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Performance and egg quality of laying hens fed pequi pulp flour

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Abstract
The objective of this study was to evaluate the effects of including pequi pulp flour (PPF) in laying hen diets on performance and egg quality. A total of 240 Hisex Brown strain were randomly assigned into 5 groups and were fed diets containing PPF at the level of 0%, 0.125%, 0.250%, 0.375%, and 0.5%. The experiment lasted 63 days (3 periods of 21 days). The variables of performance and egg quality were submitted to linear and quadratic regression analysis at the level of 5% probability. The outcomes suggest that neither performance nor egg quality was affected ($p > 0.05$) by different levels of PPF. Dietary inclusion of PPF tested in the present study was not sufficient to demonstrate improvement in the productive performance of birds as well as in egg quality. Because of the lack of responses, additional research is needed to evaluate higher levels of PPF in the diets for laying hens.
Keywords: alternative feedstuffs; by-product; *Caryocar brasiliense;* poultry.

Desempenho e qualidade de ovos de galinhas poedeiras alimentadas com farinha da polpa de pequi

Resumo: O objetivo deste estudo foi avaliar os efeitos da inclusão da farinha de polpa de pequi (FPP) em dietas de galinhas poedeiras sobre o desempenho e a qualidade dos ovos. Um total de 240 galinhas Hisex Brown foram aleatoriamente atribuídas em 5 grupos e alimentadas com dietas contendo FPP ao nível de 0%, 0,125%, 0,250%, 0,375% e 0,500%. O experimento durou 63 dias (3 períodos de 21 dias). As variáveis de desempenho e qualidade dos ovos foram submetidas à análise de regressão linear e quadrática no nível de probabilidade de 5%. Os resultados sugerem que o desempenho e a qualidade dos ovos não foram afetados ($p > 0,05$) por diferentes níveis de PPF. A inclusão dietética dao FPP testado no presente estudo não foi suficiente para demonstrar melhora no desempenho produtivo das aves, bem como na qualidade dos ovos. Devido à ausência de respostas, são necessárias pesquisas adicionais para avaliar níveis mais elevados de FPP em dietas para poedeiras.
Palavras-chave: alimentos alternativos; avicultura; *Caryocar brasiliense,* coprodutos.

Introduction

In response to concerns about food safety, consumers demand more and more for the quality of the products that they consume. The market for direct egg consumption is influenced by visual characteristics of products that trigger the preference for eggs with darker yolk. In another words, eggs with orange pigmentation result from the deposition of xanthophylls, which belong to the group of carotenoid pigments (VALENTIM *et al.*, 2019). Such feature combined with shells dark brown color of some laying hens (e.g., Isa Brown, Lohmann Brown) reflects an extensive production, or "hillbilly type", becoming the consumer's preference.

Egg yolk colors vary from one country to another or even from region to region. In the United States, the preference is for yolk coloring from 7 to 10 on the RYCF scale (MENDONÇA *et al.*, 2018), while in other countries like in Europe or Asia, it is preferred yolk coloring ranging from 10 to 14 (BITTENCOURT *et al.*, 2019). The product analysis made by consumers associate the observed yolk coloring with vitamins that are egg

constituents, and at the same time to the laying hen health (MORALECO *et al.*, 2019). Although this has no scientific support, consumers have a great preference for more orange yolk eggs due to the better colors distribution in mixtures where eggs are added, such as in bakery products, pasta making, alcoholic beverages, among others (FERRONATO *et al.*, 2020; SOUZA *et al.*, 2020).

Carotenoids are the natural pigments produced by plants, algae, and photosynthetic microorganisms (TERAMUKAI *et al.*, 2020). Dietary inclusion of feedstuffs with high content of carotenoids has been responsible for the coloring of feathers, skin, and egg yolk (SILVA *et al.*, 2019). The yellow color is typical of carotenes (MENDONÇA *et al.*, 2018). Plant extracts are available in nature and are easy to reproduce and plant, besides being less costly (SOARES *et al.*, 2008). Boka *et al.* (2014) found that plant extracts have a positive effect due to their antioxidant activity that improves nutrient digestibility and stimulates enzyme secretion, helping to preserve the intestinal microbiota, improving the feed conversion ratio, production, and internal egg quality traits.

Brazil is a country rich in wild flora, being a great center of extractive exploration. In that regard, the Brazilian Cerrado outstands (OLIVEIRA *et al.*, 2016) due to its wide array of flora. The pequi *(Caryocar brasiliense Camb*.) is a Cerrado tree with high commercial value. Its fruit is usually used and appreciated in regional cuisine, besides having antioxidant activity due to the presence of vitamins A and E, and carotenoids (LIMA *et al.*, 2020).

