EFFECT OF INCREASING LEVELS OF EGGSHELL POWDER ON THE PRODUCTION PERFORMANCE, CARCASS CHARACTERISTICS, AND BONE PROPERTIES OF BROILER CHICKEN

Kevin Roland A. Maranan[1], Cristy M. Bueno[1], Christine B. Adiova[1] and Mariam C. Recuenco[2]

ABSTRACT

The study was conducted to determine the effect of increasing levels of eggshell on the production performance, carcass characteristics, and bone properties of broiler chickens. Two hundred fifty-six (256) chicks were randomly distributed into four (4) treatments following a completely randomized design. Each treatment was replicated eight (8) times with eight (8) birds per replicate. The dietary treatments include control (0% eggshell) and increasing levels of eggshell powder as a replacement to limestone at 50%, 75%, and 100%, respectively. Commercial booster feeds were fed to the birds from day 0-10 while the experimental diets were given from day 11-35. At the starter stage (day 11-24), birds fed eggshell-containing diets had higher ADG (*P*=0.04) compared to the control group. An increasing trend was observed on BW24 (*P*=0.05), ADG (*P*=0.01), and ADFI (*P*=0.04) as the level of eggshells was increased from 0 to 100%. However, birds fed 75% eggshell had lower ADG (*P*=0.02) and poorer Feed:Gain (*P*=0.02) than the control group during the finisher stage (day 25-35). For the overall performance (day 11-35), no differences were observed among treatments on body weights, ADG, ADFI, Feed:Gain, and mortality. Birds fed diets containing eggshells had a lower dressing percentage (*P*=0.03) compared with the control group. For the bone properties, birds fed 100% eggshell had lower bone breaking strength (*P*=0.03) compared with the birds fed 0% and 50% eggshell. Although birds fed diets with eggshells had higher tibia ash content (*P*=0.04), a quadratic trend (*P*=0.04) on bone calcium has been observed as the level of eggshell in the diet increases. Replacing limestone with eggshell powder resulted in similar overall production performance of broilers but lower dressing percentage and bone breaking strength. Further studies must be conducted to establish the optimum level of eggshell powder in broiler diets.

Key words: broiler, eggshell, production performance, carcass, bones

[1]Institute of Animal Science, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna, Philippines 4031; [2]Institute of Chemistry, College of Arts and Sciences, University of the Philippines Los Baños, College, Laguna, Philippines 4031 (email: kamaranan@up.edu.ph).

INTRODUCTION

Eggshell has significant functions in maintaining the quality of the egg as well as the survival of the embryo. However, after utilizing the egg contents, eggshells no longer have value and become wastes that significantly contribute to environmental pollution. According to Swamiappan and Vijayaraghavan (2006), about 11% of the egg's weight comes from the eggshell. In 2018 alone, approximately 63,800 metric tons of eggshell waste were generated in the country which is based on the 580,000 metric tons of egg production reported by the PSA (2019a, 2019b). The huge volume of eggshell waste contributes to the waste disposal problem in various countries due to limited dumping sites, high cost of disposal, and some environmental issues (Glatz and Miao, 2009). Thus, recycling the eggshell into valued products will provide avenues in addressing economic and environmental issues and concerns on its disposal.

Eggshell is composed of high levels of calcium and other minerals (Ali and Badawy, 2017) as well as some proteins (Gautron *et al*., 2001; Hincke *et al*., 1995) making it possible to utilize it in numerous applications. This includes electro-ceramics, cement replacement, anti-corrosive component of paint, bio-filler for intumescent coating, catalyst for biodiesel production, fertilizer, calcium supplement for human nutrition, bone graft substitute, and material base for enamel mineralization and protection, among others. In the field of animal nutrition, several studies show the potential of eggshells as a calcium source for cock, cockerels, and layer diets. The effects of using eggshells were observed on the production performance, bone and blood properties, semen characteristics, and egg quality of the chickens (Gongruttananun, 2011a; Lertchunhakiat *et al*., 2016). However, limited studies were reported on broiler chickens. Thus, the objective of this study was to determine the production performance, carcass characteristics, and bone properties of broiler chicken fed diets with increasing levels of eggshell powder as a substitute calcium source for limestone.

MATERIALS AND METHODS

The protocol used for the raising and handling of experimental animals was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of the Philippines Los Baños (UPLB), College, Laguna.

