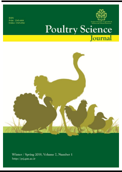

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Ileal Digestibility and Bone Retention of Calcium in Diets Containing Eggshell, Oyster Shell or Inorganic Calcium Carbonate in Broiler Chickens

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Abstract
This study was conducted to compare ileal digestibility of calcium, dry matter and ash, growth performance, gut characteristics, bone parameters and retention of calcium in broiler chickens fed diets containing different calcium sources. A total of 96 commercial Ross 308 broiler chickens were allocated to three experimental diets. The experimental diets included a diet containing inorganic calcium carbonate powder (1.43% in grower and 1.37% in finisher), a diet with oyster shell powder (1.48% in grower and 1.42% in finisher), and a diet with eggshell powder (1.52% in grower and 1.45% in finisher). Each treatment had four replicates with eight birds per each. Results showed that the treatments had no significant effect on daily growth performance of broiler chickens during grower (d 11 to 25), finisher (d 25 to 39) and entire experimental period (d 11 to 39). The average relative weight of the intestine, gizzard and pancreas, as well as the intestinal length, live body weight and dressing percentage of broiler chickens were not affected by the treatments. Dry matter digestibility was higher in broilers received eggshell compare to those birds received oyster shell ($P < 0.05$). Also, digestibility of calcium and ash were higher in eggshell treatment compared to the inorganic calcium carbonate treatment ($P < 0.05$). The digestibility of organic matter, calcium retention, dry matter, ash and diameter of femur and tibia were not affected by the treatments. This study suggested that eggshell could be added as a calcium source in broiler chicken diets with a positive effect on the apparent calcium digestibility and no negative effects on broiler chicken performance.

Introduction

Providing the right level of high-grade minerals in an appropriate balance is very important for the successful growth of broiler chickens. High-grade minerals include calcium, magnesium, sodium, potassium, and chlorine. Calcium in broiler chicken diets is effective on growth, feed efficiency, bone growth, foot health, and function of the nervous and immune systems. Providing sufficient calcium for human and animals has attracted the attention of nutritionists (Bao *et al.*, 1997). Animal health and production with the desired efficiency requires the provision of nutrients through diets in sufficient quantities and in a way that is biologically usable. Many of the nutrients found in the food are no t usable ompletely for animals; therefore, knowledge of the digestibility of nutrients in the diet is essential to accurately meet the requirements (Zaghari and Riahi, 2006). The needed calcium for birds comes from various organic and inorganic sources, which are always used in their diet formulation. Organic sources of calcium can include alfalfa powder, bone powder and inorganic calcium sources include inorganic calcium carbonate, oyster shell, monocalcium and dicalcium phosphates. Inorganic calcium sources contain between 17 to 38.5 percent of calcium and organic sources of calcium contain less than 1 percent of calcium (Gonzalez Vega, 2012). Although inorganic calcium carbonate or oyster shell are the first and most common used rich sources of calcium in laying hen nutrition, but

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today other sources of calcium are also used (Roland, 1989).

Common calcium sources have high cost and low bioavailability, for example the low bioavailability of dolomite calcium carbonate is due to the fact that this source contains at least 10% magnesium, which can compete with calcium in the intestine and causes deficiency of calcium in the birds (Leeson & Summers, 2009), also calcium in natural foods with herbal origins is combined with phytate and oxalate, which reduces its bioavailability. Therefore, to supply accurate needs of calcium for the birds, we should be aware of the amount of calcium retention in calcium supplements (Poneros-Schneier and Erdman, 1989). Eggshell is produced as a cross-cutting material from many egg processing factories, hatcheries, confectioneries and even houses at the lowest cost, and using the correct management as an available and inexpensive source of calcium in human nutrition (Schaafsma *et al*., 2000), animals (Tsugawa *et al*., 1995) and uses as a substitute for other sources (Dupoirieux *et al*., 1995). Eggshell is a biological substance which has a structure like bones of the body, therefore, in the body, it is easily digestible and its calcium content is easily absorbed in the bird's gastrointestinal tract (Mine *et al*., 2003). The objective of this study was to determine the apparent digestibility and calcium retention in broiler chickens fed diets with different calcium sources included inorganic calcium carbonate, oyster shell, and eggshell.

