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Calcium anacardate as source of phenolic compounds in diet for New Zealand White female rabbits

Abstract – The objective of this work was to evaluate the effect of the dietary addition of calcium anacardate as a source of phenolic compounds on the reproductive performance and blood antioxidant parameters of New Zealand White female rabbits. Twenty-seven New Zealand White female rabbits were distributed in a randomized complete block design with three treatments and nine replicates, considering one animal as the experimental unit. The treatments consisted of pelleted diets with different inclusion levels of calcium anacardate (0, 0.5, and 1.0%). The inclusion of calcium anacardate in the diets increased the concentration of phenolic compounds and the antioxidant capacity in the blood of lactating female rabbits. Calcium anacardate at the level of 1.0% improves the feed conversion ratio during gestation and increases the phenolic compounds and antioxidant capacity in the blood serum of female rabbits at 21 days of lactation.

Index terms: *Oryctolagus cuniculus*, gestation, lactation, natural antioxidant, phenolic compounds.

Anacardato de cálcio como fonte de compostos fenólicos na dieta para coelhas Nova Zelândia Branco

Resumo – O objetivo deste trabalho foi avaliar o efeito da adição dietética de anacardato de cálcio como fonte de ácido anacárdico sobre o desempenho reprodutivo e os parâmetros antioxidantes sanguíneos de coelhas Nova Zelândia Branco. Vinte e sete fêmeas da raça Nova Zelândia Branco foram distribuídas em delineamento em blocos ao acaso, com três tratamentos e nove repetições, tendo-se considerado um animal como unidade experimental. Os tratamentos consistiram em rações peletizadas, com diferentes níveis de inclusão de anacardato de cálcio (0, 0,5 e 1,0%). A inclusão de anacardato de cálcio nas dietas aumentou a concentração de compostos fenólicos e a capacidade antioxidante no sangue das coelhas fêmeas lactantes. O anacardato de cálcio ao nível de 1,0% melhora a conversão alimentar durante a gestação e aumenta os compostos fenólicos e a capacidade antioxidante no soro sanguíneo das coelhas aos 21 dias de lactação.

Termos para indexação: *Oryctolagus cuniculus*, gestação, lactação, antioxidante natural, compostos fenólicos.

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Introduction

In rabbit breeding, one of the main ways to increase the profitability of the production system is by improving the productivity of female rabbits through reproductive performance optimization (Castellini

et al., 2010). According to Miranda et al. (2021), the aim are female rabbits with a high prolificacy and maternal ability that are able to wean heavy and numerous litters for commercialization and replacement of the herd.

However, due to the intense reproductive cycle of female rabbits, there is an overlap between gestation and lactation, associated with metabolic and physiological changes that occur during reproduction, which could cause an increase in the production of free radicals, resulting in oxidative stress and, consequently, in a compromised health and fertility (Pereira & Martel, 2014). Oxidative stress is characterized by an increase in reactive oxygen species (ROS), which are metabolites that, when in excess, can damage organic molecules, specifically causing negative changes in the maturation and fertilization processes of oocytes in females and disturbances during the various stages of spermatogenesis in males (Andrade et al., 2010).

To combat feed oxidation and neutralize free radicals from metabolic activity, a common practice is adding synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, to rabbit feed. However, because of the potential risks of synthetic antioxidants, natural antioxidants from plants are being researched as alternatives to combat oxidative stress (Miranda et al., 2021).

Among natural antioxidants, anacardic acid, a phenolic compound found in different parts of the cashew (*Anacardium occidentale* L.) tree, mainly in its nuts, stands out due to its several biological activities (Hamad & Mubofu, 2015), particularly to its antioxidant capacity that can prevent physiological damages induced by oxidative stress (Hemshekhar et al., 2012). Gomes Júnior et al. (2018) concluded that anacardic acid reduced oxidative stress in rats by decreasing malondialdehyde concentration and increasing glutathione and catalase activity. These authors also observed that the antioxidant action in inflammatory processes is related to the neutralization of free radicals through the formation of phenolic and allylic radicals.

