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Original Article

Productive Performance, Egg Quality, and Pigments on Sorghum-Based Feed for Japanese Quail

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ABSTRACT

The study aimed to determine evaluate the use of sorghum as a substitute for maize in the laying quail diet and its effect on performance and quality of the eggs produced the best levels of lutein (Marigold flower extract) and canthaxanthin inclusion in sorghum based Japanese quail feed as well as its influence in storage time of the eggs. A total of 680 Japanese laying quails (*Coturnix coturnix japonica*), with 64 weeks of age was distributed in a 4 × x4 factorial + 1 control scheme, with four levels of lutein (LUT), and four levels of canthaxanthin (CTX) and control group with no addition of pigments, with five replicates and eight birds per experimental unit. The experiment was divided into three cycles of 21 days, where the performance and egg quality parameters were evaluated. To evaluate the time of deposition and permanence of the pigments in the yolk, three eggs were evaluated per treatment for 12 days at the beginning of the experiment and 12 days at the end, from the last day of consumption of rations containing pigments. For performance and egg quality, no maybe only differences ($p > 0.05$) were observed, except for the yolk color, with higher color scores according to the increase in lutein and canthaxanthin consumption. Whereas color parameters, it was observed that at the beginning of the period of consumption, canthaxanthin levels influenced the red color reading and lutein levels influenced the yellow color readings, while in the final period the interaction of the levels of 5.4 ppm of lutein and 1.3 ppm of canthaxanthin provided better color parameters up to 10 days after the end of consumption of rations with pigment.

INTRODUCTION

The use of alternative ingredients to corn in diets of laying quails has been increasing for providing a reduction in spending on food. Sorghum can be cited as one of those alternative foods to corn, but its composition has a deficient amount of pigments such as carotene and xanthophylls and for this reason its supply to the bird induces the depigmentation of the egg yolk entailing the need to use sensory additives that provide pigments to color the yolk without affecting production (Moura *et al*., 2011).

Normally, pigments are used by combining compounds of yellow and red color, which may be carotenes or xanthophylls obtained by natural extracts or synthetic pigments. Among the options for use are canthaxanthin and lutein xanthophylls that give the product red and yellow colors respectively (Amaya, 2014).

Canthaxanthin and lutein are pigments in the group of xanthophylls commonly used to obtain orange or reddish colors in animal products. Its deposition and transport as well as other carotenoids occur through lipid compounds and can be used according to the objective and product (Faruk, 2017).

The color of the quail egg yolk that receives sorghum-based ration containing canthaxanthin has a visual difference when compared to the use of these rations without any addition of pigments, and rations containing Marigold Flower extract as a source of lutein, have a higher colorimetric score. In a shorter time of supplement, thus showing how the use of these pigments in sorghum-based diets is necessary to maintain consumer acceptance standards (Moura *et al*., 2011).

The use of sorghum as a substitute for maize in the laying quail diet may affect the quality of the egg produced referring to the yolk color and consequently the commercialization of it. Therefore, the use of pigments associated with this diet makes it possible to use sorghum without affecting the quality of the egg produced. In this context, the objective of this study was to determine evaluate the use of sorghum as a substitute for maize in the laying quail diet and its effect on the quality of the egg referring to the yolk color. the best levels of canthaxanthin and lutein in sorghum-based diets for Japanese quails in the laying phase aiming at improvements in egg quality, thus optimizing the use of alternative foods to corn, without the production of eggs and the viability of marketing this product being compromised.

MATERIALS AND METHODS

The experiment was carried out at the Coturniculture Sector, Experimental Farm of Iguatemi, Paraná, belonging to the State University of Maringá (UEM), according to the Ethics Committee on the Use of Animals (CEUA/UEM) (Protocol number 3296140619).

Animals, facilities and design

The birds were housed in a laying shed, in a conventional system. The reference diet was formulated with sorghum and soybean meal, taking into considering consideration the values of the chemical and energetic composition of foods proposed by Rostagno *et al*. (2017) and for nutritional requirements of quails, the recommendations of NRC (1994) for laying quails were adopted (Table 1). The products were incorporated in combinations of different levels totaling 16 treatments and control that consisted of a reference diet without pigments addition.

