Effect of using different levels of egg shell as calcium sources in broiler diet on growth performance, blood parameters, and bone characteristics

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Abstract

The study was conducted at Grdarasha field in Salahaddin University –Erbil during the period from 23 November 2021 to 5 January 2022, to determine the effect of using different calcium sources in a broiler diet on growth performance, blood parameters, bone characteristics, and immunity organs. Three hundred (300) chicks have randomly distributed into three (3) treatments following a completely randomized design (CRD). Five replications for each treatment, each replicate contains 20 birds. The dietary treatments include T1: control (0% eggshell), T2 and T3 increasing levels of eggshell powder as a replacement to limestone at 50% and 100%, respectively. The results showed no significant ($P > 0.05$) differences in broiler live body weight, body weight gain (BWQ), feed intake, feed conversation ratio, and mortality % among different weeks of experiments and different treatments used. The results of blood parameters did not note any significant variations among different treatment groups ($P > 0.05$) in TG, HDL, LDL and P, however, the significant differences ($P < 0.01$)was found in the Cholesterol, VLDL, and Calcium . Bone ash mainly consists of calcium and phosphorous, and their level of them differ significantly ($P < 0.01$) among the different treatments. Also, the results of the effect of dietary treatments on the broilers' bone properties indicated that no significant differences in bone length (cm), diaphysis diameter (mm), Lateral wall thickness (mm), Medial wall thickness (mm), Medullary canal diameter (mm), Tibiotarsal index. However, the significance was found in bone weight and bone-breaking strength which is considered to affect bone fractures in poultry. Nevertheless, the results showed no significant differences in immunity organs, including spleen % and bursa %.

Keywords: broiler, eggshell, production performance, blood parameters, bone, immunity organs.

Introduction

Nevertheless, once the egg's contents have been extracted, the eggshells become waste that significantly contributes to environmental degradation. (33) estimate that the eggshell comprises about 11% of the egg's total mass. Due to limited dumping sites, high disposal costs, and environmental concerns, the massive quantity of eggshell waste contributes to the waste disposal problem in many nations (16). Consequently, transforming eggshells into valuable products will provide opportunities to address economic and environmental concerns. Eggshell is rich in calcium, other minerals, and particular proteins (4, 15 and 20).

The principal source of calcium in poultry diets is limestone, which is abundant and affordable (11). It has been observed that limestone in feed can supply more than fifty percent of the calcium in broiler diets (22). However, limestone has disadvantages such as poor solubility and, thus, poorer bioavailability due to the diet's more potent acid-binding properties (6). Limestone and oyster shells are essential sources of calcium in poultry feed, containing 380 mg Ca per kilogram (28). However, research indicates that the availability of calcium in broiler chickens varies considerably (6, 7 and10). However, few studies have been conducted on the influence of eggshells on broiler chicken diets. Consequently, the objective of this study was to assess the performance, serum characteristics, bone qualities, and immunological organs of broiler chickens fed diets with increasing levels of eggshell powder as a calcium substitute for limestone.

Material and Methods

The research was conducted at the Grdarasha field farm of the University of Salahaddin-Erbil/College Iraq's of Agricultural Engineering Sciences during the period from 23 November 2021 to 5 January 2022. 300 one-day-old, straight-run, commercial (Ross 308) broiler chicks obtained from a local hatchery were raised from day 1 to 42 days old. The chicks were distributed randomly into three treatments arranged in a completely randomized design (CRD). Five replications for each treatment, each replicate contains 20 birds. Chicks were reared under standard management practices (32)

The research employed a two-phase feeding strategy (the starter and finisher began from 1 to 21 and 22 to 42 days of age, respectively). Diets formulated to meet the nutritional requirements of broiler chicks using the Feed LIVE software (Feed LIVE 1.52, Thailand). All of the experimental diets included the same amount of calories and nitrogen. The birds will be offered different diets comprising two calcium sources: eggshell, eggshell, and limestone.

T1 is a diet of 100% Limestone (100 LS).

T2 is a diet of 50% Limestone and 50% Eggshell (50:50 LS-ES).

T3 is a diet of 100% Eggshell (100 ES) (100 ES).

