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Cholesterol reducing and bile-acid binding properties of *taioba* (*Xanthosoma sagittifolium*) leaf in rats fed a high-fat diet☆,☆☆

Elisa de Almeida Jackix [a,*], Elisa Bernardes Monteiro [a], Helena Fonseca Raposo [b], Jaime Amaya-Farfán [a]

[a] Department of Food and Nutrition, Faculty of Food Engineering, University of Campinas, Unicamp, R. Monteiro Lobato, 80, PO Box 6121, Campinas, SP 13083-862, Brazil
[b] Department of Physiology and Biophysics, Institute of Biology University of Campinas, Unicamp, R. Monteiro Lobato, 80, PO Box 6109, Campinas, SP 13083-862, Brazil

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ABSTRACT

The consumption of vegetables has been correlated with reduced risk of chronic non-communicable diseases due to the high fiber content and bioactive compounds found in vegetables. The arrowleaf elephant ear (*Xanthosoma sagittifolium*), which is known in Brazil as *taioba*, is a common plant in tropical America. Although its leafy portion possesses a high nutritional value, it is not widely consumed and has not been well studied. This study assessed the effect of lyophilised *taioba* leaf (LTL) as a hypolipidemic and prebiotic agent. Thirty-two Wistar rats were assigned to four groups: group 1 was fed a high-fat diet containing 3.67% (w/w) cellulose (low cellulose — LCEL); group 2 received a high-fat diet supplemented with 10% (w/w) cellulose (CEL); group 3 received a high-fat diet supplemented with 10% (w/w) inulin (INU); and group 4 was fed a high-fat diet supplemented with 28.4% LTL (TAI) to provide 10% (w/w) *taioba* fiber. The groups were fed their respective diets for 4 weeks. The addition of LTL to the diet resulted in reduced weight gain, reduced liver fat, and increased fecal mass and lipid, in addition to higher fecal short chain fatty acid and bile salt concentrations, compared to the LCEL group. Additionally, only the TAI group exhibited a lower serum cholesterol concentration and a higher body ash content ($p<0.05$) than the LCEL group. Both the high bile salt binding capacity and high fermentability of LTL suggest that this plant may have a protective effect against cardiovascular diseases and bowel cancer.

1. Introduction

Consumer interest in foods with functional properties such as vegetables is increasing. The beneficial properties of fruits and vegetables are attributed to the presence of vitamins, minerals, dietary fiber, phenolic acids, flavonoids, sterols, carotenoids and others, most of which are essential for health and contribute to the high nutritional quality of the vegetable products (Bumgarner, Scheerens, & Kleinhenz, 2012; Giampieri, Alvarez-Suarez, Mazzoni, et al., 2012; Hounsome, Hounsome, Tomos, & Edwards-Jones, 2008). *Xanthosoma sagittifolium* (commonly known as *taioba*) is an edible aroid widely grown as a tuber crop in many parts of Africa, America, and Asia. The tubers are commonly consumed and constitute an important energy and nutrient source in the diet of some populations. The leaves of this plant are underutilized and found only occasionally in small markets as a green vegetable (Pérez, Gutiérrez, & Dedelahaye, 2007). Although the leaves also grow rapidly and have a significant nutritional value, their use is less widespread (Pérez et al., 2007). In South America, the *taioba* leaves are consumed steamed or boiled.

Dietary fiber, which is a major constituent of the *taioba* leaf, is not digested by enzymes in the gastrointestinal tract of mammals. Thus, dietary fiber can pass unaltered through the large intestine or undergo fermentation by colonic microflora. These characteristics result in positive physiological effects, including reduced cholesterol, delayed stomach emptying and eased transit of the fecal bolus (Institute of Medicine of the National Academies, 2005). Nevertheless, it is generally agreed that the overall beneficial effect is the result of a number of substances that are present in vegetables, including the fiber, and that when the benefits are attributed to dietary fiber, the food matrix as a whole should be taken into consideration and not only the fiber (Fardet, 2010; Institute of Medicine of the National Academies, 2005).

Fiber may be classified as soluble and insoluble, and the insoluble fraction is predominant in the *taioba* leaf (Monteiro, 2011). Several studies have reported that the insoluble fiber type has beneficial effects, including an increase in intestinal motility and a shorter contact time between nutrients and non-nutrients, and the absorptive mucosa.

Abbreviations: BA, bile acid; CEL, cellulose; CNCD, chronic non-communicable diseases; CYP7A1, cholesterol 7α-hydroxylase; FER, feed efficiency ratio; FFM, fat-free mass; INU, inulin; LCEL, low cellulose; LTL, lyophilised *taioba* leaf; RAF, relative abdominal fat; SCFA, short chain fatty acid; TAI, *taioba*; TC, total cholesterol; TG, triacylglycerol.

