Growth, physiological, and molecular responses of broiler quail to dietary source, particle size, and choice feeding of calcium

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ABSTRACT
Two experiments were performed to explore the effect of dietary supplemental different sources and particle sizes of calcium (Ca) on growth performance, bone mineralisation, blood attributes, and calbindin gene expression in broiler quail. In experiment 1, 480-day old broiler Japanese quail (Coturnix japonica) were allotted to four dietary treatments based on a factorial arrangement (2x2) consisting of basal diets formulated either with limestone or oyster shell with coarse or fine particles. Supplemental coarse oyster shell impaired growth performance and Ca intake compared to coarse limestone (p < .05). The concentration of blood Ca was lower in birds who received supplemental coarse or fine oyster shell than coarse limestone (p < .05). Higher expression of calbindin gene observed in birds fed on the coarse oyster shell (p < .05). In experiment 2, a total of 360 Japanese quail assigned to experimental treatments of feeding: (1) control: a basal diet; (2) choice feeding between a Ca deficient diet and limestone with coarse (granulated) and fine particles; (3) choice feeding between a Ca deficient diet and oyster shell with coarse and fine particles. All birds preferred to use fine Ca particles across the first week of the experiment (p < .05) whereas tended to consume higher fine oyster shell during the entire rearing period (p < .05). Feed conversion ratio impaired in birds subjected to choice feeding (p < .05). Overall, coarse oyster shell decreased the growth of birds in the first experiment. The overall growth of choice-fed birds depressed compared to when received one conventional feed.

HIGHLIGHTS
• Growth of quail decreased when they fed on diets containing coarse oyster shell.
• Higher expression of calbindin gene observed in quail received coarse oyster shell.
• Quail tended to use a separate source of Ca when fed with a Ca deficient diet.

Introduction
Quail has been considered an economic bird due to its positive features, such as proper meat quality, high egg production, and fast returns on investment (Silva et al. 2018). On the other side, birds need an accurate determination of nutritional requirements to reach their maximum genetic potential. Calcium (Ca) is a macro mineral with a critical function for the formation, growth, and rapid regeneration of bone. Thereby, Ca deficiency may irreversibly damage the skeletal-system development and result in severe skeletal and particularly leg disorders (Proszkowiec-Weglarz and Angel 2013). On the other hand, excess of Ca in the diet can lead to undesirable effects so that impede the availability of the other minerals (NRC 1994), chelate lipids (Edwards et al. 1960), and also can bind with inorganic phosphorus (Pi) with a detrimental effect on Pi availability (Hurwitz and Bar 1971). Limestone and oyster shell are major sources of Ca used in poultry diets and contain ~380 g/kg Ca (NRC 1994) while studies have shown a huge difference in Ca availability of them for broiler chickens (Augspurger and Baker 2004; Anwar et al. 2016, 2017). Source and particle size of limestone and oyster shell have been reported to affect the Ca availability, measured in terms of growth and tibia ash in broiler
chickens (McNaughton et al. 1974; Reid and Weber 1976). It is well-documented that Ca needs to be solubilised to be reactive; hence Ca solubility, as well as digestibility in broiler intestines, is highly correlated with source and particle size (Shih et al. 2000; Anwar et al. 2017; Kim et al. 2018). There are studies in the literature evaluated the effect of Ca source or particle size on broiler chickens but their results were contradictory. For example, reports have shown that finer Ca particles led to the better growth performance of broiler chickens than coarse particles (McNaughton et al. 1974; Guinotte et al. 1991) while some of the other findings failed to express similar results (Bradbury et al. 2018; Majeed et al. 2020). Also, Guinotte and Nys (1991) reported that feed intake and daily weight gain (DWG) were increased in chicks fed coarse oyster shell when compared with finely ground particles. In this context, apparent ileal Ca digestibility coefficient, true ileal Ca digestibility coefficient and apparent Ca retention coefficient increased in birds received coarse (1–2 mm) compared to fine (<0.5 mm) Ca particles (Anwar et al. 2017). On the other hand, the effect of particle size of limestone in the feed did not affect performance and egg quality of laying Japanese quail. Regarding the source of Ca, dietary supplemental limestone was shown to induce improvement in feed conversion ratio (FCR) of broiler chickens compared to oyster shell (Barshan et al. 2019; Fallah et al. 2019). Although the effect of Ca source and Ca particle size on laying hens and broiler chickens have been studied to some extent, scanty information is available on broiler quail. In other words, nutrient requirements of Japanese quail are often simple extrapolations of results from the other poultry (Minvielle 2004) but they are not the same with the chickens in terms of poultry production and what is true for chickens is not always true for quail (Cheng and Kimura 1990).

