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### ANIMAL SCIENCE

# Calcium anacardate as source of anacardic acid in laying Japanese quail diet

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Abstract: Anacardic acid is naturally found in various parts of the cashew tree (Anacardium occidentale L.) and marketed as calcium anacardate. This product has antibacterial, antifungal and antioxidant activity, and has been used in humans in the treatment and prevention of cardiovascular and cerebrovascular diseases and tumors. In meat and egg production systems, anacardic acid is used as a substitute for growth-promoting antibiotics. The study objective was to evaluate effects of adding calcium anacardate as source of anacardic acid in laying Japanese quail diet on bird performance and eggs quality. A total of 252 Japanese quail with 22 week-old were studied, using a completely randomized design composed of six treatments with six replicates and seven quails per experimental unit. Treatments applied were: diet without growth promoter; diet with 0,1% growth promoter; and four diets without growth promoter and addition of 0.25; 0.50; 0.75 and 1.0% calcium anacardate (CA), respectively. The data were submitted to analysis of variance and treatment means compared with a SNK test (5%). The data from treatments containing CA were submitted to regression analysis. Treatments did not influence feed intake, egg production, egg weight, egg volume, feed conversion ratio and eggs quality parameters. The addition up to 1% of calcium anacardate in laying Japanese quail diet, does not influence their performance and egg quality.

Key words: antioxidant, egg, organic acid, performance.

# INTRODUCTION

The routine use of antimicrobials in animal production opened the possibility of development or increase of microbial resistance in human medicine. The response, a recent restriction or total ban on the use of antibiotics to promote growth in animal production, had led to important changes in poultry production systems. These include research that looks at alternative boosting-growth substances and develops products with similar properties that can be used as bird feed additives. Of the various substances available as alternatives to growthpromoting antibiotics, organic acids show great promise; they have been used as feed additives for laying hens and have possible microbial inhibitory effects (Hinton & Linton 1988, Iba & Berchieri 1995) and can influence raw material availability (Dixon & Hamilton 1981).

Among the various organic acid options, anacardic acid, a phenolic compound naturally found in various parts of the cashew tree (*Anacardium occidentale* L.), but most prominent in cashew nut shell extract, is notable for its antitumor, antibacterial, antifungal and antioxidant activity, and its ability to inhibit the enzymes tyrosinase, prostaglandin synthase and lipoxygenase (Toyomizu et al. 2003). In humans it has been used in the treatment and prevention of cardiovascular and cerebrovascular diseases and tumors (Wang et al. 1998, Kubo et al. 2003). The action of anacardic acid in inhibiting microbial growth has been studied for some time with positive results reported for the control of a variety of infectious agents, especially bacteria, with inhibitory effects described for such Gram positive forms as *Streptococcus mutans, Staphylococcus aureus* and *Helicobacter pylori* (Gellerrnan & Schlenk 1968, Muroi & Kubo 1996, Kubo et al. 1999, 2003, Lima et al. 2000, Green et al. 2007, Achanath et al. 2008, Narasimhan et al. 2008, Hamad & Mubofu 2015).

According to Murata et al. (1997), anacardic acid prevents lipid synthesis in bacterial cells by inhibiting glycerol-3-phosphate dehydrogenase. Kubo et al. (2003) suggest that it acts as a surfactant, inducing physical disruption of the bacterial membrane followed by possible interference in the electron transport chain and ATPase, so resulting in inhibition of the respiratory chain.

In addition to its antimicrobial action, anacardic acid has been shown to have antioxidant activity, preventing transition metal ions from initiating the oxidation process, acting in the inactivation of intermediates in this process and inhibiting many enzymes involved (Trevisan et al. 2006). Similar results were reported by Braz et al. (2019) who observed reducing lipid oxidation in eggs yolk from laying hens fed with diet containing 0.75% of liquid from cashew nut shells, rich in anacardic acid.

