



# Morphometric parameters and adipose tissue mass of rats fed with cactaceae flour

Izabel Cristina Ferreira<sup>1</sup>, Beatriz Barakat<sup>1</sup>, Martha Elisa Ferreira de Almeida<sup>2\*</sup>, Keidy Lara Ferreira<sup>3</sup> and José Antônio de Souza Cruz Ramos<sup>4</sup>

<sup>1</sup>Universidade Federal de Viçosa, Campus Rio Paranaíba, Rio Paranaíba, Minas Gerais, Brasil. <sup>2</sup>Curso de Nutrição, Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Viçosa, Campus Rio Paranaíba, Km 7, MG-230, 38810-000, Rio Paranaíba, Minas Gerais, Brasil. <sup>3</sup>Curso de Ciências Biológicas, Universidade Federal de Viçosa, Campus Rio Paranaíba, Rio Paranaíba, Minas Gerais, Brasil. <sup>4</sup>Técnico em Vigilância Epidemiológica e Ambiental da Comarca de Rio Paranaíba, Rio Paranaíba, Minas Gerais, Brasil. \*Author for correspondence. E-mail: martha.almeida@ufv.br

**ABSTRACT.** The aim of this study was to evaluate the morphometric parameters and the mass of adipose tissues of rats fed with cactacea flour. The animals were divided into five groups: C (Control), H (Hypercaloric diet), HP (Hypercaloric diet with 5% *Pereskia grandifolia* flour), HO (Hypercaloric diet with 5% *Opuntia ficus-indica* flour), and HA (Hypercaloric diet with 5% *Agave tequilana* flour). Feed intake and apparent digestibility, capillary glycemia, liver weight and Hepato-Somatic Index were evaluated. Adipose tissue mass and the Visceral Fat Index (VFI) and Epididymal Fat Index (EFI) were determined. The data were compared using the Tukey test at 5% significance level. There was no statistical difference between the groups for body weight, BMI and Lee Index. The HP and HA groups did not differ from the C group regarding weight gain, the C group had lower apparent digestibility of the diets, the HA group presented lower blood glucose when compared to the H and HO groups, and the H group had higher liver weight. The HP and HA groups gained the lowest adipose tissue mass, VFI and EFI than the H and HO groups. Among the cactaceae studied, *Pereskia grandifolia* showed better effects on morphometric and adipose tissue parameters.

**Keywords:** *Pereskia grandifolia*; *Opuntia ficus-indica*; *Agave tequilana*; cafeteria diet; adipose tissue.

Received on January 11 2022.

Accepted on May 20 2022.

## Introduction

The sedentary lifestyle, associated with an inadequate feeding and sleep periods (Wang, Yin, & Shao, 2021), has contributed to the emergence of various diseases, especially overweight and obesity, which is a reality in almost the entire world, and presents a great challenge in relation to its prevention and treatment. Of a total of 5 billion adults worldwide, about 2 billion are overweight, and among children under 5 years of age, 41 million are overweight, and consequently have a greater chance of becoming obese adults (International Food Policy Research Institute [IFPRI], 2016). Thus, reducing overweight in all age groups is important as it increases the risk for developing other chronic non-communicable diseases such as diabetes mellitus, hypertension, cardiovascular diseases, cancer, and chronic kidney disease (World Health Organization [WHO], 2016; Zhou, 2021).

In Brazil, data from the Surveillance of risk and protection factors for chronic diseases by telephone survey (Vigitel) 2019, showed that 57.1% of adult-aged men and 53.9% of adult-aged women were overweight, and 20.3% among adult-aged men and women were classified with obesity (Brasil, 2020). Estimates indicate that such data tend to increase, since in 35 years overweight has tripled in the adult population (Instituto Brasileiro de Geografia e Estatística [IBGE], 2010).

Increased consumption of unhealthy high-energy food and a decrease in the intake of those that are sources of fiber, vitamins and minerals, as well as lack of physical activity, have contributed to overweight (Almeida, 2017; Goswami, Trozic, Fredriksen, & Fredriksen, 2021). In this sense, Non-Conventional Food Plants (NCFPs), plants and part of plants used in food that are still little consumed have stood out due to their sustainable practice, low cost and aid in reducing body weight (Almeida, 2017; Medeiros et al., 2021).

The Cactaceae are Non-Conventional Food Plants that can be used in various recipes, among them are the ora-pro-nobis (*Pereskia grandifolia*), agave (*Agave tequilana*) and palm or prickly pear (*Opuntia ficus-indica*). *Pereskia grandifolia* has high protein content with high digestibility, fiber, calcium, and iron (Almeida, Junqueira, Simão, & Corrêa, 2014; Almeida, Simão, Corrêa, & Fernandes, 2016). *Opuntia ficus-indica* has been used in food for its carbohydrates, proteins, fibers (for example, mucilage, cellulose, and pectin), calcium,

iron and vitamin C (Loayza & Chávez, 2007; Silva et al., 2015; Figueirôa et al., 2021). *Agave tequilana* stands out for the minerals calcium, iron, magnesium, potassium, zinc and fibers (for example, cellulose, hemicellulose, and lignin) (Romero-López, Osorio-Díaz, Flores-Morales, Robledo, & Mora-Escobedo, 2015; Huerta-Cardoso, Durazo-Cardenas, Longhurts, Simms, & Encinas-Oropesa, 2020).

Plants of the genera *Pereskia* and *Opuntia* are used in folk medicine for the treatment of diabetes mellitus, obesity, neoplasms and heart disease (Almeida et al., 2016; Díaz, Rosa, Héliès-Toussaint, Guéraud, & Nègre-Salvayre, 2017). In view of the potential of these plants in the treatment of obesity, the aim of this study was to evaluate the morphometric parameters and the mass of adipose tissues of rats fed with cactaceae (*Pereskia grandifolia*, *Opuntia ficus-indica*, and *Agave tequilana*) flour.

## Material and methods

### Preparation of the cactaceae flours

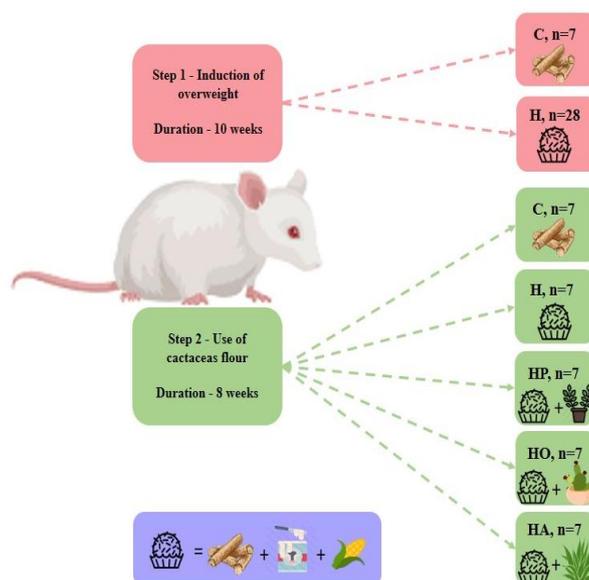
The leaves of *Pereskia grandifolia* and *Agave tequilana*, and the cladodes of *Opuntia ficus-indica* were collected in a farm in the municipality of São Gonçalo do Abaeté, Minas Gerais, Brazil. They were first sanitized in running water to remove visible dirt, and then sanitized with active chlorine for 10 minutes, and again immersed in a container of water for 2 minutes to remove excess chlorine.

