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BIOMEDICAL SCIENCES

Acrocomia aculeata (Jacq.) improves the antioxidant system but induces lipid accumulation in the liver of rats

FRANCYELE DOS S. CORREIA, ELAINE C.L. SPADA, BRUNA C.P. ESTEVAM, THAYANNE C.O. CONCEIÇÃO, GUILHERME N. DA CRUZ, WÉLITON B. DA SILVA, MAÍSA P.S. ELIAS & SUÉLEM A.F. LEMES

Abstract: *Acrocomia aculeata* pulp (ACP) is a source of oleic acid, phenolic compounds, and flavonoids that protect against diseases and improve antioxidant capacity. We evaluated whether regular intake of ACP, in combination with a standard diet, improves the antioxidant system and physiological parameters. Male Wistar rats were divided into: control (C), 250 mg/kg ACP, and 500 mg/kg ACP groups. Rats received either water or the respective *A. aculeata* solution doses for 28 days. We observed increased food intake, lower carcass protein levels, and higher carcass lipid levels in the 500 mg/kg ACP group than in the other groups. Postprandial glucose, oral glucose tolerance test results, and the area under the curve were greater, while urea was lower in the 500 mg/kg ACP group. Total liver lipids were increased, and PPAR-α, PPARγ, and carbonylated protein levels were reduced in the 500 mg/kg ACP group. NRF2 contents and glutathione reductase, superoxide dismutase, and catalase activities were increased in the 500 mg/kg ACP group. In the 250 mg/kg ACP group, only glutathione system activity increased. Thus, ACP intake improved the enzymatic antioxidant system in the liver at the evaluated doses, although the 500 mg/kg dose induced alterations in lipid, protein, and carbohydrate metabolism.

Key words: *Acrocomia aculeata*, antioxidant System, bioactive Compounds, lipid Metabolism.

INTRODUCTION

Acrocomia aculeata (Jacq.) Lodd. ex Mart. is a species belonging to the family Arecaceae, popularly known as *macaúba* or *bocaiuva,* and is distributed in Central, North, and South America (Diaz et al. 2021). The plant reaches up to 20 meters in height and produces spherical fruits, yellowish green in color, which are edible and have a pleasant aroma. The stem, leaves, and fruits can be used for different purposes (Souza et al. 2020). The pulp of the fruit of *A. aculeata* is consumed by humans *in natura* or in the form of flour, jelly, and cakes, among other products (Silva et al. 2018). In addition, the oil obtained from the mesocarp and seed of *A. aculeata* has aroused commercial interest due to its use in food, pharmaceuticals, cosmetic products, and biodiesel production (Valério et al. 2019, Teixeira et al. 2017).

The oil extracted from the fruit pulp is a source of monounsaturated fatty acids (MUFA), particularly oleic acid (accounting for 50–70% of the oil) (Costa et al. 2020). Previous studies have shown an association between the consumption of MUFA and a reduction in risk factors for coronary heart disease and other chronic diseases (Gillingham et al. 2011, Lotfi et al. 2021),

in addition to improving the fatty acid oxidation capacity and antioxidant defense system of cells (Haeiwa et al. 2014, Lim et al. 2013). High concentrations of bioactive compounds, such as carotenoids, in particular β-carotene (Schex et al. 2018) and phenolic compounds (Correia et al. 2022), are found in *A. aculeata* pulp (ACP). These compounds have several biological activities, such as anti-inflammatory and antimicrobial activities, and are potent antioxidants used in the inhibition of reactive species that act directly on the human antioxidant system (Lotfi et al. 2021). The antioxidant and anti-inflammatory activities of polyphenols and carotenoids are attributed to their ability to induce antioxidant and anti-inflammatory gene expression (Rosillo et al. 2014, Piroddi et al. 2017), which down-regulate NF-kB activity and expression of inflammatory genes and upregulate peroxisome proliferator-activated receptor (PPAR)α and protein content of nuclear factor erythroid-related (NRF)2, improving the antioxidant defense and fatty acid oxidation, leading to cell protection (Valenzuela et al. 2017, Valenzuela & Videla 2018, Mucha et al. 2021).

Previous studies by our research group showed that the mesocarp of *A. aculeata* harvested in the State of Mato Grosso is a significant source of lipids, carbohydrates, phenolic compounds, and flavonoids. In vitro analysis also revealed a significant ability of *A. aculeata* mesocarp to reduce 2,2-difenil-1-picril-hidrazil free radicals (Correia et al. 2022).

Considering the composition of the *A. aculeata* mesocarp in terms of bioactive compounds and their relationship with the antioxidant system and fatty acid oxidation, we investigated the metabolic and antioxidant effects of supplementation with the lyophilized mesocarp of *A. aculeata* in adult rats. We hypothesized that regular intake of *A. aculeata* mesocarp would be associated with intake of a standard lipid diet and would improve the antioxidant system and long-term anthropometric, biochemical, and metabolic parameters. We considered that supplementation in the form of flour is the most accessible form for the population and is economically viable. However, the impact of the consumption of this flour as part of a habitual diet needs to be clarified. Therefore, to test this hypothesis, we evaluated the effects caused by the intake of *A. aculeata* mesocarp: (i) on nutritional, metabolic, and biochemical parameters; (ii) on oxidative damage markers and the activity of antioxidant enzymes; and (iii) on the NRF2, PPARα, and PPARγ contents in the liver.

