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Article

Tithonia diversifolia meal in diets for first-cycle laying hens and its effect on egg yolk color

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Abstract:

Consumers in Mexico expect egg yolks to have a certain color. Attaining this color in intensive production systems requires addition of natural pigments to laying hen diets. Feed is the largest input cost in egg production and added pigments increase costs. Use of alternative natural pigment sources, such as tree marigold *Tithonia diversifolia*, can help to control costs. An evaluation was done of how addition of *T. diversifolia* meal (TDM), as a yellow pigment source, to diets for first-cycle laying hens affected productive variables and egg yolk color. Over a six-week experimental period meal made from *T. diversifolia* leaves and petioles was added to poultry diets at four percentages (1.77, 5, 10 and 15 %). A total of 240 chickens were distributed in five treatments (Control, and 1.77 %, 5 %, 1 0% and 15 % TDM) tested in two trials: Trial 1 (weeks 1-3), no red pigment added; Trial 2 (weeks 4-6), red pigment added. Measured variables were laying percentage, egg weight and mass, and feed intake and conversion. At the end of each trial, 20 eggs/treatment were collected for quantification of xanthophylls by HPLC, and measurement of yolk color based on DSM fan colors and reflectance colorimetry. The design was completely random and differences between means were identified with a Tukey test. Egg weight and feed conversion did not differ between treatments (P>0.05). Laying percentage and egg mass in the 10 and 15% TDM treatments, and feed intake in the 15% TDM treatment were lower than in the Control (P<0.05). Yolk color was most intense in the 10 and 15% TDM treatments in both trials. *Tithonia diversifolia* meal is a promising alternative natural source of yellow pigment in laying hen diets at up to a 10% inclusion level, and does not affect productive variables.

Key words: *Tithonia diversifolia*, Laying hens, Eggs, Productive variables, Pigment.

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Introduction

Mexico's poultry industry currently produces more than 5 million tons of products (e.g. eggs, chicken and turkey) annually to meet national demand[1]. This represents 63.8 % of the country's total annual livestock production, eggs alone accounting for 29 %. Projected egg production for 2018 is 2.806 million tons. Feed costs are 60 to 70 % of the input costs in poultry production[1].

Eggs are an excellent food due to their high biological value, ease of handling, various preparation options, especially in combination with other foods, and their accessible cost. Consumers expect egg yolks to have a certain color. In intensive production systems laying hens are not exposed to natural pigments through their feed, meaning these must be added for egg yolks to have the expected color. This represents a substantial increase in input costs and affects final product price[2,3]. Natural source yellow and red pigments, including carotenoids, are added to feed for laying hens[2,3]. Research is ongoing into new sources of natural pigments that provide carotenoids, are easy to use, and have minimal impact on production costs.

Tithonia diversifolia, known as tree marigold among many other common names, is native to Central America and Mexico. Its almost 15,000 species can be found worldwide in tropical and subtropical areas(4). It grows quickly, even in unfavorable conditions such as roadsides, and multiplies easily. In soils this plant is known to improve nutrient recycling, prevent erosion, reduce the effects of animal trampling and produce high biomass productivity without agrochemical inputs(5,6). It is a multipurpose plant that can be used as a living fence, green manure, grazing forage in silvopastoral livestock systems, and cut forage in poultry and ruminant systems. *Tithonia diversifolia* has reported values of 23.0 g/100 g dry matter, 14.8-28.7 g/100 g crude protein, 21.4 g/100 g ash, and 78.6 g/100 g organic matter(7). Of note is its high carotenoid content, suggesting its use as a pigment source in egg production systems; indeed its inclusion in feed for laying hens at 15% results in good egg yolk color(8). The present study objective was to evaluate the effect of different inclusion levels of *Tithonia diversifolia* meal in diets for first-cycle laying hens on yolk color and egg quality.

Material and methods

Study area, sampling and pigment quantification

Tithonia diversifolia was collected at the Veterinary Medicine and Zootechny Academic Unit, Autonomous University of Nayarit, in Compostela, Nayarit, Mexico. Regional climate is tropical, with summers much rainier than winters, a 22.4 °C average annual temperature and 1,060 mm approximate average annual rainfall(9).

