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Turmeric Root and Annato Seed in Second-Cycle Layer Diets: Performance and Egg Quality

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Annato, layers, second cycle, sorghum, turmeric.

ABSTRACT

The objective of this study was to evaluate the effects of the inclusion of annato (*Bixa orellana* L.) and turmeric (*Turmeric longa* L.) in layer feeds on live performance, egg quality, and yolk pigmentation and depigmentation time. A number of 144 layers were distributed in a completely randomized experimental design, with four treatments with six replicates of six birds each. In the basal diet, sorghum replaced 50% of corn, and was supplemented or not with natural pigments to composse the following treatments: Control (0% pigments), AS (2.0% annato), TR (2% turmeric) and ASTR (1% annato and 1% turmeric). Egg weight (g), egg production (%), egg mass (%), feed intake (g), feed conversion ratio (kg/dz and kg/kg) and mortality were evaluated. The following egg quality parameters were evaluated: specific gravity (SG); yolk, albumen, and eggshell percentages, and yolk color. The treatments did not influence layer performance or egg quality parameters, except for egg production and yolk color. The dietary inclusion of 1% turmeric root and 1% annato seed promoted higher egg production. Diets containing annato resulted in more saturated, more intense, and redder yolk color, with increasing pigment deposition after day 10, with maximum values obtained on day 28. Dried turmeric root did not promote good yolk pigmentation, resulting in higher presence of white in the yolk, which was stabilized on day 4. Three days after pigments were withdrawn from the feeds, yolk color faded in the treatments with annato inclusion.

INTRODUCTION

Corn is the main ingredient of poultry diets, accounting for 60-70% of feed costs. The use of feedstuffs alternative to corn and soybean meal is a strategy used by poultry nutritionists to reduce feeding costs, while ensuring the safety and the quality of the final product.

Some studies with layers have shown that sorghum can partially or completely replace corn in layer feeds with no negative effects on performance; however, a carotenoid source must be added to ensure egg yolk pigmentation (Casartelli *et al.*, 2005; Assuena *et al.*, 2008). Chickens are not capable of synthesizing color pigments, but can transport about 20-60% of the ingested dietary pigments to the yolk (Bartov & Bornstein, 1980).

Yolk color intensity is a purchase decision-making criterion for consumers, as it is usually associated with vitamin content (Oliveira, 1996).

The use of natural pigments has increased due to consumer preference and to legal restriction in countries that have banned the addition of synthetic coloring in feeds and foods.

One important natural pigment is annato (*Bixa orellana* L.) because tan. Studies have been carried out on the effects of annato, which is rich in bixin, on yolk pigmentation of layer eggs, particularly when the main dietary energy sources used instead of yellow corn have low pigmenting activity, such as sorghum, broken rice, millet, cassava meal, etc.(Oliveira, 2004).

Curcumin is the main pigment present in turmeric roots (*Turmeric longa* L.), and it is also known and saffron. In addition of its utilization as food color and spice, it has antioxidant and antimicrobial properties, which allows it to be applied in cosmetics, textiles, pharmaceutics, and foods (Pruthi, 1980). Turmeric has been increasingly used in food applications, such as in starch for cake preparations and as color in pasta, mustard, ice cream, cheese, egg, margarine, and meats.

Considering the few studies carried out in Brazil on the use of pigments in eggs, this study aimed at evaluating the effect of curcumin and bixin on the performance and egg quality of second-cycle layers fed diets with 50% low-tannin sorghum replacing corn and the time how long these natural pigments take to pigment and disappear, when removed from the diet, from the egg yolk.

MATERIALS AND METHODS

In this experiment, 144 Hy-Line® Brown layers, were evaluated after forced molting, to three 28-day periods. Birds were distributed in 24 cages (1.00m long x 0.45m deep x 0.40m high) divided in three compartments, housing two birds each. After forced molting, all birds were submitted to the same management and feeding conditions until the peak of lay, after which they grouped as a function of similar body weight and egg production, around 90 weeks of age. Layers were then distributed in a completely randomized experimental design with six replicates of six birds each per treatment, and fed diets formulated with low-tannin sorghum replacing 50% corn with the addition or not of natural pigments. The following treatments were applied: Control – reference diet based on 50% corn and 50% low-tannin sorghum; AS - Reference diet + 2% annato seed; TR - Reference diet + 2% turmeric root; and ASTR - Reference diet + 1% annato seed + 1% turmeric root.

