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Production and egg quality in chicken layers fed with *Tithonia diversifolia*

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Abstract

We evaluated the effect of different dietary *Tithonia diversifolia* (TD: 0, 10, 20, and 30 %) on productive traits, egg quality, and economic efficiency in laying hens. Feed intake was higher in hens fed the 20 and 30 % TD diets (120.6 and 119.0 g/day) compared with those fed the 0 or 10 % TD diets ($P < 0.0010$). Feed egg weight conversion increased with dietary TD inclusion ($P < 0.0010$). The laying rate was higher ($P < 0.0020$) in hens on 0 and 10 % TD diets (94.5 and 94.6 %, respectively) than those on 20 and 30 % TD diets (89.7 and 84.9 %). Eggs and yolks were heavier in hens fed 0, 10, and 20 % TD compared with those fed the 30 % TD diet ($P < 0.0020$ and $P < 0.0040$, respectively), which also had poorer albumen width ($P < 0.0010$). The yolk had a more intense yellow as the level of dietary TD increased ($P < 0.0001$). Dietary TD did not affect economic efficiency. In summary, the inclusion of up to 10 % TD in laying hen diets seems feasible as it did not affect productive traits, economic efficiency, and egg quality traits.

Keywords: Keywords: Laying hens; Egg quality; Tree forage; Economic efficiency.

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Additional information and declarations can be found on page 10

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Study contribution

In the south of Mexico, there are many plants natural resources that could be used to fed poultry instead of the traditional food commonly used in poultry diets. Some of those plant resources like *Tithonia diversifolia*, have great content of protein and carotenoids necessary to pigment the egg yolk. However, there is little information on the contribution of *T. diversifolia* to chicken layer nutritional requirements and egg yolk pigmentation. This study evaluated some productive and egg quality traits in laying hens fed diets with *T. diversifolia*. The results indicate that it is possible the use of up to 10 % of *T. diversifolia* in the diets without any effect on productive traits, increasing yolk yellowness and keeping the economy efficiency of the flock. Therefore, *T. diversifolia* is a promising resource to fed poultry layers.

Introduction

The success and profitability of poultry production depends largely on the cost of feed, which represents 70 % to 80 % of the total cost of production, mainly due to the use of conventional sources of protein and energy.(1) Therefore, the use of alternative inputs in the poultry ration is a good option for successful poultry production,(2) and profitability. In laying poultry, energy and protein in the feed represent the highest nutritional requirements of diets, where corn grain and soybean meal have been the conventional inputs for the supply of those nutrients.(3) The high costs of corn and soybean, which are widely used in animal feed, has driven to the search for feeding alternatives in developing countries, which could help to decrease production costs and improve profitability, without affecting the productive performance of animals.(4) In this regard, Abou-Elezz et al.(5) points out that the shortage in the production of animal protein for human consumption in developing countries and the high cost of conventional inputs for animal feed, have accelerated research into the use of new low-cost and easily available food resources. Likewise, it has been signaled that there is a need for the use of cheaper, highly available food ingredients that do not compete with human consumption; *v.gr.*, leaf flours from trees, which are gaining acceptance as feed in the poultry diet due to their high nutrient content.(1)

In the tropics, numerous trees and shrubs could be used as a practical and economically viable alternatives to guarantee sustainable animal production. *Tithonia diversifolia* is a non-leguminous promising plant for the feeding of different animal species. It is forage with great potential, high nutritional value, high protein and mineral contents, high digestibility of dry matter, presence of oils and a high percentage of total sugars.(6) It has an excellent nutritional quality with reported values of 16.3 % to 22.5 % of crude protein, 44.4 % to 70.4 % of carbohydrates and 15.8 % to 21.8 % of raw fiber.(7)

The ability to digest fibrous feed in birds has been reported to be low, limiting the inclusion of forage tree meal in their diets.(8,9) However, when chickens are fed high fiber diets, they can increase intake to meet their nutrient requirements.(5) There is little information on the use of tropical forages in the feeding of laying hens, especially on the use of *T. diversifolia*. In previous studies, up to 20 % inclusion in the diet of laying hens was tested, but the productive traits were negatively affected.(6) Therefore, this study evaluated the effect of the inclusion in the diet of

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up to 30% of *T. diversifolia* leaf meal on productive traits, egg quality and economic efficiency of laying hens.

