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Performance, Egg Characteristics, Hematological and Serum Biochemical Profiles of Laying Hens Fed Varying Levels of Cerium Chloride and Oxide

Research Article

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ABSTRACT

The effects of feeding varying levels of cerium chloride and oxide on the performance, egg qualities, hematological and serum biochemical parameters of 240 laying hens at 24 weeks of age were studied. The cerium chloride (CeCl) and cerium oxide (CeO) experimental diets were allotted to 120 pullets each, randomly divided into 4 treatments (0, 50, 100 and 150 mg/kg diet each of CeCl and CeO) with each treatment replicated 5 times, respectively, in a 2 × 4 factorial arrangement. The experiment lasted for a period of sixteen weeks. It was observed that CeCl and CeO significantly (P<0.05) enhanced the various performances of the laying hens. The inclusion levels of the cerium sources significantly (P<0.05) improved most of the external and internal egg quality parameters studied with the best results recorded among the pullets fed CeCl and CeO at 150 mg/kg diet. The hematological and most of the serum biochemical indices of the pullets fed the experimental diets were not significantly (P>0.05) affected. However, significant (P<0.05) increase in both alanine aminotransferase and aspartate aminotransferase concentrations were observed when the inclusion level of the cerium sources was elevated to 150 mg/kg diet. Hence, the use of cerium chloride and oxide as feed additives in place of antibiotics would be a welcome development if the inclusion level does not exceed the tolerable limit of 100 mg/kg diet.

KEY WORDS cerium, egg, hematology, layers.

INTRODUCTION

The potentials of rare earth elements (REEs) in poultry nutrition to replace the use of antibiotics have been severally stressed. REEs and calcium have been reported to share a common resemblance in size, bonding, coordination geometry, and donor atom preference (Redling, 2006). These similarities have been explained to make them capable of replacing calcium ion in various biological processes. The REEs are the elements in group III-A of the periodic table. They are the 15 lanthanide elements with atomic numbers 57 Lanthanum through 71 Lutetium (Reka *et al.* 2019). Cerium and lanthanum have been reportedly used as organic or inorganic feed micro-additives in monogastric animal diets (Pagano *et al.* 2015). The use of organic REEs in monogastric animal nutrition was reported to significantly enhance the serum concentration of 3, 5, 3′ triiodothyronine, nutrient digestibility, and growth performance (Cai *et al.* 2018). The serum minerals such as Ca and P concentrations were also reported to be significantly increased in a 22-week-old laying hen fed diets containing 100 mg/kg CeO (Bölükbaşi *et al.* 2016). Furthermore, Adu and Olarotimi (2020) reported that CeCl significantly improved the hen day egg production (HDEP) and the external egg qualities of 16-week pullets fed diets supplemented with 50-150 mg CeCl/kg diet. The inclusions of REEs in breeders' diets have also been noted to increase the rate of egg production, fertilization, and hatching in poultry production (Abdelnour *et al.* 2019). Cai *et al.* (2018) equally observed an improved embryo development survival rate of chicks produced by breeder hens fed diets supplemented with REEs. REEs also play a vital role in the reproductive performance enhancements of non-ruminant animals. Akinmuyisitan *et al.* (2015) recorded that CeO at different inclusions significantly enhanced the reproductive potentials of female rabbits, as well as the embryo survival rate at 200 mg/kg level of inclusion. The objective of this study was to further evaluate the effects of cerium chloride (CeCl) and cerium oxide (CeO) on the performance, egg qualities, hematological, and serum biochemical parameters of laying hens.

MATERIALS AND METHODS

The experiment was conducted at the poultry unit, teaching and research farm, the Federal University of Technology Akure, Nigeria. The research was approved by the research ethics and guidelines committee of the Animal Production and Health Department of the institution. Eight experimental diets were constituted in a 2 × 4 factorial arrangement containing varying inclusion levels of cerium chloride (CeCl) and cerium oxide (CeO). Each experimental diet (Table 1) was supplemented at four levels of inclusion (0, 50, 100, and 150 mg/kg diet). The diets were prepared to meet the nutrient requirements of laying hens according to NRC recommendations (NRC, 2005). A total of two hundred and forty (240) point-of-lay (POL) Isa Brown pullets of sixteen (16) weeks old purchased from a reliable farm were used for the study. They were placed on a commercial grower mash until they have reached 20% laying performance (24 weeks of age) before being placed on the experimental rations for sixteen weeks. The pullets were randomly divided into two (2) experimental groups of one hundred and twenty (120) birds per experimental group. Each group contains four (4) treatments and five (5) replicates per treatment with six (6) pullets randomly assigned per replicate in a completely randomized design. Feed was given according to body weight and age twice daily in line with the Isa Brown management manual and drinking water was also provided *ad libitum*.

Performance evaluation of layers fed different levels of two rare earth elements

The weekly average feed intake of the birds per treatment was determined by carefully packing and weighing the leftovers at the end of each week and subtracting from the total quantity of feed to given the birds at the beginning of the week.

Average feed intake (g)= quantity of feed given – leftover

Total weight gains (TWG) of the birds were determined by subtracting the initial weight (IWT) of the birds at the beginning of the experiment from the final weight (FWT) at the end of the experiment.

TWG (g)= FWT – IWT

Feed conversion ratio (FCR) was determined as the ratio of the average feed intake to kg egg mass.

FCR (per kg egg mass)= feed Intake (g) / egg Mass (g)

The percentage hen day egg production (% HDEP) was determined by dividing the total number of eggs laid per day by the number of birds alive multiplied by a hundred.

% HDEP= (total number of eggs produced per day×100) / (total number of hens alive on that day)

Assessment of egg quality parameters

Egg collection was commenced when the pullets reached 50% laying performance for the determination of egg quality parameters. The eggs were collected and analyzed weekly for both the internal and external egg qualities with twelve (12) eggs randomly selected per replicate (i.e. 60 eggs/treatment) for the assessments.

