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Effect of strontium ranelate and cerium oxide addition in the diet on egg production and eggshell quality in laying hen

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ABSTRACT

Minerals are used widely as feed additives in the diets of poultry to enhance bone and egg quality and productivity. Therefore, the current study aimed to evaluate the impact of strontium ranelate and cerium oxide addition in the diet on egg production and eggshell quality in laying hens. In this experiment, a total of 240 laying hens at the age of 44 weeks old, were divided into six treatments, with four replicates per treatment (10 hens per replicate). The hens were fed a standard diet for five weeks as an introductory period of preparation. The design of the dietary treatments was formulated as: control (T1) basal diet, Strontium ranelate (SrR) 400 and 800 mg/kg in basal diet (T2 and T3), Cerium oxide (CeO) 250 and 500 mg/kg in basal diet (T4 and T5) and their combination 400 mg/kg of Strontium ranelate + 250 mg/kg of Cerium oxide (T6) in the basal diet. Laying hen's performance and, egg quality (eggshell strength, eggshell thickness, eggshell percentage, minerals in eggshell, yolk index and Haugh unit), were analyzed. The results showed that the addition of SrR and CeO did not affect performance, yolk index, Haugh unit, ratio of Ca, P, and Ce in the eggshell. However, supplementing 800 mg/kg SrR significantly increased eggshell strength, thickness, percentage, Sr in the eggshell compared to the control, while supplementing 250 or 500 mg/kg CeO significantly increased eggshell strength and thickness. The combination of SrR and CeO significantly increased eggshell strength compared to the control group. In conclusion, supplementing SrR (800 mg/kg) and CeO significantly improved the quality of the eggshell.

Keywords: Cerium oxide, Egg production, Eggshell, Strontium ranelate

Osteoporosis and bone fracture due to genetic improvement for high egg production have serious animal welfare implications in commercial layer flocks (Meseret S 2016). Also, osteoporosis represents 6% of broilers' abnormally developed skeletal or musculoskeletal disease (Baracho *et al.* 2019).

Modern laying hens have been selected to lay eggs almost daily for consecutive months, which requires significant amounts of calcium. Unsurprisingly, this places physiological strain on the hen's skeleton (Whitehead 2004). Approximately 10% of a hen's total body calcium volume daily is used in producing eggshells, of which half is sourced from the diet, and half is sourced from medullary bone (Kerschnitzki *et al.* 2014). For several decades, osteoporosis in laying hens has been a recognized problem that results in bone fractures, layer fatigue, and production loss, and is a significant welfare concern (Whitehead and Fleming 2000).

Strontium (Sr) is the alkaline element rare earth metal (Browning and Cowieson 2013) and its physical and chemical properties are similar to those of calcium in the periodic table (Skoryna 1981). Strontium ranelate (SrR) consist of two strontium atoms coupled by ranelic acid. Clinically, therapeutic uses of Sr salts such as SrR are involved in preventing osteoporotic fractures, bone mass/quality and increased bone strength in osteoporotic patients through changes in bone matrix characteristics and bone mineral density (Meunier *et al*. 2004, Bakhit *et al.* 2018). In addition, using SrR is approved in the U.S., Europe, and Australia to stimulate bone formation in humans (Suzuki *et al.* 2008).

Cerium oxide (CeO) is an oxide of the rare-earth element considerd metal cerium with the chemical formula CeO_2. CeO has many uses either commercially or biologically and their impacts on poultry are approved (Reka *et al*. 2019 and Adu 2020). Numerous studies showed that Ce, as nanoparticles, gave a potential therapeutic tool in preventing and treating oxidative stress in animals and poultry (CórdobaJover 2019). In biological contexts, it has been reported that CeO nanoparticles can mimic enzymatic antioxidants such as superoxide dismutase and it has beneficial effects on animal and bird health (Heckert 2008, Shanmugam and Naik 2017). Thus, the aim of the present study was to determine the effect of strontium ranelate and cerium oxide addition in the diet on egg production and eggshell quality in laying hen.

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MATERIALS AND METHODS

Animal welfare: This study was conducted in accordance with animal ethics guidelines of the Research Policy of Salahaddin University, Erbil, College of Agriculture Engineering Sciences, Department of Animal Resources.

For this experiment, 240 commercial layer chickens (ISA Brown) at age of 44 weeks old were weighed individually and allocated in 24 boxes (10 hens per box) with similar average weights in each box in a well environmentally controlled hall. The first five weeks were used as an introductory period for adaptation and installation of each box for weight and egg production. The hall temperature was maintained at approximately 24°C during the experiment period.

