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Nano-calcium carbonate: Effect on performance traits and egg quality in laying hens

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Abstract A ten-week trial was carried out to determine the effect of different levels of nano-calcium carbonate (NCC) on egg production performance and egg quality. A completely randomized experiment was designed comprising 6 treatments, each with 4 replicates of 5 hens. Groups 1 and 2 were fed diets containing 8.06% and 6.045% calcium carbonate (CC) as positive and negative control respectively. Groups 3, 4, 5 and 6 received diets with 4.03% CC plus 2.015, 1.01, 0.252 and 0.126% NCC respectively. Egg production at 32 wks of age was significantly reduced by the lowest dietary supplementation with NCC (T6) ($P<0.05$). However, dietary treatments had no significant effect on egg weight, egg mass and FCR ($P>0.05$). The lowest egg shape index was recorded for the birds fed T6 at 31 and 33 wks of age ($P<0.05$). Hens fed T6 had the lowest egg specific gravity at 29 wks of age compared to those fed other diets. However, the lowest egg specific gravity at 33 wks of age belonged to the birds which received T3. Dietary treatments had no effect on the Haugh unit. Hens on T6 had the highest yolk color at 33 wks of age compared to those receiving other treatments ($P<0.05$). It was concluded that it is possible to utilize NCC at the level of 0.252 to 2.015% instead of 4.03% CC in laying hen diets. However, longer term studies are suggested to determine the potential of nano-calcium carbonate, as a new calcium source, on egg production performance and egg quality in laying hens.

Keywords: nano-calcium carbonate, laying hen, performance traits, egg quality

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Introduction

In developing countries, poultry eggs and meat are accepted as appropriate sources of nutrients (Oluyemi and Roberts, 2000). The eggshell protects the egg contents against environmental damage, therefore it must be strong enough to resist challenges during the processes of laying, collection, grading and transport, until the egg reaches the consumer (Pizzolante et al., 2009). Calcium is a key element in laying hens, as 98% of eggshell is composed of calcium carbonate. Calcium source, particle size and supplementation level affect eggshell quality (Pelicia et al., 2007). Conventional sources of calcium in laying hens are oyster shell and calcium carbonate (Leeson and Summers, 2001).

In the field of poultry nutrition, it is possible to apply nanotechnology for many purposes; obtaining information regarding a mineral, its liberation in site-specific actions, maintaining adequate levels for a longer duration, avoiding its degradation and providing better availability (Ross et al., 2004). Using nanotechnology it is possible to produce particles measuring from 1 to 100 nm, with the potential for new applications. Nanoparticles with smaller size have better properties than larger ones (Sekhon, 2012). Nanoparticles can effectively supply the requirements of poultry, promote growth, decrease the environmental contamination and produce pollution-free poultry products (Schmidt, 2009). There is a growing interest in the application of nanotechnology to produce a supplemental source of minerals in poultry diets. Although a large number of studies have emphasized the importance of nanotechnology in poultry production (Saffa et al., 2008; Schmidt, 2009; Sekhon, 2012; Siriat et al., 2013), there is no evidence regarding the application of calcium nanoparticles in laying hens. Therefore, the aim of our study was to evaluate the effects of nano-calcium carbonate (NCC) on egg production performance and egg quality in lay ing hens.

Materials and methods

The experiment was conducted at a research laying hen house in Rezvan College in Kerman, Iran. The Animal Welfare Committee of the University of Jiroft approved the animal care protocol used in this experiment.

Birds, diets and experimental design

A ten-week trial was carried out to determine the effect of different levels of nano-calcium carbonate (NCC) on egg production performance and egg quality in Bovans laying hens. A completely randomized experimental design was applied with 6 treatments, each having 4 replicates of 5 hens. Groups 1 and 2 were fed diets containing 8.06% and 6.045% calcium carbonate (CC) as positive and negative controls, respectively. Groups 3, 4, 5 and 6 received diets with 4.03% CC plus 2.015, 1.01, 0.252 or 0.126% NCC respectively. The calcium content of diets is shown in Table 1. Isoenergetic and isonitrogenous corn-soybean meal-based diets were formulated using the UFFDA software. The birds were allotted to 24 cages, 5 birds per cage (59.5 cm × 55.5 cm). Each cage was equipped with a nipple drinker and a feeder. Feed and water were offered *ad libitum*.

