T1 and T4, three birds from T2 and the livability per cent ranged from 93.75 to 100.00. The lowest per cent livability was noticed with the group offered low available P with citric acid alone (T2). However, the livability of group fed diet supplemented with phytase alone (T3) was 100 per cent. Groups maintained on standard diet (T1) as well was low available P diet supplemented with phytase

and citric acid (T4) showed a livability of 97.92 per cent. Necropsy findings did not reveal any ill effects on the physiological well being of the birds.

Vogt (1993), Denbow *et al.* (1995), Ravindran *et al.* (1995), Kanagaraju (1998), Sohail and Roland (1999), Waldroup *et al.* (2000) and Yan *et al.* (2000) also reported improvement in survivability of birds fed P deficient diets supplemented with phytase.

Sifri *et al.* (1977) observed citric acid had no pronounced effect on mortality in growing chicks and are in agreement with the present study.

In the present trial, the available P was reduced by 40 per cent in citric acid and/or phytase supplemented groups (T2, T3 and T4) without affecting the health of broiler chicken and are in partial agreement with the findings of Huff *et al.* (1998) who reported that total P could be reduced by at least 25 per cent in diets supplemented with phytase at 500 U/kg without affecting health and performance. Enhanced availability and better utilization of nutrients could have contributed for the better livability percentage in the above trials.

5.15   ECONOMICS

An assessment of the cost of different rations used in this trial, presented in Table 27 indicated that low available P diet with citric acid (T2) was costlier due to high cost of citric acid and the ration supplemented with phytase (T3) was cheaper, with respect to both starter and finisher rations. The cost difference between these two rations was Rs.2.48/kg feed for both starter and finisher rations. The cost of feed/kg for T1, T2, T3 and T4 were Rs.9.39, 11.72, 9.24 and 10.50 and 8.89, 11.24, 8.76 and 10.02 for starter and finisher rations, respectively.

In order to assess the economics of production, cost-benefit analysis was done and the relevant details are set out in Table 28. The cost of production/kg live weight gain (when feed cost alone was considered) was worked out to be Rs.19.63, 23.67, 19.12 and 21.07 at sixth week of age for the treatments, T1, T2,

T3 and T4 respectively. When the birds were marketed at eighth week of age the corresponding figures were Rs.21.73, 26.53, 20.91 and 23.69, respectively. It showed that the cost of production/kg live weight gain was higher at eighth week of age. So it is better to rear and market the birds at sixth week of age than eighth week of age (Fig. 15). The cost of production was lower in phytase (T3) group. While comparing with control (T1), phytase (T3) group has 51 and 82 paise lesser cost of production with respect to sixth and eighth week of age.

The cost of production was higher for T2 group due to high feed cost. Even though inclusion of citric acid (T2) resulted in better performance and enhanced the phytase activity on performance (T4), the cost of production/kg live weight gain is high due to higher cost of citric acid. So either cheaper source of citric acid or reduced level of citric acid inclusion or both have to be identified to get better economics of production. However, the beneficial effects by the way of minimizing the environmental pollution have to be considered before arriving at a final conclusion.

Newman (1993) reported that one kg of phytase enzyme per tonne of feed can replace 6 to 7 kg of mono calcium phosphate, thus allowing a significant reduction in P supplementation cost. Kanagaraju (1998) reported that the net profit per kg live weight (when feed cost alone was considered) was 13 paise higher in groups maintained on 0.4 per cent available P diet supplemented with phytase at 750 U/kg compared to standard broiler ration and are in partial agreement with the present study, whereas Balasubramanian (2000) observed that economics was good for standard diet than phytase supplemented diets.

Summary

6. SUMMARY

An investigation was conducted in the Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy using 192 day-old commercial broiler chicks (Ven cob) to study the effect of citric acid and microbial phytase (Natuphos®-5000G) on nutrient utilization and subsequent growth performance. The chicks were randomly divided into 16 groups of 12 chicks each. The groups were allotted randomly to four dietary treatments viz., T1, T2, T3 and T4 with four replicates in each treatment. The dietary treatments consisted of a standard broiler ration (SBR) with 0.5 per cent available P (T1), low available P broiler ration with 0.3 per cent available P (LAPBR) and 3.0 per cent citric acid (T2), LAPBR supplemented with 700 U· of phytase/kg diet (T3) and LAPBR with 1.5 per cent citric acid and 350 U of phytase/kg diet (T4). All the rations were formulated as per BIS specifications except in the level of available P.

