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The Effects of Dietary Calcium Sources and Vitamin D_3 on Egg Quality and Performance in Laying Hens

Research Article

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ABSTRACT

This experiment was designed to evaluate the effects of different sources of calcium at different rates and cholecalciferol (Vitamin D_3) on the performance and egg quality of Hy-line W-36 Laying hens. In this study a total of 648 hens were managed in a completely randomized design using a factorial arrangement of 3 × 3 × 2 treatments, with three calcium sources (oyster shell, OS; ground limestone, GLI; fine limestone, FLI) and three different rates (70:30, 30:70, 50:50) and two levels of vitamin D_3 (3300 and 5000 IU/kg diet). At 33-wk of age, hens received 18 treatments (3 replicates per treatment) until 45 wk. The results showed that egg weight (g), egg production (%), egg mass (g), feed intake (g), feed conversion ratio and egg quality traits which involve egg gravity, yolk index, shell weight (g), and Haugh unit were not effected by calcium sources and different levels of vitamin D_3. The high level of vitamin D_3, resulted in an increase of eggshell thickness, ash and calcium percentage of eggshell ($P<0.05$). Moreover, replacement of 50-70% FLI or GLI with OS improved eggshell quality ($P<0.05$). There was a significant ($P<0.05$) interaction between Ca sources and vitamin D_3 level on eggshell quality. Hens fed with diets containing OS/GLI at 70:30 and vitamin D_3 at 5000 IU/kg showed the highest values for eggshell thickness, calcium and ash ($P<0.05$). These results clearly indicated that supplying calcium sources, with at least 1 / 2 OS (large particle) with or without high level of D_3, can significantly improve eggshell quality without adverse effects on the laying performance in first cycle of the laying period.

KEY WORDS calcium source, eggshell quality, laying hens, performance, vitamin D_3.

INTRODUCTION

The influence of dietary calcium sources on performance and eggshell quality has been a concern to egg producers for many years. Eggshell quality is important in poultry production because a large number of eggs with defective shells cause great economic loss to the producer (Lavelin *et al.* 2000). Farmer *et al.* (1986) reported that egg production with good shell quality depends on the Ca in the feed. Due to genetic improvement of hens, Ca requirements have changed (Bolden and Jenson, 1985). The source of Ca might affect hen productivity and egg quality. Scott *et al.* (1971) reported that shell quality was improved when part of the fine limestone (FLI) in the diet was substituted by particulate LI or oyster shell (OS). Witt *et al.* (2009) suggested that the larger particle limestone (>1.0 mm) has a beneficial effect on improvement of mechanical traits of bone in older layers.

The improved eggshell quality from feeding oyster shell may be more a factor of particle size of the Ca source, rather than the Ca source per se. Scott *et al.* (1971) speculated that the larger particles remain in the upper digestive tract (crop and gizzard) for a longer period of time than the ground Ca sources, resulting in Ca being available to the

hen for a longer period of time. Contrary to this result, Roland (1986) concluded that large particle size has no effect on shell quality when Ca levels in the diet are adequate. Recently, Lichovnikova (2007) recommended supplying two-thirds of the Ca in the diet as large particles to ensure eggshell quality in the last third of the laying period, but he fed experimental diets for 2 weeks when laying hens were 56 and 57 weeks old.

Scheideler (2004) recommended that laying hens be fed at least 25% of their calcium from a large particle calcium source. The source and particle size of Ca play an important role in maintaining eggshell quality and bone mineralization (Keshavarz *et al.* 1993; Brister *et al.* 1981). Saunders-Blades *et al.* (2009) found that the majority of ground limestone (GLI) solubilize in the first hr and remain unchanged thereafter. Some studies have reported that OS is superior to GLI for eggshell quality (Grizzle *et al.* 1992; Keshavarz and Nakajima, 1993).

It is well established that in laying hens a 1, 25-dihydroxycholecalciferol-dependent Ca-binding protein is involved in the active transport of Ca across the intestinal membrane (Wasserman and Taylor, 1968) and probably across the uterine membrane (Arlington *et al.* 1973). The active form of vitamin D_3 known as 1, 25 (OH) 2-D3 is involved in the biosynthesis of Ca-binding protein, which is involved in active transport of Ca across the intestinal wall (Hansen *et al.* 2004).

