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Influence of pigments on the shelf life of eggs from layers hens in the final phase of production

Influência de pigmentantes na vida de prateleira de ovos de poedeiras em fase final de produção

Influencia de los pigmentos en la vida útil de los huevos de gallinas ponedoras en la etapa final de producción

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Abstract
The objective of this work was to determine the influence of natural (marigold flower extract) and synthetic (canthaxanthin) pigments on the shelf life of eggs from light laying hens in the final stages of production. 288 birds were distributed in a completely randomized design, with eight treatments, consisted in four levels of marigold flower extract (2.1; 2.4; 2.7 and 3 ppm) and four levels of canthaxanthin (0.4 ; 0.7; 1,0 and 1.3 ppm), each treatment with six replicates of six birds/experimental unit. In the last ten days of the experimental period (75-78 weeks of age) a total of 960 eggs were collected, selected and randomly distributed, which 480 eggs were stored at ambient temperature and 480 under refrigeration. Ten different storage periods were analyzed (0, 5, 10, 15, 20, 25, 30, 35, 40, and 45 days), one egg each repetition (six eggs per treatment). Internal and external quality parameters were evaluated in the eggs. Egg yolk color was evaluated using the La Roche colorimetric fan (LCR), showing a significant interaction between canthaxanthin level and storage period. Yolk height (YH), albumen percentage (%A) and yolk percentage (%Y) showed significant interaction between marigold flower level and storage period. It is concluded that the use of natural (marigold flower) and synthetic (canthaxanthin) pigments for the shelf life of eggs from hens in the final stage of production did not influence most of the egg quality variables in the present study, however, eggs stored in a refrigerated environment had better results for egg conservation.
Keywords: Antioxidant action; Storage; Canthaxanthin; Egg yolk color; Marigold flower.

Resumo
O objetivo deste trabalho foi determinar a influência de pigmentantes natural (extrato da flor de marigold) e sintético (cantaxantina) na vida de prateleira de ovos de galinhas poedeiras leves em fase final de produção. Foram distribuídas 288 aves em um delineamento inteiramente casualizado, com oito tratamentos, no qual quatro foram níveis de extrato de flor de marigold (2,1; 2,4; 2,7 e 3 ppm) e quatro níveis de cantaxantina (0,4; 0,7; 1,0 e 1,3 ppm), cada tratamento com seis repetições de seis aves/unidade experimental. Nos últimos dez dias do período experimental (75-78 semanas de idade) foram coletados e selecionados um total de 960 ovos, que foram distribuídos aleatoriamente, onde 480 ovos foram armazenados em temperatura ambiente e 480 sob resfriamento. Foram analisados 10 períodos de estocagem diferentes (0, 5, 10, 15, 20, 25, 30, 35, 40, e 45 dias), onde foram avaliados um ovo cada repetição (seis ovos por tratamento). Foram avaliados nos ovos os parâmetros de qualidade interna e externa. A coloração da gema dos ovos foram avaliadas utilizando o Leque colorimétrico de La Roche (LCR), apresentou interação significativa entre nível de cantaxantina e período de armazenamento. A altura da gema (AG), porcentagem de albúmen (%A) e porcentagem de gema (%G) apresentaram interação significativa entre nível de flor de marigold e período de armazenamento. Conclui-se que o uso de pigmentantes naturais (flor de marigold) e sintéticos (cantaxantina), para a vida de prateleira de ovos de galinhas em fase final de produção, não influenciou a maioria das variáveis de qualidade de ovos do presente estudo, no entanto os ovos armazenados em ambiente refrigerado obtiveram melhores resultados para conservação dos ovos.
Palavras-chave: Ação antioxidante; Armazenamento; Cantaxantina; Cor da gema de ovos; Flor de marigold.

Resumen
El objetivo de este trabajo fue determinar la influencia de pigmentos naturales (extracto de flor de caléndula) y pigmentos sintéticos (cantaxantina) en la vida útil de huevos de gallinas ponedoras livianas en etapa final de producción. Se distribuyeron un total de 288 aves en un diseño completamente al azar, con ocho tratamientos, en los cuales cuatro fueron niveles de extracto de flor de caléndula (2.1; 2.4; 2.7 y 3 ppm) y cuatro niveles de cantaxantina (0.4; 0.7; 1.0 y 1.3 ppm), cada tratamiento con seis repeticiones de seis aves/unidad experimental. En los últimos diez días del período experimental (75-78 semanas de edad) se colectaron y seleccionaron un total de 960 huevos, los cuales fueron distribuidos al azar, donde 480 huevos fueron almacenados a temperatura ambiente y 480 en refrigeración. Se analizaron diez periodos de almacenamiento diferentes (0, 5, 10, 15, 20, 25, 30, 35, 40 y 45 días), donde se evaluó un huevo por réplica (seis huevos por tratamiento). Se evaluaron los parámetros de calidad interna y externa en los huevos. El color de la yema de huevo se evaluó utilizando el ventilador colorimétrico de La Roche (LCR), mostrando una interacción significativa entre el nivel de cantaxantina y el período de almacenamiento. La altura de la yema (AG), el porcentaje de albúmina (%A) y el porcentaje de yema (%G) mostraron una interacción significativa entre el nivel de flor de caléndula y el período de almacenamiento. Se concluye que el uso de pigmentos naturales (flor de caléndula) y sintéticos (cantaxantina) para la vida útil de huevos de gallinas en etapa final de producción no influyó en la mayoría de las variables de calidad de huevo del presente estudio, sin embargo, los huevos almacenados en ambiente refrigerado obtuvieron mejores resultados para la conservación del huevo.
Palabras clave: Acción antioxidante; Almacenamiento; Cantaxantina; Color de la yema; Flor de calêndula.

