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Yolk color and sensory analysis of eggs of common quails (*Coturnix coturnix coturnix*) fed sorghum-based diet with the addition of annatto seed meal (*Bixa orellana* L.)

Coloração da gema e análise sensorial de ovos de codornas europeias (Coturnix coturnix coturnix) alimentadas com ração à base de sorgo com adição de farelo de urucum (Bixa orellana L.)

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ABSTRACT

This study has aimed to assess the effect of increasing levels of annatto (*Bixa orellana* L.) seed meal (AM) on yolk pigmentation and the sensory analysis of eggs of common quails fed sorghum-based diets when compared to corn. Eighty female common quails (*Coturnix coturnix coturnix*) in the laying phase were used, being them aged from 251 to 316 days. There were three experimental periods of 21 days each (251-272; 273-294; 295-316 days). The quails were distributed in a completely randomized design with five treatments (T1 - corn-based feed; T2 - feed with 100% of sorghum instead of corn without the addition of AM; T3, T4, and T5 - feed with 100% of sorghum replacing corn with the addition of 0.5, 1.0, and 1.5% AM, respectively) and four repetitions. One hundred eggs from each treatment were used for analysis. Sensory evaluation was applied with an untrained panel with 20 evaluators. The eggs were boiled, peeled, and served. The panelists assessed the appearance, flavor, color, aroma, texture, and overall evaluation of the eggs. Annatto seed meal added to sorghum-based diets promoted linear increases in appearance, flavor, color, aroma, texture, and overall evaluation, evaluated during the sensory analysis (p=0.001). The addition of 1.5% AM in the sorghum-based diets of the common quails benefited the characteristics of appearance (4.50), flavor (4.50), color (4.55), aroma (4.25), and texture (4.55) of the assessed quail eggs, being considered more attractive and with greater acceptance (overall evaluation = 5.97) in relation to the other treatments tested. The inclusion of AM in the sorghum-based diets improved the yolk pigmentation of quail eggs in relation to the control treatments. The AM triggers positive effects on yolk pigmentation and sensory characteristics of common quail eggs.

KEYWORDS: additives; bixin; carotenoids; natural pigments; yolk pigmentation.

RESUMO

Objetivou-se avaliar o efeito de níveis crescentes do farelo do resíduo da semente de urucum (*Bixa orellana* L.) (FU), sobre a pigmentação da gema e análise sensorial dos ovos de codornas europeias alimentadas com dietas à base de sorgo em substituição ao milho. Foram utilizadas 80 codornas europeias (*Coturnix coturnix coturnix*) fêmeas, na fase de postura, no período de 251 a 316 dias de idade. Foram três períodos experimentais de 21 dias cada (251-272; 273-294; 295-316 dias). As codornas foram distribuídas em delineamento inteiramente casualizado, com cinco tratamentos (T1 - ração à base de milho; T2 - ração com 100% de sorgo em substituição ao milho sem a adição de FU; T3, T4 e T5 – ração com 100% de sorgo em substituição ao milho com a adição de 0,5; 1,0 e 1,5% de FU) e quatro repetições. Cem ovos de cada tratamento foram utilizados para a análise. A avaliação sensorial foi aplicada em painel não treinado de 20 avaliadores. Os ovos foram cozidos, descascados e servidos. A aparência, sabor, cor, odor, textura e avaliação global, foram avaliadas pelos painelistas. A farinha de semente de urucum adicionada à ração à base de sorgo promoveu aumentos lineares nos atributos aparência, sabor, cor, odor, textura e avaliação global, avaliados durante a análise sensorial (p=0,001). A adição de 1,5% de FU em dietas a base de sorgo beneficiou as características de aparência (4,50), sabor (4,50) cor (4,55),

aroma (4,25) e textura (4,55) dos ovos de codorna avaliados, sendo considerados mais atrativos e com maior aceitação (avaliação global = 5,97), em relação aos demais tratamentos testados. A inclusão do FU nas rações à base de sorgo melhorou a pigmentação da gema dos ovos de codorna em relação aos tratamentos controle. O AM desencadeia efeitos positivos na pigmentação da gema e nas características sensoriais de ovos de codornas comuns.

