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Effects of Using Eggshell Waste as a Calcium Source in the Diet of Rhode Island Red Roosters on Semen Quality, Gonadal Development, Plasma Calcium and Bone Status

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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 breeder male diets. Live performance, semen quality, gonadal and visceral organ characteristics, plasma calcium concentration and bone mineralization were evaluated. Forty-five Rhode Island Red cockerels aged 25 wk were caged individually in a conventional open-sided house. The birds were randomly divided into three groups, each group consisting of 15 birds. Group 1 (Control) was provided with a standard breeder ration which contained all the calcium carbonate as fine limestone, whereas Groups 2 and 3 were placed on diets in which 50% and 100%, respectively, of the limestone was replaced with ground eggshell. The birds were given free access to food and water, and exposed to a 16L:8D photoperiod. Throughout the study, body weight, feed consumption, spermatocrit, semen volume, sperm concentration and total sperm number/ejaculation of the three bird groups were not significantly ($P > 0.05$) different. Seminal plasma pH and Ca^{++} concentration were not affected by dietary treatments. Autopsies at the end of the study showed that comb weight, testes weight, ductus deferens weight and length, and giblets weight of the birds did not differ among the treatment groups. In addition, packed cell volume and plasma calcium and phosphorus concentrations did not exhibit a difference among cockerels fed the different calcium sources. No significant differences were found in any aspect of the tibia parameters (weight, length, breaking strength and ash content). The weights and lengths of the humerus from the three bird groups were comparable whatever the calcium source. Similar observations were also found in the parameters of the tarsometatarsus and keel bones. It was apparent that eggshell can be fully used as a calcium source in diets without any detrimental effects on the live performance, semen quality, reproductive development and calcium homeostasis of breeder male chickens.

Keywords: eggshell, calcium, rooster, semen, testes, tibia

INTRODUCTION

Increased global awareness has identified serious problems worldwide. Currently, this has increased public interest regarding organic waste materials and pollution problems. Chicken eggshell waste has been a concern of the egg industry. The waste eggshells are sometimes spread on land as a fertilizer source. Many studies have looked for ways to utilize the eggshell waste

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by for example, using eggshell powder as a stabilizing material for improving soil properties (Amu *et al*., 2005), a source of calcium (Ca) for piglets (Schaafsma and Beelen, 1999), and a source of Ca in human nutrition (Schaafsma and Pakan, 1999; Schaafsma *et al*., 2000). Vandepopuliere *et al*. (1975) reporting on the nutritive value of eggshells stated they have a calcium level comparable to that of limestone, with the benefit of small amounts of protein. Froning and Bergquist (1990) reported that extruded eggshell including membranes can 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 on egg weight, egg production, feed conversion and egg specific gravity in laying hens fed diets containing extruded eggshell meal as a source of calcium. These workers pointed out that high temperature-short time extrusion is an alternative method for converting poultry industry residues into high quality poultry feedstuffs. Drying the eggshell product at a high temperature is critical to decrease the potential for contamination by pathogens. Scheideler (1998) reported that eggshells could be fully used as a calcium source in layer diets without adverse effects on body weight, feed intake, egg production and eggshell quality. The source and particle size of Ca play an important role in maintaining bone mineralization (Guinotte and Nys, 1991; Keshavarz *et al*., 1993). Lichovnikova (2007) recommended that a diet mixture containing two-thirds large particle size of Ca should be used for improving eggshell quality, especially in aged hens. Recently, in a previous study conducted with Rhode Island Red hens, the author of the present study found that ground, sterilized eggshell could be used as a sole calcium source in diets without any adverse effects on live performance, egg production, eggshell quality or hatchability (Gongruttananun, 2011). Therefore, the aim of the present study was to determine the effect on breeder Rhode Island Red roosters of using ground, sterilized eggshell as a calcium source in their diet on their body weight, feed intake, semen quality, reproductive development, plasma calcium and bone mineralization.

MATERIALS AND METHODS

Experimental birds and diets

A 10 wk trial was conducted with 45 Rhode Island Red breeder males. The birds aged 25 wk were housed in individual wire cages with 1,521 cm^2 of floor space/bird, situated in a conventional open-sided shed. Birds were randomly divided into three groups, with each group consisting of 15 cockerels. Group 1 (Control) received a standard control breeder ration using ground fine limestone as the sole source of calcium carbonate in the diet. Group 2 (50% ES) was placed on the control diet that contained a combination of 50% limestone and 50% ground eggshell as the calcium source, whereas Group 3 (100%ES) was provided the control 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.