The lighting program was adapted to management guides of commercial layers (ISA-brown). The light was switched on for 17 h daily during the experimental period. The light: dark cycle was 17L:7D.

The design of the dietary treatments was formulated as: control (T1) basal diet, Strontium ranelate (SrR) 400 and 800 mg/kg in basal diet (T2 and T3), Cerium oxide (CeO) 250 and 500 mg/kg in basal diet (T4 and T5) and their combination 400 mg/kg of Strontium ranelate + 250 mg/kg of Cerium oxide (T6) in the basal diet.

The experimental diet formulations formulated according to the nutritional guides of commercial layers (ISA-brown) are shown in Table 1.

Table 1. Feed formula and calculated nutrients analyses

Ingredient	Composition (kg/tonne of diet)	
	44–50 Weeks	51–59 Weeks
Soybean meal 45%	243	267
Corn (Maize)	443	522
Wheat flour	100	40
Wheat bran	69	0
Premix*	9	9
Limestone	93.5	120
Soybean oil	29	26.5
Methionine	1.6	2.1
Sodium Bicarbonate	2.15	2
Mono Calcium Phosphate	5.3	7
T. Salt	2.25	2.25
Toxin binder	2	2
Enzyme**	0.5	0.5
Total	1000.30	1000.35
Calculated values[1]		
ME (kcal/kg)	2796	2800
CP%	16.96	16.96
Methionine%	0.399	0.447
Lysine%	0.820	0.828
Methionine+Cysteine%	0.646	0.684
Threonine%	0.626	0.628
Valine%	0.704	0.696
Leucine%	0.651	0.660
Isoleucine%	1.252	1.291
Arginine%	1.086	1.070
Tryptophan%	0.184	0.177
Ca%	3.525	4.454
P%	0.352	0.380
Na%	0.171	0.171
Cl%	0.182	0.180
K%	0.844	0.821
Ash%	12.83	15.39
Fiber%	2.86	2.25
Fat%	2.32	2.31

*Vitamins and trace minerals contained in the premix were; arginine, 3.5%; threonine, 6%; tryptophan, 0.5%; BHT, 830 mg/kg; propylgallate, 70 mg/kg; citric acid, 125 mg/kg; betaine hydrochloride, 6000 mg/kg; ** (Kemzyme malty enzyme) Xylanase and Pectinase Complex, Cellulase, Protease, Beta-Glucanase, Alpha Amylase, from KEMIN company (USA).

Laying hen performance

Feed intake: Everyday 120 g of feed was given to each bird (1200 g for each box) and freshwater was offered daily *ad lib*. The feed intake was determined weekly by subtracting the remaining feed in the feeder from the sum of the given feed to the feeder.

Feed conversion ratio: FCR was calculated by dividing the feed consumption (intake) by the egg mass (egg weight × percentage of egg production) each week (Ibrahim 2000).

Egg weight (g): Eggs were weighed individually everyday using a sensitive balance.

Egg production: Eggs were collected manually at the same time (6 PM) each day. The weight and number of eggs laid were recorded daily. The egg weight was determined by an electronic scale. The abnormal eggs (broken eggs, shell-less, or soft shells) were excluded when measuring the egg weight. The egg production percentage was calculated as the hen-day egg production (HDEP) including the abnormal eggs.

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

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

Egg quality parameters: Every week, the egg quality indices were determined by randomly collecting 8 eggs per treatment. Egg weight and eggshell weight were measured by the electronic scale.

Eggshell thickness: The shell thickness was measured with eggshell membranes and dried for about 24 h at room temperature. Shell thickness was measured from the three different parts of the egg (equator, sharp end and broad end) of each eggshell using a digital Vernier micrometer screw gauge (Peacock dial gauge, P-1 Model, Meg Co Ltd., Ozaki, Japan) and the average was calculated.

Eggshell weight: The eggshell with membranes for each egg per replicate was weighed by a digital balance and the eggshell percentage was calculated as follows (Ibrahim 2000):

Eggshell (%) = Shell weight (g) / Egg weight (g) × 100

Eggshell strength: Eggshell strength was measured using eggshell testing equipment (Egg Force Reader, OC-SPA, France Technical Services and Supplies) and expressed as a unit of compression force that was exposed to the unit eggshell surface area (g /cm^2).

The Haugh units (HU) and Yolk index were measured by using an Egg Multi Tester EMT-5200 (Robotmation Co. Ltd., Tokyo, Japan). The Haugh Unit (HU) was estimated according to the following equation (Haugh 1937):

$$HU = 100 \log (H + 7.57 - 1.7W^{0.37})$$

Where, H, albumen height; W, egg weight.

