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Internal egg quality of hens fed diets supplemented with natural pigments and canthaxanthin

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Abstract – The objective of this work was to evaluate the influence of the supplementation with natural pigments and canthaxanthin in wheat- and corn-based diets on the internal egg quality of laying hens. A total of 820 ISA Brown hens, at 78 weeks of age, were subjected to the following 20 supplementation treatments: turmeric, paprika, and annatto at 0.1, 0.2, 0.3, and 0.4%, combined with canthaxanthin at 0.003, 0.006, 0.009, and 0.012% in wheat-soybean meal based diets; canthaxanthin at 0.003 and 0.006% in corn-soybean meal based diets; and two control groups without pigment addition to the diets. Yolk color, the Haugh unit, and the yolk index were evaluated in ten fresh eggs per treatment at 0, 3, 7, 14, 21, and 28 days after supplementation. The addition of turmeric, paprika, and annatto up to 0.4% in wheat-based diets reduced yolk color, without affecting the Haugh unit and yolk index. The addition of canthaxanthin starting at 0.003%, irrespectively of the diet, increased yolk color without affecting the Haugh unit and yolk index. Although the supplementation with canthaxanthin in wheat and corn-based diets of laying hens greatly increases yolk color, both the natural pigments and the synthetic one do not affect internal egg quality.

Index terms: annatto, paprika, pigmentation, turmeric, yolk color.

Qualidade interna de ovos de poedeiras alimentadas com dietas suplementadas com pigmentos naturais e cantaxantina

Resumo – O objetivo deste trabalho foi avaliar a influência da suplementação com pigmentos naturais e cantaxantina em dietas à base de trigo e milho, na qualidade interna de ovos de poedeiras. Um total de 820 galinhas ISA Brown, com 78 semanas de idade, foram submetidas aos seguintes 20 tratamentos de suplementação: cúrcuma, páprica e urucum a 0,1, 0,2, 0,3 e 0,4% com cantaxantina a 0,003, 0,006, 0,009 e 0,012%, em dietas à base de trigo-soja; cantaxantina a 0,003 e 0,006%, em dietas à base de milho-soja; e dois grupos controle sem adição de pigmentos nas dietas. Avaliaram-se a cor da gema, a unidade Haugh e o índice de gema em dez ovos por tratamento, aos 0, 3, 7, 14, 21 e 28 dias pós-suplementação. A adição de cúrcuma, páprica e urucum até 0,4% em dietas à base de trigo reduziu a cor da gema, sem afetar a unidade Haugh e o índice de gema. A adição de cantaxantina a partir de 0,003%, independentemente da dieta, aumentou a cor da gema sem afetar a unidade Haugh e o índice de gema. Embora a suplementação com cantaxantina nas dietas à base de trigo e milho para poadeiras aumente muito a coloração da gema, tanto os os pigmentos naturais como o sintético não afetam a qualidade interna dos ovos.

Termos para indexação: urucum, páprica, pigmentação, cúrcuma, cor da gema.

Introduction

From an economical point of view, wheat (*Triticum aestivum* L.) can replace corn (*Zea mays* L.) in poultry nutrition, mainly in periods of corn scarcity. However, although wheat is suitable as poultry feed, it has slightly lower energy contents (5.0–7.0%) than corn and is deficient in some amino acids and carotenoid pigments, requiring supplementation in diets for commercial laying hens, in order to avoid the discoloration of the egg yolk (Fassani et al., 2019).

The allocation of pigments to specific tissues depends on the birds' digestive capacity, absorption efficiency, and metabolic processes, as well as on the rate of deposition and the quantity of pigments present in their diets (Chaves et al., 2022). Briefly, following digestion, pigments are assimilated from the chicken's intestinal tract and integrated into triglyceride-rich lipoproteins (chylomicrons), subsequently released into the circulatory system and transported to the yolk, affecting its color (Salma et al., 2007, Lokaewmanee et al., 2010, Oliveira et al., 2021).

Regarding yolk colors, scores from 7 to 10 in the DSM yolk color fan (Maastricht, the Netherlands) are preferred by consumers in Brazil and the United States, but from 10 to 14, i.e., more pigmented, in Europe and Asia (Galobart et al., 2004; Moura et al., 2016). To intensify yolk pigmentation, pigments can be added to the rations of hens through synthetic or natural additives, or a combination of them (Papadopoulos et al., 2019). However, the use of these additives depends on the country's regulations.

In Brazil, for example, the current legislation establishes that only natural pigments should be used in the colony egg production system (ABNT, 2016). In addition, the search for natural pigments has increased due to the recent prohibition of most artificial pigments in diets for laying hens by the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives, on which the Brazilian legislation is based on (Valentim et al., 2019). In this scenario and with the current consumer demand for healthier and natural products, the main sources of natural pigments used in poultry nutrition are paprika (*Capsicum annuum* L.), calendula (*Tagetes erecta* L.) flower, annatto (*Bixa orellana* L.), and turmeric (*Curcuma longa* L.), while canthaxanthin is the reference synthetic pigment (Fassani et al., 2019; Valentim et al., 2019). Natural pigments can be extracted from plant parts and require a higher concentration than synthetic ones in the diet of hens, in order to obtain the desired color in egg yolks.

