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Effects of Using Ground Eggshells as a Dietary Calcium Source on Egg Production Traits, Hatching Performance and Eggshell Ultrastructure in Laying Hens

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ABSTRACT

A 10 wk trial was conducted to investigate the effect of using ground, sterilized eggshell as a calcium source in the diet of laying hens. Egg production, egg and eggshell quality, hatching characteristics and eggshell ultrastructure were evaluated. A total of 144 hens aged 25 wk were housed in floor pens, located in a conventional open-sided layer shed. The birds were randomly divided into three groups, each group represented by four replicates, consisting of twelve birds each. Group 1 (Control) was provided with a layer ration that contained all the calcium carbonate as fine limestone, whereas Groups 2 and 3 were placed on a layer diet with 50 and 100%, respectively, of the limestone substituted with ground eggshells. The birds were given free access to food and water, and exposed to a 16L:8D photoperiod daily. Throughout the entire 10 wk test period, body weight, egg production, egg weight and egg quality of the three bird groups were not significantly ($P > 0.05$) different. Eggshell weight, eggshell thickness and egg specific gravity were not affected by the treatment diets. Hatching performance was determined at 4 and 8 wk after feeding the experimental diet. There were no significant differences in average fertility, hatchability, egg weight loss, chick yield and hatchling weight among the bird groups. In addition, there were no significant differences between treatments in growth and livability of chicks aged 1 wk. Scanning electron microscopy was used to study the morphology of the eggshell. The photographs illustrated a little variation in the formation of the mammillary layer of the shell produced by hens fed diets containing eggshell calcium. Neither density nor diameter of the mammillary knobs were affected by the treatments. The results indicated that eggshells can be fully utilized as a calcium source in layer diets without detrimental effects on productive performance, fertility, hatchability, chick quality and the fine structure of the eggshell.

Keywords: eggshell, calcium, laying hen, hatchability, eggshell ultrastructure

INTRODUCTION

Chicken eggshell is a waste material from domestic sources such as hatcheries, poultry farms, egg product factories, homes and restaurants. Many researchers have been looking for ways to utilize the eggshell waste, for example, by using eggshell powder as a stabilizing material for improving soil properties (Amu *et al.*, 2005), as a source of calcium (Ca) for piglets (Schaafsma and Beelen, 1999) and as a source of Ca in human nutrition (Schaafsma and Pakan, 1999; Schaafsma

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et al., 2000). Vandepopuliere *et al.* (1975) reported that the nutritive value of eggshells had a calcium level comparable to that of limestone, with the benefit of a small amount of protein. Froning and Bergquist (1990) reported that extruded eggshell including membranes could be used as a calcium source in layer diets without adverse effects on egg production. Tadtiyanant *et al.* (1993) demonstrated that there were no detrimental effects in egg weight, egg production, feed conversion and egg specific gravity in laying hens fed diets containing extruded eggshell meal as a source of Ca. This study pointed out that high temperature-short time extrusion was an alternative method for converting poultry industry residues into high quality poultry feedstuffs, as drying the eggshell product at a high temperature is critical to decrease the potential for contamination by pathogens. Scheideler (1998) worked with first-cycle laying hens by feeding the birds with diets containing either fine limestone or ground eggshell as a calcium source and did not find any significant effects on body weight, feed intake, egg production, egg weight or egg specific gravity between the different sources of calcium. The source and particle size of Ca play an important role in maintaining eggshell quality and bone mineralization (Blister *et al.*, 1981; Guinotte and Nys, 1991; Keshavarz *et al.*, 1993). Recently, Lichovnikova (2007) recommended that a diet mixture containing two-thirds large-sized particles of Ca should be used to improve eggshell quality, especially in aged hens.