1. Introduction

Egg is a food with high nutritional value, offering the consumer essential nutrients with proteins, vitamins, minerals and fatty acids. Thus, like all foods from animal origin, the egg also has perishable characteristics, with the loss of internal quality starting from the moment of laying by the hen until consumption (Honorato et al., 2016).

To avoid premature perishability, conservation methods become important, with emphasis on refrigeration, which tends to preserve the internal quality of eggs, since it delays their deterioration. However, in Brazil, refrigeration is not mandatory (Resolution number 35, of June 17, 2009, prepared by the National Health Surveillance Agency - ANVISA), so eggs can be conditioned at room temperature at all stages, until reaching the final consumer, which can affect the internal quality of eggs, highlighting changes in the albumen and yolk.

The factors that most affect the internal quality of eggs are temperature and relative humidity, as the high temperature during the storage period is directly linked to the reduction in albumen quality, which can be related to the loss of water and carbon dioxide, which leads to the albumen fluidification, as well as the rupture of the yolk membrane becoming easier during the breaking of the eggs. The relative humidity of the air, the lower than 99.6%, the faster the egg will lose water, reducing its weight due to the increase in the air chamber (Silva et al., 2015).

Another characteristic related to internal quality is the yolk color, which is a purchase decision criterion, as the consumer usually associates the color with the quality and nutritional composition of eggs. Corn stands out for being the main source of xanthophylls (responsible for the yellowish color of the yolk), however, it can vary during the year, mainly from one harvest to another, which can influence the amount of these pigments in the grain, making it necessary the use of pigments in the diet of laying hens in order to keep the yolk color constant, considering that the birds are not able to synthesize xanthophylls, which come exclusively from food (Volp et al., 2009).

Egg yolk pigmentation is a result from the deposition of xanthophylls (carotenoid pigments), which may be natural, such as those present in marigold flower extract, or synthetic, such as canthaxanthin. Canthaxanthin is a natural carotene in some bird species, however for yolk pigmentation its synthetic form is used in food (Rocha et al., 2011).

Carotenes such as canthaxanthin are bioactive molecules, precursors of vitamin A, which can act as immunomodulators, and have antioxidant activity, as they remove free radicals, absorb and dissipate their excess energy and recycle vitamin E, influencing positively in eggs by improving its shelf life, due to its high concentration of unsaturated fatty acids, which are more liable to lipid oxidation (Surai, 2014; Silva et al., 2010).

The use of pigments can help to maintain not only the yolk coloration but also the internal quality of the eggs for a longer time, due to its antioxidant action (Detofol et al., 2018). Given these aspects, the objective of this work was to determine the influence of natural (marigold flower extract) and synthetic (canthaxanthin) pigments on the shelf life of eggs from light laying hens in the final stages of production.

2. Methodology

The experiment was carried out in a Brazilian poultry farm located in the city of Mandaguari in the state of Paraná. The experiment was approved by the Animal Use Ethics Committee (CEUA-UEM), protocol number: 8244200418.

Two hundred and eighty-eight birds of the commercial lineage Hisex, 75 weeks old, were distributed in a completely randomized design, with eight treatments, where four were levels of marigold flower extract (2.1; 2.4; 2.7 and 3 ppm) and four levels of canthaxanthin (0.4; 0.7; 1 and 1.3 ppm), each treatment with six replicates of six birds/experimental unit, totaling 48 experimental units.

The laying hens were housed in conventional laying cages (50 cm x 45 cm x 45 cm), arranged in two overlapping rows, using a manual gutter-type feeder and a nipple drinker.

Water and feed were provided *ad libitum*. The lighting program used was 16 hours of light throughout the experimental period. The experimental diets were based on corn, soybean meal and wheat, formulated to meet the nutritional requirements of light laying hens according to Rostagno et al. (2017), with variation only in the inclusion of pigments and inert in the diet.

