Effects of Zinc Oxide nanoparticles and Zeolite on cage-free hens' egg physical quality

Efecto de las nanopartículas de óxido de zinc y zeolita sobre la calidad física del huevo de gallinas libres de jaula

Julieta Ziomara Ordoñez-Morales[1], Martín Alfredo Legarreta-González[2], Rafael Rodríguez-Martínez[3], Ma. De los Ángeles De-Santiago-Miramontes[4], José Antonio Quintana-López[5], Dalia Ivette Carrillo-Moreno[4]*

[1]Universidad Autónoma Agraria Antonio Narro, Programa de Doctorado en Producción Agropecuaria, Periférico Raúl López Sánchez, Colonia Valle Verde, CP. 27054. Torreón, Coahuila, México.
[2]Tecnológico de Monterrey, Escuela de Ingeniería y Ciencias, Avenida Eugenio Garza 2501 Sur, Col. Tecnológico de Monterrey, CP. 64700. Monterrey, Nuevo León, México.
[3]Universidad Autónoma Agraria Antonio Narro, Departamento de Ciencias Médico Veterinarias; Periférico Raúl López Sánchez, Colonia Valle Verde, CP. 27054. Torreón, Coahuila, México.
[4]Universidad Autónoma Agraria Antonio Narro, Departamento de Producción Animal, Universidad Autónoma Agraria Antonio Narro, México, Periférico Raúl López Sánchez, Colonia Valle Verde, CP. 27054. Torreón, Coahuila, México.
[5]Universidad Nacional Autónoma de México, Departamento de Medicina y Zootecnia de Aves, Facultad de Medicina Veterinaria, Ciudad Universitaria, Av. Universidad #3000, Colonia, C.U., CP. 04510. Coyoacán, Ciudad de México, México.

*Corresponding author: dalia.ivettecm@gmail.com

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ABSTRACT. This study evaluates the effect of zinc oxide nanoparticles and zeolite added to the diet of cage-free hens on productive parameters and egg physical quality. A total of 400 hens, aged 44 to 60 weeks, were randomly assigned to four treatments: T1: 90 mg of zinc oxide nanoparticles per kg of feed (ZnO-NPs); T2: 90 mg of ZnO-NPs and 4% zeolite per kg of feed; T3: 4% zeolite per kg of feed; T4: Fed only the basal diet. Productivity variables (egg weight, number of eggs, percentage of egg production, egg mass) and physical quality variables (shape index, shell thickness and weight, albumen weight and height, yolk weight and diameter, and Haugh units) were measured. The data were analyzed using a one-way ANOVA, and principal component analysis (PCA) was used to identify variables associated with productivity and physical quality. Among the productivity variables, only feed intake showed significant differences, being higher in group T3 ($p < 0.05$). Regarding physical quality parameters, shell thickness was greater in T1 ($p < 0.05$) compared to the other experimental groups; for the remaining variables, no differences were observed between treatments ($p > 0.05$). These findings suggest that zinc oxide nanoparticles may influence shell thickness.
Keywords: Cage free system, eggshell quality, mineral additives.

RESUMEN. El presente estudio evalúa el efecto de las nanopartículas de óxido de zinc y la zeolita adicionada a la dieta de gallinas criadas en un sistema libre de jaula, sobre los parámetros productivos y la calidad física del huevo. Un total de 400 gallinas, de entre 44 y 60 semanas de edad, se asignaron aleatoriamente en cuatro tratamientos: T1: 90 mg de nanopartículas de óxido de zinc por kg de alimento (ZnO-NPs); T2: 90 mg de ZnO-NPs y 4% de zeolita por kg de alimento; T3: 4% de zeolita por kg de alimento; TT: Alimentadas únicamente con la dieta basal. Se midieron variables de productividad (peso del huevo, cantidad de huevo, porcentaje de postura, masa del huevo) y variables de calidad física (índice de forma, grosor y peso del cascarón, peso y altura de la albúmina, peso y diámetro de la yema y unidades Haugh). El análisis de los datos se realizó a través de un ANOVA de una vía, además de un análisis de componentes principales (PCA) para identificar las variables asociadas con la productividad y la calidad física. Las variables productivas, sólo el consumo de alimento presentó diferencias significativas, siendo mayor en el grupo T3 ($p < 0.05$). Los parámetros de calidad física, el grosor del cascarón fue mayor en T1 ($p < 0.05$) comparado con los otros grupos experimentales; en el resto de las variables analizadas, no se detectaron diferencias significativas entre los tratamientos ($p > 0.05$). Los hallazgos sugieren que las nanopartículas de óxido de zinc pueden influir en el grosor del cascarón.
Palabras clave: Sistema libre de jaula, calidad del cascarón, aditivos minerals.