Canthaxanthin ($C_{40}H_{52}O_2$) is used to intensify the red-orange color of the egg yolk, and its pigmentation capacity has been reported in studies on its supplementation in combination with yellow carotenoids (Faruk et al., 2018; Maia et al., 2022). However, there is a lack of knowledge about how internal egg quality is affected when replacing synthetic pigments by natural ones, such as paprika, annatto, and turmeric, mainly in wheat-based diets.

Paprika is considered one of the most consumed natural condiments worldwide and the most used in laying-hen diets. Its extract is obtained by grinding dehydrated chili (*C. annum*) and has a red coloration, with a red-orange pigmentation capacity (Galobart et al., 2004; Moura et al., 2011). For ISA Brown laying hens, Galobart et al. (2004) observed an improved yolk color with the use of paprika in diets with 50% wheat.

Annatto is one of the main sources of dye used around the world, mainly in the food and pharmaceutical industries, also being included in poultry nutrition. Its extract is obtained from the seed, whose main pigment is bixin, which has a red-orange staining capacity (Braz et al., 2007). Some studies have shown the positive results of the supplementation with annatto in corn and sorghum [*Sorghum bicolor* (L.) Moench]-based diets in the color of the egg yolks of laying hens (Martínez et al., 2021; Oliveira et al., 2021).

Turmeric presents, in its composition, the pigment curcumin, which is widely used as a dye and food condiment. Its extract has been studied as a pigment in animal feed, particularly for poultry due to its yellow-orange polyphenol (Khan et al., 2012), used for yolk pigmentation (Abbas et al., 2010). Although the positive influence of pigments on egg yolk color has already been reported (Valentim et al., 2019; Martínez et al., 2021), the effects of most natural pigments added to 100% wheat diets are not yet known.

The objective of this work was to evaluate the influence of the supplementation with natural pigments and canthaxanthin in wheat- and corn-based diets on the internal egg quality of laying hens.

Materials and Methods

The experiment was carried out at a laying farm located in the municipality of Ouro, in the state of

Santa Catarina, Brazil. The animals were housed at a density of 7 hens per square meter, in groups of 41, in 20 experimental pens. Each pen, measuring 1.5×4.0 m, contained a tube feeder, five nipple drinkers, three perching platforms, and eight nesting boxes. The pen floor was covered with 10 cm of pine shavings. The protocol and methods for this experiment were approved by the commission for ethics in animal experimentation of Embrapa Suínos e Aves, under registration number 13/2022.

A total of 820 ISA Brown laying hens, with 78 weeks of age and an average body weight of 2.1 kg, were used. The hens were subjected to the following 20 supplementation treatments: turmeric, paprika, and annatto at the levels of 0.1, 0.2, 0.3, and 0.4% each, combined with canthaxanthin at the levels of 0.003, 0.006, 0.009, and 0.012% in wheat and soybean meal-based diets; canthaxanthin at the levels of 0.003 and 0.006% in corn and soybean meal-based diets; and two control groups without the addition of pigments in corn and soybean meal- and in wheat and soybean meal-based diets.

The experimental design was completely randomized, in a split-plot arrangement, with treatments assigned to the main plots and supplementation time assigned to the split-plots.

Before the experiment, the birds received a standard diet based on corn and soybean meal, without any pigments. The experimental diets were formulated based on corn and soybean meal or on wheat and soybean meal (Table 1), aiming to meet the nutritional requirements of laying hens over 70 weeks of age and with a laying rate higher than 80%, based on the nutritional requirements of poultry and pigs in Brazil (Rostagno et al., 2017).

The laying hens were weighed weekly and received the experimental diets through a fixed food-supply program of 125 g per bird per day during 30 days. The water supply was ad libitum, and the light program was 16 hours a day. Feed supply and egg collection were performed manually. The field experiment lasted four weeks.

The experimental diets based on wheat received the addition of threonine, to balance essential amino acids. The powder extracts of the pigments were, then, included in the experimental diets by replacing the inert in the amounts of 0.003, 0.006, 0.009, 0.012, 0.1, 0.2, 0.3, or 0.4%.

Yolk color density, the Haugh unit, and the yolk index were determined using the DET6000 digital Egg Tester equipment (Nabel Co., Ltd., Kyoto, Japan). The analyses were performed on two fresh eggs per treatment at day 0 and, then, on ten eggs per treatment on days 3, 7, 14, 21, and 28 after the beginning of the experiment. Therefore, a total of 1,040 eggs were collected and analyzed individually. Day 0, before the supplementation with pigments, was considered the reference value for the variable responses.