After draining the excess water, the *Pereskia grandifolia* leaves were cut into thin slices (Chiffonade) to speed up their drying. Due to the presence of thorns on the leaves of *Agave tequilana*, they were removed by peeling the edges of the leaves. To avoid contact dermatitis, caused by chemical substances present in the leaves of the agave (Salinas, Ogura, & Soffchi, 2001), gloves, boots, goggles and long-sleeve lab coat were used during all the handling of this plant, whose leaves were cut into cubes of 0.5 cm<sup>2</sup> (cut - Gardener). Because the *Opuntia ficus-indica* cladodes are also covered with spines, it was necessary to remove the spines by peeling them and then cutting them into cubes of 0.5 cm<sup>2</sup> (cut - Gardener).

After cutting, the leaves were placed separately in an oven with air circulation at 50°C, where they remained for 5 days until they were completely dry. After drying, the leaves were ground in a domestic blender, sieved, and their flours stored at room temperature, protected from light in aluminum foil covered jars, until they were used in the rats' diets.

### Animal and diet protocols

Was used 30 days old male rats (*Rattus norvegicus*), Wistar strain, obtained from the Central Animal Laboratory of the Biological and Health Sciences Center of the Federal University of Viçosa (UFV), Viçosa Campus, Brazil, with a mean weight of 50 g. The animals were kept in individual cages at 22°C with 12 hours light/dark cycle (7:00 to 19:00h), and received food and water *ad libitum*. The experiment lasted 18 weeks and occurred in two stages, as shown in Figure 1.



**Figure 1.** Experimental scheme using rats submitted to different step, diets and cactacea flour.

In step 1, animals in the control group (C) received commercial feed in pellet form, while animals in the hypercaloric diet group (H) received a diet composed of 46% ground commercial feed, 46% condensed milk, and 8% corn oil (Almeida, Medeiros, Figueiredo, Coelho, & Sena, 2011). In step 2, the animals were divided into 5 groups with the administration of a commercial feed in pellet form, hypercaloric diet, and the diets with cactacea flour as described below:

- C group (Control, n = 7): commercial feed in pellet form;
- H group (Hypercaloric diet, n = 7): 46% ground commercial feed, 46% condensed milk, and 8% corn oil;
- HP group (Hypercaloric diet and *Pereskia grandifolia* flour, n = 7): 41% commercial milled feed, 46% condensed milk, 8% corn oil, and 5% *Pereskia grandifolia* flour;
- HO group (Hypercaloric diet and *Opuntia ficus-indica* flour, n = 7): 41% ground commercial feed, 46% condensed milk, 8% corn oil, and 5% *Opuntia ficus-indica* flour;
- HA group (Hypercaloric diet and Agave tequilana flour, n = 7): 41% commercial milled feed, 46% condensed milk, 8% corn oil, and 5% Agave tequilana flour.

### Parameters assessed

Body weight and naso-anal distance were measured weekly (Almeida et al., 2018). From these data the Body Mass Index (BMI) = [body weight (g) / length<sup>2</sup> (cm)], and the Lee Index (LI) = [cube root of body weight (g) / naso-anal size (cm)] was calculated. The BMI for eutrophic adult rats ranges from 0.45 to 0.68 g·cm<sup>-2</sup> (Novelli et al., 2007), and for animals lacking statural growth, the Lee Index value is classified as normal ( $\leq$  0.300) or obese ( $>$  0.300) (Bernardis & Patterson, 1968).

Weekly feed intake was calculated by the difference between the amount of diet offered and the leftover in the feeders (Almeida et al., 2018). At weeks 4 and 8 of cactaceae flour use, feces were collected for calculation of apparent digestibility (Almeida et al., 2018) according to the formula: [(ingested amount of food - excreted amount of feces) / ingested amount of food] x 100.

At the last week of the experiment, blood samples were taken from the caudal portion of the rats after fasting for 12 hours to determine glucose levels using a G-TECH Free1 portable glucometer.

### Animal sacrifice and sample collection

At the end of the experiment, the rats were submitted to general anesthesia (intraperitoneal) by overdose of ketamine (300 mg·kg<sup>-1</sup>) + xylazine (30 mg·kg<sup>-1</sup>), which were euthanized after confirmation of loss of consciousness and death. Approximately 4 mL of blood was collected by cardiac puncture, which caused death by exsanguination under anesthesia.

After euthanasia, the liver was removed by necropsy to calculate the Hepato-Somatic Index (HSI) (Almeida et al., 2018) according to the equation: (liver weight / final body weight) x 100. The epididymal adipose tissue was collected adhered to the epididymis to calculate the Epididymal Fat Index (EFI) (Almeida et al., 2018) according to the equation: (epididymal fat mass / final body weight) x 100. The visceral adipose tissue that is adhered to the cavity wall was removed, next to the parietal peritoneum in the pelvic region to calculate the Visceral Fat Index (VFI) (Almeida et al., 2018) according to the equation: (visceral fat mass / final body weight) x 100.

The reduction in adipose tissue mass of animals fed with cactaceae flours (HP, HO, and HA) compared to the hypercaloric group (H) was calculated, and it was formed as: ineffective (0 to 4.99%), effective (5.00 to 9.99%), and excellent ( $\geq$  10.00%) (Almeida et al., 2018).

### Statistical analysis

Analysis of variance (ANOVA) was used with a post hoc by Tukey Test, with a significance of 5%, in the Statistical Package for Social Sciences (SPSS), version 22.0.

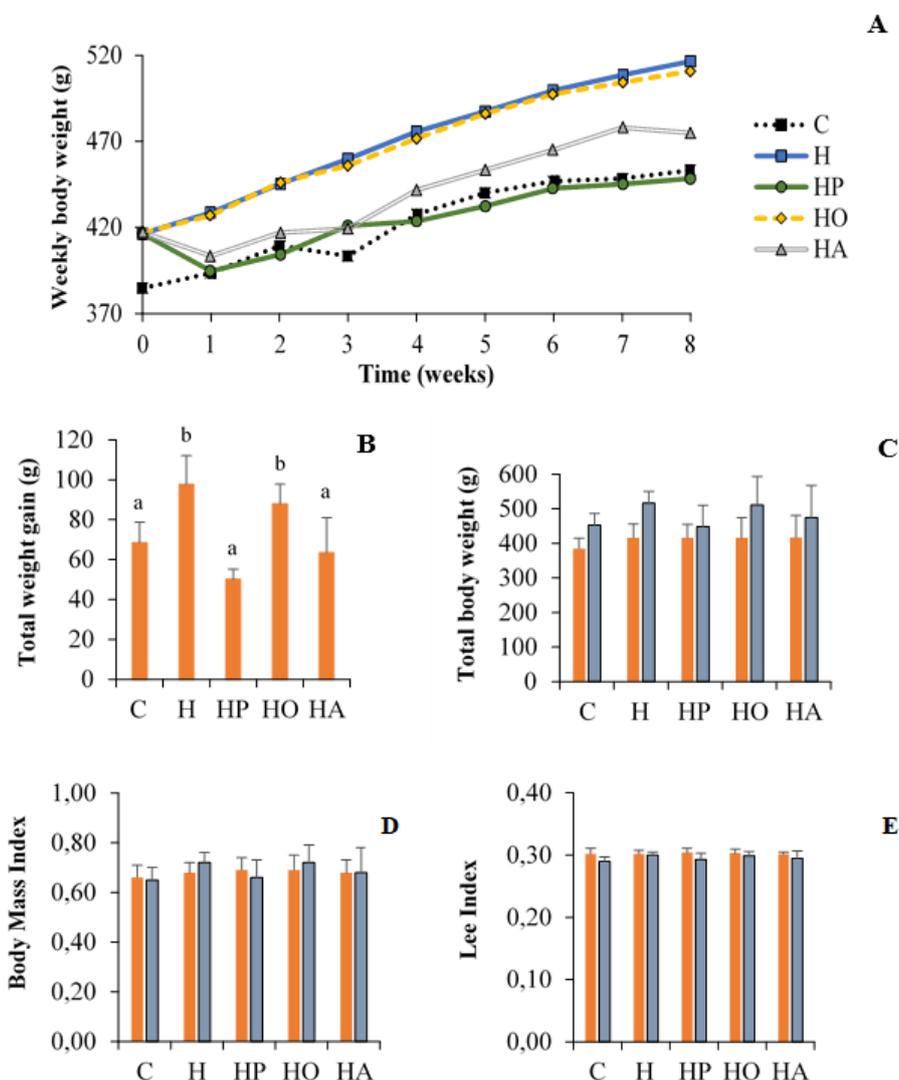
### Ethical care

The experiment was based on ethical principles in Brazilian Animal Experimentation, in accordance with Law nº 11,794, of October 8, 2008 (Brasil, 2008), and took place after approval by the Ethics Committee on the Use of Animals, of the University Federal of Viçosa (UFV) (Protocol 100/2017).

