TIP Revista Especializada en Ciencias Químico-Biológicas, 26: 1-14, 2023.
https://doi.org/10.22201/fesz.23958723e.2023.564

Evaluation of the gastrointestinal and metabolic effect in a murine model of a high-fiber snack

Sarahí Cruz-Valderrama[1], Aracely Evangelina Chávez-Piña[2], Yadira Rivera-Espinoza[1] and Rosalva Mora-Escobedo[1]*

[1]Departamento de Ingeniería Bioquímica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Miguel Othón de Mendizábal # 699, 07360, Alcaldía Gustavo A. Madero, Ciudad de México, México. [2]Departamento de Investigación y Posgrado. Lab. de Farmacología, Escuela Nacional de Medicina y Homeopatía, Instituto Politécnico Nacional, Guillermo Massieu Helguera # 239, 07320, Alcaldía Gustavo A. Madero, Ciudad de México, México. E-mail: *rosalmorae@gmail.com

Abstract

This work aimed to evaluate the gastrointestinal and metabolic effect in a murine model of a snack rich in fiber (corn, chickpea, sesame, and chia) (HFS) and the comparison with the consumption of a commercial corn snack (CMS). Unlike the commercial snack, the mixture of corn, chickpea, sesame, and chia improved the nutritional quality of the final product. In the evaluation of the intake of the different diets in the mouse model, high-fiber snack (HFS) decreased cholesterol and triglyceride levels after 30 days of consumption, while CMS increased glucose levels and caused metaplasia in gastric tissue, in addition to decreasing the height villus in small intestinal tissue. The reformulation of snacks, using in addition to cereals, legumes, and oilseeds, can be a functional food easily accessible to the population, being a better option than snacks of low nutritional quality that are on the market.
Key words: snacks, dietary fiber, legumes and oilseeds, gastrointestinal damage, antioxidant activity.

Evaluación del efecto gastrointestinal y metabólico en un modelo murino, de una botana rica en fibra.

Resumen

El objetivo de este trabajo fue evaluar el efecto gastrointestinal y metabólico en un modelo murino de una botana rica en fibra (maíz, garbanzo, ajonjolí y chía) (BRF) y la comparación con el consumo de una botana comercial de maíz (BCM). La mezcla de maíz, garbanzo, ajonjolí y chía mejoraron la calidad nutricional del producto final a diferencia de la botana comercial. En la evaluación de la ingesta de las diferentes dietas en el modelo murino, la botana rica en fibra (BRF) disminuyó los niveles de colesterol y triglicéridos luego de 30 días de consumo, y la BCM aumentó los niveles de glucosa, provocó metaplasia en el tejido gástrico y redujo la altura de las vellosidades en el tejido del intestino delgado. La reformulación de las botanas, al utilizar además de cereales, leguminosas y oleaginosas, puede ser un alimento funcional de fácil acceso para la población, y una mejor opción que las botanas de baja calidad nutricional que se encuentran en el mercado.
Palabras clave: botanas, fibra dietética, leguminosas y oleaginosas, daño gastrointestinal, actividad antioxidante.

Artículo recibido el 06 de febrero del 2023.
Artículo aceptado el 26 de junio del 2023.

Introduction

Snacks have become a food that is part of the daily nutritional diet of the majority population worldwide since they have an affordable cost, they are palatable and their consumption generates momentary satiety (Rivera-Mirón, Torruco-Uco, Carmona-García & Rodríguez-Miranda, 2020). However, to achieve these characteristics, the industry tends to use ingredients of low nutritional quality such as refined flour, saturated fats, and excessive amounts of sugar and sodium, which promote poor nutrition if consumed excessively (Hess *et al*., 2019). When consumed these foods in the absence of hunger and only as a supplement or meal replacement, they can override the hunger response and induce hyperphagia, eliminating the homeostatic energy balance and causing weight gain (Clawson *et al*., 2019). In addition, energy-dense, nutrient-poor snacks, which are rich sources of simple sugars, sodium, saturated and trans fatty acids, are associated with metabolic syndrome in children and adults, including type II diabetes, hypertension, excess waist fat, and abnormal cholesterol levels, as well as excessive amounts of sweeteners such as sucrose, which can contribute to increased energy intake, elevated glycemic indice, induce lipogenesis, dysfunction of β cells (insulin-producing), and increase obesity and metabolic disorders (Asghari, Yuzbashian, Mirmiran, Bahadoran & Azizi, 2016).