The eggs were sent to the Laboratory of Physicochemical Analysis of Embrapa Suínos e Aves, where they were stored at a controlled temperature of 17°C until their analysis on the day after collection. All analyses were performed in the same place, time, and environment.

The easyanova package (Arnhold, 2013) of the R statistical software (R Core Team, 2022) was used. The statistical analyses were carried out according to the following model: $Y_{ijk} = \mu + d_i + s_j + w_k + dw_{ik} + \varepsilon_{ijk}$, where μ is the overall mean, d_i is the fixed effect of treatments ($i = 1$ to 20), s_j is the random effect of subject ($j = 1$ to 20), w_k is the fixed effect of supplementation time ($k = 0$, 3, 7, 14, 21, and 28 days), dw_{ik} is the interaction between treatment and supplementation time, and ε_{ijk} is the random error (residual error).

The residual covariance matrix structures for model adjustment were selected in function of the lowest value obtained in the Akaike information criterion. The analysis of variance was used to test contrasts between treatment means, and, afterwards, the multi-comparison procedure was executed, comparing the adjusted means through Tukey's test, at 5% probability. Linear and quadratic regression analyses were also performed to verify the relationship between the inclusion levels of each pigment in the diets and the response variables.

To determine the relationship between time of supplementation and yolk color, the quadratic plateau polynomial model was applied using the nonlinear procedure of the easynls package of the R software (R Core Team, 2022), with the following argument: $y \sim ((a + b) \times (x + c) \times I(x^2)) \times (x \leq -0.5 \times b/c) + (a + I(-b^2/(4\ c))) \times (x > -0.5 \times b/c)$. The models were fitted using the averages of the doses obtained in the initial analysis of variance. The hypothesis was that, according to time of supplementation, a higher pigment supply in the diets should correlate to a more intense color in yolk yield, but that this relationship will most likely not be linear. For this reason, the quadratic plateau model, a type of segmented model that has a curved component that meets a zero-slope plateau at the joining point, was used. All analyses were executed using the R software (R Core Team, 2022).

Table 1. Ingredients and nutritional composition of the experimental diets.

Ingredient (kg)	Corn-based diet	Wheat-based diet
Ground corn	61.287	-
Ground wheat	-	65.560
Soybean meal	23.583	17.837
Calcitic limestone	9.654	9.727
Soybean oil	3.333	4.733
Dicalcium phosphate	0.767	0.637
Salt	0.401	0.403
Vitamin/mineral mix(1)	0.300	0.300
Mycotoxin binder(2)	0.100	0.100
DL-methionine	0.111	0.110
L-lysine	0.010	0.103
L-valine	0.042	0.042
L-threonine	-	0.036
BHT	0.010	0.010
Phytase(3)	0.003	0.003
Inert	0.400	0.400
Total	100.000	100.000
Calculated nutritional composition	Corn-based diet	Wheat-based diet
Metabolizable energy (kcal kg^{-1})	2.8500	2.8500
Crude protein (%)	15.6189	15.9384
Crude fiber (%)	2.2064	2.4206
Fat (%)	6.1148	6.1171
Calcium (%)	4.0000	4.0000
Available phosphorous (%)	0.3400	0.3400
Total phosphorous (%)	0.5352	0.5419
Sodium (%)	0.1700	0.1700
Digestible methionine (%)	0.4069	0.4000
Digestible lysine (%)	0.7300	0.7300
Digestible methionine + cystine (%)	0.5300	0.6619
Digestible threonine (%)	0.5463	0.5100
Digestible tryptophan (%)	0.1730	0.1821
Digestible isoleucine (%)	0.6000	0.6000
Digestible valine (%)	0.7000	0.7000
Digestible arginine (%)	0.9496	0.9034
Linoleic acid (%)	3.0809	2.9353

(1)Minimum composition per kilogram of the commercial product Ovotec Matriz P-3 (Agrifirm Brasil, Curitiba, PR, Brazil): 3,330 mg copper, 16.65 g iron, 33 g manganese, 100 mg selenium, 33.3 g zinc, 4,000,800 IU vitamin A, 1,000,200 IU vitamin D3, 30,000 IU vitamin E, 1,674 mg vitamin K3, 980.2 mg vitamin B1, 4,000 mg vitamin B2, 1,633.7 mg vitamin B6, 10,000 mcg vitamin B12, 1,060 mg folic acid, 4,980 mg pantothenic acid, 16 g niacin, 100 mg biotin, 140.6 g choline, and 660 mg iodine. (2)Mastersorb Gold (VitallTech, Sarandi, RS, Brazil). (3)10,000 FTU Natuphos (BASF Brasil, São Paulo, SP, Brazil). BHT, butylated hydroxytoluene.

