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Egg characteristics of Japanese quail fed diets containing guava extract (*Psidium guajava* L.)

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ABSTRACT. The main purpose of this study was to evaluate the characteristics of eggs produced by Japanese quails fed with guava extract. A total of 400 eggs collected from the quails fed with experimental diets, were distributed in a completely randomized design in factorial arrangement 4 x 4 with main effects including four dietary levels of guava extract (0, 3, 6 and 9 g kg^{-1}) and four storage periods (0, 9, 18 and 27 days) totalizing 16 treatments with five replicates of five eggs. The eggs were produced by Japanese quails, fed with experimental diets. The diets were isonutritive formulated by corn-soybean basis. The eggs were stored for different periods, at the same conditions, to constitute the treatments. The parameters evaluated were egg weight loss (g and %), yolk color, Haugh unit, specific gravity, eggshell thickness and pH of the yolk and albumen. Data were submitted to ANOVA, and means were compared using Scott-Knott test, using $\alpha = 0.05$. The specific gravity of fresh eggs was better with the use of 6 g kg^{-1} of guava extract in the diet. The use of 3 g kg^{-1} of guava extract resulted in smaller eggshell thickness of fresh eggs. The Haugh unit was affected just by the storage periods. The dietary guava extract (3, 6 or 9 g kg^{-1}) increased the pH of albumen in eggs stored for 27 days. The use of 6 g kg^{-1} of guava extract in quails diet increase the specific gravity in fresh eggs and increase the yolk color when the eggs are stored.

Keywords: additive; antioxidant; egg quality; poultry.

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Introduction

The quail yolk egg is constituted by 31.5 of fat and 25.1% of polyunsaturated fatty acid (Tolik, Polawska, Charuta, Nowaczewski, & Cooper, 2014), becoming susceptible to lipid oxidation. Some factors are responsible for fast lipid oxidation and internal egg quality, such as temperature-humidity index (El-Tarabany, 2016), temperature and time storage (Liu, Chen, Wu, Lee, & Tan, 2016) and laying nutrition (Garcia, Cruz, Kiefer, Avila, & Souza, 2015). According to Fellenberg and Speisky (2006), the oxidative rancidity represents one of the major causes of deterioration in food for human consumption.

The use of natural antioxidant substances in poultry diets has been studied as an alternative to synthetic antioxidants, aiming to attend the consumer demands, including apple peel waste (Heidarisafar, Sadeghi, Karimi, & Azizi, 2016), tomato, orange, green tea (Marzoni et al., 2014), ginger (Youssef, Selim, Abdel-Salam, & Nada, 2016), mango extract (Freitas et al., 2012) and as antimicrobial effects (Rahman, Siddiqui, Khatun, & Kamruzzaman, 2013). The guava extract is a sub product of industry, rich in phenolic compounds that shows good antioxidant activity (Haida et al., 2015). According to Oliveira et al. (2018), guava byproduct can be used as an alternative antioxidant additive in broiler diets in an early stage because it improves thigh meat quality of broilers.

Surai et al. (2003) studied the canthaxanthin supplementation of the maternal diet on the antioxidant system of the developing chick and concluded that the canthaxanthin was transferred from the egg yolk to the developing embryo. So, it is an important form to increase the antioxidant capacity in eggs. In this context, the hypothesis is that the use of guava extract could constitute a natural antioxidant in the diets of Japanese quails, capable of improving the characteristics of the eggs.

The main purpose of this study was to evaluate the characteristics of eggs produced by Japanese quails fed with guava extract and stored in different periods.

Material and methods

The experiment was conducted in Goiânia, state Goiás, Brazil (16° 35' 33.0" S 49° 16' 51.4" W). All procedures in this study were conducted according to the protocol registration number 075/14 and they were approved by the Ethics Committee on the Use of Animals (Ceua).

A total of 400 eggs, collected from the quails fed with experimental diets, on the day of laying were distributed in a completely randomized design in factorial arrangement 4 x 4 with main effects including four dietary levels of guava extract (0, 3, 6 and 9 g kg[1]) and four storage periods (0, 9, 18 and 27 days) totalizing 16 treatments with five replicates of five eggs.

The statistical model used was Equation 1:

$$y_{ijk} = m + a_i + b_j + (ab)_{ij} + e_{ijk} \quad (1)$$

where:
y_{ijk}: an observation in level i of factor a (i = 1, 2, 3, 4), level j of factor b (j = 1, 2, 3, 4) in repetition k (k = 1, 2, ..., 5);
m: the overall mean;
a_i: the fixed effect of level i of factor a (i = 1, 2, 3, 4);
b_i: the fixed effect of level j of factor b (j = 1, 2, 3, 4);
$(ab)_{ij}$: the effect of the interaction of level i of factor a (i = 1, 2, 3, 4) with level j of factor b (i = 1, 2, 3, 4);
e_{ijk}: the random error with mean 0 and variance σ^2.

