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Age and Calcium Sources in Laying Hen Feed Affect Calcium Digestibility

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Abstract

The apparent calcium (Ca) digestibility coefficient (ADC) and true digestibility coefficient (TDC) of different inorganic calcium sources were determined in laying hens of different ages. Three Ca digestibility tests were carried out, each assessing 240 Lohmann Brown lineage laying hens distributed in a completely randomized design. Nine dietary treatments were arranged in a 3 × 3 factorial design consisting of three ages (40, 50 and 70 weeks) × three Ca (dicalcium phosphate (DCP) sources, fine (FL) and coarse (CL)) limestone, comprising eight replicates per treatment of six birds per experimental unit. Regarding the DCP, the ADC was higher ($P < 0.05$) in 40-week-old birds. The DCP ADC for 40-, 50- and 70-week-old birds was 0.889, 0.613 and 0.712, respectively. No effect ($P > 0.05$) of age on the ADC was noted for either FL or CL. Comparing Ca sources, DCP exhibited a higher ($P < 0.05$) ADC (0.889), followed by FL (0.699) and CL (0.515), in 40-week-old birds. DCP (0.712) and FL (0.652) presented ($P < 0.05$) higher ADC compared to CL (0.482), in 70-week-old birds. No effect of Ca sources at 50 weeks on the ADC was observed ($P > 0.05$). Endogenous loss values of 790, 860 and 930 $mg \cdot kg^{-1}$ of consumed dry matter were observed at 40, 50 and 70 weeks, respectively. For the TDC, no interaction ($P > 0.05$) was observed between Ca sources and bird age. The highest TDC value ($P > 0.05$) was found in birds fed DCP (0.786) followed by FL (0.637) and CL (0.534). In addition, birds at 40 weeks of age (0.714) exhibited higher TDC values ($P < 0.05$) compared to animals at 50 weeks of age (0.608). The findings reported herein demonstrate that the true digestibility is greater in the youngest birds and that consumed the DCP and the FL in relation to the birds that consumed the CL.

Keywords

Dicalcium Phosphate, Digestion, Endogenous Calcium, Granulometry, Limestone

1. Introduction

The main inorganic calcium (Ca) sources used in bird diets are calcitic limestone and calcium phosphates, which exhibit variable Ca bioavailability [1]. However, the determination of Ca digestibility in these sources has received little attention, mainly due to their low cost, abundant availability and surplus global reserves [2] [3].

Recently, the initiative to determine digestible phosphorus (P) in feed has led to further attention concerning Ca digestibility [3]. In addition, young birds tend to absorb Ca more efficiently than older birds [4]. According to Albino *et al.* [5], older laying hens exhibited a 20% reduction in intestinal Ca absorption, increasing bone Ca mobilization and reducing carbonic anhydrase activity, leading to lower eggshell calcification.

Previous studies on broilers reported low true Ca digestibility coefficients for fine limestone [6] [7]. There was evidence that the Ca digestibility was due to the physical and solubility characteristics of the applied Ca sources [8]. Zhang and Coon [9] reported that laying hens displayed the ability to maintain larger food particles in their gizzards for longer periods of time, thus increasing solubility and *in vivo* use. Limestone particle size influences *in vitro* solubility [9] [10] [11] and a negative correlation between *in vitro* and *in vivo* Ca solubility in laying hens has been noted [9] [12].

Given the above, the hypothesis of this study was that laying hen age and inorganic Ca source may influence Ca digestibility. Thus, the aim herein was to determine the apparent and true Ca digestibility coefficients of different inorganic Ca sources for laying hens of different ages.

2. Materials and Methods

This research was authorized by the Animal Care and Use Committee of the *Universidade Federal de Viçosa* (process number 06/2020) and was carried out per the ethical principles from the Brazilian College of Animal Experimentation.