External egg qualities such as egg weight, length, width, egg index, shell weight, thickness, ratio and surface area as well as internal egg qualities like yolk weight, height, diameter, ratio, index and albumen weight, length, height, diameter, index, and ratio were determined as described by Adu and Olarotimi (2020). Egg surface area (ESA) was determined as reported by Lewis and Perry (1987) using the formula:

ESA/SSA= $W^{0.667} \times 4.67$

Where:
W: average egg weight.
0.667 and 4.67: constants.

Yolk index was calculated using as the relationship between yolk height and width:

Yolk index= yolk height / yolk width

Haugh Unit (HU) was estimated as HU= $100 \log (H+7.57-1.7W^{0.37})$

Where:
H: albumen height.
W: egg weight (as reported by Oluyemi and Roberts, 2000).

The use of egg mass rather than egg numbers is to ensure better comparisons of flocks. It is estimated as:

Average egg mass= HDEP × average egg weight in grammes (Fikru *et al*. 2015).

Egg specific gravity (ESG) proposed by Harms (1991):

ESG= (1.9754EW) / (1.9140EW) – ESW

Where:
EW: egg weight.
ESW: egg shell weight.
1.9754 and 1.9140: constants.

Shell weight per unit egg surface area (SWUSA)= $(3.9782EW)^{0.666}$

Other egg qualities parameters were estimated using the equations postulated by Paganelli *et al*. (1974):

Area *vs*. egg volume (V, cm^3):
$SA= 4.951\ EV^{0.666}$

Egg density (ED, g cm^{-3}) *vs*. egg weight:
$ED= 1.038\ EW^{0.006}$

Shell density (ShDgcm^{-3}) *vs*. egg weight:
$SD= 1.945\ EW^{0.014}$

Shell volume (SV, cm^3) *vs*. egg weight:
$SV= 2.48 \times 10^{-2}\ W^{1.118}$

Determination of hematological and biochemical components

Data were also collected for hematological and serum biochemical indices. At the end of the feeding trial, three birds per replicate (i.e. 15 birds per treatment) were randomly selected for blood collection to determine the serum and blood biochemical properties.

Blood samples were taken from the jugular veins on the last day of the experiment. Blood samples for serum biochemistry were collected into plain bottles and kept at room temperature for two hours in a slanting position before being transferred into a refrigerator and kept overnight at 4 ˚C.

It was thereafter centrifuged at 3000 rpm/15 min. Serum was then collected and stored at -20 ˚C until further analyses for serum biochemical indices. The blood samples for hematological analyses were collected into heparinized bottles.

Packed Cell Volume, Red Blood Cell, Hemoglobin, White Blood Cell, Total Protein, Globulin, and Albumin were determined as described by Tietz (1995). The serum creatinine and urea nitrogen were estimated by deproteinization and Urease-Berthelot colorimetric methods respectively, using a commercial kit (Randox Laboratories Ltd., U.K.). Also, the free cholesterol was determined by nonane extraction and enzymatic colorimetric methods respectively using commercial test kits (Quimica Clinica Aplicada, S.A.), while the serum enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were obtained using the Randox Laboratories Ltd, UK test kits.

Statistical analysis

All data were subjected to a 2 × 4 factorial analysis with the following model: $Y_{ijk}= \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$ in a completely randomized design using SAS (2008) statistical package.

Where:
Y_{ijk}: any of the response variables.
μ: overall mean.
a_i: effect of the i^{th} treatment (*i*=CeCl and CeO).
B_j: effect of the j^{th} level (*j*=A, B, C and D).
$(\alpha\beta)_{ij}$: effect of the interaction between the treatment and the level.
ε_{ijk}: random error due to experimentation.

Mean separation, where applicable, was done with Duncan’s multiple range test of the same statistical package, and significance was accepted at a 5.00% level.

RESULTS AND DISCUSSION

There was a significant treatment effect (P<0.05) only in the FCR. The pullets fed diets supplemented with CeO had higher FCR than those on CeCl supplemented diets. All other performance indicators such as IWT, FWT, TWG, AFI, and HDEP were not significantly (P>0.05) influenced by the two rare earth elements supplemented in the experimental diets. There were noticeable significant (P<0.05) effects of the varying inclusion levels of the two cerium sources in a dose-dependent manner in all the performance parameters considered in Table 2 with the best results recorded among the birds fed diets containing 150 mg/kg REE. The FWT, TWG, and HDEP were significantly (P<0.05) enhanced among the layers fed diets containing varying inclusion levels of CeCl and CeO when compared with the pullets on the control diet.

Table 1 Percentage composition of experimental diets

Ingredients	Diets with CeCl inclusion (kg)				Diets with CeO inclusion (kg)			
	T1	T2	T3	T4	T5	T6	T7	T8
Maize	430.00	430.00	430.00	430.00	430.00	430.00	430.00	430.00
Soya meal	230.00	230.00	230.00	230.00	230.00	230.00	230.00	230.00
Wheat offal	220.00	219.95	220.00	220.00	220.00	219.95	220.00	220.00
Limestone	90.00	90.00	90.00	90.00	90.00	90.00	90.00	90.00
Bone meal	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Lysine	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Methionine	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Layer premix[1]	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Salt	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
CeCl	0.00	0.05	0.10	0.15	0.00	0.00	0.00	0.00
CeO	0.00	0.00	0.00	0.00	0.00	0.05	0.10	0.15
Total	1000	1000	1000	1000	1000	1000	1000	1000
Calculated values								
Crude protein (%)	17.76	17.76	17.76	17.76	17.76	17.76	17.76	17.76
Metabolizable energy (kcal/kg)	2508.16	2508.07	2507.97	2507.88	2508.16	2508.07	2507.97	2507.88
Ca (%)	3.97	3.97	3.97	3.97	3.97	3.97	3.97	3.97
Total phosphorus (%)	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Crude fibre (%)	4.53	4.53	4.53	4.52	4.53	4.53	4.53	4.52
Crude fat (%)	3.30	3.29	3.29	3.29	3.30	3.29	3.29	3.29
Lysine	1.24	1.24	1.24	1.24	1.24	1.24	1.24	1.24
Methionine	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46

[1] Composition of premix: 2.5 kg of premix contains: vitamin A: 10000000 IU; vitamin D_3: 2500000 IU; vitamin E: 12000 IU; vitamin B_1: 2000 mg; vitamin B_6: 1500 mg; vitamin B_{12}: 10 mg; vitamin K_3: 2000 mg; Niacin: 15000 mg; Biotin: 20 mg; Folic Acid: 600 mg; Panthothenic acid: 7000 mg; Chlorine chloride: 150000 mg; Manganeso: 80000 mg; Iron: 40000 mg; Copper: 0 mg; Zinc: 60000 mg; Selenium: 150 mg; Iodine: 1000 mg; Magnesium: 100 mg; Ethoxyquine: 500 g; BHT: 700 g and ME: metabolizable energy.