Studies on the relationship between the microstructure of the eggshell and hatchability in domestic fowls have been carried out by many investigators. Bone *et al.* (1988) reported that the mammillary core was the major site of erosion during incubation, which would be a calcium source for development of the embryos. Lunam and Ruiz (2000) did not find any correlation between the thickness of the mammillary layer and hatchability of chicken egg shells. Mroz *et al.* (2007) found a relationship between the mammillae size and hatchability of turkey eggs. They reported that the mammillae size of good shell quality eggs that had higher hatchability was smaller than that of poor shell quality eggs. The consensus is that eggshell defects inhibit embryo growth, disturb the water loss mechanism and adversely affect the hatching process. Fathi *et al.* (2007) examined ultrastructural variations of the eggshells produced from different breeds of Egyptian local hens using scanning electron microscopy and observed mammillary bodies type B in poor shell quality eggs. Recently, Rayan *et al.* (2010) reported that percentages of types A and B mammillary bodies, including mammillary alignment in the eggshells produced by aged hens, were significantly higher than those of eggshells produced by younger birds. The aim of the present study was to determine the effect of using ground, sterilized chicken eggshells as a calcium source in layer diets on the productive performance, egg and eggshell quality, hatching characteristics and the fine structure of the eggshell in laying hens.

MATERIALS AND METHODS

Stocks and housing

A total of 144 Rhode Island Red hens, aged 25 wk, were housed in a conventional open-sided shed containing 12 individual floor pens, with hens randomly assigned to three treatments. Each treatment consisted of four replications (12 birds per replication). The hens in each replication were naturally mated with one Rhode Island Red male, aged 38 wk. Each flock was separately housed at a stocking density of 1.44m^2/bird in each individual pen. Spare males were maintained to replace dead and culled birds. Treatment 1 (Control) was provided with a standard layer diet used ground fine limestone as the whole calcium carbonate in the diet. Treatment 2 (50%ES) was placed on the layer diet that contained a combination of 50% limestone and 50% ground eggshell as the calcium source, whereas Treatment 3 (100%ES) received the layer diet containing 100% ground eggshell as the calcium source in the diet. All diets were isocaloric and isonitrogenous. The ingredients and calculated chemical analyses of the feeds are shown in Table 1.

After 3 wk for acclimatization, the birds were fed the experimental diets for 10 wk. Throughout the experimental period, the birds were provided with feed and water at all times and exposed to a 16 h photoperiod (16L:8D) daily. Fluorescent light was provided for 4 h/d, between 6:00 p.m. and 10:00 p.m. The lights were turned off by an automatic timer.

Experimental diets

Eggshells were collected from food vendors on the Kasetsart University campus and washed with tap water without removing any shell membranes. The eggshells were sterilized in boiling water for 2 h and then dried in an oven at

Table 1 Ingredient composition and calculated nutrient analysis of the experimental diets.

Item	Ration 1[A]	Ration 2[B]	Ration 3[C]
		(%)	
Corn	47.05	46.93	47.11
Rice bran	10.00	10.00	9.69
Extruded soybean	4.00	4.00	4.00
Soybean meal	25.53	25.04	24.56
Vegetable oil	2.63	2.82	3.00
Vitamin mineral premix[1]	0.50	0.50	0.50
DL-Methionine 99%	0.11	0.11	0.12
Salt	0.38	0.39	0.39
Fine limestone	8.22	4.31	-
Ground eggshell	-	4.31	9.06
Monocalcium phosphate (Biofoss P 21%)	1.68	1.68	1.69
Total	100.10	100.09	100.12
Calculated analysis			
ME (kcal/kg)	2,800.31	2,800.00	2,799.76
Crude protein (%)	18.00	18.00	18.00
Crude fiber (%)	2.62	2.60	2.57
Moisture (%)	10.03	9.99	9.97
Fat (%)	7.16	7.34	7.46
Calcium (%)	3.50	3.50	3.50
Total phosphorus (%)	0.82	0.82	0.81
Available phosphorus (%)	0.45	0.45	0.45

Total diet percentages do not equal 100% due to rounding errors in reporting constituent items to 2 decimal places.

