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D Reka
PG Research Scholar, Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

V Thavasiappan
Sheep Breeding Research Station, Sandynallah, Tamil Nadu, India

P Selvaraj
Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

A Arivuchelvan
Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Tirunelveli, Tamil Nadu, India

P Visha
Department of Veterinary Physiology and Biochemistry, Veterinary College and Research Institute, Orathanadu, Tamil Nadu, India

Manju G Preedaa
Ph.D Research Scholar, Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Correspondence
D Reka
PG Research Scholar, Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Effect of dietary REE supplementation on intestinal enzyme activities in layer chicken

D Reka, V Thavasiappan, P Selvaraj, A Arivuchelvan, P Visha and Manju G Preedaa

Abstract
A biological study was conducted to determine the effects of dietary supplementation of different levels of rare earth elements (lanthanum and cerium) on intestinal enzymes amylase, lipase and trypsin in laying hens. A total of 96 White Leghorn laying hens of 52 weeks of age were used in 8-week feeding trial. Birds were randomly allotted to three dietary treatments each with four replicates and 8 hens per replicate. Treatments consist of basal diet supplemented with 0, 250mg (lanthanum 100mg and cerium 150 mg) and 500mg/kg (lanthanum 200mg and cerium 300 mg) of rare earth elements. At the end of 60th week, six birds per treatment were randomly selected and Sacrificed. Intestinal contents were collected in sterile vials for the enzyme activities and stored in airtight containers at -4°C. The results of this study showed that supplementation of rare earth elements (lanthanum and cerium) Significantly (P<0.05) enhanced the intestinal amylase and lipase, whereas the level of tryptic activities did not alter. Hence it was concluded that low dose of rare earth elements, the birds had a significant effect in intestinal amylase and lipase level.

Keywords: Rare earth elements, intestinal enzymes, amylase, lipase, laying hens

1. Introduction
Probiotics, prebiotics, organic acids and enzymes are already known as the 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 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 [1].

The effect of dietary rare earth elements varies with the animal species. Yet, the concentration and type of rare earth elements as well as the composition of individual rare earth elements have also been shown to be important factors influencing performance enhancing effects of rare earth elements on animals [2-4] concluded that rare earth elements could promote the secretion of digestive fluids [5]. Showed that lanthanum increased gastric acid secretion dose-dependently in isolated mice stomachs. Supplementation of rare earth elements changed the activity of lipase and amylase in pancreas from fish [6, 7] 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 [8]. reported that inclusion of rare earth elements mixture (nitrate of lanthanum, cerium, praseodymium, neodymium and samarium) at the level of 100, 200, 300 and 400 mg/kg diet in Common carp showed improved intestinal enzymes trypsin and lipase activities by 29.9 and 32.8 per cent in 200 and 300mg/kg REE mixture supplemented groups compared to control diet. 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 [3].

Therefore, the aim of this study was to investigate the effects of dietary rare earth element on intestinal enzyme activities in laying hens.

2. Materials and Methods

A total of 96 White Leghorn layers of 52 weeks of age were randomly assigned to three dietary treatment groups for 8-week feeding trial and the experiment was conducted at the Poultry Farm Complex, Department of Poultry Science, Veterinary College and Research Institute, Namakkal, Tamil Nadu. Laying hens were randomly assigned to three treatments with four replicates per treatment, and 8 hens in each replicate. The layers were reared in cages in gable roofed open sided, elevated platform house. All the birds were provided with a uniform cage floor, feeder and water space and were reared under standard management conditions throughout the experimental period. The experimental layer diets (table 1) were formulated according to the breeder's specification (Venkateshwara Hatcheries Private Limited). Basal diet supplemented with 0, 250 (La 100mg, Ce 150mg) and 500mg/kg (La 200, Ce 300 mg) of REE.

2.1 Collection of intestinal contents

At the end of 60th week, six birds per treatment were randomly selected and sacrificed. Intestinal contents were collected in sterile vials for the enzyme activities and stored in airtight containers at -4°C.

2.2 Intestinal enzyme activities

2.2.1 Amylase

Estimation of amylase activity of the digesta was assessed as per the method of [9]. The digesta was homogenized, centrifuged and the supernatant was collected. The supernatant was taken and diluted to 1:1000 with 0.9 per cent saline.

Buffered starch substrate was prepared by the addition of 13.3 g of anhydrous disodium phosphate and 4.3 g of benzoic acid to 250 ml of triple glass distilled water and boiled. A soluble starch (200 mg) was prepared separately in 5ml of cold distilled water and mixed to the boiling mixture. The beaker was rinsed with the additional cold water, boiled, centrifuged for one min, cooled at room temperature and diluted to 500 ml with triple glass distilled water. One ml of 0.02 per cent buffered starch substrate (pH 7.0) was incubated at 37°C exactly for 8.5 min along with 0.1 ml of diluted supernatant solution and one ml of working iodine solution (25 g of potassium fluoride with 50ml 0.1N iodine stock solution). The stock solution contained 3.567 g of potassium iodate, 45 g of potassium iodide, and 9ml of concentrate hydrochloric acid in one liter of triple glass distilled water. The working iodine solution was made up to 500ml with triple glass distilled water. The mixture was thoroughly mixed and the volume was made up to 10ml with triple glass distilled water. The OD was read at 660 nm in UV-VIS double beam spectrophotometer (SYSTRONICS, Model 2202, India)

$$\text{Amylase activity (Unit/ml)} = \frac{\text{OD of control - OD of test}}{\text{OD of control}} \text{ X volume of supernatant X dilution factor}$$

