# Biological activities of lanthanum oxide in laying hens

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**Primary Audience**: Poultry Researchers

## SUMMARY

The aim of the present study was to determine the effects of dietary lanthanum oxide levels (0, 100, 200, 300, or 400 mg/kg) on the laying performance, egg quality, fatty acids composition of yolk, egg lipid peroxidation, and some blood serum parameters of laying hen. One hundred twenty 22-wk-old brown Lohman LSL laying hens were randomly assigned to 5 groups with 6 replications. Dietary supplementation of 400 mg/kg lanthanum oxide significantly ($P < 0.05$) increased egg production. The addition of lanthanum oxide to the diet of laying hens' had no effect on feed intake and egg weight ($P > 0.05$). Supplementation of 100 and 200 mg/kg lanthanum oxide to diet caused a significant ($P < 0.05$) increase in Haugh unit and eggshell breaking strength. Blood parameters were not affected by supplementing lanthanum oxide. Malondialdehyde (MDA) concentration of serum decreased significantly ($P < 0.05$) with supplementation of 300 mg/kg lanthanum oxide to laying hen diets. It was also observed that thiobarbituric acid reactive substance (TBARS) values in egg yolk decreased significantly ($P < 0.01$) with supplementation of lanthanum oxide in diets. Supplementation of 100 mg/kg lanthanum oxide increased saturated fatty acid (SFA). In conclusion, dietary supplementation of lanthanum oxide had positive effects on feed conversion ratio, egg production, and egg shelf life. According to these results, it might be advised to supplement laying hens feed with lanthanum oxide as a feed additive.

**Key words:** egg production, lanthanum, laying hens, malondialdehyde, SOD, TBARS

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## DESCRIPTION OF PROBLEM

The use of antibiotics as growth promoters has been banned in many developed countries. Therefore, many scientists have searched for alternatives to in-feed antibiotics. Growth promoters such as prebiotics, probiotics, organic acids, essential oils, and enzymes are widely used. Rare earth elements (REE) have been shown to influence growth performance in animal production [1]. He et al. [2] reported that REE might be candidates as new natural feed additives to improve animal performance. Rare earth elements comprise a group of metallic elements that have similar properties. Scandium, yttrium, lanthanum, cerium, praseodymium, neodymium, promethium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, and lutetium are rare earth elements called lanthanides [3].

It is reported that many results have published in the literature, especially Chinese literature, on the growth promoting effect of REE in poultry [4]. Also, it is found that REE have

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antimicrobial and antioxidant effects for animals [5–7]. In laying hens, it was found that supplementation 200, 400, 600, and 800 mg/kg REE to the diet significantly increased egg production and egg weight [8]. Zhang et al., [9] studied the effect of supplemented diets with 300, 400, and 500 mg/kg of REE-nitrate on 53-wk-old laying hens. The result showed that the diets with 300 and 400 mg/kg of REE significantly improved laying rate ($P < 0.05$).

Some researchers reported that supplementation of the diet with REE has improved body weight gain in broilers [2, 10, 11]. In contrast to these studies, Xie and Wang [6] found that growth rate and feed conversion ratio were negatively affected with high levels (300 mg/kg) of REE in the diet. Schuller et al. [12] and Agbede et al. [13] showed REE and lanthanum respectively had no effect on production parameters of broiler chickens.

There have been none or few studies in literature that explore antioxidant effects of lanthanum oxide in laying hens. Therefore, this study was designed to determine the effect of dietary lanthanum oxide supplementation on laying performance, egg quality, some blood serum biochemical parameters and glutathione peroxidase (GPx), superoxide dismutase (SOD) and malondialdehyde (MDA) amount in serum, as well as egg lipid oxidation and fatty acid composition of yolk.

## MATERIAL AND METHOD

### *Birds, Housing, Design, and Diets*

In this study, 120 22-wk-old Lohman LSL hybrid laying hens (browns) with an average weight of 1717.98 ± 19.74 g were divided into 5 groups, each of which comprised 6 cages (50 × 46 × 46 cm) each with 4 animals.

