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Performance and egg quality of light laying hens fed with canthaxanthin and marigold flower extract

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Abstract

The aim of this study was to determine the best level of inclusion of natural (marigold flower extract) and synthetic (canthaxanthin) pigments in the diet of light laying hens from 75 to 85 weeks old in terms of effects on performance, egg quality, and economic viability of production. A total of 288 laying hens were used in a completely randomized design, with a 4 x 4 factorial arrangement, with four levels of marigold flower extract (2.10; 2.40; 2.70; 3.00 ppm) and four of canthaxanthin (0. 40; 0.70; 1.00; 1.30 ppm), with three replications and six hens per experimental unit. The feed conversion by mass of eggs, egg mass, and egg laying rate showed linear improvement with the inclusion of canthaxanthin. The yolk index showed a quadratic effect with the inclusion of marigold and canthaxanthin, presenting a better estimate with diets containing 2.60 ppm/kg of marigold feed and 0.95 ppm/kg of canthaxanthin feed. The percentage of yolk and the Haugh unit increased linearly with the rising levels of marigold, whereas the percentage of albumen decreased linearly. In the evaluation of the YolkFan DSM® and the redness/yellowness, chroma (a*) presented a quadratic effect for the inclusion of marigold (2.73 and 2.80 ppm/kg of feed) and linear increase with canthaxanthin. It was concluded that the best yolk index was with 2.60 ppm/kg marigold flower extract and 0.95 ppm/kg canthaxanthin in the diet of light laying hens from 75 to 85 weeks old.

Key words: pigments, xanthophylls, yolk colour, yolk fan
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Introduction

The diets of laying hens are based mostly on corn and soybean meal. In addition to being an energy source, corn contains xanthophylls, which are responsible for the pigmentation of the yolks, skins, and beaks of birds (Curvelo *et al.*, 2009). However, a significant reduction may occur in yolk colour (Carneiro, 2013) when this cereal is stored and processed improperly.

Carotenoid supplementation in the diet of laying birds has been used to intensify the colour of the egg yolk. Not only does it increase the amount of pigment in the yolk (Papadopoulos *et al.*, 2019), but it has benefits for human health, such as an attenuation of muscle degeneration (Zeheer, 2017), reduction in cardiovascular disease (Gammone *et al.*, 2015), diminution of oxidative stress (Fiedor & Burda, 2014), and decrease in the risk of cancer (Mares-Perlman *et al.*, 2002).

In Brazil, the natural pigments most commonly used in the production of poultry species such as broilers, laying hens, and laying quails include extract of marigold flower *(Tagetes erecta),* red pepper paprika (*Capsicum annuum*), and annatto *(Bixa orellana).* The most common synthetic pigment is canthaxanthin (Golabart *et al.*, 2004; Fassani *et al.*, 2019; Valentim *et al.*, 2019).

The marigold flower is the only natural pigmentation that is sold as a source of lutein, which is used to intensify the yellow colour of the egg yolk (Volp *et al.*, 2009). Canthaxanthin is a natural carotenoid that is

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present in some bird species. It is responsible for the red colour in flamingos, for example, and can be used in its synthetic form to feed broilers and laying hens to pigment the skin and egg yolk (Garcia *et al.*, 2009).

To produce good quality eggs that are more economically viable, it is necessary to conduct research to improve the nutrition of laying hens and their products. The objective of this study was therefore to determine the best level of inclusion of natural pigments (marigold flower extract, yellow pigment) and synthetic (canthaxanthin, red pigment) in the diet of light laying hens from 75 to 85 weeks old on productive performance, the physical and chemical qualities of the eggs, and the economic viability of the enterprise.

Materials and Methods

The experiment was carried out at a laying hen farm called Granja Figueiredo, in the northwest of Paraná State, Brazil. The research followed all the rules proposed by the Ethics Committee on the Use of Animals (ECUA) from the State University of Maringá (UEM) (Protocol number 8244200418/2018).

A total of 288 light laying hens (commercial hens of the Hisex lineage) were used, 75 weeks old, with an average weight of 1.640 ± 0.224 kg. They had been raised until the beginning of the experiment in a conventional system, following the recommendations of the breeder manual, and were housed in the rearing and laying phases in conventional cages.

