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Effect of annatto and alfalfa on egg yolk pigmentation in Creole hens

Rodríguez-Ortega, Leodan T.[1]**; Segovia-Azpeitia, Sergio**[1]**; Hernández-Guzmán Filogonio J.**[2]**; Rodríguez-Ortega, Alejandro**[1*]**; Pro-Martínez Arturo**[3]**; Estrada-Hernández, María de la Luz**[1]

[1] Universidad Politécnica de Francisco I. Madero, Tepatepec, Hidalgo, México, C.P. 42660.
[2] Departamento de Ingeniería Agroindustrial. Universidad Autónoma Chapingo, Carretera México-Texcoco km. 38.5, Chapingo, Estado de México, C.P. 56230.
[3] Colegio de Postgraduados Campus Montecillo, Montecillo, Texcoco, Estado de México, México, C.P. 56264.
* Correspondence: arodriguez@upfim.edu.mx

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ABSTRACT

Objective: To evaluate the effect of an alfalfa- and annatto-enriched diet on the external and internal characteristics of eggs laid by Creole hens.

Design/Methodology: A set of 22 Creole hens was divided into two treatment groups. The first group (n=11 females and one male) was fed alfalfa, while the second (n=11 females and one male) consumed alfalfa and annatto. For the study, 116 eggs (alfalfa=58 and annatto=58) were collected and stored in refrigeration until further analysis. The data encompassed external physical characteristics (egg weight, length, width, and shell thickness) and internal physical characteristics (yolk weight, white weight, and yolk pigmentation). Yolk color was evaluated as per the DSM color spectrum.

Study Limitations/implications: Backyard poultry farming does not use artificial pigments to color egg yolks due to their high cost and low availability. Annatto and alfalfa are low-cost, handy alternatives.

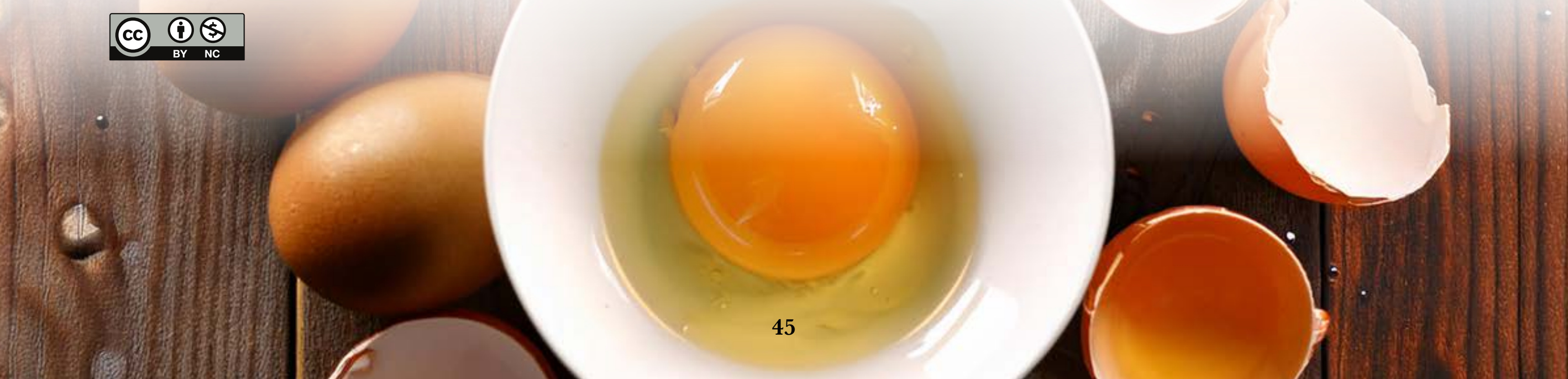
Results: Adding fresh alfalfa or annatto to the Creole hens' diet did not significantly affect ($P>0.05$) egg weight, length, or width, nor ($P>0.05$) eggshell thickness. Yolk and white weight were not affected ($P>0.05$) either. However, supplementing the hens' diet with annatto intensified yolk pigmentation.

Conclusion: Adding annatto to the Creole hens' diet intensifies yolk color without affecting the external or internal physical characteristics of eggs. Annatto (known in Mexico as achiote) is a natural pigment with no harmful effects on human health compared to synthetic alternatives. It is also inexpensive and easily accessible.

Keywords: Egg, Yolk, Color, Natural pigment.