Materials and Methods

In this study, 96 one-day-old broiler chickens of the commercial Ross 308 strain were used in a completely randomized design with three treatments. Each treatment had four replicates and each replicate included eight broiler chickens. The treatments included: corn-soy based diet containing inorganic calcium carbonate powder (1.43% in grower period and 1.37% in finisher period), corn-soy based diet containing oyster shell powder (1.48% in grower period and 1.42% in finisher period), and corn-soy based diet containing eggshell powder (1.52% in grower period and 1.45% in finisher period). The nutritional requirements of the broiler chickens were determined based on the standard requirements of the commercial Ross 308 strain and the diets were formulated by the UFFDA software (Table 1).

The energy and protein density of the diets were the same for all treatments in each period. Birds were vaccinated against infectious bronchitis, Newcastle disease, and Infectious bursal disease. Also, lighting program was operated and followed the Ross 308 strain commercial recommendations. Feed and water were provided *ad libitum*. To adapt the broiler chickens to the environment, in the first 11 days of the broiler chicken's life, all the birds were reared on the bed and all of them were fed with the same diet. On the 11^{th} day, every 8 birds were weighed and randomly were allocated to one of the 12 pens.

Table 1. Composition of grower and finisher diets

Ingredients (%)	Grower(11 to 25 d)			Finisher (25 to 39 d)		
Corn	60.03	59.92	59.85	68.17	68.20	68.22
Soybean meal	33.62	33.64	33.65	26.27	26.12	26.02
Soybean oil	1.63	1.67	1.69	0	0	0
Inorganic calcium carbonate	1.43	0	0	1.37	0	0
Concentrate biofin	2.5	2.5	2.5	2.5	2.5	2.5
Oyster shell powder	0	1.48	0	0	1.42	0
Eggshell Powder	0	0	1.52	0	0	1.45
Dicalcium Phosphate	0.11	0.11	0.11	0.33	0.33	0.33
Salt	0.12	0.12	0.12	0.06	0.06	0.06
Vitamin premix [1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.02	0.02	0.02	0	0	0
L-Lysine HCl	0.04	0.04	0.04	0.08	0.09	0.09
Rice hull	0	0	0	0.72	0.78	0.83
Nutrients composition						
ME (kcal/kg)	2950	2950	2950	2950	2950	2950
CP (%)	20.60	20.60	20.60	18.90	18.90	18.90
Calcium (%)	0.84	0.84	0.84	0.78	0.78	0.78
Available phosphorus (%)	0.42	0.42	0.42	0.39	0.39	0.39
Potassium (%)	0.61	0.61	0.61	0.60	0.60	0.60
Chlorine (%)	0.19	0.19	0.19	0.18	0.18	0.18
Sodium (%)	0.19	0.19	0.19	0.17	0.17	0.17
Lysine (%)	1.16	1.16	1.16	0.98	0.98	0.98
Methionine (%)	0.42	0.42	0.42	0.37	0.37	0.37
Methionine + Cystine (%)	0.89	0.89	0.89	0.77	0.77	0.77

[1]each kg of vitamin supplements including: Vitamin A, 7500 IU; Vitamin D3, 3000 IU; Vitamin E, 10 IU; Vitamin K, 2 mg; Vitamin B12, 12.5 µg; folic acid, 0.5 mg.; pantothenic acid, 8 mg; pyridoxine 1.8 mg; riboflavin, 5.3 mg; thiamine, 2 mg; biotin, 0.15 mg.

[2]each kg of mineral supplements including: iodine; 1 mg; selenium, 0.15 mg; niacin, 24 mg; choline, 350 mg; copper; 6 mg; iron, 30 mg; zinc, 50 mg; manganese, 80 mg.

Broiler chickens were weighed at the end of each week and feed consumption was recorded weekly. Body weight gain and feed conversion ratio were calculated. At the end of the experiment, the chickens of each pen were sent to the slaughterhouse. One broiler chicken from each pen was weighed and after slaughtering, weight of gizzard, intestine (from Meckel's diverticulum up to 2 cm before the junction of the ileum to the cecum), pancreas and carcass weight were measured. Also, left femur and tibia were taken from each broiler chickens and after separation those from meat for more analyses were kept at -20°C. Other broiler chickens of each pen were slaughtered and length of the intestine from Meckel's diverticulum up to 2 cm before the cecum was measured, and pooled digesta samples from the ileum of each pen separately were kept in the freezer at -20°C to perform the proximate analysis of nutrients afterwards.

