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Blood parameters and enzymatic and oxidative activity in the liver of chickens fed with calcium anacardate[1]

Parâmetros sanguíneos e atividade enzimática e oxidativa no fígado de frangos alimentados com anacardato de cálcio

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ABSTRACT - The aim of this research was to evaluate the inclusion of calcium anacardate (CAC) as a source of anacardic acid in the diet of broiler chickens on blood parameters, and enzymatic and oxidative activity in the liver. A total of 840 male chicks, one day old, were kept in a completely randomised experimental design, with six treatments and seven replications of 20 birds, totalling 140 birds per treatment. The treatments consisted of feed without the addition of growth promoter (GP), feed with GP, and feed with no GP and the addition of CAC at levels of 0.25, 0.50, 0.75 and 1%. The biochemical blood variables to be analysed were uric acid, total cholesterol, HDL, LDL, creatinine, AST, ALT, triglycerides, total erythrocytes, haemoglobin, haematocrit, mean corpuscular volume, corpuscular haemoglobin concentration, total plasma protein, total leukocytes, heterophils, lymphocytes, platelets and heterophil/lymphocyte ratio. The concentrations of superoxide dismutase, glutathione peroxidase and malondialdehyde were analysed for the enzymatic and oxidative parameters in the liver. There were no significant differences between treatments in the blood parameters or the enzymatic and oxidative activity in the liver of the chickens, demonstrating that the use of calcium anacardate as a source of anacardic acid is non-toxic, and does not affect these parameters.

Key words: Additives. Anacardic acid. Haemogram. Leukogram.

RESUMO - Com essa pesquisa objetivou-se avaliar a inclusão de anacardato de cálcio (ACC) como fonte de ácido anacárdico na dieta de frangos de corte sobre os parâmetros sanguíneos, atividade enzimática e oxidativa do fígado. Foram alojados 840 pintos machos de um dia de idade em um delineamento experimental inteiramente casualizado com seis tratamentos e sete repetições de 20 aves, totalizando 140 aves por tratamento. Os tratamentos aplicados foram: ração sem adição de promotor de crescimento (PC), ração com PC e, os demais, rações sem PC e adição de ACC nos níveis de 0,25; 0,50; 0,75 e 1%. As variáveis bioquímicas do sangue analisadas foram: ácido úrico, colesterol total, HDL, LDL, creatinina, AST, ALT, triglicérides, número total de hemácias, hemoglobina, hematócrito, volume corpuscular médio, concentração de hemoglobina corpuscular, proteína plasmática total, leucócitos totais, heterófilos, linfócitos, plaquetas e a relação heterófilos/linfócitos. Para os parâmetros enzimáticos e oxidativos do fígado das aves foram analisados: concentração da superóxido desmultase, glutationa peróxidase e malondialdeído. Não houve diferenças significativas entre os tratamentos nos parâmetros sanguíneos e na atividade enzimática e oxidativa do fígado dos frangos, indicando que o uso do anacardato de cálcio, como fonte de ácido anacárdico, não é tóxico e não afeta esses parâmetros.

Palavras-chave: Aditivos. Ácido anacardico. Hemograma. Leucograma.

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INTRODUCTION

The constant use of subclinical doses of antibiotics in animal feed has been a cause for concern, and as a result, several countries have banned the use of antibiotics as performance enhancers in feed (BRENES, ROURA, 2010, KOYAMA *et al*., 2014). However, the search for natural additives as alternatives to antibiotics has increased, and products extracted from plants show great potential for this purpose.

Phytogenic additives act by controlling potential pathogenic agents and benefitting the modulation of intestinal microbiota. In addition to antimicrobial action, several plant extracts are known to have antiviral, anticoccidial and fungicidal action, as well as their antioxidant properties (MURUGESAN *et al*., 2015). Some studies have demonstrated the benefits of the antioxidant activity of plant extracts, reducing the negative effects of oxidative damage in birds submitted to heat stress, and improving performance, meat quality, immune response and bone quality (HOSSEINI-VASHAN *et al*., 2005; TAWFEK *et al*., 2014).