Table 2 Performance of the laying birds fed diets containing varied levels of CeCl and CeO

Treatment		Levels	Production performance (Means±SEM)					
			IWT (g)	FWT (g)	TWG (g)	AFI (g)	HDEP (%)	FCR
CeCl			1247.44±2.31	1982.81±10.91	735.38±10.90	113.59±0.00	73.65±1.44	2.55±0.10^b
CeO			1251.56±3.15	1982.19±10.82	730.63±10.53	113.60±0.00	73.80±1.46	2.69±0.08^a
		0	1246.25±3.09^b	1912.50±8.18^b	666.25±9.00^b	113.60±0.01^a	64.05±0.13^b	3.21±0.03^a
		5	1244.25±2.47^b	2000.63±0.63^a	756.38±1.96^a	113.61±0.01^a	76.82±0.27^a	2.53±0.06^b
		100	1248.75±3.63^{ab}	2010.00±0.94^a	761.25±3.75^a	113.59±0.00^b	76.73±0.15^a	2.49±0.05^b
		150	1258.75±4.60^a	2006.87±1.88^a	748.13±5.17^a	113.59±0.00^b	77.28±0.08^a	2.26±0.06^c
Treatments × levels								
CeCl	×	0	1246.25±4.73	1912.50±12.50	666.25±13.75	113.60±0.01	64.05±0.20	3.21±0.05
CeCl	×	5	1246.00±4.49	2001.25±1.25	755.25±3.30	113.60±0.01	76.64±0.55	2.39±0.05
CeCl	×	100	1247.50±4.79	2008.75±1.25	761.25±4.27	113.58±0.00	76.72±0.21	2.43±0.07
CeCl	×	150	1250.00±6.12	2008.75±3.15	758.75±5.15	113.59±0.00	77.17±0.10	2.17±0.08
CeO	×	0	1246.25±4.73	1912.50±12.50	666.25±13.75	113.60±0.01	64.05±0.20	3.21±0.05
CeO	×	5	1242.50±2.50	2000.00±0.00	757.50±2.50	113.61±0.01	77.00±0.09	2.60±0.02
CeO	×	100	1250.00±6.12	2011.25±1.25	761.25±6.88	113.59±0.00	76.75±0.24	2.55±0.05
CeO	×	150	1267.50±3.23	2005.00±2.04	737.50±4.79	113.59±0.00	77.39±0.11	2.34±0.05
P-values								
Treatments			0.2301	0.8919	0.4086	0.4285	0.4031	< 0.0001
Levels			0.0257	< 0.0001	< 0.0001	0.0129	< 0.0001	0.0014
Treatments × levels			0.1555	0.9693	0.4289	0.8077	0.8821	0.1273

IWT: initial weight; FWT: final weight; TWG: total weight gain; AFI: average feed intake; HDEP: hen day egg production and FCR: feed conversion ratio.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

However, the varying inclusion of CeCl and CeO significantly (P<0.05) resulted in a decreased AFI and FCR among the treated pullets when compared with those on the control diet. The interaction between treatments and levels of inclusion, however, did not have a significant (P>0.05) influence on all the performance traits studied.

For the external egg quality characteristics (Tables 3a and 3b), significant (P<0.05) treatment effects were recorded in egg weight (EW), egg surface area (ESA), egg mass (EM), egg density (ED), egg volume (EV), shell density (ShD), shell weight per unit egg surface area (SWUSA) and shell volume (ShV) while the inclusion of CeCl and CeO did not significantly (P>0.05) influenced the egg specific gravity (ESG), shell weight (ShW), shell thickness (ShT), shell ratio (ShR) and shell index (ShI). For instance, it was observed that the eggs from the pullets fed diets containing CeCl had higher values in the parameters where significant differences occurred compared to those fed CeO. The levels of inclusion of the REE significantly (P<0.05) had enhancing effects on the parameters such as EW, ESA, EM, ED, EV, ShW, ShT, ShD, SWUSA and ShV dose-dependently with pullets on diets containing 150 mg/kg recording the highest values for the studied parameters. The levels of inclusion, however, did not significantly (P>0.05) influenced ESG, ShI, and ShR among the pullets fed varying inclusion levels of CeCl and CeO when compared with those on the control diets. The interactions between the two treatments and the varying inclusion levels were equally observed not to have any significant (P<0.05) effect on all the egg quality parameters studied in the present study.

The internal egg quality characteristics (Table 4) of the eggs from the birds fed varying inclusion levels of the REE showed no significant (P>0.05) treatment effects on all the parameters studied except the albumen weight (AW) where the pullets fed CeCl had significantly (P<0.05) higher values than their counterparts fed CeO-treated diets. The varying levels of inclusion of both CeCl and CeO significantly (P<0.05) increased the albumen height (AH), yolk weight (YW), AW and HU of the eggs from the pullets fed the REE when compared with those on the control diets. The interactions of the REE and the inclusion levels did not also significantly (P>0.05) affect the internal egg qualities.