ABBREVIATIONS

ACP	Aculeata pulp
ALT	Alanine aminotransferase
AP	Alkaline phosphatase
AUC	Area under the curve
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CAT	Catalase
EDL	Extensor digitorum longus
GPx	Glutathione peroxidase
GR	Glutathione reductase
IBAT	Interscapular brown adipose tissue
MUFA	Monounsaturated fatty acids
OGTT	Oral glucose tolerance test
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid-reactive substances

MATERIALS AND METHODS

Preparation of lyophilized pulp of bocaiuva (*A. aculeata*)

Fruit collection took place in the municipalities of Poconé and Cuiabá-MT, located in a region of Cerrado Mato Grosso (16° 16'21.6" S, 56°

37'34.5" W; and 15.6º 97'47.0" S, 56.06º 00'12.0" W, respectively). The formal identification of the plant material used in this study was confirmed by Dr. Suélem Aparecida de França Lemes (only the Cuiabá voucher specimen was deposited in the herbarium center of UFMT [number: 44.463]). Access to botanical materials was registered in the Management System of Genetic Patrimony and Associated Traditional Knowledge (SISGEN), Brazil (no. AC676AC). Ripe fruits were picked, and rotten fruits were removed. The remainder was cleaned with water and a neutral detergent and allowed to dry in a cool place at room temperature. After drying, fruits were manually peeled and frozen to facilitate pulp removal. After freezing, the pulp was removed manually, stored in plastic bags, duly identified, frozen at -20 °C, and lyophilized. The proximate composition of the pulp and the bioactive compounds present in the flour, such as total phenolic compounds, flavonoids, and antioxidant potential, were previously described by Correia et al. (2022).

Animals, treatment, and sample collection

Initially, a pilot study [unpublished] was conducted to determine the appropriate doses for supplementing animals. In this pilot study, 30 adult rats (180–200 g) were given *A. aculeata* solution (ACP) at doses of 500 and 1,000 mg/kg for 30 days. The control group was administered distilled water. Body weight, water intake, and food intake were monitored daily for 30 days. At the end of the experimental period, biochemical parameters in the blood of the rats were evaluated. There were no changes related to mortality and toxicity. Furthermore, studies of the oil of *A. aculeata* by Traesel et al. (2014) demonstrated the absence of toxicity at a dose of 2,000 mg/kg in rats and subaggressive (28-day) treatment at doses of 125, 250, 500, or 1,000 mg/kg. Based on these studies, we selected doses of 250 and 500 mg/kg.

To evaluate the effects of supplementation with *A. aculeata*, male Wistar rats weighing 200–220 grams (7 weeks old) were made available by the Central Bioterium of the Federal University of Mato Grosso. The animals were divided into three groups (n = 8 per group): control (C), 250 mg/kg *A. aculeata* pulp (250 mg/kg ACP), and 500 mg/kg *A. aculeata* pulp (500 mg/kg ACP). Group C received vehicle (distilled water) orally by gavage, while the 250 mg/kg ACP and 500 mg/kg ACP groups received aqueous solutions of ACP at their respective concentrations (250 and 500 mg/kg body weight, by oral gavage, respectively). The solutions were prepared in the morning after weighing the animals to determine the amount of pulp necessary to prepare the concentration for each group. Distilled water was used to prepare the solutions.

Supplementation was given for 28 days, and the animals in all groups were fed normal rat chow (Labina Nuvilab®) (Table I). Water intake, urine volume, body weight, and food intake were monitored daily. Glycemia was monitored every 5 days during and again at the end of the supplementation period. Animals of both groups were housed individually in metabolic cages, under a 12-h light/dark cycle, at a temperature of 25 ± 2 °C, and had access to water and food *ad*

Table I. Ingredients and chemical composition* (g/ Kg) of Labina feed.

Ingredient	g/Kg	
Labina feed	1,000	
Chemical composition*	g/Kg	KJ/Kg
Carbohydrate	504	8434.9
Lipid	59	2221.7
Protein	237	3966.4
Water	105	
Ash	95	
Total	1,000	14623.0

*** Values obtained by laboratory analysis performed by Correia F.S.**

libitum. All experimental procedures followed the guidelines of the National Council for Animal Experimentation Control. The project was approved by the Animal Use Ethics Committee under protocol number 23108.013689/2019-58.

At the end of the experiment (day 29), the animals were fed or fasted for 12 h, sedated (ketamine: xylazine: 150:30 mg/kg, intraperitoneally), and then sacrificed by decapitation. Blood samples were collected in glass tubes with sodium heparin to obtain plasma and in tubes without anticoagulants to obtain serum. The serum and plasma were separated by centrifugation at 131 × *g* for 10 min. Laparotomy was performed to collect the kidneys; liver; retroperitoneal, epididymal, and perirenal white adipose tissues; interscapular brown adipose tissue (IBAT); soleus and extensor digitorum longus (EDL) muscles; and carcasses, which were weighed and frozen at -80 °C for future analysis.

Glycogen, total lipid content, cholesterol, and triglyceride content in the liver

Liver and muscle glycogen contents were determined according to the spectrophotometric method described by Carroll et al. (1956). Total lipid from the liver and adipose tissues was extracted using a chloroform:methanol (2:1) solution and determined using the gravimetric method described by Folch et al. (1957). Cholesterol and triglycerides from the liver were extracted with isopropyl alcohol according to Catta-Preta et al. (2011) and spectrophotometrically determined using the Bioclin® cholesterol and triglyceride monoreagent kits (Quibasa; Belo Horizonte, Minas Gerais, Brazil).

Biochemical parameters in the plasma or serum

The glucose concentration was determined from total blood using an Accu-Chek Active® (Roche) Glucometer Kit. Plasma urea, creatinine, alanine aminotransferase (ALT), and alkaline phosphatase (AP) were measured. Total proteins, albumin, total cholesterol, high-density lipoprotein cholesterol, and triglycerides were determined in the serum. These analyses were performed by spectrophotometric methods using Labtest® kits (Lagoa Santa, Minas Gerais, Brazil) and Gold Analisa Diagnóstica (Belo Horizonte, Minas Gerais, Brazil) kits.