A total of 680 laying quails, 64-week old 64-week-old were used, distributed in a completely randomized design, in a 4 × 4 factorial scheme, with four levels of lutein (LUT) (5.4; 5.7; 6.0 and 6.3 ppm) and four levels of canthaxanthin (CTX) (0.4; 0.7; 1 and 1.3 ppm), and a control group with no pigment added, totaling 17 treatments, with 5 repetitions of 8 birds / experimental unit, counting 85 experimental units. The birds were initially weighed individually and distributed according to the average weight of the parcel.

Table 1 – Composition of the reference diet.

Ingredients	Percent composition of the feed (%)
Sorghum (low tannin)	54.71
Soybean meal (45%)	29.95
Dicalcium phosphate	1.12
Limestone	7.62
Soybean oil	4.88
Min/vit supplement1	0.40
Common salt	0.33
L-Lysine HCL (78%)	0.29
DL-Methionine (99%)	0.46
L-Threonine (99%)	0.11
L-Tryptophan (99%)	0.02
Antioxidant[2]	0.01
Inert+pigments[3]	0.10
Total	100
Calculated composition (%)	
ME (kcal/kg)	2,900
Crude protein (%)	19.00
Calcium (%)	2.99
Available phosphorus (%)	0.31
Sodium (%)	0.15
Potassium (%)	0.19
Chlorine (%)	0.24
Digestible methionine + cis cystine (%)	0.94
Digestible lysine (%)	1.15
Digestible threonine (%)	0.70
Digestible tryptophan (%)	0.24

[1]Mineral/vitamin supplement (guarantee levels per kg product); Vit. A – 18,000 IU; Vit. D3 – 5,000 IU; Vit. E – 16 mg; Vit. B1 – 1,112 mg; Vit. B2 – 8 mg; Vit. B6 – 2,100 mg; Vit. B12 – 20 mcg; Vit. K3 – 4,028 mg; Ca pantothenate – 16 mg; Niacin – 40 mg; Biotin – 50.0 mg; Choline – 60 mg; Antioxidant – 20 mg; Zn – 126 g; Fe – 98 mg; Mn – 155 mg; Cu – 30.624 mg; Co – 0.4 mg; I – 1.936 mg; Se – 0.508 mg; Vehicle Q.S.P. – 4 g.

2BHT (Butyl Hydroxy Toluene); 3Washed sand; Metabolizable energy (ME).

The natural source of lutein used was the extract of Marigold Flower, with levels of 3% lutein, thus being used in proportional amounts to achieve the desired amount for each treatment. For canthaxanthin, the synthetic pigment was used, both sold in powder form.

Performance

The experimental period was divided into three production cycles, with 21 days each. The dead birds were counted daily to correct feed intake. The eggs were harvested daily to calculate the laying rate (%) and the mass production of eggs (gram eggs/bird/day), counting all eggs produced including broken, cracked, abnormal, and soft-shelled.

The laying rate was considered to be the product of dividing the number of eggs produced by the total number of birds in the experimental unity, given by:

Laying rate (%) = (number of eggs produced / number of birds housed) × 100.

The egg mass was obtained by multiplying egg production by the average egg weight, according to the equation: egg mass (gram eggs/bird/day) = (average egg weight (g) × number of eggs produced) / number of housed birds.

The quails were weighed at the beginning and end of the experimental period to determine body weight (g) and the rations were weighed at the end of each cycle to determine feed intake (g), in addition to calculating feed conversion per kg of eggs, which expresses the relationship between feed consumption and egg production by weight, being calculated by the equation: FCkg (kg/kg of eggs) = feed consumption (kg) / weight of eggs (kg). Along with the feed conversion per dozen eggs - FCdz (kg dz of eggs), which expresses the relationship between feed consumption and egg production in dozens, was calculated by the equation: FCdz (kg/dz of eggs) = consumption of ration (kg) / dozen eggs.

Quality and storage of eggs

In the last three days of each cycle, the internal and external quality analyzes of the eggs were carried out. The characteristics evaluated were: average egg weight (g), specific gravity (g / ml), yolk index, % of shell, % of yolk, % of albumen, shell weight per surface area, and yolk color.