Nano-calcium carbonate (NCC) was obtained from the American Elements Company (Los Angeles, California 90024, USA). The average particle size of CaCO3 white nano powder (99.99% calcium carbonate nanoparticle, product code, CA-CB-04-NP) was 15-40 nm. The desired amount of NCC for each treatment was calculated and then added to the diet.

Experimental measurements

Egg weight and feed intake were recorded bi-weekly, and feed conversion ratio (FCR) was calculated (Catli et al., 2012).

$$FCR=\frac{\text{Feed intake (kg)}}{\text{Egg weight (kg)}} \quad (1)$$

Bi-weekly egg production (%) was calculated as follows (Pelicia et al., 2009):

$$\text{Cage egg production (\%)}=\frac{\text{Total number of produced egg}}{\text{Number of birds}}\times 100 \quad (2)$$

The egg weight was measured and egg mass was calc-

Table 1. Feed ingredients and calculated chemical compositions (%) of the experimental diets

Ingredients (%)	Treatments					
	T1	T2	T3	T4	T5	T6
Corn	61.5	61.5	61.5	61.5	61.5	61.5
Soyabean meal	26.64	26.64	26.64	26.64	26.64	26.64
Corn oil	1.29	1.29	1.29	1.29	1.29	1.29
Dl-Methionine	0.09	0.09	0.09	0.09	0.09	0.09
Dicalcium phosphate	1.64	1.64	1.64	1.64	1.64	1.64
Calcium carbonate	8.06	6.045	4.03	4.03	4.03	4.03
Calcium carbonate nanoparticle	0	0	2.015	1.01	0.252	0.126
Mineral supplements[1]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin supplements[2]	0.25	0.25	0.25	0.25	0.25	0.25
NaCl	0.28	0.28	0.28	0.28	0.28	0.28
Sand	0	2.015	2.015	3.02	3.778	3.904
Total	100	100	100	100	100	100
Calculated nutritive value						
ME(Kcal/Kg)	2800	2800	2800	2800	2800	2800
CP(%)	17	17	17	17	17	17
Calcium (%)	3.66	2.55	2.54	1.68	1.54	1.43
Phosphorus (%)	0.5	0.5	0.5	0.5	0.5	0.5
Na (%)	0.15	0.15	0.15	0.15	0.15	0.15
Lysine (%)	0.55	0.55	0.55	0.55	0.55	0.55
Methionine (%)	0.244	0.244	0.244	0.244	0.244	0.244
Methionine + cystine (%)	0.493	0.493	0.493	0.493	0.493	0.493

[1]Provided per kg of diet: 8,989,200 mg vitamin A from retinyl acetate; 880,000 µg cholecalciferol; 35,200 mg vitamin K from menadione sodium biosulphite; 1320 mg vitamin B12; 798,336 mg riboflavin; 498,960 mg niacin from nicotinic acid; 323,855.4 mg pantothenic acid from calcium pantothenate; 99,792 mg folic acid; 821,286.4 mg vitamin B6; 58,666.67 mg vitamin E from DL-tocopheryl acetate; 598,400 mg choline; 88,000 mg thiamin from thiamine mononitrate; 2,200 mg of biotin.

[2]provided per kg of diet: copper, 8.75 mg from copper sulfate, Zn 35 mg from zinc sulfate; iodine, 0.035 mg from organic iodine; Mn, 20 mg from manganese sulfate, iron 45 mg from iron sulfate.

ulated as follows:

$$\text{Egg mass} = \text{Egg weight} \times \text{Egg production} \quad (3)$$

Mortality was recorded and performance parameters were adjusted during the experiment.