Erbil's Evan Hatchery provided the eggshell utilized in the diet. Before being ground with an electric miller, the eggshells were sun-dried for three days. Or the proximate analysis of eggshell powder, the (9) .In contrast, the sample preparation for calcium and phosphorus analysis was based on the (8) Official Methods of Analysis

Vaccinated Programs**:** The vaccination program for broilers was used to protect the birds.

Table -1: Ingredient's composition and calculated nutrient analysis of experimental starterdiets.

Item		Dietary Treatments1		
		100 LS	50:50 LS-ES	100 ES
Corn		47.000	47.000	47.000
Soybean oil		5.000	5.000	5.000
Soybean meal 44 %		42.800	42.700	42.600
L-Lysine		0.300	0.300	0.300
DL-Methionine		0.300	0.300	0.300
Monodicalciumphosphate21		1.850	1.850	1.850
Calcium carbonate		2.000	1.000	-----
Salt		0.300	0.300	0.300
Vitamin Premix2		0.250	0.250	0.250
Mineral Premix3		0.150	0.150	0.150
Toxin Binder		0.100	0.100	0.100
Choline Chloride		0.100	0.100	0.100
Egg Shell		-----	1.100	2.200
Total		100.00	100.00	100.00
Calculated Analysis	Unit			
ME. for Poultry	Cal/Kg	2,997.480	2,995.230	2,992.980
Protein	%	23.050	23.062	23.073
Fat	%	7.070	7.070	7.070
Fiber	%	4.171	4.164	4.157
Calcium	%	1.199	1.183	1.167
Total Phosphorus	%	0.784	0.784	0.784
Avail. P for Poultry	%	0.447	0.448	0.448
Salt	%	0.318	0.318	0.318
Arginine	%	1.600	1.597	1.594
Lysine	%	1.505	1.502	1.499
Methionine + Cystine	%	0.981	0.980	0.978
Methionine	%	0.637	0.636	0.636
Threonine	%	0.886	0.884	0.882
Tryptophan	%	0.303	0.302	0.301

1LS, 100% limestone (Control diet); LS-ES, 50% limestone + 50% eggshell; ES, 100% eggshell.

2Vitamin premix provided the following per 1gm. diet: Vitamin A (retinal acetate) 2000 I.U.; Vitamin D3 500 I.U.; Vitamin E (DL-tocopherol acetate) 400 mcg; Vitamin B1 200 mcg; Vitamin B2 400 mcg; Nicotinamide 1000 mcg; Folic acid 50 mcg; Ca-D-Pantothenate 500 mcg.

3Mineral premix provided the following per 1gm. diet: Nux Vomica 20 mg; Iron 40 mg; Gentiana 150 mg; Sod. Carbonate 400 mg; manganese 20 mcg; Zinc 25 mcg; Cobalt 20 mcg; Copper 100 mcg.

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

Item		Dietary Treatments[1]		
		100 LS	50:50 LS-ES	100 ES
Corn		52.450	52.450	52.450
Soybean oil		5.500	5.500	5.500
Soybean meal 44 %		37.150	37.000	36.900
L-Lysine		0.300	0.300	0.300
DL-Methionine		0.300	0.300	0.300
Monodicalciumphosphate21		1.900	1.900	1.900
Calcium carbonate		1.600	0.800	-----
Salt		0.300	0.300	0.300
Vitamin Premix2		0.100	0.100	0.100
Mineral Premix3		0.150	0.150	0.150
Toxin Binder		0.150	0.150	0.150
Choline Chloride		0.100	0.100	0.100
Egg Shell		-----	0.950	1.850
Total		100.00	100.00	100.00
Calculated Analysis	Unit			
ME. for Poultry	Cal/Kg	3,096.112	3,092.738	3,090.487
Protein	%	21.000	20.982	20.984
Fat	%	7.705	7.704	7.704
Fiber	%	3.912	3.901	3.894
Calcium	%	1.039	1.049	1.043
Total Phosphorus	%	0.772	0.784	0.771
Avail. P for Poultry	%	0.450	0.448	0.451
Salt	%	0.318	0.318	0.318
Arginine	%	1.436	1.597	1.428
Lysine	%	1.365	1.502	1.359
Methionine + Cystine	%	0.928	0.980	0.925
Methionine	%	0.613	0.636	0.611
Threonine	%	0.804	0.884	0.799
Tryptophan	%	0.271	0.302	0.269

1LS, 100% limestone (Control diet); LS-ES, 50% limestone + 50% eggshell; ES, 100% eggshell.