### Morphometric parameters

There was no statistical difference between the groups in terms of body weight during the weeks of use of cactaceae flours (Figures 2A and 2B), demonstrating that such flours did not change the total body weight of

the animals, differing from other studies carried out with rodents (Rodríguez-Rodríguez et al., 2015; Almeida et al., 2016; Sánchez-Tapia et al., 2017), in which the cactaceae reduced this morphometric parameter. However, groups H and HO showed a greater total weight gain ( $p < 0.05$ ) when compared to the other groups that were equal to each other (Figure 2C). The groups that received *Pereskia grandifolia* (HP) and *Agave tequilana* (HA) flours did not differ ( $p > 0.05$ ) from the control group (C) in terms of total weight gain, suggesting that the bioactive compounds present in these flours made the weight gain similar to that of animals that received the commercial feed in pellet form.



**Figure 2.** A = Weekly body weight; B = Total weight gain; C = Total body weight (Orange = Initial step; Blue = Final step); D = Body Mass Index (Orange = Initial step; Blue = Final step); and E = Lee Index (Orange = Initial step; Blue = Final step) of rats fed different diets and flours. Mean  $\pm$  standard deviation followed by the same letters between groups do not differ by Tukey Test at 5% probability. Absence of letters between groups did not differ by the Tukey test at 5%. C = Commercial ration, H = Hypercaloric diet, HP = Hypercaloric diet and *Pereskia grandifolia* flour, HO = Hypercaloric diet and *Opuntia ficus-indica* flour, HA = Hypercaloric diet and *Agave tequilana* flour.

Rats ingesting a hypercaloric cafeteria diet plus 5 and 10% *Pereskia grandifolia* flour decreased final body weight (Salinas, Ogura, & Soffchi, 2001), in another study with rats ingesting a hypercaloric diet and *Pereskia* cactaceae juice, the group that received *Pereskia grandifolia* juice showed less weight gain (Brasil, Val, Ramos, & Almeida, 2020). C57BL/6 mice fed a high-fat diet supplemented with 0.3 and 0.6% of *Opuntia ficus-indica* extract had a lower body weight than those that ate only the high-fat diet (Rodríguez-Rodríguez et al., 2015). Rats fed a diet rich in lipids and sucrose added with 5% dehydrated *Opuntia ficus-indica* reduced body weight (Sánchez-Tapia et al., 2017).

Groups H (Hypercaloric diet) and HO (Hypercaloric diet and *Opuntia ficus-indica*) had a greater weight gain when compared to the other groups that were equal to each other. Such data are similar to those of calves fed with hay plus 20, 40 and 60% of *in natura* crushed *Opuntia ficus-indica* cladodes, which increased weight gain

compared to the control group with hay (Tegegne, Kijora, & Peters, 2007), as well as the cows who ingested a mixture of pasture, palm and soybean flour that gained more weight compared to the other groups (Albuquerque et al., 2002), demonstrating that among cattle this type of cactus promoted weight gain, justifying its use in feeding these animals from regions of dry climates (semi-arid and arid). However, some studies carried out with cactaceae have reduced weight gain in rodents. In a study with diabetic rats, there was an improvement in weight gain in those treated with *Opuntia ficus-indica* compared to untreated rats (Elshehy, Salah, Mawla, & Agamy, 2020).

*Pereskia aculeata* flour decreased weight gain in rats (Barbalho et al., 2016; Agostini-Costa, 2020). A high-fat diet plus 10% *Agave tequilana* provided less weight gain (Franco-Robles & López, 2016), as well as the ingestion, via gavage, of 5 g·kg<sup>-1</sup> of *Agave tequilana* fructans reduced weight gain in obese C57BL/6 mice (Márquez-Aguirre et al., 2013). Rats ingesting a high-fat diet plus agave obtained less weight gain (Franco-Robles & López, 2016), and it has been described that agavins (Urías-Silvas et al., 2008) and saponins (Sidana, Singh, & Sharma, 2016), found in such cactaceae, have beneficial effects in the treatment of obesity. Diabetic and non-diabetic obese rats fed *Agave tequilana* had a reduction in weight gain (Kang et al., 2016), as did C57BL/6 mice when treated with *Agave salmiana* extract and a high-fat diet (Leal-Díaz et al., 2016).

There was no difference between groups ( $p > 0.05$ ) in the initial and final analyzes regarding the Body Mass Index (BMI) and Lee Index (Figures 2D and 2E) differing from other study (Almeida et al., 2016), in which rats fed a hypercaloric cafeteria diet plus 5 and 10% *Pereskia grandifolia* flour reduced these two indices when compared to the control group. At the beginning of step 2, groups C, H and HA showed eutrophic BMI, and at the end of this step groups C, HP and HA obtained such classification, demonstrating that *Agave tequilana* flour did not change this parameter, while *Pereskia grandifolia* flour changed the classification of the group from obese to eutrophic. As for the Lee Index, at the beginning of step 2, all groups were classified as obese, demonstrating that this parameter is not the most suitable for assessing obesity in rats, as the animals in the control group were obese according to the classification.

The daily administration of 96 mg·kg<sup>-1</sup> of *Agave tequilana* powdered fructans to obese humans ingesting a diet of 1500 kcal decreased the Body Mass Index compared to the group that consumed only the diet (Padilla-Camberos, Barragán-Álvarez, Diaz-Martinez, Rathod, & Flores-Fernández, 2018).