Egg characteristics (egg shape index, egg specific gravity, Haugh unit and yolk color) were determined bi-weekly, from 27 to 33 weeks of age. For each treatment, two eggs per replicate (8 eggs per treatment with the nearest weight to the average egg weight) were analyzed at 27, 29, 31 and 33 weeks. The egg was weighed, and the maximum width and length of the egg were measured by a caliper for determination of shape index (width divided by length, multiplied by 100). To determine egg specific gravity, eggs were immersed in saline solutions at different concentrations (1.064, 1.068, 1.072, 1.076, 1.080, 1.084, and 1.088, 1.092, 1.096 and 1.1 mg/mL) and the floating concentration for each egg was recorded as its specific gravity. Saline solutions were adjusted using an oil densitometer, regularly calibrated. Finally, the egg was broken and its contents poured onto a flat glass plate. The albumen height was measured by a tripod micrometer and the Haugh unit calculated according to the following formula:

$$\text{Haugh unit} = 100 \log (\text{Albumen height} - 1.7\ \text{Egg weight}^{0.37} + 7.57) \quad (4)$$

The yolk color evaluated visually by the COLOR FAN method, was determined against a white, non-reflective surface, in order to eliminate the influence of adjacent colors, using indirect daylight and no artificial light. The use of indirect light prevents distracting reflections from the glossy surface of the yolk. The blades of the DSM Yolk Fan™ were held immediately above the egg yolk and viewed vertically from above, with the blade numbers facing down and the yolk positioned between the tips of the blades (DSM, 2016).

Statistical analysis

The data were analyzed using the GLM procedure of SAS for a completely randomized design (SAS Institute, 2012), according to the following model:

$$X_{ij} = \mu + T_i + E_{ij} \quad (5)$$

in which, X_{ij} represents the individual observation, μ is the mean, T is the treatment effect and E_{ij} is the error term.

Results and discussion

Egg production

Dietary treatments had no effect on egg production in weeks 24, 26, 28, 30 and over the whole experimental period (Table 2). Our results are consistent with findings of Pizzolante et al. (2009), who reported that different levels of calcium and limestone particle size had no effect on egg production. However, Ahmed et al. (2013) found that the application of limestone instead of oyster shell increased egg production. Pelicia et al. (2007) also reported that the inclusion of different levels of limestone into the diet had no effect on egg production. In the current experiment, egg production was significantly ($P<0.05$) reduced at 32 wks of age in hens receiving T6 compared to those fed other treatments. Calcium level in this treatment was 1.43% and, parallel to our results, Guo and Kim (2012) reported that reducing calcium content in the diet had a negative impact on egg production. Unfortunately, there was no report in the literature about the application of calcium nanoparticles in laying hens with which to compare our findings.

Egg weight

Dietary treatments had no significant effect on egg weight in any week or over the whole experimental period (Table 3). The main factor which can affect egg weight is the protein content of the diet and as all the treatments were isonitrogenous, these results were therefore predictable, since neither CC nor NCC have any inhibitory protein binding characteristics. However, Roland et al. (1996) reported that reducing calcium content of the diet resulted in a decrease in egg weight. Although no report was found in the literature regarding the influence of calcium nanostructures on egg weight, Siriat et al. (2013) reported that supplementation of laying hen diets with 500 ppb nano-chromium picolinate had no effect on egg weight.

Egg mass

Dietary treatments had no significant effect on egg mass during the experiment (Table 4). However, many publications have shown that the inclusion of more calcium in laying hen diets could enhance egg mass (Catli et al., 2012; Safaa et al., 2008; Pelicia et al., 2009). According to our findings, the inclusion of NCC to the diet provided enough calcium in order to maintain a similar egg mass to the control group.