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INTRODUCTION

The global poultry industry increasingly adopting cage-free systems to meet animal welfare standards (Rokade *et al.* 2024). In Mexico, approximately 10% of eggs are produced in alternative systems, including backyard, cage-free, and free-range farms (Romo *et al*. 2022). Nevertheless, increased physical activity in these systems raises maintenance energy requirements, leading to higher feed intake without a corresponding increase in egg mass. This shift negatively affects the Feed Conversion Ratio and increases production costs (Rokade *et al*. 2024, Susilo *et al.* 2025). Within the context of egg production, a key challenge is the high cost of feed (Barzegar *et al*. 2021). Increasing egg weight and production are the primary goals of laying hen breeding programs (Monika *et al*. 2021). Consequently, diets are meticulously formulated to satisfy the nutritional demands of hens across all life stages while minimizing costs (Moss *et al*. 2021). There is a clear correlation between nutrient deficiencies and production issues, including thin, fragile shells that are prone to breakage (Cuca-García *et al*. 2008), especially in layers older than 40 weeks. To enhance the nutritional quality of poultry diets, mineral additives have been employed (Abedinn *et al*. 2018), with zeolite being a notable example due to its capacity to improve egg quality and optimize feed conversion ratio (Vieira *et al*. 2023, Elsherbeni *et al*. 2024). A total of 40 distinct zeolite types have been used in livestock and poultry farming, with a high degree of similarity in their chemical composition (Taghiyev *et al*. 2025). Zeolite is an aluminum silicate mineral that is characterized by the presence of microscopic pores (Mondal *et al.* 2021). A study carried out on 40-week-old Fayoumi layer hens indicates that the addition of 20 g kg^{-1} zeolite can be a natural additive, since they observed improvement in productivity, egg quality, general health and intestinal microbiota (Medras *et al*. 2026). Research has demonstrated that incorporating zeolite into feedstuffs can mitigate the adverse effects of mycotoxins, as evidenced by improved growth in broiler chickens (Ibrahim *et al*. 2025). Another mineral used as a feed supplement in poultry is zinc (Zn); its role is considered important to the growth and bone development of chickens. As is well documented, the presence of this element is frequently observed in inorganic structures, such as zinc oxide (ZnO), and clinical and biochemical manifestations have been associated with deficiencies in dietary intake of this element. In poultry, such deficiencies have been shown to cause growth retardation, reduced appetite, and increased mortality in the flock (Naz *et al.* 2016).