Anacardic acid can be used as calcium anacardate, a calcium salt product formed by the precipitation of liquid from cashew nut shells with calcium hydroxide. This product is easier to add to animal diet as it comes in powder form and is non-toxic to birds, according to Cruz et al. (2019).

In view of the above, the objective of this study was to evaluate the effects of calcium

anacardate as anacardic acid source in laying Japanese quail diet.

## MATERIALS AND METHODS

The experimental protocols used were approved by the Animal Research Ethics Committee of the Federal University of Ceará under protocol 20/2013 of July 24, 2013, and were in accordance with the Ethical Principles on Animal Experimentation adopted by the Brazilian College of Animal Experimentation.

The experiment used 252 Japanese quail with 22 week-old and mean body weight of 181 g, housed in galvanized wire cages (33 cm x 23 cm x 16 cm). Birds were distributed in a completely randomized design, composed of six treatments with six replicates and seven birds per experimental unit.

The treatments applied were: diet without growth promoter (Without GP); diet with 0,1% growth promoter (With GP); and diets without growth promoter, but with the addition of 0.25; 0.50; 0.75 and 1.0% calcium anacardate (CA), respectively.

Used experimental diets (Table I) were isonutrient and isoenergetic, according to the nutritional requirements recommended by NRC (1994). For their calculation, nutritional values of ingredients given by Rostagno et al. (2011) were used.

Anacardic acid was added to the diets in the form of CA, an intermediate product in the process of obtaining pure acid, using liquid derived from the cashew nut shells. This liquid was initially obtained from the cashew nut shells by heating in an oven at 120°C for 1 hour and was immediately collected and stored while it accumulated in a glass container. The anacardic acid was isolated at the Organic Chemistry Laboratory, Department of Organic

	Experimental diets							
Ingredients	Without GP <sup>1</sup>		With GP	0.25% CA <sup>2</sup>	0.50% CA	0.75% CA	1.00% CA	
Corn	53.13		53.13	53.13	53.13	53.13	53.13	
Soybean meal (45%)	34.76 0.00		34.76	34.76	34.76	34.76	34.76	
Calcium anacardate			0.00	0.25	0.50	0.75	1.00	
Soybean oil	3.55		3.55	3.55	3.55	3.55	3.55	
Bicalcium phosphate	1.58		1.58	1.58	1.58	1.58	1.58	
Limestone	5.23		5.23	5.23	5.23	5.23	5.23	
Premix <sup>3</sup>	0.20		0.20	0.20	0.20	0.20	0.20	
Common salt	0.34		0.34	0.34	0.34	0.34	0.34	
Inert	Inert1.00nethionine0.16ne chloride0.05alquinol0.00BMD40.00Total100.00		0.90	0.75	0.50	0.25	0.00	
DL-methionine			0.16	0.16	0.16	0.16	0.16	
Coline chloride			0.05	0.05	0.05	0.05	0.05	
Halquinol			0.05	0.00	0.00	0.00	0.00	
BMD <sup>4</sup>			0.05	0.00	0.00	0.00	0.00	
Total			100.00	100.00	100.00	100.00	100.00	
		Nutritional composition						
Metabolizable e	2,900							
Crude pro	20.00							
Calciu	2.50							
Available pho	0.40							
Sodiu	0.15							
Total lys	1.10							
Total meth+	0.77							
Total meth	0.45							
Total three	0.78							
Total trypto	0.25							

## Table I. Percentile and nutritional composition of experimental diets.

<sup>1</sup>GP - growth promotor; <sup>2</sup>CA - calcium anacardate; <sup>3</sup>Premix-levels per kg of product: Vitamin A (min) 5,500,000 UI, Vitamin B1 (min) 500mg, Vitamin B12 (min) 7,500mcg, Vitamin B2 (min) 2,502mg, Vitamin B6 (min) 750mg, Vitamin D3 (min) 1,000,000 UI, Vitamin E (min) 6,500 UI, Vitamin K3 (min) 1,250mg, Biotine (min) 25mg, Niacin (min) 17.5g, Folic acid (min) 251 mg, Pantenoic acid (min) 6,030mg, Cobalt (min) 50mg, Copper (min) 3,000mg, Iron (min) 25g, Iodine (min) 500mg, Manganese (min) 32.5g, Selenium (min) 100,05mg, Zinc (min) 22.49g. 4BMD- Bacitracin Methylene Disalicylate.