Materials and methods

Ethical statement

At the Instituto Tecnológico de la Zona Maya there is not an animal care review board. However, the management of the birds was carried out according to the official Mexican rules related to technical specifications for animal management care and use of experimental animals (NOM-062-ZOO-199). Moreover, the birds used in this experiment were housed under the double of space recommended in poultry layer manuals (800 cm^2 per bird, instead of 400 cm^2 per bird).

Location

The study was conducted at the "Instituto Tecnológico de la Zona Maya," located at the geographic coordinates of 18° 31´ 58" N and 88° 29´ 19" W. The climate of the region is warm subhumid type AW1, the average annual temperature fluctuates between 24.5° and 25.8 °C, and with an average altitude of 15 meters above sea level.

Animals and management

Seventy-two Bovans white hens, 30 weeks of age, were used, which started laying at 18 weeks of age. The initial live weight was 1.57 ± 0.087 kg. The hens were housed in pairs, in an open barn with natural ventilation, in metal cages (1 600 cm^2), equipped with linear feeders and automatic pivot-type drinkers. From week 18 of age until the start of the experimental period, the hens were kept in metal cages, and fed commercial feed, based on corn and soybean meal

Treatments and experimental management

The vegetative material *Tithonia diversifolia* (TD) was harvested in a 45-day regrowth plot. Leaves and tender stems were cut, dried in a forced air oven at 60 °C until constant weight, and then ground with a 3 mm sieve. The flour of the TD was stored in plastic containers with lids until it was used. Dry matter, organic matter, crude protein, neutral detergent-fiber were determinate. Additionally, total tanins by Folin–Ciocalteau method suggested by Makkar(10) and condensed tannins by Vainillina method recommended by Price et al.(11) were determinate (Table 1).

Diets included 0%, 10%, 20%, and 30% TD flour. The treatments were distributed in a completely randomized design with nine replicates (cages) with two hens per cage. The diets were formulated according to the requirements proposed by the nutrition management guide for Bovans white hens from Hendrix genetics® for laying hens of 30 to 43 weeks of age. In the elaboration of the diets, TD flour, cassava flour, soybean meal, ground corn, vitamin and mineral premixes, synthetic amino acids and additives were used (Table 2).

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Table 1. Nutritional composition of the leaf flour of *T. diversifolia*

Components	*T. diversifolia* meal
Dry matter (%)	96.8
Organic matter (%)	84.1
Crude protein (%)	24.7
Neutral detergent fiber (%)	50.1
Total tannins (mg/g)[1]	3.8
Condensed tannins (mg/g)[2]	28.1

[1] Method Folin-Ciocalteu method
[2] Vainillina method

Table 2. Diet conformation and chemical composition

	Level of *T. diversifolia* meal in diet (%)			
Ingredients (%)	0	10	20	30
Corn	29.8	24.6	20.0	17.7
Soybean meal	33.5	28.9	24.1	22.5
Cassava flour	20.0	20.0	20.0	20.0
T. diversifolia	0.0	10.0	20.0	30.0
Calcium carbonate	8.9	8.5	8.1	7.9
Orthophosphate 20:20[1]	3.3	3.2	3.1	3.1
Soybean oil	3.5	3.5	3.2	3.1
Salt	0.3	0.3	0.3	0.3
DL-Methionine	0.4	0.5	0.5	0.6
Mycotoxin binding	0.1	0.1	0.1	0.1
Pigment (Yemix®)	0.1	0.1	0.1	0.1
L-Lysine HCl	0.0	0.2	0.3	0.4
Chlorine chloride[2]	0.05	0.05	0.05	0.05
Fungicide	0.05	0.05	0.05	0.05
Minerals and vitamins[3]	0.08	0.08	0.08	0.08
Antioxidant	0.01	0.01	0.01	0.01
Chemical composition (%)				
Dry matter	90.6	90.7	90.0	90.2
Organic matter	75.9	76.5	74.7	73.8
Crude protein	17.5	17.5	17.5	17.6
Neutral detergent fiber	8.4	9.7	12.1	12.4
Metabolizable energy (Mcal/kg)	2.7	2.7	2.7	2.7
Price per kg of feed (dollars)	0.36	0.34	0.31	0.30

[1] 20 % Calcium, 20% Phosphorus.
[2] 700 g/kg of Choline chloride.
[3] Minerals and vitamins: Mn, 65 mg; I, 1 mg; Fe, 55 mg, Cu, 6 mg; Zn, 55 mg; Se, 0.3 mg; vitamin A, 8000 UI; vitamin D, 2500 UI; vitamin E, 8 UI; vitamin K, 2 mg; vitamin B, 0.002 mg; riboflavin, 5.5 mg; calcium pantothenate 13 mg; niacin, 36 mg; Choline chloride, 500 mg; folic acid, 0.5 mg; thiamin, 1 mg; pyridoxine, 2.2 mg; biotin, 0.05 mg.

