Influence of the calcium and nutrient content of the prelay diet on egg production, egg quality, and tibiae mineralization of brown egg-laying hens from 16 to 63 wk of age

A. F. de Juan ,* R. Scappaticcio ,* L. Aguirre,* G. Fondevila ,* J. García,† L. Cámara ,* and G. G. Mateos*,[1]

*Departamento de Producción Agraria, Universidad Politécnica de Madrid, 28040 Madrid, Spain; and
†Camar Agroalimentaria S. L., Toledo, Spain

ABSTRACT The influence of the Ca and nutrient content of the diet fed from 16 to 19 wk of age, on egg production, egg quality, and tibiae mineralization, was studied in brown egg-laying hens from 16 to 63 wk of age. The experimental design was completely randomized with 4 prelay diets organized as a 2 × 2 factorial with 2 levels of Ca (2.5 vs. 3.8%) and 2 standardized ileal digestible Lys (g/kg) to AMEn (Mcal/kg) ratios (DLys:ME; 2.84 vs. 3.13) as main effects. From 20 to 63 wk of age, all hens received a common diet with 2.75 Mcal AMEn/kg, 0.75% DLys, and 3.8% Ca. Each treatment was replicated 18 times and the experimental unit was a cage with 10 hens. Hen production and egg quality traits were measured by period (4 wk), feeding phase (prelay and lay), and cumulatively (16−63 wk of age) and tibiae mineralization was measured at 63 wk of age. During the prelay phase, an increase in Ca delayed egg production (P = 0.065), reduced feed intake (P < 0.05), and increased BW gain (P < 0.01) and percentage of shell of the egg (P < 0.05). An increase in the DLys:ME ratio increased feed intake (P < 0.01) and reduced egg size (P < 0.01). Nutrient content of the prelay diets did not affect hen production during the lay phase, except egg weight that increased (P < 0.05) in hens previously fed the low DLys:ME ratio. Eggshell quality (weight, percentage, strength, and thickness) in this phase was better (P < 0.05) in hens previously fed 3.8% Ca. Cumulatively (16−63 wk of age), hens fed the high Ca prelay diets had better shell quality but tibiae mineralization was not affected. In conclusion, an increase in Ca content of the prelay diet from 2.5 to 3.8% improved shell quality for the entire egg cycle without showing any negative effect on hen production.

Key words: calcium, egg production, prelay diet, shell quality, tibiae ash

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INTRODUCTION

Current genetic programs for brown egg-laying hens aim to improve egg mass production by controlling mature BW while maintaining egg quality for extended periods of time (Anderson et al., 2013). Prelay diets low in Ca, increase Ca mobilization from the bones at the onset of the laying period, which may affect bone strength and shell quality at the end of the egg cycle (Fleming et al., 1998; Bar et al., 1999), reducing the advantage of the greater number of eggs laid (Bain et al., 2016). On the other hand, prelay diets high in Ca, might reduce feed intake (**FI**) and egg weight (**EW**) at the onset of egg production, a period in which egg size is of critical economical interest (Scott et al., 1971; Bolden and Jensen, 1985).

The relative low BW of modern hens reduces the size and the physical capacity of the gastrointestinal tract (**GIT**), which in turn affects voluntary FI in young hens (Leeson and Summers, 2009; Joly, 2012; Scappaticcio et al., 2021, 2022). In contrast, energy and nutrient requirements of the birds increase sharply at the onset of egg production to meet the high demands for BW gain, egg mass production, and the development of the reproductive tract (Grobas et al., 1999a; Lohmann, 2021; Hy-Line, 2022). Diets high in energy, CP, and Ca, however, are usually low in fiber, which might affect the development of the GIT and the capacity of the pullets to increase FI in the initial states of the laying period (Jiménez-Moreno et al., 2009; Guzmán et al., 2016). Consequently, the relation among energy, amino acids (**AA**), fiber, and Ca contents of the

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[1]Corresponding author: gonzalo.gmateos@upm.es

prepeak diets needs to be optimized to maximize egg production while feed cost and eggshell quality are controlled and maintained, respectively, for the whole egg cycle. Most of the research conducted to estimate the standardized ileal digestible Lys (**DLys**) requirements of the hens, however, has been conducted during the peak production phase or at the end of the laying cycle (Bregendahl et al., 2008; Rocha et al., 2009; Kumar et al., 2018; Scappaticcio et al., 2021). The objectives of this research were to study the effects of the Ca content and DLys to AMEn (**DLys:ME**) ratio in the diet fed from 16 to 19 wk of age, on egg production, egg quality, and tibiae mineralization of brown hens from 16 to 63 wk of age.

MATERIALS AND METHODS

Husbandry and Diets

The experimental procedures were approved by the animal Ethics Committee of the Universidad Politécnica de Madrid and were in compliance with the Spanish Guidelines for the care and use of animals in research (Boletín Oficial del Estado (BOE), 2013). In total, 720 Hy-Line Brown pullets were selected at random from a commercial flock (110,000 birds) at 15 wk of age and housed in cages in the second floor of an environmentally controlled laying barn. At 16 wk of age, the birds were weighed individually and allotted in groups of 10 to 72 adjacent enriched cages (120 cm × 63 cm × 45 cm; Facco S.p.A., Padova, Italy) provided with an open trough feeder and 2 low pressure nipple drinkers. Barn temperature was recorded daily at the hen level, with a minimum of 19 ± 3°C in January and a maximum of 24 ± 3°C in July. The original lighting program (11 h light/d at 15 wk of age) was modified gradually to reach 16 h light/d at 21 wk of age and then maintained constant until the end of the experiment. Birds had free access to feed in mash form and water throughout the experiment.