A number of studies demonstrated that the improvements in eggshell quality and bone mineralization are related to the increasing level of dietary cholecalciferol above the requirement level (Wasserman and Taylor, 1968; Cesar *et al.* 2010). Most egg producers are not aware of optimum combinations of common calcium sources or whether adding supplemental vitamin D3 (in excess of NRC recommendations) would be effective. Furthermore, the cost fluctuation of calcium sources is another important factor that is effective in selecting the kind of calcium source and combinations of thereof. The composition of different Ca sources may vary in the amount of Ca, particle size and the presence of other nutrients (phosphorous, magnesium, manganese) which can affect the utilization of Ca source by laying hens. To understand the significance of these problems, industry feed formulas should be evaluated to determine if common calcium sources or various combinations of calcium sources at different rates with supplemental vitamin D_3 have same effect on eggshell and performance at first cycle of lay. Because of this practice, the present experiments were designed to determine the effects of different sources of calcium at different rates and to investigate the effect of adding supplemental vitamin D_3 and their interactions on the performance and egg quality traits of Hy-line W-36 layer hens.

MATERIALS AND METHODS

A total of 648 Hy-line W-36 layer hens (33-wk of age) were randomly assigned into a 3 × 3 × 2 factorial arrangement of treatments: with three calcium sources (oyster shell, OS; ground limestone, GLI; fine limestone, FLI), three rates of calcium sources (70:30, 30:70 and 50:50) and two levels of vitamin D3 (3300 as control represented by CD and 5000 IU/kg diet) (Table 1).

Table 1 Different rations of calcium sources

Treatments	OS %	GLI %	FLI %	Vitamin D_3
T1	-	70	30	Control
T2	-	70	30	High
T3	70	-	30	Control
T4	70	-	30	High
T5	70	30	-	Control
T6	70	30	-	High
T7	-	30	70	Control
T8	-	30	70	High
T9	30	-	70	Control
T10	30	-	70	High
T11	30	70	-	Control
T12	30	70	-	High
T13	-	50	50	Control
T14	-	50	50	High
T15	50	-	50	Control
T16	50	-	50	High
T17	50	50	-	Control
T18	50	50	-	High

OS: oyster shell; GLI: ground limestone; FLI: fine limestone.

Hens were randomly distributed among 18 treatments with three replicates (n=36). Three hens at a time were put into one cage (36×45 cm). The hens were housed in layer cages (n=3) in one environmentally controlled room. The room temperature was set at 24 ˚C. The hens consumed corresponding experimental diets *ad libitum* and mash form. Lighting schedule followed a 16 h light and 8 h dark cycle. All animal care procedures were carried out in accordance to the Animal Care Council Guidelines of Azad University, Maragheh. The Ca content of feed and eggshell were analyzed using atomic absorption spectrometer (Varian AA240FS, Lake Forest, CA). Eighteen *iso caloric* and *iso nitrogenous* corn-soybean test diets containing 15.51% CP and 2.780 kcal ME/kg were utilized for feeding layers with the same amounts of calcium (Table 2). The experimental diets were fed for 12 weeks (33 to 45-wk of age). GLI from a mine in eastern Iran was used. This source of calcium contained 37% calcium. The particle size of the GLI was measured by a sieve separation test using 100 g of GLI and it was replicated 3 times.

Table 2 Composition of experimental diets[1] (%)

Ingredients	T 1	T 3	T 5	T 7	T 9	T 11	T 13	T 15	T 17
Corn	68.65	68.65	68.65	68.65	68.65	68.65	68.65	68.65	68.65
Soybean meal	21.23	21.23	21.23	21.23	21.23	21.23	21.23	21.23	21.23
OS[2]	0	5.74	5.74	0	2.45	2.45	0	4.095	4.095
GLI	5.74	0	2.45	2.45	0	5.74	4.095	0	4.095
FLI	2.45	2.45	0	5.74	5.74	0	4.095	4.095	0
Di calcium phosphate	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin permix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral permix[4]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Dl-Methionin	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Lysin (HCL)	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Calculated analysis[5]									
ME (kcal/kg)	2780	2780	2780	2780	2780	2780	2780	2780	2780
Crude protein (%)	15.51	15.51	15.51	15.51	15.51	15.51	15.51	15.51	15.51
Calcium[6] (%)	3.22	3.22	3.22	3.22	3.22	3.22	3.22	3.22	3.22
Available phosphorus (%)	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Methionine (%)	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44
Met+Cys (%)	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68
Lysine (%)	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
Chlorine (%)	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Sodium (%)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

