A modernistic challenge in poultry production is to exploit the use of specific dietary supplements to boost the intrinsic potential of poultry birds to perform better (Adil et al., 2010). Probiotics, enzymes, amino acid supplements and highly available minerals are all relatively new additions to the harmony of poultry nutritionists and have a very positive effect on nutrient utilization when used with appropriate feed ingredients.

The poultry industry readily accepts enzymes as a standard dietary component (Khattak et al., 2006). In addition to poultry, enzymes are also used in pig and turkey feeds. Enzymes are proteins or protein-based substances that speed up or catalyze chemical reactions. Nowadays enzyme production is a growing field of biotechnology (Greiner and Konietzny, 2006) and the market value of world enzyme industry is 5.1 billion US dollars as reported by Freedonia group Inc, USA (2009).

Enzymes are added to feed in order to promote one or more of four beneficial effects - to degrade antinutritional factors in feed that can interfere with the digestive process, to make available/increase the availability of nutrients poorly degraded/undegraded by endogenous digestive enzymes, to supplement digestive activity in young/sick animals and to make nutrient absorption more efficient, thereby decreasing the pollutive effect of animal manure (Bedford, 2000).

These days food safety is more seriously considered than before. On the other hand economy of food production is also a factor which is not ignorable. In applied physiology, nutrition and growth are two closely related and complementary subjects which are considered with each other and applying different strategies in this relation have considerably improved animal production (Rahmani and Speer, 2005).
Poultry feeds are said to be "complete," because they are designed to contain all the energy, proteins, vitamins, minerals and other nutrients necessary for proper growth, egg production and health of the birds. But feed manufacturers are facing challenges associated with nutritional aspects of feed as most feed ingredients of plant origin are rich in phosphorus with only one third present in inorganic form, which is easily digestible. The remaining two third is present as organic phosphorus especially in the form of salts of phytic acid (myoinositol hexakisphosphate) that cannot be utilized by monogastric animals as they lack phytate degrading enzyme resulting in the excretion of undigested phytate phosphorus (Viswanathan et al., 2007).

In order to prevent dietary phosphorus deficiency, monogastric diets are supplemented with more readily available inorganic phosphates. This supplementation is not only expensive but also fails to address the problem of over supplementation, contributing further to phosphate pollution (Vats and Banerjee, 2004). Phosphates are not highly soluble in water but accumulate in soil that may eventually result in runoff and, along with nitrogen, lead to eutrophication of surface waters, a condition that is detrimental to aquatic animals (Dilger et al., 2004).

Decrease in phosphorus excretion into the environment has become one of the most important tasks in modern poultry production. As phosphorus has an important role in bone, blood, metabolism of energy, carbohydrates, protein, fatty acid transportation, membrane phospholipids and enzymatic processes, it is of special interest that diets fulfill poultry needs for phosphorus (Bryden et al., 2007).

Additionally, nutritional problems arise because of phytic acid. It consists of an inositol, which is a hexahydroxycyclohexane in chair conformation, with six phosphate ester bonds. The phosphate groups confer on it a high negative charge and therefore it chelates mineral cations such as calcium, magnesiu,
manganese, zinc, iron, copper and reduces the availability of these cations for animals (Fredlund et al., 2006).

Phytate can also bind with proteins at a broad range of pH to form a phytate-protein complex, which is less soluble, resulting in decreased protein stability and digestibility (Kumar et al., 2010). It also reduces the availability of dietary carbohydrates and amino acids in pigs and poultry directly through interaction with digestive enzymes (Pirgozliev et al., 2007). Phytate also forms lipophytins (complexes with lipid and its derivatives) along with other nutrients (Vohra and Satyanarayana, 2003).

Most of the toxic and antinutrient effects of certain compounds in plants could be removed by several processing methods such as soaking, germination, boiling, autoclaving and other processing methods (Soetan, 2008). However, application of these methods causes a partial loss of nutrients such as proteins and minerals.

Nowadays two approaches dominate to solve this problem. First, addition of phytase enzyme that hydrolyses phytic acid and second, use of genetically modified cereals with lower phytate phosphorus content (Li et al., 2000). The development of enzyme technology based on supplementing diets with sources of microbial phytase has proved to be a practical and effective method of improving phytate digestibility in monogastric animal diets (Maenz, 2000).

