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Effect of Dietary REE Supplementation on Intestinal Microbial Count and Ileal Digestibility in Post peak Layer Chicken

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ABSTRACT

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A biological study was conducted to determine the effects of dietary supplementation of different levels of rare earth elements (lanthanum and cerium) on intestinal microbial count, ileal digestibility in post peak 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 intestinal microbial count, ileal digestibility and stored in airtight containers at -4°C. The results of this study showed that supplementation of rare earth elements (lanthanum and cerium) did not change the mean intestinal microbial count (log₁₀ cfu/g) among the treatment groups. The ileal nutrient digestibility (%) of crude protein, crude fibre and ether extract of post peak layers showed 1-2 per cent improvement among the treatment groups over the control.

Introduction

Rare earth elements are a set of seventeen chemical elements in the periodic table, specifically the fifteen lanthanides plus scandium and yttrium. REE can improve

digestibility and utilization of nutrients in the diet (Li *et al.*, 1992) which may be achieved through influencing the development of selected bacterial groups in the intestinal tract, or through stimulating activities of the hormones such as growth hormone and

triiodothyronine (Redling, 2006). Because there is a special relationship between REE and calcium in both animal and plant cells, it is also suggested that REE may affect activities of the hormones or enzymes by inhibiting or replacing calcium (Takada *et al.*, 1999). Rare earth elements have been shown to promote the animal growth by influencing the growth of bacterial species within the gastrointestinal tract selectively, by inhibiting undesired bacteria (Rambeck and Wehr, 2005). Hence, Rare earth elements maintain the micro-flora of the intestinal tract, which is involved in digestion processes, and prevent the disease onsets (Redling, 2006).

Rare earth elements cause bacterial flocculation by altering the structure and surface charge of bacterial membranes (Bentz *et al.*, 1988). By this same manner, rare earth elements promoted cell aggregation and membrane fusion (Cassone and Garaci, 1974). In general, Gram negative bacteria usually have a peptidoglycan layer beneath the lipopolysaccharide which makes them less sensitive to lysozyme. Peng *et al.*, (2004) showed that La^{3+} caused damage to the Gram negative bacteria by changing the structure of outer cell membrane.

Muroma, (1958) reported that the presence of lanthanum increased the susceptibility of bacteria to lysozymes. REE reduce the bacterial metabolism by inhibiting the respiration process (Brooks, 1921). Wenhua *et al.*, (2003) reported that lower concentrations (0.5 - 30 $\mu\text{g}/\text{kg}$), La^{3+} could inhibit the absorption of external DNA by *E. coli*, thereby effectively decreasing its transformation effectively. Ou *et al.*, (2000) suggested that rare earth elements additives lowered the pH value in the digestive tract of piglets, thus suppressed the growth of pathogenic bacteria by its acid character. Cerium inhibits the growth of several bacteria including *E. coli*, *Bacillus pyocyaneus*,

Staphylococcus aureus, *Leuconostoc* and *Streptococcus faecalis*, when applied at concentrations ranging from 10^{-3} mol/l to 10^{-2} mol/l (Zhang *et al.*, 2000).

Rare earth elements supplementation in feed improved the utilization of dietary nutrients such as total energy, crude protein and crude fat in chicken (Xie and Wang, 1998). Supplementation of rare earth elements at the level of 200, 400 or 600 mg/kg diet in weaning pigs showed significantly improved apparent digestibility of energy and protein, digestibility of total amino acids and total essential and non-essential amino acids in 400 and 600 mg/kg of REE supplemented groups compared to control (Hu *et al.*, 1999).

Therefore the aim of this study was to investigate the effects of dietary rare earth element on intestinal microbial count and ileal digestibility in post peak laying hens.

Materials and Methods

Experimental birds

A total of 96 White Leghorn layers of 52 weeks of age were randomly assigned to three dietary treatment groups for 8-weeks 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 there were 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.

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 microbial count. Samples for ileal digestibility studies were taken from the ileal region lying 18 cm below the Meckels diverticulum and stored in airtight containers at -4° C.

Microbial count

Immediately after slaughter, the intestinal content was transferred to sterile sample container and transferred to the laboratory. One gram of intestinal content from each experimental group was pooled, thoroughly mixed in the laboratory and made into duplicate samples. A serial dilution up to 10⁻⁸ was prepared. Each sample was diluted with 9ml of sterile physiological saline solution and it was thoroughly mixed. From each dilution 100 µl of aliquot was spread on the appropriate selective agar plates and incubated at 37° C for 24 h. Total plate count agar, MacConkey agar, MRS agar were used as the medium for estimation of total bacterial count, *Escherichia coli* count and *Lactobacillus* respectively by spread plate method. After incubation, the colonies were counted and expressed as the numbers of colony forming (cfu) per gram of ingesta content as per the method of Quinn *et al.*, (1992).

