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Use of various sources of calcium in the diets of broiler and its effects on carcass and some meat quality

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ABSTRACT
The study was aimed to assess the impact of adding eggshells as calcium sources in broiler diets on carcass traits, meat quality, and chemical composition, three hundred one-day-old broiler chicks were randomly designed CRD. Each treatment included five replications and each replicate containing twenty birds. The dietary of first treatment was control (0% eggshell) the second and the third treatments were eggshell powder as a replacement for limestone at 50% and 100%, respectively. .The chickens were fed a basal diet during the starting and finished periods. Ten broilers were chosen randomly from each treatment group and slaughtered at age 42 days to evaluate meat quality. The results showed that no significant differences ($p \leq 0.05$) between treatments regarding pre-slaughter in live body weight, carcass weight, and percentage of each carcass cuts weight, as well as immunological organs such as the spleen and bursa. However, a substantial difference in dressing percentage was observed. Except for pH, yellowness, and chroma, no significant differences were noticed in drip loss, cooking loss, lightness, redness, color, tenderness. Meanwhile, the bird chemical composition treatments had no significant differences in moisture, protein, and ash percentages. However, there was a considerable change in fat %. Except for broilers' pH, yellowness, and chroma, the substitution of eggshell powder for limestone resulted in comparable carcass characteristics, chemical composition, and meat quality. However, there was a significantly differ in fat %. Except for broilers' pH, yellowness, and chroma, the substitution of eggshell powder for limestone resulted in comparable of some carcass characteristics, chemical composition, and meat quality.

استخدام مصادر مختلفة من الكالسيوم في علائق فروج اللحم واثرها في صفات الذبيحة ونوعية اللحم

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خلاصة

هدفت الدراسة على تقييم تأثير إضافة قشر البيض كمصدر للكالسيوم في عليقة فروج اللحم على صفات الذبيحة و نوعية اللحم والتركيب الكيميائي. تم توزيع 300 فرخا بعمر يوم واحد غير مجنسة عشوائيا على ثلاث معاملات وبواقع خمس مكررات لكل مكرر20 فرخا استمرت لمدة 42 يوما. غذيت الافراخ على ثلاث معاملات : المعاملة الاولى مصدر كالسيوم فيها هو حجر الكلس اما المعاملة الثانية فقد استخدم كل من حجر الكلس و قشرة البيض و بنسب (50 %) لكل منهم و المعاملة الثالثة استخدم فقط قشرة البيض بالنسبة (100 %). وتم استخدام تصميم العشوائي الكامل لتحليل البيانات. لتقييم نوعية اللحوم، تم اختيار 10 طيور عشوائيا من كل المعاملة و ذبحها عند عمر ة 42 يوما. اظهرت نتائج الدراسة عدم وجود فروقات معنوية بين المعاملات في وزن الجسم الحي قبل الذبح ووزن الذبيحة ونسبة وزن كل جزء من الذبيحة وكذلك الأعضاء المناعية مثل الطحال والفابريشا. .ومع ذلك ، لوحظ وجود فروق معنوية في نسبة التصافي باستثناء الأس الهيدروجيني ولون اللحم، لم يلاحظ أي اختلافات في قابلية اللحم لحمل الماء والطراوة. لم يكن لمعاملات تأثير معنوي على التركيب الكيميائي للحم الفروج كنسب الرطوبة والبروتين والرماد. الا ان التغيير كانت معنوية في نسبة الدهون. أدى استبدال مسحوق قشر البيض بالحجر الكلس إلى تحسين بعض صفات الذبيحة ، والتركيب الكيميائي و نوعية اللحم .

الكلمات المفتاحية: فروج اللحم ، قشرالبيض ، الذبيحة ، نوعية اللحوم ، التركيب الكيميائي للحوم

INTRODUCTION

The eggshell is essential in protecting the egg's integrity and the embryo's survival. However, once the egg's contents have been extracted, eggshells become wastes that significantly contribute to environmental degradation. According to (Swamiappan and Vijayaraghavan., 2006), the eggshell accounts for around 11% of the whole egg. Due to limited dumping parts, the high cost of disposal, and environmental concerns, the enormous quantity of eggshell trash contributes to the waste disposal problem in numerous nations (Glatz and Miao, 2009). Therefore, recycling eggshells into valuable items will provide opportunities to address economic and environmental difficulties and concerns. Eggshell is rich in calcium and other minerals (Ali and Badawy, 2017) and contains low proteins (Gautron et al., 2001; Hincke et al., 1995).