The hematological parameters of the pullets fed diets containing varying levels of CeCl and CeO are shown in Table 5. There were no significant (P>0.05) effects of varied inclusions of CeCl and CeO used in the present study on all the hematological indices studied. In a similar way, the levels of inclusion of CeCl and CeO only had a significant (P<0.05) effect on the eosinophil among all the studied parameters. It was observed that inclusion of cerium in the diets significantly (P<0.05) reduced the eosinophil components of the white blood cells of the birds fed 150 mg/kg diet though inclusions at 50 and 100 mg/kg diet also recorded a decrease in this parameter which was not statically (P>0.05) different from what was obtained among the birds on the control diets. Furthermore, the interactions of the treatment and the levels of inclusion did not significantly (P>0.05) influence the studied hematological parameters in the present study.

For the serum biochemical parameters (Table 6), varied inclusions of CeCl and CeO did not significantly (P>0.05) affect all the serum biochemical indices studied. Moreover, serum glucose, albumin, globulin, albumin: globulin, urea, cholesterol, and creatinine of the treated birds were not significantly (P>0.05) influenced by the varied inclusion levels of the rare earth elements used in the present study. However, the serum total protein, ALT and AST concentrations of the treated birds were significantly (P<0.05) affected by the varied inclusion levels of the rare earth elements supplemented in the diets. The varying inclusion levels of cerium significantly (P<0.05) increased the serum total protein concentrations of the treated birds. Furthermore, the serum ALT and AST concentrations increased dose-dependently with birds fed 150 mg/kg diet of the REEs recording the higher significant (P<0.05) values compared to the control. The interactions between the CeCl and CeO each with their respective levels of inclusion did not have significant (P>0.05) influences on the serum glucose, total protein, albumin, globulin, albumin:globulin, creatinine, and ALT concentrations. The interactions between CeCl treatment and varied inclusion levels did not show any significant (P>0.05) difference for all the parameters when compared with the control diet.

However, the treatment interaction with the level of inclusion at 150 mg CeCl/kg diet significantly (P<0.05) lowered the serum cholesterol concentrations of the treated birds when compared with those on the control diet. Furthermore, the interactions between the CeO treatment and the varied inclusion levels followed the same trend as reported for the interactions between the CeCl treatment and the varied inclusion levels.

The performance parameters such as FCR, TWG, feed intake, and hen day egg p HDEP are reliable indicators of feed economy in layers production. The difference in the FCR of the pullets fed the two rare earth elements (Table 2) could be partly linked to the differences between the individual rare earth elements. This has been explained to be due to the variations in chemical and biochemical structures which might result in variations in both absorption and bioavailability of chemical compounds and in turn, might affect their efficacy (Redling, 2006). The significant improvement observed in the TWG, AFI, and FCR by the varied inclusion levels (50-150 mg/kg diet) of the REEs used in the present study has revealed that the rare earth elements are capable of improving the metabolic pathway, nutrient utilization and digestive enzymes secretion and the results obtained here further strengthened the assertion of He *et al*. (2010). A similar trend was reported by Adu *et al.* (2015) in rabbits fed varied inclusion levels of CeO.

Table 3a External egg qualities of the laying birds fed diets containing varied levels of CeCl and CeO

Treatments	Levels (mg/kg diet)	External egg quality triats (Means±SEM)					
		EW (g)	ESA (cm^2)	EM (g/hen/day)	ESG	ED (gcm^{-3})	EV (cm^3)
CeCl		61.65±1.40^{a}	73.95±1.10^{a}	45.60±1.71^{a}	1.08±0.00	1.06±0.00^{a}	58.04±1.31^{a}
CeO		57.91±0.90^{b}	70.98±0.73^{b}	42.82±1.28^{b}	1.09±0.00	1.06±0.00^{b}	54.53±0.84^{b}
	0	55.35±0.45^{c}	68.92±0.37^{c}	35.46±0.36^{c}	1.09±0.00	1.06±0.00^{c}	52.14±0.42^{c}
	5	58.78±1.48^{b}	71.68±1.19^{b}	45.13±1.03^{b}	1.09±0.00	1.06±0.00^{b}	55.35±1.39^{b}
	100	59.59±1.09^{b}	72.35±0.88^{b}	45.73±0.89^{b}	1.09±0.00	1.06±0.00^{b}	56.11±1.02^{b}
	150	65.40±1.68^{a}	76.93±1.30^{a}	50.53±1.26^{a}	1.08±0.00	1.06±0.00^{a}	61.55±1.57^{a}
Treatments × levels							
CeCl ×	0	55.35±0.68	68.92±0.56	35.46±0.54	1.09±0.00	1.06±0.00	52.14±0.64
CeCl ×	5	62.13±1.59	74.38±1.26	47.59±0.89	1.08±0.00	1.06±0.00	58.48±1.48
CeCl ×	100	61.08±1.65	73.54±1.32	46.86±1.35	1.09±0.00	1.06±0.00	57.50±1.55
CeCl ×	150	68.05±2.51	78.98±1.94	52.51±1.89	1.08±0.00	1.06±0.00	64.03±2.35
CeO ×	0	55.35±0.68	68.92±0.56	35.45±0.54	1.09±0.00	1.06±0.00	52.14±0.64
CeO ×	5	55.43±0.48	68.80±0.39	42.68±0.32	1.09±0.00	1.06±0.00	52.21±0.44
CeO ×	100	58.10±1.16	71.16±0.94	44.60±0.96	1.08±0.00	1.06±0.00	54.72±1.08
CeO ×	150	62.75±1.47	74.88±1.17	48.56±1.13	1.09±0.00	1.06±0.00	59.07±1.38
P-values							
Treatments		0.0011	0.001	0.0011	0.3581	0.0009	0.0011
Levels		< 0.0001	< 0.0001	< 0.0001	0.8446	< 0.0001	< 0.0001
Treatments × levels		0.1248	0.1194	0.1352	0.5423	0.1087	0.1247

EW: egg weight; ESA: egg surface area; EM: egg mass; ESG: egg specific gravity; ED: egg density and EV: egg volume.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3b External egg qualities of the laying birds fed diets containing varied levels of CeCl and CeO