Besides these pathologies, the habit of consuming this type of food also contributes to diseases of the gastrointestinal tract, such as gastroesophageal reflux disease where the intake of these causes an additional secretion of gastric acid, which produces irritation in the esophagus resenting in symptoms such as chronic cough, inflammation, shortness of breath, difficulty swallowing and in more serious cases ulcers and bleeding (Fiorentino, 2019). In the same way, the excessive consumption of these snacks can induce atrophies in the small intestine and colon, reducing the concentration of intraepithelial lymphocytes of the intestine and lamina propria, weakening the intestinal immune system, also causing dysbiosis in the composition of the intestinal microbiota (Tanaka *et al*., 2020).

An economic alternative in the production of snacks is the use of cereals, legumes, and oilseeds. Recent studies have proven that the incorporation of flour from this group of foods in extruded or baked snacks, significantly improves the nutritional and functional properties of the final product, favoring the health of those who consume them (López-Martínez, Azuara-Pugliese, Sánchez-Macias, Sosa-Mendoza, Dibildox-Alvarado & Grajales-Lagunes, 2019). For example, Hernández-Nava, Bello-Pérez, San Martín-Martínez, Hernández-Sánchez & Mora-Escobedo (2011) studied the effect of extrusion cooking on the functional properties and starch components of lentil/banana blends, the results of this study indicated that extrusion cooking induced desirable functional characteristics to lentil/banana blends by increasing their resistant starch content, while Cruz-Ortiz *et al*. (2020) optimized a germinated soybean/corn starch extrudate obtained a product with high protein and resistant starch content. Also, the use of sesame seed powder and chia seeds in puffed snacks and chips, respectively, improved the content of omega 9 and omega 3 fatty acids and phenolic compounds in the final product (Giaretta, Lima & Carpes, 2018; Hashempour-Baltork, Torbati, Azadmard-Damirchi & Savage, 2018). Whereas, in a randomized crossover study of repeated measures, 26 adults consumed an extruded corn snack added with chickpea flour and pinto bean flour, and an improvement in postprandial glycemic response was observed, plus an increase in protein and fiber consumption in the participants was noted (Johnston *et al*., 2021).

In addition to this, the carbohydrates present in oilseeds and legumes such as soluble and insoluble fiber, resistant starch, oligosaccharides, and some phenolic compounds also contribute to health for example, chickpeas, besides being an excellent source of protein, have a high carbohydrate content, which makes them food with high energy supply, providing a significant amount of dietary fiber which favors intestinal transit, while sesame seeds are rich in unsaturated fatty acids, mainly oleic and linoleic, phenolic compounds (sesamol, sesamolin, and furaneol), and lignan precursors such as sesamin that may have antihypertensive, lipid-lowering and anticancer activities (Hashempour-Baltork, Torbati, Azadmard-Damirchi & Savage, 2018). Moreover, chia seeds have a high content of insoluble dietary fiber, lipids, and proteins, as well as antioxidant compounds (rosmaniric, caffeic, and gallic acids), and omega 3 and 6 fatty acids such as α-linolenic acid and α-linoleic acid; therefore, these characteristics give it excellent nutraceutical properties (Scapin, Schmidt, Prestes & Rosa, 2016).

However, there is scarce research in the evaluation of the effect of a high-fiber snack (corn, chickpea, sesame chia) (HFS) and the comparison with the intake of a commercial corn snack (CMS) that is highly consumed by the Mexican population, on the gastrointestinal and metabolic effect in a murine model.

Materials and methods

Preparation of flour and snack

For the development of this work, yellow corn (*Zea mays* L.), chickpea (*Cicer arietinum* L.), sesame (*Sesamum indicum* L.), and chia seeds (*Salvia hispanica* L.) from a local market in Mexico City were used. For the elaboration of the snack, previously, yellow corn flour (YCF) (Bello-Pérez, Flores-Silva, Camelo-Méndez, Paredes-López & Figueroa-Cárdenas, 2015), chickpea flour (CKF) (Ouazib, Dura, Zaidi & Rosell, 2016), sesame flour (SSF) (Kajihausa, Fasasi & Atolagbe, 2014) and chia flour (CHF) Steffolani, Martinez, León & Gómez, 2015) were obtained. The snack was developed with a mixture of flours in proportions of 10% YCF, 40% CKF, 10% SSF, and 40% CHF, salt (NaCl, 2g/100 g flour), and water (85 mL/100 g flour). They were kneaded in a blender (mod. KSM96ER, KitchenAid, USA)

at a constant speed for 10 minutes, and laminated to later mold them in a circular way to obtain pieces of 1.0 mm thick and 5.0 cm diameter. Baking was done at 135 °C for 15 minutes; then the snacks were cooled and stored in airtight plastic bags at room temperature in a cool dry place until the time of analysis. For the proximal composition of the snack, the percentage of moisture (A.O.A.C. 925.55), lipids (A.O.A.C 922.06), ashes (A.O.A.C. 923.03), protein (A.O.A.C. 928.08), carbohydrates (by difference), and total dietary fiber (A.O.A.C 991.43) were determined (AOAC, 1990).