Feed conversion ratio

Dietary treatments had no significant effect on FCR (Table 5). The FCR is a function of feed consumption in relation to egg production in laying hens. Less calcium in the diet leads to increased feed intake to compensate for the lack of calcium. Research has shown that limestone feeding causes better FCR compared to oyster shell (Ahmed et al., *2*013). However, the results of other studies have revealed that the source of calcium did not influence FCR in laying hens. Pelicia et al. (2009) and Catli et al. (2012) reported that the inclusion of variable amounts of calcium in hen diets in the second phase of egg production resulted in a similar FCR. No evidence was found in the literature regarding the effect of calcium nanoparticles in laying hens with which to compare our results. El-Shuraydeh et al. (2014) reported that aqueous nano-suspensions of 1% of clay minerals in broilers improved FCR. Supplementation of broiler diets with 50% calcium phosphate-nanoparticles improved FCR (Vijayakumar and Balakrishnan, 2014). In agreement with our results, Cai et al. (2012) reported that nano-selenium had no effect on FCR in broilers.

Egg shape index

The minimum egg shape index at 31wks of age was observed in the T6 group while the maximum egg shape

Table 2. Egg production (%) in Bovans laying hens from 23 to 33 weeks of age fed diets containing different levels of NCC

	Age (week)					
Treatments	24	26	28	30	32	Total
1	90.7	96.4	94.3	92.6	97.1[a]	94.3
2	90.0	96.4	94.3	95.7	93.6[a]	94.4
3	85.7	87.9	92.6	91.4	94.3[a]	90.4
4	85.0	85.7	90.0	90.7	95.9[a]	89.5
5	88.6	95.0	91.4	90.7	96.4[a]	92.4
6	87.9	89.3	88.6	87.6	88.3[b]	88.4
SEM	4.84	4.51	3.04	3.40	1.41	2.74
P-value	0.948	0.269	0.711	0.709	0.004	0.528

[ab]In each column, means with common superscript (s), do not differ (P>0.05). T1: Basal diet (BD, 8.06 CC), T2: (negative control, 6.045 CC), T3: (4.03 CC + 2.015 NCC), T4: (4.03 CC + 1.01 NCC), T5: (4.03 CC + 0.252 NCC), T6: (4.03 + 0.126 NCC). CC: Calcium carbonate, NCC: Nano calcium carbonate.

Table 3. Egg weight (g) in Bovans laying hens from 23 to 33 weeks of age fed diets containing different levels of NCC

	Age (week)					
Treatments	24	26	28	30	32	Total
1	53.7	56.3	57.2	56.2	56.0	55.9
2	53.2	56.1	56.4	56.7	57.9	56.0
3	53.3	56.8	57.5	58.1	58.6	57.1
4	52.5	55.1	54.7	56.4	56.9	55.1
5	52.8	54.6	55.7	57.1	57.6	55.6
6	54.7	56.3	56.8	57.3	58.2	56.7
SEM	0.69	0.73	0.86	0.73	1.06	0.65
P-value	0.251	0.337	0.257	0.539	0.527	0.366

Table 4. Egg mass (g/hen/d) in Bovans laying hens from 23 to 33 weeks of age fed diets containing different levels of NCC

	Age (week)					
Treatments	24	26	28	30	32	Total
1	48.7	54.3	53.9	52.1	54.4	52.7
2	47.8	55.2	53.1	54.2	54.2	52.9
3	46.5	49.9	53.3	53.0	55.3	51.6
4	46.5	47.2	49.2	51.1	53.2	49.1
5	46.8	51.8	50.9	51.7	55.6	51.4
6	48.0	50.1	50.2	50.3	51.1	49.9
SEM	2.61	2.64	1.56	1.77	1.64	1.57
P-value	0.903	0.317	0.241	0.681	0.432	0.487

T1: Basal diet (BD, 8.06 CC), T2: (negative control, 6.045 CC), T3: (4.03 CC + 2.015 NCC), T4: (4.03 CC + 1.01 NCC), T5: (4.03 CC + 0.252 NCC), T6: (4.03 + 0.126 NCC). CC: Calcium carbonate, NCC: Nano calcium carbonate.