During the entire experimental period, layers were submitted to the same management and feeding conditions, with water and fee offered *ad libitum*.

Feeds were formulate to contain equal protein, sulfur amino acid, and lysine levels, according to the recommendations of Rostagno *et al*. (2005), except for pigment content (Table 1). Sorghum replaced 50% of corn in all diets. Annato seeds and turmeric roots were washed, dried for 24h in a forced-ventilation oven at 75±2 ºC, ground, and sieved.

Table 1 – Ingredient composition and calculated nutritional composition of the experimental diets

Ingredient (%)	Treatment[1]			
	Control	AS	TR	ASTR
Corn (8% CP)	32.24	31.78	31.86	31.67
Sorghum (11% CP)	33.95	32.79	32.72	32.89
Soybean meal (45% CP)	22.88	22.93	22.89	22.91
Annato (11% CP)	_	2.0	_	1.0
Turmeric (8% CP)	_	_	2.0	1.0
Calcitic limestone	7.99	7.98	7.97	7.97
Dicalcium phosphate	1.54	1.52	1.52	1.52
Salt	0.35	0.37	0.37	0.37
Vitamin supplement[2]	0.10	0.10	0.10	0.10
Mineral supplement[3]	0.10	0.10	0.10	0.10
DL-Methionine 99%	0.16	0.17	0.17	0.17
Washed sand	0.69	0.26	0.30	0.30
TOTAL	100.00	100.00	100.00	100.00
Calculated composition				
Metabolizable energy (kcal/kg)	2800	2800	2800	2800
Crude protein (%)	16.09	16.09	16.09	16.09
Methionine (%)	0.56	0.56	0.56	0.56
Lysine (%)	0.74	0.74	0.74	0.74
Methionine + Cystine (%)	0.68	0.82	0.82	0.82
Calcium (%)	4.00	4.00	4.00	4.00
Available phosphorus (%)	0.38	0.38	0.38	0.38

1 - Reference diet + 2% turmeric root and ASTR - Reference diet + 1% annato seed + 1% turmeric root. 2 - Product composition: Vitamin A, vitamin D3, vitamin E, vitamin K, vitamin B1, vitamin B2, vitamin B6, vitamin B12, niacin, folic acid, pantothenic acid, sodium selenide, vehicle Q.S.P. Guaranteed levels per kg product: Vitamin A 10,000,000 IU, Vitamin D3 2,500,000 IU, Vitamin E 6,000 IU, Vitamin K 1,600 mg, Vitamin B12 11,000 mg, niacin 25,000 mg, folic acid 400 mg, pantothenic acid 10,000 mg, selenium 300 mg, antioxidant 20 g. 3 - Product composition: manganese monoxide, iron sulfate, copper sulfate, calcium iodide, vehicle Q.S.P. Guaranteed levels per kg product: manganese 150,000 mg, zinc 100,000 mg, iron 100,000 mg, copper 16,000 mg, iodine 1.500 mg.

Eggs were collected daily in the morning and average egg production was obtained by dividing the total number of produced eggs (intact, broken, cracked, and misshapen eggs) by the number of birds alive in each experimental unit. Feed conversion ratio per kg was determined by dividing total feed intake by egg weight, and expressed as kg feed/kg eggs. Feed conversion ratio per dozen was obtained by dividing average feed intake per dozen of eggs produced. Average egg weight of an experimental unit was calculating by dividing total egg weight by the number subtracting the number of hens alive at the end of the experiment from the number of housed hens, and expressed as percentage.

In order to determine yolk, albumen, and eggshell weights and eggshell thickness, two intact eggs per experimental unit were randomly collected every 28 d for three consecutive days. After duly identified and weighed in a 0.01 g precision scale, eggs were broken, and their yolks were manually separated and weighed. Eggshells were dried in a forced-ventilation oven for 24 h at 105 °C and weighed. Albumen weight was determined as the difference between egg weight and eggshell and yolk weights. Egg specific gravity was obtained by immersing the eggs in different saline solution, with densities ranging from 1.050 g/cm^3 to 1.100 g/cm^3, and recording the solution in which the egg floated.

