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Calcium in the diet of light laying hens: comparison between two tables of nutritional requirements and two sources of dietary limestone

Cálcio na dieta de galinhas poedeiras leves: comparação entre duas tabelas de necessidades nutricionais e duas fontes de calcário dietético

Calcio dietético para gallinas ponedoras ligeras: comparación de dos tablas de requerimientos nutricionales y dos fuentes de calcio dietético

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ABSTRACT
There is a discrepancy regarding the nutritional requirements of laying hens, making it difficult to prepare and establish adequate nutritional programs. The objective of this study was to compare two tables of nutritional requirements for laying hens and two sources of limestone on performance, egg quality, and digestibility of calcium and phosphorus. Ninety-six laying hens of the Hy-Line W36 lineage were used in five production cycles of 21 days each, randomly assigned in a 2x2 factorial design with four treatments and eight replications. Hens fed with limestone A showed higher production in the 2nd and 5th cycles. Eggshell breaking strength was greater in laying hens that received the diet suggested by the Hy-Line Guidelines between 33 and 35 weeks, regardless of the limestone type. The Haugh unit increased in eggs from the third cycle of the Hy-Line/B group compared to those fed with limestone A. The percentage of calcium in the excreta was higher in hens that received limestone A. Laying hens fed according to the lineage manual excreted less phosphorus, even though they ingested higher daily amounts. It is concluded that both nutritional programs and limestone sources promoted good productive performance and internal and external egg quality.

Keywords: Digestibility, Egg, Quality, Layer Production, Poultry.

RESUMO
Há uma discrepância em relação às exigências nutricionais de poedeiras, o que dificulta a formulação e o estabelecimento de programas nutricionais adequados. O objetivo deste estudo foi comparar duas tabelas de exigências nutricionais para poedeiras e duas fontes de calcário sobre o desempenho, a qualidade dos ovos e a digestibilidade do cálcio e do fósforo. Foram utilizadas noventa e seis poedeiras da linhagem Hy-Line W36, distribuídas aleatoriamente em um delineamento fatorial 2x2, com quatro tratamentos e oito repetições, ao longo de cinco ciclos de produção de 21 dias cada. As poedeiras

alimentadas com o calcário A apresentaram maior produção no 2º e 5º ciclos. A resistência da casca dos ovos foi maior nas aves que receberam a dieta sugerida pelas Diretrizes da Hy-Line entre as 33 e 35 semanas, independentemente do tipo de calcário. A unidade Haugh aumentou nos ovos do grupo Hy-Line/B a partir do terceiro ciclo, em comparação com aqueles alimentados com o calcário A. A porcentagem de cálcio nas excretas foi maior nas aves que receberam o calcário A. As poedeiras alimentadas conforme o manual da linhagem, excretaram menos fósforo, mesmo ingerindo maiores quantidades diárias. Conclui-se que ambos os programas nutricionais e fontes de calcário promoveram bom desempenho produtivo e qualidade interna e externa dos ovos.

Palavras-chave: Digestibilidade, Ovo, Qualidade, Produção de Poedeiras, Aves.

RESUMEN

Existe una discrepancia en los requerimientos nutricionales de las gallinas ponedoras, lo que dificulta la formulación y el establecimiento de programas nutricionales adecuados. El objetivo de este estudio fue comparar dos tablas de requerimientos nutricionales para gallinas ponedoras y dos fuentes de caliza en cuanto al rendimiento, la calidad del huevo y la digestibilidad del calcio y el fósforo. Noventa y seis gallinas ponedoras Hy-Line W36 fueron asignadas aleatoriamente a un diseño factorial 2x2 con cuatro tratamientos y ocho réplicas durante cinco ciclos de producción de 21 días cada uno. Las gallinas ponedoras alimentadas con caliza A mostraron una mayor producción en el segundo y quinto ciclo. La resistencia de la cáscara fue mayor en las gallinas alimentadas con la dieta sugerida por las Guías Hy-Line entre las 33 y 35 semanas, independientemente del tipo de caliza. La unidad Haugh aumentó la cantidad de huevos del grupo Hy-Line/B a partir del tercer ciclo, en comparación con las gallinas alimentadas con piedra caliza A. El porcentaje de calcio en las excretas fue mayor en las gallinas alimentadas con piedra caliza A. Las gallinas ponedoras alimentadas según el manual de la raza excretaron menos fósforo, incluso con ingestas diarias más altas. Se concluye que tanto los programas nutricionales como las fuentes de piedra caliza promovieron un buen rendimiento productivo y una buena calidad interna y externa del huevo.

Palabras clave: Digestibilidad, Huevo, Calidad, Producción de Postura, Aves de Corral.

1 INTRODUCTION

A well-structured nutritional program enhances egg production efficiency, increases egg quantity and quality, and supports bird health by reducing disease risks. Evaluating different programs helps identify the most effective and cost-efficient diets, including optimal combinations of minerals and nutrients, ultimately improving productivity and profitability.

There is a discrepancy in the nutritional recommendations for light laying hens, leading to potential misunderstandings between producers and nutritionists. The two main references used are the Brazilian Tables (Rostagno *et al.*, 2017) and specific lineage guidelines. A key point of disagreement involves calcium and phosphorus levels, essential minerals for metabolism (Bertechini, 2007), with calcium being the primary component of eggshells (Ahmed *et al.*, 2013). While Rostagno *et al.* (2017) suggest a constant daily intake of these minerals, lineage guidelines recommend inversely proportional levels over time, resulting in significant differences.

