Effect of different concentrations of chia (*Salvia hispanica* L.) on the productive variables of Creole hens and internal characteristics of the egg

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ABSTRACT
Objective: To evaluate the effect of chia seed inclusion on productive variables and internal characteristics of eggs from Creole hens.
Methodology: Sixty 24-week-old Creole hens were selected, and five concentrations of whole chia seed (*Salvia hispanica* L.) 0, 5, 10, 15, and 20% were evaluated, incorporated into a diet consisting of corn, soybean meal, corn starch, vitamins, and minerals. The analyzed variables included feed intake (g), egg production (g), feed conversion (g/g), egg mass (g), egg weight (g), Haugh units, yolk percentage, albumen percentage, yolk color, and eggshell thickness (mm).
Results: Significant differences ($p \leq 0.05$) were found only in average egg weight, Haugh units, and yolk color.
Study limitations: This research focused on Creole hens; future studies could compare these results with commercial strains or other breeds to validate and broaden the findings.
Conclusions: The addition of whole chia seed to the diets increased egg weight and improved Haugh units compared to the control diet, without affecting the productive variables in Creole hens.

Keywords: chia, hens, Creole, egg.

INTRODUCTION

The composition of hen eggs is highly valued for its content of proteins, lipids, vitamins, and minerals, making it a fundamental food in the human diet. However, despite its high nutritional value, egg consumption has declined in recent decades due to concerns related to its cholesterol and saturated fatty acid content (Simopoulos, 2002; Ayerza & Coates, 2000). In this context, chia seeds represent an interesting alternative due to their high

oil (13.5-34.4%) and protein (18-26.5%) content (Weber *et al.*, 1991). Nevertheless, their use in the diet of laying hens has been scarcely explored. Currently, at the Colegio de Postgraduados, a breeding and improvement program for a flock of Creole hens is being conducted, whose nutritional requirements for energy and protein have recently been determined (Matus-Aragón *et al.*, 2021). Therefore, the aim of the present study was to evaluate the effect of chia seed inclusion in the diet on productive variables and internal egg characteristics in Creole hens.

MATERIALS AND METHODS

Study location

The experiment was conducted at the poultry facilities of the Colegio de Postgraduados, Montecillo Campus, located in Texcoco, State of Mexico (19° 29' N, 98° 53' W; 2,247 masl) (García, 2004). The poultry house features natural ventilation, regulated by lateral curtains.

Experimental animals

A total of 60 Creole hens, 24 weeks old, with an average initial weight of 1866±36.8 g, were used. The birds were housed individually in cages measuring 37×43×30 cm (height×length×width), with free access to feed and water. Animal management was conducted in accordance with the guidelines of the Animal Welfare Committee of the Colegio de Postgraduados (COBIAN/008/24; COLPOS, 2016).

Proximal analysis of chia

Chia seeds (*Salvia hispanica* L.), sourced from a supplier in Cholula, Puebla, were analyzed at the Nutrition Laboratory of the Universidad Autónoma Chapingo. The proximal composition was as follows: dry matter 94.9%, ash 4.22%, crude protein 23.00%, ether extract 32.21%, crude fiber 9.39%, and nitrogen-free extract 26.1%.

Experimental diets

Five diets were formulated with whole chia seed inclusion at levels of 0, 5, 10, 15, and 20%, based on corn, soybean meal, corn starch, vitamins, and minerals. These treatments were designated as T0, T5, T10, T15, and T20. The composition and calculated analysis of the diets are shown in Table 1.

The linolenic acid content of the diets (%) was: 3.08, 19.84, 24.76, 36.39, 39.09 according to the order of the treatments.

Response variables

The productive variables included feed intake (g), egg production (g), feed conversion (g/g), and egg mass (g). Data were recorded weekly. The internal egg characteristics evaluated were egg weight (g), Haugh units, yolk percentage (%), albumen percentage (%), yolk color expressed as DSM color fan intensity, and eggshell thickness (mm); these data were measured during three periods (14, 49, and 84 days).

Table 1. Composition (%) and calculated analysis of the experimental diets.

