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Egg yolk colour and retinol concentration of eggs from laying hens fed diets containing carrot and beetroot meal

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Abstract: Compounds such as pigments, antioxidants, and pro-vitamins A are found in carrots and beetroots and could increase the nutritional value of eggs when added to diets for laying hens. This research evaluated retinol concentrations and egg yolk colour with addition of freeze-dried carrot (*Daucus carota* L.) and beetroot (*Beta vulgaris* L.) meal in the diets of 240 Hisex Brown laying hens. The hens were distributed in a completely randomized design in five groups according to five experimental diets: (1) maize and soybean meal; (2) sorghum and soybean meal; (3) sorghum and soybean meal plus 0.8% of freeze-dried carrot meal; (4) sorghum and soybean meal plus 0.8% of freeze-dried beetroot meal; (5) sorghum and soybean meal plus 0.4% of freeze-dried carrot meal and 0.4% freeze-dried beetroot meal. The diet containing maize and soybean meal resulted in a more intense egg yolk colour and higher retinol levels in comparison to other diets ($P < 0.05$). However, carrot meal increased egg yolk colour and beetroot meal increased egg yolk retinol concentration in comparison to sorghum and soybean meal diets ($P < 0.05$). The inclusion of 0.8% of the carrot and beetroot meal in the diet is not enough to reach the intensity of yolk colour and yolk retinol concentration obtained through the diet containing maize, however, it increases retinol level and yolk colour in comparison to the diet containing sorghum without carrot and beetroot meal.

Keywords: additive; *Beta vulgaris* L.; *Daucus carota* L.; sorghum; photography

In Brazil, maize (Zea mays) is used as a dietary energy source in poultry production, it is a source of xanthophylls, lutein, and zeaxanthin, carotenoids with a high pigmentation efficiency (Kufel et al. 2019; Titcomb et al. 2019). However, due to the use of maize in ethanol production, it cannot always be used in poultry diets, leading to increased production costs of chicken meat and table eggs. Thus, total or partial replacement of maize by sorghum (Sorghum bicolor) may be a viable alternative (Freitas et al. 2014). However sorghum has no carotenoids and when added to the diets of laying hens, egg yolk colour can be too pale. An intense egg yolk colour meets the preferences of the consumers and provides an appearance of health; therefore, when using sorghum instead of corn, it is necessary to add pigments to the diet of laying hens (Freitas et al. 2014; Kufel et al. 2019).

Regarding yolk colour, a study conducted in Araçatuba, Brazil reported that consumers prefer an

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egg yolk colour that corresponds to level 9 on the colorimetric fan. As for the colour variations of egg yolk, differences in preference according to region and country could occur, and such preferences determine the use of pigments in animal diets (Attia et al. 2013; Sandeski et al. 2014; Grashorn 2016).

Nowadays, there is a demand for healthy and functional foods, and any technologies developed in that sense are of interest to the industry (Guldiken et al. 2016). Therefore, the use of pigments from natural sources as an alternative to synthetic ones may be a way of achieving satisfactory egg yolk pigmentation as well as preventing nutritional deficiencies such as retinol deficiency and secondary nutritional deficiencies as well as diseases related to oxidative processes (Grashorn 2016).

Significant amounts of interesting nutritional compounds are abundant in beetroot and carrot, such as antioxidants and vitamins (Hammershoj et al. 2010; Guldiken et al. 2016), and therefore can be used as a natural alternative to fortify food.

Substances present in plant tissue can act like antioxidants in the bird's body, such as ascorbic acid, tocopherol, retinol, polyphenols such as phenolic acid, flavonoids and anthocyanins, pigments such as carotenoids and betalains, and minerals such as selenium, zinc, and iron (Sharma et al. 2012; Nimalaratne and Wu 2015).

The carrot (*Daucus carota* L.), belonging to the family *Apiaceae*, is the main vegetable source of β-carotene, among other carotenoids. Carotenoids are liposoluble substances widely known for the pigmenting action of their xanthophylls, antioxidants, and pro-vitamin A, especially the carotenoids α-carotene, β-carotene, and β-cryptoxanthin, giving carrots their characteristic colour (Hammershoj et al. 2010).