The experiment for laying hens extended from 75 to 85 weeks old (egg-laying phase), which was divided for analysis into three periods of 21 days. The hens were distributed in a completely randomized design, in a 4 x 4 factorial scheme, with four levels of marigold flower extract (2.10; 2.40; 2.70; 3.00 ppm), and four levels of canthaxanthin (0, 40; 0.70; 1.00; 1.30 ppm), with 16 treatments and three repetitions of six hens/experimental unit, totalling 48 experimental units. During the experiment, laying hens were housed in an open Californian-type aviary, with 2.00 m high ceilings, covered with clay tiles, opened at the upper part of the roof, and equipped with conventional laying cages (50 cm × 45 cm × 45 cm). They were laid out in simple rows with six hens per cage, trough-type feeders and nipple drinkers, with feed distribution and manual egg collection.

Water and feed were provided *ad libitum*, and the lighting programme was 16 hours of light throughout the experiment. The diets were based on corn, soybean meal, and wheat meal to meet the nutritional requirements of laying hens in the final laying stage, according to Rostagno *et al.* (2017), varying only in the inclusion of pigments in the diet (Table 1). Ambient temperature and relative humidity were recorded twice a day at 08h00 and 16h00, using two thermo-hygrometers, placed at the beginning and end of the aviary, and were the average of all periods, with a maximum ambient temperature of 28 °C and minimum of 18.97 °C and relative humidity of maximum 56% and minimum 26.44%.

Productive performance (feed intake, oviposition rate, egg mass (EM), feed conversion per dozen (FC, kg/dz) and egg mass (FC, g/g)) were evaluated throughout the experiment. The oviposition rate of housed birds was calculated by dividing the total number of eggs produced in the period by the number of birds, multiplied by 100. Laying hens and diets were weighed at the beginning and end of each cycle to determine bodyweight, feed consumption, and feed conversion. Weight gain was calculated as the difference between the initial and final weights, and feed consumption as the difference between the feed provided and that left over.

The laying rate was assessed by dividing the total number of eggs produced in the period by the number of hens, multiplied by 100. The egg mass was measured by dividing the total egg weight by the total number of hens in each unit. The feed conversion per dozen was calculated by dividing feed consumption by the number of dozens of eggs produced. The egg mass after feed conversion was measured by dividing feed consumption by the mass of eggs produced.

The average egg weight (EW) was determined by dividing the total egg weight on each of the last three days of each period by the number of weighed eggs. In the last three days of each period, three eggs per replication were used to evaluate specific gravity (SG), yolk index (YI), Haugh unit (HU), pigmentation of egg yolks by subjective Roche colorimetric fan and objective methods, components of the eggs, pH of egg white and egg yolk, and thickness of the eggshell.

Table 1 Composition (%) of experimental diets for laying hens in the final laying stage (from 75 to 85 weeks old) with inclusions of natural (marigold flower extract) and synthetic (canthaxanthin) pigments

Ingredients	**Quantity (kg)**
Corn (7,50%)	57,38
Soybean meal (46%)	18,50
Soybean oil	1,00
Meat meal (44% GF)	2,00
Wheat bran	9,00
Limestone ground (38%)	4,00
Limestone gravel (38%)	6,00
Salt (38%)	0,22
[1]Core	1,80
[2] Mixture of pigments and inert	0,10
Total	100
Calculated composition (%)	
Metabolizable energy (kcal/kg)	2.793,89
Crude protein	16,42
Calcium	4,21
Available phosphorus	0,39
Sodium	0,17
Potassium	0,65
Chlorine	0,2
Digestible methionine + cystine	0,65
Digestible lysine	0,70
Digestible threonine	0,54
Digestible tryptophan	0,18
Crude fibre	3,18
Electrolytic balance (mEq/kg)	182,61

[1]Core (guaranteed levels per kg of product): 0,012 soybean meal 46%; 0,0054 dicalcium phosphate 18,5%; 0,0036 sodium bicarbonate; 0,0009 Propimpex (98% calcium propionate, 0,12 L-lysine 78%); 0,0009 DL-methionine 99%; 0,0108 choline chloride 60%; 0,0015 Mycosorb A+; 0,000225 yecnase (biogenic); 0,000054 Natuphos E 10000 laying 30 g (2E); 0,1 NucleoMix laying GF; 0,1 NucleoMix 09 Aminoblend GF