2.1. Experimental Design and Diets

Three Ca digestibility tests were carried out for laying hens of different ages (40, 50 and 70 weeks). A total of 240 Lohmann Brown lineage laying hens were used in each trial, distributed in a completely randomized design, comprising eight replicates per treatment of six birds per experimental unit. A 3 × 3 factorial scheme (three ages × three Ca sources) was adopted. The birds were distributed according to their body weight (1.938 ± 190 g) and egg production. The interval of each test, the birds were fed with ration according to their requirement, according to the one proposed by Rostagno *et al.* [13]. The tested Ca sources were dicalcium phosphate and fine and coarse calcitic limestone.

A basal diet containing 1.10 g·Kg^{-1} total Ca and 0.89 g·Kg^{-1} available P was formulated. The tested Ca sources replaced basal diet starch in varying amounts (DCP – basal diet + dicalcium phosphate; FL: basal diet + fine-grained calcitic limestone; CL: basal diet + coarse-grained limestone) (**Table 1**). A diet free of available Ca and P was also formulated to determine endogenous Ca losses (**Table 2**).

Table 1. Ingredient composition and analysis (g·kg^{-1}) of experimental diets.

Ingredients	Basal	DCP	FL	CL
Corn	598.08	598.08	598.08	598.08
Soybean meal	279.60	279.60	279.60	279.60
Soy oil	10.00	10.00	10.00	10.00
Sugar	40.00	40.00	40.00	40.00
Common Salt	4.97	4.97	4.97	4.97
Starch	60.83	40.83	-	-
Dicalcium phosphate	-	20.00	-	-
Calcite Limestone	-	-	60.83	60.83
Vitamin Supplement[1]	1.10	1.10	1.10	1.10
Mineral Supplement[2]	1.10	1.10	1.10	1.10
DL-Methionine	2.91	2.91	2.91	2.91
L-Threonine	0.31	0.31	0.31	0.31
BHT3	0.10	0.10	0.10	0.10
Choline chloride	1.00	1.00	1.00	1.00
Calculated composition				
Crude Protein	175.9	175.9	175.9	175.9
Metabolisable energy (MJ·Kg^{-1})	12.14	12.14	12.14	12.14
Calcium	1.1	6.0	24.0	24.0
Available Phosphorus	0.89	4.59	0.89	0.89
Ca: Available Phosphorus	1.23	1.30	26.96	26.96
Analysed composition				
Dry matter	894.9	921.6	909.6	926.6
Calcium	1.23	6.63	27.03	26.66
Available Phosphorus	0.91	4.64	0.94	0.97
Ca: Available Phosphorus	1.35	1.43	28.75	27.49

[1]Containing per kg: Vit. A—15,000,000 International Unite (IU); Vit. D3—1,500,000 IU; Vit E—15,000 IU; Vit B1—2.0 g; Vit B2—4.0 g; Vit B6—3.0 g; Vit B12—0.015 g; Nicotinic acid—25.0 g; B.C. Pantothenic—10.0 g; Vit. K3—3.0 g; B.C. folic-1.0 g; Zinc bacitracin—10.0 g; Selenium—0.25 g and antioxidant—10.0 g. [2]Containing per kg: Manganese—80 g; Iron—80 g; Zinc—50 g; Copper—10 g and Cobalt—2 g; Iodine-1 g. [3]Butyl Hydroxy Toluene. MJ = Mega Joule.

Table 2. Composition and analysis of ingredients (g·kg^{-1}) of the Ca and P free feed.

Ingredients	Amounts
Corn cob	166.90
Starch	800.00
Soy oil	10.00
Potassium carbonate	6.50
Common Salt	4.79
Vitamin Supplement[1]	0.50
Mineral Supplement[2]	0.50
DL-Methionine	0.51
L-Lysine HCl	3.22
L-Threonine	0.70
L-Valine	0.42
L-Arginine	2.17
Glycine	1.50
L-Tryptophan	0.42
L-Isoleucine	0.28
BHT3	0.10
Choline chloride	1.00
Calculated Composition	
Crude Protein	70.93
Metabolisable energy (MJ·Kg^{-1})	12.14
Calcium	0.00
Available Phosphorus	0.00
Total Phosphorus	0.00
Analysed composition	
Dry matter	917.9
Calcium	0.00
Total Phosphorus	0.02