Determination of total phenolic compounds and antioxidant capacity

The total phenolic compounds (TPC) concentration was carried out according to Singleton, Orthofer & Lamuela-Raventós (1999), and the antioxidant capacity was done by DPPH (Brand-Williams, Cuvelier & Berset, 1995) and the ABTS method (Re, Pellegrini, Proteggente, Pannala, Yang & Rice-Evans, 1999). Initially, an organic-aqueous extraction was standardized, where 2.0 g of sample were suspended in a solution of HCl, methanol, and distilled water in a ratio of 1.1:87.9:11.0, respectively, and placed in a water bath at 20 ± 2 °C with constant stirring for 2 h. Subsequently, they were centrifuged at 1900 g for 10 minutes and filtered on Whatman paper (no. 40). The extracts were stored at -18 °C in an amber flask until use, not exceedingly more than 5 days. The total phenolic compounds were reported in milligrams of gallic acid equivalents (mg GAE; standard curve 0.0-0.1 mg/mL), while the DPPH and the ABTS antioxidant capacity were reported in Trolox-equivalent micromoles (µmol ET; standard curve 0.0-250 µmol/L).

Analysis of the snack effect in rats

To evaluate the effect of the snack at the small intestinal level, 20 female Wistar rats in adulthood (220 to 250 g of weight) from Centro de Investigación y Estudios Avanzados (CINVESTAV) del Instituto Politécnico Nacional (IPN. Mexico City, Mexico) were used.

The animal care and all the procedures carried out were made according to the guidelines of the NOM-062-ZOO-1999 for the Care and Management of Animals (DOF, 1999), and the institutional code of ethics (Escuela Nacional de Medicina y Homeopatía, IPN, protocol code: CBE/021/2019) maintaining access to food and water *ad libitum* with consumption monitoring, under temperature-controlled conditions (22 ± 2 °C) and a light-darkness cycle (7:00 a.m.–19:00 p.m.) for 45 days of treatment. As a comparison, a commercial high-fat corn-based snack was also used, obtained from a local market in Mexico City; due to these types of snacks being highly consumed by the Mexican population, they are sold without nutritional regulation (Aguilera-Aburto, Rodríguez-Aguilar, Sansores-Martínez & Gutiérrez-Delgado, 2017) and excessive intake can induce atrophies in the intestinal tract (Tanaka *et al*., 2020).

The animals were acclimatized for two weeks before the allocation of the diets and subsequently randomly distributed into 3 groups: BC (basal control, administered only with standard rodent diet [Purina, Mexico], n=6), HFS (high-fiber snack, administered with 50% of the developed snack + 50% of standard diet [Purina, Mexico], n=7) and CMS (commercial snack, administered with 50% of the commercial high-fat corn snack + 50% of standard diet [Purina, Mexico], n=7). To maintain access on demand, 100 g of each diet (BC, HFS, or CMS) was initially placed, and the amount of food that the rats ingested (standard rodent, high-fiber snack, or commercial high-fat corn snack) was monitored daily. The amount (g) of each diet was increased as the rats grew to ensure that all three groups remained *ad libitum* throughout the study time.

Body weight, feed intake, and daily caloric intake

The data of the body weight variable (g) were obtained every 7 days. While the feed intake was calculated as the difference (g) between the amount of food placed the previous day and the amount of food not consumed per following day, multiplied by the energy intake (kcal/g) of the corresponding diet (Clawson *et al.*, 2019).

Determination of glucose, cholesterol, and triglyceride levels

With the previous 12-hour fasting, blood samples were obtained from the tail vein every 15 days, and glucose, cholesterol, and triglyceride (mg/dL) values were recorded using the test strips of the Accu-Chek® Performa and Accu-Trend® kit (Roche Roche Diagnostics, Mannheim, Germany). At the end of the treatment, the animals were anesthetized intramuscularly with ketamine (100 mg/kg) and xylazine (7.5 mg/kg); blood samples were taken by cardiac puncture, and after that, they were euthanized by exsanguination (Pineda-Peña *et al.*, 2018) to finally collect the small intestinal tissue samples for assessment of damage and inflammatory activity.