The recommended concentration of Zn in poultry rations ranges from 40 and 75 mg kg^{-} (NRC 1994). However, in the poultry feed industry, it is recommended to supplement Zn at levels 20-30 times the common requirement, as the bioavailability of inorganic Zn is low (Lai *et al*. 2010). On the other hand, organic Zn is preferable due to its greater bioavailability compared to inorganic salts. In an effort to identify cost-effective alternatives, researchers have directed their attention towards sources of nano-Zn within the animal feed industry (Kumar *et al*. 2021), including nano-Zn, the third most produced nanomaterial due to its potent antimicrobial activity (Hassan *et al.* 2020). Current research indicates that dietary inclusion of nano-Zn at 55 mg kg^{-1} can effectively replace traditional zinc sulphate ($ZnSO_4$) administered at 110 mg kg^{-1} without compromising the growth or productive traits of broiler chickens (Kumar *et al.* 2021), but egg quality can also be affected, and it is important to consider that high-quality eggs are a prerequisite for profitable marketing (Kumar *et al*. 2024) Several egg quality (EQ) traits are being studied due to their economic importance and because they are affected by various factors, including nutrition (Lonkar *et al*. 2023). Housing conditions significantly impact the welfare, health, behavior, and productivity of cage-free laying hens (Susilo *et al*. 2025). Therefore, the aim of this study was to evaluate the effects of dietary supplementation with zinc oxide nanoparticles and zeolite on production parameters and egg physical quality in cage-free laying hens.

MATERIALS AND METHODS

Location

The experimental work was performed at the "Unidad Academica de Produccion Avicola" of the "Universidad Autonoma Agraria Antonio Narro", located in Torreon, Coahuila, Mexico (25° 33' 3.38" LN 103° 22' 25.6" LW; 1 124 masl). The region is characterized by a dry climate, with an average annual temperature of 22.3 °C, extreme temperatures of 40 °C in summer and -3 °C in winter outdoors, and an average annual rainfall of 225 mm (INEGI 2015).

Animals

Four hundred Rhode Island hens, aged 44 weeks, with similar weights and egg-yield levels, were selected and randomly assigned to four treatments, with four replicates of 25 hens each, for a total of 100 hens per treatment. The experimental phase was from week 44 to week 60 of the production period. The hens were housed in a cage-free production system, with no access to the outdoors, in pens measuring 1.5 m x 2.0 m, each housing 25 hens. Each space was equipped with nesting boxes and perches, and *ad libitum* water was provided. The hens were fed a basal diet of 120 g per hen per day, which was served manually in two daily feedings. The diet consisted of corn, distillers dried grains, and soybeans and was formulated to meet nutritional recommendations, with 17% crude protein and 2 790 kcal (NRC 1994) (Table 1). Hens were housed under a controlled 16:8 h (light:dark) photoperiod.

Experimental treatments

The treatments involved the utilization of a locally prepared natural zeolite (Zeolita,1318-02-1 PSP, San Luis Potosi, Mexico), the chemical analysis of which is presented in Table 2. Furthermore, the nanoparticle used in the experiment comprised semiconductor zinc oxide (ZnO) particles with a purity of 99.7%, an average size of 50 nm, and a physical appearance of fine white powder with yellow tones and no characteristic aroma, with an apparent density of 5.610 g cm^{-3} and medium solubility in water and alcohols. The following treatments were administered: T1, 90 mg of zinc oxide nanoparticles per kg of feed (NPs-ZnO, 1314-13-2); T2, 90 mg of NPs-ZnO and 4% zeolite per kg of feed; T3, 4% zeolite per kg of feed (Emam *et al*. 2019); and T4, control treatment where only the basal diet was administered. The variables evaluated were divided into productive and quality-of-egg variables.

Productive variables

1) The quantity of feed intake (FI) was calculated on a daily basis by deducting the amount of feed that was rejected per pen (g) from the total amount of feed provided (g); 2) The feed conversion ratio (FCR) was determined by dividing the feed consumed per week (kg) by the egg mass

produced per week (kg); 3) The calculation of egg weight (EW) was conducted utilizing an analytical balance scale (Ohaus digital scale Model E02130, USA, with a capacity of 210 g and a precision of 0.001 g); 4) The number of eggs (EN) for each treatment was recorded during the course of the experiment, which involved collecting the eggs twice a day; 5) Subsequent to the determination of the individual weights, the average egg weight (aEW) per treatment was calculated. 6) The egg mass (EM) was calculated by adding the total number of eggs laid per week in each experimental group during the study period. 7) The percentage of laying (PL) was calculated on a weekly basis in each experimental group using the following formula:

$$Laying\ \% = \frac{Total\ eggs\ collected}{(Total\ initial\ hens)x100}/7$$

Where: Laying rate: the percentage of egg production per hen. Total eggs collected: the sum of all eggs collected during the day. Total initial hens: total number of hens at the start (100 hens for each experimental group). The value 100: factor used to determine the percentage. 7 = Days of the week.

