**Jaboticaba (Myrciaria jaboticaba (Vell.) Berg.) peel improved triglycerides excretion and hepatic lipid peroxidation in high-fat-fed rats**

**Consumo de casca de jabuticaba (Myrciaria jaboticaba (Vell.) Berg.) melhorou a excreção de triglicerídeos e a peroxidação lipídica hepática de ratos alimentados com dieta hiperlipídica**

**ABSTRACT**

Objective

The aim of this study was to evaluate the influence of high-fat diets with 1%, 2%, and 4% freeze-dried jaboticaba peel on the serum, liver, and fecal lipid profile of obese rats.

Methods

Thirty male Sprague-Dawley rats were divided into 5 groups. Obesity was induced in four groups using a high-fat diet (35% lipids). One group was used as a high-fat diet control (High-fat group - HF). The other three high-fat-diet groups were given 1%, 2%, and 4% freeze-dried jaboticaba peel (High-Fat Jaboticaba - HfJ1, HfJ2, and HfJ4, respectively) in the last 40 experimental days. Blood and the liver were collected after 70 days of treatment and feces were collected in the last experimental week. Total cholesterol, triglycerides, and lipids were measured in the serum, liver, and dried feces.
Results

In the second phase of the experiment, HFJ4 group consumed more food and calories than the high-fat group. Total serum cholesterol and triglyceride levels did not differ in the experimental groups. HFJ2 group had the highest hepatic and fecal lipid contents compared with the group fed a diet with normal fat content (N), but low hepatic lipid peroxidation. HFJ4 group had the highest mean hepatic and fecal cholesterol levels. Hepatic triglyceride levels did not differ among the groups, and groups HFJ1 and HFJ4 presented the highest fecal triglyceride content.

Conclusion

The amounts of jaboticaba peel used by this study did not protect against hepatic steatosis or undesired levels of other studied lipids, but it did increase fecal triglycerides. Lipid peroxidation in the liver decreased in the HFJ2 group.


R E S U M O

Objetivo
O objetivo deste estudo foi avaliar o efeito de dietas hiperlipídicas adicionadas de 1%, 2% e 4% de casca de jabuticaba liofilizada sobre os perfis lipídicos do fígado, soro e fezes de ratos.

Métodos
Trinta ratos, machos, Sprague-Dawley, foram divididos em cinco grupos. A obesidade foi induzida em quatro grupos, com dieta hiperlipídica ou High-Fat - HF (35% de lipídeos). Três desses grupos receberam a dieta hiperlipídica adicionada de 1%, 2% e 4% de casca de jabuticaba liofilizada (Jabuticaba - HFJ1, HFJ2 e HFJ4, respectivamente) nos últimos 40 dias de experimento. Sangue e fígado foram coletados após 70 dias de tratamento e as fezes na última semana experimental. Colesterol e triglicerídeos totais foram avaliados no soro, fígado e fezes secas, bem como lipídeos totais.

Resultados
No segundo período do experimento, a ingestão dietética e energética dos animais HFJ4 foi maior em relação ao grupo High-Fat. Não houve diferença significativa entre os grupos experimentais para colesterol total e triglicerídeos séricos. O grupo HFJ2 demonstrou maiores níveis de lipídeos hepáticos e fecais em relação a N, apesar de a peroxidação lipídica ter diminuído nesse grupo. O HFJ4 mostrou a maior média de colesterol hepático e fecal. Não houve diferenças significativas para triglicerídeos hepáticos, e os grupos HFJ1 e HFJ4 excretaram mais triglicerídeos pelas fezes.

Conclusão
As doses utilizadas de casca de jabuticaba não mostraram efeitos benéficos contra a esteatose hepática ou outro parâmetro lipídico avaliado, com exceção para a excreção de triglicerídeos. O índice de peroxidação lipídica hepática diminuiu nos ratos alimentados com 2% de casca de jabuticaba liofilizada.


