

EFFECT OF LIMESTONE SIZE PARTICLES ON IMPROVING EGGSHELL AND BONE PHYSICAL AND MECHANICAL CHARACTERISTICS AT THE PEAK OF EGG PRODUCTION IN GOLDEN MONTAZAH CHICKEN

MOSA, Sahar E.¹ and Khosht Abeer R.²

ABSTRACT

This study investigated the influence of different particle sizes of limestone in Golden Montazah layers diets on physical and mechanical properties of egg production, eggshell quality and breaking strength of tibia from 32 to 44 weeks of age. Limestone consisted of fine ($F < 0.6$ mm) and coarse ($C > 2.8$ mm-4.5mm). All hens take a basal ration of the same composition. The differences between the four equal groups were in the replacement percent of fine pulverised limestone by granular limestone, of coarser particle size. The control group takes the basal diet with only pulverised limestone as a calcium supplement. In the diet ration of the second group, 60% of the pulverised limestone was replaced with granular limestone, in the third group percent 80 % and in the last group 100% of fine limestone replaced with coarse particles of limestone. Significantly higher breaking force (31.69N), shell weight (5.65 g), shell thickness (0.46 mm) were found for eggs from the fourth group of hens than for eggs from the control group (29.46 N; 5.33 g ; 0.37 respectively). Tibia breaking strength was significantly higher (350.66 N) than those in control group (252.82 N). The results obtained in our investigation showed some possibilities of eggshell quality improvement using limestone of coarser particle size as a Ca supplement in the hen diet. Replacing 60-100% of fine limestone by coarser particle size limestone had positive effects on eggshell quality.

INTRODUCTION

Optimize the machines design and the effective utilization of the machines used in the transportation, processing, packaging and storage of agricultural products, the physical and mechanical properties of agricultural (animal and plant) materials must be known.

¹ Senior Researcher, Agricultural Engineering Research Institute (AEnRI), Agricultural Research Center (ARC), Dokki, Egypt.

² Researcher, Animal Production Research Institute, (ARC), Dokki, Egypt.

To preserve eggs during transport from farm to market, eggshells must be strong enough to prevent cracking (**Altuntas and Sekeroglu 2008**). Eggshell strength has been described using various variables such as thickness of eggshell, shell stiffness and rupture force. Eggshells must be strong enough to prevent broking in order to preserve the embryo until hatching. (**Altuntas, E. 2010**). The various egg physical properties such as egg mass and volume, surface area, shell thickness and weight affect the mechanical properties of chicken eggs. The quasi-static, undestructive compression of an egg between two parallel steel plates is a common technique for the measurement of the shell strength of a chicken egg. The strongest correlation was found between the physical and the mechanical properties of chicken eggs (**De Ketelaere *et al.* 2002**). Calcium is one of the essential minerals in poultry nutrition. In addition to its vital functions as the main component of bone structure and participation in acid-base balance and enzymatic system, calcium is also the main component of the eggshell. It is estimated that each egg contains 2.2g of calcium, present mainly in the eggshell. Ca supplementation is a key for eggshell quality, each eggshell contains up to 3g of Ca, so the diet of hens must contain adequate amount of Ca in efficiently utilizable form (**Roberts 2010**). Mineralized eggshell is formed of calcium carbonate (96%); the remaining components include organic matrix (2%), magnesium, phosphorus, and a variety of trace elements. The eggshell membranes will secrete and assemble during approximately one hour, resulting meshwork of interlaced fibers is composed of roughly 10% collagen and 70–75% other proteins and glycoproteins containing lysine-derived cross-links which are organized into morphologically distinct inner and outer sheets that enclose egg albumen. Egg size and the eggshell thickness are strongly related to each other (**Hincke *et al.* 2012**). While egg weight increases during the production period, eggshell thickness and breaking strength usually decrease. Eggshell quality depends on egg size and weight. Egg properties such as SI and shell thickness affect the proportion of damaged eggs during handling and transport (**Anderson *et al.* 2004**). These physical properties of eggs, and their resistance to damage through mechanical shock, can be characterized by measures such as rupture force, specific deformation, rupture energy and firmness (**Altuntas and Yildiz, 2007**). Calcium