Eggshells were collected from food vendors on the campus of Kasetsart University, and washed with tap water without removing the shell membranes. The eggshell was sterilized in boiling water for 2 h and then dried in an oven at 95 °C for 24 h. The dried eggshell was 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 and replicated three times. Table 2 shows the particle size separation for the shell tested. The chemical composition of the eggshell was analyzed by proximate analysis (Table 3).

After 3 wk for acclimatization, the cockerels were fed the experimental diets for 10

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

Item	Ration 1[a]	Ration 2[b]	Ration 3[c]
Ingredient			
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*	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 (kg)	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
Moister (%)	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

[a]Ration 1 = Commercial ration (Control) with limestone as the calcium source.

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

[c]Ration 3 = Commercial ration with eggshell as the calcium source.

*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.

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

wk. Throughout the experimental period, the birds were provided feed and water at all times and exposed to a 16L:8D photoperiod. Fluorescent lighting was provided for 4 h per day, between 6:00 P.M. and 10:00 P.M. The lights were turned off by a clock timer.

Live performances

Birds were weighed at the beginning and the end of the study. Feed consumption was determined biweekly. Individual plastic feed troughs were utilized to measure the feed consumption per bird.

Semen characteristics

Semen analysis was conducted twice a week. Semen was collected by means of the conventional abdominal massage technique (Burrows and Quinn, 1937). All of the ejaculates were collected between 8:30 A.M. and 9:00 A.M. Semen was pooled from three roosters to form each experimental unit to reduce the probability of missing data from urate contamination or lack of ejaculation. Any semen sample that was contaminated was not incorporated into the experimental unit. Within 30 min after collection, the semen volume, spermatocrit, motility and concentration of spermatozoa were evaluated. The volume of semen was determined with a conical graduated centrifuge tube. The spermatocrit value of each pooled semen sample was determined by the method of microcapillary as is used for blood hematocrit determination. For each determination, duplicate semen samples were centrifuged at 15,000× *g* for 5 min (Hettich, HAEMATOCRIT 24). Motility and sperm concentration were determined according to the procedure described by Sexton (1976). Microscopic examination of the motility of the fresh semen was performed immediately after semen collection. A standard hemocytometer (AO Scientific Instruments, Warner-Lambert Technologies, Inc., Buffalo, NY) and light field microscope (Olympus Corp., Tokyo, Japan) were used to determine the sperm concentration and motility at magnification 400×. The total number of sperm/ejaculation was obtained by multiplying the semen concentration value by the average semen volume/bird/ejaculation.

Seminal plasma pH and ionized calcium concentrations

Determination of the pH value and ionized calcium concentration of the seminal fluid were also performed. The semen sample from each experimental unit was centrifuged at 13,000× *g* for 5 min, and the seminal plasma was separated from the sperm pellet. The seminal plasma pH and Ca^{++} concentration were measured using a CyberScan pH 5500/5000 (Eutech Instruments Pty Ltd., Ayer Rajah Crescent, Singapore).

Hematocrit and plasma calcium

Blood samples (5 mL) were obtained from the brachial vein from eight birds of each treatment at 10 wk after feeding the experimental diets. The hematocrit was determined using the microhematocrit technique. Next, the blood samples were centrifuged at 1,096× *g* for 15 min. The plasma ionized calcium was recorded immediately on a CyberScan pH 5500/5000 (Eutech Instruments Pty Ltd., Ayer Rajah Crescent, Singapore). The plasma total calcium and inorganic phosphate were determined using a colorimetric method on a COBAS INTEGRA Model 400/700/800 (Roche Diagnostics Corporation, Indianapolis, IN). The concentration of total plasma calcium was measured according to the method suggested by Schwarzenbach (1955).

Euthanasia and gonadal morphology

At the termination of the trial, aged 38 wk, five birds of each group were euthanized by cervical dislocation. Testes, ductus deferens, combs, and giblets (heart, liver and gizzard) were

excised for morphological study. The right and left testes of each sacrificed bird were weighed. The right and left ductus deferens were also removed, and measured for weight and length. Combs and giblets were removed and weighed. The weights of the organs were expressed relative to body weight (BW).

Bone studies

Immediately after euthanasia, the left tibia, humerus and tarsometatarsus were removed and cleaned of tissues. The keel bone was also examined. The weight and length of the bones were recorded. The tibiae were wrapped individually in cling wrap to exclude air, labeled, sealed in a double plastic bag and stored at -20 °C for subsequent bone strength and bone ash measurements. Breaking strengths were measured on frozen and thawed left tibia bones. Bones were taken out of the freezer and placed in a single layer on an aluminum tray, and equilibrated at a room temperature (28 °C) overnight (16 h). These tests were carried out by 3-point bending using a Stepless Compression Test Machine (Wykeham Farrance Ltd., England). The center of each bone was aligned with the breaking probe (10 mm diameter) which approached at 0.5 mm/min (modified from the method of Nϕrgaard-Nielsen, 1990). The supports for each bone were 30 mm apart. The breaking strength was determined from the failure point (peak) of each loading curve.