I N T R O D U C T I O N

Myrciaria jaboticaba (Vell.) Berg., commonly known as Brazilian berry, is a plant native to South America. It produces globose fruits with a deep purple peel and sweet white pulp known as jaboticaba. Its popularity in Brazil is comparable to that of grapes in Europe or in the United States of America. Although not widely consumed, most of its polyphenols, such as ellagic acid, quercetin, and anthocyanins are concentrated in the peel, which has an expressive antioxidant activity1-3.

High-polyphenol/anthocyanin diets reduce Cardiovascular Diseases (CVD) because they affect the regulation of lipid metabolism4-6. These diets may reduce total serum cholesterol and triglyceride levels7 and increase High Density Lipoprotein-cholesterol (HDL-cholesterol) levels in obese
animals. The mechanism underlying these findings may regard the ability of these diets to increase biliary cholesterol excretion and fecal lipid excretion, reducing plasma lipid levels. Anthocyanins may influence the expression of hepatic enzymes. One such example is the powerful anthocyanin called cyanidin 3-glucoside, capable of suppressing hepatic enzymes involved in the synthesis of fatty acids and triglycerides, which could increase hepatic β-oxidation and decrease fatty acid synthesis. These mechanisms may also promote weight and fat loss in obese rats.

*Jaboticaba* peel is also a source of dietary fibers, nutrients with hypocholesterolemic role. High-polyphenol and high-fiber diets are capable of reducing total cholesterol and Low Density Lipoprotein-cholesterol (LDL-cholesterol), suggesting that both substances may act synergistically on the regulation of blood lipids. Moreover, diets supplemented with 1% or 2% freeze-dried *jaboticaba* peel improved the antioxidant capacity of rat plasma, indicating the potential of freeze-dried *jaboticaba* peel compounds to attenuate oxidative stress and related damages.

Previous *in vivo* investigations on the addition of 1%, 2%, and 4% freeze-dried *jaboticaba* peel to the diets of obese animals reported that it could not effectively reduce energy intake, weight gain, and body fat. However, obese animals fed diets with 2% and 4% freeze-dried *jaboticaba* peel showed an increase in HDL-cholesterol level and a decrease in insulin levels. Changes in hepatic lipid content and their excretion may have promoted these findings. Hence, the aim of this study was to assess the influence of high-fat diets with added 1%, 2%, and 4% freeze-dried *jaboticaba* peel on the serum, liver, and fecal lipid profile of obese rats.

**METHODS**

Thirty weaned, male, Sprague-Dawley (SD) rats weighing 58±18.77g were obtained from the Centro Multidisciplinar para investigação Biológica (CEMIB, Multidisciplinary Center for Biological Research) of Universidade Estadual de Campinas (Unicamp). This experiment was approved by the Animal Research Ethics Committee (CEUA/Unicamp) under protocol number 2,226-1. The study was performed as required by the Colégio Brasileiro de Experimentação Animal (COBEA, Brazilian Council on Animal Experimentation). The animals were randomly assigned to five groups (n=6) and kept in individual cages with free access to food and water, temperature of 22±1°C, humidity of 60%-70%, and controlled light and dark cycles of 12 hours throughout the experiment.

Two control diets were given during the experiment: a diet with normal-fat content (N), (AIN-93G), prepared as recommended by the American Institute of Nutrition, and one with High-Fat (HF) content, (AIN-93G with 35% fat by weight, being 4% soybean oil and 31% lard). The study also included another three experimental diets made by adding different concentrations (1%, 2%, and 4% w w⁻¹) of freeze-dried *jaboticaba* peel to the high-fat diet mentioned above. All diets were adjusted to have the same total fiber content. Table 1 shows the anthocyanin and dietary fiber contents of freeze-dried *jaboticaba* peel. The two control groups, N and HF, were fed the respective diets throughout the experiment. HFJ1, HFJ2, and HFJ4 groups were fed the HF diet during the first 4 weeks followed by diets with the respective amounts of freeze-dried *jaboticaba* peel until the end of 10 weeks. Food intake was monitored every 2 days and weight gain once a week.