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* Corresponding author. Tel./fax: +55 19 32874672.
E-mail addresses: elisajackix@yahoo.com.br (E. de Almeida Jackix), elisacirj@hotmail.com (E.B. Monteiro), raposohelena@yahoo.com.br (H.F. Raposo), jaf@fea.unicamp.br (J. Amaya-Farfán).

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Table 1
Composition of experimental diets (g/kg diet).

Ingredient composition	LCEL	CEL	INU	TAI
Cellulose	36.7	100	–	–
Inulin	–	–	100	–
Taioba fiber	–	–	–	100
Lard	100	100	100	100
Coconut fat	170	170	170	170
Cholesterol	5.6	5.6	5.6	5.6
AIN-93 mineral mixture	50	50	50	50
AIN-93 vitamin mixture	10	10	10	10
L-cystine	1.8	1.8	1.8	1.8
Choline bitartrate	3	3	3	3
Tert-butylhydroquinone	0.02	0.02	0.02	0.02
Casein (89%)	135	135	135	135
Corn starch	149	130	130	130
Sucrose	339	295	295	295

LCEL: 3.67% of cellulose; CEL: 10% of cellulose; INU: 10% of inulin; TAI: 10% of *taioba* fiber.

These effects can include the eased excretion of toxic and co-carcinogenic compounds of dietary or endogenous origin, such as secondary bile acids, as well as the elimination of residual energy that was not utilized in the foregut (Brownlee, 2011; Isken, Klaus, Osterhoff, et al., 2010). These phenomena are associated with the prevention of colon cancer and obesity (Ferguson, Chavan, & Harris, 2001; Hamauzu & Mizuno, 2011), provided that the greater motility does not result in the loss of efficiency in nutrient utilization. The decreased availability of triglycerides, cholesterol, and bile salts, however, may result in a reduction of the oversupply of these substances and is part of the mechanism by which insoluble fiber manifests its hypocholesterolemic effect (Hamauzu & Mizuno, 2011). An adequate intake for total fiber in foods has been set at 38 and 25 g/d for young men and women, respectively, based on the intake level observed to protect against coronary heart disease. Levels of 10 to 12 g of dietary fiber/1000 kcal have been suggested as safe, but adverse effects of overconsumption (such as a reduced bioavailability of minerals and gastrointestinal distress) have been observed at levels of up to 75 to 80 g/day (IOM, 2005).

The fact that *taioba* is grown in humid tropical and sub-tropical regions, and that it can constitute a subsistence food, makes this plant a target vegetable for a study of this nature. To date, no studies have been published on the *taioba* leaf, particularly on its functional properties *in vivo*. Therefore, the aim of this investigation was to assess some of the *in vivo* nutritional and functional properties associated with the consumption of *taioba* leaves, especially concurrently with high-fat fed diets.

2. Material and methods

2.1. Production of LTLs

Taioba leaves were obtained from Central Supply of Campinas S.A. (CEASA, Campinas, SP, Brazil), washed, chopped, boiled in water, lyophilised (Model LP 1010, Liobrás, São Carlos, SP), and subsequently ground in a micro-grinder (Model TE 048, Tecnal, Piracicaba, SP) to obtain a powder with a particle size of 10 μm.

2.2. Chemical composition of lyophilized taioba leaf (LTL)

The nitrogen content was estimated by the Kjeldhal method, based on the assumption that plant proteins contain 16% nitrogen. Protein content of the LTL was then calculated using the formula, % protein = % nitrogen × 6.25. Fat and ash (minerals) were determined using the Bligh and Dyer and the AOAC methods (Association of Official Analytical Chemists, 1995; Bligh & Dyer, 1959). Insoluble and soluble dietary fibers were determined after separation of non-starch polysaccharides by the enzymatic–gravimetric method of Prosky, Asp, Schweizer, De Vries, and Furda (1988). Moisture was determined by standard methods (Association of Official Analytical Chemists, 1995). All analyses were performed in triplicate. The total carbohydrate content was estimated by difference.

