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# Effects of nanoencapsulated essential oil blend in diet of Lohmann Brown hens on posture percentage, quality and egg oxidative stability

## Efecto de la suplementación con una mezcla de aceites esenciales nanoencapsulados en dietas de gallinas Lohmann Brown sobre el porcentaje de postura, calidad y estabilidad oxidativa del huevo

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### ABSTRACT

Essential oils have demonstrated beneficial effects on the productive parameters of poultry. However, the impact of nanoencapsulated essential oil blends (N–EOs) in the diet of laying hens has been little described. The objective of the research was to evaluate the effect of mixtures of N–EOs in diets of Lohmann Brown hens on the percentage of laying, quality, and oxidative stability of the egg product. 600 birds were randomly distributed into five groups (n=150 per group), according to a completely randomized design, receiving the control group (T0) a conventional diet without N–EOs while the experimental groups received mixtures of N–EOs based on soursop (S), lemon (L) and eucalyptus (E) in different proportions: T1 (S: 33.4 , L:33.3%, E:33.3%), T2 (S:50%, L:25%, E:25%), T3 (S:25%, L:50%, E:25%) and T4 ( S:25%, L:25%, E:50%). The results showed that the T4 group showed the highest posture rate compared to the T2 and T3 groups ($P<0.05$), although it was similar to T0 and T1. Comparatively, the feed conversion was better in the T4 treatment compared to T0 ($P<0.05$). The analysis of egg quality showed that the T1 and T2 treatments reached a greater shell thickness (mm) compared to the group with conventional diet ($P<0.05$). The oxidative stability of the egg yolk evaluated through the levels of malondialdehyde (MDA), showed that both the T1 and T4 groups had lower levels of MDA ($P>0.05$) compared to the standard diet (T0). In conclusion, diets with N–EOs constitute a promising option that favors feed conversion, laying percentage, and greater shell thickness of the eggs of laying hens.

**Key words:** Laying hens; soursop; lemon; eucalyptus; productive parameters

### RESUMEN

Los aceites esenciales han demostrado efectos beneficiosos en los parámetros productivos de las aves de corral. Sin embargo, el impacto de las mezclas de aceites esenciales nanoencapsulados (N–EOs) en la dieta de las gallinas ponedoras ha sido poco descrito. El objetivo de la investigación fue evaluar el efecto mezclas de N–EOs en dietas de gallinas Lohmann Brown sobre el porcentaje de postura, calidad y estabilidad oxidativa del producto huevo. 600 aves fueron distribuidas aleatoriamente en cinco grupos (n=150 por grupo), según un diseño completamente al azar. El grupo control recibió una dieta convencional no suplementada, mientras que los grupos experimentales recibieron mezclas de N–EOs a base de guanábana (S), limón (L) y eucalipto (E) en diferentes proporciones: T1 (S:33,4 %; L:33,3 %; E:33,3 %); T2 (S:50 %; L:25 %; E:25 %); T3 (S:25 %; L:50 %; E:25 %) y T4 (S:25 %; L:25 %; E:50 %). Los resultados mostraron que el grupo T4 tuvo la mayor tasa de postura en comparación a los grupos T2 y T3 ($P<0,05$), aunque fue similar a T0 y T1. Comparativamente la conversión alimenticia fue mejor en el tratamiento T4 con respecto al T0 ($P<0,05$). El análisis de la calidad del huevo mostró que los tratamientos T1 yT2 alcanzaron un mayor espesor de cascara (mm),en comparación al grupo con dieta convencional ($P<0,05$). La estabilidad oxidativa de la yema de huevo evaluada a través de los niveles de malondialdehído (MDA), mostraron que tanto el grupo T1 y T4 presentaban niveles más bajos de MDA ($P>0,05$) en comparación a la dieta estándar (T0). En conclusión, dietas con N–EOs constituyen una opción prometedora que favorece la conversión alimenticia, el porcentaje postura, y un mayor espesor de cáscara de los huevos de gallinas ponedoras.