To the best of our knowledge, this is the first study considering the dietary inclusion of pequi pulp flour (PPF) for laying hens. Therefore, the current study was conducted to evaluate the inclusion of PPF up to 0.5% in the diets of laying hens on performance and egg quality. We hypothesized that the inclusion of PPF would improve the performance and egg quality (especially yolk color).

Methodology

Pequi pulp flour

To prepare the pequi pulp flour, the pequi pulp was extracted from the fruit and then dried with the ad of forced air circulation greenhouse at 40 °C for 48 h. In the end, the pulp dry matter was 42%. After drying, the pequi pulp was beaten by an industrial food blender (Camargo, SP, Brazil) and sifted. Thus, according to each treatment, the pequi pulp flour was ready to be added to the diets.

Experimental design, diets, and management

The study was carried out at the Poultry Facilities of the Federal Institute of Minas Gerais - *Campus* Bambuí (IFMG - *Campus* Bambuí), Bambuí, MG, Brazil. A total of 35-week-old two hundred and forty Hisex Brown laying hens were randomly assigned in 5 treatments for 63 days, following a completely randomized design There were 6 replicates per treatment. Each replicates consists of 4 laying cages measuring 25 cm wide × 45 cm deep × 35 cm high and capacity for 2 hens, therefore, 8 birds per replicate. The cages were equipped with nipple drinkers and trough feeders. The light program consisted of a 16 h light per day and was kept constant to the end of the experiment. Feed and water were provided for *ad libitum* consumption.

The dietary treatment groups were the control treatment (basal diet, no PPF; control), a basal diet with 0.125% PPF, a basal diet with 0.250%, a basal diet with 0.375% PPF, and basal diet with 0.500% PPF. All diets were formulated to be approximately isocaloric, isocalcic, and isophophoric and meet or exceed the recommendations of Rostagno *et al.* (2017). Diets were composed of corn and soybean meal-based. The composition and nutrient concentrations of the experimental diets are presented in Table 1.

Table 1. Ingredients and nutrient composition of experimental diets (as-fed basis)

Ingredients, %	Control	0.125% PPF	0.250% PPF	0.375% PPF	0.500% PPF
Corn	58.190	58.190	58.190	58.190	58.190
Soybean meal	28.600	28.600	28.600	28.600	28.600
Soy oil	1.500	1.500	1.500	1.500	1.500
Limestone	4.300	4.300	4.300	4.300	4.300
Coarse limestone	4.400	4.400	4.400	4.400	4.400
Salt	0.450	0.450	0.450	0.450	0.450
Dicalcium phosphate	1.347	1.347	1.347	1.347	1.347
Kaolin	0.800	0.675	0.550	0.425	0.300
PPF[1]	0.000	0.125	0.250	0.375	0.500
DL-Methionine 99%	0.160	0.160	0.160	0.160	0.160
L-Lysine 78%	0.050	0.050	0.050	0.050	0.050
Phytase	0.003	0.003	0.003	0.003	0.003
Mineral premix [1]	0.100	0.100	0.100	0.100	0.100
Vitamin premix [2]	0.100	0.100	0.100	0.100	0.100
Calculated nutrient content					
Crude Protein, %	17.632	17.632	17.632	17.632	17.632
Linoleic acid, %	2.127	2.127	2.127	2.127	2.127
Calcium, %	3.900	3.900	3.900	3.900	3.900
Available phosphorus, %	0.460	0.460	0.460	0.460	0.460
Potassium, %	0.692	0.692	0.692	0.692	0.692
Sodium, %	0.181	0.181	0.181	0.181	0.181
Chlorine, %	0.305	0.305	0.305	0.305	0.305
ME, kcal/kg	2.773.96	2.773.96	2.773.96	2.773.96	2.773.96
SID[3] Lys, %	0.889	0.889	0.889	0.889	0.889
SID Met, %	0.404	0.404	0.404	0.404	0.404
SID Met+Cys, %	0.649	0.649	0.649	0.649	0.649
SID Try, %	0.197	0.197	0.197	0.197	0.197
SID Thr, %	0.601	0.601	0.601	0.601	0.601

[1] PPF: pequi pulp flour

[2] Mineral Premix composition (per kg of product): manganese 75000 mg, iron 50000 mg, iode 1500 mg, zinc 70000mg, copper 8500mg, cobalt 200 mg.

[2] Vitamin Premix composition (per kg of product): vitamins: A 800000 µ, B12 1000 mg, D3 200000 µ, 15000mg, K3 2000 mg, B2 4000 mg, B6 1000 mg, niacin 19900 mg, pantothenic acid 5350 mg, ac. folic 200 mg, selenium 2500 mg, antioxidant 100000 mg.