[1] T2, T4, T6, T8, T10, T12, T14, T16 and T18 are T1, T3, T5, T7, T9, T11, T13, T15 and T17 supplemented with cholecalciferol at 5000 IU/kg diet, respectively.
[2] OS: oyster shell; GLI: ground limestone; FLI: fine limestone.
[3] The vitamin mixture provided the following in milligrams per kilogram of diet: vitamin A (Retinyl acetate): 8800 IU; cholecalciferol: 3,300 IU; DL-α-tocopheryl acetate: 11 IU; menadione sodium bisulfite: 2.2 mg; riboflavin: 4.4 mg; D-calcium pantothenate: 8.8 mg; nicotinic acid: 44 mg; pyridoxine hydrochloride: 2.2 mg; folic acid: 0.55 mg; D-biotin: 0.11 mg; thiamine hydrochloride: 2.5 mg; vitamin B12: 6.6 µg; choline: 220 mg; ethoxyquin: 125 mg.
[4] The mineral mixture provided the following per kilogram of diet: Mn: 60 mg; Zn: 50 mg; Fe: 30 mg; Cu: 5 mg; I: 1.06 mg and Se: 0.1 mg.
[5] Based on tables of feed composition of Hy line W-36 catalogue (2008).
[6] Different rations include: 5.74 percent from first source of calcium and 2.45 percent from second source of calcium (70:30), 4.095 percent from first source of calcium and 4.095 percent from second source of calcium (50:50) and 2.45 percent from first source of calcium and 5.74 percent from second source of calcium (30:70).

Table 3 shows the particle size separation for the GLI tested. Limestone was supplied as fine limestone in the fine diets. The FLI was supplied as powder.

Table 3 Particle size distribution of ground limestone (GLI) used in the experiment

Particle size (mm)	Amount (% by weight)
3 to 5	37.1
2 to 3	88
1 to 2	90
<1	13

The OS contained 37.5% Ca and it was supplied in particles with an expected mean particle size of 3750 µm. Mineral analysis of each Ca source was performed with samples prepared using the AOAC dry ash method 968.08 (AOAC, 1990).

Egg production (% hen-day) and egg weight (g) were recorded daily. Daily production was determined on a shelled egg weight basis. Feed intake (g/hen/day) was recorded weekly. Feed conversion ratio (FCR) was calculated as gram feed consumption per day per hen divided by gram egg mass per day per hen. Every 25 days until end of trial, 16 eggs per replicate were individually weighed and specific gravity (g/mL) was also evaluated.

During the 25 day periods, eggs from each treatment group were taken to determine eggshell quality parameters. Eggshell weight and shell thickness were determined by randomly collecting eggs from each replicate.

After breaking the eggs, the shells were washed and dried in room temperature to determine shell weight. The shell thickness was measured by a micrometer Gange (Measure, 2421/1 type) in three parts of the shell from the equator of each egg. Then, before analysis, these measurements were pooled.

The shell weight/surface area (SWUSA) was also recorded and shell ash was determined after drying at room temperature for 3 days.

Albumen quality was evaluated in Haugh units. Using egg weight (g) and albumen height (mm) data, Haugh units were determined according to the formula suggested by Standelman and Cotterill (1986):

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

Where:
HU: Haugh unit
H: albumen height (mm)
W: egg weight (g)

Data were analyzed by the General Linear models procedure of SAS Institute (SAS, 2004). The model used is as follows:

$$Y_{ijk} = \mu + D_i + L_j + P_k + Ld_{ij} + PD_{ik} + LP_{jk} + DLP_{ijk} + e_{ijk}$$

Where:
Y_{ijk}: observation,
μ: general mean,
D_i: effect of Vitamin D_3 level (i=1, 2 and 3)
L_j: effect of Ca source (j=1, 2 and 3)
P_k: effect of proportion of Ca sources (k=1, 2 and 3)
e_{ijk}: random error effect.

Arcsine transformation was used for all percentage data. Means for treatments showing significant differences in the analysis of variance were compared using Tukey Test. All statements of significance were based on the probability level of 0.05.