Among the quality measures, specific gravity was obtained using the immersion method of all eggs in different concentrations of saline (Baumé densimeter ranging 0.005 g/mL from 1.060 to 1.090 g/mL) according to the methodology described by Hamilton (1982).

For internal quality analyzes, three eggs were selected according to the experimental unity average weight, identified and weighed individually. After weighing, they were broken to determine the height (mm) and diameter (mm) of the yolk and albumen using a digital caliper.

The determination of the yolk height was carried out at its highest point and for the albumen, closest to the yolk. The diameter was obtained by the average of two transversal measurements of both the yolk and the albumen. Later, through these data it was possible to determine the yolk index (YI), given by YI = (yolk height (mm) / yolk diameter (mm)) × 100 and the Haugh Unit (HU), calculated according to Haugh (1937), considering albumen height (AH) and egg weight (EW): HU = 100log (AH + 7.57 - 1.7 × EW0.37).

Subsequently, the yolk and albumen were separated for weighing the yolk on a precision scale, and the weight of the albumen was obtained by subtracting the weight of the egg, the yolk and shell weights. The weight data allowed quantifying the percentages of yolk, albumen, and shell related to the weight of the egg, according to the equation: % of the component = (weight of the component (g) / weight of the egg (g)) × 100.

After the egg section, the shells were washed, dried, and stored for later weight determination (on a precision scale). The shell weight per area (SWPA) was also determined, calculated, and using the formula adapted by Rodrigues et al. (1996), where: SWPA = (shell weight (g) / 3.9782 × egg weight (g)) × 100.

The color of the yolk was analyzed by the subjective method using the Roche Colorimetric Fan, which evaluates the color on a color scale from 1 to 15 and by the objective method, measuring the following parameters: L* (luminosity), a* (intensity of green/red), b* (intensity of blue/yellow) using a portable colorimeter (CR400 - MINOLTA) previously standardized in the colors black (0) and white (100), using illuminant D65 and 10º for the angle of the observer.

To evaluate the egg quality during the storage all the eggs produced were harvested and selected at the end of the last cycle, for five days, based on the absence of cracks, spots, or dirt in the shell, 54 eggs from each treatment. These were subsequently identified, placed on a cardboard tray, and stored in different environments (room temperature 17° C; refrigerated temperature 5° C) and subjected to different storage periods (15, 30, and 45 days).

In each period and conservation environment, six eggs were selected per treatment to evaluate internal quality. The characteristics assessed were average egg weight (g), specific gravity (g / ml), yolk index, % shell, % yolk, % albumen, pH of albumen and yolk.

Color analysis

To evaluate the time of deposition and permanence of pigments in the yolk during the time of consumption, three eggs were collected and evaluated per treatment for 12 days at the beginning of the experiment, counting from the first day of supply of the experimental diets. Moreover for 12 days at the end of the experimental period from the last day of consumption by birds in diets containing pigments. These eggs were used for color evaluation by the objective method.

Statistical analysis

The statistical analysis of the data was performed using the statistical program R (R Studio), according to the model: $Y_{ijkl} = b_0 + b_1L_i + b_2C_j + b_3L_{i2} + b_4C_{j2} + b_5LC_{ij} + FA + e_{ijkl,.}$

Regression analyzes of the inclusion levels of lutein and canthaxanthin were performed, and the estimates were obtained using the quadratic model as described by Sakomura and Rostagno (2016). To compare the levels with the control treatment without the addition of pigments, a Tukey test was performed, with a significance level of 5%.

For storage time data the model used was: $Y_{ijklm} = b_0 + b_1L_i + b_2C_j + b_3T_k + b_4A_l + b_5L_{i2} + b_6C_{j2} + b_7T_{k2} + b_8A_{l2} + b_9LCTA_{ijkl} + LA + e_{ijklm}$.

The comparison between the quality averages was performed by the Tukey test at the level of 5% significance.

RESULTS

There was no significant effect (p> 0.05) of interaction between pigments on the birds' performance, showing that the levels of LUT and CTX provided, acted independently on these variables (Table 2). For feed intake (FI), there was a linear effect depending on the level of lutein in the diet. For the other performance variables, no significant effects were observed.