- NucleoMix laying GF: 108 IU vitamin A; 36 IU vitamin D3; 0,162 IU vitamin E; 0,0198 mg vitamin B; 0,0558 mg vitamin B2; 0,0234 mg vitamin B6; 162 mg vitamin B12; 0,0198 mg vitamin K3; 126 mg calcium pantothenate; 0,288 g niacin; 0,0285 mg folic acid, biotin; 0,99 mg ethoxy; 0,792 mg butylated hydroxyanisole BHA; 0,608 g zinc; 0,72 g iron; 0,864 g manganese; 163,8 mg copper; 18 mg iodine; 3,42 mg cobalt; 4,68 mg selenium; 18 g excipient q.s.p.; NucleoMix 09 Aminoblend GF: 0,00306 zinc; 0,0072 lysine; 4.5 g threonine; 0.45 g manganese; 1.8 g biotin; 0.9 mg ethoxyquin; 0,72 mg BHA; 18 g excipient q.s.p.

[2] Mixture of pigments and inert: dilutions of pigments formed the desired combinations of marigold flower extract (2.1; 2.4; 2.7; 3.0 g/t) and canthaxanthin (4; 7; 10; 13 g/t), supplemented with the inert (rice straw), so that close to 100 g was included in the diet, with 16 combinations, using four levels of marigold flower extract (2.1; 2.4; 2.7; 3 ppm) and four levels of canthaxanthin (0,4; 0,7; 1; 1,3 ppm)

Hamilton (1982) measured SG from the immersion of eggs in containers with saline solutions (densities of 1.065, 1.070, 1.075, 1.080, 1.085, 1.090, and 1.095). The height and diameter of the albumen and yolk were measured with a digital calliper (Harnder *et al.*, 2008), which consisted of breaking the eggs into a smooth flat glass plate. The formula used to calculate the Haugh unit was

$$HU = 100 \log (H + 7{,}57 - 1{,}7\ W^{0{,}37}),$$

where H is the height of the albumen (HA) in millimetres, and W is the weight of the egg in grams (Haugh, 1937). A digital calliper was used to calculate YI, in which the height and width of the yolk were measured. These values were applied to the equation described by Sharp & Powell (1930),

$$YI = (HY/WY),$$

where HY is the height of the yolk (mm), and WY is the width of the yolk.

The eggshells were dried out for 24 h at room temperature (22 ℃), then placed in an oven for 72 h at 60 ℃. Two points were selected in the centre-transversal area to measure the thickness of the shell with a micrometer with divisions of 0.01 mm (Lin *et al.*, 2004).

The colour of the egg yolk was assessed subjectively with the Roche yolk colour fan, which evaluates colour on a scale from 1 to 15, where 1 is the most depigmented and 15 is the most pigmented. The egg white, yolk, and albumen were weighed separately to determine egg components (Ahn *et al.*, 1997). The pH of the egg white and yolk were measured with a digital pH meter (Instituto Adolfo Lutz,1985).

The colour of the egg yolk was analysed by an objective method by measuring these parameters: L*, which represents luminosity (L* = 0, black; L* = 100, white); a* and b*, which are the coordinates of the colours responsible for chromaticity (+ a * = red; - a * = green; + b * = yellow; - b * = blue) with a portable colorimeter (CR400m, Minolta), which was previously calibrated in black (0) and white (100), using D65 illuminant and a 10º observer's angle. To analyse the stability of the colour of the eggs, an egg was collected by replication during the first 21 days of the experiment, and the colour evaluation of the yolks was carried out subjectively using the Roche colour fan.