Chicks in each replicate were housed randomly in individual but identical pens and reared under deep litter system of management. Standard managemental procedures were adopted throughout the experimental period of eight weeks. Feed and water were provided *ad libitum*. Birds were fed with broiler starter ration up to six weeks of age and then switched over to broiler finisher ration till the end of the experiment. The body weight of individual birds was recorded at day-old age followed by fortnightly intervals. Feed consumption of the birds was recorded replicate wise at weekly intervals.

At the end of sixth and eighth week, one bird from each replicate were randomly selected and used to study the processing yields viz., dressed yield, ready-to-cook yield, giblet yield and abdominal fat yield and tibial parameters such as weight of dried tibia, tibial ash, tibial mineral content viz., Ca, P, Mg, Zn

and Mn and serum biochemical parameters such as serum Ca, inorganic P, Mg, Zn, Mn and alkaline phosphatase.

A three-day metabolism trial was conducted towards the end of the experiment using one bird from each replicate selected randomly and housed in individual metabolism cages with facilities for feeding, watering and excreta collection. Total collection method was employed for droppings collection. Per cent livability of the birds during the course of experiment was also worked out in each treatment. The economics of gain was also calculated from the data on feed intake and body weight gain of birds.

The criteria adopted for evaluation of dietary treatments were average body weight and weight gain, feed intake, feed conversion ratio, protein efficiency ratio, DM and N retention, availability of Ca, P, Mg, Zn and Mn, excretion of N and P, data on tibial parameters, serum biochemical parameters, processing yields and livability.

The overall performance of the birds offered different dietary treatments are summarized in Table 29. The salient observations made during the present study and the inferences drawn from the results are summarized below:

1) The birds maintained on all the experimental rations (T2, T3 and T4) recorded higher body weight and weight gain, compared to birds fed on standard broiler ration (T1), indicating that inclusion of citric acid and/or phytase can improve body weight and weight gain. The highest weight gain and body weight being observed in T4 with significant difference both at sixth week ($P<0.01$) and eighth week ($P<0.05$), indicating the synergistic effect of citric acid and phytase on growth performance.

2) The mean daily, fortnightly and cumulative feed intakes differed significantly between treatments. The birds fed on all the experimental rations (T2, T3 and T4) consumed more feed, than control (T1) group.

The highest cumulative feed consumption was observed in T4 at sixth week (P<0.01) and in T2 as well as T4 at eighth week (P<0.01). The results indicate that inclusion of citric acid or phytase improve feed intake and can cause further improvements in feed intake when these additives were added together (T4).

3)   Cumulative FCR recorded by the birds were 2.09, 2.02, 2.07 and 2.01 at sixth week and 2.36, 2.30, 2.30 and 2.29 at eighth week for T1, T2, T3 and T4, respectively. Though the birds maintained on different dietary treatments registered no significant difference in cumulative FCR, an increasing trend in feed efficiency was noticed in T2, T3 and T4 over the control group (T1) in the sixth week and almost similar feed efficiency for the various groups during eighth week, indicating that citric acid or phytase favours the nutrient utilization.

4)   Regarding the cumulative protein efficiency ratio, the birds belonging to various treatments T1, T2, T3 and T4 recorded similar PER (P>0.05) at eighth week and significantly higher PER (P<0.05) in T2 as well as T4 at sixth week. The results indicate that birds maintained on low P diets with additives (T2, T3 and T4) performed equally good compared to control group maintained on SBR, suggesting the favouring effect on protein utilization.

5)   The dry matter retention did not differ significantly between groups. However, an increasing trend was observed in DM retention due to citric acid and/or phytase supplementation.

6)   The mean N balance (g/day) recorded by birds were 1.67, 2.18, 1.86 and 2.27 in T1, T2, T3 and T4, respectively with a corresponding per cent retention of 42.08, 46.38, 44.72 and 45.43, respectively. It can be noticed that even though, there was no significant difference between treatments, N balance and per cent retention was higher and excretion was lower in

additive added groups than control (T1) group, indicating that dietary citric acid and/or phytase enhances the N utilization.

7) Percentage Ca availability recorded by groups of T1, T2, T3 and T4 were 38.05, 35.81, 46.57 and 28.23, respectively without any significant difference between treatments. The results revealed that phytase supplemented group (T3) had the highest Ca availability.

8) P availability recorded by experimental birds was higher in all supplemented groups with low available P diet compared to control. The values being 26.56, 30.19, 36.38 and 32.82 per cent for T1, T2, T3 and T4, respectively. P excretion was also significantly reduced ($P<0.05$) in T2, T3 and T4 compared to T1 and the results indicate that both citric acid and phytase enhance P utilization.