[A] Ration 1. Commercial ration with limestone as the calcium source; served as the control.

[B] Ration 2. Commercial ration (control) with limestone and eggshell (50:50) as the calcium source.

[C] Ration 3. Commercial ration (control) with eggshell as the calcium source.

[1] Provided the following (per kg of diet): vitamin A, 7,500 IU; vitamin D_3, 1,500 IU; vitamin E, 6 IU; vitamin K_3, 1mg; vitamin B_1, 0.75 mg; vitamin B_2, 3 mg; vitamin B_6, 1.25 mg; vitamin B_{12}, 0.75 mg; pantothenic acid, 6.9 mg; nicotinic acid, 12.5 mg; folic acid, 0.25 mg; biotin, 0.013 mg; choline, 195.3 mg; copper 14 mg; manganese, 45 mg; iron, 11 mg; zinc, 37.5 mg; cobalt, 0.15 mg; and iodine, 1 mg.

95 °C for 24 h. The dried eggshells were pulverized by a hammermill to create ground eggshell. The particle size of the eggshell was measured by a sieve separation test, using 100 g of eggshell, replicated three times. Table 2 shows the particle size separation for the eggshell tested. The chemical composition of the ground eggshell was analyzed by proximate analysis (Table 3).

Productive traits and egg quality measurements

Hens were weighed at the beginning of the study and reweighed at the end of the study. Eggs were collected twice daily at 9:30 a.m. and 3:30 p.m. The number of eggs laid by birds in each replication was recorded daily and expressed as a percentage of hen-day egg production. Eggs from each replication laid on three consecutive days in each week were weighed and the average was calculated for each replication. All eggs from each replication laid on the last day of the week were collected in each 2 wk period for egg and eggshell quality measurement. The eggs were broken at the equatorial region and the interior contents were allowed to drain out. The internal quality of eggs was assessed according to albumen height, Haugh unit and yolk color using specialized equipment (Technical Services and Supplies (TSS), York, UK.). The yolk weight was determined after it was separated from the adhering albumen and then weighed on an electric balance (Model PB 1501 Mettler, Toledo). The eggshell along with membranes was washed with tap water and dried at room temperature (around 28 °C) for 1 wk. After drying, the eggshell was weighed and the shell thickness was measured using a digimatic micrometer (Mitutoyo Corporation, Kanagawa, Japan) in millimeters. Three measurements were taken on the equatorial region for each egg shell, and the mean of three measurements was calculated. The albumen weight was determined by subtracting the yolk plus shell weight from the total egg weight. The specific gravity of eggs was determined using sodium chloride solutions ranging in specific gravity from 1.064 to 1.100 in increments of 0.004 units as described by Keshavarz and Nakajima (1993).

Hatching eggs and incubation

Hatching performance was determined after feeding the experimental diet for 4 wk and repeated at 8 wk of the feeding period; the average of the two batches was calculated for each replication. In each batch, eggs laid between 10:00 a.m. and 1:00 p.m. and between 1:00 p.m. and 4:00 p.m. were picked up from the nests, placed in incubator trays and received at the hatchery at 1:30 p.m. and 4:30 p.m., respectively. Then, eggs that were very dirty, cracked, or misshapen were

Table 2 Particle size distribution of ground eggshell used in the experiment.

Particle size (mm)	Amount (% by weight)
6˘10	0.54
2˘5	40.46
<2	59.00

Table 3 Chemical composition of ground eggshell analyzed by proximate analysis.