Limestone is the principal source of calcium in poultry diets; it is abundant and affordable (Blount, 2013). It has been observed that limestone in feed can give more than fifty percent of the total Ca in broilers' diets (Kim et al., 2019). Limestone is the predominant inorganic source of Ca in the diet of broilers. However, limestone has drawbacks, such as limited solubility, thus low bioavailability due to the enhanced acid-binding capability of the diet (Anwar et al., 2016a). Limestone and oyster shells are substantial sources of Ca used in poultry feeds and contain 380 g/kg Ca (NRC, 1994). However, studies indicate that the Ca increase availability for broiler chickens varies considerably (Augspurger and Baker 2004; Anwar et al. 2016, 2017). However, few studies have been conducted on eggshells' effects on broiler chickens' diets.

Consequently, this study aimed to assess the carcass features, meat quality, and chemical composition of meat in broiler chickens fed diets containing increasing quantities of eggshell powder as a calcium substitute for limestone.

MATERIALS AND METHODS

The research was conducted at the Grdarasha field farm of the University of Salahaddin-Erbil/College Iraq's of Agricultural Engineering Sciences. Between November 23th, 2021, and January 5th, 2022. Over 42 days (6 weeks), 300 one-day-old Ross 308 straight-run commercial broiler chicks were randomly assigned to three treatments in a completely randomized design (CRD). Each treatment included five replications, each replication consisting of twenty chicks. The chickens were grown according to industry specifications (Ross Management Guide, 2009). The research employed a two-phase feeding strategy (the starter and finisher rations began from 1 to 21 and 22 to 42 days of age, respectively). Using the Feed LIVE software, diets that meet the nutritional requirements of broiler chicks were formulated (Feed LIVE 1.52, Thailand). All of the experimental diets included the same amount of calories and nitrogen. The birds will be provided alternative diets that combine eggshells and limestone as calcium sources. The experimental diets were T1: 100% Limestone (100 LS), T2: 50% Limestone-50% Eggshell (50:50 LS-ES), and T3: 100% Eggshell (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. The AOAC (1993) Official Methods of Analysis were utilized for the proximate analysis of eggshell powder, while the AOAC (1984) Official Methods of Analysis were utilized for sample preparation for calcium and phosphorus analysis.

Vaccine Program

At six days, the birds were vaccinated against Newcastle disease virus (NDV) or (Lasota vaccine) via drinking water and spraying, infectious bronchitis virus (IBV) via drinking water and spraying, and Newcastle disease (N.D.) via drinking water and spraying at 19 days.

Table 1: Ingredients composition and calculated nutrients analysis of experimental starter diets

Item		Dietary Treatments %		
		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 Premix[2]		0.250	0.250	0.250
Mineral Premix		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
Fibre	%	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

[1]LSlimestone (Control diet); LS-ES, 50% limestone + 50% eggshell; ES, 100% eggshell. [2]Vitamin 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.

Table 2: Ingredients composition and calculated nutrients analysis of experimental finisher diets

Item	Unit	Dietary Treatments % 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 Premix[2]		0.100	0.100	0.100
Mineral Premix		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
Fibre	%	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

[1]LSlimestone (Control diet); LS-ES, 50% limestone + 50% eggshell; ES, 100% eggshell. [2]Vitamin 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.

Carcass Traits

After the feeding experiment, two birds were randomly selected for carcass evaluation. The birds were starved for 12 hours before slaughter, and their live weight was determined by using a platform scale. The birds were outfitted in the same field. After slaughtering the birds, they were scalded for 30 seconds in a 55°C to 60°C water tank before being plucked and eviscerated. To determine the post-slaughter hot carcass weighted without giblets, the feet, shanks, neck, and head were removed. Giblets represent the total yield of the removed and weighed liver, heart, and gizzard, as well as the fat pad concerning body weight. The carcasses were weighed relative to their living weight the yields of various commercial sections were assessed by dissecting carcasses (breasts, thighs, and wings). Calculated cuts yield expressed as a percentage of carcass weight (Abdulla, 2016). The breast fillets evaluated the meat quality metrics of pH, color, water-holding capacity, cooking loss, and shear force.