and Inorganic Chemistry, Federal University of Ceará. Isolation of the anacardic acid was carried out by precipitation calcium anacardate formed by the reaction of anacardic acid and calcium hydroxide according to methods Trevisan et al. (2006), adapted from Paramashivappa et al. (2001). The calcium anacardate was extracted in a 4L Becker by the addition of 550mL liquid from cashew nut shells, 150mL of distilled water and 2850mL of ethanol which after mixing, were heated to 50°C under agitation and with temperature constantly monitored. During the procedure, 250 g of calcium hydroxide were added to the mixture. After 4 hours of stirring and heating, the mixture was allowed to stand for 1 hour, after which the supernatant was removed. An additional 800 mL of ethanol was then added, and the mixture was again stirred for 1 hour and heated for 1 hour. At the end of this process, the calcium anacardate was ovendried for 72 hours, then ground and sieved.

Determination and quantification of the anacardic acids present in the produced calcium anacardate was performed by HPLC (High Performance Liquid Chromatography) with an Agilent brand DAD detector (Diode Array Detector) operating in the ultraviolet range with an analytical column C-18 Latek (5 µm, 250 x 4 mm) in a stream of mL.mim<sup>-1</sup> with an injection volume of 10µL. The DAD detector was set at wavelengths of 254, 278, 325 and 340 nm, where anacardic acids have higher absorption peaks in UV-VIS. For this analysis the calcium anacardate sample was previously converted to anacardic acids according to the procedure described by Paramashivappa et al. (2001). The amount of anacardic acids present in calcium anacardate was 94.5%: the anacardic acids triene (15:3), diene (15:2) and monoene (15:1) were detected at the relative proportions of 3:1.6:1.1, respectively.

For the inclusion of calcium anacardate in the diets preparation, anacardate was premixed with soybean meal in Y-blender, and this was then added and homogenized to the final mixture.

The experimental period lasted 84 days, divided into four periods of 21 days. Throughout the experiment, feed and water were available *ad libitum*, and birds were raised under a 16 hours light program. Egg collecting and counting was performed daily in the morning.

The following performance variables were evaluated: feed intake (g/bird/day), eggs production (%/bird/day), egg weight (g), egg mass (g/bird/day) and feed conversion ratio (g of feed/g of egg). Feed intake was calculated by the difference between the amount of feed offered and the remainder at the end of each day. Egg production was recorded daily by cage, and the laying percentages were calculated at the end of each period per replicate. The average egg weight (g) was determined by dividing the total weight of collected eggs by the number of eggs laid per replicate. The eggs were weighed once weekely on a semi-analytic balance (sensitivity 0.01g). Egg mass was calculated by multiplying the average egg weight by the laying percentage. The feed conversion ratio was calculated based on feed intake and egg mass produced.

To evaluate egg quality, once-a-week during the experimental period all eggs from each replicate were collected, identified and evaluated for the following parameters: percentages (%) of yolk, albumen and shell, Haugh Units (HU), specific density (g/cm<sup>3</sup>), shell thickness (mm) and yolk color. For the analyses of quality, three eggs per replicate were selected, all within the average weight range calculated for the group. Initially, the specific density (SD) of the egg was determined, in which the egg weighing system was assembled on a semi-analytic balance (sensitivity 0.01g) to weigh the egg in air and water, following the protocol of Freitas et al. (2004). To obtain egg weight in the air and in the water, an egg weighing system was installed on a semi-analytical balance (sensitivity 0.01 g). Egg weight values in air and water were calculated from SD, with the equation:  $SD = \frac{AW}{(WW \times T)}$ , where: AW = egg weight in air (g), WW = egg weight in water (g), and T = temperature correction factor (°C).