2.3. Animals and diets

A total of 32, 28-day old, specific-pathogen free Wistar rats, received from the Multidisciplinary Center for Biological Research/UNICAMP, were grown with commercial chow in the animal quarters until a mean weight of 270 ± 18.2 (SD) g was attained (8 weeks of age, when the high fat diet was fed for 4 more weeks). From this point on, the animals were fed the experimental diets. They were maintained under a light/dark cycle of 12/12 h and a temperature of 22 ± 2 °C throughout the experiment. The study was approved by the Ethics Committee of the Institute of Biology at UNICAMP (University of Campinas) (Protocol 2404-1). An experimental diet rich in saturated fat was used because diets with high fat and low fiber contents are known to induce hypercholesterolemia in both rats and humans (Buttner, Parhofer, Woenckhaus, et al., 2006). Both pork lard and coconut oil were sources of dietary fat, and further supplemented with cholesterol in order to magnify the hypercholesterolemic effect (Merat, Casanada, Sutphin, et al., 1999). All of the animals received a high-fat and low-fiber diet containing 3.67% (w/w) cellulose (LCEL) diet for 4 weeks, before the stating of the experimental diets. At the end of this period, the animals were assigned to 4 groups (n = 8/group) with different experimental diets: group 1 was fed a high-fat, low-fiber diet containing 3.67% (w/w) cellulose (low cellulose — LCEL); group 2 was used as the negative control, received a high-fat diet supplemented with 10% (w/w) cellulose (CEL); group 3 was used as the positive control, was fed a high-fat diet supplemented with 10% (w/w) inulin (INU); and group 4 was fed a high-fat diet supplemented with 10% (w/w) *taioba* fiber, corresponding to 28.4% lyophilised *taioba* leaf — LTL (TAI). Tables 1 and 2 show the compositions of the diets and LTL, respectively. Throughout the experiment, food and water were provided *ad libitum*. Feed efficiency ratio (FER) was estimated by the ratio between body weight increase and daily consumption. Feces were collected, fresh weights recorded and moisture contents determined, 1 week before the animals were sacrificed. After fasting for 12 h, the animals were decapitated, and their blood was collected in heparinized tubes.

2.4. Determination of total cholesterol (TC), triglycerides (TGs), lipid fractions — HDL-c (high density lipoprotein) and LDL-c (low density lipoprotein), insulin and the glucose tolerance test (GTT)

The TC and lipid fractions were determined in serum, which was obtained by centrifuging blood at 12,235 *g* at 4 °C for 15 min. The TC and TG levels were determined in triplicate, by colorimetric reactions using specific enzymatic kits (CHOD-PAP; Roche Diagnostic GmbH®, Mannheim, Germany). The variation of TC over time, was the difference between the final (end of the experiment) TC and initial (after 4 weeks of induction period) TC levels. HDL-c was quantified using a commercial precipitation kit, in triplicate (Wiener Lab®). LDL-c was estimated using

Table 2
Proximate composition of lyophilised *taioba* leaf (LTL) (g per 100 g dry matter).

Total carbohydrate (%)	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	TDF (%)[a]	SDF (%)[a]	IDF (%)[a]
49.0 ± 0.82	29.4 ± 1.76	10.6 ± 0.26	8.24 ± 0.06	2.72 ± 0.07	35.2 ± 0.26	6.82 ± 0.06	28.4 ± 0.21

[a] % of total carbohydrate; TDF: total dietary fiber, SDF: soluble dietary fiber, IDF: insoluble dietary fiber.

the Friedewald formula (Friedewald, Levy, & Fredrickson, 1972): LDL-c = TC − HDL-c − (TG/5). The GTT was performed one week prior to sacrifice, after the rats were subjected to fasting for 6 h as well as 30, 60, 90, and 120 min after a glucose solution (2 g/kg of rat weight, intraperitoneal) was administered. The blood glucose concentration was measured using the Accu-Chek-Performa® system (Roche). Insulin was measured with a radioimmunoassay using Coat-a-Count® kits (Diagnostic Products Corporation, USA).

2.5. Determination of total lipids in liver

After sacrifice, the livers of the animals were frozen at 18 °C/2 weeks. After thawing, the livers of the animals were macerated and homogenized with a Polytron® (PRO200) for 3 min. Total lipids in the liver were determined in triplicate according to the method developed by Bligh and Dyer (1959). A 3 g sample was homogenized with 10 mL chloroform, 200 mL methanol and 8 mL of distilled water for 30 min. The solution was re-homogenized with 10 mL chloroform and sodium sulfate (1.5%). This mixture was stirred for 2 min, producing three separated layers. The upper layer contained a methanol–water mixture. The middle layer contained nonlipid substances and the clear lower layer contained the tissue lipids dissolved in chloroform. This bottom layer was isolated, filtered and the lipid content determined gravimetrically after evaporating a measured aliquot.