PALAVRAS-CHAVE: aditivos; bixina; carotenoides; pigmentantes naturais; pigmentação da gema.

INTRODUCTION

The increase in the costs of feeding birds because of the increase in the prices of ingredients such as corn, or the low availability of this commodity in some Brazilian regions, has driven research to seek alternative foods that are affordable and nutritious to make up the diet offered to birds (NIAMAT 2017, SAKI et al. 2017, IBRAHIM et al. 2018, SAYED et al. 2019, MASENYA et al. 2021). In this context, sorghum is considered a promising ingredient, as it is a good source of energy to replace corn in poultry feed with nutritional characteristics similar to those of corn (MASENYA et al. 2021). However, sorghum contains reduced amounts of the xanthophyll pigment compared to corn, which provides the yellow color for egg yolk pigmentation (FARAHAT et al. 2020).

The quality of the egg produced is a very important factor, since it will determine the success of the product in the consumer market (SILVA et al. 2019). Yolk color is a sensory attribute considered an important characteristic of egg quality. Its yellow color comes from the absorption of carotenoid pigments present in the feed (SALEH et al. 2021). Therefore, when alternative ingredients low in xanthophylls are used, some pigments must be included in the feed to improve the characteristic coloring of the egg yolk (ORTIZ et al. 2021). Thus, an alternative pigment additive that can be used in the feeding of quails is the meal from the annatto seed residue (*Bixa orellana* L.), which is a by-product of the extraction of bixin, a natural dye widely used by the food, pharmaceutical, and cosmetics industries (PIMENTEL-MANI et al. 2020).

Studies seek to determine optimal levels of inclusion of annatto seed meal in the diets of laying hens (MELO et al. 2021) and broiler chickens (PARENTE et al. 2018, LUIGGI et al. 2020) in order to improve the quality characteristics of the generated products. However, to the best of our knowledge, there are few studies with the inclusion of annatto seed meal in the diets of quails (PIMENTEL-MANI et al. 2020), especially in relation to yolk color and sensory analysis of common quail eggs. Thus, we hypothesized that the use of annatto seed meal in sorghum-based diets given to common quails improves yolk color, thus positively influencing their acceptability by the consumer market.

The objective of this study has been to evaluate the effect of increasing levels of annatto (*Bixa orellana*) seed meal on yolk pigmentation and the sensory analysis of eggs of common quails fed diets based on sorghum instead of corn.

MATERIAL AND METHODS

Experiment location and ethical aspects

The experiment was carried out in the facilities of the Poultry Shed belonging to the Agricultural Sciences Center of the Federal University of Vale do São Francisco (CCA/UNIVASF), Petrolina, Pernambuco, Brazil (9º 19' 28" South latitude, 40º 33' 34" West longitude, 393 m altitude). The region's climate is classified as hot semiarid (BSh) with summer rains.

The rainy season begins in November and ends in April with an average annual rainfall of 376 mm, relative humidity of 36.73%, and average annual temperatures, maximum and minimum, of 32.0 and 26.9 °C, respectively.

The Ethics Committee approved the study for the Use of Animals (CEUA) of the Federal University of Vale do São Francisco (UNIVASF), protocol number 0002/100614.

Animals and accommodation

A total of 80 female common quails (*Coturnix coturnix coturnix*), in the laying phase, aged 251 days and with a mean initial body weight of 272.5 g were used in this experiment. The experimental period lasted 63 days divided into three 21-day experimental cycles. The first cycle corresponded to the period from 251 to 272 days of age, the second cycle corresponded to the period from 273 to 294 days of age, and the third cycle corresponded to the period from 295 to 316 days of age.