Table 1. Nutritional composition of the basal diet.

Materials	Percentage
Ingredients	
Ground corn	52.0
Soybean	20.0
Distiller's grain	10.0
Fine calcium	5.0
Coarse calcium	5.0
Orthophosphate	2.5
Mineral-vitamin premix*	2.5
Vegetable oil	2.7
Salt	0.3
Total	100.0
Chemical composition of the feed	
Dry matter (DM)	92.25
Ash	7.47
Ether extract (EE)	4.40
Crude fiber (CF)	2.54
Crude protein (CP)	16.63
Lysine	0.72
Methionine + cysteine	0.46
Metabolizable energy	2790 kcal kg^{-1}.

° vitamins and minerals: Vit. At 10 000 000 IU, Vit. D3 2 000 000 IU, Vit. E 10 mg, Vit. K3 2 mg, Vit. B1 1 mg, Vit. B2 5 mg, Vit. B6 1.5 mg, Vit. B12 10 mg, pantothenic acid 10 mg, Niacin 30 mg, Folic acid 1 mg, Biotin 0.50 mg, Iron 30 mg, manganese 60 mg, Iodine 1 mg, Copper 10 mg, Zinc 30 mg, and Selenium 0.1 mg.

Table 2. Microanalysis of the percentage of elements of the zeolite-Clinoptilolite.

Ingredients	Weight (%)
Carbon	5.39
Oxygen	44.25
Sodium	0.13
Magnesium	0.40
Aluminum	7.26
Silicon	33.94
Phosphorus	0.16
Potassium	3.35
Calcium	3.12
Iron	2.00
Total	100

Quality of egg variables

At weeks 44, 47, 51, 54, 58, and 60 of the hens' production cycle, 10 eggs were collected from each experimental group in order to evaluate the following parameters: 1) Shape index (SI) was calculated by dividing the minor diameter (width) of the egg by its major diameter (length) and then multiplying the result by 100. 2) Shell weight (SW) was determined using the technique of Anderson *et al.* (2004). 3) Shell thickness (ST) was measured. 4) Yolk diameter (YD) was determined by measuring the longest part of the yolk. 5) Yolk color (YC) was recorded using the DSM yolk color spectrum (which determines the color intensity from lightest to darkest on a scale of 1 to 15); 6) yolk pH (YpH), and; 7) Albumen pH (ApH), were measured using a potentiometer (Hanna, model HI98128, Mexico, with a resolution of 0.01 pH); 8) Yolk weight (YW), this procedure involved the separation of the yolk from the albumen, followed by weighing the yolk using an Ohaus digital scale (model E02130, USA) with a capacity of 210 g and an accuracy of 0.001 g. 9) Then, the albumen weight (AW) was calculated using the following formula: AW = W -YW – SW, where W denotes the egg weight, YW the yolk weight, and SW the shell weight. 10) The calculation of the Haugh units (HU) was performed using the following formula: HU = 100 log (AH+7.57−1.7×W0.37), where AH denotes the albumen height and W means the egg weight. 11) Albumen height (AH). The thick albumen surrounding the yolk was measured. To measure the variables SI, ST, YD, and AH, a digital vernier caliper (generic brand, model RM-KC-BXG) was used.