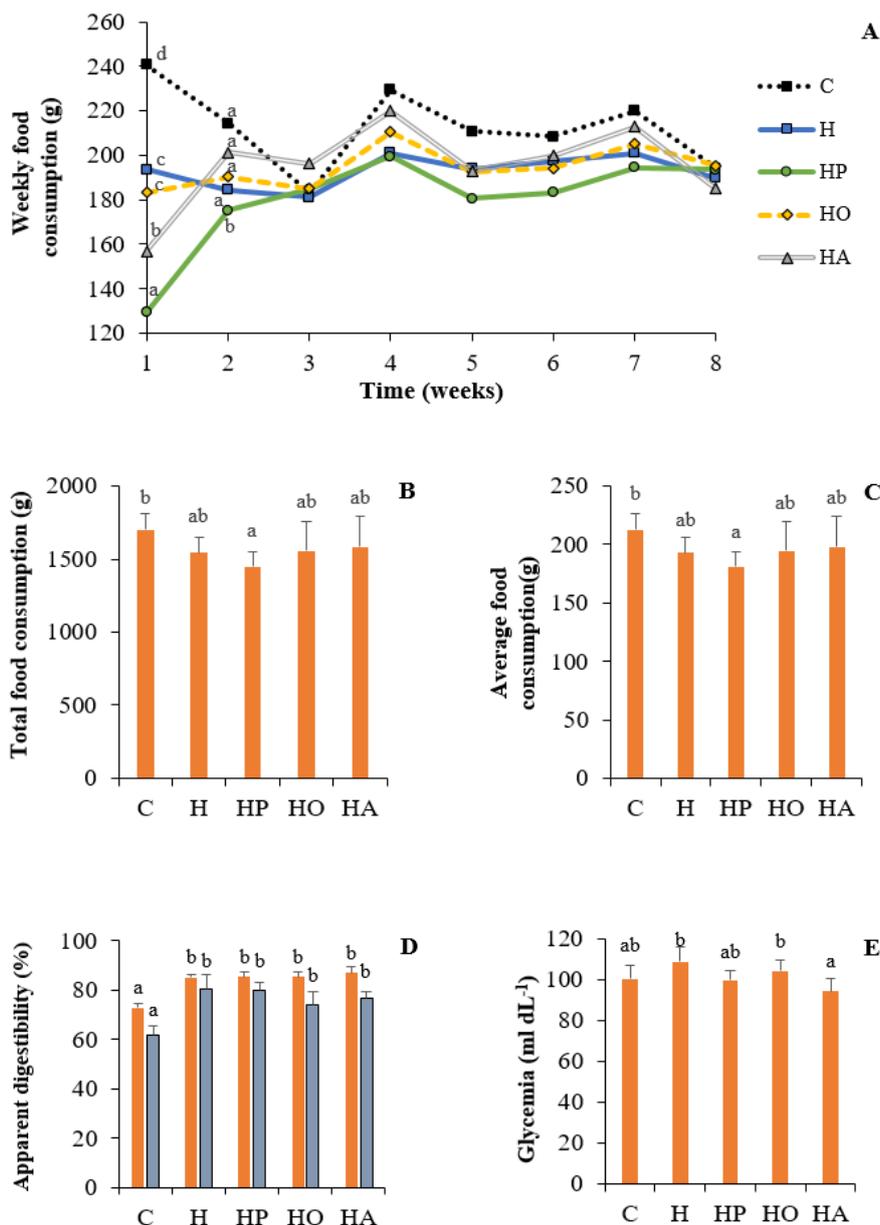
### Analysis of food consumption and apparent digestibility

At week 1, the animals in the control group (C) had a higher food consumption ( $p < 0.05$ ) than the other groups, with the H and HO groups being equal to each other and higher than the HP and HA groups. At week 2, only the HP group had a statistically lower food consumption than the control group (C), differing from the study (Almeida et al., 2016) in which the animals that received *Pereskia grandifolia* flour (5 and 10 %) had a lower food consumption during the 4 weeks evaluated.

For the other weeks, there was no statistical difference between the groups regarding food consumption (Figure 3A), however, when the total food consumption and the average consumption were evaluated, it was found that the control group (C) was higher than the HP group (Figures 3B and 3C), and such difference did not affect the final body weight between the groups. Eutrophic (Barbalho et al., 2016) and obese diabetic and non-diabetic rats (Kang et al., 2016) ingesting a high-fat diet plus agave had a lower food intake (Franco-Robles & López, 2016).

The control group (C) showed lower apparent digestibility ( $p < 0.05$ ) in both stages when compared to other groups (Figure 3D), and the high-calorie diets, even with the addition of cactaceae flours, showed greater digestibility and use of nutrients, this result was also observed in the study carried out with lychee flour (Almeida et al., 2018).

The group that received *Agave tequilana* (HA) had a lower blood glucose value when compared to groups H and HO (Figure 3E), as observed in some studies with rodents and cactaceae. Rats fed with *Pereskia grandifolia* flour (5 and 10%) reduced blood glucose when compared to the control group (Almeida et al., 2016). A decreased of glycemia and increased number of pancreatic  $\beta$  cells (Yoon, Lee, Kim, & Son, 2011) was observed in db/db mice receiving 5% *Opuntia ficus-indica* flour. Those db/db mice that received the *Opuntia ficus-indica* extract (1 and 2 g·kg<sup>-1</sup> body weight) presented lower blood glucose levels and improved glucose tolerance when compared to the diabetic control group that did not receive the extract (Leem, Kim, Hahm, & Kim, 2016). There was a reduction in blood glucose in diabetic rats fed a high-fat diet and *Opuntia ficus-indica* extract (Hwang, Kang, & Lim, 2017), as well as in rats treated with an aqueous extract of *Opuntia ficus-indica* (6 mg kg<sup>-1</sup>) (Butterweck et al., 2011).

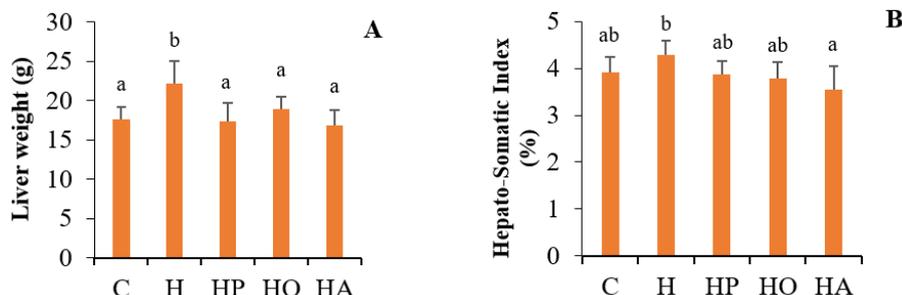


**Figure 3.** A = Weekly food consumption; B = Total food consumption; C = Average food consumption; D = Apparent digestibility (Orange = Step 1; Blue = Step 2); and E = Glycemia of rats fed with different diets and flours. Mean  $\pm$  standard deviation followed by the same letters between groups do not differ by Tukey Test at 5% probability. C = Commercial ration, H = Hypercaloric diet, HP = Hypercaloric diet and *Pereskia grandifolia* flour, HO = Hypercaloric diet and *Opuntia ficus-indica* flour, HA = Hypercaloric diet and *Agave tequilana* flour.

Rats fed 10% agave fructans decreased glycemia compared to those fed the control diet, suggesting that these oligosaccharides increased the hormone GLP-1 (glucagon-like peptide-1) (Urías-Silvas et al., 2008). Another study with rats fed the diet hyperlipidic associated with the ingestion of agave fructans and *Hibiscus sabdariffa* shows the expression of this same hormone (Sáyago-Ayerdi, Zamora-Gasga, & Venema, 2020). C57BL/6 mice treated with a high-fat diet and *Agave tequilana* with a lower degree of polymerization of branched fructans had its glycemia reduced, while those treated with a higher degree of polymerization of branched fructans had triacylglycerols decreased (Márquez-Aguirre et al., 2016). Rats fed a diet supplemented with *Agave fourcroydes* presented decreased blood glucose, total cholesterol and triacylglycerols, and increased bone calcium and magnesium (García-Curbelo, Bocourt, Savón, García-Vieyra, & López, 2015), and in non-diabetic rats *Agave tequilana* fructans reduced blood glucose (Rendón-Huerta, Juárez-Flores, Pinos-Rodríguez, Aguirre-Rivera, & Delgado-Portales, 2012). C57BL/6 mice treated with *Agave salmiana* extract and a high-fat diet showed decreased blood glucose (Leal-Díaz et al., 2016), as well as mice fed a high-fat diet with *Agave tequilana* and *Hibiscus sabdariffa* (Moyano et al., 2016).

