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Determination of apparent and true ileal digestibility of calcium in limestone with different particle sizes in broilers and pullets

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Abstract A feeding experiment was performed to determine the effect of limestone particle size on apparent (AIDC) and true (TIDC) ileal digestibility coefficients of calcium (Ca) in broilers and pullets. With this motivation, four dietary treatments were developed based on a 2×2 factorial arrangement, that included two limestone particle sizes (fine vs. coarse) and two bird types (broilers vs. pullets). Each treatment was replicated six times (eight birds/replicate). Two corn-based diets containing limestone with either fine (<0.5 mm) or coarse (1-2 mm) particles as the sole Ca source were developed and fed to broilers and pullets from 16 to 20 days of age. A Ca-unsupplemented diet was used to determine the ileal endogenous Ca losses. Titanium dioxide was incorporated in all diets as an indigestible marker. Digesta were collected on day 20 from all birds per pen. The AIDC of Ca was determined by marker and the TIDC values were determined by correcting for endogenous Ca losses. Endogenous Ca losses were obtained to be 420 and 696 mg/kg of dry matter intake for broilers and pullets, respectively. Broilers consumed more feed (P=0.001) and had higher body weight gain (P<0.05) than the pullets. Broilers had higher AIDC and TIDC of Ca than pullets (P<0.001). Coarse limestone particles increased the AIDC and TIDC of Ca regardless of bird type. Feeding coarse limestone increased the Ca concentration in the gizzard content of broilers but not in pullets, resulting in a significant (P<0.05) interaction between limestone particle size and bird type. Regardless of bird type, increasing limestone particle size enhanced AIDC and TIDC of Ca in limestone.

Keywords: broiler, calcium, digestibility, limestone, pullet

Introduction

Phosphorus (P) and calcium (Ca) are two important minerals significance in poultry nutrition. Various inorganic (limestone and calcium phosphates) and organic (oyster shell and bone and meat meal) Ca sources are used in poultry diets to supply the Ca (Kiarie et al., 2010). Among all the Ca sources, limestone is a major Ca supplement used in poultry diets worldwide. The Ca availability of limestone varies extensively depending on its origin, solubility and particle size (Saunders-Blades et al., 2009). Considerable variation in the concentration and solubility of Ca in limestone is documented even within the same region or location (Shih et al., 2000; Sa et al., 2017; Kim et al., 2018).

Limestone particle size is known to affect its solubility and, therefore, can influence Ca digestibility by changing the rate of Ca release into the gastrointestinal tract (GIT)

(Majeed et al., 2020). As reported by Anwar et al. (2016, 2017), Ca digestibility for coarse limestone compared to fine limestone was significantly higher (about 1.64 times) in broilers. In addition, Kim et al. (2018) reported a greater Ca digestibility when coarse limestone was included in the broiler diet.

Understanding the complexity of interaction between Ca and P in poultry diets is important to ensure that P and Ca dietary requirements are precisely met (David et al., 2019). Because high dietary Ca adversely affects the utilization of P (Plumstead et al., 2008; Anwar et al., 2015, 2016, 2018) a popular idea has been the application of digestible Ca and P systems for poultry feed formulations (WPSA, 2013).

Improving growth rate, meat yield and feed efficiency for broilers and maximizing egg production for pullets is now at the forefront of current genetic selection programs. Due to the divergent selection targets, modern broilers and pullets differ in the physiological and anatomical characteristics of the GIT (Rideau et al., 2014). Consequently, broilers and pullets differ not only in their weight gain and voluntary feed intake (FI) but also in GIT development and capacity, and nutrient retention, especially protein turnover (Kimiaeitalab et al., 2017). Data on the effect of bird type on the digestibility of Ca from major Ca sources are scant; however, some evidence exists that the absorption and excretion of Ca varies among different breeds and between strains within the same breed that might be related to metabolism and requirements of the minerals (Shafey et al., 1990).