**Palabras clave:** Gallinas de postura; guanábana; limón; eucalipto; parámetros productivos

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## INTRODUCTION

Eggs have a diversity of nutrients such as essential lipids, proteins, vitamins, minerals and trace elements of high digestibility and accessible price, being a basic food in people's diet. The egg industry has been expanding in response to the rising demand for a safe, reliable, and high–quality product. Therefore, the industry needs to respond to the different challenges, perceptions and preferences of consumers [1]. The increasing demand for animal protein has led to intensified scientific advances in aviculture, such as the frequent use of antibiotic growth promoters. These are used in constant subinhibitory concentrations to limit the population of pathogenic microorganisms in the intestinal microbiota [2]. Egg contamination by microorganisms such as *Salmonella* spp., *Escherichia coli*, *Campylobacter jejuni* and *Listeria monocytogenes* is possible, generating different complications, the most serious of which is the multi–resistance of these microorganisms to different antibiotics [3]. This situation is the same for carcass/meat [4], manure and soil fertilizer [5]. This situation poses a public health risk due to the potential spread of resistance genes, highlighting the need to restrict the non–therapeutic use of antibiotic growth promoters (AGP) [6].

Currently, consumer preferences are trending toward the purchase of animal products raised without antibiotics. Hence, there is a critical need for effective alternative growth promoters and treatment methods (plant–derived products, organic acids, probiotics, among others) for common poultry diseases [7]. Among the new alternatives, the scientific evidence confirms that essential oils (EO) have positive effects on the productive performance of a variety of poultry such as quail (*Coturnix coturnix japonica*), turkeys (*Meleagris gallopavo*), broilers or laying hens (*Gallus gallus domesticus*), Peking ducks (*Anas platyrhynchos*), among others [8, 9, 10]. Essential oils mixtures of chemical compounds produced by plants. They possess antibacterial, antifungal and antiviral activities [11]. Evidence suggests that the use of EO alone or in combination affects bird performance due to their effect on digestive processes, although these results depend significantly on the number and type of birds [12, 13]. However, their action is limited due to their volatile nature, reactivity and hydrophobicity. It is necessary to use encapsulation methods to protect them [14]. Nanoencapsulation emerges as a suitable method to preserve the stability, bioactivity and bioavailability of bioactive agents, besides, it is a practical and reproducible method [15]. In recent years, several studies have reported that nanoencapsulation of EO, in addition to being a cost–effective method for protecting bioactive compounds, also enhances their beneficial effects compared to EO in their free form [16, 17, 18]. However, despite these advances, there are few or no information on the effects of nanoencapsulated essential oil (N–EOs) blends.

Therefore, the objective of the present study was to evaluate the effects of dietary supplementation of nanoencapsulated EO mixtures on production values, oxidative stability and egg quality of laying hens.

## MATERIALS AND METHODS

### Experimental design and diets

The birds were handled in accordance with the guidelines of the code of ethics for research of the Universidad Nacional de Trujillo. A total of 600 20-week–old Lohmann Brown hens were randomly assigned (completely randomized design) to five dietary treatments (T) with 5 replicates per treatment and 24 birds per replicate. During the study, the birds were housed in metal cages (3.75 $m^2$, Alaso U.S.A. Corp., Florida, USA) on a local farm at a temperature 23 ± 3°C and 82–90% humidity with a 12-h light regime. The control group (T0) was fed a commercial diet without growth promoter, while the other treatments (T1, T2, T3 and T4) received a diet supplemented with a 75 $mg \cdot kg^{-1}$ dose of nanoencapsulated EO–based mixture of three local plants such as soursop (*Annona muricata*), lemon (*Citrus limon*) and eucalyptus (*Eucalytus globulus*). A detailed description of the treatments is described in TABLE I. The basal diet was formulated according to the nutrient requirements of the Hy–line Brown user manual [19]; feed components and nutrient compositions are shown in TABLE II. Feed and water were provided *ad libitum*, and eggs were collected at 8:00 am and 5:00 pm. Feeding, egg collection, and egg weighing were performed daily for ten weeks.