Ingredients	T0[1]	T5	T10	T15	T20
Yellow corn	29.23	37.52	44.61	49.92	54.05
Starch	20.00	15.00	10.00	5.00	0.00
Chia seed	0.00	5.00	10.00	15.00	20.00
Soybean meal	12.80	12.33	12.27	18.29	11.49
Corn gluten	2.60	1.87	1.09	0.80	3.38
Wheat bran	12.17	5.40	0.00	0.00	0.00
Canola meal	12.00	12.00	11.20	0.00	0.00
Fine calcium carbonate	4.38	4.38	4.38	4.46	4.44
Coarse calcium carbonate	4.37	4.37	4.37	4.37	4.37
Dicalcium phosphate	1.22	1.15	1.09	1.14	1.12
Soybean oil	0.44	0.20	0.20	0.20	0.20
Common salt	0.33	0.34	0.34	0.35	0.35
Vitamin and mineral premix[2]	0.30	0.30	0.30	0.30	0.30
Sodium bicarbonate	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.07	0.05	0.04	0.06	0.02
L-lysine HCl	0.00	0.00	0.00	0.03	0.17
Total	100.0	100.0	100.0	100.0	100.0
Calculated analysis					
Dry matter	91.10	90.80	90.50	90.10	90.10
Ash	12.60	12.50	12.50	12.40	12.30
Crude protein	16.00	16.00	16.00	16.00	16.00
Ether extract	2.30	3.70	5.30	6.90	8.60
Crude fiber	3.40	3.40	3.40	3.00	3.30
Linoleic acid	1.00	1.10	1.50	1.80	2.20
Calcium carbonate	3.70	3.70	3.70	3.70	3.70
Available phosphorus	0.35	0.35	0.35	0.35	0.35
Na	0.18	0.18	0.18	0.18	0.18
Cl	0.24	0.23	0.23	0.24	0.27
K	0.72	0.67	0.64	0.68	0.57
EMAn[3] (kcal/kg)	2600	2600	2600	2600	2600
Digestible amino acids					
Lysine	0.66	0.63	0.60	0.58	0.55
Methionine	0.31	0.28	0.26	0.24	0.21
Methionine + cystine	0.54	0.50	0.47	0.41	0.37
Threonine	0.48	0.45	0.43	0.39	0.34
Tryptophan	0.15	0.14	0.13	0.12	0.09
Isoleucine	0.54	0.51	0.48	0.46	0.41
Valine	0.63	0.59	0.56	0.52	0.46

[1] T0, T5, T10, T15, and T20: indicate the percentage concentration of chia seed included in the diet. [2] Contribution per kg of feed: vitamin A, 12,000 IU; vitamin D_3, 5,000 IU; vitamin E, 85 IU; vitamin K_3, 3 mg; vitamin B_1, 3 mg; vitamin B_2, 8 mg; vitamin B_3, 62 mg; vitamin B_6, 4 mg; vitamin B_{12}, 0.017 mg; pantothenic acid, 14.5 mg; folic acid, 1.8 mg; biotin, 0.2 mg; Zn, 100 mg; Mn, 120 mg; I, 1.25 mg; Se, 0.3 mg; Cu, 15 mg; Fe, 80 mg. [3] AMEn: Apparent Metabolizable Energy corrected for nitrogen retention.

Experimental design

The experiment was conducted following a completely randomized design. The statistical model used was as follows:

$$Y_{ijk} = \mu + T_i + E_k + T_i * E_k + \varepsilon_{ijk}$$

Where: Y_{ijk}=Response variable measured in the j-th replicate (j=1-12) of the i-th treatment (i=1-5) during the k-th period (k=1-12 weeks for productive variables and k=1-3 for internal egg characteristics); μ=Common population constant; T_i=Effect of the i-th treatment, i=1-5; E_k=Effect of the k-th period, k=1-12 or k=1-3; $T_i * E_k$=Interaction effect between treatment and period; ε_{ijk}=Random error effect associated with the i-th treatment, the j-th replicate, and the k-th period.