Treatments	Levels (mg/kg diet)	External egg qualities traits (Means±SEM)						
		ShW (g)	ShT (mm)	ShR (%)	ShI	ShD (gcm^{-3})	SWUSA (gcm^{-2})	ShV (cm^3)
CeCl		5.69±0.11	0.32±0.01	9.26±0.18	7.70±0.12	2.06±0.00^{a}	61.86±0.93^{a}	2.49±0.06^{a}
CeO		5.52±0.15	0.32±0.01	9.53±0.22	7.77±0.18	2.06±0.00^{b}	59.36±0.61^{b}	2.32±0.04^{b}
	0	5.18±0.14^{b}	0.28±0.01^{b}	9.36±0.29	7.51±0.22	2.06±0.00^{c}	57.62±0.31^{c}	2.20±0.02^{c}
	5	5.64±0.18^{ab}	0.32±0.02^{a}	9.62±0.26	7.87±0.17	2.06±0.00^{b}	59.95±1.00^{b}	2.36±0.07^{b}
	100	5.56±0.17^{ab}	0.34±0.01^{a}	9.33±0.24	7.68±0.20	2.06±0.00^{b}	60.51±0.74^{b}	2.39±0.05^{b}
	150	6.04±0.17^{a}	0.34±0.01^{a}	9.27±0.35	7.86±0.26	2.06±0.00^{a}	64.36±1.10^{a}	2.66±0.08^{a}
Treatments × levels								
CeCl ×	0	5.18±0.21	0.28±0.02	9.36±0.44	7.51±0.34	2.06±0.00	57.62±0.47	2.20±0.03
CeCl ×	5	5.78±0.13	0.33±0.01	9.32±0.33	7.78±0.23	2.06±0.00	62.22±1.06	2.51±0.07
CeCl ×	100	5.78±0.15	0.35±0.01	9.45±0.37	7.86±0.26	2.06±0.00	61.51±1.11	2.46±0.07
CeCl ×	150	6.03±0.09	0.33±0.02	8.89±0.34	7.64±0.20	2.06±0.00	66.09±1.63	2.78±0.11
CeO ×	0	5.18±0.21	0.28±0.02	9.36±0.44	7.51±0.34	2.06±0.00	57.62±0.47	2.20±0.03
CeO ×	5	5.50±0.19	0.31±0.03	9.93±0.37	7.98±0.29	2.06±0.00	57.67±0.33	2.21±0.02
CeO ×	100	5.35±0.30	0.34±0.02	9.19±0.34	7.51±0.32	2.06±0.00	59.51±0.79	2.33±0.05
CeO ×	150	6.05±0.35	0.35±0.01	9.66±0.60	8.09±0.49	2.06±0.00	62.64±0.98	2.54±0.07
P-values								
Treatments		0.2873	0.7796	0.3604	0.7464	0.0009	0.001	0.0011
Levels		0.0066	0.0057	0.8434	0.6381	< 0.0001	< 0.0001	< 0.0001
Treatments × levels		0.6889	0.8126	0.5451	0.6434	0.1089	0.1195	0.1267

ShI: shell index; ShW: shell weight; ShV: shell volume; ShT: shell thickness; ShR: shell ratio; ShD: shell density; SWUSA: shell weight per unit of surface area.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Liu (2005) equally observed improved body weight gain and feed conversion ratio in pigs and poultry chickens fed rare earth elements at varied inclusion levels. The enhanced growth performance in this study could partly be due to the anti-bacterial and anti-inflammatory properties of CeCl and CeO (Redling, 2006) as well as their function in the stabilization of the intestinal microflora (Zhao *et al.* 2002).

For the HDEP, the varied inclusion levels of the REEs used in this study brought about an increased production rate. The increased egg production rate observed in this study might not be unconnected with the activities of the REEs acting as cofactors which emulate Ca and thereby tend to replace Ca in various biological processes in layers (Tariq *et al.* 2019) to facilitate oviduct motility.

Table 4 Internal egg qualities of the laying birds fed diets containing varied levels of CeCl and CeO