Results and Discussion

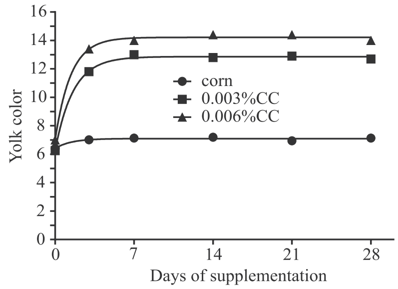
In the corn-based diets, the color of the yolk was within the score of 7 to 10 in the DMS yolk color fan preferred by Brazilian consumers, even without the addition of pigments; however, in the wheat-based diets, it only reached values close to 3.0 at 28 days from supplementation, at higher pigment doses (Table 2). Gurbuz et al. (2003) observed that the main carotenoid found in corn is lutein, which increases the yellow pigmentation of the yolk, but does not adequately enhance the red one, limiting a superior pigmentation. Despite this, according to Moura et al. (2011), the corn and soybean meal-based feed meets satisfactorily the yolk pigmentation preferred by Brazilians and Americans.

The values of the coefficient of determination (R^2) were high and similar, indicating that the used models had a good fit to the data (Figure 1). In the corn- and wheat-based diets, an initial increase in yolk pigmentation could already be observed in the plots on the third day from canthaxanthin supplementation. Yolk color began to stabilize on the fourth day, stabilizing from the sixth day at maximum values close to 14.5 with the addition of canthaxanthin levels above 0.006%. However, in the corn-based diets, the color of the yolk does not appear to increase anymore from about five days after the beginning of the canthaxanthin supplementation, reaching a plateau of 12.85 and 14.2 for the 0.003 and 0.006% levels of the synthetic pigment, respectively. The canthaxanthin level of 0.003%, just after three days of supplementation, was sufficient to produce eggs with a yolk color that met the standard for pigmentation intensity of the most demanding consumers, both in the corn- and wheat-based diets. These results align with those reported by

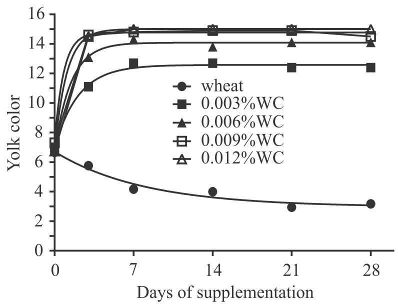
Table 2. Yolk color of the eggs of commercial laying hens fed with corn- and wheat-based diets with supplementation levels of natural and synthetic pigments[(1)].

Treatment[(2)]	0 days (n=2)	3 days (n=10)	7 days (n=10)	14 days (n=10)	21 days (n=10)	28 days (n=10)	Total mean
Corn	6.42dA	7.01dA	7.13dA	7.18dA	6.94dA	7.13dA	6.97d
0.003%CC	6.24aC	11.83cB	13.01cA	12.81cA	12.94cA	12.70cA	11.58c
0.006%CC	6.98aC	13.42bB	13.96bAB	14.38abAB	14.41abA	13.99bAB	12.85b
Wheat	6.64aA	5.76bE	4.16cEG	3.98cEF	2.93dEG	3.17dEG	4.44eh
0.003%WC	7.00cA	11.13cB	12.73cA	12.66cA	12.39cA	12.40cA	11.38c
0.006%WC	6.75cA	13.10bB	14.28abA	13.78bAB	14.05bA	14.06abA	12.67b
0.009%WC	7.28bA	14.57aA	14.85abA	14.90aA	14.95abA	14.54abA	13.51a
0.012%WC	6.75bA	14.53aA	15.00aA	15.00aA	15.00aA	15.00aA	13.54a
0.1%WT	7.17aA	6.12deB	4.52egC	3.40efD	3.53egD	3.16egD	4.65efg
0.2%WT	7.23aA	6.18deB	5.01eC	2.96eD	3.58egD	2.93egD	4.64efg
0.3%WT	7.19aA	6.05deB	4.85eC	3.56efD	3.35egD	3.36egD	4.72ef
0.4%WT	6.95aA	6.00eB	4.10egC	3.21efD	2.95egD	2.44gD	4.27fh
0.1%WP	6.75aA	5.84eB	3.68gC	3.15efCD	2.85fgD	2.88egCD	4.19gh
0.2%WP	7.53aA	5.85eB	4.24egC	3.23efD	3.28egD	3.34egD	4.58eh
0.3%WP	7.02aA	5.98eB	4.55cEG	3.94fCD	3.43egD	3.54efD	4.74ef
0.4%WP	6.37aA	6.12deA	4.89eB	3.95fC	3.84eC	3.69eC	4.81e
0.1%WA	6.25aA	5.82eA	3.84fgB	3.44efBC	2.60gD	2.74fgCD	4.11h
0.2%WA	7.13aA	5.85eB	4.40egC	3.58efD	3.70efCD	3.18egD	4.64efg
0.3%WA	7.14aA	5.84eB	4.60egC	3.83efCD	3.30egD	3.36egD	4.68efg
0.4%WA	6.51aA	5.91eB	4.69efB	3.55efC	3.64efC	3.38egD	4.61eh
Total mean	6.87C	8.14A	7.42B	6.82CD	6.68DE	6.54E	-
SEM	0.42	0.18	0.18	0.18	0.18	0.18	-