The rate of growth in broilers and pullets is distinctly different, driven by the differences in their digestive and absorptive systems (Uni et al., 1995). It was hypothesized that because of a lower rate of feed passage, limited digestive capacity, and reduced potential for voluntary FI in pullets (Kimiaeitalab et al., 2017), broilers and pullets may react differently to Ca digestion and absorption regarding limestone particle size. Moreover, the availability of Ca from inorganic sources varies widely depending on their origin and particle size (Saunders-Blades et al., 2009). There is a lack of information on the possible interaction between limestone particle size and bird type on Ca digestibility. To the authors' knowledge, no research exists comparing the digestibility of Ca in limestone for broilers and pullets. Therefore, the aim of the present work was to determine the apparent (AIDC) ileal digestibility coefficient and TIDC of Ca in limestone with different particle sizes in broilers and pullets.

Materials and methods

The experiment was approved by Ethics Committee on Biomedical Research of Bu-Ali Sina University, Hamedan, Iran.

Diets, birds and experimental design

A corn-based diet containing limestone, was formulated to maintain a dietary Ca concentration of 9.6 g/kg, with a Ca to non-phytate phosphorus ratio of 2:1, for both bird types (Table 1). Limestone from a commercial source (Alborz Company, Qazvin-Iran) was obtained, and passed through a set of sieves to obtain their particle sizes. A set of sieves (DG Scientific products Co, Iran) and a sieve mixer were used to determine the particle size of limestone (Baker and Herrman, 2002). The sample was passed using the sieve stack, sized 2.0, 1.7, 1.4, 1, 0.5, 0.3 and 0.18 mm, on shakers for 10 min, and the weight of sample retained on each sieve was recorded. Geometric Mean Diameter (GMD) and Geometric Standard Deviation (GSD) were calculated (ASAE, 1994). Limestone particles that passed through 2 mm sieve and were retained on 1.0 mm sieve were considered as coarse (1-2 mm), and those passed the 0.5 mm sieve were considered as fine particles in this experiment.

The Ca content of coarse and fine limestone particles (388 g/kg) was within the range (360–428 g Ca/kg) reported in the literature (NRC, 1994; Browning and Cowieson, 2013; Wilkinson et al., 2013; Anwar et al., 2017). Even though the calculated dietary Ca values were based on the analyzed Ca concentration of limestone and corn samples, the analyzed Ca content of the experimental diet and Ca-unsupplemented diet were higher than the calculated values by 0.30 and 0.01 g/kg, respectively (Table 1). Titanium dioxide (5 g/kg) was incorporated into all diets as indigestible indicator. Corn contains negligible amounts of Ca (0.2 g/kg; NRC, 1994) and therefore, the use of corn-based diets in pig (Gonzalez-Vega et al., 2015) and poultry (David et al., 2019) for digestible Ca assays has been well justified. A Ca-unsupplemented diet, also based on corn, was used to calculate the ileal endogenous Ca losses (David et al., 2019). In total, 288 one-day-old chicks, 144 Ross 308 female broilers and 144 Hy-Line pullets were obtained from a commercial hatchery. All birds were fed a corn-based diet, in mash form, until the introduction of the assay diets on day 16. On day 16, the broilers and pullets were individually weighed and assigned to 36 pens on weight basis so that the average bird weight per pen was similar within each bird type (average body weight, 369 ± 4.3 g for broilers and 119 ± 2.6 g for pullets). Each treatment was randomly allotted to six replicate pens (eight birds per replicate) in a completely randomized design arranged as 2 × 2 factorial arrangements including two bird types (broilers and pullets) and two particle size of limestone (fine and coarse). A Ca-unsupplemented diet was also formulated and fed to the broilers and pullets with six replicate pens (eight birds per replicate). The water was freely available, and mash diets were offered ad libitum from d 0 to 20 post-hatch. The average temperature was maintained at 32 °C on day 1 and gradually declined to 24 °C at 20 days of age. The light program consisted of 20 h of light: 4 h of dark. Body weight and FI were recorded on days 16 and 20.

Table 1. Ingredients and composition (g/kg as fed basis) of the experimental diets for broilers and pullets.