Chemical composition	Amount (%)
Crude protein	5.35
Calcium	34.89
Phosphorus	0.001
Dry matter	99.20

removed. All eggs were fumigated with formaldehyde gas for 30 min and stored in the holding room for 5 d at 18.0 ± 2.0 °C with 75.0 ± 5.0% relative humidity. On the 5th day after collection, the eggs were pre-warmed for 6 h at room temperature and 65% relative humidity, allowed to sweat and dry, and weighed to 0.01 g accuracy just before the incubation period. All eggs were fumigated again in the incubator on the day of setting. Incubating trays representing each treatment were distributed throughout all positions in the setter (Model Multiplo electric incubator, The Multiplo Incubator & Brooder PTY. LTD.) to minimize any possible machine position effects. The eggs were turned hourly through 90 ° and incubated at 37.4 ± 0.2 °C dry bulb temperature and 28.9 ± 0.2 °C wet bulb temperature. The hatcher (Model Multiplo) was operated at 37.2 ± 0.2 °C dry bulb temperature and 30.0 ± 0.2 °C wet bulb temperature.

Hatching parameters and hatchling measurements

On the 8th day of incubation, the eggs were candled in the candling room (around 28.5 °C and 80% relative humidity). Clear*f* eggs were removed and broken in order to identify early dead embryos from infertile eggs as described by Lourens *et al.* (2006). The eggs were candled repeatedly on the 19th day of incubation to remove middle embryonic mortalities and weighed in order to determine egg weight loss at the transferring time. About 2 h after removal from the setter, eggs with apparently living embryos were transferred to hatching baskets and randomly distributed in the hatcher to reduce any possible machine position effects. All chicks were taken off at 21 d and 12 h postincubation. Hatched chicks were weighed on a treatment basis to 0.01 g accuracy. Chick weight was then expressed as the percentage of egg weight for each treatment (percentage of chick yield). The fertility (number of fertile eggs per all eggs set × 100) and hatchability (number of chicks hatched per all fertile eggs × 100), and early, middle and late embryonic mortalities were calculated.

For determination of chick quality, the hatched chicks were placed by treatment in 12 identical brooding rooms (1.95 × 1.5 m), under the same environmental, managerial, and hygienic conditions. Water and a standard corn-soybean meal starter diet (2,900 kcal of ME/kg, 22% CP) were supplied *ad libitum* under continuous lighting. At age 1 wk, the chicks were weighed; the weight changes were calculated and expressed as the percentage of relative growth (body weight gain per hatched chick weight × 100). The mortality rate was recorded daily.

Preparation of samples for eggshell ultrastructural examination

At the termination of the trial, at age 38 wk, two unincubated but fertile eggs of each replication laid on the same day were randomly collected to examine the structure of the eggshell. The selected eggs were broken, the interior contents were removed and the shells were cleaned with tap water. The specimens were prepared by cutting a piece (1 cm^2) of shell from the equatorial region of each egg. The shell membranes were carefully removed by soaking in tap water. The loosely adhering membranes were peeled from the edge of the sample inwards. The samples were then soaked in 1.0N NaOH for 72 h prior to gold coating and study according to the method of Kaplan and Siegesmund (1973). The purpose of this treatment with a base was to eliminate any proteinous materials incorporated in the shell. Thereafter, the samples were rinsed in distilled water and left to dry at room temperature. All samples were mounted inner side uppermost on aluminum stubs, and coated with gold using an ion coater (Engineering IB-2) for direct observation by scanning electron microscope (SEM). These specimens were examined by a JEOL JSM-5600LV SEM (JEOL Ltd., Oxford, England) operated at 10 kV, at magnifications of ×100 and ×500. The incidence of ultrastructural variants at the level of the mammillary layer was assessed according to the method and terminology developed by the Poultry Research Unit, University of Glasgow (Solomon, 1991). Photographs of replica surfaces were made to facilitate counting the number of mammillary knobs per unit eggshell interior surface area. The density of mammillae of each shell was expressed as the number of knobs per unit. The average diameter of the mammillary knobs was estimated from the measured mammillary knob density, assuming regular circular geometry according to the method of Van Toledo *et al*. (1982).