Eggshell thickness, including egg membranes was measured in three different points in the egg equatorial region using an external micrometer (Mitutoyo, model 103-137), with 0-25 mm range, 0.01 mm reading, and 0.002 mm accuracy. Eggshell thickness was determined as the average value of three measurements, according to the methodology described by Nordstrom & Ousterhout (1982).

Yolk color was analyzed at Laboratório do Pólo Regional do Leste Paulista, APTA, using a subjective and a direct (instrumental methodologies). In the subjective method, two yolks per replicate were placed in Petri dishes on a white background on days 0, 3, 7 10, 14, 21, 28 and 56. At the end of the experiment, the pigments were withdrawn from the diets, and eggs were collected again on days 0, 3, 7, 10 and 28 after withdrawal to determine the time required for yolk depigmentation. Yolk color was determined using the DSM® yolk color fan by the same trained observer, who attributed an average score between 1 and 15, according to the description of Galobart *et al*. (2004).

Yolk color was determined by direct method in three yolks per replicate from eggs collected on day 56, using tristimulus colorimetry of the CIELAB system (portable spectrophotometer MINOLTA, model CM 508d), with D65 illuminant and 10° illuminating angle. The results are expressed in the coordinates L* (lightness), h (tone) and C* (chromaticity).

Yolk pigmentation and depigmentation data were submitted to analysis of variance and analysis of regression, and means compared by the test of Tukey at 5% significance level. Data obtained for other parameters were submitted to analysis of variance (ANOVA), and means compared by the test of Tukey at 5% significance level, using SISVAR statistical package.

RESULTS AND DISCUSSION

Average minimum and maximum environmental temperatures inside the poultry house during the experimental period were 19.5 ± 2.11 and 31.7 ± 2.74 °C, respectively.

The inclusion of annato seed and turmeric root in the diet did not affect ($p > 0.05$) egg weight, egg mass, feed intake, FCR/dz, FCR/kg or mortality (Table 2). These results are consistent with the findings of Garcia *et al*. (2009), who used 0.5, 1, 1.5, 2.0, and 2.5 % annato seed inclusion levels in diets based on corn and sorghum. Braz *et al*. (2007) added 0.0, 0.5, 1.0, 1.5 or 2% residual annato seeds to diets based on sorghum, replacing 100% corn, and did not observe any differences in layer performance. On the other hand, Queiroz (2006) and found reduced feed intake when the dietary inclusion of annato exceeded 3%. That author attributed the low feed intake of the birds fed sorghum with 6, 9 and 12% annato meal to the longer dwelling time of the feed in the digestive tract due to the high fiber content on annatto. Curvelo *et al*. (2009) did not find any significant differences ($p<0.05$) in feed intake, feed conversion ratio, average egg weight, or egg loss when annato extract and turmeric were added to layer feeds at 0.1 and 0.2% inclusion levels. In a study where broilers were fed 20 mg/bird/day turmeric and annato, Silva *et al*. (2001) concluded that these ingredients did not influence weight gain and feed conversion ratio on days 7, 14 or 21.

Egg production percentage was significantly affected by the treatments

Table 2 - Performance of layers fed diets based on corn and low-tannin sorghum containing different inclusion levels of annato seed and turmeric root.

