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Effects of Dietary Clinoptilolite and Calcium Levels on the Performance and Egg Quality of Commercial Layers

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ABSTRACT

Among the different feed additives studied in poultry production, clinoptilolite, an aluminosilicate capable of adsorbing harmful substances and of improving live performance and egg and meat quality, was evaluated. The objective of the present study was to evaluate the influence of dietary clinoptilolite and calcium levels on the performance and egg quality of layers. In total, 576 layers were distributed according to a completely randomized experimental design in a 3 x 4 factorial arrangement (three calcium levels – 2.5, 3.1, or 3.7% and four clinoptilolite levels - 0.0, 0.15, 0.25, or 0.50%), with 12 treatments of six replicates of eight birds each. The experiment included four 28-d cycles. The experimental diets were based on corn and soybean meal. Results were submitted to analysis of variance and means were compared by the test of Tukey at 5% significance level using SISVAR statistical package. There was a significant interaction between the evaluated factors for egg production and feed conversion ratio per dozen eggs and egg mass. The lowest calcium level resulted in worse performance and eggshell quality. Clinoptilolite levels affected albumen and yolk content. It was concluded that up to 0.50% inclusion of clinoptilolite in layer diets does not benefit layer performance or eggshell quality. Although the inclusion of only 2.5% calcium in layer diets is not recommended, it is possible to add 3.1% because it promoted similar results as the recommended level of 3.7%.

INTRODUCTION

Optimizing poultry performance requires applying adequate management and health practices, as well as having efficient genetic improvement and nutrition programs. The supply of adequate diets depends on several factors, and the inclusion of feed additives may improve nutrient utilization. One of these additives is clinoptilolite, a natural zeolite, classified as a tectosilicate (Armbruster, 2001). Due to its physical structure, it has high cation-exchange capacity (Mumpton, 1999), it is chemically neutral and it inflates in the presence of water (Armbruster, 2001). It is capable of adsorbing gases and vapors, mycotoxins, ammonia, water, heavy metals, and radioactive elements (Luz, 1995; Santúrio *et al.*, 1999), which may be harmful to animals, and their adsorption by zeolites may improve the performance and egg quality of commercial layers.

Ammonia is very harmful to animals and may be adsorbed by clinoptilolite (Shurson *et al.*, 1984), differently from other aluminosilicates. High ammonia concentrations may damage the intestinal mucosa, which repair requires high energy and nutrient levels (Macari *et al.*, 2002), thereby impairing animal performance. In addition to its adverse effects on the intestines, and consequently, on dietary nutrient absorption and utilization, high ammonia levels may negatively affect calcium metabolism, possibly to a reduced activation of vitamin D in the liver and in the kidneys. Such organs have regulatory mechanisms to try to eliminate toxic substances from the body (Macari *et al*., 2002) and may be overwhelmed by high ammonia levels.

Changes in calcium absorption are a matter of concern in commercial egg production, considering the importance of this mineral for eggshell formation.

Therefore, this study aimed at evaluating the effects of different dietary clinoptilolite and calcium levels on the performance and egg quality of commercial layers.

MATERIALS AND METHODS

In total, 576 Hisex Brown® layers, 67 weeks old in the beginning of the experiment, were housed in laying cages. Birds were distributed according to a completely randomized experimental design with a 3X4 factorial arrangement (three calcium levels x four clinoptilolite levels) into 12 treatments with six replicates of eight birds each. Calcium was added to the diet at 2.5, 3.1, or 3.7% and clinoptilolite at 0.0, 0.15, 0.25, or 0.50%.

The experiment included four 28-d cycles, in a total of 112 days. An intermittent lighting program of 17 hours of light daily was applied. Birds were offered feed and water *ad libitum* during the entire experimental period. The experimental diets were based on corn and soybean meal and were formulated according to feedstuff composition and the nutritional requirements of semi-heavy layers in lay recommended by Rostagno *et al*. (2005), except for calcium levels (Table 1).