Freeze-dried *jaboticaba* peel is a powder made by freeze-drying *M. jaboticaba* peels, as described elsewhere. Freeze-dried *jaboticaba* peel contains the following compounds of interest: soluble fibers (5%), insoluble fibers (20%), total polyphenols (556.30g GAE kg⁻¹), delphinidin-3-O-glucoside (634.75mg 100g⁻¹), cyanidin-3-O-glucoside (1963.57mg 100g⁻¹), and total anthocyanins (2538.32mg 100g⁻¹).

Blood was obtained from the rats by decapitation after a 12-hours fasting. After
collection, the blood samples were centrifuged at 4000 rpm for 20 minutes. Serum was collected and stored at -80°C until analyses. The livers were removed, rinsed, frozen, and dried in a freeze-dryer (LP1010, Liobras, São Carlos, São Paulo, Brazil). The livers were manually ground and kept at -20°C until analyses.

**Biochemical analyses**

*Total lipids*: liver lipid content was determined by the Bligh and Dyer method and total fecal lipid content was determined by a Soxhlet extractor and petroleum ether.

*Lipid hydroperoxide assay*: determination of hydroperoxides produced by primary lipid autoxidation in freeze-dried liver was preceded by modified lipid extraction. Three milliliters of the extract containing chloroform and lipids were used for hydroperoxide analysis. The peroxide value was measured as recommended by the official method, which is based on the oxidation of iodide into iodine by peroxides in the sample. The hydroperoxide concentration of each sample in mEq kg\(^{-1}\) was calculated based on the amount of lipids found.

*Cholesterol and triglyceride analyses*: liver and fecal lipids were extracted as recommended by Folch. The total cholesterol and triglyceride contents of the serum and extracts were determined by enzyme assay kits (Laborlab, São Paulo, Brazil).

*Fecal pH*: dried feces was diluted with deionized water (25 mg mL\(^{-1}\)) and homogenized. While stirred, the pH was measured by a pH meter (Tecnal model TEC-5, Piracicaba, SP, Brazil).

**Statistical analyses**

Body weight, weight gain and food/calorie intake were treated by two-way Analysis of Variance (Anova) and the Bonferroni test, a posteriori (\(p<0.05\)). Total food intake, total weight gain, lipid profile, dry fecal weight, lipid peroxidation, and pH were treated as follows: parametric data by one-way Anova followed by
the Tukey’s multiple comparisons test; nonparametric data by the Kruskal-Wallis’ and Dunn’s multiple comparisons tests. The significance level was set at \( p < 0.05 \). The parametric results were expressed as means ± Standard Error of the Mean (SEM) and the nonparametric results as medians and ranges. The data were treated by the software GraphPad Prism 5.0 (GraphPad Software, Inc. La Jolla, CA, USA).

**RESULTS**

The serum and liver were collected after 40 days of dietary treatment and the feces in the last week of intervention. The lipid profiles of the samples were determined. As detailed below, the diets with 1% and 4% *jaboticaba* peel increased triglyceride excretion, although no changes were observed in the liver or serum.

![Graphs](image-url)
**Body weight, food intake and fecal parameters:** The experimental groups did not differ (p>0.05) concerning total weight gain (117.6g to 192.8g) and total food intake (650.47g to 745.16g). However, in the second phase of the experiment (last 6 weeks), food intake of the HFJ4 group (441.02g) was higher than that of the HF group (378.84g). The animals fed the high-fat diets presented higher body weights from experimental week five (p<0.001, Figure 1A) and weight gain was similar in the second phase of the experiment (p>0.05, Figure 1B). The HFJ4 group consumed more food than the HF group on the last week (p<0.05, Figure 1C). The calorie intake of N group was lower than those of HF and HFJ groups throughout the experiment except for week eight, when it differed only from that of HFJ4 group. The calorie intake of HFJ4 group in the last week was also higher than that of HF group (p<0.05, Figure 1D).