Treatment	Egg weight	% lay	Egg mass	Feed intake (g/bird/day)	FCR/dz[1]	FCR/kg[2]	Mortality
Control	70.62	68.16 a	49.22	123.35	2.17	2.51	1.86
AS	69.83	70.00 ab	50.29	123.14	2.12	2.46	2.73
TR	69.91	71.83 ab	50.40	122.63	2.05	2.44	0.71
ASTR	70.89	74.83b	52.81	127.71	2.05	2.42	1.29
P	NS	<0.05	NS	NS	NS	NS	NS
CV (%)	2.45	4.77	5.37	3.15	3.65	4.06	98.46

annato seed presenting higher egg production as compared to the control birds, which were not fed these natural pigments. The other treatments presented intermediate values and were not different. These results are contrasting with those obtained by Curvelo *et al*. (2009), who did not find significant differences in egg production when lower levels of annato extract and turmeric were added to layer diets. Conversely, Garcia *et al*. (2009), observed lower egg production when dietary annato seed inclusion exceeded 1.5%, whereas, Silva *et al*. (2000) comparing the effect of annato residue dietary supplementation annato (4, 8 and 12%) in sorghum-based diets (40%) obtained better results with the level of 12%. Those authors did not observe any effect on egg specific gravity or egg weight; however, a linear increase in egg production was obtained, with the best results when 12% annato was added to the diet.

Egg quality parameters were not influenced (p>0.05) by the dietary treatments (Table 3). The results obtained in the present experiment are similar to those found by Silva *et al*. (2000) and Garcia *et al*. (2009). Braz *et al*. (2007) worked with different annato byproducts and levels, and did not find statistical differences in the studied egg quality parameters. In a study with annato extract and turmeric, Curvelo *et al*. (2009) also did not observe any influence on egg quality traits. In a study on the inclusion of paprika, marigold flowers and annato in layer diets, Lokaewmanee *et al*. (2010) did not observe any significant effects on Haugh units, egg weight, eggshell weight, yolk weight, albumen weight, or eggshell thickness.

Table 3 – Egg quality of layers fed diets based on corn and low-tanning sorghum containing different inclusion levels of annato seed and turmeric root.

Treatment	SEG[1]	Yolk %	Albumen %	Eggshell %
Control	1.077	25.52	65.84	8.88
AS	1.077	25.31	66.20	8.55
TR	1.074	25.79	66.05	8.16
ASTR	1.077	25.75	65.67	8.84
P	NS	NS	NS	NS
CV (%)	0.67	8.42	3.15	9.90

1 - SEG = specific egg gravity. Non-significant effect (P>0.05).

Results of the analyses of variance detected significant effects of all treatments (p<0.05) on the parameter yolk color. The analysis of regression for the effect of the number of days of pigment supply indicated an increasing linear effect on yolk color of the treatments AS and ASTR, which contained annato (Table 4). Yolk color intensity increased with duration of pigment supply in these treatments (p<0.05). The treatment that included the highest level of annato (AS) promoted more intense pigmentation on day 28 as compared to day 10 and day 7, suggesting higher pigment deposition in the yolk after day 10 of 2% annato supplementation in the feeds. The treatment ASTR, which included 1% annato and 1% turmeric, yolk pigmentation was also more intense on day 28 than on days 21 or 7, indicating that the use of curcumin together with annato, or that a lower levels of annato (1%) also pigments egg yolk.

The control and TR treatments, which did not contain annato, despite their significant effects on yolk color as detected by analysis of variance, presented quadratic regression results, indicating that there was no increasing linear effect on yolk color during pigment supply, as occurred when annato was supplemented in the diet. The curve starts with yolk color score 6.5 on day zero (due to the effect of the corn-soybean meal diet supplied until the day before the experiment started); however, this score was reduced and became stable on day 4, which suggests that when the corn-soybean meal diet was changed for corn-sorghum-soybean meal turmeric was not able to maintain the previous yolk pigmentation, presenting the same behavior as the control treatment, which was not supplemented with pigments. Oliveira *et al*. (2007) included annato in Japanese quail diets and observed an intensification of yolk color when pigments were continuously added to the feed for at least 21 days. The authors also observed that yolk pigmentation on day 28 was higher than on days 21, 7 and 14, independently of pigment dietary level. Curvelo *et al*. (2009) only observed changes in yolk color (score 8.39) when using 0.2% extrato annato extract; the other treatments (0.1% annato and 0.1 and 0.2% turmeric) resulted in the same yolk

Table 4 – Regression equations of yolk color of the eggs of layers fed diets based on corn and low-tannin sorghum containing different inclusion levels of annato seed and turmeric root.