Ingredient	0 to 16 days of age	Limestone diet (16-20 days of age)	Ca-unsupplemented diet (16-20 days of age)
Corn	564.8	936.40	977.40
Soybean meal	321.8	-	-
Gluten meal	50.7	-	-
Soybean oil	16.7	10.00	10.00
Monocalcium phosphate	13.7	-	-
Monosodium phosphate	-	16.90	1.00
Limestone	14.7	25.40	-
Sodium bicarbonate	3.1	1.50	2.30
Sodium chloride	2.1	2.50	2.00
Titanium dioxide	-	5.00	5.00
Vitamin premix[1]	2.5	1.15	1.15
Trace mineral premix[2]	2.5	1.15	1.15
L-Lysine (78%)	3.4	-	-
DL-Methionine (99%)	2.7	-	-
L-Threonine (99%)	1.3	-	-
Calculated composition			
Apparent metabolizable energy (kcal/kg)	3060	3202	3341
Crude protein	223	70.7	73.8
Digestible methionine + cysteine	8.5	2.90	3.03
Digestible lysine	11.3	2.02	2.11
Digestible threonine	7.6	2.24	2.34
Calcium (Ca)	9.4	9.60	0.29
Total phosphorus	6.3	6.30	2.40
Phytate phosphorus	1.6	1.50	1.60
Non-phytate phosphorus (NPP)	4.7	4.80	0.80
Ca: NPP	2.00	2.00	0.38
Sodium	1.8	1.60	1.60
Chloride	2.3	1.90	1.60
Potassium	8.2	2.82	2.93
Analyzed composition			
Dry matter	905	910	890
Calcium	9.6	9.90	0.30

[1]Supplied per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 5000 IU; vitamin E, 80 IU; vitamin K (menadione), 3.2 mg; thiamine, 3.2 mg; riboflavin, 8.6 mg; niacin, 65 mg; pantothenic acid, 20 mg; pyridoxine, 4.3 mg; biotin, 0.22 mg; folic acid, 2.2 mg; vitamin B12, 0.017; choline, 1700 mg .[2]Supplied per kilogram of diet: Copper (CuSo4), 16 mg; Iodine (Ca (IO3)2), 1.25 mg; Selenium (Na2SeO3), 0.26 mg; Manganese (MnO), 120 mg; Zinc (ZnO), 110 mg; Iron (FeSo4), 20 mg. [1,2]Vitamin and trace mineral premixes were calcium-free.

Collection of ileal digesta and gizzard contents

On day 20, all birds in a pen (eight birds) were euthanized by carbon dioxide asphyxiation and ileal digesta was collected and treated as described by Ravindran et al. (2005). The ileum was defined as the portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileocaecal junction. The digesta samples were collected from the lower half towards the ileocaecal junction after gently flushing with distilled water into plastic containers (Ravindran et al., 2005). The gizzard content was also collected for Ca determination. The content of gizzard and ileal digesta were immediately frozen at -20°C and then freeze-dried (DCNA Vacuum, Model MVP12, Korea). Dried digesta samples were ground using 0.5 mm sieve and stored in airtight containers at 4 °C pending chemical analysis.

Chemical analysis

The limestone sample was wet acid digested with perchloric and nitric acid mixture, and concentrations of Ca, potassium, cadmium, sodium, mercury, arsenic, magnesium and lead were determined, in duplicate, by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) (Varian Vista MPX CCD Simultaneous Inductively Coupled Plasm ICP-OES Spectrometer, Australia).

The *in vitro* solubility of limestone samples (fine and coarse) were determined based on Zhang and Coon method (hydrochloric acid 0.2 N; 200 mL) (Zhang and Coon, 1997).

Experimental diets and ileal digesta were analyzed, in duplicate, for titanium dioxide, Ca and dry matter (DM). Dry matter was determined according to the standard method (Method 930.15; AOAC, 2005). Titanium dioxide was determined using the method described by Short et al. (1996). Calcium was determined using an atomic absorption spectrometer (GBC 932 PLUS; Method 985.01; AOAC, 2006) after wet digestion with 30 ml HNo3 and 10 ml HCLO4 at 70-72%.

Calculations

The following equation, based on titanium dioxide ratio in the diets and digesta (Ravindran et al., 2005), was used for determination of AIDC of Ca:

$$AIDC = 1 - ((TiD/TiI)(CaI/CaD)) \quad (1)$$

where, AIDC is the apparent ileal digestibility coefficient of Ca, TiD is the titanium dioxide concentration in the diet, TiI is the titanium dioxide concentration in the ileal digesta, CaI is the Ca concentration in the ileal digesta, and CaD is the Ca concentration in the diet.

The following formula was used to calculate the ileal endogenous Ca losses (g/kg DM intake):

$$IECaL = CaI(TiD/TiI) \quad (2)$$

where, IECaL is ileal endogenous Ca losses, CaI is the Ca concentration in the ileal digesta, TiD is the titanium dioxide concentration in the diet, TiI is the titanium dioxide concentration in the ileal digesta.