2Vitamin premix provided the following per 1gm. diet: Vitamin A (retinyl acetate) 2000 I.U.; Vitamin D3 500 I.U.; Vitamin E (DL-tocopheryl acetate) 400 mcg; Vitamin B1 200 mcg; Vitamin B2 400 mcg; Nicotinamide 1000 mcg; Folic acid 50 mcg; Ca-D-Pantothenate 500 mcg.

3Mineral premix provided the following per 1gm. diet: Nux Vomica 20 mg; Iron 40 mg; Gentiana 150 mg; Sod. Carbonate 400 mg; manganese 20 mcg; Zinc 25 mcg; Cobalt 20 mcg; Copper 100 mcg.

Growth performance

Weekly measurements included feed intake (FI), feed conversion ratio (FCR), body weight (BW), and body weight gain (BWG) (BWG). Each day, mortality was monitored and recorded; all deceased birds were weighed, and the weight was used to adjust both FI and FCR. To calculate adjusted FCR, the total feed intake per replicate was divided by the weight gain of surviving birds and the weight of birds that died or were removed from the pen. (29).

Sample collection for blood sampling

On day 42 of the feeding test, birds fasted for 12 hours. Two birds per replicate (10 chicks per treatment) whose BW was closest to the replicate's mean BW were chosen, weighed, and slaughtered following Iraqi animal welfare laws.

Blood samples (3 mL) were collected following slaughter and centrifuged at 3000 rpm for 10 minutes to separate serum. Serum samples were analyzed using commercial kits to determine blood levels of aspartate cholesterol, triglyceride, low-density lipoprotein (LDL), high-density lipoprotein (HDL), calcium (Ca), and phosphorous (P) (LDL-C) was determined by Abdulla *et al*. (2)'s Fried Ewald Equation: VLDL cholesterol is equal to triglycerides/5.

Bone characteristics**:**

After the carcass was evaluated, the left tibia was separated. The flesh and cartilage were removed to ensure that only tibia bones were utilized for further analysis. Before evaluation, thirty tibia bones were gathered and frozen. The drumsticks were then thawed, labelled, and submerged for 10 minutes in 100°C boiling water. After the drumsticks had cooled to room temperature, they were deflated by hand, and the bone cap and patella were removed. They were then air-dried at room temperature for 24 hours. Using an electronic digital measuring device, the length of the tibiotarsal bones was determined, and the bones were weighed using a precision balance. Before breaking each bone, the midpoint was marked, and the external diameters were measured.

The tibia bone-breaking strength was determined using a universal testing machine (INSTRON® 4411 Universal Testing Machine, Buckinghamshire, UK) at a mechanical engineering college. Using 500 Newton load cells and a test speed of 10 mm/min, the maximum force (N) required to cause the initial fracture was determined. In order to account for differences in bone size, the maximum load (N) was converted into the maximum stress (MPa) required to fracture the bones. At the midpoint, the thickness of the medial and lateral walls was measured using a dial caliper. To determine the diameter of the medullary canal, the thicknesses of the medial and lateral walls were subtracted from the diameter of the diaphysis. The tibiotarsal index was computed with the following formula:

Tibiotarsal index = [(diaphysis diameter − medullary canal diameter)/ (diaphysis diameter)] × 100 (1)

Bone samples were oven-dried for two weeks and then soaked overnight in petroleum ether to determine tibia ash content. The samples were then placed back in the oven overnight. The samples were crushed with a mortar and pestle. Following (9) Official Methods of Analysis, the ash content of the ground tibia bones was determined. The bone ash samples were prepared for calcium analysis following the Official Analysis Methods of the AOAC (8). The calcium content was determined using a graphite furnace-atomic absorption spectrophotometer (Shimadzu® AA-7000).