Table 2 shows that the animals given freeze-dried *jaboticaba* peel showed a dose-dependent response to anthocyanin and soluble fiber (p<0.001). In addition, higher food intake by HFJ4 group resulted in higher total fiber intake, when compared with HF group (p<0.05). Soluble fiber intake may have contributed to the higher fecal dry weight of HFJ2 and HFJ4 groups in last week. The fecal pH of HF, HFJ1 and HFJ2 groups, were lower than that of N group (p<0.001). HFJ2 group had the lowest fecal pH (Table 2).

**Serum analyses:** Total serum cholesterol (69.24mg dL^{-1} to 80.38mg dL^{-1}) and triglyceride levels (39.67mg dL^{-1} to 49.49mg dL^{-1}) did not differ among the experimental groups (p>0.05).

**Total lipid analyses:** The animals fed the high-fat diet had higher hepatic lipid content, especially HFJ2 group, which had the highest mean relative to N group. The hepatic lipid contents of the groups fed the high-fat diets (HF, HFJ1, HFJ2, and HFJ4) did not differ. As expected, lipid excretion was higher in all high-fat-diet groups and highest in HFJ2 group, which was different from N group and similar to HF group (Figure 2A and 2B).

**Liver and feces cholesterol analyses:** Differently from N group, HFJ4 group had the highest mean hepatic and fecal cholesterol levels, similar to those of HF group. On the other hand, the hepatic cholesterol levels of HFJ1 and HFJ4 groups were generally lower (Figure 2C and 2D). Except for HFJ2 group, the groups fed high-fat diets also excreted more cholesterol than N group (p<0.05).

**Triglyceride analyses:** Hepatic triglycerides did not differ (p>0.05) among the groups, but according to their absolute values, animals fed high-fat diets had higher liver triglyceride contents than animals in N group (Figure 2E). HFJ1 and HFJ4 groups presented higher fecal triglycerides than HF group (18.55% and 47.22% higher, respectively; p<0.05). The fecal triglyceride contents of HFJ4 and N groups were similar (Figure 2F).

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**Table 2.** Anthocyanins (ACN) and total (TF), insoluble (IF), and soluble (SF) fiber intakes during the second phase of the experiment. Fecal dry weight and pH of the experimental groups. **Campinas** (SP), Brazil, 2011.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N (M SEM)</th>
<th>HF (M SEM)</th>
<th>HFJ1 (M SEM)</th>
<th>HFJ2 (M SEM)</th>
<th>HFJ4 (M SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACN intake (mg)</td>
<td>19.31 ± 0.35b</td>
<td>19.94 ± 0.46b</td>
<td>19.19 ± 0.52ab</td>
<td>19.99 ± 0.76bc</td>
<td>22.05 ± 1.06*</td>
</tr>
<tr>
<td>TF intake (g)</td>
<td>4.66 ± 0.52</td>
<td>5.39 ± 0.36c</td>
<td>5.58 ± 0.26bc</td>
<td>6.93 ± 0.41ab</td>
<td>7.69 ± 0.59*</td>
</tr>
<tr>
<td>Fecal pH</td>
<td>6.62 ± 0.02a</td>
<td>6.19 ± 0.08bc</td>
<td>6.16 ± 0.04bc</td>
<td>6.13 ± 0.03c</td>
<td>6.44 ± 0.07a</td>
</tr>
</tbody>
</table>

Note: Different letters in lines indicate statistically different among the experimental groups (p<0.05). Parametric data (analysis of variance and Tukey’s test) were expressed as mean ± Standard Error of the Mean (SEM) (n=6). *N*= Normal diet (AIN-93G) group; HF: High-Fat control diet group; HFJ1: High-Fat diet + 1% Freeze-dried *Jaboticaba* Peel (FJP); HFJ2: High-Fat diet + 2% FJP; and HFJ4: High-Fat diet + 4% FJP.
Figure 2. Total liver (A) and fecal (B) lipids; total liver (C) and fecal (D) cholesterol; total liver (E) and fecal (F) triglycerides of the experimental groups. Campinas (SP), Brazil, 2011.