Statistical analyzes

Principal component analysis (PCA) was used to achieve three main objectives: first, to reduce dimensionality due to the large volume of data generated; second, to identify the components that retain the most information; and third, to transform the correlated variables into a smaller set. The analysis was performed using R version 4.5.1. To facilitate a meaningful comparison of treatments, a One-way ANOVA test was performed, considering a trend at $p < 0.10$ and a statistically significant difference at $p < 0.05$. All data are expressed as the mean and its standard error.

RESULTS

As demonstrated in the analysis of the data (Figure 1), principal components 1 (PC1) and 2 (PC2) account for 19.2% and 14.7% of the observed variation. The variation for PC3 and PC4 is 19.2% and 14.7%, respectively. Collectively, these components account for 54% of the accumulated variation. Two aspects are illustrated by the graph. Firstly, it is demonstrated that each variable makes a unique contribution to the components. Secondly, the illustration clearly shows the grouping of the variables. This analysis suggests that, despite subtle T1 variations, the ellipses overlap, indicating no differences in the multivariate pattern of the variables studied. Therefore, analysis of variance (ANOVA) was employed to substantiate the results obtained from PCA.

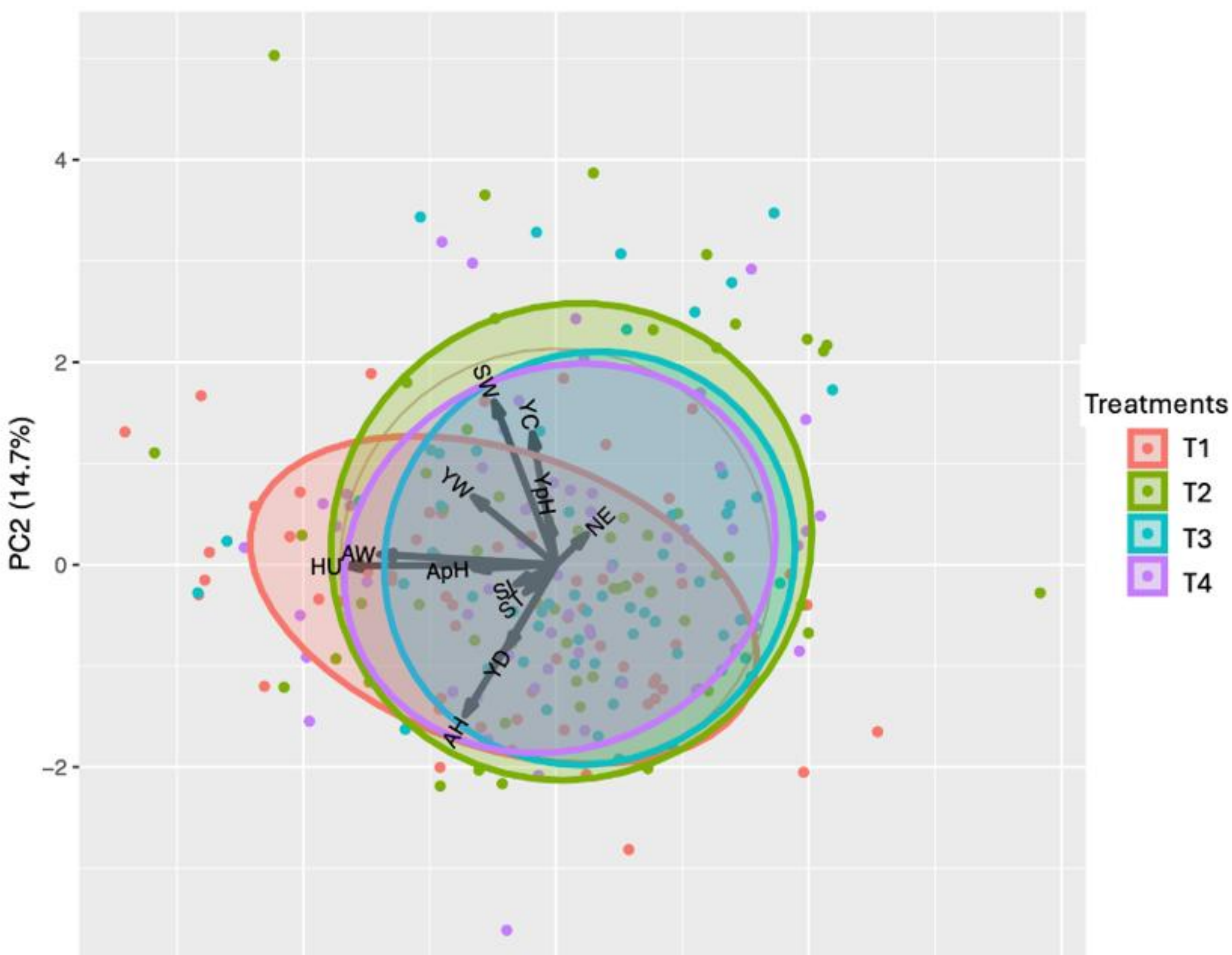

Figure 1. Principal component analysis of egg physical quality assessed over 20 weeks of production in a cage-free system. PC1: principal component 1, PC2: principal component 2. Treatments*:* T1 = 90 mg kg^{-1} per feed of zinc oxide nanoparticles (ZnO-NPs), T2 = 90 mg kg^{-1} per feed of ZnO-NPs and 4% zeolite, T3 = 4% kg^{-1} per feed zeolite, and T4 = control treatment (only basal diet), in the diet of hens in a cage-free system. *Variables:* SI = Shape index, SW = Shell weight, ST = Shell thickness, YD = Yolk diameter, YC = Yolk color, YpH = yolk pH, ApH = Albumen pH, YW= Yolk weight, AW = albumen weight, HU = Haugh units, AH = Albumen height.