The TIDC of Ca was then calculated using the following formula:

$$TIDC = AIDC + (IECaL(g/kg\ of\ DMI)/CaD(g/kg\ of\ DMI)) \quad (3)$$

where, TIDC and AIDC represent the true and apparent ileal digestibility coefficients of Ca, respectively, while IECaL represents the ileal endogenous Ca losses (g/kg of DMI) and CaD represents the Ca concentration in the diet (g/kg).

Statistical analysis

The data were analyzed by a two-way ANOVA using the GLM procedure of SAS (2012) to determine the main effects of limestone particle size and bird type, and their interaction. The differences among treatment means were analyzed by Tukey's test. Differences were considered significant at P<0.05.

Statistical model:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk}$$

where, y_{ijk} is an observation related to the i-th level of factor A (bird type) and the j-th level of factor B (particle size of calcium) in repetition k, μ is overall average, A_i is the effect of the i-th level of factor A (bird type), B_j is the effect of the j-th level of factor B (particle size of calcium), AB_{ij} is the interaction of two factors A (bird type: broiler vs. pullet) and B (particle size of calcium: <0.5 vs. 1-2 mm) and e_{ijk} is the residual error term assumed to follow a normal distribution with zero mean and variance σ^2.

Results

The AIDC and TIDC of limestone were calculated using the analyzed Ca values. The Ca concentrations of limestone and corn (388 and 0.3 g/kg, respectively) were used for the calculation of Ca concentration in the diets. The mineral contents were similar for the two particle size of limestone. The mineral content of limestone for potassium, cadmium, sodium, mercury, arsenic, magnesium and lead were 0.8, 3.0, 0.6, 2.0, 10.0, 0.5 and 9.0 g/kg, respectively.

Particle size and solubility coefficient of limestone

The GMD and GSD of limestone samples were 467 ± 1.29 µm for fine sample and 1745 ± 1.40 µm for coarse sample. The *in vitro* solubility of fine and coarse limestone particles in 0.2 N, 200 mL HCl was 0.71 and 0.64%, respectively.

Growth performance

Broilers consumed more feed (P=0.001) and gained more weight (P<0.01) than the pullets during the 4-day experimental period (Table 2). Limestone particle size had no effect on FI, weight gain and feed conversion ratio (FCR). There was no interaction between bird type and particle size of limestone for performance parameters (P>0.05).

Table 2. Influence of bird type and limestone particle size on feed intake (FI; g/bird), weight gain (WG; g/bird) and feed conversion ratio (FCR, g:g) from 16 to 20 d of age.

Treatment		Limestone diet		
Bird type	**Limestone particle size**	**FI[1]**	**WG[2]**	**FCR[3]**
Broiler	Fine	721	171.2	4.89
	Coarse	733	179.6	4.91
Pullet	Fine	441	87.8	5.37
	Coarse	435	80.4	5.63
SEM[4]		20.9	29.22	0.821
Main Effect				
Bird type				
	Broiler	728[a]	175.4[a]	4.90
	pullet	438[b]	84.1[b]	5.50
Limestone particle size				
	Fine	581	129.5	5.13
	Coarse	585	130	5.27
SEM[4]		14.8	20.66	0.581
Probabilities				
Bird type		0.001	0.006	0.476
Limestone particle size		0.877	0.986	0.867
Bird type × Limestone particle size		0.693	0.790	0.884

[1]FI, feed intake;[2]WG, weight gain; [3]FCR: Feed conversion ratio; [4]SEM: Standard error of the mean.
a,b: Within columns, mean values with common superscript (s) are not different (P>0.05).
Each value represents the mean of six replicate pens (eight chickens per replicate).