Another important point in laying hen nutrition is the use of limestone to meet dietary calcium requirements. Calcitic limestone, the most commonly used source, shows significant variation in composition and digestibility—factors often overlooked in feed formulation. These variations can greatly impact nutritional programs and are frequently not anticipated by professionals. Each calcium source has unique chemical and physical properties that influence its digestibility, absorption, and retention. According to Humer *et al.* (2015), differences in calcium solubility among sources may explain the variation in recommended requirement values.

Evaluating different limestone sources for laying hens is essential, as limestone is the main calcium source in poultry diets, crucial for eggshell formation and bone development. The quality and composition of limestone can vary by source, affecting calcium availability. Low-quality limestone may contain impurities or insufficient calcium levels, leading to poor eggshell quality, weaker bones, and reduced egg production (Humer *et al.*, 2015).

Evaluating different limestone sources helps poultry producers identify high-quality options that meet birds' calcium needs, supporting health and productivity, and ultimately improving profitability. High-quality limestone can also reduce the reliance on costly supplements, lowering feed costs and enhancing feed efficiency. Moreover, better utilization of calcium reduces its excretion, minimizing environmental impacts such as soil and water pollution (Pelicia *et al.*, 2009). Thus, a deeper understanding of feed ingredients is essential for more efficient and sustainable egg production.

Variations in calcium digestibility among different limestone sources affect their individual absorption in poultry diets. Considering potential differences in calcium and phosphorus requirements, absorption, and utilization by laying hens, it is important to study their daily nutritional needs—identifying the most suitable nutritional guideline for the region—and to evaluate the effects and digestibility of various limestone sources on performance, egg quality, and calcium excretion in commercial laying hens.

Therefore, the aim of this study was to compare two nutritional programs commonly recommended and used for laying hens along with two different sources of limestone considering animal productive performance, egg quality, and calcium digestibility.

2 METHODOLOGY

2.1 ANIMALS AND EXPERIMENTAL DESIGN

This research was conducted in the facilities of the poultry sector of UNOESC Xanxerê. This experimental protocol was approved by the Institutional Ethics Committee on the Use of Animals (CEUA/UNOESC) under protocol number 66/2018. Ninety-six Hy-Line W36 laying hens were used in five production cycles of 21 days each, starting at 33 weeks of age and ending at 47 weeks of age. The laying hens were distributed in a completely randomized experimental design, in a 2x2 factorial arrangement, consisting of four treatments (Table 1) and eight replicates with three animals each. The birds were bred according to the rules and management of commercial farms and the lineage manual.

Table 1. Treatments.

Treatments	Nutritional Requirement Table	Dietary Limestone Source
Rostagno A	Brazilian Tables*	A
Hy-Line A	Hy-Line Lineage W36 Guidelines**	A
Rostagno B	Brazilian Tables*	B
Hy-Line B	Hy-Line Lineage W36 Guidelines**	B

Legend: *Rostagno *et al*. (2017); **Hy-Line (2016).
Source: Authors

In the pre-experimental period, the animals were housed receiving feed and water ad libitum, with rations formulated for each stage of rearing, with uniformity control. Also, before starting the experiment, the geometric mean diameter (GMD), geometric standard deviation (GSD), and the calcium, phosphorus and magnesium contents of the calcium and phosphorus sources were determined (Table 2). At 31 weeks of age, the hens were placed in batteries of galvanized wire cages, with three animals per cage, fitted with a trough-type feeder, nipple-type drinker, and a frontal egg catcher.

Table 2. Composition of the different contentes of the calcium sources used in the experimental diets.

Item	GMD (µm)	GSD	Ca (g/kg)	P (g/kg)	Mg (g/kg)
Limestone A	243	2.61	314.56	0.26	20.95
Limestone B	153	2.45	323.12	0.14	27.01
Dicalcium phosphate	467	2.98	213.81	146.95	11.44

Legend: Geometric mean diameter (GMD); geometric standard deviation (GSD); calcium (Ca); phosphorus (P), magnesium (Mg).
Source: Authors

The experimental period lasted 105 days, divided into five cycles of three weeks each (21 days) in addition to two weeks for adaptation. They received water and feed ad libitum, formulated according to the recommendations of the two tested requirement guidelines (Table 3). The feeding program was divided into two phases, comprising the period from 33 to 41 weeks as Period I and the period from 42 to 47 weeks as Period II.

Table 3. Feed ingredients and nutritional compositions.

Ingredient	Period I (weeks 33 to 41)		Period II (weeks 42 to 47)	
	Rostagno*	Hy-Line**	Rostagno*	Hy-Line**
Corn, g/kg	535.66	463.76	587.46	591.74
Soybean meal (46%), g/kg	266.91	325.44	240.64	238.91
Soybean oil, g/kg	49.45	63.10	36.14	36.38
Dicalcium phosphate, g/kg	17.27	26.00	15.37	21.47
Limestone, g/kg	114.85	108.78	105.01	100.35
Salt (NaCl), g/kg	4.98	4.37	4.48	3.93
Premix Min/Vit[1]	4.00	4.00	4.00	4.00
DL- Methionine (99%), g/kg	3.73	3.00	3.49	2.04
L- Lysine HCl, g/kg	0.93	0.62	1.17	0.71
L-Threonine, g/kg	1.02	0.35	1.01	0.14
L-Tryptophan, g/kg	0.12	0.00	0.16	0.00
L- Valine, g/kg	1.06	0.62	1.04	0.31
Calculated values				
Metabolizable energy, kcal/kg	2.90	2.90	2.90	2.90