Meat quality

pH, drip loss, cooking loss, meat tenderness, and meat color were evaluated for meat quality assessment according to the method specified by Abdulla et al. (2017) and Kareem et al. (2015); nevertheless, a quick overview of all the above tests/measurements is provided below.

Measurement of muscle pH

The pH of the frozen breast muscle solution was determined (Mettler Toledo, AG 8603, and Switzerland). Approximately 30 grams of the crushed meat were extracted. According to American Meat Science Association, the pH of each sample was determined for each replicate (AMSA 2012).

Measurement of drip loss

At day 0, an approximately 40 g fresh breast muscle sample was obtained, weighed, and the weight was recorded as the beginning weight (W1). The meat sample was vacuum-sealed in a plastic bag and stored at 4°C in a refrigerator. After precise postmortem storage conditions were established, the samples were removed from the bags, dried with tissue, and the final weight (W2) was determined. According to Honikel (1998), the percentage of drip loss was calculated in the following manner: Drip loss%=[(W1-W2)/W1^{X} 100

Cooking loss

The weight of the breast muscle samples was recorded as the beginning weight (W1). The muscle samples were then placed in a plastic bag, vacuum-packed, and cooked in an 80°C water bath for 20 minutes. The samples were weighed after being dried using tissue paper without being pressed (W2). The following is how the cooking loss was calculated:

Cooking loss %=[(W1-W2)/W1^{X} 100

Measurement of meat tenderness

Using the same breast muscle sample used to evaluate the cooking loss, the softness of the meat was determined. To prevent evaporation, the sample was placed in a plastic bag and refrigerated overnight at 4 C°. After one day, the cooked sample was split into at least three subsamples (blocks) with the long axis aligned with the orientation of the muscle fibers (Kareem et al. 2015). According to (Cavitt et al.,2004) each subsample was sheared perpendicular to the muscle fibre utilising a T.A. H.D. plus® texture analyser with a Volodkevitch blade set (2004). The average shear force value was recorded for each sample block.

Measurement of meat color

Before color analysis, breast muscle samples were flowered for 25 to 30 minutes at 27c^{o}. The color coordinates were determined using an AMSA technique and a Color Flex spectrophotometer (Hunter Lab Reston, VA, USA). The device was calibrated against black and white reference tiles prior to use. Each sample received three L*(lightness), a* (redness), b* (yellowness), c, and h measurements (the cup rotated 90 degrees in the second and third readings). The average value for each sample was then calculated (Hunt 1980).
Chemical composition of meat
According to AOAC procedures, the proximate composition of the broiler meat samples was analysed in triplicate (2000).

Moisture determination in meat
Individual meat samples were weighed (about 20 g), and their initial weight was recorded (W1). The samples were dried in an oven at 75 C^{O} for 48 hours. After achieving a stable weight, samples were immediately weighed, and W2 was recorded. The moisture percentage was calculated by dividing the difference between the initial sample weight and the sample weight after 48 hours of drying.

$$\text{Moisture (\%)} = [(W1- W2) \div W1] \times 100$$

Determination of protein in meat
The tested for protein concentration. The Kjeldahl method was used to measure crude protein concentration. The process consisted of three straightforward steps: digestion, neutralisation, and titration. The organic component of the meat sample (1 g) was digested with solid sulfuric acid at 420 0C for two hours in the presence of two catalyst tablets VST (code A00000277; 3.5 g K_2 SO_4, 0.0035 g Se) in order to convert the total nitrogen to ammonium sulphate (digestion stage). During the neutralisation or distillation stage, ammonium hydroxide changed the nitrogen in the digested solution to ammonia hydroxide, which was then distilled with a boric acid solution and converted to ammonium borate, which was titrated with concentrated hydrochloride acid (titration stage). Since the Kjeldahl method does not directly measure protein concentration, the following equation was used to determine the nitrogen (N) concentration of a meat sample weighing m grammes and titrated with xM HCl acid solution: % N =x moles × (Vs - Vb) cm3 × 14g / cm3×mg ×100
Where Vs and Vb are the titration volumes of the sample and blank, and 14g is the molecular weight of nitrogen N. Once the nitrogen content was determined, it was converted to a protein content using the following equation:

$$\text{Protein (\%)} = N \times 6.25 \text{ (equivalent to 0.16 g nitrogen per gram of protein)}$$