supplementation is required in animal feeds, as most consist of grains and it's by products, which have very low calcium. Adequacy of the recommended quantities of dietary Ca for layers is still being studied. (**Castillo et al. 2004**). perceiving the valid NRC recommendations (1994) contradictory, carried out a research establishing the effect of 5 levels of Ca (from 2.93% to 4.82%) on production and quality of egg shell in a modern layer hybrid –white eggs, to determine the biologically optimal level to realize maximum production and egg shell quality. Based on research results, the authors reported that under given conditions, biologically optimal level of Ca necessary to realize the maximum production was 4.38%, for maximum egg shell quality 4.64%, and economically optimal level for maximum profit was 4.35% of Ca in diet. Many researchers in their studies start with the assumption which is becoming scientific proven fact, that the adequate Ca nutrition of laying hens does not include only the enough level of Ca in diet, but also enough source and particle size of Ca. Namely, it is considered that Coarser particles of Ca source are retained longer in the gizzard and are slowly being degraded and absorbed during this longer passage through digestive tract, whereas the smaller Ca particles pass faster through the digestive tract and therefore they get degraded/absorbed partially (**Svihus, 2011**). Limestone is the principle supplemental Ca source used in laying hen rations due to its abundant natural reserves, low cost and easy incorporation into layer feeds. Different aspects of shell quality were reported to be improved by the partial replacement of limestone with oyster shells in the diet (**Roberts, 2010**). Many factors influence eggshell quality, including the age of hens, strain of bird, nutrition, ambient conditions and diseases. About 95 % of the dry eggshell is calcium carbonate and, because of that, laying hens have a high dietary calcium requirement for shell calcification. The main source of calcium in hen diets is pulverized limestone. However, eggshell formation usually occurs at night when the hen is not eating, and satisfaction of needs is not adequate. It has been suggested that calcium absorption might be more sustained if the hen is given access to particulate calcium which might persist for longer in the gut after eating ceases (**Roberts 2010**). Coarser particle size of limestone led to an increase the soluble Ca at the end of

eggshell calcification in laying hens (**Zhang and Coon, 1997**). Replacing 50 % of ground limestone by coarse particle size limestone was found to be quite adequate in order to optimize eggshell quality (**Lichovnikova (2007)** recommended supplying two-thirds of the Ca in the diet as coarse particles to ensure eggshell quality in the last third of the laying period. (**Zhang and Coon 1997**) concluded that coarser particle size limestone with lower in vitro solubility was retained in the gizzard for a longer time, which may increase Ca retention. The change in the particle size of calcium sources has been studied with some positive results for the quality of eggshell (**Lichovnikova, 2007; Skřivan et al., 2010**) and bird bones when coarser particles of calcium sources are offered in the rations. (**Saunders-Blades et al., 2009**) presented the positive effects on eggshell quality in hens receiving a diet in which 60 % of pulverized limestone was replaced by two granular forms of limestone. By substituting 60 to 80% of lime with Coarse size lime particles in mixtures for nutrition of older layers, positive effects on the egg shell quality can be realized. (**Skřivan et al., 2010**) showed some possibilities of eggshell quality improvement using limestone of coarser particle size as a Ca supplement in the hen diet. Replacing 60-80 % of pulverised limestone by coarser particle size limestone had positive effects on eggshell quality. A slower solubilisation of sources of Ca would make Ca available during the time eggshell calcification and diminish bone Ca and p mobilization (**Skřivan et al., 2010**). **Wang et al., (2014)** Was writing a report that limestone with a Coarse particle size provided for Productive performance superior, egg quality and bone characteristics and is more suitable than oyster shell for practical applications in laying ducks. The aim of the present study was to compare the effects on egg production, eggshell and bone qualities using diets with different amounts of fine limestone replaced by coarse particles limestone at peak.