2.6. Determination of fat, relative abdominal fat (RAF), fat-free mass (FFM), and ash in animal carcasses

After thawing, the carcasses were sectioned, lyophilised, and homogenized in a blender. The fat and ash contents were obtained using the Bligh and Dyer and the AOAC methods and analysis was performed in triplicate (Association of Official Analytical Chemists, 1995; Bligh & Dyer, 1959). After sacrifice, the abdominal fat was removed, weighed and weights recorded adjusted by body weight to estimate the RAF. Therefore, RAF was defined as the abdominal fat weight (g)/body weight (g). The FFM was calculated by subtracting body fat percentage from total body weight and expressed as grams of fat-free mass.

2.7. Determination of short chain fatty acids (SCFA) in feces

The method used was adapted from AOAC (1997) (Association of Official Analytical Chemists, 1997). A 150-mg of feces was homogenized with 2.5 mL of pure acetone (Sigma-Aldrich®) by vortexing and then incubated for 15 min. The samples were filtered through a 0.45-μm membrane and transferred to a vial. Then, 5-μL aliquots were injected into a gas chromatograph (Young Lin, model 6000 series GC System Controller, FID detector, Anyang, Korea) equipped with a SGE® (USA) capillary column (BD20 60 m, ID: 0.25 μm; film: 0.25 μm; International Pty. Ltd., Australia). The chromatographic conditions were as follows: injector and detector temperatures of 260 °C and a flow of 1.4 mL/min. The oven temperature was set to 70 °C during the first 4 min, increased to 175 °C at a rate of 13 °C/min, maintained at 175 °C for 1 min, increased to 208 °C at a rate of 4 °C/min, maintained at 208 °C for 25 min, increased to 240 °C at a rate of 5 °C/min, and maintained at 240 °C for 30 min. Helium was used as the carrier gas, and the split ratio was 10:1. The SCFAs were quantitatively determined by a comparison of the retention times and peak areas of the samples with those of standards (acetic, propionic, and butyric acids; Sigma, Missouri, USA). Analyses were performed in triplicate.

2.8. Determination of bile acids (BAs) in feces

The method was adapted from Batta et al. (1999). A 150-mg of feces was homogenized in 0.8 mL n-butanol (Sigma-Aldrich®) and 0.15 mL hydrochloric (Sigma-Aldrich®) acid by vortexing and than incubated at 60 °C for 4 h. The samples were centrifuged (4000×*g* 5 min), and 0.2-mL of aliquots was transferred to 2-mL Eppendorf tubes and dried under nitrogen. Then, 0.2 mL of hexamethyldisilazane: trimethylchlorosilane:pyridine derivatization solution (3:1:9 v/v) was added (Sigma, Steinheim, Germany) to the residue. The mixture was incubated at 55 °C for 30 min and then dried under nitrogen. To the trimethylsilyl derivative formed 0.2 mL of hexane (Fisher Scientific, New Jersey, USA) was added, followed by stirring and filtration through a 0.45-μm membrane. A 2-μL of the filtrate was injected into a gas chromatograph (Young Lin, model 6000 series GC System Controller, FID detector) equipped with an Ohio Valley capillary column (OV-1 Bonded 30 m, ID: 0.32 μm, film: 0.50 μm; Ohio, USA). The chromatographic conditions were: injector and detector temperatures 285 °C; helium gas flow of 2.0 mL/min, with a split ratio of 5:1. The oven temperature was set to 150 °C for the first 2 min, increased to 278 °C at a rate of 5 °C/min, and maintained at 278 °C for 50 min. The BAs were quantitatively determined by a comparison of the retention times and peak areas of the samples with those of standards (cholic, chenodeoxycholic, deoxycholic, and lithocholic acids; Sigma, Missouri, USA). Analysis were performed in triplicate.

2.9. Statistical analysis

The results are expressed as the mean ± standard error (SE). Analysis of variance (ANOVA) was performed. The Duncan test was then applied using the program SPSS® (Chicago, USA), version 18. Differences were considered significant if $p \leq 0.05$.

3. Results and discussion

The feed efficiency ratio (FER) was lower in the TAI group compared to the LCEL and CEL groups. Thus, although consumption of the TAI diet was higher, consumption did not lead to a higher weight gain of the rats (Table 3). Owing to its bulky consistency, the diet containing LTL had a lower energy density than the other diets, leading to a higher feed intake of the animals in this group.