In the last ten days of the experimental period (75-78 weeks of age) a total of 960 eggs were collected, 96 eggs were selected each day (two eggs per repetition), by the average repetition weight, identified, weighed individually and randomly distributed in trays, where 480 eggs were stored at room temperature and 480 under cooling in a refrigerator, the temperature of both being checked once a day.

Ten different storage periods were analyzed (0, 5, 10, 15, 20, 25, 30, 35, 40 and 45 days), in each period and storage temperature, one egg per repetition (six eggs per treatment) was evaluated, totaling 48 eggs that were evaluated at room temperature and another 48 eggs that were evaluated under cooling, in a total of 96 eggs in each period. The parameters of internal and external quality of eggs evaluated were: yolk index, Haugh unit, albumen height, yolk and albumen pH, egg components, shell thickness, average egg weight, specific weight and yolk coloration by subjective method.

To calculate the Haugh unit (HU), in order to correlate the egg weight with the height of the thick white, the formula described by Haugh (1937) was used.

To determine height, albumen diameter and yolk, was used the methodology reported by Hander et al. (2008). To calculate the yolk index (YI) a digital caliper was used where the height and width of the yolk were measured, then the values were applied in the equation described by Sharp and Powell (1930).

To measure the specific weight or relative density, the methodology described by Thompson and Hamilton (1982) was used, from the submersion of eggs in containers with saline solutions (densities of 1,065, 1,070, 1,075, 1,080, 1,085, 1,090 and 1,095).

Yolk coloring was performed by the subjective method using the La Roche Colorimetric Fan, which assesses coloration on a color scale from 1 to 15, where 1 is the most depigmented and 15 is the most pigmented.

Albumen and yolk were weighed separately to determine the egg components (Ahn et al., 1997). The pH of the egg white and yolk was measured using a digital pH meter (Gerber and Van Gullik, 1985).

The shell thickness was performed after drying for 24 hours at room temperature and then in an oven at 60ºC for 72 hours and then selecting two different points in the center-transverse area to verify the measurement using a micrometer with 0.01mm divisions (Lin *et al*., 2004).

Statistical analysis of data was performed using the SAS statistical program (SAS Inst. Inc., Cary, NC), according to the model (equation 1):

$$Y_{ikl} = b_0 + b_1M_i + b_2M_i + FA + e_{ikl} \quad (1)$$

Where: Y_{ikl} = variable measured in the experimental unit k, fed diet containing the level *i* of marigold flower extract, b_0 = general constant, b_1 = linear regression coefficient as a function of marigold flower extract level, M_i = marigold flower extract level for light laying hens: M_1 = 2,1 ppm, M_2 = 2,4 ppm, M_3 = 2,7 ppm e M_4 = 3 ppm, b_2 = quadratic regression coefficient as a function of marigold flower extract level, FA = lack of adjustment of the regression model, e_{ikl} = random error associated with each observation.

A similar model was used for the pigment canthaxanthin, with the following levels: (C1= 0,4 ppm, C2= 0,7 ppm, C3= 1,0 ppm, C4=1,3 ppm).

Regression analyzes of canthaxanthin and marigold flower inclusion levels were performed and estimates of the best levels of these additives in the laying hens' diet were obtained using the quadratic model as described by Sakomura and Rostagno (2016).

3. Results

The average room temperature was 28ºC and relative humidity of 55% and in the refrigerated storage it was 9.7ºC and relative humidity of 70%.

Eggs quality using canthaxanthin in diets

For the interactions between storage period, canthaxanthin level and storage environment and between canthaxanthin level and storage environment, it was observed that no variable evaluated was significantly influenced.

There was interaction between environment and storage period for the variables AH, YH, AD, YD, LCR, ApH, YpH, HU, YI, AI, %Y, %S and %A (P<0.01). The AW variable showed a significant interaction at 5%. The variables EW, YW, ShW, ST and SW were not significantly influenced.

For effect of storage environment, the variables YW, AW, AH, YH, AD, YD, LCR, ApH, YpH, HU, YI, AI, %Y, %S and %A were significantly influenced (P<0.01) and for effect of storage period, the following variables EW, AW, AH, YH, AD, YD, LCR, ApH, YpH, HU, YI, AI, %Y, %S and %A were significantly influenced (P<0 .01) (Table 1).

Table 1 – Egg quality during storage under different environmental conditions (ambient temperature and refrigerated) of light laying hens fed with different levels of canthaxanthin from 75 to 85 weeks of age.