A total of 256 broiler chicks were distributed randomly into four (4) treatments arranged in a completely randomized design. Eight (8) replicate cages were prepared per treatment containing eight (8) birds each. The birds were given *ad libitum* access to feed and water through the feeders and drinkers that were provided in each cage. For two weeks, a source of heat was placed in the cages and electrolytes were added to the drinking water. Newcastle Disease vaccine B1 Type, B1 Strain was administered on day 7 while La Sota Strain was administered on day 21. The experiment concluded after 35 days.

A three-phase feeding strategy was employed in the study. The chicks were first fed with a commercial chick booster diet for 10 days without treatment. This was followed by starter and finisher diets that were given from day 11 to 24 and day 25 to 35, respectively. For the starter and finisher phase, four experimental diets with different levels of eggshell powder that are isocaloric and isonitrogenous were formulated to meet the nutritional requirements of birds. The experimental diets include: 1) 100% Limestone, control (100 LS); 2) 50% Limestone-50% Eggshell (50:50 LS-ES); 3) 25% Limestone-75% Eggshell (25:75

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

Item	Dietary Treatments			
	100 LS	50:50 LS-ES	25:75 LS-ES	100 ES
Yellow corn	58.78	58.78	58.78	58.78
Soybean meal	33.52	33.52	33.52	33.52
Coconut oil	4.30	4.30	4.30	4.30
MDCP	1.59	1.59	1.59	1.59
Limestone	0.90	0.45	0.23	0.00
Egg Shell	0.00	0.45	0.68	0.90
L-Lysine HCl	0.04	0.04	0.04	0.04
DL- Methionine	0.20	0.20	0.20	0.20
L-Threonine	0.02	0.02	0.02	0.02
Refined Iodized Salt	0.25	0.25	0.25	0.25
Choline chloride 50%	0.15	0.15	0.15	0.15
Mineral Premix[1]	0.15	0.15	0.15	0.15
Vitamin Premix[2]	0.10	0.10	0.10	0.10
Antioxidant	0.01	0.01	0.01	0.01
Total	100.00	100.00	100.00	100.00
Calculated Analysis				
ME, kcal/kg	3156	3156	3156	3156
Crude Protein, %	20.65	20.68	20.70	20.71
Calcium, %	0.84	0.83	0.82	0.81
Available P, %	0.45	0.45	0.45	0.45
Ash, %	2.98	2.98	2.98	2.98
Dig. Lysine, %	1.06	1.06	1.06	1.06
Dig. Met + Cys, %	0.82	0.82	0.82	0.82
Dig. Methionine, %	0.51	0.51	0.51	0.51
Dig. Threonine, %	0.72	0.72	0.72	0.72
Dig. Tryptophan, %	0.23	0.23	0.23	0.23
Crude Fiber, %	2.47	2.47	2.47	2.47
Crude Fat, %	7.10	7.10	7.10	7.10

[1]The mineral premix contains 10,000 mg of Copper, 80,000 mg of Ferrous, 70,000 mg of Manganese, 80,000 mg of Zinc, 200 mg of Cobalt, 800 mg of Iodine, 200 mg of Selenium and 20 g anti-caking carrier.

[2]The vitamin premix contains 11,000,000 IU of vit. A, 5,00,000 IU of vit. D3, 75,000 mg of vit. E, 3,000 mg of vit K3, 3,000 mg of vit. B1, 8,000 mg of vit. B2, 4,000 mg of vit. B6, 16 mg of vit. B12, 2,000 mg of Folic Acid, 60,000 mg of Niacin, 15,000 mg of Pantothenic Acid, and 150 mg of d-Biotin.

Table 2. Ingredient composition and calculated nutrient analysis of finisher experimental diets.

Item	Dietary Treatments			
	100 LS	50:50 LS-ES	25:75 LS-ES	100 ES
Yellow corn	61.90	61.90	61.90	61.90
Soybean meal	30.80	30.80	30.80	30.80
Coconut oil	4.32	4.32	4.32	4.32
MDCP	1.27	1.27	1.27	1.27
Limestone	0.85	0.43	0.21	0.00
Egg Shell	0.00	0.43	0.64	0.85
L-Lysine HCl	0.02	0.02	0.02	0.02
DL- Methionine	0.16	0.16	0.16	0.16
L-Threonine	0.01	0.01	0.01	0.01
Refined Iodized Salt	0.25	0.25	0.25	0.25
Choline chloride 50%	0.15	0.15	0.15	0.15
Mineral Premix[1]	0.15	0.15	0.15	0.15
Vitamin Premix[2]	0.10	0.10	0.10	0.10
Antioxidant	0.01	0.01	0.01	0.01
Total	100.00	100.00	100.00	100.00
Calculated Analysis				
ME, kcal/kg	3192	3192	3192	3192
Crude Protein, %	19.58	19.61	19.63	19.64
Calcium, %	0.76	0.75	0.74	0.73
Available P, %	0.38	0.38	0.38	0.38
Ash, %	2.85	2.85	2.85	2.85
Dig. Lysine, %	0.98	0.98	0.98	0.98
Dig. Met + Cys, %	0.76	0.76	0.76	0.76
Dig. Methionine, %	0.46	0.46	0.46	0.46
Dig. Threonine, %	0.68	0.68	0.68	0.68
Dig. Tryptophan, %	0.22	0.22	0.22	0.22
Crude Fiber, %	2.47	2.47	2.47	2.47
Crude Fat, %	7.21	7.21	7.21	7.21