Statistical analysis

The experiment was conducted as a completely randomized design with three treatments. Data were analyzed using the statistical software package SAS, version 9.0 (SAS Institute, 2002). The GLM procedure was used to analyze the effect of the experimental diet on productive performance, egg and eggshell quality, hatching parameters and the fine structure of the eggshell. The arcsine transformation was used for all percentage data. When the means of the GLM procedure were statistically different, these means were further compared between the control and the experimental groups using Duncan s multiple range test. Significance was based on $P < 0.05$. The experimental unit was a group of 12 hens for all traits studied. For the determination of morphology of the eggshell, only two samples of shell per replicate were used. Data were presented as means and the pooled SEM.

RESULTS AND DISCUSSION

Egg production traits

Table 4 shows the effects of the experimental diets on body weight, productive performance and egg quality. No effect of dietary treatment was found for body weight, egg production, egg weight or egg and eggshell quality among the bird groups. The results supported the reports of other researchers (Froning and Bergquist, 1990; Scheideler, 1998), in which

Table 4 Effects of dietary treatment on body weight and egg production traits during the experimental period (age 28 to 38 wk).

Parameters	Treatment[1]			SEM[2]	*P-value*
	Control	50%ES	100%ES		
Initial body weight (kg)	1.69	1.69	1.70	0.03	0.7347
Final body weight (kg)	1.78	1.83	1.89	0.06	0.1121
Egg production (%)	61.18	69.24	68.28	7.87	0.3320
Egg weight (g)	47.47	47.56	47.81	1.13	0.9098
Yolk weight (%)	25.17	25.23	25.46	0.54	0.7293
Yolk color (Roche scores)	5.84	5.84	5.94	0.29	0.8570
Albumen weight (%)	65.98	65.79	65.65	0.61	0.7571
Albumen height (mm)	6.22	6.36	6.62	0.32	0.2543
Haugh units	81.69	82.79	84.39	2.33	0.3048
Shell weight (%)	8.93	8.89	8.83	0.14	0.6549
Shell thickness (mm)	0.333	0.329	0.325	0.006	0.3305
Egg specific gravity	1.084	1.084	1.083	0.001	0.8876

[1] Control = 100% fine limestone ; 50%ES = 50% fine limestone and 50% ground eggshell ; 100%ES = 100% ground eggshell.
[2] SEM = Pooled standard error of the mean (4 replicates of 12 hens each per treatment).

feeding chicken eggshells as a calcium source to laying hens did not have detrimental effects on body weight and egg production. From the present study, the bodyweight for each group was initially comparable and remained not significantly different at the end of the trial. Throughout the 10 wk period, the average egg production of the control group was 61.18%, which was not significantly ($P > 0.05$) different from that of the 50%ES (69.24%) and 100%ES groups (68.28%). Nevertheless, the means of egg production in all treatments were considerably low and variable, perhaps due to the housing system used in the present study. Throughout the course of the trial, brooding behavior was observed among the treatment groups. This phenomenon might have led to cessation of laying by the broody hens, consequently reducing overall egg production.

The results from the present study also confirmed the findings of Scheideler (1998) who reported that dried chicken eggshells could be used as the sole calcium source in layer diets without detrimental effects on egg weight, albumen weight, yolk weight and egg specific gravity. Over the entire experimental period, the averages of egg weight were 47.47, 47.56 and 47.81 g for the control, 50%ES and 100%ES groups, respectively. Hens in all groups produced eggs that had similar quality of eggshell in terms of shell percentage (8.83 to 8.93%), shell thickness (0.325 to 0.333 mm) and egg specific gravity (1.083 to 1.084). The findings indicated that Ca in the eggshells can be utilized by the birds as efficiently as Ca in limestone. Scheideler (1998) demonstrated that calcium digestibility in particulate eggshells was comparable to that in fine limestone. In addition, the particle size of eggshells used in the current study would contribute to the utilization of Ca by the hens. In fact, the proportion of the eggshells that had a diameter size ranging from 2 to 5 mm was approximately 40% of the total used amount, suggested that resulted in enough retention time for the Ca in the digestive tract. From the autopsies, a large quantity of particulate eggshells was found in the gizzards of the sacrificed birds fed eggshell calcium diets, especially in the 100%ES group. Zhang and Coon (1997) demonstrated that large particle size calcium was retained in the gizzard for a longer time which enhanced the utilization of Ca for eggshell formation during the nighttime. Rao and Roland (1990) stated that the particle size of limestone required for the retention in the gizzard should not be less than 1.00 mm in diameter. Recently, Lichovnikova (2007) indicated that not only the source of Ca but also the particle size influenced eggshell quality and also recommended that a layer diet should contain two-thirds large particles of Ca to maintain the quality of the eggshell.

