Aus dem Institut für
Physiologie, Physiologische Chemie und Tierernährung der Tierärztlichen Fakultät
der Ludwigs-Maximilians-Universität München
Geschäftsführender Vorstand:
Prof. Dr. H.-J. Gabius

Arbeit angefertigt unter der Leitung von
Prof. Dr. W.A. Rambeck

The Effect of Rare Earth Elements on Growth Performance, Tibia Mineralization and Blood Serum of Japanese Quails

Inaugural-Dissertation
zur Erlangung der tiermedizinischen Doktorwürde
der tierärztlichen Fakultät
der Ludwig-Maximilians-Universität München

von
Maryam Zohravi
aus
Iran

München 2006

Gedruckt mit Genehmigung der Tierärztlichen Fakultät der
Ludwig-Maxximilians-Universität München

Dekan: Univ.-Prof. Dr. E.P. Märtlbauer

Referent: Prof. Dr. Rambeck

Korreferent: Prof. Dr. Hoffmann

Tag der Promotion: 9. Februar 2007

For my Parents

Contents

List of Figures

List of Tables

1. Introduction

The remaining antibiotic feed additives used in food-producing animals were banned from use in the European Union at the beginning of 2006. So there is a need for developing an alternative which promotes growth and also enhances the feed efficiency. Probiotics, prebiotics, organic acids and enzymes are already known as replacement for antibiotic feed additives but rare earth elements might be the new generation of growth promoters. Rare earth elements (REE) are 15 lanthanide elements with atomic numbers 57 (lanthanum) through 71 (lutetium), which are in group III A of the periodic table. Despite their name, the REE are in fact not especially rare. In China REE are used for 40 years as performance enhancers in agricultural plant production and remarkable results were reported from Chinese agricultural operations (wan et al., 1998). In animal production, as with plants, amazing results have been achieved by supplying REE in animal diets in Chinese literature. It was reported that proper concentrations of REE in diet can improve animal growth performance without affecting quality of products. Feeding experiments performed under Western conditions showed that dietary supplementation of rare earths had positive effects on both animal growth and feed conversion of pigs and poultry kept and fed under Western conditions (Rambeck et al., 1999a; He et al., 1999, 2001, 2006a and 2006b). However, some studies showed no positive effect of REE or showed negative effect of high concentrations of REE on growth performance (Schuller, 2001, 2002), (Kraatz et al., 2004).

Based on results obtained from Western feeding experiments, the effect of dietary rare earth elements varies with the animal species. Yet, the concentration and type of rare earths as well as the composition of individual rare earth elements have also been shown to be important factors influencing performance enhancing effects of rare earths on animals (He et al., 2003a, 2006a, 2006b) and (Redling, 2006).

The present study was designed to investigate the effect of dietary rare earth elements on poultry under western condition. Three experiments were carried out during this study to test the effect of low and high concentrations of REE-citrate and the effect of different kinds of Rare earth elements on growth performance, bone mineralization and blood serum minerals of Japanese quails.

2. Literature review

2.1. Feed additives

Feed additives are used world-wide for many different reasons. Some help to cover the needs for essential nutrients and others to increase animal performance, feed intake and thereby optimize feed utilization. The consumer demands for safer and more organic feed. So there is restricted use of some substances like antibiotics and hormones. Therefore, there is strong need for alternative growth promoters which are acceptable to the consumer and not be harmful environmentally, such as organic acids, enzymes, probiotics, prebiotics as well as herb extracts (Wenk, 2003). A very new approach in this respect is the supplementation of feed with “Rare Earth Elements”.

Feed additives categorized as:

- Technical additives, which include preservatives, acidity regulators and silage additives
- Sensory additives, including colourants and flavoring compounds
- Nutritional additives such as vitamins, pro-vitamins, compounds of trace elements, amino acids, urea and its derivatives
- Zootechnical additives like digestibility enhancers, gut flora stabilizer, environment enhancers and other zootechnical additives
- Antibiotics like coccidiostats and hisotomonostats

(Khan, 2004)

2.1.1. Antimicrobial agents

Antimicrobial agents that are banned from use in European Union, were used as feed additives which develop their activity in the digestive tract (in non-ruminants mainly in small intestine). They suppress undesired microorganisms that utilize nutrients and produce undesirable or toxic substances. The consequence is an optimal environment for the intestinal mucosa, which allows efficient nutrient absorption. Therefore nutrient utilization, feed conversion ratio and growth rate are in

most cases improved. Furthermore the health status of animals that are reared under sub-optimal conditions becomes better (Wenk, 2003).

2.1.1.1. Definition

The term “antibiotic growth promoter” is used to describe any medicine that destroys or inhibits bacteria and is administered at a low, subtherapeutic dose. The use of antibiotics for growth promotion had arisen with the intensification of livestock farming. Infectious agents reduce the yield of farmed feed animals and the administration of subtherapeutic and antimicrobial agents has been shown to be effective. The use of growth promoters is a problem of intensive farming methods and the problems caused by those used in developed countries rather than developing countries (Hughes and Heritage, 2001).

2.1.1.2. Growth promoting effect

According to the national office of animal health (NOAH, 2001), antibiotic growth promoters have been used to help growing animals digest their feed more efficiently, and allow them to develop into strong and healthy individuals. Although the mechanism supporting their action is unclear, it is believed that antibiotics suppress sensitive population of bacteria in the intestine (Hughes and Heritage, 2001).

It is probable that they allow animals to express their natural potential for growth, and that the 'growth promotion' is achieved by antibiotics exerting their effects through a direct influence on bacteria in the animal gut, since there is no response in germ-free animals. Antibiotics used as routine feed additives also appear to prevent some diseases (Gill et al, 2005).

Thomke and Elwinger (1998) hypothesize that cytokines released during the immune response may also stimulate the release of catabolic hormones, which would reduce muscle mass. So a reduction in gastrointestinal infections would result in the subsequent increase in muscle weight. The result of the using growth promoters is an improvement in daily growth rates between 1 to 10 percent resulting in meat of better quality with less fat and increased protein content (Hughes and Heritage, 2001).

Most antibiotics, around 60 percent are used for therapeutic purposes in humans. The farming industry is the second largest consumer of antibiotics after medical practitioners. About 40 percent of antibiotics are used as growth promoters although antibiotics are also used therapeutically for animals. Currently there is controversy surrounding the use of growth promoters for animals destined for meat production, as overuse of any antibiotic over a period of time may lead to the local bacterial populations becoming resistant to the antibiotic (Hughes and Heritage, 2001).

2.1.1.3. Problems

The medical exploitation of antimicrobial chemotherapy, particularly to treat human infections, has imposed an enormous selection pressure on formerly sensitive bacteria to acquire genetic elements that code for resistance to antibiotics. Human health can either be affected directly through residues of an antibiotic in meat, which may cause side-effects, or indirectly, through the selection of antibiotic resistance determinants that may spread to a human pathogen (Hughes and Heritage, 2001).

Selection may occur in microbes that are pathogenic for human. Alternatively resistance may be selected in zoonotic bacteria which cause human disease. On another level the resistance determinant may be selected in a bacterium that is member of commensal flora of the animal being fed a growth promoter if such a resistance determinant is mobilisable, it may subsequently transfer to human or animal pathogens. The consequence of selection of resistance can range from prolonged illness and side effects, due to the use of alternative, and possibly more toxic, drugs, to death, following complete treatment failure. To reduce the risk of selecting resistant bacteria the use of antibiotics must be restricted. The most attractive area for reducing the use of antibiotics is to ban their used as growth promoters (Hughes and Heritage, 2001).

2.1.1.4. Past use of antibiotic growth promoters

The initial use of antibiotics in diets arose from the discovery in the late 1940's, in the United States, that including the fermentation products of Streptomyces aureofaciens (a strain of bacteria) in the diets of simple-stomached animals, such as

pigs and poultry, resulted in growth responses (Gill et al, 2005). By 1949, antibiotics had been approved for growth promotion in experimental, and many different groups of antibacterial have subsequently been approved for on-farm use as growth promoters in many European countries and United States (Karaoglu and Durdag, 2005). In the next fifty years, the use of antibiotics as feed additives in pig and poultry production became virtually universal (Gill et al, 2005).
On world scale the use of antibiotics as animal growth promoters differs dramatically. Sweden now makes no use of antibiotics for growth promotion purposes but USA uses a wide range of antibiotics including some considered to be medically important. The animal health institute of America (AHI, 1999) has estimated that without use of growth promoting antibiotics, the USA would require an additional 452 chickens, 23 millions more cattle and 12 millions more pigs to reach the level of production attained by the current practices (Hughes and Heritage, 2001).

A general ban on antibiotics as antimicrobial performance promoters in animal nutrition has been enforced since 1986 in Sweden and since 1999 in Switzerland. Following the ban of all feed animal growth-promoting antibiotics by Sweden in 1986, The European Union (EU) banned the use of avoparcin, a widely used antibiotic feed additive in food-producing animals in 1997. Two years later, the EU banned the use of bacitracin, spiramycin, tylosin and virginiamycin (Casewell et al., 2003 and Cervantes, 2005). Under pressure from consumer organizations and supermarket chains, the complete ban of all antimicrobial performance promoters in farm animals is presently discussed (Wenk, 2003). On January 1, 2006 the remaining antibiotic feed additives used in food-producing animals were banned from use in the European Union (Cervantes, 2005).

The ban of growth promoters has, however, revealed that these agents had important prophylactic activity and their withdrawal is now associated with a deterioration in animal health, including increased diarrhea, weight loss and mortality due to *Escherichia coli* and *Lawsonia intracellularis* in early post-weaning pigs, and clostridial necrotic enteritis in broilers (Casewell et al., 2003). The surge in enteric diseases of food-producing animals was followed by a surge in antibiotic use in food-producing animals for therapeutic purposes. The antibiotics used to treat food-producing animals belong to the various classes of antibiotics most frequently used in human medicine; this might have actually had a more adverse effect on the creation of antibiotic resistance in people than the use of the antibiotic feed additives.

The surge in use of antibiotics for therapeutic purposes in food-producing animals has clearly proven that the prior use of antibiotic feed additives had a health promotional and disease prevention effect in food-producing animals even when used at concentrations labeled for "growth promotion" (Cervantes, 2005).

In considering phasing out or banning antibiotic growth promoters, the quality of any alternatives, either on the market, that could be developed or that are available illegally, must be assessed. There are two ways in which we can reduce our dependence on antibiotic use in animals. An obvious choice is the development of alternatives to antibiotics that work via similar mechanisms, promoting growth and also enhancing the efficiency of feed conversion. Like pronutrient available as organic acids, dietary fibers, pro- and prebiotics and highly available nutrients (Hughes and Heritage, 2001; Wenk, 2003).

Any substance which is intended to replace the role of antibiotics in farm animals will be subject to the intense scrutiny that antibiotics have been subjected to over the past 40 years. Since the growth benefit found from feeding antibiotics is achieved through many different effects on the gut, the strategy for replacing them will depend on a combination of nutritional, housing and husbandry factors (Gill et al, 2005).

2.1.2. Probiotics

2.1.2.1. Definition

A “probiotic” by the generally accepted definition, is a “live microbial feed supplements which beneficially affects the host animal by improving its intestinal microbial balance”. The other definition given by Green and Sainsbury (2001) says probiotic is living microorganism which given to animals help in establishment of an intestinal population which is beneficial to the animal and antagonistic to harmful microbes (Denli et al., 2003).

Live bacterial cultures or direct-fed microbial or probiotics or competitive exclusion cultures, have been proposed to improve the balance of the gut microflora. Thus, the bacterial cultures added to the feed will compete with pathogenic bacteria for nutrients and binding sites on the intestinal wall promote an effective immune system. Bacterial cultures may include Lactobacillus, Bacillus, Streptococcus, yeast, or other

microorganisms. Considering the mode of action, direct-fed microbial would be a logical choice as antibiotic replacement strategies (North Carolina University, 2005).

Although referring to the supplementation of animal feeds for farm animals, the definition are easily applied to the human situation. The major consumption of probiotics by human is in the form of dairy-based foods containing intestinal species Lactobacilli and bifidobacteria. It is implicit in the definition that consumption of the probiotic affects the composition of the intestinal microflora (Tannock, 1999).

2.1.2.2. Antimicrobial effect

According to Hughes and Heritage (2001), probiotics improve the overall health of an animal by improving the microbial balance in its gut. The way they work has not been established, although it has been hypothesized that their action can be summarized in three ways. The first proposal is a reiteration of the competitive exclusion principle: by colonizing the gut in large numbers, the probiotic bacteria exclude pathogens and thus prevent them from causing infection. The second possibility is that they act as a stimulus for the immune system. As the immune system is engaged following exposure to probiotic bacteria, any hostile bacteria are also noticed, following increased surveillance by leukocytes, and thus potential pathogens are eliminated. Koenen et al., (2004) found that probiotic lactobacilli can have a positive effect on humoral and cellular immune responses in layer- and meat-type strain chickens, but the lactobacillus strain to be used, the age of the animals and effective dose of lactobacilli to be administered need to be optimized. Galvao et al, (2005) showed that while calves receiving live yeast products had higher levels of antibiotic resistance than controls.

The third suggestion proposes that probiotics have strong, positive influence on intestinal metabolic activities, such as increased production of vitamin B12, bacteriocins, and propionic acid. Other mechanisms have been proposed but remain to be confirmed (Hughes and Heritage, 2001). This effect of probiotics on the intestinal ecosystem impacts in some beneficial way on the consumer. A number of potential benefits arising from changes to the intestinal milieu through the agency of probiotics have been proposed, including: Increased resistance to infectious disease, particularly of the intestine, decreasing duration of diarrhea, Reduction in blood pressure, Reduction in serum cholesterol concentration, reduction in allergy,

stimulation of phagocytosis by peripheral blood leucocytes, modulation of cytokine gene expression, adjuvant effect, regression of tumors, reduction in carcinogen or co-carcinogen production. Perhaps despite this impressive list of therapeutic attributes, probiotics are not commonly art of the medical prescription drugs. Instead, probiotics are available from retail outlets, usually supermarkets, and grocery and health food stores. The probiotics are available to the consumer as powders or tablets, but most commonly as milk-based products (Tannock, 1999).

2.1.2.3. probiotics for farm animals

Micro-organisms have been used since the end of the 1980s in animal feeds and were strictly regulated in 1993, when they were introduced under the scope of Council Directive 70/524/EEC of 23 November 1970 on additives in animal nutrition on feed additives. After a transition period, which ended in the year 2000, every microbial strain must now be assessed by the EU bodies and authorized by a Commission Regulation, before it can be placed on the market for use in feeding stuffs. Council Directive 70/524/EEC on feed additives is based on three main principles: (1) pre-market authorization, (2) positive list principle, and (3) thorough risk assessment of the effect of a particular strain on human and animal health as well as on the environment (Becquet, 2003). Observations indicate a positive effect of probiotics on the gut flora, especially for young animals or during feed transition phases. This favorable effect is perceived by the farmers as a means of maintenance of the health status of the animals (e.g. less diarrhea) and results in significant improvement of animal performance (Becquet, 2003).

The development of probiotics for farm animals is based on the knowledge that the gut microflora is involved in resistance to disease. The stressful conditions experienced by the young animal cause changes in the composition or activity of the gut microflora. Probiotics supplementation repairs these deficiencies and provides the type of microflora which exists in feral animals uninfluenced by modern farm rearing methods. The products available are of varying composition and efficiency but the concept is scientifically based and intellectually sounds. Under the right conditions the claims made for probiotic preparations can be realized (Fuller, 1999). The growth promoting effect of subtherapeutic level probiotics used in animal feed,

also been ascribed to suppressing of urea hydrolysis and subsequently reduced ammonia production in the gastrointestinal tract. (Yeo and Kim, 1997)

Kurtoglu et al., (2004) reported that supplementation of 250, 500 and 750 mg /kg probiotic in layer hens diet increased egg production, but decreased the damaged egg ratio ($p < 0.05$), egg yolk cholesterol and serum cholesterol ($p < 0.001$) levels. In addition, serum triglyceride levels were reduced by using 500 and 750 mg /kg probiotic supplementation ($p < 0.001$) and feed conversion ratios were positively affected by supplementation of 250 and 500 mg /kg probiotic compared with controls ($p < 0.05$).

Stanley et al., (2004) found that yeast culture residue (YCR) can be a viable alternative for antibiotic growth promoters in broiler chicks because YCR increased body weight compare to lasalocid and bacitracin (antibiotic feed additives). Zhang et al., (2005) concluded that dietary yeast components, such as Whole yeast (WY) or Saccharomyces cerevisiae cell wall (CW) supplementation improves growth performance in broiler chicks ($p<0.05$). They also showed that Meat tenderness could be improved by the Whole yeast (WY) or Saccharomyces cerevisiae extract (YE). Both YE and CW have oxidation-reducing effects and Yeast cell wall may improve ileal villous development.

2.1.2.4. Problems

The problem with probiotics is the lack of evidence as to their mechanism of action and of the effects on host animals. Shahani et al. (1983) showed that the growth of experimentally induced tumors could be inhibited in mice fed with fermented colostrums, but only in animals dosed before tumor growth started. Kato et al (1985) confirmed these experiments, showing that intraperitoneal administration of Lactobacillus casei inhibited tumor growth. Unfortunately these results could not be replicated on farms. Most probiotics would not be administered via the intraperitoneal route on a working farm. It is also discovered that some strains could be harmful. (Hughes and Heritage, 2001)

Probiotics are effective in certain cases (Like new born animals and animals treated by antibiotics) that they have the same effect as competitive exclusion products. An additional problem caused by the use of live bacterial products is that there may be dangers concerning antibiotic resistance and virulence factors. A recent

report from the scientific committee for animal nutrition (2001) concerning the safety of probiotic product found that two of the principal strains (Pediococcus acidilactic and Lactobacillus plantarum) were resistant to tetracycline. So it was concluded that because of the possible dissemination of tetracycline resistance genes in animal bacterial populations, the food chain and the environment, the use of that product is risky when used in animal nutrition. (Hughes and Heritage, 2001)

Currently available probiotic organisms and known issues regarding their safety are briefly summarized. While most of species and genera, particularly lactobacilli and bifidobacteria, are apparently safe, certain micro-organisms may be problematic particularly the enterococci, which are associated with nosocomial infections and harbor transmissible antibiotic resistance determinants. At present, probiotic human foods are not governed under specific EU regulatory frameworks, although the Novel Food Regulation EU 258/97 could be relevant in some specific cases (Von Wright, 2005). Particular attention is focused on the presence of transmissible antibiotic resistance markers, and to the potential for production of harmful metabolites. The guidelines do not differentiate between species and strains with long histories of safe use and other micro-organisms. This has caused some concern regarding overregulation, if the same principles are to be applied to probiotics or starter cultures intended for human food use (Von Wright, 2005).

2.1.3. Prebiotics

2.1.3.1. Definition

A range of non-digestible dietary supplements have now been identified that modify the balance of the intestinal microflora, stimulating the growth or activity of beneficial organisms and suppressing potentially deleterious bacteria already resident in the digestive tract and attempt to improve host health (Gibson and Roberfroid, 1995; Crittenden, 1999). In summary, prebiotics are nondigestible food ingredients that encourage proliferation of selected groups of the colonic microflora, thereby altering the composition toward a more beneficial community (Tzortzis, et al., 2005).

These supplements include lactulose, lactitol, a variety of oligosaccharides, and inulin (Crittenden, 1999). Another major aspect of the use of prebiotics in diets for young animals is the benefit due to the competitive exclusion of pathogenic microorganisms such as *E. coli* or salmonella (Savage and Zakrewska, 1995; Spring, 1996)

2.1.3.2. Antimicrobial and growth promoting effect

Prebiotics promote the proliferation of bifidobacteria in the colon (Crittenden, 1999). Prebiotics are recognized for their ability to increase levels of health promoting bacteria in the intestinal tract of humans or animals. These bacteria involve bifidobacteria and/or lactobacilli. Non digestible oligosaccharides such as fructo-oligosacharides, lactulose and trans-galacto-oligosacharides seem as effective prebiotics. Other oligomers are used as prebiotics in Japan like xylo-oligosacharides, soybean oligosaccharides, and isomalto-oligosacharides (Rastall and Gibson, 1999).

Compare to probiotics, which introduce exogenous bacteria into the colonic microflora, a prebiotic stimulates the growth of one or a limited number of potentially health-promoting indigenous micro-organisms which means modulate the composition of the natural ecosystem. The other possible beneficial effects of prebiotics can be anti-colon cancer properties, negative modulation of colon carcinogenesis, lipid lowering action, modulation of metabolism of triacylglycerol, modulation of insulinemia, improved calcium bioavailability (Cashman, 1999; Roberfroid, 2000).

Solis et al., (2005) suggested that a feed ration supplemented with prebiotic may reduce the effect of hypoxia on broiler gut development and ascites incidence. Chen et al., (2005) found that supplementation laying hens diet with prebiotics (Oligofructose and Inulin) improved egg production, cumulative egg weight per bird and feed conversion ratio. They explained that prebiotic products could change the microflora in the layer's intestine which would have effect on its development and absorption capacity with, as a consequence the observation of an improved feed conversion ratio. Improvement in egg production might be due to healthier birds whose feed efficiency and mineral absorption have been improved by the prebiotics.

2.1.4. Organic acids

2.1.4.1. Definition

This name is used for all acids whose structures are based on a carbon framework. These acids are also referred to as “carboxyl acids”. The carboxyl acids include formic acid (derived from methane) and propionic acid (derived from propane). These acids occur naturally and are completely metabolized. Their energy content can be included in estimated energy content of a compound feed. This energy is not negligible, especially with high Propionic acid contents. Propionic acid and formic acid are used in animal nutrition in particular for preserving and ensiling feedstuffs. In addition, they may have nutritional effects (BASF Corporation, 2001).

Organic acids are often included in diets as mold inhibitors, but they also have antimicrobial properties that can position them as potential replacements for antibiotics. In addition, they may reduce gut pH and stimulate digestibility, which can in turn improve feed efficiency. Examples of organic acids are acetic, propionic, butyric, formic, citric, fumaric, lactic, sorbic, and malic acids or commercial acid blends (North Carolina University, 2005).

The most efficient way to keep compound feed at hygienic status is the use of organic acids. Bacteria and yeast in compound feed have a negative impact on the intestinal flora, resulting in nutrient losses and negatively influencing the feed intake of animals. All these effects result in a reduction of animal performance. With the use of organic acids the microbial contamination levels of feed will be reduced, proliferation of pathogens prevented and animal performance improved. In compound feed or their single components, basic contamination of moulds, bacteria and yeast are always present (Çelik et al., 2003; HeindI, 2002).

2.1.4.2. Antimicrobial effect

Organic acids control microorganisms by intervention in the energy metabolism by blocking the enzyme pyruvatedecarboxylase and influencing DNA synthesis. (Adams, 1999) organic acids which have strong effect in PH reducing, are extremely efficient against bacteria and yeast. This results in a reduction of number of caliform germs in

feed, which lowers the microbial stress for the animal (Çelik et al., 2003). Organic acids act in three different areas: in the feed, in the intestinal tract and n the metabolism. Feed even under favorable conditions always has a certain load of microorganisms. The application of organic acids depresses the metabolic activity of susceptible germs and therefore reduces the microbial load of feed (Çelik et al., 2003).

It has been suggested that benefits of including organic acids and their salts are related to antimicrobial properties of their cations and anions when acids dissociate after passing the bacterial cell wall and that causes a disruptive effect on bacterial protein synthesis (Roth and Kirchgessner 1997, 1998; Partanen and Mroz, 1999). Undissociated organic acids can diffuse freely through the semi permeable membrane of microorganisms into their cell cytoplasm. Once inside the cell, where PH is maintained near 7, the acid will dissociate and suppress cell enzymes and nutrient transport system (Lueck, 1980; partanen and Mroz, 1999). This effect is thought to be important in the proximal gastrointestinal tract, and it requires the presence of the acid in the gastrointestinal lumen (Canibe et al., 2001).

In the presence of high numbers of bacteria in the cecum and colon, the animal is able to utilize a part of energy from the nutrients being resistant to hydrolysis by host enzymes. Therefore the use of organic acids or their salts to reduce pathogenic and nonpathogenic bacteria in the proximal gastrointestinal tract would be desirable for dietary energy utilization by the animal (Canibe et al., 2001).

2.1.4.3. Growth promoting effects

The antimicrobial and growth promoting effects of organic acids and their salts make them a possible alternative to feed additive antibiotics in starter diets for pigs (Canibe et al., 2001). The addition of organic acids to diets for pigs has been reported to increase their performance and to decrease the intraluminal concentration of caliform bacteria, involved in digestive disorders and other microorganism in the gastrointestinal tract (Paulicks et al., 1996; Kirchgessner et al., 1997). Lowering dietary buffering capacity, via acidification with organic acids or their salts, has been indicated to inhibit luminal growth of eterotoxigenic microflora and to enhance swine performance (Ravindran and kornegay, 1993; Gabert and sauer, 1994). According to Kirchgessner and Roth (1982), the growth promoting effects of organic acids may

result from decreased bacterial growth and increased nutrient digestibility. Studies on the mode of action of organic acids in pigs indicates that they cause a higher protein and energy digestibility and retention, change the bacterial populations and metabolites in the gastrointestinal tract and possibly have effect on metabolism(Roth and Kirchgessner, 1998). In other study by Mroz et al., (2000), it was reported that organic acids have a beneficial effect on the apparent ileal/total tract nutrient digestibility.

A series of studies with poultry indicates that organic acids in their undissociated forms are able to pass through the cell membrane o bacteria. Once inside the cell the acid dissociates to produce H+ ions which lower the PH of the cell causing the organism to use its energy in trying restoring the normal balance (Nursey, 1997). But the effectiveness of organic acids in poultry may also depend on the composition of the diet and its buffering capacity (Çelik et al., 2003). Denli, et al., (2003) indicate that Organic acids inhibit pathogen bacteria growth in the feed and at the opening of the digestive tract. They concluded organic acid may enhance growth performance and carcass quality of broiler chick. The other comment given by Langhout (2000) is organic acids reduces production of toxic components by bacteria and changes the morphology of the intestinal wall which reduces colonization of pathogens on the intestinal wall. This prevents damage to the epithelial cells. Rafacz-Livingston et al., (2005) showed that gluconic acid and citric acid (but not fumaric acid or EDTA) improve phytate phosphorus utilization on chicks fed a corn-soybean meal diet which results in increasing weight gain and tibia ash.

2.1.5. In feed enzymes

In feed enzymes are routinely added to pig and poultry feeds and work by helping to break down certain components of the feed, such as glucans, proteins and phytates, which animal may have problems digesting. They are produced as fermentation products from fungi and bacteria and seem only having a positive effect on the animal. Some ethicists, however, have argued that adding enzymes to animals merely shows that we think of them as “factory beasts”. Apart from ethical objections, in-feed enzymes are very effective at maximizing feed conversion and few draw backs (Hughes and Heritage, 2001). BASF Corporation (2001) indicates it is

only recently that enzymes have been used in animal nutrition, mostly for monogastric animals, such as pigs and poultry. When enzymes used through feed, generally catalyze in the gastrointestinal tract, chemical reactions which lead to degradation of the feed ingredients. Enzymes and enzyme complexes which are obtained by fermentation are primarily used for following purposes:

- Increasing the availability or convertibility of feed ingredients (as phytase for breaking phytate, amylase for breaking amylase)
- Degrading or destroying anti nutritive substances (as degradation of beta-glucans in barely by the use of beta-glucanase)
- Supplementation/supporting endogenous enzymes (as proteases in young animals with an incompletely developed enzyme pattern).

Research showed that enzyme supplementation can improve digestion, prevent or reduce gas production and bloat, reduce adverse feed reaction, control some feed allergies in animal body, leading to better utilization of feed nutrients (Esonu et al., 2005). Availability and digestibility of nutrients play the major role in poultry feedstuffs to lead a desirable production. Numerous studies have cleared the enzyme influence in increasing protein digestibility. Saki et al., (2005) concluded that enzyme supplementation in broiler diets improves protein digestibility and decrease uric acid excretion which results in reduce ration price and save environmental aspects by nitrogen contamination.

Midilli and Tuncer (2001) showed that addition of enzyme and enzyme with combination of probiotics to the poultry diet, significantly increased body weight gain ($p<0.001$) and improved feed conversion ratio ($p<0.001$). Also they showed that enzyme significantly decreased intestinal viscosity ($p<0.001$) and decreased abdominal fat weight ($p<0.05$). In other research (Silversides et al., 2004) supplementation phytase enzyme in broiler chicks diet, increased performance and calcium and phosphorus digestibility.

Dilger et al., (2004) reported that supplementation microbial phytase in broiler diets increased weight gain, feed intake, feed efficiency, tibia and toe ash significantly ($p<0.001$). Snow et al., (2004) showed that phytase enzyme in young chicks diet increases weight gain and tibia ash. They indicated that enzyme increase phytase phosphorus use. Lázaro et al., (2004) and Wang et al., (2005) got result with supplementation of broiler diet with xylanase and β-glucanase. These enzymes improved performance, average daily gain and feed conversion ratio until 46 days in

broiler chicks and the beneficial effect of enzyme was more evident when birds were fed ad libitum. Iyayi and Davis (2005) showed that enzyme supplementation in broiler chicks diet, significantly ($p<0.05$) enhanced performance as weight gain. They reported that enzyme supplementation decreases the viscosity of the digesta which results in enhancing apparent digestibility of protein and fat and increase in feed intake and weight gain.

2.1.6. Botanical feed additives

Plant secondary metabolites are a natural resource that is largely unexploited in 'conventional' animal production systems. They have in the past been generally considered as a source of antinutritional factors, and not as a source of exploitable performance-enhancing compounds. Recent and continuing changes to legislation controlling the use of animal feed additives have stimulated interest in bioactive secondary metabolites as alternative performance enhancers. They are broadly compatible with current thinking on the future of agriculture and food in Europe, and with consumer opinion. Interest has been largely on their manipulative role in the digestive and absorptive processes of the hindgut. Use of plants and their extracts manipulate the rumen microbial ecosystem to improve the efficiency of rumen metabolism. So the bioavailability of secondary metabolites and their actions on peripheral metabolism improve animal performance (Greathead, 2003).

Wallace (2004) used two classes of plant secondary compounds, i.e. essential oils and saponins, as feed additives in 'Rumen-up' project. Dietary inclusion of essential oils caused markedly decreased NH3 production from amino acids in rumen fluid taken from sheep and cattle. Saponin-containing plants and their extracts suppress the bacteriolytic activity of rumen ciliate protozoa and thereby enhance total microbial protein flow from the rumen. Saponins also have selective antibacterial effects that may prove useful in, for example, controlling starch digestion. The result of project showed that plant secondary compounds, of which essential oils and saponins comprise a small proportion, have great potential as 'natural' manipulators of rumen fermentation to benefit the farmer and the environment in the future.

Hernandez et al., (2004) studied the influence of 2 plant extracts on performance, digestibility, and digestive organ weights in broilers which were 200 ppm essential oil

extract (EOE) from oregano, cinnamon, and pepper; and 5,000 ppm Labiatae extract (LE) from sage, thyme, and rosemary. Both plant extracts improved the digestibility of the feeds for broilers. The effect of different additives on digestibility improved the performance slightly, but this effect was not statistically significant.

Dugenci et al., (2003) showed immunostimulant effects of the dietary intake of various medicinal plant extracts in fish, rainbow trout. Fish were fed with diets containing aqueous extracts of mistletoe (Viscum album), nettle (Urtica dioica), and ginger (Zingiber officinale). Plant materials tested for immunostimulatory feed additives caused an enhanced extracellular respiratory burst activity ($P<0.001$) compared to the control group. Phagocytosis and extracellular burst activity of blood leukocytes were significantly higher in this group than those in the control group.

2.2. Rare Earth Elements

2.2.1. Definition

Rare earth elements (REE) are 15 lanthanide elements with atomic numbers 57 (lanthanum) through 71 (lutetium), that are in group III A of the periodic table. They are named in order Lanthanum (La), Cerium (Ce), Praseodymium (Pr), Neodymium (Nd), Promethium (Pm), Samarium (Sm), Europium (Eu), Gadolinium (Gd), Terbium (Tb), Dysprosium (Dy), Holmium (Ho), Erbium (Er), Thulium (TM), Ytterbium (Yb), and Lutetium (Lu). The REE are represented by the single square of lanthanum in the main part of the periodic table and listed in a separate sub-table below the main grouping Figure 1.

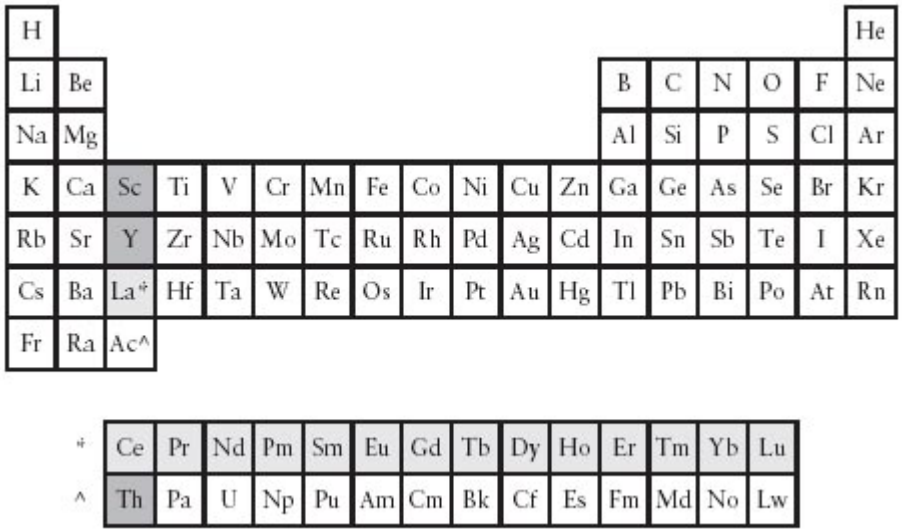


Figure 1: Periodic table with REE (light shading) and scandium, yttrium and thorium highlighted.

Yttrium (Y, atomic number 39) and scandium (Sc, atomic number 21) are sometimes included in the group of rare earth elements. Attempts have also been made to split the lanthanides into various subgroups. One of these, based upon their occurrence in different minerals, distinguishes the light or cerium subgroup (cerium earths), comprising the first seven elements (atomic numbers 57-63) and thorium; and the heavy or yttrium subgroup (yttrium earths), comprising the elements with atomic numbers 64-71 as well as yttrium and scandium. Despite its low atomic weight, Yttrium is categorized with heavy REE, because its properties are closer to those of the heavier REE than to the lighter group (Evans, 1990; Wald, 1990; SpyLOG, 2004).

Others have divided the series into three, making reference to the cerium group (La-Sm), the terbium or transitional group (Eu, Gd, Tb), and the yttrium group (Dy-Lu, Y). One of the advantages of the lanthanides is the relatively smooth and progressive nature of the changes in their chemical properties throughout the series (Evans, 1990).

2.2.2. Distributions and abundance

Despite their name, the REE are in fact not especially rare. Each is more common in the earth's crust than silver, gold or platinum, while Cerium, Yttrium, Neodymium and Lanthanum are more common than lead. Their average content in the earth crust is approximately 0.015%, which matches with that of copper, lead and zinc, and is much higher than that of tin, cobalt, silver, and mercury (Hu et al., 2004).The light REE (La through Eu) are more abundant than heavy REE (Gd through Lu), and furthermore, the elements of even atomic number are more abundant than neighbors of odd atomic number, because of the greater relative stability of nuclei. Rare earth elements are never found as free metals in the earth and all their naturally occurring minerals consist of mixture of various REE and nonmetals (Wald, 1990; SpyLOG, 2004). Table 2.2-1 summarizes the natural occurrence of rare earths compare to other important metals.

Table 2.2-1: Natural occurrence of rare earths and other elements (Considine, 1984)

Rare earths	ppm	other	ppm
Thulium	0.5	Gold	0.015
Terbium	0.9	Silver	0.1
Europium	1.2	Mercury	1.0
Holmium	1.2	Lead	16
Erbium	2.8	Cobalt	23
Dysprosium	3.0	Tin	40
Ytterbium	3.0	Bromine	50
Gadolinium	5.4	Nickel	80
Neodymium	28	Copper	100
Lanthanum	30	Zinc	130
Yttrium	33		
Cerium	60		

Rare earth elements are separated from other elements in a mineral by precipitation with a suitable reagent. Separation of the rare earth elements from each other by ordinary chemical means is difficult because their chemical properties are

similar, and the isolation of individual elements may involve hundreds of fractional crystallizations. But with ion-exchange methods the separation of an individual REE can be accomplished with greater ease and precision (Wald, 1990; Luminescent lanthanides, 1997). More than 250 kinds of minerals containing rare earths are known. Only some of them are important for industrial production of rare earth materials (metals, alloys, compounds, fertilizers):

Monazite, a thorium-rare-earth phosphate (Th, RE) PO_4
Bastnaesite, a rare earth fluorocarbonate, $REFCO_3$
Xenotime, a rare earth phosphate $REPO_4$
LoParit, (Na, Ce, RE) $_2$(Ti, Nb)$_2O_6$
Euxenit (RE, Ca, U, Th) (Nb, Ta, Ti)$_2O_6$
Parisit, Yttroparisit, RE_2Ca $(CO_3)_3F_2$

The so-called- "ion adsorption" ores (Longnan clay and Xunwu clay both found in the Jiangxi Province in China) and residues from uranium mining in Canada are also important sources for industrial production of metals and compounds of REEs. The common commercial rock phosphates, which are used for the production of phosphoric acid, elemental phosphorus and different types of phosphorus fertilizers, also contain different amounts of REEs (Hu et al., 2004).

Oxides of the rare earth elements are called rare earth, and are found in minerals that are actually more abundant than those of some other metals, such as those in the platinum group (Luminescent lanthanides, 1997). Bastnaesite [(Ce, La) (Co3) F], Monazite [(Ce, La, Nd, Th) (Po_4)] and Xenotime [YPo_4] are the three most economically significant minerals of the more than 200 minerals known to contain essential for significant REE (SpyLOG, 2004). The contents of REEs expressed in equivalent to oxide forms (REO) in rock phosphates was reported to 0.015% in Morocco/Khourigba, 0.14% in Tunisia/Gafsa, 0.06-0.29% in USA/Florida, 0.21% in Russia/Kovdor, 0.46--0.58% in South Africa/Phalaborwa, and 0.8-1.0% in Russia/ Kola-Peninsula (Hu et al., 2004).