No literature pertaining to the safety of consumption of the *taioba* leaf by animals or humans was found. However, since the tubers have been traditionally consumed by small communities and there was a tendency of the animals to ingest greater volumes of food in a apparent attempt to compensate for the lower energy density of the LTL diet, this was understood as a sign of food acceptance or a lack of depression of food intake. The slightly lower weight gain observed in the animals that ingested LTL (Table 3) was associated with the presence of large amounts of insoluble fiber (Table 2). This is supported by earlier studies showing that diets with high content of insoluble fiber have less metabolizable energy and significantly higher energy loss *via* the feces, in contrast with diets containing high soluble fiber (Isken et al., 2010; Kristensen, Jensen, & Aarestrup, 2012). This also explains the high fecal mass and the lower FER observed in the TAI group because of the high ingestion by this group of compounds with zero energy contribution (Table 3). Recent studies have reported

Table 3
Average daily consumption, weight gain, fecal mass (g) and food efficiency ratio (FER) (means ± SE) of rats fed high fat diets containing cellulose, inulin or *taioba* fiber.

Groups	Consumption (g/day)	Weight gain (g)	Fresh fecal mass (g)*	FER (body weight increase/ daily consumption: g)
LCEL	21.34 ± 1.61^{b}	74.84 ± 5.42^{ab}	13.04 ± 0.65^{c}	0.12 ± 0.03^{a}
CEL	19.92 ± 1.41^{bc}	82.40 ± 9.75^{a}	21.20 ± 1.97^{b}	0.13 ± 0.02^{a}
INU	18.26 ± 0.92^{bc}	63.25 ± 9.01^{ab}	11.35 ± 0.76^{c}	0.11 ± 0.04^{ab}
TAI	24.83 ± 0.74^{a}	61.07 ± 9.67^{bc}	25.31 ± 1.22^{a}	0.08 ± 0.03^{b}

Different superscripted letters in columns indicate statistical differences ($p<0.05$). N = 8.
* No significant differences were found in moisture contents among the groups.

Table 4
Final fasting glucose, total area under the curve (AUC), AUC after 30 min., insulin levels, initial cholesterol (after 4 weeks with diet LCEL) and final cholesterol (initial and final total cholesterol — TC) of rats fed high fat diets containing cellulose, inulin or *taioba* fiber.

Experimental groups				
	LCEL	CEL	INU	TAI
Final glucose (mg/dL)	130 ± 3.36^{ab}	118 ± 3.45^{bc}	109.5 ± 3.40^{c}	136 ± 6.49^{a}
Total AUC	139 ± 35.6^{a}	123 ± 16.9^{a}	91.0 ± 25.9^{a}	77.6 ± 22.16^{a}
AUC after 30 min	55.8 ± 15.8^{a}	49.9 ± 3.76^{ab}	39.6 ± 8.76^{ab}	34.1 ± 10.1^{b}
Insulin (ng/dL)	3.08 ± 0.41^{a}	1.83 ± 0.69^{ab}	1.37 ± 0.20^{b}	2.65 ± 0.48^{ab}
Initial TC (mg/dL)	71.0 ± 5.4^{a}	63.5 ± 1.5^{a}	70 ± 3.7^{a}	62.3 ± 2.86^{a}
Final TC (mg/dL)	74.1 ± 4.5^{a}	68.6 ± 2.8^{ab}	70 ± 3.9^{ab}	62.7 ± 3.11^{bc}

Means with different superscripted letters in the same row differ significantly ($p<0.05$). N = 8.

Table 5
Fat content in feces, liver and body (g/100 g), free fat mass (FFM) (g/100 g), body ash (g/100 g), concentration of total of SCFA in feces (mg/g), and relative abdominal fat (RAF) (g) of rats fed high fat diets containing cellulose, inulin or *taioba* fiber.

Experimental groups				
	LCEL	CEL	INU	TAI
% Fat in feces*	9.24 ± 0.42^{c}	7.20 ± 0.93^{d}	12.5 ± 0.61^{b}	14.2 ± 0.54^{a}
Total SCFA†	0.35 ± 0.04^{c}	0.36 ± 0.04^{c}	1.30 ± 0.09^{a}	1.05 ± 0.21^{b}
Acetate†	0.21 ± 0.04^{c}	0.22 ± 0.05^{c}	0.59 ± 0.13^{b}	0.83 ± 0.11^{a}
Propionate†	0.07 ± 0.00^{b}	0.07 ± 0.00^{b}	0.47 ± 0.15^{a}	0.12 ± 0.01^{b}
Butyrate†	0.08 ± 0.00^{b}	0.07 ± 0.00^{b}	0.24 ± 0.04^{a}	0.10 ± 0.00^{b}
Liver fat content*	28.6 ± 1.40^{a}	25.0 ± 1.94^{b}	24.0 ± 1.94^{bc}	21.3 ± 0.90^{c}
Body fat*	51.6 ± 2.59^{a}	48.3 ± 6.12^{abc}	43.9 ± 2.93^{c}	49.8 ± 2.83^{ab}
FFM*	48.4 ± 2.46^{c}	51.7 ± 2.53^{bc}	56.1 ± 2.86^{ab}	50.2 ± 3.09^{bc}
Ash*	5.69 ± 0.64^{c}	6.03 ± 0.67^{ac}	6.96 ± 0.53^{ac}	7.33 ± 0.77^{ab}
AFR*	0.043 ± 0.003^{a}	0.044 ± 0.004^{a}	0.033 ± 0.003^{b}	0.038 ± 0.003^{ab}