RESULTS AND DISCUSSION

No significant differences were observed to study different sources of calcium at different rates and vitamin D_3 levels in egg weight, egg production, egg mass, feed intake, and feed conversion ration (Table 4). These results are consistent with those previous experiments that evaluated different calcium sources (Farmer *et al.* 1986; Florescu *et al.* 1986; Ahmad and Balander, 2003). These findings agree also with other investigations (Florescu *et al.* 1986; Cheng and Coon, 1990) that did not find any difference in egg production with different calcium sources including 4.5% OS meal or limestone replacement with OS, respectively. Furthermore, our results agree with the report of Scheideler (1998), who did not find any effect on productivity when 25 or 50% FLI in the diet was substituted with either OS or GLI in Single Comb White Leghorn (SCWL) hens in their first or third production cycle. In contrast, Ahmad and Balander (2003) reported that the replacement of 50% FLI with OS significantly increased egg production in SCWL hens from 28 to 64-wks of age. However, increase in egg production observed in this trial was only 3.11 percentage units. In contrast to our results, Hamilton *et al.* (1985) found an increase in egg production with OS supplementation. In the current study, feed consumption (Table 4) was similar among Ca sources. Although energy is the largest determinant of feed intake, hens have a specific appetite for Ca and may vary feed intake to accommodate Ca needs (Mongin and Sauveur, 1974; Sauveur and Mongin, 1974). Hens on low Ca diets (1%) have been shown to consume more feed than hens on adequate Ca diets (3.15%) (Mongin and Sauveur, 1974). Results of the current study indicated that all Ca sources used in this study were able to supply the hens with sufficient Ca, so as to not to have to alter feed consumption to compensate for low Ca bioavailability. Egg production was not different among Ca sources, indicating that satisfactory amounts of Ca and other ingredients were available to support and sustain similar egg production. These results support previous research that also found egg production not to differ among hens fed limestone or OS (Guinotte and Nys, 1991; Grizzle *et al.* 1992). Miller and Sunde (1975) found egg weight not to be affected by Ca source when fed to laying hens over production periods ranging from 20 to 60 wk. In this study, neither Ca sources not adding Vitamin D affected body weight during 12-weeks of experiment which is consistent with results reported by Guinotte and Nys (1991). Abdulrahim *et al.* (1979) found no effect on eggshell weight and egg production in young laying hens fed diets with 3 or 9 µg of l, 25-$(OH)_2D_3$/kg; at the higher concentration there was a significant decrease in food consumption and a slight decrease in egg production.

Similarly, egg specific gravity (SG), yolk index, eggshell weight, and Haugh unit values were not influenced by experimental diets or the interactions between main effects (Table 5) ($P>0.05$). Lack of difference in egg weights by different calcium sources is in agreement with Cheng and Coon (1990), who concluded, through a series of experiments, that switching from limestone to OS, in short-term laying trails, caused no significant differences in eggshell quality or layer performance including egg weight. Improving eggshell quality causes a decrease in the high gas interchanges through eggshell pores that finally improve albumin quality and the Haugh unit, which is high numerically in diets containing OS ($P>0.05$) (Table 5).

These results are also in agreement with those of Oluyemi *et al.* (1979) and Richter *et al.* (1999), who found no significant difference in eggshell weight and Haugh unit when hens were fed with 70 percent OS. Some other researchers have contradictory results (Guinotte and Nys, 1990). Based on these results, using OS as a calcium source or as the part of calcium carbonate with OS in layers diet significantly improved the eggshell weight ($P<0.05$) (Wasserman and Taylor, 1968).

Generally, the higher specific gravity value is related to thicker eggshell and shell weight per unit of surface area (Hamilton, 1982), which is a desirable characteristic in the egg industry (Keshavarz and Quimby, 2002). In our trial, SG did not differ among Ca sources (Table 5). Previous research have reported conflicting results regarding the effect of different Ca sources on eggshell quality when comparing similar dietary levels of Ca within each study, although ranging from around 2 to 5.5% among the different studies.

Table 4 The interaction and main effects of different calcium sources, various rations and D_3 on performance[1]