[1]The mineral premix contains 10,000 mg of Copper, 80,000 mg of Ferrous, 70,000 mg of Manganese, 80,000 mg of Zinc, 200 mg of Cobalt, 800 mg of Iodine, 200 mg of Selenium and 20 g anti-caking carrier.
[2]The vitamin premix contains 11,000,000 IU of vit. A, 5,00,000 IU of vit. D3, 75,000 mg of vit. E, 3,000 mg of vit K3, 3,000 mg of vit. B1, 8,000 mg of vit. B2, 4,000 mg of vit. B6, 16 mg of vit. B12, 2,000 mg of Folic Acid, 60,000 mg of Niacin, 15,000 mg of Pantothenic Acid, and 150 mg of d-Biotin.

LS-ES); and 4) 100% Eggshell (100 ES). Table 1 and Table 2 present the composition of the diets.

The eggshell used for the diet preparation was collected from a bakeshop in Los Baños, Laguna. The eggshells were sun-dried for three days and pulverized using a hammermill. Powdered eggshell samples were allowed to pass through a set of sieves to determine the particle size. For the proximate analysis of eggshell powder, the AOAC (1993) Official Methods of Analysis was followed while the sample preparation for calcium and phosphorus analysis was based on the AOAC (1984) Official Methods of Analysis.

The birds and feeds were weighed at the beginning and end of the starter and finisher phase to determine bodyweights, ADG, ADFI, and Feed:Gain. The number of mortalities was recorded and data were adjusted for mortalities. For the diet economics, the feed cost per broiler, the value of gain per broiler, feed cost per kg of gain, and margin over feed cost were calculated for each treatment.

At the end of the feeding trial, two birds per replicate were randomly selected for carcass evaluation. The birds were fasted for 12 hours prior to slaughter and the liveweight was determined using a platform scale. The birds were dressed in the Animal Product Science and Technology Division, IAS, CAFS, UPLB. The weights of the carcass, giblets, and cut-ups were determined using a digital weighing scale. The cut-up parts include the breast, leg quarters, wings, and back. Based on the weights obtained, the dressing, giblets and cut-ups percentages were calculated.

After the carcass evaluation, the left tibia was separated. The flesh was removed together with the cartilage to ensure that only tibia bones would be used for further analysis. A total of 64 tibia bones were collected and placed in a freezer before evaluation. The tibia bone breaking strength was determined using a universal testing machine (INSTRON® 4411 Universal Testing Machine, Buckinghamshire, UK) at the Agricultural, Food and Bio-Process Engineering Division (AFBED), Institute of Agricultural and Biosystems Engineering, College of Engineering and Agro-Industrial Technology, UPLB. The maximum load (N) necessary to incur first breaking was measured with 500 Newton load cell at a test speed of 10 mm/minute. To take into account the difference in the sizes of bones, the maximum load (N) recorded was converted into maximum stress (MPa) which is the amount of stress necessary to break the bones.

For the determination of tibia ash content, six tibia bones per treatment were randomly selected. The bone samples were oven-dried for two weeks then soaked overnight in petroleum ether. After which, the samples were again placed in the oven overnight. The dried samples were ground using mortar and pestle. The ash content of the ground tibia bones was determined following the AOAC (1993) Official Methods of Analysis. The bone ash samples were then prepared for calcium analysis based on the AOAC (1984) Official Methods of Analysis. The calcium content was determined using a graphite furnace-atomic absorption spectrophotometer (Shimadzu® AA-7000).

The collected data were analyzed using the GLM procedure of SAS University Edition with the cage as the experimental unit. The least-square means were calculated for each independent variable. Tukey's Honest Significant Difference Test was used for pairwise mean comparison, except for bone breaking strength that used Duncan's Multiple Range Test. Orthogonal polynomial contrasts were performed to check the difference between the control group and the eggshell-containing treatments and to determine linear and quadratic effects of eggshell level in the diet. The Pearson correlation procedure was used to determine the correlation between the bone characteristics parameters. The α-level of ≤0.05 was used to determine significant differences between means while the α-level of <0.10 was used for tendencies.