Anacardic acid is a phenolic compound found in the different parts of the cashew tree (*Anacardium occidentale* L.), the highest proportion being in the cashew nut shell liquid. The biological activities of anacardic acid have been studied, and among them, antimicrobial and antioxidant action has been reported (HA; KUBO, 2005; HAMAD; MUBOFU, 2015; MORAIS *et al*., 2010; TREVISAN *et al*., 2006). The antioxidant action of anacardic acid has been associated, among others, with the inhibition of enzymes such as xanthine oxidase. Anacardic acid could therefore contribute to a reduction in uric acid synthesis (TREVISAN *et al*., 2006). This effect would be important for human health; however, it may harm the birds, as the action of this enzyme is the most important route of excretion of excess metabolic nitrogen, which occurs mainly in the form of uric acid. Furthermore, there is also the possibility that if consumed in excess, anacardic acid can lead to problems of toxicity (ACHANATH *et al*., 2010; TREVISAN *et al*., 2006).

The evaluation of blood parameters, enzyme activity and the oxidative status of some organs, allow a precise estimation of the health and nutritional status of the birds, together clarifying the effects of additives on the organism. In this respect, several studies into the use of organic acids in poultry feed have evaluated the effects on blood parameters and the enzymatic and oxidative activity in the liver (ABDEL-FATTAH *et al*., 2008; KAYA; TUNCER, 2009; NOURMOHAMMADI *et al*., 2010; ÖZEK *et al*., 2011; SOLTAN, 2008; WANG *et al*., 2009; YALCIN; ONBASILAR; KOCAOGLU, 1997).

In view of the above, the aim of this study to evaluate the effects of including calcium anacardate as a source of anacardic acid in broiler feed on blood parameters, and on enzyme activity and the oxidative status of the liver.

MATERIAL AND METHODS

In this research, analyses were made of blood and liver samples from broiler chickens at 35 days of age, obtained from an experiment carried out in the Poultry Sector of the Department of Animal Science at the Universidade Federal do Ceará (UFC).

For the experiment, 840 male Ross 308 chicks, each one day old, vaccinated in the hatchery against Marek's disease and Gumboro disease, were housed in a 15 m x 10 m brick shed, with clay roof tiles, a cement floor and a ceiling height of 3.5 m, which was oriented longitudinally in an east-west direction and contained 48 boxes, 1.5 mx 1.0 m in size.

On the first day, the chicks were weighed, and based on the initial weight (48 ± 0.81 g), distributed in a completely randomised experimental design with six treatments and seven replications of 20 birds each, giving a total of 140 birds per treatment. The birds from each experimental plot were all housed in one box, which was equipped with a hanging drinker and tubular feeder. The floor of the box was covered with reused litter to increase the challenge imposed on the animals.

The applied treatments were poultry feed without the addition of growth promoter (GP), feed with GP, and the remainder, feed with no GP and the addition of CAC at levels of 0.25, 0.50, 0.75 and 1%. The feeding program for the chicks up to 35 days old was divided into starter feed (1 to 21 days) and grower feed (22 to 35 days). The experimental feeds were formulated to be isonutritive and isoenergetic, according to the nutritional requirements recommended by the lineage handbook (Tables 1 and 2). In calculating these diets, the chemical compositions of the ingredients presented by Rostagno *et al*. (2011) were considered.

Anacardic acid was added to the feed in the form of calcium anacardate, the intermediate product in the process to obtain pure acid from cashew nut liquid (CNL). The CNL and calcium anacardate were obtained as per the methodologies described by Trevisan *et al*. (2006), with modifications.

To obtain the CNL, cashew nuts were purchased in the local market and taken to the Natural Products Laboratory of the Department of Organic and Inorganic Chemistry at the Centre for Science of the Universidade

Federal do Ceará. The CNL was extracted by subjecting the cashew nuts to oven heating at a temperature of 120 °C for up to one hour. During this period, as the CNL drained from the nuts and accumulated in the container, it was collected and stored in glass jars.

To extract the calcium anacardate, 550 mL of CNL, 150 mL of distilled water, and 2,850 mL of ethanol were added to a 4 L beaker. This mixture was placed in a heated stirrer (QUIMIS® Q0261-22) and stirred for 4 h at 50 °C, with the temperature constantly monitored. Over

Table 1 - Calculated percentage and nutritional composition of the experimental diets used for broilers from 1 to 21 days of age