Glycemic response to glucose administration

An oral glucose tolerance test (OGTT) was performed on animals in the C, 250 mg/kg ACP, and 500 mg/kg ACP groups on the 25th day of treatment. The animals were fasted for 12 h and then administered a load of 2.5 grams of glucose/kg of body weight via orogastric gavage. Blood glucose was determined before (0 times) and after a load of glucose (15, 30, 45, 60, 90, and 120 min). Whole blood was used to determine glucose levels using an Accu-Chek Active® Glucometer Kit.

Carcass composition and energy intake

The methodology adopted for the determination of water and ash in the carcasses was described by Aparecida de França et al. (2009). Total lipids in the carcass were determined according to Leshner et al. (1972). The difference between the mass of the fatty sample and that of the defatted sample was equal to the lipid content. The results were expressed as the percentage of lipids in fresh carcasses. Subtraction of the water, lipid, and ash weights from the weight of the fresh carcass determined the protein content of the carcass. Body composition was determined in other group of animals to establish a baseline for the experimental animals' body composition on the first day of the experiment, before the treatment. Carcass energy was calculated based on the protein and

carbohydrate contents (equivalent to 23.8 kJ/g) and lipid contents (equivalent to 39.6 kJ/g), as described by Emmans (1994). The energy gain was obtained from the difference between the energy of the carcass and the control baseline. Energy expenditure was obtained from the difference between energy intake and energy gain. Energy intake was calculated according to total food intake (14.623 kJ/g of diet, Table I) and *A. aculeata* intake according to the dose administered per group (13.21 kJ/g of pulp) (Correia et al. 2022).

Liver antioxidant status

Thiobarbituric acid-reactive substances and carbonylated protein content in the liver

Lipid peroxidation in the liver was determined according to the method described by Percário et al. (1994), using a thiobarbituric acid solution (0.144%) solubilized in a phosphate buffer (75 mM pH 2.5). The results were expressed in µmol/g of liver. The formation of carbonylated proteins in liver samples, mainly as a result of oxidative deamination catalyzed by metals, was determined using the method proposed by Levine et al. (1994). The results were expressed in nmol of carbonylated proteins/mg of protein. The total protein content of the homogenates was determined using the Bradford method (1976).

Enzymatic antioxidant system in the liver

Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and catalase (CAT) activities were measured in the liver. For the determination of SOD, GPx, and GR activity, approximately 250 mg of the tissue was homogenized in 2.5 mL of sodium phosphate buffer (0.1 M, pH 7.0). The homogenate was centrifuged at 10,000 × *g* for 10 min at 4 °C. The supernatant was used to determine antioxidant enzyme activity. For catalase, a homogenate was prepared using 200 mg of tissue and 2 mL of Tris-HCl buffer (50 mM, pH 7.4) and centrifuged at 1,026 × *g* for 10 min at 4 °C; the supernatant was used for the test. Proteins were measured in all homogenates using the Bradford method (1976). The SOD, GPx, and GR activities were determined using commercial kits from Randox® (Randox Laboratories Ltd., Antrim, UK). The results were expressed as U/mg protein.

The CAT activity was determined according to the methodology described by Aebi (1984) using hydrogen peroxide (0.3 M). The results were expressed as U/mg protein.

Western blotting

Western blotting was used to determine the protein content of PPARα (Sigma–Aldrich, St. Louis, MO, USA), PPARγ (Santa Cruz Biotechnology, Dallas, TX, USA), NRF2 (Sigma–Aldrich), and β-actin (Santa Cruz Biotechnology) proteins in the liver. A tissue homogenate was prepared using a specific extraction buffer (50 mM Tris-HCl buffer, pH 7.4, at 4 °C) containing protease and phosphatase inhibitors. The homogenate was centrifuged for 40 min at 2,533 × *g* at 4 °C. The supernatant was used for electrophoresis and protein measurement by the Bradford (1976) method. Laemmli's (1970) buffer was added to the supernatant in a 4:1 ratio. For electrophoresis, the samples were boiled for 5 min, and then 100 µg of protein per lane was applied to a 10% polyacrylamide gel for electrophoretic separation. The proteins were subsequently transferred to a nitrocellulose membrane, which was incubated in a blocking solution for 4 h. Next, the membrane was incubated overnight with the primary antibody corresponding to each of the proteins, and then with a peroxidase-conjugated secondary antibody. The intensity of the bands on the membrane was revealed using ChemiDoc Imaging Systems equipment and quantified

with ImageJ software (Wayne Rasband, National Institute of Health, New York, NY). The results are expressed as a relative proportion, using the internal control (β-actin) for standardization.

Statistical analysis

Statistical analyses were performed using the GraphPad Prism 5.0 program for Windows (GraphPad Inc., San Diego, CA, USA). The normality of the data was tested using the Kolmogorov–Smirnov test. The results are expressed as the mean ± standard error of the mean. Statistical comparisons were performed using a one-way analysis of variance, followed by Tukey's or a Newman–Keuls post-hoc test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Body weight, food and water intake, and urinary volume

The body weight increase in animals in all groups during the experimental period was similar, as shown in Table II. The daily and total food intake (g/100 g body weight) were approximately 10% and 9% higher in the 500 mg/kg ACP group than in the C group and 250 mg/kg ACP group, respectively. No significant differences were observed in water intake or urinary volume among the evaluated groups (Table II).

Organs and tissue mass, total lipids, cholesterol, triglycerides, and glycogen contents in the liver

Organ mass (g/100 g) was similar among the groups (Table III). The total lipid content in the livers of animals in the 500 mg/kg ACP group was 30% higher than that in the C group and 34% higher than that in the 250 mg/kg ACP group. The cholesterol and triacylglycerol contents in the liver were similar among the groups. No differences were observed in the total lipid content of white adipose tissues (retroperitoneal, epididymal, and perirenal) or glycogen content (liver and muscle tissues).

Table II. Body parameters, food and water intake, and urinary volume of animals treated with *A. aculeata* at different doses, measured after 28 days of treatment.