The birds were placed in a galvanized wire battery cage containing cages with length, width, and height of 100 x 33 x 15 cm, respectively. Each cage consisted of four divisions measuring 23 x 55 x 15 cm,

respectively, amounting to 759 cm², with a spacing of 189.75 cm²/birds. The cages were equipped with trough feeders and drinkers, with four birds per cage. Throughout the entire experimental period, the maximum and minimum temperatures and relative humidity of the environment were recorded daily, at 8:00 AM and 5:00 PM, using digital thermo-hygrometers (Incoterm, São Paulo, SP, Brazil) placed at the height of the birds (Table 1).

Table 1. Mean values of maximum and minimum temperatures and relative humidity during the experimental cycles.

Periods	Temperatures		Relative humidity (%)
	Maximum (°C)	Minimum (°C)	
251 to 272 days	33.42	25.87	41.53
273 to 294 days	32.36	24.80	45.64
295 to 316 days	33.86	24.56	43.33

Experimental treatments and diets

The quails were distributed in a completely randomized design with five treatments and four replications, and four birds per experimental unit. The treatments consisted of: T1 - corn-based feed (positive control); T2 - feed with 100% sorghum replacing corn without the addition of pigment (negative control); T3, T4, and T5 - feed with 100% sorghum replacing corn with the addition of 0.5, 1.0, and 1.5% of annatto seed meal (AM), respectively (Table 2).

Table 2. Percentage and chemical composition of experimental diets.

Ingredients (natural matter %)	Feed				
	Annatto seed meal (AM) levels				
	T1	T2	T3	T4	T5
Corn (7.8%)	42.90	0.00	0.00	0.00	0.00
Sorghum	0.00	40.43	40.15	39.35	39.40
Annatto seed meal (AM)	0.00	0.00	0.50	1.00	1.50
Soybean meal (45%)	41.00	41.50	41.00	41.00	40.14
Soybean oil	5.43	7.36	7.64	7.93	8.23
Calcitic Limestone	7.89	7.89	7.89	7.89	7.89
Dicalcium phosphate	1.29	1.28	1.28	1.28	1.29
Vitamin Mineral Premix[1]	0.50	0.50	0.50	0.50	0.50
Choline Chloride	0.15	0.15	0.15	0.15	0.15
DL-methionine (99%)	0.25	0.28	0.28	0.28	0.28
L-lysine HCL (78.4%)	0.06	0.08	0.08	0.08	0.08
Common salt	0.53	0.53	0.53	0.54	0.54
Calculated chemical composition[2]					
Apparent Metabolizable Energy (Mcal/Kg)	2,900	2,900	2,900	2,900	2,900
Crude protein (%)	22.0	22.0	22.0	22.0	22.0
Available phosphorus (%)	0.37	0.37	0.37	0.37	0.37
Total phosphorus (%)	0.60	0.60	0.60	0.60	0.60
Calcium (%)	3.50	3.50	3.50	3.50	3.50
Digestible lysine (%)	1.15	1.15	1.15	1.15	1.15
Total lysine (%)	1.26	1.26	1.26	1.26	1.26
Digestible methionine + cystine (%)	0.84	0.84	0.84	0.84	0.84
Total methionine + cystine (%)	0.91	0.91	0.91	0.91	0.91
Digestible methionine (%)	0.54	0.54	0.54	0.54	0.54
Total methionine (%)	0.59	0.59	0.59	0.59	0.59
Sodium (%)	0.23	0.23	0.23	0.23	0.23
Chemical composition					
Dry matter (natural matter %)	91.5	92.1	92.4	92.3	92.4
Mineral matter (dry matter %)	0.54	0.49	0.49	0.50	0.53
Crude protein (dry matter %)	22.4	21.6	22.2	22.5	22.7
Ether extract (dry matter %)	5.83	4.10	5.05	5.53	4.97