The following performance parameters were evaluated: egg production, percentage of intact eggs, average egg weight, egg mass, feed intake, and feed conversion ratio per dozen eggs and per kg eggs. Therefore, egg production was daily recorded, whereas feed intake and egg weight were weekly determined.

Egg quality was evaluated at the end of each 28-d period for three consecutive days, with two eggs per replicate collected daily. The following egg quality parameters were evaluated: yolk and albumen percentages, Haugh units, egg specific gravity according to Stadelman & Cotterill (1990), and eggshell percentage, thickness, and breaking strength.

Eggshell percentage was determined by drying the shells in an oven at 65°C for 72h and then weighed again. Eggshell thickness was calculated as the average of three measurements performed at the egg equator using a digital pachymeter. Eggshell breaking strength

Table 1 – Ingredients and calculated nutritional composition of the experimental diets.

	Treatments											
Ingredients	1	2	3	4	5	6	7	8	9	10	11	12
Corn	56.34	58.38	60.43	56.52	58.57	60.61	56.65	58.69	60.74	56.95	58.99	61.04
Soybean meal	15.21	16.52	17.84	15.33	16.65	17.96	15.41	16.72	18.04	15.60	16.92	18.24
Wheat	20.83	15.90	10.96	20.39	15.46	10.52	20.09	15.16	10.23	19.36	14.42	9.49
Limestone	5.66	7.19	8.72	5.65	7.17	8.70	5.64	7.17	8.69	5.62	7.15	8.67
Dicalcium phosphate	1.00	1.07	1.13	1.01	1.07	1.14	1.01	1.08	1.14	1.02	1.09	1.15
Methionine	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Lysine	0.16	0.15	0.13	0.16	0.14	0.13	0.16	0.14	0.12	0.16	0.14	0.12
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Mineral supplement[1]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin supplement[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Adsorbent	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Clinoptilolite	0.00	0.00	0.00	0.15	0.15	0.15	0.25	0.25	0.25	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Estimated nutritional composition												
Metabolizable energy (kcal/kg)	2800	2800	2800	2800	2800	2800	2800	2800	2800	2800	2800	2800
Crude protein (%)	14.78	14.78	14.78	14.78	14.78	14.78	14.78	14.78	14.78	14.78	14.78	14.78
Calcium (%)	2.50	3.10	3.70	2.50	3.10	3.70	2.50	3.10	3.70	2.50	3.10	3.70
Available phosphorus (%)	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
Total methionine (%)	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Total methionine + cystine (%)	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66
Total lysine (%)	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73

[1] Mineral levels per kg of feed: Cu: 8 mg; Fe:50 mg; Mn: 70 mg; Zn:50 mg; I: 1.2 mg; Se: 0.2 mg. [2] Vitamin levels per kg of feed: Vit. A: 7000 IU; vit. D3: 2000 IU; vit. E: 5 mg; vit. K3: 1.6 mg; vit. B2: 3mg; vit. B12: 8 mcg; Niacin: Niacin: 20 mg; pantothenic acid: 5 mg; antioxidant: 15 mg. * Calcitic limestone 100% fine.

was evaluated at the egg equator using a specific cell coupled to a Texture Analyser TA. XT Plus, with the use of a 2mm Cyl Stainless probe, P/2 code, pre-test velocity of 2mm/sec, test velocity of 1mm/sec, and post-test velocity of 40mm/sec. A software program recorded the strength required to break the eggshell in kg.

Results were statistically analyzed using SISVAR statistical package, according to Ferreira (1998). The obtained data were submitted to analysis of variance and means were compared by the test of Tukey at 5% significance level.

RESULTS AND DISCUSSION

The interaction between clinoptilolite and calcium levels affected egg production and feed conversion ratio per dozen egg and kg egg (Tables 2 and 3).