Leaves and petioles of *T. diversifolia* were harvested manually after 60 d regrowth (644.5 kg/fresh). All foreign matter was removed from the material, which was pre-dried in shade at the harvest site. The pre-dried leaves and petioles were stored in black plastic bags and transported to the Dr. Fernando Pérez-Gil Romo Department of Animal Nutrition, Salvador Zubirán National Institute of Medical Sciences and Nutrition. Here the material was oven dried at 60 °C/24 h. Once dried it was ground in a hammer mill with a 1 mm mesh to produce *Tithonia diversifolia* meal (TDM) and stored for later analysis.

Pigment content in the TDM was quantified by high-resolution HPLC (Industrias VEPINSA, S.A. de C.V.; Research and Development Office)[10].

Diets and experimental design

All feed trials were done at the Poultry Production Teaching, Research and Extension Center (Centro de Enseñanza, Investigación y Extensión en Producción Avícola - CEIEPAv) of the Faculty of Veterinary Medicine and Zootechny (FMVZ), National Autonomous University of Mexico (Universidad Nacional Autónoma de México – UNAM). All experimental procedures involving animals were reviewed and approved by the Institutional Subcommittee on Experimental Animal Care and Use (FMVZ, UNAM), and met applicable federal regulations[11].

Experimental animals were 240 Bovans White line laying hens. The diets were formulated to meet animal nutritional needs by production phase using the Allix2 ver. 5.37.1 software. Five treatments were tested using a completely random design with four replicates per treatment and twelve birds per replicate: 1) Control, 15 ppm yellow pigment; 2) 1.77% TDM + 15 ppm total xanthophylls; 3) 5% TDM + 42.5 ppm total xanthophylls; 4) 10% TDM + 85 ppm total xanthophylls; and 5) 15% TDM + 127.5 ppm total xanthophylls. Water and feed were freely available. Two trials were run: weeks 1-3, no red pigment in diets; and weeks 4-6, red pigment included in diets (Tables 1 and 2).

Table 1: Experimental diets in first trial (weeks 1-3) using *Tithonia diversifolia* meal and no red pigment (kg)

		***Tithonia diversifolia* meal (%)**			
Ingredients	**Control**	**1.77**	**5**	**10**	**15**
Sorghum	660.500	647.950	621.800	539.921	456.946
Soy paste	221.390	213.960	202.402	222.920	242.914
Calcium carbonate	101.791	100.435	98.000	94.033	90.112
TDM	0.000	17.700	50.000	100.000	150.000
Calcium phosphate	4.568	4.659	4.553	4.361	4.183
Salt	3.026	3.033	3.046	3.057	3.068
Vit + min premix[1]	2.400	2.400	2.400	2.400	2.400
DL-Methionine (84%)	2.289	2.327	2.401	2.042	1.704
Vegetable oil	1.482	5.552	13.277	30.391	47.798
L-Lysine HCl	1.179	1.209	1.246	0.000	0.000
Choline chloride (60%)	0.500	0.500	0.500	0.500	0.500
Yellow pigment[2]	0.500	0.000	0.000	0.000	0.000
Antioxidant[3]	0.150	0.150	0.150	0.150	0.150
Bambermycin	0.125	0.125	0.125	0.125	0.125
Phytase[4]	0.100	0.100	0.100	0.100	0.100
Total	1000	1000	1000	1000	1000
Calculated analysis					
Metabolizable energy, kcal/kg	2800	2800	2800	2800	2800
Crude protein, %	17.400	17.400	17.400	18.970	20.480
Total Met + Cys, %	0.730	0.730	0.730	0.730	0.730
Total lysine, %	0.860	0.860	0.860	0.866	0.967
Total threonine, %	0.622	0.623	0.625	0.691	0.754
Total tryptophan, %	0.205	0.199	0.189	0.196	0.201
Crude fiber, %	2.446	2.583	2.831	3.331	3.824
Total calcium, %	4.100	4.100	4.100	4.100	4.100
Available phosphorous, %	0.420	0.420	0.420	0.420	0.420
Sodium, %	0.180	0.180	0.180	0.180	0.180