After determining the SD, the eggs were broken on a glass surface to determine albumen

height, using a micrometer. Albumin height and egg weight data were used to calculate HU, using the equation:  $HU = 100_{log}(H + 7.57 - 1.7W^{0.37})$ where: H = albumen height (mm), and W = eggweight (g). After measuring albumin height, the yolk was separated from the albumen and weighed on semi-analytic balance (sensitivity 0.01g). To obtain percentages, yolk weight was divided by egg weight, and the value obtained then multiplied by 100. Shells were washed, then dried at ambient temperature for 72 hours. After drying, shells were weighed on a semianalytic balance (sensitivity 0.01g). To obtain percentages, shell weight was divided by egg weight, multiplying the value obtained by 100. Albumen percentage was obtained by difference, Where: % albumen = 100 - (%yolk + %shell).

To determine eggshell thickness, after shell weighing, shell fragments were removed from the major, minor and equatorial regions of eggs to measure eggshell thickness in each region. Measurements were taken using digital calipers with 0.01mm divisions. Overall shell thickness was taken as the average of values obtained from the three regions. After the yolks were weighed, they were evaluated for color by visual comparison with Roche's colorimetric range.

Statistical analysis of data was performed using the "Statistical Analyzes System" (SAS 2008). The data were submitted to analysis of variance, according to a completely randomized model, and treatment means compared with a SNK test (5%). To determine the optimum inclusion level, data from treatments containing 0.25%; 0.50%; 0.75% and 1.0% calcium anacardate were submitted to regression analysis.

# **RESULTS AND DISCUSSION**

According to the results, supplementing diet with calcium anacardate produced no

significant effect on the variables: feed intake, egg production, egg weight, egg mass and feed conversion ratio at any inclusion level (Table II).

Among those factors associated with feed intake variation by birds, changes in metabolizable energy levels and diet palatability have been emphasized.

As birds usually regulate feed intake to meet energy needs, feed modification often decreases their metabolizable energy, resulting in increased feed intake, while increasing diet metabolizable energy may result in lower uptake. Thus, considering that the experimental diets were formulated to be isoenergetic, it is likely that addition of calcium anacardate up to 1%, or of antibiotic growth promoter, did not influence energy use by the birds tested, since there was no detectable difference in feed intake levels between the different treatments.

The relationship between energy requirement and feed intake is very important for diet composition, because nutrient intake is regulated by predetermined energy levels. Pinto et al. (2002) carried out tests on laying Japanese quail using diets with three differing energy levels (2,850; 2,950 and 3,050 kcal de EM/kg) and recommended 2,850 kcal ME/kg as the optimal diet level. As the current diets were considered to be isoenergetic, quails were able to meet nutritional requirements energetic needs without altering feed intake.

Palatability is an additional factor that may influence poultry feed intake rates (Leeson & Summers 2001). Some ingredients can modify the taste and odor of foods, making them less palatable, and consequently reducing their feed intake by animals (Racanicci et al. 2004). However, in the current study, that addition of calcium anacardate had no significant effect on feed intake indicates that levels up to 1% of diet had no effect on feed palatability to the quail under study.

	Treatments	Feed intake (g/ bird/day)	Eggs production (%/bird/day)	Egg weight (g)	Egg mass (g/bird/day)	Feed conversion ratio (g/g)		
	Without GP <sup>1</sup>	22.02	85.94	11.16	9.59	2.30		
	With GP	22.78	89.18	11.22	10.00	2.28		
	0.25% CA <sup>2</sup>	22.82	88.83	11.40	10.12	2.25		
	0.50% CA	23.00	89.58	11.31	10.14	2.27		
	0.75% CA	23.02	89.73	11.14	10.00	2.30		
	1.0% CA	22.96	88.85	11.24	9.98	2.30		
	Mean	22.78	88.76	11.25	9.98	2.28		
	CV <sup>3</sup> (%)	4.20	5.31	2.11	5.65	2.33		
Ì	ANOVA <sup>4</sup>	<i>p</i> -value						
ĺ	Treatments	0.5539	0.8060	0.4618	0.6688	0.6135		
ĺ	Regression	gression <b>p</b> -value						
ľ	Linear	0.6992	0.6236	0.2788	0.9838	0.5306		
ľ	Quadratic	0.7374	0.6304	0.4029	0.9357	0.7252		