There was no effect (p>0.05) of clinoptilolite or calcium levels on intact egg percentage or average egg weight. Consistent results were obtained by Frost *et al*. (1992), who did not observe any differences in egg weight in layers fed diets containing different zeolite levels (0.0 or 0.75%). The absence of influence of calcium levels on egg weight was also detected by Roland (1988), when feeding 2.75 or 3.75% calcium to Shaver layers. Pelícia *et al*. (2011) did not observe any effect of dietary calcium levels on the weight of eggs of semi-heavy layers.

Clinoptilolite levels did not affect egg mass, which, however, was influenced by calcium levels (p ≤ 0.05). Layers fed 2.5% calcium produced lower egg mass compared with those fed 3.1 and 3.7% calcium.

Table 3 – Details of the effects of the interaction between clinoptilolite and calcium levels on egg production (EP) and feed conversion ratio per dozen eggs (FCR/dz) and per kg eggs (FCR/kg).

Calcium, %	Clinoptilolite (%)			
	0.00	0.15	0.25	0.50
	EP (%)			
2.5	79.17	79.71	71.93B	72.83B
3.1	83.80	76.17	82.27A	83.72A
3.7	77.08	82.69	82.38A	80.45AB
	FCR/dz			
2.5	1.84ab	1.79b	1.98abA	2.00aA
3.1	1.73	1.88	1.78B	1.72B
3.7	1.79	1.76	1.71B	1.80B
	FCR/kg			
2.5	2.33	2.35	2.54A	2.55A
3.1	2.15	2.38	2.27B	2.17B
3.7	2.26	2.24	2.20B	2.27B

Means followed by different capital letters in the same column and different small letters in the same row are statistically different by the test of Tukey (p ≤ 0.05).

Feed intake was not influenced (p>0.05) by dietary clinoptilolite or calcium levels. Consistent and contradictory results have been reported in literature (Nakaue e Koelliker, 1981; Roland, 1988; Roland *et al*., 1991; Frost *et al*., 1992; Shariatmadari, 2008; Pelícia *et al*., 2011). The reported differences may be due to the use of different layer genetic strains as well as different

Table 2 – Egg production (EP), intact eggs (IE), egg weight (EW), egg mass (EM), feed intake (FI), feed conversion ratio per dozen eggs (FCR/dz) and per kg eggs (FCR/kg) of layers fed diets with different clinoptilolite (CLINO) and calcium (Ca) levels.

	EP (%)	IE (%)	EW (g)	EM (g/hen/d)	FI (g/hen/d)	FCR/dz	FCR/kg
CLINO (%)							
0.00	80.02	98.17	65.58	52.45	117.83	1.78	2.25
0.15	79.52	98.46	64.88	51.61	118.63	1.81	2.32
0.25	78.86	97.77	65.19	51.37	117.98	1.82	2.33
0.50	79.00	96.57	65.69	51.89	119.32	1.84	2.33
Ca (%)							
2.5	75.91	96.78	64.77	49.15B	118.46	1.90	2.44
3.1	81.49	98.21	65.79	53.61A	119.50	1.78	2.24
3.7	80.65	98.24	65.44	52.73A	117.35	1.76	2.24
Average	79.35	97.74	65.33	51.83	118.44	1.81	2.31
CV (%)	7.67	3.43	2.28	7.95	4.08	7.29	7.17
P value							
CLINO	0.938	0.357	0.344	0.875	0.782	0.686	0.353
Ca	0.004	0.234	0.061	0.000	0.310	0.001	0.000
CLINO X Ca	0.015	0.941	0.414	0.053	0.135	0.018	0.048

Means followed by different capital letters in the same column are statistically different by the test of Tukey (p ≤ 0.05) zeolite and calcium levels and sources applied in the diets.

Clinoptilolite levels did not affect egg production in none of the tested calcium levels, in agreement with Roland *et al*. (1985), who also did not find any effect of zeolite levels on egg production when layers were fed different calcium levels. However, Roland (1988) obtained a quadratic effect of clinoptilolite at 2.75% dietary calcium level, with 0.75% clinoptilolite promoting better egg production.