The experiment (12 periods of 4 wk each) was conducted in 2 phases: prelay from 16 to 19 wk and lay from 20 to 63 wk of age. In the prelay phase, there were 4 diets arranged as a 2 × 2 factorial, with 2 levels of Ca (2.5 vs. 3.8%) and 2 DLys:ME ratios (2.84 vs. 3.13). The low and high DLys:ME ratios corresponded to diets with 7.8 vs. 8.2 DLys g/kg and 2.75 vs. 2.62 Mcal AMEn/kg, respectively. All the diets contained 0.39% digestible P (FEDNA, 2018; Lohmann, 2021; Hy-Line, 2022). From 20 to 63 wk of age, all hens were fed a common diet based on FEDNA (2018) recommendations. The ingredient composition and the calculated (FEDNA, 2021) and determined nutrient content of the experimental diets are presented in Table 1.

Laboratory Analysis

Experimental Diets. Mean particle size and particle size distribution of the diets, expressed as geometric mean diameter (**GMD**) ± geometric standard deviation (**GSD**), were determined in 100 g samples using a shaker equipment (Retsch, Stuttgart, Germany) provided with 8 sieves ranging in mesh from 5,000 to 40 μm as outlined by the ASAE (2003). Representative samples of the diets were ground with a laboratory mill (Retsch Model Z-I, Stuttgart, Germany) equipped with a 0.75-mm screen and analyzed for gross energy using an adiabatic bomb calorimeter (model 1356, Parr Instrument Company, Moline, IL) with benzoic acid as the calibration standard, moisture by oven-drying (method 930.15), nitrogen by combustion (method 968.06) using a Leco analyzer (Model FP-528, Leco Corp., St. Joseph, MI), total ash in a muffle furnace (method 942.05), ether extract by Soxhlet after 3N HCl hydrolysis (method 920.39), and crude fiber by sequential extraction with diluted acid and alkali (method 962.09) as described by AOAC International (2019). Calcium and P content of the feeds were determined as described by Hermida et al. (2006). All the analyses were conducted in duplicate except for GMD ± GSD that were determined in triplicate.

Tibiae Mineralization. At the end of the experiment (63 wk of age), all hens were euthanized by CO_2 inhalation and the right tibiae was excised, cleaned of connective tissue, and stored in individual plastic bags at −20°C. Before analysis, the frozen tibiae were thawed for 24 h at room temperature, oven-dried at 103°C for 24 h, submerged in diethyl ether for 48 h, and dried again to determine the weight of the defatted tibiae. The tibiae were ashed at 600°C for 8 h and weighed, and the ash, Ca, and P contents were determined.

Measurements

Hen Production. All eggs were collected daily. EW was measured in all the eggs laid the first d of each wk of the 12 experimental periods (4 wk each) and the average value was used for further analyses. Feed disappearance and BW of the hens were determined by replicate and period. Mortality was recorded and weighed as it occurred. From these data, egg production, EW, egg mass, FI, feed conversion ratio (**FCR**) per kilogram of eggs, and BW gain were determined by period (4 wk), phase (prelay from 16 to 19 wk and lay from 20 to 63 wk), and cumulatively (16−63 wk of age). In addition, DLys intake, expressed in mg per day, energy intake (**EI**), expressed as kcal AMEn ingested per hen per day, and energy conversion ratio (**ECR**), expressed as kcal AMEn per g of egg, were determined at the same times.

Egg Quality. Egg quality traits were measured from 19 wk of age to the end of the experiment at 63 wk of age. Because of the small size and low production rate, eggs produced from 16 to 18 wk of age were not controlled. The percentage of shell-less, cracked, and dirty eggs were recorded by replicate in all the eggs produced. An egg was considered as dirty when a spot of any kind or size was detected on the shell (Lázaro et al., 2003). Other egg quality traits, including shell weight in absolute (g) and relative (% EW) terms, shell strength, and shell thickness, were measured in 10 fresh eggs collected randomly from each replicate for the last 2 d of each of the 12 experimental periods. Shell strength, expressed in

Table 1. Ingredient composition (% as fed basis) and chemical analyses of the experimental diets.

	Prelay phase (16−19 wk)				Lay phase (20−63 wk)
Calcium	2.50%		3.80%		3.80%
DLys:ME[1]	2.84	3.13	2.84	3.13	2.73
Ingredient					
Wheat	36.0	25.0	36.0	25.0	36.2
Barley	10.8	30.0	8.20	30.0	10.2
Corn	15.0	7.10	15.0	4.20	15.0
Soybean meal (47% CP)	19.8	19.8	25.0	25.0	15.6
Sunflower meal (35% CP)	8.28	8.80	1.09	1.70	8.46
Soy oil soapstocks	2.50	1.70	3.47	2.91	3.40
Calcium carbonate[2]	5.50	5.53	9.13	9.13	9.45
Dicalcium phosphate	1.00	0.91	1.00	0.93	0.60
Sodium chloride	0.32	0.34	0.33	0.33	0.34
DL-Met (99%)	0.19	0.19	0.22	0.23	0.16
L-Thr (98%)	0.02	0.01	0.03	0.02	0.01
L-Lys (78%)	0.09	0.12	0.03	0.05	0.13
Vitamin-mineral premix[3]	0.50	0.50	0.50	0.50	0.50
Determined analyses					
Moisture	10.7	10.3	10.4	10.0	9.82
Gross energy (Mcal/kg)	3.80	3.86	3.69	3.73	3.66
Crude protein	18.0	18.8	17.7	18.6	17.0
Ash	9.43	9.16	12.4	12.2	12.8
Calcium	2.58	2.47	3.76	3.82	3.85
Total phosphorus	0.59	0.58	0.53	0.52	0.47
Calculated analyses[4]					
AMEn (Mcal/kg)	2.75	2.62	2.75	2.62	2.75
Digestible amino acid					
Lys	0.78	0.82	0.78	0.82	0.75
Met	0.45	0.47	0.45	0.47	0.41
Met + Cys	0.71	0.74	0.71	0.74	0.67
Thr	0.56	0.58	0.56	0.58	0.53
Ether extract	4.20	4.03	5.03	4.34	4.27
Crude fiber	4.29	5.82	2.88	3.65	4.17
Neutral detergent fiber	11.8	14.9	9.32	11.7	11.5
Linoleic acid	2.01	1.91	2.44	2.06	2.17
Digestible phosphorus	0.39	0.39	0.39	0.39	0.33
Sodium	0.16	0.16	0.16	0.16	0.14
GMD ± GSD[5] (μm)	1,120 ± 2.3	1,118 ± 2.0	1,153 ± 2.2	1,145 ± 2.3	1,150 ± 2.1

[1]Standardized ileal digestible Lys (g):AMEn (Mcal) per kg of diet.