In animal diets, anacardic acid is included as calcium anacardate, obtained by the reaction between cashew nut liquid and calcium hydroxide, resulting in a powder form that is easier to add to the feed (Matos et al., 2017). As an additive of recent use, some studies have shown that it improves animal productive performance and presents antioxidant activity (Ferreira et al., 2020). Vizzari et al. (2021), for example, evaluating the chemical characteristics of phenolic compounds of plant origin, observed improvements in the antioxidant status of male rabbits, indicating a possible effect on their reproductive performance.

The objective of this work was to evaluate the effect of the dietary addition of calcium anacardate as a source of phenolic compounds on the reproductive performance and blood antioxidant parameters of New Zealand White (*Oryctolagus cuniculus* Linnaeus, 1758) female rabbits.

Materials and Methods

The experimental procedures followed the protocols approved by the ethics committee on animal use of Universidade Federal do Ceará.

Twenty-seven New Zealand White female rabbits, with an average age of 18.5 months (two to five parity orders) and initial weight of 2.94+0.613 kg, were used. The animals were distributed in a completely randomized design, with three treatments (0, 0.5, 1.0% calcium anacardate) and nine replicates, considering one animal as the experimental unit.

The experimental diets were pelleted and formulated to be isonutrient and isoenergetic, following the nutritional requirements of the animals at the reproduction stage as described by De Blas & Wiseman (2010). The diets contained corn, soybean meal, wheat meal, and alfalfa hay (Table 1), plus the three levels of calcium anacardate replacing the inert ingredient. Anacardic acid in the form of calcium anacardate was obtained using cashew nut liquid, distilled water, ethanol, and calcium hydroxide through stirring and heating according to Trevisan et al. (2006).

The rabbits were housed individually in galvanized steel wire cages, with 80×60×45 cm length, width, and height, containing an automatic nipple drinker and a semi-automatic feeder. A period of 15 days was used for the adaptation of the animals to the diets. During the entire experimental period, the female rabbits received feed and water ad libitum. The rations were administered twice a day. The females were naturally mated using four New Zealand White males. On the fifteenth day after mating, gestation was confirmed through abdominal palpation, and non-pregnant females were mated in the subsequent estrus. At 27 days of gestation, the pregnant females received a wooden nest box with wood shavings to make their nests.

The animals were weighed at the beginning of the experiment, at 28 days of gestation, and at 1, 7, 14, 21, and 35 days after parturition. The feeds were collected and weighed at the end of the gestation (28 days) and lactation (35 days postpartum) stages. During gestation, the following performance variables were evaluated: daily feed intake, daily weight gain, and feed conversion. After 35 days of lactation, daily feed intake and weaning weight of the female rabbits were determined.

Data on the reproduction parameters included number of rabbits born alive, stillborn, dead (mortality), and weaned. The rabbits were weighed at birth, at 7, 14, and 21 days, and at weaning (at 35 days). Milk production was estimated through the weight gain of the litter, considering the weight differences between 0–7, 0–14, and 0–21 days according to Maertens et al. (2006).

At 21 days after parturition, 4.0 mL blood samples were collected from all females, through jugular puncture, for the analysis of phenolic compounds and antioxidant capacity. The samples were centrifuged at 1,500 *g*, for 10 min, to obtain the serum supernatant. To perform the analyses, the serum was deproteinized to avoid the influence of serum proteins (Ferreira et al., 2014). The phenolic components were evaluated as described by Parker et al. (2007) and quantified based on the standard curve generated with gallic acid, with results expressed in micrograms per milliliter of gallic acid equivalents. To evaluate the antioxidant potential of the serum, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was used, which consists of the percentage of DPPH free radical capture, following Janaszewska & Bartosz (2002).

The statistical analysis of the data was performed using the GLM procedure of the SAS University Edition software (SAS Institute Inc., Cary, NC, USA), and means were compared by the Student-Newman-Keuls test, at 5% probability.

Table 1. Percentage and nutritional composition of experimental diets for New Zealand White (*Oryctolagus cuniculus*) female rabbits.