To determine the digestibility of the experimental diets, the measured parameters in the laboratory was performed with two and three replicates, also their means and CVs were calculated and those with a CV higher than 5% were re-measured. The measured parameters were multiplied by converse of dry matter according to the percentage of dry matter, then were entered in formula to calculate digestibility. To measure the digestibility of dry matter percentage and ash content of feed and ileal samples by proximate analysis method were measured and their calcium percentage by flame photometer and atomic absorption devices were determined. Chromic oxide (Cr_2O_3) was added to the diets at 0.5% as an external marker for 5 days in order to measure the nutrients digestibility. Then, chromium oxide was measured by Williams *et al*. (1962) method and the apparent ileal digestibility value (AD) of dietary nutrients was calculated based on the equation1 (Scott *et al*., 1976):

(Equation 1)

$$AD = 100 - (100 \times ((\text{diet nutrients}/\text{ileal nutrients}) \times (\text{ileal}Cr_2O_3 / \text{diet } Cr_2O_3))$$

Also, to determine the bone parameters (femur and tibia) after preparation of bone samples, the percentage of bone dry matter was also calculated by equation 2:

(Equation 2)

$$\text{Percentage of dry matter} = \frac{\text{Weight of sample after oven}}{\text{Weight of wet sample}} \times 100$$

The bone diameter of bone samples (femur and tibia) without fat and dried was measured by Caliper (Oso *et al*., 2011), percentage of bone ash based on AOAC (2000; method 942.05) was obtained. Also, bone calcium retention was calculated by equation 3:

(Equation 3)

$$\text{Retention of bone calcium} = \text{weight of bone dry matter} \times \text{Percentage of bone calcium}$$

Statistical analysis

Data were statistically analyzed in a completely randomized design. Weight of broilers at 11 day was considered as covariate for digestibility and growth performance, while live body weight of the birds was considered as covariate for bone parameters (bone calcium retention, bone diameter, ash percentage, and bone dry matter). The experimental data were analyzed using general linear model of SAS software (SAS, 2004) and least squares of means (LSM) of treatments were compared at a significance level at 5%.

Results

The inorganic calcium carbonate, oyster shell, and eggshell had no significant effect on daily body weight gain of broiler chickens in grower, finisher, and entire experimental periods, as well as average body weight of chickens at the end of each period (Table 2). There was also insignificant difference between calcium sources for daily feed intake and feed conversion ratio (Table 2). The results of this study showed that the inorganic calcium carbonate, oyster shell, and eggshell calcium sources had no significant effect on the relative body weight (based on live body weight) of intestine, gizzard, pancreas, as well as dressing percentage, live body weight and intestinal length of broiler chickens (Table 3).

Digestibility of dry matter was significantly higher in eggshell treatment compared to oyster shell ($P < 0.05$), while it was similar between eggshell and inorganic calcium carbonate treatments as well as oyster shell and inorganic calcium carbonate treatments. Calcium digestibility was higher in eggshell and oyster shell treatments compared to the inorganic calcium carbonate treatment ($P < 0.05$). The digestibility of ash in eggshell treatment was higher than oyster shell treatment and inorganic calcium carbonate ($P < 0.05$), digestibility of ash was not significantly different in oyster shell treatment with digestibility of ash in inorganic calcium carbonate treatment (Table 4).

Table 2. Effect of experimental diets on growth performance of broiler chickens (Mean ± SE)

Daily weight gain (g)	Inorganic calcium carbonate [1]	Oyster shell [2]	Eggshell [3]	*P*-value
11 to 25 d	39.29±3.80	41.02±3.77	39.76±3.80	0.946
25 to 39 d	69.7±2.97	67.7±2.95	69.7±2.98	0.860
11 to 39 d	56.6±1.90	54.2±1.89	57.8±1.90	0.432
Average weight (g)				
25 d	861.3±22.30	802.5±22.14	877.9±22.33	0.095
39 d	1837.1±17.23	1750.3±17.11	1853.7±22.04	0.084
Daily feed intake (g)				
11 to 25 d	71.6±0.92	70.3±0.91	73.8±0.92	0.076
25 to 39 d	142.2±5.06	145.0±5.02	152.3±5.07	0.396
11 to 39 d	112.1±3.47	114.4±3.45	120.2±3.48	0.297
Feed conversion ratio				
11 to 25 d	1.8±0.06	1.7±0.06	1.8±0.06	0.152
25 to 39 d	2.0±0.11	2.1±0.11	2.2±0.11	0.615
11 to 39 d	1.9±0.08	2.1±0.08	2.0±0.08	0.534

[1]Inorganic calcium carbonate treatment (1.43% in grower and 1.37% in finisher), [2]oyster shell treatment (1.48% in grower and 1.42% in finisher), and [3]eggshell treatment (1.52% in grower and 1.45% in finisher).