Crude protein, g/kg	171.40	190.50	164.00	161.50
Lysine dig., g/kg	8.56	9.60	8.19	7.80
Met. + Cys. dig., g/kg	8.39	8.10	8.03	6.60
Threonine dig., g/kg	6.59	6.70	6.31	5.50
Tryptophan dig., g/kg	1.97	2.10	1.88	1.72
Valine dig., g/kg	7.96	8.40	7.62	6.90
Linoleic acid, g/kg	38.86	45.28	32.47	32.67
Calcium, g/kg	49.40	49.40	45.08	44.80
Available phosphorous, g/kg	4.13	5.80	3.77	4.90
Sodium, g/kg	2.32	2.10	2.12	1.90
Chlorine, g/kg	3.26	2.87	2.99	2.66
Analyzed composition				
Crude protein, g/kg	171,45	190,51	164,12	161,48
Calcium, g/kg	49,60	49,02	45,17	44,90
Phosphorus, g/kg	4,04	5,75	3,79	4,89

Legend: [1]Vitamin supplement containing per kg: Vit. A - 10,000,000 I.U.; Vit. D3 - 2,000,000 I.U.; Vit. E - 30,000 I.U.; Vit. B1 - 2.0g; Vit. B2 - 6.0g; Vit. B6 - 4.0g; Vit. B12 - 0.015g; Pantothenic acid - 12.0g; Biotin - 0.1g; Vit. K3 - 3.0g; Folic acid - 1.0g; Nicotinic acid - 50.0g. Iron - 150.0mg; Copper - 30.0mg; Manganese - 240.0mg; Zinc - 150.0mg; Iodine - 3.0mg; Selenium – 0.40mg. *Rostagno *et al.* 2017. ** Hy-Line Lineage W-36 Guidelines (2016).

Source: Authors

2.2 PERFORMANCE

Hens were kept under a 16-hour light program, with daily egg production recorded and laying percentage calculated at the end of each 21-day cycle. Feed intake was determined by weighing feed provided and leftovers weekly, allowing calculation of daily intake (g/hen/day) and feed conversion. Eggs from the last three days of each cycle were weighed to calculate average egg weight and egg mass (g/hen/day). Feed conversion was expressed as kilograms of feed per dozen eggs (kg/dz) and kilograms of feed per kilogram of eggs produced (kg/kg).

2.3 EGG ANALYSIS

At the end of each cycle, fresh eggs were weighed and broken to assess shell, albumen, and yolk quality. Shell and yolk were weighed individually, and albumen weight was calculated by subtracting shell and yolk weights from the total egg weight. Albumen quality was measured using the Haugh unit (HU), calculated based on albumen height and egg weight (Card and Nesheim, 1968). Yolk quality was evaluated by the yolk index, the ratio of yolk height to diameter. Shell quality was assessed by measuring breaking strength with a texturometer, and shell weight was recorded after breaking.

2.4 DIGESTIBILITY

To assess calcium and phosphorus balance, total excreta were collected twice daily for five days starting at 35 weeks of age. Excreta were stored frozen, then thawed, homogenized, and dried at 55°C for 48 hours. Dried samples were ground to measure dry matter and calcium content (Silva and Queiroz, 2002). Calcium digestibility coefficients were calculated based on feed intake and excreta output using equations adapted from Jongbloed and Kemme (1990).

2.5 STATISTICAL ANALYSIS

The experimental results were submitted to the Shapiro Wilk normality test. Since all data were considered normal, the analysis of variance used was parametric, whose data were subjected to analysis of variance, and in the event of a significant effect, the data were submitted to Tukey's test, at 0.05 significance, for using the statistical software R.

3 RESULTS

3.1 PERFORMANCE

Egg production percentage differed between limestone sources in the second (36–38 weeks) and final (45–47 weeks) production cycles, with limestone A resulting in higher productivity than limestone B ($P = 0.016$), regardless of the nutritional guideline used. Additionally, during the fourth cycle (42–44 weeks), hens fed limestone A showed higher production ($P = 0.025$) than those fed limestone B when following the Hy-Line (2016) recommendations.

Table 4. Performance of laying hens fed with different nutritional programs.

	Production (%)				
	33-35 week	36-38 week	39-41 week	42-44 week	45-47 week
Rostagno A	89	92 a	92	89 AB	91 A
Hy-Line A	92	96 a	89	94 a	93 A
Rostagno B	90	91B	91	92 AB	86 B
Hy-Line B	89	91B	91	88 B	90 B
C.V (%)	5.76	3.61	4.44	5.03	4.31
Value P Limestone	0.535	0.014	0.617	0.429	0.016
Value P Table	0.535	0.193	0.322	0.929	0.108
Valor P calc x tab	0.282	0.157	0.278	0.025	0.605
	Feed intake (g)				
	33-35 week	36-38 week	39-41 week	42-44 week	45-47 week
Rostagno A	98	94	84	85	91
Hy-Line A	98	90	83	91	95
Rostagno B	95	89	84	86	91
Hy-Line B	97	91	85	83	89
C.V (%)	5.91	5.19	6.65	6.15	7.44
Value P Limestone	0.313	0.352	0.800	0.204	0.333
Value P Table	0.598	0.733	0.857	0.544	0.789
Value P calc x tab	0.751	0.209	0.541	0.051	0.279
	Feed conversion (kg of feed/kg of eggs)				
	33-35 week	36-38 week	39-41 week	42-44 week	45-47 week
Rostagno A	1.94	1.80	1.65	1.68	1.74 A
Hy-Line A	1.87	1.76	1.69	1.69	1.80 A
Rostagno B	1.89	1.78	1.66	1.67	1.91 B
Hy-Line B	1.99	1.81	1.68	1.70	1.83 B
C.V (%)	9.10	7.04	7.29	6.41	6.04
P Value Limestone	0.621	0.761	0.921	0.970	0.034
P Value Table	0.844	0.962	0.575	0.709	0.855
P Value calc x tab	0.252	0.513	0.867	0.765	0.135
	Feed conversion (kg of feed/dozen of eggs)				
	33-35 week	**36-38 week**	**39-41 week**	**42-44 week**	**45-47 week**
Rostagno A	1.48	1.36	1.21	1.27	1.32
Hy-Line A	1.42	1.32	1.26	1.29	1.35
Rostagno B	1.41	1.31	1.22	1.25	1.41
Hy-Line B	1.46	1.35	1.24	1.28	1.32
C.V (%)	7.86	5.91	7.06	5.75	5.96
P Value Limestone	0.736	0503	0.945	0.528	0.455
P Value Table	0.957	0.330	0.348	0.428	0.398
P Value calc x tab	0.215	0.053	0.763	0.890	0.751
	Egg Mass (g/hen/day)				
	33-35 week	36-38 week	39-41 week	42-44 week	45-47 week
Rostagno A	56.51	58.29	56.29	56.13	57.84 A
Hy-Line A	58.39	57.16	55.20	59.94	58.51 A
Rostagno B	55.85	55.74	55.92	57.30	53.05 B
Hy-Line B	54.25	56.00	56.19	54.71	53.73 B
C.V (%)	7.55	5.25	6.54	7.20	5.85
P Value Limestone	0.181	0.144	0.838	0.239	0.001
P Value Table	0.937	0.727	0.786	0.721	0.618
P Value calc x tab	0.329	0.573	0.654	0.071	0.997