One approach, to combat adverse effects of dietary extra Ca content and also to optimise the hydrolysis and availability of phytate phosphorus without compromising skeletal integrity might be the separation of major inorganic Ca sources from the diet. Previous studies have shown that broiler chickens tended to consume a separate source of Ca when offered with a Ca deficient diet (Wilkinson et al. 2014a; Abdollahi et al. 2015). Also, they have shown that the coefficient of apparent ileal digestibility increased when birds fed on a low Ca diet with access to a separate source of Ca. Additionally, there are numerous studies, indicating Ca preference in Ca deprived broiler chickens (Wood-Gush and Kare 1966; Hughes and Wood-Gush 1971; Joshua and Mueller 1979). However, less published data are available on choice feeding of Ca as a separate source in broiler quail. Thus, in the first experiment, we hypothesised that different source and particle size of supplemental Ca may affect growth performance, blood and bone mineral-related indexes, and calbindin gene expression in broiler quail. In the second experiment, it was hypothesised that choice feeding of Ca as a separate ingredient might determine the broilers’ tendency to source and size of Ca as well as growth performance, and mineral bone and blood-related parameters. The objective of experiment 1 was to determine the influence of dietary source (limestone and oyster shell) and particle size (coarse and fine) of Ca on growth performance, bone mineralisation, blood attributes, and expression of calbindin gene in broiler quail. In experiment 2, we aimed to investigate the effect of choice feeding of Ca with different sources (limestone and oyster shell) and particle sizes (coarse and fine) on noted parameters in broiler quail.

Materials and methods
All experimental procedures were evaluated and approved by the Institutional Animal Care and Ethics Committee (No: 25496) of the Islamic Azad University, Isfahan (Khorasgan) Branch. The experiment was conducted according to the regulations and guidelines established by this committee.

Experiment 1

Birds, diets, and management
A total of 480 one-day-old broiler Japanese quail (Coturnix japonica) were weighed and randomly distributed in 24 pens in a power-ventilated house during a five-week period. Six replicate pens with 20 broiler Japanese quail each were randomly allotted to four dietary treatments based on a factorial arrangement of treatments (2 × 2) in a completely randomised design. Experimental treatments consisted of basal diets formulated either with one of two Ca sources (limestone and oyster shell), of two-particle sizes (coarse = 2–2.8 mm or fine = 0.5–1 mm). The coarse (granulated) or fine limestone (Carbonate Atlas Co., Tehran, Iran) contained 980 g/kg Ca carbonate, 367 g/kg Ca, and 0.9 g/kg of phosphorus. The oyster shell (Pouya Sadaf Production Group, Gonbad Kavous, Golestan Province, Iran) contained 970–980 g/kg Ca carbonate, 380–400 g/kg Ca, and 1 g/kg of phosphorus. Diets were formulated to meet or exceed the
nutrient requirement of broiler quail according to Rostagno et al. (2011) and were fed during 35 days of the experiment (Table 1). Dietary inclusion rates of limestone and oyster shell were set to maintain the recommended dietary Ca concentration for broiler quail with a Ca to non-phytate phosphorus ratio of 2:1. The feed ingredient and diet samples from all the treatments were taken and submitted for crude protein (Method 990.03; AOAC 200) analysis. Also, Ca and P analyses (Method 2011.14; AOAC 1990) were performed for feed ingredients, feed samples, and Ca sources before the initiation of the trial to confirm Ca and P contents in diets. Dietary treatments were fed in mash form and offered ad libitum throughout the study. All birds had free access to water during the experiment. Ambient temperature was kept at 34 °C (at floor level) from one to 3 days of age, at 31 °C from 4 to 7 days of age, at 28 °C from 8 to 14 days of age, and then gradually decreased to 25 °C until the end of the experiment. The lighting program consisted of 23-h light and 1-h darkness.

Data collection and sampling

Daily feed intake (DFI) and DWG of broiler quail in each pen were recorded during the entire rearing period and FCR (feed intake/weight gain) was calculated accordingly. Ca intake was recorded for pens containing quail receiving Ca across the whole experiment (g/d/chick). On day 35 of the experiment, two quail close to the mean body weight of the pen were slaughtered after 4 h feed deprivation to evaluate carcass and digestive organs. Carcase, proventriculus, gizzard, duodenum, jejunum, ileum, and caecum were collected, weighed, and expressed as a percentage of live body weight. The length of intestinal segments consisting of the duodenum, jejunum, ileum, and caecum, also were measured and recorded.