Control group was fed basal diet (Table 1). The basal diet was formulated according to recommendations of NRC [14]. Experimental diets were prepared from the basal diet by adding different lanthanum oxide levels: 100, 200, 300, 400 mg/kg, respectively. Lanthanum oxide (99% pure) was purchased from a commercial company (Sigma-Aldrich). During the experiment (10 wk in duration), hens were fed and watered ad libitum.

**Table 1.** Composition and calculated content of experimental diet.

| Item | Composition (%) |
|---|---|
| Corn | 52.736 |
| Soybean meal 46% | 15.628 |
| Sunflower meal 36% | 11.00 |
| Limestone | 8.940 |
| Wheat middlings | 7.50 |
| Meat and bone meal | 2.50 |
| Vitamin-mineral premix[a] | 0.20 |
| Soybean oil | 1.00 |
| Salt | 0.216 |
| Dicalcium phosphate | 0.205 |
| $NaHNO_3$ | 0.075 |
| Total | 100 |
| Analysis results (%) | |
| ME (MJ/kg) | 2720 |
| Crude protein (%) | 17.50 |
| Lysine (%) | 0.85 |
| Met (%) | 0.42 |
| Met + Cys (%) | 0.73 |

[a]Per kg diet was supplemented with: 12,000 IU vitamin A, 2 400 IU vitamin D3, 30 IU vitamin E, 2.5 mg vitamin K3, 3 mg vitamin B1, 6 mg vitamin B2, 30 mg niacin, 10 mg calcium D-pantothenate, 5 mg vitamin B6, 0.015 mg vitamin B12, 1 mg folic acid, 0.050 mg D-biotin, 50 mg vitamin C,125 mg choline chloride 80 mg manganese, 60 mg iron, 60 mg zinc, 5 mg copper, 0.5 mg cobalt, 0.2 mg iodine, 0.15 mg selenium.

### *Data Collection*

Feed intake and egg production were recorded, and feed conversion ratio was calculated daily. Feed conversion ratio was calculated as grams of feed intake per gram of egg mass produced. Egg weight, Haugh units, specific gravity, shell thickness, shell breaking strength and weight of albumen, yolk and shell were measured biweekly using eight eggs from each dietary treatment.

### *Chemical Analyses*

At the end of the study, each group blood samples (n = 6) were taken from wing vena into additive free blood tubes. The study protocol was approved and conducted in accordance with the Animal Ethics Committee Guidelines of Atatürk University (protocol number: 2014/116). Serum was obtained following centrifugation at 4,000

$\times g$ for 10 minute at +20°C. Cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG), glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), phosphorus (P), and calcium (Ca) levels of serum were determined by biochemical automatic analysis. Superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA) in serum were determined by commercial kits. Also, all eggs were sampled according to date of laying and hen number, and stored at 4°C for thiobarbituric acid reactive substance (TBARS) analysis at 1, 21, and 42 d. TBARS were determined according to Tarladgis et al. [15]. The TBARS values were described as mg malonaldehyde/kg yolk. Also, 5 egg samples were taken from each treatment group to determine fatty acid composition of yolk at the end of experiment [16].

### *Statistical Analyses*

Data were analyzed with one-way and two-way analysis of variance (ANOVA) by using the statistical package SPSS for Windows [17], version 10.0. Significant means were subjected to a multiple comparison test (Duncan) at $\alpha = 0.01$ and 0.05 level.

## RESULTS

Egg production, egg weight, feed intake and feed conversion ratio of the layers fed diets containing lanthanum oxide are presented in Table 2. There were no significantly differences between the groups in terms of feed intake and egg weight. Egg production was significantly ($P < 0.05$) increased by supplementation of 400 mg/kg lanthanum oxide in the experiment. The level of 400 mg/kg lanthanum oxide improved feed conversion ratio compared to 100 mg/kg and 300 mg/kg lanthanum oxide.