Ileal digestibility

The ileal digestibility was studied using titanium dioxide (TiO₂) as marker added at the rate of 10 g per kg of feed. Six birds from each group were fed with TiO₂ containing

diets for five days to study the ileal digestibility. Birds were slaughtered on day six and ileal contents were milked out immediately into a container. These samples were pooled across treatments. The ileal digesta immediately after collection were transferred into the oven and dried at 80°C for 24 h. The dried ileal digesta samples were stored in airtight containers at -4° C and were analyzed for crude protein, crude fiber and ether extract. The TiO₂ content of the feed and ileal digesta were estimated according to the method of Myers *et al.*, (2004).

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 Snedecor and Cochran (1994).

Results and Discussion

The influence of Rare earth elements at different levels on intestinal microbial count and ileal digestibility in post peak layers are presented in tables 2 and 3.

The mean intestinal microbial count (log₁₀ cfu/g) of post peak layer did not vary significantly among the treatment groups.

The results of the present study concurred with that of Cai *et al.*, (2015) and Cai *et al.*, (2016), who reported no change in *lactobacillus* and *E. coli* count when rare earth element enriched yeast (0.05 to 1.0 %) fed in birds compared to control. Similarly, rare earth elements did not affect the composition of faecal bacterial populations in pigs, which was investigated in biomolecular studies using PCR-DGGE methods (Polymerase Chain Reaction Amplification (PCR) - Denaturing Gradient Gel

Electrophoresis (DGGE)) (Kraatz *et al.*, 2004). The ileal nutrient digestibility (%) of crude protein, crude fibre and ether extract of post peak layer showed 1-2 per cent improvement among the treatment groups. These results are in concurrence with the finding of Xie and Wang, (1998) reported that layer diets influenced positively the nutrient digestibility of crude protein, crude fibre and crude fat by supplementation of organic rare earth elements compared to control diet. Similarly, improved apparent digestibility in

piglets by addition of Rare earth elements mixture (Hu *et al.*, 1999), improved dry matter digestibility in broilers chicken at different levels of dietary rare earth element enriched yeast (Cai *et al.*, 2015) in treatment groups compared to control. However, Cai *et al.*, (2016) reported no changes in dry matter, gross energy and increased nitrogen digestibility when rare earth element enriched yeast (0.05 to 1.0 %) fed in birds compared to control.

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 (¹Hyblend) - 10 g, trace mineral (²Ultra TM) - 100 g, toxin binder - 25 g, Vitamin B-complex (³Meriplex) - 10 g, liver stimulant (hepatocare) - 25 g, choline chloride (60 %) - 50 g, oxytetracycline (10 %) - 50 g

¹Hyblend – 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.

²Ultra 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

³Meriplex - each gram contains vitamin B₁ - 8 mg, vitamin B₆ - 16 mg, vitamin B₁₂ - 80 mcg, vitamin E₅₀ - 80 mg, niacin - 120 mg, folic acid - 8 mg, calcium D pantothenate - 80 mg, calcium - 86 mg.

Table.2 Mean (\pm SE) Intestinal microbial count (log₁₀ cfu/g) of White Leghorn layers fed with different levels of REE during 60 weeks of age

Treatment	Total Microbial count (log ₁₀ cfu/g)	<i>E.coli</i> count (log ₁₀ cfu/g)	<i>Lactobacillus spp</i> count (log ₁₀ cfu/g)
T1 Control	9.12 \pm 0.03	5.69 \pm 0.27	5.19 \pm 0.31
T2 250 mg (La 100 mg + Ce 150 mg)	8.89 \pm 0.3	5.36 \pm 0.18	5.28 \pm 0.36
T3 500 mg (La 200 mg + Ce 300 mg)	9.40 \pm 0.16	5.66 \pm 0.18	5.33 \pm 0.27
Each value is a mean of six observations			

Table.3 Effect on nutrient digestibility (%) in White Leghorn layers fed different levels of REE in 60th week of age

Treatment	Digestibility coefficient (%)		
	Crude protein	Crude fibre	Ether extract
T1 Control	77.87	19.53	69.46
T2 250 mg (La 100 mg + Ce 150 mg)	78.35	20.05	71.31
T3 500 mg (La 200 mg + Ce 300 mg)	79.30	20.45	71.14

Each value is a mean of two observations

In conclusion the results of the experiment revealed that supplementation of rare earth elements (lanthanum and cerium) did not change the mean intestinal microbial count (log₁₀ cfu/g) among the treatment groups. The ileal nutrient digestibility (%) of crude protein, crude fibre and ether extract of post peak layers showed 1-2 per cent improvement among the treatment groups over the control. Growth performance enhancing effects due to

dietary supplementation of rare earth elements at low concentrations are described for nearly all categories of farming animals, including beef cattle, sheep, pigs, rabbits, ducks, chickens, shrimps and fish (Redling, 2006). 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.

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