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REGULAR ARTICLES

Calcium anacardate and its association with citric acid in diets for meat-type breeding quails

Ednardo Rodrigues Freitas[1] · Cleane Pinho da Silva[1] · Thalles Ribeiro Gomes[2] · Rafael Carlos Nepomuceno[1] · Edibergue Oliveira dos Santos[1] · Valquíria Sousa Silva[1] · Luana Ledz Costa Vasconcelos Rocha[1] · Maria Teresa Salles Trevisan[3]

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Abstract

The purpose of this study was to evaluate the effects of using calcium anacardate (CaA) as a source of anacardic acid and its association with citric acid (CA) in diets for breeding quails on the performance, the egg quality, incubation parameters, and progeny performance. Were used 540 quails European quails (*Coturnix coturnix coturnix*) that were 21 weeks old, housed in laying cages based on a completely randomized design, with nine treatments and six replications of 10 quails per parcel, with each experimental unit having eight females and two males. The following additions to the diet were evaluated: 1, no addition (control diet); 2, 0.25% CaA; 3, 0.25% CaA and 0.25% CA; 4, 0.50% CaA; 5, 0.50% CaA and 0.25% CA; 6, 0.50% CaA and 0.50% CA; 7, 0.75% CaA; 8, 0.75% CaA and 0.25% CA; and 9, 0.75% CaA and 0.50% CA. The treatments had no significant effects on the performance of the breeding quails, incubation parameters, and progeny performance. For egg quality, there was only an effect on yolk lipid oxidation, which was lower for eggs from quails fed the diets containing 0.50% CaA and 0.25% CA, 0.50% CaA and 0.50% CA, or 0.75% CaA alone, when compared with the control group. Considering that including CaA with or without CA in diets for breeding quails only affected yolk lipid oxidation, it can be recommend including 0.50% CaA and 0.25% CA or 0.75% CaA alone to mitigate oxidative damage in the yolk of fertile eggs.

Keywords Cashew nut liquid · Feed additive · Lipid oxidation · Organic acid · Phenolic compounds

Introduction

Aspects such as use restriction or total exclusion of growth-promoting antibiotics and synthetic antioxidants in poultry feeding have driven the search for alternative substances. This endeavor has led to studies investigating phytogenic additives that, in addition to their antimicrobial action (Mitsch et al. 2004) that affects animal performance, can also present antioxidant activity (Ciftci et al. 2010; Zhang et al. 2013), benefiting the birds and the quality of their products. Anacardic acid is a

✉ Thalles Ribeiro Gomes
thalles.gomes@ufrr.br

Ednardo Rodrigues Freitas
ednardo@ufc.br

Cleane Pinho da Silva
cleanepinho@gmail.com

Rafael Carlos Nepomuceno
rafael.zootecnista@ufc.br

Edibergue Oliveira dos Santos
edibergueoliveira@gmail.com

Valquíria Sousa Silva
walzoouft@gmail.com

Luana Ledz Costa Vasconcelos Rocha
ledzluana3@gmail.com

Maria Teresa Salles Trevisan
trevisan@ufc.br

[1] Department of Animal Science, Federal University of Ceará, Mister Hull Ave, 2.977, Block 808, Campus do Pici, Fortaleza, CE 60356-000, Brazil

[2] Department of Animal Science, Federal University of Roraima, BR 174, Km 12, Campus Cauamé, Boa Vista, RR 69301-970, Brazil

[3] Department of Organic and Inorganic Chemistry, Federal University of Ceará, Mister Hull Ave, 2.977, Block 940, Campus do Pici, Fortaleza, CE 60356-000, Brazil

phenolic compound naturally found in different parts of the cashew tree (*Anacardium occidentale* L.) but in a greater proportion in the cashew nut shell liquid (CNSL). It exhibits antitumor, antibacterial, antifungal, and antioxidant activities, as well as the ability to inhibit the activity of enzymes such as tyrosinase, prostaglandin synthase, and lipoxygenase (Toyomizu et al. 2003; Trevisan et al. 2006).

Anacardic acid can be added to poultry feed by including CNSL, where it is found mixed with other compounds (cardol and cardanol). The addition of up to 1% CNSL in in the feed of laying hens did not influence blood biochemical parameters; the activity of enzymes related to oxidative stress in the liver, ovary, magnum, and uterus (Braz et al. 2018); or the performance of the birds (Braz et al. 2019). There was no impairment in the evaluated egg quality variables; however, there was improvement in yolk color and yolk lipid stability (Braz et al. 2019) and ovary (Braz et al. 2018) with the addition of 0.75% CNSL. This level of addition was also effective in reducing lipid oxidation in fresh and stored yolks (Abreu et al. 2017).