The effect of dietary treatments on some egg quality characteristics is presented in Table 3. The addition of lanthanum oxide to the diet had no impact on percent of albumen, yolk and shell, or on specific gravity and shell thickness in this study. Haugh unit was significantly ($P < 0.05$) influenced by treatment. The highest Haugh unit was obtained in eggs of hens fed the diet containing 100 mg/kg lanthanum oxide compared to control group, 200 mg/kg and 400 mg/kg lanthanum oxide. Shell breaking strength of egg was significantly ($P < 0.05$) increased by supplementing 200 mg/kg of lanthanum oxide compared to other groups. Blood metabolites of laying hens are shown in Table 4. None of the dietary factors did affect serum ALT, AST, glucose, TG, total cholesterol, LDL, HDL, Ca, and P concentration.

The effects of dietary treatments on MDA, SOD and GPx of serum of laying hens are shown in Table 5. The differences among groups were not significant for SOD and GPx values in the present study. The addition of 300 mg/kg lanthanum oxide to the basal diet decreased significantly ($P < 0.05$) serum MDA value.

The effects of dietary lanthanum oxide on TBARS values of egg yolk are shown in Table 6. Supplementation of dietary lanthanum oxide did not affect the TBARS values at 1 and 21 d. But on d 42, egg yolk of hens fed 200, 300 and 400 mg/kg lanthanum oxide diets had

**Table 2.** Effects of dietary supplementation lanthanum oxide on performance of the laying hens (df = 5).[1]

| Groups | Feed intake (g/hen/d) | Egg production (%) | Feed conversion ratio (g:g) | Egg weight (g) |
|---|---|---|---|---|
| Control | 131.75 | 83.91[b] | 2.57[a,b] | 61.10 |
| Lanthanum 100 mg/kg | 133.20 | 80.91[b] | 2.86[a] | 58.22 |
| Lanthanum 200 mg/kg | 133.30 | 80.83[b] | 2.53[a,b] | 65.86 |
| Lanthanum 300 mg/kg | 134.88 | 80.83[b] | 2.76[a] | 61.08 |
| Lanthanum 400 mg/kg | 133.41 | 90.24[a] | 2.39[b] | 61.88 |
| SEM | 1.24 | 1.54 | 0.11 | 0.89 |
| *P* value | NS | 0.05 | 0.04 | NS |

[a,b]Column means with no common superscript differ significantly ($P < 0.05$). NS: Not significant.
[1]df: degree of freedom.

**Table 3.** Effects of dietary supplementation lanthanum oxide on egg quality of the laying hens (df = 7).[1]

| Groups | Albumen (%) | Yolk (%) | Shell (%) | Specific gravity | Haugh units | Shell thickness ($\mu$m) | Shell breaking strength (kg/cm$^2$) |
|---|---|---|---|---|---|---|---|
| Control | 64.93 | 24.29 | 10.77 | 1.08 | 76.48$^{b}$ | 0.473 | 2.06$^{b}$ |
| Lanthanum 100 mg/kg | 64.70 | 23.95 | 11.34 | 1.09 | 84.89$^{a}$ | 0.444 | 2.20$^{b}$ |
| Lanthanum 200 mg/kg | 65.48 | 24.00 | 10.51 | 1.06 | 79.47$^{b}$ | 0.460 | 2.64$^{a}$ |
| Lanthanum 300 mg/kg | 64.30 | 24.70 | 10.98 | 1.06 | 81.03$^{a,b}$ | 0.443 | 2.13$^{b}$ |
| Lanthanum 400 mg/kg | 63.85 | 25.04 | 11.10 | 1.10 | 78.90$^{b}$ | 0.416 | 2.15$^{b}$ |
| SEM | 0.54 | 0.45 | 0.35 | 0.014 | 0.83 | 0.023 | 0.064 |
| *P* value | NS | NS | NS | NS | 0.02 | NS | 0.03 |

$^{a,b}$Column means with no common superscript differ significantly ($P < 0.05$). NS: Not significant.

[1]df: degree of freedom.

