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Guava extract as an antioxidant additive in diets of Japanese breeder quails to mitigate the effect of egg storage time on newly hatched quality

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Abstract: An experiment was carried out to evaluate the effect of guava extract in the diets of quail breeders and storage time on the quality of newly hatched quails. Four hundred and thirty-two eggs were randomly distributed in a 4 × 2 factorial scheme, with the main factors being the dietary levels of guava extract (0.0, 3.0, 6.0, and 9.0 g kg^{-1}) and the storage time (three and nine days), totaling eight treatments and 54 replicates. The egg was considered the replicate. The eggs were produced by Japanese quail breeders at 28 weeks of age that had been fed the experimental diets. The quail breeders were randomly distributed according to body weight and laying percentage into 24 cages, with 12 females and four males per cage, totaling 288 female and 96 male Japanese quail breeders. The parameters of incubation, embryodiagnosis, intestinal development and quality of newly hatched quail were assessed. The eggs were incubated in a single-stage incubator at a temperature of 37.5 °C and 65 % humidity. The incubation period lasted 432 h. There was no significant interaction between the factors on incubation parameters. The incubation parameters, the embryodiagnosis, and the newly hatched quality were not affected by dietary guava extract. The quail produced by eggs stored for nine days presented lower body weight and body length at hatch. Dietary levels of 6.0 and 9.0 g kg^{-1} resulted in similar intestinal development of quail produced from eggs stored for nine or three days. We concluded that the use of 6.0 g kg^{-1} extract guava in the diets of quail breeders is sufficient to improve the intestinal development of newly hatched quail when eggs are stored for nine days.

Key words: Embryology, embryo metabolism, hatchery, incubation, breeder nutrition

1. Introduction

The lipids present in the egg yolk are used by the embryo during the incubation period in its development. Egg lipids are susceptible to oxidation due to the high concentration of polyunsaturated fatty acids. Qingling et al. [1] observed the decrease of phospholipid during egg storage and concluded that the lipid degradation is a result of both hydrolysis and oxidation.

Oxygen consumption and the production of free radicals in many tissues are high in the final stage of embryonic development, making embryos susceptible to oxidative damage [2]. Embryonic development causes the greatest demand for antioxidant protection in an animal's life [3].

Antioxidants are substances that act to reduce or block oxidation reactions induced by free radicals [4]. Reactive oxygen species (ROS) are naturally formed in the metabolic processes of enzymatic or non-enzymatic reactions. They are responsible for inducing oxidative damage in biomolecules, such as carbohydrates, proteins, lipids, and DNA [5].

Surai [6] verified that canthaxanthin is transferred from the egg yolk in embryonic development. Oliveira et al. [7] observed that the guava extract was able to decrease the lipid oxidation of the yolk in commercial eggs stored for up to nine days. Therefore, the use of antioxidant additives in the diet of the breeder may improve the quality of the progeny at hatch and consequently result in better performance of the bird in the production phase.

Guava is rich in vitamins, fibre, minerals, and antioxidants [8]. Guava fruit contains ellagic acid, which has an antioxidant effect that is effective in inhibiting lipid peroxidation [9]. The ellagic acid was found in guava fruit to neutralize reactive oxygen species (ROS) such as hydroxyl radicals, peroxyl radicals, nitrogen dioxide radicals and peroxynitrite at rate constants that are comparable with those of many well-known antioxidants such as vitamin E and vitamin C [10].

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The objective was to evaluate the effect of storage time and the use of guava extract in quail breeders' diets on incubation parameters, intestinal development, and the quality of newly hatched quails.

2. Material and methods

The experiment was conducted at the poultry facilities of the Department of Animal Science at the Federal University of Goiás, Goiânia, Goiás, Brazil (latitude: 16°35'33.0"S; longitude: 49°16'51.4"W; altitude: 730 m). All procedures in this study were conducted according to the protocol with registration number 052/15 and were approved by the Ethics Committee for Animal Use of the Federal University of Goiás.