Likewise, the results for egg quality, in the experimental conditions, did not show a significant difference (Table 3). The parameters of quality consider data of weight and dimensions of its components; this does not necessarily correlate with the levels of pigments in the diet.

Table 2 – Average performance values of laying quails according to lutein and canthaxanthin levels in sorghum-based diets.

LUT	CTX	FI (g)[a]	FCdz[a]	FCEM[a]	EW[a] (g)	EM[a] (g)	PR[a] (%)
0,0	0.0	32.95	0.49	3.62	11.33	9.85	84.29
5,4	0.4	29.87	0.43	3.40	11.46	9.04	86.56
	0.7	30.10	0.52	3.70	11.72	9.43	73.01
	1.0	31.64	0.51	3.45	11.38	10.03	76.04
	1.3	30.73	0.45	3.19	11.46	9.53	83.92
5,7	0.4	28.83	0.47	3.52	11.52	10.20	77.79
	0.7	31.11	0.43	3.11	11.57	9.28	85.10
	1.0	31.98	0.51	3.23	11.92	9.98	77.67
	1.3	31.32	0.47	3.17	11.77	9.63	78.94
6,0	0.4	30.90	0.45	3.22	11.77	8.93	78.85
	0.7	33.73	0.56	3.78	11.56	9.00	74.35
	1.0	31.44	0.54	3.65	11.58	9.67	74.85
	1.3	32.46	0.48	3.58	11.56	9.91	81.65
6,3	0.4	32.14	0.50	3.70	11.39	10.58	77.80
	0.7	33.46	0.50	3.25	11.53	10.17	80.85
	1.0	34.11	0.49	3.36	11.46	9.73	84.12
	1.3	33.55	0.49	3.41	11.33	9.63	83.89
			p value				
LUT		<.0001(L)	0.112	0.352	0.286	0.547	0.348
CTX		0.478	0.596	0.848	0.112	0.946	0.275
LUT x CTX		0.531	0.847	0.725	0.143	0.914	0.254
Standard error		0.292	0.007	0.048	0.035	0.124	0.991
Regression equation			R^2				
FI =29.91862+29.91862LUT			0.38				

LUT: Lutein; CTX: Canthaxanthin; FI: feed intake; FCdz: feed conversion per dozen eggs; FCEM: feed conversion by egg mass; EW: egg weight; EM: egg mass; PR: posture rate; R2: coefficient of determination; L: linear effect; p (>0.05)(p> 5%). [a]n = 680 birds.

For yolk color values, evaluations were made within the period of the experimental cycles, with readings in the range of colors and the objective method, for values of L* a* and b*, as expected, there was a significant effect depending on the levels of pigments in the diet. There was a negative linear effect for the levels of canthaxanthin in L*, for canthaxanthin interaction: lutein in a* and color in the fan (CSF), and positive linear effect for lutein in b* (Table 4).

The average values of L* in digital colorimeter correspond to the level of luminosity found in the sample, in comparison to the control group, without the addition of pigments, there was a difference in comparison to the levels of 5.4 ppm of LUT +1.3 ppm

Table 3 – Average quality values of Japanese quail eggs, according to lutein and canthaxanthin levels in sorghum-based diets.