The statistical analysis of the data was performed with the SAS statistical program (SAS Inst. Inc., Cary, NC), according to the model:

$$Y_{ijkl} = b_0 + b_1M_i + b_2C_j + b_3M_{i2} + b_4C_{j2} + b_5MC_{ij} + FA + e_{ijkl}$$

where Y_{ijkl} = variable measured in experimental unit k, fed with a diet containing level i of marigold flower extract and level j of canthaxanthin;

b_0 = general constant;

b_1 = linear regression coefficient as a function of the level of marigold flower extract;

M_i = marigold flower extract content for laying hens (from 75 to 85 weeks old): M1 = 2.10 ppm; M2 = 2.40 ppm; M3 = 2.70 ppm and M4 = 3.00 ppm;

C_j = canthaxanthin content for laying hens (from 75 to 85 weeks old): C1 = 0.40 ppm; C2 = 0.70 ppm; C3 = 1.00 ppm and C4 = 1.30 ppm;

b_2 = linear regression coefficient as a function of canthaxanthin level;

b_3 = quadratic regression coefficient as a function of marigold flower extract level;

b_4 = quadratic regression coefficient as a function of canthaxanthin level;

b_5 = linear regression coefficient as a function of the interaction between the level of marigold flower extract and the level of canthaxanthin;

FA = lack of adjustment of the regression model;

e_{ijkl} = random error associated with each observation

Regression analyses of the levels of inclusion of marigold flower extract and canthaxanthin were performed and estimates of these levels were obtained using a quadratic model (Sakomura & Rostagno, 2016).

Results and Discussion

No interaction effect was observed between the levels of marigold and canthaxanthin flower extract on the variables, indicating that they acted independently (Tables 2, 3, 4, 5, and 6).

Table 2 Average values of three periods of productive performance of light commercial laying hens from 75 to 85 weeks old, depending on the levels of marigold and canthaxanthin flower extract

Marigold (ppm)	Canthaxanthin (ppm)	FI (g/bird)	FC (kg/dz)	FC (g/g)	EM (g)	PR (%)
2,10	0,40	111,41	1,58	1,97	56,59	87,96
	0,70	112,96	1,89	2,20	51,99	79,04
	1,00	116,64	1,66	2,05	57,06	88,47
	1,30	112,92	1,64	1,99	56,98	88,97
2,40	0,40	111,78	1,83	2,18	51,9	79,80
	0,70	117,54	1,76	2,12	55,62	86,70
	1,00	114,58	1,78	2,07	55,24	84,01
	1,30	115,66	1,75	2,13	54,37	84,43
2,70	0,40	114,44	1,83	2,09	55,72	84,68
	0,70	114,83	1,84	2,17	54,59	84,26
	1,00	113,52	1,87	2,11	54,44	83,16
	1,30	114,81	1,67	1,89	60,73	91,25
3,00	0,40	113,85	1,96	2,24	51,58	79,71
	0,70	117,10	1,62	1,95	60,22	84,02
	1,00	109,61	2,00	2,17	43,79	67,34
	1,30	116,58	1,69	2,00	58,34	88,38
P value						
Marigold		NS	NS	NS	NS	NS
Canthaxanthin		NS	NS	0,029(L)	0,017(L)	0,029(L)
Marigold x canthaxanthin		NS	NS	NS	NS	NS
Standard error		0,522	0,028	0,032	0,775	1,117
Regression equation					R^2	
EMFC = 2,23430-0,178029 canthaxanthin					0,98	
EM = 51,3010+4,85449 canthaxanthin					0,89	
PP = 79,8419+6,29555 canthaxanthin					0,77	

Marigold: marigold flower extract; FI: feed consumption; FC: feed conversion per dozen; FC: feed conversion by egg mass; EM: egg mass; PR: oviposition rate; *P*-value: coefficient of determination; NS: not significant for $P < 0.05$; L: linear effect and R^2: coefficient of determination

Feed consumption (g/g) improved linearly with the inclusion of canthaxanthin in the diet, whereas EM and PR showed an increasing linear effect for inclusion of canthaxanthin. The levels of marigold and canthaxanthin flower extract did not influence FI and FC (kg/dz) during the experiment. Yolk index showed a quadratic effect for the inclusion of marigold and canthaxanthin flower extract

The thickness of the shell and SG showed a decreasing linear effect with the inclusion of marigold and canthaxanthin flower extract. The variables, average egg weight (AEW) and the percentage of eggshell, showed a linear effect because of the inclusion of canthaxanthin in the feed, with this effect increased for AEW and decreased for the percentage of eggshell. The percentage of egg yolk and HU variables showed a linear effect with increasing levels of canthaxanthin. The percentage of albumen showed a linear reduction when the extract of the marigold flower was included. The levels of marigold and canthaxanthin flower extract did not influence the pH of albumen and yolk during the experiment.