[1]Containing per kg: Vit. A—15,000,000 IU; Vit. D3—1,500,000 IU; Vit E—15,000 IU; Vit B1—2.0 g; Vit B2—4.0 g; Vit B6—3.0 g; Vit B12—0.015 g; Nicotinic acid—25.0 g; B.C. Pantothenic—10.0 g; Vit. K3—3.0 g; B.C. folic—1.0 g; Zinc bacitracin—10.0 g; Selenium—0.25 g and antioxidant—10.0 g. [2]Containing per kg: Manganese—80 g; Iron—80 g; Zinc—50 g; Copper—10 g and Cobalt—2 g; Iodine—1 g. [3]Butyl Hydroxy Toluene. MJ = Mega Joule.

2.2. Birds

The chicks used were acquired from a 1-day-old local hatchery and were fixed on the floor in an open shed and, at the 17th week of age, were transferred to a laying shed (60 × 9 m) covered with clay tiles, housed in cages [25 cm wide, 35 cm long and 40 cm high; two birds per cage (three cages combined with the experimental unit)].

During the rearing, rearing and production phases up to 40 weeks of age, the birds were managed as described in the lineage manuals and fed with rations formulated according to the recommendations of Rostagno *et al.* [13]

In the 40th week, the birds were fed with the experimental rations for nine days, being five days for adaptation to the cage and the experimental diet and four days for total excrement collection.

At each end of the experimental phase (nine days), the birds received the feed formulated according to the recommendations of Rostagno *et al.* [13], until they are submitted to a new digestibility test.

Ambient temperature (˚C) was monitored through three maximum and minimum thermometers located throughout the shed. The maximum and minimum mean shed temperatures varied between 29.08˚C and 18.33˚C, respectively. A 16 h light/8h dark photoperiod was applied. Food and water were provided *ad libitum*.

2.3. Sample Collection and Processing

Hen excreta were collected and stored in plastic bags in a freezer (−18˚C) until the end of the collection period of each test. The hens received a diet free of calcium and phosphorus to assess endogenous losses. At the end of the collection period, the excreta were thawed, weighed, homogenized, dried in a ventilated oven at 55˚C for 72 h and ground and stored in plastic containers for subsequent analyses.

The following data was collected: feed intake (g) (FI), dry matter intake (g) (DMI), Ca intake (g) (CaI), basal feed diet and Ca sources (g), Ca supply by the basal diet and food ($g \cdot Kg^{-1}$), Ca content in the diets and excreta ($g \cdot Kg^{-1}$), Ca excretion (g) and Ca excreted by birds receiving a diet with low Ca content ($g \cdot Kg^{-1}$). These data were used to obtain the apparent and true Ca digestibility coefficients values and apparent dry matter digestibility coefficient, using the equations adapted by Rostagno and Featherston [14].

2.4. Calculations

Apparent Ca Digestibility Coefficient (ADC).

$$\text{ADC} = \frac{\text{Ingested Ca}(\text{g}) - \text{Excreted Ca}(\text{g})}{\text{Ingested Ca}(\text{g})}$$

True Ca Digestibility Coefficient (TDC).

$$\text{TDC} = \frac{\left[\text{Ingested Ca}(\text{g}) - \left(\text{Excreted Ca}(\text{g}) - \text{Endogenous Ca}\right)\right]}{\text{Ingested Ca}(\text{g})}$$

Apparent Dry Matter Digestibility Coefficient (ADMDC).

$$\text{ADMDC} = \frac{\text{Ingested dry matter}(\text{g}) - \text{Excreted dry matter}(\text{g})}{\text{Ingested dry matter}(\text{g})}$$

2.5. Chemical Analyses

The mean geometric diameter (MGD) determinations of the feed were performed at the Embrapa—CNPSA Animal Nutrition laboratory, in Concordia, SC, using the Softgran Granucalc® program [15]. This is an alternative method that approximates the applied screen meshes (Table 3).

Ca content was determined by Flame Atomic Absorption Spectrometry [16]. *In vitro* solubility was determined according to Cheng and Coon [10], through the percentage of weight loss, where 100 mL of 0.1 N hydrochloric acid was added.