The beetroot (*Beta vulgaris* L.) is a plant of the family *Chenopodiaceae* and a source of betalains, the water-soluble pigments that characterize the colour of the tuber. Betalains are nitrogenous water-soluble compounds similar to flavonoids and anthocyanins. In addition, the beta-aldehydes and the phenolic compounds have antioxidant activity and inhibit lipid peroxidation (Attia et al. 2013; Chandran et al. 2014; Guldiken et al. 2016).

In this context, this work aimed at evaluating internal egg quality, retinol concentration, and egg yolk colour in Hisex Brown laying hens fed diet containing sorghum by including 0.8% of freeze-dried carrot meal, beetroot meal, or a blend of both.

MATERIAL AND METHODS

The research was conducted under the guidelines of the animal welfare and safety current in Brazil (law No. 11.794 of August 8th, 2008), and approved by the Ethics Committee from the Federal University of Mato Grosso (process No. 23108.007745/14-8).

Animals, diets and management. The experimental period lasted 63 days, divided into three 21-day phases. We used 240 Hisex Brown laying hens at 55 weeks of age, with an initial weight of 1.71 ± 0.08 kg and egg production average of 53.69 ± 4.66% at the beginning of the experiment. The laying hens were distributed in a completely randomized design, with five treatments corresponding to experimental diets and eight replicates per treatment, 40 experimental units in total. Each experimental unit consisted of three cages with two birds each, six birds per experimental unit in total.

Hens were housed in galvanized steel cages (25 cm width × 46 cm depth × 47 cm height), with a housing density of 575 cm^2/bird. The cages were equipped with trough-type feeders and nipple-type waterers. Food and water were provided *ad libitum*, and feeding was performed twice a day. The lighting program consisted of 16 hours daily, with 12 hours of natural light plus 4 hours of artificial light. The thermal conditions of the aviary were also recorded daily by means of a digital thermo-hygrometer. During the experimental period, the averages of maximum and minimum temperature and relative humidity were 36.5 and 25.5°C and 69.0 and 32.5%, respectively.

The experimental groups received iso-proteic and iso-energetic diets, formulated to meet the nutritional requirements recommended by Rostagno et al. (2011). A maize and soybean meal diet (Maize) and another diet based on sorghum and soybean meal (Sorghum) were used as control diets. To the other diets, we added 0.8% of the freeze-dried carrot meal (Sorghum + carrot) or beetroot meal (Sorghum + beetroot) or a blend containing 0.4% of both (Sorghum + carrot + beetroot) (Table 1).

Performance and egg quality. Feed consumption was calculated at the end of each cycle, by means of the difference between the quantity supplied and the leftovers from each experimental unit. The result obtained was divided by the number of birds and by the days the birds consumed the feed, the consumption is expressed in g/bird/day. The eggs were collected daily and the number of eggs was recorded to determine the average

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Table 1. Percentage composition of the experimental diets

Ingredients (%)	Experimental diets				
	maize	sorghum	sorghum + carrot	sorghum + beetroot	sorghum, carrot + beetroot
Maize	62.00	–	–	–	–
Sorghum meal	–	64.00	63.20	63.20	63.20
Soybean meal	25.0	23.00	23.00	23.00	23.00
Soybean oil	1.50	1.50	1.50	1.50	1.50
Limestone	8.10	8.10	8.10	8.10	8.10
Dicalcium phosphate	1.10	1.10	1.10	1.10	1.10
Salt	0.50	0.50	0.50	0.50	0.50
Premix1	1.80	1.80	1.80	1.80	1.80
Carrot meal	–	–	0.80	–	0.40
Beetroot meal	–	–	–	0.80	0.40
Calculated and analyzed composition	100	100	100	100	100
Metabolizable energy (kcal/kg)	2900	2900	2900	2900	2900
Crude protein (%)	16.74	16.68	16.17	16.95	16.57
Digestible lysine (%)	0.77	0.77	0.77	0.77	0.77
Digestible methionine + cystine (%)	0.70	0.70	0.70	0.70	0.70
Digestible tryptophan (%)	0.18	0.18	0.18	0.18	0.18
Digestible threonine (%)	0.56	0.56	0.56	0.56	0.56
Calcium (%)	3.90	3.90	3.90	3.90	3.90
Disponible phosphorus (%)	0.29	0.29	0.29	0.29	0.29
Sodium (%)	0.22	0.22	0.22	0.22	0.22
Crude fibre (%)	2.45	2.45	2.45	2.45	2.45