Parameters	Groups		
	C	250mg/kg ACP	500mg/kg ACP
Initial weight (g)	202.0 ± 8.2	203.2 ± 10.2	203.3 ± 5.1
Final weight (g)	350.2 ± 18.7	324.1 ± 17.5	348.6 ± 7.6
Weight gain (g/ 100g)	41.8 ± 2.3	36.9 ± 2.5	41.4 ± 2.3
Daily food intake (g/100g)	8.4 ± 0.2[a]	8.5 ± 0.2[a]	9.3 ± 0.2[b]
Total food intake (g/100g)	236.1 ± 6.6[a]	238.4 ± 4.9[a]	260.1 ± 5.3[b]
Daily water intake (mL/100g)	12.7 ± 0.5	13.2 ± 0.9	13.7 ± 0.6
Total water intake (mL/100g)	356.9 ± 13.8	370.3 ± 24.4	384.5 ± 17.4
Daily urinary volume (mL/100g)	3.2 ± 0.1	3.4 ± 0.1	3.5 ± 0.1
Total urinary volume (mL/100g)	87.2 ± 4.0	96.8 ± 2.8	98.7 ± 5.4

C (control group), 250mg/kg ACP (group supplemented with *A. aculeata* pulp at a dose of 250 mg/kg of the animal) and 500mg/kg ACP (group supplemented with *A. aculeata* pulp at a dose of 500 mg/kg of the animal). Values are expressed as the means ± standard mean error. Different letters represent statistical differences among groups. p<0.05 ANOVA-one way, n= 8 per group.

Carcass composition and energy balance components

The lipid content in the carcass in the 500 mg/kg ACP group was approximately 57% higher than in the C group and 111% higher than that in the 250 mg/kg ACP group (Table IV). The protein content in the 500 mg/kg ACP group was 30% lower than in the 250 mg/kg ACP group. Carcass mass, water content, and ash content were similar among groups. The animals in the 500 mg/kg ACP group had a 17% higher energy intake than those in the C group and 12% higher than those in the 250 mg/kg ACP group. Furthermore, the animals in the 500 mg/kg ACP group showed an increase in carcass energy (18% and 11%, respectively) and energy gain (36% and 52%, respectively) as compared with the C and 250 mg/kg ACP groups. The energy expenditures of the groups were similar.

Biochemical parameters in the plasma

Postprandial glycemia was 10% higher in the 500 mg/kg ACP group than in the C group. Serum urea was approximately 21% lower in the 500 mg/kg ACP group than in the C group. The other parameters did not differ between the groups (Table V).

During treatment, on the 15th and 20th days, the blood glucose of the animals in the 500 mg/kg ACP group was 12% and 10% higher, respectively, than in the C group (Figure 1a). The area under the curve (AUC) evaluated during the whole treatment period was 8.5% higher in the 500 mg/kg ACP group than in the C group (Figure 1b).

Oral glucose tolerance test

Figure 1c shows the glucose levels determined by the OGTT. Glucose levels were mostly similar, except at 30 min, when it was observed that the animals in the 500 mg/kg ACP group presented

Table III. Organs and tissue mass, total lipid, cholesterol, triacylglycerol, and glycogen contents in the liver of animals supplemented with *A. aculeata* at different doses, measured after 28 days of treatment.

	Mass (g/100g)			Lipids content (mg/g of tissue)			Glycogen content (mg/g of tissue)		
	C	250mg/kg ACP	500mg/kg ACP	C	250mg/kg ACP	500mg/kg ACP	C	250mg/kg ACP	500mg/kg ACP
Liver	3.9 ± 0.10	3.9 ± 0.10	3.9 ± 0.18	29.8 ± 2.07^{a}	28.7 ± 0.47^{a}	38.6 ± 1.67^{b}	14.77 ± 4.09	13.93 ± 1.37	13.66 ± 1.02
Kidney	0.81 ± 0.01	0.83 ± 0.03	0.79 ± 0.01						
Retroperitoneal	0.80 ± 0.08	0.73 ± 0.12	1.03 ± 0.07	674.7 ± 8.36	629.3 ± 22.0	668.1 ± 12.87			
Epididymal	0.83 ± 0.080	0.79 ± 0.07	0.91 ± 0.04	646.9 ± 13.06	659.4 ± 14.32	650.3 ± 34.71			
Perirenal	0.21 ± 0.020	0.16 ± 0.03	0.20 ± 0.02	664.7 ± 13.52	661.9 ± 19.35	695.3 ± 20.71			
EDL muscle	0.085 ± 0.002	0.088 ± 0.001	0.084 ± 0.003				0.345 ± 0.07	0.460 ± 0.06	0.314 ± 0.06
Soleus muscle	0.092 ± 0.005	0.088 ± 0.004	0.085 ± 0.002				0.602 ± 0.06	0.635 ± 0.08	0.577 ± 0.04
IBAT	0.135 ± 0.009	0.153 ± 0.008	0.131 ± 0.004						
Liver cholesterol (mg/g of tissue)				2.07 ± 0.08	1.79 ± 0,13	2.14 ± 0.12			
Liver Triacilglycerol (mg/g of tissue)				9.14 ± 0.41	9.02 ± 0.06	10.18 ± 0.57			

C (control group), 250mg/kg ACP (group supplemented with *A. aculeata* pulp at a dose of 250 mg/kg of the animal) and 500mg/kg ACP (group supplemented with *A. aculeata* pulp at a dose of 500 mg/kg of the animal). Values are expressed as the means ± standard mean error. Different letters represent statistical differences among groups. p<0.05 ANOVA-one way, n= 8 per group. (Interscapular brown adipose tissue- IBAT)

glycemia that was 14% and 8.5% higher than in the C and 250 mg/dL ACP groups, respectively. The AUC was higher in the 500 mg/kg ACP group than those in the C (8%) and 250 mg/kg ACP (13%) groups (Figure 1d).