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Production parameters

The production parameters are shown in Table 3. No statistically significant differences were observed among the treatments in egg weight, egg number, egg mass, laying percentage, or feed conversion ratio ($p \geq 0.05$). Only a higher feed intake was observed in group T3 [10.95 ± 1.37] in comparison to the other experimental groups [T1: 10.60 ± 1.56, T2: 10.68 ± 1.44, T3 10.95 ± 1.37 and T4 10.33 ± 1.83] ($p \leq 0.05$).

Table 3. Evaluation of production parameters with the inclusion of four different zinc oxide nanoparticle and zeolite treatments.

Variables	T1	T2	T3	T4
Egg weight (g)	$59.42^{a} \pm 1.40$	$59.39^{a} \pm 2.54$	$59.35^{a} \pm 1.09$	$59.77^{a} \pm 1.31$
Number of eggs	$538.06^{a} \pm 34.86$	$549.72^{a} \pm 51.07$	$540.05^{a} \pm 34.61$	$510.83^{a} \pm 55.98$
Laying percentage (%)	$76.87^{a} \pm 4.98$	$78.53^{a} \pm 7.30$	$77.15^{a} \pm 4.94$	$72.98^{a} \pm 8.00$
Egg mass (kg)	$31.99^{a} \pm 2.65$	$32.05^{a} \pm 1.86$	$32.11^{a} \pm 2.37$	$30.58^{a} \pm 3.82$
Feed consumption (kg)	$10.60^{b} \pm 1.56$	$10.68^{b} \pm 1.44$	$10.95^{a} \pm 1.37$	$10.33^{c} \pm 1.83$
Feed conversion ratio	$2.35^{a} \pm 0.22$	$2.35^{a} \pm 0.21$	$2.44^{a} \pm 0.27$	$2.37^{a} \pm 0.15$

T1, basal diet plus 90 mg of zinc oxide nanoparticles (ZnO-NPs); T2, basal diet plus 90 mg of ZnO-NPs and 4% zeolite; T3, basal diet and 4% zeolite; and T4, control treatment (only basal diet), in the diet of hens in a cage-free system. Different letters in the same row indicate significant differences ($p < 0.05$). One-way ANOVA test.