### Liver weight and adipose tissue mass

Group H presented greater liver weight when compared to the other groups (Figure 4A), demonstrating that the cactaceae flours attenuated the increase in the weight of this organ, as in the study (Almeida et al., 2016) in which animals ingesting a hypercaloric diet showed higher liver weight resulting from lipid deposition compared to groups fed a hypercaloric diet plus *Pereskia grandifolia* flour.



**Figure 4.** A = Liver weight; and B = Hepato-Somatic Index of rats fed different diets and flours. Mean  $\pm$  standard deviation followed by the same letters between groups do not differ by Tukey Test at 5% probability. C = Commercial ration, H = Hypercaloric diet, HP = Hypercaloric diet and *Pereskia grandifolia* flour, HO = Hypercaloric diet and *Opuntia ficus-indica* flour, HA = Hypercaloric diet and *Agave tequilana* flour.

In this study, only groups H and HA differed from each other in terms of Hepato-Somatic Index (Figure 4B), as referred by other study who did not identify any statistical difference between the control and flour groups of *Pereskia grandifolia* (Almeida et al., 2016). However, in another study they identified that the control group had a higher Hepato-Somatic Index when compared to groups fed a high-calorie diet and lychee flour (Almeida et al., 2018).

The addition of *Opuntia ficus-indica* seed extract to a high-fat diet improved hepatic steatosis in rats, reduced the expression of nuclear factor  $\kappa$ B (NF- $\kappa$ B), tumor necrosis factor (TNF- $\alpha$ ) and interleukin 6 (IL-6) (Kang et al., 2016), activity. The anti-inflammatory effect of *Opuntia ficus-indica* demonstrated by other studies that also obtained a reduction in TNF- $\alpha$  (El-Hawary et al., 2020; Elswawi, Radwan, Elbatany, El-Feky, & Sherif, 2020). Rats fed a diet rich in lipids and sucrose, plus 5% *Opuntia ficus-indica* had a decrease in hepatic steatosis and oxidative stress in adipose tissue and in the brain (Sánchez-Tapia et al., 2017). C57BL/6 mice that consumed a high-fat diet added with *Agave salmiana* extract decreased liver lipids, suggesting that the saponins present in the extract increased fatty acid oxidation (Leal-Díaz et al., 2016). C57BL/6 mice treated with *Agave tequilana* fructans reduced hepatic steatosis (Rendón-Huerta, Juárez-Flores, Pinos-Rodríguez, Aguirre-Rivera, & Delgado-Portales, 2012; Márquez-Aguirre et al., 2016).

There was no difference ( $p > 0.05$ ) between the groups that received *Pereskia grandifolia* and *Agave tequilana* flours (HP and HA) and the control group (C) regarding the mass of the epididymal and visceral adipose tissues (Table 1). However, the group that received *Opuntia ficus-indica* flour (HO) did not differ from group H in terms of adipose tissue mass, both were superior to the control group (C).

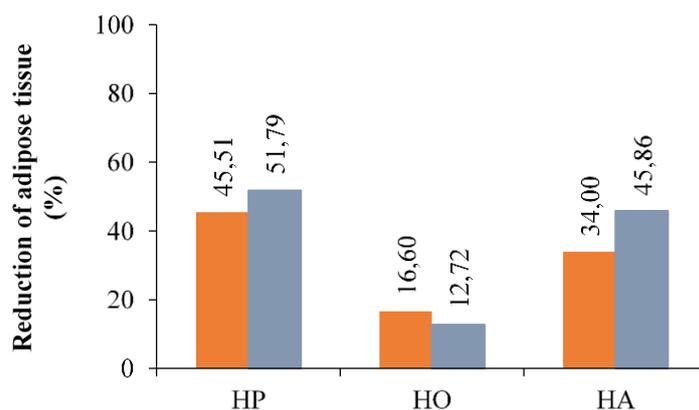
The groups that received the hypercaloric diet (H) and the diet added with *Opuntia ficus-indica* flour (HO) had the highest Fat Indexes (VFI and EFI), and the other groups did not differ from each other (Table 1).

**Table 1.** Values of adipose tissues (epididymal and visceral), Epididymal Fat Index (EFI), and Visceral Fat Index (VFI) of rats fed with different diets and flours.

Parameters evaluated	GROUPS				
	C	H	HP	HO	HA
Epididymal adipose tissue (g)	7,42 $\pm$ 0,78 <sup>a</sup>	14,94 $\pm$ 2,08 <sup>b</sup>	8,14 $\pm$ 1,87 <sup>a</sup>	12,46 $\pm$ 3,64 <sup>b</sup>	9,86 $\pm$ 2,24 <sup>a</sup>
Visceral adipose tissue (g)	9,95 $\pm$ 1,94 <sup>a</sup>	18,71 $\pm$ 2,49 <sup>b</sup>	9,02 $\pm$ 3,85 <sup>a</sup>	16,33 $\pm$ 4,78 <sup>b</sup>	10,13 $\pm$ 4,01 <sup>a</sup>
Total (g)	17,38 $\pm$ 2,59 <sup>a</sup>	33,65 $\pm$ 3,74 <sup>b</sup>	16,97 $\pm$ 3,33 <sup>a</sup>	28,48 $\pm$ 2,53 <sup>b</sup>	19,94 $\pm$ 1,57 <sup>a</sup>
EFI (%)	1,67 $\pm$ 0,23 <sup>a</sup>	2,85 $\pm$ 0,28 <sup>b</sup>	1,76 $\pm$ 0,24 <sup>a</sup>	2,45 $\pm$ 0,62 <sup>ab</sup>	2,07 $\pm$ 0,35 <sup>a</sup>
VFI (%)	2,23 $\pm$ 0,47 <sup>a</sup>	3,61 $\pm$ 0,29 <sup>b</sup>	1,91 $\pm$ 0,66 <sup>a</sup>	3,19 $\pm$ 0,77 <sup>b</sup>	2,09 $\pm$ 0,66 <sup>a</sup>
Total (%)	3,89 $\pm$ 0,67 <sup>a</sup>	6,38 $\pm$ 0,72 <sup>b</sup>	3,67 $\pm$ 0,90 <sup>a</sup>	5,65 $\pm$ 1,34 <sup>b</sup>	4,16 $\pm$ 0,91 <sup>a</sup>

Mean  $\pm$  standard deviation followed by the same letters between groups do not differ by Tukey Test at 5% probability.

When compared to group H, the groups fed with cactaceae flours (HP, HO, HA) were classified as excellent for reducing the mass of the epididymal and visceral adipose tissues (Figure 5). The *Pereskia grandifolia* and *Agave tequilana* flours showed the greatest reduction in adipose tissue, although there was no statistical difference regarding the final body weight between the groups (Figure 2A).



**Figure 5.** Reduction of adipose tissue in rats fed with different diets and flours. (Orange = Epididymal; Blue = Visceral). HP = Hypercaloric diet and *Pereskia grandifolia* flour, HO = Hypercaloric diet and *Opuntia ficus-indica* flour, HA = Hypercaloric diet and *Agave tequilana* flour.

The groups that received the *Pereskia grandifolia* (HP) and *Agave tequilana* (HA) flours had a lower adipose tissue mass (epididymal and visceral), as well as lower Visceral and Epididymal Fat Indexes when compared to group H, as observed in some animal and human studies. Rats fed a hypercaloric diet plus 5 and 10% *Pereskia grandifolia* flour (Almeida et al., 2016) did not differ from the control group regarding the amount of total body lipids, as identified in this study, where the control (C) and *Pereskia grandifolia* (HP) flour groups obtained statistically similar values for the adipose tissue masses and the evaluated adiposity indexes. *Pereskia aculeata* flour decreased visceral fat in rats (Barbalho et al., 2016).