Treatments	Levels (mg/kg diet)	Internal egg quality traits (Means±SEM)									
		YD (cm)	YH (cm)	YW (g)	YR (%)	YI	AH (mm)	AW (g)	AR (%)	Y:A	HU
CeCl		43.38±0.35	1.41±0.00	14.88±0.48	24.17±0.65	3.25±0.03	56.38±6.22	38.81±1.08^{a}	62.97±1.04	0.39±0.01	164.53±9.85
CeO		43.63±0.43	1.42±0.00	14.96±0.47	25.81±0.66	3.25±0.03	59.50±6.58	36.13±0.74^{b}	62.40±0.84	0.42±0.02	166.63±10.10
	0	43.75±0.49	1.42±0.00	14.03±0.39^{b}	25.35±0.73	3.25±0.04	22.75±10.31^{b}	34.23±0.63^{b}	61.83±0.95	0.41±0.02	110.09±16.24^{b}
	5	44.38±0.60	1.41±0.00	13.65±0.54^{b}	23.30±1.00	3.18±0.05	67.13±1.61^{a}	38.04±1.19^{a}	64.71±1.18	0.36±0.02	182.55±1.10^{a}
	100	43.25±0.41	1.41±0.00	15.23±0.56^{ab}	25.53±0.71	3.26±0.02	71.38±1.63^{a}	37.79±0.80^{a}	63.43±0.80	0.40±0.02	185.20±1.03^{a}
	150	42.63±0.56	1.41±0.00	16.78±0.59^{a}	25.77±1.19	3.32±0.05	70.50±1.95^{a}	39.83±1.89^{a}	60.78±1.87	0.43±0.03	184.47±1.20^{a}
Treatments × levels											
CeCl ×	0	43.75±0.75	1.42±0.01	14.03±0.59	25.35±1.12	3.25±0.06	22.75±15.75	34.23±0.96	61.83±1.46	0.41±0.03	110.09±24.81
CeCl ×	5	44.25±1.11	1.41±0.01	13.45±1.12	21.60±1.53	3.19±0.09	65.25±2.87	40.70±0.98	65.61±1.98	0.33±0.03	181.17±1.94
CeCl ×	100	42.75±0.25	1.40±0.00	15.48±0.74	25.33±0.97	3.28±0.02	69.75±2.90	38.85±1.17	63.61±0.78	0.40±0.02	184.12±1.88
CeCl ×	150	42.75±0.25	1.41±0.00	16.58±0.71	24.38±0.84	3.30±0.01	67.75±1.75	41.45±3.08	60.83±3.26	0.40±0.02	182.73±1.06
CeO ×	0	43.75±0.75	1.42±0.01	14.03±0.59	25.35±1.12	3.25±0.06	22.75±15.75	34.23±0.96	61.83±1.46	0.41±0.03	110.09±24.81
CeO ×	5	44.50±0.65	1.41±0.00	13.85±0.30	25.00±0.61	3.17±0.04	69.00±1.22	35.38±0.97	63.81±1.43	0.39±0.02	183.92±0.77
CeO ×	100	43.75±0.75	1.42±0.01	14.98±0.93	25.72±1.19	3.24±0.04	73.00±1.47	36.73±0.93	63.24±1.53	0.41±0.03	186.28±0.84
CeO ×	150	42.50±1.19	1.42±0.01	16.98±1.05	27.17±2.16	3.34±0.10	73.25±3.09	38.20±2.32	60.73±2.37	0.46±0.06	186.20±1.90
P-values											
Treatments		0.6547	0.1659	0.8949	0.0799	0.9295	0.5918	0.0277	0.6793	0.1718	0.8142
Levels		0.1719	0.1553	0.0027	0.213	0.1995	< 0.0001	0.0156	0.2068	0.1941	< 0.0001
Treatments × levels		0.868	0.4894	0.9321	0.4595	0.914	0.9891	0.4331	0.962	0.6959	0.9991

YD: yolk diameter; YH: yolk height; YW: yolk weight; YR: yolk ratio; YI: yolk index; AH: albumen height; AW: albumen weight; AR: albumen ratio; Y:A: yolk:albumen ratio and HU: Haugh unit.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Haematological parameters of the laying birds fed diets containing varied levels of CeCl and CeO

Treat-ments	Levels (mg/kg diet)	Haematological parameters (Means±SEM)											
		ESR (mm)	PCV (%)	RBC (x10^{6} mm^{3})	Hb (g/dL)	LYM (%)	MONO (%)	BASO (%)	EOSINO (%)	HETERO (%)	MCHC (g/dL)	MCH (pg)	MCV (fL)
CeCl		3.44 ±0.34	25.31 ±0.55	2.72 ±0.15	8.43 ±0.18	59.19 ±0.74	11.94 ±0.62	2.06 ±0.06	1.44 ±0.13	25.38 ±0.50	33.31 ±0.03	32.37 ±1.69	97.13 ±5.04
CeO		4.31 ±0.31	24.25 ±0.56	2.69 ±0.18	8.08 ±0.18	58.25 ±0.76	13.19 ±0.65	2.25 ±0.11	1.40 ±0.1	25.13 ±0.48	33.30 ±0.03	31.78 ±1.79	95.39 ±5.33
	0	4.25 ±0.41	23.750 ±0.82	2.89 ±0.27	7.90 ±0.26	58.75 ±1.15	12.00 ±0.71	2.00 ±0.00	1.75 ±0.16^{a}	25.50 ±0.87	33.28 ±0.05	29.09 ±2.57	87.34 ±7.58
	5	4.00 ±0.53	25.25 ±0.62	2.35 ±0.17	8.43 ±0.21	59.00 ±1.15	12.88 ±1.27	2.25 ±0.16	1.38 ±0.18^{ab}	24.75 ±0.53	33.36 ±0.03	36.76 ±1.93	110.17 ±5.77
	100	3.50 ±0.38	25.13 ±0.52	2.86 ±0.21	8.38 ±0.17	58.13 ±0.97	13.00 ±0.80	2.13 ±0.13	1.50 ±0.19^{ab}	25.25 ±0.82	33.33 ±0.04	30.32 ±2.18	90.94 ±6.55
	150	3.75 ±0.62	25.00 ±1.13	2.71 ±0.24	8.31 ±0.37	59.00 ±1.13	12.38 ±0.91	2.25 ±0.16	1.00 ±0.00^{b}	25.50 ±0.57	33.25 ±0.02	32.14 ±2.46	96.61 ±7.36
Treatments × levels													
CeCl ×	0	4.25 ±0.63	23.75 ±1.25	2.89 ±0.42	7.90 ±0.40	58.75 ±1.75	12.00 ±1.08	2.00 ±0.00	1.75 ±0.25	25.50 ±1.32	33.28 ±0.08	29.09 ±3.93	87.34 ±11.57
CeCl ×	5	3.25 ±0.85	25.75 ±1.18	2.29 ±0.18	8.60 ±0.40	60.50 ±0.96	10.75 ±1.31	2.00 ±0.00	1.25 ±0.25	25.50 ±0.65	33.39 ±0.04	37.84 ±1.26	113.33 ±3.86
CeCl ×	100	3.75 ±0.63	24.75 ±0.85	2.87 ±0.29	8.25 ±0.28	59.00 ±1.08	12.00 ±1.08	2.00 ±0.00	1.75 ±0.25	25.25 ±1.38	33.34 ±0.06	29.57 ±2.98	88.70 ±8.93
CeCl ×	150	2.50 ±0.50	27.00 ±0.71	2.83 ±0.30	8.98 ±0.24	58.50 ±2.22	13.00 ±1.68	2.25 ±0.25	1.00 ±0.00	25.25 ±0.95	33.24 ±0.03	32.98 ±3.88	99.18 ±11.59
CeO ×	0	4.25 ±0.63	23.75 ±1.25	2.89 ±0.42	7.90 ±0.40	58.75 ±1.75	12.00 ±1.08	2.00 ±0.00	1.75 ±0.25	25.50 ±1.32	33.28 ±0.08	29.09 ±3.93	87.34 ±11.57
CeO ×	5	4.75 ±0.48	24.75 ±0.48	2.42 ±0.32	8.25 ±0.17	57.50 ±1.94	15.00 ±1.68	2.50 ±0.29	1.50 ±0.29	24.00 ±0.71	33.33 ±0.05	35.68 ±3.88	107.01 ±11.57
CeO ×	100	3.25 ±0.48	25.50 ±0.65	2.85 ±0.34	8.50 ±0.22	57.25 ±1.65	14.00 ±1.08	2.25 ±0.25	1.25 ±0.25	25.25 ±1.11	33.33 ±0.05	31.06 ±3.60	93.19 ±10.81
CeO ×	150	5.00 ±0.71	23.00 ±1.68	2.59 ±0.42	7.65 ±0.55	59.50 ±0.96	11.75 ±0.85	2.25 ±0.25	1.00 ±0.00	25.75 ±0.75	33.27 ±0.04	31.29 ±3.58	94.04 ±10.66
P-values													
Treatments		0.0593	0.1746	0.8929	0.1667	0.4159	0.1749	0.1625	0.7066	0.7418	0.7976	0.8137	0.8145
Levels		0.6638	0.4866	0.4023	0.445	0.9395	0.8484	0.4822	0.0327	0.88	0.1868	0.163	0.1621
Treatments × levels		0.0902	0.1591	0.9626	0.1542	0.6087	0.1721	0.4822	0.4299	0.8013	0.8731	0.9511	0.9505