[1]composition per kg diet: calcium 148 g, phosphorus 35 g, sodium 40 g, phytase 10 000 IU/g, methionine 35 g, zinc 1500 mg, copper 190 mg, iron 1250 mg, fluorine 350 mg, manganese 1300 mg, cobalt 0.5 mg, iodine 25 mg, selenium 6.2 mg, choline 6000 mg, folic acid 6.17 mg, niacin 350 mg, biotin 2.5 mg, pantothenic acid 175 mg, retinol 156 250 IU, thiamine 37 mg, cobalamin 500 µg, riboflavin 85 mg, pyridoxine 24.75 mg, cholecalciferol 50 000 IU, tocopherol 3125 IU, menaquinone 24.5 mg, zinc bacitracin 500 mg

egg production, including broken, cracked and abnormal eggs, being represented as a percentage of the average number of birds in the period (egg/bird/day).

To assess internal egg quality, nine eggs per experimental unit were collected at the end of each period, totaling 360 eggs per period and 1080 eggs analyzed over 63 days. The eggs were weighed individually on a precision analytical scale (0.0001 g) and the total weight obtained was divided by the number of eggs used for weighing, resulting in the average egg weight in grams (g). After weighing, the eggs were submitted to analysis of specific gravity (g/cm^3), which consisted of their immersion in saline solutions at concentrations of 1.065, 1.070, 1.075, 1.080, 1.085, 1.090, and 1.095 g/cm^3, and determination using a densimeter.

Subsequently, the egg yolks were separated from the albumen and weighed. The shells were then air-dried for 72 h and weighed. Based on the difference between the weights of the whole egg, the yolk, and the eggshell, the weight of the albumen was obtained. Thus, weight averages and percentages of the egg components were calculated.

The egg mass (g/bird/day) was obtained by multiplying the average egg weight by egg production, and the result divided by 100. Feed conversion by egg mass (kg/kg) was calculated by the average feed intake (kg) divided by the egg mass produced. The feed conversion per dozen eggs (kg of feed/dozen eggs) was calculated by the total feed consumption (kg) divided by the production of dozen eggs.

Colorimetric analysis. During each period, 120 egg yolks were submitted to colorimetric analy-

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sis, three egg yolks per experimental unit, totaling 360 samples. Samples were deposited on the flat surface of Petri dishes and all analyses were performed maintaining the integrity of the egg yolks. To evaluate the yolk colour, three different methods were used.

First, egg yolks were submitted to a previously calibrated Konica Minolta digital colorimeter, (model CR-410, Konica Minolta, Japan), to obtain the parameters L* (lightness), a* (redness) and b* (yellowness) (Titcomb et al. 2019). The second method analyzed the samples using a Yolk Color Fan DSM (DSM Nutritional Products Ltd.) (Englmaierova et al. 2013). The third method consisted of digital photography of the samples, using a Sony DSC-HX300 camera (Sony, Japan). For standardization, the same settings were applied in all analyses. The specific settings of the equipment were 50× zoom, light 4, temperature 2 and intensity 2 for better sharpness and resemblance to visual observations according to the laboratory environment. The distance between the camera lens and the samples was 25.2 cm, and the images were originated in JPEG (Joint Photographic Experts Group) format.

The photographs were analyzed by ImageJ® scientific image analysis software (Image Processing and Analysis in Java, Maryland, USA). In each photo, a central circle of a fixed size was selected in the sample image, without visible interference of reflections and shadows and with a standard deviation close to 5. The software returned the red, green, and blue colour averages of the selected area, which can vary in the RGB (Red, Green, and Blue) system from 0 to 255 for each one of the parameters.

Retinol concentration. Twenty-four eggs from each experimental group were sent to the specialized laboratory for egg yolk retinol (RE) quantification by High-Performance Liquid Chromatography (HPLC), according to Manz and Philipp (1988). The results were expressed in μg RE and IU.