*TABLE I*
**Experimental design for the inclusion of nanoencapsulated essential oils (EOs) in feed, (% content in the composition)**

| Group | T0 | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|
| Normal diet | √ | √ | √ | √ | √ |
| Soursop | 0 | 33.4 | 50 | 25 | 25 |
| Lemon | 0 | 33.3 | 25 | 50 | 25 |
| Eucalyptus | 0 | 33.3 | 25 | 25 | 50 |

### Nanoencapsulation of essential oils

The EO of soursop seeds was obtained by the extrusion method. Briefly, after collecting the soursop seeds, they were washed and dried in an oven (SLN 15, Pol–Eko, Poland) at 70°C for two days, then taken to a screw to be pressed (Oil Expeller, Mill Power, India) at 100–105°C [20]. On the other hand, the essential oils of lemon and eucalyptus leaves were extracted through steam distillation (Essential oil extractor 04, Figmay, Argentina).

Once the oils were obtained, nanoencapsulation was carried out as previously described [21] with some modifications. The modifications included the use of casein as the aqueous phase material, high–pressure homogenization with a microfluidizer (LM10, Microfluidizer, USA), adjustment of the pH with HCl, and the use of a Nano Spray Dryer (Laboratory Nano Spray Dryer, Techno Search Process & Systems, India) for encapsulation. In detail, a primary emulsion was obtained by mixing 18% (w/w) oil phase with 82% (w/w) of a 4% casein aqueous phase at pH 7 using a high–speed mixer (HG-15D, Witeg, Germany) for 30 s at the highest speed. This pre–emulsion was then subjected to two passes of high shear fluid homogenizer at room temperature (25°C) using a microfluidizer to reduce the droplet size to nanometer levels. The primary emulsion was then diluted, and the pH was adjusted with 0.3 N HCl to form secondary emulsions. The interfacial double layer was formed by diluting the secondary emulsions with the addition of maltodextrin solutions to increase the solids content. Finally, nanocapsules of mixtures of soursop, lemon and eucalyptus essential oil powders were obtained by dehydration of secondary nanoemulsions by spray drying using a Nano Spray Dryer.

*TABLE II*
**Ingredients and nutrient composition of the experimental diets**

| Ingredients | % |
|---|---|
| Corn | 62.34 |
| Soybean meal (48%) | 15.80 |
| Bran | 4.60 |
| Palm kernel meal | 4.60 |
| Calcium carbonate (0.85 – 2 mm) | 2.50 |
| Calcium carbonate (2 – 4 mm) | 7.50 |
| Dicalcium phosphate | 0.80 |
| Salt | 0.25 |
| Vegetable oil | 1.00 |
| Choline chloride | 0.02 |
| Lysine | 0.05 |
| Methionine | 0.08 |
| Enzyme complex | 0.01 |
| Sodium bicarbonate | 0.20 |
| Mycotoxin binder | 0.15 |
| Vitamin and mineral premix* | 0.10 |
| **Nutrient Composition** | |
| Crude protein (%) | 17.0 |
| Metabolizable energy ($Kcal·Kg^{-1}$) | 3150.8 |
| Phosphorus (%) | 0.38 |
| Calcium (%) | 4.36 |
| Methionine + Cysteine (%) | 0.70 |
| Methionine, Met (%) | 0.40 |
| Lysine, Lys (%) | 0.84 |

*Per kg contains 8.000.000 IU vitamin A, 250.000 IU vitamin D3, 12.000 IU vitamin D, 1,8 g vitamin K3, 1 g vitamin B1, 4 g vitamin B2, 2,5 g vitamin B6, 0,025 g vitaminB12, 10 g vitamin B5, 0,5 g vitamin B9, 25 g vitamin B3, 80 g manganese, 72 g zinc, 55 g iron, 10 g copper, 1 g iodine, 0,3 g selenium, and excipients. DM: Dry Matter.

### Laying performance and egg quality

Eggs were collected daily and egg production rate was recorded for a period of 21 to 30 weeks. During the experiment, egg weight and mass were recorded daily. Feed intake was recorded daily, and the feed conversion ratio rate was calculated. Each week, the live weight of the hens was taken, and the daily weight gain was calculated.