Four hundred and thirty-two eggs were randomly distributed in a 4 × 2 factorial scheme, with the main factors being the dietary levels of guava extract (0.0, 3.0, 6.0, and 9.0 g kg^{-1}) and the storage time (three and nine days), totaling eight treatments, and 54 replicates. The eggs were produced by Japanese quail breeders at 28 weeks of age and fed with the experimental diets. The quail breeders were randomly distributed according to body weight and laying percentage into 24 cages, with 12 females and four males per cage, totaling 288 female and 96 male Japanese quail breeders. Eggs produced by the breeders were collected and distributed in an entirely randomized design, with eight treatments and 54 replicates.

The diets provided were iso-nutritive and iso-energetic based on corn and soybean meal, according to Rostagno et al. [11] (Table 1). To reach the proposed levels of guava extract, the experimental diets were obtained by supplementing this extract in a basal diet, replacing the

Table 1. Composition and nutritional values of experimental diets.

	Treatments (Guava extract g kg^{-1})			
Ingredients	0.0	3.0	6.0	9.0
Corn grain	546.81	546.81	546.81	546.81
Soybean meal	326.21	326.21	326.21	326.21
Guava extract	0.00	3.00	6.00	9.00
Starch	9.00	6.00	3.00	0.00
Soy oil	14.70	14.70	14.70	14.70
Limestone	57.80	57.80	57.80	57.80
DL-Methionine	3.52	3.52	3.52	3.52
L-Lysine HCL	1.89	1.89	1.89	1.89
L-Tryptophan	0.06	0.06	0.06	0.06
Premix[1]	40.00	40.00	40.00	40.00
TOTAL	1000.0	1000.0	1000.0	1000.0
Calculated composition (g kg^{-1})				
Metabolizable energy (Kcal/kg^{-1})	2.800	2.800	2.800	2.800
Sodium	1.88	1.88	1.88	1.88
Calcium	30.99	30.99	30.99	30.99
Available phosphorus	4.06	4.06	4.06	4.06
Crude protein	199.40	199.40	199.40	199.40
Lysine	10.83	10.83	10.83	10.83
Methionine + Cystine	8.88	8.88	8.88	8.88
Threonine	6.62	6.62	6.62	6.62
Tryptophan	2.27	2.27	2.27	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; Fe 1.125 mg; Mn 2.500 mg; iodo 22.5 mg; Zn 750 mg; Se 12.5 mg; zinc bacitracin 550 mg; methionine 28.7 g.

starch. Birds received feed and water ad libitum. Birds were fed for 12 weeks with experimental diets.

To obtain the guava extract, the residue from the industrial processing of guava fruits was used, supplied by Predilecta Alimentos LTDA (Matão - SP, Bazil), which was stored in a cold chamber at –20 °C. Subsequently, it was defrosted over 48 h, dehydrated in a forced air circulation oven at 40 °C until a constant mass was obtained, and ground in a knife and hammer mill. The powder obtained was stored free of light and moisture. The hydroalcoholic extract was obtained through the percolation of the plant material contained in the powder, using 50 % alcohol (v/v) as a solvent. In a percolator containing filter paper and cotton, 2 kg of plant material and 10 L of solvent were added, and the material was subjected to maceration for 24 h.

After percolation started, there was intense dripping until the solvent was depleted. Care was taken to avoid leaving the powder without solvent, to prevent it from drying out. Afterwards, the liquid obtained from the percolated powder was retrieved in ten rounds, based on the method adapted from the Formulário de Fitoterápicos da Farmacopeia Brasileira [12]. After percolation, the liquid extract was concentrated through solvent evaporation using a fan at room temperature until a solid content of 3.7% was obtained. The processing of the concentrated extract was performed at the Laboratory of Research, Development and Innovation of Bioproducts at the Faculty of Pharmacy at the Federal University of Goiás. The composition is presented in Table 2. The ellagic acid was considered the antioxidant component of guava extract.