Determination of fat in meat
The fat content of samples of dried meat samples was determined using hexane and the Soxhlet extraction technique. The sample of dried meat was weighed individually (about 1 g) and recorded as the initial weight (W1) before being put onto a pre-weighed and dried filter paper (W2). The substance was then placed into a distillation path or extraction tube. After the water passing through the condenser was drained, the cleaned distillation flask was filled with hexane to a level of 34, then connected to other components of the Soxhlet

apparatus and put on a heat source. After the hexane began to evaporate in the condenser and was added to the meat sample in the distillation path, the fat extracted from the sample and the hexane packed with fat returned to the distillation flask, reaching the end of the side tube of the distillation path (siphon). The syphon is performed five to ten times per hour for three hours. After hexane extraction, the meat sample was dried, refrigerated, and reweighed (W 3). The following formula is used to calculate the fat concentration percentage:

$$\text{Fat (\%)} = [(W2 - W3) \div W1] \times 100$$

Determination of ash in meat

For ash determination, fresh and frozen meat samples were individually weighed and recorded as initial weight (W1) and placed into a dried and pre-weighed porcelain crucible (W2). The samples were then burned in a muffle furnace at a temperature of 550°C for 48 h. The burned samples were removed from the muffle furnace, equilibrated to room temperature in a desiccator and reweighed (W3). The ash percentages were calculated using the following equation:

$$\text{Ash (\%)} = [(W3 - W2) \div W1] \times 100$$

Data 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 three treatments' main effects were tested with five replications on carcass yield and meat quality. The overall level of statistical significance was set at $p<0.05$ for carcass composition and meat quality.

RESULT AND DISCUSSION

The effect of eggshell experimental diets on the carcass characteristics of broiler chickens is shown in Table 3. Except for the dressing percentage, no significant differ ($P<0.05$) were seen for any of the analyzed characteristics, including carcass weight and percentage of carcass weight (breast%, thigh%, wing%, back%, neck%, heart%, gizzard%, and liver%). These results concurred with those of Maranan et al., 2021; they demonstrated that increasing eggshell as a replacement for limestone does not affect carcass weight or any of its components. This result was consistent with the findings of Omole et al. (2005). In their trial, broiler hens were fed escalating amounts of gypsum as an oyster shell substitute. Their findings suggested that no significant changes existed between treatments.

In carcass evaluation and production, Al Daraji et al. (2011) reported that birds fed an extremely low or excessively high amount of dietary Ca had a reduced carcass weight due to a drop in BW and BWG, which was validated in T3 birds. However, when the eggshell content of the meal grew, the dressing % fell linearly. Comparing the groups revealed that the birds fed eggshell-containing treatments had a reduced dressing % compared to the control group. This is related to the poor performance of broilers fed diets containing eggshells during the finisher stage, which may reduce carcass quality. At the finisher stage, these birds gained less weight and were less productive than the control group.

Table 3: Effects of calcium on carcass characteristics in broiler birds at the finisher phase