In the final week of the experiment, 30 eggs were randomly selected daily from each treatment for a total of 210 eggs. The eggs were used to determine fracture resistance, albumen height, haugh unit, yolk color, yolk height, yolk diameter, yolk index and shell thickness (Digital Egg Tester DET 6500, NABEL Co. Ltd., Kyoto, Japan).

### Oxidative stability

The methodologies described by Loyaga–Cortéz *et al.* [9] and Romero *et al.* [22] were used. Two grams (g) of egg yolk was homogenized with 8.0 mL of 5% trichloroacetic acid in a vortex (Heidolph Instruments GmbH & Co, Germany) at 770 G for 30 s. Then, it was centrifuged (Eppendorf, Centrifuge 5430R, Germany) at 4435 G for 15 min at 4°C 1.5 mL of supernatant was taken and added to 1.5 mL of 0.8% (w/v) aqueous 2-thiobarbituric acid (TBA) 0.8% (w/v). Finally, this mixture was incubated (BINDER GmbH, Model ED56, Germany) at 70°C for 30 min. The tubes were cooled, and the reading was recorded at 532 nm (Fisher Scientific, Microplate reader AccuSkan GO UV/Vis, USA).

### Statistical analysis

To test the effect of dietary treatment, data on indicators of egg quality and oxidative stability were analyzed by one–way ANOVA, as appropriate. Differences between means were evaluated by Tukey's multiple comparisons test. A $P \leq 0.05$ was considered statistically significant. Values are expressed as the mean ± standard error of the mean (SEM), and all statistical analyses were performed using GraphPad Prism software (San Diego, CA, EE. UU.).

## RESULTS AND DISCUSSION

### Laying Performance

According to TABLE III, egg production rate was significantly increased in the T4 group compared to the T2 and T3 groups ($P<0.05$), however, it was similar to the T0 and T1 groups. In addition, the T4 treatment obtained a higher value of egg mass than the control group ($P<0.05$) that received a non–supplemented diet. The feed conversion was significantly lower in T4 treatment compared to the control and the T2 and T3 treatments ($P<0.05$). Only the T1 group had a feed conversion rate that was statistically comparable to that of the T4 group. ($P>0.05$). The feed intake, weight gain, and egg weight were similar among all treatments ($P>0.05$). Essential oils have been shown to possess bioactive compounds with antibacterial, antifungal, antiparasitic and antiviral activities [23]. Previously, Xiao *et al.* [24] showed that 300 $mg·kg^{-1}$ of a mixture of carvacrol, thyme and cinnamaldehyde in the diet of laying hens did not significantly affect egg production rate, feed intake, egg weight and feed conversion ratio. This result does not agree with this present research, where significant differences were found in egg production rate and feed conversion ratio. This may be because nanoencapsulation increases the efficiency of production parameters by delivering the active compounds of the EO directly to the absorptive cells of the small intestine mucosa in a slow and controlled manner [25].

On the other hand, Eucalyptus powder has been reported to have positive effects on production parameters such as egg production rate, egg mass and feed conversion ratio in hens [26] as in quails [27], showing that the treatment with the highest concentration of eucalyptus EO has the best results. This could be attributed to the fact that the active compounds of eucalyptus essential oil have the ability to stimulate the secretion of digestive and pancreatic enzymes [28]. Likewise, this effect could be enhanced by mixing different essential oils [23].

### Egg quality

The effects of supplementation of dietary N–EOs combinations on egg quality indicators of laying hen eggs are shown in TABLE IV. Treatments T1 and T2 were significantly higher in eggshell thickness

*TABLE III*
**Effects of nanoencapsulated EO supplementation on productive performance**

| Indicators | Treatments | | | | | *P* value[2] | SEM[1] |
|---|---|---|---|---|---|---|---|
| | T0 | T1 | T2 | T3 | T4 | | |
| Egg production rate (%) | 76.78[ab] | 77.35[ab] | 72.44[b] | 75.37[b] | 81.13[a] | 0.004 | 0.825 |
| Feed intake (hen·day$^{-1}$·g$^{-1}$) | 109.19 | 109.22 | 109.17 | 109.19 | 109.17 | 1.000 | 0.148 |
| Egg weight (g) | 59.58 | 62.04 | 60.39 | 62.08 | 61.76 | 0.106 | 0.375 |
| Feed conversion ratio | 2.40[ab] | 2.32[bc] | 2.51[a] | 2.34[b] | 2.19[c] | 0.000 | 0.027 |
| Egg mass (g·d$^{-1}$) | 55.76[bc] | 57.98[ab] | 53.73[c] | 56.78[abc] | 60.09[a] | 0.001 | 0.598 |
| Weight gain (g·d$^{-1}$) | 2.17 | 3.86 | 2.88 | 2.89 | 3.68 | 0.385 | 0.293 |