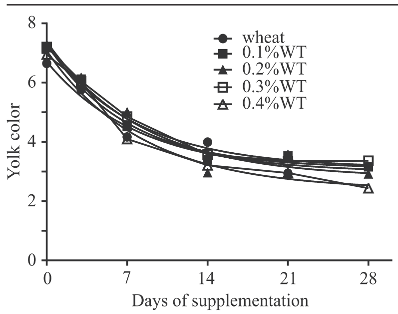
[(1)]Means followed by equal letters, lowercase in the columns and uppercase in the lines, do not differ for the effect of diets and supplementation time, respectively, by Tukey's test, at 5% probability. [(2)]CC, corn + canthaxanthin ($C_{40}H_{52}O_2$); WC, wheat + canthaxanthin; WT, wheat + turmeric (*Curcuma longa*); WP, wheat + paprika (*Capsicum annuum*); and WA, wheat + annatto (*Bixa orellana*). SEM, standard error of the mean.



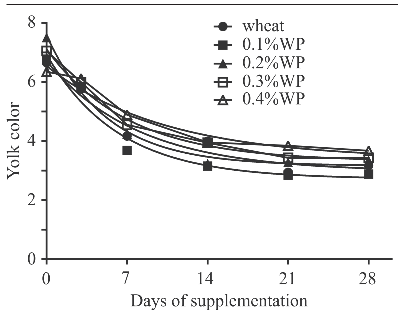
Level	Equation	R^2	Plateau	Critical point in x
0	$y= 6.4+0.3003033x-0.03232331x^2$	0.91	7.09	4.64
0.003	$y= 6.25+2.645007x-0.2650024x^2$	0.99	12.85	4.99
0.006	$y= 7+3.25x-0.3555556x^2$	0.99	14.20	4.50



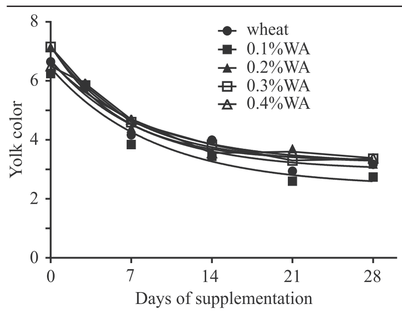
0	$y= 6.5505503-0.3201058x+0.0074868x^2$	0.95	3.12	21.37
0.003	$y= 7+1.808792x-0.1473751x^2$	0.99	12.55	6.13
0.006	$y= 6.75+3.101714x-0.3283491x^2$	0.99	14.07	4.72
0.009	$y= 7.3+4.220845x-0.5958371x^2$	0.99	14.77	3.54
0.012	$y= 6.75+4.147921x-0.521529x^2$	0.99	14.99	3.97



0	$y= 6.5505503-0.3201058x+0.0074868x^2$	0.95	3.12	21.37
0.1	$y= 7.277422-0.4847506x+0.01490029x^2$	0.99	3.33	16.26
0.2	$y= 7.382662-0.4659824x+0.01282012x^2$	0.96	3.14	18.17
0.3	$y= 7.200672-0.4159935x+0.01124302x^2$	0.98	3.35	18.50
0.4	$y= 7.017218-0.4586244x+0.01227868x^2$	0.97	2.73	18.67



0	$y= 6.5505503-0.3201058x+0.0074868x^2$	0.95	3.12	21.37
0.1	$y= 6.9338-0.5621194x+0.01978191x^2$	0.97	2.94	14.20
0.2	$y= 7.510625-0.6281749x+0.002333061x^2$	0.99	3.28	13.46
0.3	$y= 7.02077-0.3985504x+0.0112876x^2$	0.98	3.50	17.65
0.4	$y= 6.540226-0.2679362x+0.006393016x^2$	0.97	3.73	20.95



0	$y= 6.550503-0.3201058x+0.00748682x^2$	0.95	3.13	21.37
0.1	$y= 6.356161-0.3408907x+0.00796921x^2$	0.95	2.71	21.38
0.2	$y= 7.174668-0.5153407x+0.01797784x^2$	0.98	3.48	14.33
0.3	$y= 7.0555025-0.4126726x+0.011654374x^2$	0.99	3.40	17.70
0.4	$y= 6.630733-0.3365242x+0.00892x^2$	0.98	3.45	18.85

Figure 1. Yolk color with the addition of different doses of turmeric (*Curcuma longa*), paprika (*Capsicum annuum*), annatto (*Bixa orellana*), and canthaxanthin ($C_{40}H_{52}O_2$) at different supplementation times in corn- and wheat-based diets, using nonlinear quadratic models with a plateau. CC, corn + canthaxanthin; WC, wheat + canthaxanthin; WT, wheat + turmeric; WP, wheat + paprika; and WA, wheat + annatto.