Parameters treatments[1]	Egg weight (g)	Egg production (%)	Egg mass (g)	Feed intake (g)	Feed conversion ratio (g/g)
Different proportion × Calcium sources × D3					
70:30 GLI/FLI + HD	60.6	85.1	55.2	103.2	1.85
70:30 GLI/FLI + CD	61.9	85.1	55.1	103.2	1.84
70:30 OS/FLI + HD	61.4	86.3	56.3	104.2	1.90
70:30 OS/FLI + CD	60.9	86.3	55.9	104.2	1.89
70:30 OS/GLI + HD	61.7	88.2	57.2	105.7	2.11
70:30 OS/GLI + CD	61.9	87.9	56.6	105.3	2.08
70:30 FLI/GLI + HD	61.1	84.3	54.2	102.4	1.82
70:30 FLI/GLI + CD	61.4	83.9	54.0	102.3	1.81
70:30 FLI/OS + HD	61.5	85.5	55.6	103.0	1.88
70:30 FLI/OS + CD	61.1	85.4	55.3	103.4	1.88
70:30 GLI/OS + HD	60.8	87.2	56.5	104.5	1.93
70:30 GLI/OS + CD	61.6	86.9	56.5	104.4	1.91
50:50 FLI/ GLI + HD	61.2	85.0	55.0	102.9	1.84
50:50 FLI/GLI + CD	61.8	84.7	54.9	102.8	1.84
50:50 FLI /OS + HD	61.3	85.9	55.9	104.0	1.89
50:50 FLI /OS + CD	62.4	85.6	55.7	103.9	1.88
50:50 GLI/OS + HD	61.8	87.7	56.6	104.6	1.98
50:50 GLI/OS + CD	61.6	87.2	56.6	104.5	1.97
SEM[2]	0.536	1.532	1.079	0.649	0.09
P-value interaction	NS	NS	NS	NS	NS
Different proportion × Calcium sources					
P-value	NS	NS	NS	NS	NS
Different proportion × D_3					
P-value	NS	NS	NS	NS	NS
Calcium sources × D_3					
P-value	NS	NS	NS	NS	NS
The effect of D_3					
P-value	NS	NS	NS	NS	NS
Different calcium sources					
P-value	NS	NS	NS	NS	NS
Different proportion					
P-value	NS	NS	NS	NS	NS

[1] OS: oyster shell; GLI: ground limestone; FLI: fine limestone; HD: high level of vitamin D_3 (5000 IU/kg); CD: Control level of D_3 (3300 IU/kg).
[2] SEM: standard error of the mean.
NS: non significant (P>0.05).

Other studies have reported eggshell quality to be similar between hens fed OS or those fed large particle limestone (Miller and Sunde, 1975; Muir *et al.* 1976). The differing results can be attributed to the different Ca sources being used within the different studies as well as the form (large particle *vs.* finely ground), age of hens and level in which they were administered. Eggshell thickness, was significantly (P<0.05) affected by calcium source and D_3 (Table 6).

The highest eggshell thickness was obtained from eggs of hens fed the diet containing 70:30 OS/GLI with high level of D_3. However, the lower shell thickness was observed for birds fed diet containing 70:30 GLI/FLI (P<0.05). Also, there was an important negative relationship between eggshell thickness and broken eggs (Table 6).

As the eggshell thickness increased, the percentage of broken eggs decreased and vice versa. The increase in eggshell thickness may be because of the presence of the coarse particles of OS and high level of D_3 (5000 IU/kg). It is well established that in laying hens a vitamin D_3-dependent Ca-binding protein is involved in the active transport of Ca across the intestinal membrane (Lichovnikova, 2007) and probably across the uterine membrane (Wasserman and Taylor, 1968). Vitamin D_3 stimulates the synthesis of intestinal calcium binding protein, thereby increasing calcium absorption and increasing eggshell thickness. It appears that the addition of D_3 may have caused increased utilization of Ca.

Increasing levels of cholecalciferol have been shown to increase intestinal phytase and alkaline phosphatase activity

Table 5 The interaction and main effects of different calcium sources[1], various rations and D_3 on egg quality

Parameters treatments[1]	Specific gravity (g/cm^3)	Yolk index	Eggshell weight (g)	Haugh unit
Different proportion × Calcium sources × D_3:				
70:30 GLI/FLI + HD	1.081	40.0	5.69	100.8
70:30 GLI/FLI + CD	1.080	39.3	5.68	99.7
70:30 OS/FLI + HD	1.082	40.9	5.97	102.7
70:30 OS/FLI + CD	1.082	40.6	5.96	102.7
70:30 OS/GLI + HD	1.084	42.5	6.23	105.0
70:30 OS/GLI + CD	1.083	41.5	6.21	105.0
70:30 FLI/GLI + HD	1.077	38.6	5.55	98.0
70:30 FLI/GLI + CD	1.077	38.3	5.49	96.6
70:30 FLI/OS + HD	1.081	40.3	5.82	101.0
70:30 FLI/OS + CD	1.081	40.2	5.79	100.6
70:30 GLI/OS + HD	1.082	41.0	6.06	103.2
70:30 GLI/OS + CD	1.082	40.9	6.01	102.8
50:50 FLI/ GLI + HD	1.079	39.3	5.65	99.6
50:50 FLI/GLI + CD	1.078	38.7	5.58	99.6
50:50 FLI /OS + HD	1.081	40.6	5.85	102.4
50:50 FLI /OS + CD	1.081	40.5	5.82	102.0
50:50 GLI/OS + HD	1.082	41.2	6.10	104.2
50:50 GLI/OS + CD	1.082	41.1	6.09	103.9
SEM[2]	0.0017	1.274	0.248	2.340
P-value	NS	NS	NS	NS
Different proportion × Calcium sources				
P-value	NS	NS	NS	NS
Different proportion × D_3				
P-value	NS	NS	NS	NS
Calcium sources × D_3				
P-value	NS	NS	NS	NS
The effect of D_3				
P-value	NS	NS	NS	NS
Different calcium sources				
P-value	NS	NS	NS	NS
Different proportion				
P-value	NS	NS	NS	NS