Hatchability performances and chick quality

Hatching egg traits, incubation-related parameters and hatchling characteristics are summarized in Table 5; clearly, fertility, hatchability, embryonic mortality and setting egg weight were not affected by the experimental diet. The hatchability of eggs in the control group was 70.29%, which was not significantly different from that of eggs in the 50%ES (76.37%) and 100%ES (77.10%) groups. These findings reflect that hatching eggs produced from all treatment groups had similar quality of shells (Table 4). In previous studies, a number of investigators reported that the quality of eggshells was closely related to fertility and hatchability (McDaniel *et al*., 1979, 1981; Bennett, 1992; Roque and Soares, 1994). Wilson (1997) stated that maternal nutrition had an important role in subsequent development and hatching of avian embryos.

Table 5 shows that hatchling weight, chick yield, chick growth and livability of the control treatment were similar to those of the other two treatments ($P > 0.05$). The results show that the experimental diet did not affect any aspect of hatchling characteristics or viability at age 1 wk.

Table 5 Influence of dietary treatment on hatching parameters and growth of posthatch chicks.

Item	Control	50%ES	100%ES	SEM[8]	*P-value*
n[1]	202	223	194		
Fertility (%)	96.44	97.51	96.18	3.31	0.8408
Hatchability[2] (%)	70.29	76.37	77.10	14.12	0.7618
Setting egg weight (g)	46.93	46.52	47.33	1.38	0.7195
Egg weight loss (%)	8.39	5.56	8.66	2.76	0.2651
Embryonic mortality (%)					
Early dead[3]	9.73	8.17	6.17	4.95	0.6129
Middle dead[4]	7.22	6.69	7.93	7.00	0.9694
Late dead[5]	11.83	8.70	8.30	5.79	0.6539
Chick weight (g)	32.51	32.85	33.50	1.11	0.4712
Chick yield[6] (%)	69.27	70.53	70.74	2.00	0.5545
Chick weight on day 7 (g)	54.56	54.57	52.45	4.29	0.7320
Relative growth[7] (%)	67.71	66.54	56.64	9.53	0.2497
Livability (%)	95.17	98.68	95.73	3.03	0.2633

[1] n = Number of setting eggs.
[2] Hatchability of fertile eggs = (number of chicks/ number of fertile eggs) × 100.
[3] Early dead = (number of embryos dying from day 1 to 7 / number of fertile eggs set) × 100.
[4] Middle dead = (number of embryos dying from day 8 to 18 / number of fertile eggs set) × 100.
[5] Late dead = (number of embryos dying from day 19 to 21 / number of fertile eggs set) × 100.
[6] Chick yield = (chick weight/setting egg weight) × 100.
[7] Relative growth = after 1 wk = (chick weight on day 7 - chick weight at hatching) × 100/ chick weight at hatching.
[8] SEM = Pooled standard error of the mean.