Treatments include – Control group (T0): received a conventional diet. Experimental groups (T1-T4) received a diet supplemented with N–EO from soursop (S), lemon (L) and eucalyptus (E) in different proportions (%).: T1 (S:33.4%, L:33.3% y E:33,3%), T2 (S:50%, L:25% y E:25%), T3 (S:25%, L:50% y E:25%) y T4: (S:25%, L:25% y E:50%). [a,b,c]Means with different superscripts within the columns differ significantly ($P$<0.05). [1]SEM, standard error of the means. [2]*P*–value, significance associated with dietary treatment

*TABLE IV*
**Effects of nanoencapsulated EO supplementation on egg quality**

| Indicators | Treatments | | | | | *P* value[2] | SEM[1] |
|---|---|---|---|---|---|---|---|
| | T0 | T1 | T2 | T3 | T4 | | |
| Fracture resistance (kgf) | 4.34 | 4.88 | 4.85 | 4.89 | 4.92 | 0.150 | 0.084 |
| Albumen height (mm) | 8.98 | 8.50 | 8.79 | 8.70 | 9.02 | 0.665 | 0.118 |
| Haugh unit | 94.26 | 93.16 | 92.42 | 91.71 | 93.33 | 0.708 | 0.758 |
| Yolk color | 6.29 | 6.02 | 6.18 | 5.95 | 6.02 | 0.395 | 0.060 |
| Yolk height (mm) | 18.02 | 18.01 | 18.05 | 18.59 | 18.27 | 0.295 | 0.099 |
| Yolk diameter (mm) | 39.76 | 41.82 | 40.42 | 40.37 | 39.81 | 0.601 | 0.429 |
| Yolk index | 0.47 | 0.44 | 0.46 | 0.46 | 0.46 | 0.223 | 0.010 |
| Shell thickness (mm) | 0.39[b] | 0.42[a] | 0.42[a] | 0.40[b] | 0.39[b] | 0.003 | 0.003 |

Treatments include – Control group (T0): received a conventional diet. Experimental groups (T1-T4) received a diet supplemented with N–EO from soursop (S), lemon (L) and eucalyptus (E) in different proportions (%).: T1 (S:33.4%, L:33.3% y E:33,3%), T2 (S:50%, L:25% y E:25%), T3 (S:25%, L:50% y E:25%) y T4: (S:25%, L:25% y E:50%). [a,b]Means with different superscripts within the columns differ significantly ($P$<0,05). [1]SEM, standard error of the means. [2]*P*–value, significance associated with dietary treatment

than treatments T0, T3 and T4 ($P$<0.05). The supplementation of different types of oil has increased shell thickness, except for the treatments with a higher proportion of lemon and eucalyptus oil. It has been reported that supplementation for 8 weeks of up to 3 g of Eucalyptus powder per kilogram of diet in laying hens has no influence on shell thickness [26]. Also, supplementation of EO blends of oregano, bay leaf oil, sage leaf oil, myrtle leaf oil, fennel seed oil and citrus peel oil in laying hens has not shown significant effects on shell thickness [29, 30, 31].

Based on our findings, equal proportions of non–aromatic essential oils enhance eggshell thickness, though this effect is also observed with a higher proportion of essential oil from soursop seed. To gain a better understanding of the metabolism of essential oils, it is essential to identify their chemical composition and primary deposition sites in the birds' bodies. This would help elucidate how nanoencapsulated essential oils (N–EOs) or their metabolites contribute to the observed benefits [32].