The discovery of the total rare earth elements took 135 years as much. On account of their similarities, it is not 40’s of the century, until that the pure rare earth individuals were not prepared in commercial scale except a small quantity obtained in laboratory. In 1947, some American scientists developed an effective separation

process was used to separate adjacent rare earth element and successful. In 1958, solvent-solvent extraction commenced coming to be used to group separation in a large scale production line. During the period of 1958-1970 in China, 16 individual rare earth oxides and individual rare earth metals were successfully produced, and the complete technical reports were prepared in details (Chengdu Beyond chemical Co., 2005). Up to 1980, American mining amount was over 253000 tons (REO), but China exceeded America in separation capacity for the first time in 1986. During the period of 1971-1990 in China, The first-, second and third-generation methods of sulphatizing calcination for producing rare earth chlorides from Baotou rare earth concentrates had been invented. And the separation techniques for individual rare earth and those for the Southern ionized rare earth ores were successfully developed. The precedent of the research of rare earth for agricultural use in China was initiated in this period of time (Chengdu Beyond chemical Co., 2005). In 1994, the production of processed RE products in China amounted to 28000 ton REO (Richter, 1996). China’s mining quantity of rare earth in 1995 accounted for 70% of total production in the world. In 1998 total production of rare earth concentrates in China was 65000 ton REO, UP 22% from that in 1997. The turnout of the processed rare earth products was 52000 ton REO, 12% from the previous year. And the total rare earth consumption in China was 15300 ton REO, 2% up from that in 1997 (China rare earth information, 1999). Now China has become the biggest supplier of rare earth over the world (Chengdu Beyond chemical Co., 2005).

Total amounts of reserves of REEs in the world are estimated to be 100 million tons of REO. In the People's Republic of China's territory the reserves are estimated to be in the amount of 43 million tons of REO. China's mine production was 73,000 tons REO in 2000 and 75,000 -tons REO in 2001. This corresponded to 87% and 90% of the world's total production in the same periods (Hu et al., 2004). China has done much research work and made great progress in the application of RE in metallurgy, chemical industry, electronics, and agriculture and other fields (Richter, 1996).

2.2.3. Chemistry and biochemistry of Rare Earth Elements

The REEs have very similar chemical and physical properties. With few exceptions the characteristic oxidation state is +3. Cerium forms a stable +4 species, which is a very strong oxidizing agent in aqueous solution. Praseodymium and Th are known to form higher valence oxides. The +2 oxidation state is also important for some REEs. The EU^{2+} and Yb^{+2} are the most stable dipositive species (Hu et al., 2004).

2.2.3.1. Electronic configurations and their consequences

The outer electronic configurations of the lanthanides, yttrium, and calcium and their most common ions are given in Table 2.2-2. In progressing from La, the lightest lanthanide, to Lu, the heaviest, there is progressive filling of the *4f* orbital. As we shall see, it is these *4f* electrons which endow the lanthanides with the special properties of such importance to structural biochemical analysis. Of great significance is the electronic configuration whereby the *4f* electrons are not in the outermost orbital but are shielded by the electrons of higher orbital (Table 2.2-2). All the lanthanides have filled 5s, 5p, and 6s orbital, while La, Gd, and Lu each have 5d electrons. As the outermost electrons are the valence electrons, the most important properties resulting from the inner, *4f* electronic behavior are not lost upon ionization. Furthermore, upon coordination with ligands, the *4f* electrons remain sufficiently shielded that complexing groups have only minor effects upon their behavior. Thus the magnetic and spectroscopic properties of the lanthanides are not lost upon ionization or upon binding.

The sum of the first three ionization potentials is shown in Table 2.2-2. Screening of the 4f electrons is such that the *6s* and 5d orbital change little in energy across the series, as indicated by the relatively constant values of the ionization potentials. The lanthanides are highly electropositive, and their compounds are essentially ionic in nature. The predominant ionic form existing under the conditions appropriate to biochemical investigation is the trivalent cation, Ln^{3+}. Tetravalent states are known for Ce, Pr, Nd, Th, Dy, and Ho, while Sm, Eu, Er, Tm, and Yb have divalent forms. Of these, only Ce^{4+} and Eu^{2+} are stable enough to exist for any length of time in aqueous solution, and neither of these is as stable as its respective trivalent form under physiological conditions (Evans, 1990).

Table 2.2-2: some pertinent chemical properties of Lanthanides, Calcium and Yttrium

				Outer electronic configuration											Ionic radious (Å)				
				Atomic (Ln^0)								Ionic (Ln^{3+})							
Element	Symbol	Atomic number	Atomic weight	4s	4p	4d	4f	5s	5p	5d	6s	4f	5s	5p	CN6	CN7	CN8	CN9	ΣI^a (eV)
Calcium	Ca	20	40	[b]											1.00	1.06	11.2	1.18	-
Lanthanum	La	57	139	2	6	10	2		6	1	2		2	6	1.03	1.10	1.16	1.22	36.2
Cerium	Ce	58	140	2	6	10	2	2	6		2	1	2	6	1.01	1.07	1.14	1.20	36.4
Praseodymium	Pr	59	141	2	6	10	3	2	6		2	2	2	6	0.99	-	1.13	1.18	37.55
Neodymium	Nd	60	144	2	6	10	4	2	6		2	3	2	6	0.98	-	1.11	1.16	38.4
Promethium	I'm	61	147	2	6	10	5	2	6		2	4	2	6	-	-	-	-	-
Samarium	Sm	62	150	2	6	10	6	2	6		2	5	2	6	0.%	1.02	1.08	1.13	40.4
Europium	Eu	63	152	2	6	10	7	2	6		2	6	2	6	0.95	1.01	1.07	1.12	41.8
Gadolinium	Gd	64	157	2	6	10	7	2	6	1	2	7	2	6	0.94	1.00	1.05	1.11	38.8
Terbium	Tb	65	159	2	6	10	9	2	6		2	8	2	6	0.92	0.98	1.04	1.10	39.3
Dysprosium	Dy	66	162.5	2	6	10	10	2	6		2	9	2	6	0.91	0.97	1.03	1.08	40.4
Holmium	Ho	67	165	2	6	10	11	2	6		2	10	2	6	0.90	-	1.02	1.07	40.8
Erbium	Er	68	167	2	6	10	12	2	6		2	11	2	6	0.89	0.95	1.00	1.06	40.5
Thulium	Tm	69	169	2	6	10	13	2	6		2	12	2	6	0.88	-	0.99	1.05	41.85
Ytterbium	Yb	70	173	2	6	10	14	2	6		2	13	2	6	0.87	0.93	0.99	1.04	43.5
Lutetium	Lu	71	175	2	6	10	14	2	6	1	2	14	2	6	0.86	-	0.98	1.03	40.4
Yttrium	Y	39	89	2	6	1		2			[c]				0.90	0.%	1.02	1.08	-

[a] ΣI= sum of the first three ionization potentials.
[b] outer electronic configuration: $3s^2 3p^6 3d^0 4s^2$
[c] outer electronic configuration: $4s^2 4p^6$

In general terms, the magnetic moments of the lanthanides arise from the presence of *4f* electrons with unpaired spins. However, there is more to it than this, as Gd^{3+}, which has the highest number of unpaired electrons, does not have the highest magnetic moment (Table 2.2-3). This occurs because the *4f* electrons are sufficiently well screened that both their spins and their orbital motions about their nuclei contribute to the magnetic moment. As a result, the lanthanide series contains two magnetic maxima (Table 2.2-3). Tb^{3+}, Dy^{3+}, Ho^{3+}, and Er^{3+} are among the strongest paramagnetic ions known and-, as such, are of great practical use, especially with regard to nuclear magnetic resonance (NMR) spectroscopy (Evans, 1990).

Table 2.2-3: Magnetic moments and colors of Yttrium and the Lanthanide Ions

Ion	Unpaired 4f electrons	Magnetic moment (Bohr magnetons)	color
Y^{3+}	0	0	Colorless
La^{3+}	0	0	Colorless
Ce^{3+}	1	2.39	Colorless
Pr^{3+}	2	3.47	Yellow-green
Nd^{3+}	3	3.62	Reddish
PM^{3+}	4	2.83	Pink; yellow
Sm^{3+}	5	1.54	Yellow
Eu^{3+}	6	3.61	Nearly colorless
Gd^{3+}	7	7.95	Colorless
Tb^{3+}	6	9.6	Nearly colorless
Dy^{3+}	5	10.5	Yellow
Ho^{3+}	4	10.5	Pink; yellow
Er^{3+}	3	9.55	Reddish
Tm^{3+}	2	7.5	Pale green
Yb^{3+}	1	4.4	Colorless
Lu^{3+}	0	0	Colorless

2.2.3.2. Comparison between Ln^{3+} and Ca^{2+} and Mg^{2+} Ions

REEs have similar characteristics as Ca. REEs have a similar ionic radius (9.6-11.5 nm) as the Ca. ion (9.9 nm). Consequently, many chemical characteristics of REEs conform to that of Ca. REEs locate at the same binding sites in organisms as Ca, and thus show a similar effect with that of Ca (Hu et al., 2004). The effects of REEs on physiological functions of Ca in plants have been summarized by Brown et al., (1990). They have concluded that:

- REEs are thought to be analogous to Ca, especially to La, which therefore was nicknamed "super-calcium."
- Many enzymes and other functional-proteins have been demonstrated to be inhibited by La^{3+}
- La^{3+} can displace Ca^{2+} from extra-cellular binding sites and can inhibit the efflux of extra-cellular, and part of the intracellular Ca^{2+}.

Much of the justification for using Ln^{3+} ions in biochemical investigations turns on their ability to replace Ca^{2+} in a specific, isomorphous manner. Table 2.2-4 compares the properties of Ca^{2+} and Ln^{3+} which are of greatest biochemical relevance. In their sizes, bonding, coordination geometry, and donor atom preference, these ions are remarkably similar. It is these similarities which permit Ln^{3+} ions to replace Ca^{2+} so specifically. Nature has tailored many Ca^{2+}-binding sites to exclude competing metal

ions, especially Mg^{2+}. This is particularly important intracellular, where certain proteins can selectively bind Ca^{2+} which is present at submicromolar concentrations, while immersed in fluids containing millimolar concentrations of Mg^{2+}. Research with tRNA has confirmed that Ln^{3+} ions can occupy Mg^{2+} sites in a specific manner. This is also true for certain Mg^{2+}-requiring enzymes. There are also instances of the replacement of Fe^{3+}, Fe^{2+}, and Mn^{2+} by Ln^{3+} ions. The lanthanides also bear certain chemical similarities to the actinides. For this reason, they are finding use as nutritional markers in studies of actinide metabolism (Evans, 1990).

Table 2.2-4: Salient properties of Ca^{2+} and Ln^{3+}

Property	Ca^{2+}	Ln^{3+}
Coordination number	6-12 reported 6 or 7 favored	6-12 reported 8 or 9 favored
Coordination geometry	Highly flexible	Highly flexible
Donor atom preference	0 >> N >> S	0 >> N >> S
Ionic radius (Å)	1.00- 1.18 (CN 6-9)	0.86-1.22 (CN 6-9), depending on species
Type of bonding	Ionic	Ionic
Hydration number	6	8 or 9
Water exchange rate constant (s^{-1})	~5 X 10^8	~5 x 10^7
Diffusion coefficient (cm^2/S X 10^5)	1.34	La $^{3+}$, 1.30
Crystal-field stabilization	None	Negligible

More than one author has commented that Ca^{2+} sites cannot be designed to exclude Ln^{3+} ions. However, although there are indeed numerous examples of the specific replacement of Ca^{2+} by Ln^{3+} ions, exceptions exist. There are several biochemical examples of Ca^{2+}-binding sites that do not accept Ln^{3+} ions, and vice versa: Ln^{3+} ions cannot replace Ca^{2+} in scallop myosin, for example, and are excluded from the Ca^{2+} site on concanavalin A. Furthermore, high concentrations of Ca^{2+} cannot displace Gd^{3+} from IgG or Tb^{3+} from ferritin. Both the acetylcholine and insulin receptors have two classes of Tb^{3+}-binding site, only one of which accepts

Ca^{2+}. If a Ca^{2+}-binding site is spatially restricted so as to exclude larger ions, and is surrounded by a hydrophobic environment, Ln^{3+} ions will be excluded. The basis for this conclusion is the observation that when the local dielectric constant falls below that of water, Ln^{3+} ions, but not Ca^{2+} ions, bind nonspecifically to counterions. Under these conditions, the Ln^{3+}-counterion adduct would be too large to occupy the Ca^{2+} site. It is not clear whether this phenomenon exists for biological ligands. If such sites do exist biochemically, extracellular proteins may provide the most productive hunting ground for examples of this kind (Evans, 1990). X-ray intensity data to 2.8 A° resolution were collected from each of three crystals of turkey skeletal troponin C (TnC) that had been soaked individually in solutions containing the lanthanide ions, europium, thulium and lutetium. Each of the resulting difference electron density maps computed for the three data sets showed three lanthanide ion-binding sites on the TnC molecule. Two of the sites were approximately coincident with the Ca^{2+} positions in binding loops III and IV and the third site was near the Ca^{2+} free loop I. the mode of ion binding in loop I was different from that commonly observed for Ca^{2+} in the Ca^{2+} binding proteins (Herzberg and James, 1986).

As the larger Ln^{3+} ions are closer in ionic radius to the Ca^{2+} ions they replace, they are better able to interfere with Ca^{2+}-dependent functions. In other molecules, size specificity appears to have been carried to an extreme degree, with a peak of activity appearing within the lanthanide series at the ionic radius closest to that of Ca^{2+}. Under spatially restricted conditions, large ions would be sterically excluded while small ions would not be able to coordinate to all ligands in the binding site. Thus, maximum binding would occur at an optimum ionic radius. With Ca^{2+}-specific binding sites, this would be the Ln^{3+} ion whose radius is closest to that of Ca^{2+} (Table 2.2-5).

Table 2.2-5: The Lanthanides whose Ionic radii equal or are closest to that of Calcium at different coordination numbers[a]

Coordination number of Calcium	Coordination number of lanthanide			
	6	7	8	9
6	Ce-Pr	Sm-Eu	Er	<Lu[b]
7	>La[c]	Ce-Pr	Eu-Gd	Er
8	>La	>La	Pr-Nd	Eu
9	>La	>La	>La	Pr

[a] Based upon the ionic radii of Shannon (1976).

[b] <Lu: Ca^{2+} smaller than all Ln^{3+} at these coordination numbers.

[c] > La: Ca^{2+} bigger than all Ln^{3+} at these

The major difference between Ca^{2+} and Ln^{3+} complexes is the greater stability of the latter. This is to be expected from the higher charge-to volume ratios of the Ln^{3+} ions and their greater coordination numbers (Evans, 1990).

La^{3+} shows a similar function as Ca^{2+}, with respect to ion uptake and translocation in plants. Both La and Ca inhibit K uptake in plants treated by them for a short time. However, they accelerate K^+ uptake in plants with increasing time of treatment. This indicates that both, La^{3+} and Ca^{2+}, operate at the same sites. Lanthanum combined with binding sites of Ca^{2+} in the outer cell membranes impact translocation of Ca^{2+}. Therefore, La^{3+} is considered an obstructer for Ca^{2+} ion channels (Hu et al., 2004).

2.2.3.3. The interaction of Lanthanides with Amino acids and Proteins

The functional properties of the protein upon substitution by a lanthanide should be retained if the role of Ca^{2+} is purely structural, yet be lost if Ca^{2+} has an important functional role. In many investigations it is assumed that Ln^{3+} ions replace Ca^{2+} isomorphously. This assumption is supported by X-ray diffraction studies of thermolysin, parvalbumin, and troponin C, showing that Ln^{3+} ions can indeed occupy certain Ca^{2+} -binding sites in a nondisruptive manner. Minor differences do exist between the Ca^{2+}- and Ln^{3+}-containing forms, but, overall, a high degree of isomorphism is conserved. Further evidence that Ln^{3+} ions specifically replace Ca^{2+} at precise locations has come from binding studies which demonstrate the competitive

nature of the substitution. Often the conformational changes in the protein produced by metal ion binding are the same for Ca^{2+} and Ln^{3+} ions. Functional assays also support the claim of specificity in the attachment of Ln^{3+} ions to proteins. For example, enzymic activity is sometimes retained following the substitution of Ca^{2+} by Ln^{3+} ions. In addition, in cases where the substitution inhibits enzymic activity, kinetic analyses often show that inhibition by lanthanides is competitive with respect to Ca^{2+} (Evans, 1990).

Lanthanides coordinate with the unprotonated carboxyl groups of amino acids. Weak interaction with the hydroxyl oxygen of serine, and presumably also threonine and tyrosine, can occur too. Whether or not lanthanides are also capable of coordinating with unprotonated amino groups remains an unresolved controversy. The problem is complicated, as the pH values at which coordination to the α-amino nitrogen atom becomes a reasonable proposition are also those at which Ln^{3+} hydrolysis occurs. Both the amino and carboxyl components of amino acids contribute to complexing Ln^{3+} ions and uses this as an explanation of why amino acids form stronger complexes than monocarboxylic acids with Ln^{3+} (Evans, 1990). Evidence that the N-terminal amino groups of peptides also coordinate Ln^{3+} ions comes from NMR studies of the interaction of Gd^{3+} with various small glycopeptides at pH 6-7. However values for the deprotonation of α-amino nitrogen ensure that hydrolysis of Ln^{3+} ions occurs before uncharged N donors are generated. Nevertheless, interaction between the acetylated N-terminus of a peptide and Ln^{3+} ions has been detected. Some binding values are given in Table 2.2-6.

Angiotensin II is an octapeptide with the sequence Asp-Arg-Val-Tyr-Ile-His-Pro-Phe. Its association with Ca^{2+} and Tb^{3+} has been monitored by measuring the changes in its Tyr fluorescence and Tb^{3+} luminescence. The pentapeptide Arg-Lys-Asp-Val-Tyr is a biologically active fragment of the thymic hormone thymopoietin. Tb^{3+} binds to this fragment, undergoing luminescence enhancement upon irradiation at 270nm Due to energy transfer from the C-terminal Tyr residue (Evans, 1990).

Table 2.2-6: Binding data for certain complexes between Lanthanides and Amino acids

Amino acid	Binding parameter	Conditions	Lanthanide^{3+} La^{3+}	Ce^{3+}	PO^{3+}	Nd^{3+}	Pm^{3+}	Eu^{3+}	Tb^{3+}	Reference
Glycine	Log stability constant	PH 3.64, 25°C, 2 *M* NaC104	-	3.4	-	-	4.7	5.0	-	Tanner and Choppin, 1968
Glycine	Log stability Constant	*0. 1 M KCI,* 30°C	3.2	3.4	3.6	3.7	-	-	-	Moeller el al., 1965
Aspartate	Log K_1, $[Ln\text{-}Asp]^+$	0. *1 M* KCI, 25°C	5.0	5.2	5.4	5.5	-	-	-	Moeller et al 1965
	Log K_2, $[Ln(ASP)_2]^-$	0. 1 M KCI, 25°C	4.2	4.6	4.8	4.9	-	-	-	Sinha, 1966
γ-Carboxyglutamate	K_d	PH 6.5	-	-	-	-	-	-	~50 *IAM*	Sperling et at.,1978
Alanine	Stability constant	PH 3	All Ln^{3+} approx. 0.7 ± *0.1 M*							Sherry and Pascnol. 1977
	Stability constant	PH 4	-	-	-	6.5	-	-	-	Sherry et al., 1973
Histidine	Stability constant	PH 4	-	-	-	1.8	-	-	-	Sherry et al., 1973
	Stability constant	PH 7	-	-	-	123.0	-	-	-	Sherry et al., 1973
Threonine	Stability constant	PH 4	-	-	-	7.6	-	-	-	Sherry et al., 1973
Serine	Stability constant	PH 4	-	-	-	12.6	-	-	-	Sherry et al., 1973

Small peptides containing certain amino acids found at the Ca^{2+}-binding sites of proteins have been synthesized and studied. In this way, N-acetyl-Asp and N-acetyl-Asp-Gly-aspartylamide, which are associated with the EF Ca^{2+}-binding site of parvalbumin, are examined. Acetyl-Asp-Val-Asp-Ala has been shown to bind Pr^{3+} via both carboxyls of each Asp, in a bidentate fashion. La^{3+} interactions with synthetic peptide analogs of the Ca^{2+}-binding site III of troponin C have also been studied (Evans, 1990).

REEs can replace Ca from many enzymes, and participate in enzymatic reactions, and thereby interfere with the normal physiological functions of Ca (Hu et al., 2004). α-amylase [α (1→ 4) -glucan 4-glucanohydrolase] degrades α 1→4 linkage in starch, yielding a mixture of glucose and maltose. α-amylase binds four to five ions of Ca^{2+}; this means it has an absolute requirement for Ca^{2+}. Nd^{3+} inhibits α-amylase and is able partially to reactivate the Ca^{2+}-free apoenzyme. It seems likely that Nd3+ replaces Ca^{2+} in the holoenzyme, forming a substituted species with lower enzymic activity. The ability of Ln^{3+} to reactivate Ca^{2+}-free α-amylase is inversely proportional

to ionic radius, with Lu^{3+}, the smallest Ln^{3-} ion, restoring full enzymic activity. This enzyme undergoes no major conformational change in the presence or absence of Ca^{2+} or Ln^{3+} ions (Evans, 1990). Wang et al., (2000) showed that Ce^{3+} at the high concentration displaces Ca^{2+} from α-amylase competitively. The equilibrium dialysis showed that α-amylase have five Ca^{2+} binding sites with different affinities and the fluorescence titration showed that Ce^{3+} can bind to Ca^{2+} binding sites.

The influences of mono-, bi- and trivalent metal ions on the activity of dihydrofolate reductase (DHFR) from chicken liver have been studied by Wu (2000) to elucidate the mechanism of ion-activation of this enzyme. The results show that monovalent ions (Na^+ and K^+) activate DHFR at low concentration. Ca^{2+} shows similar activation but at lower concentration. The trivalent lanthanide ions show a dramatic inhibition of activity of DHFR even at very low concentration. The activity of DHFR declines to 50% of that of the control at 0.02 mM $EuCl_3$. The ion-dependent activation in the presence of mono- and bivalent metal ions is due to conformational changes in the protein. Energy transfer phenomenon suggests that the specific interaction of Eu^{3+} with Trp24 located in a loop at the active site of DHFR is responsible for the strong inhibition. Ghosh et al., (1991) found that acute single dose administration of two rare earth elements like lanthanum chloride (250 mg/kg body weight) and neodymium chloride (200 mg/kg body weight) to chicks reduce the activity of certain erythrocyte membrane bound enzymes like acetylcholinesterase, NADH dehydrogenase, Mg^{2+}-ATPase, p-nitrophenyl phosphatase. Erythrocyte membrane bound glycosidase, beta-D-glucosidase, beta-D-galactosidase and beta-D-glucuronidase were also reduced. Other components such as cholesterol and phospholipids residues were reduced but their ratio remaining unchanged. Membrane sulfhydryl groups were also significantly inhibited by these rare earth elements.

Cells need to regulate very closely their cytosolic Ca^{2+} concentrations, usually maintaining them at submicromolar levels. This is achieved despite the presence of an extracellular milieu where the Ca^{2+} concentration is often in the millimolar range. Such regulation is aided by specialized Ca^{2+} "pumps" which harness the energy released by hydrolysis of the terminal phosphodiester bond of ATP to transport Ca^{2+} across cellular membranes. These enzymes hence show ATPase activity; as they require both Ca^{2+} and Mg^{2+}, they are referred to as Ca^{2+}/Mg^{2+}-ATPases. Four distinct steps within the overall ATPase, Ca^{2+}-transporting reaction have been identified, as shown in Figure 2:

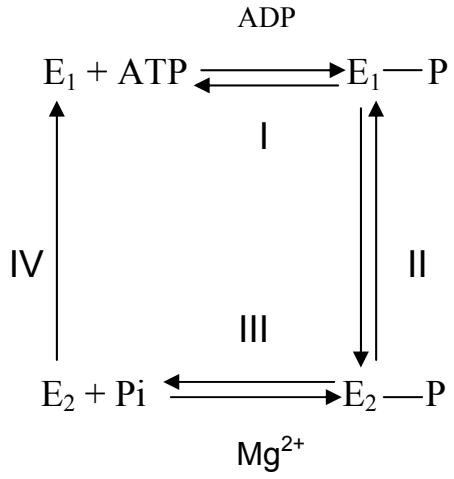


Figure 2: Four steps of ATPase, Ca^{2+} reaction

The first reaction is the ATPase step, which produces ADP and a phosphorylated form of the enzyme (E_1 — P). This reaction can be reversed by adding ADP. During the second reaction, the E_1—P intermediate undergoes a conformational shift to form an altered intermediate designated E_2— P, which cannot be dephosphorylated by ADP. Phosphate is cleaved from E_2 during the Mg^{2+}-requiring third reaction, and the original form of the enzyme (E_1) is subsequently regenerated. Only the last reaction is irreversible. Lanthanides inhibit the overall reaction. It is reported that Ln^{3+} ion compete with Ca^{2+} for the Ca^{2+}-binding (transporter) sites on the enzyme, forming a Ln^{3+}-substituted derivative which undergoes reaction I to form the phosphorylated enzyme intermediate but which fails dephosphorylate. Although confirming that Gd^{3+} and Tb^{3+} are able to compete with Ca^{2+} for the transporter site, inhibition of ATPase activity occurred at Ln^{3+} concentrations well below those needed to displace Ca^{2+} (Evans, 1990).

The insulin-like growth factor binding proteins (IGFBPs) modulate IGF actions because of their ability to bind IGF-I and IGF-II. IGFBPs have been reported to enhance and to inhibit IGF actions. Generally the soluble high affinity IGFBPs are inhibitory and the cell-associated lower affinity forms enhance activity. Several studies support the idea that there is a delicate balance between the IGF inhibiting activity and IGF potentiating activity. Slight changes in culture conditions can result in a change in this equilibrium. These changes in some cases probably reflect differences in levels of IGFBP at the cell surface. Therefore it is important, when

measuring IGF activity, to quantify the amount of IGFBPs present in extracellular fluids and on the cell surface. With the use of La^{3+}, IGFBP release from cell surfaces was limited and the number and affinity of binding sites on the cell surface can be accurately quantified. Type I IGF receptor binding can be assessed with insulin displacement and IGFBP binding with IGF-I displacement beyond that of insulin. There are numerous other reports of insulin competing for all of the [^{125}I] -IGF-I binding sites. Binding affinity cannot be used to distinguish binding between the receptor and IGFBPs. La^{3+} increases [^{125}I] -IGF-I binding to the type I IGF receptor. This is not a direct effect on either receptor affinity or number. Instead, by preventing the release of IGFBPs and their strong IGF binding activity, more [^{125}I] -IGF-I is available for receptor binding (McCusker and Clemmons, 1997).

2.2.4. Rare Earth elements as growth promoters

In China REE are used for 40 years as performance enhancers in agricultural plant production and remarkable results were reported from Chinese agricultural operations (wan et al., 1998). The Chinese data were confirmed in different other countries as in Australia (Diatloff et al., 1995) and the United Kingdom (Andrew et al., 1983). REE supplemented fertilizer may increase productivity by up to 15 % (Hu et al., 2004). In animal production, as with plants, amazing results have been reported by supplying REE in animal diets in Chinese literature as well as in Europe (Rambeck et al., 1999a), (He et al., 1999, 2001, 2003a, 2006a, 2006b). Schuller (2001) pointed out that most studies in the Chinese literature are hardly comparable, as differing REE compounds and samples with varying purity were often used. The performance enhancing effect of REE has been described in the Chinese literature for long time but it was never tested under “western conditions”. Rambeck et al., (1999a) for the first time tested the growth promoting effect of rare earth elements in animal under western conditions.

Rare earth elements (REE) may have important values for animal production as well as for high technology industries and clinical application. It was reported that proper concentrations of REE in diet can improve animal growth performance without affecting quality of products. However the effect is not stable and may be affected by

concentration and type of applied REE (He et al., 2001). The following results show the effect of rare earth elements in different kinds of animals.

2.2.4.1. Lab animals

He et al., (2006b) studied the effect of dietary supplementation of $LaCl_3$ and REE mixture with concentrations (75 mg/kg and 150 mg/kg) on growth performance of rats. The result showed that supplementation of REE improved the body weight by 4 to 9% and feed conversion ratio by 3 to 11 percent compare to those of control ($p<0.05$). Also the feed intake in rats fed diets supplemented with REE was 3-5% less than that in control group. He concluded that oral administration of REE has positive effect on the growth performance and blood serum biochemical parameters of rat. This result suggested that concentration and type of REE in the animal diet are two important factors. Xu et al., (2004) showed that La^{3+} could increase secretion of gastric acid of isolated mice stomach. The mechanism might involve in release of gastrin by stimulating G cell, release of histamine of ECL (Enterochromaffin-like) cell, or activation of gastrin receptor and histamine H_2 receptor of parietal cell which augments secretion function of parietal cell of stomach. In a research carried out by Liu et al., (2004), rabbits were fed with hyperlipids for two months. Results showed that hyperlipids induced obviously lipids deposition (which is the characteristic of early atherosclerosis), decreased bone density, reduced bone trabecula, increased surficial lacuna and accelerated lose of minerals and organic matter at a ratio of normal bone. Administration of $LaCl_3$ lessened pathological change of arteriosclerosis, to some extent, reversed the above bone alteration. These results showed that lanthanum plays an important role in inhibiting early lipids deposition, cell damage and improving bone structure.

Lu et al., (2003) studied the effect of rare earth elements on the activities of seven enzymes (ALT, ICD, AST, LAP, ALP, γ-GT, CHE) in rat liver. The results indicated that Hormesis effect on most enzymes occurred and Hormesis dose-response range was dependent on the type and character of the enzyme. Zhong et al., (2003) studied the effect of lanthanum chloride expressions of type I, II, III and IV collagen proteins and possibility to prevent and treat scar by injecting 50 mmol/l lanthanum chloride to adult female rats. The result showed the expressions of type I, III and IV collagen proteins reduced significantly ($p<0.05$). They concluded that lanthanum chloride can

certainly prevent and treat scar by inhibiting expressions of collagen proteins. Wang et al., (2003) found that the apoptosis of thymocytes in mice induced by LPS was decreased by lanthanum chloride (10 mg/kg). Li et al., (2002) studied the dynamic distribution patterns of rare earth elements Sm and Yb in Wistar rats using enriched activable isotope tracer technique. Intravenously injected ^{152}Sm and ^{168}Yb in chloride form were distributed in almost all rat organs and tissues studied, including liver, skeleton, kidney, spleen, heart, lung and testicles. Liver and skeleton were the main deposition sites for REEs, only small to moderate amounts of Sm and Yb were found in other organs. Marciniak et al., (1996) examined the transport of lanthanides in milk of contamined rats into their suckling and the retention of lanthanides in the suckling. This study showed that in the period of lactation the transport in milk from the mother to the offspring of the lanthanides under examination (^{144}Ce, ^{147}Nd, ^{152}Sm, ^{155}Eu and ^{160}Tb) increased with their mass numbers: Ce<Nd<Sm<Eu<Tb, and varied from 0.01% for ^{144}Ce to 17.7% for ^{160}Tb of the administered dose per litter. It was demonstrated that lanthanides were not absorbed from the digestive tract of suckling because they were not detected beyond its area. Terbium-160 accumulation in suckling increased whereas its elimination decreased with the age of infant.

He et al., (2006c) showed that supplementation of $LaCl_3$, $CeCl_3$ or mixture of lanthanum chlorides to the media significantly stimulated proliferation of 3T3-L1 cells. The supplementation of REE also decreased the concentration and composition of monosaturated fatty acids in the differentiating adipocytes. These results indicated that REE may affect adipogenesis and lipogenesis rates of 3T3-L1 cells. But the effects may depend on the dose or type of REE. In another study He et al., (2006d) showed that the inhibitory effects of conjugated linoleic acids (CLAs) on the differentiation and fatty acids accumulation of 3T3-L1 cells are depended upon the isomer type, treatment period and dose. When they applied in the early differentiation period a high dose of CLA c9, t11 and C18:3 as well as CLA t10, c12 could also depress the differentiation and fatty acids accumulation in 3T3-L1 adipocytes.

2.2.4.2. Fish

Tang et al., (1997) studied the effect of rare earth element-amino acid compounds with different concentrations (300 and 400 mg/kg) on carp and rainbow trout. Both concentrations of REE supplement improved body weight gain in carp and rainbow

trout but the diet supplemented with 400 mg/kg of REE had better effect. Shao et al., (1998) used different concentrations of REE solutions (1, 5, 10, 50, 500 and 1000 mg/kg) to study the effect of REE on egg embryo development in carp. The result suggested that some levels of REE (less than 100 mg/kg) had significant positive effect on embryo development of carp while high content of REE inhibited embryo development. Tang et al., (1998) studied the effect of REE-AA (consisting of 50% REE-Met and 50% REE-Lys), REE-Vitamin C, REE-citrate, REE-Glu and REE-gluconate on growth performance of carp fries. After 60 days supplementation of different REE compounds improved weight gain by 28.9%, 27.2%, 24.1%, 23% and 20.1% respectively compare to the same control group. Also they indicated that among the REE compounds, REE-AA was the best. In a research carried out by Shao et al., (1999), crossbred carp fries fish and adult carp fish fed with diets supplemented with different levels of REE mixture ranging from 100 mg/kg to 1500 mg/kg and the enzyme activities in liver, pancreas and blood were studied. The result showed REE supplement improved the activities of proteinase, lipase and amylase in liver and pancreas of fries. The highest activities were seen in groups fed 100, 200 or 300 mg /kg of REE supplement. Also the activities of catalase in liver and pancreas of fries and blood of adult fish were increased by REE supplement. Wang et al., (1999) found about bioaccumulation of two kinds of REE (Gadolinium and Yttrium) in different internal organs of *Carassius auratus* (goldfish) and effect of those REE on activities of liver enzymes. The result indicated that bioaccumulation order of Yttrium (Y) in internal organs of fish was liver> gallbladder> kidney> eggs> spleen and the order for Gadolinium (Gd) was eggs> liver> gallbladder> spleen> kidney. Also the result showed both types of REE had inhabitation effect on the activities of catalase and superoxide dismutase in livers of fish. Yang and Chen (2000, 2002) found the positive effect of rare earth elements ions on hatching the eggs of fish and shrimp. They showed that La^{3+} at the concentration range of 1.2-4.8 mg/l increased egg hatching rate of river shrimp by 22.8-27.7% and Pr^{3+} at the concentration range of 2.4-4.8 mg/l increased egg hatching rate of grass carp by 18.5-27.5%. They indicated that egg hatching was promoted by the absorbed ions. Yang et al., (2001) also found that RE-citrate at the concentration range of 1.2-4.8 mg/l improved egg hatching rate of river shrimp (*Macrobrachium nipponense*) by 10.6%-26.2% but with concentration of more than 9.6 mg/l significantly decreased hatching rate.

2.2.4.3. Nonruminant animals

2.2.4.3.1. Pigs

Chen and Xiong (1994) studied the effect of organic REE (RCT-3) on pigs at different ages. The results showed that: 1) supplementation of 200 mg/kg RCT-3 for 123 days significantly ($p<0.01$) improved weight gain of finishing pigs by 24.1% and decreased feed consumption by 16.4%. 2) Supplementation of RCT-3 to 20 days old suckling pigs for 40 days significantly improved weight gain by 17.9% and feed conversion ratio by 13.9% and 3) supplementation of RCT-3 to weanling piglets for 21 days improved weight gain by 25% and feed conversion ratio by 17%. Ming et al., (1995) used a certain amount of rare earth complex (STV-2, CL-1, and CL-3) in growing pig diet. The result showed that rare earth complex improved daily gain and feed conversion ratio by 11.3% and 6.8% respectively compare to those of control group. Hemoglobin, urea nitrogen, cholesterol and blood sugar contents, GPT and GOT activities and liver function in treatment groups were normal. The rare earth residual was more in bone, lung and stomach respectively but it was very small in muscles. They concluded that rare earth complex was not harmful to pig and man and it can be used as growth promoter in pig production. Fan et al., (1997) showed that applying organic REE additives with concentrations of 100, 150 and 200 mg/kg in 40-50 days old pigs, significantly improved daily body weight gain ($p<0.05$, $p<0.01$).

In a study by Hu et al., (1999) weaning pigs were allotted to four experimental groups including a control group and three REE groups. The REE treatments were 200, 400 or 600 mg/kg of a REE mixture containing oxides of La, Ce, Pr, Nd, Sm, Eu and Gd. The result showed that supplementation of 400 and 600 mg/kg of REE mixture significantly improved daily body weight gain, feed conversion ratio, apparent digestibility of energy and protein, digestibility of total amino acids and total essential and non-essential amino acids ($p<0.05$, $p<0.01$). Xu et al., (1999) studied the effect of supplemented diet with 100 mg/kg Lanthanum on growing pigs for 30 days. The result showed that ADG and ADFI of growing pigs were increased by 13.3% ($p<0.05$) and 5.4% ($p<0.05$) but feed gain ratio was decreased by 8.5% ($p<0.05$). They showed that the peak amplitude level and mean level of growth hormone in serum were elevated by 103.4%, 88.9% and 90.9% respectively ($p<0.05$). They also found

that the levels of serum thyroid hormones (T3 and T4) were increased by 36.7% and 28.9% in growing pigs fed Lanthanum. In an experiment carried out by He and Xia (2000), the Duroc×Landrace×Large pigs were fed with 75 mg/kg of REE oxide during starting, growing and finishing periods. The result showed that daily body weight gain (BWG) of starters, growers and finishers were improved by 22.9%, 20.3% and 13% respectively compare to control group of each period. Ou et al., (2000) used a kind of REE additive which was a liquid product of one REE company in Guangxi. They fed 20 days old piglets with this REE additive for 40 days. The results suggested that supplementation of this product in feed improved daily body weight gain (BWG) about 7-8.5% and feed efficiency about 16.8-18.4%. There was a significant difference on feed efficiency of 20-60 days between control group and REE supplemented group. Wang et al., (2000, 2002) showed that the activity of α-amylase from porcine pancreas was enhanced under the treatment by Ce^{3+} of the low concentration (0.5 to 10 μmol/l), but was inhibited by Ce^{3+} of the high concentration (> 10 μmol/l). They indicated that Ce^{3+} at the high concentration displaces Ca^{2+} from α-amylase competitively.