C57BL/6 mice ingesting a high-fat diet and *Agave tequilana* fructans decreased adipose tissue mass compared to those that consumed only the high-fat diet (Márquez-Aguirre et al., 2013), and when consuming a high-fat diet added with *Agave salmiana* extract, they decreased adipose tissue mass, being highlighted by Leal-Díaz et al. (2016) that the saponins present in this extract increase the oxidation of fatty acids. *Agave tequilana* in association with *Hibiscus sabdariffa* reduced adiposity and pro-inflammatory cytokines in the adipose tissue of C57BL/6 mice submitted to a high-fat diet (Moyano et al., 2016). Rats treated with a high-fat diet supplemented with agavins from *Agave tequilana* showed a significant reduction in adipose tissue and smaller adipocytes compared to those fed only with a high-fat diet (Huazano-García et al., 2020).

The groups that received the Hypercaloric diet (H) and the *Opuntia ficus-indica* flour (HO) had greater adipose tissue mass (Epididymal and Visceral), differing from other studies whose cactaceae promoted a decrease in adipose tissue mass or size of adipocytes. Glycosides from *Opuntia ficus-indica* decreased the size of adipocytes in rats (Rodríguez-Rodríguez et al., 2015), the anti-adipogenic effect of *Opuntia ficus-indica* and other plants of the genus *Opuntia* was also demonstrated in 3T3-L1 cells, in which the reduction of lipid concentration in cells after treatment with cactaceae (Camarena-Rangel et al., 2020). Consumption of a high-fat diet with 4% fiber from dehydrated *Opuntia ficus-indica* decreased body fat and adipocyte hypertrophy in rats (Moran-Ramos et al., 2017). Among humans with metabolic syndrome who received a lipid-controlled diet and 1.6 g of Neopuntia/flour (compound obtained from *Opuntia ficus-indica*), 39% were no longer diagnosed with such syndrome (Linarès, Thimonier, & Degre, 2007). The daily administration of 96 mg·kg<sup>-1</sup> of *Agave tequilana* powdered fructans to obese humans ingesting a 1500 kcal diet decreased body fat and triacylglycerols, compared to the group that consumed only the diet (Padilla-Camberos, Barragán-Álvarez, Diaz-Martinez, Rathod, & Flores-Fernández, 2018). *Agave tequilana* and *Hibiscus sabdariffa* reduced adiposity in C57BL/6 mice submitted to high-fat diet (Moyano et al., 2016).

When compared to group C, the groups fed with cactaceae flours (HP, HO and HA) did not differ statistically regarding the Epididymal Fat Index, as well as the HP and HA groups for the Visceral Fat Index, diverging from other study (Almeida et al., 2018), who identified that the control group had lower indices (EFI and VFI) than the groups that received lychee flour.

## Conclusion

Among the studied cactaceae, *Pereskia grandifolia* flour showed the best results, since it did not differ statistically from the control group (C) regarding morphometric parameters, in the mass of adipose tissues, and in blood glucose. However, *Opuntia ficus-indica* flour promoted a result similar to that of the hypercaloric (H) diet, increasing weight gain, visceral adipose tissue mass and liver weight.

It is suggested that *Pereskia grandifolia* flour be used in the prevention and/or treatment of obesity, since its phytochemicals in association with the high-calorie diet attenuated the increase in adipose tissue.

## References

- Agostini-Costa, T. S. (2020). Bioactive compounds and health benefits of Pereskioideae and Cactoideae: A review. *Food Chemistry*, 327, 126961. DOI: <https://doi.org/10.1016/j.foodchem.2020.126961>
- Albuquerque, S. S. C., Lira, M. A., Santos, M. V. F., Dubeux Júnior, J. C. B., Melo, J. N., & Farias, I. (2002). Utilização de três fontes de nitrogênio associadas à palma forrageira (*Opuntia ficus-indica*, Mill.) Cv. gigante na suplementação de vacas leiteiras mantidas em pasto diferido. *Revista Brasileira de Zootecnia*, 31(Suppl 3), 1315-1324. DOI: <https://doi.org/10.1590/S1516-35982002000600001>
- Almeida, M. E. F. (2017). Non-Conventional Food Plants of the family Cactaceae: a healthy food option. *EC Nutrition*, 7, 84-85.
- Almeida, M. E. F., Ferreira, J. T., Augusto-Obara, T. R., Cruz, R. G., Arruda, H. S., Santos, V. S., ... Botrel, R. V. B. F. (2018). Can lychee reducing the adipose tissue mass in rats? *Brazilian Archives of Biology and Technology*, 61, 1-12. DOI: <https://doi.org/10.1590/1678-4324-2018160483>
- Almeida, M. E. F., Junqueira, A. M. B., Simão, A. A., & Corrêa, A. D. (2014). Caracterização química das hortaliças não-convencionais conhecidas como ora-pro-nobis. *Bioscience Journal*, 30, 431-439.
- Almeida, M. E. F., Medeiros, R. S., Figueiredo, F. J. B., Coelho, E. J. B., & Sena, M. P. T. (2011). Effect of auditory stress and high calorie diet on body weight, lipids and glycemia in wistar rats. *Alimentos e Nutrição*, 22(3), 359-365.
- Almeida, M. E. F., Simão, A. A., Corrêa, A. D., & Fernandes, R. V. B. (2016). Improvement of physiological parameters of rats subjected to hypercaloric diet, with the use of *Pereskia grandifolia* (Cactaceae) leaf flour. *Obesity Research and Clinical Practice*, 10(6), 701-709. DOI: <https://doi.org/10.1016/j.orcp.2015.10.011>
- Barbalho, S. M., Guiguer, E. L., Marinelli, P. S., Bueno, P. C. S., Pescinini-Salzedas, L. M., Santos, M. C. B., ... Goulart, R. A. (2016). *Pereskia aculeata* Miller flour: metabolic effects and composition. *Journal of Medical Food*, 19(9), 890-894. DOI: <https://doi.org/10.1089/jmf.2016.0052>
- Bernardis, L. L., & Patterson, B. D. (1968). Correlation between 'Lee index' and carcass fat content in weanling and adult female rats with hypothalamic lesions. *Journal of Endocrinology*, 40(4), 527-528. DOI: <https://doi.org/10.1677/joe.0.0400527>
- Brasil, D. C. M., Val, R. M. M., Ramos, J. A. S. C., & Almeida, M. E. F. (2020). Juice from leaves of cacti of the genus *Pereskia*: effect on the physiological parameters of Wistar rats. *Ciência Animal Brasileira*, 21, e-58061. DOI: <https://doi.org/10.1590/1809-6891v21e-58061>
- Brasil. Lei nº 11.794, de 8 de outubro de 2008. (2008). Procedimentos para o uso científico de animais. *Diário Oficial União*, Brasília, DF, 9 out. 2008. Retrieved from [http://www.planalto.gov.br/ccivil\\_03/\\_ato2007-2010/2008/lei/111794.htm](http://www.planalto.gov.br/ccivil_03/_ato2007-2010/2008/lei/111794.htm)
- Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Análise em Saúde e Vigilância de Doenças não Transmissíveis (2020). *Vigitel Brasil 2019: vigilância de fatores de risco e proteção para doenças crônicas por inquérito telefônico: estimativas sobre frequência e distribuição sociodemográfica de fatores de risco e proteção para doenças crônicas nas capitais dos 26 estados brasileiros e no Distrito Federal em 2019*. Brasília, DF: Ministério da Saúde. Retrieved from [https://bvsms.saude.gov.br/bvs/publicacoes/vigitel\\_brasil\\_2019\\_vigilancia\\_fatores\\_risco.pdf](https://bvsms.saude.gov.br/bvs/publicacoes/vigitel_brasil_2019_vigilancia_fatores_risco.pdf)
- Butterweck, V., Semlin, L., Feistel, B., Pischel, I., Bauer, K., & Verspohl, E. J. (2011). Comparative evaluation of two different *Opuntia ficus-indica* extracts for blood sugar lowering effects in rats. *Phytotherapy Research*, 25(3), 370-375. DOI: <https://doi.org/10.1002/ptr.3271>
- Camarena-Rangel, N. G., Antunes-Ricardo, M., Gutiérrez-Urbe, J., Velarde-Salcedo, A. J., Rosa, A. P. B., & Santos-Díaz, M. (2020). Identification of metabolites present in *Opuntia callus* and study of their antioxidant, anti-inflammatory and anti-adipogenic properties. *Plant Cell, Tissue and Organ Culture*, 143, 31-43. DOI: <https://doi.org/10.1007/s11240-020-01893-4>
- Díaz, M. S. S., Rosa, A-P. B., Héliers-Toussaint, C., Guéraud, F., & Nègre-Salvayre, A. (2017). *Opuntia* spp.: characterization and benefits in chronic diseases. *Oxidative Medicine and Cellular Longevity*, 2017, 8634249. DOI: <https://doi.org/10.1155/2017/8634249>