INTRODUCTION

Chicken eggs are considered one of the most complete foods for humans, since they offer a well-balanced source of protein. The most abundant proteins in egg whites are ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), lysozyme (3.5%), and ovomucin (3.5%) (Nurliyani *et al.*, 2023). The yolk —separated from the white by the vitelline membrane— contains primarily lipids (Benedé and Molina, 2020). Due to their high nutrient digestibility and low cost, eggs are one of the most consumed animal products in Mexico (López-Sobaler *et al.*, 2017; Mendoza *et al.*, 2016).



The first physical characteristic of eggs noticed by consumers is eggshell color, with shades ranging from white and brown to olive green and blue, depending on the chicken breed (Rodríguez-Ortega *et al.*, 2021). Yolk color is another key feature that influences consumer preferences and approval. Consumers favor eggs with deep yellow yolks, while pale- or light-yellow yolks are perceived as lower in quality and tend to be rejected. Yolk color is directly linked to the carotenoid content in the hens' diet (Shevchenko *et al.*, 2021).

Carotenoids —natural pigments found in seeds, fruits, plants, and fungi— are divided into two groups: carotenes and xanthophylls. The most prevalent carotenes are α-carotene, β-carotene, and lycopene. Xanthophylls include β-cryptoxanthin, lutein, zeaxanthin, astaxanthin, fucoxanthin, and peridinin (Maoka, 2019). Birds and mammals cannot synthesize carotenoids —only plant tissues can (Jaswir *et al.*, 2011). Therefore, hens must obtain carotenoids through their diet. In hen egg yolks, the main carotenoids are lutein and zeaxanthin (Zaheer, 2017), along with small amounts of β-cryptoxanthin, while β-carotene is found but not as a major pigment.

The carotenoids in yolks are highly bioavailable due to their lipid solubility, rendering eggs an excellent source of carotenoids for human dietary enrichment (Kavtarashvili *et al.*, 2019). Eggs enriched with xanthophylls can serve as a functional source of carotenoids in human diets. Annatto or *Bixa orellana* L. (family Bixaceae), known as achiote in Mexico, is a carotenoid-rich plant, particularly high in bixin and norbixin, which provide its orange to red color (Rivera-Madrid *et al.*, 2016). In Mexico, annatto is used as a natural colorant in foods like bread, beverages, regional sweets, and marinated meats such as enchilada or al pastor. Annatto is non-toxic (Scotter, 2009) and has antioxidant and anti-inflammatory properties (Giridhar *et al.*, 2014).

Alfalfa is a highly digestible forage with low cellulose content, making it a suitable alternative feed for backyard poultry. Vera-Vázquez *et al.* (2021) note that this legume is rich in carotenoids, with a total carotenoid content of 257 mg/g MS^{-1}. In small-scale poultry farming, the use of artificial pigments to enhance yolk color is scarce because of high costs and limited availability. However, annatto offers a cost-effective and accessible alternative.

This study aims to evaluate the effect of fresh alfalfa and annatto supplementation in Creole hens' diet on the external and internal physical properties of eggs.

MATERIALS AND METHODS

The study was conducted at the Poultry Unit of the Universidad Politécnica de Francisco I. Madero, located in Tepatepec, Hidalgo, Mexico, at an altitude of 1,900 masl. The site has a temperate-cold climate with an average annual temperature of 17 °C and an average annual precipitation of 540 mm (Rodríguez-Ortega *et al.*, 2020).

Experimental Design

A completely randomized design was applied in this study, using 116 eggs —58 from the alfalfa group and 58 from the annatto group. The eggs were collected from 22 hens divided into two treatment groups. One group was fed commercial feed supplemented with minced fresh alfalfa (n=11 females plus one male), while the other group received the same feed supplemented with both alfalfa and annatto (n=11 females plus one male).

Bird Feeding

The commercial feed met the physiological requirements established by the National Research Council (1994). Each hen received 20 g of fresh alfalfa daily. For the second group, a commercial annatto paste was used to provide 10.41 g of annatto per hen per day. A solution was prepared by dissolving 25 g of annatto paste in 100 ml of water and mixing it with 240 g of fresh alfalfa (20 g of alfalfa/hen/day for 12 hens per treatment). The mixture was provided in free-access feeders measuring 1 m in length, 30 cm in width, and 15 cm in height.

External Physical Characteristics

The collected eggs were stored under refrigeration until further analysis. Egg weight, length, width, and eggshell thickness were measured using a digital caliper (HER-411 model, STEREN, Mexico) with a measuring range of 0 to 150 mm and a resolution of 0.1 mm.

Internal Physical Characteristics and Yolk Color

Yolk, egg white, and eggshell weights were measured using a digital scale with a 200 g capacity and 0.01 g resolution (MH-200 model, MKS Tools, China). Yolk pigmentation was visually assessed by comparing it with the DSM YolkFan (Figure 1). Three evaluators reached a consensus on the closest matching color. The color scale ranged from 1 (light yellow) to 15 (closer to orange).