V	CPL	SE	SP	CPLxSE	CPL x SP	SE x SP	CPLxSExSP
YW	NS	**	*	NS	NS	NS	NS
EW	NS	NS	**	NS	NS	NS	NS
AW	NS	**	**	NS	NS	*	NS
ShW	NS	NS	NS	NS	NS	NS	NS
ST	NS	NS	NS	NS	NS	NS	NS
AH	NS	**	**	NS	NS	**	NS
YH	NS	**	**	NS	NS	**	NS
AD	NS	**	**	NS	NS	**	NS
YD	NS	**	**	NS	NS	**	NS
LCR	**	**	**	NS	*	**	NS
ApH	NS	**	**	NS	NS	**	NS
YpH	NS	**	**	NS	NS	**	NS
SW	NS	NS	NS	NS	NS	NS	NS
HU	NS	**	**	NS	NS	**	NS
YI	NS	**	**	NS	NS	**	NS
AI	NS	**	**	NS	NS	**	NS
%Y	NS	**	**	NS	NS	**	NS
%S	NS	**	**	NS	NS	**	NS
%A	NS	**	**	NS	NS	**	NS

YW: yolk weight; EW: egg weight; AW: albumen weight; ShW: shell weight; ST: shell thickness; AH: albumen height; YH: yolk height; AD: albumen diameter; YD: yolk diameter; LCR: La Roche fan color; ApH: albumen pH; YpH: yolk pH; SW: specific weight; HU: Haugh unit; YI: yolk index; AI: albumen index; %Y: yolk percentage; %S: shell percentage; %A: albumen percentage; V: variables; CPL: canthaxanthin pigment level; SE: storage environment; SP: storage period; NS: non-significant for p<0,05; ** Significant for p<0,01; * Significant for p<0,05. Source: Authors.

It was observed that for eggs stored at room temperature the variables EW, AW, AH, YH, LCR, HU, YI, AI and %A had a decreasing linear effect, while the variables YW, AD, YD, ApH, YpH, %Y and %S showed an increasing linear effect as a function of storage days.

For refrigerated storage, it was analyzed that the variables YW, ApH, %S, and %Y had an increasing linear effect, while the variables EW, AW, AH, YH, HU, YI and %A had a decreasing effect and the variables AD, YD, LCR, YpH and AI were not significant (p>0.05).

ST, ShW and SW had no significant effect for both storage environments (Table 2).

Table 2 – Unfolding of the interaction between different storage times of eggs stored in two environments (9.7°C and 70% and 28°C, 55% RU) on the variables of egg quality of light laying hens fed with canthaxanthin from 75 to 85 weeks of age.

Storage time x refrigerated environment (9,7ºC e 70% RU)					
V	L	Q	R²	P value	Equations
YW	*	NS	0,9756	0,0486	YW=17,7214+0,0451x
EW	*	NS	0,9403	0,0019	EW=65,3979-0,1007x
AW	*	NS	0,8993	<0,0001	AW=41,5310-0,1077x
ShW	NS	NS	-	-	-
ST	NS	NS	-	-	-
AH	*	NS	0,9055	<0,0001	AH=6,4713-0,0245x
YH	*	NS	0,8931	<0,0001	YH=16,4902-0,0310x
AD	NS	NS	-	-	-
YD	NS	NS	-	-	-
LCR	NS	NS	-	-	-
ApH	*	NS	0,8452	<0,0001	ApH=8,7644+0,0068x
YpH	NS	NS	-	-	-
SW	NS	NS	-	-	-
HU	*	NS	0,9410	0,0020	HU=77,4901-0,1561x
YI	*	NS	0,9743	0,0507	YI=0,3790-0,0005x
AI	NS	NS	-	-	-
%Y	*	NS	0,9211	0,0003	%Y=28,1728+0,0471x
%S	*	NS	0,9088	0,0001	%S=8,4642+0,0179x
%A	*	NS	0,8810	<0,0001	%A=63,3668-0,0652x
Storage time x ambient temperature (28ºC e 55% RU)					
YW	*	NS	0,8947	<0,0001	YW=18,9661+0,0417x
EW	*	NS	0,8795	<0,0001	EW=65,4602-0,1044x
AW	*	NS	0,7624	<0,0001	AW=40,8959-0,1539x
SW	NS	NS	-	-	-
ST	NS	NS	-	-	-
AH	*	NS	0,5274	<0,0001	AH=5,5148-0,0817x
YH	*	NS	0,5620	<0,0001	YH=14,3670-0,1541x
AD	*	NS	0,6850	<0,0001	AD=95,8064+0,4177x
YD	*	NS	0,5995	<0,0001	YD=47,2359+0,1872x
LCR	*	NS	0,7845	<0,0001	LCR=8,6434-0,0594x
ApH	*	NS	0,7756	<0,0001	ApH=8,9190+0,0108x
YpH	*	NS	0,7745	<0,0001	YpH=5,9630+0,0073x
SW	NS	NS	-	-	-
HU	*	NS	0,6041	<0,0001	HU=66,6140-0,8085x
YI	*	NS	0,5297	<0,0001	YI=0,3032-0,0037x
AI	*	NS	0,5696	<0,0001	AI=0,0583-0,0017x
%Y	*	NS	0,7670	<0,0001	%Y=29,0282+0,1171x
%S	*	NS	0,8743	<0,0001	%S=8,5873+0,0264x
%A	*	NS	0,7353	<0,0001	%A=62,3793-0,1431x