[1]Content per kilogram: Vit. A, 4.0 MUI; Vit. D_3, 666,666.7 UI; Vit. E, 10,000.0 UI; Rovimix HyD 5 kg: Vit. K_3, 1.67 g; Vit. B_1, 0.83 g; Vit. B_2, 2.33 g; Vit. B_6, 1.17 g; Vit. B_{12}, 6.666.67 mg; niacin, 10 g; D-pantothenic acid, 3.33 g; folic acid, 0.33 g; biotin, 33.33 mg; choline, 100 g; Fe, 20 g; Zn, 26.67 g; Mg, 36.67 g; Cu, 5 g; I, 0.33 g; Se, 0.1 g.

[2]Florafil 93 Powder (Vepinsa): 30 g/kg (minimum) total xanthophylls.

[3] BHA, 1.2%; BHT, 9.0%; Ethoxyquin, 4.8%; chelating agents, 10.0%.

[4]Quantum Blue 5000 FTU/kg derived from *E. coli*.

Table 2: Experimental diets in second trial (weeks 4-6) using *Tithonia diversifolia* meal, with added red pigment (kg).

		***Tithonia diversifolia* meal (%)**			
Ingredients	**Control**	**1.77**	**5**	**10**	**15**
Sorghum	660.090	647.550	621.400	539.721	456.936
Soy paste	221.000	213.860	202.002	222.720	242.414
Calcium carbonate	101.791	100.435	98.000	94.033	90.112
TDM	0.000	17.700	50.000	100.000	150.000
Calcium phosphate	4.568	4.559	4.553	4.361	4.183
Salt	3.026	3.033	3.046	3.057	3.068
Vit + min premix[1]	2.400	2.400	2.400	2.400	2.400
DL-Methionine (84%)	2.289	2.327	2.401	2.042	1.704
Vegetable oil	1.482	5.252	13.277	29.991	47.508
L-Lysine HCl	1.179	1.209	1.246	0.000	0.000
Choline chloride (60%)	0.500	0.500	0.500	0.500	0.500
Red pigment[2]	0.800	0.800	0.800	0.800	0.800
Yellow pigment[3]	0.500	0.000	0.000	0.000	0.000
Antioxidant[4]	0.150	0.150	0.150	0.150	0.150
Bambermycin	0.125	0.125	0.125	0.125	0.125
Phytase[5]	0.100	0.100	0.100	0.100	0.100
Total	1000	1000	1000	1000	1000
Calculated analysis					
Metabolizable energy, kcal/kg	2800	2800	2800	2800	2800
Crude protein, %	17.400	17.400	17.400	18.970	20.480
Total Met + Cys, %	0.730	0.730	0.730	0.730	0.730
Total lysine, %	0.860	0.860	0.860	0.866	0.967
Total threonine, %	0.622	0.623	0.625	0.691	0.754
Total tryptophan, %	0.205	0.199	0.189	0.196	0.201
Crude fiber, %	2.446	2.583	2.831	3.331	3.824
Total calcium, %	4.100	4.100	4.100	4.100	4.100
Available phosphorous, %	0.420	0.420	0.420	0.420	0.420
Sodium, %	0.180	0.180	0.180	0.180	0.180

[1]Content per kilogram: Vit. A, 4.0 MUI; Vit. D_3, 666,666.7 UI; Vit. E, 10,000.0 UI; Rovimix HyD 5 kg: Vit. K_3, 1.67 g; Vit. B_1, 0.83 g; Vit. B_2, 2.33 g; Vit. B_6, 1.17 g; Vit. B_{12}, 6.666.67 mg; niacin, 10 g; D-pantothenic acid, 3.33 g; folic acid, 0.33 g; biotin, 33.33 mg; choline, 100 g; Fe, 20 g; Zn, 26.67 g; Mg, 36.67 g; Cu, 5 g; I, 0.33 g; Se, 0.1 g.