Ingredient	Without additives	With additives	Level of Anacardate (%)			
			0.25	0.50	0.75	1\
Corn	55.83	55.83	55.83	55.83	55.83	55.83
Soybean meal	36.05	36.05	36.05	36.05	36.05	36.05
Soybean oil	3.10	3.10	3.10	3.10	3.10	3.10
Dicalcium phosphate	1.90	1.90	1.90	1.90	1.90	1.90
Limestone	0.83	0.83	0.83	0.83	0.83	0.83
Inert	1.00	0.91	0.75	0.50	0.25	-
DL-Methionine	0.30	0.30	0.30	0.30	0.30	0.30
L-lysine HCl	0.29	0.29	0.29	0.29	0.29	0.29
Vit. + mineral supp.[1]	0.20	0.20	0.20	0.20	0.20	0.20
Cholin	0.05	0.05	0.05	0.05	0.05	0.05
Common salt	0.45	0.45	0.45	0.45	0.45	0.45
Calcium anacardate	-	-	0.25	0.50	0.75	1.00
Nicarbazin	-	0.004	-	-	-	-
Monteban	-	0.040	-	-	-	-
BMD11%[2]	-	0.046	-	-	-	-
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutritional and energetic composition*						
Energy Metab. (kcal/kg)	3,000	3,000	3,000	3,000	3,000	3,000
Crude protein (%)	21.16	21.16	21.16	21.16	21.16	21.16
Dry matter (%)	88.80	88.80	88.80	88.80	88.80	88.80
NDF (%)	11.63	11.63	11.63	11.63	11.63	11.63
ADF (%)	4.80	4.80	4.80	4.80	4.80	4.80
Calcium (%)	0.89	0.89	0.89	0.89	0.89	0.89
Avail. phosphoros (%)	0.46	0.46	0.46	0.46	0.46	0.46
Sodium (%)	0.20	0.20	0.20	0.20	0.20	0.20
Chlorine (%)	0.32	0.32	0.32	0.32	0.32	0.32
Total lysine (%)	1.36	1.36	1.36	1.36	1.36	1.36
Total methionine (%)	0.60	0.60	0.60	0.60	0.60	0.60
Total Meth.+ cystine (%)	0.94	0.94	0.94	0.94	0.94	0.94
Total threonine (%)	0.82	0.82	0.82	0.82	0.82	0.82
Total tryptophan (%)	0.26	0.26	0.26	0.26	0.26	0.26

[1]Mineral vitamin supplement (composition per kg of product): vit. A - 5,500,000 IU; vit. B1 - 500 mg; vit. B12 - 7,500 mcg; vit. B2 = 2,502 mg; vit. B6 - 750 mg; vit. D3 - 1,000,000 IU; vit. E - 6,500 IU; vit. K3 = 1,250 mg; biotin - 25 mg; niacin -17.5 g; folic acid - 251 mg; pantothenic acid - 6,030 mg; cobalt - 50 mg; Copper - 3,000 mg; Iron - 25 g; Iodine - 500 mg; Manganese - 32.5 g; Selenium - 100.50 mg; Zinc - 22.49 g; [2]*Bacitracin methylene disalicylate* 11%**;**
* Value obtained by multiplying the amount of the ingredient in the diet and the value of its composition as proposed by Rostagno *et al*. (2011)

Table 2 - Calculated percentage and nutritional composition of the experimental diets used for broilers from 22 to 35 days of age

Ingredient	Without additives	With additives	Level of Anacardate (%)			
			0.25	0.50	0.75	1
Corn	60.90	60.90	60.90	60.90	60.90	60.90
Soybean meal	30.93	30.93	30.93	30.93	30.93	30.93
Soybean oil	3.67	3.67	3.67	3.67	3.67	3.67
Dicalcium phosphate	1.66	1.66	1.66	1.66	1.66	1.66
Limestone	0.76	0.76	0.76	0.76	0.76	0.76
Inert	1.00	0.90	0.75	0.50	0.25	-
DL-Methionine	0.24	0.24	0.24	0.24	0.24	0.24
L-lysine HCl	0.23	0.23	0.23	0.23	0.23	0.23
Vit. + mineral supp.[1]	0.15	0.15	0.15	0.15	0.15	0.15
Cholin	0.05	0.05	0.05	0.05	0.05	0.05
Common salt	0.41	0.41	0.41	0.41	0.41	0.41
Calcium anacardate	-	-	0.25	0.50	0.75	1.00
Salinomycin	-	0.05	-	-	-	-
BMD11%[2]	-	0.05	-	-	-	-
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutritional and energetic composition*						
Energy Metab. (kcal/kg)	3,100	3,100	3,100	3,100	3,100	3,100
Crude protein (%)	19.15	19.15	19.15	19.15	19.15	19.15
Dry matter (%)	88.75	88.75	88.75	88.75	88.75	88.75
NDF (%)	11.53	11.53	11.53	11.53	11.53	11.53
ADF (%)	4.55	4.55	4.55	4.55	4.55	4.55
Calcium (%)	0.80	0.80	0.80	0.80	0.80	0.80
Avail. phosphoros (%)	0.41	0.41	0.41	0.41	0.41	0.41
Sodium (%)	0.18	0.18	0.18	0.18	0.18	0.18
Chlorine (%)	0.30	0.30	0.30	0.30	0.30	0.30
Total lysine (%)	1.18	1.18	1.18	1.18	1.18	1.18
Total methionine (%)	0.52	0.52	0.52	0.52	0.52	0.52
Total Meth.+cystine (%)	0.83	0.83	0.83	0.83	0.83	0.83
Total threonine (%)	0.75	0.75	0.75	0.75	0.75	0.75
Total tryptophan (%)	0.23	0.23	0.23	0.23	0.23	0.23