YW: yolk weight; EW: egg weight; AW: albumen weight; ShW: shell weight; ST: shell thickness; AH: albumen height; YH: yolk height; AD: albumen diameter; YD: yolk diameter; LCR: La Roche fan color; ApH: albumen pH; YpH: yolk pH; SW: specific weight; HU: Haugh unit; YI: yolk index; AI: albumen index; %Y: yolk percentage; %S: shell percentage; %A: albumen percentage; V: variables; L: linear effect; Q: quadratic effect; R^2: determination coefficient; NS: non-significant for p<0,05. Source: Authors.

The LCR variable showed a significant interaction between storage level and period, with an increasing linear behavior (P<0.05) (Table 3).

Table 3 - Unfolding of the interaction between storage time and canthaxanthin levels inclusion.

Storage time x level					
V	L	Q	R²	P value	Equations
LCR	*	NS	0,94	<0,0001	LCR= 6,977+1,229x

V: variables; L: linear effect; Q: quadratic effect; R^2: determination coefficient; LCR: la roche fan color; *Significant for p<0,05; NS: non-significant for p<0,05. Source: Authors.

Eggs quality using marigold flower in diets

For the interactions between the storage period, marigold flower level, storage environment and between marigold flower level and storage environment, it was observed that none of the evaluated variables was significantly influenced.

There was an interaction between environment and storage period for the variables AH, YH, YD, LCR, ApH, YpH, HU, YI, AI, %Y, %S and %A with significance at 1%, the variables AW and AD showed interaction significant at 5% and the variables EW, YW, SW, ST and SW showed no interaction.

For level effect, the variables EW, AW, YD and YI were significantly influenced at 1%, the variables AD, %Y and %A showed significance at 5% while the others were not significantly influenced. For effect of storage environment, the variables YW, AW, AH, YH, AD, YD, LCR, ApH, YpH, HU, YI, AI, %Y, %S and %A were significantly influenced (P<0.01) and for effect of storage period, the variables YW, EW, AW, AH, YH, AD, YD, LCR, ApH, YpH, SW, HU, YI, AI, %Y, %S and %A were significantly influenced (P<0.01), with interaction between both (Table 4).

Table 4 – Egg quality during storage under different environmental conditions (ambient temperature and refrigerated) of light laying hens fed with different levels of marigold flower from 75 to 85 weeks of age.

V	CPL	SE	SP	CPLxSE	CPL x SP	SE x SP	CPLxSExSP
YW	NS	**	**	NS	NS	NS	NS
EW	**	NS	**	NS	NS	NS	NS
AW	**	**	**	NS	NS	*	NS
ShW	NS	NS	NS	NS	NS	NS	NS
ST	NS	NS	NS	NS	NS	NS	NS
AH	NS	**	**	NS	NS	**	NS
YH	NS	**	**	NS	*	**	NS
AD	*	**	**	NS	NS	*	NS
YD	**	**	**	NS	NS	**	NS
LCR	NS	**	**	NS	NS	**	NS
ApH	NS	**	**	NS	NS	**	NS
YpH	NS	**	**	NS	NS	**	NS
SW	NS	NS	**	NS	NS	NS	NS
HU	NS	**	**	NS	NS	**	NS
YI	**	**	**	NS	NS	**	NS
AI	NS	**	**	NS	NS	**	NS
%Y	*	**	**	NS	**	**	NS
%S	NS	**	**	NS	NS	**	NS
%A	*	**	**	NS	*	**	NS

YW: yolk weight; EW: egg weight; AW: albumen weight; ShW: shell weight; ST: shell thickness; AH: albumen height; YH: yolk height; AD: albumen diameter; YD: yolk diameter; LCR: La Roche fan color; ApH: albumen pH; YpH: yolk pH; SW: specific weight; HU: Haugh unit; YI: yolk index; AI: albumen index; %Y: yolk percentage; %S: shell percentage; %A: albumen percentage; V: variables; CPL: canthaxanthin pigment level; SE: storage environment; SP: storage period; NS: non-significant for p<0,05; ** Significant for p<0,01; * Significant for p<0,05. Source: Authors.

In the unfolding of the interaction between storage environment and period, it was observed that for room temperature the variables EW, AW, AH, YH, LCR, SW, HU, YI, AI and %A showed a decreasing linear effect, while the variables YW, ShW, AD, YD, ApH, YpH, %Y and %S showed an increasing linear effect, depending on the days of storage. ST had no significant effect (P>0.05).