2.6. Statistical Analyses

The analysis of variance was performed according to the statistical model to a completely randomized design:

$$Y_{ij} = \mu + t_i + \beta_j + (t\beta)_{ij} + \varepsilon_{ij},$$

in which Y_{ij} = observed value for treatment i, in repetition j, μ = average of the experiment, t_i = effect of level i of factor t, β_j = effect of level j of factor β, $(t\beta)_{ij}$ = effect of the interaction between t_i and β_j, and ε_{ij} = random error associated to each observation.

The data were submitted to an ANOVA test, and the means were compared by the Tukey test at a 5% probability using the statistical package R Core Team [17].

3. Results

An interaction ($P < 0.05$) was observed between Ca sources and bird age (weeks), for FI and DMI (Table 4). The highest FI and DMI were found in 40- and 50-week-old birds relative to 70-week-old birds when consuming CL and in 40- and 70-week-old birds when consuming FL relative to DCP and CL ($P < 0.05$).

Like FI and DMI, CaI also showed an interaction ($P < 0.05$) between Ca sources and bird age. Birds at 40 and 50 weeks of age showed higher CaI ($P < 0.05$) when consuming CL compared to birds at 70 weeks of age. The highest CaI ($P < 0.05$) was found in the 40- and 70-week-old birds that consumed FL followed by CL and DCP, and at 50 weeks of age ($P < 0.05$) that consumed FL and CL followed by DCP.

For ADMDC, there was no effect ($P > 0.05$) of Ca sources, bird ages and their interaction.

Concerning the ADC, an interaction ($P < 0.05$) between Ca sources and bird age was detected (Table 5). Regarding DCP, the ADC was higher ($P < 0.05$) in

Table 3. Mean geometric diameter (MGD), geometric standard deviation (GSD), solubility and Ca and P concentrations of the test foods analyzed in the laying bird experiments.

Test feeds	MGD (μm)	GSD	Solubility (%)	Ca ($g \cdot Kg^{-1}$)	P ($g \cdot Kg^{-1}$)
DCP	560.00	2.58	-	266.10	184.90
FL	558.00	1.98	21.55	365.50	-
CL	1998.50	1.29	15.52	363.50	-

Table 4. Means for feed intake (FI), dry matter intake (DMI), Ca intake (CaI) and apparent dry matter digestibility coefficient (ADMDC) of Ca sources at different ages for laying birds[1].

	Sources	Age (weeks)			$Means^{Sources}$	SEM	P value		
		40	50	70			Age	Sources	Age × Sources
FI (g/hen d)	DCP	89.340Ba	90.430Aa	78.334Ba	86.035	10.613	0.001	<0.001	0.004
	FL	106.201Aa	101.916Aa	107.041Aa	105.053				
	CL	94.764ABa	99.139Aa	74.354Bb	96.952				
	$Mean^{Age}$	96.768	97.162	86.576					
DMI (g/ hen d)	DCP	81.588Ba	83.352Aa	72.850Ba	79.263	9.722	0.001	<0.001	0.004
	FL	96.110Aa	93.131Aa	97.407Aa	95.549				
	CL	87.258ABa	92.119Aa	69.138Bb	82.838				
	$Mean^{Age}$	88.319	89.534	79.798					
CaI (g/hen d)	DCP	0.543Ca	0.568Ba	0.463Ca	0.524	0.221	<0.001	<0.001	<0.001
	FL	2.681Aa	2.466Aa	2.600Aa	2.582				
	CL	2.380Ba	2.411Aa	1.822Bb	2.204				
	$Mean^{Age}$	1.868	1.871	1.628					
ADMDC	DCP	0.864	0.887	0.874	0.874	0.029	0.080	0.495	0.752
	FL	0.866	0.882	0.886	0.878				
	CL	0.861	0.883	0.860	0.868				
	$Mean^{Age}$	0.864	0.883	0.874					

Means followed by different lowercase letters on the line indicate a statistically significant difference by the Tukey test (P < 0.05). Means followed by different capital letters in the column indicate a statistically significant difference by the Tukey test (P < 0.05). [1]Each value represents the mean of eight replicates (six birds per replicate). SEM = standard error mean.