RESULTS AND DISCUSSION

The particle size distribution of the powdered eggshell after sieving is shown in Table 3 while the chemical composition of the eggshell used in the experiment is presented in Table 4. The effect of experimental diets on the production performance of broiler chickens is presented in Table 5. In the starter stage (day 11 to 24), no significant differences were observed on all parameters based on pairwise mean comparisons. Adeyemo (2013) studied the effect of using increasing levels of fossil shell powder on broiler chicken performance. Similarly, no significant differences were found in the feed intake and feed:gain ratio of broiler chickens at the starter phase. However, providing a high percentage of fossil shell powder at 1.8% led to lower weight gain compared to a low percentage at 0.9%.

For the group comparison, it was revealed that the broiler chickens fed eggshell containing treatments had higher (P=0.04) ADG and tended (P=0.09) to have heavier BW24

Table 3. Particle size distribution of the ground eggshell used in the experiment.

Sieve Opening, mm	Amount (% by weight)	
	Starter	Finisher
>1.91	0.10	0.01
0.86-1.91	33.78	86.50
0.38-0.86	44.20	10.48
0.18-0.38	14.36	1.74
0.11-0.18	3.19	0.61
0.07-0.11	2.31	0.41
<0.07	2.06	0.25

Table 4. Chemical composition of the ground eggshell used in the experiment (as-fed basis).

Chemical Composition	Amount (%)
Proximate Analysis	
Moisture[1]	1.21
Crude Protein[2]	5.26
Crude Fiber[1]	1.02
Ether Extract[1]	0.30
Ash[1]	90.70
NFE[1]	1.52
Calcium[3]	40.67
Total Phosphorus[4]	0.66

[1]Weende method, [2]Kjeldahl method, [3]Atomic Absorption Spectrophotometric method, [4]Molybdate method

Table 5. Effect of increasing levels of eggshell powder on the production performance of broilers[1].

Parameters	Dietary Treatment				SEM	*P*-value			
							Contrast		
	100 LS	50:50 LS-ES	25:75 LS-ES	100 ES		Diet	T1 vs. T2, T3, T4	Linear	Quadratic
Starter Stage (day 11-24)									
BW10, g	213.63	214.32	214.51	214.76	6.12	1.00	0.90	0.89	0.99
BW24, g	883.35	916.47	944.06	956.56	27.40	0.26	0.09	0.05	0.99
ADG, g	46.91	49.24	52.11	52.99	1.82	0.10	0.04	0.01	0.89
ADFI, g	80.32	82.15	82.43	90.14	2.89	0.10	0.18	0.04	0.21
Feed:Gain	1.74	1.67	1.59	1.70	0.05	0.25	0.19	0.32	0.20
Mortality, no. of birds	0.00	0.13	0.00	0.00	0.06	0.41	0.57	0.87	0.21
Finisher Stage (day 25-35)									
BW35, g	1745.83	1736.45	1690.94	1764.42	39.13	0.60	0.74	0.96	0.39
ADG, g	78.41^{a}	74.54^{ab}	67.90^{b}	70.92^{ab}	2.32	0.02	0.01	0.01	0.48
ADFI, g	135.54	135.55	133.31	136.37	3.05	0.91	0.90	0.98	0.70
Feed:Gain	1.73^{b}	1.82^{ab}	1.97^{a}	1.95^{ab}	0.06	0.02	0.01	0.004	0.83
Mortality, no. of birds	0.13	0.00	0.00	0.00	0.06	0.41	0.09	0.14	0.43
Starter to Finisher Stage (day 11-35)									
ADG, g	60.58	60.28	59.06	60.88	1.44	0.82	0.76	0.90	0.56
ADFI, g	104.38	105.46	104.82	110.47	2.66	0.35	0.42	0.18	0.34
Feed:Gain	1.73	1.75	1.78	1.82	0.04	0.41	0.23	0.11	0.61
Mortality, no. of birds	0.13	0.13	0.00	0.00	0.09	0.58	0.42	0.24	0.74

[1]Values are least square means of 8 replicates per treatment with 8 birds per replicate.

[ab]Means in the same row with different superscript differ significantly at *P*-value ≤0.05.

than the birds fed with 100 LS diet. An increasing linear trend was also observed for ADG (*P*=0.01), ADFI (*P*=0.04), and BW24 (*P*=0.05) as the level of eggshell substitution increases.