Evaluation of gastric and intestinal tissue in rats

To check the possible damage caused by the consumption of the snacks, and to visualize the structure of the gastric tissue and the small intestinal barrier, a histological test with hematoxylin and eosin staining was performed. The stomachs were removed and opened by the major curvature and thoroughly rinsed with saline solution (Pineda-Peña *et al*., 2018). Small intestine samples (distal jejunum and ileum) from each rat were removed and opened along the antimesenteric border removing fecal content with saline solution (Wallace *et al.*, 2011). For histological testing, a small section of gastric and intestinal tissue was fixed with 10% formaldehyde in phosphate-buffered saline (PBS) until analysis (Pineda-Peña *et al.*, 2018). The tissues were then washed with distilled water, dehydrated into alcohol, and embedded in paraffin taking 5 µm sections to mount on a silane-coated glass slide; then, hematoxylin and eosin staining was performed (Carson & Hladik, 2015). Each slide was

examined under an optical microscope (Nikon Eclipse Slog, Nikon Instruments Inc. New York, USA) equipped with a high-resolution digital camera (Nikon Digital Sight DS-2mv, Nikon Instruments Inc. New York, USA). The gastric fossa damage, height villus, and crypt depth were analyzed using the ImageJ software (version 1.53).

Quantification of myeloperoxidase (MPO) levels in gastric and small intestinal tissue

Myeloperoxidase (MPO) is a lysosomal protein that is released into the phagosome of neutrophils and that reacts with hydrogen peroxide and a halide to form hypochlorous acid or with tyrosine to form tyrosyl radicals; these products can damage normal tissue and contribute to the production of pro-inflammatory cytokines (Clawson *et al.*, 2017). To assess MPO levels in gastric and small intestinal tissue, a modified version of the method previously described by Fornai *et al.* (2014) was carried out. The MPO concentration was calculated by measuring the absorbance of the samples at 620 nm on a plate spectrophotometer (Epoch™, BioTek Instruments, Vermont, USA), reporting the results as µmol/g of tissue and interpolating on a standard MPO curve (0.0 µmol/L at 0.001 µmol/L).

Statistical analysis

Statistical analyses were performed with Graph Pad Prism version 8.4.1 (Graph Pad Software; La Jolla, CA, USA). Data were reported as mean ± standard deviation or mean ± standard error. An analysis of variance (ANOVA) with a Tukey test was used to determine significant differences in the values of the proximal composition, total phenols, and antioxidant capacity of the flours. To determine the significant differences in the measurements of weight, caloric intake, glucose, cholesterol, and triglyceride levels of the groups of study during treatment time, a two-way ANOVA with a Geisser-Greenhouse correction followed by a Tukey test was used. In all cases, a significance level of $p \leq 0.05$ was applied.

Results

Characterization and determination of the antioxidant capacity of corn, chickpea, sesame, and chia flours

Table I shows the characterized flours used to obtain the high-fiber snack, observing that CKF had a higher concentration of protein (19.73 ± 0.28 g/100 g), while SSF had a concentration of total lipids higher than other flour (38.48 ± 1.68 g/100 g). On the other hand, CHF had a greater amount of dietary fiber (27.68 ± 0.65 g/100 g) presenting a significant difference ($p \leq 0.05$) from the rest of the samples. Regarding the analysis of TPC, a higher proportion of these was also observed in CHF (513 ± 41.0 mg GAE), which was reflected in the results of antioxidant capacity using the DPPH and ABTS methods (Table I).

Characterization and determination of total phenolic compounds and antioxidant capacity in the administered diets

Table II presents the nutritional composition of HFS, CMS, and BC of the murine model. The results showed that the amount obtained of dietary fiber and protein in HFS and BC diets is higher than the content declared on the label of the CMS, while the energy content of HFS is similar to that of the BC and lower than that declared in CMS. It is also observed that HFS presented the highest proportion of TPC being statistically like BC, but statistically different from CMS ($p \leq 0.05$). The antioxidant capacity values measured by DPPH and ABTS for HFS were statistically higher than those found in BC and CMS.

Table I. Characterization of corn, chickpea, sesame and chia flours (g /100 g d. b.)