For refrigerated storage, it was observed that the variables ApH, %S and %Y had an increasing linear effect, while the variables EW, AW, AH, YH, SE, HU, YI, AI and %A had an effect decreasing. YW, ShW, AD, YD, AI, LCR, YpH and ST did not show a significant effect (p>0.05) (Table 5).

Table 5 – Unfolding of the interaction between different storage times of eggs stored in two environments (9.7ºC and 70% and 28ºC, 55% RU) on the variables of egg quality of light laying hens fed with marigold flower in the period from 75 to 85 weeks old.

Storage time x refrigerated environment (9,7ºC e 70% RU)					
V	L	Q	R^2	P value	Equations
YW	NS	NS	-	-	-
EW	*	NS	0,9403	0,0019	EW=65,3982-0,1007x
AW	*	NS	0,8995	<0,0001	AW=41,5306-0,1077x
ShW	NS	NS	-	-	-
ST	NS	NS	-	-	-
AH	*	NS	0,9066	<0,0001	AH=6,4668-0,0243x
YH	*	NS	0,8917	<0,0001	YH=16,4941-0,0313x
AD	NS	NS	-	-	-
YD	NS	NS	-	-	-
LCR	NS	NS	-	-	-
ApH	*	NS	0,8593	<0,0001	ApH=8,7667+0,0064x
YpH	NS	NS	-	-	-
SW	*	NS	0,6363	<0,0001	SW=1,0773-0,0002x
HU	*	NS	0,9409	0,0020	HU=77,4933-0,1563x
YI	*	NS	0,9411	0,0018	YI=0,3768-0,0005x
AI	*	NS	0,9487	0,0038	AI=0,0714-0,0002x
%Y	*	NS	0,9204	0,0003	%Y=28,1721+0,0473x
%S	*	NS	0,9077	<0,0001	%S=8,4605+0,0179x
%A	*	NS	0,8808	<0,0001	%A=63,3677-0,0652x
Storage time x ambient temperature (28ºC e 55% RU)					
YW	*	NS	0,8770	<0,0001	YW=18,8185+0,0460x
EW	*	NS	0,8919	<0,0001	EW=65,1981-0,0968x
AW	*	NS	0,7647	<0,0001	AW=40,7971-0,1510x
ShW	*	NS	0,9658	0,0193	ShW=5,5825+0,0081x
ST	NS	NS	-	-	-
AH	*	NS	0,5282	<0,0001	AH=5,4722-0,0805x
YH	*	NS	0,4345	<0,0001	YH=14,3176-0,1528x
AD	*	NS	0,6800	<0,0001	AD=95,8354+0,4169x
YD	*	NS	0,5856	<0,0001	YD=46,9603+0,1951x
LCR	*	NS	0,7584	<0,0001	LCR=8,7548-0,0627x
ApH	*	NS	0,7673	<0,0001	ApH=8,9048+0,0109x
YpH	*	NS	0,7679	<0,0001	YpH=5,9647+0,0071x
SW	*	NS	0,6969	<0,0001	SW=1,0755-0,0001x
HU	*	NS	0,6041	<0,0001	HU=66,4915-0,8084x
YI	*	NS	0,4477	<0,0001	YI=0,3100-0,0041x
AI	*	NS	0,5142	<0,0001	AI=0,0591-0,0010x
%Y	*	NS	0,7595	<0,0001	%Y=28,9287+0,1200x
%S	*	NS	0,8680	<0,0001	%S=8,5805+0,0264x
%A	*	NS	0,7265	<0,0001	%A=62,4916-0,1465x

YW: yolk weight; EW: egg weight; AW: albumen weight; ShW: shell weight; ST: shell thickness; AH: albumen height; YH: yolk height; AD: albumen diameter; YD: yolk diameter; LCR: La Roche fan color; ApH: albumen pH; YpH: yolk pH; SW: specific weight; HU: Haugh unit; YI: yolk index; AI: albumen index; %Y: yolk percentage; %S: shell percentage; %A: albumen percentage; V: variables; L: linear effect; Q: quadratic effect; R^2: determination coefficient; NS: non-significant for p<0,05. Source: Authors.

There was a significant interaction between storage level and period for the variables YH and %A ($P<0.05$) and for %G ($P<0.01$), both with an increasing linear behavior (Table 6).

Table 6 - Unfolding of the interaction between storage time and inclusion level of the marigold flower pigment.

Storage time x level					
V	L	Q	R²	P value	Equations
AG	*	NS	0,85	<0,0001	AG=15,405+0,0920x
%G	*	NS	0,86	<0,0001	%G=28,550+0,083x
%A	*	NS	0,83	<0,0001	%A=62,929+0,105x

V: variables; L: linear effect; Q: quadratic effect; R^2: determination coefficient; NS: non-significant for p<0,05; *Significant for p<0,05; YH: yolk height; %Y: yolk percentage; %A: albumen percentage. Source: Authors.