[2]Avired 5 g/kg (minimum) xanthophylls from *Capsicum annum*.

[3]Florafil 93 Powder (Vepinsa): 30 g/kg (minimum) total xanthophylls.

[4]BHA, 1.2%; BHT, 9.0%; Ethoxyquin, 4.8%; chelating agents, 10.0%.

[5]Quantum Blue 5000 FTU/kg derived from *E. coli.*

During the six-week experimental period weekly records were kept of laying percentage, egg weight and mass, and feed intake and feed conversion. At the end of trial 1 (week 3) and trial 2 (week 6), 20 eggs were collected per treatment and yolk color measured with a TSS QCC Yolk Color automated device (Technical Service and Supplies, Inc., England, UK). Readings were transformed to absolute values of the DSM color fan, which range from 1 (light yellow) to 15 (yellow-orange). At the same time, eight eggs per trial were collected and egg yolk pigments quantified by HPLC[10]. Yolk color was also measured by refraction colorimetry applying a three-dimensional definition scale based on the CIE system, which quantifies luminosity (L*), yellow hue (b*) and red hue (a*).

Statistical analysis

A completely random design was used with the model[12]:

$Y_{ij} = \mu + T_i + e_i(_j)$

i = 1, 2, 3, 4 and 5 j = 1, 2, 3 and 4

Where:

Y_{ij} = Response variable (laying percentage, feed intake/bird/day (g), egg weight (g), egg mass/day (g), feed conversion (kg:kg), yolk color and pigment quantification;

μ = General mean;

T_i = Effect of i-th treatment;

$e_i(_j)$ = Experimental error.

Differences between the means were identified with a Tukey test using a 0.05 significance level, and applied with the SPSS for Windows ver. 21.0 software package. Box-Cox transformations were applied to create variance homogeneity by transforming the pigment quantification variables (i.e. total carotenoids and lutein)[12]:

$$\text{Total carotenoids} = \frac{(Total\ carotenoids\ ug^{-0.04})-1}{-0.0001554438364}$$

$$\text{Lutein} = \frac{(Lutein\ ug^{-0.04})-1}{-0.0002864746461}$$

Results

Pigment quantification of the TDM showed the majority component to be lutein (50.67 %), followed by zeaxanthin (0.65 %) and total carotenoids (0.92 %) (Table 3). Neither egg weight nor feed conversion were affected (P>0.05) by TDM inclusion levels during the six-week (42-d) experimental period (Table 4). Laying percentage was lower in the 15% TDM treatment (89.8 %) versus the Control and the other inclusion levels. Feed intake/d decreased by an average of 5 g in the 15% TDM treatment versus the other treatments (P<0.05). Egg mass was lowest (P<0.05) in the 15% TDM treatment (52.5 g), higher in the 10% TDM treatment (55.1 g), and did not differ between the 1.77% TDM (55.9 g) and 5% TDM treatments (56.3 g) and the Control (56 g).

Table 3: *Tithonia diversifolia* meal pigment composition

Pigments (g/kg)	Wet base	Dry base
Total carotenoids	0.85	0.92
Carotene esters	28.10	30.49
β-cryptoxanthin	5.90	6.40
Trans-lutein	46.70	50.67
Trans-zeaxanthin	0.60	0.65
Epoxy-trans-lutein	1.80	1.95

Mean of n = 3.

Table 4: Productive variables, color, pigments and colorimetric values in egg yolk at different levels of *Tithonia diversifolia* meal inclusion

	***Tithonia diversifolia* meal (%)**					
	Control	**1.77**	**5**	**10**	**15**	**SME**
Productive variables[1]						
Laying, %	94.8^a	94.3^a	94.8^a	92.7^{ab}	89.8^b	0.52
Egg weight, g	59.1	59.2	59.4	59.5	58.5	0.24
Feed intake, bird/day/g	105^a	105^a	106^a	105^a	100^b	0.64
Feed conversion, kg:kg	1.876	1.892	1.895	1.910	1.908	0.011
Egg mass, bird/day/g	56.0^a	55.9^a	56.3^a	55.1^{ab}	52.5^b	0.44