**Table 4.** Effects of dietary supplementation lanthanum oxide on blood serum biochemical parameters of the laying hens (df = 5).[1]

| Item | Control | Lanthanum 100 mg/kg | Lanthanum 200 mg/kg | Lanthanum 300 mg/kg | Lanthanum 400 mg/kg | SEM | *P* value |
|---|---|---|---|---|---|---|---|
| ALT (U/L) | 27.66 | 9.5 | 15.83 | 17 | 12.5 | 4.2 | NS |
| AST (U/L) | 198.83 | 176.6 | 195.3 | 188.6 | 206.6 | 11.55 | NS |
| Glucose (mg/dL) | 114.5 | 139 | 128.6 | 129.6 | 152.3 | 14.17 | NS |
| TG (mg/dL) | 1046.83 | 1115.3 | 1295.16 | 1240 | 1071.6 | 124.92 | NS |
| Total cholesterol (mg/dL) | 139.16 | 82 | 113.3 | 113.5 | 125.5 | 32.45 | NS |
| LDL (mg/dL) | 47.66 | 43.6 | 51.66 | 49.5 | 50 | 3.7 | NS |
| HDL (mg/dL) | 30.66 | 14.6 | 23.3 | 22.6 | 49.83 | 6.97 | NS |
| Ca (mg/dL) | 24.63 | 20.48 | 22.73 | 22.83 | 22.5 | 1.09 | NS |
| P (mg/dL) | 6.23 | 4.6 | 5.35 | 4.76 | 5.12 | 0.36 | NS |

[1]df: degree of freedom.

NS: Not significant.

**Table 5.** Effects of dietary supplementation lanthanum oxide on MDA, SOD, GPx in serum of the laying hens (df = 5).[1]

| Groups | MDA (M/L) | SOD (U/L) | GPx (M/L) |
|---|---|---|---|
| Control | 0.78$^{a}$ | 144.49 | 25.18 |
| Cerium 100 mg/kg | 0.69$^{a,b}$ | 125.31 | 11.54 |
| Cerium 200 mg/kg | 0.69$^{a,b}$ | 120.80 | 14.50 |
| Cerium 300 mg/kg | 0.66$^{b}$ | 97.69 | 18.31 |
| Cerium 400 mg/kg | 0.77$^{a}$ | 110.10 | 21.78 |
| SEM | 0.015 | 6.26 | 1.88 |
| *P* value | 0.02 | NS | NS |

$^{a,b}$Column means with no common superscript differ significantly ($P < 0.05$).

NS: Not significant.

[1]df: degree of freedom.

**Table 6.** Effects of dietary supplementation lanthanum oxide on TBARS (mg MDA/kg) values in egg yolk of the laying hens (df = 5).[1]

| Groups | 1 d | 21 d | 42 d |
|---|---|---|---|
| Control | 0.09 | 0.26 | 0.90$^{a}$ |
| Lanthanum 100 mg/kg | 0.15 | 0.26 | 0.80$^{a}$ |
| Lanthanum 200 mg/kg | 0.09 | 0.27 | 0.50$^{b}$ |
| Lanthanum 300 mg/kg | 0.10 | 0.25 | 0.50$^{b}$ |
| Lanthanum 400 mg/kg | 0.15 | 0.25 | 0.40$^{b}$ |
| SEM | 0.008 | 0.011 | 0.004 |
| *P* value | NS | NS | 0.001 |
| Days | | 0.000 | |
| Diet x Days | NS | | |

$^{a,b}$Column means with no common superscript differ significantly ($P < 0.05$).

NS: Not significant.

[1]df: degree of freedom.

significantly ($P < 0.01$) lower TBARS values than the other groups. TBARS values increased depending on the time in all dietary treatments. The interaction between Diet × Days was not significant ($P > 0.05$) with respect to the TBARS values of egg yolk.

Supplementation of dietary lanthanum oxide did not affect the palmitic, palmitioleic, stearic, oleic, linoleic, linolenic, oktadecatetraeonic, eicosenic, eicosatrienoic, arachidonic,