[1]Mineral vitamin supplement (composition per kg of product): vit. A - 5,500,000 IU; vit. B1 - 500 mg; vit. B12 - 7,500 mcg; vit. B2 = 2,502 mg; vit. B6 - 750 mg; vit. D3 - 1,000,000 IU; vit. E - 6,500 IU; vit. K3 = 1,250 mg; biotin - 25 mg; niacin -17.5 g; folic acid - 251 mg; pantothenic acid - 6,030 mg; cobalt - 50 mg; Copper - 3,000 mg; Iron - 25 g; Iodine - 500 mg; Manganese - 32.5 g; Selenium - 100.50 mg; Zinc - 22.49 g; [2]*Bacitracin methylene disalicylate* 11%**;**
* Value obtained by multiplying the amount of the ingredient in the diet and the value of its composition as proposed by Rostagno *et al*. (2011)

the course of the procedure, 250 g of calcium hydroxide was added to the mixture. After this step, the mixture was allowed to rest for 1 h to help remove the supernatant. After removing the supernatant, an additional 800 mL of ethanol was added to the material contained in the beaker, and this was again subjected to stirring with heat for another hour. Once this step was finished, the supernatant was drained and the calcium anacardate was placed in a forced air circulation oven at 55 °C and dried for 72 hours. After drying, the material was ground in a mill and stored in plastic pots until mixed with the feed. The amount of anacardic acids present in calcium anacardate is 94.5%.

During the experimental period, the data for maximum and minimum temperature and relative humidity were collected in the early morning and late afternoon by means of maximum and minimum thermometers and a psychrometer respectively. The mean minimum and maximum ambient temperatures in the shed during the experiment were 26.0 and 28.9 °C respectively; relative humidity was 69%.

To collect the blood samples, two birds from each experimental plot were randomly selected at 35 days of age. The birds continued to receive both feed and water until the blood was collected.

For the haemogram, the blood of each bird was collected through a puncture of the brachial vein, located on the wing, using a needle with a 3 mL disposable syringe. After aspirating, the blood was placed into suitable sample vials containing EDTA (BD Microtrainer). Haemoglobin was determined with a veterinary automatic blood cell counter (Hemascreen 18). Globular volume was determined by means of the microhaematocrit technique using Perfecta® capillary tubes and a microhaematocrit centrifuge. Total plasma proteins were determined by refractometry, using a hand refractometer (QUIMIS® Q767). Haematimetry, global leucometry and thrombocytometry were determined with a single dilution to display erythrocytes, leukocytes and thrombocytes using Natt and Herrick's solution. The particular leucometry technique was carried out by haematoscopy at a 1000 x magnification (immersion) of the smears stained by May-Grunwald-Giemsa solution (MGG).

For the biochemical analysis, another blood sample was collected by cardiac puncture, placed in suitable tubes, and left to coagulate at room temperature, with subsequent centrifugation at 3,000 rpm for 15 minutes. After centrifugation, the supernatant (serum) was removed, and the sample placed into 1.5 mL eppendorf tubes, so that each aliquot was suitably packed for subsequent use in the respective determinations.