Morphology of the eggshell

Table 6 summarizes the density and diameter of the mammillary knobs of selected eggshells. It was apparent that there were no significant differences in the microstructure of the eggshell produced by birds that had received different sources of Ca. However, the mammillary knob density of the shells in the 50%ES and 100%ES groups tended to be higher than that of the shells in the control group. On the other hand, the diameter of the mammillary knobs of the shells produced by hens in the two experimental groups tended to be less than that of the shells produced by the control birds. The density of the mammillary knobs of the control shells was 176.68 knobs/mm^2 which was not significantly different from that of the 50%ES (196.95 knobs/mm^2) and 100%ES shells (203.13 knobs/mm^2). The diameter of the mammillary knobs of the shells for the control, 50%ES and 100%ES groups were 2.69, 2.54 and 2.49 µm, respectively.

Table 6 Mammillary knob density and diameter of eggshell produced by laying hens fed diets differing in calcium sources.

Parameter	Treatment			SEM[1]	*P-value*
	Control	50%ES	100%ES		
Number of samples	8	8	8		
Mammillae density (knobs/mm^2)	176.68	196.95	203.13	17.48	0.1365
Mammillae knob diameter (µm)	2.69	2.54	2.49	0.12	0.1301

[1] SEM = Pooled standard error of the mean.

Scanning electron microscopy inspection at a magnification of ×100 revealed normal formation of mammillae with rounded caps in all samples of the shells taken from all experimental groups (Figures 1, 2 and 3). Nonetheless, the examination at a magnification of ×500 showed evidence of mammillary bodies type B in one sample of the selected shell produced by the 50%ES hens (Figure 4). The mammillary bodies type B are small and round, and make no contribution to the thickness of the true shell. They are often found in shells produced by young birds and in those of hens exposed to stress (Solomon, 1991). In addition, good mammillary cap confluence could be detected in shell samples taken from both the control and the 100%ES

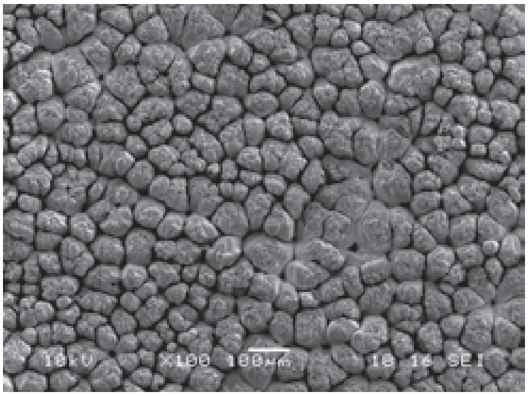

Figure 1 Scanning electron micrograph showing ultrastructure of the mammillary layer of birds in the control group; ×100. (Scale bar = 100 mm).

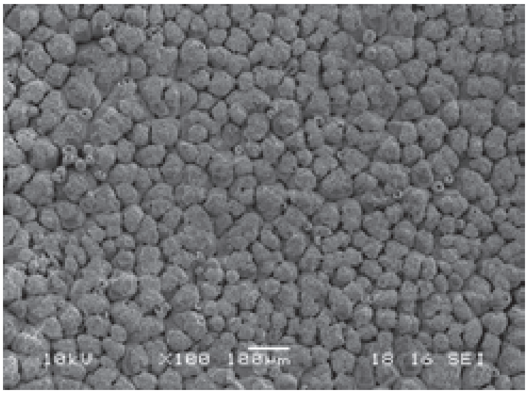

Figure 2 Scanning electron micrograph showing ultrastructure of the mammillary layer of birds in the 50%ES group; ×100. (Scale bar = 100 mm).

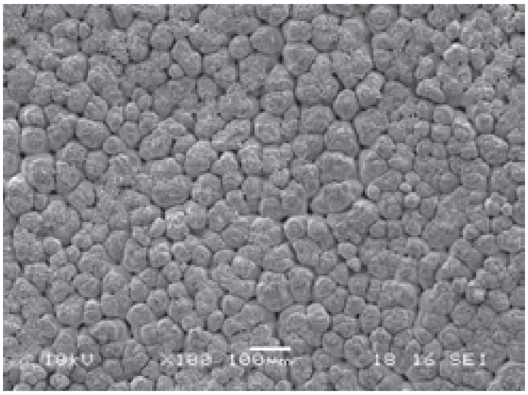

Figure 3 Scanning electron micrograph showing ultrastructure of the mammillary layer of birds in the 100%ES group; ×100. (Scale bar = 100 mm).