9) The citric acid supplemented groups T2 and T4 and the control group (T1) recorded similar Mg balance and availability, whereas phytase supplemented group (T3) recorded significantly higher ($P<0.05$) Mg availability, suggesting significant influence of phytase on Mg availability.

10) Availability of Zn recorded by birds of T1, T2, T3 and T4 were 8.32, 15.46, 26.25 and 14.66 per cent, respectively. Even though there is no statistical significance, an increasing trend could be noticed in Zn availability in supplemented groups, the increase being nearly two times higher for T2 and T4 and three times higher for T3 compared to T1.

11) Regarding the availability of Mn also, citric acid and phytase supplemented groups registered higher per cent availability of 18.99, 31.71 ($P<0.05$) and 19.25 for T2, T3 and T4, respectively when compared to control (T1), the value being 9.06.

12) An increasing trend in weight of dried tibia and tibial ash was noticed for T2, T3 and T4 when compared to control group (T1) with a significant

increase in weight of dried tibia at sixth week for T2 and T4 tending to suggest that citric acid and/or phytase favours P utilization and bone development. No significant difference could be observed in the serum and tibial mineral contents viz., Ca, P, Mg, Zn and Mn of the various groups both at sixth and eighth week except for a higher tibial Mg ($P<0.05$) for T4 and serum Zn ($P<0.05$) for T3 at sixth week. These results indicate that dietary available P level could be reduced by 0.2 per cent from 0.5 per cent without affecting tibial parameters and serum mineral content which can be due to citric acid and/or phytase added as additives.

13) Significant reduction ($P<0.01$) in serum alkaline phosphatase was noticed in additive supplemented groups over the non-supplemented control (T1), the lowest value being recorded in T4 followed by T3, T2 and T1, indicating that dietary citric acid and/or phytase favours better P utilization and subsequent performance.

14) Birds of experimental groups T2, T3 and T4 recorded a per cent dressed yield, ready-to-cook yield, giblet yield and abdominal fat yield similar to that of control group without any significant difference. Per cent livability was also not influenced by different dietary treatments. This results showed that reduction of available P did not affect the processing yields and livability, when supplemented with either citric acid (3.0 per cent) or phytase (700 U/kg diet) or its combination (1.5 per cent citric acid and 350 U phytase /kg diet)

15) When the cost of feed/kg live weight gain was calculated, the phytase supplemented group (T3) recorded the lowest. This may be due to the high cost of citric acid in citrate supplemented groups.

On scrutiny of the results of the present study, it can be concluded that inclusion of citric acid (3.0 per cent) or phytase (700 U/kg diet) or its combination

(1.5 per cent citric acid + 350 U phytase/kg diet) as additives in low available P diets (0.3 per cent) of broiler chicks from day-old to eight weeks, significantly enhanced body weight, weight gain and weight of dried tibia, significantly reduced P excretion and serum alkaline phosphatase and tended to improve feed conversion efficiency, protein efficiency, retention of DM and N, mineral availability, processing yields, tibial and serum mineral contents.

Based on the overall conclusion of the present study, it can be inferred that the available P level can be reduced by 40 per cent in diets of broiler chicks by inclusion of either 3.0 per cent citric acid or phytase (700 U/kg diet) or both together (1.5 per cent citric acid + 350 U phytase/kg diet) without affecting the health and performance of broiler chicken.

Considering the economics of gain, supplementation of phytase (700 U/kg) in low available P diet is advantageous than addition of citric acid due to high cost of citric acid. So, cheaper source and lower levels of citric acid in combination with phytase have to be investigated to get better economics of production.

Table 29. Effect of dietary citric acid and microbial phytase on nutrient utilization and subsequent performance in broiler chicken