Ileal Ca digestibility and Ca concentration in gizzard contents

Table 3 summarizes the AIDC and TIDC of Ca in limestone. Ileal endogenous Ca losses (mean ± SE) were 420 ± 0.006 and 696± 0.014 mg/kg DM intake in broilers and pullets, respectively, and these values were used to calculate the TIDC of Ca. The main effect of bird type on the AIDC and TIDC of limestone was significant (P<0.001), with broilers having higher AIDC and TIDC than pullets. Birds fed with coarse limestone particles had higher AIDC (0.49 vs. 0.42) and TIDC (0.54 vs. 0.48) than fine limestone particles (P<0.05), regardless of bird type. No interaction between limestone particle size and bird type was observed for the AIDC and TIDC of Ca. Feeding coarse limestone significantly increased the Ca concentration in the gizzard content of broilers but not in pullets, resulting in a significant (P<0.05) interaction between bird type and limestone particle size. Therefore, concentration of calcium in the gizzard was higher in broilers fed with coarse particle size.

Table 3. Influence of bird type and limestone particle size on the apparent and true ileal calcium digestibility of limestone, and calcium concentration in gizzard contents.

Treatment		Limestone diet		
Bird type	**Limestone particle size**	**Apparent ileal calcium digestibility coefficient**	**True ileal calcium digestibility coefficient[2]**	**Calcium concentration in gizzard contents (mg/g of DM)**
Broiler	Fine	0.53	0.58	108[b]
	Coarse	0.64	0.68	330[a]
Pullet	Fine	0.32	0.38	196[b]
	Coarse	0.33	0.39	226[b]
SEM[1]		0.022	0.022	39.4
Main Effect				
Bird type				
	Broiler	0.58[a]	0.63[a]	220
	pullet	0.32[b]	0.39[b]	211
Limestone particle size				
	Fine	0.42[b]	0.48[b]	153
	Coarse	0.49[a]	0.54[a]	278
SEM[1]		0.015	0.015	27.9
Probabilities				
Bird type		0.0001	0.0001	0.837
Limestone particle size		0.020	0.038	0.007
Bird type × Limestone particle size		0.065	0.060	0.031

[1]SEM: Standard error of the mean.

[2]Endogenous calcium losses in broilers and pullets were determined to be 420 and 696 mg/kg dry matter intake, respectively.

a,b: Within columns, mean values with common superscript (s) are not different (P>0.05).

Table 4 summarizes the correlations between AIDC and TIDC of Ca in broilers and pullets with Ca concentration in gizzard contents. The Ca concentration in gizzard contents of broilers were positively correlated (P<0.05) to AIDC (r=0.83) and TIDC of Ca (r=0.79).

Discussion

The calculated Ca value in the limestones specified by Rostagno et al. (2017) (377 g/kg) and by the NRC, 1994 (380 g/kg) were close to the analyzed values in the present study (388 g/kg). *In vitro* solubility is an indicator of Ca availability in limestone (Cheng and Coon, 1990). An inverse relationship between the *in vitro* and *in vivo* solubilities of limestone has been observed (Zhang and Coon, 1997). The *in vitro* solubility values for the fine and coarse limestone particles were 0.71 and 0.64%, respectively. Similar to previous studies (Cheng and Coon, 1990; Zhang and Coon, 1997; Anwar et al., 2016, 2017), fine limestone particles in the current study showed higher *in vitro* solubility than those of coarse particles. This occurs because highly soluble limestone results in greater amounts of ionizable Ca in solution (kim et al., 2018). A larger surface area of fine limestone particles may increase the reactivity of hydrochloric acid and, thus the *in vitro* solubility conditions (Guinotte et al., 1991). The two limestones were exactly the same with the only difference being particle size. Thus, the difference in solubility found in this trial has to be related only to the particle size and not the composition of or limestone source (Kim et al., 2018).

As expected, broilers outperformed pullets by having greater FI and WG. Broilers consumed 290 g more feed and gained 91.3 g more weight than pullets during the digestibility assay. The current finding is in agreement with the objectives of the genetic selection programs of the broilers and pullets and with those of Walugembe et al. (2014) and Kimiaeitalab et al. (2017, 2018) who reported higher FI and WG in broilers than pullets. Broilers are more voracious than pullets, and secrete more pancreatic enzymes for each unit of feed with more developed ileal mucosa than pullets (Uni et al., 1995; Kimiaeitalab et al., 2017). Limestone particle size had no effect on FI and WG; a finding which is consistent with those reported by Bradbury et al. (2016). Little information is available about the effect of particle size of

Ca sources on performance of broilers and pullets, and the published data are conflicting (Guinotte et al., 1991;- Bradbury et al., 2016), which could be due to the use of different sources of Ca with varying particle sizes and possibly adaptation time to the experimental diets (Walk et al., 2021).