Faruk et al. (2018) through a meta-analysis, revealing a linear relationship (R^2=0.79) between the quantity of canthaxanthin present in the feed and egg yolk; in their study, the supplementation range of this synthetic pigment was from 0.0 to 8.0 mg per kilogram of feed. To ensure consumer safety, the European Union (2015) establishes an upper limit of 8.0 mg per kilogram of canthaxanthin in the feed of laying hens (European Union, 2015). However, the use of synthetic pigments requires the application of minimal quantities to achieve the desired color intensity (Moraleco et al., 2019). Therefore, to elucidate the observed effects, the mechanisms associated with carotenoid metabolism and functions in avian species represent a new research area (Esatbeyoglu & Rimbach, 2017).

The models used to evaluate turmeric, paprika, and annatto, at all supplementation levels, presented very similar curves, showing the depigmentation of the egg yolk in wheat-based diets throughout the experimental period (Figure 1). The addition of natural pigments was not enough to maintain or intensify the initial staining of the yolks at the beginning of the experiment or equivalent to the values observed in corn-based diets with or without the addition of synthetic pigments.

With the used models, the obtained estimates ranged from 2.71 to 3.5 for the plateau values for yolk color and from 13.48 to 21.38 for critical points. A significant decrease in yolk pigmentation was observed up to 14 days after the beginning of supplementation with turmeric, paprika, or annatto in the wheat-based diets. Yolk color started to stabilize between 14 and 21 days, finally stabilizing from 21 days from supplementation at minimum values close to 3.0. In the literature, the days to reach the plateau were: 14 days for various natural pigments in the diet of light-laying hens (Hammershøj et al., 2010), 15 days for yellow and red synthetic pigments (Sandeski et al., 2014), and 18 days in diets that contained synthetic and natural pigments (Chaves et al., 2022). In general, the efficiency of yolk pigmentation depends on several factors, such as carotenoid amount ingested, period of consumption, and absorption by birds (Amaya et al., 2014).

Yolk color was more intense in the diets with canthaxanthin than with the natural pigments. Similarly, Valentim et al. (2019) found that canthaxanthin resulted in a higher average yolk color than the used paprika extract, whose dose would need to be 0.8% in the diet to ensure a yolk color close to that of the synthetic pigment.

The saponified extract from paprika contains from 25 to 90 g per kilogram of diverse carotenoids, with the predominance (35% of the total carotenoids) of capsanthin, a brightly orange-red-colored pigment present in paprika fruits, belonging to xanthophylls, i.e., oxygen-containing carotenoids (EFSA et al., 2020). According to these authors, the recommended maximum usage level of 40 mg total carotenoids per kilogram of feed is considered safe for hens intended both for fattening and laying. In their research, egg yolk color exhibited a significant increase in intense red color with higher levels of the saponified extract over the entire range of 15–240 mg total carotenoids per kilogram, but a decreased lightness and yellowness. In addition, a satisfactory egg yolk pigmentation, with a score around 14 in the DSM yolk color fan, was achieved with 15 mg total carotenoids per kilogram of feed, while higher values were obtained with 80 and 240 mg total carotenoids per kilogram of complete feed, surpassing consumer expectations for table eggs.

Although some studies have consistently shown a dose-dependent effect of paprika extract on egg yolk color (Lai et al., 1996; Galobart et al., 2004), others have confirmed this effect with the lowest effective dose (Baiao et al., 1999; González et al., 1999), as that of 4.0 mg total carotenoids per kilogram of feed (Lai et al., 1996; Santos-Bocanegra et al., 2004). In a recent study, Saleh et al. (2021) verified that the color of the egg yolk of hens fed a diet with 4.0 kg per milligram of natural paprika appeared yellower than that of those fed the control diet without any colorant supplementation.

The diets supplemented with annatto showed a poorer pigmentation than that obtained with the control corn-based diet and the wheat-based diets with the addition of canthaxanthin. The seed of annatto, a nontoxic native plant, is a natural pigment known for its safety and is abundant in the carotenoids bixin and norbixin, whose ratios vary among cultivars, being typically higher for bixin (Preston & Rickard, 1980). For laying hens, Silva et al. (2000) found that the addition of 0.1% annatto extract to diets containing 40% sorghum resulted in an egg yolk pigmentation comparable to that of diets using corn as the main energy source. Similarly, Garcia et al. (2010) stated that the incorporation of 0.89% ground annatto seeds into sorghum-based feeds for layers is sufficient to obtain a yolk pigmentation comparable to that of corn-based diets. However, Braz et al. (2007) observed that the inclusion of 2.0% of an annatto seed extract in sorghum-based commercial layer diets failed to produce the same egg yolk color as that achieved with the corn-based diet. Likewise, Silva et al. (2006) verified that supplementing feeds with 4.0, 8.0, or 12% annatto extract in combinations containing up to 40% sorghum did not result in a yolk color equivalent to that obtained with the diet based on corn and soybean meal. According to Garcia et al. (2009), these findings could be explained by the lower pigmentation efficiency of natural sources compared with synthetic pigments.