Immune Organ Indexes

Immune organ indexes were calculated according to the change in the immune organ weight. The spleen and bursa indexes were calculated. (6).

Statistical Analysis

Data were submitted to analysis of variance (ANOVA) according to a completely randomized design using the PROC Mixed procedure of SAS (Version 9.4, SAS Institute Inc.). Pairwise differences between means were determined using Duncan s multiple-range test. The main effects of the three treatments, with five replications on growth performance, bone quality, and blood parameters, were tested. The overall level of statistical significance was set at $p<0.05$ for performance traits, blood profile, and bone quality.

A linear model was used to analyze the data:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = the observed data of the ijth traits,

μ = the overall mean,

T_i = the effect of the ith treatments

e_{ij} = the effect of random error

Results and Discussion

The effect of experimental diets on the production performance of broiler chickens is presented in Table 3. There were no significant differences ($P > 0.05$) on all parameters, such as body weight (BW), body weight gain (BWG), and feed intake (FI) expected feed conversation ratio based on mean comparisons during the starter stage (days 1 to 21). A decreasing linear trend was also observed for body weight, weight gain, and feed intake as the level of eggshell substitution increased. Control treatment T1 recorded the highest value in the above traits. Otherwise, the minimum value was recorded by T3, and T2 was intermediate.

Nevertheless, significant differences ($P > 0.05$) were noted in FCR among treatments. The best value was recorded in the second treatment (50LS:50 ES). Researchers conducted several studies. One of them, (25) revealed the effect of experimental diets of eggshells on the production performance of broiler chickens in the starter stage (days 11 to 24). No significant differences were observed on all parameters based on pairwise mean comparisons.

No significant differences ($P > 0.05$) were noted for the finisher stage (days 22 to 42) on all production traits, including BW, BWG, FI, and FCR. The BW and FI linearly decreased with increasing dietary eggshell. Meanwhile, the control treatment recorded a high value in BWG and FCR. These results agreed with Adeyemo (3), who revealed no significant differences ($P > 0.05$) 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 (30). 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 why the eggshell-containing group had lower ADG and poorer Feed: Gain. Similarly, increasing eggshell levels as a replacement to limestone also did not affect the production performance of layer chickens and roosters (17 and 18). 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 (12 and 13).

On the other hand, (25) indicated that significant differences ($P < 0.05$) were noted in ADG and Feed Gain at the finisher stage (day 25 to 35), while no significant differences or trends were observed for the BW35 and ADFI. 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, when the level of eggshell substitution increases, a decreasing linear trend in ADG (P=0.01) and an increasing linear trend in Feed: Gain (P=0.004) were observed.

Overall, from the starter to the finisher stage (days 1-42), no significant differences ($P > 0.05$) or trends were observed in all parameters. Similarly, increasing eggshell levels as a replacement for limestone also did not affect the production performance of broiler chicks (25). 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 (12, 13 and 26)

The effects of dietary eggshell on triglycine total cholesterol, HDL, LDL, Ca, and P of broiler chicks at 42 days are presented in Table (4). The significant differences ($P< 0.05$) among treatments in triglyceride (TG) which T1 recorded the highest value while the lowest was in the T2 treatment group ,however no significant differences ($P > 0.05$) was found in cholesterol levels,. Furthermore, the broiler chicks in T3 showed no significant differences ($P > 0.05$) as compared with T1 and T2. Inspite of decreased TG and total cholesterol. On the other hand, none were significant among treatments in HDL and LDL. (24) conducted a study to compare the effects of feeding limestone, cockle shell, oyster shell, fine ES, and coarse ES as the sole Ca source on blood biochemical constituents in ISA-Brown laying hens. The results showed that no significant changes in the blood biochemical constituents among the dietary treatments (P>0.05) in LDH lactate dehydrogenase, TG triglycerides, TC total cholesterol, LDL low-density lipoprotein and VLDL very low-density lipoprotein, except for high-density lipoprotein (HDL) (%). The HDL proportion decreased in response to dietary ESC and OS supplementation compared to LS, ESF, and CS treatments ($P<0.01$).