ESR: erythrocyte sedimentation rate; PCV: parked cell volume; RBC: red blood cell; Hb: haemaglobin; LYM: lymphocyte; MONO: monocytes; BASO: basophils; EOSINO: eosinophils; HETERO: heterophils; MCHC: mean corpuscular haemaglobin concentration; MCH: mean corpuscular haemaglobin and MCV: mean corpuscular volume.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Serum biochemical parameters of the laying birds fed diets containing varied levels of CeCl and CeO

Treatment	Levels (mg/kg diet)	Serum biochemical parameters (Means±SEM)									
		Glucose (mg/dL)	TP (g/dL)	Alb (g/dL)	Glo (g/dL)	Alb:Glo	Urea (mg/dL)	Chol (mg/dL)	Crt (mg/dL)	ALT (U/L)	AST (U/L)
CeCl		168.04±5.61	44.21±1.47	25.38±1.13	18.83±1.02	1.42±0.12	9.74±0.45	136.00±3.86	1.01±0.03	213.11±10.80	43.88±6.15
CeO		166.62±5.63	43.86±1.41	25.34±1.12	18.51±0.96	1.43±0.11	10.08±0.40	138.31±3.72	0.98±0.02	210.26±9.83	44.97±5.75
	0	169.33±5.73	38.73±0.69[b]	22.13±0.56	16.60±0.97	1.38±0.11	9.73±0.18	145.20±5.39	1.00±0.027	176.68±9.59[b]	33.33±9.54[b]
	5	178.26±8.66	45.54±1.73[a]	26.40±1.68	19.14±1.42	1.48±0.21	9.40±0.78	136.93±4.99	1.03±0.05	208.64±11.46[ab]	41.30±6.62[ab]
	100	163.71±9.77	47.95±1.61[a]	27.40±1.83	20.55±1.51	1.43±0.21	11.09±0.16	138.34±5.55	0.98±0.03	224.25±15.70[ab]	42.59±10.18[ab]
	150	158.03±5.96	43.93±2.28[a]	25.53±1.46	18.40±1.42	1.43±0.09	9.44±0.79	128.13±4.25	0.98±0.04	237.18±12.41[a]	60.48±2.99[a]
Treatments × levels											
CeCl ×	0	169.33±8.75	38.73±1.06	22.13±0.86	16.60±1.47	1.38±0.17	9.73±0.28[ab]	145.20±8.23[ab]	1.00±0.04	176.68±14.65	41.30±10.11[ab]
CeCl ×	5	175.20±8.88	44.80±1.92	25.03±1.40	19.78±2.01	1.33±0.22	7.98±1.18[b]	127.28±6.30[bc]	1.05±0.10	259.30±18.08	22.68±14.25[b]
CeCl ×	100	171.78±17.20	46.70±3.05	27.88±3.10	18.83±2.44	1.62±0.38	11.08±0.15[a]	148.68±3.28[a]	1.03±0.03	210.08±10.27	58.93±4.74[a]
CeCl ×	150	155.88±10.29	46.63±3.84	26.50±2.70	20.13±2.49	1.37±0.17	10.20±0.92[ab]	122.80±3.86[c]	0.95±0.07	206.40±22.70	52.60±13.26[a]
CeO ×	0	169.33±8.75	38.73±1.06	22.13±0.86	16.60±1.47	1.38±0.17	9.73±0.28[ab]	145.20±8.23[ab]	1.00±0.04	176.68±14.65	41.30±10.11[ab]
CeO ×	5	181.33±16.28	46.28±3.13	27.78±3.16	18.50±2.27	1.63±0.37	10.83±0.25[ab]	146.58±3.78[ab]	1.00±0.04	215.05±8.11	62.50±4.05[a]
CeO ×	100	155.65±10.31	49.20±1.33	26.93±2.42	22.28±1.65	1.25±0.20	11.10±0.31[a]	128.00±7.86[bc]	0.92±0.048	207.20±22.49	62.03±4.21[a]
CeO ×	150	160.18±7.54	41.23±2.17	24.55±1.43	16.68±1.09	1.48±0.10	8.68±1.30[bc]	133.45±7.10[abc]	1.00±0.04	242.10±20.56	14.05±1.24[b]
P-values											
Treatments		0.8626	0.8359	0.9808	0.8166	0.9424	0.5198	0.613	0.5152	0.8165	0.8643
Levels		0.3602	0.0062	0.1143	0.2498	0.9808	0.0949	0.0922	0.7542	0.0112	0.0384
Treatments × levels		0.7636	0.3813	0.7345	0.3576	0.5834	0.0448	0.0272	0.5457	0.1733	0.0024

TP: total protein; Alb: albumin; Glo: globulin; Chol: cholesterol; Crt: creatinine; ALT: alanine aminotransferase and AST: aspartate aminotransferase.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The result was not in variance with the report of Reka *et al.* (2019) who also observed an increased percentage hen day egg production in the layers fed REEs.