[2]The ratio between coarse (2−4 mm ø) and fine (≤1 mm ø) calcium carbonate was 70% for all the diets.

[3]Provided per kilogram of diet: vitamin A (*trans*-retinyl acetate), 10,000 IU; vitamin D3 (cholecalciferol), 3,750 IU; vitamin E (dl-α-tocopheryl acetate), 20 mg; vitamin B_1, 1.3 mg; vitamin B_2, 5 mg; vitamin B_6, 2 mg; vitamin B_{12} (cyanocobalamin), 15 μg; niacin, 25 mg; pantothenic acid (d-calcium pantothenate), 10 mg; folic acid, 1 mg; D-biotin, 0.15 mg; choline (choline chloride, 60%), 250 mg; manganese (MnO), 90 mg; zinc (ZnO), 60 mg; iron ($FeCO_3$), 40 mg; copper ($CuSO_4 \cdot 5H_2O$), 8 mg; iodine [$Ca(IO_3)_2$], 0.7 mg; selenium (Na_2SeO_3), 0.3 mg; Roxazyme, 200 mg [1,600 U endo-1,4-β-glucanase (EC 3.2.1.4), 3,600 U endo-1,3 (4)-β-glucanase (EC 3.2.1.6), and 5,200 U endo-1,4-β-xylanase (EC 3.2.1.8)] supplied by DSM S.A., Madrid, Spain; Axtra PHY, 30 mg [300 U of 4a24 6-phytase (EC 3.1.3.26)] supplied by IFF, Madrid, Spain.

[4]According to FEDNA (2021).

[5]Geometric mean diameter ± geometric standard deviation.

kg/cm^2, was evaluated using a press meter (Egg Force Reader, SANOVO Technology A/S, Odense, Denmark) applying increased pressure to the broad pole of the egg. Shell thickness (μm) was measured at the 2 pole ends and at the middle section of the eggshell, using a digital micrometer (model IT014UT, Mitotuyo, Kawasaki, Japan), and the average of the 3 measurements was used for further analyses (Safaa et al., 2008b). Haugh units were analyzed in the same eggs using a Multitester equipment (QCM System, Technical Services and Supplies, Dunnington, York, UK) as indicated by Pérez-Bonilla et al. (2012a). Data are presented by period, phase, and cumulatively.

Statistical Analysis

The experiment was conducted as a completely randomized design with 4 prelay diets arranged as a 2 × 2 factorial, with Ca level and DLys:ME ratio as main effects. Each treatment was replicated 18 times and the experimental unit was an enriched cage with 10 birds for all traits. The data were analyzed by period, feeding phase, and cumulatively, using the MIXED procedure of SAS (SAS Institute, 2004). When significant differences among treatments were detected, means were separated using the Tukey test. The effects of age (12 periods of 4 wk each) and the interaction between age and dietary effects (Ca content and DLys:ME ratio) on egg production and egg quality traits, were tested as indicated by Littell et al. (1998). Mortality did not follow a normal distribution and consequently, the data (number of dead birds) was analyzed as a binomial distribution, using the LOGISTIC procedure of SAS (SAS Institute, 2004). Results in tables are presented as means and differences were considered significant at $P < 0.05$.

Table 2. Effects of diet composition on hen production from 16 to 19 wk of age[1] (prelay phase).

	Calcium (%)		DLys:ME[2]		SEM	P value[3,4]	
	2.50	3.80	2.84	3.13	(n = 36)	Calcium	DLys:ME
Feed intake (g/d)	80.1	78.0	77.8	80.3	0.605	0.019	0.005
Energy intake (kcal/d)	215	209	214	210	1.62	0.018	0.130
DLys intake (mg/d)	641	624	607	659	4.85	0.019	<0.001
Egg production (%)	14.0	12.3	13.3	13.0	0.667	0.065	0.722
Egg weight (g)	50.2	49.6	50.3	49.5	0.364	0.241	0.010
Egg mass (g/d)	7.03	6.10	6.69	6.44	0.353	0.059	0.576
Feed conversion (g/g)	11.4	12.8	11.6	12.5	0.749	0.222	0.237
Energy conversion ratio (kcal/g)	30.4	34.3	31.8	32.6	2.00	0.207	0.558
BW gain[5] (g)	228	241	238	231	3.49	0.009	0.136

[1]No mortality occurred in this period.
[2]Standardized ileal digestible Lys (g/kg):AMEn (Mcal/kg). The DLys and AMEn contents of the diets were 7.8 and 8.2 g/kg and 2.75 and 2.62 Mcal/kg, respectively.
[3]Age effect was significant for all the variables studied ($P < 0.001$).
[4]The interactions between main effects of the diets and between age and diet were not significant ($P > 0.10$).
[5]The BW of the pullets at 16 wk of age was 1,310 ± 35 g.

RESULTS

The analytical values of the experimental diets were in reasonable agreement with the calculated values. Health status of the birds was good, and the average mortality was 6.4%, a value considered acceptable for hens kept under commercial conditions.

Prelay Phase (16–19 Wk of Age)

Hen Production. Feed (80.1 vs. 78.0 g/d; $P < 0.05$), energy (215 vs. 209 kcal AMEn/d; $P < 0.05$), and DLys (641 vs. 624 mg/d; $P < 0.05$) intake, and egg rate (14.0 vs. 12.3%; $P = 0.065$) and egg mass production (7.07 vs. 6.11 g/d; $P = 0.059$), were higher, and BW gains (228 vs. 241 g; $P < 0.01$) were lower in pullets fed 2.5% Ca than in pullets fed 3.8% Ca (Table 2 and Figure 1). Hens fed the low DLys:ME ratio ate less feed (77.8 vs. 80.3 g/d; $P < 0.01$) and less DLys (607 vs. 659 mg/d; $P < 0.001$) but laid heavier eggs (50.3 vs. 49.5 g; $P < 0.01$) than hens fed the high DLys:ME ratio. Energy intake, egg mass production, FCR, and BW gain, however, were not affected by diet.