T1 - corn-based feed (positive control); T2 - feed with 100% sorghum replacing corn without the addition of pigment (negative control - 0% AM); T3 - Sorghum + 0.5% AM; T4 - Sorghum + 1.0% AM; T5 - Sorghum + 1.5% AM; [1]Nutritional levels of vitamins and microminerals/kg of feed; Iron (min) 50 mg; copper (min) 8.00 mg; zinc (min) 65.00 mg; manganese (min) 9.0 mg; iodine (min) 1.30 mg; selenium (min) 0.5 mg; cobalt (min) 0.25 mg; vitamin A (min) 9600.00 IU; vitamin D3 (min) 2400 IU; vitamin E (min) 18 IU; vitamin K3 (min) 2.40 mg; niacin (min) 12.00 mg; pantothenic acid (min) 12.00 mg; vitamin B1 (min) 3.60 mg; vitamin B2 (min) 4.80 mg; vitamin B6 (min) 3.60 mg; vitamin B12 (min) 0.0096mg; [2]Composition calculated according to SILVA (2009).

The proximate composition of the feed used in the diets is shown in Table 3. The annatto seed meal came from the local market in Senhor do Bonfim, Bahia, Brazil. The bixin extraction process is carried out by centrifuging in water, and the residue from this extraction undergoes a drying operation, being subsequently ground and packaged for commercialization.

The feed was formulated to be isonutritive based on the nutritional requirements for common quails described by SILVA (2009). Samples of the experimental diets were collected to determine the contents of dry matter (DM; method 967.03), mineral matter (MM; method 942.05), crude protein (CP; method 981.10). and ether extract (EE; method 920.29) using the procedures described by AOAC (2016) (Table 3).

Table 3. Proximate composition of ingredients.

(%)	Corn*	Sorghum*	Annatto seed meal**	Soybean meal*	Soybean oil*
Dry matter	87.48	87.90	90.20	88.75	99.60
Mineral matter	1.27	1.41	-	5.83	-
Crude protein	7.88	8.97	13.13	45.22	-
Fat	3.65	2.96	2.10	1.69	99.60

Source: ROSTAGNO et al. (2017) *, PIMENTEL-MANI et al. (2020) **.

Daily management

Feed and water were provided ad libitum throughout the experimental period. A lighting programming equipment was installed in the shed, which provided a total of 16 hours of light every day, being them 13 hours of natural light coming from the sun and three hours of artificial lighting (40 watt fluorescent lamps).

Sensory analysis

To evaluate the sensory attributes, the eggs were collected, classified according to treatments, and stored under refrigeration until testing. A total of 100 eggs from each treatment were used in this analysis. The Quantitative Descriptive Analysis (QDA) test for sensory evaluation was applied with an untrained panel composed of 20 evaluators with previous experience in the consumption of quail eggs. All volunteers signed an informed consent (BRASIL 2012).

The eggs were prepared for evaluation by cooking them in boiling water for 10 minutes, then being cooled in running water. Once at room temperature, the eggs were peeled and served in plastic containers coded with three random digits. The tests were carried out in individual cabins under controlled temperature and lighting conditions. Each evaluator received five quail eggs, one from each treatment. The samples were tasted and the intensity of the sensorial attributes of appearance, flavor, color, odor, texture, and overall evaluation were analyzed. The evaluators were instructed to, between tasting one sample and another, drink water to clean the palate from sample residues that could interfere with their evaluative response.

The intensity of each sensory attribute was assessed in a structured manner. Each attribute was scored on a scale from 1 to 5, in which 1 corresponded to the least favorable condition and 5 to the most favorable condition. The analysis was performed in the morning and then the mean values of each characteristic were calculated for the overall quality of each treatment by using the following formula (CAMPOS et al. 2007): Overall quality = (appearance x 0.1) + (color x 0.1) + (smell x 0.15) + (flavor x 0.4) + (texture x 0.25).