For the finisher stage (day 25 to 35), significant differences were noted on ADG and Feed:Gain while there were no significant differences or trends observed for the BW35, ADFI, and mortality. Birds from 25:75 LS-ES treatment had significantly lower (*P*=0.02) ADG and higher (*P*=0.002) Feed:Gain value than the birds fed with 100 LS. In addition, a decreasing linear trend in ADG (*P*=0.01) and an increasing linear trend in Feed:Gain (*P*=0.004) were observed when the level of eggshell substitution increases. These results contradicted that of Adeyemo (2013) wherein no significant differences were observed in the production performance of broiler chickens at the finisher phase despite using increasing levels of fossil shell powder. Likewise, broiler chickens given finisher diets with increasing levels of gypsum did not differ in production performance (Omole *et al*., 2005).

Group comparisons also showed that the broiler chickens under the eggshell treatments had lower (*P*=0.01) ADG and poorer (*P*=0.01) feed efficiency than the control group. The larger particle size of the eggshell powder used in the finisher diets could have been the factor why the eggshell-containing group had lower ADG and poorer Feed:Gain. The proportion of coarse particles in the finisher eggshell powder is higher compared to the proportions of coarse particles in fine limestone and starter eggshell powder. In the study of McNaughton *et al*. (1974), broilers fed with larger particle sizes have lower body weight gain. Similarly, the results obtained by Guinotte *et al*. (1991) suggested that larger particle size leads to lower body weight gain and poorer feed efficiency while finer particle size translated to more efficient broiler chickens with higher intake and weight gain.

Overall, from starter to finisher stage (day 11-35), no significant differences or trends were observed in all parameters. Similarly, the use of increasing levels of eggshell as a replacement to limestone also did not affect the production performance of layer chickens and roosters (Gongruttananun, 2011a, 2011b). There were also no significant differences in the production performance of quails when increasing levels of cuttlefish bones, mussel shells, and quail eggshells were utilized (Bugdayci *et al*., 2019, 2020; Moura *et al*., 2020).

Table 6 shows the effect of experimental diets on the carcass characteristics of broiler chickens. No significant differences were observed on all parameters evaluated. This is in accordance with the study of Omole *et al*. (2005). In their study, broiler chickens were fed with increasing levels of gypsum as a substitute for oyster shells. The results of carcass analysis also yielded no significant differences among treatments.

However, the increasing levels of eggshell in the diet resulted in a linear decrease in dressing percentage (*P*=0.02). Group comparison also revealed that birds fed eggshell-containing treatments had a lower dressing percentage (*P*=0.03) as compared to the control group. This can be attributed to the poor performance of the broilers fed eggshell-containing diets during the finisher stage that could have also affected carcass quality. Compared to the control group, these birds had lower weight gain and are less efficient at the finisher stage. Meanwhile, a tendency for a quadratic trend (curving downward then upward) was observed on % breast (*P*=0.06).

The effect of dietary treatments on the broilers' bone properties is summarized in Table 7. One of the bone parameters observed is the bone breaking strength which is considered to affect bone fractures in poultry. It was observed that the decrease in bone strength increases the occurrence of fractures in hens (Bishop *et al*., 2000). Bone fractures are not

Table 6. Effect of increasing levels of eggshell powder on the carcass characteristics of broilers[1].

Parameters	Dietary Treatment				SEM	P-value			
							Contrast		
	100 LS	50:50 LS-ES	25:75 LS-ES	100 ES		Diet	T1 vs. T2, T3, T4	Linear	Quadratic
Live weight, kg	1.92	1.85	1.80	1.87	0.05	0.30	0.12	0.20	0.24
Dressing percentage	75.22	74.03	73.99	73.36	0.54	0.12	0.03	0.02	0.83
%Giblet[2]	4.38	4.58	4.71	4.66	0.14	0.36	0.10	0.10	0.59
%Breast[3]	33.95	33.69	33.09	35.14	0.56	0.09	0.97	0.37	0.06
%Leg[3]	27.27	27.45	27.17	26.59	0.31	0.26	0.59	0.17	0.15
%Wing[3]	11.58	11.62	11.91	11.49	0.23	0.60	0.74	0.92	0.43
%Back[3]	27.20	27.25	27.83	26.78	0.42	0.39	0.86	0.83	0.27

[1]Values are least square means of 8 replicates per treatment with 2 birds per replicate.
[2]Percentages were computed based on the live weight.
[3]Percentages were computed based on the dressed weight.

Table 7. Effect of increasing levels of eggshell powder on the bone properties of broilers.

