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Natural and synthetic pigments in diet of Japanese quails

Jean Kaique Valentim[1*], Tatiana Marques Bittencourt[2], Heder José D'Àvilla Lima[2] Fernanda Keller Queiroz Barros[2], Maira Santos Cruz[2], Isabelli Dias Brito Pereira[2] , Nayara Emanoelle Matos e Silva, Guilherme Resende de Almeida[2] and Henrique Momo Ziemniczak[1]

[1]Universidade Federal da Grande Dourados, Rua João Rosa Góes, 1761, 79825-070, Vila Progresso, Dourados, Mato Grosso do Sul, Brasil. [2]Universidade Federal de Mato Grosso, Cuiabá, Mato Grosso, Brasil. *Author for correspondence. E-mail: kaique.tim@hotmail.com

ABSTRACT. The objective of this study was to evaluate productive performance and egg quality of Japanese quails (*Cortunix japonica*) fed with synthetic and natural pigments. The treatments used were: 0.8% paprika extract (*Capsicum annuum*), 0.8% marigold flower extract, 4% paprika extract and 4% marigold flower extract and 0.045% synthetic pigment (*Canthaxanthin*) in feed. A total of 240 Japanese quails at 20 weeks of age were distributed in a completely randomized experimental design with five treatments and six replicates, with eight quails per experimental unit (30 experimental units). There was no difference ($p > 0.05$) for the evaluated parameters, except for yolk color which showed significant difference ($p < 0.05$) between the treatments used. The inclusion of 0.8% natural pigments in diet of Japanese quails can be used to substitute canthaxathin because it is effective on improving yolk color without affect performance and the quails egg quality.

Keywords: antioxidants; canthaxanthin; yolk color; plant extracts.

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Introduction

Coturniculture has increased exponentially in the last years and have a great importance on agricultural economy due to technified productions and new ways of commercializing quail eggs (Hosseini, Mehraei Hamzekolaei, Moghaddam, Arabha, & Tohidifar, 2017). The benefits of quail production are the fast profitable return, consumer acceptance and nutritional quality on humans' diet, low investments and labor necessity, small area to house the birds.

Consumers are increasing the demand on market products and eggs showing a strong yolk color have a better acceptance because of society's food behavior (Mendonça, Correa, Benevides, Mota, & Franca, 2018). The use of pigments being natural or synthetic is important to comply with market demand (Englmaierová, Bubancová, & Skřivan, 2014).

Boka, Mahdavi, Samie, and Jahanian (2014) found that plant extracts have a positive effect because of its antioxidant activity which improves nutrient digestibility and stimulates enzymes secretion, helping to preserve intestine microbiota, impacting on better feed conversion, production and egg's internal quality. According to Silva et al. (2012) the use of natural pigments has increased because of costumer needs in developed countries which have banned the use of synthetic-pigment additives such as canthaxanthin and astaxanthin in human and animal feed. Annatto extract (*Bixa orellana*), saffron (*Curcuma longa*), marigold flower extract (*Tagetes erecta*) and paprika extract (*Capsicum annum*) are the most used natural pigments in feed (Moura et al., 2011). Many researches were carried out using synthetic products (Rocha et al., 2013; Víctor Rojas, Callacna, & Arnaiz, 2015). However, after synthetic-pigments such as azo was prohibited, European and North American countries increased the search for natural- pigments.

Brazilian law is based on FAO/OMS Committee to determine the use of synthetic pigments. This law prohibits a significant number of synthetic pigments on animal's feed because of toxic effects in products consumed by humans (Carvalho, Cipolli, Ormenese, Carvalho, & Silva, 2009).

The objective of this research was to assess the effects of three pigments: canthaxanthin (synthetic), extract of marigold and paprika (natural) in performance and egg quality of Japanese quails.

Material and methods

The experimental protocol was approved by the University's Ethics Committee on Animal Use under protocol number 23108.187860/2016-11. The experiment was carried out at Experimental Farm of Federal University of Mato Grosso – Quail Department, for 63 days, split in three 21- day measurements. A total of 240 Japanese quails (*Coturnix japonica*) of 20 weeks old were housed in galvanized wire cages receiving *ad libitum* feed and water. Temperature and relative humidity (RH) were daily recorded using a digital hygrometer placed in center of the barn. The maximum and minimum temperatures and relative humidity were 32.10 and 19.2ºC and 82.3 e 35.5%, respectively. A lighting schedule with 16h of light per day was provided.