[3] SID = standardized ileal digestible.

Hen productivity and egg quality

Different variables related to egg performance and quality were evaluated according to the methodologies of several authors (BITTENCOURT *et al.*, 2019; SELIM; HUSSEIN, 2020; VAN DER HEIDE *et al.*, 2021).

From the beginning (35 weeks of age) to the end of the study (44 weeks of age), hen productivity was measured weekly per replicate. Eggs from each hen group were collected and recorded in spreadsheets twice a day. Eggs that showed deformation in the shell, as well as broken and cracked was considered as egg loss. The viable eggs were calculated by subtracting the total eggs collected from the egg loss. Egg weight was calculated by dividing the total weight by the collected number of eggs.

Feed intake was measured by subtracting the weight of feed remaining in each hen group from the sum of feed supplied weekly to each hen group. Egg production was calculated by dividing the total number of eggs by the housed hens. Egg mass was calculated by multiplying egg weight by egg production. Feed conversion ratio (FCR) was calculated as a gram of feed intake divided by a gram of egg weight.

Regarding egg quality, in the last week of the study (9th week) all intact eggs produced per replicate were used to perform the specific gravity according to the method proposed by Freitas *et al.* (2004). The method is based on the Archimedes principle. After analyzing the specific gravity, two fresh eggs per replicate (12 eggs/treatment) were collected randomly from each replicate to perform egg components. Each egg was analyzed individually. First of all, the egg was weighed (digital scale, 0.05 g) and then broken. The yolk color was analyzed by a single observer in the same ambient conditions of light by comparison with the color pattern scale (comparative scale from 1 to 15, Yolk Color Fan colorimetric disc). Using a caliper, the albumen height was measured in the middle region of the thick albumen, to determine the Haugh unit. After that, the yolk was carefully removed and then weighed separately.

The shell was washed in running water and was dried at room temperature for 24 h and then weighted. Albumen weight was calculated by subtracting the sum of yolk and shell weight from the egg weight. The egg components were calculated considering the percentage that each component represents in the egg weight. Shell thickness was measured with the aid of a micrometer (accuracy of 1 µm) at 3 different places around the equator of the shell.

The assumption of the normality of the studentized residuals was checked using the Shapiro Wilk test while Levene's test was used to verify the homogeneity of the variances. When needed, the response variable was transformed into a normal distribution approximation. Regression analysis was performed using linear and quadratic models and outcomes were considered to be significant if $p < 0.05$.

Resultados and Discussions

During the study, no mortality was recorded. There was no effect of different inclusion levels of PPF on the performance and egg quality variables. Only for the variables Shell (%) and Albumen (%) that had quadratic effect with value of better inclusion the levels of 0.28% and 0.278 %, according to the regression equations presented in the table 2.

Table 2. Performance and egg quality of laying hens fed with different levels of inclusion of pequi pulp flour in the diet.

Items	Pequi pulp flour levels					EPM	p-linear value	p-quadratic value
	Control	0.125%	0.250%	0.375%	0.500%			
Egg production, %	95.69	95.19	96.77	95.60	96.16	1.026	0.680	0.872
Feed intake, g/d	121.53	120.04	120.80	118.14	118.93	2.395	0.699	0.900
Fed conversion ratio, kg feed/dz egg	1.565	1.555	1.537	1.527	1.53	0.029	0.559	0.764
Fed conversion ratio, g feed/g egg	2.038	2.011	1.999	1.982	1.968	0.049	0.654	0.910
Egg weight, g	62.44	62.74	62.49	62.48	63.16	0.565	0.787	0.634
Viable eggs, %	99.75	99.11	99.19	99.63	99.24	0.234	0.497	0.329
Specific gravity, g/cm³	1.091	1.091	1.091	1.090	1.092	0.001	0.882	0.169
Yolk color	4.62	4.97	4.87	5.07	4.82	0.162	0.333	0.142
Yolk, %	24.39	24.65	24.91	24.99	24.47	0.293	0.596	0.113
Shell, %*	9.48	10.04	9.99	9.71	9.86	0.102	0.185	0.007
Albumen, %	66.13	65.3	65.1	65.3	65.67	0.295	0.324	0.013
Shell thickness, mm	0.464	0.477	0.477	0.466	0.474	0.073	0.699	0.465
Haugh unit	94.57	93.77	94.90	93.88	95.05	1.160	0.770	0.684

2* quadratic effect ($P < 0.05$); Regression equation: Shell (%): $Y= 9{,}579 + 2{,}777x - 4{,}864x^2$; R^2: 87.59% (0.28% inclusion). Albumen (%): $Y = 66.088 - 7.190x + 12.897 x^2$; R^2: 97.04% (0.278 % inclusion).