Although T2 recorded the high values in Ca ($p=0.0032$) compared to T3, which recorded the low values and control treatment are intermediate, on the other hand there were no significant differences ($p = 0.1121$) among treatments in P Similarity. (19) found the same results in 2011 when he conducted a study with Rhode Island Red females to determine the effects of replacing limestone in the diet with the ground, sterilized eggshell. For Plasma Ca, he used three treatments include. Group 1 (control) was provided with a layer ration that contained fine limestone. In contrast, groups 2 and 3 were placed on diets in which 50 and 100% of the limestone were substituted with ground eggshell. Results also showed no effect of treatment for total plasma P among the bird groups. However, the significance was found in Ca. Although (14) conducted a study by using seven dietary calcium (Ca) at different concentrations and two calcium sources (oyster shell and limestone) on broiler chicken from 1 to 21 days of age. Results indicated no significant differences ($P > 0.05$) in serum Ca concentrations at 21 days of age.

The effect of dietary treatments on the broilers' Bone properties is presented in table (5). The results showed no significant differences ($P > 0.05$) in bone length (cm), diaphysis diameter (mm), Lateral wall thickness (mm), Medial wall thickness (mm), Medullary canal diameter (mm), and Tibiotarsal index. However, the significance was found in bone weight and bone-breaking strength which is considered to affect bone fractures in poultry. Based on the results, bone weight (gm) and bone-breaking strength were higher in T3 birds fed 100 ES ($P=0.03$) than in T1 and T2 treatments. The tibia bones of the limestone-containing groups also tend ($P=0.06$) to break easily compared to the other group. With the substitution of limestones in the diet. (5) showed that the tibial breaking strength tended to increase with an increase in dietary Ca levels. This result is consistent with (21), who reported that the hens fed a diet with 4.4% Ca had similar bone density and strength compared to those with a diet with 3.7% Ca.

Contrary to these results, a study has shown that increasing dietary Ca level linearly increased bone strength (31). Also, (23) also suggested that an increasing which decrease in bone strength increases the occurrence of fractures in the bone. Although (26), 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 why the broiler chickens fed with higher levels of eggshell had lower bone-breaking strength. Although bone ash mainly consists of calcium and phosphorous and their level of them differ significantly ($P < 0.01$) among the different treatments.

The higher calcium % observed in that fed diet T1 (0 % eggshell) followed by T3 that fed a diet of (replaced 100 % limestone) and the lower calcium observed in T2 (replaced 50 %

limestone). While the higher phosphorus % was observed in T1 fed diet (0 % eggshell) followed by T3 that fed a diet of (replaced 100 % limestone) and the lower calcium observed in T2 that, fed a diet (replaced 50 % limestone). This result agreed with the results of (1), who reported that the primary minerals constituent of the bone is calcium and phosphorus, which are responsible for the healthy one of the broilers. That content indicates bone strength and calcification (35). Therefore, some researchers suggested that diets should contain more Ca and nPP than the current recommendations of (28) to maintain skeletal integrity, especially during the finisher period for modern strains (34).

Nevertheless, the results showed no significant differences in immunity organs, including the spleen and bursa presented in table (6).

Table -3: Effects of different calcium sources on broiler growth performance at 42 days.

Parameters	Treatments1 T1	T2	T3	SEM	P-value
Body weight (g)					
Initial	35.80	35.90	36.00	0.18	0.9184
Week 3	565.50	564.50	546.90	6.36	0.4372
Week 6	2232.68	2162.07	2201.77	25.93	0.5727
Body weight gain (g)					
Week 1-3	529.70	528.60	510.90	6.38	0.4335
Week 4-6	1667.18	1597.57	1654.87	24.77	0.5067
Overall	2196.88	2126.17	2165.77	25.85	0.5697
Feed intake (g/bird)					
Week 1-3	698.70	691.00	673.50	9.73	0.5909
Week 4-6	2805.92	2777.21	2777.17	27.30	0.8991
Overall	3504.62	3468.21	3450.67	33.51	0.8221
FCR (feed/gain)					
Week3	1.320	1.306	1.318	0.004	0.3025
Week6	1.684	1.746	1.680	0.026	0.5350
Overall	1.596	1.636	1.594	0.018	0.6100

1T1: 100% Limestone (Control diet); T2: 50% Limestone + 50% Eggshell; T3: % 100 Eggshell.