After testing the breaking strength, the bones were dried in an oven at 105 °C for 24 h and after cooling in a desiccator, the dry weight was recorded. The bones were fat extracted with ether for 24 h, weighed again and ashed in a muffle furnace at 600 °C for 24 h to obtain the ash weight. Results were expressed as the percentage ash of the dry fat-free weight according to the method described by Elaroussi *et al*. (1994).

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 body weight, feed consumption, semen characteristics, hematocrit, plasma electrolyte concentrations, morphology of organs and bone traits. The arcsine transformation was used for all percentage data. When the means of the GLM 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 $P < 0.05$. Data were presented as means and the pooled standard error of the mean (SEM).

RESULTS AND DISCUSSION

Live performances

Table 4 shows the effects of dietary treatment on body weight, feed consumption and the feed conversion ratio of the experimental birds. No effect of dietary treatment was found for feed intake, feed conversion ratio or body weight among the bird groups. The results are in agreement with the results found in laying hens in previous studies (Froning and Bergquist, 1990; Scheideler, 1998). From the present study, the body weight for each group was initially comparable and no difference was found up to the end of the trial. Throughout the 10 wk period, the average feed intake of the control group was 114.99 g/bird/day, which was not significantly ($P > 0.05$) different from those in the groups fed 50%ES (110.83 g/bird/day) and 100%ES (118.56 g/bird/day).

Semen quality

The semen characteristics of the experimental birds are shown in Table 5. There were no significant differences among the three bird groups regarding pH value and the ionized

Table 4 Effects of dietary treatment on live body weight, feed intake and feed conversion ratio of the experimental cockerels.

Treatment[1]	Body weight[2]			Feed intake[2]	FCR[3]
	Initial wt. (kg)	Final wt. (kg)	Weight gain (kg)	(g/bird/day)	(g/g)
Control	2.52	2.90	0.43	114.99	19.01
50%ES	2.52	2.81	0.31	110.83	24.72
100%ES	2.48	2.82	0.39	118.56	20.70
SEM[4]	0.19	0.19	0.17	8.95	4.33

[1]Control = 100% fine limestone; 50%ES = 50% fine limestone and 50% ground eggshell; 100%ES = 100% ground eggshell.
[2]Each value represents the mean of 15 observations.
[3]FCR = Feed conversion ratio (grams of feed intake per gram of body weight gain) from 28 to 38 wk of age.
[4]SEM = Pooled standard error of the mean.
There were no significant differences among treatment groups ($P > 0.05$).

Table 5 Effects of using different amounts of ground eggshell as the calcium source in diets on semen quality parameters.

Treatment	Seminal plasma		Spermatocrit value (%)	Semen volume (mL)	Sperm concentration (× 10^9cells/mL)	Sperm number /ejaculation (×10^9cells)
	pH	Ca^{++} (m*M*/L)				
Control	7.9	1.34	10.50	0.31	7.23	2.24
50%ES	7.8	1.59	11.23	0.40	7.60	3.15
100%ES	7.9	1.45	11.27	0.37	8.07	3.07
SEM	0.3	0.87	0.67	0.09	1.85	1.17

There were no significant differences among treatment means ($P > 0.05$).
SEM = Pooled standard error of the mean.

calcium concentration of the seminal fluid, spermatocrit, semen volume, sperm concentration or total sperm number per ejaculation. Spermatocrit was in the range 10.50–11.27%. The average sperm concentration of the control birds was 7.23 × 10^9cells/mL whereas those of the 50%ES and 100%ES cockerels were 7.60 × 10^9 and 8.07 × 10^9 cells/mL, respectively. Seminal plasma Ca^{++} concentration in the present study was in the range 1.35–1.59 m*M*/L which was similar to the normal value (1.51 m*M*/L) of broiler breeder males reported by Karaca *et al*. (2002).

Apart from the characteristics mentioned above, the sperm motility scores of the semen were also similar among the three bird groups (data not shown). The motility scores were mainly at level 4 or 5 and occasionally at level 3 of the 0–5 scale (Wishart and Wilson, 1997). The results indicated that the cockerels in the 50%ES and 100%ES groups were able to produce normal quality semen that was similar to the control roosters.