Morphology of small intestine

Two birds of each pen were slaughtered on day 35 of age. Segments of intestine were sampled from duodenum; intestine from the gizzard to pancreatic and bile ducts and jejunum; midway between the point of

Table 1. Dietary composition and nutrients (as fed basis).

| Ingredients (g/kg) | Experiment 1 | | Experiment 2 | | |
|-------------------|--------------|----------------|--------------|----------------|
| Limestone Oyster shell | | | | |
| Coarse Fine Coarse Fine Choice diet Basal diet |
| **Ingredients (g/kg)** | | | | |
| Corn (850 g/kg crude protein) | 569.10 | 569.10 | 569.10 | 569.10 |
| SBM (440 g/kg crude protein) | 360 | 360 | 360 | 360 |
| CGM (600 g/kg crude protein) | 25 | 25 | 25 | 25 |
| Soybean oil | 10 | 10 | 10 | 10 |
| Dicalcium phosphate | 13.60 | 13.60 | 13.60 | 13.60 |
| Calcium carbonate | 12.20 | 12.20 | – | – |
| Oyster shell | – | – | 12.20 | 12.20 |
| Silica sand | 1.40 | 1.40 | 1.40 | 1.40 |
| DL-Methionine | 1.30 | 1.30 | 1.30 | 1.30 |
| L-Threonine | 0.90 | 0.90 | 0.90 | 0.90 |
| Vitamin and mineral premix | 2.50 | 2.50 | 2.50 | 2.50 |
| Sodium chloride | 2.50 | 2.50 | 2.50 | 2.50 |
| Sodium bicarbonate | 1.50 | 1.50 | 1.50 | 1.50 |
| **Calculated nutrient level (as fed basis)** | | | | |
| ME (Kcal/kg) | 12.14 | 12.14 | 12.14 | 12.14 |
| Crude protein (g/kg) | 217 | 217 | 217 | 217 |
| Digestible lysine (g/kg) | 11.10 | 11.10 | 11.10 | 11.10 |
| Digestible Met + Cys (g/kg) | 7.70 | 7.70 | 7.70 | 7.70 |
| Digestible threonine (g/kg) | 8.10 | 8.10 | 8.10 | 8.10 |
| Calcium (g/kg) | 9.00 | 9.00 | 9.00 | 9.00 |
| Available phosphorous (g/kg) | 3.89 | 3.89 | 3.89 | 3.89 |
| **Analysed values (as fed basis)** | | | | |
| Crude protein (g/kg) | 216.40 | 216.50 | 216.40 | 216.60 |
| Calcium (g/kg) | 8.95 | 8.95 | 8.95 | 8.96 |
| Total phosphorus (g/kg) | 5.85 | 5.84 | 5.85 | 5.85 |

SBM: soybean meal; CGM: corn gluten meal; ME: metabolisable energy.

*Vitamin premix provided per kg of diet: vitamin A (retinol), 2.7 mg; vitamin D3 (Cholecalciferol), 0.05 mg; vitamin E (tocopheryl acetate), 18 mg; vitamin K3, 2 mg; riboflavin, 6.6 mg; pantothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; antioxidant 100 mg; Mineral premix provided per kg of diet: Fe (FeSO4·7H2O, 20.09% Fe), 50 mg; Mn (MnSO4·H2O, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu (CuSO4·5H2O), 10 mg; I (KI, 58% I), 1 mg; Se (Na2SeO3, 45.56% Se), 0.2 mg.
entry of the bile ducts and Meckel’s diverticulum. Noted intestinal samples were taken to evaluate the villus height, crypt depth, and ratio of villus height to crypt depth. Segments that were 1.5 cm in length were flushed with saline (1% NaCl) and fixed in 100g/l-1 buffered formalin (PH = 7.0). The fixed intestinal samples embedded in paraffin were then sectioned (5 μm) and stained with hematoxylin-eosin and examined by light microscope (Olympus CX31, Tokyo, Japan). A total of 10 intact, well-oriented villus–crypt units were selected for each intestinal cross-section (24 cross-sections/treatment, for a total of 240 measurements/treatment). Villus height (μm) was measured from the tip of the villus to the villus crypt junction and crypt depth was measured from the base upward to the region of transition between the crypt and villus. Villus height to crypt depth ratio (V/C) was then calculated. The average of values for each cross-section was used for further analysis.

**Blood parameters**

Two birds from each pen were selected randomly and blood samples were collected into heparinised tubes by puncturing the brachial vein after 4 h starvation on day 35. Samples were immediately centrifuged for 10 min at 3000 × g at 20 °C, and frozen at −20 °C. Plasma Ca and P concentrations were determined with a Sysmex KX-21N automated haematology analyser using a colorimetric method. Ca was determined with a Bionik Diagnostic kit (Lot no. 140335) measuring the colour intensity at wavelength 630 nm at 37 °C. Plasma P concentrations were determined with a Bionik Diagnostic kit (catalog no. A. 110537) measuring the colour intensity at 405 nm.