Legend: Means followed by uppercase letters in the column indicate a significant difference regarding the different limestones and followed by lowercase letters in the column indicate a significant difference when comparing the Nutritional Requirements Tables, differing statistically by the Tukey test at 0.05 of significance.
A – Dietary Limestone “A”. B – Dietary Limestone “B”.
Source: Authors

Feed intake and feed conversion (kg/dozen) were not affected by the nutritional tables or limestone sources over five cycles (P > 0.05). However, in the last cycle (45–47 weeks), feed conversion (kg feed/kg egg) was better (P = 0.034) for hens fed limestone A. Additionally, egg mass (g/hen/day) was higher (P = 0.001) in hens receiving limestone A compared to limestone B during the final period.

3.2 EGGS ANALYSIS

Table 5 shows the data for the weight of egg parts. During the second productive period, eggs of the laying hens fed with limestone B (P < 0.05) were heavier. In the same period, the total egg weight of laying hens fed the diet from the Rostagno table was higher (P < 0.05) than that of the laying hens fed the diet formulated from the Hy-line Guidelines (Hy-Line 2016).

Table 5. Weight of egg and egg parts of laying hens are submitted to different nutritional programs.

		Whole Egg Weight (g)			
	33-35 week	36-38 week	39-41 week	42-44 week	45-47 week
Rostagno A	64	63 Aab	61	63	63 a
Hy-Line A	63	60 Bb	62	64	63 a
Rostagno B	62	61 ABb	61	62	62 B
Hy-Line B	61	62 Aba	62	63	60 B
C.V (%)	4.33	2.93	5.37	4.63	4.23
P Value Limestone	0.099	0.823	0.761	0.339	0.041
P Value Table	0.506	0.851	0.585	0.492	0.288
P Value calc x tab	0.940	0.008	0.855	0.679	0.645
		Albumen Weight (g)			
	33-35 week	36-38 week	39-41 week	42-44 week	45-47 week
Rostagno A	38.81 a	37.08	35.73	35.36	36.39 A
Hy-Line A	38.45 a	35.33	37.40	37.36	36.52 A
Rostagno B	37.11 B	36.46	35.86	34.58	35.26 B
Hy-Line B	36.14 B	36.44	35.63	35.35	34.12 B
C.V (%)	5.17	4.05	5.97	5.66	5.62
P Value Limestone	0.038	0.688	0.364	0.106	0.043
P Value Table	0.605	0.155	0.422	0.108	0.540
P Value calc x tab	0.494	0.167	0.290	0.463	0.444
		Yolk Weight (g)			
	33-35 week	36-38 week	39-41 week	42-44 week	45-47 week
Rostagno A	16.91	17.34 a	16.91	18.45	18.22
Hy-Line A	16.56	16.01 b	16.56	17.95	17.60
Rostagno B	16.79	16.55 a	16.90	18.82	17.73
Hy-Line B	17.14	17.21 a	17.41	18.18	17.44
C.V (%)	3.94	2.82	6.51	430	3.79

P Value Limestone	0.406	0.312	0.360	0.363	0.255
P Value Table	1.000	0.101	0.855	0.921	0.113
P Value calc x tab	0.205	<0.001	0.355	0.818	0.550
Shell Weight (g)					
	33-35 week	36-38 week	39-41 week	42-44 week	45-47 week
Rostagno A	8.48	8.63	8.56	9.10	8.67
Hy-Line A	8.25	8.13	8.40	8.73	8.60
Rostagno B	8.14	8.41	8.51	8.77	8.51
Hy-Line B	7.89	8.28	8.56	9.05	8.24
C.V (%)	4.99	5.38	5.86	5.10	5.12
P Value Limestone	0.050	0.840	0.808	0.972	0.159
P Value Table	0.172	0.101	0.790	0.838	0.332
P Value calc x tab	0.933	0.324	0.600	0.951	0.555

Legend: Means followed by uppercase letters in the column indicate a significant difference regarding the different limestones and followed by lowercase letters in the column indicate a significant difference when comparing the Nutritional Requirements Tables, differing statistically by the Tukey test at 0.05 of significance. A – Dietary Limestone “A”. B – Dietary Limestone “B”.
Source: Authors

Egg white weight was higher (P = 0.038) in hens fed limestone A during cycles one and five, with no differences in other periods. Yolk weight in the second cycle showed an interaction between nutritional tables and limestone sources, with higher yolk weight (P < 0.001) in hens fed diets with limestone A following Rostagno *et al*. (2017), while the opposite occurred with limestone B under Hy-Line guidelines (Hy-Line 2016). Eggshell weight did not differ across cycles (P > 0.05). For egg quality, egg white height and Haugh unit differed only in cycle three, favoring limestone A under Hy-Line guidelines (P < 0.05). Hens fed Rostagno’s formulation had higher yolk height in cycles two (P = 0.017) and four (P < 0.001) compared to those fed Hy-Line guidelines.