MATERIALS AND METHODS

The experimental work was done at El-Fayoum Research Station, Animal Production Research Institute, ARC, MOLAR The physical and mechanical properties of eggshells and bones were carried out at the Agricultural Engineering Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. One hundred and twenty eight 32

weeks old Golden Montazah chickens were randomly allocated to four groups (32 pullets per group were divided into four replicates) and housed in cages individually from 32 to 44 weeks of age.

The differences between the groups were in the replacement percent of fine pulverized limestone by granular limestone of coarser particle size. The two particle sizes of limestone grit were obtained from a commercial supplier of limestone to the poultry industry. Fine (F) and coarse (C) particles were ($F < 0.6$ mm) and ($C > 2.8-4.5$ mm), respectively. Limestone was screened through sieves which used (0.6 mm, 2.8 mm and 4.5 mm) Particle sizes of the limestone were measured manually to obtain samples with appropriate diameters. The first group (control) received the basal diet with only pulverized limestone as a calcium supplement. In the diet of the second group, 60 % of pulverized limestone was replaced with granular limestone, in the third group 80 % was replaced by granular limestone and in the last group 100%. All groups received a basal diet (Table 1) of the same composition.

Experimental diets were balanced and formulated to be is caloric and is nitrogenous with only the Ca particle sizes of limestone into diets. The diet was analyzed according to (AOAC, 1990).

An amorphous limestone that contained 90% CaCO_3 and 36% calcium was used. At 17 weeks of age the hens were subjected to 16.30 hours of light. Body weight was obtained by weighing hens at beginning and at the end of the experimental period. Egg production was recorded daily. Feed intake and egg weight were recorded weekly. Egg weights were recorded for all eggs produced and used to calculate mean egg weight for the experimental period. Egg mass was calculated from collecting data of egg production and egg weight at biweekly via the following formula:

$$\text{EM (egg mass)} = \text{EP (egg production)} \times \text{EW (an egg weight)}.$$

From these samples three eggs were selected per plot for determining quality and characteristics (avoiding broken, cracked, or dirty eggs). The eggshell quality parameters, the geometric mean diameter (D_g), sphericity (Φ), volume (V) and surface area (S) of eggs sampled were determined.

The length and width (thickness) of the chicken eggs were measured by a dial micrometer with an accuracy of 0.01 mm (Mitutoyo, 0.01 mm, Japan). To obtain the unit mass of the hens' eggs, they were measured by an electronic balance with an accuracy of 0.001 g. The geometric mean

diameter (D) of the chicken eggs was calculated by using the following relationship as seen in Eq. (1) (**Mohsenin 1970**)

$$D_g = (LW^2)^{1/3} \dots\dots\dots(1)$$

where L is the length and W is the width (thickness) in mm.

The sphericity (Φ) is defined as the ratio of the geometric mean diameter of egg sample to the length of the egg (**Mohsenin 1970**). The surface area of the chicken eggs was found by analogy with a sphere of the same geometric mean diameter, using an expression cited by **Altuntas and Sekeroglu (2008)**.

$$S_a = \pi (D_g)^2 \dots\dots\dots(2)$$

$$\phi = \{ (LW^2)^{1/3} / L \} \times 100 \dots\dots\dots(3)$$

Where S_a is the surface area in mm and D is the geometric mean diameter in mm. Egg shape index is defined as the ratio of the width of a sample of egg to the length of the egg (**Anderson et al. 2004**). The shell thickness was determined according to **Monira et al (2003)**. Eq. 2 and 3

The volume of the egg was determined by two methods: (1). Measurement of the volume of eggs with a graduated measuring 100 ml volumetric flask and (2), using the method of **Baryeh and Mangope (2003)** by measuring the dimensions of length and width and calculated as follows:

$$V = \pi / 6 \times LW^2 \dots\dots\dots(4)$$

Eggshell breaking strength was measured using The digital force gauge (model: FGN-50) to measure shear strength and breaking force for eggshells and bones

Eggs were then broken, and eggshell, and yolk were separated and weighed. Eggshells were dried in air in order to determine the eggshell thickness (including the membrane). In three points on the eggs (one point on air cell or the randomized two points of equator), using a digital micrometer. Eggshells were weighed using a 0.001g precision scale.