Note: N: Normal-fat diet (AIN-93G) group; HF: High-Fat control diet group; HFJ1: animals fed HF + 1% Freeze-dried Jaboticaba Peel (FJP); HFJ2: animals fed HF + 2% FJP; and HFJ4: animals fed HF + 4% FJP. Different letters in columns indicate statistical difference among the experimental groups. Parametric data (analysis of variance and Tukey’s test) were expressed as mean ± Standard Error of the Mean (SEM); nonparametric data in B, D and E (Kruskal-Wallis’ and Dunn’s tests) were expressed as median and ranges (n=6); p<0.05.
Liver lipid peroxidation: The peroxide values indicated that HFJ2 group had 28.36% fewer primary lipid peroxidation products in the liver than HF group (Figure 3).

DISCUSSION

This study indicates that *M. jacobtaca* peel may increase fecal triglycerides in obese rats, but does not reduce hepatic and serum lipid contents. Freeze-dried *jacobtaca* peel contains bioactive compounds that could be responsible for these findings, such as dietary fibers and polyphenols. Although the studied diets had the same fiber content, the differences may stem from the soluble fiber content of freeze-dried *jacobtaca* peel, from its polyphenol content, and/or from other yet unknown substances. Moreover, the lard added to the high-fat diets is high in saturated fatty acids, which could hinder lipid metabolism in rats. Additionally, in the present study lard promoted obesity in SD rats.

The impact of anthocyanins on the serum lipids of rats fed high-fat diets is controversial. Kwon et al. found that SD rats fed 10% black soybean or 0.037% anthocyanin extract presented lower total serum cholesterol and triglyceride levels. However, studies with obese mice treated with a high-anthocyanin beverage or diet corroborate our findings. Polyphenols have been found to reduce the total cholesterol of animals fed atherogenic diets and even healthy diets. Another study found that HFJ2 and HFJ4 diets were capable of increasing HDL-cholesterol. Thus, although the total cholesterol levels of animals given freeze-dried *jacobtaca* peel did not decrease, an increase in HDL-cholesterol could reduce the risk of cardiovascular disease.

Fatty liver and steatorrhea were expected in animals fed the high-fat diets. The freeze-dried *jacobtaca* peel did not reduce the total lipid content of the liver and/or increase fecal lipid content. Studies have shown that anthocyanins could reduce the lipid content of the liver but not change the lipid content of the feces. Although HFJ2 group had a higher liver lipid content than N group, it had a lower lipid peroxidation value. The presence of polyphenols from freeze-dried *jacobtaca* peel in the liver may explain these findings. Polyphenols, especially anthocyanins, can donate electrons or hydrogen atoms and neutralize the peroxyl radicals that attack the lipids in cell membranes. In addition, these compounds also correlate with better performance of the enzymes of the endogenous antioxidant system. Bioavailability studies found that anthocyanins and their metabolites are largely present in the liver, being associated with high hepatic antioxidant capacity. Thus, the addition of 2% freeze-dried *jacobtaca* peel to the high-fat diet might have protected the liver from obesity-induced oxidative stress by providing exogenous and endogenous antioxidants, preventing lipid oxidation.

Different anthocyanin doses did not affect the hepatic cholesterol levels of animals fed high-fat diets. In our work, HFJ4 and HF groups reached the highest hepatic cholesterol levels. However, this could imply that freeze-dried *jacobtaca* peel is protective, since HFJ4 group...
consumed more food than HF group and did not experience proportional damage.

The higher food intake of HFJ4 group in the second phase of the experiment could also indicate that the HF4 diet was more palatable than the HF diet. On the other hand, these animals might have a dysfunction in the reward system in response to fat intake26, or an obesity-related disorder regarding leptin and anorexigenic peptide signaling in the hypothalamus27. Further studies on changes in the mechanisms of leptin signaling are needed to investigate this hypothesis.