Feedstuff (%)	Calcium anacardate level (%)		
	0	0.50	1.00
Alfalfa hay	37.98	37.98	37.98
Corn	27.76	27.76	27.76
Wheat meal	16.06	16.06	16.06
Soybean meal	10.44	10.44	10.44
Soybean oil	3.50	3.50	3.50
Dicalcium phosphate	1.30	1.30	1.30
Limestone	0.79	0.79	0.79
Salt	0.55	0.55	0.55
DL-methionine	0.21	0.21	0.21
L-lysine HCL	0.16	0.16	0.16
Vitamin supplement[(1)]	0.15	0.15	0.15
Mineral supplement[(2)]	0.10	0.10	0.10
Calcium anacardate	0.00	0.50	1.00
Inert[(3)]	1.00	0.50	0.00
Total	100.00	100.00	100.00
Calculated nutritional composition			
Digestible energy (kcal kg^{-1})	2,560	2,560	2,560
Crude protein (%)	16.50	16.50	16.50
Acid detergent fiber	16.50	16.50	16.50
Available phosphorus (%)	0.60	0.60	0.60
Calcium (%)	1.05	1.05	1.05
Lysine (%)	0.81	0.81	0.81
Methionine + cystine	0.63	0.63	0.63
Sodium (%)	0.23	0.23	0.23

[(1)]Composition per kilogram of product: 9000000.00 UI vitamin A, 2500000.00 UI vitamin D3, 20000.00 mg vitamin E, 2500.00 mg vitamin K3, 2000.00 mg vitamin B1, 6000.00 mg vitamin B2, 15.00 mg vitamin B12, 35000.00 mg niacin, 12000.00 mg pantotenic acid, 8000.00 mg vitamin B6, 1500.00 mg folic acid, 250.00 mg selenium, and 100.00 mg biotin. [(2)]Composition per kilogram of product: 100000.00 mg iron, 20.00 g copper, 130000.00 mg manganese, 130000.10 mg zinc, and 2000.00 mg iodine. [(3)]Celite 545.

Results and Discussion

The New Zealand White female rabbits fed with the diet with 1.0% calcium anacardate showed a better feed conversion ratio in the gestation stage than those that did not receive the additive (Table 2), but did not differ from those fed 0.5% calcium anacardate. In addition, no significant differences were observed for the other measured variables.

During the early weeks of gestation, female rabbits tend to increase feed intake, which decreases in the last week of gestation when the nutritional requirements for fetal development increase considerably, resulting in a negative energy balance (Fortun-Lamothe, 2006; Nawito et al., 2016). These high metabolic activities can cause oxidative stress, which can be reduced by dietary antioxidants, such as anacardic acid, which are expected to improve animal performance and, consequently, feed conversion. Chen et al. (2022) concluded that the effect of dietary phenolic compounds on the performance of rabbits may be related to improvements in their intestinal morphology

when fed a diet with chlorogenic acid, whose phenolic compounds could favor the structure of the mucosa and increase the absorptive area of the jejunum, resulting in a better feed efficiency. As a phenolic compound that presents antimicrobial activity against several pathogenic microorganisms (Konda et al., 2019), anacardic acid favors beneficial bacteria that produce short-chain fatty acids, mainly propionate and succinate (Watanabe et al., 2010), promoting a better intestinal health and feed efficiency (Elghalid et al., 2020). Although studies about the use of natural antioxidants on the reproduction performance of female rabbits are still scarce, similar results to those of the present work have been reported for antioxidant compounds from plant extracts (Azoz & Basyony, 2012; Bakeer et al., 2022).

The dietary inclusion of calcium anacardate at 1.0% in the diets of female rabbits promoted lower feed intake compared to 0 and 0.5%, although no effect on weaning weight was observed (Table 3). During the lactation period, female rabbits tend to increase feed intake and body weight in the first and second week postpartum, but, due to the high requirements for milk maintenance and production, they may lose weight mainly in the lactation peak that occurs in the third week, representing a negative energy balance that promotes the mobilization of body reserves to meet the nutritional demands of this stage (Fortun-Lamothe, 2006; Mahmoud, 2013; Machado et al., 2020). As the used diets were formulated to be isonutrient and isoenergetic, the differences in feed intake could be related to the presence of calcium anacardate, but also to other factors such as litter size and body condition of the female rabbits (Mahmoud, 2013). Contrastingly, Azoz & Basyony (2012) observed an increase in the feed intake of female rabbits during lactation with the supplementation of 1.0 and 1.5% dehydrated pomegranate residue, whose phenolic substances were able to act as enzyme modulators and metal chelators, reducing oxidative stress and improving the productive performance of the animals.