**Bone characteristics**

Two quail from each pen were slaughtered on day 35 of the experiment and the left and right tibias of each bird were excised, sealed in plastic bags, and stored at −20 °C until analysis. Tibia length was measured using a digital calliper (Neiko 01407A, Japan). The tibia ash content was determined using a muffle furnace for 4 h at 600 °C. The ash content of the tibia was digested with hydrochloric and nitric acids and phosphorus was determined by ammonium molybdate method using a spectrophotometer (UNICO 2150, Germany) at an absorbance of 340 nm (method 946.06; AOAC 2000). Determination of Ca content in tibia ash was conducted by digestion in perchloric acid and Ca was quantified by Triethanolamine (50%), and KOH 4N, using a spectrophotometer (UNICO 2150, Germany) with measuring absorbance at 600 nm.

**Quantitative real-time PCR**

On day 21 of the experiment, two quail from each pen were slaughtered after 4 h of feed withdrawal. Samples from duodenum were collected and quickly snap-frozen in liquid nitrogen for further measurement of calbindin mRNA levels. Total RNAs were extracted from duodenum using IRAIZOL reagent (RNA Biotechnology Co., Isfahan, Iran). RNA concentration was quantified by spectrophotometer nano-drop (MD-1000) in wavelength of 250 nm. Complementary (c) DNA was synthesised from 1 μg of RNA samples with an MiScript II RT kit (QIAGENE, Germany), according to the manufacturer’s recommended protocol. All primers were synthesised and purified by Sigma Company. The β-actin was used as a reference gene to normalise the expression of the target gene. The primer pairs for the amplification of Calbindin and β-actin cDNA fragments are listed in Table 2. Quantitative real-time PCR (qRT-PCR) was performed to determine the levels of inducible Calbindin mRNA. Two microliters of 10-fold dilution reverse transcription products were used for PCR in a final volume of 25 μL containing 0.4–0.8 μM primers and 12.5 μL of QuantiTect SYBR Green master mix (Life Technologies, Cat # 4367659). Cycling parameters were as follows: 10 min at 95 °C, then 40 cycles of 95 °C for 30 s, annealing temperature for 30 s, and 72 °C for 30 s, and extension for 2 min at 72°C. To

| Target gene | Gene bank accession | Forward sequence (5’ to 3’) | Reverse sequence (5’ to 3’) |
|-------------|---------------------|-----------------------------|-----------------------------|
| Calbindin   | XM_015855985.2      | AATCTGCCGTTCGGGACGAT      | CATTATGTTGCTCGTACACCT       |
| β-actin     | XM_015876619.1      | AAGGACCGACCACCGTCGTGAT    | TGAGTCAAGCCGCAAAAGAAAA     |
confirm amplification specificity, the PCR products from each primer were subjected to a melting curve analysis and subsequent agarose gel electrophoresis. The final Calbindin concentrations were calculated as an arbitrary unit of band density relative to the total protein concentration of each sample.

Experiment 2

Birds, management, and data collection
Three hundred and sixty day-old broiler Japanese quail were allotted to three dietary treatments, with six replicates and 20 birds per replicate in this experiment. Dietary treatments included: (1) CTL: a basal diet; (2) CH-Lim: choice feeding between a Ca deficient diet and limestone with coarse (granulated) and fine particles; (3) CH-OS: choice feeding between a Ca deficient diet and oyster shell with coarse and fine particles. In this respect, three identical, adjacent feeder troughs were located on each side of the pen so that birds were offered by one basal diet and two different particle sizes of Ca source. The particle size of limestone or oyster shell was similar to experiment 1. Although the first dietary treatment (control group) contained only a basal diet, it was offered in three separate trough feeders to present a similar floor area to birds in the other treatments. A calcium-deficient diet was formulated to contain adequate concentrations of all nutrients with the exception of Ca (Rostagno et al. 2011). Silica sand was used as a filler in a Ca deficient diet. Consumption of each Ca source was recorded for pens containing quail receiving Ca sources at different periods. Further data collection and sampling, intestinal morphology, blood parameters as well as bone characteristics were assayed exactly as shown in experiment 1. Other experimental procedures also were adjusted similarly to the first experiment.

Statistical analysis
Data were analysed considering all birds in a pen as an experimental unit for different parameters (SAS 9.2; SAS Institute Inc., Cary, NC, USA). In the first experiment, when a significant F-test was detected, corresponding means were separated by Tukey’s test, and the interaction between treatments was analysed using a least-square means test adjusted for Tukey’s test. Whenever the interaction effects of main factors were significant, the main effects were not further discussed. In the second experiment, data were subjected to the analysis of variance appropriate for a completely randomised design using the General Linear Model (GLM) procedure. If a significant effect was detected, differences among treatments were separated using the LSD test. Statements of statistical significance in both experiments are based on a probability of \( p < .05 \) and means are presented with their standard error of means (SEM).