Analysis	YCF	CKF	SSF	CHF
Moisture (%)	10.27 ± 1.20^{a}	9.09 ± 0.94^{a}	5.07 ± 0.85^{c}	6.53 ± 0.94^{b}
Ash (g)	1.76 ± 0.28^{c}	2.79 ± 0.23^{b}	5.85 ± 0.31^{a}	5.31 ± 0.73^{a}
Protein (g)	3.64 ± 0.46^{c}	19.73 ± 0.28^{a}	17.45 ± 0.51^{b}	17.42 ± 0.21^{b}
Total lipids (g)	8.90 ± 0.62^{d}	14.19 ± 0.68^{c}	38.48 ± 1.68^{a}	33.06 ± 0.58^{b}
Total Carbohydrates (g)	69.24 ± 0.89^{a}	38.56 ± 1.06^{b}	18.56 ± 2.42^{c}	9.99 ± 1.37^{d}
Dietary fiber (g)	6.16 ± 0.06^{c}	15.61 ± 0.40^{b}	14.57 ± 0.69^{b}	27.68 ± 0.65^{a}
TPC (mg GAE)	102.78 ± 22^{b}	83.58 ± 12.0^{b}	133.31 ± 17.0^{b}	513.77 ± 41.0^{a}
DPPH (µm TE)	1037.13 ± 129^{b}	843.72 ± 50.0^{d}	909.06 ± 77.0^{c}	1278.0 ± 60.0^{a}
ABTS (µm TE)	542.21 ± 69.0^{b}	222.67 ± 28.0^{c}	573.06 ± 22.0^{b}	760.16 ± 36.0^{a}

YCF: yellow corn flour, CKF: chickpea flour, SSF: sesame flour, and CHF: chia flour. TPC: Total phenolic compounds reported in milligrams of gallic acid equivalent (mg GAE). DPPH and ABTS antioxidant capacity reported in Trolox-equivalent micromoles (µm TE). Data are presented as mean ± standard deviation; n= 4. ANOVA analysis, followed by a Tukey test. The means within the same row that do not share a letter are significantly different ($p \leq 0.05$).

Table II. Nutritional composition of diets administered (per 100 g).

Analysis	HFS	BC	CMS
Energy content (kcal)	342 ± 27.36	327.0*	493.0*
Moisture (%)	10.76 ± 1.88	12.0*	7.0*
Ash (g)	5.95 ± 0.17	-	-
Protein (g)	13.74 ± 0.72	23.0*	5.0*
Total lipids (g)	10.31 ± 0.72	3.0*	25.0*
Total carbohydrates (g)	36.90 ± 0.75	49.0*	61.0*
Total Dietary fiber (g)	24.12 ± 0.14	6.0*	2.0*
Insoluble dietary fiber	16.25 ± 0.08	-	-
Soluble dietary fiber	07.87 ± 0.06	-	-
TPC (mg GAE)	181.18 ± 8. 08[a]	164.13 ± 3.98[a]	19.00 ± 0.43[b]
DPPH (µM TE)	1221.16 ± 58. 37[a]	157.63 ± 19.10[b]	50.07 ± 17.42[c]
ABTS (µM TE)	480.70 ± 21.0[a]	97.30 ± 1.20[b]	82.00 ± 2.10[c]

HFS (high-fiber snack), BC (basal control), and CMS (commercial corn snack). TPC: Total phenolic compounds reported in milligrams of gallic acid equivalent (mg GAE). DPPH and ABTS antioxidant capacity reported in Trolox equivalent micromoles (µm TE). The data are presented as mean ± standard deviation; n= 4. ANOVA analysis, followed by a Tukey test. The means within the same row that do not share a letter are significantly different ($p \leq 0.05$). * Data declared on the product label.