To determine egg traits, yolks were separated and weighed on the precision scale AD1000. Eggshells were washed, left to dry for 48 hours, and then weighed. Percentages of yolk and shell were obtained as the ratio between the weight of each portion and egg weight; and the percentage of albumen was determined as following:

$$\% \text{ albumen} = 100 - (\% \text{ yolk} + \% \text{ shell}) \dots\dots\dots(5)$$

The analyzed variables were volume, density, color and haugh unit, whereas the egg trait variables were shell thickness and percentages of yolk, shell, and albumen (%).

After weighing, eggs were cracked and put onto a glass surface, for measuring the height of thick albumen using a micrometer. Albumen - height and egg weight were utilized in the calculation of Haugh units (HU) by the equation

$$HU = 100 \log (H + 7.57 - 1.7W^{0.37}) \dots \dots \dots (6)$$

Where: H is the albumen height (mm); and W is the egg weight (g).

Tibia of the right leg was removed, cleaned of flesh, and air dried (72 h) for determination of mean tibia weight and strength. Bone-breaking strength is commonly used as a response criterion for assessing bone mineralization.

Table (1): percentage and calculated compositions of the experimental diets.

Ingredients	%
Corn	63.56
Soybean meal 44%	24.65
Wheat bran	2.55
Limestone	6.97
Salt	0.3
Vit. &Min.	0.4
Di calcium phosphate	1.51
DL- Methionine	0.06
Calculated analysis :	
Protein	16.15
Met. Energy(kcal/kg)	2714
Calcium %	3.02
Av. Phosphorus	0.409
Lysine %	0.895
Methionine	0.351

* Vitamin and mineral premix at (0.1 % Vit. and 0.3% Min.) of the diet supplies the following per kg of the diet: Vit. A 14000000 IU, Vit. D3 3000000 IU, Vit. E 8000 mg, Vit K 4000 mg, Vit B1 3500 mg, Vit B310000 mg, Vit. B6 3500mg, Vit. B12 30 mg, Biotin 300 mg, Pantothenic acid 20000 mg, Nicotinic acid 5000 mg, Folic acid 2000 mg , & Colene 500000 mg , Mn 100000 mg , Zn 80000 mg, Fe 50000 mg, Cu 12000 mg, I 1000 mg, Se 300 mg, Co 300 mg .

Breaking strength of the middle of the bone was determined with a materials tester (Instron 4411, Instron Corporation, Grove City, PA) using

software version 8.09, a standard 50-kg load cell, and a modified shear plate (8cm in length and 1 mm in width), as described by **Riczu et al., (2004)**. The digital force gauge (model: FGN-50) was used to measure breaking force and shear strength for eggshells and bones

Statistical Analysis:

Data were subjected to ANOVA using General Linear Model procedure (GLM) in SPSS16.0. Duncan’s multiple range tests were applied to separate of different treatment. Statements of statistical significance are based on a probability of $p < 0.05$.

RESULTS AND DISCUSSION

Table (2) shows the effects of different particle sizes on egg production and egg weight during the experimental period.

A significantly higher egg production % (69.20, 68.40%) and egg weight (50.57, 50.29g) occurred in eggs from the fourth and first groups than in those from the second and third groups fed a diet without (60% and 80%) granular limestone.