Table 3 shows the regression equations for egg quality parameters of light hens aged between 75 and 85 weeks fed with marigold and canthaxanthin flower extracts.

Table 3 Average quality of light commercial laying eggs from 75 to 85 weeks old for various inclusions of dietary marigold and canthaxanthin flower extract

Marigold (ppm)	Canthaxanthin (ppm)	EW (g)	SG (g/cm³)	TS (mm)	Eggshell (%)	HU	pH albumen	Albumen (%)	YI (mm)	pH yolk	Yolk (%)
2,1	0,4	63,61	1,082	0,598	7,75	73,45	7,99	65,41	0,364	5,91	26,63
	0,7	63,77	1,082	0,591	7,73	79,69	7,8	65,07	0,385	6	26,83
	1	64,31	1,083	0,594	7,67	82,52	7,78	65,7	0,391	6,02	26,41
	1,3	64,73	1,082	0,593	7,62	83,33	7,81	65,16	0,387	5,95	27,09
2,4	0,4	65,02	1,082	0,599	7,58	81,59	7,82	64,87	0,392	5,96	27,43
	0,7	65,31	1,083	0,593	7,55	82,86	7,77	64,55	0,378	5,96	27,65
	1	65,08	1,081	0,592	7,58	83,37	7,81	64,87	0,39	6,02	27,19
	1,3	64,88	1,08	0,588	7,6	79,56	7,79	65,25	0,388	6,05	26,78
2,7	0,4	64,33	1,082	0,592	7,67	81,01	7,87	64,63	0,386	6,01	27,28
	0,7	64,85	1,081	0,592	7,6	82,28	7,87	64,96	0,388	5,95	27,01
	1	66,49	1,079	0,577	7,42	80,64	7,81	65,58	0,396	5,97	26,45
	1,3	64,77	1,079	0,578	7,63	79,63	7,91	64,95	0,383	5,87	26,93
3	0,4	64,85	1,079	0,597	7,63	86,56	7,77	64,52	0,376	5,96	27,23
	0,7	62	1,082	0,584	7,97	83,16	7,88	63,45	0,353	5,95	28,28
	1	64,07	1,081	0,587	7,71	84,52	7,92	64,39	0,391	5,95	27,54
	1,3	63,95	1,081	0,591	7,72	83,36	7,72	63,45	0,38	5,99	26,96
P value											
Marigold		NS	0,012(L)	0,022(L)	NS	0,005(L)	NS	0,022(L)	0,025(Q)	NS	0,020(L)
Canthaxanthin		0,024(L)	0,007(L)	0,003(L)	0,013(L)	0,002(L)	NS	NS	0,012(Q)	NS	NS
Marigold x canthaxanthin		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Standard error		0,276	0,003	0,014	0,033	0,575	0,136	0,138	0,024	0,127	0,128

Marigold: marigold flower extract; EW: average egg weight; YI: yolk index; Yolk: percentage of yolk; Albumen: percentage of albumen; Eggshell: percentage of shell; TS: shell thickness; SG: specific weight; HU: Haugh unit; *P*-value: coefficient of determination; NS: not significant for $P < 0.05$; Q: quadratic effect, L: linear effect

Table 4. Regression equations for quality parameters of eggs from light hens aged from 75 to 85 weeks old fed with marigold and canthaxanthin flower extracts

Regression equation	R²	Estimation	
		Marigold	Canthaxanthin
EW = 63,3335 + 1,62261 CANT	0,91	---------	---------
YI = 0,016.8763 + 0,253311 EFM - 0,0486649 EFM² + 0,107255 CANT - 0,0566701 CANT²	0,59	2,60 ppm	0,95 ppm
Yolk = 25,5062 + 0,77814 EFM	0,75	---------	---------
Albumen = 66,3938 - 0,705418 EFM	0,63	---------	---------
Eggshell = 7,79345 - 1,98251 CANT	0,91	---------	---------
TS = 0,617613 - 0,00731039 EFM - 0,0100169 CANT	0,81	---------	---------
SG = 1,08741 - 0,00175595 EFM – 0,00191389 CANT	0,97	---------	---------
HU = 70,7102 + 4,31198 EFM	0,71	---------	---------