Yolk color

Visual assessment of yolk pigmentation intensity was performed using the yolk color fan (DSM 2016), which has a range of color intensity values ranging from 1 (pale yellow) to 16 (dark orange) points. The test was always performed by the same person and under the same lighting conditions to avoid changing patterns. The fan blades were positioned immediately above the yolk and viewed vertically, top to bottom, with the blade numbers facing down and the yolk positioned between the tips of the blade (FASSANI et al. 2019).

Statistical analysis

The data were submitted to analysis of variance and regression at the 5% probability level using the PROC GLM of the Statistical Analysis System (SAS University). The significance of the parameters estimated by the models and the values of the coefficients of determination (R^2) were adopted as criteria for choosing the linear and quadratic regression models, according to the best fit obtained for each variable.

The comparison of the mean values obtained in the parameters evaluated in relation to the use of corn and sorghum in the feed was carried out using the Tukey test at the 5% probability level.

RESULTS AND DISCUSSION

Eggs tend to change their organoleptic characteristics according to the type of food offered to the birds (BERKHOFF et al. 2020). Thus, the addition of additives in the feed should be evaluated regarding their interference in the sensory characteristics of quail eggs (VALENTIM et al. 2020). Because of market requirements, natural pigment additives have been increasingly used in the feed offered to livestock and poultry for the production of attractive products of animal origin in order to make them well accepted by the consumer market (OLIVEIRAS et al. 2021).

In this sense, we have observed that annatto seed meal added to sorghum-based feed promoted linear increases in the attributes of appearance, flavor, color, odor, texture, and overall evaluation, which were evaluated during the sensory analysis (p=0.001; Table 4); thus, we recommend the inclusion of annatto seed meal in the feed offered to common quails. Panelists have judged that the addition of 1.5% AM to the sorghum-based diets of common quails benefited the characteristics of appearance (4.50), flavor (4.50), color (4.55), aroma (4.25), and texture (4.55) of the evaluated quail eggs (Table 4), being this amount considered as more attractive and with greater acceptance (overall evaluation = 5.97) in relation to the other treatments tested.

Table 4. Sensory evaluation of eggs from common quails fed sorghum-based diets with the addition of increasing levels of annatto seed meal (AM).

	Feed					SEM	P Value (Source Effect)	P Value	
	T1	Annatto seed meal levels							
Variables	(Control)	T2	T3	T4	T5			L	Q
Appearance	3.35	2.25	3.40	3.90	4.50	0.23	0.001	0.001	0.131
Flavor	3.80	2.75	3.50	3.80	4.50	0.17	0.003	0.001	0.033
Color	3.05	1.90	3.25	3.95	4.55	0.21	0.002	0.001	0.330
Aroma	3.80	3.20	3.30	3.80	4.25	0.17	0.023	0.001	0.899
Texture	3.65	3.25	3.60	3.95	4.55	0.26	0.278	0.001	0.533
Overall Assessment	4.97	3.93	4.61	5.19	5.97	0.22	0.001	0.001	0.833

T1 - corn-based feed (positive control); T2 - feed with 100% sorghum replacing corn without the addition of pigment (negative control - 0% AM); T3 - Sorghum + 0.5% AM; T4 - Sorghum + 1.0% AM; T5 - Sorghum + 1.5% AM; SEM - standard error of the mean; L - linear effect; Q - quadratic effect; Equations: $\hat{Y}^1 = 2.42 + 1.45x$; $R^2 = 50\%$; $\hat{Y}^2 = 2.80 + 1.11x$; $R^2 = 34\%$; $\hat{Y}^3 = 2.11 + 1.73x$; $R^2 = 61\%$; $\hat{Y}^4 = 3.09 + 0.73x$; $R^2 = 21\%$; $\hat{Y}^5 = 3.20 + 0.85x$; $R^2 = 23\%$; $\hat{Y}^6 = 3.92 + 1.34x$; $R^2 = 41\%$, Significant at the 5% probability level.