LUT	CTX	HU[b]	SG[b] (g/ml)	YI[b] (mm)	S[b] (%)	Y[b] (%)	A[b] (%)	SWA[b]
0,0	0.0	86.62	1.07	0.47	7.83	32.30	60.07	4.10
5,4	0.4	87.06	1.06	0.45	7.63	31.48	60.90	3.93
	0.7	87.93	1.06	0.46	7.78	31.21	61.09	4.05
	1.0	86.13	1.06	0.46	7.64	32.29	60.05	3.93
	1.3	86.77	1.07	0.45	7.87	31.79	60.32	4.09
5,7	0.4	86.97	1.07	0.46	7.59	31.33	60.86	3.91
	0.7	88.03	1.07	0.46	7.79	31.58	60.83	4.00
	1.0	88.22	1.06	0.46	7.64	31.23	61.03	4.02
	1.3	87.79	1.06	0.45	7.71	30.96	60.96	4.01
6,0	0.4	87.38	1.07	0.45	7.82	31.75	60.22	4.08
	0.7	86.76	1.07	0.46	7.90	31.85	60.29	4.07
	1.0	86.90	1.07	0.45	7.57	30.85	61.70	3.91
	1.3	86.40	1.06	0.46	7.87	31.72	60.34	4.06
6,3	0.4	86.62	1.07	0.47	7.72	31.35	61.02	3.97
	0.7	89.00	1.07	0.46	7.73	31.19	61.06	3.95
	1.0	87.89	1.07	0.46	7.88	30.44	61.28	4.13
	1.3	88.38	1.07	0.47	7.84	31.55	61.10	4.05
				p value				
LUT		0.081	0.278	0.363	0.736	0.220	0.494	0.353
CTX		0.939	0.572	0.973	0.736	0.673	0.904	0.592
LUT x CTX		0.199	0.962	0.805	0.482	0.924	0.706	0.293
Effect		NS	NS	NS	NS	NS	NS	NS
Standard error		0.158	0.001	0.001	0.033	0.105	0.110	0.018

LUT: Lutein; CTX: Canthaxanthin; HU: Haugh Unit; SG: specific gravity; YI: yolk index; S: percentage of the shell; Y: percentage of yolk A: percentage of albumen; SWA: shell weight per area; *p*: significant effect at(> 0.05). [b]n = 255 eggs5%.

Table 4 – Average color values of Japanese quail egg yolk according to lutein and canthaxanthin levels in sorghum-based diets.

LUT	CTX	L*[c]	a*[c]	b*[c]	RFC[c]
0,0	0,0	50.17 [a]	-3.22 [g]	13.03 [b]	3.00 [e]
5,4	0.4	46.27 [ab]	-0.29 [ef]	18.52 [a]	5.50 [de]
	0.7	46.87 [ab]	1.33 [cd]	18.71 [a]	6.33 [cd]
	1.0	46.02 [ab]	2.40 [abc]	18.25 [a]	8.83 [abc]
	1.3	45.14 [b]	2.95 [a]	17.57 [a]	8.50 [abc]
5.7	0.4	46.34 [ab]	-0.55 [f]	18.43 [a]	6.50 [bcd]
	0.7	46.78 [ab]	0.89 [de]	19.07 [a]	7.33 [abcd]
	1.0	46.26 [ab]	2.34 [abc]	18.31 [a]	9.16 [ab]
	1.3	45.60 [ab]	3.24 [a]	17.86 [a]	9.83 [a]
6.0	0.4	46.70 [ab]	-0.52 [f]	20.70 [a]	6.33 [cd]
	0.7	47.03 [ab]	0.62 [def]	19.82 [a]	7.66 [abcd]
	1.0	44.35 [b]	2.51 [abc]	16.56 [ab]	9.33 [a]
	1.3	45.27 [ab]	2.81 [ab]	16.99 [ab]	9.83 [a]
6.3	0.4	45.89 [ab]	-0.46 [f]	17.25 [ab]	7.50 [abcd]
	0.7	46.98 [ab]	0.84 [de]	17.04 [ab]	7.50 [abcd]
	1.0	45.96 [ab]	1.59 [bcd]	20.34 [a]	8.83 [abc]
	1.3	46.06 [ab]	2.89 [a]	18.46 [a]	10.00 [a]
			p value		
LUT		0.235	0.001	0.008 (L)	0.015
CTX		0.023 (L)	<0.001	0.079	0.013
LUT x CTX		0.281	<0.001 (L)	0.062	0.045 (L)
Standard error		0.251	0.243	0.291	0.215
Regression equation					R^2
L* = 48.0579-0.3800LUT-0.7452CTX					0.41
a* = -2.6082+0.3577LUT+1.6428CTX-0.1629LUT×*CTX					0.98
b* = 15.7521+1.0722LUT					0.76
RFC=3.26947+0.76972LUT+1.52663CTX-0.15460LUT×*CTX					0.91

LUT: Lutein; CTX: Canthaxanthin; RFC: fan color; L*: luminosity; a *: red / see coordinate; b *: yellow / blue coordinate; R^2: coefficient of determination; L: linear effect; *p* (>0.05)(*p*> 5%). [c]n = 255 eggs.