Table 5. Feed conversion ratio in Bovans laying hens from 23 to 33 weeks of age fed diets containing different levels of NCC

	Age (week)					
Treatments	24	26	28	30	32	Total
1	1.97	1.77	1.81	1.96	1.91	1.88
2	2.00	1.75	1.87	1.89	1.91	1.88
3	1.94	1.89	1.86	1.93	1.89	1.90
4	2.16	2.02	2.00	2.01	1.95	2.03
5	2.04	1.79	1.92	1.97	1.86	1.92
6	2.03	1.80	1.89	2.05	2.04	1.96
SEM	0.12	0.08	0.06	0.07	0.06	0.06
P- value	0.405	0.390	0.235	0.306	0.334	0.450

T1: Basal diet (BD, 8.06 CC), T2: (negative control, 6.045 CC), T3: (4.03 CC + 2.015 NCC), T4: (4.03 CC + 1.01 NCC), T5: (4.03 CC + 0.252 NCC), T6: (4.03 + 0.126 NCC). CC: Calcium carbonate, NCC: Nano calcium carbonate.

index at 33 wks of age belonged to T3 group (P<0.05,Table 6). The most balanced egg shape index is 74 and, if this number is increased or decreased, the egg will become more orbicular or oblong, respectively. The egg shape index is also affected by the hen's age, with young laying hens producing more orbicular eggs. In this study, almost all egg shape indexes were greater than 74, which may be related to the young age of our laying hens. Catli et al. (2012) and Wang et al. (2014) reported no effect of different calcium sources (bone, limestone or oyster shell meal) on egg shape index.

Egg specific gravity

As shown in Table 7, the control group (containing the highest amount of calcium) had the highest egg specific gravity at 29 and 33 wks of age compared to other treatments (P<0.05). Confirming this trial, many previous scientific studies have reported that calcium supplementation to the laying hens' diet significantly improves egg specific gravity (Roland et al., 1996). Pelicia et al. (2007) reported that calcium source had a significant effect on egg specific gravity. Pizzolante et al. (2009) found that calcium level and limestone particle size had no significant effect on egg specific gravity. According to the mentioned studies, an egg specific gravity above 1.08 shows appropriate egg shell quality and all the treatments in the current experiment produced egg specific gravity above this value. Therefore, it suggests that when using NCC, reduction of calcium concentration in the diet (to the level provided by T6) will not markedly decrease the egg specific gravity.

Haugh unit

Data on the Haugh unit, for which no differences between treatments were observed, are shown in Table 8. Increased Haugh unit indicates better albumen quality. The Haugh unit is affected by many parameters, including hen's age and strain, nutrition, especially protein and amino acids, disease, egg storage time and tempera-

Table 6. Egg shape index in Bovans laying hens from 23 to 33 weeks of age fed diets containing different levels of NCC

	Age (week)			
Treatments	27	29	31	33
1	76.6	77.2	79.0[a]	76.7[b]
2	77.2	77.9	77.5[a]	77.1[b]
3	76.8	77.3	78.8[a]	80.1[a]
4	75.6	75.9	78.6[a]	77.4[b]
5	76.2	75.7	77.9[a]	75.7[b]
6	75.0	75.5	75.3[b]	75.5[b]
SEM	1.06	1.25	0.79	0.83
P-value	0.710	0.854	0.040	0.013

[ab] In each column, means with common superscript (s), do not differ (P>0.05). T1: Basal diet (BD, 8.06 CC), T2: (negative control, 6.045 CC), T3: (4.03 CC + 2.015 NCC), T4: (4.03 CC + 1.01 NCC), T5: (4.03 CC + 0.252 NCC), T6: (4.03 + 0.126 NCC). CC: Calcium carbonate, NCC: Nano calcium carbonate.