However, the variations in the proportion of anacardic acid in relation to the other compounds of CNSL due to several factors (Trevisan et al. 2006), as well as the synergistic or antagonistic action between compounds, can cause problems to obtain the expected results. These problems can be minimized if the addition is made in the form of calcium anacardate, which is the product of the reaction between anacardic acid within CNSL and calcium hydroxide. This reaction forms calcium salts that allow separating anacardic acid from the other compounds. By using this approach, researchers can study the isolated effects of anacardic acid.

In broiler and laying bird research, inclusion of 0.75% calcium anacardate in the diet reduced lipid oxidation in fresh meat (Freitas et al. 2022) as well as frozen and processed meat, while the addition of 1% calcium anacardate had a pro-oxidant effect (Abreu et al. 2019). This addition did not compromise the performance of birds, carcass parameters, and egg quality (Freitas et al. 2022; Santos et al. 2022). Moreover, it caused no toxic effects (Cruz et al. 2018).

The efficiency by which an organic acid promotes growth in birds depends on the chemical properties of the acid or salt. The dissociative capacity is the main characteristic involved in the magnitude of this response (Dibner and Buttin 2002). The buffering capacity of the diet is also important because diets high in minerals, such as diets for laying hens, with high calcium concentrations, promote greater resistance to gastric pH reduction (Jung and Bolduan 1986). In the laboratory, the dissociation of calcium anacardate into anacardic acid and calcium ions occurs by hydrolysis in 11 M hydrochloric acid (Paramashivappa et al. 2001) with a pH equivalent of 1.04. Cruz et al. (2019) related the absence of a significant effect of the addition of calcium anacardate on the bone parameters of broilers to impairment of its hydrolysis and, consequently, the release of anacardic acid, at the normal pH of the digestive tract of the birds.

In this context, one can hypothesize that it is possible to add calcium anacardate along with an organic acid to poultry diets. This approach would favor the dissociation of calcium anacardate during the digestive process and thus release free anacardic acid. Once free, anacardic acid could express its growth-promoting and antioxidant effects in the birds.

Citric acid is a good option for the organic acid because it is the most common organic acid used in poultry diets due to its growth-promoting action. It acidifies the gastrointestinal content, preventing the development and growth of pathogenic microorganisms; improves the solubility of the feed ingredients, digestion, and absorption of nutrients; and exerts antioxidant action (Centeno et al. 2007; Deepa et al. 2011; Islam 2012; Nourmohammadi and Khosravinia 2015; Fikry et al. 2021).

The overall antioxidant capacity in the egg can help in optimal embryo development (Deeming and Pike 2013). Thus, the development of the bird embryo depends on antioxidants accumulated in the egg yolk via the diet of the female. This practice also provides oxidative protection of the newly hatched chick, with possibilities to improve post-hatch performance. Considering the antioxidant activity of phenolic compounds and the possibility of their benefits for breeding birds, this research aimed to evaluate the use of calcium anacardate associated with citric acid in the diet of meat-type breeding quails.

Material and methods

Experimental design, diets, and quail management

This experiment used a total of 540 quails (*Coturnix coturnix coturnix*) housed in a conventional shed for rearing laying quails, equipped with galvanized wire cages (35 cm long × 25 cm wide × 20 cm high) arranged in a pyramidal system and equipped with a linear trough feeder, a nipple drinker, and an egg tray. At 21 weeks of age, the quails were selected based on body weight and egg production and were uniformly distributed in cages, so that all replications were composed of quails of similar weight and egg production, according to the recommendations of Sakomura and Rostagno (2007).

The quails were distributed in cages based on a completely randomized design comprising nine treatments and six replications of 10 quails per parcel, with the experimental unit composed of eight females and two males. We evaluated the following additions to the diet: 1, no addition of acids (control diet); 2, 0.25% calcium anacardate; 3, 0.25% calcium anacardate and 0.25% citric acid; 4, 0.50% calcium anacardate; 5, 0.50% calcium anacardate associated with 0.25% citric acid; 6, 0.50% calcium anacardate associated with 0.50% citric acid; 7, 0.75% calcium anacardate; 8, 0.75% calcium anacardate associated with 0.25% citric acid; and 9, 0.75% calcium anacardate associated with 0.50% citric acid.

To obtain the experimental diets, the control diet was formulated (Table 1) considering the nutritional requirements presented by Silva and Costa (2009) and the values of nutritional and energy composition of the ingredients proposed by Rostagno et al. (2017), corrected by the dry matter content determined in the laboratory according to method 934.01 described by AOAC International (2005), and using in its composition the inclusion of 1.25% of an inert ingredient (washed sand). The other diets were obtained by replacing the inert ingredient with calcium anacardate and citric acid in the appropriate proportion of each treatment. Rations were prepared by grinding, weighing, and mixing processes, and the diets were obtained in the mash form.