The quails were housed in galvanized wire cages (experimental unit) measuring 50 x 38 x 21cm (length x width x height). In each experimental unit, an area of 271 cm^2/bird was provided. Quails were distributed in a completely randomized experimental design of five treatments and six replicates (8 birds per cage) with a total of 30 experimental units.

The amount of extracts added in feed followed the manufacture's guidelines. The treatments used were: control feed (without pigments), 0.8% inclusion of paprika extract (*Capsicum annuum*), 0.8% inclusion of marigold flower extract, 4% inclusion of paprika extract 4% inclusion of marigold flower extract and 0.045% inclusion of canthaxanthin (synthetic pigment) in feed.

Experimental diets (Table 1) were formulated based on corn and soybean meal and according to nutritional requirements established by Rostagno et al. (2011).

Table 1. Calculated composition of experimental diets.

Ingredients (kg^{-1})	Control	Paprika	M. flower	Mix	Canthaxanthin
Corn	54.17	53.37	53.37	53.37	53.37
Soybean meal	34.70	34.70	34.70	34.70	34.70
Limestone	7.01	7.01	7.01	7.01	7.01
Dicalcium phosphate	1.15	1.15	1.15	1.15	1.15
Sodium chloride	0.36	0.36	0.36	0.36	0.36
Vitamin-mineral supplement (1)	1.50	1.50	1.50	1.50	1.50
Soybean oil	1.11	1.12	1.11	1.11	1.11
Paprika	0.0	0.8	0.0	0.4	0.0
Marigold flower	0.0	0.0	0.8	0.4	0.0
Canthaxanthin	0.0	0.0	0.0	0.0	0.045
Composition					
Metabolizable energy (kcal kg^{-1})	2800.00	2800.00	2800.00	2800.00	2800.00
Crude protein (%)	19.46	19.46	19.46	19.46	19.46
Digestible lysine (%)	1.080	1.080	1.080	1.080	1.080
Digestible methionine+ Cystine (%)	0.94	0.94	0.94	0.94	0.94
Digestible tryptophan (%)	0.23	0.23	0.23	0.23	0.23
Digestible threonine (%)	0.68	0.68	0.68	0.68	0.68
Calcium (%)	3.07	3.07	3.07	3.07	3.07
Available phosphorus (%)	0.30	0.30	0.31	0.32	0.33
Sodium (%)	0.16	0.16	0.16	0.16	0.16
Crude fiber (%)	2.74	3.49	4.21	4.93	5.65

[1]Vitamin-mineral supplement composition: Calcium (min) 80 g kg^{-1} , Calcium (max) 100 g kg^{-1} Phosphorus (min) 37 g kg^{-1} , Sodium (min) 20 g kg^{-1} , Methionine (min) 21.5 g kg^{-1}, Lysine (min) 18 g kg^{-1}, Vitamin A (min) 125000 UI kg^{-1}, Vitamin D3 (min) 25000 UI kg^{-1}, Vitamin E (min) 312 UI kg^{-1} , Vitamin K3 (min) 20 mg kg^{-1}, Vitamin B1 (min) 20 mg kg^{-1}, Vitamin B2 (min) 62.5 mg kg^{-1}, Vitamin B6 (min) 37.5 mg kg^{-1} , Vitamin B 12(min) 200 mcg kg^{-1}, Folic Acid (min) 6,25 mg kg^{-1}, Pantothenic acid (min) 125 mg kg^{-1}, Biotin (min) 1.25 mg kg^{-1}, Choline (min) 1700 mg kg^{-1}, Niacin (min) 312 mg kg^{-1}, Copper (min) 125 mg kg^{-1}, Iron (min) 680 mg kg^{-1}, Iodine (min) 8.75 mg kg^{-1}, Manganese (min) 937 mg kg^{-1}, Selenium (min) 3.75 mg kg^{-1}, Zinc (min) 500 mg kg^{-1}, Fluorine (max) 370 mg kg^{-1}.

Performance and egg quality were evaluated in the end of each 21-day period. The performance parameters assessed were: egg production, marketable eggs, individual feed intake (g/quail/day), feed conversion per egg mass (kg kg^{-1}), feed conversion per dozen eggs (kg dz^{-1}), viability and weight gain, according to Sakomura and Rostagno (2007) methodology.