PPARα and PPARγ contents in the liver

The PPARα and PPARγ contents in the liver were both around 40% less in the 500 mg/kg ACP group than those in the C group (Figures 2a and 2b). There were no significant differences in the PPARα and PPARγ contents between the 250 mg/kg ACP group and the C and 500 mg/kg ACP groups.

Oxidative damage markers, enzymatic antioxidant status, and NRF2 content in the liver

Carbonylated protein levels in the 500 mg/kg ACP group were 32% lower than in the C group. The 250 mg/kg ACP group showed a concentration of carbonyl proteins similar to that in the C and 500 mg/kg ACP groups. There were no significant differences in the concentrations of thiobarbituric acid-reactive substances (Table VI).

The animals in the 250 mg/kg ACP and 500 mg/kg ACP groups showed 62% and 64% increased GPx activity, respectively, as compared to the C group (Table VI). On the other hand, the glutathione reductase activity was increased by 49% and 46% in the 250 mg/kg ACP group as compared with the C and 500 mg/kg ACP groups, respectively. Superoxide dismutase and catalase activities were, respectively, 79% and 70% higher in the 500 mg/kg ACP group as compared with those in the C group (Table VI). NRF2 content was 116% higher in the 500 mg/kg ACP group than in the C group (Figure 2c).

DISCUSSION

The fruits of *A. aculeata* (Jacq) Lodd palm. ex Mart. is distinguished from other tropical fruits by its high concentration of starch, fibers, lipids

Table IV. Carcass composition and energy balance components of animals treated with *A. aculeata* at different doses, measured after 28 days of treatment.

Parameters	C	250mg/kg ACP	500mg/kg ACP
Carcass mass (g)	260.4 ± 18.3	229.2 ± 10.2	259.9 ± 6.8
Water (%)	62.2 ± 1.5	61.5 ± 2.1	60.4 ± 1.7
Lipids (%)	10.5 ± 0.4^{a}	7.8 ± 0.9^{a}	16.5 ± 2.2^{b}
Proteins (%)	24.8 ± 1.5^{ab}	29.2 ± 2.7^{a}	20.6 ± 1.4^{b}
Ashes (%)	2.3 ± 0.2	2.9 ± 0.3	2.4 ± 0.2
Energy intake (kJ/100g)	3393 ± 100.9^{a}	3446 ± 76.5^{a}	3984 ± 121.8^{b}
Baseline carcass (kJ)	1412.5 ± 45.0	1412.5 ± 45.0	1412.5 ± 45.0
Carcass energy (kJ/100g)	1021 ± 40 ^{a}	1020 ± 48 ^{a}	1165 ± 59 ^{b}
Energy gain (kJ/100g)	469 ± 73a	399 ± 67^{a}	620 ± 64^{b}
Energy expenditure (kJ/100g)	3521 ± 415	3524 ± 166	3794 ± 151

C (control group), 250mg/kg ACP (group supplemented with *A. aculeata* pulp at a dose of 250 mg/kg of the animal) and 500mg/kg ACP (group supplemented with *A. aculeata* pulp at a dose of 500 mg/kg of the animal). Values are expressed as the means ± standard mean error. Different letters represent statistical differences among groups. p<0.05 ANOVA-one way, n= 8 per group.

(of which 70% comprises oleic acid), and high bioavailability of β-carotene, vitamin C, calcium, magnesium, zinc, and copper (Ramos et al. 2007). Associations between the consumption of these components and the reduction of risk factors for chronic diseases, including coronary heart disease, and improved oxidation capacity and cellular antioxidant systems have been defined. Therefore, in this study, we investigated the effects of supplementation of *A. aculeata* pulp at two different concentrations (250 and 500 mg/kg) on rodents fed a standard diet for 28 days. Our study showed an increase in food and energy intake in animals that received higher doses of *A. aculeata* without changes in body mass, water intake, or urinary volume. The increased energy intake by the 500 mg/kg ACP group led to an increase in energy gain and lipid content, with a reduction in the protein content in the carcass and preservation of the mass of the adipose tissue and skeletal muscles. The alteration in the carcass lipid and protein contents likely contributed to the maintenance of the body and carcass mass of these animals. Our results also showed a reduction in serum urea levels without changes in total protein or serum albumin levels. Therefore, in the 500 mg/kg ACP group, it appears that the use of amino acids as substrates for the synthesis of non-protein compounds or the production of energy was reduced, although not due to a lack of amino acids, as serum total protein and albumin levels were not altered. We attribute this finding to a reduction in the liver content of PPARα in the 500 mg/kg ACP group since this protein is involved in the transcriptional

Table V. Biochemical parameters in the plasma of animals treated with *A. aculeata* at different doses, measured after 28 days of treatment.

Fed	C	250mg/kg ACP	500mg/kg ACP
Glucose (mg/dL)	103 ± 3[a]	112 ± 3[ab]	114 ± 1[b]
Total cholesterol (mg/dL)	122 ± 7	133 ± 8	128 ± 3
HDL cholesterol (mg/dL)	49 ± 4	54 ± 6	54 ± 3
Triglycerides (mg/dL)	158 ± 36	155 ± 31	146 ± 8
Albumin (g/dL)	25 ± 1	25 ± 1	23 ± 0.3
Total proteins (g/L)	73 ± 4	79 ± 2	74 ± 2
Urea (mg/dL)	48 ± 3[a]	47 ± 1[ab]	41 ± 1[b]
Creatinine (mg/dL)	0.410 ± 0.05	0.334 ± 0.07	0.420 ± 0.09
Alanine aminotransferase (U/L)	36 ± 3	38 ± 3	32 ± 3
Alkaline phosphatase (U/L)	172 ± 23	153 ± 14	139 ± 10
Fasting	C	250mg/kg ACP	500mg/kg ACP
Glucose (mg/dL)	80 ± 4	76 ± 3	81 ± 3
Total cholesterol (mg/dL)	125 ± 4	122 ± 6	126 ± 5
HDL cholesterol (mg/dL)	56 ± 6	41 ± 7	57 ± 4
Triglycerides (mg/dL)	96 ± 9	84 ± 13	75 ± 9