Table- 4: Effects of different calcium sources on broiler blood parameters at 42 days.

Blood Parameters1	Treatments2 T1	T2	T3	SEM	P-value

TG1 (mg/dl)	187.17a	161.63b	170.42ab	4.27	0.0306
CHOL (mg/dl)	214.62	210.77	198.46	3.39	0.1223
HDL (mg/dl)	116.31	116.39	114.11	0.98	0.5935
LDL (mg/dl)	60.87	62.05	50.27	3.47	0.3359
VLDL (mg/dl)	37.43a	32.33b	34.08ab	0.85	0.0307
Ca (mg/dl)	13.32a	13.83a	8.78b	0.77	0.0032
P (mg/dl)	4.36	4.84	4.54	0.10	0.1121

a-b Values in the same row with different letters are significantly different ($p < 0.05$). 1TG, triglyceride; CHOL, total cholesterol; HDL, high-density lipoprotein; LDL, low density lipoprotein; VLDL, very low density. lipoprotein; mg/dl, milligram/decilitre. 2T1: 100% Limestone (Control diet); T2: 50% Limestone + 50% Eggshell; T3: % 100 Eggshell.

Table -5: Effects of different calcium sources on broiler Bone characteristics at 42 days.

Parameters	Treatments1			SEM	P-value
	T1	T2	T3		
Length (cm)	9.815	9.819	10.01	0.06	0.3024
Weight (g)	7.89b	8.25b	9.10a	0.17	0.0082
Diaphysis diameter (mm)	7.82	7.89	7.69	0.10	0.7544
Medullary canal diameter (mm)	5.02	5.03	4.91	0.08	0.8113
Lateral wall thickness (mm)	1.53	1.56	1.53	0.024	0.8673
Medial wall thickness (mm)	1.26	1.30	1.25	0.023	0.6610
Tibiotarsal index	35.75	36.27	36.17	0.43	0.8829
Bone-breaking strength, N	390b	409ab	450a	10.08	0.0396
Ash (%)	41.34	41.00	40.48	0.25	0.4180
Ca (%)	26.51a	21.18c	23.69b	0.61	0.0001
P (%)	12.92a	9.04c	10.83b	0.44	0.0001

a-b Values in the same row with different letters are significantly different ($p < 0.05$). 1T1: 100% Limestone (Control diet);

T2: 50% Limestone + 50% Eggshell; T3: % 100 Eggshell.

Table- 6: Effects of different calcium sources on broiler immunity organ at 42 days.

immunity organ	Treatments1			SEM	P-value
	T1	T2	T3		
Spleen %	0.14	0.12	0.15	0.01	0.5531
Bursa %	0.22	0.18	0.20	0.01	0.3164

1T1: 100% Limestone (Control diet); T2: 50% Limestone + 50% Eggshell; T3: % 100 Eggshell.

Means within a column, row and their interactions followed with the same letters are not significantly different from each other according to Duncan multiple ranges test at significant level of 5%.

Conclusion

The present study demonstrated that the use of eggshell as a source of Ca could be used instead of limestone without any negative effects on broiler performance, moreover it improves bone quality, broiler carcass and healthy bones

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

References

1- **Abdulla, N.R.; T. C. Loh; H. Akit; A. Q. Sazili; H. L. Foo; K. Y. Kareem; R. Mohamad and Abdul Rahim, R., 2017**. Effects of dietary oil sources, calcium and phosphorus levels on growth performance, carcass characteristics and bone quality of broiler chickens. Journal of Applied Animal Research, 45(1):423-429.

2- **Abdulla, N.R.; T. C. Loh; H. L. Foo; M. L. Alshelmani and Akit, H. 2019**. Influence of dietary ratios of n-6: n-3 fatty acid on gene expression, fatty acid profile in liver and breast muscle tissues, serum lipid profile, and immunoglobulin in broiler chickens. Journal of Applied Poultry Research, 28(2):454-469. https://doi.org/10.3382/japr/pfz008.