CTX and 6.0 ppm LUT + 1.0ppm CTX, with the latter showing lower luminosity compared to the control, because higher levels of canthaxanthin (red pigment), provides a darker intensity color to the yolk, thus leading to a lower luminosity and therefore its effect inversely proportional to canthaxanthin level.

For RCS (Roche colorimetry scale) and a* (green/red intensity) the linear effect as a function of the interaction between pigments shows that the color of the yolk is the result of the associated effect of products, as well as the other color parameters the group control differed from the others.

The reading of a* showed that the control had a lower intensity of red color, as well as the treatments with lower levels of canthaxanthin compared to the others. While the reading of the colorimetric scale (CSF) had the lowest value for the control group, indicating that it had lower color intensity, and treatments with lower levels of canthaxanthin also showed lower values of color intensity compared with other treatments.

For b* reading, corresponding to the yellow color intensity, the values showed that the control group differed from the others with a lower proportion of yellow compared to the other treatments and its positive linear effect, as a function of the level of lutein in the diet shows that the yellow color intensity is proportional to the increase in lutein consumption.

For color parameters analyzed over the initial consumption time by the birds, in the first 15 days of the experiment, the results of the reading in a digital colorimeter showed that for luminosity (L*) there was no significant effect (p>0.05) for consumption time, while for the readings of the colors red (a*) and yellow (b*) there were effects of the levels of canthaxanthin and lutein respectively. The regression equations and p values are shown in Table 5.

The reading of a* corresponds to bands of green (less than zero) and red (greater than zero), so the addition of canthaxanthin, red pigment, increased values of a*, that is, greater intensity of red color over the time of the experimental diets consumption. The control treatment showed a drop after ten days of consumption, considering that the lack of red pigment in the diet without the addition of pigments resulted in less color intensity of the yolk.

For b* values corresponding to the blue (-) and yellow (+) color bands, the results showed that for control, without the addition of pigments there was a drop after the tenth day of consumption, and up to 15 days of consumption the lowest level of lutein presented the lowest values in comparison to the other levels.

At the end of the experimental period, the color readings were made according to a decrease in the deposition of pigments in the yolk due to the pause in the consumption of the experimental diets. For values of luminosity (L*), red (a*), and yellow (b*) the results were significant (p>0.05), for the interaction of levels of lutein and canthaxanthin over the time of consumption of sorghum-based rations without the addition of pigments.

For L* the comparison of means within the levels of addition of lutein and canthaxanthin, showed that the treatment containing 5.5 ppm of lutein and 1.3 ppm of canthaxanthin showed values that differed with greater luminosity for the control and both showed close values after the tenth day of feed intake without pigments.

At the end of the consumption period of the experimental rations, the values of a* and b* decreased over time without consumption of pigments, matching the control values to the fifteenth day of consumption, showing that the intensities of red and yellow colors were affected by consumption of feed without the addition of pigments, presenting a more visible fall after the fifth day.

For the parameters of yolk index (YI), and pH of yolk and albumen the effects (p>0.05) were observed as a function of time and environment storage, where for yolk index the results for the group kept under refrigeration showed constant close to 0.4 while for the group kept at room temperature were below 0.2 after forty days of storage.

Table 5 – Yolk color according to pigments period of consumption.

Parameter	Regression equation	*p* value	R^2	Error
a* start [d]	a* = -1.784-0.107CTX-0.107T+0.504CTX×*T	<0.001	0.69	0.146
b* start [d]	b* = 19.128-0.889LUT-2.403T+0.555LUT×*T	0.010	0.17	0.176
L* final [d]	L* = 53.241-1.336LUT-1.883CTX-1.650T-0.164LUT×*CTX×*T	0.046	0.10	0.160
a* final [d]	a* = 4.328-0.262+LUT0.144CTX+1.260T+0.001LUT×*CTX×*T	<0.001	0.80	0.139
b* final [d]	b* = 18.484+1.340LUT+1.372CTX-2.110T+0.117LUT×*CTX×*T	0.028	0.69	0.289

LUT: lutein; CTX: canthaxanthin; T: storage time; A: storage environment; L*: luminosity; a*: green and red band; b*: blue and yellow band; Significant p value at(> 0.05). [d]n = 612 eggs5%.