Table 4 Pearson correlations (probability values in parentheses) between calcium concentration in gizzard contents and apparent and true ileal calcium digestibility in broilers and pullets.

	Broiler			Pullet		
	CaGizz[1]	AIDC[2]	TIDC[3]	CaGizz	AIDC	TIDC
CaGizz	1.00			1.00		
AIDC	0.826 (0.011)	1.00		0.032 (0.939)	1.00	
TIDC	0.793 (0.018)	0.993 (0.0001)	1.00	0.114 (0.787)	0.946 (0.0004)	1.00

[1]CaGizz, Calcium concentration in gizzard contents; [2]AIDC, Apparent ileal calcium digestibility coefficient; [3]TIDC, True ileal calcium digestibility coefficient.
Note: Pearson correlations probability values are significant at $P<0.05$.
Each value represents the mean of six replicate pens (eight chickens per replicate).

Ileal endogenous Ca losses obtained for broilers in our experiment (420 ± 0.006 mg/kg DM intake) are higher than those previously reported (Anwar et al., 2015, 2017; David et al., 2019; Anwar and Ravindran, 2020). David et al. (2019) determined ileal endogenous Ca losses in broilers fed either corn-based or purified diets (based on dextrose and corn starch) and reported lower ileal endogenous Ca losses compared to the current study. These researchers reported ileal endogenous Ca losses of 131 ± 25 and 253 ± 65 mg/kg DM intake in broilers fed Ca-free purified diets and corn-based, respectively. The ileal endogenous Ca losses obtained for pullets in our experiment was 696 ± 0.014 mg/kg DM intake. Diana et al. (2021) reported that endogenous loss values of 790, 860 and 930 mg/kg of consumed dry matter were observed at 40, 50 and 70 weeks in laying hens, respectively, and birds at 40 weeks of age (0.714) exhibited higher true calcium digestibility values compared to hens at 50 weeks of age (0.608). Overall, the present findings indicated that the composition of basal diet could affect the Ca digestibility measurement in broilers and that the use of different diets should be considered in future experiments. Adedokun and Adeola, (2013) reported that the amount of excreted Ca is affected by many factors, including the source of the nutrient (which affects digestibility or availability), concentration of Ca, P, and vitamin D_3 in the diet, the status of parathyroid hormone, bird and blood pH. To the authors' knowledge, no previous published data are available on the ileal endogenous Ca losses in pullets, and therefore, no comparable data are available in the literature. Whilst it is difficult to explain higher ileal endogenous Ca losses in pullets than broilers, the differences in organ development, rate of feed passage, and digestive capacity between two bird types, and the lower potential for voluntary FI in pullets (Kimiaeitalab et al., 2017, 2018), might partly explain the observed differences in ileal endogenous Ca losses.

Little data are available comparing the AIDC and TIDC of Ca in limestone between broilers and pullets. The AIDC (0.58 vs. 0.32) and TIDC (0.63 vs. 0.39) of limestone obtained in this study were higher in broilers than in pullets. Differences in the digestibility of dietary nutrients have previously been reported between broilers and layers (Mtei et al., 2019a), and between broilers, pullets and layers (Mtei et al., 2019b). A better understanding of the growth processes in pullets is needed as part of the effort to improve the Ca digestibility in these birds. A general belief is that layers digest Ca more efficiently than broilers due to their more developed digestive tract (Mtei et al., 2019a). Differences in digestibility of nutrients between broilers and layers are known (Mtei et al., 2019a). Kimiaeitalab et al. (2017, 2018) reported that broilers showed greater DM and N digestibility than pullets at 21 d, that was more pronounced for N (64.1% vs. 55.0%). The lower FI and consequently longer retention time for digestion in the GIT and the lower gizzard pH values in the layers and pullets (Mtei et al., 2019a) compared to broilers, described better nutrient utilization in the layers. However, the digestive capacity in layers can be constrained by the limited digestive enzyme secretion and villus absorptive capacity in laying hens than broilers, as reported by Uni et al. (1995). Mtei et al. (2019b) found a significant effect of bird type on Ca digestibility in a corn-based diet with layers showed considerably higher ileal Ca digestibility compared to pullets and broilers. These differences in responses in nutrient digestibility among broilers and layers may be partly reflective of the age effects. Layers are mature birds, whereas pullets and broilers are growing birds (Mtei et al., 2019b). A trend towards broilers digesting -

Ca more efficiently (0.248) than pullets (0.192) also existed, though the differences were non-significant (Mtei et al., 2019b).