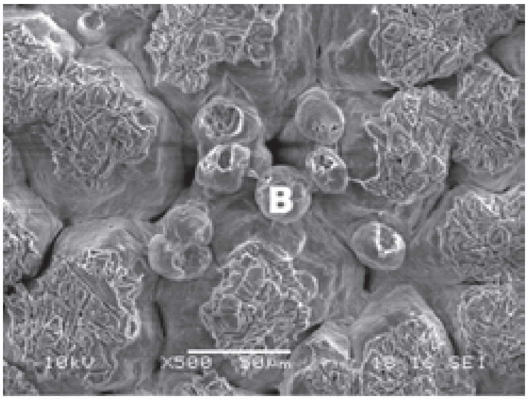

Figure 4 Scanning electron micrograph showing ultrastructure variations of the mammillary layer of birds in the 50%ES group. Note that the mammillary knobs showing Type B mammillary bodies (B); ×500. (Scale bar = 50 mm).

groups (Figures 5 and 6, respectively). Confluence refers to the condition where the mammillary caps join up with one another to form large areas of fusion at the cap sites (Solomon, 1991). Roberts and Brackpool (1994) pointed out that confluence either made the region itself stronger or possibly altered the distribution of pores and influenced the formation of the palisade layer of the shell. In the present study, however, mammillary variation was found in only one sample of each treatment group. Furthermore, the sample size of shells was too small; therefore, the results were assumed to be due to individual variations of the experimental birds rather than the effect of the treatment.

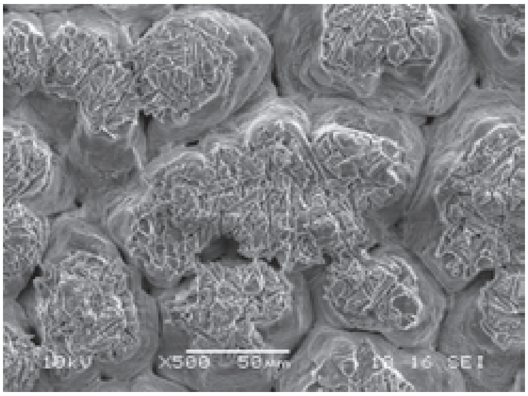

Figure 5 Scanning electron micrograph showing ultrastructure of the mammillary knobs of birds in the control group. Note that the mammillary knobs showing confluence; ×500. (Scale bar = 50 mm).

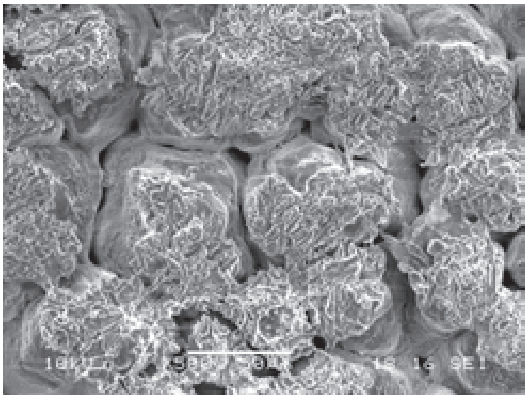

Figure 6 Scanning electron micrograph showing ultrastructure variations of the mammillary layer of birds in the 100%ES group. Note that the mammillary knobs showing confluence; ×500. (Scale bar = 50 mm).

CONCLUSION

Ground chicken eggshells can be used as the sole calcium source in layer diets without detrimental effects on any aspect of egg production traits, hatching performance, posthatch chick quality and morphology of the eggshell. Nevertheless, suitable attention should be given to the appropriate particle size and sterilization when using eggshells as animal feed ingredients.

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