4. Discussion

With the increase in the storage period of the eggs, both the eggs of hens that received the synthetic pigment canthaxanthin and the eggs with the presence of the pigment of marigold flower extract showed a linear increase in yolk weight (YW), which can be explained by the fact that water present in albumin, cross the yolk membrane, being retained in the yolk, which process occurs by osmosis and causes a weakening of the yolk membrane (Lemos et al., 2014), this also reflects in the %Y variable, that is correlated, having increased linearly. However, for the YI variable there was a decreasing linear effect, because when a significant amount of water enters the interior of the yolk, it loses its original spherical shape, becoming elliptical, thus reducing the YI.

The loss of the original yolk shape influenced the variable YH in a linear and decreasing way also for both pigments, that is, as the yolk became elliptical, the height observed was smaller, and likewise there was an influence on the YD, which presented a increasing linear behavior, thus as it lost its original shape due to higher water permeability. Similar results were found by Pissinati et al. (2014), when evaluating the internal quality of eggs from Shaver White lineage hens during the 35-day egg storage period at a temperature of (25ºC).

The variable AW had a decreasing linear effect, this effect was observed for the two pigments, that is, over the days the albumen weight decreased significantly, probably due to transformation reactions that occur in the eggs when stored for long periods, when there is a loss of CO_2 through the shell, which allows this reaction to transform ovalbumin into S-ovalbumin, causing the destruction of the ovomucin gel that makes the albumen more liquefied. This reaction can increase the evaporation of water through the pores present in the shell, and also has an effect on the %A variable, which also presented a decreasing linear behavior, considering that they have a correlation.

For the variable AI, the loss of CO_2 through the shell also influenced it in a decreasing way, because for the determination of the AI the ratio between AH/AD was used, since the variable AH had a decreasing linear behavior, that is, with the denaturation of the ovomucin gel there was a reduction in the albumen height and, in contrast, the variable AD showed a linear increasing behavior in eggs stored at ambient temperature both with presence of canthaxanthin and marigold flower, that is, according to the albumen became more liquefied, the greater the diameter observed, and these data are consistent with the results found by Lana et al. (2017), when evaluating the internal quality of eggs from hens of the Dekalb White strain, for an experimental period of 30 days at an ambient temperature of 26.5°C.

The EW variable had a decreasing linear effect, as the days went by, there was significant weight loss, because in the shell there is the presence of pores (on average from 6000 to 8000 pores with a diameter of 1 to 10 µm), which allows gas exchange and loss of moisture, this occurs in the egg after oviposition, progressively over the days of storage, causing an increase in the air chamber, and a decrease in egg weight.

This increase in the air chamber is also correlated with the decrease in SW as observed for the marigold flower pigment, whereas for the pigment canthaxanthin the variable SW was not significant in this study, obtaining an average density of 1,060 since the fifth day of storage at ambient temperature, this characteristic being more accentuated in eggs stored at ambient temperature than in refrigerated ones.

These results for the SW of the present work are in agreement with those obtained by Santos et al. (2009), that when analyzing eggs from commercial laying hens of the Hy-line W-36 lineage for a storage period of 28 days at room temperature (27, 84°C and 71.14% RH) and 28 days at refrigerated temperature (4.65°C and 78.5% RH) observed that for both environments there was a significant loss of egg weight, and for those stored at room temperature this loss was more pronounced when compared to eggs kept under refrigeration. Silva et al. (2013), when working with storage for 28 days at room temperature (21.2°C and 57% RH), and with fresh eggs from laying hens of the Hy-line lineage, observed that the weight loss of eggs was increasing because the water evaporation from the egg is a process that does not stop, starting at laying until the egg is completely dehydrated.

The ApH and YpH variables showed an increasing linear behavior, which can be explained by the loss of CO_2 through the pores present in the shell, which caused the pH of the albumen and yolk to increase linearly, corroborating the data found by Xavier et al. (2008), who, when evaluating the pH of the albumen and yolk for a storage period of 30 days, also obtained a linear increase over the days of storage.

The HU variable presented a decreasing linear behavior for both pigments, and this was a process accelerated by the high temperature of the environment (28ºC), corroborating the results found by Figueiredo et al. (2011), in an experiment carried out with hens of the Hy-line strain, storing eggs for 15 days, with the quality of the eggs performed daily, at a temperature of 25.6°C ±1.7°C, finding HU values for storage at room temperature of 58.5, which does not characterize an excellent quality standard, corroborating the results found by Feddern et al. (2017), who, when evaluating the eggs of two strains of laying hens White Leghorn and Rhode Island Red for up to 9 weeks at room temperature (20 to 35°C) and refrigerated (5 to 6.5°C), observed that for both in the strains there was a greater decrease in HU in eggs that were kept at room temperature than in those that were refrigerated.