Statistical analysis. The SAS software package (Statistical Analysis System, Version 3.5, 2016) was used for all statistical analyses. The MEANS procedure was used for the descriptive analyses, the ROBUSTREG procedure to verify outliers, the GLM procedure to perform analysis of variance and Student-Newman-Keuls test at 5% significance ($P < 0.05$); and the UNIVARIATE procedure to test the normality of the residue. The variables that did not follow a normal distribution were submitted to data transformations (square root, hyperbolic, and logarithmic) and reanalyzed through the latter two procedures. The variables which, after tests with all the mentioned data transformations, did not meet the normality assumption of the residue, were analyzed through the NPAR1WAY procedure through the Kruskal-Wallis test for non-parametric data analysis.

RESULTS AND DISCUSSION

There was no difference ($P > 0.05$) in feed intake, egg production, feed conversion per dozen eggs, feed conversion per egg mass, egg mass, and egg weight among the experimental groups (Table 2). Heat limits the performance of laying hens (Abd El-Hack et al. 2019), and under high-temperature conditions, birds reduce their feed intake to lower heat production from metabolic processes (Farghly et al. 2018). Average feed intake in our study was 98.94 g/bird/day, approximately 13 g/bird/day less in comparison to the value described in the lineage manual for 55-week-old laying hens. Possibly, feed consumption and egg production were adversely affected by the high temperatures recorded during the experimental period, affecting, in turn, egg mass, feed conversion by egg mass, feed conversion per dozen eggs, and egg weight. The inclusion of 0.8% of beetroot or carrot meal, or the blend of both, did not negatively affect poultry performance in diets replacing maize with sorghum. Similarly, in research evaluating the effects of vitamin A sources and the use of sorghum on the performance of laying hens, no performance loss was observed (Garcia et al. 2009; Freitas et al. 2014; Ilhan and Bulbul 2016).

No differences were observed ($P > 0.05$) among treatments for albumen weight, albumen percentage, eggshell percentage, and specific gravity (Table 3). The content of proteins present in the albumen is related to the ingestion of proteins by the laying hens (Fuente-Martínez et al. 2012). Thus, the addition of carrot and beetroot meal in the diets probably did not represent a protein increase, nor did any losses to the digestion and the use of the proteins. Therefore, the weight and percentage of the albumen did not differ among the experimental groups. Similarly, in a study conducted by Garcia et al. (2010), the use of urucum in laying hen diets

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Table 2. Performance of laying hens fed carrot and beetroot meal

	Maize	Sorghum	Sorghum + carrot	Sorghum + beetroot	Sorghum, carrot + beetroot	CV (%)	Pr > *F*
FI (g/bird/day)	100.47 ± 9.65	100.08 ± 4.12	96.46 ± 2.58	97.97 ± 5.08	99.73 ± 7.19	4.66	0.4040
EP (egg/bird/day)	0.523 ± 0.05	0.559 ± 0.03	0.526 ± 0.05	0.548 ± 0.04	0.526 ± 0.05	8.69	0.4368
FC/Dz (kg/dz)	2.92 ± 0.45	2.62 ± 0.46	2.56 ± 0.19	2.44 ± 0.23	2.64 ± 0.26	12.84	0.1540
FC/EM (kg/kg)	3.29 ± 0.46	3.07 ± 0.27	3.20 ± 0.38	3.01 ± 0.35	3.21 ± 0.51	12.78	0.6337
EM (g/bird/day)	29.94 ± 3.17	32.78 ± 2.26	30.55 ± 3.35	32.81 ± 2.78	30.81 ± 3.31	9.57	0.2034
EW (g)	57.26 ± 2.19	59.25 ± 2.68	57.98 ± 1.84	59.82 ± 1.94	58.47 ± 1.62	3.29	0.0945

FI = feed intake, EP = egg production, FC = feed conversion, Dz = dozen egg, EM = egg mass, EW = egg weight, CV = coefficient of variation

with total replacement of maize by sorghum did not result in differences in albumen percentage.