Table 6 – Regression equations for egg quality parameters.

Parameter	Regression equation	*p* value	R^2	Error
Haugh Unit [e]	HU = 88.080+3.131LUT-4.367T-0.394A+1.807LUT×*T×*A	0.009	0.56	0.308
Yolk index [e]	YI = 0.559-0.184T-0.046A+0.089T×*A	< 0.001	0.90	0.006
% Yolk [e]	%Y =23.557+0.715LUT+8.675T+3.851A+0.652LUT×*T×*A	0.047	0.55	0.325
pH of yolk [e]	pHy = 5.711+0.394T+0.208A-0.168T×*A	0.012	0.43	0.018
pH albumen [e]	pHa = 9.104+3.790T+2.938A-4.910T×*A	0.028	0.11	0.009

LUT: lutein; CTX: canthaxanthin; T: storage time; A: storage environment; Significant *p* value at (>0.05)5%. [e]n = 255 eggs.

For pH values, for yolk up to thirty days of storage at room temperature the values remained close to 6.3 while under refrigeration the values were close to 6.1 within the same storage period. For albumen, the pH dropped after the fifteenth day of storage from 9.3 to 8.9 at room temperature and under refrigeration, within the same period the values showed a less pronounced drop from 9.2 to 9.0 at thirty days.

There was a significant effect for HU parameters, between lutein levels within different egg storage times and environments, and for storage conditions at room temperature, the treatment containing 5.4 ppm of lutein showed higher averages than the other levels in the same storage conditions, and in general, the eggs kept under refrigeration obtained Haugh Unit values higher than the eggs kept at room temperature.

Significant results for lutein levels as a function of environment and storage time were also observed for yolk percentage. For the treatment of 5.4 ppm of lutein kept refrigerated for up to 45 days of storage, it presented lower values of yolk percentage compared to other treatments under the same conditions. Higher values of yolk percentage indicate higher yolk weights, that is, higher water concentration from albumen.

DISCUSSION

Although some carotenoids are precursors of vitamin A, this factor was not relevant to the performance of birds, considering that despite the wide variety of natural carotenoids available, only 10% of these can be converted into vitamin A in birds (Surai, 2003). It is important to note that the vitamin levels provided in the feed were above that required by the birds (NRC, 1994), and therefore the carotenoids present in the feed were metabolized and directed to the yolk synthesis (target organ) (Pérez-Vendrell *et al*., 2001).

Moura *et al*, (2010), observed that the use of sorghum in total replacement for corn did not result in a drop of laying quails quail performance, thus showing that substitution is viable, without affecting productivity. This proves to be a positive factor for producers, who has one more option to use in animal diets, especially when corn has higher prices, usually in the off-season.

Previous works have also shown similar results, using pigments in sorghum-based diets, without affecting egg production and quality parameters, except for yolk color (Silva *et al*., 2000; Santos-Bocanegra *et al*., 2004; Curvelo *et al*., 2009; Moura *et al*., 2011).

The colorimetric score of yolk as a function of the carotenoids had similar effects observed by Baião *et al*. (1999), in research with commercial products derived from marigold and paprika, the authors found greater efficiency of synthetic pigments concerning natural sources in yolk pigmentation and reported that synthetic pigments with a higher concentration of xanthophylls are more stable.

In general, the pigments are deposited from a yellow base, in this case, lutein, associated with the red color, given here by canthaxanthin, proportionally the deposition of the two pigments will give an orange color to the yolk of greater or lesser intensity. Therefore, the fact that the yolk is paler or more intense in color is directly associated with the proportional consumption of yellow and red pigments by the bird (Amaya, 2004).

The deposition efficiency can also change according to the type of carotenoid, Gonzales & Sartori (1999) evaluated the deposition of carotenoids in the egg yolk and observed greater deposition obtained with apo-ester (50%), followed by canthaxanthin (45%), lutein (20%), zeaxanthin (22%), capsanthin (11%) and β-carotene (1%).