[1] OS: oyster shell; GLI: ground limestone; FLI: fine limestone; HD: high level of vitamin D_3 (5000 IU/kg); CD: Control level of D_3 (3300 IU/kg).
[2] SEM: standard error of the mean.
NS: non significant (P>0.05).

in chicks (Davies *et al.* 1970), and as a resultant in utilization of calcium. It has also been shown that elevated 1, 25-$(OH)_2$ D_3 concentrations in the blood induces increased absorption and retention of Ca and raises serum Ca (Norman, 1987; Soares *et al.* 1988). These results were consistent with reports from Florescu *et al.* (1986) and Scott *et al.* (1971).

This experiment showed that the highest eggshell thickness, calcium, ash and SWUSA were obtained from diets which contain OS as a part of calcium source compared to different combination of GLI/FLI (P<0.05), which was not significant in diets containing 3300 IU/kg D_3. This was in contrast with the reports of Cheng *et al.* (1990) that using OS had no effect on eggshell thickness. When compared with other groups, egg-shell calcium increased in 70% OS and 30% GLI with high D_3 or control D_3 that is in concurrent with the effect of interaction between calcium source and vitamin D_3 and the main effects of calcium sources and vitamin D_3 (P<0.05).

These improvements suggest that the coarse particles of OS affected the deposition of calcium carbonate in eggshell, which is the same form of calcium carbonate existing in diet and eggshell structure, and this was also related to the existence of high vitamin D_3 in diets as well. Due to longer period digestion in gastrointestinal tract of OS, because of its particle size, can supply high amount of body's requirements for calcium. Vitamin D_3, by biosynthesis of Ca-binding protein, can also increase the active transport of calcium across the intestinal wall and then it could be assumed that it could promote absorption of calcium for shell formation.

The results of the present experiment agree with the findings of Solomon *et al.* (1991) who studied the effects of different sources of calcium on eggshell quality and they observed significantly differences in calcium percentage of eggshells.

They reported that using OS in a diet can significant increase calcium percent of eggshell (P<0.05).

Table 6 The interaction and main effects of different calcium sources, various rations and D_3 on egg quality