The use of 0.8% carrot and beetroot meal in diets containing sorghum did not cause any loss the specific gravity of the egg. In a similar study, the replacement of maize by sorghum in diets for laying hens promoted no differences in the specific gravity among the treatments (Garcia et al. 2009).

Eggshell weight was higher ($P < 0.05$) for the diet containing beetroot meal (Table 3). Despite this, eggshell percentage did not vary among treatments and no losses in egg quality were observed because of the experimental diets. It is possible that eggshell weight was higher for the diet containing beetroot meal due to the high amount of manganese in beetroot in comparison to the carrot. The minerals present in the diet can cause structural changes in the eggshell (Ketta and Tumova 2016), and 100 g of carrot contain 0.05 mg of manganese, whereas 100 g of beetroot contain 1.23 mg of manganese (UNICAMP 2011). Manganese supplementation from organic or inorganic sources increases eggshell weight and improves eggshell quality without any losses in egg production (Zhang et al. 2017).

There was a significant difference ($P < 0.05$) for yolk weight and yolk percentage, which were lower for diets containing maize. The increase in yolk weight and yolk percentage is often associated with a decline in egg quality (Carvalho et al. 2007). This can be an indicator that the diets containing sorghum led to an earlier onset of the deterioration process compared to diets containing maize, since maize is a source of antioxidant carotenoids, such as β-cryptoxanthin (Titcomb et al. 2019), which are absent in sorghum. However, diets with beetroot meal and carrot meal plus beetroot presented lower yolk percentages than diets containing only sorghum and sorghum with carrot meal. It is possible that the antioxidant properties of the compounds present in the beetroot promoted a reduction in the oxidative process in relation to the other diets containing sorghum.

There was a significant difference ($P < 0.05$) in the colour of the yolk among the experimental groups,

Table 3. Egg quality from laying hens fed carrot and beetroot meal

	Maize	Sorghum	Sorghum + carrot	Sorghum + beetroot	Sorghum, carrot + beetroot	CV (%)	Pr > *F*
Yolk weight (g)	$13.22^{b} \pm 0.75$	$14.43^{a} \pm 0.68$	$13.91^{a} \pm 0.62$	$14.56^{a} \pm 0.46$	$13.99^{a} \pm 0.66$	3.79	0.0003
Yolk (%)	$24.03^{b} \pm 0.78$	$25.00^{a} \pm 0.47$	$24.92^{a} \pm 0.56$	$24.75^{ab} \pm 0.61$	$24.57^{ab} \pm 0.75$	2.62	0.0395
Albumen weight (g)	37.81 ± 1.40	38.66 ± 1.68	38.31 ± 1.53	39.18 ± 1.67	38.68 ± 1.38	3.99	0.4974
Albumen (%)	65.88 ± 0.63	64.96 ± 0.59	65.11 ± 0.62	65.08 ± 0.72	65.28 ± 0.74	1.02	0.0684
Eggshell weight (g)	$5.69^{b} \pm 0.29$	$5.73^{b} \pm 0.26$	$5.55^{b} \pm 0.32$	$6.05^{a} \pm 0.20$	$5.76^{b} \pm 0.22$	4.03	0.0035
Eggshell (%)	10.09 ± 0.33	10.02 ± 0.30	9.98 ± 0.30	10.14 ± 0.22	9.92 ± 0.28	2.84	0.5224
Specific gravity (g/cm3)	1.088 ± 0.0016	1.087 ± 0.0021	1.086 ± 0.0016	1.087 ± 0.0012	1.087 ± 0.0010	0.14	0.1220

CV = coefficient of variation

[a,b]different letters in a row indicate statistical difference according to Student–Newman–Keuls test ($P < 0.05$)

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and the yolks of the group of birds that received the diet containing maize showed a more intense colour than the others (Table 4). Colour analysis using the colorimetric fan showed that the yolks from the group fed maize obtained a more intense colour than those with a diet based on sorghum. In previous studies, the average values of colour obtained by colorimetric fan in diets based on maize and soybean meal ranged from 5.10 to 7.44; and from 1.16 to 1.93 in diets where maize was replaced by sorghum (Garcia et al. 2009; Rojas et al. 2015; Kufel et al. 2019). These values are similar to the results obtained in this research.