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The experimental period lasted 14 weeks due to the amount of TD flour available. The productive variables recorded were feed intake, laying hen rate, feed conversion and egg weight, measured every week for 14 weeks.

Egg quality

At 15, 30, 45, 60, 75, 90, and 105 days of the experimental period, two eggs were collected from each cage. Immediately after collection, the eggs were identified and transferred to the laboratory and sampled. They were individually weighed on an Ohaus® CS 200 scale and the length and width of the egg were measured with a digital vernier to calculate the egg shape index. Immediately after, the eggs were carefully broken, and the contents were deposited on a plastic plate. After removal of the inner membrane of the shell with dissecting forceps, the thickness of the shell at the middle, tip and the base of the egg was measured with a digital vernier and the data were averaged. The weight of the shell was determined after drying in a forced air oven for four hours at 60 °C.

The width and height of the albumen were measured in the middle portion of the thick albumin, between the edge of the albumen and the membrane of the yolk using a digital vernier. The yolk was carefully removed with the help of a spoon and weighed on a garnet scale. The weight of the albumen was calculated as the difference between the total weight of the egg and the weight of the shell plus the weight of the yolk. With those values, the percentages of albumen, yolk and shell weight were estimated based on the weight of the egg. The length, width and height of the yolk were measured with a digital vernier. The yolk index was calculated by dividing the height by the diameter, and the result was multiplied by 100. The color of the yolk was measured using the scale from Roche ® yolk color fan, with 1 to 15 yellow scale. To calculate the Hugh units (UH), the height of the egg white and the weight of the egg were used, using the formula proposed by Eke et al.[12]

Economic efficiency

The economic efficiency for each treatment was calculated as the difference between the economic income per kilogram of egg produced per hen/day, and the average daily feeding cost/hen. The income per kilogram of egg produced per hen per day was estimated using the average egg weight in each treatment, adjusted to the respective laying percentage (Table 3), and multiplied by the price per kilogram of egg at that time (1.80 dollars per kg). The daily cost of feed per hen was calculated by multiplying the average feed consumption by the price per kilogram of feed for each treatment. To calculate the cost of feed, the price of the ingredients at that time and a price of 0.10 dollars per kilogram of *Tithonia diversifolia* meal were considered.[13] The economic efficiency was expressed as the dollars returned for each dollar invested.

Statistical analysis

As has been mentioned before, the 72 hens were housed in pairs, so, there were 9 replicates per treatment. Normal distribution of the data were tested using

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Table 3. Productive traits of hens fed different levels of *T. diversifolia*

Traits	Level of *T. diversifolia* in diet (%)				SEM[1]	P value
	0	10	20	30		
n	9	9	9	9		
Initial weight of hens (kg)	1.57	1.57	1.55	1.58	0.030	0.9614
Final weight of hens (kg)	1.51	1.45	1.44	1.41	0.027	0.1140
Feed intake (g/hen/day)	109.8[a]	114.0[a]	120.6[b]	119.0[b]	2.647	0.0010
Laying rate (%)	94.5[a]	94.6[a]	89.7[ab]	84.9[b]	2.662	0.0020
Egg weight (g)	59.4[a]	57.7[ab]	57.5[ab]	56.7[b]	0.638	0.0020
Feed conversion (kg feed/kg egg)	2.0[a]	2.1[b]	2.4[c]	2.5[d]	0.079	0.0010

[1] SEM = Standard error of the means
[a, b, c, d] Means with equal letters per row are significantly different ($P < 0.05$)

univariate procedure and the Shapiro-Wilks test.(14) Initial and final weight of the hens were subjected to an one way analysis of variance.(14)

The production and quality of the egg traits were subjected to an analysis of variance for a completely randomized design with repeated measures using the GLM procedure of SAS program.(14) The means of the treatments were compared using the Tukey test.

Results

Productive traits

The productive performance of hens non-given and given TD in the diets is shown in Table 3. The initial weight of the hens was similar ($P > 0.9614$). The hens lost weight at the end of the experiment in all treatments, however, no statistical differences were found between treatments ($P > 0.1140$). The hens fed 20 and 30 % TD flour consumed more feed than those fed 0 and 10 % TD ($P < 0.0010$). The laying rate and the weight of the egg gradually decreased as the level of TD in the diet increased ($P < 0.0020$), being the lowest in diet with 30 %. The feed conversion increased as did the level of TD, the highest feed- egg weight conversion being in the diet belonging to the diet with 30 % TD ($P < 0.0010$).