Parameters treatments[1]	Eggshell thickness (mm)	Broken eggs (%)	Eggshell calcium (%)	Eggshell ash (%)	SWUSA[2] (mg)
Different proportion × Calcium sources × D_3:					
70:30 GLI/FLI + HD	0.445^{ab}	1.93^{a}	70.67^{dexho}	74.11^{be}	77.16^{ab}
70:30 GLI/FLI + CD	0.440^{ab}	2.00^{a}	70.33^{dxgho}	73.23^{be}	76.94^{ab}
70:30 OS/FLI + HD	0.470^{ab}	1.48^{ab}	72.67^{bcx}	76.02^{bef}	79.06^{ab}
70:30 OS/FLI + CD	0.469^{ab}	1.53^{ab}	72.33^{bcx}	75.29^{bef}	79.02^{ab}
70:30 OS/GLI + HD	0.572^{a}	1.00^{b}	82.67^{a}	85.88^{ac}	82.25^{a}
70:30 OS/GLI + CD	0.496^{ab}	1.14^{b}	80.00^{ab}	82.17^{cf}	80.94^{ab}
70:30 FLI/GLI + HD	0.420^{b}	2.25^{a}	68.67^{xh}	72.21^{bf}	73.49^{ab}
70:30 FLI/GLI + CD	0.405^{b}	4.06^{a}	68.33^{xh}	70.87^{be}	72.79^{b}
70:30 FLI/OS + HD	0.463^{ab}	1.85^{ab}	70.67^{dexho}	74.32^{b}	78.74^{ab}
70:30 FLI/OS + CD	0.461^{ab}	1.91^{ab}	70.67^{dexho}	74.18^{be}	78.49^{ab}
70:30 GLI/OS + HD	0.471^{ab}	1.39^{b}	75.00^{abcx}	77.32^{bef}	79.90^{ab}
70:30 GLI/OS + CD	0.470^{ab}	1.46^{b}	74.33^{bch}	76.46^{bef}	79.70^{ab}
50:50 FLI/GLI + HD	0.432^{ab}	2.03^{a}	69.33^{fxh}	72.78^{be}	74.65^{ab}
50:50 FLI/ GLI + CD	0.428^{ab}	2.04^{a}	68.67^{xh}	72.55^{b}	73.69^{ab}
50:50 FLI /OS + HD	0.464^{ab}	1.68^{ab}	72.00^{dexh}	75.10^{bef}	78.85^{ab}
50:50 FLI /OS + CD	0.464^{ab}	1.78^{ab}	71.67^{dexh}	74.79^{bd}	78.75^{ab}
50:50 GLI/OS + HD	0.490^{ab}	1.30^{b}	77.67^{acdeo}	80.18^{cd}	80.94^{ab}
50:50 GLI/OS + CD	0.486^{ab}	1.35^{b}	77.00^{bcefg}	79.24^{cde}	80.83^{ab}
SEM[3]	0.018	0.496	1.507	1.369	2.848
P-value[4] interaction	*	*	*	*	*
Different proportion × Calcium sources:					
70:30 OS/GLI	0.517^{a}	1.24^{b}	78.83^{a}	81.60^{a}	80.42^{a}
70:30 GLI/OS	0.471^{a}	1.60^{ab}	73.50^{b}	78.17^{a}	79.71^{ab}
70:30 FLI/GLI	0.417^{b}	3.15^{a}	70.50^{b}	72.05^{bc}	73.59^{b}
70:30 GLI/FLI	0.457^{ab}	1.77^{ab}	71.00^{b}	73.78^{bc}	76.75 ^{ab}
70:30 FLI/OS	0.462^{ab}	1.72^{ab}	71.33^{b}	73.82^{b}	78.75^{ab}
70:30 OS/FLI	0.468^{ab}	1.69^{ab}	72.67^{b}	76.68^{b}	79.66^{ab}
50:50 OS/GLI	0.488^{a}	1.41^{b}	75.33^{b}	78.32^{a}	80.32^{ab}
50:50 GLI/FLI	0.426^{b}	1.82^{ab}	70.67^{b}	73.26^{c}	74.86^{ab}
50:50 FLI/OS	0.467^{ab}	1.72^{ab}	72.50^{b}	75.66^{bc}	79.04^{ab}
SEM	0.013	0.350	1.065	0.968	2.014
P-value	*	*	*	*	*
Different proportion × D_3					
P-value	NS	NS	NS	NS	NS
Calcium sources × D_3:					
GLI/OS + HD	0.518^{a}	1.42^{b}	77.55^{a}	79.45^{a}	81.34^{a}
GLI/OS + CD	0.468^{b}	1.56^{ab}	77.11^{b}	78.89^{a}	78.85^{ab}
GLI/FLI + HD	0.437^{b}	2.05^{ab}	69.89^{b}	74.21^{b}	77.08^{ab}
GLI/FLI + CD	0.432^{b}	2.41^{a}	69.89^{b}	74.10^{b}	75.73^{b}
OS/FLI + HD	0.464^{b}	1.60^{b}	72.11^{b}	74.63^{b}	78.52^{ab}
OS/FLI + CD	0.462^{b}	1.71^{ab}	71.00^{b}	74.28^{b}	77.19^{ab}
SEM	0.010	0.286	0.870	0.790	1.644
P-value	*	*	*	*	*
The effect of D_3:					
5000 IU/kg (HD)	0.473^{a}	1.74	75.59^{a}	77.52^{a}	78.19
3300 IU/kg (CD)	0.454^{b}	1.85	70.26^{b}	74.34^{b}	78.05
SEM	0.006	0.165	0.502	0.456	0.949
P-value	*	NS	*	*	NS
Different calcium sources:					
OS/GLI	0.491^{a}	1.51^{b}	74.28^{a}	76.77^{a}	79.93^{a}
FLI/GLI	0.435^{c}	2.23^{a}	71.00^{b}	74.42^{b}	76.46^{b}
OS/FLI	0.465^{b}	1.63^{ab}	73.50^{a}	76.59^{a}	77.98^{ab}
SEM	0.007	0.202	0.615	0.559	1.163
P-value	*	*	*	*	*
Different proportion					
P-value	NS	NS	NS	NS	NS