The experimental period lasted 210 days, divided into 10 periods of 21 days each, when diets and water were offered at will. The light program provided 16 h of light per day, including 12 h of natural light and 4 h of artificial light.

Table 1 Composition and calculated nutritional levels of the control group diet for breeding quails

Ingredients	%
Corn, grain	49.15
Soybean meal, 45% CP	37.20
Soybean oil	3.24
Calcitic limestone, 37.7% Ca	7.15
Dicalcium phosphate, 25% Ca, 19% P	0.99
Common salt	0.54
DL-methionine, 99%	0.14
L-lysine HCl, 78%	0.08
L-threonine, 98.5%	0.11
Vitamin supplement[1]	0.10
Mineral supplement[2]	0.05
Washed sand (inert)	1.25
Nutritional and energetic calculated composition	
Metabolizable energy (kcal/kg)	2800
Dry matter	90.56
Crude protein (%)	21.00
Digestible lysine (%)	1.100
Digestible methionine + cystine (%)	0.700
Digestible methionine (%)	0.418
Digestible cystine (%)	0.282
Digestible threonine (%)	0.810
Calcium (%)	3.000
Available phosphorus (%)	0.300
Sodium (%)	0.230

[1]Composition per kg of diet: vitamin A (min), 13,500 IU; vitamin B1 (min), 3 mg; vitamin B12 (min), 0.025 mg; vitamin B2 (min), 9 mg; vitamin B6 (min), 4.5 mg; vitamin D3 (min), 3750 IU; vitamin E (min), 40 IU; vitamin K3 (min), 3.75 mg; biotin (min), 0.15 mg; niacin (min), 50 mg; folic acid (min), 2.25 mg; pantothenic acid (min), 20 mg

[2]Composition per kg of diet: cobalt (min), 0.2 mg; copper (min), 10 mg; iron (min), 50 mg; iodine (min), 1.5 mg; manganese (min), 80 mg; selenium (min), 0.38 mg; zinc (min), 50 mg

Obtention of calcium anacardate

First, CNSL was extracted from cashew nut shells with 99.5% GL ethyl alcohol, according to the methodology presented by Trevisan et al. (2006). Then, anacardic acid was separated by precipitation in the form of calcium anacardate, according to a method adapted from Paramashivappa et al. (2001).

To obtain calcium anacardate from CNSL, 550 mL of CNSL, 150 mL of distilled water, and 2850 mL of ethanol were added to a 4-L beaker. This solution was heated under stirring until it reached a temperature of 50°C and was incubated for 4 h. Throughout the procedure, at 10-min intervals, 250 g of calcium hydroxide was added to the mixture. This procedure promotes the reaction of anacardic acids with calcium hydroxide to form calcium anacardate, which precipitates at the bottom of the container and is thus separated from the other compounds in the mixture. The solution was left to decant for 1 h at room temperature. Then the supernatant was removed, and 800 mL of ethanol was added; the mixture was subjected to the same conditions of stirring and heating for 1 h. Afterwards, it was left to decant for 1 h, and then the supernatant was removed. This procedure was carried out once more to obtain a more effective washing. The precipitated material, which was calcium anacardate, was left to dry in a forced ventilation oven at 55°C for 72 h and was then ground and sieved.

Determination and quantification of the anacardic acids present in the produced calcium anacardate were performed by high-performance liquid chromatography (HPLC) with an Agilent diode array detector (DAD) operating in the ultraviolet range with an analytical column C-18 Latek (5 µm, 250 × 4 mm) in a stream of mL/min with an injection volume of 10 µL. The DAD was set at wavelengths of 254, 278, 325, and 340 nm, where anacardic acids have higher absorption peaks in the ultraviolet–visible light range. For this analysis, a calcium anacardate sample was previously converted to anacardic acids according to the procedure described by Paramashivappa et al. (2001). The calcium anacardate sample comprised 94.5% of the triene (15:3), diene (15:2), and monoene (15:1) anacardic acids, with a relative proportion of 3:1.6:1.1.

Performance of the breeding quails

To evaluate the performance of the breeding quails, the following variables were determined: feed intake (g/quail/day), which was calculated as the difference between the amount of diet supplied at the beginning of the phase and the leftovers obtained at the end of the phase; laying percentage (%/quail/day), which was obtained based on the daily record

of egg production per cage, calculated at the end of each period, as well as the laying percentages per replication; egg mass (g/quail/day), which was calculated by multiplying the number of eggs produced by the average egg weight of each replication in the period; and the feed conversion ratio (kg of diet/kg of egg), which was calculated from the ratio of feed intake by the egg mass produced by each replication per period.