Table 6. Egg quality of laying hens submitted to different nutritional programs.

Albumen Height (mm)					
	33-35 week	36-38 week	39-41 week	42-44 week	45-47 week
Rostagno A	7.49	7.74	8.16 AB	8.41	7.62
Hy-Line A	7.16	7.95	7.67 B	8.19	7.87
Rostagno B	7.48	8.20	7.90 AB	8.54	7.56
Hy-Line B	7.03	7.93	8.41 A	8.55	7.83
C.V (%)	6.53	6.36	6.49	5.12	4.46
P Value Limestone	0.722	0.309	0.273	0.185	0.739
P Value Table	0.058	0.896	0.957	0.557	0.085
P Value calc x tab	0.754	0.254	0.028	0.533	0.958
Haugh Unit					
	33-35 week	36-38 week	39-41 week	42-44 week	45-47 week
Rostagno A	85	87	90 A	91	87
Hy-Line A	84	89	86 B	89	88

Rostagno B	86	90	88 AB	92	87
Hy-Line B	84	88	90 A	92	89
C.V (%)	3.30	3.05	3.59	2.58	2.36
P Value Limestone	0.828	0.416	0.261	0.132	0.626
P Value Table	0.107	0.941	0.528	0.494	0.509
P Value calc x tab	0.828	0.982	0.041	0.494	0.626
Yolk Height (mm)					
	33-35 week	36-38 week	39-41 week	42-44 week	45-47 week
Rostagno A	17.82	17.03 a	16.89	19.08 a	17.60
Hy-Line A	18.11	16.63 b	16.51	17.92 b	17.60
Rostagno B	17.98	17.56 a	17.15	18.71 a	17.51
Hy-Line B	17.81	16.24 b	16.79	17.43 b	17.48
C.V (%)	3.74	4.81	5.05	3.62	3.71
P Value Limestone	0.805	0.846	0.451	0.123	0.681
P Value Table	0.820	0.017	0.299	<0.001	0.958
P Value calc x tab	0.405	0.183	0.966	0.829	0.958
Yolk Index					
	33-35 week	36-38 week	39-41 week	42-44 week	45-47 week
Rostagno A	0.42	0.40 aB	0.39	0.46 a	0.42
Hy-Line A	0.44	0.40 bB	0.40	0.43 b	0.43
Rostagno B	0.42	0.43 aA	0.40	0.46 a	0.42
Hy-Line B	0.42	0.38 bA	0.30	0.42 b	0.42
C.V (%)	3.57	4.22	5.41	2.85	3.41
P Value Limestone	0.225	0.802	0.881	0.270	0.985
P Value Table	0.408	0.005	0.681	<0.001	0.735
P Value calc x tab	0.152	0.004	0.323	0.698	0.212
Yolk Diameter (mm)					
	33-35 week	36-38 week	39-41 week	42-44 week	45-47 week
Rostagno A	42.34	42.37	43.00	41.43	42.37
Hy-Line A	41.67	41.36	41.46	41.68	41.35
Rostagno B	42.65	41.30	42.51	40.96	41.33
Hy-Line B	42.58	42.47	43.00	41.32	41.82
C.V (%)	1.52	1.46	1.87	1.90	1.52
P Value Limestone	0.330	0.934	0.124	0.206	0.276
P Value Table	0.175	0.755	0.124	0.345	0.318
P Value calc x tab	0.267	0.210	0.095	0.857	0.108
Shell Breaking Strength (kgf)					
	33-35 week	36-38 week	39-41 week	42-44 week	**45-47week**
Rostagno A	4.68 b	4.43	4.54	4.51	4.20
Hy-Line A	5.68 a	4.23	4.49	4.23	4.23
Rostagno B	4.77 b	4.21	4.57	4.44	3.67
Hy-Line B	5.12 a	4.56	4.08	4.31	4.16
C.V (%)	13.49	10.38	13.80	12.25	11.46
P Value Limestone	0.402	0.749	0.451	0.999	0.129
P Value Table	0.024	0.693	0.298	0.352	0.184
P Value calc x tab	0.262	0.153	0.386	0.741	0.238

Legend: Means followed by uppercase letters in the column indicate a significant difference regarding the different limestones and followed by lowercase letters in the column indicate a significant difference when comparing the Nutritional Requirements Tables, differing statistically by the Tukey test at 0.05 of significance. A – Dietary Limestone “A”. B – Dietary Limestone “B”.

Source: Authors

In the second cycle, the yolk index was higher (P = 0.005) in hens fed diets based on Rostagno's table, while hens fed limestone B showed higher yolk index values (P < 0.05) than those fed limestone A. This trend continued in the fourth cycle, with Rostagno's formulation resulting in higher yolk index (P < 0.001). Yolk diameter was not affected by any treatment (P > 0.05). Eggshell breaking strength was greater (P = 0.024) in hens fed according to the Hy-Line guidelines during weeks 33 to 35, regardless of limestone source.