C (control group), 250mg/kg ACP (group supplemented with *A. aculeata* pulp at a dose of 250 mg/kg of the animal) and 500mg/kg ACP (group supplemented with *A. aculeata* pulp at a dose of 500 mg/kg of the animal). Values are expressed as the means ± standard mean error. Different letters represent significant differences (p <0.05) according to one-way ANOVA. n=8 per group.

regulation of genes related to transamination, oxidative deamination, the urea cycle, and the oxidation of alpha-keto acids by suppressing the expression of the relevant genes, leading to a reduction in amino acid degradation (Wahli 2002). In addition, supplementation with 500 mg/kg ACP also induced adaptations in the metabolism of carbohydrates and lipids in these animals. Glycemia in the absorptive period in the 500 mg/kg ACP group was higher than that in the controls from the 15th day of treatment and remained high until the end (28th day). The response to oral glucose overload in the 500 mg/kg ACP group included a higher glycemic peak at 30 min after glucose overload with a higher AUC, suggesting a lower glucose tolerance in this group. This may be due to impaired insulin secretion or resistance to insulin signaling. Similarly, Almeida et al. (2020) found a lower glucose tolerance in rats fed a hyperproteic diet supplemented with *A. aculeata*. When supplementation was accompanied by physical

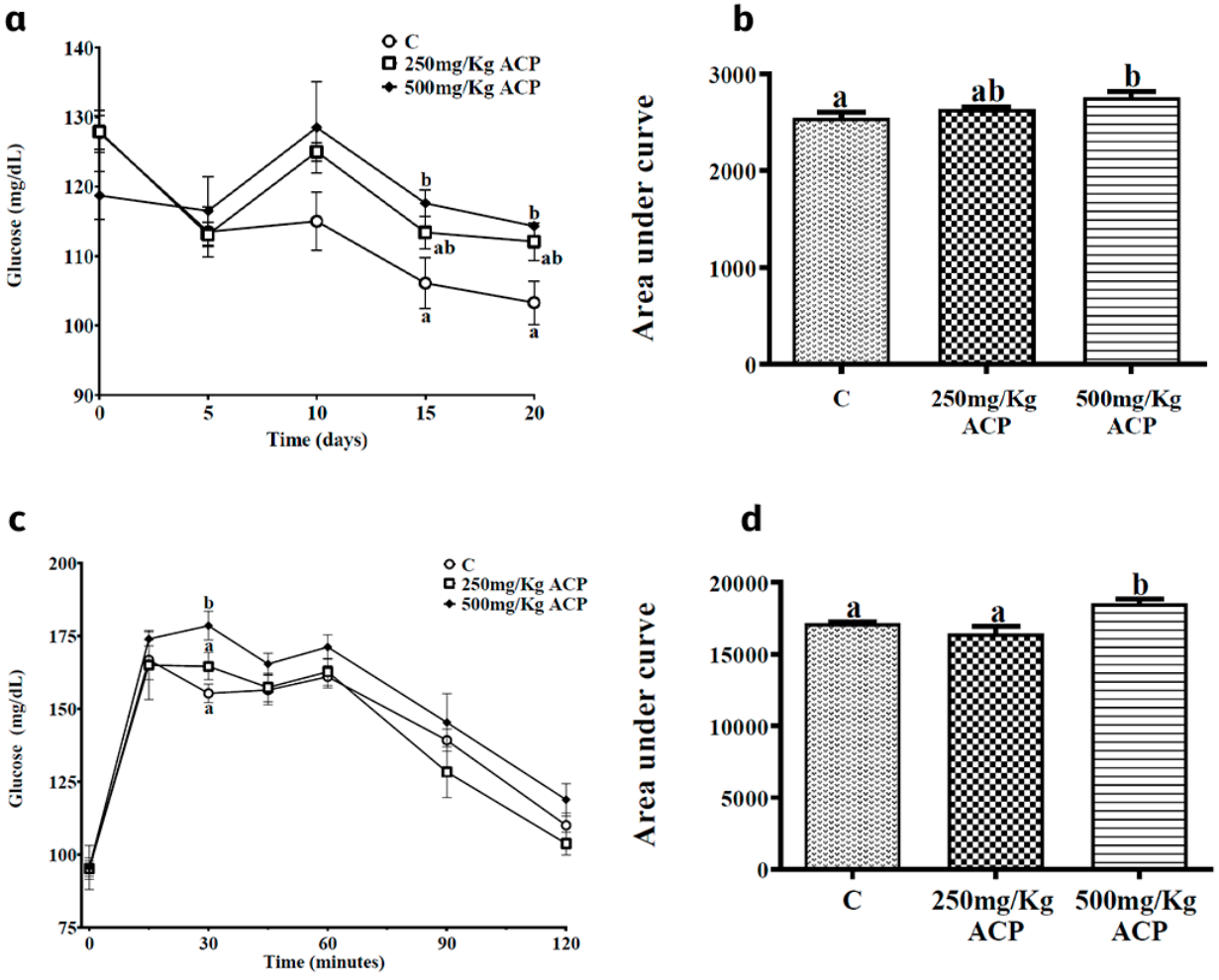

Figure 1. (a) Glycemic levels during the supplementation period, (b) area under the curve of the period, (c) serum glucose (mg/dL), and (d) area under the curve of the oral glucose tolerance test (OGTT) for the C (control group), 250 mg/kg ACP (group supplemented with *A. aculeata* at a dose of 250 mg/kg body weight), and 500 mg/kg ACP (group supplemented with *A. aculeata* at a dose of 500 mg/kg body weight) groups. Values are expressed as the means ± standard mean error. Different letters represent significant differences ($p < 0.05$) according to a one-way analysis of variance (n = 7 per group).

training, a reduction in fasting glucose levels and greater tolerance to glucose were observed, which may be associated with the capacity of exercise to improve insulin sensitivity.