Haugh Unit index: The score of HUI was recorded as AA 72 or more, A 71-60, B 59-31 and C 30 or less (Abdurehman and Urge 2016).

Yolk Index: The egg was broken on clean flat glass, the height of yolk was measured by Tripod micrometer on the center point of the yolk and albumen height was measured in two points (thick and thin) by Ames micrometer to calculate the average of them. Yolk diameter was measured by digital vernier in two edges. Egg yolk index was measured, according to following equation (Ibrahim 2000):

$$\text{Yolk index} = \text{Yolk height (mm)} / \text{Yolk diameter (mm)} \times 100$$

Eggshell mineral analysis: In the last week of the experiment, the eggshell was collected to measure the mineral content (Ca, P, Sr, Ce, Mg, Na, Al, Cl) using Energy-dispersive X-ray spectroscopy (EDX) and the eggshell was scanned by Scanning Electron Microscope (SEM) under 500x resolution to take an image of eggshell surface structure.

Statistical analysis: Statistical analysis of the data is presented as means ± SE. One-way ANOVA followed by a CRD test was performed using (SAS) program version (SAS 9.3, 2013). Differences were reported as significant in all instances with a $P<0.01$. Duncan's multiple range test was employed to determine differences between individual treatment means and interactions were observed ($P<0.01$).

RESULTS AND DISCUSSION

Laying hens performance: The impact of SrR, CeO and their combination on the performance of layer chickens is demonstrated in Table 2. There were no significant (P>0.05) differences observed among treatments except T3 compared with T5 for egg production. A higher value was observed in T3 followed by T4 while the lowest value was recorded in group T5, this may be due to fact that some eggs were missed because of health issue during the experimental time. The addition of SrR (800 mg/kg feed) resulted in 1.35% increase in egg production compared to the control and the addition of CeO (300 mg/kg feed) resulted in 0.97% increase.

No significant (P>0.05) differences were observed among treatments on egg weight, egg mass, FI and FCR. It seemed that the addition of SrR (800 mg/kg) and CeO (T4 and T5) lead to a decrease in feed intake compared to other groups except T3.

Also, the addition of SrR (800 mg/kg feed) resulted in 4.23% improvement in FCR compared to the control and the addition of CeO (300 mg/kg feed) resulted in 0.94% improvement, but not significantly.

The results of the present study agree with Shahnazari *et al*. (2007) who showed that the addition of Sr (3000, 4500, and 6000 mg/kg feed) to the diet of laying hen for 11 months had no significant effect on feed intake, egg production and egg weight. Also, Browning and Cowieson (2014) showed that using Sr (500 and 1000 mg/kg feed) with Vitamin D_3 (2500 and 5000 iu/kg) in a laying hen's diet had no significant effect on egg production, egg weight, egg mass, FI and FCR.

Reka *et al*. (2019) showed lanthanum100 mg and cerium 150 mg in the diet of layers significantly (P<0.05) decreased feed intake at 53-60 weeks of age, and increased hen day egg production, feed efficiency and egg weight, whereas feeding 200 mg lanthanum and 300 mg cerium significantly (P<0.05) decreased feed intake and increased egg weight during 53-56 weeks of age.

As well as the results of T4 and T5 for performance agreed with Olarotimi (2021) who used 50, 100, 150 mg/kg feed CeO to the hens at 24 weeks of age. Bolukbası *et al.* (2016) showed no significant effect on FI and egg weight by using 100, 200, 300 and 400 mg/kg feed Ce in laying hens (brown Lohman LSL) and a significant increase in egg production.

Egg quality: Effect of SrR, CeO and their combination on egg quality in (ISA Brown) laying hen is shown in Table 3. All additives except T2 significantly (P<0.01) increased eggshell strength compared with control group (T1). The highest value was obtained by the addition of 800 mg/kg feed SrR (12.3% improvement), this result could be attributed to the high ratio of Sr in the eggshell

Table 2. Effect of Strontium Ranelate, Cerium Oxide and their combination on layer performance

Treatment	Egg production (%)	Egg weight (g)	Egg mass (g/hen/day)	FI (g/hen/day)	FCR (g feed/egg mass)
T1	88.68 ± 2.97 ^{ab}	63.2 ± 0.39	56.17 ± 1.45	119.03 ± 0.43	2.13 ± 0.06
T2	88.36 ± 1.46 ^{ab}	62.1 ± 0.31	54.87 ± 0.85	119.91 ± 0.09	2.19 ± 0.03
T3	90.03 ± 2.23 ^{a}	63.04 ± 0.46	54.54 ± 2.27	110.37 ± 2.91	2.04 ± 0.03
T4	89.65 ± 1.81^{ab}	62.07 ± 0.61	55.97 ± 1.37	116.51 ± 1.28	2.11 ± 0.05
T5	83.10 ± 2.22 ^{b}	63.36 ± 0.79	52.72 ± 1.51	113.13 ± 2.48	2.16 ± 0.06
T6	88.69 ± 2.23 ^{ab}	62.91 ± 0.41	55.81 ± 1.49	119.04 ± 0.26	2.14 ± 0.06
L.S.	*	NS	NS	NS	NS