Egg Quality. An increase in Ca content of the diet from 2.5 to 3.8% improved the percentage of shell of the eggs (10.22% vs. 10.36%; $P < 0.05$) and tended to increase shell thickness (392 vs. 397 μm; $P = 0.093$). However, shell weight, shell strength, and the incidence of cracked eggs were not affected (Table 3). An increase in the DLys:ME ratio of the diet did not affect any egg quality trait, except the percentage of dirty eggs that was reduced (0.37 vs. 0.07%; $P < 0.05$).

Lay Phase (20–63 Wk of Age)

Hen Production. The effects of Ca content and DLys:ME ratio of the prelay diet on hen production during the lay phase, once all the birds received a common commercial diet, were of limited effect (Table 4). In fact, the only trait affected was EW that was greater (62.4 vs. 61.8 g; $P < 0.05$) in hens previously fed the low DLys:ME ratio but egg mass production was not affected.

Egg Quality. The effects of Ca content of the prelay diet on egg quality traits in this phase are shown in Table 5. An increase in Ca of the prelay diets reduced the incidence of cracked (0.77 vs. 0.55%; $P < 0.05$) and shell-less (0.85 vs. 0.48%; $P < 0.01$) eggs and improved shell thickness (394 vs. 390 μm; $P < 0.001$), shell strength (4.360 vs. 4.271 kg/cm^2; $P < 0.05$), and shell weight, in absolute (6.28 vs. 6.20 g; $P < 0.001$) and relative (10.1 vs. 10.0%; $P < 0.001$) terms. The DLys:ME ratio of the prelay diet did not affect any egg quality trait, except eggshell percentage that increased (10.09 vs. 10.01%; $P < 0.01$) in hens previously fed the higher ratio.

Whole Egg Cycle (16–63 Wk of Age)

An increase in Ca of the prelay diets did not affect hen production for the whole cycle but improved the quality of the shells, with most of the benefits observed for the first (<27 wk of age) and last (>51 wk of age) periods of the experiment (Table 6 and Figures 1–3). A decrease in the DLys:ME ratio increased EW (60.0 vs. 60.6 g; $P < 0.05$), with most of the effects observed for the last part of the experiment (Figure 4).

Tibiae Mineralization. The nutrient content of the prelay diet did not affect ash, Ca, or P content of the tibiae at 63 wk of age (Table 7).

DISCUSSION

Prelay Phase (16–19 Wk of Age)

Hen Production. The effects of the Ca content of the diet on hen production at the onset of the egg cycle are a subject of debate. In the current research, an increase in Ca from 2.5 to 3.8% delayed egg production, reduced FI, and increased BW gain of the birds, in agreement with data of Miller and Sunde (1975) in pullets fed 1.5 vs. 3.0% Ca from 18 to 20 wk of age and of Roland et al. (1985) in pullets fed 1.70 vs. 3.75% Ca from 19 to 21 wk of age. Hawes and Kling (1993), Rodrigues et al. (2013), and Khanal et al. (2019), however, did not find any effect of an increase in Ca on egg production in this phase. In

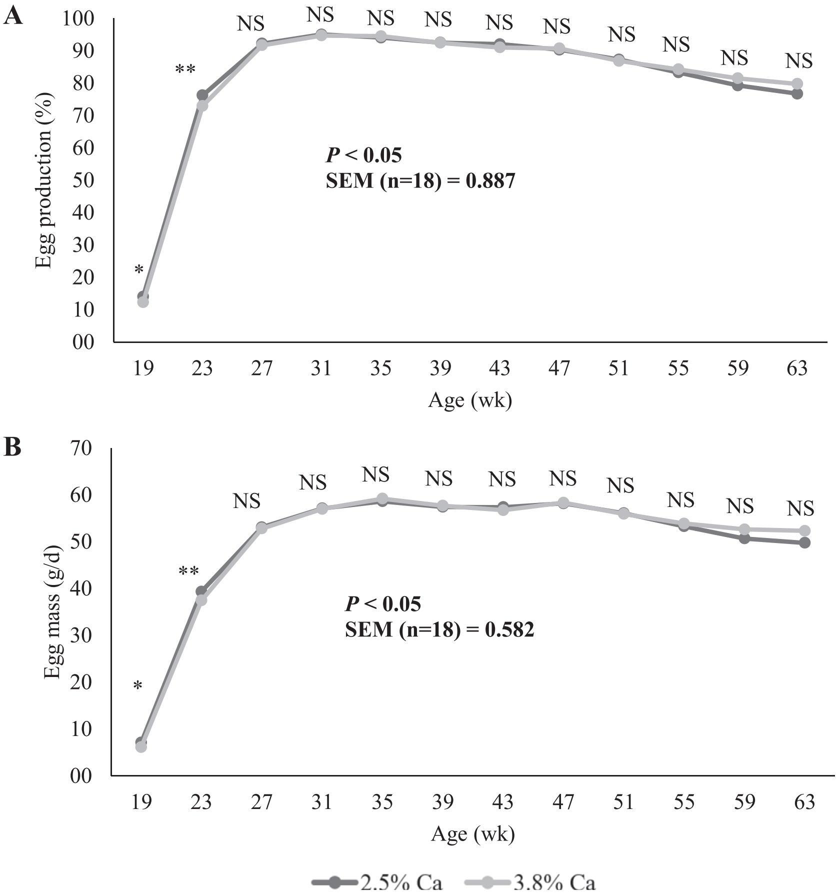

Figure 1. Effects of Ca content of the prelay diet (16−19 wk of age) on (A) egg production and (B) egg mass from 19 to 63 wk of age[1]. [1]Age effect was significant ($P < 0.001$) for all the variables. $^{NS}P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$.

contrast, Keshavarz (1987) observed that an increase in Ca from 0.8 to 3.5% in white hens, increased egg production from 6.7 to 12.2% from 16 to 20 wk of age, without showing any effect on FI. The reasons for the discrepancies among authors in this phase of egg production are not evident but are probably related to the age of the hens at the start of egg production, the length of the experimental period, and the management of the hens during the trial that might affect the development of the reproductive tract and the egg production process (Rodrigues et al., 2013; Khanal et al., 2019).