Table 3. The effect of experimental diets on the weight of some internal organs, length of the intestine, live body weight, and dressing percentage of broiler chickens (Mean ± SE)

	Inorganic calcium carbonate [1]	Oyster shell [2]	Eggshell [3]	*P*-value
Pancreas (g/Kg live weight)	0.2±0.01	0.2±0.01	0.2±0.01	0.427
Gizzard (g/Kg live weight)	1.5±0.15	1.4±0.15	1.6±0.15	0.863
Intestine (g/Kg live weight)	7.3±0.70	7.5±0.70	7.7±0.70	0.931
Intestine Length (cm)	81.5±1.87	80.6±1.87	85.7±1.87	0.177
Live body weight (g)	2392.6±152.89	2491.8±152.89	2600.9±152.89	0.644
Dressing percentage (%)	63.4±0.9	63.9±0.9	64.4±0.9	0.792

[1]Inorganic calcium carbonate treatment (1.43% in grower and 1.37% in finisher), [2]oyster shell treatment (1.48% in grower and 1.42% in finisher), and [3]eggshell treatment (1.52% in grower and 1.45% in finisher).

Table 4. Effect of experimental diets on nutrients digestibility in broiler chickens (Mean ± SE)

	Inorganic calcium carbonate [1]	Oyster shell [2]	Eggshell [3]	*P*-value
Dry matter	66.4±2.70[ab]	60.8±2.68[b]	72.4±2.70[a]	0.046
Calcium with atomic absorption device	64.4±4.07	78.6±4.04	75.3±4.07	0.089
Calcium with flame photometer device	22.8±6.92 [b]	54.0±5.63[a]	56.1±5.78[a]	0.017
Ash	29.8±3.14[b]	26.5±3.11[b]	49.5±3.14[a]	0.001
Organic matter	68.7±2.77	63.4±2.75	73.7±2.77	0.080

[1]Inorganic calcium carbonate treatment (1.43% in grower and 1.37% in finisher), [2]oyster shell treatment (1.48% in grower and 1.42% in finisher), and [3]eggshell treatment (1.52% in grower and 1.45% in finisher).
[a-b] Values within the same row with no common superscripts differ significantly ($P \leq 0.05$).

The effect of treatments on bone calcium retention was not significantly different either based on the atomic absorption or the flame photometry. Moreover, the diameter of the femur and tibia were not affected by the different calcium sources. Bone dry matter (femur and tibia), and bone ash (femur and tibia) had no significant difference among the different calcium sources (Table 5).

Table 5. The effect of experimental diets on some of the bone parameters of broiler chickens (Mean ± SE)

	Inorganic calcium carbonate[1]	Oyster shell[2]	Eggshell[3]	*P*-value
Bone calcium retention (g; atomic)	2.37±0.13	2.62±0.13	2.07±0.17	0.114
Bone calcium retention (g; flame)	0.6±0.06	0.7±0.06	0.5±0.08	0.505
Tibia bone diameter (mL)	7.7±0.35	7.5±0.34	7.4±0.35	0.858
Femur bone diameter (mL)	8.5±0.29	8.5±0.28	8.8±0.29	0.752
Bone dry matter (g)	9.8±0.55	9.7±0.54	8.8±0.55	0.421
Bone ash (g)	4.6±0.19	4.5±0.18	4.0±0.19	0.186

[1]Inorganic calcium carbonate treatment (1.43% in grower and 1.37% in finisher), [2]oyster shell treatment (1.48% in grower and 1.42% in finisher), and [3]eggshell treatment (1.52% in grower and 1.45% in finisher).

Discussion

McNaughton (1981) showed that broiler chickens fed inorganic calcium carbonate or oyster shell had similar body weight when the particle size and the level of these supplements are the same in the diet. These results are consistent with our results, because in our study, the particle size of calcium sources used in the diet was the same. Saunders-Blades *et al*. (2009) by investigating the effect of different calcium sources on the performance of laying hens; reported that calcium sources have insignificant effect on the body weight. Ajakaiye *et al*. (2003) reported that different calcium sources (inorganic calcium carbonate and oyster shell) had no effect on the feed intake or body weight of broiler chickens. Scheideler (1998) also reported that the feed intake was not affected by the dietary calcium sources that our findings were consistent with the findings of these researchers.