The birds that produced the eggs were 19 weeks old and they were in peak of production. The experimental diets were formulated from a basal diet (Table 1) according to Rostagno et al. (2011), based on corn and soybean meal, differing only in the dietary level of guava extract.

The eggs were stored in an identified commercial plastic packages and kept under room temperature. The maximum and minimal temperatures were recorded once a day at 8am, by a digital thermometer hygrometer. The maximum and minimal temperatures during the experimental period were 31.0 and 24.3°C, respectively.

To the eggs' quality analysis, five eggs were used, which represented the corresponding experimental unity, in which it was evaluated: egg weight, egg weight loss, eggshell thickness, albumen height, yolk color, specific gravity, Haugh unit, yolk's pH and albumen.

Table 1. Composition and calculated nutritional value of the basal diet.

Ingredient	$g\ kg^{-1}$ as fed
Corn	546.82
Soybean meal 45%	326.21
Limestone	57.80
Inert	9.0
Guava extract	0.0
Soybean oil	14.7
DL-Methionine	3.52
Premix[1]	40.0
L-Tryptophan	0.06
L-Lysine	1.89
Total	1000
Nutrient	Calculated composition ($g\ kg^{-1}$)
Metabolizable energy ($kcal\ kg^{-1}$)	2.800
Crude protein	199.40
Methionine + Cystine	8.88
Lysine	10.83
Calcium	30.99
Available phosphorus	4.06
Threonine	6.62
Tryptophan	2.27

[1]Premix: Provided per kg of product: calcium – 189.65 g; sodium – 37.5 g; phosphorus – 75 g; fluorine – 750 mg; retinol – 67.5 mg; cholecalciferol – 1.75 mg; tocopherol – 1.27 mg; menadione – 50 mg; thiamine – 82,5 mg; riboflavin – 212,5 mg; pyridoxine – 125 mg; cobalamin – 0.50 mg; pantothenic acid – 375 mg; niacin – 875 mg; folic acid – 37,5 mg; biotin – 5 mg; choline – 4.875 mg; Cu – 225 mg (as $CuSO_4$); Fe – 1.125 mg (as $FeSO_4$); Mn – 2.500 mg (as $MnSO_4$); iodo – 22,5 mg [as $Ca(IO_3)_2$]; Zn – 750 mg (as $ZnSO_4$); Se – 12,5 mg (as Na_2SeO_3); zinc bacitracin – 550 mg; methionine 28,7 g.

In every storage period which has passed (fresh eggs, nine, 18 and 27 days of storage), the five eggs that represented the experimental unity were individually identified and weighted in a precision electronic scale

(± 0,001g). The specific gravity (SG) was determined according to the methodology described by Hamiltom (1982), where eggs were immersed in saline solutions with densities varying from 1.050 to 1.090 with interval of 0.005. After the SG determination, the eggs were broken, and the internal content was put in a levelled glass surface to obtain albumen heights (mm) with a micrometer.

Then, the yolk was separated from the albumen to determine its color by an evaluator who performed the same role in all the periods using the Roche® colorimetric spectrum. When the measures were done, a sample was made by five albumens and five yolks, separately, to determine the pH by reading the identified value in the bench pH meter. After that, the eggshells were washed and dried in room temperature for 72 hours to measure its thicknesses using a digital pachymeter in two points in the center-transversal area of the eggshell, obtaining the mean, expressed in millimeters (mm) to the statistical analysis.

To calculate the egg's weight loss, the weight from day zero was used, after each storage period and in the end of the experiment, by the difference between the weight in the beginning and in the end of the storage period, the weight loss was determined in grams (g). Subsequently, this value was divided by the egg's weight in the beginning of the storage period, creating the weight loss data, in percentage. The Haugh unit (HU) was calculated according to the following equation: $HU = 100 \times \log (H - 1.7P0.37 + 7.57)$, where: HU = Haugh unit; H = albumen height; P = egg weight (Silva, 2004).

The quality data were evaluated by analysis of variance and Scott-Knott test, using the software R. It was adopted $\alpha = 0{,}05$.

Results and discussion

The levels of guava extract and stored time showed no interaction for egg weigh loss (g and %). However, the egg weight loss was influenced by the egg storage period, meaning that the biggest the storage period resulted in the biggest weight loss (Table 2). Interaction was observed between levels guava extract and stored time to the specific gravity ($p < 0.001$) and eggshell thickness ($p = 0.0358$; Table 2). In the fresh eggs, the specific gravity was higher in the quail eggs fed 6 g kg^{-1} of guava extract. When the eggs were stored, independent of the storage period, no difference was found in the specific gravity according to the use of guava extract in the feed (Table 3). It was verified that independent of the guava extract dosage, as the egg's storage period increased, the specific gravity decreased. Therefore, the guava extract use was not efficient to improve the specific gravity.