Table 7. Egg specific gravity (g/cm^3) in Bovans laying hens from 23 to 33 weeks of age fed diets containing different levels of NCC

	Age (week)			
Treatments	27	29	31	33
1	1.09	1.09[a]	1.09	1.09[a]
2	1.09	1.08[b]	1.09	1.08[b]
3	1.08	1.08[b]	1.09	1.08[b]
4	1.08	1.08[b]	1.09	1.08[b]
5	1.09	1.08[b]	1.09	1.08[b]
6	1.08	1.08[b]	1.09	1.08[b]
SEM	0.001	0.001	0.001	0.001
P-value	0.116	0.047	0.638	0.014

[ab] In each column, means with common superscript (s), do not differ (P>0.05). T1: Basal diet (BD, 8.06 CC), T2: (negative control, 6.045 CC), T3: (4.03 CC + 2.015 NCC), T4: (4.03 CC + 1.01 NCC), T5: (4.03 CC + 0.252 NCC), T6: (4.03 + 0.126 NCC). CC: Calcium carbonate, NCC: Nano calcium carbonate.

Table 8. Haugh unit in Bovans laying hens from 23 to 33 weeks of age fed diets containing different levels of NCC

	Age (week)			
Treatments	27	29	31	33
1	99.2	95.1	94.9	86.7
2	99.7	95.4	91.1	87.2
3	97.8	97.3	95.4	89.3
4	96.9	93.4	95.0	88.1
5	97.7	95.9	91.6	87.9
6	97.7	92.3	89.3	86.8
SEM	1.34	2.45	1.99	2.71
P-value	0.682	0.755	0.202	0.984

T1: Basal diet (BD, 8.06 CC), T2: (negative control, 6.045 CC), T3: (4.03 CC + 2.015 NCC), T4: (4.03 CC + 1.01 NCC), T5: (4.03 CC + 0.252 NCC), T6: (4.03 + 0.126 NCC). CC: Calcium carbonate, NCC: Nano calcium carbonate.

ture, etc. Cheng and Coon (1990) did not observe any significant effects of the inclusion of different amounts of calcium to the diet on the Haugh unit. However, several researchers showed that the origin of dietary calcium source (Catli et al., 2012) and the amount of calcium in the diet (Wang et al., 2014) could influence the Haugh unit.

Yolk color

Dietary treatments had a significant effect ($P<0.05$) on yolk color (Table 9). In week 27, the best (highest) yolk color was recorded for hens receiving T4 while in week 29, the lowest yolk color was recorded for the birds fed T2. In week 31, the best yolk color belonged to birds receiving T1 and finally, in week 33 the best yolk color was recorded for birds receiving T6. A number of studies have reported that increased level of calcium in laying hens' diets will lead to a decrease in yolk color (Guo and Kim, 2012; Ahmed et al., 2013). High dietary concentration of calcium would reduce feed consumption, and as a result, less oxy-carotenoid intake leads to a reduction in yolk color (Seemann, 2000). However, in contrast to our finding, Pelicia et al. (2007) and Pizzolante et al. (2009) reported, respectively that calcium source and level or limestone particle size had no significant effect on yolk color in laying hens. There was no report in the literature regarding the effect of nanoparticles on yolk color in laying hens.