These results are in accordance with the findings of **Phirinyane et al., (2011)** reported a significant ($p < 0.05$) increase in egg production from 18-21 weeks of age and thereafter egg production remained constantly high. Contrary to the present findings **Cheng and Coon (1990)** and **Kuleile et al., (2009)** who concluded that limestone particle size had no effect on egg production performance. **Mc Daniel (1983)** reported a non-significant ($p < 0.05$) increase in egg production for hens fed oyster shell from 21 to 30 weeks of age.

Table (2): The effect of different limestone particle sizes on the production parameters of the layers during the experimental period.

Parameters	Particle size ratios (Coarse : fine)			
	0%	60%	80%	100%
Egg production %	69.20 ± 1.804 ^a	60.69 ± 1.161 ^c	62.17 ± 1.251 ^b	68.40 ± 1.137 ^a
Egg weight (g)	50.57 ± 0.123 ^a	49.49 ± 0.156 ^b	50.78 ± 0.223 ^a	50.29 ± 0.103 ^a

There was no mortality during the experiment. Cracked and shell-less eggs presented 3-4% of the total egg production during the experimental period. **Phirinyane et al., (2011)** reported that most of cracked or shell-

less eggs were recorded for treatment with fine particles, indicating that smaller particle sources such as pulverized CaCO_3 pass quickly through the digestive tract resulting in the bird not able to sufficiently extract enough calcium to meet its needs. The influence of limestone Particle size distribution on physical characteristics of egg length, Egg diameter (mm), egg volume (cm^3), egg weight (g) and density (g/cm^3) are presented in Figs 1-5 and table 3.

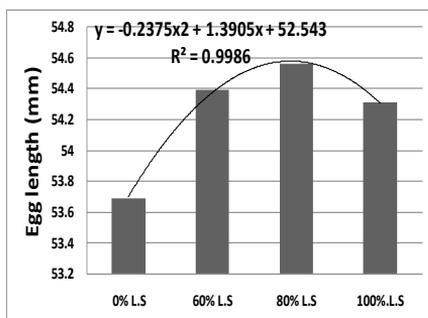


Fig.1.The effect of particle size ratios (coarse : fine) on egg length (mm)

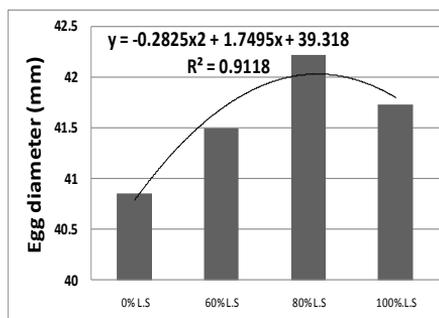


Fig.2.The effect of particle size ratios (coarse : fine) on egg diameter (mm)

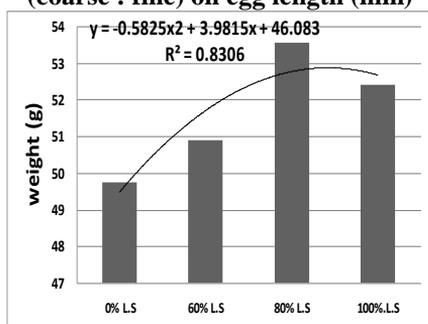


Fig.3.The effect of particle size ratios (coarse : fine) on egg weight (g)

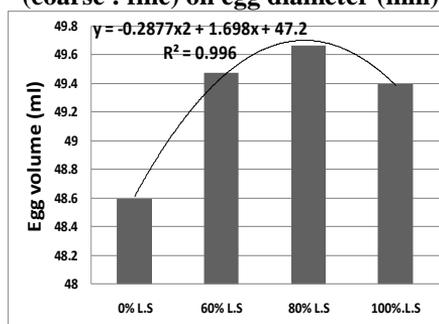


Fig.4.The effect of particle size ratios (coarse : fine) on egg volume (ml)