Data were analyzed using the MIXED procedure of the SAS statistical software (SAS Institute Inc., 2013), with period evaluated as a repeated measure. For the repeated measures analysis, four covariance structures were tested, and the most appropriate was selected based on Akaike's and Schwarz's Bayesian Information Criteria.

Each treatment (experimental diet) was administered to 12 hens, each considered an experimental unit.

The adjusted means obtained were compared using Tukey's test with an α=0.05.

RESULTS AND DISCUSSION

Productive variables

Throughout the 12-week study, the productive variables evaluated feed intake per hen, egg production, feed conversion, egg mass, and laying percentage showed no significant differences between treatments ($p>0.05$), both over the course of the experiment and in the overall average (data not shown). The only exception was feed intake in week 12, where hens fed the diet containing 20% chia seed had significantly lower consumption ($p\leq0.05$) compared to the other treatments.

Average egg weight

Figure 1 shows the trend in egg weight over the 12-week period. Significant differences ($p\leq0.05$) were observed between treatments in most weeks, except for weeks 3 and 8. On average for the experimental period, the diets with 10% and 15% chia inclusion promoted greater egg weight. These treatments were significantly superior to the control, as well as to the treatments with 5% and 20% chia. Notably, the treatment with 20% chia seed showed values similar to the control in most of the weeks evaluated.

Internal egg characteristics

The average egg weights used to evaluate these variables were 48.3, 49.4, 50.7, 51.3, and 50.3 g for the treatments with 0%, 5%, 10%, 15%, and 20% chia, respectively. Regarding Haugh units, during the first period, the diet with 5% chia showed values similar to the control treatment, while the 20% chia diet yielded comparable results to

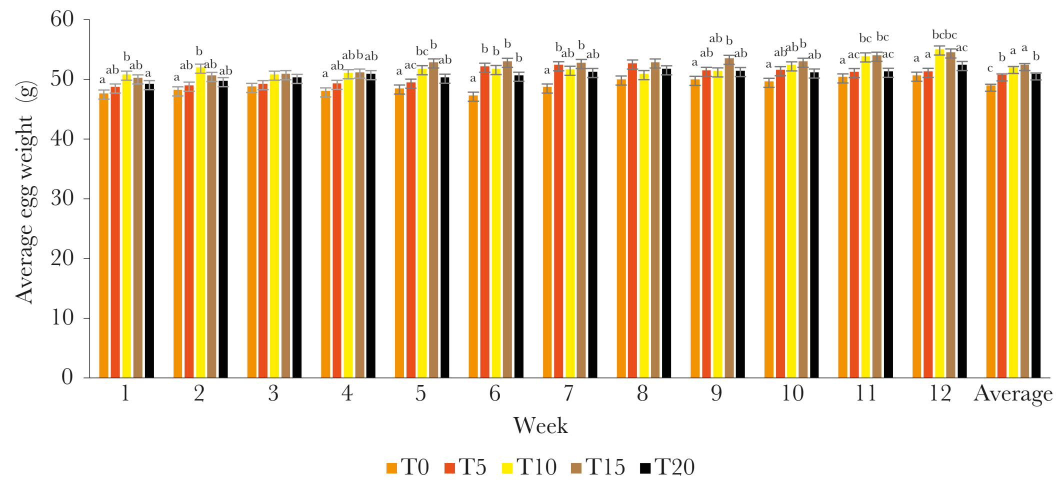

Figure 1. Average egg weight (g) of Creole hens fed for 12 weeks with different concentrations of chia. The numbers 0, 5, 10, 15, and 20 indicate the level of chia inclusion (%) in the diet. [abc]Means with different superscript letters within each week indicate significant differences (p≤0.05).

the 5% group. The diets with 10% and 15% chia did not differ from each other, but were significantly higher than those with 0% and 5% chia. In periods two and three, no significant differences were observed among treatments (p>0.05). However, the overall average across the three periods indicated that all chia-inclusive diets significantly outperformed the control in this variable. Concerning yolk color, during the first period, the 20% chia diet produced the most intense yolk color compared to the control. No significant differences were found among treatments in periods two and three; however, the overall average showed that only the 20% chia treatment significantly increased yolk color intensity (p≤0.05) (Figure 3).