3- **Adeyemo, G. O.2013**.Growth performance of broiler chickens fed fossil shell growth promoter. Food and Nutrition Sciences, 4:16–19. DOI:10.4236/fns.2013.41004

4- **Ali, M. and W. Z. Badawy.2017**. Utilization of eggshells by-product as a mineral source for fortification of bread strips. Journal of Food and Dairy Sciences, Mansoura Univ., 8(11):455-459. DOI:10.21608/jfds.2017.38960

5- **An, S.H.; D. W. Kim and An, B. K.2016**. Effects of dietary calcium levels on productive performance, eggshell quality and overall calcium status in aged laying hens. Asian-Australasian Journal of Animal Sciences, 29(10):1477-1482. doi: 10.5713/ajas.15.0655 .

6- **Anwar, M.N.; V. Ravindran; P. C. Morel; G. Ravindran and Cowieson, A. J.2016**.Apparent ileal digestibility of calcium in limestone for broiler chickens. Animal Feed Science and Technology, 213:142-147. DOI: 10.1016/j.anifeedsci.2016.01.014

7- **Anwar, M. N.; V. Ravindran; P. C. H. Morel; G. Ravindran and Cowieson, A. J.2017**.Effect of calcium source and particle size on the true ileal digestibility and total tract retention of calcium in broiler chickens. Animal Feed Science and Technology, 224:39-45.

https://doi.org/10.1016/j.anifeedsci.2016.12.002.

8- **Association of Official Analytical Chemists. 1984**. AOAC Official Method of Analysis. USA.
9- **Association of Official Analytical Chemists. 1993**. Official Methods of Analysis of the AOAC. USA.
10- **Augspurger, N. R. and D. H. Baker.2004**.Phytase improves dietary calcium utilization in chicks, and oyster shell, carbonate, citrate, and citrate-malate forms of calcium are equally bioavailable. Nutr. Res., 24(4):293-301. https://doi.org/10.1016/j.nutres.2003.11.005.

11- **Blount, W.2013**.Poultry Nutrition, Food and Feeding. Page 103 in Intensive Livestock Farming. Elsevier. Amsterdam. Netherlands.
12- **Bugdayci, K. E.; H. Gümüş; M. N. Oğuz; F. Karakaş Oğuz 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.
13- **Bugdayci K. E; M. N. Oguz; F. Karakas Oguz; I. C. Uyguralp; and Akinci I. Y.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.
14- **Fallah, H.; A. Karimi; G. H. Sadeghi; N. Behroozi-Khazaei; H. Fallah; A. Karimi; G. H. Sadeghi and N. Behroozi-Khazaei.2018.**The effects of calcium source and concentration on performance, bone mineralisation and serum traits in male broiler chickens from 1 to 21 days of age. Animal Production Science 59(6):1090-1097. DOI: [10.1071/AN18166](https://doi.org/10.1071/AN18166)

15- **Gautron, J.; M. T. Hincke; K. Mann; M. Panhéleux; M. Bain; M. D. McKee; S. E. Solomon and Nys, Y.2001**.Ovocalyxin-32, a novel chicken eggshell matrix protein: isolation, amino acid sequencing, cloning, and immunocytochemical localization. Journal of Biological Chemistry, 276(42):243-252. https://doi.org/10.1074/jbc.M104543200.
16- **Glatz P and Z. Miao.2009**.High-value products from hatchery waste. Rural Industries Research and Development Corporation. Australia: Rural Industries Research and Development Corporation. Australia.

17- **Gongruttananun N.2011a**. Effects of eggshell calcium on productive performance, plasma calcium, bone mineralization, and gonadal characteristics in laying hens. Poult Sci., 90(2):524-529. doi: 10.3382/ps.2010-01003.

18- **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. Kasetsart Journal - Natural Science 45(3):413-421

19- **Gongruttananun, N.2011**. Effects of eggshell calcium on productive performance, plasma calcium, bone mineralisation, and gonadal characteristics in laying hens. Poultry Science, 90(2), pp.524-529. doi:10.3382/ps.2010-01003

20- **Hincke, M. T.; C. P. W. Tsang; M. Courtney; V. Hill and Narbaitz, R.1995**.Purification and immunochemistry of a soluble matrix protein of the chicken eggshell (ovocleidin 17). Calcif. Tissue Int., 56(6)578-583. doi: 10.1007/BF00298593.