Treatment	Yolk color score on different observation day							Equation	R^2 (%)
	0	3	7	10	14	21	28		
Control	6.5	5.8	4.6	4.4	4.1	4.0	4.0	$Y = 6.4355 - 0.2595 X + 0.006312 X^2$	97.02
AS	6.5	8.5	9.2	9.3	9.5	9.7	11.8	$Y = 7.5125 + 0.1434 X$	81.45
TR	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

color, but it must be mentioned that the basal diet was based in corn and soybean meal.

When the pigments were withdrawn from the feed, the analysis of variance detected significant effects only in treatments AS and ASTR, which contained annato ($p<0.05$) only on the parameter yolk color. The analysis of regression for the effect of days of supply of diets with pigments indicated a decreasing linear effect on the yolk color of the eggs of layer fed treatments AS and ASTR (Table 5). Three days after pigments were withdrawn from the feeds, yolk color faded ($p<0.05$).

h angles mean that yolk color is closer to yellow (TR), whereas lower angles indicate that the color is closer to red. The results showed that yolks resulting from the treatments containing annato presented lower h angle values, and therefore, their color was closer to red as annato dietary inclusion increased, whereas it increased in the other treatments, going from orange in treatment ASTR from yellow in the Control and TR treatments.

The color parameter C, which represent average sample chromaticity, defined the saturation and

Table 5 – Regression equations of yolk color of the eggs of layers fed diets based on corn and low-tannin sorghum containing different pigment inclusion levels.

Treatment	Yolk color score according to day of observation					Equation	R^2 (%)
	0	3	7	10	28		
AS	12.1	10.5	4.9	4.2	4.0	$Y= 12.6239 – 1.1877 X + 0.0314 X^2$	95.73
ASTR	9.8	9.1	4.4	4.1	4.0	$Y= 10.3226 – 0.8804 X + 0.0233 X^2$	92.38

The control and TR treatments did not maintain yolk color and therefore, did not present significant results.

Treatments AS and ASTR presented decreasing linear effect on yolk color after 7 days of withdrawal from the feeds. Egg yolks maintained their color up to three days after pigment withdrawal and then stabilized at colorimeric score 4.0. This shows that when annatto was removed from the diet, only the diet 50% corn replacement by sorghum was not able to maintain the previous yolk pigmentation after day 3.

The coordinate L* significantly decreased ($p<0.05$) as a function of increasing dietary annato levels. High luminosity values (L*) indicate lighter yolks, which are opposite to consumers' demands as darker yolks are associated to healthier, more natural eggs.

Table 6 – Means of the coordinates luminosity, tone, and chromaticity of raw eggs of layers fed diets with different natural pigments.

Treatment	Raw yolk		
	L*	h	C*
Control	55.24c	78.90b	47.22ab
TR	54.66c	83.57a	38.38b
AS	45.55a	65.64d	49.38a
ASTR	49.89b	72.82c	47.18ab
P	>0.05	>0.05	>0.05
CV	4.52	5.68	3.97

L* (luminosity), h (tone) and C* (chromaticity). Means followed by different letters in the same column are statistically different by the test of Tukey.

The parameter h, which defines the basic color of

intensity of the color defined in h (McGuirre, 1992). High chromaticity indicates higher color saturation and intensity. Chromaticity in the AS treatment was significantly higher as compared to the the treatment using only turmeric in the diet (TR), indicating that the color is more intense in yolk of the eggs of layers fed diets with 2% annato. The lowest saturation values (C) indicate the presence of white color in the yolks, as observed in the eggs of layers fed feeds containing turmeric as pigment.

Similar results were observed by Harder *et al*. (2008) with the inclusion of 2% annato seeds in layer diets.

CONCLUSIONS

In diets where sorghum replaced 50% of corn, the inclusion of turmeric and annato affected egg production and yolk color of the eggs of layers in their second production cycle. Yolk color in treatments containing annato seed was more saturated, more intense, and closer to red. Yolk pigmentation started on day 7 and was intensified on day 28. Dried turmeric root did not provide good yolk pigmentation, with a higher presence of white color in the yolk. The withdrawal of pigments from the diet results in color fading after 3 days.

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