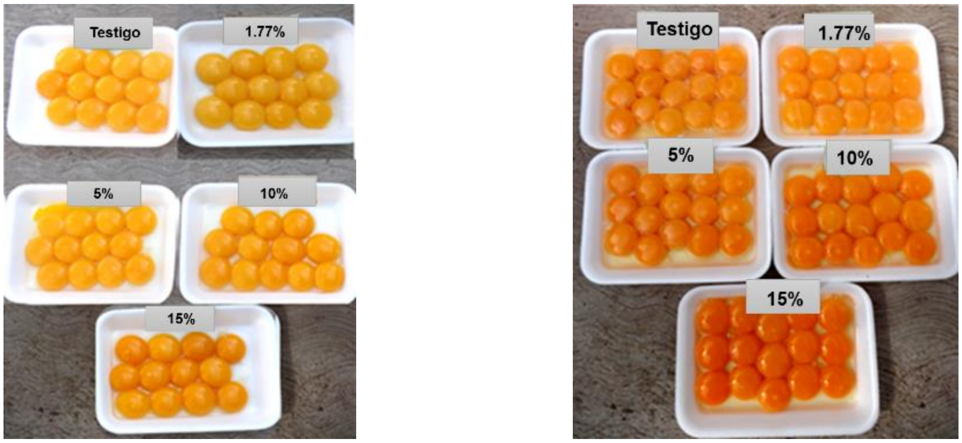
Color[2]						
Yolk color, no red pigment	9^{b}	8^{c}	9^{b}	10^{a}	10^{a}	0.12
Yolk color, red pigment	11^{a}	10^{b}	11^{a}	11^{a}	11^{a}	0.78
Pigments (µg/100g)[3]						
Total carotenoids	235^{b}	142^{c}	269^{ab}	367^{ab}	440^{a}	31.66
Lutein	152^{b}	87^{c}	173^{ab}	245^{ab}	291^{a}	21.06
Zeaxanthin	16.5	9.75	13.25	13.0	11.75	0.92
Capsanthin	5.5	5.25	5.25	6.5	5.5	0.49
Colorimetric values[2]						
L*	67.38^{ab}	69.40^{a}	66.02^{ab}	65.21^{b}	64.30^{b}	0.92
a*	-2.66^{bc}	-4.29^{c}	-2.71^{bc}	-1.37^{b}	0.44^{a}	0.42
b*	49.49^{ab}	44.01^{c}	47.47^{bc}	49.28^{abc}	5.53^{a}	1.30

[1] n=48 birds/treatment.
[2] n=20 eggs/ treatment.
[3] n=3 samples/ treatment.
SME= Standard mean error.
[abc] Different letter superscripts in the same row indicate significant difference (P<0.05).

Yolk color based on DSM values, with no added red pigment (Figure 1 A), was lowest in the 1.77% TDM treatment (DSM= 8) and highest in the 10 and 15% TDM treatments (DSM= 10). When red pigment was added to the diet the values followed a similar pattern to when no red pigment was added, with the lowest value (DSM= 8) in the 1.77% TDM treatment (value 8) and higher values in the other treatments and the Control (DSM= 11) (P<0.05) (Figure 1 B).

Total carotenoids content was lowest in the 1.77% TDM treatment (142 µg/100 g) and then increased from the Control (235 µg) to the 5% TDM treatment (269 µg), the 10% (367 µg) and the 15% (440 µg). Lutein values were also lowest in the 1.77% TDM treatment (87 µg/100 g) and highest (P<0.05) in the 15% TDM treatment (291 µg). No differences between treatments were observed for zeaxanthin and capsanthin contents.

Figure 1: Egg yolks from treatments with increasing amounts of *Tithonia diversifolia* meal



A B

A= without added red pigment; B= added red pigment.

Discussion

Studies of how inclusion of natural ingredients in livestock forages affects animal productive behavior and product quality traits have been done largely in ruminants, particularly in agrosilvopastoral systems. Very little research of this type has been done in monogastric species such as chickens since this type of digestive system does not degrade high fiber diets, although fiber can be used in poultry diets(13). There is therefore only a very limited literature on the use of *T. diversifolia* in poultry systems and even less comparing it to ruminant systems.