Similar to our results, Brister *et al*. (1981) showed insignificant difference between calcium sources of oyster shell and inorganic calcium carbonate for feed conversion ratio (2.37 vs 2.44 kg of feed to produce one kg of egg). Cufadar (2014) investigated the effect of inorganic calcium carbonate, oyster shell, and eggshell in the diet on the performance of laying hens and showed that the different calcium sources did not have any significant effect on the feed conversion ratio. In the present study, levels of 1% (starter), 0.9% (grower) and 0.85% (finisher) calcium from different sources were added in the diet. Talpur *et al*. (2012) investigated the effect of dietary calcium levels (1%, 2%, and 3%) on the performance of broiler chickens and they reported that the dressing percentage was significantly higher in the group fed with 1% of calcium supplementation than the two other groups ($P < 0.05$). The live body weight, gizzard, intestine, heart and liver were higher in the group fed with 3%of calcium supplementation compared to the two other groups ($P < 0.05$). It was shown that the diet supplemented with 3% of calcium resulted in improvement in the growth performance. Bintvihok and Kositcharoenkul (2006) also showed that the addition of calcium in the diet had a positive effect on the body weight gain. In a research project 0, 50 and 100% oyster shell in the broiler chicken finisher diet was substituted with chalkstone as a calcium source, the results showed that treatments had no significant effect on the dressing percentage of broiler chickens. The range of the dressing percentage was between 74.9% and 75.2%. The weight of gizzard, heart, liver and live body weight were not significantly different between experimental groups and control groups (Omole *et al*., 2005).

Alu (2013) reported that the digestibility of dry matter, crude protein, crude fiber, ether extract, and ash were not affected by replacing bone powder with eggshell powder in broiler chicken diets. These findings regarding the effect of eggshell on digestibility of nutrients is indicative of suitability the use of eggshell in the diet without the negative effect on health and digestibility in broiler chickens. There was not any significant difference between treatments in terms of their effects on calcium digestibility (based on the atomic absorption device) and organic matter. Scheideler (1998*)* also reported that there was not any significant difference in the calcium digestibility between eggshell and inorganic calcium carbonate treatments. These results were consistent with our results based on the atomic absorption device, but inconsistent with our results based on the flame photometry device. However, our results based on the flame photometric and atomic absorption methods were slightly different, nevertheless, a similar trend was observed in both methods in terms of more digestibility of eggshell treatment than inorganic calcium carbonate treatment.

Roland and Bryant (2000) reported that the use of oyster shell in the laying hen diet improved calcium retention and improved the quality of eggshell so that supplying calcium from oyster shell resulted in the increase of digestibility and calcium retention compared to the same amount of supplied calcium from inorganic calcium carbonate powder. McDonald *et al*. (1992) reported that the digestibility of nutrients is affected by the feed composition, as feed components affect digestion rate in animals, so it can be said that in the present study feed had the potential effectiveness on the apparent digestibility of calcium, dry matter and ash. In consistent with our results, Lichovnikova (2007) by investigating the effect of dietary calcium sources and particle sizes on the calcium retention in laying hens, reported that calcium retention in the ratio of 50 to 50 small particle sizes of calcium carbonate to large particle sizes of calcium carbonate and small particle sizes of calcium carbonate to the eggshell in comparison with the ratio of 29 to 71 small particle sizes of calcium carbonate to large particle sizes of calcium carbonate and small particle sizes of calcium carbonate to the oyster shell were significantly higher ($P < 0.05$), and the highest calcium retention and other nutrients (energy, nitrogen, and ash) were observed in the diets containing the eggshell. Scheideler (1998) also consistent with these findings reported that the highest calcium retention is related to eggshell treatment, the results of these researchers were not consistent with the results of our study.

Results of current experiment are consistent with the results of Oso *et al*., (2011) who reported that different calcium sources had no effect on the diameter and length of the femur and tibia in broiler chickens. Guinotre and Nys (1991) investigated the effect of calcium sources (oyster shell and inorganic calcium carbonate) and the size of particles (powdery and large) on bone mineralization in laying hens.

They showed that the strength of tibia bone and ash percentage under the influence of large particles of calcium sources were greatly increased, but calcium sources had not any effect on these factors. Our results indicate that the calcium retention in relation to the bone characteristics under the influence of different calcium sources did not differ significantly and this means that the same performance achieved with the same amounts of each of these sources. McNaughton (1981) and Saunders-Blades *et al.* (2009) showed that the oyster shell and inorganic calcium carbonate as two calcium sources have the same calcium retention.

Conclusion

There was no significant difference between inorganic calcium carbonate, oyster shell and eggshell in terms of their effects on the performance (daily body weight gain, daily feed intake, and feed conversion ratio), also gut and bone parameters of broiler chickens. Eggshell increased digestibility of dry matter, ash and calcium in broiler chickens, but did not affect the digestibility of organic matter. Eggshell can be used as a biological source of calcium instead of chemical sources in broiler chicken diets without having a negative effect on the broiler chicken performance and calcium bioavailability.

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