Calcium levels influenced ($p \leq 0.05$) egg production when 0.25 and 0.50% clinoptilolite was added to the diet. The dietary addition of 0.25% clinoptilolite associated with 2.5% calcium decreased egg production. When clinoptilolite was supplemented at 0.50%, egg production was different between layers fed 2.5% and 3.1% calcium. There was no effect of calcium levels when clinoptilolite was added at 0.00 or 0.15%.

Feed conversion ratio per dozen eggs was only affected by clinoptilolite levels at 2.5% calcium level. It was worse when 0.50% clinoptilolite was fed compared with 0.15%, which was not different than 0.0 or 0.25%. Calcium levels affected FCR/dz only at 0.25 and 0.50% clinoptilolite levels, with the worst FCR obtained when 2.5% calcium was added to the diet.

There was no influence of clinoptilolite levels at any calcium levels on feed conversion ratio per egg mass. The lack of effects on feed conversion ratio per egg mass and the results obtained for feed conversion ratio per dozen eggs indicate that clinoptilolite dietary inclusion did not benefit the performance of layers fed low calcium levels. These results are opposite to those reported by Leach Jr. *et al*. (1990), who concluded that this zeolite is more effective when included in diets with low levels of calcium and other minerals. There were significant effects of calcium levels only when 0.25 and 0.50% clinoptilolite was supplemented, and the worse feed conversion ratios were obtained with 2.5% calcium. Hens fed 2.5% calcium presented the worst feed conversion ratio per dozen eggs and per egg mass, as well as the lowest egg production, even when 0.25 and 0.50% were included in the feed, opposite to the results of Leach *et al*. (1990) mentioned above. The feed conversion ratio per dozen eggs and per egg mass, nor egg production of hens fed 0.15% or no clinoptilolite were not influenced by calcium levels. This response is probably an attempt of the birds to maintain constant calcium blood levels. Therefore, high aluminosilicate levels may negatively affect egg production and feed conversion ratio of layers fed low calcium levels. This may be explained by the adsorption of minerals and other dietary nutrients by clinoptilolite, in agreement with Santin (2000) and contradicting the hypothesis that this zeolite has beneficial effects of the intestinal absorption of calcium.

No evaluated internal egg quality parameter was influenced by the interaction between the studied factors.

Table 4 – Internal quality of the eggs of commercial layers fed diets containing different clinoptilolite (CLINO) and calcium (Ca) levels.

	Albumen (%)	Yolk (%)	Haugh unit
CLINO (%)			
0.00	65.87A	24.96B	80.78
0.15	64.89B	25.76A	80.28
0.25	65.21AB	25.49AB	80.74
0.50	65.57AB	25.21AB	81.52
Ca (%)			
2.5	65.65A	25.47	81.60
3.1	65.43AB	25.19	80.48
3.7	65.08B	25.40	80.40
Average	65.39	25.36	80.83
CV (%)	1.22	3.01	2.51
P value			
CLINO	0.003	0.016	0.333
Ca	0.051	0.424	0.079
CLINO X Ca	0.898	0.932	0.146

Means followed by different capital letters in the same column are statistically different by the test of Tukey ($p \leq 0.05$)

Clinoptilolite dietary levels influenced ($p \leq 0.05$) albumen and yolk percentages, whereas dietary calcium levels affected ($p \leq 0.05$) albumen percentage.

The lowest albumen percentage, and consequently, the highest yolk percentage, were obtained when 0.15% clinoptilolite was fed, whereas the highest albumen percentage and the lowest yolk percentage were observed in the eggs of hens not fed clinoptilolite. The other treatments presented intermediate values and were not different in terms of albumen or yolk percentage.