In the current research, the average egg production from 16 to 19 wk of age was 13% and consequently, most of the pullets needed very little extra Ca for egg production. An excess of Ca of the diet fed to nonlaying pullets, however, might reduce feed palatability and FI (Hughes and Wood-Gush, 1971) as occurred in the present study.

Pullets fed the 3.13 DLys:ME ratio diet (8.2 g DLys/kg and 2.62 Mcal AMEn/kg) ate similar amount of energy and more DLys but produced the same amount of egg mass than hens fed the 2.84 ratio (7.8 g DLys/kg and 2.75 Mcal AMEn/kg). The DLys requirements of

Table 3. Effects of diet composition on egg quality traits at 19 wk of age (prelay phase).

	Calcium (%)		DLys:ME[1]		SEM	P value[2,3]	
	2.50	3.80	2.84	3.13	($n = 36$)	Calcium	DLys:ME
Cracked eggs (%)	1.34	1.06	1.51	0.89	0.333	0.551	0.198
Shell-less eggs (%)	1.90	2.63	2.19	2.34	0.501	0.308	0.826
Dirty eggs (%)	0.21	0.23	0.07	0.37	0.091	0.658	0.035
Haugh units[4]	90.9	89.5	89.9	90.5	0.623	0.122	0.450
Eggshell quality[4]							
Shell weight (g)	5.98	6.01	5.99	6.00	0.039	0.619	0.871
Percentage of shell (% egg)	10.22	10.36	10.25	10.33	0.051	0.047	0.290
Shell strength (kg/cm^2)	4.518	4.583	4.563	4.538	0.057	0.433	0.761
Shell thickness (μm)	392	397	395	394	2.10	0.093	0.916

[1]Standardized ileal digestible Lys (g/kg):AMEn (Mcal/kg). The DLys and AMEn contents of the diets were 7.8 and 8.2 g/kg and 2.75 and 2.62 Mcal/kg, respectively.
[2]Age effect was significant for all the variables studied ($P < 0.001$).
[3]The interactions between main effects of the diets and between age and diet were not significant ($P > 0.10$).
[4]Average of 10 eggs per replicate collected at 19 wk of age.

Table 4. Effects of the composition of the prelay diet (16–19 wk of age) on hen production from 20 to 63 wk of age[1] (lay phase).

	Calcium (%)		DLys:ME[2]		SEM	*P* value[3,4]	
	2.50	3.80	2.84	3.13	($n = 36$)	Calcium	DLys:ME
Feed intake (g/d)	108.8	108.9	108.3	109.2	0.453	0.773	0.103
Egg production (%)	87.1	87.9	86.7	88.4	0.765	0.436	0.120
Egg weight (g)	62.0	62.2	62.4	61.8	0.202	0.530	0.030
Egg mass (g/d)	54.1	54.7	54.1	54.6	0.491	0.339	0.453
Feed conversion (g/g)	2.011	1.991	2.002	2.000	0.016	0.284	0.890
BW gain (g)	332	339	340	330	10.0	0.614	0.493
Mortality[5]	0.14	0.13	0.15	0.12		0.754	0.268

[1]All hens received a common diet in this phase.
[2]Standardized ileal digestible Lys (g/kg):AMEn (Mcal/kg). The DLys and AMEn contents of the diets were 7.8 and 8.2 g/kg and 2.75 and 2.62 Mcal/kg, respectively.
[3]Age effect was significant for all the variables studied ($P < 0.001$).
[4]The interactions between main effects of the diets and between age and diet were not significant ($P > 0.10$).
[5]Expressed as the proportion of dead birds with respect to total number of birds per cage.

young hens are approximately 100 mg/kg of $BW^{0.75}$, 20 mg/g of BW gain, and 12.9 mg/g of egg produced (Fisher, 1998; Joly, 2012; Rostagno et al., 2017). In the current research, $BW^{0.75}$, BW gain, and egg mass production of the hens at 19 wk of age, were as an average, 1.38 kg (1.54 kg BW), 8.50 g/d (from 16 to 19 wk of age), and 7.50 g/d, respectively. Consequently, the requirement of the hens for DLys was below intake (405 vs. 633 mg/d) and thus, it was unlikely that a deficiency in DLys was responsible for the reduction in EW observed. On the other hand, EI was similar for both groups of hens, an observation that was expected because egg mass production and BW gain were similar for the 2 groups of hens, and birds tend to eat to satisfy their energy requirements (Summers and Leeson, 1993; Grobas et al., 1999a; Pérez-Bonilla et al., 2012b). In the current research, the percentage of fat added to the diet was higher for the low than for the high DLys:ME ratio and an increase in supplemental fat increases EW (Grobas et al., 1999b, 2001; Safaa et al., 2008a; Bouvarel et al., 2011; Herrera et al., 2018). The information provided herein suggests that the increase in EW observed in hens fed the diets with the low DLys:ME ratio should be attributed primarily to an increase in the level of supplemental fat rather than to an increase in the consumption of DLys.

Egg Quality. An increase in the Ca content of the diet from 2.5 to 3.8%, increased shell thickness and the percentage of shell of the eggs at 19 wk of age, consistent with data of Brooks (1986) in pullets fed 1.0 vs. 3.0% Ca from 17 to 21 wk of age. An increase in the DLys:ME ratio, however, did not affect egg quality, except the incidence of dirty eggs that increased. Probably, the higher level of barley used in these diets was responsible for the increase in dirty eggs observed (Al Bustany and Elwinger, 1988; Lázaro et al., 2003).