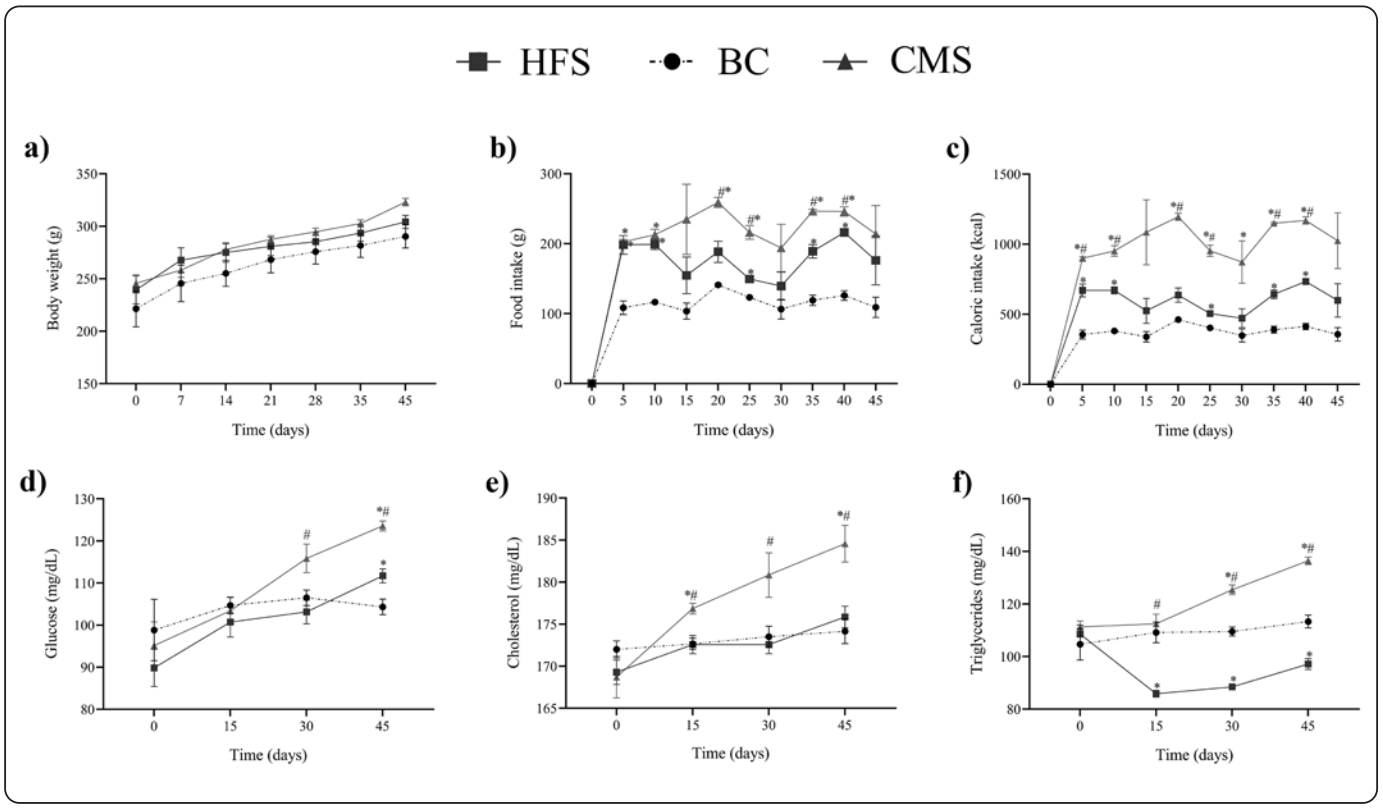

Figure 1. a) Comparison of body weight (g), b) food intake (g), c) caloric intake (kcal), d) glucose, e) cholesterol, and f) triglycerides (mg/dL) of the study groups. HFS group (high-fiber snack + standard diet, n= 7), BC (basal control administered with a standard diet, n= 6), and CMS (commercial corn snack + standard diet, n= 7), for a treatment time of 45 days. The data are expressed as a mean ± standard error. ANOVA with a Geisser-Greenhouse correction followed by a Tukey test. Bars denoting * indicate a statistically significant difference ($p \leq 0.05$) against BC and # $p \leq 0.05$ against HFS.

Analysis of the snack effect on rats

Assessment of body weight, feed, and caloric intake

There was no significant difference in the initial body weight of the rats that were randomly assigned to one of the three experimental groups. Growth curves during the 7-week dietary intervention period are shown in Figure 1a, where the change in body weight of the HFS and CMS diet-fed groups was observed to be moderately different from the BC during the first 14 days; however, there was no significant difference ($p \leq 0.05$) between the three groups throughout the treatment. At the end of the intervention, the CMS-fed rats final body weight was higher than the BC-fed. On the other hand, the amount of food was measured every week and no significant changes ($p \geq 0.05$) were observed between HFS and CMS during the first 10 days of consumption (Figure 1b); nevertheless, caloric intake did differ between both groups, being higher in CMS (Figure 1c). After 20 days of consumption and until the end of the treatment, significant changes ($p \leq 0.05$) were observed between the 3 groups, except on the days when the groups were subjected to the fasting period (day 15, 30, and 45). In this case, the CMS group consumed more food, which increased the number of calories ingested weekly because 78.59% of these calories came from the high-fat commercial snack, while in the HFS group, 75.84% of the calories ingested were provided by the high-fiber snack, highlighting the preference that the rats had over the administered snacks and not over the standard rodent food.