Egg quality and yolk lipid stability

The egg quality was evaluated based on specific gravity; Haugh units; percentages of albumen, yolk, and shell; yolk color; and shell thickness. To determine these parameters, all eggs from each plot were collected, identified, and taken to the egg quality assessment laboratory in the Poultry Sector of the Federal University of Ceará once a week throughout the experimental period. There, the eggs were individually weighed on a semi-analytical scale with a sensitivity of 0.01 g to determine the average egg weight. After weighing the eggs, three eggs per plot were selected and subjected, in sequence, to the determinations according to the steps described below.

Initially, the specific gravity (g/cm^3) of the eggs was determined according to procedures described by Freitas et al. (2004). Then, the albumen quality was assessed by determining the Haugh units. The eggs were broken on a flat glass surface, and the height (in millimeters) of the dense albumen was measured with a depth micrometer. The Haugh units were determined with the equation: $HU = 100 \times \log(H - 1.7 \times W^{0.37} + 7.6)$, where HU is Haugh units, H is the albumen height in millimeters, and W is the egg weight in grams (Haugh 1937).

Next, the yolk was separated from the albumen and weighed on a semi-analytical scale with a sensitivity of 0.01 g, and its percentage was calculated by dividing the yolk weight by the egg weight and then multiplying by 100. The yolk color was assessed by comparison through the Digital YolkFan™ app. After breaking the egg, the shell was washed and dried for 72 h and then weighed on a semi-analytical scale with a sensitivity of 0.01 g. The shell percentage was determined by dividing the shell weight by the egg weight, and then multiplying by 100. The percentage of albumen was calculated as % albumen = 100 – (% yolk + % shell).

To determine the shell thickness, measurements were taken in three regions of the eggs: the greater and lesser poles and the equatorial region, using a digital micrometer (Mitutoyo Company, Kawasaki, Japan) with 0.01-mm divisions. The average of the obtained values was calculated.

In the sixth experimental period, the yolk lipid stability was evaluated. Yolks were evaluated for lipid oxidation by determining the concentration of thiobarbituric acid reactive substances (TBARS) through the aqueous acid extraction method (Cherian et al. 1996). Three eggs with intact shells were selected from each plot, all of which were identified, placed in cardboard trays, and stored at an average temperature of 18 ± 2.55°C and relative humidity of 79.75% ± 9.11% until the moment of analysis (7 days). The trial was carried out at the Laboratory of Natural Products of the Chemistry Department of the Federal University of Ceará.

In a 15-mL tube, approximately 2 g of in natural yolk (without skin) was weighed. Then, 6.75 mL of perchloric acid (3.86%) and 18.75 µL of BHT (4.5%) were added, and the content was homogenized in a vortex for 30 s. Then, the tubes were centrifuged at 8500 rpm for 10 min at 4°C. The supernatant was filtered on filter paper (Whatman No. 1). Subsequently, 1 mL of the filtrate was placed in an Eppendorf tube, followed by the addition of 1 mL of liquid TBA solution (20 mM). The tubes were heated in a heater (Eppendorf ThermoMixer) for 30 min at 95°C without agitation. To reduce the temperature, the tubes were placed in a refrigerated centrifuge at 4°C. Then, the optical density was read at 531 nm in a spectrophotometer. The TBARS concentration was calculated through a standard malonaldehyde curve (MDA), and the results were expressed in µg of MDA per gram of yolk.

Incubation parameters

To evaluate the incubation parameters, four incubations were performed with eggs collected during the second, fourth, sixth, and eighth experimental periods. During the last 10 days of each period, all eggs from each plot were collected daily and sent to the hatchery, where they were subjected to selection for incubation, going through ovoscopy and discarding cracked, broken, and irregularly shaped eggs (rounded and elongated). The selected eggs were identified and stored in an environment with controlled temperature of 18°C.

The day after the last day of collection, all stored eggs were placed at room temperature for 8 h, weighed on a semi-analytical scale with a sensitivity of 0.01 g, placed in incubator trays previously identified according to the treatments, and taken to an incubator at 38°C, where they remained for 15 days. After this period, the eggs were placed in polyethylene nets and transferred to the hatcher at 36.8°C, where they remained until hatching (17 days). At the end of each incubation, the unhatched eggs were broken to determine the number of unfertilized eggs.

The data from all incubations were pooled, making it possible to evaluate the following parameters: (a) the average weight of incubatable eggs (g/egg), obtained from the arithmetic mean considering the total weight of the eggs from the plot and the number of incubated eggs; (b) the average chick weight at hatching (g/quail), obtained after hatching, when

all the chicks were weighed on a semi-analytical scale with a sensitivity of 0.01 g; (c) hatching (%), calculated as (number of chicks/number of incubated eggs) × 100; (d) hatchability (%), calculated as (number of chicks/number of fertile eggs) × 100; and (e) fertility (%), calculated as (number of fertile eggs/number of incubated eggs) × 100.