For the pigments evaluated in this study, the variable LCR showed a linear decreasing behavior when the eggs were stored at room temperature, that is, over the days the yolk became more depigmented even with the addition of the synthetic pigment (canthaxanthin) and the pigment natural (marigold flower) in the diet of light laying hens from 75 to 78 weeks of age, whose eggs for shelf life were collected in the last ten days. The fact that the yolk loses its color over time can be explained by the permeability of the water that passes from the egg white into the yolk, which makes it more depigmented and watery (Barbosa, 2009).

For the interaction between temperature in a refrigerated environment and storage time with the addition of canthaxanthin pigment and marigold flower extract pigment in the laying hens' diet, the variables AD, YD, AI and YpH were not influenced, showing that when keeping the eggs under a refrigerated environment the quality of yolk and albumen is maintained.

The variable SW, as well as at room temperature, showed a linear decreasing effect for the pigmentation of marigold flower extract, while for canthaxanthin it did not show a significant effect, and this process was less accentuated, taking longer for these results to be observed when eggs were stored in a refrigerated environment, demonstrating the beneficial effect of refrigerated storage on the maintenance of internal egg quality. Similar results were obtained by Souza (2016), with Hisex White laying hens in 4 storage periods (7, 14, 21 and 28 days) at room temperature (27°C) and refrigerated (4°C), showing that the eggs in a refrigerated environment showed better quality parameters.

For both pigments, the variable LCR was not significantly influenced when the eggs were stored in a refrigerated environment, with no yolk depigmentation, the albumen properties were also maintained (also had no significant effect on the AD), reducing the loss of water to the yolk and by evaporation. Indicating that if eggs are stored under recommended conditions (low temperatures) there is an improvement in their shelf life.

However, for the pigment canthaxanthin, the LCR variable presented a significant interaction for the storage period and inclusion level, with an increasing linear behavior. According to Rosa (2018), this is due to the fact that canthaxanthin is a carotenoid that has its metabolism directed towards the synthesis of yolk (pigmenting) and antioxidant activity, having no effect on albumen quality. Corroborating Garcia et al. (2002), that when working with layers of the Hisex Brown lineage, including 60 ppm of canthaxanthin for a period of 56 days, they obtained an improvement in the color and shape of the yolk.

The pigment of marigold flower extract, unlike canthaxanthin, did not show significant interaction for LCR, that is, the yolk color was not influenced, which can be explained by the fact that they are different compounds and the chemical pigmentation capacity of canthaxanthin is greater when compared to the marigold flower pigment (Sandeski, 2016), this result being similar to that found by Valentim et al. (2019), who carried out a research with four treatments, namely: control feed; ration with 0.8% paprika extract (*Capsicum annuum*); ration with addition of 0.8% of marigold flower extract (*Tagetes erecta*) and ration with addition of 0.045% of canthaxanthin, obtained a larger yolk color scale (12.62) with the la Roche colorimetric fan for canthaxanthin.

The greater efficiency of synthetic pigments in relation to yolk pigmentation is a well-established fact, Baião et al. (1999), in a research with commercial products derived from marigold and paprika flower extract, already found this efficiency of synthetic pigments in relation to natural sources in yolk pigmentation and also observed that synthetic pigments with higher concentration of xanthophylls present a smaller colorimetric variation.

But for the marigold flower pigment there was a significant interaction for the storage period and inclusion level for the variables YH, %Y and %A, both with increasing linear behavior, which may have happened because the marigold flower pigment has approximately 12 g/ kg of xanthophylls, 80 to 90% of lutein, presenting antioxidant properties that may have significantly influenced these variables (Skrivan et al., 2015). Aquino (2019), when working with the storage of quail eggs for a period of 45 days in a refrigerated environment and at room temperature, found results similar to the present study, with the addition of 5.4 ppm of lutein in the sorghum-based diet, obtained a significant %Y that slowed down the albumen degradation process, maintaining the quality for a longer storage period.

5. Conclusion

The use of natural (marigold flower) and synthetic (canthaxanthin) pigments for the shelf life of eggs from laying hens in the final stage of production (75-78 weeks of age) did not influence most of the internal and external quality variables of eggs in the present study, however eggs stored in a refrigerated environment had better results for egg conservation.

In view of what was observed in the present study for future research, there is a suggestion of analyzes that can measure the concentration of lutein deposited in the yolk in order to correlate with its antioxidant power in fact, and adjust the levels that were added to the feed in order to find an ideal level that helps to delay the degradation of egg components, especially when they are stored at room temperature and/or with temperature variation.

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