The biochemical blood analysis was automated (Metrolab 2300 plus) using Weiner Kinetic kits, as per the manufacturer's instructions. Each analyte under test was individually processed by spectrophotometry with regulated temperature of the incubation cuvettes, the results being printed by the device itself immediately after conclusion of the analysis. The following were measured: Uric acid, creatinine (Cr), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol, HDL, LDL and triglycerides (TAG).

After collecting the blood, the birds were euthanised by electro-narcosis, followed by bleeding with a cut to the jugular. The livers were then removed, immediately frozen in liquid nitrogen, and stored at -80 °C until analysis of the enzymatic activity with a mixture of superoxide dismutase (SOD) and the non-protein sulfhydryl groups (NP-SH).

SOD activity was determined as per Beauchamp and Fridovich (1971). The tissue was homogenised in a 50 mM potassium phosphate buffer at pH 7.8 to obtain a 10% homogenate, and then centrifuged at 3,600 rpm for 10 minutes at 4 °C. The supernatant was removed and centrifuged again at 12,000 rpm for 20 minutes at 4 °C. In a dark room, 1 mL of the reaction medium (50 mM potassium phosphate buffer, 100 nM EDTA, and 13 mM L-methionine, pH 7.8), 150 µL of 750 µM NBT, and 300 µL of 2 µM riboflavin were added to 10 µL of the supernatant. The tubes containing the resulting solution were exposed to a fluorescent lamp (15 W) for 15 minutes. Absorbance was measured at 560 nm. The results were expressed in enzyme units, which is the amount of SOD required to inhibit the rate of NBT reduction by 50%.

Glutathione (GSH) concentration in the liver was evaluated using the test for non-protein sulfhydryl groups - NP-SH (SEDLAK; LINDSAY, 1968). The tissue was homogenized in ice-cold EDTA solution (0.02 M) to prepare the 10% homogenate. Then, 0.4 mL of distilled water and 0.1 mL of 50% trichloroacetic acid were added to a 0.5 mL aliquot of the homogenate to precipitate the proteins. Following this step, the material was centrifuged at 3,000 rpm for 15 min at 4 °C. Fresh 0.5 mL aliquots of the supernatant were then mixed into 1 ml of 0.4 M Tris buffer, pH 8.9, and 25 µl 0.01 M Dithiobisnitrobenzoate (DTNB). The absorbance was measured within 5 min at 412 nm against a white reagent (with no homogenate). The NP-SH concentration was calculated using a standard reduced glutathione (GSH) curve and the results expressed in µg NP-SH/g tissue.

Lipid peroxidation was determined by estimation of malondialdehyde (MDA) using the thiobarbituric acid test (AGAR *et al*., 1999). The tissue and blood serum were homogenised in 10% KCl buffer (pH 7.4) to prepare the 10% homogenate. Then, 250 µL of the homogenate was incubated in a water bath at 37 °C for 60 min. After incubation, 400 µL of 35% perchloric acid was added, and the samples centrifuged at 14,000 rpm for 10 min. Two hundred µL of 1.2% thiobarbituric acid was added to 600 µL of the supernatant. The mixture was placed in a water bath at 95-100 °C for 30 min. The solution was then removed and left at room temperature. The absorbance reading was carried out at 532 nm. The standard curve was obtained using 1,1,3,3-tetramethoxypropane. The results were expressed as nanomoles of MDA per gram of tissue (nmol/g tissue).

The data obtained in all treatments were submitted to analysis of variance by the ANOVA procedure of the SAS software (2009), and to a comparison of means

by SNK test. To determine the best inclusion level for calcium anacardate in the feed, data from the treatments with calcium anacardate (0.25, 0.50, 0.75 and 1% CAC) were submitted to regression analysis. All the analyses considered a significance level of 5%.

RESULTS AND DISCUSSION

For the values for uric acid, total cholesterol, HDL, LDL, creatinine, AST, ALT and triglycerides (Table 3) there was no significant effect ($p>0.05$) from the treatments on any variable under analysis.

Generally, serum biochemical constituents reflect the health, nutrition, climate and management conditions to which the animals are submitted (MINAFRA *et al*., 2010). The levels of biochemical parameters in the blood can therefore be used as an indication of the productive performance of the birds and of metabolic diseases (ROTAVA *et al*., 2008). As the blood uric acid and creatinine levels of the birds were not influenced by the CAC levels in the feed, it can be said that there was no impairment of renal function, as per Konan *et al*. (2007). Furthermore, no impairment of hepatic function was found, as there was no change in the activity of the enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Based on these results, it can be inferred that CAC, at the levels used in the feed, was not toxic to the chickens.