Progeny performance

To evaluate the progeny performance, after hatching and weighing, the chicks were housed according to the treatments, in 60 × 60 cm boxes, with a floor covered with sawdust and equipped with a pressure cup drinker, a tubular feeder, and a 100-watt light bulb. The progeny from the breeding quails of the different treatments was fed with the same type of feed (Table 2) formulated for the growth phase, considering the nutritional requirements presented by Silva and Costa (2009) and the values of nutritional and energy composition of the ingredients proposed by Rostagno et al. (2017), corrected by the dry matter content determined in the laboratory according to method 934.01 described by AOAC International (2005).

For the performance evaluation, quails and feed were weighed on the first day of life and at 21 days of age to obtain feed intake (g/quail), weight gain (g/quail), and feed conversion. Feed intake was calculated as the difference between the amount of feed supplied at the beginning of the experiment and the amount left over at the end of each period in each plot. Weight gain was calculated as the difference in the mean weight of the plot. Finally, the feed conversion ratio was obtained by dividing the feed intake by the weight gain of each plot. The variables were corrected for mortality.

Table 2 Composition and calculated nutritional levels of the diet for growth phase

Ingredients	%
Corn, grain	54.94
Soybean meal, 45% CP	40.84
Soybean oil	1.26
Calcitic limestone, 37.7% Ca	1.18
Dicalcium phosphate, 25% Ca, 19% P	0.94
Common salt	0.36
DL-methionine, 99%	0.28
Vitamin supplement[1]	0.15
Mineral supplement[2]	0.05
Nutritional and energetic calculated composition	
Metabolizable energy (kcal/kg)	2950
Dry matter	89.65
Crude protein (%)	23.00
Digestible lysine (%)	1.14
Digestible methionine + cystine (%)	0.89
Digestible methionine (%)	0.58
Digestible cystine (%)	0.31
Digestible threonine (%)	0.78
Calcium (%)	0.75
Available phosphorus (%)	0.29
Sodium (%)	0.16

[1]Composition per kg of diet: vitamin A (min), 13,500 IU; vitamin B1 (min), 3 mg; vitamin B12 (min), 0.025 mg; vitamin B2 (min), 9 mg; vitamin B6 (min), 4.5 mg; vitamin D3 (min), 3750 IU; vitamin E (min), 40 IU; vitamin K3 (min), 3.75 mg; biotin (min), 0.15 mg; niacin (min), 50 mg; folic acid (min), 2.25 mg; pantothenic acid (min), 20 mg

[2]Composition per kg of diet: cobalt (min), 0.2 mg; copper (min), 10 mg; iron (min), 50 mg; iodine (min), 1.5 mg; manganese (min), 80 mg; selenium (min), 0.38 mg; zinc (min), 50 mg

Statistical analysis

The Statistical Analyses System software (version 8.2; SAS Institute Inc., Cary, NC, USA) was for data analysis with analysis of variance followed by the post hoc Student–Newman–Keuls test. A P-value < 0.05 was considered to be statistically significant.

Results and discussion

Performance of the breeding quails

The treatments did not affect any of the quail performance variables, indicating that the levels of addition of calcium anacardate and its association with citric acid do not affect feed intake, egg production, egg weight, egg mass, and the feed conversion ratio of the quails (Table 3).

In birds destined for egg production, meeting nutrient requirements is related to daily feed intake and nutrient utilization by the birds. Thus, factors that negatively influence nutrient intake or nutrient utilization may lead to performance problems, because egg production depends primarily on meeting the energy requirements, while protein and amino acid requirements influence egg weight (Leeson and Summers 1997). Thus, considering that the diets were formulated to be iso-energetic, iso-proteinic, and iso-aminoacidic, and that there was no significant difference between treatments for feed intake, we can infer that the addition of calcium anacardate as well as its association with citric acid at the levels used had no influence on the energy and nutrient utilization of the feed. It met the requirements in terms of metabolizable energy, protein, and amino acids, ensuring that the percentage of laying, egg weight, and egg mass and the feed conversion ratio did not vary significantly between the treatments. These results agree with studies with calcium

Table 3 Performance of meat-type quail breeders fed diets containing calcium anacardate (CaA) associated or not associated with citric acid (CA)