2.2.2 Tryptic activity

The tryptic activity of the digesta was assessed by the method of [10]. The digesta was homogenized, centrifuged and the supernatant was collected. It was serially diluted at the rate of 1, 1/2, 1/4, 1/8, 1/16 and 1/32. One drop of phenolpthalein solution was added to each tube followed by the addition of 2 per cent sodium bicarbonate solution drop by drop until the development of light pink colour. To all the tubes, 0.5ml of casein solution (0.4 g of casein in 40ml of 0.1N NaOH, 130 ml of triple glass distilled water and 30ml of 0.1N hydrochloric acid) was added and incubated at 40°C for 5h. The undigested casein was precipitated by drop wise addition of a precipitating solution (glacial acetic acid 1ml; alcohol (95%) 50ml and distilled water 50ml). Development of a clear solution indicated complete enzymatic digestion. The boiled supernatants and distilled water were taken in separate tubes with casein solution followed by the reagents served as control which showed turbidity on addition of the precipitating solution. The tryptic values were expressed in terms of dilution.

2.2.3 Lipase

The lipase activity of the digesta was determined as per the method of [11]. The substrate buffer mixture was prepared by stirring five volumes of 0.067 M phosphate buffer (pH 7.0) along with one volume of olive oil emulsion. Twelve milliliters of the substrate buffer mixture was equally transferred into two tubes warmed in a water bath at 37°C. To one tube 5ml of the intestine tissue homogenate was added and mixed thoroughly by gentle inversion. The second tube served as a blank. Both the tubes were incubated at 37°C for 24h. At the end of incubation, 5ml of tissue homogenate was added to the blank. The mixture was titrated along with 4-6 drops of phenolphthalein against 0.05N sodium hydroxide (NaOH) to get a distinct pink colour.

The lipase unit was the quantity of enzyme required to release an acid equivalent to one ml of 0.05N from an olive oil substrate in 24h.

$$\text{Lipase activity (Units/ml)} = \frac{\text{Titre value of test} - \text{Titre value of Blank}}{\text{Weight of intestinal content (g)}} \text{ X 5 (dilution factor)}$$

3. Statistical analysis

The data collected were analysed using SPSS® 20.0 software package. Post hoc analysis was done by Duncan's multiple descriptive significant difference. All the statistical procedures were performed based upon [12].

4. Results and discussion

The effect of supplementation of rare earth elements on the intestinal enzymes amylase, lipase, trypsin activities of post peak layers is presented in Table 2.

The mean (± S.E.) intestinal amylase activities in the post peak layer were significantly (p<0.05) increased in the treatment groups compare to control. The supplementation of

REE enhanced the intestinal amylase and lipase, whereas the level of tryptic activities did not alter. In this present study intestinal amylase activity significantly (p<0.05) increased in rare earth element supplemented groups. These findings are agreement with [13] who reported greater activity of amylase by the addition of nano ceria at 30mg/kg in hens diet compared to control.

The mean intestinal activity of trypsin (U/ml) of post peak layers did not show any significant difference among the treatment groups. On the contrary, [8] recorded 29.9 per cent more trypsin in Common carp by adding a rare earth element mixture at 300 mg/kg diet.

The results of the present study showed significantly (p<0.05) increased intestinal lipase activity, agreement with the observation of increased lipase activities by addition of organic rare earth compounds (1 to 3 %) in chickens [14], 32.8 per cent in 200 and 300mg/kg REE mixture supplemented groups in [8] Common carp compared to control birds.

[3] reviewed literature on rare earth elements and concluded that enzymes involved in the digestive system were also shown to be affected by rare earth element supplementation. It is known that the conversion of trypsinogen into its active form trypsin is catalyzed by calcium, whereas at the same time, calcium also prevented the autodigestion of trypsin. Thus, by replacing calcium, rare earth elements were able to accelerate the autocatalytic activation of trypsinogen. Though the requirement of rare earth concentrations was 100 times lower, their outcome was more effectual than that of calcium. In a similar way, rare earths can also affect several other enzymes, which are important for digestion and utilization of nutrients, such as α-amylase, lipase, proteinase and catalase. The gastrointestinal motility, nutrient absorption and secretion of digestive juices may be influenced by rare earth elements via the nervous system.