Rambeck et al., (1999a, 1999b) studied the effect of rare earth elements under western conditions on piglets. In this study 72 crossbred piglets (Deutsche Landrasse × Pietrain) with average body weight of 7.3 kg were allotted to five dietary treatment groups which include a control group, two REE groups with low (75 mg/kg) and high (150 mg/kg) content of lanthanum chloride ($LaCl_3$) and two REE groups with low (75 mg/kg) and high (150 mg/kg) content of an REE mixture containing mainly chlorides of lanthanum (La), cerium (Ce) and praseodymium (Pr). The experimental period lasted five weeks. The results showed that the weight gain of piglets in different REE groups increased by 2 to 5% and the feed conversion ratio in those groups improved by up to 7 percent. They also showed the content of lanthanum and cerium in liver, muscle and kidney were very low even when they were calculated in dry matter. In other study carried out by He and Rambeck (2000), 14 crossbred piglets (Deutsche Landrasse × Pietrain) with average body weight of 17 kg were allotted to two dietary treatments including control group and a group with high content (150 mg/kg) of REE mixture containing mainly chlorides of lanthanum (La), cerium (Ce) and praseodymium (Pr). The experimental period lasted eight weeks. The results showed that REE treatment significantly ($p<0.01$) improved both body weight gain and feed conversion ratio compare to control group by 19% and 10% respectively. In the first

two weeks of experiment, REE supplement significantly ($p<0.01$) improved daily weight gain by 25% and feed conversion ratio by 21% compare to control group. Rambeck et al., (2004) repeated the last experiment on 14 piglets of the same crossbred for 12 weeks and determined serum parameters and meat quality as well as REE content in muscle, kidney and liver. In the last 6 weeks the daily weight gain and feed conversion ratio significantly increased by 18% and 9% respectively. The results from slaughtering and meat quality control showed that according to the class of quality "EUROP" all animals were graded E or U (the two best classes) and the animals in highest grade (E) were more from REE group than control group. The REE contents in the samples of muscle, liver and kidneys were very low. Although the content of La in the REE group was higher than those in the control group, generally the accumulation rates in all the experimental pigs were very low and close to the limit of detection. Schuller et al., (2002) showed that in pigs receiving feed supplemented with 150 to 300 mg/kg REE, an increase in daily weight gain of up to 19% and an improvement in feed conversion of up to 11% can be achieved. In a study carried out by He et al., (2001), fourteen crossbred piglets (Deutsche Landrasse × Pietrain) were allotted to two dietary treatments: a control group without REE and the REE-treated group which was supplemented with 300 mg/kg of an REE mixture. The REE mixture contained mainly chlorides of lanthanum (La), cerium (Ce) and praseodymium (Pr). The feeding period consisted of a 2 months *ad libitum* feeding period (M-I) and a one month restricted feeding period (M-II). The result showed that REE supplement increased daily weight gain by 19% ($p<0.05$) in the period M-I and 12% in the period M-II compare to control group. Also the REE supplement improved feed conversion ratio compare to control group by 11% and 3% in periods M-I and M-II respectively. They showed that REE had no significant effect on blood serum thyroxine (T_4), aspartate-amino-transferase (AST), Alanine-amino-transferase (ALT), alkaline-phosphatase (AP), total cholesterol, triglyceride, total protein, albumin, and glucose, Ca, P, Na, K and Cl. However, serum triiodothryonine (T_3) in REE group was significantly ($p<0.01$) lower than that in control group. The accumulation rate of La and Ce in the muscle, liver and kidneys was very low after feeding the REE diet for 3 months. Halle et al., (2003a) showed that various REE supplements had no significant effect on nutrient digestibility in pigs. But crude fiber showed tendency towards higher values when REE-ascorbate was supplemented. In other study carried by Liu et al., (2003), growing pigs were fed with different amino

acid-metal coordination compound additives. The result suggested that all amino-metal coordination compound additives have well-promoting effects on improving the quality and chemical components of pork, with more obvious effect of amino acid-microelements-rare earth element coordination compound additives. Wang and Xu (2003) studied the effect of supplemental Lanthanum (La) on growth performance of pigs. In this study crossbred growing pigs were fed with basal diet supplemented with 0 or 100 mg/kg of Lanthanum for 30 days. The results showed that ADG and FCR of growing pigs were significantly ($p<0.05$) improved by 13.06% and 6.63% respectively with supplementation of lanthanum. Blood sample analysis showed that lanthanum supplement significantly ($p<0.05$) elevated peak amplitude, base-line level and mean level of growth hormone (GH) in serum by 103.35%, 89.88% and 90.91% respectively.

In a study carried out by Prause et al., (2005b and 2005c), 40 piglets with the average weight of 8.5 kg were assigned to three treatments including a control group with 100 mg citrate and two REE groups with 150 mg and 300 mg of REE-citrate (VG1 and VG2). The result showed that daily body weight gain was affected by REE supplement, like seen in a few studies (Böhme et al., 2002). But this was in opposition of result of Knebel (2004) and Kessler (2004), for example. The low dose of REE-citrate (VG1) significantly ($p<0.01$) reduced feed conversion rate almost 8% because daily feed intake was 9% lower than control group. The high dose of REE-citrate (VG2) reduced feed intake by 5% and feed conversion ratio by 2% compare to control group. During both periods of respiration digestibility of energy (BE), nitrogen (N) and carbon (C) was more efficient in pigs fed low dose of REE (VG1) than control group but the digestibility and nutrient retention in pigs fed high dose of REE (VG2) was not so different than control group. The result showed analyzed blood parameters (Urea, Creatinin, AP, Triglycerides, Cholesterol, Ca^{2+}, P^{-}, Total protein and Albumin) were not significantly affected by REE. But calcium (Ca) to phosphorus (P) ratio in ashes of metatarsus decreased in favor of phosphorus compare to control group (Ca/P: control=2.79, VG1=2.72, VG2=2.65). Also magnesium (Mg) ash content in control group was significantly ($p<0.05$, $p<0.001$) reduced compare to low dose REE group and high dose REE group (ash mg content: control=6mg/kg, VG1=5.2mg/kg, VG2=4.6mg/kg). Förster et al., (2006) used 100, 200, 400 and 800 mg/kg of citrate-bound rare earth elements in a study on 21 days' old weaned piglets. They found only low concentrations of REE increased weight gain and higher

concentrations of REE had negative effect on animal growth performance. Also they showed that thyroid hormone levels (T3 and T4) were increased by REE supplementation.

2.2.4.3.2. Poultry

The effect of diets supplemented with different levels of REE (200, 400, 600 and 800 mg/kg) on 6 month old laying hens was studied by Wu et al., (1994). The result showed that the diet supplemented with 600 mg/kg of REE significantly ($p<0.05$) increased egg production, egg weight and fertilization rate of hatching eggs. Supplementation 1000-1500 mg/kg of REE in diets of black-bone silky fowl improved weight gain by 1-9% and feed conversion ratio by 9-11%. The optimum level of REE was 1500 mg/kg according to weight gain and 1000 mg/kg according to the other index (Fang et al., 1994). A research by Zhou (1994) carried out on ducks showed that supplemented diet with 180 mg/kg of REE improved weight gain of meat-type ducks by 16-25% and advanced sex maturity by 5-7 days. This research also showed that supplemented diets with 60 mg/kg of REE improved total egg production of laying ducks by 12-15% and prolonged the peak of egg production. In a study carried out by Zhang and Shao (1995), broiler chicks at 10 days of age fed with four experimental diets. The diets included a control diet and three REE diets containing 300, 400 and 500 mg/kg of REE nitrate compound (mainly containing oxides of lanthanum, cerium and neodymium). The result showed the positive effect of REE supplement on body weight gain ($p<0.01$) and the optimum level of REE was 300 mg/kg. Zhang et al., (1996) studied the effect of supplemented diets with 300, 400 and 500 mg/kg of REE-nitrate on 53 weeks old laying hens. The result showed that the diets with 300 and 400 mg/kg of REE significantly improved laying rate ($p<0.01$, $p<0.05$) and the diets with 400 and 500 mg/kg of REE significantly improved individual egg weight ($p<0.05$). Gong et al., (1996) showed that supplementation of 100 mg/kg REE in diets of broiler-type breeding bird improved laying rate by 4.7% and decreased damaged egg rate by 1.5%. The REE supplement also improved fertilization rate, hatching rate and healthy chicken rate. Duan et al., (1998) used 100 mg/kg of REE supplement in diets of broiler type breeding birds during growing and egg-laying period. They showed that REE supplement improved survival rate and laying rate and decreased individual feed intake.

Xie and Wang (1998) applied 1%, 2% and 3% of organic rare earth compounds (PREC) in the diet of one week old Liang Feng Hua chickens. The result showed that average daily gain of chicken fed 1% and 2% of REE compound was significantly ($p<0.01$) increased compare to control group. The result of blood analysis showed the activities of several hormones and enzymes such as GH, T_3, Lipase, and SOD and peroxidase trend to increase. Also they indicated that there was no contamination of rare earth elements in muscles and liver and the meat quality was improved by rare earth element compound. Schuller (2001) studied the effect of rare earth elements on broiler chicks and Japanese quails with feeding experiments in which the broiler experiment was designed to gather fattening data, whilst in the quails experiment both fattening and laying data were studied. The animals were fed a normal commercial feed which was supplemented either with highly purified lanthanum chloride or a mixture of different rare earth elements containing mainly chlorides of lanthanum (La), cerium (Ce) and praseodymium (Pr) at dosages of 0, 75, 150 and 300 mg/kg feed. No positive effects of REE supplements on growth or productivity in either the broilers or the quails could be shown by these experiments. There was even some evidence of possible negative effects on growth and feed conversion in broilers supplemented with 300 mg/kg of REE mixture. The measurement of the REE content of the inner organs showed a mild accumulation of lanthanum, predominantly in the liver and bones. An analysis of the intestinal microflora did not show any direct effects of the supplementation of the feed with REE on the quantitative or qualitative composition of the microorganic populations in the gut.

Halle et al., (2002, 2003a, 2003b and 2004) studied the effect of supplemented diets with 100 mg/kg of REE-citrate, REE-nitrate, REE-ascorbate and Lanthanum chloride on day-old broiler chicks. They found clear growth promoting effect of REE-ascorbate and REE-citrate. These two REE supplement significantly ($p<0.05$) improved body weight by 5-7% and feed conversion ratio by 1-3% in broiler chicks at 35 days of age. The REE supplements had no effect on carcass quality of broilers. He et al., (2006a) carried out two experiments to study the effect of REE-chloride and REE-citrate on male Ross broiler chicks. In the experiment 1 it was found that supplementation of 40 mg/kg REE-chloride improved significantly ($p<0.05$) both feed intake and body weight gain (BWG) during the day 22-35 period by 4.2 and 4.9% respectively. Also the supplementation of REE-citrate improved significantly ($p<0.05$)

both feed intake and BWG during the period of overall and day 22-35 by more than 5%. In experiment 2 it was found that supplementation of 70 mg/kg REE-chloride improved significantly ($p<0.05$) both feed intake and BWG during the period of day 4-21 by 8.8 and 8.3% respectively. Also supplementation of REE-citrate with a dose of 100 mg/kg improved significantly ($p<0.05$) feed intake, BWG and FCR during the period of day 4-21 by 10.1%, 13.2% and 3% respectively. During the overall period supplementation of 70 and 100 mg/kg REE-citrate improved significantly ($p<0.05$) feed conversion ratio by 2.6 and 3.4% respectively.

2.2.4.4. Ruminant animals

Zhang et al., (1994) studied the effect of REE nitrate containing mainly La2O3, CeO2 and Nd2O3 on adult fattening bulls at the age of six years. The result showed that supplementation of 600 mg/kg REE improved weight gain and feed conversion ratio. Meyer et al., (2006) showed that preruminant calves fed milk replacer supplemented with 200 mg/kg of REE-Citrate, had higher average daily gain and lower feed intake compare to control group. But the difference between control group and treatment groups was not significant.

Effects on ruminal fermentation was performed following the assumption that rare earths may exert their performance enhancing effects by influencing the micro flora of the gastrointestinal tract since only very small amounts are absorbed (Evans, 1990). Yet, in ruminants, concentrations of 150 ppm, 750 ppm, 3750 ppm rare earth citrates (TREO 32.2 %) did not influence ruminal fermentation in a in-vitro study using an artificial rumen (RUSITEC - rumen stimulation technique) (Wehr et al., 2005), (Knebel, 2004). This may indicate that rare earths are not able to affect microbial organisms in the gastrointestinal tract of animals at all. But it may reflect the inability of rare earths to enhance the performance of ruminants under Western conditions (Redling, 2006).

2.2.5. Possible Mode of Action of Rare Earths

Though rare earths have been shown to enhance animal performance under both Chinese and Western conditions, the underlying mechanism has not been clarified

yet. But several proposals have been made. The fact is that orally applied rare earths are only poorly absorbed from gastrointestinal tract (Durbin et al., 1956), (Haley, 1965), (Haley, 1979), (Eisele et al., 1980), (Ji, 1985), (Evans, 1990), (Fiddler et al., 2003), (D'Haese et al., 2003), (Rambeck et al., 2004), (Hutchison and Albaaj, 2005) (He et al., 2006a). Yet, little amounts absorbed, on the other hand, may still be physiological available, thus, able to affect the intermediate metabolism in various manners (Redling, 2006).

2.2.5.1. Antimicrobial Effects

The early study showed that performance enhancing effect of REE in pigs may be achieved through their antimicrobial properties (Muroma, 1958; Evans, 1990). It has been reported that REE have been used as a performance enhancer in fresh-water fish farming by protecting the fish from diseases and they also improved the growth of the fish (Yeng, 1990) (He et al., 2001). It was assumed that REE might be involved to change the gut-flora like those antibiotics did. So it has been suggested that rare earth elements may influence the development of selected bacterial groups in the intestinal tract (He et al., 1999), (Feldmann, 2003), (Flachowski, 2003), (Rambeck and Wehr, 2005). Also Ou et al., (2000) suggested that acid characteristic of REE additives could decrease the pH value in digestive tracts of piglets and suppress the harmful bacterial increasing. It has been reported that La^{3+} at lower concentration (0.5-30 $\mu g.kg^{-1}$) could inhibit E. coli-absorbing external DNA and effectively decrease transformation. La^{3+} could change the structure of outer cell membrane and cause damages in it (Wenhua et al., 2003), (Peng et al., 2004), (He at al., 2006). However a previous study found that oral administration of a relative higher REE (300 mg/kg) had no significant effect on both gut-flora and growing performance of the broilers (Schuller et al., 2002). Also Böhme et al. (2002a), Kraatz et al. (2004) and Knebel (2004) have not been able to demonstrate significant antibacterial actions of rare earths in gut flora of piglets or in in-vitro studies on rumen microorganism (Redling, 2006).

2.2.5.2. Improvements in Digestibility and Utilization of Nutrients

Related to those results found in the previous studies (He et al., 2001, 2003a, 2006b) that showed REE improved the body weight gain and feed conversion ratio without increasing feed intake of pigs and rats, it is suggested that REE might improve the utilization of nutrients in the diets (He et al., 2006a). This is supported by those early studies in pigs (Li et al., 1992; Cheng et al., 1994; Zhu et al., 1994) and broilers (Lu and Yang, 1996). Ming et al., (1995) showed that rare earths were capable of improving the digestibility of dry matter, total energy and protein in pigs, thereby enhancing feed utilization. Hu et al. (1999) observed significant better apparent digestibility of energy, crude protein as well as total essential and non-essential amino acids in pigs whose diet was supplemented with rare earth elements. Xie and Wang (1998) showed that Rare earth elements improved the utilization of dietary nutrients such as total energy, crude protein and crude fat in chickens. Ou et al., (2000) concluded that REE could form complex with proteins and affect their metabolism in body. They also indicated that rare earth elements could promote the secretion of digestive fluids. Xu et al., (2004) showed that lanthanum increased gastric acid secretion dose-dependently in isolated mice stomachs. Prause et al., (2004) revealed that rare earth elements at concentration of 150 mg/kg increased the digestibility and retention of energy, nitrogen and carbon nitrogen uptake in piglets. But higher concentration (300 mg/kg) didn't have effect on those parameters (Prause et al., 2005a), (Prause et al., 2005c). Enhanced fat accretion was also observed. Together with increased protein accretion, this might be ascribed to enhanced feed intake (Redling, 2006). while increased nitrogen intake and utilization may account for the fact that pigs supplemented with rare earths put on more protein (Kyriazakis and Emmans, 1992), (Bikker et al., 1995), (Tome and Bos, 2000). Performance enhancing effects might be ascribed to the modification of phosphorous compounds by rare earths, thereby; allowing better utilization of these compounds (Fleckenstein et al., 2004). Due to anti-oxidative effects, rare earths may also be able to protect fatty acids, such as omega-3 fatty acids, present in the diet from oxidization. Rare earths could thereby preserve nutrients within the feed or, moreover, enhance their uptake (Redling, 2006).

2.2.5.3. Interaction with Calcium

A wide range of physiological and biochemical processes in both human and animal bodies has already been shown to be affected by rare earth elements, whereas most of these processes are known to be Ca^{2+} - depending. To that effect, several pharmacological or biochemical properties of rare earths, such as inhibiting coagulation (Jakupec et al., 2005), muscle contraction (Triggle and Triggle, 1976) and transmission of nervous impulses (Vaccari et al., 1999) or influencing hormonal responses (Enyeart et al., 2002) or the release of histamine from mastcells (Beaven et al., 1984), are ascribed to their high resemblance to calcium ions. Rare earths not only present a marked similarity in both size and bonding but also in coordination geometry and donor atom preference, which allows them to replace Ca^{2+} specifically in various physiological processes (Redling, 2006).

2.2.5.4. Influence on Hormones and Enzymes

It has been reported that REE can improve protein and other nutrient utilization through stimulating activities of the hormones such as growth hormone and T_3 (Xie et al., 1991, Yang et al., 1992, Xie et al., 1995, He et al., 2006a). It has been suggested that because of relationship between REE and calcium in both animal and plant cells, REE may affect activities of the hormones or enzymes by inhibiting or replacing calcium which plays a very important role in metabolism (Nayler, 1975; Hanioka et al., 1994; Takada et al., 1999; Rambeck et al., 1999a). Xie and Wang (1998) reported Rare earth supplement increased the activities of several hormones and enzymes, such as growth hormone (GH), T_3, lipase, SOD and peroxidase in chickens. Xu et al., (1999) found that growth hormone (GH) level in pigs was significantly increased by rare earths supplement ($p < 0.05$).

Förster et al., (2006) showed that rare earth elements increased the thyroid hormones (T_3 and T_4) levels in blood of piglets. However, He et al., (2001) found that REE significantly decreased the level of triiodothyronine (T_3) and increased the level of thyroxine (T_4) in blood serum of pigs. Thyroid hormones T_3 and T_4 are very important for growth rate, nitrogen and lipid metabolism, so alterations in their balance affects energy turnover and body weight (Rosenbaum et al., 2000), (He et

al., 2001), Wiesner and Ribbeck (1991). Although T_4 is much higher than T_3 in the blood serum, almost all T_4 is eventually converted to T_3, which in turn has a very high binding affinity to the cellular thyroid hormone receptors (He at al, 2001). The increased thyroid hormone can stimulate almost all aspects of carbohydrate metabolism, increase both lipogenesis and lipolysis, and increase basal metabolism rate (King and May, 1984; Guyton, 1991). A proper concentration of T_3 is important to maintain an optimum basal metabolic rate that is more economically efficient for animal growth and fattening (He et al., 2001). Yet other hormones such as insulin and sex hormones may also be influenced by rare earths. Rare earths may also affect the intermediate metabolism by influencing the carbohydrate metabolism via insulin (Redling, 2006).

Ou et al., (2000) concluded that rare earth elements can improve the activity of proteinase in stomach and pancreas of piglets. Wang et al., (2002) demonstrated that the activity of α-amylase was enhanced by low concentration of Ce^{3+} and was inhibited by high concentration of Ce^{3+}. That is because Ce^{3+} at high concentration displaces Ca^{2+} from α-amylase competitively. α-amylase has five Ca^{2+}- binding sites with different affinities and Ce^{3+} can bind to these sites. It has also been suggested that rare earth elements are able to replace calcium junctions and could so impact intestinal enzymes and after absorption enzymes and hormones of metabolism (Prause et al., 2005b). Xu et al., (2004) showed that La^{3+} can increase the release of gastrin in stomach of isolated mice by stimulating G cells or activation of gastrin receptor. Lu et al., (2003) found hormesis effect of rare earth elements on seven enzymes (ALT, ICD, AST, LAP, ALP, γ-GT, CHE) in rat liver. Recurrently, increased liver enzyme activities were also observed in pigs (Borger, 2003) and mice (Kawagoe et al., 2005) after oral application of rare earths. There are also feeding trials in which no significant influence on aspartate transaminase (AST), alanine transaminase (ALT) or alkaline phosphatase (AP) occurred after dietary rare earth supplementation (He et al., 2001).

2.2.5.5. Influence on Certain Cellular Functions

Powis et al. (1994) found out that rare earths could be transported by sodium - calcium channels, hence, triggering hormone releases. It has been reported that rare

earth elements can stimulate the proliferation of preadipocytes (3T3-L1 cells) decrease the concentration and composition of monounsaturated fatty acids in differentiating adipocytes. This indicates that rare earths may have affect adipogenesis and lipogenesis rates in adipose tissue (He et al., 2003b, 2006c). Liu et al., (2004) also demonstrated that lanthanum plays an important role in inhibiting early lipids deposition and cell damage.

2.2.5.6. Immune System Stimulating Abilities

Apart from influencing the immune system indirectly by presenting anti-oxidative properties, other immunomodulating abilities have also been ascribed to rare earths (Ni, 1995), (Li et al., 1998), (He et al., 2003b). Accordingly, it was reported that rare earths can stimulate the immune system (Ni, 1995) and the histamine secretion of mast cells (Foreman and Mongar, 1973) dose dependently. So it has been suggested that oral intake of less or slightly more may strengthen the immune system. Positive effects on the immune system due to rare earth application have already been considered as possible mode of action explaining performance enhancing effects in animals (Feldmann, 2003). recent investigations on cell lines demonstrated dose-dependent effects of lanthanum and cerium on the production of blood cells within the bone marrow (Flachowski, 2003), as to synthesis and reduction of white blood cells (Redling, 2006).

3. Material and methods

3.1. Experimental animals

Japanese quails (Coturnix coturnix japonica) were used as experimental animals in this research consisting three experiments. The quails were from the breed produced in institute of animal physiology and nutrition of Munich University. These animals are used for poultry research in institute rather than broiler chickens, because of some advantages. They have low need for feed and space. They have short incubation period and reach to mature age in less than two months. Also these quails are less susceptible to infectious diseases and have a high growth rate.
In the following research, hatching eggs from breeder flock were collected and put inside a small hatching machine for 3 weeks. The day after hatching, the new born quails were used for the experiments. The one day old Japanese quails were raised for four weeks in each experiment.

- In the first experiment 120 one day old Japanese quails with the average weight of 8.34gr ± 0.39gr divided randomly into five groups, one control group and four treatment groups with 24 quails per group.
- In the second experiment 300 one day old Japanese quails with the average weight of 7.70gr ± 0.43gr were divided randomly into eight groups, one control group with 20 quails and seven treatment groups with 40 quails per group.
- In the third experiment 225 one day old Japanese quails with the average weight of 8.10gr ± 0.49gr were divided randomly into five groups, one control group and four treatment groups with 45 quails per group.

3.2. Rearing condition

After hatching, the new born quails were transferred to brooder house and were put in three or four plastic boxes until the next day when experiment started. The brooder house was a building without window and with size of 40 square meters which also was used for broiler chicks experiment. At the first day of experiment the one day old quails were randomly divided between experimental groups after measuring weight. The quails were kept in plastic reticular boxes with the size of 60 ×

80 cm. the boxes were covered on the top by lattice metal covers to prevent quails from going out by jumping. In each box there were one bell drinker and one trough feeder and by the second week of experiment one more drinker was added to each box. After hatching the quails were feed with corn meal and the next day were feed with experimental diets until four weeks old. Feed and water were *ad libitum* during the experiment.

The heat was provided by electric heater with thermostat and air circulation was provided by ventilators. The temperature in house was set at 38 °C at the first day and was decreased by 2°C every two days during the first week. Then the temperature was weekly decreased by 4°C to reach the room temperature (22 ±2 °C) by the end of fourth week. Light was provided for 24 hours per day during the experiment.

3.3. Composition of experimental rations

In each of the three trials, the one day Japanese quails were fed with control diet and treatment diets until four weeks old. The control diet was the basal diet with no additives and the treatment diets were basal diet plus Rare Earth Elements. Rare earth elements were used as feed additive to study their effect on growth factors. The basal diet was formulated according to NRC nutrient requirements of poultry 1994. The basal diet composition is shown in Table 3.3-1. The treatment diets which contained rare earth elements are defined based on each trail.

Table 3.3-1: Composition of basal diet

Ingredients	composition (gr/kg)
Corn meal	350
Soybean meal	420
Ground wheat	145
Soybean oill	47
Starch	5
Calcium carbonate, CaCo3	14
Calcium phosphate, dibasic form (DCP)	10
DL-methionine	1
Salt (NaCl)	3
Vitamin premix	2.5
Trace mineral premix	2.5
Total	1000
Analyzed nutrient composition	
Dry matter (%)	90
ME (MJ/Kg)	12.13
Crude protein (%)	23.9
Crude fiber (%)	2.5
Calcium (%)	0.82
Available Phosophor, AP (%)	0.33

3.3.1. Experiment 1

In the first experiment, REE-Citrate was used as rare earth element with different concentrations in treatment diets. REE-Citrate was a Lanthanoid mixture containing 5.5% La, 17.5% Ce and 3.28% Pr. This REE was used with two low concentrations (50 ppm and 100 ppm) and two high concentrations (400 ppm and 800ppm) in four treatment diets. The REE with mineral and vitamin supplements were added and mixed with diet at the end of making ration.

There were five experimental diets in the first experiment which were fed to 120 Japanese quails. Each experimental group was divided into two subgroups (replicates) comprising 12 quails each. The experimental diets were: 1) Control diet which was basal diet without REE. 2) Basal diet with 50 mg/kg REE-Citrate. 3) Basal diet with100 mg/kg REE-Citrate. 4) Basal diet with 400 mg/kg REE-Citrate. 5) Basal diet with 800 mg/kg REE-Citrate. The experimental diets are shown in Table 3.3-2.

Table 3.3-2: Diets of the first experiment

Diet	Statement
1	Control (basal diet)
2	Basal diet + 50 mg/kg REE-Citrate
3	Basal diet + 100 mg/kg REE-Citrate
4	Basal diet + 400 mg/kg REE-Citrate
5	Basal diet + 800 mg/kg REE-Citrate

3.3.2. Experiment 2

In the second experiment four different types of REE were used in treatment diets. The REE supplements consisting of: REE-Citrate (type A), Lanthanum-Acetate (type B), Lanthanum-Chloride (type C) and Lanthanum-Carbonate (type D). REE-Citrate was a Lanthanoid mixture containing 5.5% La, 17.5% Ce and 3.28% Pr, but the other types of REE were Lanthanum salts not the mixtures. The REE-Citrate was used with concentration of 50 ppm and the Lanthanum salts were used with two concentrations (50 ppm and 100 ppm) in seven treatment diets. The REE with mineral and vitamin supplements were added and mixed with diet at the end of making ration.

There were eight experimental diets in the second experiment which were fed to 300 Japanese quails. Each experimental group (except the control group) was divided into two subgroups (replicates) comprising 20 quails each. Because of the limited number of plastic boxes which were used for raising quails, the control diet was assigned to one group of 20 quails without replication. The experimental diets were: 1) Control diet which was basal diet without REE. 2) Basal diet with 50 mg/kg REE-Citrate. 3) Basal diet with 50 mg/kg Lanthanum-Acetate. 4) Basal diet with 100 mg/kg Lanthanum-Acetate. 5) Basal diet with 50 mg/kg Lanthanum-Chloride. 6) Basal diet with 100 mg/kg Lanthanum-Chloride. 7) Basal diet with 50 mg/kg Lanthanum-Carbonate. 8) Basal diet with 100 mg/kg Lanthanum-Carbonate. The experimental diets are shown in Table 3.3-3.

Table 3.3-3: Diets of the second experiment

Diet	Statement
1	Control (basal diet)
2	Basal diet + 50 mg/kg REE-Citrate
3	Basal diet + 50 mg/kg Lanthanum-Acetate
4	Basal diet + 100 mg/kg Lanthanum-Acetate
5	Basal diet + 50 mg/kg Lanthanum-Chloride
6	Basal diet + 100 mg/kg Lanthanum-Chloride
7	Basal diet + 50 mg/kg Lanthanum-Carbonate
8	Basal diet + 100 mg/kg Lanthanum-Carbonate

3.3.3. Experiment 3

The third experiment was carried out to examine the accuracy of results of the first experiment. So the treatment diets in this experiment were the same as in the first experiment, the only difference was the number of animals. There were five experimental diets in the third experiment which were fed to 225 Japanese quails. Each experimental group was divided into three subgroups (replicates) comprising 15 quails each.

3.4. Measured parameters

3.4.1. Body weight and weight gain (gr)

At the beginning of each experiment the one day old Japanese quails were weighed and divided into experimental groups. The weight measurement was done every week. In the first experiment the quails were weighed by group but in the other experiments were weighed one by one. The average body weight was calculated by dividing the sum of the body weight in each group into the number of quails in that group. The weekly average weight gain was calculated by subtracting the average body weights of each group in two consecutive weeks.

3.4.2. Feed intake (gr)

At the beginning of each experiment the feeder of each box was weighed, and some of dietary feed from relevant group was weighed and put in the feeder. Every week the weight of the feeder with remaining feed from each box was measured and if it was needed some feed from the relevant experimental diet was weighed and added to feeder. During the experiment the feeders were checked and if the feed was not enough, more feed from that group was weighed and added to the feeder. The amount of feed consumption in each group was calculated by subtraction the sum of added feed to feeder from the remained feed in feeder.

Despite of high weight gain of the fifth group quails in the first experiment, the quails in group five didn't grew well during the first two weeks of the third experiment. It was assumed there was a technical problem in making ration, so the ration for fifth experimental group was made again. The new ration was given only to the second and third replicates of group five since the quails were two weeks old. The results showed the quails were fed new ration grew better than quails of the first replicate so there might be a technical error in making ration.

3.4.3. Feed conversion ratio

Feed conversion ratio was calculated by dividing the feed consumption (intake) into weight gain. This parameter which doesn't have unit indicates how much weight is gained by quails with consuming certain amount of feed.

3.4.4. Mortality

Since the experiment began, every day the boxes were checked for dead or sick quails. Until the 3rd day of each experiment, the dead quails replaced with new quails. After the third day the dead quails were removed without replacement and the remained feed in the feeder was measured. During each experiment quails mortality was recorded as it occurred and percentage of mortality was determined at the end of study. In general there was no disease which resulted in high mortality.

3.4.5. Calcium, Phosphorus and Magnesium measurement

At the end of each experiment four Japanese quails (two male and two female) were randomly chosen from each box (replicate) and slaughtered by cervical dislocation. Tibia bones, breast and liver were taken from each slaughtered bird. Also blood sample was taken from each quail during cutting the neck. Blood samples and tibia bones were sent to the lab fro measuring Calcium, Phosphorus and Magnesium. The liver and breast samples were kept in refrigerator to send them to other institute for measuring REE content if it was necessary.

3.4.5.1. Tibia bone

The removed tibia bones were kept in refrigerator at -20°C until doing the laboratory tests. One bone from each slaughtered quail was used for mineral assay. The bones were cleaned of surrounding flesh then they were weighed and were put in electric kiln at the temperature of 550°C for 48 hours. The weight of bone ashes was measured and each bone was put in a glass pipe to be prepared for chemical procedure.

3.4.5.1.1. Chemical procedure

To prepare the bones ash for mineral assessment, 1 cm^3 of 37% hydrochloride acid solution was added to each pipe containing bone ashes. Then 1 cm^3 of distilled water was added to ashes and the pipes were put on the shaker to mix the solution. To make a 1:10 diluted solution, distilled water was added to each pipe until the volume of each solution reach to 10 cm^3. Before using the samples for mineral assessment, they should be diluted by ten times. So 1 cm^3 of acid solution from each bone sample was transferred to other pipe and 9 cm^3 distilled water was added to it. This 1:100 diluted acid solution was used for bone calcium, phosphorus and magnesium determination.

3.4.5.1.1.1 Calcium determination

Calcium content in bones was determined by Flame Photometric method. To prepare for calcium assessment, 1milt of standard urine solution and 1milt flame photometer standard solution were put in the flame photometer system. 500 micro liter of 1:100 diluted acid solution of each bone sample were purred in small caps and set into the system. Besides the calcium, sodium and potassium content of bone ashes were determined by flame photometer system. The minerals were determined by system based on millimolar per liter (mmol/lit), so the following formula was used to calculate grams of calcium in kilogram bone ash:

$$\text{Ca (g/kg)} = \frac{40.08[\text{g/mol}] * \text{reading [mmol/lit]} * \text{dilution rate (10[milt] of bone ash)}}{1000 * \text{bone ash weight [gr]}}$$

To calculate the percentage of calcium in bone ash, the amount of calcium based on gram per kilogram of bone ash was divided into ten.

3.4.5.1.1.2 Phosphorus determination

Phosphorus content in bones was determined by spectrophotometric method. To prepare for phosphorus assessment it was needed to make a 1:1 solution of amoniumvanadat and ammoniummolybdat. 50 micro liter of 1:100 diluted acid solution of each bone sample were transferred to plastic pipes then 2milt of 1:1 mixture and 1milt TCA (trichloroacetic acid) were added to bone acid solutions. Also two blank solutions were needed for testing the spectrophotometer system. In blank pipes 2milt of 1:1 molybdat mixture and 1milt TCA were added without bone acid solution. Before placing samples in phosphor assessment system, they were put on shaker with the speed of 3000 round per minute and put aside for 10 minutes. The samples transferred to special rectangular cylinders which worked as prism in the spectrophotometer system. First the blank samples were put in the system and their phosphor content was determined. Then the blank sample with higher phosphor was removed and the other blank sample with the bone samples was placed in the spectrophotometer system for phosphor assessment. The phosphor content of bone

samples were assayed based on 366 nm phosphor absorption, so the following formula was used to calculate grams of phosphor in kilogram bone ash:

$$P\ (g/kg) = \frac{\text{reading} * 10.5 * \text{dilution rate (100)}}{\text{Standard} * 100 * \text{ash weight (gr)}}$$

Standard = 0.34

To calculate the percentage of phosphor in bone ash, the amount of phosphor based on gram per kilogram of bone ash was divided into ten.

3.4.5.2. Blood serum

The blood samples were set in centrifuge system to separate the serum. Serum from each blood sample separated and transferred to small cap which was labeled with the name of treatment, replicate, sex and number of slaughtered bird. The serum samples were stored in refrigerator at -20°C until doing the mineral assessment at laboratory.

3.4.5.2.1. Calcium determination

Calcium content in blood serum was determined by Flame Photometric method. To prepare samples for calcium assessment, it was needed to make 1:10 diluted acid solution. 1milt of 37% hydrochloride acid solution was poured in a pipe and with distilled water the volume of acid was increased to 10milt. The serum samples were taken out from fridge and were put at room temperature for half an hour. 100 micro liter of each serum sample was poured in small caps and 100 micro liter of 1:10 diluted acid solution was added to serum. Also 1milt of standard urine solution and 1milt flame photometer standard solution were poured in two separate small caps. The serum samples with the standard solutions were set into the flame photometer system to determine the calcium content of blood serum. The output data from system was multiplied to dilution rate (10) to calculate the calcium content of blood serum based on millimolar per liter (mmol/lit).

3.4.5.2.2. Phosphorus determination

Phosphorus content in bones was determined by spectrophotometric method. To prepare for phosphorus assessment it was needed to make a 1:1 solution of amoniumvanadat and ammoniummolybdat. The serum samples were taken out of the fridge and were put at room temperature for half an hour. 100 milt of each serum sample was poured in plastic pipes and 2milt of TCA (trichloroacetic acid) was added to it. The mixture of serum samples and TCA make crystals so they were put in a centrifuge system at the speed of 3000 round per minute for 10 minutes. The mixture was divided into two separate parts after centrifugation, the clear part at the top and the unclear part at the bottom. One milliliter of clear part from each serum sample was transferred to the plastic pipes, and then 2milt of 1:1 molybdat mixture was added to it. Also two blank solutions were needed for testing the spectrophotometer system. In blank pipes 2milt of 1:1 molybdat mixture and 1milt TCA were poured. Before placing samples in phosphor assessment system, they were put on shaker with the speed of 3000 round per minute and put aside for 10 minutes. The samples transferred to special rectangular cylinders which worked as prism in the spectrophotometer system. First the blank samples were put in the system and their phosphor content was determined. Then the blank sample with higher phosphor was removed and the other blank sample with the serum samples was placed in the spectrophotometer system for phosphor assessment. The phosphor content of serum samples were assayed based on 366 nm phosphor absorption, so the following formula was used to calculate milligrams of phosphor per liter serum:

$$P\ (g/kg) = \frac{\text{reading} * 10.5 * \text{dilution rate (1)} * 1000}{\text{Standard} * 100 * 1}$$

Standard = 0.34

3.4.5.3. Magnesium determination

Magnesium content of bone ash and blood serum was determined by Flame Atomic Absorption Spectrometry method. The procedure was completely automatic. For determining magnesium content of bone ash, the 1:1000 diluted acid solution of

bone ash sample was used. To determine serum magnesium content, 50 micro liter of each serum sample was transferred to glass pipe and the volume of it was increased to 5milt by adding distilled water. The diluted serum samples and diluted acid solution of bone ash samples were set in the atomic absorption spectrometer system to determine their magnesium content. The output of system was based on milligram magnesium per kilogram bone ash and milligram magnesium per liter serum. To calculate the percentage of magnesium in bone ash, the magnesium content determined by system was divided into ten thousand.