At end of the 12-week period during which the birds were fed with experimental diets, the eggs were collected. The egg collection for incubation occurred over two days. On the first day, 216 eggs were selected (54 eggs from each group of breeders fed with the four levels of guava extract), collected, and stored at room temperature (with a minimum and maximum of 24.3 and 31 °C, respectively) and, six days later, the same egg collection procedure was performed; another 216 eggs were collected and stored together with the eggs from the first collection. The eggs were selected, and the uneven, dirty, or cracked eggs were discarded. After completing nine days of storage of the first eggs and, consequently, three days of storage of the others, the 432 eggs were weighed, identified, and incubated.

The eggs were incubated in a single-stage incubator. The incubator was monitored and regulated to maintain an average temperature of 37.5 °C and 65 % relative humidity.

The eggs were randomly distributed in an incubator. The incubation period lasted 432 h. At the end of the first 360 h, the eggs were placed individually in air-permeable fabric bags for treatment identification, and transferred to the birth trays. The incubator was set to hatcher mode. The hatch window lasted 40 h.

Table 2. Guava extract composition.

Parameter	(g kg^{-}1)
Humidity	963.0
Crude protein	26.0
Ethereal extract	30.0
Ellagic acid	21.3

Newly hatched quails were evaluated for body weight, body length and quality score and immediately euthanized. The weights of the yolk sac residue of the total intestine with pancreas were measured, and a segment of the duodenum was collected for histological analysis. The variables studied were egg weight, the weight, and length of the newly hatched quail, quality score, intestine weight, the weight of the yolk sac residue, the percentage of quail in the egg, the percentage of the intestine in the quail, the percentage of sac residue yolk in the quail, the net weight of the quail, hatchability, the hatching window, embryodiagnosis, and the histomorphometry of the duodenum. All quails hatched in the hatch window were weighed, received a quality score, and their length was measured using a ruler graduated in centimeters and millimeters.

For intestinal development, the duodenum was collected, washed in a saline solution, and fixed in 10% buffered formaldehyde for 24 h. Then, the tissues were washed in running and distilled water and dehydrated in increasing concentrations of alcohol (70%–95%). The sections were fixed on histological slides and stained by the HE technique [13]. The slides were made in the Department of Pathology of the Federal University of Goiás. Optical microscopy examination for histological evaluation was performed in the Department of Animal Pathology of the EVZ/UFG with a clear field optical microscope (Carl Zeiss Jevenal model, Carl Zeiss Microscopy GmbH, Oberkochen, Germany) coupled to an Axio Vision 3.0 (Oberkochen, Germany). The captured images were later investigated by the Software Image J. The villi/crypt relationship was calculated by dividing the height of the villi by the depth of the crypts.

To perform the embryodiagnosis, the eggs were opened and evaluated for the presence of an embryo. If absent, fertility was evaluated; if present, evaluation was made of the loss of moisture and the position of the embryo in the egg to record cases of inversion; thus, the embryo was classified with a numerical evaluation from one to four, which indicated that at which stage of development of the embryo was interrupted. Phase one was used to register

embryos that had only formed the cardiac and blood system; phase two was used to record embryos that were already visible but small and with the yolk sac still outside the abdominal cavity; phase three indicated embryos that had about half of the yolk sac outside the abdominal cavity, and phase four was used to register embryos that had absorbed almost 100% of the yolk sac [14].

The data were evaluated using analysis of variance and the Tukey test. Data that originated from the embryo diagnosis and the score percentage were evaluated using the Fisher's exact test. It was adopted that $\alpha = 0.05$. The R Development software package was used in all analyses.