Parameters	Dietary Treatment				SEM	*P*-value			
						Diet	Contrast		
	100 LS	50:50 LS-ES	25:75 LS-ES	100 ES			T1 vs. T2, T3, T4	Linear	Quadratic
Bone Breaking Strength, MPa[1]*	3.70[a]	3.66[a]	3.37[ab]	3.28[b]	0.11	0.03	0.06	0.01	0.35
Tibia Ash Content, %[2]	47.44	48.53	48.77	48.31	0.43	0.18	0.04	0.09	0.15
Calcium Content, %[2]	7.33	7.68	7.58	7.28	0.15	0.21	0.31	0.99	0.04
%Back[3]	27.20	27.25	27.83	26.78	0.42	0.39	0.86	0.83	0.27

[1]Values are least square means of 8 replicates per treatment with 2 tibia per replicate.
[2]Values are least square means of 6 replicates per treatment with 1 tibia per replicate.
*The Duncan's Multiple Range Test was used for the pairwise mean comparison of this parameter

only painful for the birds but also affect meat processing and the quality of processed products (Gregory and Wilkins, 1989). Based on the results, the bone breaking strength of birds fed 100 ES treatment was lower (P=0.03) compared to those fed 100 LS and 50:50 LS-ES treatments. The tibia bones of the eggshell-containing groups also have the tendency (P=0.06) to break easily compared to that of the control group. With the substitution of eggshells in the diet, a linear decrease (P=0.01) in the maximum stress needed to crack the bones was observed.

In the study of Guinotte *et al*. (1991), they found out that particle size is the factor that affects the bone breaking strength and not the calcium source. They reported that the bones of broiler chicks fed with fine particle size (80% of particles = 0.075 mm and lower; 20% of particles = 0.075 to 0.15 mm) had higher stiffness and yield stress while those fed with coarse particle size (80% of particles = 1.18 to 2.36 mm; 20% of particles = 2.36 to 4.75 mm) needed lower ultimate force to break the bone. The difference in the particle size of the calcium sources used in the present study could have also affected the bone breaking strength. Based on the particle size provided by Molnár *et al*. (2017), 87.49% of the fine limestone particle size is less than 0.30 mm. In contrast, the eggshell used in the present study has 78-97% of its particle size measuring greater than 0.38 mm. The larger particles of eggshell compared to limestone could be possibly the reason why the broiler chickens fed with higher levels of eggshell had lower bone breaking strength.

Tibia ash content was also determined as one of the indicators of bone mineralization in poultry (Hall *et al*., 2003; Huff, 1980). Based on the results, the replacement of limestone with increasing levels of eggshell had no significant effect on the tibia ash of broilers. This is consistent with the results obtained for layers and roosters (Gongruttananun, 2011a, 2011b). In the case of quails that received increasing levels of cuttlefish bones and mussel shells, the crude tibia ash percentage was also similar among treatments (Bugdayci *et al*., 2019, 2020).

However, group comparison in the present study showed that the birds fed with eggshells have higher (P=0.04) tibia ash content than the birds that received the control treatment. There was also a tendency (P=0.09) for tibia ash content to increase linearly when eggshell was added to the diet. In the study of McNaughton *et al*. (1974), lower tibia ash percentage was measured on broilers fed fine particle size than medium particle size. It is possible that the finer particle size of limestone compared to eggshells that were used in this study may have contributed to the lower tibia ash content of the control group.

While tibia ash provides the estimated mineral content of the sample, separate analysis can also be done to determine the specific mineral content of bones such as calcium and phosphorus content (Skinner and Waldroup, 1995). In the present study, the calcium content of tibia bones showed a quadratic trend (P=0.04) curving upward up to 50% ES then downward as the level of eggshell was further increased. However, differences among the treatments were not significant. Likewise, the different levels of fossil shell powder and quail eggshell powder did not affect the bone calcium content of broilers and quails, respectively (Adeyemo, 2013; Moura *et al*., 2020). This is in contrast with the results obtained by Kismiati *et al*. (2018) in laying hens. Based on their study, replacing 7.5% of limestone with eggshell flour led to a lower calcium percentage in tibia bones compared to 5% substitution. The authors attributed this to the mobilization of calcium from the tibia to produce eggs.

Summary of Pearson's correlation coefficient of the level of eggshell substitution, maximum stress for bone breaking, tibia ash content, and tibia calcium content are presented in Table 8. The correlation of eggshell substitution level with maximum stress (r=-0.52) was moderate and negative while it tends to have a weak and positive correlation with tibia ash content (r=0.35). This suggests that higher levels of eggshell substitution reduce the amount of stress needed to break the tibia but tends to increase tibia ash content. In addition, tibia ash content also tended to have a weak and positive correlation with tibia calcium content (r=0.39). This means that the tibia bones containing high ash content could also have high calcium content.