3.4.6. Statistical model

The experimental design was random complete block. Data was analyzed in General Linear Model by SigmaStat from SAS software. Statistical model is as bellow:

$$Y_{ij} = \mu + \alpha_i + \beta_i + \varepsilon_{ij}$$

Y_{ij} = amount of each data

μ= mean

α_i = effect of block

β_j= effect of treatment

ε_{ij}= error of experiment

4. Results

4.1. Experiment 1

The results of the first experiment are shown in Table 4.1-1 to Table 4.1-15.

4.1.1. Body weight

The average body weights of Japanese quails at different ages with their standard deviations are shown in Table 4.1-1. There was no significant difference between experimental groups at one day old. The result shows that quails were fed REE-citrate had higher body weight than quails in control group. REE-citrate supplement significantly ($p<0.05$) increased body weight for 10.41% to 23.16% at 7 days old, 20.6% to %29.4% at 14 days old and 21.52% to 28.94% at 21 days old. At the age of 28 days old, REE supplemented diets increased the body weight for 16.7% to 20.22% but the total effect was not significant.

Table 4.1-1: Body weight per bird (gr) in experimental groups at different ages

Treatment	REE addition mg/kg	r	n	Average body weight (gr/bird)				
				1 day old	7 days old	14 days old	21 days old	28 days old
1	-	2	24	8.25 ±0.00	21.03 ^{a} ±0.81	36.86 ^{a} ±1.47	59.75 ^{a} ±0.35	81.85 ±1.77
2	50	2	24	8.63 ±1.00	25.90 ^{b} ±0.03	47.71 ^{b} ±0.65	77.04 ^{b} ±0.77	98.40 ±0.03
3	100	2	24	8.33 ±0.12	23.26 ^{ac} ±0.62	44.74 ^{b} ±3.48	72.61 ^{b} ±5.23	95.52 ±5.49
4	400	2	24	8.17 ±0.00	23.22 ^{ac} ±1.73	44.45 ^{b} ±2.82	73.36 ^{b} ±5.14	96.13 ±7.51
5	800	2	24	8.33 ±0.35	24.72 ^{bc} ±0.78	45.82 ^{b} ±0.64	75.32 ^{b} ±1.35	97.55 ±3.86
n			120	120	105	104	104	103
SEM				±0.36	±0.74	±1.69	±2.63	±3.56
significance				n.s	*	*	*	n.s

r= repetition.

n= number of animals.

a-b: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

* $p<0.05$

4.1.2. Mortality

The average percentage of mortality (dead and eliminated animals) during the whole period of experiment was 2.7% which mostly hapened during the first week of experiment. As there was not special disease during the experiment, the number of dead or eliminated quails significantly dropped to 0.6% after first week of trial.

4.1.3. Weight gain

The average weight gain at different ages based on gram per bird is shown in Table 4.1-2. The Japanese quails fed diets supplemented with REE-citrate gained more weight compare to quails of control group during experiment. REE supplement significantly ($p<0.05$) increased weight gain for 33.3% to 37.8% during second week and 21.76% to 28.87% during the third week of trial. There was no significant difference between control group and groups fed REE supplement during fourth week of experiment. The quails fed with 50 mg/kg REE gained the highest weight than quails in other groups during 28 days of age.

Table 4.1-2: Average weight gain (gr) per bird in experimental groups at different ages

Treatment	REE addition mg/kg	r	Average weight gain (gr/bird)								
			age (days old)								
			1-7	7-14	14-21	21-28	1-21	1-28	7-21	7-28	14-28
1	-	2	12.78 ±0.81	15.83 ^{a} ±0.66	22.89 ^{a} ±1.83	22.10 ±2.12	51.50 ^{a} ±0.35	73.60 ±1.77	38.72 ^{a} ±1.16	60.82 ±0.96	44.99 ±0.30
2	50	2	17.27 ±0.97	21.81 ^{b} ±0.62	29.33 ^{b} ±0.12	21.35 ±0.74	68.42 ^{b} ±0.24	89.77 ±0.97	51.15 ^{b} ±0.74	72.50 0.00	50.69 ±0.62
3	100	2	14.93 ±0.74	21.48 ^{b} ±2.86	27.87 ^{b} ±1.74	22.91 ±0.26	64.27 ^{b} ±5.34	87.19 ±5.61	49.35 ^{b} ±4.60	72.26 ±4.87	50.78 ±2.00
4	400	2	15.06 ±1.73	21.23 ^{b} ±1.09	28.91 ^{b} ±2.32	22.77 ±2.37	65.20 ^{b} ±5.14	87.97 ±7.51	50.14 ^{b} ±3.41	72.91 ±5.78	51.68 ±4.69
5	800	2	16.39 ±1.14	21.10 ^{b} ±0.14	29.50 ^{b} ±0.71	22.23 ±2.51	66.99 ^{b} ±1.70	89.21 ±4.21	50.60 ^{b} ±0.57	72.83 ±3.07	51.73 ±3.21
SEM			±0.89	±1.09	±1.13	±1.43	±2.68	±3.66	±2.08	±2.86	±2.00
significance			n.s	*	*	n.s	*	n.s	*	n.s	n.s

r= repetition.

a-b: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

* p<0.05

4.1.4. Feed consumption

The average feed consumption based on gram per bird at different ages is shown in Table 4.1-3. As the result shows REE-citrate supplement had no significant effect on the amount of feed consumption. But the Japanese quails in REE supplemented group consumed 3.27% to 20% more feed than the quails in control group.

Table 4.1-3: Average feed consumption (gr) per bird in experimental groups at different ages

Treatment	REE addition mg/kg	r	Average feed consumption (gr/bird)								
			age (days old)								
			1-7	7-14	14-21	21-28	1-21	1-28	7-21	7-28	14-28
1	-	2	42.26 ±2.07	60.39 ±3.95	104.70 ±14.57	109.99 ±16.81	207.36 ±20.58	317.34 ±37.39	165.09 ±18.51	275.08 ±35.32	214.69 ±31.38
2	50	2	44.69 ±1.34	64.88 ±0.18	110.44 ±14.76	107.71 ±21.15	220.01 ±15.92	327.72 ±15.24	175.31 ±14.58	283.02 ±16.57	218.15 ±16.39
3	100	2	44.60 ±0.84	65.24 ±8.43	109.30 ±11.74	108.93 ±10.96	219.13 ±19.33	328.07 ±30.29	174.54 ±20.17	283.47 ±31.13	218.23 ±22.7
4	400	2	43.44 ±0.39	66.29 ±3.36	127.24 ±8.43	143.83 ±14.39	236.97 ±10.53	380.80 ±40.04	193.53 ±9.92	337.36 ±40.43	271.08 ±43.80
5	800	2	47.27 ±4.40	68.44 ±5.47	112.41 ±2.38	138.73 ±2.31	228.12 ±20.49	366.85 ±22.80	180.85 ±11.09	319.57 ±13.41	251.14 ±13.06
SEM			±3.24	±5.45	±8.35	±18.54	±12.75	±22.24	±11.14	±21.52	±20.29
significance			n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

r= repetition.

n.s: no significant difference.

4.1.5. Feed conversion ratio

The feed conversion ratio (gram of feed intake per gram gained weight) at different ages is shown in Table 4.1-4. REE-citrate supplement had no significant effect on the feed conversion ratio. But the result showed that Japanese quails were fed REE supplemented diets had lower feed conversion ratio than the ones in control group for up to 21.98% at one week old, up to 22% at two weeks old, up to 17.32% at three weeks old and up to 5.75% at four weeks old respectively. REE-citrate decreased

feed conversion ratio up to 20.1% by the end of third week of experiment and up to 15.51% by the end of experiment. The Japanese quails were fed 50 mg/kg REE-citrate had the lowest feed conversion ratio. So it can be said that REE-citrate improved feed efficiency in Japanese quails.

Table 4.1-4: Average feed conversion ratio per bird in experimental groups at different ages

Treatment	REE addition mg/kg	r	Feed conversion ratio								
			age (days old)								
			1-7	7-14	14-21	21-28	1-21	1-28	7-21	7-28	14-28
1	-	2	3.32 ±0.37	3.82 ±0.41	4.56 ±0.27	5.04 ±1.24	4.03 ±0.37	4.32 ±0.61	4.26 ±0.35	4.53 ±0.65	4.77 ±0.73
2	50	2	2.59 ±0.07	2.98 ±0.08	3.77 ±0.52	5.06 ±1.17	3.22 ±0.22	3.65 ±0.10	3.43 ±0.34	3.90 ±0.09	4.31 ±0.18
3	100	2	2.99 ±0.21	3.04 ±0.01	3.92 ±0.18	4.75 ±0.42	3.41 ±0.02	3.76 ±0.11	3.53 ±0.08	3.92 ±0.17	4.29 ±0.28
4	400	2	2.90 ±0.31	3.12 ±0.00	4.41 ±0.33	6.41 ±2.55	3.64 ±0.22	4.36 ±0.76	3.87 ±0.18	4.65 ±0.90	5.30 ±1.30
5	800	2	2.91 ±0.78	3.24 ±0.62	3.81 ±0.01	6.29 ±0.81	3.41 ±0.39	4.12 ±0.45	3.58 ±0.26	4.40 ±0.37	4.86 ±0.30
SEM			±0.31	±0.26	±0.21	±1.11	±0.2	±0.37	±0.19	±0.41	±0.53
significance			n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

r= repetition.

n.s: no significant difference.

4.1.6. Tibia ash weight

The average weight of tibia ash in male and female Japanese quails at 28 days old is shown in Table 4.1-5. There was no significant difference between control group and the REE supplemented groups. The tibia ash weight of quails fed 50 mg/kg and 800 mg/kg of REE-citrate were 6.54% to10.28% higher than the quails in other experimental groups. The female Japanese quails fed 50 mg/kg and 800 mg/kg of REE-citrate had 10-14 percent higher tibia ash than control group. The REE-citrate supplement with different concentrations (except 400 mg/kg) increased tibia ash in male Japanese quails for 3 to 15 percent compare to control group.

Table 4.1-5: Average tibia ash weight (gr) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	Ash weight (gr)		
				female	male	total
1	-	2	8	0.108 ±0.01	0.105 ±0.020	0.107 ±0.015
2	50	2	8	0.123 ±0.018	0.114 ±0.012	0.118 ±0.015
3	100	2	8	0.091 ±0.012	0.121 ±0.003	0.106 ±0.018
4	400	2	8	0.107 ±0.022	0.100 ±0.019	0.104 ±0.019
5	800	2	8	0.119 ±0.010	0.108 ±0.020	0.114 ±0.016
n			40	20	20	40
SEM				±0.004	±0.004	±0.006
significance				n.s	n.s	n.s

r= repetition.

n= umber of samples.

n.s: no significant difference.

4.1.7. Calcium content of tibia ash

The average calcium content of tibia ash based on gram per kilogram and percentage of calcium in tibia ash in male and female Japanese quails are shown in Table 4.1-6 and Table 4.1-7. There was no significant difference between experimental groups. But the diets supplemented with 50 mg/kg and 100 mg/kg of REE-citrate increased calcium content and percentage of calcium of tibia ash compare to control group for 11.81% to 14.3% in Japanese quails. This increase was 6.43% to 10.22% in female quails and 17.76% to 18.81% in male quails respectively. Without considering gender the high levels of REE-citrate (400 mg/kg and 800 mg/kg) decreased percentage of calcium and calcium content of tibia ash compare to control group for 1.73% to 9.76%. The female quails fed 400 mg/kg of REE-citrate and male quails fed 800 mg/kg of REE-citrate had 1% more calcium in tibia ash than quails in control group.

Table 4.1-6: Average calcium content of tibia ash (gr/kg) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	Calcium in tibia ash (gr/kg)		
				female	male	total
1	-	2	8	320.37 ±33.78	289.99 ±75.11	305.18 ±56.307
2	50	2	8	340.98 ±12.74	341.49 ±13.46	341.23 ±12.138
3	100	2	8	353.11 ±6.40	344.53 ±11.46	348.82 ±9.738
4	400	2	8	323.51 ±60.89	276.29 ±48.48	299.90 ±56.861
5	800	2	8	304.28 ±58.58	291.41 ±46.06	297.85 ±49.267
n			40	20	20	40
SEM				±9.06	±11.182	±15.37
significance				n.s	n.s	n.s

r= repetition.

n= umber of samples.

n.s: no significant difference.

Table 4.1-7: Percentage of calcium in tibia ash per bird and sex within experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	%Ca per kg tibia ash		
				female	male	total
1	-	2	8	32.04 ±3.38	29.00 ±7.51	30.518 ±5.63
2	50	2	8	34.10 ±1.27	34.15 ±1.35	34.123 ±1.21
3	100	2	8	35.31 ±0.64	34.45 ±1.15	34.882 ±0.97
4	400	2	8	32.35 ±6.09	27.63 ±4.85	29.99 ±5.69
5	800	2	8	30.43 ±5.86	29.14 ±4.61	29.785 ±4.93
n			40	20	20	40
SEM				±0.97	±0.97	±1.54
significance				n.s	n.s	n.s

r= repetition.

n= umber of samples.

n.s: no significant difference.

4.1.8. Phosphor content of tibia ash

The average phosphor content of tibia ash based on gram per kilogram and percentage of phosphor in tibia ash in male and female Japanese quails are shown in Table 4.1-8 and Table 4.1-9. The effect of REE supplement was significant in male Japanese quails ($p<0.05$) and without considering gender ($p<0.01$). Without considering gender, the Japanese quails fed 50 mg/kg and 100 mg/kg of REE-citrate had significantly 19.2 to 20 percent higher phosphor content and percentage of phosphor of tibia ash than the ones in control group ($p<0.01$). The high levels of REE-citrate (400 mg/kg and 800 mg/kg) decreased percentage of phosphor and phosphor content of tibia ash compare to control group for 1.5% to 1.9%.

The male Japanese quails fed 50 mg/kg and 100 mg/kg of REE-citrate had significantly 25.6 to 26 percent higher phosphor content and percentage of phosphor of tibia ash than the ones in control group ($p<0.05$) . The female quails fed 400 mg/kg of REE-citrate and male quails fed 800 mg/kg of REE-citrate had 1.22% and 7.87% more phosphor in tibia ash than quails in control group respectively. The phosphor content and percentage of phosphor of tibia ash in female Japanese quails was increased up to 14.5% by low concentrations of REE-citrate (50 mg/kg and 100 mg/kg).

Table 4.1-8: Average phosphor content of tibia ash (gr/kg) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	Phosphor in tibia ash (gr/kg)		
				female	male	total
1	-	2	8	155.31 ±16.97	139.05 [a] ±40.11	147.18 [a] ±29.81
2	50	2	8	177.813 ±4.62	175.14 [b] ±5.02	176.48 [b] ±4.69
3	100	2	8	176.24 ±3.0	174.65 [b] ±3.50	175.45 [b] ±3.14
4	400	2	8	157.20 ±36.40	132.83 [a] ±24.31	145.01 [a] ±31.48
5	800	2	8	140.24 ±25.71	149.99 [ab] ±6.97	145.12 [a] ±18.20
n			40	20	20	40
SEM				±5.445	±5.872	±7.607
significance				n.s	*	**

r= repetition.

n= umber of samples.

a-b: means within columns with no common superscripts are significantly different (p<0.05).
n.s: no significant difference.
* p<0.05
** p<0.01

Table 4.1-9: Percentage of phosphor in tibia ash per bird and sex within experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	%P per kg tibia ash		
				female	male	total
1	-	2	8	15.53 ±1.7	13.90 [a] ±4.0	14.72 [a] ±2.981
2	50	2	8	17.781 ±0.46	17.51 [b] ±0.50	17.65 [b] ±0.469
3	100	2	8	17.624 ±0.30	17.47 [b] ±0.35	17.55 [b] ±0.314
4	400	2	8	15.72 ±3.64	13.28 [a] ±2.43	14.50 [a] ±3.148
5	800	2	8	14.02 ±2.64	14.99 [ab] ±0.7	14.51 [a] ±1.89
n			40	20	20	40
SEM				±0.55	±0.587	±0.76
significance				n.s	*	**

r= repetition.
n= umber of samples.
a-b: means within columns with no common superscripts are significantly different (p<0.05).
n.s: no significant difference.
* p<0.05
** p<0.01

4.1.9. Calcium to phosphor ratio

The calcium to phosphor ratio in tibia of Japanese quails at 28 days old is shown in Table 4.1-10. There was no significant effect between control group and the REE supplemented groups. Supplemented diets with 100 mg/kg and 50 mg/kg of REE-citrate decreased calcium to phosphor ratio of tibia compare to control group for 4.33% to 7.21% in Japanese quails.

The low concentrations of REE-citrate decreased the tibia calcium to phosphor ratio in female Japanese quails up to 7% compare to control group. The female

Japanese quails fed high concentrations of REE-citrate had up to 5% higher calcium to phosphor ratio in tibia ash. The REE supplemented diets decreased the calcium to phosphor ratio of tibia ash in male Japanese quails up to 7.6% compare to control diet.

Table 4.1-10: Calcium to phosphorus ratio in tibia per bird and sex within experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	Ca/P ratio in tibia		
				female	male	total
1	-	2	8	2.06 ±0.08	2.10 ±0.07	2.08 ±0.07
2	50	2	8	1.92 ±0.03	1.95 ±0.04	1.93 ±0.04
3	100	2	8	2.00 ±0.04	1.97 ±0.05	1.99 ±0.05
4	400	2	8	2.08 ±0.22	2.08 ±0.03	2.08 ±0.14
5	800	2	8	2.17 ±0.08	1.94 ±0.25	2.05 ±0.21
n			40	20	20	40
SEM				±0.03	±0.03	±0.04
significance				n.s	n.s	n.s

r= repetition.

n= umber of samples.

n.s: no significant difference.

4.1.10. Magnesium content of tibia ash

The magnesium content and percentage of magnesium in tibia ash of Japanese quails at 28 days old is shown in Table 4.1-11. The effect of REE supplement was significant in male Japanese quails ($p<0.01$) and without considering gender ($p<0.05$). The diets supplemented with 50 mg/kg of REE-citrate significantly ($p<0.05$) increased magnesium content and percentage of magnesium in tibia ash of Japanese quails for 26.4% compare to control diet and 24.8 to 36.7 percent compare to the diets with high levels of REE-citrate. The supplemented diet with 100 mg/kg of REE-citrate significantly ($p<0.05$) increased the magnesium content and percentage of magnesium in tibia ash of Japanese quails compare to diet with 400 mg/kg of REE-citrate. The magnesium content and percentage of magnesium in Japanese

quails fed 100 mg/kg of REE-citrate was 16.75% higher than the quails in control group and 15.3% to 26.3% higher than the ones fed high levels of REE-citrate.

The low concentrations of REE-citrate (50 and 100 mg/kg) significantly increased magnesium content and percentage of magnesium in male Japanese quails compare to control group and compare to high concentrations of REE-citrate ($p<0.01$, $p<0.001$). The male Japanese quails fed low concentrations of REE-citrate (50 and 100 mg/kg) had between 27 to 62 percent higher magnesium content in tibia ash than the ones in other experimental groups. The REE supplement with different concentrations (except 50 mg/kg), decreased the magnesium content and percentage of magnesium of tibia ash in female Japanese quails compare to control group. There was no significant difference between experimental groups within female Japanese quails.

In general tibia magnesium content was higher in quails fed low level of REE supplement than the quails in other groups.

Table 4.1-11: Average magnesium content of tibia ash (mg/kg) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	Magnesium in tibia ash (mg/kg)		
				female	male	total
1	-	2	8	7763 ±1895	6616 ^{a} ±1372	7190 ^{ab} ±1650
2	50	2	8	9277 ±1488	8895 ^{b} ±969	9086 ^{c} ±1180
3	100	2	8	7399 ±1072	9390 ^{b} ±531	8394 ^{ac} ±1321
4	400	2	8	7492 ±2661	5801 ^{a} ±857	6646 ^{b} ±2041
5	800	2	8	7645 ±1444	7003 ^{a} ±1642	7278 ^{ab} ±1470
n			40	20	20	40
SEM				±348	±337	±541
significance				n.s	**	*

r= repetition.

n= umber of samples.

a-b: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

* $p<0.05$

** $p<0.01$

Table 4.1-12: Percentage of magnesium in tibia ash per bird and sex within experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	% Mg per Kg tibia ash		
				female	male	total
1	-	2	8	0.78 ±0.19	0.66 [a] ±0.14	0.72 [abc] ±0.16
2	50	2	8	0.93 ±0.15	0.89 [b] ±0.1	0.91 [a] ±0.12
3	100	2	8	0.74 ±0.11	0.94 [b] ±0.05	0.84 [ab] ±0.13
4	400	2	8	0.75 ±0.27	0.58 [a] ±0.08	0.67 [bc] ±0.20
5	800	2	8	0.57 ±0.14	0.70 [a] ±0.16	0.64 [c] ±0.29
n			40	20	20	40
SEM				0.05	0.04	0.07
significance				n.s	**	*

r= repetition.

n= umber of samples.

a-c: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

* $p<0.05$

** $p<0.01$

4.1.11. Calcium content of blood serum

The average calcium content of blood serum of Japanese quails at 28 days old is shown based on millimolar per liter (mmol/lit) in Table 4.1-13. Without considering gender REE-citrate supplement had significant effect ($p<0.05$) on serum calcium content. The diets supplemented with 400 mg/kg and 800 mg/kg of REE-citrate significantly ($p<0.05$) decreased average calcium content of serum for 30.45% to 31% compare to diet with 50 mg/kg REE-citrate supplement. The serum calcium content in Japanese quails fed highest level (800 mg/kg) of REE-citrate was significantly ($p<0.05$) lower than the Japanese quails fed control diet. In general all levels of REE supplement except the lowest level (50 mg/kg), decreased serum calcium content compare to control group. This decrease was 33% to 46% in female quails and 6.3% to 15% in male quails.

Table 4.1-13: Average calcium content of blood serum (mmol/lit) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	Calcium in serum (mmol/lit)		
				female	male	total
1	-	2	8	14.75 ±0.58	9.55 ±0.32	12.15 [ab] ±5.16
2	50	2	8	13.40 ±0.45	12.45 ±0.60	12.93 [a] ±4.91
3	100	2	8	9.90 ±0.52	8.60 ±0.05	9.25 [abc] ±3.46
4	400	2	8	7.95 ±0.14	8.95 ±0.12	8.45 [bc] ±1.31
5	800	2	8	8.65 ±0.22	8.13 ±0.12	8.39 [c] ±1.66
n			40	20	20	40
SEM				±1.03	±0.71	±1.30
significance				n.s	n.s	*

r= repetition.

n= umber of samples.

a-c: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

* $p<0.05$

4.1.12. Phosphor content of blood serum

The average phosphor content of blood serum of Japanese quails at 28 days old is shown based on milligram per liter (mg/lit) in Table 4.1-14. There was no significant different between Japanese quails in control group and the ones fed REE-citrate. The effect of different levels of REE-citrate was not the same in female and male Japanese quails. The effect of REE supplement was not significant in each of female or male Japanese quails separately. In female quails low levels of REE supplement decreased the serum phosphor content for 16.2% to 26.9% and the high levels of REE supplement increased the serum phosphor content for 3.83% to 45.73% compare to control group. In male quails low levels and the highest level (800 mg/kg) of REE supplement increased the serum phosphor content for 7.2% to 29.3% and the 400 mg/kg level of REE supplement decreased the serum phosphor content for 11% compare to control group.

Table 4.1-14: Average phosphorus content of blood serum (mg/lit) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	Phosphor in serum (mg/lit)		
				female	male	total
1	-	2	8	74.43 ±18.45	80.68 ±33.16	77.55 ±25.07
2	50	2	8	54.43 ±23.30	104.31 ±50.48	79.37 ±45.12
3	100	2	8	62.38 ±8.05	86.47 ±68.49	74.43 ±46.95
4	400	2	8	108.47 ±85.76	71.80 ±44.90	90.14 ±66.33
5	800	2	8	77.28 ±20.92	89.46 ±66.66	82.50 ±41.74
n			40	20	20	40
SEM				±10.68	±11.03	±16.89
significance				n.s	n.s	n.s

r= repetition.

n= umber of samples.

n.s: no significant difference.

4.1.13. Magnesium content of blood serum

The average magnesium content of blood serum of Japanese quails at 28 days old is shown based on milligram per liter (mg/lit) in Table 4.1-15. The low levels of REE supplement decreased the serum magnesium content for 6.63% to 7.51% and the high levels of REE supplement increased the serum magnesium content for 10.37% to 43.87% compare to control group. The magnesium content of serum in Japanese quails fed 400 mg/kg REE-citrate was significantly ($p<0.05$) higher than quails fed other experimental diets. The effect of REE supplement was not significant in each of female or male Japanese quails separately. In male quails the low levels of REE-citrate decreased the serum magnesium content for 6.33% to 22.55% and the high levels of REE-citrate increased the serum magnesium content for 30% to 56% compare to control group. In female quails the 50 mg/kg and 400 mg/kg levels of REE-citrate increased serum magnesium content compare to control group. Also the 100 mg/kg and 800 mg/kg levels of REE-citrate decreased serum magnesium content compare to control group. Within replicate 1, the female Japanese quails fed400 mg/kg of REE supplement had significantly ($p<0.05$) higher serum magnesium content than the ones fed 100 and 800 mg/kg of REE supplement.

Table 4.1-15: Average magnesium content of blood serum (mg/lit) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	Magnesium in serum (mg/lit)		
				female	male	total
1	-	2	8	29118 ±17942	28195 ±7589	28722 [a] ±13431
2	50	2	8	31800 ±14266	21836 ±4754	26818 [a] ±11193
3	100	2	8	26719 ±8840	26409 ±5055	26564 [a] ±6669
4	400	2	8	35651 ±17240	46993 ±44012	41322 [b] ±31532
5	800	2	8	26739 ±8364	36665 ±11735	31702 [a] ±10824
n			40	20	20	40
SEM				±4025	±4157	±5314
significance				n.s	n.s	*

r= repetition.

n= umber of samples.

a-b: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

* $p<0.05$

4.2. Experiment 2

4.2.1. Body weight

The average body weight of Japanese quails with standard deviation at different ages is shown in Table 4.2-1. The result shows that REE supplements had significant effect on quails body weight at 7 days old ($p<0.01$), at 14 days old ($p<0.001$), at 21 days old ($p<0.01$) and at 28 days old ($p<0.05$). High level (100 mg/kg) of REE-type B and REE-type and both levels (50 mg/kg and 100 mg/kg) of REE-type D significantly ($p<0.05$) increased average body weight compare to control group at 7 days and 14 days old. This increase was 9.54% to 13.05% at 7 days old and 7.74% to 13.6% at 14 days old respectively.

At the age of 21 days old the 50 mg/kg of REE-type C and both levels of REE-type D significantly ($p<0.05$) increased average body weight for 5.84% to 10% compare to

control group. At the age of 28 days old the Japanese quails fed high level (100 mg/kg) of REE-type B and both levels of type C and D of REE, had significantly ($p<0.05$) about 4.8% to 6.3% higher body weight compare to quails in control group. There was no significant difference between the effect of two levels (50 mg/kg and 100 mg/kg) of each type of REE supplement on average body weight. Also the average body weight in Japanese quails fed high level (100 mg/kg) of REE-type C and both levels of REE- type D was significantly ($p<0.05$) higher than quails fed REE-type A.

Table 4.2-1: Average body weight (gr) per bird in experimental groups at different ages

Treatment	REE addition mg/kg	REE [1] type	r	n	Average body weight (gr/bird)				
					age (days old)				
					one day old	7 days old	14 days old	21 days old	28 days old
1	-	-	1	20	7.33 ±0.38	19.92 ^{a} ±2.69	42.24 ^{a} ±4.41	70.53 ^{a} ±6.26	95.82 ^{a} ±7.75
2	50	A	2	40	7.44 ±0.57	21.28 ^{abc} ±3.00	43.67 ^{ab} ±7.02	71.76 ^{ab} ±7.74	97.71 ^{ab} ±8.14
3	50	B	2	40	7.45 ±0.43	20.88 ^{ab} ±2.59	45.08 ^{abc} ±4.96	74.11 ^{abc} ±6.49	99.75 ^{abc} ±7.42
4	100	B	2	40	7.28 ±0.50	21.82 ^{bcd} ±2.23	45.51 ^{bcd} ±4.62	74.03 ^{abc} ±5.53	100.54 ^{bc} ±5.87
5	50	C	2	40	7.56 ±0.50	20.98 ^{ab} ±2.86	45.08 ^{abc} ±4.25	74.65 ^{bcd} ±5.60	100.38 ^{bc} ±5.92
6	100	C	2	40	7.72 ±0.55	21.98 ^{bcd} ±3.04	47.03 ^{cde} ±4.91	73.97 ^{abc} ±5.04	101.84 ^{c} ±7.31
7	50	D	2	40	9.38 ±11.63	22.40 ^{cd} ±2.66	47.75 ^{de} ±5.15	77.35 ^{d} ±6.32	101.50 ^{c} ±6.10
8	100	D	2	40	7.18 ±0.55	22.52 ^{d} ±2.30	47.98 ^{e} ±4.67	75.6 ^{cd} ±7.95	101.35 ^{c} ±7.42
n				300	300	300	300	300	300
SEM					±0.70	±0.45	±0.88	±1.12	±1.20
significance					n.s	**	***	**	*

1) REE-type A: REE-Citrate (5.5% La, 17.5% Ce and 3.28% Pr); REE-type B: Lanthanum-Acetate; REE-type C: Lanthanum-Chloride; REE-type D: Lanthanum-Carbonate.

r= repetition.

n= number of animals.

a-b: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

* $p<0.05$

** $p<0.01$

*** $p<0.001$

4.2.2. Mortality

The average percentage of mortality (dead and eliminated animals) during the whole period of experiment was 1.3%. The weak quails which had much lower weight than the average weight of replicate were eliminated. The rate of mortality in Japanese quails was higher during the first week of experiment (3.7%). As there was no special disease during the experiment, the number of dead or eliminated quails significantly dropped to 0.9% after first week of trial.

4.2.3. Weight gain

The average weight gain of Japanese quails with standard deviation at different ages is shown in Table 4.2-2. As the result shows, there was no significant difference between average body weight of Japanese quails fed REE supplement and the quails fed control diet. But the different types of REE supplement increased average body weight up to 8.26% until the age of three weeks old and up to 6.41% until the age of four weeks old. During the experiment the Japanese quails fed REE-type C and REE-type D gained more weight than the quails in other groups. By increasing the level of REE supplement from 50 mg/kg to 100 mg/kg, the average weight gain increased during the experiment.

Table 4.2-2: Average weight gain (gr) per bird in experimental groups at different ages

Treatment	REE addition (mg/kg)	REE type[1]	r	Average weight gain (gr/bird)								
				age (days old)								
				1-7	7-14	14-21	21-28	1-21	1-28	7-21	7-28	14-28
1	-	-	1	12.60 ±0.00	22.31 ±0.00	28.29 ±0.00	25.29 ±0.00	63.20 ±0.00	88.50 ±0.00	50.61 ±0.00	75.90 ±0.00	53.59 ±0.00
2	50	A	2	13.85 ±0.22	22.42 ±2.03	28.05 ±1.26	25.95 ±1.27	64.33 ±0.98	90.28 ±0.28	50.47 ±0.76	76.42 ±0.50	54.00 ±2.53
3	50	B	2	13.43 ±0.12	24.15 ±1.51	29.09 ±1.46	25.69 ±1.27	66.66 ±0.17	92.35 ±1.10	53.23 ±0.05	78.92 ±1.22	54.77 ±2.73
4	100	B	2	14.55 ±0.79	23.67 ±0.96	28.52 ±0.13	26.51 ±0.25	66.74 ±0.04	93.25 ±0.29	52.19 ±0.83	78.70 ±1.08	55.03 ±0.11
5	50	C	2	13.43 ±0.44	24.10 ±1.10	29.51 ±1.37	25.79 ±3.28	67.03 ±2.91	92.82 ±0.37	53.60 ±2.47	79.40 ±0.81	55.30 ±1.92
6	100	C	2	14.26 ±0.05	25.05 ±0.15	26.94 ±0.15	27.62 ±6.62	66.25 ±0.25	93.87 ±6.87	51.99 ±0.30	79.61 ±6.91	54.56 ±6.77
7	50	D	2	13.02 ±2.27	25.35 ±0.80	29.60 ±1.20	24.15 ±0.35	67.97 ±1.87	92.12 ±2.23	54.95 ±0.40	79.10 ±0.05	53.75 ±0.85
8	100	D	2	15.34 ±1.19	25.45 ±0.43	27.63 ±1.03	25.75 ±2.76	68.42 ±0.59	94.17 ±3.35	53.08 ±0.60	78.83 ±2.16	53.38 ±1.73
SEM				±0.80	±0.81	±0.71	±2.07	±1.01	±2.19	±0.81	±1.91	±2.27
significance				n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

1) REE-type A: REE-Citrate (5.5% La, 17.5% Ce and 3.28% Pr); REE-type B: Lanthanum-Acetate; REE-type C: Lanthanum-Chloride; REE-type D: Lanthanum-Carbonate.

r= repetition.

n.s: no significant difference.

4.2.4. Feed consumption

The average feed consumption of Japanese quails with standard deviation at different ages is shown in Table 4.2-3. There was no significant difference between amount of consumed feed by Japanese quails fed control diet and the ones fed REE supplements. During the last two weeks of experiment (14 to 28 day old) the types B, C and D of REE supplements significantly ($p<0.05$) increased the feed consumption up to 13.24% compare to control group.

Table 4.2-3 Average feed consumption (gr) per bird in experimental groups at different ages

Treatment	REE addition (mg/kg)	REE_1 type	r	Average feed consumption (gr/bird)								
				age (days old)								
				1-7	7-14	14-21	21-28	1-21	1-28	7-21	7-28	14-28
1	-	-	1	33.35 ±0.00	49.80 ±0.00	83.00 ±0.00	100.65 ±0.00	166.15 ±0.00	266.80 ±0.00	132.80 ±0.00	233.45 ±0.00	183.65 ±0.00 [a]
2	50	A	2	32.18 ±0.18	50.35 ±3.25	87.95 ±2.90	101.73 ±0.59	170.47 ±6.33	272.21 ±6.92	138.30 ±6.16	240.03 ±6.75	189.68 ±3.49 [ab]
3	50	B	2	32.91 ±1.51	51.42 ±0.07	97.57 ±3.19	110.40 ±5.47	181.90 ±4.77	292.30 ±0.70	148.99 ±3.26	259.39 ±2.21	207.97 ±2.28 [d]
4	100	B	2	34.78 ±0.93	49.87 ±7.18	91.67 ±3.30	106.38 ±5.82	176.31 ±4.81	282.69 ±10.64	141.53 ±3.88	247.92 ±9.71	198.05 ±2.52 [bc]
5	50	C	2	32.06 ±1.15	47.97 ±1.75	93.45 ±3.76	102.62 ±0.24	173.48 ±3.16	276.10 ±2.92	141.42 ±2.01	244.04 ±1.77	196.07 ±3.52 [bc]
6	100	C	2	31.79 ±1.49	48.30 ±1.07	97.55 ±0.71	105.54 ±1.90	177.64 ±3.24	283.19 ±5.15	145.86 ±1.78	251.40 ±3.68	203.10 ±2.61 [cd]
7	50	D	2	34.10 ±2.05	51.65 ±1.41	94.85 ±1.06	100.65 ±6.36	180.60 ±0.42	281.25 ±6.79	146.50 ±2.48	247.15 ±8.84	195.50 ±7.43 [bc]
8	100	D	2	35.45 ±3.54	46.55 ±3.32	93.78 ±3.00	100.38 ±3.00	175.78 ±9.86	276.15 ±6.86	140.33 ±6.33	240.70 ±3.32	194.15 ±0.00 [b]
SEM				±1.07	±2.39	±2.08	±3.02	±3.88	±4.88	±3.08	±4.56	±2.57
significance				n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	*

1) REE-type A: REE-Citrate (5.5% La, 17.5% Ce and 3.28% Pr); REE-type B: Lanthanum-Acetate; REE-type C: Lanthanum-Chloride; REE-type D: Lanthanum-Carbonate.

r= repetition.

a-d: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

* $p<0.05$

4.2.5. Feed conversion ratio

The average feed conversion ratio of Japanese quails with standard deviations at different ages is shown in Table 4.2-4. There was no significant difference between feed conversion ratio of Japanese quails fed different types of REE supplements and the ones in control group. In general during the experiment (1-28 days old) REE supplements decreased feed conversion ratio up to 9.74% compare to control diet and the highest decrease was in the group fed 100 mg/kg of REE-type D (group 8). The results also show that by increasing the level of REE supplement from 50 mg/kg to 100 mg/kg, the feed conversion ratio improved.