- El-Hawary, S. S., Sobeh, M., Badr, W. K., Abdelfattah, M. A. O., Ali, Z. Y., El-Tantawy, M. E., ... Wink, M. (2020). HPLC-PDA-MS/MS profiling of secondary metabolites from *Opuntia ficus-indica* cladode, peel and fruit pulp extracts and their antioxidant, neuroprotective effect in rats with aluminum chloride induced neurotoxicity. *Saudi Journal of Biological Science*, 27(10), 2829-2838. DOI: <https://doi.org/10.1016/j.sjbs.2020.07.003>
- Elsawi, S. A., Radwan, R. R., Elbatanony, M. M., El-Feky, A. M., & Sherif, N. H. (2020). Prophylactic effect of *Opuntia ficus indica* fruit peel extract against irradiation-induced colon injury in rats. *Planta Medica*, 86, 61-69. DOI: <https://doi.org/10.1055/a-1019-9801>
- Elshehy, H., Salah, S., Abdel-Mawla, E., & Agamy, N. (2020). Protective effect of *Opuntia ficus-indica* against diabetes in alloxan-induced diabetic rats. *Canadian Journal of Clinical Nutrition*, 8(2), 20-34. DOI: <https://dx.doi.org/10.14206/canad.j.clin.nutr.2020.02.03>
- Figueirôa, J. A., Novaes, G. U. M., Gomes, H. S., Silva, V. L. M. M., Lucena, D. M., Lima, L. M. R., ... Gomes, J. P. (2021). *Opuntia ficus-indica* is an excellent eco-friendly biosorbent for the removal of chromium in leather industry effluents. *Heliyon*, 7(6), e07292. DOI: <https://doi.org/10.1016/j.heliyon.2021.e07292>
- Franco-Robles, E., & López, M. G. (2016). Agavins increase neurotrophic factors and decrease oxidative stress in the brains of high-fat diet-induced obese mice. *Molecules*, 21(8), 998. DOI: <https://doi.org/10.3390/molecules21080998>
- García-Curbelo, Y., Bocourt, R., Savón, L. L., García-Vieyra, M. I., & López, M. G. (2015). Prebiotic effect of *Agave fourcroydes* fructans: an animal model. *Food & Functional*, 6, 3177-3182. DOI: <https://doi.org/10.1039/c5fo00653h>
- Goswami, N., Trozic, I., Fredriksen, M. V., & Fredriksen, P. M. (2021). The effect of physical activity intervention and nutritional habits on anthropometric measures in elementary school children: the health oriented pedagogical project (HOPP). *International Journal of Obesity*, 45, 1677-1686. DOI: <https://doi.org/10.1038/s41366-021-00830-5>
- Huazano-García, A., Silva-Adame, M. B., Vásquez-Martínez, J., Gastelum-Arellanez, A., Sánchez-Segura, L., & López, M. G. (2020). Highly branched neo-fructans (agavins) attenuate metabolic endotoxemia and low-grade inflammation in association with gut microbiota modulation on high-fat diet-fed mice. *Foods*, 9(12), 1792. DOI: <https://doi.org/10.3390/foods9121792>
- Huerta-Cardoso, O., Durazo-Cardenas, I., Longhurts, P., Simms, N. J., & Encinas-Oropesa, A. (2020). Fabrication of agave tequilana bagasse/PLA composite and preliminary mechanical properties assessment. *Industrial Crops and Products*, 152. DOI: <https://doi.org/10.1016/j.indcrop.2020.112523>
- Hwang, S. H., Kang, I.-J., & Lim, S. S. (2017). Antidiabetic effect of fresh nopal (*Opuntia ficus-indica*) in low-dose streptozotocin-induced diabetic rats fed a high-fat diet. *Hindawi*, 2017, 4380721. DOI: <https://doi.org/10.1155/2017/4380721>
- Instituto Brasileiro de Geografia e Estatística [IBGE]. (2010). *Pesquisa de Orçamentos Familiares 2008-2009*. Retrieved from <https://biblioteca.ibge.gov.br/visualizacao/livros/liv50063.pdf>.
- International Food Policy Research Institute [IFPRI]. (2016). *Relatório sobre a Nutrição Mundial 2016: da Promessa ao impacto: erradicar a má nutrição até 2030*. Washington, DC: IFPRI. DOI: <http://dx.doi.org/10.2499/9780896295872>
- Kang, J.-W., Shin, J.-K., Koh, E.-J., Ryu, H., Kim, H. J., & Lee, S.-M. (2016). *Opuntia ficus-indica* seed attenuates hepatic steatosis and promotes M2 macrophage polarization in high-fat diet-fed mice. *Nutrition Research*, 36(4), 369-379. DOI: <https://doi.org/10.1016/j.nutres.2015.12.007>
- Leal-Díaz, A. M., Noriega, L. G., Torre-Villalvazo, I., Torres, N., Alemán-Escondrillas, G., López-Romero, P., ... Tovar, A. R. (2016). Aguamiel concentrate from *Agave salmiana* and its extracted saponins attenuated obesity and hepatic steatosis and increased *Akkermansia muciniphila* in C57BL6 mice. *Scientific Reports*, 6(34242). DOI: <https://doi.org/10.1038/srep34242>
- Leem, K.-H., Kim, M.-G., Hahm, Y.-T., & Kim, H. K. (2016). Hypoglycemic effect of *Opuntia ficus-indica* var. saboten is due to enhanced peripheral glucose uptake through activation of AMPK/p38 MAPK pathway. *Nutrients*, 8(12). DOI: <https://doi.org/10.3390/nu8120800>
- Linarès, E., Thimonier, C., & Degre, M. (2007). The effect of NeOpuntia on blood lipid parameters--risk factors for the metabolic syndrome (syndrome X). *Advances in Therapy*, 24, 1115-1125. DOI: <https://doi.org/10.1007/BF02877717>