Regarding the reproductive performance of female rabbits (Table 4), no significant difference was observed between the treatments for number of rabbits born alive, stillborn, dead, and weaned. Litter size at birth and the number of stillbirths may be related to the mother's nutrition during pregnancy, whereas mortality in the lactation stage and litter size at weaning may be influenced by milk production. Other factors may affect the reproductive performance of the animals, such as oxidative stress, since embryonic mortality under heat stress may be related to the production of ROS (Liang et al., 2022).

The inclusion of calcium anacardate only affected the performance of female rabbits during gestation, but not their reproductive performance. Bakeer

Table 2. Productive performance of New Zealand White (*Oryctolagus cuniculus*) female rabbits fed with diet with different levels of calcium anacardate at the gestation stage.

Calcium anacardate (%)	Feed intake (g per day)	Weight gain (g per day)	Feed conversion ratio (g g^{-1})[(1)]
0	106.63	32.31	3.30a
0.5	91.10	28.38	3.21ab
1.0	85.64	27.54	3.11b
CV (%)	8.53	10.52	7.53
p-value	0.4533	0.5676	0.0324

[(1)]Means followed by equal letters, in the column, do not differ from each other by the Student-Newman-Keuls test, at 5% probability.

Table 3. Productive performance of New Zealand White (*Oryctolagus cuniculus*) female rabbits fed with diet with different levels of calcium anacardate at the lactation stage.

Calcium anacardate (%)	Feed intake (g per day)[(1)]	Weaning weight (g)
0	178.10a	3,196.30
0.5	171.83a	3,218.75
1.0	159.13b	3,163.47
CV (%)	5.20	11.35
p-value	0.0270	0.8814

[(1)]Means followed by equal letters, in the column, do not differ from each other by the Student-Newman-Keuls test, at 5% probability.

Table 4. Reproductive performance of New Zealand White (*Oryctolagus cuniculus*) female rabbits fed with diet with different levels of calcium anacardate.

Calcium anacardate (%)	Born alive	Stillborn	Mortality	Weaned rabbits
0	7.50	0.33	1.66	5.85
0.5	7.36	0.33	1.16	6.19
1.0	7.60	0.31	1.35	6.24
CV (%)	7.59	11.28	20.85	6.98
p-value	0.2574	0.8820	0.2691	0.9711

et al. (2021) also did not find significant differences between treatments when evaluating the reproductive performance of female rabbits using a dietary level of 0.5% pumpkin seed oil as a natural antioxidant. However, Azoz & Basyony (2012) reported a reduction in offspring mortality and an increase in litter size at weaning when adding pomegranate residue to the diet of heat-stressed female rabbits. The authors associated these positive effects with the phenolic compounds of the tested additive, which was able to protect the female rabbits from the damage caused by the free radicals arising from oxidative stress.

The different results found in the literature can be attributed to the chemical structure of the organic compounds with antioxidant activity, which may alter their pharmacological function (Zhong & Zhou, 2013). In this line, Ferreira et al. (2020) added that the magnitude of the benefits of an additive is related to the level of challenge to which the animal is subjected to in the field. Therefore, the effect of the addition of calcium anacardate on the reproductive performance of the rabbits may not have been observed due to the lack of environmental challenge in the present study.

Regarding estimated milk production, there was no significant effect of dietary calcium anacardate in the lactation stage (Table 5). In New Zealand White rabbits, the peak of milk production occurs in the third week of lactation, decreasing from the fourth week onwards. Mahmoud (2013) and El-Deghadi (2019) highlighted that milk production varies according to physiological, hereditary, and environmental factors and can be negatively influenced by oxidative stress, which compromises animal health. Milk production can also vary according to litter size and rabbit body weight (El-Deghadi, 2019), although no significant differences were found for these variables in the present work. Zeweil & El-Gindy (2016) observed different results, with a linear and quadratic increase in the milk production of heat-stressed rabbits fed with diets with different levels of pomegranate peel (0, 0.75, 1.5, and 3.0%). According to these authors, the antioxidant activity of the phenolic compounds present in pomegranate contribute to improving the performance and milk production of female rabbits during reproduction under unfavorable environmental conditions.