3.3 DIGESTIBILITY

During the Ca digestibility tests (Table 7), the feed intake, the amount of total Ca ingested, the excreta volume, as well as the percentage of excreted Ca and the coefficient of total tract apparent digestibility of calcium were not significantly different (P > 0.05). However, the excreted Ca content was higher (P = 0.007) in laying hens fed diets containing limestone A.

Table 7. Average of calcium balance of diets for laying hens submitted to different nutritional programs and sources of dietary limestone.

	DM Consumed (g/hen/d)	DM Excreted (g/hen/d)	Total Ingested Ca (g/hen/d)	Ca Excreted (g/ave/d)	Fecal Level of Ca (g/kg)	CTTAD Ca Total (g/kg)	Ca Excreted (g/kg)
Rostagno A	87.25	12.55	2.01	1.13	89.1 a	444.9	560.1
Hy-Line A	86.41	11.71	2.01	1.05	89.9 a	467.8	524.2
Rostagno B	91.45	13.34	1.97	1.14	85.0 b	419.6	577.5
Hy-Line B	85.60	12.94	1.89	1.03	79.7 b	446.8	549.4
CV (%)	7.27	16.60	17.81	19.24	8.07	14.12	12.10
P Limestone	0.528	0.255	0.585	0.958	0.007	0.605	0.609
P Table	0.211	0.517	0.745	0.322	0.613	0.643	0.671
P calc x tab	0.650	0.813	0.771	0.855	0.215	0.934	0.927

Legend: Means followed by uppercase letters in the column indicate a significant difference regarding the different limestones and followed by lowercase letters in the column indicate a significant difference when comparing the Nutritional Requirements Tables, differing statistically by the Tukey test at 0.05 of significance. A – Dietary Limestone "A". B – Dietary Limestone "B".DM – Dry matter. Ca – Calcium. CTTAD – Coefficient total tract apparent digestibility.
Source: Authors

Table 8 shows phosphorus balance results. Hens fed diets based on Hy-Line guidelines (Hy-Line 2016) consumed more total phosphorus daily (P = 0.001), while those fed Rostagno's formulations excreted a higher percentage of fecal phosphorus (P < 0.001). Additionally, the total tract apparent phosphorus digestibility was lower (P < 0.001) in diets following Rostagno's table. Other variables showed no significant differences (P > 0.05).

Table 8. Average of phosphorus balance of diets for laying hens submitted to different nutritional programs and sources of dietary limestone.

	DM Consumed (g/hen/d)	DM Excreted (g/hen/d)	Total Ingested P (g/hen/d)	P Excreted (g/hen/d)	Fecal Level of P (%)	CTTAD P Total (%)	P Excreted (%)
Rostagno A	87.25	12.55	0.268b	0.310	2.48	-14.76 b	115.87 a
Hy-Line A	86.41	11.71	0.349a	0.267	2.28	23.49 a	76.69 b
Rostagno B	91.45	13.34	0.269b	0.339	2.54	-26.90 b	126.08 a
Hy-Line B	85.60	12.94	0.367a	0.315	2.43	13.66 a	85.79 b
CV (%)	7.27	16.60	13.14	18.80	13.03	2.12	21.81
P Limestone	0.528	0.255	0.536	0.170	0.607	0.498	0.064
P Table	0.211	0.517	0.001	0221	0.314	<0.001	<0.001
P calc x tab	0.650	0.813	0.569	0.738	0.826	-	0.834

Legend: Means followed by uppercase letters in the column indicate a significant difference regarding the different limestones and followed by lowercase letters in the column indicate a significant difference when comparing the Nutritional Requirements Tables, differing statistically by the Tukey test at 0.05 of significance. A – Dietary Limestone "A". B – Dietary Limestone "B". DM – Dry matter. CTTAD – Coefficient total tract apparent digestibility. P – Phosphorus.
Source: Authors

4 DISCUSSION

In order to maintain the sustainability of the productive chain, it is imperative to maximize mineral use and its digestibility, reducing production costs and environmental contamination caused by waste, also reducing the need for natural exploration of new deposits, leading to the depletion of world reserves (Li *et al.*, 2016). Thus, more accurate adjustment of the nutritional requirements, their applicability in laying hens, and the mitigation of differences in use between different sources of limestone become an important element to improve performance and guarantee egg quality.

Both recommendations of nutritional requirements provided similar performance in laying hens for most parameters of productivity and egg quality, indicating that both are suitable to be used as a reference in the production of feed for laying hens. The main difference between the two is the trend of Ca and P levels throughout production, however, this element did not influence the productivity and quality of eggs in the period evaluated. This is mainly due to the fact that laying hens have the ability to physiologically regulate both the absorption of these minerals by the gut as well as bone storage and resorption (Kebreab *et al.*, 2009), allowing them to maintain constant productivity, even with nutritional fluctuations in the ingested diet. The two limestone sources also provided performance and egg quality similar to each other, in all periods evaluated, demonstrating that the two sources tested can be used in an equivalent way.

At the beginning of the experimental period, the laying hens had practically already reached the adult weight for the lineage (1.51 to 1.57 kg), and from this point onwards, the feed consumption is intended to promote maintenance, directing the rest to egg production. It was observed that the consumption recommended (98 to 105 g/hen/day) for the lineage W-36 of Hy-Line do Brasil (Hy-Line 2016) was met in the lower limit in some cycles, reaching lower levels at some points.