Calcium levels influenced ($p \leq 0.05$) albumen percentage, with the lowest value obtained with 3.7% Ca and the highest with 2.5%. The level of 3.1% was not statistically different from the others. The results of the present study are consistent with those of Pelícia *et al*. (2009) and Ito et (2006), who did not observe any significant effect of dietary calcium levels on yolk percentage, but did report effects on albumen percentage.

There was no influence of clinoptilolite or calcium levels on Haugh units, as previously found by other authors (Rodrigues *et al*., 2005; Geraldo, 2006; Pelícia *et al*., 2009).

No evaluated internal egg quality parameter was influenced by the interaction between the studied factors. Calcium levels influenced ($p \leq 0.05$) eggshell percentage, egg specific gravity, and eggshell breaking strength (Table 5).

Table 5 – Eggshell percentage (SHELL), eggshell thickness (EST), egg specific gravity (ESG), and eggshell breaking strength (EBS) of the eggs of commercial layers fed diets containing different clinoptilolite (CLINO) and calcium (Ca) levels.

	SHELL (%)	EST (mm)	ESG (g/L H_2O)	EBS (kgF)
CLINO				
0.00	9.17	0.38	1.087	2.653
0.15	9.35	0.41	1.089	2.682
0.25	9.30	0.38	1.089	2.686
0.50	9.22	0.38	1.088	2.648
Ca (%)				
2.5	8.88B	0.39	1.085B	2.534B
3.1	9.38A	0.39	1.090A	2.677A
3.7	9.52A	0.39	1.090A>	2.790A
Average	9.26	0.39	1.088	2.667
CV (%)	3.14	15.09	0.25	6.80
P value				
CLINO	0.253	0.312	0.102	0.889
Ca	0.000	0.964	0.000	0.000
CLINO X Ca	0.838	0.513	0.059	0.642

Means followed by different capital letters in the same column are statistically different by the test of Tukey ($p \leq 0.05$)

As shown in Table 5, clinoptilolite levels did not affect ($p>0.05$) any of the evaluated parameters. Opposite results were reported in literature for egg specific gravity (Roland, 1988; Roland *et al*., 1991; Frost *et al*., 1992). These authors fed hens with zeolite levels of 0.0 to 1.5% and observed significant better egg specific gravity when zeolite was added to the feed.

Considering the hypothesis that clinoptilolite may improve dietary nutrient absorption and utilization, including calcium, improvements were expected in eggshell quality, and would explain the results reported in literature. However, this hypothesis was not confirmed in the present study because none of the evaluated external egg quality parameters was significantly affected by the dietary inclusion of that zeolite.

On the other hand, calcium levels influenced ($p \leq 0.05$) eggshell percentage, specific gravity and breaking strength. Hens fed the lowest calcium levels present the worst results for these parameters, as expected, because insufficient calcium intake results in lower quality eggshells (Rodrigues *et al*., 2005).

Rodrigues *et al*. (2005) obtained significant and positive effects on eggshell percentage when increasing dietary calcium levels from 2.5 to 3.5%. On the other hand, no effect of dietary calcium levels on eggshell percentage was observed by Nunes *et al*. (2006).

The results obtained in the present study on the effects of calcium levels on egg specific gravity are consistent with those of Silva *et al*. (2008), who reported better egg specific gravity when increasing dietary calcium levels from 3.5 to 4.2%. However, Murata *et al*. (2009) did not observe any effects of calcium levels (3.75 to 4.55%) on that parameter.

Clinoptilolite levels did not influence eggshell breaking strength, differently from calcium levels that, when added at 2.5%, resulted in lower breaking strength. Eggshell breaking strength was evaluated because it is directly related to other eggshell quality characteristics. However, there are few reports on this parameter in literature, warranting further studies to allow comparisons to be made.

CONCLUSIONS

The inclusion of up to 0.50% clinoptilolite in layer diets does not benefit their performance or egg quality.

The inclusion of 2.5% calcium in the diet of Hisex Brown layer at the end of their first laying cycle is not recommended. Calcium levels higher than 3.1% promote better external egg quality.

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