- Loayza, D. G., & Chávez, J. (2007). Chemical compositional study of nopal (*Opuntia ficus-indica*) cladophyll for human consumption. *Revista de la Sociedad Química del Perú*, 73, 41-45.
- Márquez-Aguirre, A. L., Camacho-Ruiz, R. M., Arriaga-Alba, M., Padilla-Camberos, E., Kirchmayr, M. R., Blasco, J. L., & González-Avila, M. (2013). Effects of *Agave tequilana* fructans with different degree of polymerization profiles on the body weight, blood lipids and count of fecal *Lactobacilli/Bifidobacteria* in obese mice. *Food and Functional*, 4, 1237-1244. DOI: <https://doi.org/10.1039/C3FO60083A>
- Márquez-Aguirre, A. L., Camacho-Ruiz, R. M., Gutiérrez-Mercado, Y. K., Padilla-Camberos, E., González-Ávila, M., Gálvez-Gastélum, F. J., ... Ortuño-Sahagún, D. (2016). Fructans from *Agave tequilana* with a lower degree of polymerization prevent weight gain, hyperglycemia and liver steatosis in high-fat diet-induced obese mice. *Plant Foods for Human Nutrition*, 71, 416-421. DOI: <https://doi.org/10.1007/s11130-016-0578-x>
- Medeiros, P. M., Santos, G. M. C., Barbosa, D. M., Gomes, L. C. A., Santos, E. M. C., & Silva, R. R. V. (2021). Local knowledge as a tool for prospecting wild food plants: experiences in northeastern Brazil. *Scientific Reports*, 11(594). DOI: <https://doi.org/10.1038/s41598-020-79835-5>
- Moran-Ramos, S., He, X., Chin, E. L., Tovar, A. R., Torres, N., Slupsky, C. M., & Raybould, H. E. (2017). Nopal feeding reduces adiposity, intestinal inflammation and shifts the cecal microbiota and metabolism in high-fat fed rats. *PLOS One*, 12(2), e0171672. DOI: <https://doi.org/10.1371/journal.pone.0171672>
- Moyano, G., Sáyago-Ayerdi, S. G., Largo, C., Caz, V., Santamaria, M., & Taberner, M. (2016). Potential use of dietary fibre from *Hibiscus sabdariffa* and *Agave tequilana* in obesity management. *Journal of Functional Foods*, 21, 1-9. DOI: <https://doi.org/10.1016/j.jff.2015.11.011>
- Novelli, E. L. B., Diniz, Y. S., Galhardi, C. M., Ebaid, G. M. X., Rodrigues, H. G., Mani, F., ... Novelli Filho, J. L. V. B. (2007). Anthropometrical parameters and markers of obesity in rats. *Laboratory Animals*, 41, 111-119. DOI: <https://doi.org/10.1258/002367707779399518>
- Padilla-Camberos, E., Barragán-Álvarez, C. P., Diaz-Martinez, N. E., Rathod, V., & Flores-Fernández, J. M. (2018). Effects of agave fructans (*Agave tequilana* Weber var. azul) on body fat and serum lipids in obesity. *Plant Foods for Human Nutrition*, 73, 34-39. DOI: <https://doi.org/10.1007/s11130-018-0654-5>
- Rendón-Huerta, J. A., Juárez-Flores, B., Pinos-Rodríguez, J. M., Aguirre-Rivera, J. R., & Delgado-Portales, R. E. (2012). Effects of different sources of fructans on body weight, blood metabolites and fecal bacteria in normal and obese non-diabetic and diabetic rats. *Plant Foods for Human Nutrition*, 67, 64-70. DOI: <https://doi.org/10.1007/s11130-011-0266-9>
- Rodríguez-Rodríguez, C., Torres, N., Gutiérrez-Urbe, J. A., Noriega, L. G., Torre-Villalvazo, I., Leal-Díaz, A. M., ... Tovar, A. R. (2015). The effect of isorhamnetin glycosides extracted from *Opuntia ficus-indica* in a mouse model of diet induced obesity. *Food and Function*, 6, 805-815. DOI: <https://doi.org/10.1039/c4fo01092b>
- Romero-López, M. R., Osorio-Díaz, P., Flores-Morales, A., Robledo, N., & Mora-Escobedo, R. (2015). Chemical composition, antioxidant capacity and prebiotic effect of aguamiel (*Agave atrovirens*) during in vitro fermentation. *Revista Mexicana de Ingeniería Química*, 14(2), 281-292.
- Salinas, M. L., Ogura, T., & Soffchi, L. (2001). Irritant contact dermatitis caused by needle-like calcium oxalate crystals, raphides, in *Agave tequilana* among workers in tequila distilleries and agave plantations. *Contact Dermatitis*, 44(2), 94-96. DOI: <https://doi.org/10.1034/j.1600-0536.2001.440208.x>
- Sánchez-Tapia, M., Aguilar-López, M., Pérez-Cruz, C., Pichardo-Ontiveros, E., Wang, M., Donovan, S. M., ... Torres, N. (2017). Nopal (*Opuntia ficus indica*) protects from metabolic endotoxemia by modifying gut microbiota in obese rats fed high fat/sucrose diet. *Scientific Reports*, 7(4716), 1-16. DOI: <https://doi.org/10.1038/s41598-017-05096-4>
- Sáyago-Ayerdi, S. G., Zamora-Gasga, V. M., & Venema, K. (2020). Changes in gut microbiota in predigested *Hibiscus sabdariffa* L calyces and *Agave* (*Agave tequilana* weber) fructans assessed in a dynamic in vitro model (TIM-2) of the human colon. *Food Research International*, 132, 109036. DOI: <https://doi.org/10.1016/j.foodres.2020.109036>
- Sidana, J., Singh, B., & Sharma, O. P. (2016). Saponins of agave: chemistry and bioactivity. *Phytochemistry*, 130, 22-46. DOI: <https://doi.org/10.1016/j.phytochem.2016.06.010>
- Silva, A. P. G., Souza, C. C. E., Ribeiro, J. E. S., Santos, M. C. G., Pontes, A. L. S., & Madruga, M. S. (2015). Physical, chemical and bromatological characteristics of the giant forage cactus (*Opuntia ficus-indica*)

- and small forage cactus (*Nopalea cochenillifera*) from Paraíba state (Brazil). *Revista Brasileira de Tecnologia Agroindustrial*, 9(2), 1810-1820. DOI: <https://doi.org/10.3895/rbta.v9n2.1616>
- Tegegne, F., Kijora, C., & Peters, K. J. (2007). Study on the optimal level of cactus pear (*Opuntia ficus-indica*) supplementation to sheep and its contribution as source of water. *Small Ruminant Research*, 72(2-3), 157-164. DOI: <https://doi.org/10.1016/j.smallrumres.2006.10.004>
- Urías-Silvas, J. E., Cani, P. D., Delmée, E., Neyrinck, A., López, M. G., & Delzenne, N. M. (2008). Physiological effects of dietary fructans extracted from *Agave tequilana* Gto. and *Dasyilirion* spp. *British Journal of Nutrition*, 99(2), 254-261. DOI: <https://doi.org/10.1017/S0007114507795338>
- Wang, X., Yin, C., & Shao, C. (2021). Heterogeneous relationships between the health-related lifestyle and risk of overweight and obesity in urbanizing China. *Journal of Transport and Health*, 20. DOI: <https://doi.org/10.1016/j.jth.2021.101023>
- World Health Organization [WHO]. (2016). *Report of the Commission on Ending Childhood Obesity*. Retrieved from [https://apps.who.int/iris/bitstream/handle/10665/204176/9789241510066\\_eng.pdf?sequence=1](https://apps.who.int/iris/bitstream/handle/10665/204176/9789241510066_eng.pdf?sequence=1)
- Yoon, J. A., Lee, S.-J., Kim, H.-K., & Son, Y.-S. (2011). Ameliorating effects of a nopal (*Opuntia ficus-indica*) complex on blood glucose in db/db mice. *Food Science and Biotechnology*, 20, 255-259. DOI: <https://doi.org/10.1007/s10068-011-0035-4>
- Zhou, M. (2021). The shifting income-obesity relationship: Conditioning effects from economic development and globalization. *SSM - Population Health*, 15, 100849. DOI: <https://doi.org/10.1016/j.ssmph.2021.100849>