Guidelines for W-36 Hy-Line lineage highlight that the peak of egg production occurs in the 32nd week of life, and may reach 97% of egg production, decreasing linearly from the following week, reaching 92% in the 47th week. In the studied period (33 to 47 weeks), average productivity rates were close to those recommended by the lineage. The difference in feed conversion (kg of feed/kg of egg) showed by laying hens fed with limestone A, in the fifth production cycle, can be basically explained by the higher egg production, egg weight and egg mass also observed in the same period. Feed intake did not differ at this stage, thus not interfering with the result, thus, greater production with equal consumption leads to lower feed conversion.

The average egg weight observed during the experimentation period was similar to that suggested for the lineage, and, in some cycles, it was higher than that proposed by the genetics manual. This shows that the nutritional levels prescribed by the two tables were able to provide the laying hens' needs, in particular the sulfur amino acids that are correlated with the size/weight of the eggs of commercial laying hens (Pavan *et al*., 2005). To assess and express albumen quality, the HU is scientifically the most accepted parameter, since egg quality varies according to the height of the thick part of the white.

Egg quality is measured to assess and corroborate differences in fresh egg production, genetic characteristics, diet and environmental factors to which laying hens are subjected. Eggs with higher HU can ensure longer shelf life and, consequently, higher quality when reaching the consumer. In general, the higher the HU value, the better the egg quality (Chen *et al*., 2021; Alleoni & Antunes 2001). During the evaluation cycles of the present study, the values found for the HU were between 84 and 92. The Quality Control Program instituted by the United States Department of Agriculture (USDA) defines the quality conditions of the egg for consumption, being excellent quality eggs (AA) with HU values greater than 72 (USDA 2000). Therefore, the eggs obtained in this experiment, regardless of the treatments, could be classified in the highest degree of quality.

The shell is of great importance in egg quality since it may preserve its nutritional value (Pelicia *et al*., 2009), and losses caused by poor shell quality during the entire production cycle may range from 6 to 8% (Siske *et al*., 2000). Eggshell formation and mineralization in commercial laying hens is a physiological process that requires high amounts of Ca (An *et al*., 2016; Nys & Le Roy 2018). Laying hens need to dispense more than 2g of calcium per day, and this amount is equivalent to 10% of their total body Ca. In general, the necessary Ca comes from the supplementation of this mineral through the diet and the rest from the mobilization of the skeleton (marrow bone) (Barr 2008). In this context, the formulation of feeds with adequate levels of available Ca as well as the ideal particle size and the correct balance with other ingredients that contribute to the absorption and synthesis of vitamin D, P, and trace elements Mn, Zn, Cu (Barr 2008; Preda *et al*., 2013), can be evaluated on the quality, integrity, and resistance of the shell to breakage (Salajegheh *et al*., 2020).

In general, the resistance of the eggshell to breakage declines as the layer ages. For the experimental period, the Hy-line Guidelines (Hy-Line 2016) indicate 4.14 kgf in the 33rd and 4.08 kgf in the 47th week as ideal values but this study found higher values in all cycles. Therefore, it attests that both the formulation tables and the limestone brands tested and their combinations were able to meet the nutritional needs of Ca and P, as well as guaranteeing the quality, integrity, and resistance of the eggshells.

All parameters for evaluating the calcium balance show that the nutritional recommendation program did not influence the use of Ca in the diet. However, there was a difference in the calcium content in the excreta of hens when comparing the two sources of limestone used. This scenario brings to light the concern that the different sources of limestone have a different utilization profiles, which can affect performance and the production curve over time. Currently, most nutritionists consider limestone to be unique in their formulations, neglecting the differences in its use, which affect the performance of laying hens.

As for the Ca balance, the nutritional recommendations in the different tables were able to meet the daily requirement of the laying hens during the digestibility test, being attentive to the needs in order to promote correct nutritional intake, ensuring well-being and longevity for the laying hens throughout the productive life.

P balance was affected by different nutritional programs, whose laying hens fed diets formulated based on the requirements proposed by the Hy-Line Lineage Guidelines (Hy-Line 2016) generated greater P retention in the diet compared to laying hens fed the Rostagno-based diet (Rostagno *et al*., 2017). As there were changes in the Ca:P ratio between the two nutritional programs, there was this discrepancy, showing that the relationship between these two minerals must always be maintained. For the observed negative apparent digestibility coefficient, Van Soest (1994) and Sordi *et al*. (2019) also reported similar findings. Probably, the greatest excretion of P in laying hens occurred because they were not in an ideal relationship with Ca, preventing its more efficient absorption.

There is an economic importance of evaluating the relationship between nutrient consumption and their residual excretion along with egg productivity, which helps in making decisions regarding the choice of which nutritional program to use. Given this, studies like this can form part of an information base for future economic research, important and necessary for the poultry chain.

5 CONCLUSION

It is concluded that the two proposed and tested nutritional programs, as well as the two limestone brands, were able to promote good productive performance and excellent internal and external egg quality. In addition, the two limestone brands tested showed calcium digestibility compatible with the requirements of high-performance commercial light laying hens. These results contribute both to society and academia by indicating more economical and sustainable nutritional strategies that reduce costs and environmental impacts, as well as by providing comparative data that support future research on poultry nutrition and mineral metabolism.

Finally, the limitations of the present study relate to the restricted experimental period and the evaluation of only two mineral sources, aspects that, although not compromising the relevance of the results, highlight the need for additional research to broaden its applicability in different production contexts. Therefore, future studies are recommended to include different genetic strains, longer production phases, alternative calcium sources, and nutrient interactions, aiming to optimize performance and egg quality across diverse production systems.