The statistical model used was:

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

where:

y_{ijk}: an observation in level i of factor a (i=1,2,3,4), level j of factor b (j=1,2) in repetition k (k=1,2,…,54);

m: the overall mean;

a_i: the fixed effect of level i of factor a (i=1,2,3,4);

b_j: the fixed effect of level j of factor b (j=1,2);

$(ab)_{ij}$: 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);

e_{ijk}: the random error with mean 0 and variance σ^2.

3. Results

No statistical difference was observed ($p > 0.05$) among the treatments for the hatching and hatchability percentage (Table 3).

The use of the guava extract did not affect ($p > 0.05$) embryo mortality, live pecking, dead pecking, and inverted eggs for the storage period of three and nine days. However, the percentage of infertile eggs was lower ($p < 0.05$) in groups that originated from quail that received 3.0, 6.0, and 9.0 g kg^{-1} of guava extract in their diets, and stored for nine days. The higher level of inclusion of the extract provided a lower infertility rate (Table 4).

The results of the incubation parameters and the quality of newly hatched quail are shown in Table 5. Differences ($p < 0.05$) were observed between the levels of inclusion of the guava extract and storage time, for egg weight, length, and the weight of newly hatched quail. The levels of 3.0 and 9.0 g kg^{-1} provided eggs and newly hatched quail with weight similar to the control treatment and the level of 6.0 g kg^{c1}. The levels of 6.0 and 9.0 g kg^{-1} originated newly hatched quails of length similar to the control treatment and the level of 3.0 g kg^{-1}. Eggs stored for three days provided the best values from egg weight, length, and the weight of newly hatched quail.

No statistical difference was observed ($p > 0.05$) among the treatments for the percentage of quail chick in the egg, the net weight of the quail chick, and the yolk sac and small intestine of the quail chick (Table 6).

Table 3. Hatching and hatchability of eggs from Japanese quail breeders on feeding diets containing different levels of guava extract and stored for different periods.

Factor	Hatching (%)	Hatchability (%)
Dietary guava extract (g.kg^{-1}) -GE		
0.0	75.21	94.12
3.0	70.40	89.10
6.0	72.32	91.66
9.0	73.90	92.27
Storage time (days) -ST		
Nine	74.28	92.44
Three	71.63	91.12
ANOVA (p value)		
GE	0.819	0.844
ST	0.486	0.743
GE × ST	0.810	0.883
CV (%)	14.48	12.24
SEM	±3.74	±3.97

CV = coefficient of variation; SEM = standard error.

No statistical difference was observed ($p > 0.05$) in the quality score of newly hatched quails that resulted from the level at which guava extract was included in the diet of the breeders and storage time (Table 7).

Differences were observed between the levels of inclusion of guava extract for villus height and crypt depth of newly hatched quail ($p < 0.05$) (Table 8). The level of 9.0 g kg^{-1} originated newly hatched quails of crypt depth similar to the control treatment but with values higher than those of the other treatments. No differences ($p > 0.05$) were observed between the storage time.

There was an interaction between the factors for villus height and crypt depth ($p < 0.05$) (Table 9). For villus height, the results were similar, except for the level of 3.0 g kg^{-1} of the eggs stored for nine days, which presented the worst values. No differences between extract levels for eggs stored for three days; however, the inclusion of 9.0 g kg^{-1} of guava extract resulted in newly hatched quail with greater depth of crypt from eggs stored for nine days.

4. Discussion

The storage time of the eggs did not affect the hatching and the hatchability percentage, and the levels of guava extract were not able to improve them. According to Xavier et al. [10], the inclusion of an antioxidant component in the breeder's diet could improve the egg hatching rate by minimizing the quality loss of egg components owing

Table 4. Embryodiagnosis of eggs non-hatched from Japanese quail breeders feeding diets containing different levels of guava extract and stored for different time.