Sl. No.	Particulars	Dietary treatments			
		T1	T2	T3	T4
1.	Body weight (g)				
	a. Sixth week**	1867.29[b]	1974.55[ab]	1938.44[b]	2071.56[a]
	b. Eighth week*	2545.12[b]	2797.99[a]	2662.73[ab]	2806.59[a]
2.	Cumulative body weight gain (g)				
	a. Sixth week**	1826.96[b]	1934.52[ab]	1897.64[b]	2031.44[a]
	b. Eighth week*	2504.79[b]	2757.97[a]	2621.92[ab]	2766.47[a]
3.	Total feed consumption (g)				
	a. 0 to 6 weeks**	3817.29[b]	3906.54[b]	3921.87[ab]	4075.79[a]
	b. 0 to 8 weeks**	5904.33[b]	6340.86[a]	6035.73[b]	6337.33[a]
4.	Cumulative FCR				
	a. Sixth week	2.09	2.02	2.07	2.01
	b. Eighth week	2.36	2.30	2.30	2.29
5.	Cumulative protein efficiency ratio				
	a. Sixth week*	2.03[b]	2.14[a]	2.05[b]	2.13[a]
	b. Eighth week	1.89	1.97	1.94	1.96
6.	Retention				
	a. Dry matter retention (per cent)	66.07	67.87	70.06	67.19
	b. N balance (g/day)	1.67	2.18	1.86	2.27
	c. N retention (per cent)	42.08	46.38	44.72	45.43
	d. N excretion (g/DM intake)	18.76	17.46	17.88	17.80
7.	Availability (per cent)				
	a. Ca	38.05	35.81	46.57	28.23
	b. P	26.56	30.19	36.38	32.82
	c. Mg*	7.52[b]	6.19[b]	19.99[a]	6.39[b]
	d. Zn	8.32	15.46	26.25	14.66
	e. Mn*	9.06[b]	18.99[ab]	31.71[a]	19.25[ab]
	f. P excretion (g/kg DM intake)*	5.92[a]	4.11[b]	3.71[b]	3.94[b]
8.	Tibial parameters				
	(i) Weight of dried tibia (g)				
	a. Sixth week*	5.62[b]	6.98[a]	6.33[ab]	7.08[a]
	b. Eight week	10.04	11.98	11.69	11.89
	(ii) Tibial ash (per cent)				
	a. Sixth week	39.97	40.43	40.92	41.82
	b. Eight week	40.51	42.37	41.50	42.67
	(iii) Tibial Ca (per cent)				
	a. Sixth week	12.50	12.73	12.71	12.80
	b. Eight week	14.30	15.61	13.90	15.60
	(iv) Tibial P (per cent)				
	a. Sixth week	9.25	8.02	9.26	9.25
	b. Eight week	11.80	12.06	10.30	11.53
	(v) Tibial Mg (per cent)				
	a. Sixth week*	0.36[c]	0.41[ab]	0.37[bc]	0.44[a]
	b. Eight week	0.43	0.47	0.43	0.56

	(vi) Tibial Zn (ppm)	147.55	104.61	84.73	116.49
	a. Sixth week	139.73	160.79	145.10	151.17
	b. Eight week				
	(vii) Tibial Mn (ppm)	61.23	80.40	72.55	75.99
	a. Sixth week	77.88	85.94	84.73	86.25
	b. Eight week				
9.	Serum biochemical parameters				
	(i) Serum Ca (mg %)				
	a. Sixth week	9.38	8.63	10.44	8.69
	b. Eight week	8.38	9.00	9.81	10.44
	(ii) Serum inorganic P (mg %)				
	a. Sixth week	5.12	6.56	5.92	6.49
	b. Eight week	7.09	7.64	6.89	7.17
	(iii) Serum Mg (mg %)				
	a. Sixth week	2.20	2.25	2.60	2.10
	b. Eight week	2.40	2.25	2.40	2.50
	(iv) Serum Zn (ppm)				
	a. Sixth week*	1.75^b	2.25^{ab}	3.25^a	2.50^{ab}
	b. Eight week	2.50	2.50	2.00	2.63
	(v) Serum Mn (ppm)				
	a. Sixth week	0.50	0.75	1.00	0.75
	b. Eight week	0.75	1.75	2.25	1.50
	(vi) Serum ALP (U/L)				
	a. Sixth week**	446.38^a	425.31^{ab}	417.22^{bc}	401.62^c
	b. Eight week**	424.11^a	411.64^a	400.55^{ab}	380.57^b
10.	Processing yields (per cent)				
	(i) Dressed yield				
	a. Sixth week	90.12	90.44	90.58	90.33
	b. Eight week	91.91	90.90	91.56	90.67
	(ii) Ready-to-cook yield				
	a. Sixth week	69.90	70.17	71.68	70.94
	b. Eight week	72.54	70.69	72.38	71.61
	(iii) Giblet yield				
	a. Sixth week	4.18	4.33	4.24	4.15
	b. Eight week	3.77	3.91	3.87	3.90
	(iv) Abdominal fat yield				
	a. Sixth week	1.67	2.09	1.46	1.29
	b. Eight week	1.49	2.09	1.78	1.43
11.	Livability (per cent)	97.92	93.75	100.00	97.92
12.	Cost/kg of feed (Rs.)				
	a. Starter ration	9.39	11.72	9.24	10.50
	b. Finisher ration	8.89	11.24	8.76	10.02
13.	Cost of feed/kg live weight gain (Rs.)				
	a. Sixth week	19.63	23.67	19.12	21.07
	b. Eighth week	21.73	26.53	20.91	23.69

a, b, c - Means bearing different superscripts in the same row differed significantly
*   Significant (P<0.05)
** Significant (P<0.01)