Means with different superscripted letters in the same row differ significantly ($p<0.05$).
* N = 6.
† N = 4.

that, similar to the case of insoluble fiber, indigestible substances associated with the plant cell wall bind to and carry some energetic nutrients out of the body (Isken et al., 2010; Kristensen et al., 2012). *Taioba* leaves have not been studied in great detail and the possibility should not be discarded that other indigestible compounds besides fiber could eventually explain why the TAI group exhibited a lower weight gain than the CEL group.

Although there were no significant differences in final blood glucose concentration among TAI group and LCEL group, the area under the curve (AUC) obtained after the 30-minute GGT was lower ($p<0.05$) in the TAI group than in the LCEL group (Table 4). These results indicate that consumption of the leaf resulted in a lower increase in the glucose concentration during the glycemic peak, which occurred 30 min after glucose administration. No significant difference was observed among the different groups when the total AUC was evaluated after 2 h (Table 4). Although this is the first study of *taioba* leaf consumption, other studies have shown that plant compounds act as competitors for glucose in the intestine, thereby resulting in a low AUC (Baldea, Martineau, Benhaddou-Andaloussi, et al., 2010; Zheng, Shu, Yang, et al., 2012).

Both the positive control group INU and the TAI group exhibited a lower increase in the TC over time, compared to the LCEL and CEL groups (−0.5%, +0.6%, +4.1%, and +7.5%, respectively). However, at the end of the experiment, only the TAI group had a TC concentration that was significantly lower than that of the LCEL group (Table 4). There was no significant difference in the lipid and TG fractions between the groups (data not shown).

The excretion of BAs by the rats in the TAI group was approximately 2-fold higher than that in the LCEL and negative control (CEL) groups (Fig. 1, $p = 0.001$). These results suggest that there was an elevated BA binding capacity in the TAI group, which can result in a decrease of the hepatic pool of BAs and a reduction of TC (Hamauzu & Mizuno, 2011; Qiang, Lee, Ye, et al., 2012). The high quantity of fat in the feces of the TAI group (55% more than in the feces of the LCEL group, Table 5) demonstrated that the leaf has a high capacity to bind to dietary fats, thereby promoting a higher excretion of this substance from the body. Both effects may be responsible for the lower TC concentration observed in the TAI group compared with the LCEL group.

Cholesterol is converted to BA through different routes. The final products of this conversion are the primary BAs: chenodeoxycholic and cholic acids. Cholic acid is reabsorbed by hepatocytes and promotes increased activity of CYP7A1 (cholesterol 7α-hydroxylase), which in turn increases the synthesis and transport of BAs to the intestine (Qiang et al., 2012; Wu & Chen, 2011). As shown in Fig. 2, the experimental group, ingesting LTL, exhibited higher cholic acid excretion than the other groups ($p = 0.002$), with the exception of the negative control (CEL) group. This result indicates that some component of the