In the present results laying percentage, feed intake and egg mass were better in the 5% TDM treatment, which did not differ from the control. Feed conversion was better in the 10% TDM treatment. These data generally coincide with those in a study of the effects of *T. diversifolia* foliage meal inclusion (5, 10, 15 and 20% TDM, and a commercial feed control) in laying hens in which egg production did not differ between treatments, feed intake was lowest in the 20% TDM (96.3 g/bird/d) compared to the control (107 g/bird/d), and feed conversion was best in the 15% TDM(8). This response may have been due to the negative effects of high fiber content and the presence of antinutritional factors in the 20% TDM treatment, which could compromise absorption of nutrients, mainly of amino acids(14,15).

Tannins are a common antinutritional factor which can undermine feed palatability by imparting bitter flavors, but can also form complexes with proteins, starches and digestive enzymes, consequently reducing feed nutritional value, and negatively influencing animal growth, feed digestibility and protein and amino acids availability. Lower *T. diversifolia* inclusion levels do not seem to negatively affect productive performance in poultry; for example, 2 % inclusion of *T. diversifolia* leaf meal in laying hen diets is reported to improve egg mass and feed conversion(16).

Poultry do not synthesize pigments that will add color to skin or egg yolk, which therefore need to be added to diets. The present data on the pigment contents of the studied TDM can function as a standard for its use as a source of xanthophylls for improving egg yolk color. Other natural sources of carotene pigments include alfalfa leaf meal (396 mg/kg), yellow corn (22 mg/kg), dehydrated Ladino clover meal (490 mg/kg), chili meal (187 mg/kg) and *Tagetes erecta* (Mexican marigold flower) (8,000 to 10,000 mg/kg), which has the highest xanthophylls content (80 to 90 % lutein). When ingested by laying hens xanthophylls enter the blood stream and are deposited in the skin, fatty tissue, the liver and egg yolks; carotenes are incorporated in smaller quantities and are transformed into vitamin A(17). In a study of laying hens fed diets including 5, 10, 15 and 20% TDM, the *T. diversifolia* was found to be the only source of yellow pigments (lutein and zeaxanthin) in egg yolks(8). Some variation is to be expected between different studies due to study conditions such as the age and element of the plant used as pigment source, animal assay conditions, climate, temperature, etc.

Relative to the treatments without red pigment (Trial 1), addition of red pigment to the feed formulation (Trial 2) in the present study notably increased yolk red hue in the Control, 5, 10 and 15% TDM treatments, although not in the 1.77% TDM treatment. This behavior may have been due to the combination of yellow and red colors in the treatments, which resulted in an orange color. Diet yellow pigment content increased as TDM inclusion level increased. Because red pigment was added in comparable amounts to the yellow pigments yolk color differed minimally between treatments, which is why the 1.77% TDM treatment had the least orange color of the red pigment treatments. The two 1.77% TDM treatments (no red pigment, red pigment) had the lowest color values since they were formulated based on an adjusted total carotenoids content of 15 ppm, the same proportion as in the Control, considering total carotenoids as the pigment. The egg yolk pigment content results were therefore directly proportional to TDM inclusion level, with increases in lutein corresponding to approximately 50 % of total carotenoids. In the egg yolk colorimetry values the yellow hue (b*) was higher in the Control and 15% TDM treatments, confirming the majority presence of lutein in the TDM.

Conclusions and implications

The leaves and petioles of *Tithonia diversifolia* constitute a valid source of natural pigments. They can be included in laying hen diets at up to 10% to improve egg yolk color, without negatively affecting productive parameters. Combination of a natural red pigment (canthaxanthin) with the yellow pigments in the *Tithonia diversifolia* meal produced egg yolks with a stronger orange color.

Acknowledgements and conflicts of interest

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The authors declare no conflict of interest in the research reported here, and that all authors approved the final manuscript.

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