Egg production percentage was obtained by the number of eggs produced, including broken, cracked, eggs with soft shells and shell-less produced in each experimental period. To obtain marketable eggs data, the number of broken, cracked, soft-shell and shell-less eggs was subtracted from total egg production. Feed consumption (g bird-1 day-1) was determined by the difference between the ration fed and the amount leftover, in the days 1° and 21° of each period in each experimental unit.

Feed conversion per egg mass was calculated by feed consumption in kilograms divided by total egg mass (kg kg^{-1}) produced. Feed conversion per dozen eggs was calculated by the ratio of total feed consumption in kilograms divided by production and the result multiplied per 12 (kg dz^{-1}).

To determine weight gain, all quails were weighed at the beginning and end of the experimental period. Mortality was recorded and the number of deaths was subtracted from the total number of live birds, converting values into percentage at the end of the experimental period to determine viability.

The parameters used to evaluate egg quality were: average egg weight, yolk weight, shell weight, albumen weight, specific gravity, yolk color. Eggs of each day were identified and weighted individually using an analytical scale with 0.001 g precision. Specific gravity was determined by immersing the eggs in saline solutions, according to Thompson and Hamilton (1982). Eggs were immersed in solutions with density ranging from 1.070 to 1.095 g cm^{-3}. Density was measured using a calibrated densimeter (OM-5565, Incoterm). Eggs were broken to precede the intensity of the yolks' yellow analysis. Yolks were placed on a flat surface and the color of the yolk *in natura* was visually compared and classified using DSM® Color Fan (1-15 score, ranging from light yellow to orange). Those evaluations were carried out by the same researcher in the same place with the same light intensity to avoid ranges. The yolk of each egg was separated from albumen and had its weight recorded. Shell weight was registered after natural drying during 72 hours. Albumen weight was obtained by the difference of egg weight and yolk weight, plus shell weight. The results were submitted to analysis of variance and means were compared by Tukey test at 5% probability using PROC GLM of SAS® (Statistical Analysis System [SAS], 1985).

Results and discussion

Experimental diets of each treatment containing different pigments did not have an effect on individual feed intake, feed conversion per egg mass and dozen (kg dz^{-1}), egg production and marketable eggs ($p > 0.05$), according Table 2.

Table 2. Feed intake, feed conversion per mass and per dozen, egg production, marketable eggs, viability and weight gain of Japanese quails fed with different pigments in diet.

Parameters	Control	Paprika	Marigold	Mix	Canthaxanthin	P-value
FI (g $quail^{-1}$ day^{-1})	30.19	29.22	30.96	29.83	30.36	0.65
FCM (kg kg^{-1})	3.48	3.53	3.22	3.26	3.18	0.59
FCD (kg dz^{-1})	2.91	2.81	3.09	2.93	2.95	0.29
%PROD (%)	75.65	77.43	73.89	73.50	76.45	0.66
ME. (%)	72.72	74.56	71.23	71.79	75.87	0.43
Viability*	98.77	100	98.45	100	97.65	----
WG (g $quail^{-1}$)*	0.0040	0.0038	0.0046	0.0045	0.0033	----

FI: feed intake. FCM: feed conversion per mass. FCD: feed conversion per dozen. %PRO: egg production. ME: marketable eggs. WG: weight gain. *Descriptive analysis.

Gumus, Oguz, Bugdayci, and Oguz (2018) did not found effects in production, but in egg quality when using plant extracts. Moeini, Ghazi, Sadeghi, and Malekizadeh (2013) showed a positive effect in poultry's performance when using carotenoids of red pepper as feed additive (*Capsicum annuum*) and marigold flower extract (*Tagetes erectus*).

Natural or phytogenic additives have antioxidant/nutraceutical activity which impacts in physiological metabolism and productive performance; however, the administration, dose and physiological status of the animal can affect these substrates' absorption (Pessôa, Tavernari, Alves Vieira, & Albino, 2012). This fact can explain the absence of significant difference ($p > 0.05$) on performance between the treatments.

The results found in this study are in agreement with those reported by Molino et al. (2012) which used annatto (natural pigment) as a carotenoid source for laying hens and did not found significant difference on egg quality and productive performance. Natural pigments can be used as an alternative instead of using synthetic pigments for poultry because did not affect the productive performance of chickens. Alagawany and El-Hack (2015) evaluated levels of rosemary in laying hens and did not found differences on live weight, feed intake, feed conversion or egg weight, but the number of eggs and egg mass had a linear increase with rosemary supplementation.