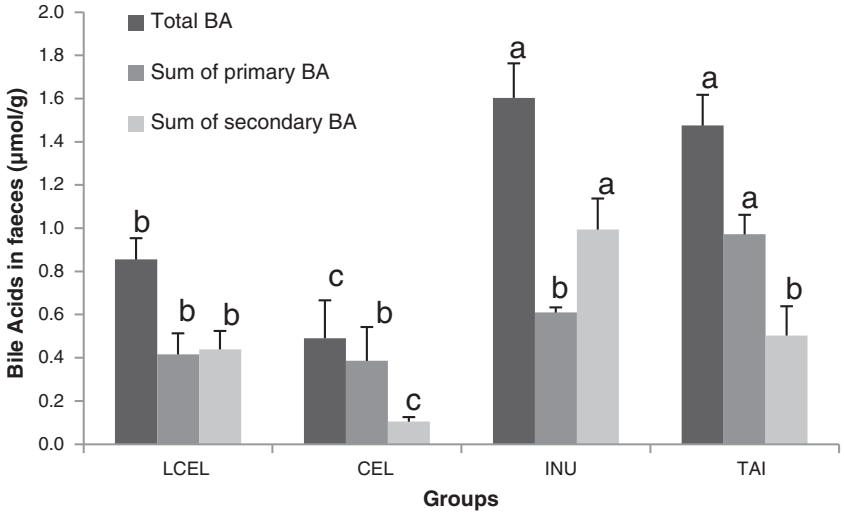

Fig. 1. Concentration of total bile acids (primary + secondary), sum of primary BA (chenodeoxycholic acid + cholic acid) and sum of secondary BA (deoxycholic acid + lithocholic acid) in feces of rats fed high fat diets containing cellulose, inulin or *taioba* fiber. The values represent the means ± SE. Different superscripted letters in columns indicate statistical differences ($p<0.05$). N = 4.

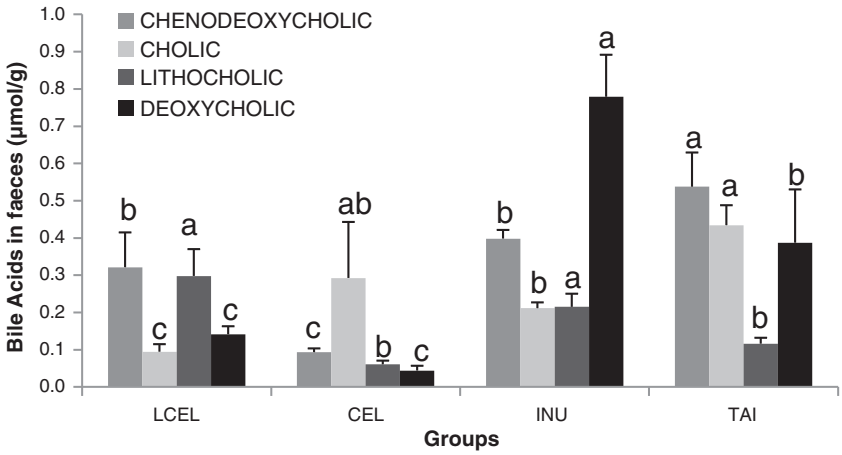

Fig. 2. Concentration of chenodeoxycholic acid, cholic acid, deoxycholic acid and lithocholic in feces of rats fed high fat diets containing cellulose, inulin or *taioba* fiber. The values represent the means ± SE. Different superscripted letters in column indicate statistical differences ($p<0.05$). N = 4.

leaf may also reduce blood cholesterol through the increased synthesis of BAs and suggests that leaf consumption reduced the reabsorption of cholic acid thus favoring its excretion.

The high excretion of BAs by the rats in the TAI group was due to increased excretion of primary rather than secondary BAs, as seen in Fig. 1. The CYP7A1 enzyme, which is responsible for the conversion of primary BAs to secondary BAs, is present in bacterial species such as *Clostridia* and *Eubacteria* but not in *Lactobacilli* and *Bifidobacteria* (Ridlon, Kang, & Hylemon, 2006; Takahashi & Morotomi, 1994; Wu & Chen, 2011). Although our data offers no direct support, the possibility that the *taioba* leaf alters the microbiota structure in a way to reduce the production and/or activity of CYP7A1, thereby reducing the conversion of primary BAs to secondary BAs, should not be discarded. Furthermore, considering the greater food ingestion and the high content of insoluble fiber in the LTL diet, and the fact that there were no significant differences in the moisture contents of the feces, it was concluded that the *taioba* leaf accelerated intestinal transit (inferred from the higher fecal weight, Table 3) and thus reduced the possibility of transforming primary acids into secondary acids. In addition, the activity of CYP7A1 is inhibited in more acidic media (Christl, Bartram, Paul, et al., 1997; Takahashi & Morotomi, 1994), and inhibition may have occurred as a result of the high fermentability of the LTL (as demonstrated by the higher fecal SCFA content, Table 4). Moreover, it was observed that the TAI group exhibited the lowest fecal concentration of lithocholic acid, while the TAI and the negative control, CEL, groups showed a lower deoxycholic acid concentration than the INU group ($p<0.05$, Fig. 2). Previous studies have shown that secondary BAs, such as lithocholic and deoxycholic acids, are toxic to cells of the intestinal mucosa and lead to a compensatory proliferation of enterocytes, which increases the risk for the development of colon cancer (Ridlon et al., 2006; Wu & Chen, 2011). Therefore, the *taioba* leaf can potentially reduce the risk of toxicity to colonocytes.