Statistical Analysis

The Proc Univariate Normal procedure was employed to assess the normality of the data through the Shapiro-Wilk test (Alonso and Montenegro, 2015) for the following variables:

Figure 1. DSM YolkFan. The DSM color fan provides a practical and cost-effective method for evaluating yolk coloration. Color intensity can be considered an indicator of good health, performance, and well-being in hens.

egg weight, yolk weight, egg white weight, egg length, egg width, eggshell thickness, and yolk color. Statistical analyses were conducted using the SAS software (version 2011). The data were analyzed using the PROC GLM (General Linear Model) procedure, and the means were compared using Tukey's test. Yolk color was further examined using GLM and the PROC FREQ procedure. The correlations between egg weight and both egg white weight and eggshell weight were determined using the PROC CORR procedure.

RESULTS AND DISCUSSION

Egg weight is an important characteristic for consumers, who tend to prefer larger eggs. In this study, egg weight was not affected (P>0.05) by adding annatto or alfalfa to the hens' diet (62.32 g *vs.* 63.19 g; Table 1). External morphological characteristics such as egg weight, length, width, color, and eggshell thickness can be influenced by the age of the hens, their genetics, and their nutritional management. Kocevski *et al.* (2011) reported that egg weight increases with the age of hens, while Juárez-Cataracheа *et al.* (2010) found that Creole hens tend to lay smaller eggs compared to commercial breeds. Segura *et al.* (2007) noted that the weight of the first egg laid by Creole hens was 45.3 g, with the weight increasing as hens aged. Segura (2021) reported annatto in drinking water did not affect egg weight in Issa Brown hens. Rodríguez-Molano *et al.* (2023) observed that feeding fresh alfalfa did not affect egg weight in Babcock Brown hens (56.63 g *vs.* 55.62 g).

In this study, egg length and width were not affected by adding annatto or alfalfa to the hens' diet (P<0.05; Table 1). Illescas-Cobos *et al.* (2022) report the following morphometric standard for eggs laid by Creole hens: average weight=55.96 g, length=5.72 cm, and width=4.18 cm. The results in this study are similar to the aforementioned standard.

Egg color and weight, along with eggshell thickness are crucial for consumers and the poultry industry. Due to their hardness and thickness, eggshells protect the egg whites and yolks inside them, so a reduction in eggshell weight leads to breaking or cracking which, in turn, increase losses. In this study, eggshell weight was not affected by feeding hens annatto or alfalfa (Figure 2). The eggshell weights observed (Figure 2) align with the findings of Kibala *et al.* (2018), who reported an average eggshell weight of 7.30±0.6 g in Rhode Island Red hens.

Egg white and yolk weights were not affected (P>0.05) by the dietary treatments (Table 2). The egg white contains highly digestible proteins such as ovalbumin, ovotransferrin,

Table 1. Weight, length, and width of eggs of Creole hens fed with annatto or alfalfa.

Treatment	Weight (g)	Length (cm)	Width (cm)
Annatto	62.32[a]	5.92[a]	4.41[a]
Alfalfa	63.19[a]	5.92[a]	4.39[a]
Standard error	0.7326	0.038	0.032
P-valor			
Shapiro-Wilk	0.0222	<0.0001	<0.0001
ANOVA	0.4059	0.9557	0.7150

Matching letters in each column indicate that there are no significant differences between treatments, according to Tukey's test (P>0.05).

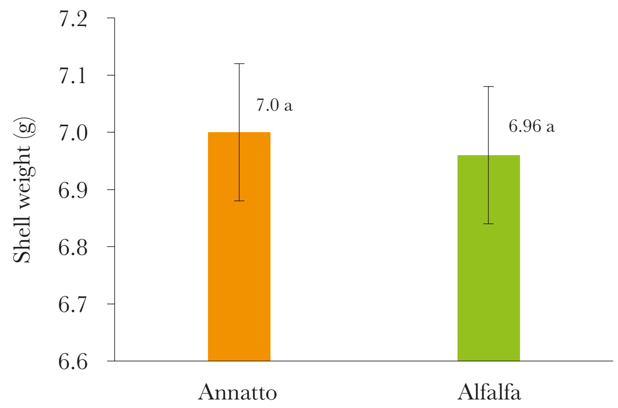


Figure 2. Eggshell weight in Creole hens fed with annatto or alfalfa. The Shapiro-Wilk test was used to determine data normality (P≤0.0001). Matching letters indicate that there are no significant differences between treatments (ANOVA; P=0.0001), according to Tukey's test (P<0.05).