The different levels of calcium anacardate did not affect weaning average weight (Table 6). During the suckling stage, body weight gain and rabbit survival depend on the milk production of the female, with a high correlation between birth weight and litter size (El-Deghadi, 2019; Szendrő et al., 2019). In the present study, the dietary inclusion of calcium anacardate did not influence the weight of the rabbits from birth until weaning, nor did the treatments affect litter size and milk production. Zeweil & El-Gindy (2016) found different results when evaluating dehydrated pomegranate peel as a natural antioxidant in the diets of rabbits stressed by heat, observing that the inclusion of pomegranate peel tended to increase quadratically the weight of the litter at weaning. According to these authors, the improvement in the reproductive performance of the rabbits may be due to the protective action of the phenolic antioxidants, and the reduction in the individual weight of the rabbits is related to the existing correlation between litter size and the weight of each rabbit.

The female rabbits fed with diets with 0.5 and 1.0% calcium anacardate had a higher content of phenolic compounds and serum antioxidant capacity than those that were not fed with this additive (Table 7). Similar results were obtained by other authors when adding

Table 5. Estimated milk production of New Zealand White (*Oryctolagus cuniculus*) female rabbits fed with diet with different levels of calcium anacardate.

Calcium anacardate (%)	Estimated milk production (g)		
	7 days	14 days	21 days
0	216.00	599.67	447.04
0.5	203.83	579.17	430.31
1.0	206.40	555.00	425.83
CV (%)	11.96	6.53	4.56
p-value	0.1997	0.2220	0.2589

Table 6. Average weight of offspring from New Zealand White (*Oryctolagus cuniculus*) female rabbits fed with different levels of calcium anacardate.

Calcium anacardate (%)	Average weight (g)				
	Birth weight	7 days	14 days	21 days	35 days
0	60.20	133.06	225.37	301.36	596.55
0.5	61.50	128.04	239.25	304.92	636.61
1.0	63.11	133.38	233.53	319.78	663.50
CV (%)	24.34	31.75	28.92	27.33	20.77
p-value	0.9499	0.9707	0.9380	0.9312	0.6994

Table 7. Serum phenolic compounds and antioxidant potential by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method of New Zealand White (*Oryctolagus cuniculus*) female rabbits fed with different levels of calcium anacardate[(1)].

Calcium anacardate (%)	Phenolic compounds (ug mL^{-1})	DPPH (%)
0	34.00b	37.66b
0.5	47.05a	44.95a
1.00	46.73a	44.91a
CV (%)	11.52	5.46
p-value	0.0016	0.0004

[(1)]Means followed by equal letters, in the columns, do not differ from each other by the Student-Newman-Keuls test, at 5% probability.

natural phenolic antioxidants to rabbit diets (Azoz & Basyony, 2012; Bakeer et al., 2021, 2022).

Lactating rabbits are susceptible to oxidative stress, when free radicals increase oxidative damage to lipids, proteins, and DNA, contributing to a reduction in their reproductive performance (Zhao & Kim, 2020). In this context, anacardic acid is an effective alternative in combating oxidative stress since it is a phenolic compound known for its antioxidant capacity, which is related to ROS neutralization and xanthine oxidase inhibition (Kubo et al., 2006; Trevisan et al., 2006).

According to the results obtained in the present study, the dietary inclusion of calcium anacardate as a source of phenolic compounds increased serum antioxidant capacity and, therefore, could contribute to the reduction of oxidative stress, although no improvement was observed in the reproductive performance of New Zealand White female rabbits.

Conclusion

Calcium anacardate at the level of 1.0% improves the feed conversion ratio of New Zealand White female rabbits during gestation, also promoting an increase in the phenolic compounds and antioxidant capacity in their blood serum at 21 days of lactation.

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