Whereas both the TAI and positive control (INU) groups exhibited high total SCFA production ($p<0.05$), both the LCEL and negative control (CEL) groups showed low fermentative capacity and the lowest SCFA production (Table 5). Inulin has been proven to be a prebiotic polymer that promotes the production of SCFAs (Beylot, 2005), but the effect of the *taioba* leaf as a prebiotic vegetable has not been studied. Prebiotic compounds target the colon and affect the environment of the gastrointestinal tract through the production of SCFAs and other bioactive substances. The production of SCFAs in the intestine has been associated with a reduced risk of some diseases, including the irritable bowel syndrome, inflammatory bowel disease, cardiovascular disease, and cancer (Jenkins, Kendall, & Vuksan, 1999; Wong, de Souza, Kendall, et al., 2006). Because the LTL was found to possess high fermentability, it may be proposed that the boiled *taioba* leaf is a prebiotic source yet without excluding the possible contribution of other fermentable substances to such an effect in addition to the soluble fiber.

As expected, the predominant fatty acid in the feces of all of the animals was acetic acid (Sembries, Dongowski, Mehrländer, et al., 2006; Wong et al., 2006), although the TAI group exhibited the highest proportion of acetic acid (78%, Table 5). This effect may be related to the presence of fermentative substrates (in high proportions in the LTL) that specifically favored the production of acetic acid compared to other SCFAs. It is suggested that the lower excretion of butyrate and propionate in the TAI group compared to the INU group may reflect the increased use of these fatty acids by the microbiota that colonize the large intestine to obtain energy for maintenance and growth. This result is consistent with the findings reported by Monteiro (2011), who observed high concentrations of butyric and propionic acids in the caecum and colons of animals receiving LTL compared with animals that received inulin. These results demonstrate a positive physiological effects of the leaf because both of these fatty acids are associated with growth inhibition and apoptosis of colon tumor cells (Ferguson et al., 2001; Mcorist, Miller, Bird, et al., 2011; Wong et al., 2006).

The percentage of liver fat was lower in the TAI and in positive control (INU) groups ($p<0.001$), although the TAI group was the only group that significantly differed from the CEL and LCEL groups, with approximately 25.5% less fat (Table 5). Therefore, LTL prevented the accumulation of fat in the liver of animals ingesting a high-fat diet, thereby also demonstrating a hepatoprotective effect. The analysis of the body composition of the animals (Table 5) revealed that only the INU group presented a fat percentage and relative abdominal fat (RAF) lower than those observed in the LCEL group.

Only the LCEL group exhibited a low proportion of FFM and ash in the carcass; however, the only significant difference found was in the ash content of the TAI group ($p<0.05$). These results indicate that *taioba* leaf consumption resulted in a greater preservation of the mineral content in the body than the other fiber sources. Other studies have shown that some plant families exhibit a protective effect on bone mass perhaps by several not yet well established mechanisms of action that include the participation of their bioactive compounds (Muhlbauer, Lozano, Palacio, et al., 2003). The high fermentability of the LTL contributed to the reduction of intestinal pH (Wong et al., 2006), which is consistent with increased bioavailability of minerals (Banu, Varela, & Fernandes, 2011). Furthermore, the high ash and calcium content of the LTL also explains the increase in the body mineral content that was observed in the animals that consumed the plant

(Brownlee, 2011; Tabela brasileira de composição de alimentos, 2011).

Although no studies were found about possible bioactive components of the *Taioba* leaf, a diverse variety of substances beneficial to human health are known to exist in practically all leaves studied, such as lettuce, spinach, cabbage and sweet potato leaves. Beyond the dietary fibers, phenolic and polyphenolic compounds (*i.e.*, flavonoids, phenolic acids, and lignans), glucosinolates, terpenoids (sterols, phytol, carotenoids) and alkaloid compounds, have been reported in edible leaves (Hounsome et al., 2008; Johnson & Pace, 2010).

4. Conclusions

The results suggest that the *taioba* leaf has high fermentability and binding capacity for bile acids, as compared to the positive and negative controls, resulting in the excretion of greater amounts of the primary acids in the feces. These properties indicate that the leaf exhibits the potential of being a dietary aid to lower blood cholesterol and the risk of bowel cancer. No negative effects were noticed after four weeks of feeding the cooked leaf to growing rats, thereby adding an extra advantage to the health benefits observed in this study. The possibility that the positive effects associated with the ingestion of the leaf are related to a synergy between the fiber and other bioactive substances is not excluded. This report shows data characterizing some nutritional and functional properties and related health-benefits of a poorly studied leafy vegetable.

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