Testicular and reproductive tract growth

The means of comb weight, testes weight, ductus deferens weight and length and giblets weight are summarized in Table 6. No differences were found among the experimental bird groups for any of the reproductive organ traits, comb weight and giblets weight. The means of relative testes weight were 7.54, 6.03 and 7.39 mg/100g BW, respectively, in the control, 50%ES and 100%ES cockerels. The mean values of relative ductus deferens weight were 0.61, 0.46 and 0.67 mg/100g BW for the control, 50%ES and 100%ES

Table 6 Effects of dietary treatment on comb weight, giblets weight and reproductive organs of the experimental cockerels.

Treatment	Comb		Giblets[2]		Testes		Ductus deferens			
	Absolute weight[1] (g)	Relative weight (g/100gBW)	Absolute weight[1] (g)	Relative weight (g/100gBW)	Total weight[1] (g)	Relative weight (mg/100gBW)	Total weight[1] (g)	Relative weight (mg/100gBW)	Total length[1] (cm)	Relative length (cm/100gBW)
Control	32.42	1.25	74.65	0.28	19.47	7.54	1.58	0.61	26.46	1.01
50%ES	29.84	1.13	77.09	0.29	15.91	6.03	1.24	0.46	26.23	0.99
100%ES	32.45	1.18	72.22	0.25	20.18	7.39	1.86	0.67	26.90	0.98
SEM	6.65	0.27	3.04	0.01	5.58	2.19	0.49	0.18	0.82	0.05

[1]Each value represents the mean of five observations.
[2]Heart, liver and gizzard.
There were no significant differences among treatment means ($P > 0.05$).
SEM = Pooled standard error of the mean.

birds, respectively. In addition, the length of the ductus deferens did not differ among cockerels fed the three dietary treatments (0.98–1.01 cm/100g BW). The results demonstrated that the experimental diet did not affect the development of the testes and reproductive tracts of the cockerels.

Plasma Ca and bone mineralization

Table 7 demonstrates the influence of the experimental diets on hematocrit and plasma Ca and P concentrations which were determined at the termination of the trial. Similar hematocrit values were noticed among the treatment groups in the present study, mirroring a comparable state of health and reproductive potential of the experimental birds.

No significantly different means were found for total plasma Ca, ionized Ca and P among the bird groups. The results indicated that substitution of all the limestone in the diet with ground eggshell did not alter the plasma-calcium balance of the cockerels. This was confirmed by the lack of any differences in the tibia breaking strength and ash content and the other bone traits summarized in Table 8. The breaking strengths of the left tibia of the control, 50%ES and 100%ES birds were 57.20, 57.65 and 50.33 kg, respectively ($P > 0.05$). In addition, the bone ash content (49.76 to 50.70%) expressed as the percentage of fat-free dry weight was not affected by the dietary treatment. Obviously, the source of Ca used in the present study did not affect any bone traits. It was concluded that chicken eggshell can be used as the sole calcium source in breeder male diets without any adverse effects on body weight, feed consumption, development of reproductive organs, semen production, plasma calcium balance and bone mineralization. However, the appropriate particle size and sterilization should be properly managed when using eggshell as an animal feed ingredient.

Table 7 Hematocrit and plasma electrolyte concentrations in the experimental birds measured at the termination of the trial.

Treatment	Hematocrit (%)	Total Ca (mg/dL)	Ca^{++} (m*M*/L)	P (mg/dL)
Control	39.29	11.40	1.04	7.90
50%ES	37.49	10.73	0.81	9.06
100%ES	34.92	10.10	1.04	8.93
SEM	3.23	0.90	0.52	1.63

There were no significant differences among treatment means ($P > 0.05$).
SEM = Pooled standard error of the mean.

Table 8 Bone traits in the cockerels determined at the end of the experiment.

Item	Treatment			SEM
	Control	50%ES	100%ES	
Tibia				
Breaking strength (kg)	57.20	57.65	50.33	8.48
Ash content (%)	50.70	50.69	49.76	1.48
Length (cm)	15.66	16.00	16.40	0.40
Weight (g)	25.56	26.11	26.96	1.35
Humerus				
Length (cm)	9.33	9.86	9.83	0.46
Weight (g)	9.18	9.42	9.91	0.55
Keel				
Length (cm)	12.80	13.33	12.96	0.53
Weight (g)	22.16	20.81	19.93	4.95
Tarsometatarsus				
Length (cm)	10.76	11.16	11.00	0.33
Weight (g)	35.56	38.76	38.07	2.88

There were no significant differences among treatment means ($P > 0.05$).
SEM = Pooled standard error of the mean.

CONCLUSION

Eggshell can be used as the sole calcium source in breeder male diets without any detrimental effects on any aspect of live performances, reproductive development, semen quality, plasma calcium balance and bone mineralization. Nevertheless, the appropriate particle size and sterilization should be properly managed when using eggshell as an animal feed ingredient.

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