Parameter	Feed intake (g/quail/day)	Egg production (%)	Egg weight (g/day)	Egg mass (g/day)	FCR[1]
Control diet	29.02	78.30	12.68	9.94	2.96
0.25% CaA	29.12	77.47	12.81	9.93	2.95
0.25% CaA + 0.25 CA	28.14	74.68	12.64	9.44	3.00
0.50% CaA	28.27	75.37	12.96	9.77	2.91
0.50% CaA + 0.25% CA	28.22	77.18	12.63	9.73	2.92
0.50% CaA + 0.50% CA	28.53	74.94	12.62	9.45	3.03
0.75% CaA	28.72	79.37	12.55	9.96	2.92
0.75% CaA + 0.25% CA	28.13	75.12	12.41	9.31	3.04
0.75% CaA + 0.50% CA	28.51	75.38	12.58	9.47	3.04
SEM[2]	0.188	0.949	0.046	0.121	0.036
P-value	0.9174	0.9548	0.2400	0.8984	0.9841

[1]Feed conversion ratio

[2]Standard error of the mean

anacardate in feeds for broilers (Freitas et al. 2022) and laying Japanese quails (Santos et al. 2022), in which inclusion at up to 1% resulted in no change in bird performance when compared with the control treatments.

The initial hypothesis was that the combined use of calcium anacardate with citric acid promotes digesta acidification in the gastrointestinal tract to the point of ensuring the dissociation of anacardic acids from calcium anacardate salts, thus enhancing the performance of the birds, which we did not observe. However, the absence of a health challenge may have contributed to these results. This factor has been reported to contribute to the variability of responses to the inclusion of organic acids (Viola et al. 2008), with reports of positive response (Gama et al. 2000; Soltan 2008; Youssef et al. 2013) and absence of response on performance and egg quality (Yesilbag and Çolpan 2006; Rahman et al. 2008; Świątkiewicz et al. 2010).

For healthy birds, changes in egg compounds and, consequently, egg quality are associated with the availability of nutrients for the formation of each compound. Thus, protein or amino acid deficiency can decrease the amount of albumen and, consequently, egg size (Costa et al. 2004). In turn, mineral deficiency, mainly calcium, can affect the proportion and quality of the shell (Świątkiewicz et al. 2010). Thus, the absence of a significant effect of the treatments on egg compounds and quality can be related to meeting the quail requirements, because the feeds were formulated to be isonutrient and there was no significant variation in feed intake.

Egg quality and yolk lipid stability

Regarding the egg quality (Table 4), there was no effect of the treatments on Haugh units; specific gravity; shell thickness; yolk color; and the percentages of yolk, albumen, and shell. Evaluation of yolk lipid oxidation showed that there were significant differences among treatments. Specifically, there was less oxidation for the yolks of eggs from quails fed the diets containing 0.50% calcium anacardate associated with 0.25% citric acid, 0.50% calcium anacardate associated with 0.50% citric acid, and 0.75% calcium anacardate alone, when compared with the control group and the group fed the diet with 0.25% calcium anacardate alone. Therefore, the association of 0.50% calcium anacardate with citric acid up to 0.50% or 0.75% calcium anacardate alone may benefit lipid stability. However, the association of 0.75% calcium anacardate with the different levels of citric acid promoted a pro-oxidative effect, increasing the TBARS values in the yolks.

The egg quality results are similar to those observed by Santos et al. (2022), who tested calcium anacardate in the feed provided for laying Japanese quails. However, some researchers have suggested that the addition of organic acids enhances some internal egg quality parameters (Soltan 2008; Özek et al. 2011) and mineral utilization by birds, improving eggshell quality (Soltan 2008; Świątkiewicz et al. 2010; Kaya et al. 2013). These effects are associated with the reduced pH of the digestive tract, increased dissociation of minerals, and the activity of digestive enzymes. Thus, the absence of significant effects on the egg compounds, internal quality, and shell quality indicate that these benefits did not occur with the addition of calcium anacardate associated or not associated with citric acid in the feed of the quails.

It is worth noting that there have been no reports of benefits of the addition of organic acids on the internal quality of eggs (Yesilbag and Çolpan 2006; Świątkiewicz et al. 2010; Kaya et al. 2013) or on the shell quality (Mahdavi et al. 2005).