Lay Phase (20–63 Wk of Age)

Hen Production. The information available on the effects of the composition and nutritional characteristics of the prelay diet on the subsequent phase of egg production, once the hens receive a common commercial layer diet, is very limited. In the current research, hen production in the lay phase was not affected by the Ca content of the prelay diet, in agreement with previous research (Hurwitz and Bar, 1971; Keshavarz, 1987; Hawes and Kling, 1993; Keshavarz and Nakajima, 1993). In this work, an increase in the DLys:ME ratio of the prelay diets did not affect egg production but reduced EW, which was also shown by Sujatha et al. (2014) in hens

Table 5. Effects of the composition of the prelay diet (16–19 wk of age) on egg quality traits from 20 to 63 wk of age[1] (lay phase).

	Calcium (%)		DLys:ME[2]		SEM	*P* value[3,4]	
	2.50	3.80	2.84	3.13	($n = 36$)	Calcium	DLys:ME
Cracked eggs (%)	0.77	0.55	0.65	0.66	0.078	0.045	0.908
Shell-less eggs (%)	0.85	0.48	0.79	0.55	0.096	0.007	0.081
Dirty eggs (%)	0.56	0.66	0.66	0.57	0.054	0.197	0.263
Haugh units[5]	89.8	89.8	89.7	89.9	0.162	0.741	0.636
Eggshell quality[5]							
Shell weight (g)	6.20	6.28	6.25	6.23	0.013	0.001	0.436
Percentage of shell (% egg)	10.01	10.09	10.01	10.09	0.017	0.001	0.005
Shell strength (kg/cm^2)	4.271	4.360	4.299	4.332	0.030	0.037	0.425
Shell thickness (μm)	390	394	392	392	0.741	0.001	0.882

[1]All hens received a common diet in this phase.
[2]Standardized ileal digestible Lys (g/kg):AMEn (Mcal/kg). The DLys and AMEn contents of the diets were 7.8 and 8.2 g/kg and 2.75 and 2.62 Mcal/kg, respectively.
[3]Age effect was significant for all the variables studied ($P < 0.001$).
[4]The interactions between main effects of the diets and between age and diet were not significant ($P > 0.10$).
[5]Average of 10 fresh eggs per replicate collected at random for the last 2 d of each of the 11 experimental periods.

Table 6. Effect of the composition of the prelay diet (16−19 wk of age) on hen production from 16 to 63 wk of age[1] (whole cycle).

	Calcium (%)		DLys:ME[2]		SEM	*P* value[3,4]	
	2.50	3.80	2.84	3.13	($n = 36$)	Calcium	DLys:ME
Feed intake (g/d)	106.4	106.2	105.8	106.8	0.394	0.739	0.073
Egg production (%)	80.5	80.1	79.9	80.7	0.558	0.632	0.296
Egg weight (g)	60.3	60.4	60.6	60.0	0.211	0.603	0.043
Egg mass (g/d)	49.4	49.3	49.3	49.4	0.375	0.889	0.811
Feed conversion (g/g)	2.158	2.155	2.148	2.166	0.014	0.892	0.382
BW gain (g)	335	346	348	334	9.57	0.423	0.293

[1]From 20 to 63 wk of age, all hens received a common diet.
[2]Standardized ileal digestible Lys (g/kg):AMEn (Mcal/kg). The DLys and AMEn contents of the diets were 7.8 and 8.2 g/kg and 2.75 and 2.62 Mcal/kg, respectively.
[3]Age effect was significant for all the variables studied ($P < 0.001$).
[4]The interactions between main effects of the diets and between age and diet were not significant ($P > 0.10$).

from 19 to 55 wk of age fed a prelay diet in which the ratio was increased from 2.67 to 2.88. In addition, Summers and Leeson (1993) and Keshavarz and Nakajima (1993) observed a positive correlation between an increase in the energy content of the prelay diet and BW of the hens at sexual maturity, with an increase EW during the laying phase.

Egg Quality. An increase in the Ca content of the prelay diet from 2.5 to 3.8% improved shell quality, in agreement with data of Hurwitz and Bar (1971) and Brooks (1986) in hens fed prelay diets containing 1.3 vs. 4.1% Ca and 1.0 vs. 3.0% Ca, respectively. Similarly, Koçbeker et al. (2017) reported an improvement in shell thickness and breaking strength of the eggs from 20 to 42 wk of age, in hens fed from 14 to 20 wk of age a diet with 3.2 vs. 1.6% Ca.

Under commercial conditions, many poultry companies recommend diets with 2.0 to 2.5% Ca, from 2 wk before expected maturity, until reaching 5 to 10% egg production (Khanal et al., 2019; Lohmann, 2021; Hy-Line, 2022). Assuming a Ca bioavailability of 60% (Ren et al., 2019) and a Ca content of 2.3 g of a 55 g egg, pullets in egg production will require at least 4 g of Ca per day to maintain Ca homeostasis (Etches, 1987; Bar, 2009). If the prelay diet contains only 2.5% Ca, pullets in egg production should consume daily a minimum of 160 g of feed to maintain their Ca status, which is not possible. Consequently,