Means ± SE. $^{a, c.}$ means with different letter within column are significantly different (P<0.05). L.S., level of significant; *, Significant in (P<0.05); N.S., Non-significant (P>0.01). n = 4. T1: control, T2: SrR (400 mg/kg), T3: SrR (800 mg/kg), T4: CeO (250 mg/kg), T5: CeO (500 mg/kg), T6: SrR + CeO (400+250) mg/kg.

Table 3. Effect of Strontium Ranelate, Cerium Oxide and their combination on egg quality (ISA Brown)

Treatment	Egg shell strength (g)	Eggshell thickness (mm)	Eggshell (%)	Yolk index	HU
T1	1320.72 ± 42.89^{b}	0.353 ± 0.003^{c}	9.04 ± 0.09^{b}	0.438 ± 0.002	90.23 ± 1.14
T2	1438.16 ± 46.02^{ab}	0.365 ± 0.004^{ab}	9.18 ± 0.13^{ab}	0.432 ± 0.004	85.93 ± 0.94
T3	1506.15 ± 45.94^{a}	0.370 ± 0.003^{a}	9.38 ± 0.08^{a}	0.439 ± 0.002	87.74 ± 0.89
T4	1498.75 ± 34.26^{a}	0.368 ± 0.005^{a}	9.39 ± 0.09^{a}	0.424 ± 0.006	87.12 ± 1.24
T5	1467.98 ± 32.79^{a}	0.365 ± 0.004^{ab}	9.16 ± 0.1^{ab}	0.437 ± 0.003	87.80 ± 0.92
T6	1481.19 ± 47.01^{a}	0.356 ± 0.003^{bc}	8.93 ± 0.08^{b}	0.432 ± 0.002	87.23 ± 0.91
L.S.	**	**	**	NS	NS

Means ± SE.$^{a-c}$means with different letter within column are significantly different (P<0.01). L.S., level of significance; **, Significant in (P<0.01); N.S., Non-significant (P>0.01). n = 8 egg/week. T1: control, T2: SrR (400 mg/kg), T3: SrR (800 mg/kg), T4: CeO (250 mg/kg), T5: CeO (500 mg/kg), T6: SrR + CeO (400+250) mg/kg.

as it is higher in the eggshell images scanned by Scanning Electron Microscope (Fig. 1.) showing eggshells crystal microstructure for all treatments, as clear in T3, the compounds of eggshells are more compact and at the same time, the fragility of eggshell in T1 is more than the other treatments. Eggshell thickness increased significantly by adding 400, 800 mg/kg feed SrR and 250, 500 mg/kg feed CeO, compared with the control group; while the combination of additives T6 has no significant effect. The