21- **Jiang, S.; L. Cui; C. Shi; X. Ke; J. Luo and Hou, J.2013.** Effects of dietary energy and calcium levels on performance, egg shell quality and bone metabolism in hens. Vet. J., 198(1):252-258. doi: 10.1016/j.tvjl.2013.07.017

22- **Kim, S.W.; W. Li; R. Angel and Plumstead, P. W.2019**. Modification of a limestone solubility method and potential to correlate with in vivo limestone calcium digestibility. Poultry Science, 98(12):6837-6848. https://doi.org/10.3382/ps/pez423.

23- **Koutoulis, K. C.; I. Kyriazakis; G. C. Perry and Lewis, P. D.2009.** Effect of different calcium sources and calcium intake on shell quality and bone characteristics of laying hens at sexual maturity and end of lay. Int. J. Poult. Sci. 8:342-348.

24- **Lee, W. D.; D. Kothari; K. M. Niu; J. M. Lim; D. H. Park; J. Ko and Kim, S. K.2021.** Superiority of coarse eggshell as a calcium source over limestone, cockle shell, oyster shell, and fine eggshell in old laying hens. Scientific Reports, 11(1):1-10.

25- **Maranan, K.R.A.; C. M. Bueno; C. B. Adiova and Recuenco, M. C.2021**. Effect of increasing levels of eggshell powder on the production performance, carcass characteristics, and bone properties of broiler chicken. Philippine Journal of Veterinary and Animal Sciences, 47(2):1-15.

26- **Molnár A.; L. Maertens; B. Ampe; J. Buyse; J. Zoons 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. Poultry.

Sciences, 96(6):1659-1671. DOI:10.3382/ps/pew424

27- **Moura, G. R. S.; R. D. S. Reis; M. D. O. Mendoca; H. R. Salgado; K. D. S. Abreu; G. D. S. Madella and Lima, M. B. D.2020.** Substitution of limestone for eggshell powder in the diet of Japanese laying quails. Rev Bras Saúde Prod., 21:1-13.

28- **NRC.1994.**Nutrient Requirements for Poultry. National Academies Press. Washington (DC). USA.

29- **Obajuluwa, O.V.; K. A. Sanwo; L. T. Egbeyale and Fafiolu, A.O., 2020**. Performance, blood profile and gut morphometry of broiler chickens fed diets supplemented with Yohimbe (*Pausynistalia yohimbe*) and Larvacide. Vet. Anim. Sci., 10:1-10. doi: 10.1016/j.vas.2020.100127.

30- **Omole, A. J.; G. F. Ogbosuka; R. A. Salako and Ajayi, O. O.2005**. Effect of replacing oyster shell with gypsum in broiler finisher diet. J. Appl. Sci. Res., 1:245-248.

31- **Roland, D. A. and M. Bryant. 1996.** Influence of Calcium on Energy Consumption and Egg Weight of Commercial Leghorns. The Journal of Applied Poultry Research, 3(2):184–189. DOI: 10.1093/japr/3.2.184

32- **Ross Management Guide. Broiler management guide 308. 2009.** Available from: www.aviagen.com.

33- **Sasikumar, S. and R. Vijayaraghavan.2006.**Low temperature synthesis of nanocrystalline hydroxyapatite from egg shells by combustion method. Trends Biomater Artif Organs, 15(2):70-73.

34- **Venalainen, E.; J. Valaja, and Jalava, T.2006**. Effects of dietary metabolisable energy, calcium and phosphorus on bone miner-alisation, leg weakness and performance of broiler chickens. Br. Poult. Sci. 47(3):301–310. DOI: 10.1080/00071660600741776

35- **Williams, B.; D. Waddington; S. Solomon and Farquharson, C. 2000.** Dietary effects on bone quality and turnover, and Ca and P metabolism in chickens. Res. Vet. Sci. 69(1):81–87. DOI: 10.1053/rvsc.2000.0392