The eggshell thickness that was not stored was influenced by the guava extract levels, whereas the use of 3 g kg^{-1} resulted in lower eggshell thickness (Table 4). When the eggs were stored, independent of the storage period, no difference was found in the eggshell according to dietary guava extract (Table 4). It was observed that the eggshell decreased as the storage time.

Table 2. Egg weight loss (g and %), specific gravity and eggshell thickness of eggs by japanese quails feed with guava extract and stored at different periods.

Factor	Egg weight loss (g)	Egg weight loss (%)	Especific Gravity	Eggshell thickness (mm)
Storage Time -T (days)				
0	0.00a	0.00a	1.0665	0.2095
9	0.376b	3.22b	1.0500	0.1960
18	0.6135c	5.17c	1.0450	0.1909
27	0.9881d	8.40d	1.0450	0.1880
Levels guava extract -L (g kg^{-1})				
0	0.4840	4.10	1.0514	0.1965
3	0.4786	4.15	1.0510	0.1951
6	0.5172	4.15	1.0524	0.1957
9	0.4980	4,38	1.0515	0.1970
ANOVA (p-value)				
T x L	0.3786	0.1749	< 0.001	0.0358
T	< 0.001	< 0.001	< 0.001	< 0.001
L	0.5339	0.6484	< 0.001	0.8579
CV (%)	18.00	17.78	0.08	3.67

CV – coefficient of variation; Means followed by different lower case at column are significantly different by Scott-knott test (0.05).

Table 3. Deployment of interaction storage time x levels of guava extract for specific gravity.

Storage Time (days)	Levels guava extract (g kg^{-1})			
	0.0	3.0	6.0	9.0
0	1.066bA	1.064cA	1.070aA	1.066bA
9	1.050aB	1.050aB	1.050aB	1.050aB
18	1.045aC	1.045aC	1.045aC	1.045aC
27	1.045aC	1.045aC	1.045aC	1.045aC

Means followed by different upper-case letter in columm and lower-case letter in lines are significantly different by Scott-knott test (0.05).

Table 4. Deployment of interaction storage time x levels of guava extract for eggshell thickness.

Storage Time (days)	Levels guava extract (g kg^{-1})			
	0.0	3.0	6.0	9.0
0	0.212aA	0.202bA	0.212aA	0.212aA
9	0.194aB	0.196aA	0.202aB	0.192aB
18	0.194aB	0.192aA	0.185aC	0.192aB
27	0.186aB	0.190aA	0.184aC	0.192aB

Means followed by different upper-case letter in columm and lower-case letter in lines are significantly different by Scott-knott test (0.05).

There was significant interaction between storage period and guava extract levels to the yolk color, Haugh unity and albumen pH (Table 5). The yolk pH was affected by the storage period, meaning that the pH reduced when the storage period increased (Table 5).

When the eggs were stored for nine days, the yolk color was higher using 6 g kg^{-1} of guava extract. When they were stored for 18 days, the greatest color was obtained with 0 or 3 g kg^{-1} of guava extract (Table 6).

Storage time affected the Haugh unit. The Haugh unit reduced according to increase of egg storage time (Table 7).

The use of guava extract (3, 6 or 9 g kg^{-1}) increased the albumen's pH only when the eggs were stored for 27 days (Table 8). The storage period also influenced the albumen's pH. It was verified that the eggs stored for 18 days presented higher albumen pH, independent of the used dosage of guava extract.

The egg weight losses were affected by the storage period. Eggs stored for 27 days presented increase of weight loss. Lee, Jung, Choi, and Sohn (2016) verified that egg weight decreased with the increasing storage period and they concluded that the deterioration of egg quality was attributed to water loss by evaporation through the pores in the shell and the escape of carbon dioxide from albumen. The use of guava extract was not effective in reducing egg weight loss, indicating that it has no effect on maintaining albumen integrity. In fact, the Haugh unit, a parameter with direct relation with albumen height and egg weight was not affected by dietary guava extract.

It was observed that the use of 6 g kg^{-1} of guava extract results in better specific gravity of fresh eggs and the use of 3 g kg^{-1} resulted in the lower specific gravity. This result agrees with the lower eggshell thickness verified with the use of 3 g kg^{-1} of the eggshell thickness of fresh eggs. The eggshell thickness is not a characteristic that have been related with storage time, because this characteristic analyzed after laid is dependent of weight and size of egg. In fact, some author did not verified effects of storage time on weigh and thickness of shell (Scott & Silversides, 2000; Lee et al., 2016).