Table 4.2-4: Average feed conversion ratio per bird in experimental groups at different ages

Treatment	REE addition mg/kg	REE_1 type	r	Feed conversion ratio								
				age (days old)								
				1-7	7-14	14-21	21-28	1-21	1-28	7-21	7-28	14-28
1	-	-	1	2.65 ±0.00	2.23 ±0.00	2.93 ±0.00	3.98 ±0.00	2.63 ±0.00	3.02 ±0.00	2.62 ±0.00	3.08 ±0.00	3.43 ±0.00
2	50	A	2	2.32 ±0.02	2.25 ±0.06	3.14 ±0.25	3.93 ±0.21	2.65 ±0.06	3.02 ±0.09	2.74 ±0.08	3.14 ±0.11	3.52 ±0.23
3	50	B	2	2.45 ±0.13	2.13 ±0.14	3.36 ±0.06	4.31 ±0.43	2.73 ±0.08	3.17 ±0.05	2.80 ±0.06	3.29 ±0.08	3.80 ±0.23
4	100	B	2	2.39 ±0.07	2.12 ±0.39	3.22 ±0.13	4.01 ±0.26	2.64 ±0.07	3.03 ±0.12	2.71 ±0.12	3.15 ±0.17	3.60 ±0.05
5	50	C	2	2.39 ±0.16	1.99 ±0.02	3.17 ±0.27	4.01 ±0.52	2.59 ±0.16	2.97 ±0.02	2.64 ±0.16	3.07 ±0.01	3.55 ±0.06
6	100	C	2	2.23 ±0.10	1.93 ±0.05	3.62 ±0.05	3.94 ±1.01	2.68 ±0.06	3.03 ±0.28	2.81 ±0.05	3.17 ±0.32	3.75 ±0.51
7	50	D	2	2.65 ±0.30	2.04 ±0.12	3.21 ±0.10	4.17 ±0.33	2.66 ±0.08	3.06 ±0.15	2.67 ±0.03	3.13 ±0.11	3.64 ±0.08
8	100	D	2	2.33 ±0.41	1.83 ±0.16	3.40 ±0.02	3.91 ±0.30	2.57 ±0.17	2.94 ±0.18	2.64 ±0.09	3.06 ±0.13	3.64 ±0.12
SEM				±0.16	±0.11	±0.1	±0.37	±0.08	±0.11	±0.07	±0.12	±0.18
significance				n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

1) REE-type A: REE-Citrate (5.5% La, 17.5% Ce and 3.28% Pr); REE-type B: Lanthanum-Acetate; REE-type C: Lanthanum-Chloride; REE-type D: Lanthanum-Carbonate.

r= repetition.

n.s: no significant difference.

4.2.6. Tibia ash weight

The average tibia ash weight of male and female Japanese quails based on gram per bird at 28 days old is shown in Table 4.2-5. There was no significant difference between control group and the REE supplemented groups. Without considering gender, the weight of tibia ash was 3.64% to 10% higher in quails fed REE-type A and high level (100 mg/kg) of REE-type B and C compare to control group. The male quails fed REE supplements (group 2 to 8) had heavier tibia ash for 8.42% to 23.16% compare to control group. In female quails except the group fed 100 mg/kg of REE-type B, all types of REE supplement decreased the weight of tibia ash compare to control group. The results show concentration the level of REE supplement from 50 mg/kg to 100 mg/kg, increased the weight of tibia ash.

Table 4.2-5: Average tibia ash weight (gr) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	REE type[1]	r	n	Ash weight (gr)		
					female	male	total
1	-	-	1	4	0.125 ±0.013	0.095 ±0.038	0.110 ±0.029
2	50	A	2	8	0.121 ±0.022	0.117 ±0.017	0.119 ±0.018
3	50	B	2	8	0.110 ±0.026	0.107 ±0.026	0.108 ±0.024
4	100	B	2	8	0.134 ±0.011	0.107 ±0.032	0.121 ±0.026
5	50	C	2	8	0.112 ±0.022	0.108 ±0.024	0.110 ±0.022
6	100	C	2	8	0.113 ±0.024	0.116 ±0.023	0.114 ±0.022
7	50	D	2	8	0.110 ±0.026	0.103 ±0.024	0.106 ±0.024
8	100	D	2	8	0.109 ±0.024	0.109 ±0.032	0.109 ±0.026
n				60	30	30	60
SEM					±0.004	±0.004	±0.010
significance					n.s	n.s	n.s

1) REE-type A: REE-Citrate (5.5% La, 17.5% Ce and 3.28% Pr); REE-type B: Lanthanum-Acetate; REE-type C: Lanthanum-Chloride; REE-type D: Lanthanum-Carbonate.

r= repetition.

n= number of samples.

n.s: no significant difference.

4.2.7. Calcium content of tibia ash

The average calcium content of tibia ash based on gram per kilogram and percentage of calcium in tibia ash in male and female Japanese quails are shown in Table 4.2-6 and Table 4.2-7. The effect of REE supplements was not significant through male or female Japanese quails but it was significant ($p<0.05$) without considering the gender. In female Japanese quails the REE-type A and the high level (100 mg/kg) of other types of REE, decreased calcium content and percentage of calcium of tibia ash up to 15.8% and the low level (50 mg/kg) of REE supplements increased calcium content and percentage of calcium of tibia ash up to 8.6% compare to control group. The male Japanese quails fed REE supplements with both low and high levels, had less calcium in tibia ash compare to quails in control group.

With or without considering the gender, increasing the level of REE supplements reduced calcium content and percentage of calcium of tibia ash in Japanese quails. The 100 mg/kg of REE-type B significantly ($p<0.05$) reduced calcium content and percentage of calcium in tibia ash compare to control group and the 50 mg/kg level of REE-type A.

Table 4.2-6: Average calcium content of tibia ash (gr/kg) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	REE type[1]	r	n	Calcium in tibia ash (gr/kg)		
					female	male	total
1	-	-	1	4	320.53 ±67.31	367.20 ±16.59	343.86 [ab] ±48.25
2	50	A	2	8	313.68 ±21.54	294.36 ±59.59	304.02 [ac] ±42.75
3	50	B	2	8	348.07 ±3.35	348.41 ±11.24	348.24 [b] ±7.68
4	100	B	2	8	269.83 ±73.39	302.72 ±60.78	286.27 [c] ±64.81
5	50	C	2	8	348.05 ±6.26	351.31 ±7.04	349.68 [b] ±6.41
6	100	C	2	8	316.46 ±42.66	315.27 ±84.37	315.86 [abc] ±61.89
7	50	D	2	8	341.62 ±26.74	344.03 ±8.78	342.82 [ab] ±18.47
8	100	D	2	8	293.18 ±72.84	309.02 ±20.67	301.10 [ac] ±50.29
n				60	30	30	60
SEM					±8.78	±8.47	±16.09
significance					n.s	n.s	*

1) REE-type A: REE-Citrate (5.5% La, 17.5% Ce and 3.28% Pr); REE-type B: Lanthanum-Acetate; REE-type C: Lanthanum-Chloride; REE-type D: Lanthanum-Carbonate.

r= repetition.

n= number of samples.

a-c: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

* $p<0.05$

Table 4.2-7: Percentage of calcium in tibia ash per bird and sex within experimental groups at 28 days old

Treatment	REE addition mg/kg	REE type[1]	r	n	%Ca per kg tibia ash		
					female	male	total
1	-	-	1	4	32.05 ±6.73	36.72 ±1.66	34.39 [ab] ±4.83
2	50	A	2	8	31.37 ±2.15	29.44 ±5.96	30.40 [ac] ±4.28
3	50	B	2	8	34.81 ±0.33	34.84 ±1.12	34.82 [b] ±0.77
4	100	B	2	8	26.98 ±7.341	30.27 ±6.08	28.63 [c] ±6.48
5	50	C	2	8	34.81 ±0.63	35.13 ±0.70	34.97 [b] ±0.64
6	100	C	2	8	31.65 ±4.27	31.53 ±8.44	31.59 [abc] ±6.19
7	50	D	2	8	34.16 ±2.67	34.40 ±0.88	34.28 [ab] ±1.85
8	100	D	2	8	29.32 ±7.28	30.90 ±2.07	30.11 [ac] ±5.03
n				60	30	30	60
SEM					±0.88	±0.85	±1.61
significance					n.s	n.s	*

1) REE-type A: REE-Citrate (5.5% La, 17.5% Ce and 3.28% Pr); REE-type B: Lanthanum-Acetate; REE-type C: Lanthanum-Chloride; REE-type D: Lanthanum-Carbonate.

r= repetition.

n= number of samples.

a-c: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

* $p<0.05$

4.2.8. Phosphor content of tibia ash

The average phosphor content of tibia ash based on gram per kilogram and percentage of phosphor in tibia ash in male and female Japanese quails are shown in Table 4.2-8 and

Table 4.2-9. The effect of REE supplements was significant through male Japanese quails ($p<0.01$) and without considering the gender ($p<0.001$). The Japanese quails fed 100 mg/kg of REE supplements had significantly ($p<0.05$) lower phosphor content and percentage of phosphor in tibia ash compare to the quails fed 50 mg/kg level of REE supplements. The low concentration (50 mg/kg) of REE supplements (except the REE-type A) increased phosphor content and percentage of phosphor of tibia ash up to 3.5% compare to control group. The high concentration (100 mg/kg) of REE supplements decreased phosphor content and percentage of phosphor of tibia ash up to 8.34% compare to control group.

The male Japanese quails fed 100 mg/kg of REE-type C and REE-type D had significantly lower phosphor content and percentage of phosphor of tibia ash compare to the ones fed 50 mg/kg of those types of REE ($p<0.01$) and compare to control group ($p<0.05$). The low concentration (50 mg/kg) of REE-type A significantly decreased phosphor content and percentage of phosphor of tibia ash in male Japanese quails compare to low concentration (50 mg/kg) of other types of REE supplement ($p<0.05$, $p<0.01$).

The REE-type A and the high level (100 mg/kg) of other types of REE, decreased phosphor content and percentage of phosphor of tibia ash up to 5.6% in female Japanese quails and up to 12.6% in male Japanese quails compare to control group. The low level (50 mg/kg) of REE supplements except the type A, increased phosphor content and percentage of phosphor of tibia ash up to 6% in female Japanese quails and up to 3% in male Japanese quails compare to control group. With or without considering the gender, increasing the concentration of REE supplements reduced phosphor content and percentage of phosphor of tibia ash in Japanese quails.

Table 4.2-8: Average phosphor content of tibia ash (gr/kg) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	REE type[1]	r	n	Phosphor in tibia ash (gr/kg)		
					female	male	total
1	-	-	1	4	169.65 ±31.48	176.43 ^{acd} ±7.51	173.04 ^{ab} ±1.91
2	50	A	2	8	160.14 ±14.85	160.38 ^{ab} ±4.64	160.26 ^{ac} ±1.02
3	50	B	2	8	179.60 ±6.69	178.73 ^{cd} ±9.35	179.16 ^{b} ±0.75
4	100	B	2	8	161.50 ±7.43	166.84 ^{abc} ±8.73	164.17 ^{ac} ±0.80
5	50	C	2	8	174.39 ±5.38	179.71 ^{cd} ±6.26	177.05 ^{b} ±0.61
6	100	C	2	8	164.67 ±15.51	155.79 ^{b} ±11.94	160.23 ^{ac} ±1.37
7	50	D	2	8	175.19 ±12.56	181.56 ^{d} ±12.98	178.38 ^{b} ±1.23
8	100	D	2	8	163.05 ±16.96	154.14 ^{b} ±13.11	158.60 ^{c} ±1.48
n				60	30	30	60
SEM					±2.53	±2.55	4.10
significance					n.s	**	***

1) REE-type A: REE-Citrate (5.5% La, 17.5% Ce and 3.28% Pr); REE-type B: Lanthanum-Acetate; REE-type C: Lanthanum-Chloride; REE-type D: Lanthanum-Carbonate.

r= repetition.

n= number of samples.

a-c: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

** $p<0.01$

*** $p<0.001$

Table 4.2-9: Percentage of phosphor in tibia ash per bird and sex within experimental groups at 28 days old

Treatment	REE addition mg/kg	REE type[1]	r	n	%P per kg tibia ash		
					female	male	total
1	-	-	1	4	16.97 ±3.15	17.64 ^{acd} ±0.75	17.30 ^{ab} ±1.91
2	50	A	2	8	16.01 ±1.48	16.04 ^{ab} ±0.46	16.03 ^{ac} ±1.02
3	50	B	2	8	17.96 ±0.67	17.87 ^{cd} ±0.94	17.92 ^{b} ±0.75
4	100	B	2	8	16.15 ±0.74	16.68 ^{abc} ±0.87	16.42 ^{ac} ±0.80
5	50	C	2	8	17.44 ±0.54	17.97 ^{cd} ±0.63	17.71 ^{b} ±0.61
6	100	C	2	8	16.47 ±1.55	15.58 ^{b} ±1.19	16.02 ^{ac} ±1.37
7	50	D	2	8	17.52 ±1.26	18.16 ^{d} ±1.30	17.84 ^{b} ±1.23
8	100	D	2	8	16.31 ±1.70	15.41 ^{b} ±1.31	15.86 ^{c} ±1.48
n				60	30	30	60
SEM					±0.25	±0.25	±0.41
significance					n.s	**	***

1) REE-type A: REE-Citrate (5.5% La, 17.5% Ce and 3.28% Pr); REE-type B: Lanthanum-Acetate; REE-type C: Lanthanum-Chloride; REE-type D: Lanthanum-Carbonate.

r= repetition.

n= number of samples.

a-c: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

** $p<0.01$

*** $p<0.001$

4.2.9. Calcium to phosphor ratio of tibia ash

The calcium to phosphor ratio of tibia ash in male and female Japanese quails at 28 days old is shown in Table 4.2-10. There was no significant difference between control group and REE supplemented groups. In female Japanese quails low level of all REE supplements increased calcium to phosphor ratio for 2.65% to 5.82% and high level of REE supplements (except REE-type C) decreased calcium to phosphor

ratio for 5.3% to 11.1% compare to control group. In male Japanese quails REE supplements decreased calcium to phosphor ratio for 1.44% to 13% compare to control group. Without considering the gender, REE supplements (except the REE-type C) decreased calcium to phosphor ratio for 1.5% to 11.6% compare to control group. The results show by increasing the concentration of REE supplement from 50 mg/kg to 100 mg/kg, the calcium to phosphor ratio decreased.

Table 4.2-10: Calcium to phosphorus ratio in tibia per bird and sex within experimental groups at 28 days old

Treatment	REE addition mg/kg	REE type[1]	r	n	Ca/P ratio in tibia		
					female	male	total
1	-	-	1	4	1.89 ±0.05	2.08 ±0.01	1.98 ±0.12
2	50	A	2	8	1.97 ±0.19	1.84 ±0.36	1.90 ±0.28
3	50	B	2	8	1.94 ±0.088	1.95 ±0.05	1.95 ±0.07
4	100	B	2	8	1.68 ±0.49	1.81 ±0.32	1.75 ±0.39
5	50	C	2	8	2.00 ±0.05	1.96 ±0.09	1.98 ±0.07
6	100	C	2	8	1.94 ±0.35	2.05 ±0.65	2.00 ±0.49
7	50	D	2	8	1.95 ±0.06	1.90 ±0.09	1.93 ±0.08
8	100	D	2	8	1.79 ±0.31	2.01 ±0.09	1.90 ±0.25
n				60	30	30	60
SEM					±0.05	±0.05	±0.1
significance					n.s	n.s	n.s

1) REE-type A: REE-Citrate (5.5% La, 17.5% Ce and 3.28% Pr); REE-type B: Lanthanum-Acetate; REE-type C: Lanthanum-Chloride; REE-type D: Lanthanum-Carbonate.

r= repetition.

n= number of samples.

n.s: no significant difference.

4.2.10. Magnesium content of tibia ash

The average magnesium content and percentage of magnesium of tibia ash in female and male Japanese quails at 28 days of age are shown in Table 4.2-11 and

Table 4.2-12. There was no significant difference between control group and REE supplemented groups. In female Japanese quails REE supplements except the high level of REE-type B, decreased Magnesium content and percentage of magnesium of tibia ash for 3.54% to 18.53% compare to control group. The male Japanese quails fed REE supplements except the ones fed REE-type D had 4.1% to 21.95% higher magnesium content and percentage of magnesium in tibia ash compare the quails in Control group. Without considering the gender the REE-type A and the high level (100mg/kg) of REE-type B and C increased magnesium content and percentage of magnesium of tibia ash up to 7.65% compare to control group. But the Japanese quails fed REE-type D and low level (50mg/kg) of REE-type B and C, had up to 13.4% lower magnesium content and percentage of magnesium in tibia ash compare the quails in control group. The results show that increasing the concentration of REE supplements (except the REE-type D) from 50 mg/kg to 100 mg/kg, the magnesium content and percentage of magnesium of tibia ash increased.

Table 4.2-11: Average magnesium content of tibia ash (mg/kg) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	REE type[1]	r	n	Magnesium in tibia ash (mg/kg)		
					female	male	total
1	-	-	1	4	10625 ±120	8154 ±2978	9390 ±2235
2	50	A	2	8	10249 ±2978	9418 ±1439	9833 ±2210
3	50	B	2	8	8705 ±2393	8509 ±2361	8607 ±2203
4	100	B	2	8	11193 ±700	9023 ±2443	10108 ±2028
5	50	C	2	8	9339 ±2047	8488 ±1556	8913 ±1744
6	100	C	2	8	9044 ±2798	9944 ±1780	9494 ±2224
7	50	D	2	8	8746 ±1940	7831 ±2294	8288 ±2027
8	100	D	2	8	8656 ±1897	7657 ±2326	8156 ±2036
n				60	30	30	60
SEM					±384	±364	±744
significance					n.s	n.s	n.s

1) REE-type A: REE-Citrate (5.5% La, 17.5% Ce and 3.28% Pr); REE-type B: Lanthanum-Acetate; REE-type C: Lanthanum-Chloride; REE-type D: Lanthanum-Carbonate.

r= repetition.

n= number of samples.

n.s: no significant difference.

Table 4.2-12: Percentage of magnesium in tibia ash per bird and sex within experimental groups at 28 days old

Treatment	REE addition mg/kg	REE type[1]	r	n	% Mg per Kg tibia ash		
					female	male	total
1	-	-	1	4	1.06 ±0.01	0.82 ±0.30	0.94 ±0.22
2	50	A	2	8	1.03 ±0.30	0.94 ±0.14	0.98 ±0.22
3	50	B	2	8	0.87 ±0.24	0.85 ±0.24	0.86 ±0.22
4	100	B	2	8	1.12 ±0.07	0.90 ±0.24	1.01 ±0.20
5	50	C	2	8	0.93 ±0.21	0.85 ±0.16	0.89 ±0.17
6	100	C	2	8	0.90 ±0.28	0.99 ±0.18	0.95 ±0.22
7	50	D	2	8	0.88 ±0.19	0.78 ±0.23	0.83 ±0.20
8	100	D	2	8	0.87 ±0.19	0.77 ±0.23	0.82 ±0.20
n				60	30	30	60
SEM					±0.04	±0.04	±0.07
significance					n.s	n.s	n.s

1) REE-type A: REE-Citrate (5.5% La, 17.5% Ce and 3.28% Pr); REE-type B: Lanthanum-Acetate; REE-type C: Lanthanum-Chloride; REE-type D: Lanthanum-Carbonate.

r= repetition.

n= number of samples.

n.s: no significant difference.

4.2.11. Calcium content of blood serum

The average calcium content of blood serum of Japanese quails at 28 days old is shown based on millimolar per liter (mmol/lit) in Table 4.2-13. The effect of REE supplements on calcium content of serum was significant ($p<0.05$) without considering the gender but was not significant in each of male or female Japanese quails. The female Japanese quails fed REE supplements except the ones fed 100 mg/kg of REE-type B and 50 mg/kg of REE-type C, had 6.74% to 41.87% higher calcium content in their blood serum compare to the quails in control group. In male

Japanese quails all types of REE supplements increased calcium content of serum for 17.5% to 97.33% compare to control group. Without considering the gender, all types of REE supplements increased calcium content of tibia ash for 8.32% to 68.1% compare to control group. The Japanese quails fed 100 mg/kg of REE-type C had significantly ($p<0.05$) higher serum calcium content compare to quails in control group. Without considering the gender and also in female Japanese quails, increasing the concentration of REE supplement (except the REE-type C) decreased the serum calcium content. But in male Japanese quails, increasing the concentration of REE supplements (except the REE-type D) increased the calcium content of serum. The calcium content of serum in male Japanese quails was significantly ($p<0.05$) for 16.9% higher than that in female Japanese quails.

Table 4.2-13: Average calcium content of blood serum (mmol/lit) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	REE type[1]	r	n	Calcium in serum (mmol/lit)		
					female	male	total
1	-	-	1	4	9.65 ±3.96	8.63 ±0.53	9.14 [a] ±2.38
2	50	A	2	8	10.30 ±0.72	14.09 ±1.15	12.19 [a] ±2.21
3	50	B	2	8	13.31 ±2.10	10.14 ±3.16	11.73 [a] ±3.00
4	100	B	2	8	8.33 ±1.08	11.48 ±3.30	9.90 [a] ±2.83
5	50	C	2	8	9.15 ±0.83	15.34 ±4.69	12.24 [ab] ±4.55
6	100	C	2	8	13.69 ±3.35	17.03 ±5.05	15.36 [b] ±4.35
7	50	D	2	8	11.73 ±1.82	13.70 ±3.24	12.71 [ab] ±2.65
8	100	D	2	8	11.68 ±5.26	12.26 ±2.63	11.97 [a] ±3.86
n				8	30	30	60
SEM					±0.55	±0.71	±1.2
significance					n.s	n.s	*

1) REE-type A: REE-Citrate (5.5% La, 17.5% Ce and 3.28% Pr); REE-type B: Lanthanum-Acetate; REE-type C: Lanthanum-Chloride; REE-type D: Lanthanum-Carbonate.

r= repetition.

n= number of samples.

a-b: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

* p<0.05

4.2.12. Phosphor content of blood serum

The average phosphor content of blood serum of Japanese quails at 28 days old is shown based on milligram per liter (mg/lit) in Table 4.2-14. The effect of REE supplements on phosphor content of serum was not significant. Without considering the gender, the REE supplements in both concentrations (except the 100 mg/kg level of REE- type D) increased the serum phosphor content for 7.8% to 32.04% compare to control group. The female Japanese quails fed REE supplements except the ones fed 50 mg/kg of REE-type C, had 12.37% to 56% higher phosphor content in their blood serum compare to the quails in control group. In male Japanese quails the REE supplements (except the REE-type A and 100 mg/kg level of REE-type D) increased the serum phosphor content for compare to control group. With or without considering the gender, increasing the level of REE supplement (except the REE-type C) from 50 mg/kg to 100 mg/kg, decreased the phosphor content of blood serum in Japanese quails.

Table 4.2-14: Average phosphorus content of blood serum (mg/lit) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	REE type[1]	r	n	Phosphor in serum (mg/lit)		
					female	male	total
1	-	-	1	4	54.97 ±7.42	67.02 ±12.23	60.99 ±10.80
2	50	A	2	8	81.45 ±15.3	60.92 ±8.92	71.18 ±23.33
3	50	B	2	8	85.70 ±13.65	75.35 ±18.80	80.53 ±26.19
4	100	B	2	8	71.03 ±14.93	69.10 ±21.66	70.06 ±26.39
5	50	C	2	8	52.19 ±12.04	79.29 ±26.46	65.74 ±23.92
6	100	C	2	8	61.77 ±10.21	91.80 ±18.52	78.93 ±21.54
7	50	D	2	8	70.95 ±24.81	74.43 ±10.57	72.69 ±40.54
8	100	D	2	8	62.31 ±14.70	56.52 ±11.08	59.41 ±12.45
n				8	30	30	60
SEM					±5.64	±3.40	±9.11
significance					n.s	n.s	n.s

1) REE-type A: REE-Citrate (5.5% La, 17.5% Ce and 3.28% Pr); REE-type B: Lanthanum-Acetate; REE-type C: Lanthanum-Chloride; REE-type D: Lanthanum-Carbonate.
r= repetition.
n= number of samples.
n.s: no significant difference.

4.2.13. Magnesium content of blood serum

The average magnesium content of blood serum of Japanese quails at 28 days old is shown based on milligram per liter (mg/lit) in Table 4.2-15. The effect of REE supplements on serum magnesium content was significant ($p<0.05$) in each female or male Japanese quails. Without considering the gender, REE supplements (except the type A and 100 mg/kg of REE-type D) decreased the serum phosphor content for 3.1% to 27% compare to control group.

In female Japanese quails, increasing the concentration of REE supplements from 50 mg/kg to 100 mg/kg decreased the magnesium content of serum. The female Japanese quails fed 50 mg/kg of REE-type C had significantly ($p<0.05$) higher magnesium content in serum than the ones fed 100 mg/kg of REE-type C. Also serum magnesium content in female Japanese quails fed 50 mg/kg of REE-type C was significantly ($p<0.05$) higher than the ones fed REE-type B and REE-type D.

In male Japanese quails, all types of REE supplements decreased magnesium content of serum up to 58% compare to control group. The male Japanese quails fed 50 mg/kg of REE-type C had significantly ($p<0.05$) lower serum magnesium content than the quails in control group and the ones fed 100 mg/kg of REE-type D. Within group 5 which were fed 50 mg/kg of REE-type C, magnesium serum content of female Japanese quails was significantly ($p<0.001$) higher than that of male Japanese quails.

Table 4.2-15: Average magnesium content of blood serum (mg/lit) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	REE type[1]	r	n	Magnesium in serum (mg/lit)		
					female	male	total
1	-	-	1	4	45480 ^{abc} ±3104	67640 ^{a} ±16751	56560 ±16138
2	50	A	2	8	69609 ^{ab} ±35517	48381 ^{ab} ±17124	58995 ±28196
3	50	B	2	8	45776 ^{ac} ±4707	40783 ^{ab} ±1107	44112 ±4493
4	100	B	2	8	44685 ^{ac} ±3012	62400 ^{a} ±18121	54808 ±16027
5	50	C	2	8	78221 ^{b} ±39018	28328 ^{b} ±454	53274 ±38944
6	100	C	2	8	37767 ^{c} ±859	44887 ^{ab} ±5171	41327 ±5119
7	50	D	2	8	46060 ^{ac} ±12203	40803 ^{ab} ±2543	43807 ±9193
8	100	D	2	8	45555 ^{ac} ±7439	66285 ^{a} ±30868	57401 ±24853
n				8	30	30	60
SEM					±3998	±4130	±8160
significance					*	*	n.s

1) REE-type A: REE-Citrate (5.5% La, 17.5% Ce and 3.28% Pr); REE-type B: Lanthanum-Acetate; REE-type C: Lanthanum-Chloride; REE-type D: Lanthanum-Carbonate.

r= repetition.

n= number of samples.

a-c: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

* $p<0.05$

4.3. Experiment 3

4.3.1. Body weight

The average body weight of Japanese quails based on gram at different ages is shown in Table 4.3-1. The effect of REE supplement on body weight was significant

(p<0.001) during the experiment. The Japanese quails fed 800 mg/kg of REE-citrate had significantly (p<0.001) lower body weight than the quails in other experimental groups. At the age of 14 days and 21 days old, increasing the concentration of REE-citrate from 50 mg/kg to 400 mg/kg increased average body weight of quails up to 4% compare to control group. At 28 days old, diets supplemented with 100 mg/kg and 400 mg/kg of REE-citrate significantly (p<0.05) increased average body weight for 4.7% to 5.5% compare to control group.

Table 4.3-1: Average body weight per bird (gr) in experimental groups at different ages

Treatment	REE addition mg/kg	r	n	average body weight (gr/bird)				
				1 day old	7 days old	14 days old	21 days old	28 days old
1	-	3	45	8.16 ±0.56	23.14 ^{a} ±2.81	46.62 ^{a} ±6.17	72.82 ^{a} ±8.16	96.86 ^{a} ±9.31
2	50	3	45	8.02 ±0.51	22.52 ^{a} ±2.72	46.73 ^{a} ±6.19	72.51 ^{a} ±7.20	100.10 ^{ab} ±9.20
3	100	3	45	8.19 ±0.51	22.50 ^{a} ±2.70	47.28 ^{a} ±6.26	75.68 ^{a} ±8.93	101.40 ^{b} ±9.80
4	400	3	45	8.09 ±0.45	23.03 ^{a} ±2.60	48.36 ^{a} ±5.80	75.71 ^{a} ±9.32	102.16 ^{b} ±9.69
5	800	3	45	7.99 ±0.45	19.19 ^{b} ±1.85	34.10 ^{b} ±4.21	57.48 ^{b} ±8.96	85.29 ^{c} ±9.91
n			225	225	225	225	225	225
SEM				±0.07	±0.39	±0.89	±1.25	±1.45
significance				n.s	***	***	***	***

r= repetition.

n= number of samples.

a-b: means within columns with no common superscripts are significantly different (p<0.05).

n.s: no significant difference.

* p<0.001

4.3.2. Mortality

The average percentage of mortality (dead and eliminated animals) during the whole period of experiment was 1.3%. The weak quails which had much lower weight than the average and also dead quails were eliminated. The rate of mortality in Japanese quails was higher during the first week of experiment. As there was no

special disease during the experiment, the rate of mortality significantly dropped to less than 0.5% after first week of trial.

4.3.3. Weight gain

The average weight gain of Japanese quails with standard deviations at different ages is shown in Table 4.3-2. The effect of REE supplement on the average weight gain was significant ($p<0.001$) during the experiment. The Japanese quails fed 800 mg/kg of REE-citrate had significantly ($p<0.001$) lower weight gain than the quails in other groups until age of 14 days old. The average weight gain of Japanese quails fed 800 mg/kg of REE-citrate was increased after 2 weeks old and was significantly ($p<0.05$) higher than average weight gain in control group during 3-4 weeks of age. During the second, third and fourth weeks of age, the Japanese quails fed 100mg/kg and 400 mg/kg of REE-citrate supplement, gained up to 10% more weight than the ones in control group. Form 3 to 4 weeks of age supplemented diets with 50 mg/kg and 800 mg/kg of REE-citrate, significantly ($p<0.05$) increased weight gain of Japanese quails compare to control group. During 7-28 days old, increasing the concentration of REE-citrate from 50 mg/kg to 400 mg/kg increased weight gain in Japanese quails up to 7.4% compare to control.

Table 4.3-2: Average weight gain (gr) per bird in experimental groups at different ages

Treatment	REE additoin (mg/kg)	r	Average weight gain (gr/bird)								
			age (days old)								
			1-7	7-14	14-21	21-28	1-21	1-28	7-21	7-28	14-28
1	-	3	14.99 ^{a} ±0.77	23.48 ^{a} ±1.06	26.22 ±0.77	24.05 ^{a} ±0.15	64.68 ^{a} ±1.75	88.73 ^{a} ±1.84	49.70 ^{a} ±1.10	73.75 ^{a} ±1.16	50.27 ±0.92
2	50	3	14.50 ^{a} ±0.09	24.23 ^{a} ±0.91	25.78 ±1.15	27.57 ^{b} ±1.45	64.51 ^{a} ±1.67	92.08 ^{a} ±0.22	50.01 ^{a} ±1.68	77.58 ^{a} ±0.26	53.35 ±0.67
3	100	3	14.32 ^{a} ±1.35	24.75 ^{a} ±0.40	28.42 ±2.13	25.61 ^{ab} ±2.26	67.48 ^{a} ±3.51	93.10 ^{a} ±3.14	53.17 ^{a} ±2.42	78.78 ^{a} ±1.83	54.03 ±1.96
4	400	3	14.95 ^{a} ±0.57	25.35 ^{a} ±1.61	27.38 ±1.99	26.46 ^{ab} ±0.10	67.67 ^{a} ±3.81	94.13 ^{a} ±3.87	52.72 ^{a} ±3.46	79.18 ^{a} ±3.51	53.83 ±2.06
5	800	3	11.20 ^{b} ±0.18	14.91 ^{b} ±1.24	23.38 ±7.393	27.81 ^{b} ±1.32	49.49 ^{b} ±6.32	77.30 ^{b} ±5.51	38.29 ^{b} ±6.16	66.10 ^{b} ±5.37	51.19 ±6.60
SEM			±0.40	±0.66	±1.61	±0.70	±1.21	±1.48	±1.19	±1.34	±1.74
significance			***	***	n.s	*	***	***	***	***	n.s

r= repetition.

a-b: means within columns with no common superscripts are significantly different (p<0.05).
n.s: no significant difference.
* p<0.05
*** p<0.001

4.3.4. Feed consumption

The average feed consumption of Japanese quails with standard deviations at different ages is shown in Table 4.3-3. Only during the first week of experiment the effect of REE supplement on the average feed consumption was significant (p<0.001). During 1-7 days of age, the REE-citrate supplement significantly (p<0.001) decreased feed consumption of Japanese quails for 5.8% to 12.76% compare to control group. From 1 to 7 days of age the average feed consumption of Japanese quails fed 50 mg/kg of REE-citrate was significantly (p<0.05) lower than that of the quails fed high concentrations (400 and 800 mg/kg) of REE-citrate. Also the Japanese quails fed 100 mg/kg of REE-citrate during 1-7 days of age, significantly (p<0.05) consumed less feed than the one fed 800 mg/kg of REE-citrate. During the experimental period (1-28 days of age) the REE-citrate supplement decreased average feed consumption of Japanese quails up to 9% compare to control group. The Japanese quails fed 100 mg/kg of REE-citrate consumed the lowest amount of feed during the experiment (1-28 days).

Table 4.3-3: Average feed consumption (gr) per bird in experimental groups at different ages

Treatment	REE addition (mg/kg)	r	Average feed consumption (gr/bird)								
			age (days old)								
			1-7	7-14	14-21	21-28	1-21	1-28	7-21	7-28	14-28
1	-	3	40.20 [a] ±1.54	77.33 ±5.58	111.04 ±4.86	137.54 ±4.059	228.58 ±11.39	366.12 ±14.77	188.38 ±10.34	325.92 ±13.92	248.59 ±8.34
2	50	3	35.07 [b] ±0.18	77.29 ±5.56	115.03 ±7.42	130.71 ±8.626	227.39 ±11.95	358.10 ±20.55	192.32 ±12.11	323.04 ±20.71	245.75 ±15.69
3	100	3	35.92 [bc] ±0.17	73.89 ±4.70	106.64 ±8.18	117.00 ±3.418	216.45 ±12.66	333.44 ±16.03	180.53 ±12.50	297.53 ±15.87	223.64 ±11.44
4	400	3	36.84 [cd] ±0.34	78.73 ±2.78	111.51 ±4.95	124.25 ±4.566	227.08 ±7.09	351.34 ±11.57	190.24 ±6.86	314.49 ±11.32	235.76 ±9.51
5	800	3	37.88 [d] ±1.12	73.41 ±1.48	115.71 ±11.20	132.10 ±20.364	226.99 ±13.37	359.09 ±32.28	189.12 ±12.63	321.21 ±31.86	247.81 ±30.64
SEM			±0.55	±2.39	±4.67	±6.26	±6.89	±12.23	±6.62	±12.06	±10.34
significance			***	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

r= repetition.

a-d: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

*** $p<0.001$

4.3.5. Feed conversion ratio

The average feed conversion ratio of Japanese quails with standard deviations at different ages is shown in Table 4.3-4. The results show during the experiment (except 14-21 days of age), the effect of REE-citrate supplement on feed conversion ratio of Japanese quails was significant ($p<0.05$, $p<0.001$). During one week and two weeks of age supplemented diets with 800 mg/kg of REE-citrate significantly ($p<0.001$) increased feed conversion ratio of Japanese quails compare to other experimental groups. But during the 3 and 4 weeks of age, feed conversion ratio in the group supplemented 800 mg/kg of REE-citrate dropped compare to control group. During 1-7 days and 7-14 days of age, REE-citrate supplement with concentrations of 50,100 and 400 mg/kg decreased the feed conversion ratio up to 10% compare to control group. From 3 to 4 weeks of age, the Japanese quails fed different levels of REE-citrate had significantly ($p<0.05$) up to 19.6% lower feed conversion ratio than the ones in control group. During the experiment (1-28 days) REE supplemented diets (except the 800 mg/kg concentration) decreased feed conversion ratio of Japanese quails up to 13.1% compare to control diet. The Japanese quails fed 100 mg/kg of REE-citrate had the lowest feed conversion ratio compare to quails in other groups.

Table 4.3-4: Average feed conversion ratio per bird in experimental groups at different ages

Treatment	REE addition (mg/kg)	r	Feed conversion ratio								
			age (days old)								
			1-7	7-14	14-21	21-28	1-21	1-28	7-21	7-28	14-28
1	-	3	2.69 [a] ±0.24	3.29 [a] ±0.13	4.24 ±0.30	5.72 [a] ±0.19	3.54 [a] ±0.22	4.13 [ab] ±0.19	3.79 [a] ±0.21	4.42 ±0.18	4.95 ±0.24
2	50	3	2.42 [a] ±0.01	3.19 [a] ±0.24	4.48 ±0.48	4.74 [b] ±0.20	3.53 [a] ±0.27	3.89 [a] ±0.23	3.85 [a] ±0.36	4.16 ±0.27	4.61 ±0.29
3	100	3	2.52 [a] ±0.24	2.98 [a] ±0.15	3.77 ±0.45	4.60 [b] ±0.53	3.22 [a] ±0.28	3.59 [a] ±0.28	3.40 [a] ±0.28	3.78 ±0.28	4.15 ±0.36
4	400	3	2.47 [a] ±0.07	3.11 [a] ±0.12	4.08 ±0.12	4.70 [b] ±0.16	3.36 [a] ±0.09	3.73 [a] ±0.04	3.61 [a] ±0.12	3.97 ±0.04	4.38 ±0.03
5	800	3	3.38 [b] ±0.07	4.94 [b] ±0.38	4.04 ±0.23	4.75 [b] ±0.71	4.65 [b] ±0.80	4.68 [b] ±0.74	5.05 [b] ±1.11	4.91 ±0.88	4.95 ±1.27
SEM			±0.09	±0.14	±0.16	±0.27	±0.19	±0.20	±0.27	±0.24	±0.34
significance			***	***	n.s	*	*	*	*	n.s	n.s

r= repetition.

a-b: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

* $p<0.05$

*** $p<0.001$

4.3.6. Tibia ash weight

The average weight of tibia ash in male and female Japanese quails at 28 days old is shown in Table 4.3-5. The effect of REE-citrate on tibia ash weight was significant ($p<0.001$) in general and in each female or male Japanese quails ($p<0.05$, $p<0.01$). The ash weight in Japanese quails fed 800 mg/kg of REE-citrate was significantly ($p<0.001$) lower than that in Japanese quails of other groups. Also the Japanese quails fed 400 mg/kg of REE-citrate had significantly ($p<0.01$) heavier tibia ash than the Japanese quails in other experimental groups. The tibia ash weight in REE supplemented groups (except the 400 mg/kg group) was lower than that in control group.