It is difficult to provide a definitive explanation for the observed discrepancy in Ca digestibility in poultry, because unlike many other minerals, intestinal Ca absorption and metabolism are strictly regulated by various factors such as breed or strain of poultry, age, feed ingredients and levels of phytase inclusion (Adedokun and Adeola, 2013). Overall, the present findings indicated that the composition of the basal diet (corn-based diet or purified diet) and methodology (regression, difference, and direct) affected the Ca digestibility measurement in broilers and pullets. In comparative experiments, the same Ca level should be used for both bird types, so the ration used was not a complete diet and its protein level was low because protein sources containing Ca were eliminated. Although dietary protein levels were low, previous experiments (David et al., 2020) showed that, the Ca digestibility of limestone was unaffected by dietary protein content. Due to the discrepancies found in the present study in relation to other published assessments, further studies related to age and Ca source effects on Ca digestibility in pullets are suggested.

The AIDC and TIDC of Ca in the current study varied depending on the limestone particle size. The consumption of coarse limestone increased both the AIDC (0.49 vs. 0.42) and TIDC (0.54 vs. 0.48) compared to fine particles, regardless of the bird type. Similarly, Anwar et al. (2016) reported lower TIDC of Ca in fine limestone than coarse limestone (0.43 vs. 0.71). In a follow-up study, in which Anwar et al. (2017) compared limestone particles with similar sizes to the current study, reducing particle size from 1-2 mm to <0.5 mm decreased TIDC of Ca from 0.62 to 0.38. Kim et al. (2018) reported similar results where Ca digestibility decreased as particle size of the limestone was reduced (402 μm vs. 75 μm).

Different limestone particle sizes vary in solubility, and fine limestone particles are more soluble *in vitro* than coarse particles (De Witt et al., 2006; Manangi and Coon, 2007; Kim et al., 2018) as reported in the current study. Calcium utilization in poultry, inter alia, depends on the solubilization of Ca source in the foregut and continued solubility into the small intestine where it is absorbed (Mutucumarana et al., 2014). The presence of coarse particles in the diet can increase the retention time of digesta in the GIT and may improve mineral availability to the bird (Amerah et al., 2007). Compared to fine particles, coarse particles, with lower *in vitro* solubility, remained longer in the gizzard of broiler (Zhang and Coon, 1997), and increasing digesta Ca concentration. This improves the gizzard function (Hetland et al., 2005) and stimulates HCl production in the proventriculus via mechanoreceptors. This results in a low pH in the upper part of the GIT, which favors the activity of pepsin and facilitates the solubility and absorption of mineral salts (Guinotte et al., 1995). In the current study, the concentration of Ca in gizzard content increased with the increase in limestone particle size only in broiler chickens. It is difficult to provide a definitive explanation for the observed findings. Due to the divergent selection targets, bird types differ not only in weight gain and voluntary FI but also in organ development (Kimiaeitalab et al., 2017). Shires et al. (1987) observed that broilers had a faster rate of feed passage and greater FI than pullets. These findings illustrated that the effect of limestone particle size on Ca concentration in gizzard content depends on the bird type and that broilers show a greater response to changes in particle size (Abdollahi et al., 2018), which may be due to their higher FI with larger limestone particles. Strong positive correlations between the TIDC of Ca and Ca concentration in gizzard contents for broilers describe the slow release and subsequent higher Ca digestibility of coarse vs fine limestone. Large particles of limestone increase the retention time in the gizzard which hinders the release of Ca, thus increasing their *in vivo* solubility and availability (De Witt et al., 2006).

Conclusion

The current data showed that the ileal endogenous Ca losses were higher in pullets than in broilers. To our knowledge, this paper is the first report on the quantification of ileal endogenous Ca losses in pullets. The current study also found that AIDC and TIDC of Ca in limestone were higher in broilers than in pullets. Although coarse limestone increased the concentration of Ca in gizzard content only in broilers, it increased the AIDC and TIDC of Ca in both bird types. Further research is warranted to establish the AIDC and TIDC of Ca in a range of Ca sources for various types of poultry.

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