Considering that the antioxidant activity of anacardic acid largely stems from its ability to inhibit various oxidative enzymes, such as xanthine oxidase, which is the enzyme responsible for the conversion of xanthine and hypoxanthine to uric acid (TREVISAN *et al*., 2006), there could a reduction in the blood levels of uric acid with the increasing inclusion of CAC in the feed, confirming the inhibition of xanthine oxidase. On the other hand, inhibition of this enzyme could be a problem for the birds, whose main form of nitrogen excretion is in the form of uric acid. It is worth noting that the inhibitory effects on this enzyme have been reported in rats, and it is not yet clear how anacardic acid or its metabolites are able to regulate this enzymatic activity in the cell; further studies are needed to understand the action of anacardic acid on this enzyme (KUBO *et al*., 2006).

The results obtained in the present study agree with those reported by Nourmohammadi *et al*. (2010), who evaluated the effects of citric acid in the diet of broilers up to an inclusion level of 6%, and saw no significant effects of this acid on the values of uric acid or triglycerides. Özek *et al*. (2011), evaluating the inclusion of organic acid, essential oil, and a mixture of essential oil and organic acid in the feed of laying hens, found no significant effects of the treatments on biochemical variables of the

Table 3 - Biochemical parameters of broilers fed calcium anacardate in the diet, at 35 days of age

Treatment	Variable							
	Uric acd mg/dL	T. Chol mg/dL	HDL mg/ dL	LDL mg/dL	Creatinin mg/dL	AST U/L	ALT U/L	Trig mg/ dL
No additives	4.90	191.17	37.333	153.83	0.2433	233.00	13.333	192.83
With additives	4.85	181.83	38.667	143.17	0.2217	211.00	14.000	168.50
0,25% CAC	4.98	198.83	38.667	160.17	0.2117	206.00	15.500	163.83
0,50% CAC	5.00	197.00	39.000	158.00	0.2500	223.17	12.333	181.17
0,75% CAC	5.12	194.67	40.833	147.17	0.2500	201.00	14.667	171.33
1% CAC	4.90	186.33	39.000	147.33	0.2400	229.83	13.667	166.33
ANOVA	p-value							
Treatment	0.9895	0.6861	0.8306	0.7485	0.4842	0.6494	0.6195	0.1927
Regression	p-value							
Linear	0.9205	0.2952	0.6759	0.2512	0.3028	0.5954	0.4786	0.9563
Quadratic	0.7024	0.7951	0.4817	0.9042	0.1858	0.4224	0.7124	0.2446
CV(%)	14.35	10.63	10.91	14.93	17.20	18.17	22.83	12.28

Mean values followed by different letters in a column differ by SNK test (5%); T. Chol. Total cholesterol; AST-aspartate aminotransferase; ALT-alanine aminotransferase; Trig-triglycerides

blood (total cholesterol and triglycerides). According to Kaya and Tuncer (2009), a mixture of organic acid with essential oils in broiler feed did not affect total cholesterol or triglyceride levels. The values reported by those authors were close to those obtained in this study.

Unlike the effects observed in the present research, the results obtained by Abdel-Fattah *et al*. (2008) demonstrated that the addition of citric acid to the feed increases the concentration of total proteins and reduces total cholesterol and triglycerides. For Al-Saad *et al*. (2014), the use of prebiotics, probiotics and organic acid as growth promoters reduced total cholesterol and triglyceride levels in the blood of broilers. Whereas for Nourmohammadi *et al*. (2010), the use of citric acid increases the total blood cholesterol of chickens, and for Yalcin, Onbasilar and Kocaoglu (1997), the use of 5% lactic acid in the feed increased the levels of total blood cholesterol in quails.

Al-Saad *et al*. (2014), studying the influence of organic acids (sorbic, propionic, benzoic and phosphoric acid) as growth promoter for broiler chickens, found no significant effects of these acids on the variables HDL and LDL when adding 1000 g per ton of feed. Nourmohammadi *et al*. (2010) saw no significant effect on the enzymatic activity of AST and ALT. The results of the authors cited above are similar to those of the present research. Biavatti *et al*. (2003) reported an increase in AST values in treatments with linseed oil, in relation to the control, a different result to that found in this study; however, no effect was reported on ALT. Those authors attributed hepatic toxicity to flaxseed oil used as an alternative additive for broiler chickens.