[1] OS: oyster shell; GLI: ground limestone; FLI: fine limestone; HD: high level of vitamin D_3 (5000 IU/kg); CD: Control level of D_3 (3300 IU/kg).
[2] Shell weight/Surface area.
[a-x] Within mean effects or interaction, means with no common superscript differ significantly (P<0.05).
[3] SEM: standard error of the mean.
NS: non significant (P>0.05); *: significant (P<0.05).

Due to positive liner relationship between the eggshell minerals (especially calcium) and ash percentage of eggshell, all factors which increase calcium percent can also increase ash percentage of eggshell and, subsequently, shell weight per unit surface area (SWUSA). Hence, the highest calcium percent of eggshell (82.66%), ash percent of egg-

shell (85.87%) and SWUSA (82.25 mg) belong to a diet which contains 70:30 of OS/FLI with high level of vitamin D3. The results of this experiment were not consistent with previous report that no beneficial effect on laying hens performance can be obtained by increasing level of vitamin D3 above the level that normally is used commercial practice (Keshavarz, 2000).

Similarly, increase in eggshell strength was reported by Richter *et al.* (1999) when the hens were fed limestone with a particle size of 0.5 to 2 mm or a mixture of one-third finely GLI and two-thirdsOS. The results of this experiment agree with the findings of Brister *et al.* (1981); Scott *et al.* (1971) who studied the effects of particle size and origin of calcium sources on eggshell quality in laying hens.

These investigations reported that using OS in the diet can significantly ($P<0.05$) improved eggshell quality. Generally in our current study, the use of a vitamin D_3 at 33-45 wks of age was adequate to support all of the production traits, in spite of positive effect of excess vitamin D_3 (5000 IU/kg) on eggshell thickness (mm), eggshell calcium (%) and eggshell ash (%). The results of this experiment were consistent with previous report that no beneficial effect on laying hens performance can be obtained by increasing the level of vitamin D_3 above the level that normally is used commercial practice (Brister *et al.* 1981).

There were no significant differences between OS/FLI and OS/GLI in eggshell quality, except that the eggshell thickness was higher in groups containing OS/GLI in comparison to FLI/GLI ($P<0.05$), showing that combination of OS/FLI or OS/GLI has a positive effect on eggshell quality rather than GLI/FLI. Cheng and Coon (1990) reported that the eggshell quality (and bone status) was more closely related with limestone *in vitro* solubility than particle size. Furthermore, current study showed considerable improvement in eggshell quality traits in diets containing 50-70% OS with GLI or FLI, when compared to FLI/GLI. Several previous studies have also found *in vitro* solubility rates of large particle sizes to be lower than those of smaller particles and ground forms of the same Ca source (Rao and Roland, 1989; Guinotte and Nys, 1991; Zhang and Coon, 1997).

The slower the *in vitro* solubility of Ca source, the longer it will remain within the gizzard of the hen, thereby increasing the retention of these slower dissolving Ca particles (Zhang and Coon, 1997).

Therefore, the results of the current study would indicate that the larger particles (OS) should remain in the gizzard longer than GLI, thereby having a greater Ca retention than the small particle.

There are conflicting results in other literatures on the beneficial effects of large-particle calcium on eggshell status. Reviews (Scott *et al.* 1971; NRC, 1994) have shown a positive effect of calcium with a coarse particle size on eggshell quality. The large scope of calcium sources and size make the comparison of these results difficult.

CONCLUSION

The present study suggests that supplying calcium sources in laying diets with 50-70% oyster shell along with Fine Limestone or Ground Limestone provide a sufficient particle size for maintaining eggshell quality and resulting in decreasing economic losses via decreasing broken eggs. Although different particle size treatments yielded similar egg production, the hens in the mixed particle size treatment groups were apparently able to do this without excess Vitamin D_3. It is also indicated that laying hens can tolerate relatively high dietary levels of vitamin D_3 without adverse effect on their performance, likewise improved some eggshell traits.

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