Although bones are composed mainly of minerals, results showed that bone breaking strength and tibia ash content are not significantly correlated. In the review of Rath *et al.* (2000), they implied that the mineral content of bones is not the sole factor affecting bone strength but also the organic matrix. Collagen is one of the components of the organic matrix and according to Martin and Boardman (1993), collagen fiber orientation can influence the bending of bones in bovines. With organic matrix also contributing to bone strength, the correlation between bone breaking strength and tibia ash may not always be significant, similar to the results of the present study.

Table 9 presents the effect of increasing the level of eggshell powder on margin over feed cost. The highest value of gain per broiler was observed in the treatment with 100% eggshell. However, the same group also had the highest feed cost per broiler and the highest feed cost per kg of gain. The 100% eggshell group only ranked second to the control group

Table 8. Pearson's correlation coefficient of the level of eggshell substitution and different bone parameters in broilers.

Variables	Eggshell Level	Maximum Stress	Tibia Ash	Calcium Content
Eggshell Level	1	-	-	-
Maximum Stress	-0.52**	1	-	-
Tibia Ash	0.35*	0.006	1	-
Calcium Content	0.004	0.26	0.39*	1

$^*P<0.10$

$^{**}P\leq0.05$

Table 9. Effect of increasing level of eggshell powder on margin over feed cost.

Parameters	Dietary Treatment			
	100 LS[a]	50:50 LS-ES[b]	25:75 LS-ES[c]	100 ES[d]
Feed Cost Per Broiler, ₱	60.12	60.66	60.23	63.06
Value of Gain Per Broiler, ₱	161.13	160.24	155.91	162.90
Feed cost per kg of gain, ₱/kg	35.45	35.97	36.70	36.78
Margin Over Feed Cost, ₱	101.00	99.57	95.69	99.84

Price per kg of starter diets – ₱ 20.21[a], ₱ 20.20[b], ₱ 20.20[c], ₱ 20.20[d]

Price per kg of finisher diets – ₱ 20.71[a], ₱ 20.71[b], ₱ 20.71[c], ₱ 20.71[d] in terms of margin over feed cost. The control group still had the highest margin over feed cost due to its lowest feed cost per broiler which is a function of feed intake and feed cost.

For the comparison of costs of the calcium sources, eggshells can be obtained for free from the bakery. However, the rent for using a hammermill to pulverize the eggshells must be considered. In this study, the computed price per kilogram of the eggshell is ₱ 2.84 which is lower than the ₱ 3.05/kg price of limestone.

In conclusion, the use of increasing levels of eggshell powder as a calcium source had similar effects with limestone in terms of the overall production performance (day 11-35). However, lower dressing percentage and lower bone breaking strength were observed on broiler chickens fed eggshell powder. Further studies must be conducted to establish the optimum level of eggshell powder in broiler diets. Different particle sizes must also be considered to determine the optimum particle size of eggshells suitable for broiler chickens.

ACKNOWLEDGEMENT

The authors would like to thank the Department of Science and Technology-Science Education Institute (DOST-SEI) for the thesis grant and the CHED-funded project entitled, "Backyard Livestock Island-Based Sustainable System (BLISS): Development of Feeds for Organic Livestock Production under the leadership of Dr. Renato SA. Vega for partially funding the experiment.

REFERENCES

Adeyemo GO. 2013. Growth performance of broiler chickens fed fossil shell growth promoter. Food Nutr Sci 4: 16–19.

Ali M and Badawy WZ. 2017. Utilization of eggshells by-product as a mineral source for fortification of bread strips. J. Food & Dairy Sci. 8: 455–459.

Association of Official Analytical Chemists. 1984. AOAC Official Method of Analysis.

Association of Official Analytical Chemists. 1993. Official Methods of Analysis of the AOAC.

Bishop SC, Fleming RH, Mccormagk HA, Flock DK and Whitehead CC. 2000. Inheritance of bone characteristics affecting osteoporosis in laying hens. *Br Poult Sci* 41:33-40.

Bugdayci KE, Gümüş H, Oğuz MN, Karakaş Oğuz F and Gülle İ. 2019. Effects of Mediterranean mussel shell (*Mytilus galloprovincialis*) on performance and egg quality in laying quails. *Acta Vet Eurasia* 45:22-29.