The hepatic triglyceride contents of the experimental groups did not differ, but the absolute contents were higher in animals fed the high-fat diets, corroborating the serum triglyceride results. However, freeze-dried jaboticaba peel increased triglyceride excretion in the HFJ1 and HFJ4 groups. The triglyceride levels of obese HFJ4 animals were comparable to those of health animals in N group. The high fecal triglyceride content of HFJ4 group prevented their high food/calorie intake to increase their serum and hepatic triglyceride levels.

High soluble fiber intake may have prevented high cholesterol/triglyceride absorption in HFJ4 group, or even cholesterol/triglyceride synthesis. Tsuda et al.10 found that mice given a high-fat diet with 2g kg⁻¹ of purple corn had lower lipogenic enzyme mRNA levels in their white adipose tissue. Additionally, Kim et al.5 found that the CYP 51 gene encoding lanosterol 14α-demethylase was down-regulated in the livers of golden Syrian hamsters fed a high-fat diet with 8% whole blueberry peel, indicating low cholesterol synthesis.

The dry weight of the fecal matter of HFJ2 and HFJ4 groups was high, probably reflecting their highest food intake during the last week. So far, there is evidence that the right proportion of soluble and insoluble dietary fibers in a meal has functional effects, such as reducing blood glucose variation and cholesterol levels. Soluble fibers are especially capable of forming complexes with dietary constituents, possibly producing larger food boluses that would increase the dry matter content of the feces28. The soluble fiber dose-dependent response shown by the groups fed freeze-dried jaboticaba peel corroborates this findings. Therefore, freeze-dried jaboticaba peel impacted the absorption of some nutrients, such as lipids, mainly triglycerides.

Curiously, the HFJ2 group had the lowest fecal pH. Figure 2 shows that this group had higher fecal fatty acid content, which may explain this result. Another hypothesis is that soluble fiber and polyphenol contents of freeze-dried jaboticaba peel promoted the production of Short Chain Fatty Acids (SCFA) by gut microbiota. Higher SCFA production and a potentially higher delivery of SCFA, specifically butyrate, to the distal colon may be protective. Moreover, the production of propionate, another SCFA, helps to inhibit cholesterol synthesis in the liver29.

The fecal pH of HFJ4 group was not as low as expected. The higher polyphenol intake stemming from higher freeze-dried jaboticaba peel intake may have promoted enzymatic changes in the gut and microbiota30 that could reduce SCFA production31. Thus, the combination of fecal lipid content and ideal polyphenol and dietary fiber intakes may be responsible for the lowest fecal pH of HFJ2 group.

**Conclusion**

The addition of 1%, 2%, and 4% freeze-dried jaboticaba peel to high-fat diets was not capable of decreasing the hepatic and serum cholesterol and triglyceride levels of obese animals. However, despite the higher lipid intake, the HFJ2 group had lower hepatic peroxide values. Fecal triglycerides increased in HFJ2 and HFJ4 groups, which showed a dose-dependent response to soluble fiber and anthocyanins intake. Concomitantly, the fecal pH of HFJ2 group decreased. More studies with different dietary fat contents and longer treatment periods with freeze-dried jaboticaba peel could better clarify the impact of this ingredient on the lipid profile of obese rats.
ACKNOWLEDGMENTS

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REFERENCES


ERRATA

No artigo “Jaboticaba (Myrciaria jaboticaba (Vell.) Berg.) peel improved triglycerides excretion and hepatic lipid peroxidation in high-fat-fed rats” publicado no número 5, volume 26, Revista de Nutrição, na página 571, onde se lê:

“Jaboticaba (Myrciaria jaboticaba (Vell.) Berg.) peel increased triglycerides excretion and hepatic lipid peroxidation in high-fat-fed rats”

Leia-se:

“Jaboticaba (Myrciaria jaboticaba (Vell.) Berg.) peel improved triglycerides excretion and hepatic lipid peroxidation in high-fat-fed rats”

No mesmo artigo, na página 577, onde se lê:

“As unidades na Figura 2 (C, D, E, F) de g 100g⁻¹”

Leia-se:

“As unidades na Figura 2 (C, D, E, F) mg 100g⁻¹”