### Oxidative stability

In addition to the production parameters, the oxidative stability of egg yolk was studied in hens that received a diet supplemented with the N–EOs mixture. As seen in FIG. 1, the control group (T0) that received a standard diet showed the highest levels of malondiandehyde (MDA), a marker of lipid peroxidation and oxidative stress [33]. The T1 group that received equal parts N–EOs supplementation (33.3%) showed a tendency to reduce MDA levels compared to the control group (T1: 0.69 ± 0.01 nmol·L$^{-1}$ vs T0 (control): 1.2 ± 0,23 nmol·L$^{-1}$). This finding is consistent with a previous study in which was reported that T1 treatment also reduced lipid peroxidation in broiler thigh meat [18], however, in the present study this difference did not reach the expected statistical significance ($P$>0.05). Similarly and following the same trend as the T1 group, the T4 group presented lower MDA levels compared to the control (0.78 ± 0.05 nmol·L$^{-1}$; $P$>0.05).

It is widely known that poultry products are particularly prone to oxidative deterioration due to their high concentrations of polyunsaturated fatty acids, and EOs are a good alternative in

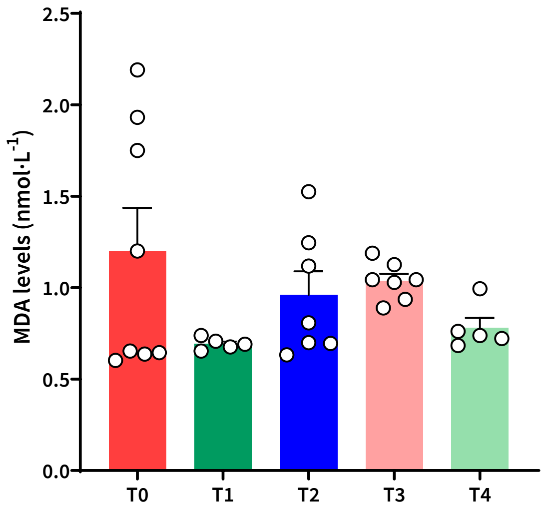


**FIGURE 1. Effect of nanoencapsulated EOs supplementation on oxidative stability of egg yolk. Control group (T0): received a conventional diet. The experimental groups (T1-T4) received a diet supplemented with N–EOs from soursop (S), lemon (L) and eucalyptus (E) in different proportions (%): T1 (S:33.33%, L:33.33% and E:33.33%), T2 (S:50%, L:25% and E:25%), T3 (S:25%, L:50% and E:25%) and T4: (S:25%, L:25% and E:50%). Each circle represents an individual value (n)**

this context [32]. In this study, the addition of N–EOs to the diet maintained a tendency to reduce the concentration of MDA in egg yolk of laying hens. EO from *Eucalyptus globulus* has been described to reduce serum MDA levels in broiler chickens [28], probably due to its radical scavenging properties and also the ability to inhibit lipid peroxidation [34]. The main component of eucalyptus EOs is the terpene 1,8-cineole, also known as eucalyptol [34], has been shown to improve total antioxidant activity in broilers by reducing MDA levels and increasing total superoxide dismutase enzyme activity [35].

In addition, compounds such as δ–cadinene, α–muurolene, β–caryophyllene, epic–α–cadinol and α–cadinol present in *Annona muricata* L. (Soursop) EO [36], as well as, d–Limonene and β–Pinene present in *Citrus limon* EO [37] could contribute to this antioxidant effect. The individual or synergistic effects of all these compounds present in each of the EOs used have a tendency to reduce of lipid peroxidation in egg yolk. Fortification of foods with bioactive compounds such as EOs serves to improve the quality characteristics of derived products and protects consumers against oxidation [38].

## CONCLUSION

In conclusion, the findings in this study indicate that dietary supplementation with nanoencapsulated essential oil (N–EO) blends significantly improves feed conversion efficiency, boosts egg production rates, and enhances eggshell thickness in laying hens. Furthermore, N–EOs contribute to reduced lipid peroxidation in egg yolk, although this reduction was not significant. These results suggest that incorporating N–EOs into poultry diets could be a valuable strategy for optimizing the health and productivity of laying hens.


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### Conflict of interests

The authors declare no conflicts of interest.


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