The values for erythrocytes, haemoglobin, haematocrit, mean corpuscular haemoglobin concentration, mean corpuscular volume, and total proteins (Table 4) did not differ significantly ($p>0.05$) as a function of the applied treatments.

The results found in this research for the number of erythrocytes agree with those presented by Al-Saad *et al*. (2014), who used a mixture of sorbic, propionic, benzoic and phosphoric acids in broiler feed, and saw no significant effects on the number of erythrocytes, haemoglobins or haematocrit when compared to the feed with no additives.

Hepatic lesions may lead to a decrease in the concentration of total plasma proteins, as the liver is the organ that synthesises proteins, especially albumin. Therefore, a reduction in these proteins with the use of a feed or an additive may be associated with the toxic effects of these diets (SCHMIDT *et al*., 2007). In this respect, the addition of calcium anacardate did not promote hepatic damage, since it did not alter the amount of total proteins in the chicken plasma, agreeing with the results for the values of AST and ALT.

The addition of phenyllactic acid (WANG *et al*., 2009) and fumaric acid plus organic salt (SOLTAN, 2008) in poultry feed resulted in an increase in the concentration of total proteins in the blood. On the other hand, Yalcin, Onbasilar and Kocaoglu (1997) found that the use of 5%

Table 4 - Erythrogram of broilers fed calcium anacardate in the diet, at 35 days of age

Tratamentos	Variáveis					
	He (106µl)	Hb (g/dL)	Ht (%)	MCV (fL)	MCHC (%)	TPP (g/dL)
No additives	2.48	9.97	30.333	156.78	31.64	7.07
With additives	2.56	9.60	30.333	154.92	32.66	7.13
0.25% ACC	2.33	9.77	30.667	156.11	31.55	7.10
0.50% ACC	2.40	9.50	30.500	151.04	32.19	7.42
0.75% ACC	2.39	9.93	32.167	157.65	31.88	7.27
1% ACC	2.45	10.02	33.500	152.75	31.76	7.40
ANOVA	p-value					
Treatment	0.8270	0.8743	0.4570	0.9837	0.9753	0.8730
Regression	p-value					
Linear	0.5376	0.4302	0.0796	0.9167	0.9504	0.5642
Quadratic	1.0000	0.6067	0.5566	0.9910	0.7397	0.8994
CV(%)	12.20	8.86	10.42	11.00	7.93	8.70

Mean values followed by different letters in a column differ by SNK test (5%); He-total number of erythrocytes; Hb-haemoglobin; Ht-haematocrit; MCV-mean corpuscular volume; MCHC-mean corpuscular haemoglobin concentration; TPP-total plasma protein

lactic acid in the feed increased the serum level of total proteins in quail blood, when compared to the feed with no organic acid.

For Wang *et al*. (2009), the concentration of white blood cells was increased by a 0.1% phenyllactic acid supplement, whereas the concentration of red blood cells increased with a supplement of 0.1, 0.2, and 0.3% of that acid. Those authors suggest that the acid can be used up to a level of 0.3% in poultry feed to stimulate the immune system, since it is well known that intestinal microorganisms are necessary for development of the intestinal immune system.

The values for MCV and MCHC found in this study agree with the normal parameters considered by Tessari *et al*. (2006) and Borsa *et al*. (2009), who reported that the lack of change in these parameters may be related to good nutrition and the absence of challenges found in the places where the experiments are conducted.

No significant effects from the treatments were seen on the variables total leukocytes, heterophils, lymphocytes, platelets or the heterophil/lymphocyte ratio (Table 5).

According to Wang *et al*. (2009), the use of organic acid (phenyllactic acid) significantly increased the concentration of leukocytes and lymphocytes, partially improving blood characteristics in the short term. These results differ from those found in the present study.

The ratio of heterophils to lymphocytes was not altered by the addition of calcium anacardate. The mean values found for this ratio show that the birds suffered moderate stress. These results agree with Gross and Siegel (1993), who classify a value for the heterophil/lymphocyte ratio of 0.2 as indicating mild stress, 0.5 moderate stress and 0.8 high stress.

The differences in some biochemical and haematological parameters found in this study in relation to other authors can be attributed to such factors as species, management, nutrition and level of stress suffered by the animals under study.