<sup>1</sup>GP – growth promotor; <sup>2</sup>CA – calcium anacardate; <sup>3</sup>CV – coeffecient of variation; <sup>4</sup> ANOVA - analysis of varience.

Metabolizable energy requirements in a laying bird is directly related to egg production (Leeson & Summers 2008), while protein and amino acid requirements are related to egg size and/or weight (Pinto et al. 2002, Figueiredo et al. 2012). In this context, considering that the diets were formulated to be isoenergetic, isoproteic and isoaminoacidic, and that the quails submitted to the different treatments showed no significant differences in laying percentage and eggs weight, it is concluded that such birds had their requirements for metabolizable energy, protein and amino acids met in all experimental circumstances. The results obtained for egg mass and feed conversion ratio directly reflect the results obtained for egg production and egg weight.

In the literature, antibacterial effects are reported to be one of the biological effects of anacardic acid (Kubo et al. 2003). Consequently, it was expected that when calcium anacardate was added to diet, it would show an antimicrobial effect once it had dissociated from the calcium during digestion.

Thus, it was expected that the addition of calcium anacardate in the diets, once it had dissociated during the digestive process in the anacardic acid in its free form, would produce antimicrobial effect and consequently improved performance of birds, so producing results similar to those reported in the literature for organic acid addition (Gama et al. 2000, Soltan 2008, Youssef et al. 2013). However, such benefits were not confirmed in the current study on quail.

According to Dibner & Buttin (2002), dissociation capacity is the main characteristic influencing the magnitude and effectiveness of the antibacterial activity of an organic acid. In the laboratory, dissociation of calcium anacardate into anacardic acid and calcium ions occurs by hydrolysis in 11 molar hydrochloric acid (Paramashivappa et al. 2001), with a pH equivalent to 1.04. As a result, the observed absence of response to anacardic acid may be a result of the standard pH values in the avian gastrointestinal tract; being equivalent to 5.5 in the throat, 2.5 to 3.5 in the proventriculus and gizzard, 5.7 to 6.4 in the duodenum, from 5.8 to 6.6 in the jejunum, and 6.3 to 7.2 in the ileum (Denbow 2015, Wilkinson et al. 2016). None of these provide an optimal dissociation medium for calcium anacardate.

Another factor influencing organic acid responses is the buffer capacity of the diet, since diets with high mineral content, such as diets for laying hens with high calcium concentrations, make gastric pH reduction difficult (Jung & Bolduan 1986). Such an effect in the current study may have compromised calcium anacardate hydrolysis, and consequently the release of anacardic acid. In addition, salts of organic acids, such as calcium anacardate, tend to increase diet buffer capacity, which may again compromise bird response capacity (Partanen & Mroz 1999).

The literature contains a number of other studies reporting no performance benefits from the use organic acids (Świątkiewicz et al. 2010, Özek et al. 2011, Kaya et al. 2013), or plant extracts (Özek et al. 2011, Braz et al. 2019), as diets additives for laying hens. Others report problems in adding acids or mixtures of organic acids to diets, especially in the determination of the optimal dose (Soltan 2008).

Another possible cause for the results may the fact that, since birds were kept under good and clean conditions, there were, in fact, insufficient health challenges to highlight the potentially positive effect of treatments with calcium anacardate without growth promoter, as growth rates were the same as those with growth promotor.

For egg quality, no significant effects of the treatments were found for yolk, albumen and shell percentages, Haugh units, specific density, shell thickness and yolk color (Table III).