In the present study, turmeric did not enhance yolk pigmentation in the wheat-based diets as reported in the literature. Ayed et al. (2018), investigating the inclusion of 0.5 to 2.0% turmeric in the diet of laying hens, found that the intensity of yolk color exhibited a linear and positive correlation with an increasing dose of turmeric (R^2=0.975). This suggests that a higher addition of turmeric leads to a more intense egg yolk color. Similarly, Park et al. (2012) observed a significant increase in yolk color with the addition of 0.5% dietary turmeric powder, when compared with a control diet. Riasi et al. (2012) also verified a significant effect on yolk color after four weeks of dietary supplementation with turmeric due to its yellowish pigments, such as curcuminoids, curcumin, and related compounds. In contrast, Gumus et al. (2018) concluded that yolk color remained unaffected by dietary supplementation with 0.5% of turmeric, which is in alignment with the findings of the present work.

The Haugh unit pattern did not differ between treatments throughout supplementation times, i.e., the interaction between these two factors was not significant (Table 3). A significantly higher total mean

Table 3. Haugh unit of the eggs of commercial laying hens fed with corn- and wheat-based diets with supplementation levels of natural and synthetic pigments[(1)].

Treatment[(2)]	0 days (n=2)	3 days (n=10)	7 days (n=10)	14 days (n=10)	21 days (n=10)	28 days (n=10)	Total mean
Corn	80.42aA	81.48aA	75.83aA	85.94abA	85.11aA	79.96aA	81.46ab
0.003%CC	81.58aA	79.55aA	79.31aA	82.00abA	80.59aA	82.36aA	80.90ab
0.006%CC	78.65aA	84.64aA	75.05aA	77.62abA	76.18aA	82.08aA	79.03ab
Wheat	84.22aA	85.06aA	79.83aA	85.19abA	83.80aA	87.88aA	84.33ab
0.003%WC	78.99aA	77.12aA	77.19aA	79.81abA	80.32aA	79.50aA	78.82ab
0.006%WC	84.60aA	85.55aA	80.79aA	81.09abA	81.69aA	80.20aA	82.32ab
0.009%WC	77.95aA	76.88aA	76.53aA	78.08abA	80.33aA	77.41aA	77.86b
0.012%WC	80.19aA	79.46aA	75.98aA	81.67abA	81.65aA	79.16aA	79.68ab
0.1%WT	77.66aA	83.39aA	86.73aA	87.84aA	84.63aA	85.06aA	84.21ab
0.2%WT	78.05aA	73.04aA	80.04aA	78.82abA	81.82aA	81.95aA	78.95ab
0.3%WT	85.75aA	83.68aA	82.65aA	81.16abA	76.95aA	82.12aA	82.05ab
0.4%WT	82.02aA	81.61aA	83.00aA	80.94abA	83.72aA	83.48aA	82.46ab
0.1%WP	80.69aAb	87.13aA	78.20aAB	72.79bB	86.39aA	89.31aA	82.41ab
0.2%WP	91.53aA	82.50aA	82.59aA	87.42aA	84.47aA	85.51aA	85.67a
0.3%WP	86.18aA	84.31aA	78.60aA	82.84abA	85.48aA	84.33aA	83.62ab
0.4%WP	81.12aA	82.04aA	76.10aA	76.59abA	84.64aA	81.41aA	80.31ab
0.1%WA	86.01aA	84.83aA	84.60aA	80.89abA	79.75aA	78.88aA	82.49ab
0.2%WA	78.93aA	84.03aA	75.92aA	80.72abA	83.19aA	80.58aA	80.56ab
0.3%WA	79.99aA	78.00aA	80.99aA	80.59abA	84.91aA	77.33aA	80.30ab
0.4%WA	76.50aA	73.43aA	80.11aA	81.18abA	81.27aA	75.33aA	77.97b
Total mean	81.55AB	81.38AB	79.50B	81.16AB	82.34A	81.69AB	-
SEM	6.47	2.89	2.89	2.89	2.89	2.89	-

[(1)]Means followed by equal letters, lowercase in the columns and uppercase in lines, do not differ for the effect of diets and supplementation time, respectively, by Tukey's test, at 5% probability. CC, corn + canthaxanthin ($C_{40}H_{52}O_2$); WC, wheat + canthaxanthin; WT, wheat + turmeric (*Curcuma longa*); WP, wheat + paprika (*Capsicum annuum*); and WA, wheat + annatto (*Bixa orellana*). SEM, standard error of the mean.

of 85.7 was obtained for 0.2% paprika, compared to those of 77.9 for 0.009% canthaxanthin and of 78 for 0.4% annatto in the wheat-based diets. Despite this, the obtained results are an indicative that canthaxanthin, turmeric, paprika, and annatto do not affect the quality of the egg albumen of hens fed with corn- or wheat-based diets. Until the end of the experimental period, the Haugh unit remained within the standard established by the U.S. Department of Agriculture (USDA, 2000), as excellent quality (AA), regardless of the pigment and cereal used. Other authors also concluded that synthetic and natural pigments do not influence Haugh unit values (Garcia et al., 2009; Fassani et al., 2019).