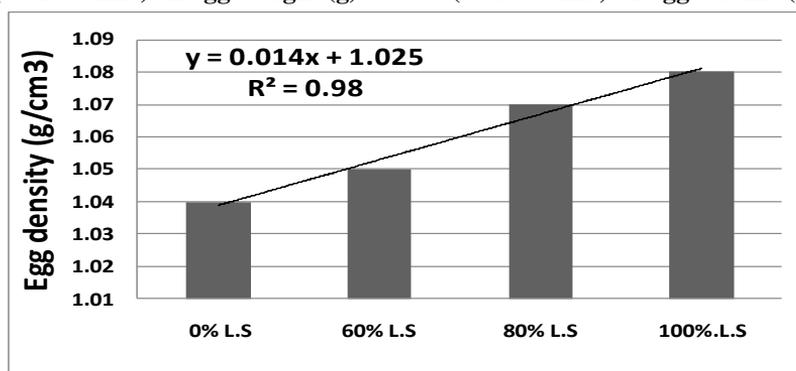


Fig.5.The effect of particle size ratios (coarse : fine) on egg density (g/cm^3)

No significant differences occurred in these characteristics ($p < 0.05$), which agreed with **Wang et al., (2014)** who found that particle size did not egg shape index. Differences in shell weight were not significant. A significantly higher shell weight (5.65, 5.55) and shell thickness (0.47, 0.46) occurred in eggs from the fourth and third groups (100%, 80% pulverized limestone replaced by granular limestone) than in those from the first group fed a diet without (0%) granular limestone Fig 6. **Krasucki et al., (2002)** confirms that particles of calcium carbonate in the diet increased the thickness of eggshells at 33 weeks of age and at 60-80% limestone supplementation, which agreed with **Koreleski and Swiatkiewicz (2004)** for the period of peak egg production in hens.

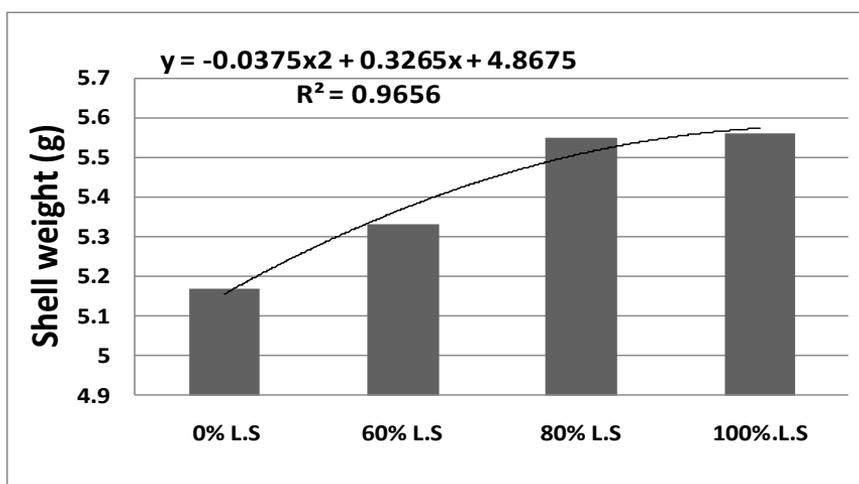


Fig.6.The effect of particle size ratios (coarse : fine) on shell weight (g)

It seems from Fig.7 that different ratios of limestone particle size influence the breaking force of the shell. A significantly ($p < 0.05$) higher shell breaking force (31.69, 30.19) were observed in eggs from the fourth and third group which received diets with (100%, 80% coarse particles replaced by fine limestone) than in those from the first group fed a diet without (0%) granular limestone.

Skrivan et al. (2010); and **Wang et al. (2014)** found that LP Coarse particle of limestone increased the breaking strength of the shell ($p < 0.05$), and improved the appearance of eggs based on increased albumen height and Haugh units ($p < 0.01$). The degree of egg yolk pigmentation was

improved under the influence of limestone grit in the diet. It is likely that the presence of coarse particles in the gizzard caused better destruction of feed cells and more efficient liberation of xanthophyll especially in the period of high laying.

Table (3): The influence of limestone particle size on egg physical characteristics at peak production.