R^2: coefficient of determination; Marigold: marigold flower extract; EW: average egg weight; CANT: canthaxanthin; YI: yolk index; EFM: from the marigold flower extract; Yolk: percentage of yolk; Albumen: percentage of albumen; Eggshell: percentage of shell; TS: shell thickness; SG: specific weight and HU: Haugh Unit

The quadratic effect of the marigold flower extract produced a linear increase for canthaxanthin on the Roche colorimetric fan (LCR) and a* variables, indicating estimates of maximum yolk colour with 2.73 and 2.80 ppm/kg of the flower extract of marigold with increased levels of canthaxanthin. The content of marigold and canthaxanthin flower extract did not influence the variables L * and b * during the experiment. Pigments from marigold flower extract and canthaxanthin are classified in Brazil as sensory additives or substances that are added to animal feed to preserve, intensify colour, taste, and odour and modify their properties, without harming the nutritional value of the feed (IN Nº13/2004 – Brazil, MAPA, 2004). Canthaxanthin may be considered pro-vitamin (Beardsworth & Henández, 2003), because of its antioxidant properties and ability to be converted into vitamin A (Surai, 2003; Surai *et al*., 2006).

Several studies have analysed the effect of the addition of pigments to the diets of poultry (Garcia *et al.*, 2002; Moeini *et al.*, 2013; Sandeski, 2013; Fernandes, 2016; Fassani, 2019; Papadopoulos et al., 2019; Valentim *et al.*, 2019). These authors claim that treatments containing synthetic and natural pigments do not influence the performance of the birds or the quality of the eggs. They merely commented on intensification of the colour of the egg yolk. However, in the present work, the authors observed that the variables of the productive performance of laying hens (FC (g/g), EM and PR) were influenced satisfactorily with the addition of marigold and canthaxanthin.

Sandeski (2013) added canthaxanthin and yellow pigmentation (lutein + zeaxanthin) to the diet of light laying hens in the egg-laying phase and observed that EW did not change because of the treatments. However, they observed that the supplementation of the diets of light laying hens from 90 to 103 weeks old with carotenoid additives based on red pepper *(Capsicum annuum)* and marigold flower extract did not have positive effects on their performance, but that EW increased.

Table 5 Average values of yolk colour in light commercial laying eggs from 75 to 85 weeks old for dietary inclusions of marigold and canthaxanthin flower extract

Marigold (ppm)[1]	Canthaxanthin (ppm)	LCR	L*	a*	b*
2,10	0,40	5,02	36,57	-1,99	28,64
	0,70	5,93	37,97	-1,56	25,71
	1,00	6,73	34,98	-1,07	26,47
	1,30	7,73	37,03	-0,77	31,58
2,40	0,40	6,07	38,79	-0,62	29,35
	0,70	6,79	34,92	-0,08	30,09
	1,00	7,05	37,10	0,28	20,87
	1,30	8,11	38,05	0,53	25,48
2,70	0,40	6,35	37,58	0,53	26,96
	0,70	7,07	35,16	0,85	31,32
	1,00	7,95	38,57	0,50	32,35
	1,30	8,67	35,04	-0,34	27,61
3,00	0,40	6,39	36,54	-0,92	29,81
	0,70	6,92	37,63	-1,03	36,53
	1,00	7,04	36,32	-0,42	30,51
	1,30	8,31	36,65	-0,21	21,00
P-value					
Marigold		0,000 (Q)	NS	0,025 (Q)	NS
Canthaxanthin		0,000 (L)	NS	0,000 (L)	NS
Marigold x canthaxanthin		NS	NS	NS	NS
Standard error		0,144	0,333	0,136	0,499