Results

Experiment 1

Growth performance
Results on growth performance are summarised in Table 3. Interaction effect of Ca source and Ca particle size on DFI, DWG, and Ca intake was observed during the entire rearing period \( (p < .05) \). Feeding quail with diets containing coarse oyster shell decreased DFI and Ca intake compared to birds fed on coarse limestone supplemented diets \( (p < .05) \). Also, DWG was higher in

Table 3. Effects of calcium (Ca) source and ca particle size on growth performance of broiler quails.

| Calcium source | Calcium particle size | Daily feed intake, g/day | Daily weight gain, g/day | Feed conversion ratio, g:g | Calcium intake, g/day |
|----------------|-----------------------|--------------------------|-------------------------|-----------------------------|-----------------------|
| Limestone      | Coarse                | 21.280 \textsuperscript{a} | 8.540 \textsuperscript{a} | 2.510                       | 0.188 \textsuperscript{a} |
| Limestone      | Fine                  | 20.480 \textsuperscript{ab} | 8.500 \textsuperscript{b} | 2.420                       | 0.184 \textsuperscript{ab} |
| Oyster shell   | Coarse                | 19.320 \textsuperscript{b} | 7.860 \textsuperscript{b} | 2.480                       | 0.174 \textsuperscript{b} |
| Oyster shell   | Fine                  | 20.400 \textsuperscript{ab} | 8.380 \textsuperscript{a} | 2.450                       | 0.184 \textsuperscript{ab} |
| SEM            |                       | 0.950                    | 0.245                    | 0.046                       | 0.009                 |
| Ca source      | Limestone             | 21.040 \textsuperscript{a} | 8.520 \textsuperscript{a} | 2.470                       | 0.186 \textsuperscript{a} |
| Oyster shell   | 19.860 \textsuperscript{b} | 8.120 \textsuperscript{b} | 2.460                   | 0.179 \textsuperscript{b}  |
| SEM            | 4.351                 | 0.254                    | 0.005                   | 0.006                       |
| Ca particle size | Coarse               | 20.300                    | 8.200                    | 2.490 \textsuperscript{a}  | 0.181                 |
| Fine           | 20.440                | 8.440                    | 2.430 \textsuperscript{b} | 0.184                       |
| SEM            | 0.085                 | 0.127                    | 0.003                   | 0.003                       |
| p-Value        | Calcium source        | .012                     | .003                    | .847                        | .047                  |
|                | Particle size         | .150                     | .052                    | .036                        | .369                  |
|                | Calcium source \times Particle size | .019 | .026 | .308 | .047 |

\( ^{ab} \)Values in the same column not sharing a common superscript differ significantly \( (p < .05) \).
birds fed diets supplemented either with fine particles of limestone or oyster shell than those received dietary supplemental coarse oyster shell \((p < .05)\). Results on main effects showed that FCR was higher in birds that received coarse than fine Ca particles in their feed \((p < .05)\).

**Morphology of small intestine**

As shown in Table 4, quail subjected to diets supplemented with Coarse Ca source had greater villus height in the duodenum than those fed on fine Ca particles \((p < .05)\). Moreover, effects of Ca particle size and Ca source interacted on jejunal villus height \((p < .05)\) whereby dietary supplemental coarse and fine limestone resulted in higher villus height than the fine oyster shell \((p < .05)\). Experimental treatments failed to induce any effect on V/C.

**Blood parameters, bone characteristics, and quantitative real-time PCR**

The effect of experimental treatments on blood-related parameters is shown in Table 5. There was a significant interaction between Ca source and Ca particle size on blood Ca level \((p < .05)\). The concentration of blood Ca was lower in birds who received supplemental coarse or fine oyster shell than coarse limestone \((p < .05)\) while blood concentrations of phosphorus or ALP were not affected by treatments. Bone-related characteristics were neither affected by Ca particle size nor by Ca source in the first experiment (Table 6). Table 7 shows the calbindin gene expression in broiler quail. Expression of the Calbindin gene was affected by the interaction of Ca source and Ca particle size \((p < .05)\). The greatest expression observed in birds fed on fine oyster shell-containing
diets was higher than the other dietary treatments \((p < .05)\). Furthermore, dietary supplementation of coarse oyster shell increased Calbindin gene expression compared to limestone supplemented diets \((p < .05)\). Moreover, feeding quail with diets containing fine limestone resulted in higher Calbindin gene expression than dietary supplemental coarse limestone \((p < .05)\).