Within the female Japanese quails, the group fed 800 mg/kg of REE supplement had significantly ($p<0.05$) lower tibia ash than other groups except the ones fed 50 mg/kg of REE supplement. The female Japanese quails fed 400 mg/kg of REE-citrate had significantly ($p<0.05$) higher tibia ash than the ones fed 50 and 800 mg/kg of

REE-citrate. The tibia ash weight in REE supplemented groups (except the 400 mg/kg group) was lower than that in control group. In male Japanese quails, the diet supplemented with 800 mg/kg of REE-citrate significantly ($p<0.05$) decreased tibia ash compare to other experimental groups. The male Japanese quails fed 400 mg/kg of REE-citrate had heavier tibia ash than the ones in other groups.

In general the tibia ash weight in Japanese quails fed 800 mg/kg of REE-citrate was the lowest and in Japanese quails fed 400 mg/kg was the highest among the experimental groups.

Table 4.3-5: Average tibia ash weight (gr) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	Ash weight (gr)		
				female	male	total
1	-	3	12	0.127 ^{ab} ±0.010	0.127 ^{a} ±0.011	0.127 ^{ab} ±0.010
2	50	3	12	0.116 ^{bc} ±0.013	0.128 ^{a} ±0.012	0.122 ^{a} ±0.014
3	100	3	12	0.126 ^{ab} ±0.020	0.123 ^{a} ±0.012	0.124 ^{a} ±0.016
4	400	3	12	0.138 ^{a} ±0.011	0.134 ^{a} ±0.006	0.136 ^{b} ±0.010
5	800	3	12	0.107 ^{c} ±0.017	0.107 ^{b} ±0.007	0.107 ^{c} ±0.012
n			60	30	30	60
SEM				±0.003	±0.002	±0.004
significance				*	**	***

r= repetition.

n= umber of samples.

a-c: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

* $p<0.05$

** $p<0.01$

*** $p<0.001$

4.3.7. Calcium content of tibia ash

The average calcium content of tibia ash and percentage of calcium in tibia ash of male and female Japanese quails at 28 days old are shown in Table 4.3-6 and Table 4.3-7. The effect of REE supplement was significant ($p<0.05$) only in female Japanese quails. Without considering gender, the REE supplemented Japanese quails except the ones fed 50 mg/kg of REE had lower calcium content and percentage of calcium in tibia ash than the quails in control group.

The female Japanese quails fed 50 mg/kg of REE-citrate had significantly ($p<0.05$) up to 5% higher calcium content and percentage of calcium in tibia ash than the ones in other groups. In female Japanese quails, REE-citrate supplement with concentrations of 100, 400 and 800 mg/kg decreased the calcium content and percentage of calcium in tibia ash compare to control group. The male Japanese quails fed 50,400 and 800 mg/kg of REE supplement, had lower calcium content and percentage of calcium in tibia ash than the ones in control group. Within group fed 50 mg/kg of REE-citrate, the female Japanese quails had significantly ($p<0.05$) higher Calcium content of tibia ash than male Japanese quails.

Table 4.3-6: Average calcium content of tibia ash (gr/kg) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	Calcium in tibia ash (gr/kg)		
				female	male	total
1	-	3	12	361.84 [ab] ±3.37	360.30 ±6.07	361.07 ±4.75
2	50	3	12	370.88 [a] ±20.24	357.43 ±6.45	364.15 ±15.95
3	100	3	12	353.34 [b] ±3.63	361.64 ±4.69	357.49 ±5.90
4	400	3	12	356.73 [b] ±6.63	359.14 ±2.78	357.93 ±5.01
5	800	3	12	356.34 [b] ±3.10	354.24 ±4.51	355.29 ±3.85
n			60	30	30	60
SEM				±2.03	±0.98	±2.45
significance				*	n.s	n.s

r= repetition.

n= umber of samples.

a-b: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

* p<0.05

Table 4.3-7: Percentage of calcium in tibia ash per bird and sex within experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	%Ca per kg tibia ash		
				female	male	total
1	-	3	12	36.18 [ab] ±0.34	36.03 ±0.61	36.11 ±0.48
2	50	3	12	37.09 [a] ±2.02	35.74 ±0.65	36.42 ±1.60
3	100	3	12	35.33 [b] ±0.36	36.16 ±0.47	35.75 ±0.59
4	400	3	12	35.67 [b] ±0.66	35.91 ±0.28	35.79 ±0.50
5	800	3	12	35.63 [b] ±0.31	35.42 ±0.45	35.53 ±0.39
n			60	30	30	60
SEM				±0.20	±0.10	±0.25
significance				*	n.s	n.s

r= repetition.

n= umber of samples.

a-b: means within columns with no common superscripts are significantly different (p<0.05).

n.s: no significant difference.

* p<0.05

4.3.8. Phosphor content of tibia ash

The average phosphor content of tibia ash and percentage of phosphor in tibia ash of male and female Japanese quails at 28 days old are shown in Table 4.3-8 and Table 4.3-9. The REE supplement had no significant effect on phosphor content and percentage of phosphor in tibia ash of Japanese quails. Without considering gender, the REE supplemented quails had lower phosphor content and percentage of phosphor in tibia ash.

The REE- citrate supplement with different concentrations (except 50 mg/kg) decreased phosphor content and percentage of phosphor up to 2.5% in female Japanese quails compare to control group. The female Japanese quails fed 50 mg/kg of REE-citrate had slightly higher phosphor content in tibia ash compare to control

group. Increasing the concentration of REE supplement decreased the phosphor content and percentage of phosphor in female Japanese quails. The male Japanese quails fed 50 and 800 mg/kg of REE-citrate, had up to 3% lower phosphor content and percentage of phosphor in tibia ash than the ones in control group. The diets supplemented with 100 and 400 mg/kg of REE-citrate slightly increased phosphor content and percentage phosphor of tibia ash in male Japanese quails compare to control diet.

Table 4.3-8: Average phosphor content of tibia ash (gr/kg) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	Phosphor in tibia ash (gr/kg)		
				female	male	total
1	-	3	12	180.21 ±3.64	179.13 ±5.20	179.67 ±4.32
2	50	3	12	183.16 ±10.20	175.19 ±7.10	179.17 ±9.35
3	100	3	12	176.04 ±4.56	179.57 ±3.50	177.80 ±4.29
4	400	3	12	179.26 ±2.82	180.29 ±2.21	179.78 ±2.47
5	800	3	12	176.41 ±3.63	173.81 ±3.75	175.11 ±3.77
n			60	30	30	60
SEM				1.10	0.93	1.65
significance				n.s	n.s	n.s

r= repetition.

n= umber of samples.

n.s: no significant difference.

Table 4.3-9: Percentage of phosphor in tibia ash per bird and sex within experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	%P per kg tibia ash		
				female	male	total
1	-	3.00	12	18.02 ±0.36	17.91 ±0.52	17.97 ±0.43
2	50	3	12	18.32 ±1.02	17.52 ±0.71	17.92 ±0.94
3	100	3	12	17.60 ±0.46	17.96 ±0.35	17.78 ±0.43
4	400	3	12	17.93 ±0.28	18.03 ±0.22	17.98 ±0.25
5	800	3	12	17.64 ±0.36	17.38 ±0.38	17.51 ±0.38
n			60	30	30	60
SEM				±0.11	±0.1	±0.17
significance				n.s	n.s	n.s

r= repetition.

n= umber of samples.

n.s: no significant difference.

4.3.9. Calcium to phosphor ratio of tibia

The calcium to phosphor ratio of tibia in Japanese quails at the age of 28 day old is shown in Table 4.3-10. As the result shows, calcium to phosphor ratio in tibia of Japanese quails was significantly ($p<0.05$) affected by REE supplement. Without considering gender, the Japanese quails fed 400 mg/kg of REE-citrate had significantly ($p<0.05$) lower calcium to phosphor ratio than the ones fed 50 and 800 mg/kg of REE-citrate. There was no significant difference between control group and the REE supplemented groups in each male or female Japanese quails.

Both male and female Japanese quails fed 400 mg/kg of REE supplement had the lowest calcium to phosphor ratio in their tibia among the experimental groups. In female Japanese quails increasing the concentration of REE-citrate decreased the calcium to phosphor ratio of tibia ash. The diets supplemented with 50 mg/kg or 800 mg/kg of REE-citrate slightly increased the calcium to phosphor ratio of tibia in male and female Japanese quails.

Table 4.3-10: Calcium to phosphorus ratio in tibia per bird and sex within experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	Ca/P ratio in tibia		
				female	male	total
1	-	3	12	2.01 ±0.03	2.01 ±0.03	2.01 [ab] ±0.03
2	50	3	12	2.03 ±0.01	2.04 ±0.07	2.03 [a] ±0.05
3	100	3	12	2.01 ±0.04	2.01 ±0.04	2.01 [ab] ±0.04
4	400	3	12	1.99 ±0.01	1.99 ±0.03	1.99 [b] ±0.02
5	800	3	12	2.02 ±0.05	2.04 ±0.04	2.03 [a] ±0.04
n			60	30	30	60
SEM				±0.03	±0.05	±0.01
significance				n.s	n.s	*

r= repetition.

n= umber of samples.

a-b: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

* $p<0.05$

4.3.10. Magnesium content of tibia ash

The average magnesium content and percentage of magnesium of tibia ash in female and male Japanese quails at 28 days of age are shown in Table 4.3-11 and Table 4.3-12. The REE supplement had significant effect ($p<0.001$) on magnesium content and percentage of magnesium in tibia ash. Without considering gender, supplemented diet with 400 mg/kg of REE-citrate significantly increased the magnesium content and percentage of magnesium of tibia ash in Japanese quails compare to that in quails fed 100 mg/kg or 800 mg/kg of REE-citrate ($p<0.05$, $p<0.001$). The Japanese quails fed 400 mg/kg of REE-citrate had the highest magnesium content of tibia among all the experimental groups. The diet supplemented with 800 mg/kg of REE-citrate significantly decreased magnesium content and percentage of magnesium of tibia ash in Japanese quails compare to that in other groups including the control group ($p<0.01$, $p<0.001$).

The male Japanese quails fed 400 mg/kg of REE-citrate had significantly up to 26% higher Magnesium content and percentage of magnesium in tibia ash than the ones fed 100 mg/kg and 800 mg/kg of REE-citrate ($p<0.01$, $p<0.001$) and than

control group ($p<0.05$). The male Japanese quails fed 800 mg/kg of REE-citrate had significantly up to 21% lower magnesium content and percentage of magnesium of tibia ash than the ones fed 100 mg/kg and 400 mg/kg of REE-citrate ($p<0.001$) and than control group ($p<0.05$).

There was no significant different between experimental groups in female Japanese quails. The diet supplemented with 400 mg/kg of REE-citrate increased the magnesium content and percentage of magnesium of tibia ash in female Japanese quails for 6% to 24% compare to other experimental groups. The female Japanese quails fed 800 mg/kg of REE-citrate had for 9% to 19.4% lower magnesium content and percentage of magnesium in tibia ash than the ones in other groups. In both male and female Japanese quails, the group fed 400 mg/kg of REE-citrate had the highest magnesium content and the group fed 800 mg/kg of REE-citrate had the lowest magnesium content in tibia ash among the experimental groups.

Table 4.3-11: Average magnesium content of tibia ash (mg/kg) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	Magnesium in tibia ash (mg/kg)		
				female	male	total
1	-	3	12	10424 ±859	9942 ^{ab} ±948	10183 ^{ab} ±898
2	50	3	12	9900 ±1549	10842 ^{ac} ±1258	10371 ^{ab} ±1433
3	100	3	12	10595 ±1799	9528 ^{bd} ±708	10061 ^{a} ±1417
4	400	3	12	11188 ±678	11072 ^{c} ±770	11130 ^{b} ±694
5	800	3	12	9014 ±1675	8792 ^{d} ±661	8903 ^{c} ±1220
n			60	30	30	60
SEM				±272	±218	±351
significance				n.s	***	***

r= repetition.

n= umber of samples.

a-d: means within columns with no common superscripts are significantly different ($p<0.05$).

n.s: no significant difference.

*** p<0.001

Table 4.3-12: Percentage of magnesium in tibia ash per bird and sex within experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	% Mg per Kg tibia ash		
				female	male	total
1	-	3	12	1.04 ±0.09	0.99 [ab] ±0.09	1.02 [ab] ±0.09
2	50	3	12	0.99 ±0.15	1.08 [ac] ±0.13	1.04 [ab] ±0.14
3	100	3	12	1.06 ±0.18	0.95 [bd] ±0.07	1.01 [a] ±0.14
4	400	3	12	1.12 ±0.07	1.11 [c] ±0.08	1.11 [b] ±0.07
5	800	3	12	0.90 ±0.17	0.88 [d] ±0.07	0.89 [c] ±0.12
n			60	30	30	60
SEM				±0.03	±0.02	±0.04
significance				n.s	***	***

r= repetition.

n= umber of samples.

a-d: means within columns with no common superscripts are significantly different (p<0.05).

n.s: no significant difference.

*** p<0.001

4.3.11. Calcium content of blood serum

The average calcium content of blood serum of Japanese quails at 28 days old is shown based on millimolar per liter (mmol/lit) in Table 4.3-13. There was no significant difference between control group and REE supplemented groups. Without considering gender, supplemented diet with 400 mg/kg of REE-citrate increased the serum calcium content of Japanese quails for 19.76% compare to control group and up to 30.5% compare to other groups. The Japanese quails fed 800 mg/kg of REE-citrate had 8% to 23.4% lower serum calcium content than the quails in other groups.

The female Japanese quails fed 400 mg/kg of REE supplement had 37.5% higher serum calcium content than control group and 36.3% to 62% compare to other groups. The supplemented diet with 800 mg/kg of REE-citrate decreased the serum

calcium content in female Japanese quails for 15.13% compare to control group and for 12.54% to 38.3% compare to other groups.

The male Japanese quails fed 50 mg/kg of REE-citrate had 9.87% higher serum Calcium content than control group and 9% to 11.2% compare to other groups. The male Japanese quails fed 100 mg/kg of REE-citrate had 11.8% lower serum calcium content than control group and up to 10% compare to other groups. Increasing the REE-citrate concentration from 50 mg/kg to 100 mg/kg and from 400 mg/kg to 800 mg/kg decreased the serum calcium content in both female and male Japanese quails.

Table 4.3-13: Average calcium content of blood serum (mmol/lit) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	Calcium in serum (mmol/lit)		
				female	male	total
1	-	3	12	11.83 ±1.01	11.04 ±3.95	11.44 ±2.78
2	50	3	12	11.48 ±1.95	12.13 ±1.59	11.80 ±1.73
3	100	3	12	11.94 ±2.35	10.91 ±2.04	11.43 ±2.17
4	400	3	12	16.27 ±3.94	11.13 ±1.80	13.70 ±6.75
5	800	3	12	10.04 ±1.37	10.95 ±2.98	10.50 ±2.26
n			60	30	30	60
SEM				±0.83	±0.45	±1.02
significance				n.s	n.s	n.s

r= repetition.

n= umber of samples.

n.s: no significant difference.

4.3.12. Phosphor content of blood serum

The average phosphor content of blood serum of Japanese quails at 28 days old is shown based on milligram per liter (mg/lit) in Table 4.3-14. There was no significant difference between control group and REE supplemented groups. With or without considering gender, the high concentrations of REE-citrate (400 mg/kg and 800 mg/kg) increased the serum phosphor content in Japanese quails compare to low concentrations of REE-citrate (50 mg/kg and 100 mg/kg). Increasing the concentration of REE-citrate from 50 mg/kg to 800 mg/kg increased the serum phosphor content of Japanese quails up to 14.04%.

The female Japanese quails fed low concentrations of REE-citrate (50 mg/kg and 100 mg/kg) had 6% to 8.6% lower serum phosphor content than control group. The supplemented diets with 800 mg/kg of REE-citrate increased serum phosphor content of female Japanese quails for 3.65% compare to control group. Increasing the concentration of REE-citrate from 50 mg/kg to 400 mg/kg increased the serum phosphor content of female Japanese quails up to 13.4%.

The low concentrations of REE-citrate (50 mg/kg and 100 mg/kg) decreased serum phosphor content of male Japanese quails for 6.5% to 14.2% compare to that in control group. The male Japanese quails fed 800 mg/kg of REE-citrate had 1.8% higher serum phosphor content than the ones in control group. The serum phosphor content of male Japanese quails was increased up to 18.6% by increasing the concentration of REE-citrate in diet.

Table 4.3-14:

Treatment	REE addition mg/kg	r	n	Phosphor in serum (mg/lit)		
				female	male	total
1	-	3	12	114.11 ±13.95	118.59 ±34.66	116.35 ±25.30
2	50	3	12	104.33 ±41.88	101.76 ±46.98	103.04 ±42.45
3	100	3	12	107.42 ±31.08	110.92 ±39.00	109.17 ±33.67
4	400	3	12	118.28 ±21.30	110.15 ±28.07	114.21 ±24.13
5	800	3	12	114.32 ±34.29	120.70 ±51.50	117.51 ±41.85
n			60	30	30	60
SEM				±5.20	±7.05	±9.85
significance				n.s	n.s	n.s

Average phosphorus content of blood serum (mg/lit) per bird and sex in experimental groups at 28 days old

r= repetition.

n= umber of samples.

n.s: no significant difference.

4.3.13. Magnesium content of blood serum

The average magnesium content of blood serum of Japanese quails at 28 days old is shown based on milligram per liter (mg/lit) in Table 4.3-15. As the result shows, the REE supplement had no significant effect on serum magnesium content of Japanese quails. With or without considering gender, the serum magnesium content of Japanese quails was increased by low concentrations of REE-citrate and was decreased by high concentrations of REE-citrate compare to control group.

The female Japanese quails fed low concentrations of REE-citrate (50 mg/kg and 100 mg/kg) had 5.86% to 17.49% higher serum magnesium content than the ones in control group. The high concentrations of REE-citrate (400 mg/kg and 800 mg/kg) decreased serum magnesium content of female Japanese quails up to 37.86% compare to that of control group.

The male Japanese quails fed low concentrations of REE-citrate (50 mg/kg and 100 mg/kg) had 14.1% to 43.3% higher serum magnesium content than the ones in control group. The highest concentration of REE-citrate (800 mg/kg) decreased serum magnesium content of male Japanese quails for 8.24% compare to that of control group. Within each of male or female Japanese quails, the group fed 100

mg/kg of REE supplement had the highest serum magnesium content among experimental groups.

Table 4.3-15: Average magnesium content of blood serum (mg/lit) per bird and sex in experimental groups at 28 days old

Treatment	REE addition mg/kg	r	n	Magnesium in serum (mg/lit)		
				female	male	total
1	-	3	12	47334 ±18269	41585 ±25596	44460 ±21821
2	50	3	12	50107 ±9928	47447 ±16971	48777 ±13329
3	100	3	12	55614 ±25102	59574 ±19770	57774 ±21254
4	400	3	12	29413 ±9535	43503 ±13141	35818 ±12988
5	800	3	12	45670 ±18500	38160 ±20831	42256 ±18976
n			60	30	30	60
SEM				±3444	±3772	±5185
significance				n.s	n.s	n.s

r= repetition.

n= umber of samples.

n.s: no significant difference.

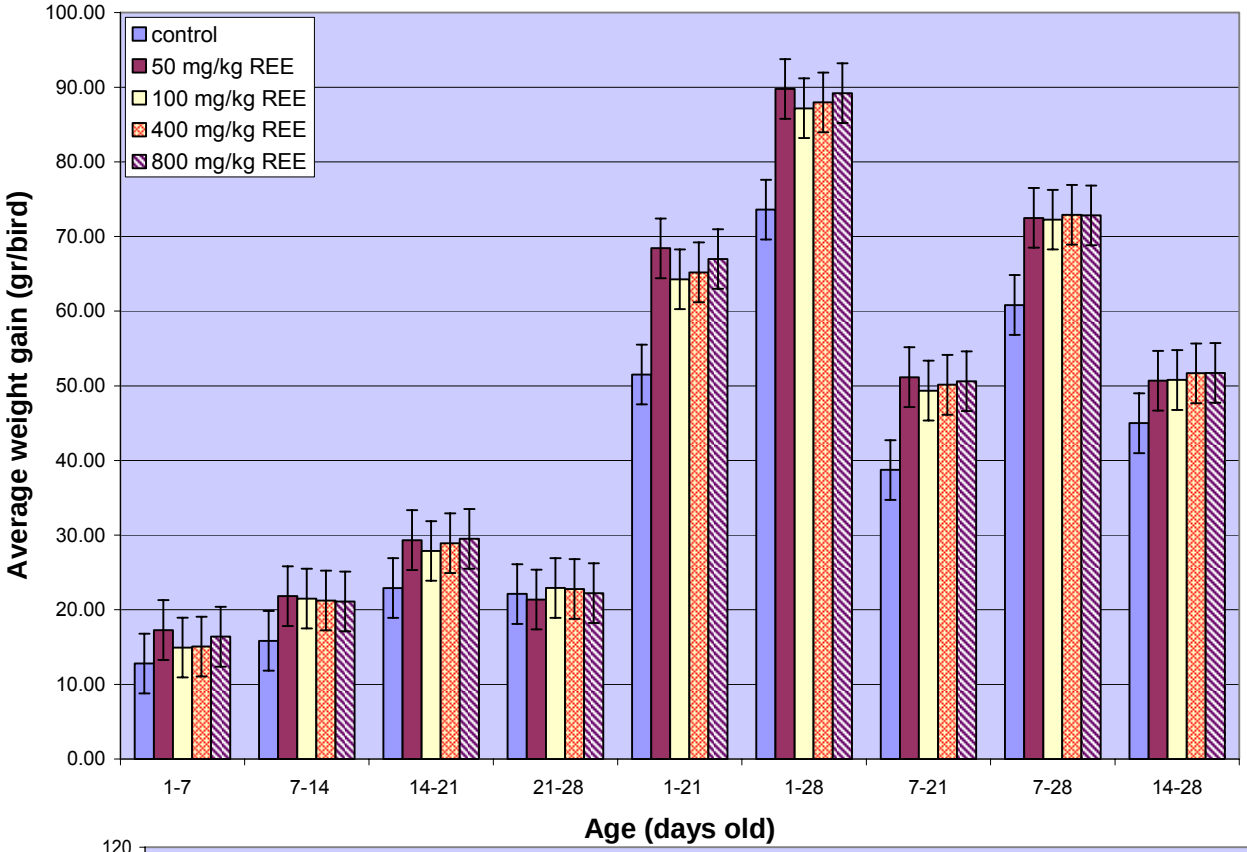
5. Discussion

5.1. Body weight gain

As the result of the first experiment (Figure 3) shows, rare earth element supplement increased body weight gains of Japanese quails compare to control group during the experiment. The growth promoting effect of REE-citrate was significant ($p<0.05$) until 21 days of age and there was no negative effect of REE on

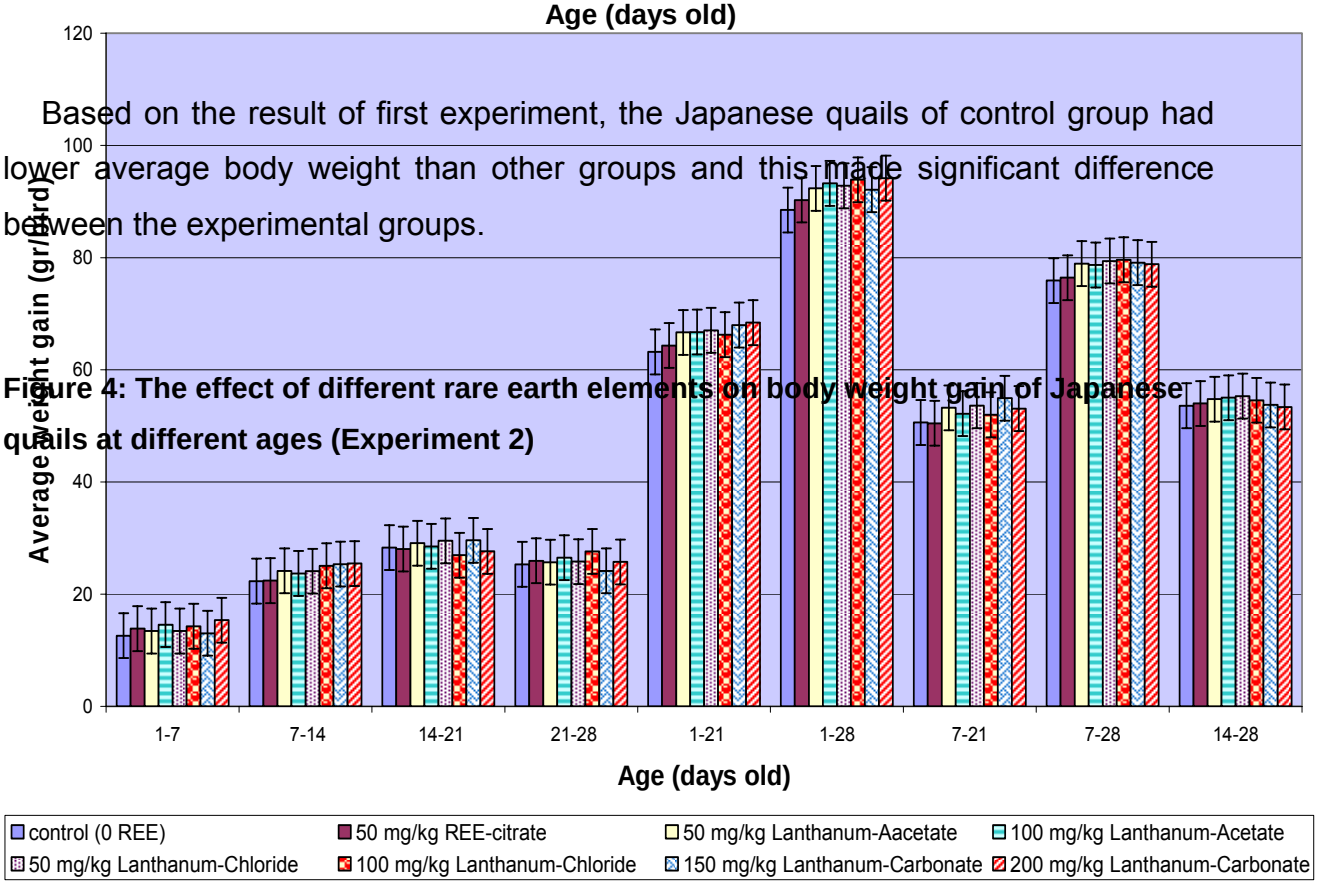
weight gain. The order of weight gained by different concentrations of REE-citrate (mg/kg) was 50>800>400>100>0 (control) during 1-21 and 1-28 days of age.

Figure 3: The effect of different concentrations of REE-citrate on body weight gain of Japanese quails at different ages (Experiment 1)



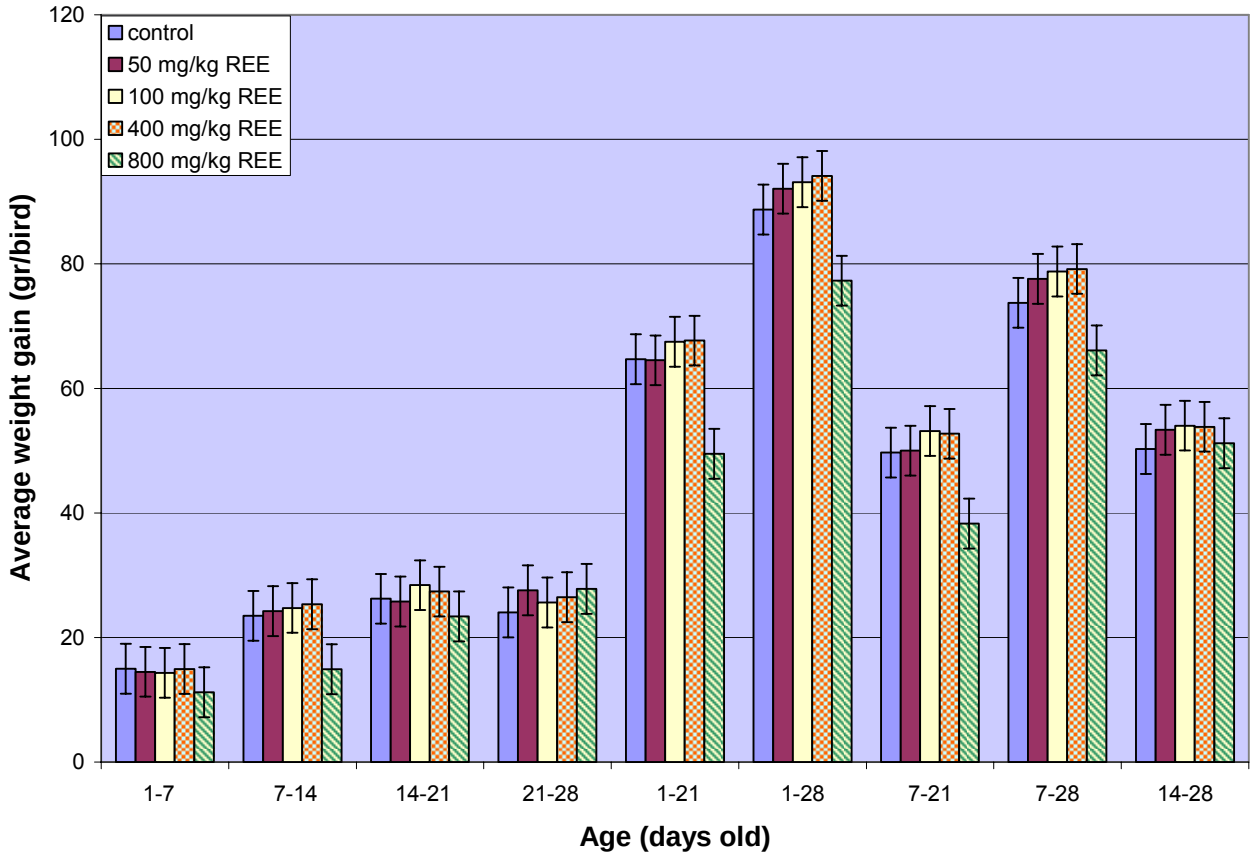
Based on the result of first experiment, the Japanese quails of control group had lower average body weight than other groups and this made significant difference between the experimental groups.

Figure 4: The effect of different rare earth elements on body weight gain of Japanese quails at different ages (Experiment 2)



The result of the second experiment (Figure 4) shows that rare earth elements had positive effect on growth of Japanese quails although this effect was not significant. The order of weight gained by different types of REE supplements during the 1-28 days of age were C>B>D>A>control and D>C>B>control at levels of 50 mg/kg and 100 mg/kg respectively. Also comparison the two levels of REE supplements showed that Japanese quails fed 100 mg/kg of any types of REE gained more weight than the quails fed 50 mg/kg of the same type of REE.

Figure 5: The effect of different concentrations of REE-citrate on body weight gain of Japanese quails at different ages (Experiment 3)



The result of the third experiment (Figure 5) also shows the positive effect of rare earth element on weight gain of Japanese quails. The order of weight gained by different concentrations of REE-citrate (mg/kg) was 400>100>50>0 (control)>800. The Japanese quails fed 800 mg/kg of REE gained significantly lower weight than other experiment groups, but this was not because of REE effect. There was a technical problem in making ration which is assumed to be the reason for low weight of Japanese quails fed with 800 mg/kg of REE. The ration with 800 mg/kg of REE was made again and it was given to two of three replicates of that group. The result shows that the Japanese quails fed with new diet grew better than the ones fed with old diet and than control group. So the order of weight gained by different concentrations of REE-citrate during 14-28 days of age changed to100>400>50>800>0 (control). Since the third experiment was the repeat of the first experiment, the results of these two experiments need to be compared. Because of the error mentioned, we have to compare the result of the first and third experiment without considering the high concentration REE (800 mg/kg) group. The other problem which makes this comparison difficult is lower average body weight of Japanese quails in control group of the first experiment compare to the control group of the third experiment. One common thing between the first and third experiment is that increasing the REE level from 100 to 400 mg/kg increased the average weight gain of Japanese quails compare to control group.

5.2. Feed conversion ratio

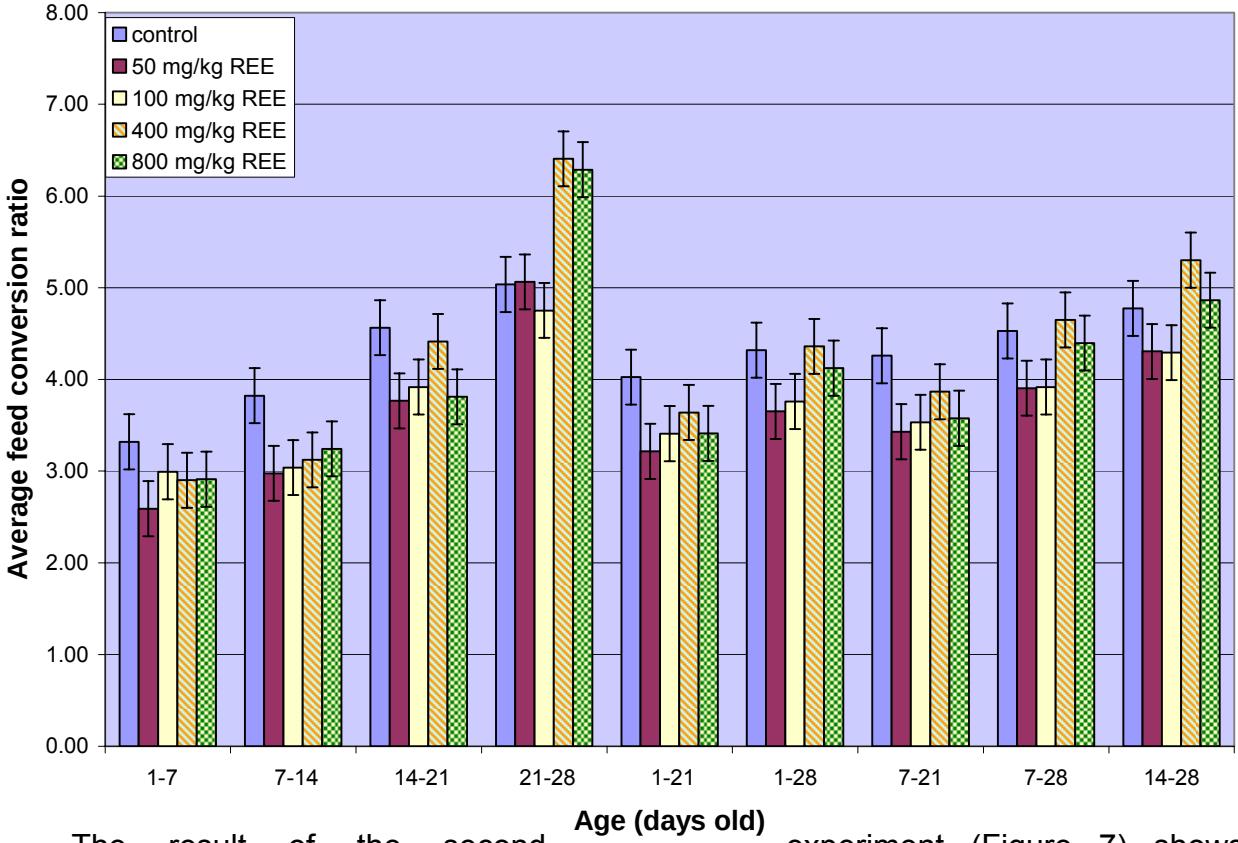
The feed conversion ratios of Japanese quails during the three experiments are shown in

Figure 6, Figure 7and Figure 8. The result of the first experiment (

Figure 6) shows that REE-citrate decreased feed conversion ratio of Japanese quails but this effect was not significant. The order of feed conversion ratio based on

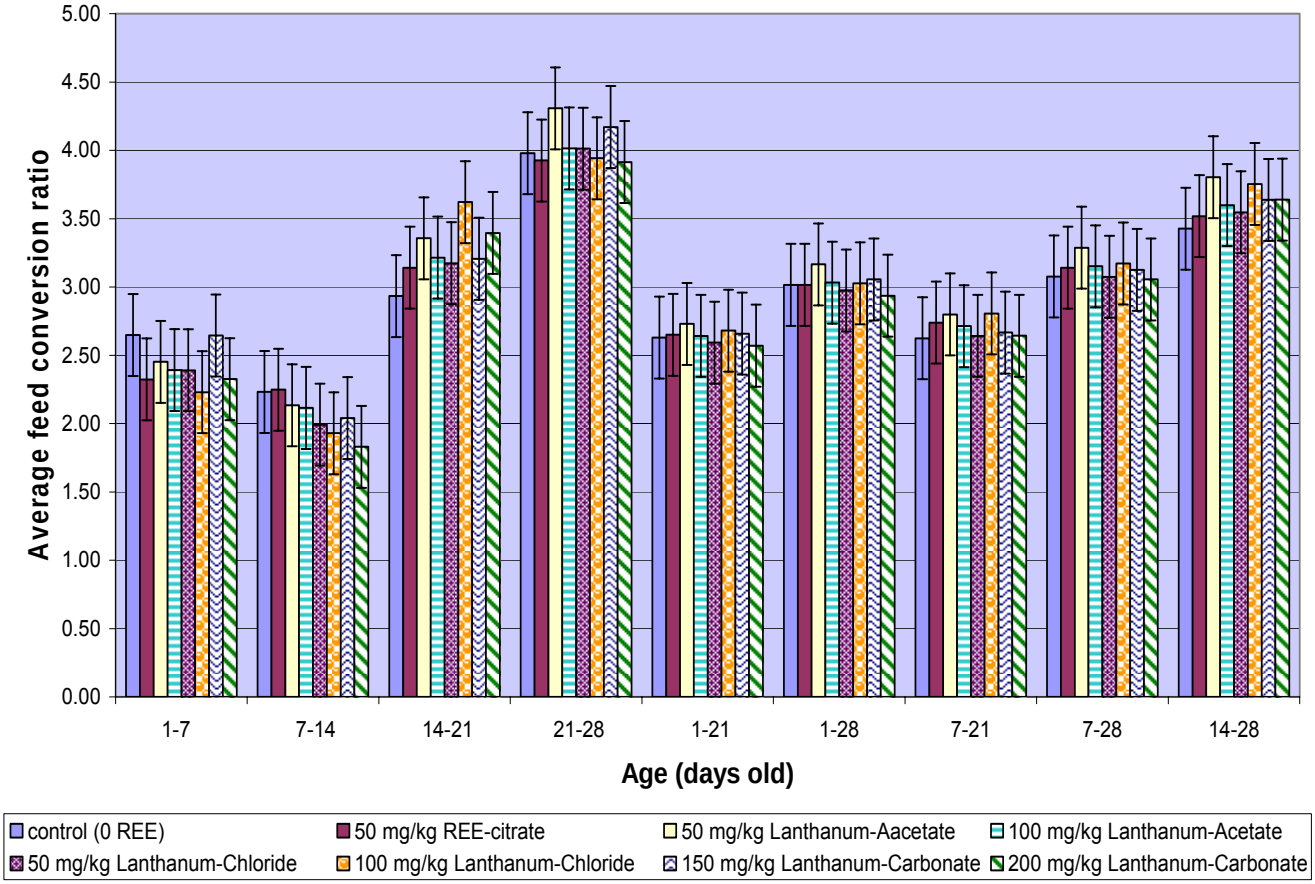
different concentrations of REE-citrate (mg/kg) was 50<100<800<0 (control) <400 during 1-28 days of age.