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EFFECT OF CITRIC ACID AND MICROBIAL PHYTASE ON PHOSPHORUS UTILIZATION AND GROWTH IN BROILER CHICKEN

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ABSTRACT

An investigation spread over a period of eight weeks was carried out to study the effect of citric acid and microbial phytase (Natuphos®-5000G) on nutrient utilization and growth performance in broiler chicken. One hundred and ninety two day-old broiler chicks (Ven cob) were divided into four identical groups having four replicates in each group with 12 birds in each replicate and allotted randomly into four dietary treatments viz., T1, T2, T3 and T4. The treatments consisted of a standard broiler ration (SBR) with 0.5 per cent available P (T1), low available P broiler ration having 0.3 per cent available P (LAPBR) and 3.0 per cent citric acid (T2), LAPBR supplemented with 700 U of phytase/kg feed (T3) and LAPBR with 1.5 per cent citric acid and 350 U of phytase/kg feed (T4). All the rations were formulated as per BIS specifications except in the level of available P. Effect on body weight, weight gain, feed efficiency, protein efficiency, DM retention, nitrogen balance, mineral availability, serum and tibial mineral contents, processing yields and livability of birds were the criteria employed for evaluation. Body weight and weight gain of the experimental birds were significantly influenced by the dietary treatments. Maximum weight and weight gain were recorded in T4 followed by T2, T3 and T1 in the descending order, indicating the positive and synergistic effects of citric acid and phytase on phosphorus utilization and growth. Cumulative feed intake of experimental birds was significantly ($P < 0.01$) enhanced in the additive supplemented groups over the control, the highest feed intake being noticed in T4 followed by T2, T3 and T1. Cumulative FCR did not differ significantly ($P > 0.05$) between treatments. However, comparatively better feed efficiency was recorded in citric acid and phytase groups (T2, T3 and T4) at sixth week and comparable values at eighth week with the control group (T1). Cumulative PER also showed similar trend in eight week with significantly high ($P < 0.05$) PER in T2 and T4 at sixth week. Though not differed significantly, better DM retention, nitrogen balance, per cent retention and reduced nitrogen excretion were noticed in T2, T3 and T4 than T1.

Availability of P, Mg, Zn and Mn were enhanced ($P>0.05$) in T2, T3 and T4 by citric acid and phytase addition compared to T1 group on SBR. Mg and Mn availability were significantly influenced by dietary treatments with highest availability of Mg and Mn noticed in T3 ($P<0.05$) followed by T4, T2 and T1. The excretion of P was significantly reduced ($P<0.05$) in T2, T3 and T4 on citric acid and /or phytase supplementation. Weight of dried tibia was significantly high ($P<0.05$) in T4 followed by T2, T3 and T1 at sixth week. Per cent tibial ash at sixth and eighth week were also high ($P>0.05$) in T2, T3 and T4 compared to T1 on SBR which could indicate that citric acid and/or phytase favour P utilization and bone development. No significant difference could be noticed in tibial and serum Ca, P, Mg, Zn and Mn except for a significantly high tibial Mg ($P<0.05$) in T4 and serum Zn ($P<0.05$) in T3 at sixth week. Serum alkaline phosphatase was significantly reduced ($P<0.01$) with maximum reduction in T4 followed by T3, T2 and T1. Birds of citric acid, phytase and combination group though maintained on low P diet registered no significant difference in per cent dressed yield, ready-to-cook yield, giblet yield, abdominal fat yield and livability, when compared to T1 fed on SBR. Regarding the cost of production as feed cost/kg gain, phytase group (T3) recorded the lowest due to high cost citric acid.

Overall evaluation of the results of the present study revealed that inclusion of either 3.0 per cent citric acid or phytase (700U/kg feed) or its combination (1.5 per cent citric acid + 350 U phytase/kg feed) in low available P diet (0.3 per cent) resulted in better nutrient utilization and growth performance in chicks than chicks maintained on SBR with 0.5 per cent available P; tending to suggest that available P level in the feed could be reduced by 40 per cent without affecting the performance and health of broiler chicks by dietary inclusion of citric acid and/or phytase.