The parameters average egg weight (AEW), yolk weight (YW), shell weight (SW), albumen weight (AW), yolk percentage (% Yolk), shell percentage (% Shell), albumen percentage (% Albumen), specific gravity (SG) did not show difference ($p > 0.05$) between the treatments tested (Table 3). Yolk color (CG) (table 3) demonstrated significant difference ($p < 0.05$) between inclusion levels of pigments in quails feed.

Table 3. Average egg weight (AEW), yolk weight (YW), shell weight (SW), albumen weight (AW), yolk percentage, shell percentage, albumen percentage, specific gravity (SG), yolk color (YC) of quails' egg fed with different pigments on diet.

Parameters	Control	Paprika	Marigold	Mix	Canthaxanthin	P-value
AEW (g)	10.37	10.38	10.02	10.19	10.28	0.69
YW (g)	3.28	3.50	3.19	3.33	3.26	0.16
SW (g)	0.82	0.83	0.81	0.81	0.81	0.96
AW (g)	6.26	5.94	6.01	6.03	6.15	0.58
% Yolk	31.72	32.05	31.83	32.82	31.43	0.68
% Shell	7.98	7.72	8.14	8.08	8.01	0.46
% Albumen	60.29	57.47	60.01	59.09	60.54	0.02
SG (g m^{-3})	1.076	1.077	1.077	1.075	1.076	0.30
YC	5.68c	7.01b	6.89b	7.10b	9.78a	0.00

Means followed by different letters on the same line differ from each other by Tukey test ($p < 0.05$).

Adding plant extracts pigments in quails' feed did not change egg quality but improves yolk color due to the higher deposition of carotenoid pigments. The canthaxanthin-based pigment showed a higher yolk color mean (9.78) and this fact can be explained by the chemical pigmentation capacity of this product, which is related to the difference in digestion rate, absorption and deposition of pigments in egg yolk. Paprika, marigold flower and paprika+marigold flower extracts can change yolk color, even in a smaller way than canthaxanthin.

Natural sources of carotenoid such as carrot, chlorella algae, marigold or lutein promotes a higher carotenoid concentration which increases carotenoid deposition in egg yolk improving egg quality. For practical use, it is necessary to analyze the price of carotenoid natural sources (Englmaierová et al., 2014).

Oliveira et al. (2017) worked with marigold flower extract and paprika for laying hens feed and reported that marigold flower extract in feed without paprika extract did not result in higher yolk height; however, when paprika extract were added with marigold flower extract yolk, yolk percentage was 8.34% higher because of synergistic activity of pigments resulting in better carotenoid deposition in egg.

Experimental diets with synthetic and natural carotenoid-pigments showed like enriched when compared with feed without pigments. The same was observed by Carvalho, Pita, Piber-Neto, Mirandola, and Mendonça-Júnior (2006) and Mendonça et al. (2018).

Moura et al. (2011) studying the inclusion of natural pigments in Japanese quails' sorghum-based diet, observed higher pigmentant efficiency of paprika extract pigment (*Capsicum annuum*) than marigold flower extract (*Tagetes erectus*), but both pigment sources used together were more efficient than using separately because improved egg yolk colorimetric score. This was not observed in the present study in which paprika extract, marigold flower extract and association of both showed the same results using colorimetric score.

According to Mendes et al. (2017) egg yolk color is one of the major characteristics that matters for consumers, after price and shell color. The yolk color is generally associated with nutritional quality which is not proved, but it is linked to visual appearance (costumers' preferences).

Englmaierová et al. (2014) mentioned that synthetic pigments are used because of its coloring effect of yolk but the use should be limited. Natural alternatives give the same coloring result when choosing eggs, without having a negative effect in egg quality and human health. As a conclusion after working with phytogenic additives for laying hens feed, Oliveira et al. (2017) described that adding paprika extract inclusion in sorghum-based diets improves egg pH and yolk color. Ghasemi, Zarei, and Torki (2010) investigating garlic and thyme as phytogenic additives for laying hens, noticed that they have a benefit effect relating to higher carotenoid deposition in yolk and consequently improves yolk color.

The present research results showed that adding different pigments in quails feed did not have an influence in performance and egg quality but can be an alternative to avoid synthetic pigments use because showed a colorful yolk which is valuable for costumers.

Conclusion

The inclusion of 0.8% of natural pigments (paprika and marigold extracts based) can be used as a way to substitute the use of 0.045% inclusion of synthetic pigment (*canthaxanthin*) in diet without affecting performance and the quails egg quality.

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