Parameters	Particle size ratios (coarse : fine)			
	0%	60%	80%	100%
Egg weight (g)	49.75±0.8 ^b	50.51±0.9 ^{ab}	53.59±1.2 ^a	25.4±0.9 ^{ab}
Egg volume (ml)	48.59±0.2	49.48±0.9	49.77±0.8	49.40±0.8
Egg length (mm)	53.69±0.4	54.39±0.5	54.56±0.6	54.31±0.3
Egg diameter (mm)	40.85±0.2 ^b	41.49±0.3 ^{ab}	42.22±0.4 ^a	41.73±0.3 ^{ab}
Egg density (g/cm ³)	1.04±0.1	1.05±0.01	1.07±0.01	1.08±0.03
Shell weight (g)	5.17±0.1 ^b	5.33±0.15 ^{ab}	5.55±0.1 ^a	5.65±0.2 ^a
Shell percentage (%)	10.93±0.1	10.56±0.5	10.70±0.5	10.03±0.3
Yolk percentage (%)	30.25±0.4 ^b	31.03±0.6 ^b	30.53±0.5 ^b	32.34±0.7 ^a
Albumen percentage (%)	58.81±0.5	58.41±0.8	58.76±0.7	57.61±0.6
Yolk color	6.80±0.3 ^b	6.80±0.5 ^b	6.82±0.3 ^a	7.21±0.5 ^a
Yolk height	17.15±0.2	16.66±0.2	16.73±0.2	17.08±0.3
Albumen height	5.42±0.2 ^{ab}	5.40±0.2 ^c	5.94±0.3 ^b	7.11±0.2 ^a
HU	74.49±1.7 ^{ab}	72.73±2.3 ^c	86.70±1.3 ^a	79.67±1.4 ^b
Shell thickness (mm)	0.37±0.02 ^b	0.43±0.01 ^a	0.47±0.01 ^a	0.46±0.02 ^a
Sharp end	0.338±0.01	0.337±0.01	0.361±0.01	0.358±0.01
Equator	0.372±0.01 ^{ab}	0.359±0.01 ^b	0.383±0.01 ^a	0.352±0.01 ^b
Blunt end	0.351±0.01 ^a	0.329±0.01 ^b	0.353±0.01 ^a	0.318±0.01 ^b

a,b,c Mean within a row for each item with different superscripts are significantly different($p \leq 0.05$)

The effect of particle size ratios on mechanical characteristics are shown in Fig.8. and table 4 the coarser particle size of limestone increased ($p < 0.05$) the breaking strength of fresh tibia at 80% and 100% replacement of fine limestone when compared with the control diet .These results are in accordance with the finding of **Koreleski and Swiatkiewicz (2004)** they showed that coarse particle limestone improved tibial strength. **Wang et al., (2014)** stated that inclusion of limestone rather oyster shell did increase the breaking strength of the tibias ($p < 0.05$), but

not the other tibial characteristics. Contrary to the present findings , **De Witt, et al., (2009)** indicated that coarse particle size limestone had no beneficial effect on egg production and egg shell quality characteristics of laying hens at later stage (>54 weeks of age) of production . (> 2.0 – 3.8 mm) limestone particles in a layer diet does not influence egg production and egg shell quality at 24 weeks of age .Overall, the best tibial indices were obtained from use of limestone of coarse particle size. The results obtained in our experiment showed the possibilities of improving egg shell quality using limestone of coarser particle size as a Ca source in diets for hens. **Phirinyane et al., (2011)** concluded that the ratio of fine (<1.0 mm) and coarse.