When comparing the results obtained between the treatments without the addition of annatto seed meal (T1 and T2), we have observed that the panelists judged that eggs from quails that received corn-based feed presented superior sensory attributes of appearance, flavor, color, and aroma, as well as overall evaluation, in relation to eggs from quails that received sorghum-based feed ($p<0.05$; Table 4). Our findings corroborate SILVA et al. (2018), who have also observed overlapping of the mean values attributed to the sensory characteristics of eggs of birds fed with corn in relation to sorghum.

When viewing the results obtained from the inclusion of annatto seed meal in sorghum-based feed, from the inclusion of 0.5% of annatto seed meal, the panelists considered that the appearance and color of the yolk of the quail eggs analyzed were superior to the eggs from quails that received corn-based feed ($p<0.05$; Table 4). Thus, we can infer that the addition of annatto seed meal in sorghum-based diets made the analyzed eggs more attractive to the panelists, which is emphasized by HANSEN et al. (2015), who in studies with supplementation of the diet of laying hens with annatto have observed that this ingredient changes the nutritional composition, appearance, and color of the egg yolk, thus making food supplementation a means of nutritionally enriching products of animal origin.

Flavor and aroma are important factors in the consumer's decision to purchase any food and their usual consumption (MARGETA et al. 2019). We could observe that the inclusion of 1.0% of annatto seed meal in the sorghum-based diet provided the quail eggs with a flavor and aroma similar (3.80) to those found in eggs from quails that received a corn-based diet (Table 4). Above this level of inclusion of annatto seed meal (1.5%), there is greater acceptance of these sensory attributes in relation to eggs from the control treatment (feed containing corn). According to the overall assessment, the panelists believe that, from 1.0% onwards, the inclusion of annatto seed meal in sorghum-based diets improves the organoleptic characteristics of flavor and aroma of the quail eggs (Table 4).

There was no effect of the control feed (corn and sorghum without pigment additive) on the texture of the analyzed quail eggs ($p = 0.278$; Table 4).

Figure 1. Egg yolk color of common quails fed sorghum-based diets with the addition of increasing levels of annatto seed meal (AM) (T1 - corn-based feed (positive control); T2 - feed with 100% sorghum replacing corn without the addition of pigment (negative control - 0% AM); T3 - Sorghum + 0.5% AM; T4 - Sorghum + 1.0% AM; T5 - Sorghum + 1.5% AM).

Yolk color is strongly correlated with the carotenoid content of the food ingested by quails and, to a lesser extent, with the physiological state of the birds. This determines the importance of achieving adequate yolk pigmentation, given that the consumer associates yolk pigmentation with the health of the birds, the farming system, and the naturalness of the products (MARTÍNEZ et al. 2021). However, the type and chemical composition of carotenoids present in the natural additives of the feed significantly affect the efficiency of carotenoid transfer to the egg yolk and its impact on yolk color (SALEH et al. 2021). Annatto has pigmenting substances that are not synthesized by the bird and, when added to feed, provide greater intensity of yolk pigmentation. This fact is seen in Figure 1, where we can observe that the inclusion of annatto seed meal in sorghum-based diets improves egg yolk pigmentation when compared to control treatments (corn and sorghum without pigment additive).

With only 0.5% inclusion of annatto seed meal in the diets, it was possible to obtain a coloration of the raw yolk similar to that presented by treatment 1 (corn-based diet). This result is consistent with the sensory evaluation of the color attribute (Table 4), in which the panelists, through sensory perception, saw that, from the inclusion of 0.5% of annatto seed meal in the feed, the color of the yolk of the boiled eggs analyzed resembled the corn treatment. The results obtained differ from those found by PIMENTEL-MANI et al. (2020), who only managed to achieve yolk color similar to that provided by corn-based diets by adding 3.0% of annatto seed meal to the sorghum-based feed offered to Japanese quails.

CONCLUSION

A minimum of 0.5% AM is recommended in diets containing sorghum instead of corn for a more intense yolk pigmentation of common quail eggs.

The inclusion of annatto seed meal improves the sensory characteristics of common quail eggs.

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