REFERENCES

AHMED, N. M.; ABDEL ATTI, K. A.; ELAMIN, K. M.; DAFALLA, K. Y.; MALIK, H. E. E.; DOUSA, B. M. Effect of dietary calcium sources on laying hens performance and egg quality. **Journal of Animal Production Advances**, v. 3, p. 226–231, 2013.

ALLEONI, A.; ANTUNES, A. Unidade Haugh como medida da qualidade de ovos de galinha armazenados sob refrigeração. **Scientia Agricola**, v. 58, n. 4, p. 681–685, 2001.

AN, S. H.; KIM, D. W.; AN, B. K. Effects of Dietary Calcium Levels on Productive Performance, Eggshell Quality and Overall Ca Status in Aged Laying Hens. **Asian-Australasian Journal of Animal Sciences**, v. 29, n. 10, p. 1477–1482, 2016.

BARR, A. Calcium homeostasis and vitamin D metabolism and expression in strongly calcifying laying birds. **Comparative Biochemistry and Physiology**, v. 151, n. 4, p. 477–490, 2008.

BERTECHINI, A. G. O ovo de codorna. In: III **Simpósio Internacional e II Congresso Brasileiro de Coturnicultura**. Lavras: UFLA/NECTA, 2007.

CARD, L. E.; NESHEIM, M. C. **Producción avícola**. Nueva York: Ithaca, 1968. 392 p.

CHEN, F.; ZHANG, H.; DU, E.; JIN, F.; ZHENG, C.; FAN, Q.; ZHAO, N.; GUO, W.; ZHANG, W.; HUANG, S.; WEI, J. Effects of magnolol on egg production, egg quality, antioxidant capacity, and intestinal health of laying hens in the late phase of the laying cycle. **Poultry Science**, v. 100, n. 2, p. 835–843, 2021.

HAUGH, R. R. A new method for determining the quality of an egg. **U.S. Egg Poultry Magazine**, v. 39, p. 27–49, 1937.

HUMER, E.; SCHWARZ, C.; SCHEDLE, K. Phytate in pig and poultry nutrition. **Journal of Animal Physiology and Animal Nutrition**, v. 99, n. 4, p. 605–625, 2015.

HY-LINE. **Manual da linhagem: Poedeiras Comerciais Hy-Line White.** 2016. Available in: https://www.hyline.com/filesimages/Hy-Line-Products/Hy-Line-Product-PDFs/Brown/BRN%20STD%20POR.pdf. Acessed in: Nov 30, 2024.

JONGBLOED, A. W.; KEMME, P. A. Apparent digestible phosphorus in the feeding of pigs in relation to availability, requirement and environment. **NJAS**, v. 38, p. 56–75, 1990.

KEBREAB, E. et al. Development and evaluation of a dynamic model of calcium and phosphorus flows in layers. **Poultry Science**, v. 88, p. 680–689, 2009.

LI, X.; ZHANG, D.; YANG, T.; BRYDEN, W. L. Phosphorus bioavailability: a key aspect for conserving this critical animal feed resource with reference to broiler nutrition. **Agriculture**, v. 6, n. 2, p. 25, 2016.

NYS, Y.; LE ROY, N. Calcium homeostasis and eggshell biomineralization in female chicken. In: FELDMAN, D. (ed.). **Vitamin D**. 4th ed. London: Academic Press, 2018. p. 361–382.

PAVAN, A. C. et al. Níveis de proteína bruta e de aminoácidos sulfurados totais sobre o desempenho, a qualidade dos ovos e a excreção de nitrogênio de poedeiras de ovos marrons. **Revista Brasileira de Zootecnia**, v. 34, n. 2, p. 568–574, 2005.

PELICIA, K. et al. Calcium and available phosphorus levels for laying hens in second production cycle. **Brazilian Journal of Poultry Science**, v. 11, n. 1, p. 39–49, 2009.

PREDA, C.; BĂLĂCEANU, R.; DOJANĂ, N. Evolution of hormonal control of calcium and phosphorus metabolism in different hen breeds during the laying cycle. **Bulletin UASVM Agriculture**, v. 70, n. 2, p. 286–292, 2013.

ROSTAGNO, H. S. et al. **Composição de alimentos e exigências nutricionais: tabelas brasileiras para aves e suínos.** Viçosa, MG: Universidade Federal de Viçosa, 2017. 488 p.

SALAJEGHEH, M. H.; YOUSEF ELAHI, M.; SALARMOINI, M.; BANIASADI, M. Efficacy of feeding various calcium source and concentration on egg quality, some blood variables, and performance of aged laying hens. **Journal of Poultry Science,** v. 8, n. 1, p. 33–42, 2020.

SILVA, D. J.; QUEIROZ, A. C. **Análises de alimentos (métodos químicos e biológicos).** 3. ed. Viçosa, MG: Editora UFV, 2002. 235 p.

SISKE, V.; ZEMAN, L.; KLECKER, D. The egg shell: a case study in improving quality by altering mineral metabolism–naturally. In: **Alltech's Annual Symposium Biotechnology in the Feed Industry**, 2000. p. 327–346.

SORDI, C. **Digestibilidade de cálcio e fósforo de fontes inorgânicas para galinhas poedeiras.** 2019. Dissertação (Mestrado) – Universidade Estadual de Santa Catarina, SC, 72 p. Avaialable in: https://sistemabu.udesc.br/pergamumweb/vinculos/000068/00006875.pdf. Accessed in: 30 out. 2024.

USDA. **Egg-Grading Manual.** Washington: Department of Agriculture, Agricultural Marketing Service, 2000. 75 p.

VAN SOEST, P. J. **Nutritional ecology of the ruminant**. Ithaca: Comstock Publishing Associates, 1994.