Diagnosis	Dietary Guava extraxt (g kg^{-1})				**p value**
	0.0	3.0	6.0	9.0	
	Nine days				
Infertile (%)	4.62 a	1.62 b	1.62 b	0.69 c	0.023
Phase 1 (%)	2.77	0.92	0.69	1.62	0.172
Phase 2 (%)	0.00	0.46	0.92	0.46	0.654
Phase 3 (%)	0.00	0.92	0.46	0.69	0.776
Phase 4 (%)	0.69	0.23	0.92	0.69	0.823
Live pecking (%)	0.00	0.00	1.38	0.00	0.983
Dead pecking (%)	0.00	0.00	0.00	0.00	1.000
Inverted (%)	0.00	0.69	0.23	0.23	0.712
	Three days				
Infertile (%)	2.31	2.31	2.31	2.41	1.000
Phase 1 (%)	1.38	0.92	0.69	0.92	0.652
Phase 2 (%)	0.00	0.23	0.46	0.00	0.772
Phase 3 (%)	0.00	0.00	0.23	0.00	0.776
Phase 4 (%)	0.00	0.46	0.46	0.46	0.912
Live pecking (%)	0.00	0.00	0.23	0.00	0.991
Dead pecking (%)	0.00	0.46	0.23	0.23	0.933
Inverted (%)	0.00	0.00	0.00	0.23	0.881

Means within the same line with different letter are significantly different by Fisher's exact ($p < 0.05$).

to oxidation during embryonic development. Similarly, Herve et al. [15] claim that phytochemicals essentially made up of the flavonoid, phenolic acid (ellagic acid), reduce oxidative stress and histological alteration in vital organs, and subsequently improve fertility.

Oyebanji and Atoki [16] verified that the inclusion of traditional medicinal plants and vitamin E in the diets of Japanese quail breeders increases the incubation parameters. However, these findings agree with the results of Hajiaghapour and Rezaeipour [17], which verified that the inclusion in diets of herbal essential oils did not affect hatchability in Japanese quail breeders. Likewise, given the egg storage time, Roriz et al. [18] concluded that the storage eggs fertility of Japanese quail breeders did not affect hatching percentage.

The infertility rate was lower in eggs from the Japanese quail breeders that received the highest level of guava extract in the diets. The sperm cell membrane is particularly rich in polyunsaturated fatty acid, which predisposes them to lipid peroxidation by reactive oxygen species (ROS), which is associated with male infertility [19]. The semen characteristics of quail, especially those related to lipids, can be affected by nutrition [10]. Khoobbakht et al. [20] concluded that supplementing diets with organic zinc (zinc-methionine) improves male Japanese quail reproductive performance by improving the semen characteristics. Similarly, Herve et al. [15], verified that supplementation of dietary ginger (*Zingiber officinale*) essential oil improves the semen characteristics in male Japanese quail because the antioxidant action of ginger essential oil subsequently reduced the lipid peroxidation responsible for apoptosis in spermatogenic cells, increasing the fertility rate. The present study suggests that the antioxidant substances in the guava extract were able to improve sperm mobility by protecting sperm against free radicals, consequently increasing the rate of fertility.

Likewise, given the egg storage time, Premavalli et al. [21] concluded that as the length of storage of the Japanese quail eggs increased, the mean percent fertility total was decreased and total embryonic mortalities were increased. In this study, the storage time did not affect the quality parameters of fertile eggs, possibly due to the temperature

Table 5. Egg weight (EW), quail chick weight (CW), quail chick length (CL), weight of yolk sac (WYS), net weight of quail chick (WQC), small intestine weight of quail chick (SIW) from Japanese quails breeders on feeding diets containing different levels of guava extract and stored for different periods.