Table 5. Means for Ca, Apparent (ADC) and True (TDC) Digestibility Coefficients of Ca sources, at different ages, for laying birds[1].

	Sources	Age (weeks)			$Means^{Sources}$	SEM	P value		
		40	50	70			Age	Sources	Age × Sources
ADC	DCP	0.889Aa	0.613Ab	0.712Ab	0.738	0.141	0.011	<0.001	0.040
	FL	0.689Ba	0.556Aa	0.652Aa	0.633				
	CL	0.515Ca	0.550Aa	0.482Ba	0.516				
	$Mean^{Age}$	0.698	0.574	0.615					
TDC	DCP	0.892	0.713	0.754	0.786A	0.125	0.013	<0.001	0.208
	FL	0.692	0.559	0.662	0.637B				
	CL	0.558	0.553	0.492	0.534C				
	$Mean^{Age}$	0.714a	0.608b	0.636ab					

Means followed by different lowercase letters on the line indicate a statistically significant difference by the Tukey test (P < 0.05). Means followed by different capital letters in the column indicate a statistically significant difference by the Tukey test (P < 0.05). [1]Each value represents the mean of eight replicates (six birds per replicate). SEM = standard error mean.

40-week-old birds, while no effect of age (P > 0.05) on ADC was observed for FL and CL (P > 0.05).

Comparing Ca sources, DCP exhibited a higher ADC ($P < 0.05$), followed by FL and CL in 40-week-old birds. DCP and FL exhibited a higher ADC ($P < 0.05$) compared to CL in 70-week-old birds, while no effect ($P > 0.05$) of Ca sources on ADC was observed at 50 weeks.

Endogenous losses of 790, 860 and 930 $mg \cdot kg^{-1}$ of consumed dry matter were obtained at 40, 50 and 70 weeks of age, respectively.

For TDC, no interaction ($P > 0.05$) was observed between Ca sources and bird age. The highest TDC ($P > 0.05$) was observed in birds fed DCP, followed by FL and CL. In addition, 40-week-old birds exhibited a higher TDC value ($P < 0.05$), compared to 50-week-old birds.

4. Discussion

The higher *in vitro* solubility of fine-grained limestone compared to coarse particles observed in the present study has also been observed in previous assessments [6] [7] [10] [18]. The larger surface area of the FL particle counts may increase the hydrochloric acid reaction and consequently, *in vitro* solubility [19].

The calculated Ca value in the limestones specified by Rostagno *et al.* [13] (377.00 $g \cdot Kg^{-1}$) and by the NRC [20] (380.00 $g \cdot Kg^{-1}$) were close to those analyzed in the thin (365.50 $g \cdot Kg^{-1}$) and coarse (363.50 $g \cdot Kg^{-1}$) limestones assessed in the present study. The present findings are consistent with previously published data, in which limestone Ca concentrations range between 360.00 and 415.00 $g \cdot Kg^{-1}$ [21] [22] [23]. Anwar *et al.* [7] state that limestone cannot exceed 400.00 $g \cdot Kg^{-1}$ of Ca based on the atomic and molecular weights of Ca carbonate, and that the aforementioned values can be attributed to analytical errors, contamination with Ca hydroxide or both.

The higher values found for FI and DMI, in the present study, with the 40 and 50-week-old birds, compared to older birds (70 weeks old), when treated with CL, may be attributed to the larger granulometry of limestone, because according to Portella *et al.* [24] and Nir *et al.* [25], older chickens tend to select and ingest larger feed particles. This greater intake of these feed particles may interfere with the dwell time of the feed in the gizzard and the rate of intestinal transit [1], leading to greater satiety of the animal and decreased FI. This finding corroborates that found by Geraldo *et al.* [26], when evaluating two limestone granulometry (0.135 mm *versus* DGM = 0.899 mm) during 8 to 12 weeks of age of Lohmann—LSL birds, observed lower IF of older birds that consumed feed with limestone with higher granulometry.