In healthy birds, changes in egg composition and, consequently, their quality are associated with availability of nutrients required for formation of each component. Costa et al. (2004) reported that egg albumen solids are almost entirely proteinaceous, so that demand for protein and amino acids is high, and therefore any protein shortage will result in a reduction in the amount of albumen and hence egg size. Mineral deficiency, especially calcium, can influence shell thickness and quality (Świątkiewicz et al. 2010). Consequently, the lack of a significant effect of experimental treatments on egg quality and composition is likely due to the isonutrient formulation of diets used, and the lack of significant variation in feed intake.

Results from some studies have suggested that addition of organic acids improves internal egg quality parameters (Soltan 2008, Özek et al. 2011), while provision of supplemental minerals improves bird egg shell quality (Soltan 2008, Świątkiewicz et al. 2010, Kaya et al. 2013). For these researchers, such effects are associated with reduced pH of the digestive tract, increased dissociation of minerals and activity of digestive enzymes. On the other hand, improvement in some aspects of egg internal quality have been reported with addition of vegetal extracts to the diet (Özek et al. 2011). Thus, the lack of any significant effect on egg composition, internal quality and egg shell, in the current study, indicates that these benefits did not occur with calcium anacardate addition, as source of anacardic acid, in quail diet.

It is worth noting that it is not only performance that has remained unaffected by such diets supplements. Adding organic acids (Mahdavi et al. 2005, Yesilbag & Çolpan 2006, Świątkiewicz et al. 2010, Kaya et al. 2013) or plant extracts (Botsoglou et al. 2005, Florou-Paneri et

Trestmente	Percentage			Haugh	Specific Density (5/sm3)	Shell Thickness	Valla Calari
Treatments	Yolk	Albumen	Shell	Units	Specific Density (g/cifi*)	(mm)	YOIK COLOF
Without GP <sup>1</sup>	30.60	61.38	8.02	90.21	1.075	0.20	4.60
With GP	30.29	61.76	7.94	90.38	1.072	0.21	4.60
0.25% CA <sup>2</sup> 0.50% CA	30.61	61.37	8.01	90.10	1.073	0.20	4.46
	30.71	61.30	7.99	90.92	1.072	0.20	4.48
0.75% CA	31.19	60.85	7.97	90.66	1.072	0.20	4.61
1.00% CA	30.79	61.19	8.02	90.27	1.073	0.20	4.61
Mean	30.70	61.31	7.99	90.43	1.072	0.20	4.56
CV <sup>3</sup> (%)	1.87	1.01	2.32	0.95	0.22	2.36	3.48
ANOVA <sup>4</sup>	<i>p</i> -value						
Treatment	0.2011	0.2550	0.966	0.5769	0.4000	0.1617	0.3300
Regression	<i>p</i> -value						
Linear	0.2642	0.3630	0.5978	0.0597	0.5627	0.9518	0.5317
Quadratic	0.3273	0.4374	0.5869	0.0599	0.5046	1.000	0.8042

Table III. Components and quality of Japanese quail eggs fed diets with calcium anacardate at	various
concentrations.	

<sup>1</sup> GP – growth promotor; <sup>2</sup>CA – calcium anarcardiate; <sup>3</sup>CV – coeffecient of variation; <sup>4</sup> ANOVA – analysis of varience.

al. 2005, Özek et al. 2011) have also been reported to have no effect on eggs internal quality.

Adding organic acids or mixtures of organic acids to the diet has also been reported to cause problems (Gama et al. 2000), including yolk color. However, use of calcium anacardate as a diets supplement was not expected to cause problems in yolk pigmentation, and, indeed, both Yesilbag & Çolpan (2006) and Park et al. (2009) have reported that the addition of organic acids had no effect on egg yolk coloration.

# CONCLUSIONS

The addition up to 1% of calcium anacardate in laying Japanese quail diet, does not influence their performance and egg quality.

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