Figure 6: The effect of different concentrations of REE-citrate on feed conversion ratio of Japanese quails at different ages (Experiment 1)



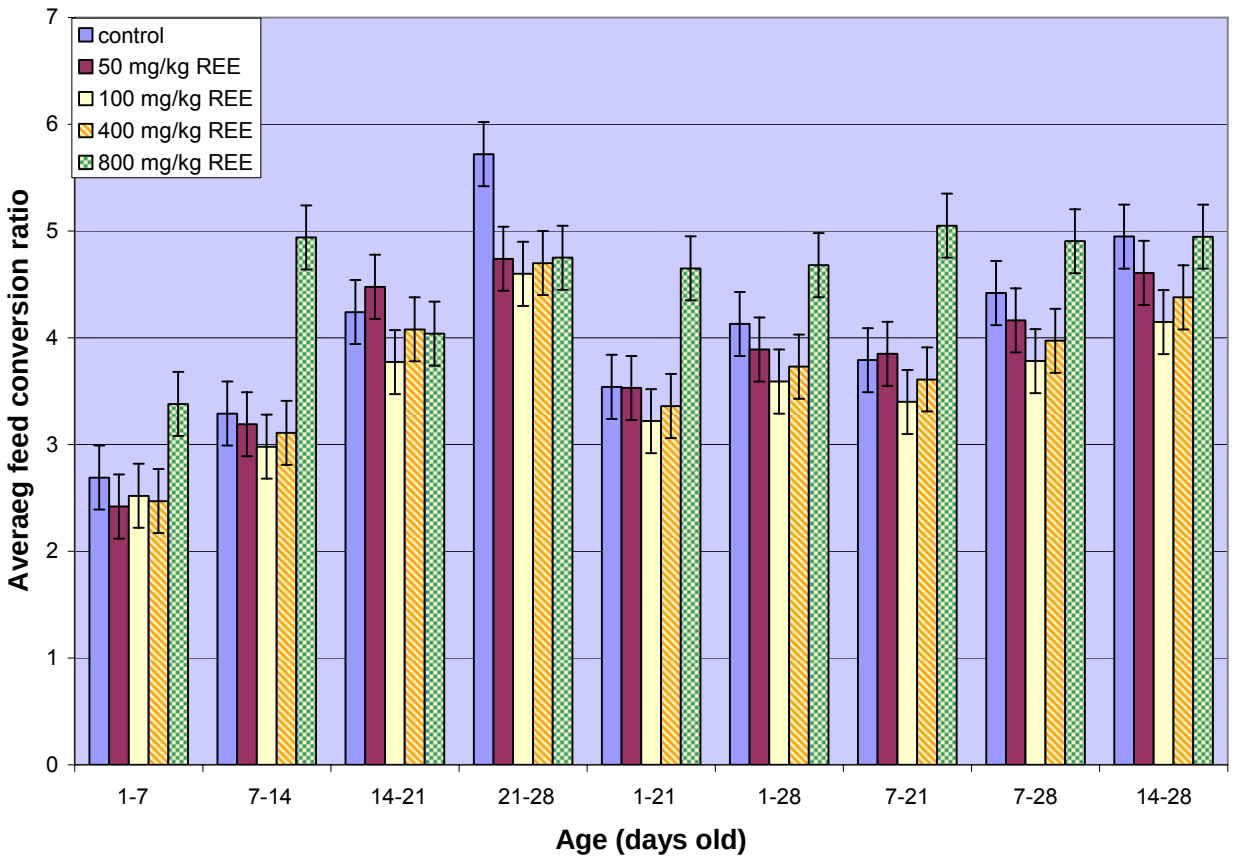
The result of the second experiment (Figure 7) shows the positive effect of some kinds of REE on improving feed conversion ratio. During 1-28 days of age, the order of feed conversion ratio based on different types of REE were C<control=A<D<B by 50 mg/kg of REE and D<control<C=B by 100 mg/kg of REE. Also the Japanese quails fed 100 mg/kg of any types of REE (except the type C) had lower feed conversion ratio than the quails fed 50 mg/kg of the same type of REE.

Figure 7: The effect of different rare earth elements on feed conversion ratio of Japanese quails at different ages (Experiment 2)



The result of the third experiment (Figure 8) which was repetition of the first experiment shows the positive effect of REE-citrate on improving feed conversion ratio. The Japanese quails fed 800 mg/kg of REE had significantly higher feed conversion ratio than other groups. As it was explained earlier the lower growth performance of quails fed high concentration of REE (800 mg/kg) was not because of REE effect but was because of the technical error in making ration for that experimental group. The order of feed conversion ratio based on different concentrations of REE-citrate (mg/kg) was 100<400<50<800=0 (control) during 1-28 days of age.

Figure 8: The effect of different concentrations of REE-citrate on feed conversion ratio of Japanese quails at different ages (Experiment 3)



According to the result of the first experiment, the lowest concentration of REE (50 mg/kg) was the best level for improving the growth parameters. Although the Japanese quails fed 100 mg/kg of REE gained less weight than the other REE groups, they consumed less feed and had the lower feed conversion ratio than the quails fed higher concentrations of REE and than control group.

According to the result of the second experiment, the best REE supplements for improving the growth performance were type C (Lanthanum Chloride) at level of 50 mg/kg and type D (Lanthanum Carbonate) at level of 100 mg/kg. Also the result of second experiment shows that increasing the level of REE from 50 mg/kg to 100 mg/kg improved performance of Japanese quails.

According to the result of the third experiment, 100 mg/kg of REE-citrate was the best level for improving growth performance. Although the Japanese quails fed 100 mg/kg of REE gained less weight than the group fed 400 mg/kg of REE, they consumed the lowest amount of feed and had the best feed conversion ration between experimental groups.

The growth promoting effect of rare earth elements has been reported for most of farming animal (mostly for pigs and poultry) in China (Xiong, 1995). Increases in body

weight gain of 8 - 23 % along with decreased feed conversion of 8 - 16 % have been reported in chickens (Zhang and Shao, 1995), (Xie and Wang, 1998), (Yang et al., 2005). Feeding experiments performed under Western conditions showed increased body weight up to 7 % and improved feed conversion ratio up to 3% in chickens (Halle et al., 2002, 2004). While recently performed studies by He et al., (2006a) showed increased body weight gain 5-13% and improved feed conversion ratio by 3% in broiler chicks.

The result of present study confirms the results of the last studies on chickens under both Chinese and western conditions. The first experiment showed great positive effect of REE supplement on weight gain and feed conversion ratio of Japanese quails which confirms the result of Chinese studies on chickens. But as it was already mentioned, the lower average body weight of Japanese quails in control group during the first experiment compare to the control groups of the second and third experiment could increase the effect of rare earth elements. Based on the results of the second and the third experiments, REE supplement increased weight gain of Japanese quails up to 6.4% which is close to results of studies on chickens under western condition. The feed conversion ratio of Japanese quails during the third experiment was improved by REE up to 13% which is higher than the results from studies under western condition. Based on results obtained to this day from Western feeding experiments, it might be concluded that the outcome of dietary rare earth application varies with the animal species. Yet, concentration and type of rare earths applied as well as the composition of individual rare earth elements have also been shown to be important factors influencing performance enhancing effects of rare earths on animals (He et al., 2003a, 2006a, 2006b) and (Redling, 2006).

According to some Chinese reports the optimum range of REE for growth promoting effect in chickens was 100-300 mg/kg of feed (Zhang and Shao, 1995), (Gong et al., 1996), (Xie and Wang, 1998). However higher concentrations (300-600 mg/kg) were reported improved the egg production in laying hens (Wu et al., 1994) and (Zhang et al., 1996). The recent study carried by He et al., (2006a) showed low concentrations of REE (40-70 mg/kg) improved both weight gain and feed conversion ratio of broiler chicks. Also studies under western conditions showed positive effect of REE on growth performance of chickens. Halle et al., (2003b and 2004) reported that 100 mg/kg of REE supplement had positive effect on growth performance of broiler chicks. The result of present study showed that the optimum range of REE

concentration for improving growth performance of Japanese quail was 50-400 mg /kg of feed which confirms the last studies on poultry under both Chinese and western conditions but it is the opposite of the result achieved by Schuller (2001) who didn't find any positive effects of REE on growth or productivity of either broiler chicks or Japanese quails. In present study, the first experiment showed that the lowest concentration (50 mg/kg) of REE made the most improvement in growth and feed conversion ratio and 100 mg/kg of REE was the next optimum level for improving feed conversion ratio and feed intake. The third experiment showed increasing the concentration of REE from 50 mg/kg to 100 and 400 mg/kg, improved body weight gain, feed intake and feed conversion ratio of Japanese quails. The result of the second experiment showed increasing the level of lanthanum salts in feed from 50 mg/kg to 100 mg/kg, improved the weight gain and feed conversion ratio.

The chemical compound, especially the anion bound to rare earths, seems to affect their performance enhancing effects. Several Chinese scientists concluded that the growth promoting effects may vary when different rare earth sources are used (Xie et al., 1991), (Lu et al., 2000). Currently, organic rare earth compounds are mainly used as feed additives in China (Xiong, 1995). In line with Chinese reports (Fang et al., 1997), (Zhang and Shao, 1995) and (Chen, 1997), slightly better growth promoting effects were observed in both pigs (Knebel, 2004) and poultry (Halle et al., 2002) due to the application of organic in stead of inorganic rare earth compounds under western condition (Redling, 2006). Another factor possibly affecting the efficacy of rare earths as to growth promotion may be the percentage of individual rare earth elements presented within the compound applied. Performance enhancing effects have been shown to be more obvious when a mixture of rare earth elements was applied instead of pure lanthanum chloride (Redling, 2006). According to Rambeck et al., (1999a) and He et al., (2003a, 2006), REE mixture containing mainly chlorides of La, Ce, Pr, Nd improved both body weight gain and feed conversion rate more than pure lanthanum chloride. But the present study showed that the Japanese quails fed 50 mg/kg of lanthanum salts gained more weight than the ones fed 50 mg/kg of REE-citrate which was a mixture. Also the result showed that between different lanthanum salts only lanthanum chloride improved feed conversion ratio compare to REE mixture. That is because the Japanese quails fed REE mixture consumed less feed than the ones fed lanthanum salts.

5.3. Mineral content of tibia ash

5.3.1. Experiment 1

The result of the first experiment in Japanese quails shows that REE-citrate supplement didn't have significant effect on calcium content of tibia ash but had significant effect on phosphor ($p<0.01$) and magnesium ($p<0.05$) content of tibia ash (Figure 8 and Figure 9). The calcium content of tibia was increased by lower concentrations and was decreased by high concentrations of REE-citrate compare to those of control group. The low concentrations of REE-citrate (50 and 100 mg/kg) significantly increased phosphor content of tibia ash compare to other groups ($p<0.05$). The calcium to phosphor ratio (Ca/P) in tibia of Japanese quails fed high concentrations (400 and 800 mg/kg) of REE-citrate was higher than that of quails fed low concentrations (50 and 100 mg/kg) of REE-citrate. That is because low concentrations of REE increased phosphor content of tibia more than the calcium content. The low concentrations of REE (50 and 100 mg/kg) increased tibia magnesium compare to other groups.

Figure 9: The effect of different concentrations of REE-citrate on phosphor content of tibia ash (gr/kg) in Japanese quails (Experiment 1)

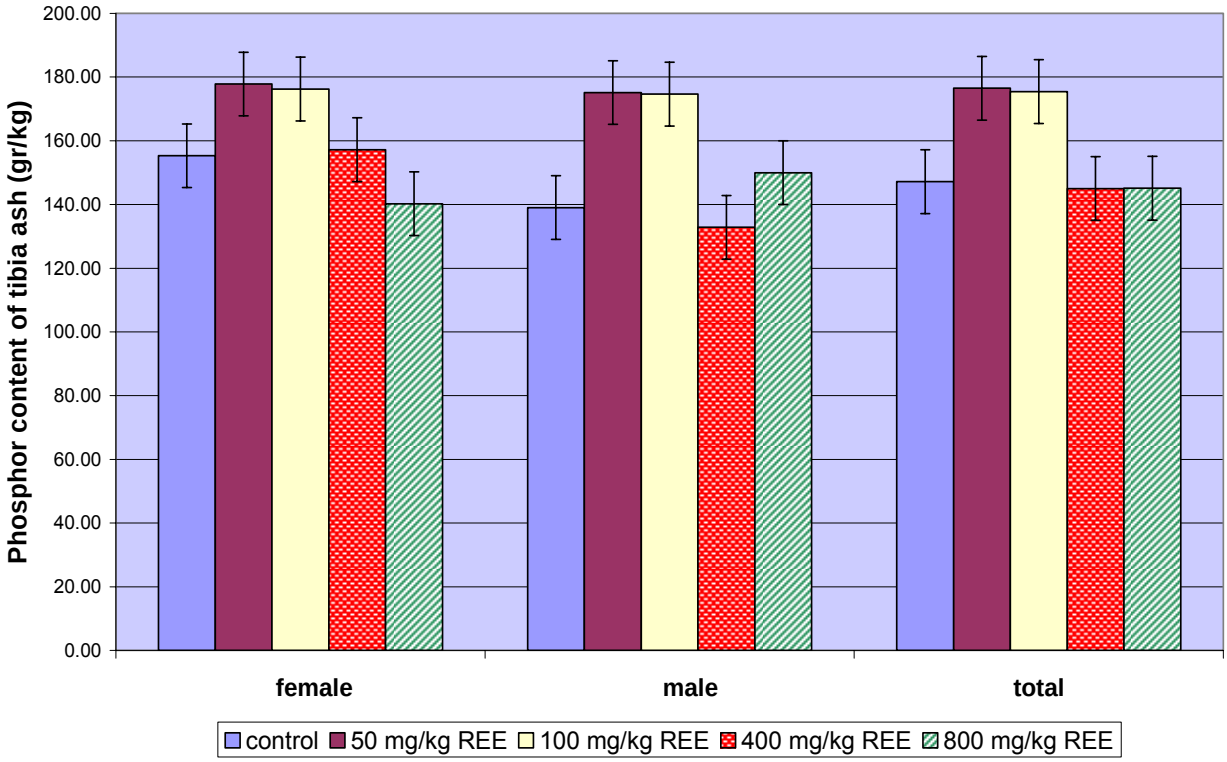
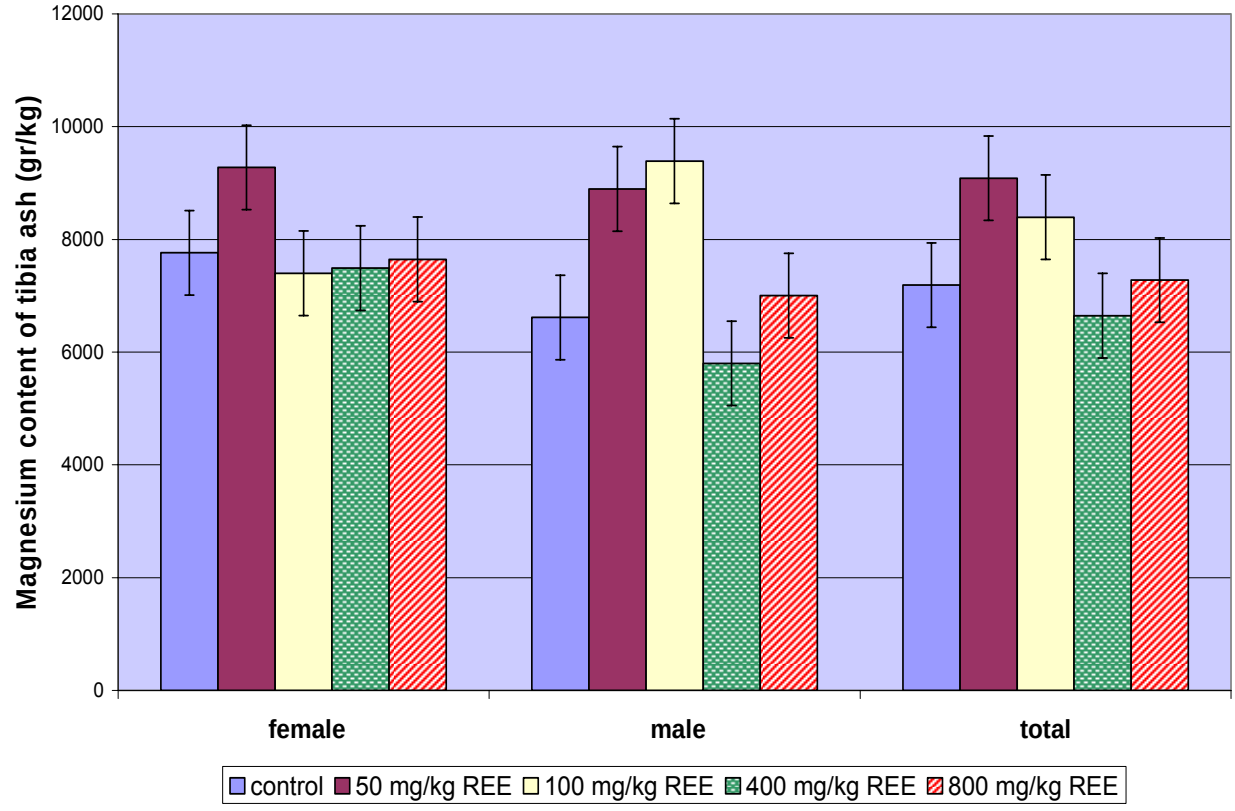


Figure 10: The effect of different concentrations of REE-citrate on magnesium content of tibia ash (mg/kg) in Japanese quails (Experiment 1)



5.3.2. Experiment 2

The result of the second experiment shows the significant effect of REE supplements on calcium ($p<0.05$) and phosphor content ($p<0.001$) of tibia ash in Japanese quails (Figure 11 and Figure 12). Increased tibia calcium by REE type C, resulted in higher calcium to phosphor ratio (Ca/P) in tibia of Japanese quails fed REE type C than other experimental groups. The Ca/P ratio in tibia was decreased by increasing the REE level from 50 to 100 mg/kg of feed. The REE supplements had no significant effect on tibia magnesium content of Japanese quails. Based on the result of the second experiment, it can be concluded that REE type C (Lanthanum-chloride) and REE type B (Lanthanum-acetate) had the best effect on mineralization of tibia. Also the result shows that low concentration (50 mg/kg) of REE supplements increased mineral content of tibia ash compare to higher concentration (100 mg/kg).

Figure 11: The effect of different types of REE supplement on calcium content of tibia ash (gr/kg) in Japanese quails (Experiment 2)

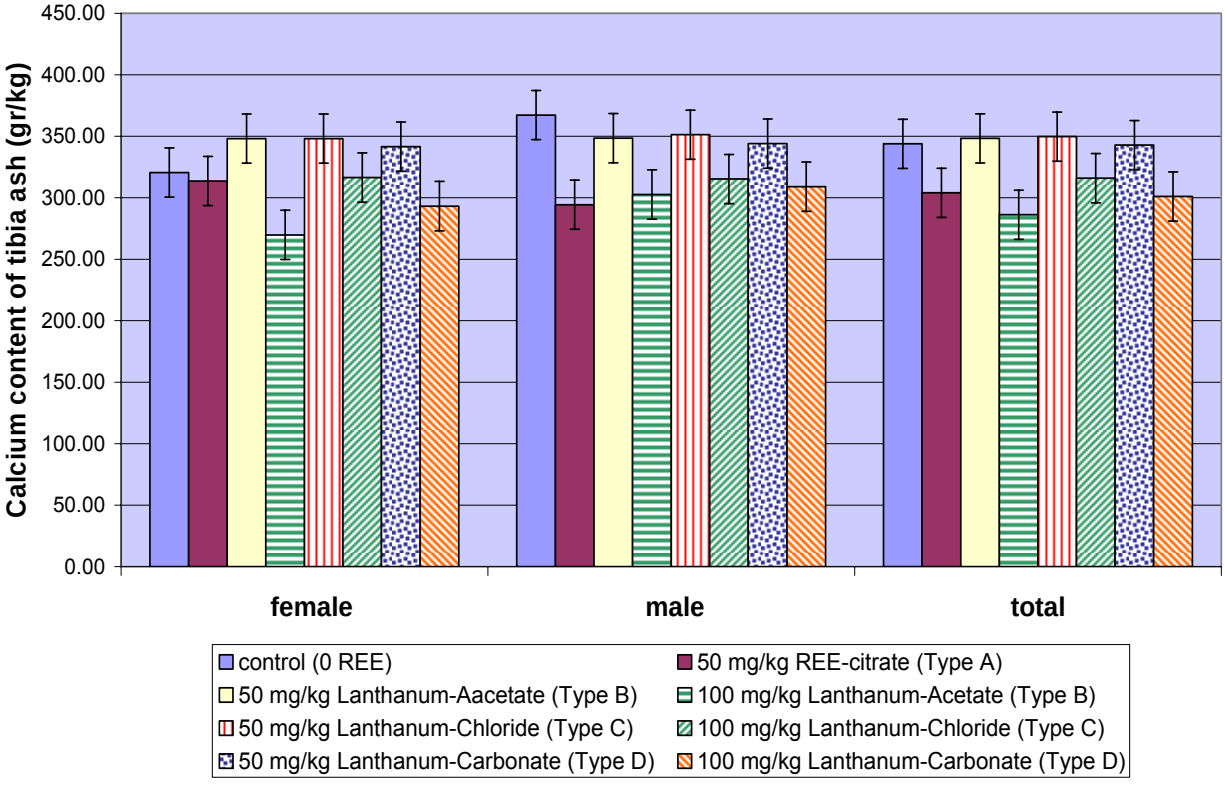
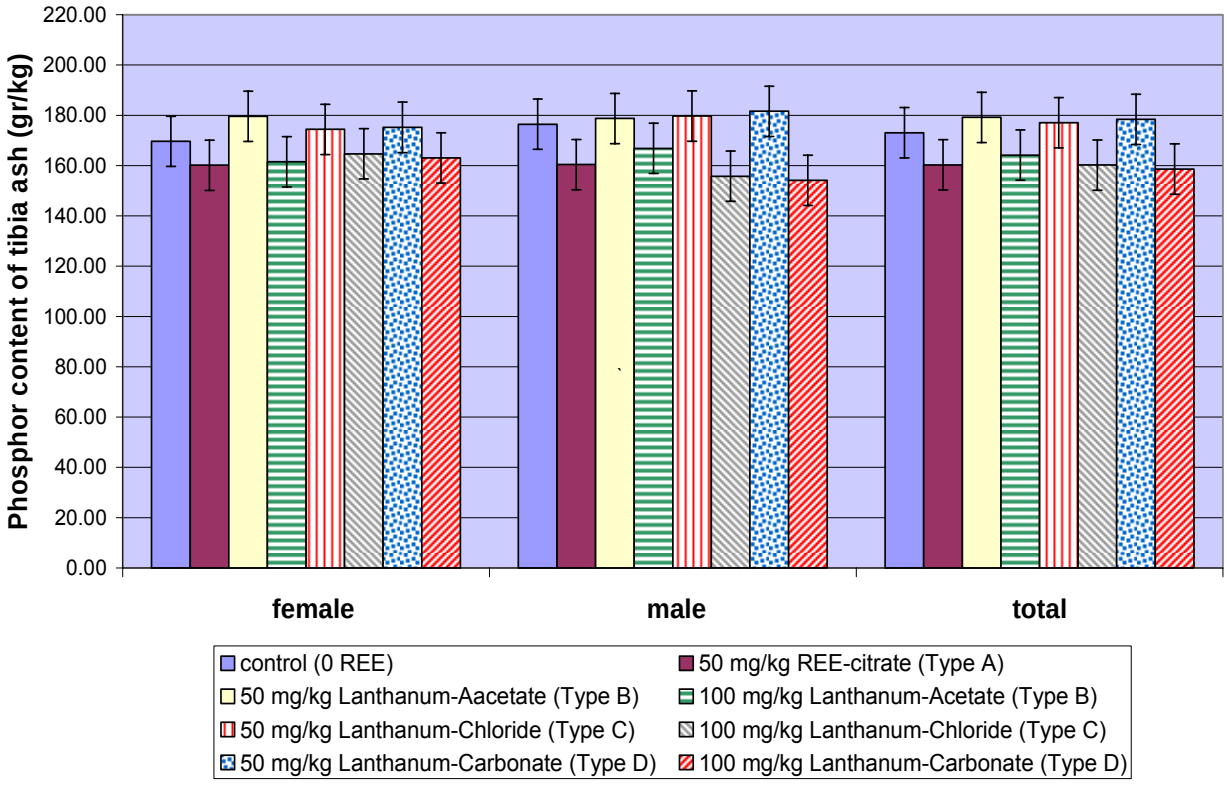


Figure 12: The effect of different types of REE supplement on phosphor content of tibia ash (gr/kg) in Japanese quails (Experiment 2)



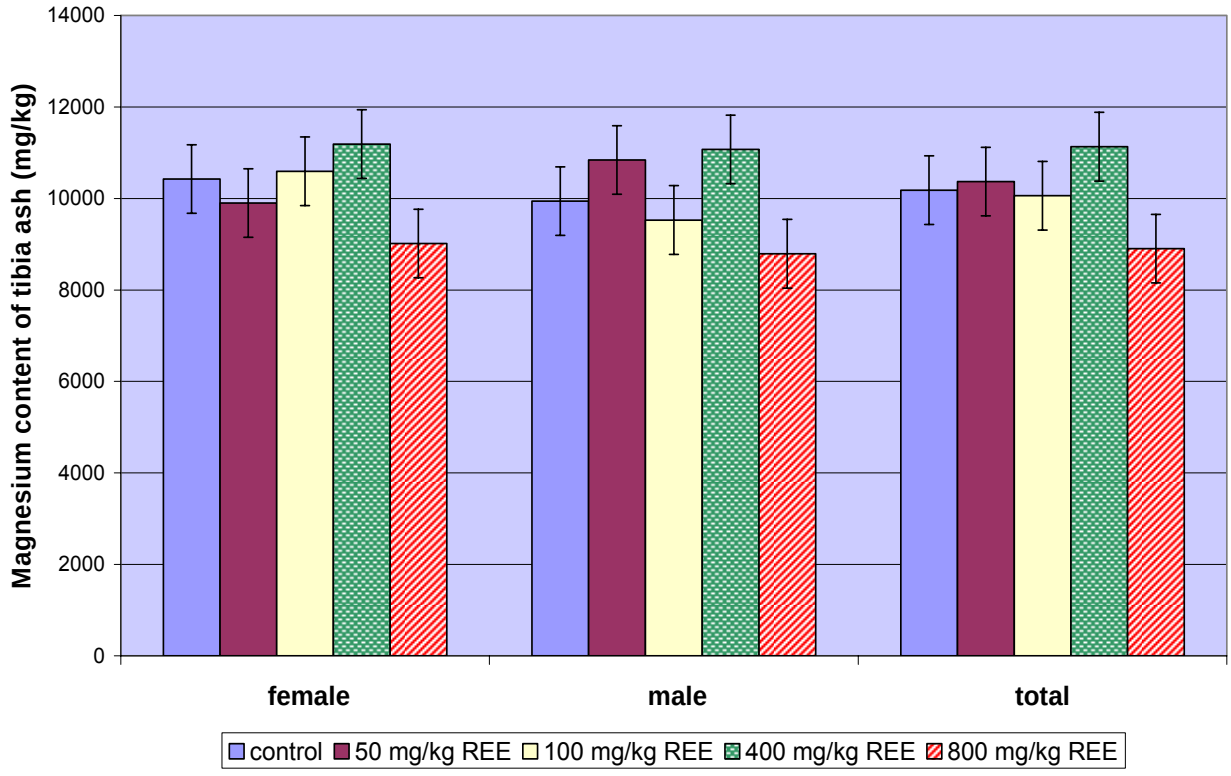
5.3.3. Experiment 3

The result of the third experiment shows that REE-citrate supplement didn't have significant effect on calcium and phosphor content of tibia ash in Japanese quails. The lowest concentration of REE-citrate (50 mg/kg) increased the calcium content of tibia compare to other groups. This difference was significant in female Japanese quails ($p<0.05$). High calcium content of tibia in group fed 50 mg/kg of REE and high phosphor content of tibia in group fed 400 mg/kg of REE resulted in the highest and the lowest Ca/P ratio of tibia in these two groups respectively. The REE supplement had significant effect ($p< 0.001$) on magnesium content of tibia ash (Figure 13). The highest concentration of REE-citrate (800 mg/kg), significantly decreased tibia magnesium compare to other experimental group including control group ($p<0.01$, $p< 0.001$).

Comparing the results from the first and the third experiments shows that the low concentrations of REE-citrate (50 and 100 mg/kg) especially the lowest concentration, were more effective on tibia mineralization compare to higher

concentrations of REE and compare to control group. However in the third experiment, the diet supplemented with 400 mg/kg of REE-citrate increased the phosphor and magnesium content of tibia ash compare to other groups. Also the results show that the highest concentration of REE (800 mg/kg) had negative effect on bone mineralization.

Figure 13: The effect of different concentrations of REE-citrate on magnesium content of tibia ash (mg/kg) in Japanese quails (Experiment 3)



According to the results of three experiments in present study, rare earth element supplements at low concentrations (50 and 100 mg/kg) especially at level of 50 mg/kg can improve bone mineralization in Japanese quails. Positive effect of REE on bone was also shown by Liu et al., (2004). He indicated that lanthanum chloride inhibited lipid deposition and cell damage and improved bone structure and mineralization in rabbits which were fed hyperlipids. On the other hand the high concentration of REE-citrate (400 and 800 mg/kg) and Lanthanum salts (100 mg/kg)

reduced calcium and phosphor in tibia ash. This confirms the results of study by Prause et al., (2005b and 2005c) who showed that administration of 150 and 300 mg/kg of REE-citrate in growing pigs, decreased ca/p ratio in metatarsus ash in favor of phosphor compare to control group. Huang et al., (2006) also showed the loss in bone mineralization by lanthanum nitrate in femur bone of rats. The negative effect of high dose rare earth elements on bone minerals was more obvious by lanthanum salts which decreased bone minerals at administration level of 100 mg/kg. The high concentrations of rare erath elements induce their effects on bone mineralization via indirect pharmacological mechanism as phosphate depletion (by phosphate binding properties) or affecting thyroid hormones (by Ca binding properties) not via direct bone toxicity (Damment and Shen, 2005) (Freemont et al., 2005).

5.4. Mineral content of blood serum

According to the results of the three experiments, the concentration of serum calcium in experiment 3, serum phosphor in all three experiments and serum magnesium in experiments 2 and 3 was not affected by REE supplement. Conforming to these results, the studies carried by other people showed no significant effect of REE on the concentration of serum calcium and phosphor in animals (Rambeck et al., 1999a), (He et al., 2001 and 2006a), (Prause et al., 2005b and 2005c). On the other hand, the result of the first and second experiments shows that concentration of serum calcium was significantly increased by low concentrations of REE supplements (50 and 100 mg/kg) but the high concentrations of REE had negative effect on serum calcium content. Kramsch et al., (1980) also showed that oral administration of $LaCl_3$ to rabbits with atherosclerosis significantly decreased the concentration of serum calcium.

6. Summary

The present study was designed to determine the effect of different types and concentrations rare earth elements on growth performance of Japanese quails. 120 and 225 one day old Japanese quails in the first and third experiment respectively were allotted to five dietary treatments: a control group and four REE groups which were supplemented with 50,100, 400 and 800 mg/kg of REE-citrate, a Lanthanoid mixture. In the second experiment 300 one day old Japanese quails were allotted to eight dietary treatments: a control group and seven REE groups which were supplemented with 50 or 100 mg/kg of REE-citrate (type A), lanthanum acetate (type B), lanthanum chloride (type C) and lanthanum carbonate (type D). Each experiment lasted four weeks and during it feed and water was *ad libitum*.

The REE supplements increased the weight gain of Japanese quails compare to control group by 18.5-22% during the first experiment ($p<0.05$), by 2-6.4% during the second experiment and by 6% during the third experiment. The feed consumption and the feed conversion improvement were not significantly affected by REE in any of the three experiments. REE-citrate improved the feed conversion ratio by 4.6-15.5% and by 5.8-13.1% during the first and third experiments respectively. In the second experiment, the REE-type C at level of 50 mg/kg and REE-type D at level of 100 mg/kg, improved the feed conversion ratio by 1.7-2.6%. Also increasing the level of different types of REE from 50 to 100 mg/kg improved weight gain and feed conversion ratio in the second experiment.

In the first experiment, the low concentrations of REE-citrate (50 and 100 mg/kg) significantly increased phosphor content of tibia ash compare to that of other groups ($p<0.05$). Also the lowest concentrations of REE-citrate (50 mg/kg) significantly ($p<0.05$) increased tibia magnesium compare to control group and the high concentrations (400 and 800 mg/kg) of REE. In the second experiment, the calcium content of tibia ash of Japanese quails fed 50 mg/kg of REE type B or C was significantly ($p<0.05$) higher than that of quails fed 50 mg/kg of REE-type A. also the diet supplemented with 100 mg/kg of REE-type B, significantly ($p<0.05$) decreased the tibia calcium compare to control group. The phosphor content of tibia ash in Japanese quails fed REE-type A was significantly lower than that in quails fed types B, C and D of REE. In the third experiment, the highest concentration of REE-citrate

(800 mg/kg) significantly decreased magnesium content of tibia ash compare to other experimental groups including control group ($p<0.01$, $p<0.001$). In the first experiment, the lowest concentration of REE-citrate (50 mg/kg) increased calcium content of blood serum compare to control group and significantly ($p<0.05$) compare to high concentrations (400 and 800 mg/kg) of REE-citrate. Also the Japanese quails fed 400 mg/kg of REE-citrate had significantly ($p<0.05$) higher magnesium in their blood serum than the quails of other groups. In the second experiment, the diet supplemented with 100 mg/kg of REE-type C significantly ($p<0.05$) increased serum calcium compare to diets with 100 mg/kg of other types of REE supplements.

Based on the results of present study, the optimum concentration range of REE-citrate for improving both growth and tibia mineralization was 50-100 mg/kg of feed. However the diet with 400 mg/kg of REE-citrate also improved growth performance and tibia minerals in experiment 3. Comparison between different rare earth elements showed that Lanthanum salts especially lanthanum-chloride had better effect on growth and tibia mineralization than REE-citrate which was a mixture. The optimum concentration of lanthanum salts for increasing growth performance was 100 mg/kg of feed and for improving bone mineralization was 100 mg/kg of feed.

References

Adams, C., 1999. Nutricines. Food components in health and nutrition, Nottingham University Press.

Andrew, J. W, 1983. Plant Growth Stimulators Comprising Metal Ions and Long-Chain Alkyl Carboxylic Acids and Alts and Derivates thereof. UK Patent GB 2118158 A, October 26 1983.

BASF Corporation, 2001. Organic acids. Copyright 2001 BASF Aktiengesellschaft.

Beaven, M. A., J. Rogers, J. P.Moore, R. Hesketh, G. A. Smith, and J. C. Metcalfe, 1984. The mechanism of the calcium signal and correlation with histamine release in 2H3 cells. Journal of Biological Chemistry, 259: 7129 – 7136.

Becquet, P., 2003. EU assessment of enterococci as feed additives. International Journal of Food Microbiology, 88(2-3): 247-54.

Bikker, P., V. Karabinas, M. W. Verstegen, and R. G. Campbell, 1995. Protein and lipid accretion in body components of growing pigs (20 to 45 kilograms) as affected by energy intake. Journal of animal science, 73: 2355 – 2363.

Böhme, H., J. Fleckenstein, Z. Hu, and E. Schnug, 2002. Bilanzversuche zum Einsatz von Seltenen Erden in der Schweinemast. In 114. VDLUFA Kongress Ressourcen und Produktsicherheit-Qualitätssicherung in der Landwirtschaft, 16. - 20. September 2002, Leipzig, Germany.

Borger, C., 2003. Alternative Methoden in der Schweinemast. Untersuchungen zum leistungsstiegernden Potential Seltener Erden und zur Jodanreicherung im Gewebe durch die Verfütterung von Meeresalgen. Dissertation, Ludwig-Maximilians-Universität, München, 2003.

Brown, P. H., A. H. Rathjen, R. D. Grahahm, and D. E. Tribe, 1990. Rare earth elements in biological systems, in: Gschneidner Jr. K. A., Eyring, L. Roy (Eds.), Handbook on the physics and chemistry of rare earths, volume 13, 92. Amsterdam, Oxford: Elsevier North-Holland, 1990.

Canibe, N., S. H. Steien, M. Øverland, and B. B. Jensen, 2001. Effect of K-diformate in starter diets on acidity, microbiota, and the amount of organic acids in the digestive tract of piglets and on gastric alterations. Journal of Animal Science, 79: 2123-2133.