Glucose, cholesterol, and triglyceride levels

In this analysis, the CMS group presented the highest values of cholesterol (176.86 ± 1.68 mg/dL) and triglycerides (112.43 ± 9.83 mg/dL) in blood after the first 15 days of consumption and during the 45 days of treatment (Figure 1d-e), while glucose levels (Figure 1c) increased at 30 days of intake (115.86 ± 9.04 mg/dL), presenting differences against HFS (103.14 ± 7.47 mg/dL) and BC (106.50 ± 4.51 mg/dL). The HFS group had a significant decrease in triglyceride levels from day 15 of consumption (85.86 ± 3.48 mg/dL) and continued with this trend until the end of treatment (97.14 ± 5.55 mg/dL), presenting a significant difference with BC (104.33 ± 4.50 mg/dL) (Figure 1e).

Assessment of gastric and small intestinal tissue

For damage assessment, gastric and small intestinal tissue (jejunum and ileum) samples from all groups were analyzed. In Figure 2a the gastric tissue samples of the HFS and BC groups show a gastric wall in good condition, with well-defined gastric folds and crypts, while the tissue of the CMS group presented a gastric wall with the absence of folds.

These changes can be seen in detail in the histological images of each tissue (Figure 2b), where HFS and BC have an intact mucosa and complete gastric foveolas that even reveal a layer of mucus secretion, unlike CMS where the cells of the superficial mucosa, the gastric foveolas and the mucous cells are partially compromised (Figure 3a). In spite of, the small intestinal tissue also presented differences in terms of the type of diet that was administered in each group. In this case, at first sight, the tissue of the CMS group was observed with less thickness and with a pale and translucent coloration, unlike HFS and BC samples, where both presented an appearance and characteristic coloration (Figure 2c).

In the same way, the histological images allowed to observe the lesions of the intestinal barrier of the rats fed with the CMS diet, where the administration of this commercial snack altered the integrity and villus height (Figure 3b), and increased Lieberkühn's crypts depth (Figure 3c), presenting statistically significant differences ($p \leq 0.05$) against HFS and BC, who, in turn, showed no significant differences ($p \geq 0.05$) in the depth crypts and villus height ratio (V/C), and whose values were higher than CMS (Figure 3d).

Evaluation of inflammation markers

The results described above are supported by the evaluation of MPO, where the tissue samples analyzed revealed an increase in the concentration of this enzyme in the CMS group (Figure 4a), mainly in gastric tissue (8.76 ± 1.02 µmol/g tissue), presenting a statistically significant difference ($p \leq 0.05$) with the levels found in the HFS and BC group (5.17±1.45 and 2.14± 0.61 µmol/g tissue, respectively). Additionally, lower concentrations of MPO were observed in the small intestinal tissue in the HFS group (2.90 ± 0.81 µmol/g tissue), being significantly different from those found in the CMS group (4.91 ± 1.43 µmol/g tissue) as shown in Figure 4b.

Discussion

Nutritional quality of the high-fiber snack

In this study, the effect of a snack made with cereals and legumes on intestinal health and function was evaluated in a mouse model.

In the first instance, HFS turned out to be a good source of protein (13.74 ± 0.72 g/100 g) and dietary fiber (21.46 ± 1.06 g/100 g) where a considerable amount is soluble fiber (07.87 ± 0.06 g/100 g); these are interesting results due to dietary fiber has an important influence on the prevention of diseases compromised with food, such as obesity, diabetes, cardiovascular problems, neurodegenerative diseases and cancer (Tornero-Martínez *et al.*, 2022). In the same way, soluble or fermentable fiber has a prebiotic effect that the food industry could use for the benefit of human health, modifying the composition of the intestinal microbiota and thus avoiding chronic-degenerative diseases, since colonic fermentation produces short-chain fatty acids (SCFAs) with beneficial influence on human health (Tornero-Martínez *et al.*, 2019).

It has also been reported that using legumes in extruded or baked products increases the content of minerals, total phenolic