**Experiment 2**

**Growth performance**
Quail preferred to consume fine Ca particles compared to the coarse source which was not consumed during 1–7 days of the experiment \((p < .05)\; Table 8\). Also, birds tended to use a higher magnitude of fine than coarse oyster shell or the other particle sizes of limestone during 7–14, 14–21, 28–35, and 1–35 days of age \((p < .05)\; Table 8\). Fine limestone was consumed as much as fine oyster shell; higher than coarse particles across 21–28 days of age \((p < .05)\). Total Ca intake was significantly greater in choice-fed birds than the CTL diet during the first week of the experiment \((p < .05)\; Table 8\). Also, total Ca intake increased in quail subjected to CH-OS compared to CTL or CH-Lim across the second week and the whole rearing period of trial \((p < .05)\; Table 8\). As indicated in Table 10, subjecting broiler quail to CH-Lim and CH-OS decreased DWG compared to those in the CTL group during the entire rearing period \((p < .05)\). Furthermore, DFI was lower in birds of CH-Lim than CTL and CH-OS \((p < .05)\). Therefore, FCR was higher in choice-fed birds than those fed on the CTL diet \((p < .05)\).

**Morphology of small intestine**
Although duodenal villus height and duodenal crypt depth increased in birds of CH-Lim treatment compared to CTL and CH-OS \((p < .05)\; Table 11\), V/C was not affected by experimental treatment \(Table 11\). In the jejunum, villus height was higher in birds of the CTL treatment group than those choice fed by different particle sizes of limestone or oyster shell \((p < .05)\; Table 11\). Likewise, V/C increased in quail of CTL than the birds in CH-Lim \((p < .05)\; Table 11\).

**Blood parameters and bone characteristics**
As shown in Table 12, none of the blood-related parameters were affected by experimental treatments \((p < .05)\). Choice feeding of Ca sources increased tibia Ca content compared to those fed on CTL diet \((p < .05)\; Table 13\). Conversely, birds in the CTL group possessed higher phosphorous content than choice-fed birds \((p < .05)\; Table 13\).
Table 8. Effects of dietary treatments on calcium intake from different sources and particle sizes of calcium in choice-fed broiler quails.

| Parameters         | Coarse limestone | Fine limestone | Coarse oyster shell | Fine oyster shell | SEM | p-Value |
|--------------------|------------------|----------------|---------------------|-------------------|-----|---------|
| Calcium source intake (g/day) |                  |                |                     |                   |     |         |
| 1–7 d              | 0.162±           | 0.156±         |                     |                   | 0.126± | <.001  |
| 7–14 d             | 0.153±           | 0.148±         |                     |                   | 0.150± | <.001  |
| 14–21 d            | 0.122±           | 0.248±         |                     |                   | 0.184± | <.001  |
| 21–28 d            | 0.204±           | 0.238±         |                     |                   | 0.209± | <.001  |
| 28–35 d            | 0.102±           | 0.198±         |                     |                   | 0.224± | <.001  |
| 1–35 d             | 0.102±           | 0.198±         | 0.184±              | 0.209±            | 0.224± | <.001  |

Means in the same row with different superscripts differ significantly (p < .05).

Table 9. Effects of dietary treatments on total calcium intake in choice-fed broiler quails.

| Parameters | Total calcium intake (g/day) |
|------------|-----------------------------|
|            | CTL | CH-Lim | CH-OS | SEM | p-Value |
| 1–7 d      | 0.073± | 0.096± | 0.101± | 0.011 | <.001 |
| 7–14 d     | 0.153± | 0.162± | 0.176± | 0.006 | .004  |
| 14–21 d    | 0.185± | 0.215± | 0.013 | .015  |
| 21–28 d    | 0.233± | 0.233± | 0.001± | .998  |
| 28–35 d    | 0.289± | 0.314± | 0.017± | .158  |
| 1–35 d     | 0.189± | 0.198± | 0.003± | .005  |

Means in the same row with different superscripts differ significantly (p < .05).

Table 10. Effects of dietary treatments on growth performance of choice fed broiler quails.

| Parameters | DWG (g/d) | DFI (g/d) | FCR |
|------------|-----------|-----------|-----|
|            | CTL | CH-Lim | CH-OS | SEM | p-Value |
| 1–35 d     | 8.29± | 7.69± | 7.90± | 0.071 | <.001 |
| 1–35 d     | 20.91± | 20.10± | 20.87± | 0.161 | <.001 |
| 1–35 d     | 2.52± | 2.61± | 2.63± | 0.014 | <.001 |

Means in the same row with different superscripts differ significantly (p < .05).