Although quail eggs are marketed and consumed as a whole, this does not diminish the fact that a depigmented yolk can generate consumer dissatisfaction, so the use of pigments in sorghum-based diets is a viable strategy to maintain yolk color without affecting consumer acceptability. The yolk staining results obtained for the levels of 5.4ppm (LUT) + 1.3ppm (CTX) show that, under these experimental conditions, this is the best way to use the associated products, considering which provided the best yolk color parameters, using the lowest level of lutein.

The initial time of pigments consumption can be a factor in the deposition of these compounds in the yolk, Garcia *et al*. (2012), observed that the addition of pigments in the quail diet promoted a higher colorimetric score within a period of 12 days of consumption, which shows the importance of considering the consumption time of pigments in obtaining the desired yolk color. Moura *et al*. (2011), observed that with 11 days of the initial consumption of carotenoids, the yolk color showed the average plateau color of 8.75 in the colorimetric range.

Likewise, the permanence of deposition of these compounds in the yolk after the ceases ceasing of consumption of the pigment in the ration shows that in a period of up to five days there may still be an influence of the consumed carotenoids in the previous days, still being deposited and giving color to the yolk. This can be a parameter considered by the producer when managing the supply of the product knowing the time that it can provide yolk coloration, thus the supply can be stopped with a period of up to five days of pigment deposition remaining from the previous consumption.

During the storage period, the gas and water loss by the egg normally occurs, however, these losses can be minimized under adequate storage conditions. This was observed in the present work, in which the storage of eggs under refrigeration improved the quality parameters over the storage period. Similar results were observed by Barbosa *et al*. (2008), Santos *et al*. (2009) and Rosa *et al*. (2018), who tested the effect of temperature and egg storage period under quality parameters, the authors observed that for the eggs kept under refrigeration the indicative values of quality were higher ~~to~~ in the eggs kept at room temperature.

The albumen liquefaction is a sign of quality loss. When a fresh egg is broken on a surface, the yolk remains in a central position surrounded by thick albumen. When an old egg is broken, the yolk is flattened and often moved to one side and the albumen becomes thinner, resulting in a larger area of liquid albumen (Karui *et al*., 2006).

The displacement of water from the albumen to the yolk, together with the loss of water from the albumen to the environment, results in less egg weight and the percentage of the yolk of the eggs stored at room temperature has a higher value than those stored under refrigeration. The water resulting from the chemical reactions of albumen, which occur more quickly when the eggs are stored at room temperature, passes to the yolk, increasing its weight (Figueiredo *et al*., 2011).

The albumen pH in a fresh egg is between 7.6 and 8.5. During storage, the albumen pH may increase at a temperature-dependent rate to a value of about 9.7. The increase in the albumen's pH is caused by the loss of carbon dioxide through the shell pores. Thus, the pH of the albumen depends on the balance between dissolved carbon dioxide, bicarbonate ions, carbonate ions, and proteins (Karui *et al*., 2006).

Regarding the effect of storage period and temperature on the physicochemical properties of the eggs, Lee *et al*. (2016), observed a significant increase in albumen pH with increasing storage time and temperature. The pH of the albumen was not affected by the storage time at 2 °C. The pH value of yolk increased significantly as the storage period increased. Other studies also reported that the pH of the yolk was significantly affected by the storage period and temperature (Samli *et al*., 2005; Akyurel and Okur, 2009; Jin *et al*., 2011).

Including the increase in storage period and temperature, the deterioration in egg quality can be mainly attributed to the loss of water through evaporation through the shell pores and the escape of carbon dioxide from the albumen (Robinson, 1987; Samli *et al*., 2005).

The egg storage temperature and storage period are important factors to be considered for quail´s egg quail quality. However, even in storage temperature conditions less favorable to conservation, the deposition of lutein in eggs at the level of 5.4 ppm helped to maintain characteristics indicative of quality such as HU and % of yolk, which can be attributed to the fact that it has antioxidant properties that aid in the conservation by slowing down the degradation processes of albumen protein, enabling quality to be maintained for longer.

CONCLUSIONS

The use of lutein and canthaxanthin in the diet did not interfere with the performance and egg quality of quail. Birds fed diets associating levels of 5.4 ppm of lutein with 1.3 ppm of canthaxanthin had eggs with better yolk color with a rating of 8 on the colorimetric scale.

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