The parameters L*, a*, and b* showed similar behaviour to that of the samples analyzed with the colorimetric fan. Values of lightness (L*) were lower for the diet containing maize, indicating that the yolk colour intensity was high compared to that in the other diets. Furthermore, chrome a* and b* were also higher, indicating a more intense yellow colour compared to diets containing sorghum. In the Lab system, the value of a* denotes the colour in the region of red (+a*) to green (–a*), while b* indicates the colour in the range of yellow (+b*) to blue (–b*). The value of L* refers to luminosity, ranging from white (L* = 100) to black (L* = 0) (Titcomb et al. 2019).

The values of L*, a*, and b* found in studies with the use of a digital colorimeter varied widely according to the type of the pigment and its level of inclusion in the diet and, therefore, are not suitable for numerical comparison with the obtained results in the present study. However, they evidenced the recurrence of lower values for L* in diets containing maize in comparison to those containing sorghum in substitution. Also, the values recorded for a* and b* were higher (Garcia et al. 2009; Rojas et al. 2015).

When analyzing the photographs, the red colour (R) was similar ($P > 0.05$) in all treatments, while the green (G) and blue (B) colours were of lower intensity ($P < 0.05$) for the diet based on maize and soybean meal. The blue colour was of higher intensity for the diet containing only sorghum in relation to the diet containing sorghum and carrot meal, indicating that, although it did not promote the intense colour reached with the diet containing maize, only the carrot meal promoted the pigmentation of the egg yolk probably due to the carotenoids present in carrot. Thus, the inclusion of 0.8% freeze-dried carrot meal in the diet containing sorghum promoted an increase in egg yolk colour intensity, although not sufficient for commercialization, indicating that the use of higher levels could promote significant results in the yolk colour. Similarly, Titcomb et al. (2019) suggested that carrot leaves may be a viable option for rural farmers to improve yolk colour and carotenoid concentration.

The betalains from beetroot had no pigmentation effect on the lipids of the yolk possibly because of its hydrophilic chemical nature. Similar results were obtained by Kopriva et al. (2014), who, when adding 1 to 2% of beet to the diets of laying hens, did not observe any effects on yolk colour.

We detected differences in yolk colour intensity using the RGB system to analyze the samples images. The applicability of this method can be improved to meet a field demand with practicality and accuracy.

Table 4. Egg yolk colour and retinol concentration

	Maize	Sorghum	Sorghum + carrot	Sorghum + beetroot	Sorghum, carrot + beetroot	CV (%)	Pr > *F*
Colour fan	5.66^{a} ± 0.86	1.65^{b} ± 0.24	1.78^{b} ± 0.39	1.76^{b} ± 0.15	1.97^{b} ± 0.33	18.09	< 0.0001
L*	77.08^{b} ± 0.94	81.27^{a} ± 1.00	79.83^{a} ± 1.65	80.90^{a} ± 0.61	80.81^{a} ± 1.26	1.43	< 0.0001
a*	4.17^{a} ± 0.78	0.31^{b} ± 0.31	0.69^{b} ± 0.72	0.33^{b} ± 0.28	0.38^{b} ± 0.29	32.55	< 0.0001
b*	48.56^{a} ± 2.94	38.42^{b} ± 1.70	38.48^{b} ± 2.55	38.50^{b} ± 1.11	39.28^{b} ± 1.45	3.94	< 0.0001
Red	217.37 ± 13.81	225.00 ± 3.93	218.50 ± 8.76	219.62 ± 5.77	222.37 ± 6.66	3.89	0.4009
Green	182.75^{b} ± 9.36	204.87^{a} ± 3.84	198.00^{a} ± 7.48	200.00^{a} ± 2.90	201.87^{a} ± 7.27	3.34	< 0.0001
Blue	24.12^{c} ± 7,44	123.12^{a} ± 6.11	111.37^{b} ± 8.43	120.75^{ab} ± 5.36	118.37^{ab} ± 10.47	7.78	< 0.0001
Retinol (μg RE)[1]	49.79^{a} ± 4.93	28.69^{d} ± 1.59	35.52^{bc} ± 1.98	40.17^{b} ± 1.53	33.20^{cd} ± 2.50	7.48	< 0.0001
Retinol (IU)[1]	166^{a} ± 16.1	96^{d} ± 5.13	119^{b} ± 6.66	134^{b} ± 5.13	111^{cd} ± 8.62	7.43	< 0.0001