Factor	EW (g)	CW (g)	CL (cm)	WYS (g)	WQC (g)	SIW (g)
Dietary guava extract (g.kg^{-1}) -GE						
0.0	12.08 a	8.16 a	12.07 a	0.66	7.71	0.37
3.0	11.99 ab	8.13 ab	11.71 b	0.63	7.53	0.32
6.0	11.71 b	7.87 b	11.99 ab	0.58	7.28	0.34
9.0	11.94 ab	8.10 ab	11.94 ab	0.63	7.18	0.35
Storage time (days) -ST						
Nine	11.80 b	7.91 b	11.80 b	0.67	7.46	0.34
Three	12.06 a	8.21 a	12.05 a	0.56	7.39	0.35
ANOVA (p value)						
GE	0.038	0.085	0.038	0.948	0.856	0.252
ST	0.007	<0.001	0.007	0.097	0.116	0.638
GE × ST	0.554	0.699	0.554	0.500	0.327	0.314
CV (%)	6.90	9.38	16.90	42.73	7.71	21.30
SEM	±0.09	±0.09	±0.12	±0.07	±0.16	±0.02

Means within the same column with different letter are significantly different by Tukey test ($p < 0.05$); CV = coefficient of variation; SEM = standard error.

Table 6. Percentage of quail chick on egg (QC), net weight of quail chick (QW), yolk sac (YS), and small intestine of quail chick (SI) from Japanese quails breeders on feeding diets containing different levels of guava extract and stored for different periods.

Factor	QC (%)	QW (%)	YS (%)	SI (%)
Dietary guava extract (g.kg^{-1}) -GE				
0.0	67.76	62.39	7.88	4.40
3.0	67.52	62.45	7.48	3.94
6.0	67.91	62.90	7.38	4.33
9.0	67.43	62.06	8.04	4.42
Storage time (days) -ST				
Nine	67.77	62.25	9.15	4.15
Three	67.64	62.84	7.07	4.44
ANOVA (p value)				
GE	0.926	0.908	0.934	0.529
ST	0.961	0.360	0.108	0.150
GE × ST	0.441	0.411	0.425	0.400
CV (%)	4.40	5.41	39.34	19.61
SEM	±0.83	±0.94	±0.84	±0.23

CV = coefficient of variation; SEM = standard error.

Table 7. Quality score (0 to 100 points*) of newly hatched quail chicks from Japanese quails breeders on feeding diets containing different levels of guava extract and stored for different periods

Score (%)	Dietary Guava extract (g.kg^{-1})				p value
	0.0	3.0	6.0	9.0	
	Nine days				
<70	0.00	0.00	0.00	0.00	0.748
71–75	0.00	2.94	0.00	0.00	0.625
76–80	2.44	0.00	0.00	0.00	0.807
81–85	4.88	0.00	2.50	0.00	0.854
86–90	12.20	2.94	5.00	5.41	0.678
91–95	14.63	17.65	22.50	16.22	0.809
96–100	65.85	76.47	70.00	78.38	0.965
	Three days				
<70	0.00	0.00	0.00	0.00	1.000
71–75	0.00	0.00	0.00	0.00	1.000
76–80	2.56	2.56	0.00	0.00	1.000
81–85	0.00	2.56	0.00	0.00	1.000
86–90	2.56	10.26	10.53	10.81	0.637
91–95	17.95	20.51	15.79	8.11	0.514
96–100	76.92	64.10	73.68	81.08	0.427

*Score adapted of Tona et al. (2003).

(minimum and maximum of 24.3 and 31 °C, respectively) to which they were submitted to enable them to maintain their physical-chemical properties.

Oliveira et al. [7] concluded that guava extract does not affect the quantitative increase of yolk and albumen content, and consequently have no effect on the increase of egg weight. Similarly, Hajiaghapour and Rezaeipour [17] verified that the inclusion of herbal essential oils in the diets of Japanese quail breeders did not affect the egg characteristics, including the weight of the shell, yolk, and albumen. The yolk is a source of nutrients that are necessary for the embryo to grow and hatch, and it contains proteins, carbohydrates, fats, vitamins, and minerals [22].