Regarding yolk percentage, albumen percentage, and eggshell thickness, no differences were observed due to treatment effects or in the overall average (p>0.05) (Table 3).

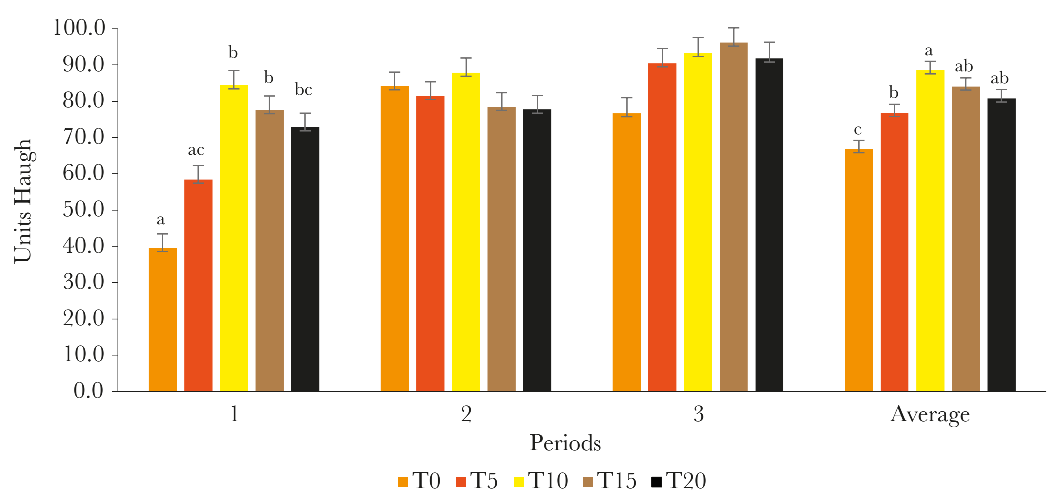

Figure 2. Haugh units of eggs from Creole hens fed diets with different concentrations of chia. The numbers 0, 5, 10, 15, and 20 indicate the level of chia inclusion (%) in the diet. [abc]Means with different superscript letters within each period indicate significant differences (p≤0.05).

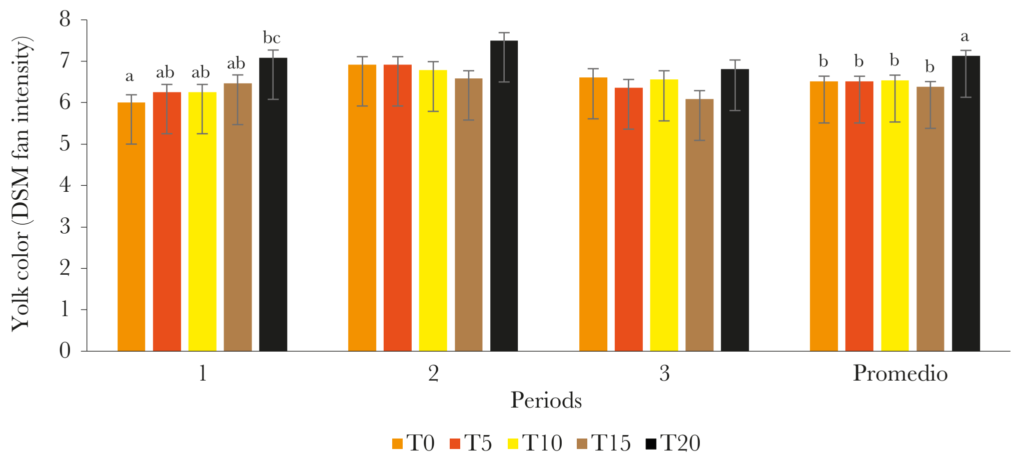

Figure 3. Yolk color expressed as DSM color fan intensity in eggs from Creole hens fed diets with different concentrations of chia. The numbers 0, 5, 10, 15, and 20 indicate the level of chia inclusion (%) in the diet. [abc]Means with different superscript letters within each period indicate significant differences (p≤0.05).