Egg quality traits

Egg quality is shown in Table 4. Most of the egg quality characteristics evaluated in this experiment were not affected by the inclusion of TD in the diets ($P > 0.0040$). However, the width of the albumen was shorter in the diet with 30 % TD ($P < 0.0010$). The weight of the yolk was similar in diets with 0 %, 10 %, and 20 % TD and lower in the diet with 30 % TD ($P < 0.0040$). The color of the yolk was more intense in the diets with 10 %, 20 %, and 30 % TD, compared with the diet with 0 % ($P < 0.0001$).

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Table 4. The egg quality trait means for hens fed different levels of *Tithionia diversifolia*

Trait	Level of *T. diversifolia* in diet (%)				SEM[1]	P value
	0	10	20	30		
n	9	9	9	9		
Egg length (mm)	56.6	56.0	56.0	55.6	0.28	0.1260
Egg width (mm)	45.2	44.8	44.6	44.4	0.22	0.2057
Shape index (%)	71.7	71.9	71.4	71.7	0.45	0.8759
Shell thickness (mm)	0.33	0.32	0.32	0.33	0.01	0.1907
Shell weight (g)	5.8	5.5	5.5	5.6	0.01	0.0763
Albumen weight (g)	39.5	38.4	38.7	37.7	0.49	0.1620
Albumen height (mm)	7.5	7.5	7.6	7.3	0.13	0.5151
Albumen length (mm)	124.0	127.5	120.6	117.0	3.62	0.3028
Albumen width (mm)	106.7[a]	109.5[a]	109.9[a]	101.5[b]	1.08	0.0010
Albumen index (%)	0.07	0.06	0.07	0.07	0.01	0.2544
Yolk weight (g)	15.4[a]	15.1[a]	14.7[ab]	14.5[b]	0.18	0.0040
Yolk height (mm)	15.0	14.9	14.7	14.4	0.17	0.0525
Yolk length (mm)	39.7	39.2	38.8	39.2	0.25	0.1901
Yolk width (mm)	37.9	37.5	37.4	37.6	0.18	0.4018
Yolk index (%)	38.8	39.0	38.6	37.5	0.52	0.2418
Yolk color[2]	4.9[c]	5.8[b]	6.0[b]	6.7[a]	0.11	0.0001
Hugh units (%)	86.7	86.7	87.6	86.1	0.79	0.5810

[1] SEM = Standard error of the means.
[2] Roche® Yolk Color Fan.
[a, b]Means with equal letters are significantly different ($P < 0.05$)

Table 5. Economic efficiency of hens fed different levels of *Tithionia diversifolia*

	Level of *T. diversifolia* in diet (%)			
Traits	0	10	20	30
Feed consumption (kg/day)	0.110	0.114	0.121	0.119
Price per kg of feed (dollar)	0.36	0.34	0.31	0.30
Cost of feed consumed (dollar)	0.040	0.039	0.038	0.036
Egg produced/hen/day (kg)[1]	0.056	0.055	0.052	0.048
Price per kg of egg (dollar)	1.80	1.80	1.80	1.80
Income per eggs sold (dollar)	0.100	0.099	0.094	0.086
Economic efficiency[2]	2.5	2.5	2.5	2.4

[1]Calculated as average egg weight, adjusted by the percentage of lay per treatment.
[2]The economic efficiency is expressed as the dollars returned for each dollar invested.

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Economic efficiency

The cost of the feed consumed, decreased as the level of TD in the diet increased (Table 5). However, the kg of egg produced per hen/day was reduced as the level of TD in the diet increased.

Therefore, income per egg sold, also decreased, which explains that the economic efficiency was similar for the diets with 0%, 10%, and 20% TD. However, economic efficiency was reduced slightly in the diet with 30% of TD because of an evident reduction in egg produced/hen/day and the consequent reduction in income from eggs sold.