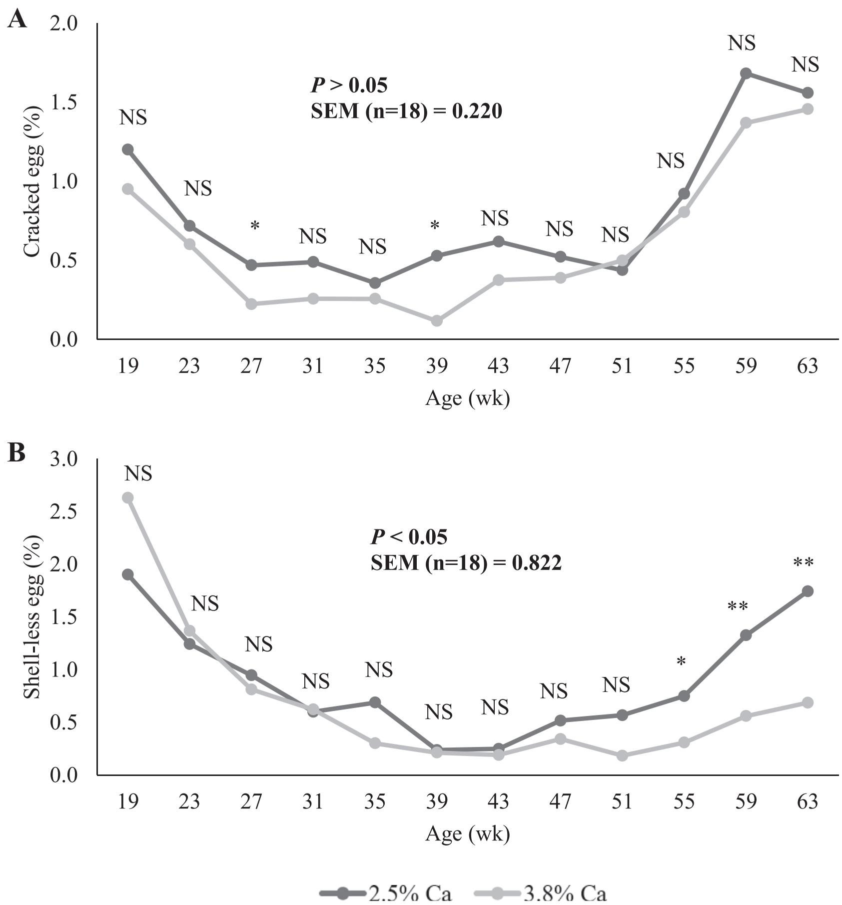

Figure 2. Effects of Ca content of the prelay diet (16−19 wk of age) on (A) percentage of cracked eggs and (B) percentage of shell-less eggs from 19 to 63 wk of age[1]. [1]Age effect was significant ($P < 0.001$) for all the variables. $^{NS}P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$.

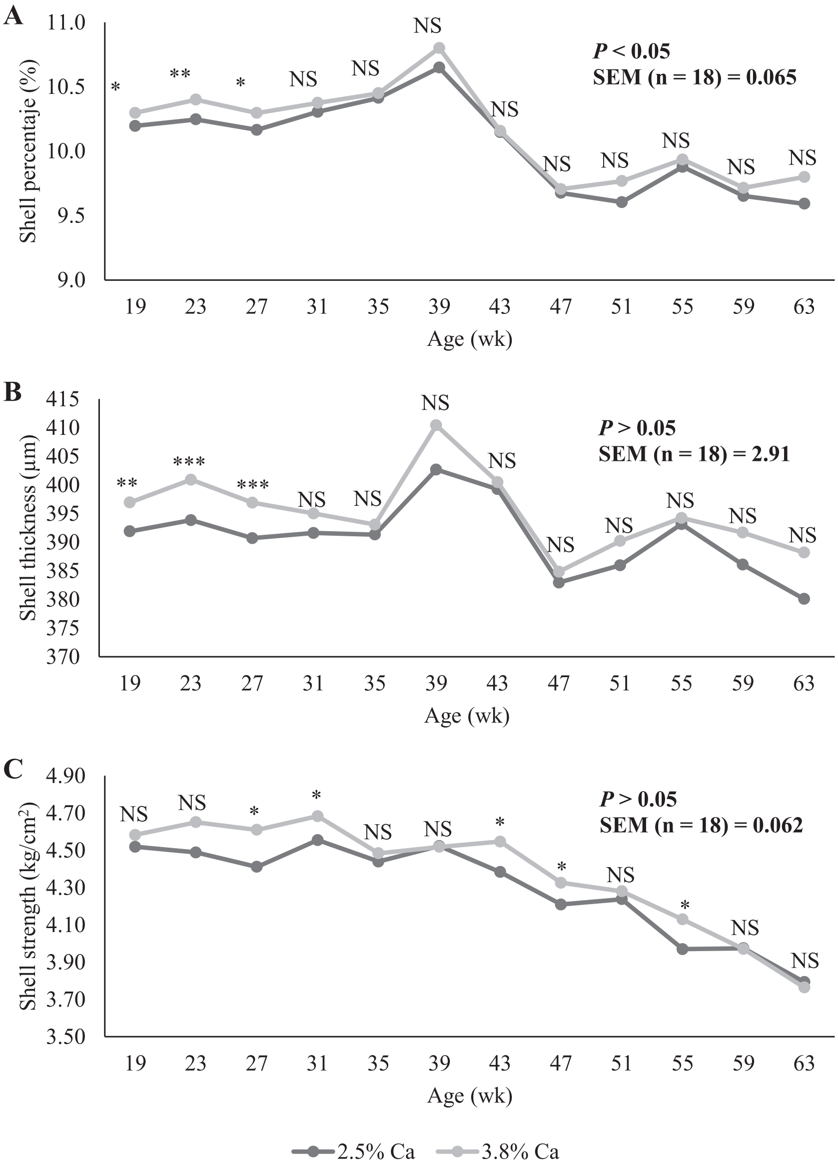

Figure 3. Effects of Ca content of the prelay diet (16−19 wk of age) on (A) percentage of shell of the egg (%), (B) shell thickness (μm), and (C) shell strength (kg/cm^2) from 19 to 63 wk of age[1]. [1]Age effect was significant ($P < 0.001$) for all variables. $^{NS}P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

young laying hens will need to mobilize extra amounts of Ca from the bones (Gilbert, 1983). However, the capacity of the hens to supply all the Ca needed for egg production from the medullary bone, is limited (Petersen, 1965; Whitehead and Fleming, 2000) which might result in the use of Ca from the structural bones for shell formation, increasing the incidence of shell quality problems at the end of the egg cycle (Korver, 2020; Alfonso-Carrillo et al., 2021).