**Table 7.** Effects of dietary supplementation lanthanum oxide on fatty acids composition (%) values in egg yolk of the laying hens (df = 4).[1]

| Fatty acids | Control | Lanthanum 100 mg/kg | Lanthanum 200 mg/kg | Lanthanum 300 mg/kg | Lanthanum 400 mg/kg | SEM | *P* value |
|---|---|---|---|---|---|---|---|
| Palmitic (C16:0) | 25.67[a,b] | 27.67[a] | 26.01[a,b] | 23.98[b] | 24.52[b] | 0.80 | 0.011 |
| Palmitoleic (C16:1) | 3.72 | 2.88 | 3.02 | 3.00 | 2.95 | 0.47 | NS |
| Stearic (C18:0) | 8.10 | 10.91 | 7.26 | 8.42 | 8.54 | 0.78 | NS |
| Oleic (C18:1) | 45.04 | 40.53 | 44.96 | 43.65 | 44.39 | 0.92 | NS |
| Linoleic (C18:2) | 12.89 | 12.93 | 13.47 | 15.43 | 14.54 | 0.57 | NS |
| Linolenic (C18:3 - 3) | 0.046 | 0.048 | 0.044 | 0.042 | 0.038 | 0.001 | NS |
| Oktadecatetraeonic (C18:4) | 0.086 | 0.088 | 0.092 | 0.074 | 0.074 | 0.003 | NS |
| Eicosenic (C20:1) | 0.228 | 0.214 | 0.258 | 0.240 | 0.246 | 0.005 | NS |
| Eicosadienoic (C20:2) | 0.116[b] | 0.182[a] | 0.202[a] | 0.198[a] | 0.200[a] | 0.010 | 0.04 |
| Eicosatrienoic (C20:3) | 0.178 | 0.222 | 0.186 | 0.212 | 0.170 | 0.07 | NS |
| Arachidonic (C20:4) | 1.99 | 2.12 | 2.42 | 2.44 | 2.22 | 0.06 | NS |
| Eicosapentaenoic (C20:5) | 0.086[a,b] | 0.126[a] | 0.048[b] | 0.080[a,b] | 0.054[b] | 0.09 | 0.04 |
| Docosapentaenoic (C22:5) | 0.140 | 0.182 | 0.182 | 0.170 | 0.134 | 0.009 | NS |
| Docosaheptaenoic (C22:6) | 0.560 | 0.660 | 0.650 | 0.792 | 0.720 | 0.00 | NS |
| SFA | 33.77[b] | 38.58[a] | 33.27[b] | 32.4[b] | 33.06[b] | 0.53 | 0.00 |
| MUFA | 48.99 | 43.62 | 48.24 | 46.89 | 47.58 | 0.65 | NS |
| PUFA | 16.09 | 16.55 | 17.29 | 19.43 | 18.15 | 0.63 | NS |
| n3 | 1.12 | 1.34 | 1.23 | 1.46 | 1.24 | 0.04 | NS |
| n6 | 15.22 | 15.51 | 16.32 | 18.33 | 17.20 | 0.61 | NS |
| n3/n6 | 0.075 | 0.089 | 0.075 | 0.080 | 0.075 | 0.003 | NS |

[a,b]Rows means with no common superscript differ significantly ($P < 0.05$). NS: Not significant.

[1]df: degree of freedom.

docosapentaenoic, docosaheptaenoic (DHA), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), n3, n6 and n3/n6 content of egg yolk (Table 7). Palmitic, eicosapentaenoic (EPA), eicosadienoic acid and saturated fatty acids (SFA) of yolk were significantly ($P < 0.05$) influenced by experimental diets. Supplementation of 100 mg/kg lanthanum oxide increased EPA compared to 200 mg/kg and 400 mg/kg and that 100 mg/kg increased palmitic compared to 300 mg/kg and 400 mg/kg. Eicosadienoic acid ratio was significantly ($P < 0.01$) higher for hens fed lanthanum oxide diets than the control group.

The greatest concentration of palmitic, EPA and SFA in yolk was obtained with the diet containing 100 mg/kg lanthanum oxide.

## DISCUSSION

It was found that lanthanum oxide (400 mg/kg) improved feed conversion ratio and egg production in this study. This also confirms earlier findings [8, 18, 19]. Zhang et al. [9] reported that REE (rare earth element)-nitrate (300, 400, and 500 mg/kg) supplementation improved laying rate and egg weight in laying hens. Similar to the present study, Duan et al. [20] found that laying rate of broiler type breeding birds fed a diet containing 100 mg/kg REE was improved. Some researchers found that dietary REE had positive effects on growth performance in broilers [10, 21, 22]. It was also reported that feed conversion ratio was affected positively by using diet containing 100 mg/kg REE-lanthanum in pigs [23]. He et al. [2] reported that REE-citrate improved broiler performance, but the mechanism how REE could improve animal performance is not yet known. On the other hand, it was reported that secretion of digestive fluids could be promoted by REE [24]. Xu et al. [25] found that lanthanum increased gastric acid secretion in the stomach of mice.