Although we did not observe any changes in liver weight, the total liver lipid content was higher in the 500 mg/kg ACP group than that in group C or the 250 mg/kg ACP group, without changes in the concentration of total cholesterol and hepatic triglycerides. Concurrently, regulatory proteins associated with fatty acid/triacylglycerol metabolism in the liver, such as PPARα, which positively regulates the expression of genes related to mitochondrial and peroxisomal oxidation of fatty acids (Rakhshandehroo et al. 2004), and PPARγ, which activates adipogenesis and lipid storage (Okamura et al. 2010), were shown to be reduced in the 500 mg/kg ACP group. The lower content of PPARα may contribute to the higher content of hepatic lipids, since the use of fatty acids in the liver may be impaired in these animals. In addition, the lower content of PPARγ showed that the synthesis of fatty acid/triacylglycerol was not the main factor contributing to the increase in liver lipids. The lack of change in the concentrations of triglycerides and total cholesterol in the liver reinforced this hypothesis. It is important to note that, although the lipid content in the

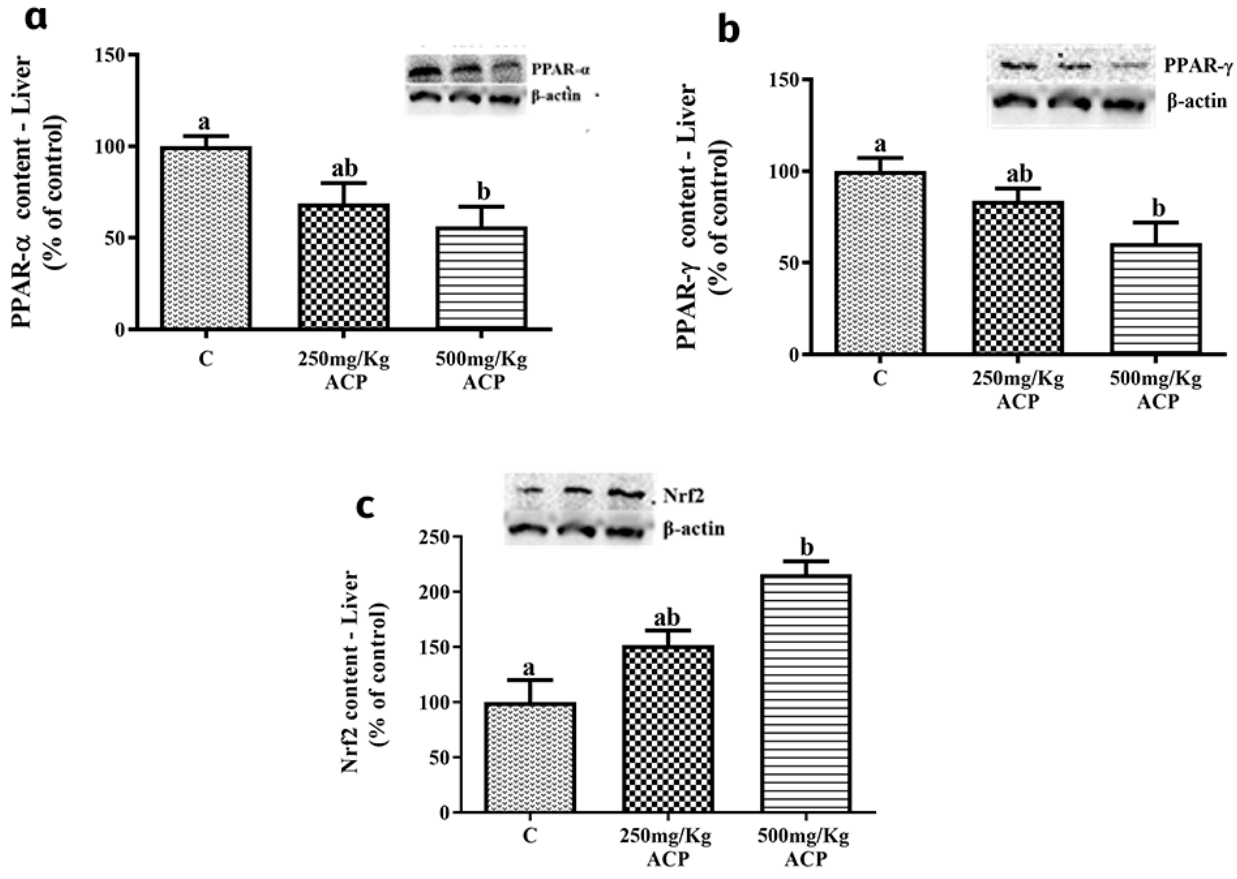

Figure 2. Peroxisome proliferator-activated receptor (PPAR)α (a), PPARγ (b), and nuclear factor erythroid-related 2 (NRF-2) (c) contents of the rat control group (C group) and groups supplemented with *A. aculeata* at doses of 250 mg/kg ACP or 500 mg/kg ACP. The values represent the means ± standard error of the number of animals. Different letters represent significant differences ($p < 0.05$) according to a one-way analysis of variance (n = 5–6 per group).

liver of the 500 mg/kg ACP group was higher, liver functionality was preserved because the enzymes ALT, and AP were not altered. We also cannot rule out that the increase in the total lipid content of the 500 mg/kg ACP group may be associated with increased hepatic vitamin A storage, which was induced by a greater bioavailability and conversion of β-carotene into vitamin A. Ramos et al. (2007) showed that the levels of hepatic retinol in rats that received *A. aculeata* were 100 times higher than those of animals that received pure β-carotene, after a period of repletion. This increase in hepatic retinol levels occurred without changes in liver mass.