Discussion

In experiment 1, DWG of broiler quail decreased in response to dietary supplemental coarse oyster shell while this effect was not seen when quail received different particles of limestone or fine oyster shell. This might be attributed to lower DFI and consequently reduction in Ca intake of them. Lower DFI of broiler quail might be related to grittiness and higher density of coarse oyster shell which resulted in the early fill of the gut. As such, the source of Ca did not seem to affect the growth of quail because only coarse oyster...
shell decreased DWG whereas fine oyster shell did not make any difference. Findings associated with the effect of Ca source on poultry are against our results, showing that FCR was lower when broiler chickens fed on diets formulated with limestone than oyster shell (Barshan et al. 2019). Likewise, Fallah et al. (2019) reported that the lowest FCR was observed when broiler chickens dietary fed on supplemental 5.5 g/kg limestone. The particle size of Ca is highly correlated with solubility, where increased solubility was reported to be associated with decreased particle size (Zhang and Coon 1997; Walk et al. 2012). Meanwhile, Saunders-Blades et al. (2009) reported that coarse oyster shell is more soluble than coarse limestone due to its higher surface area. Therefore, we expected that birds that received coarse oyster shell show better performance than those given coarse limestone. In addition to these cases, the effect of Ca particle size on the growth performance of broilers has resulted in equivocal outcomes. In this respect, McNaughton et al. (1974) reported the increased DWG of chicks dietary supplemented with fine (0.15 mm) and medium (0.25–0.85 mm) compared to the larger Ca particles (2.36–3.35 mm). Also, Guinotte et al. (1991) observed that ground Ca carbonate (<0.15 mm) improved week 4 body weight of broiler chickens compared to medium or coarse Ca particles. Otherwise, Bradbury et al. (2018) and Majeed et al. (2020) failed to show any effect of Ca source with a particle size of <0.5 and <0.5 or >0.5 mm, respectively on the growth performance of broiler chickens. It is likely that effect of Ca particle size on growth performance of poultry is variable depending on the size investigated.

The villus height and crypt depth are the most important indexes for measuring the digestive and absorption function of the small intestine. Villus height is an indicator of provided surface area for digesta absorption (Montagne et al. 2003) and crypt depth reflects the proliferative potential of the intestine (Iji et al. 2001). Accordingly, measuring V/C is a useful method to estimate the absorptive capacity of the small intestine (Montagne et al. 2003). In this experiment, jejunal and duodenal villus height increased by dietary treatments whereas V/C remained unaffected. Therefore, it could be interpreted that variations in the growth of quail have not resulted from the effect of dietary treatments on intestinal morphology. On the contrary, Xing et al. (2020) exhibited that supplementation of active dicalcium phosphate to the diet of broiler chickens increased intestinal villus height and V/C. Information on the effect of Ca source on the morphology of the small intestine is scarce and further research is needed to find its underlying mechanism.

The nutritional status of Ca and phosphorus contents in broiler quail can be reflected in serum Ca and phosphorus levels. The lower serum Ca concentration observed in quail fed on dietary supplemental coarse oyster shell might be related to their lower Ca intake. On the contrary, Mansilla et al. (2020) reported that Ca concentration of serum was unaffected by reduction of Ca content in pre-starter diet during 0–4 days of age but serum phosphorous level increased on day 4 post-hatch. Furthermore, Yang et al. (2020) have shown that reduction in dietary Ca content failed to affect serum mineral concentration of broiler chickens. Barshan et al. (2019) did not see any effect of Ca particle size on plasma Ca concentration. Moreover, the source of Ca including limestone and oyster shell failed to affect blood Ca and phosphorus concentrations (Fallah et al. 2019). In another work, plasma phosphorus content was higher when the diet of broiler chickens was supplemented with limestone than the oyster shell (Barshan et al. 2019), suggesting that blood phosphorous level is less tightly regulated than Ca (Ansar et al. 2004; Fallah et al. 2019). However, the concentration of serum phosphorous was not influenced by dietary treatments in this experiment. Limited published data are available on the effect of dietary Ca source and particle size on serum mineral contents in broiler quail and more research in this field are needed.

Tibia measurements for ash and mineral contents are a widely accepted indicator of bone mineralisation and are thought to be correlated with the overall structural strength of the skeleton. In contrast to the works of McNaughton et al. (1974), Anderson et al. (1984), and Guinotte et al. (1991) who found that the use of a larger particle size did help improve bone ossification and mineralisation, no effect of particle size on bone characteristics was observed in the present study. Also, bone-related parameters were not affected by supplemental Ca source in the feed. Calbindin-D_28k is a calcium-binding protein found in the tissues of birds and the intestinal calbindin is correlated with calcium transport capability (Bar 2009). Calbindin-D_28k could act as a Ca^{2+} buffer in Ca^{2+}-transporting epithelia (Lambers et al. 2006). Higher expression of Calbindin gene in birds dietary fed on supplemental oyster shell than limestone is assumed to result from lower feed consumption and consequently reduction in Ca intake of broiler quail. In accord with our results, Armbrrecht et al. (2003) reported that restricting dietary Ca and phosphorus to
more than 50% of the requirement stimulated calbindin gene expression of rat intestine. This stimulation might be rooted in a compensatory increase of 1,25(OH)2D3 and 1-hydroxylase activity in the kidney when fed a low Ca or low phosphorus diet (Blahos et al. 1987). Upregulation of calbindin gene expression in response to low dietary Ca and phosphorus intake in broiler chickens has been reported in the experiment of the other researchers (Li et al. 2012; Zanu et al. 2020).