Regression equation	R^2	Estimation Marigold	Estimation Canthaxanthin
LCR = - 15,2244 +15,0843EFM -2,76516EFM²+2,42233CANT	0,97	2,73 ppm	---------
a* =- 13,4879+8,47674EFM-1,51164EFM² + 3,02328CANT	0,67	2,80 ppm	---------

Marigold: marigold flower extract; LCR: Roche colorimetric fan; L*: luminosity; a*: red / green coordinate; b*: yellow / blue coordinate; P value: coefficient of determination; Q: quadratic effect; NS: not significant for *P* <0.05; L: linear effect; R^2: coefficient of determination; EFM: marigold flower extract; CANT: canthaxanthin

Fernandes (2016) evaluated the use of vitamin E, selenium, and canthaxanthin in the diet of laying hens of 40 to 55 weeks and observed that the inclusion of 6 ppm canthaxanthin provided an increase in EW compared with the other treatments (vitamin E and selenium). The same result was found by Oliveira *et al.* (2017), who studied the addition of paprika extract and marigold flower extract to the diet of 160 light laying hens aged 95 weeks, and by Fassani *et al.* (2019), who evaluated the addition of commercial pigments based on canthaxanthin and annatto to the diet of light laying hens that were 55 weeks old.

The synthesis of pigments in the egg yolk begins with the processes of digestion and metabolism, which are similar to that of cholesterol in poultry. After they are consumed, they are digested in the form of fat droplets, which are emulsified by bile salts, and transformed into micelles (Parker, 1996). They are transported through the lipoproteins in the cell membrane and accumulate in the fat-rich tissues, then are deposited in the egg yolk (Pérez-Vendrell *et al.*, 2001; Faehnrich *et al.*, 2016; Vinus *et al.*, 2018), interfering with its composition (Surai *et al.*, 2001).

The inclusion of marigold flower extract (2.60 ppm/kg) influenced the percentage of the yolk, and 0.95 ppm/kg of canthaxanthin increased YI. These results may be related to the greater amount of carotenoids transferred to the yolk and to an increase in the components that compose it, such as proteins and lipids promoted by the absorption of pigments (Carneiro, 2013).

Specific gravity is important for producers, as this variable indicates the quality of the eggshell, which is easy to analyse quickly at low cost without damaging the product (Santos *et al.*, 2016). Peebles & McDaniel (2004) and Silva (2004) consider that the SG of the eggs cannot be less than 1.080. They believe that values below this could cause costly losses for the producers. Variations in SG can occur because of porosity, the presence of cracks in the eggshells (Freitas *et al.*, 2004), and the age of laying hens (Domingues & Faria, 2019).

Studies also indicate that the older the poultry, the lower the percentage of the eggshell and TS, because the shell does not increase in the same proportion as EW, because of the lower deposition of calcium carbonate per unit area (Oliveira & Oliveira, 2013), thus increasing the quantity and thickness of the pores in the eggshell (Domingues & Faria, 2019). This may be the reason that the higher the inclusion of canthaxanthin in the current study, the greater the EW and the lower the percentage of eggshell. These variables are linked to the external quality of the eggs, i.e., the higher the SG values, percentage of shell and TS, the better the quality of the eggs, and greater their resistance to breaking and cracking (Domingues & Faria, 2019).

Oliveira *et al.* (2017) evaluated the addition of natural pigments (red pepper paprika and marigold flower extract) to the diet of light laying hens at 95 weeks old and reported a decrease in the percentage of eggshells and TS. In the current study, the addition of pigments caused a decrease in TS and SG. This can be explained by Hirsch *et al.* (2007). They reported that the lutein and zeaxanthin, which are the carotenoids present in the marigold flower extract, inhibited the activity of the hormone, oestrogen, which inhibits the action of carbonic anhydrase (an enzyme that is responsible for the formation of the eggshell) (Benesch *et al.*, 1944). This may result in eggs with a soft shell or without a shell.