The yolk index did not differ significantly according to treatments and time of supplementation, with no significant interaction between these two factors (Table 4). The range of this index was within the recommended for a good quality of fresh eggs, which is in alignment with the results obtained by Papadopoulos et al. (2019) and Valentim et al. (2019) when adding pigments to poultry diets. These authors found that the treatments only altered the intensification of egg yolk color, without influencing the other variables related to the egg quality of laying hens.

The present study highlighted the limitations of the natural pigments turmeric, paprika, and annatto, at a level of 0.4%, in achieving the desired yolk color. This underscores the need for further research to determine their optimal supplementation levels in wheat-based diets. However, in cases of corn scarcity within the conventional egg production system, the addition of 0.003% canthaxanthin to the wheat-based diet emerges as a viable solution, ensuring yolk color without compromising egg quality.

Table 4. Yolk index of eggs from commercial laying hens fed with corn- and wheat-based diets with supplementation levels of natural and synthetic pigments[(1)].

Treatment[(2)]	0 days (n=2)	3 days (n=10)	7 days (n=10)	14 days (n=10)	21 days (n=10)	28 days (n=10)	Total mean
Corn	0.49aA	0.42aA	0.44aA	0.38aA	0.42abA	0.45aA	0.43a
0.003%CC	0.26aB	0.36aAB	0.40aAB	0.39aAB	0.45abB	0.36aAB	0.37a
0.006%CC	0.41aA	0.42aA	0.37aA	0.38aA	0.34bA	0.43aA	0.39a
Wheat	0.32aA	0.38aA	0.43aA	0.38aA	0.38abA	0.38aA	0.38a
0.003%WC	0.36aA	0.40aA	0.39aA	0.38aA	0.43abA	0.39aA	0.39a
0.006%WC	0.43aA	0.40aA	0.42aA	0.41aA	0.45abA	0.41aA	0.42a
0.009%WC	0.25aB	0.43aA	0.33aAB	0.39aAB	0.33bAB	0.37aAB	0.35a
0.012%WC	0.31aA	0.40aA	0.39aA	0.39aA	0.33bA	0.37aA	0.37a
0.1%WT	0.47aA	0.36aA	0.39aA	0.33aA	0.40abA	0.34aA	0.38a
0.2%WT	0.40aA	0.42aA	0.40aA	0.38aA	0.34bA	0.41aA	0.39a
0.3%WT	0.52aA	0.35aB	0.34aB	0.36aAB	0.42abAB	0.41aAB	0.40a
0.4%WT	0.46aA	0.39aA	0.40aA	0.36aA	0.42abA	0.37aA	0.40a
0.1%WP	0.34aA	0.34aA	0.39aA	0.38aA	0.39abA	0.37aA	0.37a
0.2%WP	0.18aB	0.32aAB	0.41aA	0.41aA	0.42abA	0.41aA	0.36a
0.3%WP	0.55aA	0.42aAB	0.40aAB	0.36aB	0.44abAB	0.44aAB	0.43a
0.4%WP	0.30aA	0.32aA	0.40aA	0.41aA	0.40abA	0.41aA	0.37a
0.1%WA	0.40aA	0.37aA	0.36aA	0.40aA	0.42abA	0.31aA	0.37a
0.2%WA	0.40aA	0.35aA	0.40aA	0.38aA	0.38abA	0.40aA	0.38a
0.3%WA	0.37aB	0.35aB	0.35aB	0.41aAB	0.55aA	0.39aB	0.40a
0.4%WA	0.22aB	0.36aAB	0.39aA	0.32aAB	0.28bAB	0.40aA	0.33a
Total mean	0.37A	0.38A	0.39A	0.38A	0.40A	0.39A	-
SEM	0.83	0.37	0.37	0.37	0.37	0.37	-

[(1)]Means followed by equal letters, lowercase in the columns and uppercase in the lines, do not differ for the effect of diets and supplementation time, respectively, by Tukey's test, at 5% probability. CC, corn + canthaxanthin ($C_{40}H_{52}O_2$); WC, wheat + canthaxanthin; WT, wheat + turmeric (*Curcuma longa*); WP, wheat + paprika (*Capsicum annuum*); and WA, wheat + annatto (*Bixa orellana*). SEM, standard error of the mean.

Conclusions

1. In wheat- and corn-based diets for laying hens, the supplementation with the synthetic pigment canthaxanthin ($C_{40}H_{52}O_2$) greatly intensifies yolk color, compared with the studied natural pigments.

2. Canthaxanthin, turmeric (*Curcuma longa*), paprika (*Capsicum annuum*), and annatto (*Bixa orellana*) do not affect egg quality, maintaining the Haugh unit pattern and the yolk index regardless of the used diet.

3. Corn-based diets meet the desired yolk color preference in Brazil, even without the addition of pigments.

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