Table 2. Egg white and yolk weight in Creole hens fed with annatto or alfalfa.

Treatment	Egg white weigth	Yolk weigth
Annatto	35.16 [a]	20.15 [a]
Alfalfa	35.39 [a]	20.83 [a]
Standard error	0.62	0.24
P-valor		
Shapiro-Wilk	0.0009	0.0007
ANOVA	0.8024	0.0576

The Shapiro-Wilk test was used to determine data normality. Matching letters in each column indicate that there are no significant differences between treatments, according to Tukey's test (P>0.05).

ovomucoid, ovomucin, and lysozyme (Lomakina and Míková, 2006), with various applications in the pharmaceutical industry. The yolk is made of water, lipids, proteins, minerals, and vitamins (Abeyrathne *et al.*, 2022).

Yolk pigmentation was evaluated using the DSM YolkFan, a practical, reliable, and economical tool. This method is particularly useful in backyard poultry farming, where financial resources may be limited for purchasing advanced equipment like the Konica Minolta colorimeter. Annatto supplementation resulted in significantly heightened (P<0.05) yolk color (Figure 3) in eggs laid by Creole hens. Rojas *et al.* (2015) also observed a deeper yolk pigmentation in laying hens fed with canthaxanthin and annatto extract.

Yolk color is a key factor for consumer preference. A deeper yolk color indicates higher amounts of carotenoids, which is beneficial for human health. Segura (2021) found that adding 200 g of annatto to 4000 ml of drinking water for specialized Issa Brown laying hens did not affect yolk color. According to the Roche color fan, the treatments reached a scale of four, indicating low pigmentation. Hansen *et al.* (2015) assessed the addition of up to 2000 ppm of annatto to the diet of Hy-Line W36 hens and reported no significant changes in yolk coloration. However, in the present study, the addition of annatto resulted in an intensified yolk color, reaching high pigmentation levels. The highest percentages were

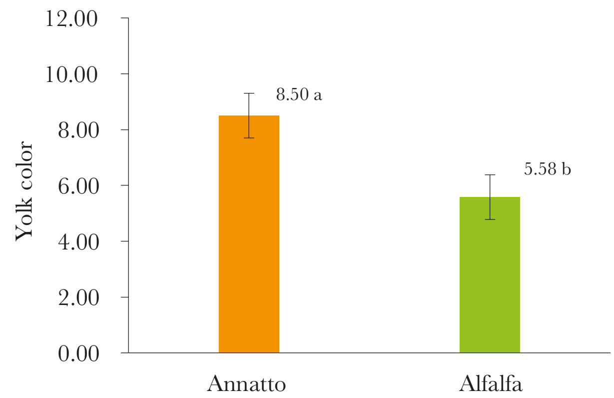


Figure 3. Yolk color in eggs laid by Creole hens fed with annatto or alfalfa.
The Shapiro-Wilk test was used to determine data normality (P=0.0016). Different letters indicate significant differences between treatments (P=0.0001), according to Tukey's test (P<0.05).

found on scales 9 and 10 (intense yellow, Figure 1), a much deeper color compared to the yolk of eggs laid by alfalfa-fed hens (Table 3). Some yolks reached tones on scales 12 and 15, showing orange hues (Figure 1). In comparison, fresh-alfalfa-supplemented feed did not significantly enhance yolk color (Figure 3 and Table 3), though it remains an accessible option for backyard poultry producers.

In this study, the main carotenoids responsible for yolk pigmentation were bixine and norbixine, which are found in annatto. Annatto or achiote is a natural pigment with no harmful effects on human health. Ofosu *et al.* (2010) reported that bixine (chemical formula C25H30O4) is oil-soluble, while norbixine (chemical formula C24H28O4) is water-soluble.

Table 3. Yolk color percentages in eggs of Creole hens fed with annatto or alfalfa, according to the scales of the DSM Yolk Fan.

Treatment	Color scale										
	3	4	5	6	7	8	9	10	11	12	15
Alfalfa	8.9	30.4	16.1	19.6	7.1	5.4	7.1	3.6	1.8		
Annatto			5.0	6.7	21.7	15.0	23.3	16.7	5.0	5.0	1.7

CONCLUSION

Including annatto in the diet of Creole hens intensifies yolk color without affecting the external or internal physical characteristics of eggs, such as weight, width, and length, weight of the egg white or yolk. Using annatto to feed Creole hens is an effective alternative for yolk pigmentation. Unlike synthetic alternatives, this natural pigment does not have harmful effects on human health. Moreover, it is cost-effective and accessible to small-scale producers.

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