To explore the preference of quail to calcium source and particle size, we evaluated the magnitude of each Ca source intake, separately. From the first week of the experiment, quail tended to use a separate source of Ca when they fed on a low Ca diet. It confirms previously reported Ca-specific appetite in poultry (Hughes and Wood-Gush 1971; Joshua and Mueller 1979; Abdollahi et al. 2015). Moreover, quail only consumed fine Ca across 0–7 days of the trial but started to use coarser particles from the second week although they still tended to consume a higher portion of fine particles. It may show that quail chicks cannot use coarse Ca before 7 days of age. Birds can distinguish differences in feed particle size by mechanoreceptors located in the beak (Gentle 1979) and preference for higher particle size is known to increase with age for optimum poultry performance (Nir et al. 1994; Amerah et al. 2007). On the other hand, birds selected more oyster shell with fine particles than fine limestone across the whole experiment whereas chose a similar amount of coarse Ca during the same period, suggesting that quail can select Ca based on both source and particle size at least from 7 days of age. It has been demonstrated that birds need a period of time to associate the different feedstuffs to their nutritional values, which can affect the adaption to choice feeding (Rose and Kyriazakis 1991; Wilkinson et al. 2014b). Total Ca intake was greater in birds offered by oyster shell and a low Ca diet during the two first weeks of the experiment but Ca intake was not different from the CTL group from day 14 to 35 of trial. This may confirm the idea that broiler chickens can learn and adapt to choice feeding of a Ca source within a short period of time (Wilkinson et al. 2014b; Abdollahi et al. 2015) so they learned to consume a lower amount of Ca after two first weeks of age. Otherwise, these variations in the two first weeks of the experiment resulted in higher total Ca intake of quail across the entire rearing period and finally lead to lower DWG of choice fed quail compared to the birds fed on one complete conventional feed. Many reports have shown the detrimental impact of extra Ca intake on the growth performance of poultry (Amerah et al. 2014; Abdollahi et al. 2016; Akter et al. 2018). Our results challenge the findings of Joshua and Mueller (Joshua and Mueller 1979) reported that broilers consumed more of a separate Ca source than control birds when offered with a Ca deficient diet but the total amount of Ca consumed per day by choice fed birds was less than birds fed the control diets.

Regarding separate feeding with different sources and particles of Ca in conjunction with a low Ca diet, evaluating variations in the morphology of the small intestine is difficult. However, it does not seem that effect of dietary treatments on the intestinal morphology influenced the growth performance of quail since the V/C in the duodenum was similar between groups and higher V/C of jejunum in birds of the control group did not lead to higher DWG than the birds in CH-OS.

The effect of dietary Ca variations on bone mineralisation has led to different consequences in the literature (Bradbury et al. 2018; Barshan et al. 2019; Fallah et al. 2019). Our results showed that growth of quail depressed in response to higher Ca intake in choice-fed birds but tibia Ca content increased. This may support the idea that bone mineralisation and growth performance may react differently following modulation of Ca in the feed as it has been demonstrated that for optimal bird performance, it is desirable to have low concentrations of Ca in the feed but it may cause negative effects on skeletal health (Bradbury et al. 2018). Furthermore, Rao et al. (2006) reported that dietary Ca: P ratio rather than the concentration of each mineral per se plays a more pivotal role in bone mineralisation.

**Conclusion**

In conclusion, the growth of quail decreased when they were fed on diets containing coarse oyster shell. This effect could be due to lower DFI and consequently lower Ca intake of them. Grittiness and higher density of coarse oyster shell probably assisted fill of gut and decreased DFI of broiler quail. The source of Ca did not seem to influence the growth of birds and results on particle size were different from what we expected. Similar to DWG, Serum Ca level decreased in birds fed on the supplemental coarse oyster shell while bone mineralisation was not affected by experimental treatments. The greatest expression of calbindin gene observed in quail fed with a coarse oyster shell containing diets. This might be a compensatory...
reaction in response to the lower DFI and Ca intake of these birds. Quail tended to use a separate source of Ca when receiving a Ca deficient diet. Moreover, data showed that quail can select Ca based on both source and particle size at least from 7 days of age. However, choice feeding of Ca resulted in higher Ca intake and consequently lower growth of broiler quail while tibia Ca content increased. It shows that bone mineralisation and growth performance react differently following modulation of Ca in the feed.

Disclosure statement
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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