Quality of egg variables

The investigation revealed no statistically significant differences between the experimental groups, and no disparities in yolk weight were detected ($p \geq 0.05$; Table 4). Regarding the shell thickness variable (Table 4), a greater increase was observed in T1 (0.24 ± 0.08) compared to the other treatments ($p \leq 0.05$). T2 (0.22 ± 0.08) was the second largest, compared to T3 (0.21 ± 0.08) and T4 (0.20 ± 0.09) ($p \leq 0.05$), while the latter two were similar to each other ($p \geq 0.05$).

Table 4. Evaluation of external and internal physical quality of eggs with the inclusion of four different zinc oxide nanoparticle and zeolite treatments in the diet of hens in a cage-free system.

Variables	T1	T2	T3	T4
Shape Index %	$78.39^{a} \pm 10.82$	$75.55^{a} \pm 6.93$	$76.42^{a} \pm 3.61$	$76.76^{a} \pm 4.65$
Shell Thickness (mm)	$0.24^{a} \pm 0.08$	$0.22^{b} \pm 0.08$	$0.21^{c} \pm 0.08$	$0.20^{c} \pm 0.09$
Shell Weight (g)	$6.66^{a} \pm 0.87$	$6.63^{a} \pm 1.51$	$6.65^{a} \pm 0.94$	$6.62^{a} \pm 1.06$
Albumen Weight (g)	$38.22^{a} \pm 5.07$	$37.78^{a} \pm 5.25$	$37.25^{a} \pm 4.48$	$37.14^{a} \pm 4.63$
Albumen Height (mm)	$12.44^{a} \pm 1.94$	$10.68^{a} \pm 2.74$	$11.12^{a} \pm 2.34$	$11.29^{a} \pm 2.20$
Albumen pH (1-14)	$7.27^{a} \pm 0.50$	$7.08^{a} \pm 0.51$	$7.18^{a} \pm 0.60$	$7.28^{a} \pm 0.62$
Yolk Weight (g)	$15.96^{a} \pm 2.41$	$15.89^{a} \pm 1.62$	$15.71^{a} \pm 2.29$	$16.15^{a} \pm 1.85$
Yolk Diameter (mm)	$42.21^{a} \pm 2.27$	$41.10^{a} \pm 3.03$	$41.46^{a} \pm 3.15$	$43.40^{a} \pm 5.52$
Yolk Color (1-15)	$10.06^{a} \pm 0.92$	$10.14^{a} \pm 0.91$	$10.04^{a} \pm 0.89$	$10.30^{a} \pm 1.05$
Yolk pH (1-14)	$6.38^{a} \pm 0.53$	$6.30^{a} \pm 0.41$	$6.35^{a} \pm 0.51$	$6.36^{a} \pm 0.55$
Haugh Units	$70.23^{a} \pm 0.50$	$69.71^{a} \pm 0.50$	$69.68^{a} \pm 0.50$	$69.77^{a} \pm 0.50$

Different letters in the same row indicate significant differences ($p < 0.05$). One-way ANOVA test.

DISCUSSION

Production parameters

Statistically significant differences were not observed between the treatments in egg weight, egg number, egg mass, laying percentage, or feed conversion ratio. These findings are consistent with those reported by Rodríguez *et al*. (2024), who stated that the use of micronized zeolite did not alter the number of eggs per week or laying intensity. Nevertheless, the outcomes of zeolite incorporation remain the subject of debate. The results show that zeolite supplementation led to the highest feed intake, contradicting the findings of Elsherbeni *et al*. (2024) and Ujilestari *et al*. (2025). These authors previously reported that zeolite improves feed efficiency in both laying hens and broilers during their initial growth stages. Similarly, feed intake in the T1 group (supplemented with zinc oxide nanoparticles) was higher than in the control group. However, no significant differences were observed in other production parameters, which aligns with Hassan *et al.* (2023), who reported that nano-ZnO supplementation was not effective ($P > 0.05$) in improving laying performance.