Table 1: Ingredients and nutrient composition of experimental layer diet (DM %)

Ingredients	Kg/100 kg diet
Maize	50.5
DORB	13.5
SFOC	6.0
SOYA	17.5
Calcite/LSP	5.5
Grit	5.0
Di calcium phosphate	1.5
Methionine	0.164
Lysine	0.117
NSP Enzyme	0.05
Salt	0.137
Nutrient compositions (%)	
Crude protein	16.67
Crude fibre	6.4
Calcium	4.0
Ether extract	3.0
Available phosphorus*	0.41
Lysine*	0.89
Methionine*	0.45
Metabolizable Energy* (kcal/kg)	2550

* Calculated values

Additives and supplements (per 100 kg): Vitamin premix ([1]Hyblend) - 10 g, trace mineral ([2]Ultra TM) - 100 g, toxin binder - 25 g, Vitamin B-complex ([3]Meriplex) - 10 g, liver stimulant (hepatocare) - 25 g, choline chloride (60 %) - 50 g, oxytetracycline (10 %) - 50 g

[1]Hyblend – nutritional value per gram- vitamin A - 82500 IU, vitamin B2 - 50 mg, vitamin D3 - 12000 IU, menaphthone sodium bisulphate and vitamin K (stabilized) - 10 mg.

[2]Ultra TM - Each 5kg contains manganese - 270 g, zinc - 260 g, iron - 100 g, iodine - 10 g, copper - 10 g, cobalt - 5 g, selenium - 1.5 g

[3]Meriplex - each gram contains vitamin B_1 - 8 mg, vitamin B_6 - 16 mg, vitamin B_{12} - 80 mcg, vitamin E_{50} - 80 mg, niacin - 120 mg, folic acid - 8 mg, calcium D pantothenate - 80 mg, calcium - 86 mg.

Table 2: Mean (± SE) small intestinal amylase (U/ml), trypsin (U/ml) and lipase (U/ml) of White Leghorn layers fed different level of REE

Treatment	Amylase	Tryptic activity	Lipase
T1 Control	53.97^{a} ±7.08	11.33 ±2.17	3.12^{a} ±0.64
T2 250 mg (La 100 mg + Ce 150 mg)	81.58^{b} ±7.40	12.00 ±4.38	4.99^{b} ±0.58
T3 500 mg (La 200 mg + Ce 300 mg)	74.69^{ab} ±7.65	10.67 ±2.46	3.88^{ab} ±0.52

Means bearing different superscript within the column differ significantly (P<0.05) Each value is a mean of six observations

5. Conclusion

In conclusion the results of the experiment revealed that supplementation of rare earth elements (lanthanum and cerium) Significantly (P<0.05) enhanced the intestinal amylase and lipase. Based on the results of this study, it can be recommended to supplement laying hens feed with low level of rare earth elements.

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7. References

1. Zohravi M. The effect of rare earth elements on growth performance, tibia mineralization and blood serum of Japanese quails. Dissertation of veterinary Doctorate of Veterinary Faculty of the Ludwig Maximilian, University of Munich, 2006.
2. He ML, Wang YZ, Xu ZR, Chen ML, Rambeck WA. Effect of dietary rare earth elements on growth performance and blood parameters of rats. Journal of animal Physiology and animal Nutrition. 2003; 87:229-235.
3. Redling K. Rare earth elements in agriculture with emphasis on animal husbandry. Dissertation, the Ludwig Maximilian University, Munich. 2006.
4. Ou X, Guo Z, Wang J. The effects of rare earth element additive in feed on piglets. Livestock and Poultry Industry. 2000; 4:21-22.
5. Xu X, Xia H, Rui G, Hu C, Yuan F. Effect of lanthanum on secretion of gastric acid in stomach of isolated mice. Journal of Rare Earths. 2004; 22:427.
6. Wang H, Sun H, Chen Y, Wang X. 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. 1999; 19:141-144.
7. Wang X, Hong F, Shen S. Effect of cerium on activity of α- Amylase from porcine pancreas. Journal of the Chinese Rare Earth society. 2002; 20(4):353-356.
8. Jiang ZY, Jia ZH, Guo YS, Zhou FW, Liu HT. Effect of

rare earth elements on digest enzyme and growth of intestinal in common carp (Cyprinus Carpio). Chinese Journal of Animal Nutrition. 2007; 19:86-90.
9. Coles. Pancreatic function test. In: Veterinary Clinical Pathology. 4th edn., W. B. Saunders Company, Philadelphia, London. 1986, 444-446.
10. Hawk PB, Oser BL, Summerson WH. Practical Physiological Chemistry, 12th edn., The Blakiston Company, Toronto. 1947, 349-350.
11. Boutwell Jr JA. Clinical Chemistry Laboratory Manual Methods. 1st edn., Lea and Febiger, Philedelphia. 1962, 212-214.
12. Snedecor GM, Cochran WC. Statistical Methods. 9th Edn. Oxford and IBM Publishing company, Mumbai, India, 1994.
13. Chen H, Di K, Wang C, Bai K, Li L, Ge S *et al*. Effect of nano-ceria on amylase activity and gene expression of hens. Journal of Food, Agriculture and Environment. 2014; 12:47-51.
14. Xie J, Wang Z. The effect of organic rare earth compounds on production performance of chicken. In 2nd International Symposium on Trace Elements and Food Chain, Wuhan, China, 1998.