Parameters	**Treatments[1]**			**SEM**	**P-value**
	T1	T2	T3		
Pre-slaughter body weight, g	2232.00	2322.00	2380.00	40.05	0.3259
Carcass weight, g	1897.00	1879.00	1907.00	30.95	0.9367
Body weight with offal, g	2024.69	1997.14	2033.03	32.45	0.9009
Dressing percentage, %	85.24^{a}	81.06^{b}	80.06^{b}	0.80	0.0143
Breast, %	37.68	39.30	37.55	0.41	0.1495
Thigh, %	27.89	27.81	29.00	0.32	0.2405
Wing, %	11.04	10.60	11.12	0.16	0.3693
Back, %	18.89	18.32	18.45	0.26	0.6681
Neck, %	4.50	3.96	3.88	0.21	0.4413
Liver, %	2.60	2.30	2.37	0.07	0.1383
Cizzard, %	2.41	2.13	2.25	0.06	0.1199
Heart, %	0.74	0.68	0.68	0.02	0.3038
Spleen, %	0.14	0.12	0.15	0.01	0.5531
Bursa, %	0.22	0.18	0.20	0.01	0.3164

$^{a-b}$ Values in the same row with different letters are significantly different ($p < 0.05$). [1]LS, 100% limestone (Control diet); LS-ES, 50% limestone + 50% eggshell; ES, 100% eggshell.

Table 4 showing the effect of eggshell levels on broiler meat quality. The drip loss, cooking loss, lightness, redness, color and tenderness parameters of all experimental broiler chicks showed no statistically significant differences.

As the eggshell replaced the limestone, the pH increased significantly while the yellowness and chroma decreased. The most effective treatments were the ones that served as control. Even though Li et al. (2016) evaluated cooking loss and found no significant differences between groups of broilers fed varying doses of Phosphor, Wang et al., 2021 demonstrated the effects of dietary Ca and NPP on meat quality-related parameters. Ca intake significantly affects the breast muscle's lightness and shear strength (quadratic). It is feasible to conclude that eggshell calcium powder could be used to enhance meat's physical and sensorial properties.

Table 4: Effects of various sources of calcium on the quality of broiler meat at the finisher phase

Parameters	Treatments[1]			SEM	P-value
	T1	T2	T3		
Drip loss, %	3.63	3.32	2.93	0.15	0.1471
Cooking loss, %	32.83	32.14	30.93	0.70	0.5482
PH	5.16^b	5.35^a	5.38^a	0.04	0.0429
Lightness	64.29	62.13	60.84	0.80	0.2084
Redness	9.25	8.39	8.60	0.37	0.6270
Yellowness	14.41^a	11.68^b	11.60^b	0.51	0.0329
Chroma	17.24^a	14.49^b	14.60^b	0.51	0.0377
Hue	56.83	54.20	53.63	1.43	0.6380
Tenderness	0.98	0.93	0.96	0.02	0.5254

[a-b] Values in the same row with different letters are significantly different ($p < 0.05$). [1]LS, 100% limestone (Control diet); LS-ES, 50% limestone + 50% eggshell; ES, 100% eggshell.

Table 5 shows effect of eggshell on the chemical cuts of the breast of broilers. According to the data, moisture %, ash %, and protein % did not differ significantly among treatments. In contrast to the other treatments, the second treatment had the highest values for ash% and protein%, although there was a substantial difference in fat%. The fat percentage declined linearly when eggshell replaced limestone; the fat percentage was lowest in the third treatment compared to other treatments. The results may be attributable to a return to the chemical composition of eggshells, which consists of 65.6% water, 11.8% proteins, 11% fat, and 11.7% ash (Kausar and Naureen,2021). Thus, eggshell calcium powder enhances meat's calcium, ash, and moisture content.

Table 5: Effects of various sources of calcium on the chemical composition of broiler meat at the finisher phase

Parameters	Treatments[1]			SEM	P-value
	T1	T2	T3		
Moisture, %	74.35	74.44	75.04	0.32	0.6794
Protein, %	20.90	21.58	20.97	0.27	0.5605
Fat, %	3.75^a	3.20^b	3.01^b	0.10	0.0008
Ash, %	1.00	0.78	0.98	0.05	0.1827

[a-b] Values in the same row with different letters are significantly different ($p < 0.05$). [1]LS, 100% limestone (Control diet); LS-ES, 50% limestone + 50% eggshell; ES, 100% eggshell.

CONCLUSION

pH, yellowness, and chroma at 42 days of age the eggshell powder had the same effect as limestone on overall carcass characteristics, meat chemical composition and meat quality attributes in the current investigation. On the other hand, birds fed eggshell powder had a reduced dressing percentage.

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