Discussion

The increase in food consumption observed in the diets with 20 % and 30 % TD compared with diets with 0% and 10% TD may be associated with increases in the percentage of NFD, going from 8.4% and 9.7% in 0% and 10% TD diets, respectively, to 12.11% and 12.4% in the diets with 20% and 30% TD, respectively, which could affect the efficiency in the use of nutrients. Additionally, the levels of TD here evaluated may have reduced the available nutrients and energy intake, increasing animal consumption to meet energy requirements.(5, 15-17) An increase in fiber and feed consumption, when TD meal was used has been previously reported in hens fed 15% TD,(6) and in Rhode Island red hens fed up to 15% of *Leucaena leucocephala* or *Moringa oleifera*.(5) Additionally, those authors mention that laying hens fed high fiber diets can increase their feed consumption to meet their nutrient requirements, particularly energy, which could explain the increase in consumption found in diets with 20% and 30% of TD compared to diets with 0% and 10% of TD.

The reduction in laying rate and egg weight ,here found, with 20% TD may directly be related to energy consumption and digestibility, since it has been reported that high fiber diets reduce energy digestibility of the diet.(15) The low capacity of the hen digestive system in the use of high fiber foods must be considered, since the cecum is the only place to digest plant fiber and this is very short.(5) Likewise, by including forage tree meal in diets for laying hens, the presence of anti-nutritional factors such as tannins is increased, which form complex products with proteins, starch and digestive enzymes, causing reduction of digestibility, mainly of proteins, which reduces the availability of amino acids for maintenance and production.(18) In the present research the TD utilized had 28.1 mg/g condensed tannins and 3.8 mg/g total tannins that increased in the experimental diets as the levels of TD increased.

The results obtained in this study indicate that the use of 10% TD in the diet of laying hens did not affect the laying rate and egg weight. These results agree with those reported by Rodríguez et al.(6) for hens fed 0% to 20% TD meal in the diet. Comparing the results, here obtained, with those in the literature, where laying hens were fed with forage trees, such as *Morus alba*(19) and *Gliricidia sepium*(1) the production was maintained using low levels of forage inclusion, as happened here.

Feed conversion increased as the level of TD in the diet increased. It was associated with a linear climb in feed consumption and the linear reduction in laying

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rate, as the level of TD increased; feed conversion was better for the control (0 %) diet, followed by 10 % TD diet. Similar results were reported by Rodríguez et al.[6] in laying hens, where the worst feed conversion was for birds consuming diets with more of 20 % TD. Better feed conversion with diets based on corn and soybean meal, compared to those with forage tree leaves, has also been reported in chickens by Abou-Ellezz et al.[5] The results found here for many of the egg traits evaluated agree with those reported by Rodríguez et al.[6] who observed that levels of 20 % TD in the diet, did not affect the thickness, weight and eggshell strength. This also agrees with that of Herrera et al.[19] results, who mention that the inclusion of 5 % of forage meals of *Gliricidia sepium*, *Cajanus cajan*, and *Morus alba* did not affect the size, weight and shape of the egg of Rhode Island Red hens.

Abou-Ellezz et al.[20] reported the reduction of the yolk weight as an effect of the inclusion of forage in the diet in hens fed with fresh *Moringa oleifera* leaves as a dietary supplement, which agrees in part with what was found here. This could be explained by a reduction in the energy density of the diet due to the inclusion of TD. It is highly probable that the metabolizable energy value assigned to the forage of TD was underestimated when designing the diets.

The most intense color of the yolk due to the inclusion of TD with respect to the control diet (0 %) may be due to, the presence of xanthophylls and carotenoid compounds in the TD meal.[21] Similar results have been reported by Mohamed et al.[22] in Rhode Island Red hens with access to natural vegetation, and by Abou-Ellezz et al.[20] in hens supplemented with fresh *Moringa oleifera* leaves. Additionally, de Koning et al.[23] mentioned that the egg yolk color from hens reared free range improved significantly when they were consuming plants like Saltbush (*Atriplex nummularia*).

The inclusion of TD in the diets here evaluated, reduced the price per kilogram of feed; however, egg produced/hen/day was also reduced and feed intake increased for 20 % and 30 % TD diets. Therefore, the economic efficiency of the diets with the highest levels of TD was not enhanced. Several authors have reported the reduction in the fixed costs of feed due to the inclusion of cheaper inputs in the feed of laying hens, although the reduction in feed costs was not necessarily reflected in higher egg production.[2,24]

Conclusion

It is feasible to use up to 10 % TD meal in laying hen diets, without affecting productive traits, economic efficiency and egg quality traits except the increase in the yellow of the yolk. With respect to the economic efficiency, the use of up to 20 % of TD could be recommended. The level of 30 % TD in the diet reduced the laying rate and the economic efficiency.

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Data availability

Datasets used in this experiment are available from the corresponding author on request.

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Conflicts of interest

The authors confirm that there are no conflicts of interest associated with this publication.

Author contributions

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