Table 4 Quality of eggs from meat-type quail breeders fed diets containing calcium anacardate (CaA) associated or not associated with citric acid (CA)

Parameter	HU[1]	Specific gravity (g/cm^3)	Egg shell thickness (mm)	Yolk color	Albumen (%)	Yolk (%)	Shell (%)	TBARS (mg MDA/g yolk)
Control diet	82.16	1.0772	25.77	6.28	63.27	28.39	7.75	0.77a
0.25% CaA	82.12	1.0748	25.69	6.25	63.02	29.05	7.93	0.75a
0.25% CaA + 0.25 CA	81.27	1.0757	25.56	6.39	63.73	28.66	7.87	0.69ab
0.50% CaA	82.46	1.0763	25.48	6.40	63.66	28.58	7.88	0.68ab
0.50% CaA + 0.25% CA	82.05	1.0767	25.39	6.34	63.95	28.13	7.96	0.59b
0.50% CaA + 0.50% CA	82.25	1.0760	25.35	6.32	62.84	29.23	7.94	0.63b
0.75% CaA	82.13	1.0745	25.48	6.35	63.17	29.30	7.79	0.57b
0.75% CaA + 0.25% CA	81.83	1.0750	25.60	6.48	62.72	29.61	7.81	0.65ab
0.75% CaA + 0.50% CA	81.27	1.0743	25.67	6.41	63.49	28.75	7.76	0.69ab
SEM[2]	0.136	0.0002	0.048	0.033	0.134	0.122	0.020	0.009
P-value	0.4049	0.1009	0.5194	0.8646	0.3697	0.0827	0.0691	0.0002

[1]Haugh units

[2]Standard error of the mean

Regarding the yolk color, considering that changes in color intensity depend on the intake of feed rich in pigments and their absorption in the gastrointestinal tract and transfer to the yolk, the inclusion of calcium anacardate associated or not associated with citric acid in the feed did not influence the previously reported factors and, consequently, did not alter this parameter. Santos et al. (2022) also observed no effect of the addition of calcium anacardate on quail egg yolk color. Other researchers (Yesilbag and Çolpan 2006; Park et al. 2009) also reported that organic acids have no influence on pigmentation of chicken egg yolks.

The yolk lipid oxidation results in this study are consistent with the literature. The benefits of the antioxidant action of anacardic acid on egg yolk have been demonstrated by other researchers with the addition of cashew nut liquid in the feed of laying hens (Abreu et al. 2017; Braz et al. 2019), as well as its pro-oxidant action when calcium anacardate was added at a level greater than 0.75% in the feed of broilers (Abreu et al. 2019). Reduced lipid oxidation of eggs from broiler breeding hens has been reported after the addition of other compounds with antioxidant activity in the feed, such as canthaxanthin (Zhang et al. 2018). Finally, considering the observed effects on yolk lipid oxidation, the decision on the best strategy for the use of calcium anacardate (alone or combined with citric acid) in the diet of breeding quails should consider the cost of the additives and the minimum dose options that produce the positive response. Based on findings, 0.75% calcium anacardate alone or 0.50% calcium anacardate associated with 0.25% citric acid provides the same benefit toward the oxidative stability of the bud yolk.

Incubation parameters

No significant effect was found of the treatments on any of the incubation parameters (Table 5). These findings indicate that the levels of addition of calcium anacardate and its association with citric acid did not influence the average weight of incubated eggs, chicks at hatching, relative chick weight, percentage of hatching, hatchability of eggs, or the fertility of the quails.

The weight of the incubated egg and chick at hatching are directly related. The weight of a good-quality chick should range from 62 to 76% of the incubated egg weight (Schimidt et al. 2002). According to results, the average chick weight at hatching was 69.40% of the incubated egg weight, which agrees with reports in the literature (Hegab and Hanafy 2019; Rocha et al. 2022; González-Redondo et al. 2023).

Considering that hatchability is determined based on the relationship between the number of chicks hatched and the proportion of fertile eggs incubated, changes in this index may be associated with embryo mortality throughout the incubation process. Thus, as the incubation conditions were similar for all eggs, the absence of significant difference between treatments indicates that the addition of calcium anacardate and its association with citric acid had no influence on embryo development. These components did not cause or prevent embryo mortality at any stage.

According to some researchers (Zhang et al. 2018; Surai, 2020), the addition of antioxidants to the feed provided to breeding females can contribute to lipid stabilization of egg yolks during egg storage until incubation. They can also reduce naturally occurring lipid peroxidation damage to embryos that can lead to embryonic malformations, which

Table 5 Incubation parameters from meat-type quail breeder eggs fed with calcium anacardate (CaA) associated or not associated with citric acid (CA)

Parameter	Weight of the incubated egg (g)	Chick weight (g)	Chick weight/egg weight (g/g)	Hatching (%)	Hatchability (%)	Fertility (%)
Control diet	12.91	8.84	68.66	62.80	66.37	94.62
0.25% CaA	12.63	8.85	70.12	60.65	66.70	87.32
0.25% CaA + 0.25 CA	12.41	8.70	70.04	59.00	65.19	90.00
0.50% CaA	12.65	8.84	69.94	61.60	66.58	90.98
0.50% CaA + 0.25% CA	12.33	8.71	70.60	63.69	67.85	91.11
0.50% CaA + 0.50% CA	12.57	8.76	69.68	63.15	71.01	87.95
0.75% CaA	12.47	8.57	68.78	57.16	62.80	89.93
0.75% CaA + 0.25% CA	12.52	8.61	68.77	67.82	73.92	88.88
0.75% CaA + 0.50% CA	12.59	8.59	68.26	56.77	61.42	91.42
SEM[1]	0.058	0.046	0.245	2.253	1.967	1.445
P-value	0.5278	0.7522	0.2695	0.9107	0.6429	0.8995

[1]Standard error of the mean

contribute to reduced hatchability and chick quality. However, the results disagree with previous reports because the reduction in yolk lipid peroxidation from the beginning of the incubation process did not result in significant differences in the evaluated incubation parameters.