The results of this experiment showed that supplementation of the diet with 100 and

200 mg/kg lanthanum oxide improved respectively Haugh unit and shell breaking strength. It was reported that some natural antioxidants had beneficial effects on to albumen quality due to their antioxidant properties [26]

It was reported that REE were considered to be as analogous to Ca, especially lanthanum (La) had been named as super calcium [27]. As reported by those researchers, a similarity between Ca and La might lead to increased shell breaking strength. These results cannot be compared with literature data since there are no reports about the effect of REE on egg quality.

The differences among the groups in blood biochemical parameters were not significant in the present study. Similarly, He et al. [28] showed no significant effect of REE on ALT and AST in pig. These results were suggests that feeding La as feed additive did not cause any adverse effects on laying hens as it was similarly reported previously by Agbede et al. [13].

In contrast to this study, it was found that blood glucose decreased with increasing levels of REE in broiler diets [11]. Unlike this study, Zohravi [29] reported that 50 and 100 mg/kg REE supplements in Japanese quails significantly increased concentration of serum calcium. On the other hand, He et al. [2] showed that supplementation of REE to the diet of broilers did not affect the concentration of serum calcium. Controversial results obtained from the present study compared to previous ones may be connected with the environmental factors, the breed effect, and the concentration of the REE.

We found that serum GPx and SOD values did not change, but MDA values were decreased by dietary 300 mg/kg lanthanum oxide. An and Chen [30] concluded that neodium as an REE increased the superoxide dismutase (SOD) activity while decreasing malonyldialdehyde (MDA) content in plants. Unlike our results, some researchers reported that addition of REE to the diet significantly increased superoxide dismutase (SOD) levels of the blood in fish [31] and chickens [6]. However, Kawagoe et al. [32] reported that dietary supplementation of cerium to mice had no effect on the amount of plasma lipoperoxide (MDA) whereas SOD activity of plasma decreased. It may be concluded that antioxidant properties of lanthanum reduced MDA values in serum. Controversial results obtained from the present study compared to previous ones may be connected with animal species.

Supplementation of the basal diet with lanthanum oxide increased the oxidative stability of the yolk at 42 d in the present study. The values of TBARS in the egg yolk were significantly decreased by dietary lanthanum oxide supplementation. It may be concluded that antioxidant properties of lanthanum has a positive effect on the shelf life of eggs. As to our knowledge, no studies were found on this topic in literature, so no comparison could be made with other publications.

It has been reported that REE had high antioxidative effects and therefore protected dietary fatty acids from oxidization and increased uptake of nutrients [33]. Supplementation of 100 mg/kg lanthanum oxide increased EPA compared to 200 mg/kg and 400 mg/kg lanthanum oxide and SFA ratio of egg yolk. Li et al. [34] reported that unsaturated fatty acids in wheat increased with lanthanum. He et al. [35, 36] suggested that MUFA was reduced by REE, and REE might affect lipogenesis rate in adipose tissue.

## CONCLUSIONS AND APPLICATIONS

1. In conclusion, the results of our trial show that especially the dietary supplementation of 400 mg/kg lanthanum oxide increased egg production and 200 mg/kg shell breaking strength.
2. Also, the addition 300, mg/kg of lanthanum oxide to the laying hen feed led to a decrease in the MDA amount in the plasma.
3. At 42 d, TBARS values of the egg yolk were significantly decreased by dietary 200, 300, and 400 mg/kg of lanthanum oxide supplementation.
4. Supplementation of 100 mg/kg lanthanum oxide increased EPA ratio of egg yolk compared to 200 mg/kg and 400 mg/kg lanthanum oxide. Based on the results of this study, it may be recommended to supplement laying hens' feed with lanthanum oxide.

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