Xavier et al. [10], using the same extract but in the feeding of Japanese quail breeders, concluded that guava residue extract increased the weight of quail chicks but did not affect the quail chick length. According to Qingling [1], the weights of eggs decreased significantly during storage, which was mainly caused by the loss of water through the porous shell.

The characteristics of one-day-old chicks are related to their yolk-free body mass and serve as an indication of embryonic growth through the transformation of yolk sac nutrients into body mass [23]. As the relation between the weight of the newborn chick and the weight of the egg diminishes, less yolk will be used by the embryo, impairing protein synthesis. The use of guava extract was not effective in reducing egg weight loss and characteristics in newly hatched quail.

The quality of neonate quails influences their growth performance. Many factors can affect the quality of newly hatched quails as breeders age [24], including egg quality characteristics [25] and the dietary levels of energy and protein of the breeder [26]. Araújo et al. [27] verified that the physical quality score in chicks originating from supplemented eggs with vitamin E were higher than that of those without supplementation. The levels of guava extract and the storage times of the eggs did not affect the quality score of newly hatched quail chicks, maybe because the incubated eggs came from Japanese quail breeders of the same age, possibly leading to non-significant differences in the quality score.

The guava extract was able to maintain the intestinal development of newly hatched quails from eggs stored for nine days compared to quails hatched from eggs stored for three days. Possibly, the antioxidant component (ellagic acid) present in the guava extract was able to preserve

Table 8. Embryonic development on intestinal mucosal morphometry of the duodenum of newly hatched quail chicks from Japanese quail breeders on feeding diets containing different levels of guava extract and stored for different periods.

Factor	Villus (µm)	Crypt (µm)	Villus:Crypt (µm)
Dietary Guava extract (g.kg^{-1})			
0.0	239.06 a	207.87 a	1.16
3.0	202.22 b	194.56 b	1.21
6.0	233.71 a	170.52 c	1.21
9.0	244.87 a	218.55 a	1.13
Storage time (days) -ST			
Nine	229.51	196.78	1.18
Three	230.42	198.97	1.17
ANOVA (p value)			
GE	<0.001	<0.001	0.143
ST	0.873	0.653	0.706
GE x ST	<0.001	<0.001	0.122
CV (%)	16.08	16.06	16.00

Means within the same column with different letter are significantly different by Tukey test ($p < 0.05$); CV = coefficient of variation.

Table 9. Unfolding interactions of inclusion levels of guava extract × storage time of embryonic development on intestinal mucosal morphometry of the duodenum of newly hatched quail chicks.

Factor	Storage time (days)	
	Three	Nine
Dietary Guava extract (g.kg^{-1})	Villus (µm)	
0.0	235.47 Aa	242.65 Aa
3.0	222.35 Aa	182.08 Bb
6.0	227.37 Aa	240.05 Aa
9.0	236.48 Aa	253.27 Aa
	Crypt (µm)	
0.0	200.26 Aa	215.49 Ab
3.0	196.88 Aa	144.15 Bc
6.0	190.31 Aa	198.81 Ab
9.0	208.45 Aa	228.65 Aa

Means within by different lowercase letters in the line and uppercase letters in the column are statistically different by Tukey test ($p < 0.05$); CV = coefficient of variation.

the integrity of intestinal development and improve the nutrient utilisation by the quail. Similarly, Mello et al. [28] verified that the use of 0.3% inclusion of guava extract, contained ellagic acid in layer quail diets, enhances the intestinal development of the neonate quail. Hajiaghapour and Rezaeipour [17] concluded that the inclusion of herbal essential oils in the diets of Japanese quail breeders improves the height of villi in the jejunum and ileum, in addition to the crypt depth in the ileum. Likewise, Surai et al. [29] observed that antioxidants in the egg yolk can control oxidation by reducing or disabling free radicals before acting on the chick's organ tissues.

We concluded that the use of 6.0 g kg^{-1} of extract guava in the diets of quail breeders improves the intestinal development of newly hatched quails when eggs are stored for nine days.

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