The DLys:ME ratio of the prelay diets had little effect on eggshell quality during the lay phase, consistent with data of Sujatha et al. (2014). In contrast, Xin et al. (2022) observed an improvement in eggshell thickness from 21 to 72 wk of age, in hens fed previously, from 15 to 20 wk of age, a prelay diet with 2.86 vs. 2.96 total Lys:ME. We do not have any clear explanation for the discrepancy of results between both experiments.

Whole Egg Cycle (16−63 Wk of Age)

The effects of the nutrient content of the prelay diet on egg production and egg quality during the entire egg cycle, reflects the changes that occurred during the lay phase, a period in which most of the eggs were produced. An increase in Ca of the prelay diet from 2.5 to 3.8% did not have any effect on hen production, in agreement with data of Keshavarz (1987) comparing prelay diets (16−20 wk of age) with 0.8 or 3.5% Ca. Overall, eggshell quality was better in hens fed prelay diets with 3.8% Ca than in hens fed diets with 2.5% Ca, with the greatest differences observed for the last part of the lay phase. The data reported herein suggest that an increase in Ca of the prelay diets from current recommendations (2.0−2.5%) to 3.8%, may increase the content and mobility of Ca to and from the medullary bones much later in the laying cycle (Leeson et al., 1993; Khanal et al., 2019).

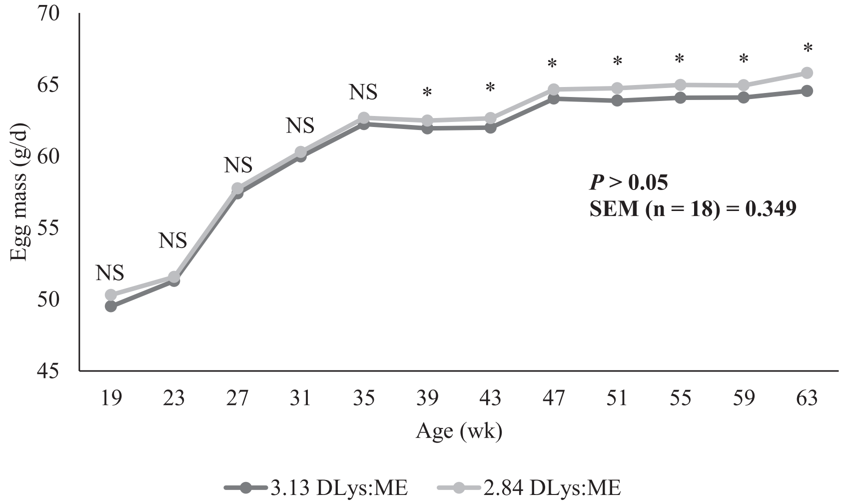

Figure 4. Effects of standardized ileal digestible Lys (g/kg):AMEn (Mcal/kg) ratio of the prelay diet (16−19 wk of age), on egg weight from 19 to 63 wk of age[1]. [1]Age effect ($P < 0.001$). $^{NS}P > 0.05$; $^{*}P < 0.05$.

Table 7. Effect of the composition of the prelay diet (16−19 wk of age) on ash, calcium, and phosphorus content of the tibiae (% DM) at 63 wk of age[1].

	Calcium (%)		DLys:ME[2]		SEM	P value[3]	
	2.50	3.80	2.84	3.13	(n = 36)	Calcium	DLys:ME
Ash content (% of the tibiae)	58.8	57.9	58.6	58.1	0.688	0.331	0.540
Calcium							
% of the tibiae	31.0	31.0	31.1	30.9	0.224	0.851	0.410
% of the ash	18.2	18.0	18.3	17.9	0.212	0.406	0.247
Phosphorus							
% of the tibiae	16.3	16.2	16.3	16.3	0.086	0.568	0.825
% of the ash	9.60	9.41	9.54	9.46	0.120	0.247	0.646

[1]From 20 to 63 wk of age, all hens received a common diet.
[2]Standardized ileal digestible Lys (g/kg):AMEn (Mcal/kg). The DLys and AMEn contents of the diets were 7.8 and 8.2 g/kg and 2.75 and 2.62 Mcal/kg, respectively.
[3]The interactions between main effects were not significant ($P > 0.10$).

Tibiae Mineralization. The medullary bone starts to develop 2 wk before the onset of sexual maturity (Whitehead and Fleming, 2000; Whitehead, 2004). Adequate levels of Ca in the prelay diets ensures that Ca reserves in the medullary bone are maximized in this phase which in turn might help to maintain eggshell quality at the end of the egg cycle (Leeson et al., 1993; Rodrigues et al., 2013; Korver, 2020). When bone Ca repletion between successive ovulations is limited, the medullary bone might be substituted by the structural cortical bone, ending in Ca deficiency (Leeson and Summers, 2009). In the current research, tibiae mineralization at 63 wk of age was not affected by the Ca content of the prelay diet, consistent with data of Akbari Moghaddam Kakhki et al. (2019) and Khanal et al. (2019). Whitehead (2004), Bello et al. (2020), and Alfonso-Carrillo et al. (2021) suggested that the inherent decrease in shell quality with age did not correlate well with tibiae mineralization, consistent with the results reported herein. In this respect, Brooks (1986) observed that bone ash at 21 wk of age, was higher in pullets fed diets with 3.0% Ca from 17 to 21 wk than in pullets fed 1.0% Ca containing diets, but no differences were detected after this age. All this information suggests that the requirements in Ca of modern laying hens, are better evaluated by studying changes in shell quality than by measuring bone ash content.

In conclusion, Ca content and DLys:ME ratio of the prelay diet have limited impact on hen production during the lay phase. Prelay diets with more Ca than currently recommended (3.8 vs. 2.5%) improves shell quality for the whole egg cycle, without showing any effect on tibiae ash content at 63 wk of age. A decrease in the DLys:ME ratio of the prelay diet from 3.13 to 2.84, resulting from a reduction in DLys and an increase in energy content, has little effect on egg production or eggshell quality. An increase in Ca content of the prelay diets over the 2.5% recommended by most genetic companies results in an improvement of the quality of the shell.

DISCLOSURES

The authors confirm that there are no conflicts of interest in this research.

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