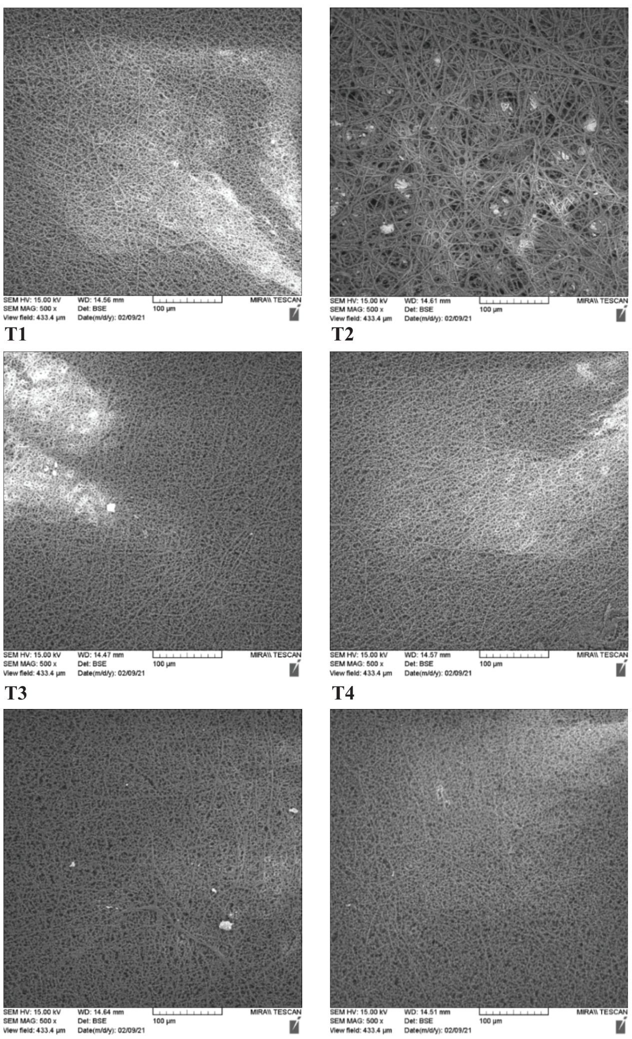

Fig. 1. Scanning electron microscope images of eggshells of different treatments.

eggshell percentage increased with the addition of SrR and CeO, with a significant increase in T3 and T4 compared to the control group. Unexpectedly supplementing the combinations of both minerals (T6) resulted in non-significant decrease. The lower eggshell percentage as recorded in T6, could be attributed to the rare earth elements that resemble calcium not only in size and bonding but also in coordination geometry and donor atom preference, which make them replace Ca^{+2} specifically in various biological processes (Reka *et al*. 2019), which means both Sr and Ce synergically strengthening the eggshell with less amount of perception in the eggshell without affecting hardness of the eggshell. No significant influence was observed on the yolk index and HU. Browning and Cowieson (2014) showed that adding Sr 500 and 1000 mg/kg with Vitamin D_3 (2500 and 500 iu/kg) in a laying hen's diet had no significant effect on eggshell percentage.

Bolukbası *et al*. (2016) showed that using 100, 200, 300, and 400 mg/kg feed Ce had no significant effect on eggshell weight, HU, and eggshell thickness but adding 200 and 300 mg/kg feed Ce significantly increased eggshell strength; at the same time adding 100 and 400 mg/kg feed Ce didn't show any effect on eggshell strength. Olarotimi (2021) also didn't show significant effect on eggshell %, eggshell thickness, yolk index and HU by using 50, 100, and 150 mg/kg feed CeO.

Effect of SeR, CeO and their combination on the ratio of minerals in the eggshell is shown in Table 4. The treatments had no effect on the ratio of Ca, P, and Ce in the eggshell. At the same time, the ratio of Sr was increased significantly (P<0.01) by adding 800 mg/kg to the diet, this increase could be attributed to the Ralenic acid bound with Sr which led to higher availiability of Sr for absorption, in which organic mineral absorption increases in form of organic minerals.

Shahnazari *et al.* (2006) showed that adding Sr (3000, 4500, and 6000 mg/kg feed) had no significant effect on the ratio of (Ca) in the eggshell ash and plasma of laying hen, but the ratio of Sr significantly increased in the eggshell by increasing the level of Sr in the diet.

The present study showed that the addition of SrR and CeO did not affect performance, yolk index, Haugh unit, ratio of Ca, P, and Ce in the eggshell. However, supplementing of SrR (800 mg/kg) and CeO significantly

Table 4. Effect of Strontium Ranelate, Cerium Oxide and their combination on the ratio of minerals eggshell

Treatment	Ca %	P %	Sr %	Ce %
T1	0.203 ± 0.032	0.128 ± 0.066	0.09 ± 0.026[b]	0.337 ± 0.122
T2	0.105 ± 0.018	0.1 ± 0.031	0.098 ± 0.031[b]	0.374 ± 0.063
T3	0.288 ± 0.085	0.353 ± 0.071	0.477 ± 0.095[a]	0.153 ± 0.015
T4	0.247 ± 0.085	0.067 ± 0.028	0.093 ± 0.044[b]	0.22 ± 0.046
T5	0.333 ± 0.031	0.208 ± 0.068	0.097 ± 0.042[b]	0.423 ± 0.064
T6	0.203 ± 0.066	0.163 ± 0.083	0.088 ± 0.042[b]	0.18 ± 0.017
L.S.	NS	NS	**	NS

Means ± SE.[a, c]means with a different letter within the column are significantly different (P<0.01). L.S., Level of significance; **, Significant in (P<0.01); N.S., Non-significant (P>0.01). n = 4. T1: control, T2: SrR (400 mg/kg), T3: SrR (800 mg/kg), T4: CeO (250 mg/kg), T5: (500 mg/kg), T6: SrR + CeO (400+250) mg/kg.

improved the quality of eggshell compared to the control group.

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