For the enzymatic and oxidative parameters in the liver of chickens fed calcium anacardate in the diet, no significant effects ($p>0.05$) from the treatments were seen on these variables (Table 6). Such results demonstrate that the use of calcium anacardate in the feed had no influence on the enzymatic and oxidative parameters in the liver of the chickens.

SOD and GSH did not present any significant differences ($p>0.05$) in their activities with the increasing levels of calcium anacardate in the feed. Different results were seen by Hosseini-Vashan *et al*. (2012), who reported an increase in SOD and GSH activity in broilers fed powdered saffron in the diet. These authors concluded that, since SOD is the first enzyme that contributes to the antioxidant defence system of the body, a high concentration of this enzyme could improve the balance of the antioxidant system of chicken meat, contributing to an increase in shelf life. For Tawfeek *et al*. (2014), the increase in the activity of the enzyme GSH with the use of antioxidants (Vit.

Table 5 - Leucogram of broilers fed calcium anacardate in the diet, at 35 days of age

Treatament	Variáveis				
	T. Leuc.(106/ µl)	Heterophils (%)	Lymphocytes (%)	Platelets (103/ µl)	H/L
No additives	14.000	22.333	56.000	40.000	0.453
With additives	14.150	24.000	57.500	41.167	0.375
0.25% ACC	15.117	23.500	53.833	40.333	0.427
0.50% ACC	14.667	24.667	55.333	42.333	0.430
0.75% ACC	15.033	24.500	55.333	42.833	0.443
1% ACC	14.833	24.000	53.333	44.000	0.452
ANOVA			p-value		
Treatament	0.8871	0.6405	0.8743	0.9559	0.6277
Regression			p-value		
Linear	0.8971	0.7387	0.8905	0.4353	0.4120
Quadratic	0.8839	0.3570	0.4802	0.9010	0.9186
CV(%)	13.36	10.49	11.18	19.84	19.80

Mean values followed by different letters in a column differ by SNK test (5%); T. Leuc-Total leukocytes; H/L-heterofil to lymphocyte ratio

Table 6 - Enzymatic and oxidative parameters in the liver of broilers fed calcium anacardate in the diet, at 35 days of age

Treatment	Variable		
	SOD (U/L)	GSH (U/L)	MDA (µ/M)
No additives	0.1666	142.85	13.88
With additives	0.1697	136.75	14.22
0.25% ACC	0.1796	137.28	14.05
0.50% ACC	0.1766	136.02	14.59
0.75% ACC	0.1698	140.74	14.29
1% ACC	0.1622	141.43	14.69
ANOVA		p-value	
Treatment	0.8194	0.8774	0.9207
Regression		p-value	
Linear	0.2173	0.2606	0.3923
Quadratic	0.8195	0.9288	0.6721
CV(%)	13.11	8.56	10.65

Mean values followed by different letters in a column differ by SNK test (5%); SOD (superóxido dismultase); GSH (glutationa peroxidase); MDA (malondialdeído)

E+C, Zn+Se and Cr) reduces the negative impact of heat stress. The same authors also report that supplementing diets with antioxidants, especially vitamins and chromium, is essential to overcoming the harmful effects of thermal stress on the oxidative status and performance of broilers.

The level of MDA is an important variable in evaluating oxidative stress (SIM *et al*., 2003). Therefore, since in this study there was no significant difference ($p>0.05$) between treatments for the amount of hepatic MDA, it can be inferred that the conditions that would increase lipid oxidation in the liver of the birds did not occur, as the control group showed no increase in hepatic MDA, and also that, in doses of up to 1%, calcium anacardate has no negative effect on this variable. The benefit of the addition of antioxidants on hepatic oxidation in chickens was reported by Hosseini-Vashan *et al*. (2012), who obtained a decrease in MDA with increasing levels of saffron powder (0.4 and 0.8%). According to the researchers, under conditions of heat stress, saffron powder reduced oxidative reactions in the body of the chickens, as well as the rates of MDA production, improving the quality of the meat due to a reduction in free radicals.

The absence of significant results from the addition of calcium anacardate in the feed of broiler chickens can be seen as an indication that this product does not promote any toxic or antinutritional effects that would influence the enzymatic and oxidative parameters in the blood of the birds.

CONCLUSION

The addition of up to 1% calcium anacardate as the source of anacardic acid in the diet of broiler chickens does not affect the blood parameters or the enzymatic and oxidative parameters in the liver of the birds.

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