Quality of egg variables

The investigation revealed no statistically significant differences in egg quality variables between the experimental groups. These findings are consistent with those reported by Demir *et al*. (2017), who observed no substantial changes in Haugh units when hens were fed diets supplemented with zeolite at various concentrations. Furthermore, no disparities in yolk weight were detected ($p \geq 0.05$), unlike the observations reported by Berto *et al*. (2013). In their study, the addition of zeolite at doses of 15, 25, and 50 g kg^{-1} to the hens' diets resulted in a significant effect on egg yolk.

The findings of this study demonstrate that shell thickness enhancement is consistent with the observations reported by Elsherbeni *et al*. (2024) in hens and Hanusova (2021) in quails. This enhancement is evident in both T1 and T2 compared with T3 and T4. This phenomenon may be attributable to the observed increase in serum aluminum and zinc concentrations in laying hens following zeolite supplementation, which has been shown to improve eggshell quality and bone development (Roland *et al*. 1993). Additionally, it has been documented that zeolite contains substantial quantities of nutrients, including calcium, within its structural composition (Rodríguez *et al*. 2024). Furthermore, zeolites have been demonstrated to incorporate and subsequently release calcium ions from their structure (Buchwald *et al*. 2020). The positive effects observed may also be attributable to the aluminum (Al), silicon (Si), or sodium (Na) content present in zeolites, which have been demonstrated to influence calcium metabolism (Roland *et al.* 1993). In addition, zeolite has been demonstrated to function as an additive in bedding and feed, facilitating the absorption and elimination of noxious waste products from the intestine and food toxins. This process subsequently helps maintain a stable gastric pH and enhances the bioavailability of essential minerals, including phosphorus, calcium, and trace elements (Taghiyev *et al.* 2025).

In a similar manner, zinc functions as a cofactor of carbonic anhydrase, which supplies carbonate ions during the process of eggshell formation. Consequently, a deficiency in zinc results in a reduction of eggshell weight and thickness (Domel *et al*. 2024). This phenomenon can be attributed to the critical role of this mineral as a cofactor of carbonic anhydrase (CA), which plays a pivotal part in the catalysis of the hydration of metabolic CO_2 to HCO^-_3 and in the provision of carbonate ions during eggshell formation (Li *et al*. 2019).

Zinc has been identified as a vital element in the deposition of calcium carbonate during eggshell formation. It functions as a catalyst, accelerating the hydration of metabolic CO_2 to HCO^-_3, the precursor of eggshell carbonate (Zhang *et al*. 2017). The findings of the present study indicated that the eggshells of the T1 group exhibited increased thickness, thereby suggesting a direct correlation between zinc levels and shell quality. This finding is consistent with the observations reported by Lilburn and McIntyre (2024), who demonstrated that a decrease in zinc content is associated with eggs that are fragile and have thin shells. In addition, there is ample documentation indicating that shell thinning becomes more pronounced in older hens, resulting in a significant increase in egg breakage rates.

In order to elucidate the function of these elements in the diet of laying hens, a comparison of the results obtained with synthetic zeolites and other levels of inclusion in the diet, as well as the use of other types of nanoparticles, is recommended. Furthermore, it is imperative to evaluate the impact of these factors on hens of different breeds and/or raised in different rearing systems (Vieira *et al*. 2023).

CONCLUSIONS

The findings of this study indicate that the incorporation of zinc oxide nanoparticles and zeolite into the diet of hens does not result in alterations to egg production parameters within a cage-free system. However, a significant enhancement in shell thickness was observed, specifically following a hierarchical trend where T1 > T2 > T3 = T4. This ranking implies that the observed effects are not limited to a single treatment but suggest a tiered response across the experimental groups. It is therefore recommended that further research be conducted to determine how the use of zinc oxide nanoparticles and zeolite may enhance production and quality parameters, with particular focus on shell thickness in hens in a cage-free system.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest

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