The hatchability and fertility results obtained in this study are consistent with those reported by Araújo et al. (2015), who obtained a hatchability rate of 63.97% and a fertility rate of 84% for Japanese quails. According to those researchers, storing eggs for more than 1 week reduces hatchability due to chemical changes that occur during the storage time and conditions. Thus, it can be inferred that the aforementioned benefits of adding antioxidants to protect the yolks may improve the incubation outcomes of eggs subjected to a longer storage time before incubation. This eventuality should be evaluated in additional studies.

Progeny performance

When assessing progeny performance, it was found that there was no effect of the treatments on any of the variables from 1 to 21 days of age (Table 6), indicating that the levels of addition of calcium anacardate and its association with citric acid did not influence feed intake, weight gain, average final weight, and the feed conversion ratio of the progeny.

The relationship between breeding bird nutrition and progeny performance has been proved (Vieira and Moran Jr 1998), indicating that additives included in the breeder's diet can be used by their progeny. In this context, the use of compounds with antioxidant action in the breeder's diet has been studied. Indeed, the absorption of these compounds by the body of the breeding birds and their transfer to the egg yolk can benefit embryonic development and the newly hatched chicks via a protective effect against oxidative damage in the tissues. This protection is reflected by improvements in fertility, embryo mortality, and hatchability, as well as chick performance during the initial phase (Surai 1999; Surai 2000; Surai et al. 2003; Koutsos et al. 2003). However, based on the literature, this effect on progeny performance is variable and depends on factors such as the nutritional status of breeding birds (Surai, 2020), the storage time of fertile eggs (Yang et al. 2021a, 2021b), and the age of the breeding bird (Koedijk et al. 2016). In this context, considering that the breeding

Table 6 Performance of the progeny at 21 days old from meat-type quail breeders fed diets containing calcium anacardate (CaA) associated or not associated with citric acid (CA)

Parameter	Feed intake (g/quail)	Weight gain (g/quail)	Feed conversion ratio	Weight at 21 days (g)
Control diet	272.50	129.24	2.11	138.08
0.25% CaA	257.97	122.57	2.11	131.42
0.25% CaA + 0.25 CA	258.70	127.26	2.04	135.99
0.50% CaA	265.14	126.26	2.10	135.12
0.50% CaA + 0.25% CA	269.25	121.51	2.22	130.22
0.50% CaA + 0.50% CA	270.64	132.32	2.06	141.07
0.75% CaA	265.19	130.22	2.05	138.79
0.75% CaA + 0.25% CA	265.83	126.36	2.11	134.96
0.75% CaA + 0.50% CA	264.62	122.43	2.18	131.07
SEM[1]	4.187	1.909	0.038	1.275
P-value	0.8889	0.4670	0.7547	0.4820

[1]Standard error of the mean

quails were the same age and were fed with isonutrient and iso-energetic diets and the incubated eggs were submitted to the same storage time, it can be inferred that the protection of the buds to the observed oxidative damage was not sufficient to promote positive effects on the incubation parameters and progeny performance.

Considering that the results obtained in this research indicated that the use of calcium anacardate and its association with citric acid in diets for breeding quails had an effect only on yolk lipid oxidation, it can be recommended including 0.50% calcium anacardate calcium associated with 0.25% citric acid or 0.75% calcium anacardate alone to mitigate oxidative damage in the yolk of fertile eggs. New research should be performed to verify whether this antioxidant action can contribute to oxidative stability in fertile eggs that remain stored for a longer time before incubation, thus improving the hatching rate and quality of the chicks.

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Data availability All data generated or analyzed during this study are included in this published article; supplementary information can be accessed on reasonable request from the corresponding author.

Code availability Not applicable.

Declarations

Ethics approval That experiments were conducted in a manner that avoided unnecessary discomfort to the animals by use of proper management and laboratory techniques, and the experimental procedures were approved by the Ethics Committee on the Use of Animals — CEUA/UFC under protocol No. 2552250718, according to the ethical principles adopted by the Brazilian Council for Control of Animal Experimentation (CONCEA).

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