Egg production was not influenced (p>0.05) by the addition of PPF. The result is in agreement with Silva *et al.* (2000) that investigating the effects of the inclusion of annatto extract as a source of carotenoids on egg yolk pigmentation did not observe a significant difference in production. Likewise, Fassani *et al.* (2019) do not report effects of annatto extract as a natural source of carotenoids not even synthetic commercial pigments Carophyll® Yellow and Red (20 + 10 ppm) and (15 + 30 ppm) on production performance. According to Valentim *et al.* (2019), the use of natural pigments has been increased due to the needs of customers in developed countries, who banned the use of synthetic pigment additives, such as canthaxanthin and astaxanthin in humans as well as in animal feed.

With the different inclusions of PPF in the feed, the variations in feed intake among different treatments were not significant. We believe that this fact can be explained by the non-variation of palatability, color appearance, and other variables since these factors are important for the determination of birds' feed intake. These results are in agreement with Oliveira *et al.* (2016) who studied the inclusion of different levels of pequi peel flour in diets for Japanese quail and also do not observe any difference in feed conversion, corroborating with our results, although we work with the pulp instead of peel.

The feed intake influences the average egg weight due to the direct effect of the daily nutrient consumption such as protein, sulfur amino acids, and fatty acids. Since the nutrient composition of the diets was similar and the feed intake at different levels of PPF inclusion was the same, the average egg weight remained in the same range, without difference among treatments.

The high egg viability in all treatments with no statistical differences can be justified by the correct feed formulation. The diets offered to laying hens through the experiment period had no interference in any of the PPF inclusion levels on egg viability. Furthermore, the adequate levels of minerals (mainly calcium and phosphorus) ensured the correct shell formation with high quality, consequently improving the egg viability.

The specific gravity was not altered at different levels of PPF. Our results are in agreement with the study of Garcia *et al.* (2002) which report that the inclusion of 10% of canthaxanthin (carophyll Red) did not influence the performance and egg quality (e.g., specific gravity) of commercial laying hens.

Pequi (*Caryocar brasiliense*) is an important source of carotenoids and has 7.25 mg 100 g^{-1} fruit of carotenoids (SILVA *et al.*, 2016). Carotenoids from pequi have been described as compounds that act as natural pigments (UENOJO; JUNIO; PASTORE, 2007). Thus, it was expected that the provision of a diet with PPF would increase the intensity of pigmentation of egg yolk. However, regardless of the inclusion level, no improvement in egg yolk color was observed. Our outcomes disagree with Valentim *et al.* (2020) which reported that the addition of pigments from plant extracts containing carotenoid in the feed for quail improves the yolk color.

No references are found on the use of PPF in the nutrition of laying hens. Based on experiments with annatto extract (EU), we observed that perhaps the level of inclusion of PPF may have been low, not reaching even 50 % of the minimum significant level in comparison with the EU.

Other works such as Carvalho *et al.* (2006) demonstrate that the presence of carotenoids in the diet for laying hens has significant gains in the yolk color. Although the pequi pulp contains different types of carotenoids (anthexanthena, zeaxanthin, violaxanthin, and lutein) as identified by Azevedo-Meleiro and Rodriguez-Amaya (2004), they were not sufficient to influence in the yolk colorat the level proposed in the current research.

The yolk percentage, and shell thickness, are in agreement with Silva *et al.* (2000) which do not observe the effect of annatto extract supplementation in these variables.

According to Carvalho *et al.* (2006), the Haugh unit will vary according to the source used for carotenoids. The use of marine sources such as seaweed demonstrates significant results, improving the internal quality of the eggs, going against that obtained in the current study where PPF was used as a source of carotenoids. Perhaps this improvement can be attributed to the properties of the seaweed, not just to the carotenoids.

Oliveira *et al.* (2016) indicated that pequi peel flour can be used in commercial diets for laying quail in up to 3%, maintaining poultry

productivity, improving yolk color, and reducing egg production costs.

Several studies (BITTENCOURT *et al.*, 2019; SILVA *et al.*, 2019; VALENTIM *et al.*, 2020) report the effectiveness of alternative pigmenting sources to those commonly used (FASSANI *et al.*, 2019). However, the inclusion of PPF may not have been enough to demonstrate its action.

Conclusion

The levels of pequi pulp flour tested in the present study were not sufficient to demonstrate improvements on the performance of laying hens as well as on the egg quality, especially in the yolk color. Further research with higher inclusion percentages is necessary to evaluate the effectiveness of this natural additive.

Ethics and biosafety committee

The project was submitted and approved under protocol number 05/2017 of UNIFENAS.

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