Table 2. Internal egg characteristics of Creole hens fed diets with different concentrations of chia.

	Treatment						Period					P>F	
	T0	**T5**	**T10**	**T15**	**T20**	**EE**	**1**	**2**	**3**	**EE**	**Treatment**	**Period**	**Treatment × Period**
Haugh Units	66.82^{c}	76.80^{b}	88.55^{a}	84.09^{ab}	80.79^{ab}	2.39	66.57^{c}	81.96^{b}	89.72^{a}	1.78	0.0001	0.0001	<.0001
Yolk (%)	35.62	34.62	35.21	34.67	35.64	0.85	33.39^{b}	36.16^{a}	35.92^{a}	0.61	0.8521	0.0001	0.0459
White (%)	51.64	52.00	51.05	52.23	51.38	1.01	50.34^{b}	50.26^{b}	54.40^{a}	0.75	0.9306	0.0001	0.2815
Yolk Color	6.51^{a}	6.50^{a}	6.53^{a}	6.38^{a}	7.13^{b}	0.12	6.41^{b}	6.94^{a}	6.49^{b}	0.08	0.0018	0.0001	0.2263
Shell Thickness (mm)	0.32	0.34	0.33	0.32	0.32	0.01	0.33	0.34	0.33	0.00	0.4562	0.0415	0.1113

The numbers 0, 5, 10, 15, and 20 indicate the level of chia inclusion (%) in the diet. [abc]Means with different literals in each row indicate significant differences (p≤0.05). SE: Standard error.

Proximal analysis

The results of the proximal analysis of chia seed conducted in this study partially align with those reported by Ayerza and Coates (1999), revealing a higher protein content (23% *vs.* 17%) and lower crude fiber content (9.4% *vs.* 22.1%), while the ether extract was similar (32.2% *vs.* 32.8%).

The average results for feed intake, egg production, egg mass, feed conversion, and laying percentage were similar throughout the 12-week study. This is attributed to the diets having comparable contents of apparent metabolizable energy, crude protein and digestible amino acids, with the exception of the 20% chia diet, which had a lower concentration of amino acids. Average egg weight was higher in hens fed diets containing 10% and 15% chia, followed by those of 20% and 5%, and lowest in the control group (T0). This effect is linked to the higher content of linoleic and linolenic acids in the chia diets, compounds that, along with amino acids, directly influence egg size (Scott *et al.*, 1976). These findings partially agree with those of Ayerza and Coates (2001), who reported no differences in egg production, yolk weight, or albumen weight; however, no significant differences in egg weight were observed in their study. It is worth noting that this discrepancy may stem from the statistical test used, as Ayerza and Coates employed Duncan's multiple range test, while the present study used Tukey's test. In a later study, Ayerza and Coates (2002) reported that the yolk-to-egg weight ratio was lower in white hens compared to brown hens, regardless of chia inclusion level in the diet. Similarly, Salazar-Vega *et al.* (2009) observed an increase in egg weight with higher chia proportions in the diet of Babcock hens, findings that align with those of the present study.

Eisen *et al.* (1962) indicated that Haugh units are a reliable indicator of internal egg quality, as they reflect albumen height. In the present study, hens fed chia-supplemented diets produced eggs with higher Haugh units compared to the control group. Yolk and white percentages showed similar trends across treatments. Regarding yolk color, an increase in intensity was observed toward the end of the study, measured using the DSM color fan scale, with the most notable enhancement in diets with higher chia inclusion. In contrast, eggshell thickness showed no significant differences among treatments.

CONCLUSIONS

The inclusion of whole chia seed in the diets of Creole hens significantly increased egg weight and improved Haugh units compared to the control diet, without negatively affecting the productive variables evaluated. These results indicate that chia can be used as a functional ingredient in the feeding of Creole hens, contributing to the improvement of internal egg quality without compromising productive performance.

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