Given the results in this study, within each age (40 and 70 weeks of chicken age), birds treated with FL compared to CL and DCP, showed higher FI and DMI. These results reinforce that due to the greater consumption of coarser particles and longer time the feed remains in the gizzard, the hens are satiated longer, which reduces FI and consequent reduction in DMI. However, the DCP, by presenting the MGD (560.00 μm) close to the MGD of FL (558.00 μm), should not be attributed to the particle size of the DCP, but in relation to its Ca digestibility coefficient, which may have favored its lower FI and DMI.

The results of this study indicate that laying hens were more efficient in digesting Ca from DCP and FL, which contributed, in part, to the higher TDC values observed for DCP and FL compared to CL. This can be explained by the particle size of these ingredients (**Table 3**), as DCP and FL exhibit lower MGD, which, in turn, affects intestinal functions and digestive processes [27] [28]. Finer particle size increases the contact surface area of the particles per unit volume, which can improve digestion efficiency and nutrient absorption [9] [30]. In addition, Ca levels in the DCP diet (6.00 $g \cdot Kg^{-1}$) were lower than in the limestone diet (24.00 $g \cdot Kg^{-1}$), where it can be demonstrated by CaI. Thus, higher Ca levels may have intensified the lower Ca absorption effect observed in the CL diet [31].

Birds consuming diets with high levels of Ca tend to have a low absorption rate for this mineral. This low absorption rate is associated with saturation of the Ca transport protein [32]. In addition, due to the increase in pH promoted by high Ca levels in the gut, the formation of insoluble calcium phosphate is reduced due to the neutralization in the gut promoted by the increase in pH [33]. Furthermore, in older birds, the capacity to absorb Ca is lower compared to younger birds [34].

Similar results were reported by Mayer [35], who observed a 10% decrease in the apparent Ca digestibility rate (0.376 *versus* 0.275) with increased limestone particles from 126 to 933 micrometers. However, intact particles (coarse limestone) were notoriously excreted, explaining the low Ca CL digestibility.

The hens were expected to exhibit greater CL digestion compared to FL. Anwar *et al.* [2] when comparing the effect of calcium source and particle size (fine (<0.5 mm) and coarse (1 - 2 mm)) on the apparent ileal Ca digestibility coefficient for broilers reported higher coefficients for birds that consumed the larger particles, 0.430, *versus* 0.710 that consumed fine particles. It is possible that the inclusion of coarser particle size particles with low *in vitro* solubility increased *in vivo* solubility and caused improvements in nutrient retention and absorption in laying hen [9] [36]. In addition, diets with larger particles positively influence the development and size of poultry gizzards [37] [38], as more rustic diets stimulate intestinal motility [39], due to the stimulus of cholecystokinin release [40]. This, in turn, acts on the release of endogenous enzymes and gastro-duodenal reflux [41] [42], which favors a decrease in the rate of digestion passage [25], and an increase in nutrient digestibility [43], differing from the results of the present study.

The TDC observed in this study was higher in 40-week-old birds compared to 50-week-old birds. A similar result was reported by Sordi *et al.* [44] when evaluating Ca digestibility for laying hens, who also noted a decrease in the true Ca digestibility coefficient of different DCP and limestone granulometries with increasing bird age. These results, in addition to being influenced by apparent digestibility, may also be due to the average endogenous Ca losses of the hens. Older birds exhibited higher endogenous losses, which may have influenced the reduction of TDC since, in the present study, endogenous loss values of 790, 860 and 930 mg·Kg^{-1} of consumed dry matter were detected at 40, 50 and 70 weeks of age, respectively. David *et al.* [3], observed a higher coefficient of true ileal digestibility for limestone (0.510) in broilers compared to DCP (0.320). These results differ from those reported herein, where DCP (0.786) presented a higher coefficient compared to FL (0.637) and CL (0.534). 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 laying hens are suggested.

5. Conclusion

It can be concluded that currently researches still disagree regarding the ideal granulometry of calcium sources associated to their digestibility, mainly related to limestone. However, in the current study, although the true digestibility was higher in younger birds when treated with DCP and FL in relation to CL, some points still need to be elucidated, such as the proof that smaller limestone particles are more efficient in calcium digestibility, especially for brown laying hens.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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