The improved treatment effects observed in EW, ESA, EM, ED, EV, ShD, SWUSA, and ShV among the hens fed CeCl supplemented diet over those fed CeO was suggestive of the better bioavailability of CeCl in the diets. The varying inclusion levels of CeCl and CeO in the present study have clearly indicated that REEs can positively have value addition on the external qualities of poultry eggs. The consistent improvement recorded in the external egg qualities in response to the increasing rate of cerium was in agreement with studies of Reka *et al.* (2019). For instance, the improvement in egg weight as occasioned by an increase in cerium addition in the diet was suggestive that the protein contents of the experimental diets were positively enhanced. According to Bezerra *et al.* (2015), egg weight is dependent on the dietary protein as it is the precursor for the synthesis of egg albumen. Smaller egg size is, however, brought about by a decrease in dietary protein. The improved eggshell quality in this study agreed with Durmuş and Bölükbaşi (2015) who equally reported a significant increase in shell breaking strength of egg from pullets fed CeO at 200 mg/kg diet and above. The eggs from the cerium-treated birds would have a better consumer appeal due to their improved shell quality. This is because shell quality is an important economic sell point. The consumers' perception is that increased shell thickness means improved shell breaking strength to enhance the reduced number of cracked eggs (Oke *et al.* 2014). Olarotimi and Adu (2020) also opined that improved shell thickness and weight were indicative of enhanced Ca absorption and utilization among the hens fed cerium-treated diets. The significant improvements in some internal egg quality parameters such as YW, AH, AW, and HU were evidence of the enhancing effects of the varied inclusion levels of cerium in the experimental diets. The improved HUs recorded agreed with the findings of Cai *et al.* (2016). Albumen and HU being the major determinants of internal egg quality (Adu and Olarotimi, 2020) have more to say about the superiority of the eggs. The enhanced yolk, albumen, and HU recorded in this study would definitely result in enhanced fertilization, egg embryo development (Cai *et al.* 2016); hatchability, and chick survival rate as previously highlighted (Reka *et al.* 2019). The interactions of the cerium treatments and the varied levels of inclusions, however, did not compromise the nutritional quality of the experimental diets as far as egg qualities are concerned.

In the present study, it is suggestive that cerium treatments, varied inclusion levels, and their interactions did not have negative effects on the health status of the treated birds. All the hematological parameters were within the normal range values for chickens (Harrison and Lightfoot, 2005). Since the inclusion doses of cerium did not affect the normal hematological values, the immune statuses of the hens were not compromised by the various experimental diets. This finding supported the earlier reports by He *et al.* (2001) and Cai *et al.* (2018) which reported no significant change in all the hematological parameters studied in pigs. However, the significant decrease in eosinophil counts observed among the hens fed 150 mg cerium/kg diet validated the finding of He *et al.* (2010) which reported a significant reduction in lymphocytes, neutrophils, and eosinophils counts of broiler chickens. The statistical similarities recorded in the erythrocyte sedimentation rate (ESR) values between hens on the control diet and those on cerium treated diets revealed there was no presence of inflammation caused by the cerium treatments, their varied inclusion levels, and interaction. A significantly lower ESR is always associated with a disease or condition that increases red blood cell production, white blood cell production, or production of abnormal red blood cells (sickle cell anemia) (Marx *et al.* 2013). For the serum biochemical parameters, the results of this study partially disagreed with Adua *et al.* (2013) as non-significant differences were reported for all the serum biochemical parameters studied in rabbits fed varying levels of CeO. The significant increase observed in serum total protein concentration disagreed with Reka *et al.* (2018) who reported an increased total protein but agreed in globulin and albumin concentrations as no significant effect was recorded. Durmuş and Bölükbaşi (2015) also reported non-significant difference in serum ALT, AST, glucose; total cholesterol in laying hens fed varied levels (100-400 mg) of LaO. The present study recorded a significant increase in ALT and AST when the inclusion levels of cerium were increased to 150 mg/kg diet. The non-significant effect observed in the globulin concentration of the treated birds indicated that cerium did not interfere with protein metabolism when the inclusion levels employed in this study. It, also, was suggestive that there were no problems in the liver or kidney. Blood proteins are primarily synthesized in the liver, and hence, the increased concentration of total protein recorded among the birds on cerium treated diets is indicative of normal hepatic function and unimpaired protein synthesis. Hepatic cell damage is characterized by a significant rise in blood enzyme activities such as ALT and AST and thus causes alterations in liver function (Kim *et al.* 2008).

Hence, the significant treatment increase in the serum concentrations of these enzymes indicated that high levels of cerium in the diets of laying birds may predispose them to liver damage. This was, however, contrary to the report of He *et al.* (2010) who reported no residual effects of La and Ce in the liver and muscles of broiler chickens fed varied inclusion levels. The difference in these studies may be occasioned by the length of exposure of the birds to these REEs as broiler chickens will, usually, spend lesser time on the diets before reaching table size as compared with laying hens.

CONCLUSION

Since the varied inclusion levels of the two cerium sources used in the present study positively enhanced productive performances of the laying hens as well as improved the internal and external qualities of their eggs without any residual effects recorded on the health status of the birds, it is, therefore, concluded that CeCl and CeO could positively enhance cell-mediated and antibody-mediated immunity of hens. Cerium could, therefore, be used as a feed additive in place of antibiotics and to improve the hens' performances without compromising the product quality and leaving any antibiotic residues on the product.

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