Bugdayci KE, Oguz MN, Karakas Oguz F, Uyguralp IC and Akinci IY. 2020. Effects of Mediterranean cuttlefish (*Sepia officinalis*) bone as an alternative calcium source on egg production, egg quality and some blood parameters in laying quails. *Fresenius Environ Bull* 29:2683-2690.

Gautron J, Hincke MT, Mann K, Panheleux M, Bain M, McKee MD, Solomon SE and Nys Y. 2001. Ovocalyxin-32, a novel chicken eggshell matrix protein. Isolation, amino acid sequencing, cloning, and immunocytochemical localization. *J Biol Chem* 276: 39243–39252.

Glatz P and Miao Z. 2009. High value products from hatchery waste. Rural Industries Research and Development Corporation. Australia: Rural Industries Research and Development Corporation.

Gongruttananun N. 2011a. Effects of eggshell calcium on productive performance, plasma calcium, bone mineralization, and gonadal characteristics in laying hens. *Poult Sci* 90:524-529.

Gongruttananun N. 2011b. 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. *Witthayasan Kasetsat* 45:413-421.

Gregory NG and Wilkins LJ. 1989. Broken bones in domestic fowl: Handling and processing damage in end-of-lay battery hens. *Br Poul Sci* 30:555-562.

Guinotte F, Nys Y and de Monredon F. 1991. The effects of particle size and origin of calcium carbonate on performance and ossification characteristics in broiler chicks. *Poult Sci* 70:1908-1920.

Hall LE, Shirley RB, Bakalli RI, Aggrey SE, Pesti GM and Edwards HM. 2003. Power of two methods for the estimation of bone ash of broilers. *Poult Sci* 82:414-418.

Hincke MT, Tsang CPW, Courtney M, Hill V and Narbaitz R. 1995. Purification and immunochemistry of a soluble matrix protein of the chicken eggshell (Ovocleidin 17). *Calcif Tissue Int* 56:578-583.

Huff WE. 1980. Discrepancies between bone ash and toe ash during aflatoxicosis. *Poult Sci* 59:2213-2215.

Kismiati S, Yuwanta T, Zuprizal S, Supadmo S and Atmomarsono U. 2018. Calcium deposition in egg due to substitution of limestone by eggshell flour in feed of laying hens. *J Indones Trop Anim Agric* 43:257-264.

Lertchunhakiat K, Saenphoom P, Nopparatmaitree M and Chimthong S. 2016. Effect of eggshell as a calcium source of breeder cock diet on semen quality. *Agric Agric Sci Procedia* 11:137-142.

Martin RB and Boardman DL. 1993. The effects of collagen fiber orientation, porosity, density, and mineralization on bovine cortical bone bending properties. *J Biomech* 26:1047-1054.

McNaughton JL, Dilworth BC and Day EJ. 1974. Effect of particle size on the utilization of calcium supplements by the chick. *Poult Sci* 53:1024-1029.

Molnár A, Maertens L, Ampe B, Buyse J, Zoons J and Delezie E. 2017. Supplementation of fine and coarse limestone in different ratios in a split feeding system: Effects on performance, egg quality, and bone strength in old laying hens. *Poult Sci* 96: 1659-1671.

Moura GRS, Reis RDS, Mendoca MDO, Salgado HR, Abreu KDS, Madella GDS and Lima MBD. 2020. Substitution of limestone for eggshell powder in the diet of Japanese laying quails. *Rev Bras Saúde Prod* 21:1-13.

Omole AJ, Ogbosuka GE, Salako RA and Ajayi OO. 2005. Effect of replacing oyster shell with gypsum in broiler finisher diet. *J Appl Sci Res* 1:245-248.

Philippine Statistics Authority. 2019a. Chicken Situation Report: January-December 2018. Retrieved on 3 July 2019 from https://psa.gov.ph/content/chicken-situation-report-january-december-2018.

Philippine Statistics Authority. 2019b. Duck Situation Report: January-December 2018. Retrieved on 3 July 2019 from https://psa.gov.ph/content/duck-situation-report-january-december-2018.

Rath NC, Huff GR, Huff WE and Balog JM. 2000. Factors regulating bone maturity and strength in poultry. *Poult Sci* 79: 1024-1032.

Skinner JT and Waldroup PW. 1995. Allometric bone development in floor-reared broilers. *J Appl Poult Res* 4:265-270.

Swamiappan S and Vijayaraghavan R. 2006. Low temperature synthesis of nanocrystalline hydroxyapatite from egg shells by combustion method. *Trends Biomater Artif Organs* 19:70-73.