L* = lightness, a* = redness, b* = yellowness, CV = coefficient of variation

[1]values obtained in 100 g of yolk

[a–d]different letters in a row indicate statistical difference (Student–Newman–Keuls test; $P < 0.05$)

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There was a difference ($P < 0.05$) in retinol concentrations in the yolk between the treatments, and the addition of carrot or beetroot meal in the diets containing sorghum was not sufficient to reach a retinol concentration similar to that of the diet containing maize and soybean meal.

The values of retinol concentration in the yolk found in the literature, presented a wide variation, being associated mainly with the different sources of supplementation and dietary levels of vitamin A (0.58 vs 0.87 mg RE/100 g/yolk for diets supplemented with 5000 and 35 000 IU/kg of retinyl acetate, respectively) used in the diets (Yuan et al. 2014; Ilhan and Bulbul 2016).

It was possible to increase retinol concentrations in the egg yolks of laying hens fed 0.8% carrot or beetroot meal compared to the diet containing only sorghum. The possible antioxidant action of the beetroot on retinol concentration and the percentage of yolk were correlated in this study because the smaller percentages of yolk occurred in the treatments with higher retinol concentrations. Higher levels of carrot and beetroot meals inclusion in the diet of the hens could possibly promote higher values of retinol and the enrichment of eggs with vitamin A and antioxidant compounds.

The antioxidant capacity of the plants is mostly derived from the phenolic compounds present in the plant tissue (Melo and Faria 2014), and the antioxidant activity decreases as the amounts of phenolic compounds present in the plants decrease (Melo et al. 2011; Sharma et al. 2012). Anthocyanins are antioxidants present in the beetroot, which are practically absent in the common carrot (Leja et al. 2013; Nimalaratne and Wu 2015; Guldiken et al. 2016). In addition, when compared to carrot, beetroot showed higher antioxidant capacity and higher concentrations of antioxidant phenolic compounds in several studies (Melo et al. 2011; Melo and Faria 2014).

The literature value for the total phenol content in the carrot was 1.2 mg of gallic acid equivalent/g of freeze-dried sample, while for beetroot, it was 2.9 mg of gallic acid equivalent/g of freeze-dried sample. In addition, the antioxidant activity was 22% for freeze-dried carrot and 85% for freeze-dried beetroot (Melo et al. 2011).

Heat reduces the amount of betanin, the major antioxidant of beetroot, by only 25%, with little loss of antioxidant action (Guldiken et al. 2016). On the other hand, retinol and carotenoids are easily destroyed by heat and therefore only slightly absorbed by animals.

Polar antioxidants, such as betalains, tend to be more effective at acting in the presence of lipid compounds since they accumulate at the air-oil interface where peroxidation reactions occur, as in lipoproteins deposited in the egg yolk (Leja et al. 2013).

In addition, retinol and carotenoids in the gastrointestinal tract may decrease the uptake of tocopherols due to competition for binding sites that occur between them (Yuan et al. 2014), and the decrement of the tocopherol deposition in the yolk can accelerate the beginning of the oxidative process of the fatty acids, also degrading the vitamin A present in the egg yolk.

In the case of carotenoids, the antioxidant activity is not necessarily related to pigmentation and therefore, the lipophilic pigments present in the carrot, which provoked a more intense yolk colour than the pigments found in the beetroot in the diets containing sorghum, were not equally efficient in depositing or preserving retinol in the yolk. On the other hand, the antioxidant action of the pigments and phenolic compounds of the beetroot possibly promoted the preservation of retinol in the egg yolk and, indirectly, a higher amount of retinol than observed in diets containing only sorghum.

CONCLUSION

Freeze-dried carrot and beetroot meal at 0.8% increased retinol concentration and yolk colour in comparison to the diet containing only sorghum, although the values were lower than those obtained through the diet with maize.

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