We also investigated the antioxidant capacity of *A. aculeata* because its pulp contains high levels of phenolic compounds and total flavonoids (Correia et al. 2022). The results obtained in our study showed that both doses of ACP induced an improvement in the antioxidant system of the liver; however, the antioxidant response of the 500 mg/kg ACP group was better than that of the 250 mg/kg ACP group. We observed an increase in the activities of GPx, SOD, and CAT in the livers of the 500 mg/kg ACP group, which was associated with a reduction in carbonylated proteins and an increase in NRF2 content (116%). On the other hand, animals that were supplemented with 250 mg/kg of *A. aculeata* showed an increase only in GPx and GR activities, without alteration in the other parameters evaluated. The increase in the activity of SOD and CAT in the 500 mg/kg ACP group was likely decisive in maintaining the integrity of the liver against reactive oxygen species. CAT has one of the highest efficacy rates among antioxidant enzymes. One CAT enzyme can convert millions of molecules of hydrogen peroxide per second into oxygen and water. The reduction in the level of protein carbonylation in the 500 mg/kg ACP group suggested a reduction in protein oxidation in the liver. The presence of vitamin A in the liver, resulting from an increase in the intake of *A. aculeata* in the 500 mg/kg ACP group, may have contributed to the reduction in protein carbonylation, as retinol can also be considered a potent antioxidant, similar

Table VI. Concentration of carbonylated proteins, thiobarbituric acid reactive substances (TBARS), and antioxidant enzyme activities in the liver, measured after 28 days of treatment.

	C	250mg/kg ACP	500mg/kg ACP
Carbonylated proteins (nmol ptns carb\mg of ptns)	0.74 ± 0.07[a]	0.63 ± 0.04[ab]	0.51 ± 0.03[b]
TBARS (µmol\g of tissue)	14.3 ± 0.81[a]	13.6 ± 0.49[a]	14.1 ± 0.33[a]
Glutathione peroxidase (U\mg of protein)	1.97 ± 0.32[a]	3.19 ± 0.22[b]	3.25 ± 0.34[b]
Glutathione reductase (U\mg of protein)	0.029 ± 0.003[a]	0.043 ± 0.003[b]	0.029 ± 0.004[a]
Superoxide dismutase (U\mg of protein)	63.2 ± 7.86[a]	81.7 ± 6.84[ab]	113.5 ± 13.1[b]
Catalase (U\mg of protein)	23.7 ± 1.55[a]	22.3 ± 3.55[a]	40.1 ± 4.85[b]

C (control group), 250mg/Kg ACP (group supplemented with *A. aculeata* pulp at a dose of 250 mg/kg of the animal) and 500mg/Kg ACP (group supplemented with *A. aculeata* pulp at a dose of 500 mg/kg of the animal). Values are expressed as the means ± standard mean error. Different letters represent significant differences (p <0.05) according to one-way ANOVA (n=5-6 per group).

to tocopherol (Kartha & Krishnamurthy 1977). Although we did not determine the nuclear content of NRF2, an increase in the total content of this protein likely contributed to the increased activation of the enzymatic antioxidant system in the 500 mg/kg ACP group as compared to that in the other groups. NRF2 is an emerging regulator of cellular resistance to oxidative stress. NRF2 controls the basal and induced expression of a network of genes dependent on antioxidant response elements to regulate the physiological and pathophysiological processes of oxidant exposure (Ma 2013). The activation of NRF2 induces the expression of an enzymatic and protein signaling network that regulates the antioxidant system and oxidative signaling, thus influencing the physiology and pathology of the oxidizing agent (Ma 2013).

In summary, the data from the present study confirmed our hypothesis that the intake of *A. aculeata* pulp improves the enzymatic antioxidant system in the liver at the evaluated doses, although the amount ingested may impact changes in body composition, components of energy balance, biochemical and metabolic parameters, and glucose tolerance.

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FRANCYELE DOS S. CORREIA[1]
https://orcid.org/0000-0002-2118-3112

ELAINE C.L. SPADA[1]
https://orcid.org/0000-0003-2939-7825

BRUNA C.P. ESTEVAM[1]
https://orcid.org/0000-0002-4298-8921

THAYANNE C.O. CONCEIÇÃO[1]
https://orcid.org/0000-0002-3863-8398

GUILHERME N. DA CRUZ[1]
https://orcid.org/0000-0002-4330-3568

WÉLITON B. DA SILVA[1]
https://orcid.org/0000-0002-7267-232X

MAÍSA P.S. ELIAS[2]
https://orcid.org/0000-0002-1400-8447

SUÉLEM A.F. LEMES[1]
https://orcid.org/0000-0002-3829-8530.

[1]Universidade Federal de Mato Grosso, Departamento de Química, Avenida Fernando Correa da Costa, 2367, 78060-900 Cuiabá, MT, Brazil

[2]Universidade Federal de Mato Grosso, Faculdade de Engenharia Florestal, Avenida Fernando Correa da Costa, 2367, 78060-900 Cuiabá, MT, Brazil

Correspondence to: **Suélem A.F. Lemes**
E-mail: suelem.lemes@ufmt.br

Author contributions

FSC performed animal experiments, biochemical determinations, western blotting, and antioxidant analyses. ECLS and BCP performed lipid and glycogen analyses. TCOC and GNC analyzed carcass composition. WBS performed animal experiments and critically read the manuscript. MPSE helped with antioxidant analyses, critically read the manuscript, and contributed to the discussion. SAFL designed the experiments, analyzed the data, wrote the manuscript, and supervised the project. We certify that all of the authors have read and approved the manuscript.