However, the dietary antioxidants have been related to eggshell characteristics. Al-Harthi (2014) reported that Vitamin E significantly increased eggshell thickness. Invernizzi et al. (2013) verified that selenium supplementation results in increasing of eggshell weight, but not differences were observed in eggshell thickness. So, it is possible that the antioxidants affect the development of eggshell.

Some antioxidants can be transferred into egg yolk and affect the yolk's color (Kang, Kim, Cho, Yim, & Kim, 2003; Martino, Haouet, Marchetti, Grotta, & Ponzielli, 2014). In this study, the yolk's color was affected by the guava extract. The utilization of 6 g kg^{-1} resulted in higher yolk's color of eggs stored for nine days. When the eggs were stored for 18 days the higher yolk's color was obtained with 0 or 3 g kg^{-1}. It was not clear how the yolk's color changed according to the dietary guava extract and storage period of eggs. The dietary antioxidants have been related to increase oxidative stability of eggs, but did not show influence the yolk color (Loetscher, Kreuzer, & Messikommer, 2014). According to Scott and Silversides (2000) there is a little or no direct relation between shell color and nutritional content of the egg.

The Haugh unit and yolk's pH were not affected using guava extract in the diet. Therefore, we concluded that the guava extract was not efficient to avoid the reduction of Haugh unit when the eggs are stored.

Table 5. Yolk color, Haugh unit, pH of yolk and albumen of eggs by Japanese quails feed with guava extract and stored at different periods.

Factor	Yolk color	UH	Yolk pH	Albumen pH
Storage Time -T (days)				
0	5.08	89.241	6.0109c	8.7040
9	4.33	75.889	6.2160b	9.1950
18	4.61	73.230	6.2770b	10.6415
27	5.23	69.208	6.7955a	9.2583
Levels guava extract -L (g kg^{-1})				
0	4.78	76.8460	6.3364	9.4125
3	4.67	76.8330	6.4085	9.4455
6	5.11	77.3995	6.2655	9.4715
9	4.69	76.4900	6.2890	9.4693
ANOVA (p-value)				
T x L	0.0058	0.0389	0.5369	0.0094
T	< 0.001	< 0.001	< 0.001	< 0.001
L	< 0.001	0.6332	0.3085	0.1338
CV (%)	8.82	2.88	3.99	0.93

CV – coefficient of variation; Means followed by different lower case at column are significantly different by Scott-knott test (0.05).

Table 6. Deployment of interaction storage time x levels of guava extract for yolk color.

Storage Time (days)	Levels guava extract (g kg^{-1})			
	0.0	3.0	6.0	9.0
0	4.96aA	5.00aA	5.48aA	4.88aA
9	3.88bB	3.96bB	5.20aA	4.28bB
18	5.08aA	4.72aA	4.44bB	4.20bB
27	5.20aA	5.00aA	5.32aA	5.40aA

Means followed by different upper-case letter in column and lower-case letter in lines are significantly different by Scott-knott test (0.05).

Table 7. Deployment of interaction between storage time x levels of guava extract for Haugh unit.

Storage Time (days)	Levels guava extract (g kg^{-1})			
	0.0	3.0	6.0	9.0
0	88.996A	89.036A	89.848A	89.084A
9	76.086B	75.910B	77.554B	74.006B
18	73.696B	71.742C	72.166C	75.318B
27	68.606C	70.644C	70.030C	67.552C

Means followed by different upper-case letter in columm are significantly different by Scott-knott test (0.05).

Table 8. Deployment of interaction storage time x levels of guava extract for pH of albumen.

Storage Time (days)	Levels guava extract (g kg^{-1})			
	0.0	3.0	6.0	9.0
0	8.726aC	8.732aD	8.678aC	8.680aD
9	9.160aB	9.160aC	9.236aB	9.224aC
18	10.662aA	10.610aA	10.686aA	10.608aA
27	9.102bB	9.280aB	9.286aB	9.365aB

Means followed by different upper-case letter in column and lower-case letter in lines are significantly different by Scott-knott test (0.05).

The guava extract (3, 6 or 9 g kg^{-1}) increased the albumen's pH when the eggs were stored for 27 days. According to Lee et al. (2016), the storage period and temperature are the major factors affecting egg quality, but the storage temperature is a more sensitive determinant of egg quality deterioration compared with the storage period.

In this study, the use of guava extracts up to 9 g kg^{-1} was not effective to maintain the quality of egg stored until 27 days. It was expected that the antioxidant propriety of guava would improve the egg's characteristics and became an alternative to synthetic antioxidants, since the guava results in greater antioxidant status in broiler meat (Oliveira et al., 2018). Some studies should be carried out to clarify if the guava can be transferred into de the egg and present the antioxidant effects.

Conclusion

The use of 6 g kg^{-1} of guava extract in quail diet increase the specific gravity in fresh eggs and increase the yolk color when the eggs are stored.

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