The Haugh unit is the variable that is most commonly used to measure the quality of the albumen. It is a mathematical expression that correlates the weight of the egg with the height of the albumen. The higher this index, the better the quality of the eggs (Oliveira & Oliveira, 2013; Domingues & Farias, 2019). According to USDA (2000), eggs can be classified into excellent (100.0 to 72.0), high (71.0 to 60.0), medium (59.0 to 30.0), and low quality (29.0 to 00.0). The values in the present study varied between 73.45 (2.10 ppm of the marigold flower extract and 0.40 ppm of canthaxanthin) and 86.56 (3.00 ppm of the marigold flower extract and 0.40 canthaxanthin ppm), indicating that the eggs were of excellent quality.

Similar values for HU were observed by Rojas *et al.* (2015), who evaluated the addition of 30 g and 60 g of canthaxanthin + annatto extract in the diet of laying hens of HyLine Brown lineage from 34 to 45 weeks old. They observed that the addition of 30 g and 60 g canthaxanthin and annatto extract showed HU values of 82 and 86, respectively. Similar values were reported by Garcia *et al.* (2002) and Garcia *et al.* (2009).

The colour intensity of the yolk of laying hens depends on the amount of carotenoids consumed in the diet, because hens are not able to synthesize these pigments. The greater the consumption of food with a higher concentration of carotenoids, the greater the deposition in the yolk and concomitant intensity, to the point of saturating the colour, such that the addition of pigment to the diet no longer has an effect (Curvelo *et al.*, 2009). Yolk deposition occurs in concentric layers. To obtain the most intense colouring, a combination of two pigments is needed, since the deposition of the egg yolk occurs in two phases.

Saturation is for the deposition of yellow carotenoids forming a base, which will present uniform deposition so that good saturation of the final colour occurs afterwards. After the deposition of the base (yellow), the second phase of pigmentation, which is the addition of red carotenoids, changes the tone (yellow) for the most reddish-orange colour. The combination of the pigments presenting these two colours is therefore more interesting when the objective is to increase the intensity of these two compounds (Fletcher & Hallo Ran, 1983). Sandeski (2013) confirmed that 15 days of yellow and red synthetic pigments are needed to achieve saturation. In Hammershoj *et al.* (2010), it took 14 days with various natural pigments in the diet of light laying hens. In the current study, it took an average of 18 days on the diets containing the various pigments for yolk colour saturation to occur.

The efficiency of yolk pigmentation depends on several factors, for example the amount of carotenoid ingested, the period of consumption of the additive (Curvelo *et al.*, 2009), and the birds' ability to absorb the carotenoids in the diet (Amaya *et al.*, 2013). It is therefore impossible to predict the pigmentation capacity of the additives accurately. Some authors have reported that synthetic pigments are more efficient than natural pigments in effective pigmentation of the yolk (Moura *et al.*, 2011; Valentim *et al.*, 2019). Inclusion of marigold flower at 2.73 ppm and canthaxanthin at 1.30 ppm in the current study showed an increase in the degree of yolk colour, reaching a score of 8 in the subjective LCR method, indicating the need to use the association of the two pigments for homogeneous yolk colouring.

Valentim *et al.* (2019) compared the use of paprika extract, marigold flower, and canthaxanthin in the diet of black laying hens (Avifran) at 60 weeks of age. The authors did not observe the influence of additives on the parameters of performance and quality of the eggs. A significant difference was observed only in the colour of the yolk, in which the inclusion of 0.045% canthaxanthin presented a greater average (12.62) according to the Roche colour fan, because the chemical capacity of canthaxanthin is greater than that of natural pigments. However, the inclusion of natural pigments showed satisfactory values, confirming that synthetic dye could be substituted, reducing the cost of production.

Conclusion

To obtain the best YI, the optimal inclusion level was 2,60 pp/kg of marigold flower extract and 0.95 ppm/kg of canthaxanthin in a diet based on corn, wheat, and soybean meal, in light laying hens from 75 to 85 weeks old.

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Authors' contributions

KMM, DOG, and SMM designed this experiment; KMM, DRA, MTFP, JBT, and DOG performed the animal experiments, measured, and acquired the data; KMM, DOG, and APST participated in result tabulation and statistics; KMM and SMM wrote the manuscript and revised it; DOG and SMM supervised all processes from performing the experiment to writing the manuscript. All authors read and approved the final manuscript.

Conflict of interest declaration

All authors declare that there are no conflicts of interest in the information provided in this paper.

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