Casewell, M., C. Friis, E. Marco, P. McMullin, and I. Phillips, 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. Journal of Antimicrobial Chemotherapy, 52: 159-161.

Cashman, K., 1999. Prebiotics and calcium bioavailability. Probiotics and prebiotics: where are we going?. Wymondham, UK: Caister Academic Press.

Çelik, K., I. E. Ersoy, A. Uzatici, and M. Erturk, 2003. The using of organic acids in California turkey chicks and its effects on performance before pasturing. International Journal of Poultry Science, 2(6): 446-448.

Cervantes, H., 2005. Assessing the results of the EU ban on antibiotic feed. http://www.thepoultrysite.com. Accessed in 2005.

Chen, H., and B. Xiong, 1994. A study of feeding new type organic Rare Earth Element compound (RCT-3) to pigs and chicks. Feed Research, 8(4): 4 – 7.

Chen, H. F., 1997. Influence of rare earth compounds on the growth of pigs. Journal of the Chinese Rare Earth Society, 15: 441 – 443.

Chen, Y.C., C. Nakthong, and T.C. Chen, 2005. Improvement of laying hen performance by dietary prebiotic chicory Oligofructose and inulin. International Journal of Poultry Science, 4(2): 103-108.

Cheng, Q., J. Gao, B. Jing, and D. Yuan, 1994. The apparent digestibility of rare earth elements and their effect on crude protein and fat digestibility in pigs. Jiangsu Agriculture Science, 1: 59 –61.

Chengdu Beyond Chemicals. Rare Earth. World Wide Web, `http://www.beyondchem.com/rarearth.htm`, 2005. Accessed in 2005.

China Rare Earth Information, 1999. CRE Information, 5(3): 1-4.

Crittenden, R. G., 1999. Prebiotics. Probiotics: a critical review. Wymondham, UK: Horizon Scientific Press.

Damment, SJ., V. Shen, 2005. Assessment of effects of lanthanum carbonate with and without phosphate supplementation on bone mineralization in uremic rats. Clinical Nephrology, 63(2): 127-37.

Denli, M., F. Okan, and K. Çelik, 2003. Effect of dietary probiotic, organic Acid and antibiotic supplementation to diets on broiler performance and carcass yield. Pakistan Journal of Nutrition, 2(2): 89-91.

Diatloff, E., C. J. Asher, and F. W. Smith, 1995. Effects of rare earth elements (REE) on the growth and mineral nutrition of plants. In Proceeding of the Rare Earths in Agriculture Seminar, 20. September 1995, pages: 11 – 24, Canberra, ACT Australia.

Dilger, R. N., E. M. Onyango, J. S. Sands, and O. Adeola, 2004. Evaluation of microbial phytase in broiler diets. Poultry Science, 83: 962-970.

Duan, S., H. Zhao, and K. Chen, 1998. A Study of feeding Rare Earth Elements to broiler-type breeding bird. Livestock and Poultry Industry, 2: 16 – 17.

Dugenci, SK, N. Arda, and A. Candan, 2003. Some medicinal plants as immunostimulant for fish. Journal of Enthnopharmacology, 88(1): 99-106.

Durbin, P.W., M. H. Williams, M. Gee, R. H. Newman, and J. G. Hamilton, 1956. Metabolism of the lanthanons in the rat. Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N. Y.), 91: 78 – 85.

Eisele, G. R., F. R. Mraz, and M. C. Woody, 1980. Gastrointestinal uptake and ^{144}Ce in the neonatal mouse rat and pig. Health Physics, 39: 185 – 192.

Enyeart, J. J., L. Xu, and J. A. Enyeart, 2002. Dual actions of lanthanides on ACTH-inhibited leak K^+ channels. American Journal of Physiology Endocrinology and Metabolism, 282(6): 1255 –1266.

Esonu, B. O., R. O. Izukanne, and O. A. Inyang, 2005. Evaluation of cellulolytic enzyme supplementation on production indices and nutrient utilization of laying hens fed soybean hull based diets. International Journal of Poultry science, 4(4): 213-216.

Evans, C. H., 1990. Biochemistry of the lanthanides, Frieden, E. (Ed.). Plenum Press, New York and London.

Fan, C., F. Zhang, Y. Wu, G. Zhang, and J. Ren, 1997. A study of feeding organic REE additives on growing pork pigs. QingHai Journal of Husbandry and Veterinary, 27(3): 23 – 24.

Fang, J., Y. Huang, and H. Gong, 1994. A study of feeding Rare Earth Elements to black-bone silky fowl. Fujian Journal of Husbandry and Veterinary, 3: 28 – 29.

Feldmann, A., 2003. Seltene Erden in der Schweinemast. Report, Gesellschaft zur Erhaltung alter und gefährdeter Haustierrassen e.V. (GEH), the society for the conservation of old and endangered livestock breeds, http://www.g- e- h.de/aktuell/news/news10.htm, 2003.

Fiddler, G., T. Tanaka, and I. Webster, 2003. Low systemic adsorption and excellent tolerability during administration of Lanthanum carbonate (Fosrenol™) for 5 days. In 9th Asian Pacific Congress of Nephrology, 19. - 20. February 2003, Pattaya, Thailand.

Flachowski, G. , 2003. Huhn und Schwein und Seltene Erden. Wirtschaft erleben, 1:1, 6 – 7.

Fleckenstein, J., I. Halle, Z. Y. Hu, G. Flachowsky, and E. Schnug, 2004. Analyse von Lanthaniden mittels ICP - MS in Futter- und Organproben im Broilermastversuch. In 22. Arbeitstagung Mengen und Spurenelemente, Jena, Germany, 2004.

Foreman, J. C., and J. L. Mongar, 1973. The action of lanthanum and manganese on anaphylactic histamine secretion. British Journal of Pharmacology, 48: 527 – 537.

Förster, D., A. Berk, H. O. Hoppen, and W. Rambeck, 2006. Effect of rare earth elements (REE) on the performance and thyroid hormone status of rearing piglets. Proceedings of the Society of Nutrition Physiology, 21. - 23. März, Göttingen, 15, 2006.

Freemont. AJ, JA. Hoyland , and J. Denton, 2005. The effects of lanthanum carbonate and calcium carbonate on bone abnormalities in patients with end-stage renal disease. Clinical Nephrology, 64(6): 428-37.

Fuller, R., 1999. Probiotics for farm animals. Probiotics: a critical review. Wymondham, UK: Horizon Scientific Press.

Galvao, KN., JE. Santos, A. Coscioni, M. Villasenor, WM. Sischo, and AC. Berge, 2005. Effect of feeding live yeast products to calves with failure of passive transfer on performance and patterns of antibiotic resistance in fecal Escherichia coli. Reprod. Nutrition Development, 45(4): 427-40.

Gabert, V. M., and W. C. Sauer, 1994. The effect of supplementing diets for weanling pigs with organic acids. A review. Journal of Animal Feed Science, 3: 73-87.

Ghosh, N., D. Chattopadhyay, and GC. Chatterjee, 1991, Chicken erythrocyte membrane: lipid profile and enzymatic activity under lanthanum chloride and neodymium chloride administration. Indian Journal of Experimental Biology, 29(3): 226-9.

Gibson, GR., and MB. Roberfroid, 1995. Dietary modulation of the human colonic microflora: introducing the concept of prebiotics. Journal of Nutrition, 125: 1401-12.

Gill, P., V. Fowler, and D. Armstrong, 2005. Alternatives to antibiotic feed additives for pigs. British Society of Animal Science- 2005. http://www.bsas.org.uk. Accessed in 2005.

Gong, Z., 1996. A study of feeding Rare Earth Elements to broiler-type breeding bird. Chinese Poultry, 7: 43.

Greathead, H., 2003. Plants and plant extracts for improving animal productivity. The Proceedings of the Nutrition Society, 62(2): 279-290.

Green, A. A., and D. W. B. Sainsbury, 2001. The role of probiotic in producing quality poultry products. XV European Symposium on the quality of poultry meat 9-12 September 2001, kusadasi, Turkey, 245-251.

Halle, I., H. Böhme, and E. Schnug, 2003a. Investigations on Rare Earth Elements as growth promoting additives in diets for broilers and growing-finishing pigs. In J. Kampheus and P. Wolf, editors, Proceedings of the 7th Conference of the European Society of Veterinary and Comparative Nutrition (ESVCN), 3. - 4. Oktober 2003, Hannover, Germany.

Halle, I., J. Fleckenstein, Z. Hu, G. Flachowsky, and E. Schnug, 2002. Untersuchungen zum Einsatz von Seltenen Erden auf dasWachstum und die Schlachtleistung von Broilern. In 114. DLUFA Kongress Ressourcen und Produktsicherheit-Qualitätssicherung in der Landwirtschaft, 16. - 20. September 2002, Leipzig, Germany.

Halle, I., J. Fleckenstein, Z. Y. Hu, G. Flachowsky, and E. Schnug, 2003b. Untersuchungen zum Einfluss von Seltenen Erden auf das Wachstum und die Ganzkoerperzusammensetzung von Broilern. In Vitamine und Zusatzstoffe in der Ernährung von Mensch und Tier, 9. Symposium, 24. und 25. September 2003, pages 376 – 379, Jena, Germany.

Halle, I., J. Fleckenstein, Z. Y. Hu, G. Flachowsky, and E. Schnug, 2004. Investigations on Rare Earth Elements as growth promoting additives in diets for broilers. In XXII World's Poultry Congress, 8. - 13. June 2004, page 491, Istanbul, Turkey.

He, M. L., and W. A. Rambeck, 2000. Rare earth elements - a new generation of growth promoters for pigs? Archives of Animal Nutrition, 53(4): 323 – 334.

He, M. L., D. Ranz, and W. A. Rambeck, 2001. Study on performance enhancing effect of rare earth elements in growing and fattening pigs. Journal of Animal Physiology and Animal Nutrition, 85: 263 – 270.

He, M. L., J. Chang, R. Arnold, R. Henkelmann, X. Lin, A. Süss, and W. A. Rambeck, 1999. Studies on the effect of rare earth elements in piglets. Mengen und Spurenelemente. 19. Arbeitstagung, 3. und 4. Dezember 1999, Jena, Germany.

He, M. L., Y. Z. Wang, Z. R. Xu, M. L. Chen, and W. A. Rambeck, 2003a. Effect of dietary rare earth elements on growth performance and blood parameters of rats. Journal of Animal Physiology and Animal Nutrition, 87: 229 – 235.

He, M. L., U. Wehr, and W. A. Rambeck, 2006a. Oral administration of a low dose of rare earth elements improved the performance of broilers. Submitted in Japan 2006.

He, M. L., U. Wehr, Z. R. Xu, and W. A. Rambeck, 2006b. Effect of dietary rare earth elements on growth performance of rats. Proceedings of the Society of Nutrition Physiology, 15, 2006.

He, M. L., W. Z. Yang, H. Hidari, and W. A. Rambeck, 2003b. Effect of rare earth elements on proliferation and fatty acids accumulation in preadipocyte cell lines. Journal of Animal Physiology and Animal Nutrition, 87(5-6): 229 – 235.

He, M. L., W. Z. Yang, H. Hidari, and W. A. Rambeck, 2006c. Effect of rare earth elements on proliferation and fatty acids accumulation of 3T3-LI cells. Asian-Australasian Journal of Animal Sciences, 19(1): 119 – 125.

He, M. L., W. Z. Yang, H. Kuwayama, H. Hidari, and W. A. Rambeck, 2006d. Effect of various C18 polysaturated fatty acids on differentiation and cellular fatty acids accumulation in 3T3-L1 adipocytes. Proceedings of the Society of Nutrition Physiology, 15, 2006.

He, R., and Z. Xia, 2000. Effect of rare earth elements on growth of growing and fattening pigs. JiangXi journal of animal science and veterinary medicine, 4(6 - 7).

HeindI, U., 2002. The choice for success. Food and Agriculture, pages: 1-31. National Research.

Hernandez F, J. Madrid, V. Garcia, J. Orengo, and M. D. Megias, 2004. Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poultry Science, 83(2): 169-174.

Herzberg, O., MN. James, 1986. Crystallographic determination of lanthanide ion binding to troponin C. FEBS Lett., 199(2): 279-82.

Hu, Z., H. Richter, G. Sparovek, and E. Schug, 2004. Physiological and biochemical effects of rare earth elements on plants and their agricultural significance: A Review. Journal of Plant Nutrition, 12(1):183 – 220.

Hu, Z., J. Wang, Y. Yang, and Y. Ma, 1999. Effect of REE on the nutrients digestibility for growing pigs. Feed World, 11(1): 29 – 31.

Huang J., TL. Zhang, SJ. Xu, RC. Li, K. Wang, J. Zhang and YN. Xie, 2006. Effects of lanthanum on composition, crystal size, and lattice structure of femur bone mineral of Wistar rats. Calcified Tissue International, 78(4): 241-247.

Hughes, P., and J. Heritage (2001). Antibiotic growth-promoters in food animals. http://www.fao.org/DOCREP/ARTICLE/AGRIPPA/555_EN.HTM. Accessed in 2004.

Hutchison, A. J., and F. Albaaj, 2005. Lanthanum carbonate for the treatment of hyperphosphataemia in renal failure and dialysis patients. Expert Opinion on Pharmacotherapy, 6(2): 319 – 328.

Iyayi, E. A., and Bina I. Davies, 2005. Effect of enzyme supplementation of palm kernel meal and brewer's dried grain on the performance of broilers. International Journal of Poultry Science 4 (2): 76-80.

Jakupec, M. A., P. Unfried, and B. K. Keppler, 2005. Pharmacological properties of cerium compounds. Reviews of Physiology, Biochemistry and Pharmacology, 153(101 - 111).

Ji, Y., 1985. Toxicological study on safety evaluation of rare earth elements used in agriculture. In G. Xu and J. Xiao, editors, New frontiers in rare earth science and applications, Proceedings of the 1st international conference on rare earth development and applications.

Kahn, N., 2004. Alternatives to in-feed antibiotics. Re-evaluation of additives in Europe. Feed magazine, Kraftfutter. (7-8): 236-239.

Karaoglu, M., and H. Durdag, 2005. The influence of dietary probiotic (*Saccharomyces cerevisiae*) supplementation and different slaughter age on the performance, slaughter and carcass properties of broilers. International Journal of Poultry Science, 4(5): 309-316.

Kato, I., T. Yokokura, and M. Mutai, 1985. Induction of tumoricidal peritoneal exudates cells by administration of *Lactobacillus casei*. International Journal of Immuno-Pharmacology, 7:103-9.

Kawagoe, M., F. Hirasawa, S. C. Wang, Y. Liu, Y. Ueno, and T. Sugiyama, 2005. Orally administered rare earth element cerium induces metallothionein synthesis and increases glutathione in the mouse liver. Life Sciences, 77: 922 – 937.

Kessler, J., 2004. Lanthanoide - Wachstumsförderer mit Zukunft. In Schweinehaltung, 04.255, 22. - 23. Juni 2004, Sursee/Oberkirch, Schweiz.

Kirchgessner, M., B. R. Paulicks, and F. X. Roth, 1997. Effects of supplementations of diformate complexes (Formi^{TM} LHS) on growth and carcass performance of piglets and fattening pigs in response to application time. Agribiology Research, 50: 1-10.

Kirchgessner, M., and F. X. Roth, 1982. Fumaric acid as a feed additive in pig nutrition. Pig News Information, 3: 259-264.

Knebel, C., 2004. Untersuchungen zum Einfluss Seltener Erd-Citrate auf Leistungsparameter beim Schwein und die ruminale Fermentation im künstlichen Pansen (RUSITEC). Dissertation, Ludwig-Maximilians-Universität, München, 2004.

Koenen, ME., J. Kramer, R. van der Hulst, L. Heres, SH. Jeurissen and WJ. Boersma, 2004. Immunomodulation by probiotic lactobacilli in layer- and meat-type chickens. British Poultry Science, 45(3):355-66.

Kraatz, M., D. Taraz, K. Männer, and O. Simon, 2004. Eine Untersuchung zur Wirksamkeit Seltener Erden bei Ferkeln. In 8. Tagung Schweine und Geflügelernährung, am Institut für Ernährungswissenschaften, 23. - 25. November 2004, Halle, Germany.

Kurtoglu, V., F. Kurtoglu, E. Seker, B. Coskun, T. Balevi, and ES. Polat, 2004. Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol. Food Additives and Contaminants, 21(9): 817-23.

Kyriazakis, I., and G. C. Emmans, 1992. The effects of varying protein and energy intakes on the growth and body composition of pigs / Part 1. British Journal of Nutrition, 68(3): 615 – 625.

Langhout, P., 2000. New additives for broiler chickens. World Poultry, 16: 22-27.

Lázaro, R., M. A. Latorre, P. Medel, M. Gracia, and G. G. Mateos, 2004. Feeding regimen and enzyme supplementation to rye-based diets for broilers. Poultry Science, 83: 152-160.

Li, D., W. She, L. Gong, W. Yang, and S. Yang, 1992. Effects of rare earth elements on the growth and nitrogen balance of growing pigs. Feed BoLan, 4: 3 – 4.

Li, F., Y. Wang, Z. Zhang, J. Sun, H. Xiao, and Z. Chai, 2002. Distribution of samarium and yttrium in rats measured by enriched stable isotope tracer technique and INAA. Journal of Radioanalytical and Nuclear Chemistry, 251(3): 437 – 441.

Li, J., L. Zhang, J. Liu, L.Wang, Z. Wang,W.Wu, and Y. Ji, 1998. Inhibiting Effect of Light Rare Earth on Pulmonary Adenomas. Journal of Chinese Rare Earth Society, 16(2):184 – 187.

Liu, H., H. Lan, H. Wang, K. Wang, S. Xu, and S. Chang, 2004. Change of bone structure induced by hyperlipid and effects of lanthanum. Advance in Nature Science, 14(4): 397 – 403.

Liu, L., T. Shan, Y. Xie, Y. Huang, and M. Huang, 2003. Effect of different amino acid-metal coordination compound additives on quality of pork. Animal Husbandry and Veterinary Medicine, 35(8):1 –3.

Lu, J., X. Zhao, Y. Yang, X. You, Y. Bai, X. Li, and Y. Cheng, 2000. Effects of different forms of trace elements additives in feed on growth and nutritional condition in common carp (*Cyprinus carpio L.*). Journal of Dalian Fisheries College/Dalian Shuichan Xueyuan Yuebao, 15(3): 181 – 185.

Lu, K.W., and W. Z. Yang, 1996. Effects of rare earth elements on availability of energy and amino acids in broilers. Acta Agriculture Shanghai, 12: 78 – 82.

Lu, Z., D. Cheng, and Y. Liu, 2003. The hormesis effect of rare earth elements on several enzymes in rat liver. Key Laboratory of Rare Earth Chemistry and Physics, Changchun Institute of Applied Chemistry, Chinese Academy of Science, Changchun 130022.

Lueck, E., 1980. Antimicrobial food additives: Characteristics, Uses and Effects. Spring-Verlag, Berlin.

Luminescent Lanthanides, 1997. The Lanthinides: what they are. http://perso.univ-rennes1.fr/martinus.werts/lanthanides.

Marciniak, M., J. Chas, and Z. Baltrukiewicz, 1996. Transport of lanthanides in milk into suckling rats. The Quarterly Journal of Nuclear Medicine, 40(4): 351 – 358.

McCusker, R. H., and D. R. Clemmons, 1997. Use of lanthanum to accurately quantify insulin-like growth factor binding to proteins on cell surfaces. Journal of cellular biochemistry, 66: 256 – 267.

Meyer, U., M. Spolders, W. A. Rambeck, and G. Flachowsky, 2006. Effect of dietary rare earth elements on growth performance of preruminant female Holstein calves. Proceedings of the Society of Nutrition Physiology, 21. - 23. März, Göttingen, 15.

Midilli, M., and S. D. Tuncer, 2001. The effects of enzyme and probiotic supplementation to diets on broiler performance. Turkish Journal of Veterinary and Animal Science, 25(6): 895-903.

Ming, Y., Z. Xiu, H. Ming, and L. Yuan, 1995. Production and physiological effect of Rare Earth complex added to growing pig diet. In Proceedings of the 3rd International Conference on Rare Earth Development and Applications, 21. - 25. August 1995, Baotou, China.

Mroz, Z., A. W. Jongbloed, K. H. Partanen, K. Vreman, P. A. Kemme, and J. Kogut, 2000. The effects of calcium benzoate in diets with or without organic acids on dietary buffering capacity, apparent digestibility, retention of nutrients and manure characteristics in swine. Journal of Animal Science, 78: 2622-2632.

Ni, J. Z., 1995. The use of rare earth elements in agriculture and medicine, in: Bioinorganic Chemistry of Rare Earth Elements. Science Press, Beijing, China.

North Carolina State University, 2005. Searching for alternatives to antibiotic growth promoters. http://www.thepigsite.com/articles/. Accessed in 2005.

Nursey, I., 1997. Control of salmonella. Kraftfutter, 10: 415-22.

Ou, X., Z. Guo, and J. Wang, 2000. The effects of rare earth element additive in feed on piglets. Livestock and Poultry Industry, 4: 21 – 22.

Partanen, K. H., and Z. Mroz, 1999. Organic acids for performance enhancement in pig diets. Nutrition Research Reviews, 12:1-30.

Paulicks, B. R., F. X. Roth, and M. Kirchgessner, 1996. Dose effects of potassium diformate (Formi^{TM} LHS) on the performance of growing piglets. Agribiol. Res., 49: 318-326.

Powis, D. A., C. L. Clark, and K. J. O'Brien, 1994. Lanthanum can be transported by the sodium calcium exchange pathway and directly triggers catecholamine release from bovine chromaffin cells. Cell Calcium, 16(5): 377 – 390.

Prause, B., S. Gebert, C. Wenk, W. A. Rambeck, and M. Wanner, 2004. Seltene Erden – alternative Leistungsförderer beim Schwein – ein Überblick und erste Ergebnisse eines Gesamtstoffwechselversuches. In 3. BOKU Symposium für Tierernährung, Fütterungsstrategien und Produktqualität, 4. November 2004, pages: 38–44,Wien, Austria.

Prause, B., S. Gebert, C. Wenk, W. A. Rambeck, and M. Wanner, 2005a. Der Einfluss Seltener Erden auf den Kohlenstoff, Stickstoff und Energiewechsel wachsender Ferkel. In M. Kreuzer, C.Wenk, and T. Lanzini, editors, Fokus Verdauung - Schlüssel für eine effiziente Tierernährung, Schriftenreihe Institut für Nutztierwissenschaften, Ernährung-Produkte-Umwelt, ETH Zürich, volume 26, pages: 192–195.

Prause, B., S. Gebert, C. Wenk, W. A. Rambeck, and M. Wanner, 2005b. Impact of rare earth elements on metabolism and energy-balance of piglets. In 9th Congress of the European Society of Veterinary and Comparative Nutrition, 22. - 24. September 2005, Grugliasco (To), Italy.

Prause, B., S. Gebert, C. Wenk, W. A. Rambeck, and M. Wanner, 2005c. The impact of rare earth elements on energy, carbon, and nitrogen balance of growing pigs. In 10th Symposium of vitamins and additives in nutrition of man and animal, 28. - 29. September 2005, Jena, Germany.

Rafacz-Livingston, K. A., C. M. Parsons, and R. A. Jungk. The effects of various organic acids on phytate phosphorus utilization in chicks. Poultry Science, 84(9): 1356-1362.

Rambeck, W. A., M. L. He, J. Chang, R. Arnold, R. Henkelmann, and A. Süss, 1999a. Possible role of rare earth elements as growth promoters. In Vitamine und Zusatzstoffe in der Ernährung von Mensch und Tier, 7. Symposium, 22. - 23. September 1999, Jena, Germany.

Rambeck, W. A., M. L. He, J. Chang, R. Arnold, R. Henkelmann, and A. Süss, 1999b. Seltene Erden als Leistungsverbesserer in der chinesischen Landwirtschaft. In Spurenelemente und Mineralstoffe- Versorgungsstatus, Bioverfügbarkeit, Analytik, 1. - 2. Oktober 1999, Graz, Austria.

Rambeck, W. A., M. L. He, and U. Wehr, 2004. Influence of the alternative growth promoter "Rare Earth Elements" on meat quality in pigs. In Proceedings of the British Society of Animal Science pig and poultry meat quality - genetic and non-genetic factors, 14. - 15. October 2004, Krakow, Poland. British Society of Animal Science.

Rambeck, W. A., and U. Wehr, 2005. Use of rare earth elements as feed additives in pig production. Pig News and Information, 26(2): 41 N – 47 N.

Rastall, R. A., and G. R. Gibson, 1999. Prebiotic oligosaccharides: evaluation of biological activities and potential future developments. Probiotics and prebiotics: where are we going?. Wymondham, UK: Caister Academic Press.

Ravindran, V., and E. T. Kornegay, 1993. Acidification of weaner pig diets: A review. Journal of the Science of Food and Agriculture, 62: 313-322.

Redling, K., 2006. Rare earth elements in agriculture with emphasis on animal husbandry. Dissertation, Ludwig-Maximilians-Universität, München.

Richter, H., 1996 Die industrielle Produktion von Seltenen Erden in China. Erzmetall, 49:134 – 141.

Roberfroid, MB., 2000. Prebiotics and probiotics: are they functional foods?. The American Journal of Clinical Nutrition, 71(6 suppl):1682S-7S.

Rosenbaum, M., J. Hirsch, and E. Murphy, and R. L. Leibel, 2000. Effects of changes in body weight on carbohydrate metabolism, catecholamine excretion, and thyroid function. The American Journal of Clinical Nutrition, 71(6): 1421 – 1432.

Roth, F. X., and M. Kirchgessner, 1997. Formic acid as a feed additive for piglets: Nutritional and gastrointestinal effects. In: J. P. Laplace, C. Février and A. Barbeau (ed.) Proc. 7th International symposium on digestive physiology in pigs, Saint Malo, France. Pages: 498-501.

Roth, F. X., and M. Kirchgessner, 1998. Organic acids as feed additives for young pigs: Nutritional and gastrointestinal effects. Journal of Animal Feed Science, 7: 25-33.

Saki, A. A., M. T. Mazugi, and A. Kamyab, 2005. Effect of mannanase on broiler performance, ileal and in-vitro protein digestibility, uric acid and litter moisture in broiler feeding. International Journal of Poultry Science, 4(1): 21-26.

Savage, T. F., and E.I. Zakrzewska, 1995. Performance of male turkeys to 8 weeks of age when fed an oligosaccharide derived from yeast cells. Poultry Science, 74(suppl.1) 53.

Schuller, S., 2001. Seltene Erden als Leistungsförderer beim Geflügel. Untersuchungen an Broilern und Japanischen Wachteln. Dissertation, Ludwig-Maximilians-Universität, München.

Schuller, S., C. Borger, M. L. He, R. Henkelmann, A. Jadamus, O. Simon, and W. Rambeck, 2002. Untersuchungen zur Wirkung von Seltenen Erden als mögliche Alternative zu Leistungsförderern bei Schweinen und Geflügeln. Berliner Münchner Tierärztliche Wochenschrift, 115: 16 – 23.

Shahani, K., B. Friend, and P. Bailey, 1983. Antitumor activity of fermented colostrums and milk. Journal of Food Protection, 46: 385-6.

Shao, H., Z. Jiang, Z. Zong, H. Liu, and W. Liu, 1999. Effect of dietary Rare Earth Elements on some enzyme activities in liver, pancreas and blood of carp. Chinese Feed, 1: 21 – 22.

Shao, H., H. Liu, Z. Jiang, Z. Zong, and W. Liu, 1998. Effect of Rare Earth Elements on egg embryo development of carp. Fresh Water Fishery, 28(4):20 – 21.

Silversides, F. G., T. A. Scott, and M. R. Bedford, 2004. The Effect of Phytase Enzyme and Level on Nutrient Extraction by Broilers. Poultry Science, 83: 985-989.

Snow, J. L., D. H. Baker, and C. M. Parsons, 2004. Phytase, citric acid, and 1α-hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a corn-soybean meal diet. Poultry Science, 83: 1187-1192.

Solis de los Santos, F., M. B. Farnell, G. Téllez, J. M. Balog, N. B. Anthony, A. Torres-Rodriguez, S. Higgins, B. M. Hargis, and A. M. Donoghue, 2005. Effect of prebiotic on gut development and ascites incidence of broilers reared in hypoxic environment. Poultry Science, 84: 1092-1100.

Spring, P., 1996. Effects of mannanoligosaccharide on different cecal parameters and on cecal concentrations of enteric pathogens in poultry. Dissertation ETH No. 11897.

SpyLOG. Rare Earth Elements on the Net. Copyright©1999-2004.

Stanely, V. G., C. Gray, M. Daley, W. F. Krueger, and A. E. Sefton, 2004. An Alternative to Antibiotic-Based Drugs in Feed for Enhancing Performance of Broilers Grown on *Eimeria* spp.-Infected Litter. Poultry Science, 83: 39-44.

Tang, Y., C. Xia, and L. Wang, 1998. A comparative study of the effect of different REE compounds on growth performance of carp. Chinese Journal of Fisheries, 11(1): 47 – 49.

Tang, Y., B. Zhang, L. Wang, and J. Li, 1997. Effect of dietary rare earth elements-amino acid compounds on growth performance of carp and rainbow trout. Chinese Journal of Fisheries, 10(2): 88 – 90.

Tannock, GW., 1999. What are probiotics?. Probiotics: a critical review. Wymondham, UK: Horizon Scientific Press.

Thomke, S., and K. Elwinger, 1998. Growth promotants in feeding pigs and poultry ii; mode of action of antibiotic growth promotants. Annales de zootechnie, 47: 153-167.

Tome, D., and C. Bos, 2000. Dietary Protein and Nitrogen Utilization. Journal of Nutrition, 130(8): 1868– 1873.

Triggle, C. R., and D. J. Triggle, 1976. An analysis of the action of cations of the lanthanide series on the mechanical responses of guinea-pig ileal longitudinal muscle. The Journal of Physiology, 254 (1): 39 – 54.

Tzortzis, G., AK. Goulas, JM. Gee, and GR. Gibson, 2005. A novel galactooligosaccharide mixture increases the bifidobacterial population numbers in a continuous in vitro fermentation system and in the proximal colonic contents of pigs in vivo. The Journal of Nutrition, 135(7): 1726-31.

Vaccari, A., P. Saba, I. Mocci, and S. Ruiu, 1999. Lanthanides stimulate (3H) tyramine binding in the rat striatum. Neuroscience Letters, 261(1): 49 – 52.

Von Wright, A., 2005. Regulating the safety of probiotics-the European approach. Current Pharmaceutical Design, 11(1): 17-23.

Wald, P.H., 1990. A review of the literature on the toxicity of Rare-Earth metals as it pertains to the engineering demonstration system surrogate testing. Lawrence Livermore national laboratory, UCID-21823 Rev. 1, pages: 1 – 27.

Wallace, R. J., 2004. Antimicrobial properties of plant secondary metabolites. The Proceedings of Nutrition Society, 63(4): 621-629.

Wan, Q., J. Tian, H. Peng, X. Zhang, D. Lee, C. Woo, J. Ryu, and C. Park, 1998. The effects of rare earth on increasing yield, improving quality and reducing agricultural chemical remained in crop production. In 2nd International Symposium on Trace Elements and Food Chain, page 25, Wuhan, China, 1998.

Wang, H., H. Sun, Y. Chen, and X. Wang, 1999. The bioaccumulation of rare earth elements in the internal organs of fish and their effect on the activities of enzymes in liver. China Environmental Science, 19(2): 141 – 144.

Wang, M. Q., and Z. R. Xu, 2003. Effect of supplemental lanthanum on growth performance of pigs and its security as a feed additive. In Asian-Australasian journal of animal sciences, volume 16, pages: 1360 – 1363.

Wang, X., F. Hong, and S. Shen, 2002. Effect of cerium on activity of _-Amylase from porcine pancreas. Journal of the Chinese Rare Earth society, 20(4): 353 – 356.

Wang, X., F. Hong, S. Shen, G. Su, and X. Pan, 2000. Effect of cerium on activity of _-Amylase from porcine pancreas. Livestock and Poultry Industry, 4, 2000.

Wang, Y., F. Guo, K. Yuan, and Y. Hu, 2003. Effect of lanthanum chloride on apoptosis of thymocytes in endotoxemia mice. Acta Academia Medicine Jiangxi, 43(3): 31.

Wang, Z. R., S. Y. Qiao, W. Q. Lu, and D. F. Li, 2005. Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology, and volatile fatty acid profiles in hindgut of broilers fed wheat-based diets. Poultry Science, 84: 875-881.

Wehr, U., C. Knebel, W. A. Rambeck, and M. Wanner, 2005. Effect of Rare Earth Elements on ruminal fermentation as measured in vitro in Rumen Simulation Technique (RUSITEC). Proceedings of the Society of Nutrition Physiology, 14:84.

Wenk, C., 2003. Growth promoter alternative after the ban on antibiotics. Pig News and Information, 24(1): 11N-16N.

Wiesner, E., and R. Ribbeck, editors, 1991. Wörterbuch der Veterinärmedizin, volume 3. Gustav Fischer.

Wu, Y., 2000. Lanthanide ions inhibit the activity of dihydrofolate reductase from chicken liver. Biometals, 13(3): 195-201.

Wu, J., Z. Zhang, and J. Yan, 1994. An initial study on effect of adding rare earth element on productivity of egg laying breeder hens. NingXia Science and Technology of Farming and Forestry, 4: 36 –38.

Xie, J., and Z. Wang, 1998. The effect of organic rare-earth compounds on production performance of chicken. In 2nd International Symposium on Trace Elements and Food Chain, 12. - 15. November 1998, page 74, Wuhan, China.

Xie, J., Z. Xia, and Z. Wang, 1995. Studies on the effects of rare earth compound added to diets of Guangxi Broiler Chickens. unpublished.

Xie, K., Y. Xing, J. Zhang, H. Zhong, N. He, and S. Deng, 1991. Effects of rare earth elements on growth of broilers. Research of Agricultural Modernization (Chinese), 12: 50 – 54.

Xiong, B. K., 1995. Application of Rare Earths in Chinese Agriculture and their perspectives of Development. In Proceeding of the Rare Earths in Agriculture Seminar, 20. September 1995, pages: 5 – 9, Canberra, ACT Australia.

Xu, X., H. Xia, G. Rui, C. Hu, and F. Yuan, 2004. Effect of lanthanum on secretion of gastric acid in stomach of isolated mice. Journal of Rare Earths, 22(3): 427.

Xu, Z., M. Wang, and L. Chen, 1999. Growth response of pigs fed supplemental lanthanum and approach of mechanism. Journal of the Chinese Rare Earth Society, 17: 53 – 59.

Yang, H. W. Zhang, J. Cheng, H. Zhang, and Y. Zhu, 2005. Effect of Supplementing Rare-earth Complex Compound with Fumaric in Ration on Live weight Gain of Yellow Hybrid Broiler. Chinese Qinghai Journal of Animal and Veterinary Sciences, 35(3): 7 – 8.

Yang, Z., and L. Chen, 2000. Effect of lanthanum on egg hatching of Macrobrachium nipponense. Aquiculture, 6(1): 31 – 32.

Yang, Z., and L. Chen, 2002. Effect of Pr3+ on egg hatching of grass carp. FuJian Agricultural Technology, 1: 15 – 16.

Yang, Z., M. Dong, C. Mao, K. Zhang, and P. Zhang, 1992. Effects of Rare Earth Elements on Serum Parameters in Broiler. Gansu Animal Science and Veterinary medicine, 22: 7 – 8.

Yang, Z., M. Zhao, and Z. Wu, 2001. Effect of Rare Earth Citrate on Egg Hatching of Macrobrachium nipponense. Journal of Zhejiang Ocean University (Natural Science), 20(3): 192 – 194.

Yeo, J., and KI. Kim, 1997. Effect of feeding diets containing an antibiotic, a probiotic, or yucca extract on growth and intestinal urease activity in broiler chicks. Poultry Science, 76(2): 381-5.

Zhang, A., X. Li, P. Tian, and Y. Liu, 1996. A study of feeding Rare Earth Elements to laying anaphase hens. Journal of Husbandry and Veterinary, 15(1): 9 – 10.

Zhang, AW., BD. Lee, SK. Lee, KW. Lee, GH. An, KB. Song, and CH. Lee, 2005. Effects of yeast (Saccharomyces cerevisiae) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Poultry Science, 84(7): 1015-21.

Zhang, B., and L. Shao, 1995. Effect of inorganic REE on growth performance of broilers. Chinese Journal of Husbandry, 31(3):38 – 39.

Zhang, J., M. He, and H. Yang, 1994. A study of feeding Rare Earth Element addition to fattening bull. Gansu Journal of Husbandry and Veterinary, 114(2): 8.

Zhong, X., S. Yu Cheng, and C. Yong, 2003. Effect of lanthanum chloride on expressions of different types of collagen proteins in wound tissue. Journal of Hangzhou medical college, 24(5):210.

Zhou, G. Rare Earth Element is useful to ducks. Journal of Husbandry and Veterinary, 18(2):48.

Zhu, X., D. Li, W. Yang, C. Xiao, and H. Chen, 1994. Effects of rare earth elements on the growth and nitrogen balance of piglets. Feed Industry, 15: 23 – 25.

Acknowledgements

At first I would like to say special and great thank to my advisor Prof. Dr. Walter Rambeck for suggesting this research as my dissertation, for helping me to get scholarship and giving me the opportunity to study in Germany. I also thank him for spending his time to discuss my research problems and giving professional advice.

I owe many thanks to H. Wilhelm Schaumann Stiftung for providing the scholarship for me to do my research in Germany.

I would like to thank Dr. Ulrich Wehr who gave me advice and helped me with my problems during the experiments. I am also indebted to Ms. Stadler and other staff in Ubervisum Feld, who helped me with performing my experiments in farm. I would like to thank Mr. Hesselbach for helping me and showing the methods of chemical analysis of the samples from experiments. I wish to thank Kerstin for sharing papers about Rare Earth Elements.

Finally, I thank my dear parents for their support and their love.