Supplementation of enzymes to animal diets has been approved because they are natural fermentation products and therefore they do not pose a threat to animal and human health. Supplemental enzymes are digested in the same way as other proteins and their residues are not present in the excreta (Pintar et al., 2005).

Initially, poultry diets were supplemented with exogenous phytase to allow better utilization of phytate phosphorus which reduces the inclusion of
inorganic phosphorus and fecal phosphate excretion. However, later studies demonstrated that the benefits of using dietary phytases are not restricted to improving phosphorus retention, but also improve performance, energy, amino acid and other nutrient availability (Cowieson et al., 2006a and 2006b).

Phytases (myoinositol hexakisphosphate phosphohydrolases) catalyze the hydrolysis of phytates to myoinositol pentakisphosphate (IP5) or to less phosphorylated myoinositol phosphates IP3 (Quan et al., 2004). The complete hydrolysis of phytate produces one molecule of inositol and six molecules of inorganic phosphate, while partial hydrolysis results in myoinositol intermediates, namely, mono, di, tri, tetra and pentaphosphates besides inorganic phosphate (Rao et al., 2009).

Phytases have a wide distribution in plants, microorganisms and in some animal tissues. Although phytases from several species of bacteria, yeast and fungi have been characterized, commercial production currently focuses on the soil fungus Aspergillus. However, due to some properties, such as substrate specificity, resistance to proteolysis and catalytic efficiency, bacterial phytases are a real alternative to the fungal enzymes (Konietzny and Greiner, 2004).

The market volume of phytases is in the range of 150 million Euros which is still raising further (Greiner and Konietzny, 2006). The increasing use of phytases as additives in many biotechnological applications, including animal feed, reinforces the interest of isolating new and efficient phytase producing microorganisms, obtaining effective phytases capable of releasing as many of the food phosphates as possible into the digestive tract and selecting phytases that remain stable during food processing and storage, with the lowest production costs (Ragon et al., 2008).

Following the ban on the use of antibiotics as growth promoters in animal nutrition by the European Union (EU) in 2006, nutritionists and researchers are attempting other alternatives claiming to enhance the
performance of broiler chicken. One such alternative was the use of organic acids as feed additives in animal production. Organic acids and their salts belong to GRAS (Genetically Recognized As Safe) group (Adil et al., 2010).

The use of organic acids has been reported to protect the young chicks by competitive exclusion (Ragione and Woodward, 2003), enhancement of nutrient utilization and growth and feed efficiency (Denli et al., 2003). Acidification with various organic acids has been reported to reduce the production of toxic components by the bacteria and colonization of pathogens on the intestinal wall, thus preventing the damage to epithelial cells (Langhout, 2000). It was also found to improve the digestibility of proteins, calcium, phosphorus, magnesium, zinc and served as substrates in the intermediary metabolism (Adil et al., 2010). Fumaric acid, propionic acid and citric acid were reported with known effects on weight gain and feed efficiency (Moghadam et al., 2006).

A typical corn soybean meal based diet using dicalcium phosphate as the inorganic phosphorus source was found to have a pH of approximately 6.0 (Radcliffe et al., 1998). The addition of organic acids, such as citric acid, is known to lower diet acidity. As most of the phytases have activity at acidic condition and since the site of phytase activity is primarily the stomach, lowering the dietary pH might reduce the pH of the stomach digesta and thereby increase the efficacy of microbial phytase in hydrolysing phytic acid (Smulikowska et al., 2010). Thus improving the efficiency of phytase could lead to reduced feed costs and to a greater use of phytase, which would be of environmental importance.

Therefore, an attempt was made in the present study to isolate, identify and characterize phytase producing organism from an environmental source and check for the efficacy of the partially purified phytase enzyme as a supplement in the poultry feed.
The objectives of the study were:

1. To isolate and identify bacteria from poultry litter having maximum phytase activity

2. To conduct a trial by supplementing phytase enzyme in broiler chick feed

The study was conducted in two phases. The first phase included isolation, screening and identification of phytase producing bacteria. The second phase included animal trial to assess the influence of phytase and citric acid supplementation individually and in combination on the growth performance and mineral retention among broilers.