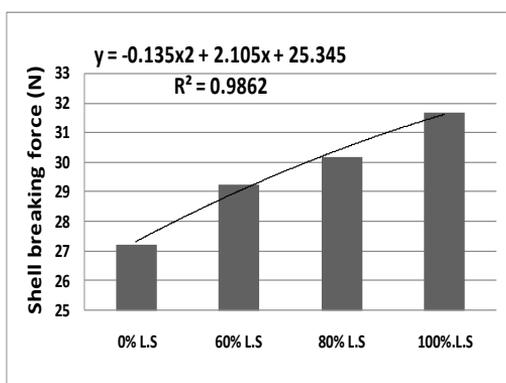


Fig. 7.The effect of particle size ratios (coarse : fine) on shell breaking force (N)

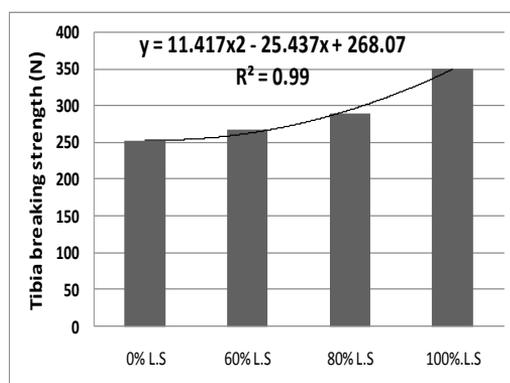


Fig.8.The effect of particle size ratios (coarse : fine) on Tibia breaking strength (N)

Table (4): The influence of limestone particle size on shell and tibia breaking strength during the experimental period.

Parameters	Particle size ratios (Coarse : fine)			
	0%	60%	80%	100%
Shell breaking force (N)	29.47 ± 1.176 ^{ab}	27.23 ± 1.011 ^b	30.19 ± 1.380 ^{ab}	31.69 ± 2.009 ^a
Tibia breaking strength (N)	252.83 ± 21.986 ^b	276.87 ± 20.633 ^b	298.51 ± 12.701 ^b	350.67 ± 20.718 ^a

a,b, Mean within a row for each item with different superscripts are significantly different(p≤0.05)

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الملخص العربي**تأثير جزيئات حجم الحجر الجيري علي تحسين جودة البيض والقشرة والعظام في ذروة إنتاج البيض في دجاج المنتزه الذهبي.**سحر السيد أحمد موسى^١ و عبير ربيع محمد خشت^٢

اجريت هذه الدراسة بمحطة بحوث الدواجن بالفيوم لمعرفة تأثير إستبدال الأحجام الجزيئية المختلفة للحجر الجيري كمصدر للكالسيوم في علائق دجاج المنتزه الذهبي البياض من عمر ٣٢ - ٤٤ اسبوع، وتأثير ذلك على الخواص الميكانيكية والفزيائية وجوده البيض والعظام، كذلك درجة صلابة كلا من قشرة البيض وعظمة القص. وأجريت القياسات الفزيائية والميكانيكية بمعهد بحوث الهندسة الزراعية. تم إستخدام أقطار مختلفة من الحجر الجيري ناعم أقل من ٠,٦ مم وكبير الحجم (أكبر من ٢,٨ مم وأقل من ٤,٥ مم بمستويات إستبدال صفر %، ٦٠%، ٨٠%، ١٠٠% من الحجر الجيري الناعم) ولقد أظهرت النتائج المتحصل عليها أن إستخدام الحجر الجيري بجزيئات كبيرة الحجم أدت إلى زيادة في كل من صفات جودة القشرة (سمك القشرة ووزن القشرة ودرجة الصلابة) هذا بالإضافة إلى زيادة قوة صلابة الساق في المعاملات المقدم لها الحجر الجيري بأحجام جزيئية كبيرة عن المجموعة الكنترول وبالتالي توصي الدراسة بإمكانية إستبدال الحجر الجيري الناعم كمصدر للكالسيوم بجزيئات كبيرة الحجم بنية إستبدال تصل إلى ١٠٠%.

^١ باحث أول ، معهد بحوث الهندسة الزراعية ، مركز البحوث الزراعية.^٢ باحث، معهد بحوث الانتاج الحيوانى ، مركز البحوث الزراعية.