Table 9. Yolk color in Bovans laying hens from 23 to 33 weeks of age fed diets containing different levels of NCC

	Age (week)			
Treatments	27	29	31	33
1	4.3^{b}	4.3^{a}	5.3^{a}	4.6^{ab}
2	4.3^{b}	3.8^{b}	4.5^{b}	4.3^{bc}
3	4.5^{b}	4.7^{a}	3.8^{b}	4.1^{cd}
4	5.1^{a}	4.4^{a}	4.1^{b}	3.8^{d}
5	4.3^{b}	4.8^{a}	4.3^{b}	4.1^{cd}
6	4.1^{b}	4.5^{a}	4.5^{b}	5.0^{a}
SEM	0.17	0.13	0.26	0.17
P-value	0.033	0.013	0.025	0.007

$^{a-d}$ In each column, means with common superscript (s), do not differ ($P>0.05$).
T1: Basal diet (BD, 8.06 CC), T2: (negative control, 6.045 CC), T3: (4.03 CC + 2.015 NCC), T4: (4.03 CC + 1.01 NCC), T5: (4.03 CC + 0.252 NCC), T6: (4.03 + 0.126 NCC).
CC: Calcium carbonate, NCC: Nano calcium carbonate.

Conclusion

It was concluded that it is possible to utilize NCC at a level of 0.252 to 2.015% instead of 4.03% CC in diets for laying hens. However, longer term studies are suggested to determine the potential of NCC, as a new calcium source, on egg production performance and egg quality in laying hens.

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نانو کربنات کلسیم: اثر بر صفات عملکردی و کیفیت تخم مرغ در مرغ های تخمگذار

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چکیده یک آزمایش ده هفته ای برای تعیین اثر سطوح مختلف نانوکربنات کلسیم بر تولیدو کیفیت تخم مرغ در مرغهای تخمگذارانجام شد. آزمایش در قالب طرح کاملا تصادفی با شش تیمار، چهار تکرار و پنج مرغ در هر تکرار انجام شد. مرغ های گروه های یک و دو به ترتیب با جیره های دارای ۸/۰۶ و ۶/۰۴۵ درصد کربنات کلسیم به عنوان شاهد مثبت و منفی تغذیه شدند. مرغ های گروه های سه، چهار، پنج و شش به ترتیب با جیره های دارای ۴/۰۳ درصد کربنات کلسیم به اضافه ۲/۰۱۵، ۱/۰۱، ۰/۲۵۲ و ۰/۱۲۶ درصد نانوکربنات کلسیم تغذیه شدند. تولید تخم مرغ در مرغ هایی که تیمار ششم را دریافت کردند در سن ۳۲ هفتگی به طور معنی داری (P<۰/۰۵) کاهش یافت. ولی تیمارهای غذایی اثری بر وزن تخم مرغ، وزن توده تخم مرغ و ضریب تبدیل خوراک نداشتند. پایین ترین شاخص شکل تخم مرغ در سن ۳۱ و ۳۳ هفتگی برای مرغ هایی ثبت شد که تیمار شش را دریافت کردند (P<۰/۰۵). مرغ هایی که تیمار شش را دریافت کردند در سن ۲۹ هفتگی نسبت به آنهایی که با دیگر جیره ها تغذیه شدند کمترین وزن مخصوص تخم مرغ را داشتند (P<۰/۰۵). کمترین وزن مخصوص تخم مرغ در ۳۳ هفتگی در مرغ های گروه سه مشاهده شد(P<۰/۰۵). تیمارهای غذایی اثر معنی داری بر واحد هاو نداشتند. اثر تیمارهای غذایی بر رنگ زرده تخم مرغ معنی دار بود و مرغ هایی که تیمار شش را دریافت کرده بودند در سن ۳۳ هفتگی کمترین رنگ زرده را داشتند نسبت به مرغ هایی که سایر تیمارها را دریافت کرده بودند (P<۰/۰۵). یافته ها نشان دادند که امکان استفاده از نانوکربنات کلسیم در سطح ۰/۲۵۲ تا ۲/۰۱۵ درصد به جای ۴/۰۳ درصد کربنات کلسیم در جیره مرغ های تخمگذار وجود دارد ولی به هر حال آزمایش های بلند مدت بیشتری پیشنهاد می شود تا پتانسیل نانوکربنات کلسیم به عنوان یک منبع جدید کلسیم برای تولید و کیفیت تخم مرغ در مرغ های تخمگذار مشخص شود.