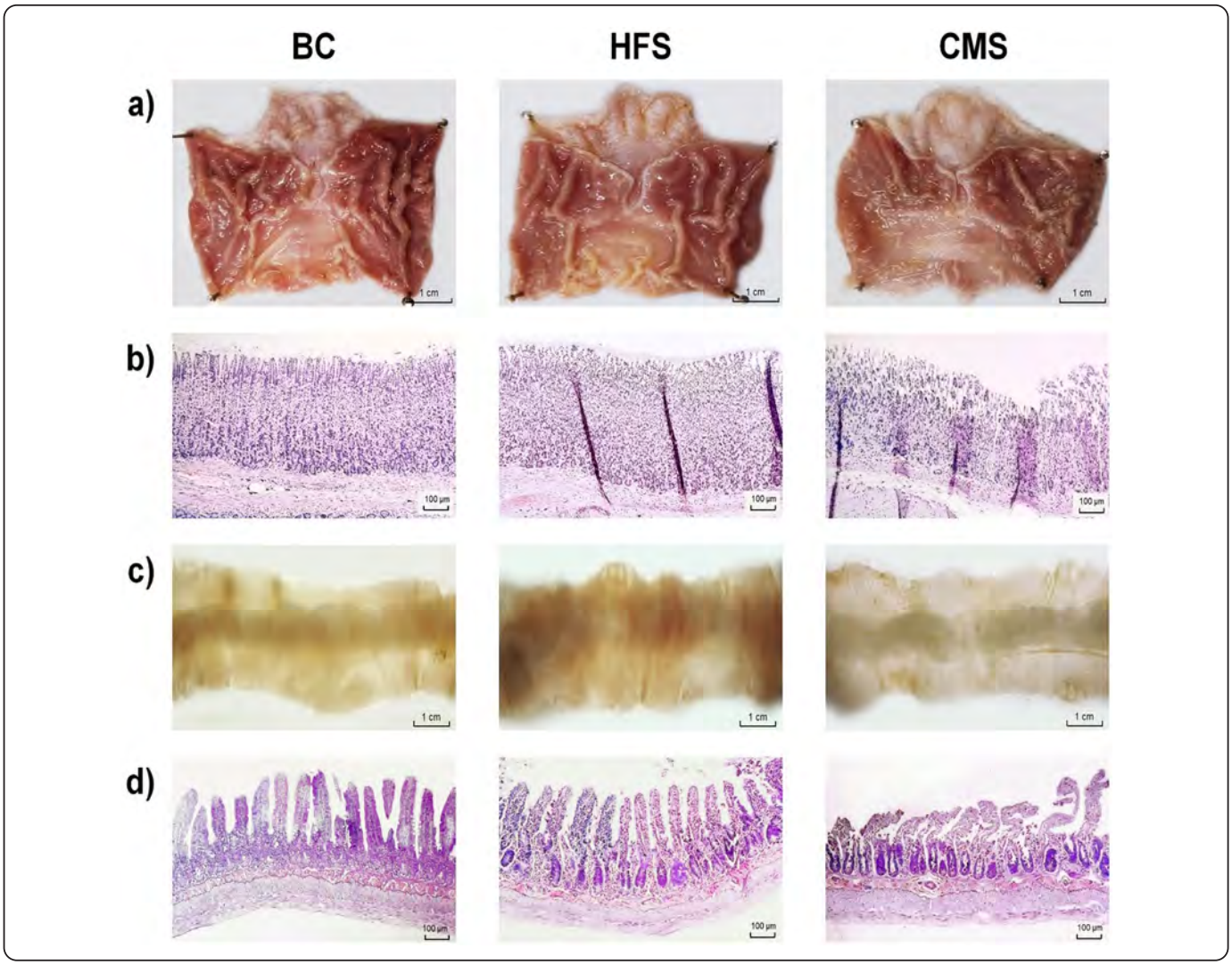

Figure 2. Comparison of gastric and small intestinal damage of the study groups a) Images and b) micrographs of gastric tissue; (c) images and (d) micrographs of small intestinal tissue. BC: basal control group (administered with standard diet), HFS group: administered with high-fiber snack + standard diet, and CMS group: administered with commercial corn-based snack + standard diet, for a treatment time of 45 days. Hematoxylin and eosin (H&E) stain, 100 microns.

compounds, and antioxidant activity of the final product and decreases the content of saturated lipids in these foods, in addition to the increase in dietary fiber (Félix-Medina *et al.*, 2020). Likewise, has been observed that the addition of seeds of the genus Salvia in corn chips has improved the nutritional value of the final product, in addition to increasing the dietary fiber content and reducing the glycemic index compared to chips made only with corn flour (Yuksel, Ilyasoglu & Baltaci, 2020).

These results are consistent with those reported by López-Martínez *et al*. (2019) since they found that snacks made from bean and soy flour had good acceptance and provided a protein content of 34.17 g/100 g and dietary fiber of 11.53 g/100 g. Therefore, it can be said that the product elaborated in the present work would be an alternative as a functional food of easy access for the consumer, being able to be a better option than the low-nutritious snacks that are already on the market; as in the case of the commercial snack used in this study whose composition, according to its label, is 25.0 g of total fat, 5.0 g of protein, 61.0 g of total carbohydrates and only 2.0 g of dietary fiber providing a total of 493 kcal, per 100 g of product.

Effect of snack intake

To evaluate the HFS intake at the gastric and small intestinal level, it was administered in Wistar female rats; in addition, it was compared with the effects produced by the intake of CMS.

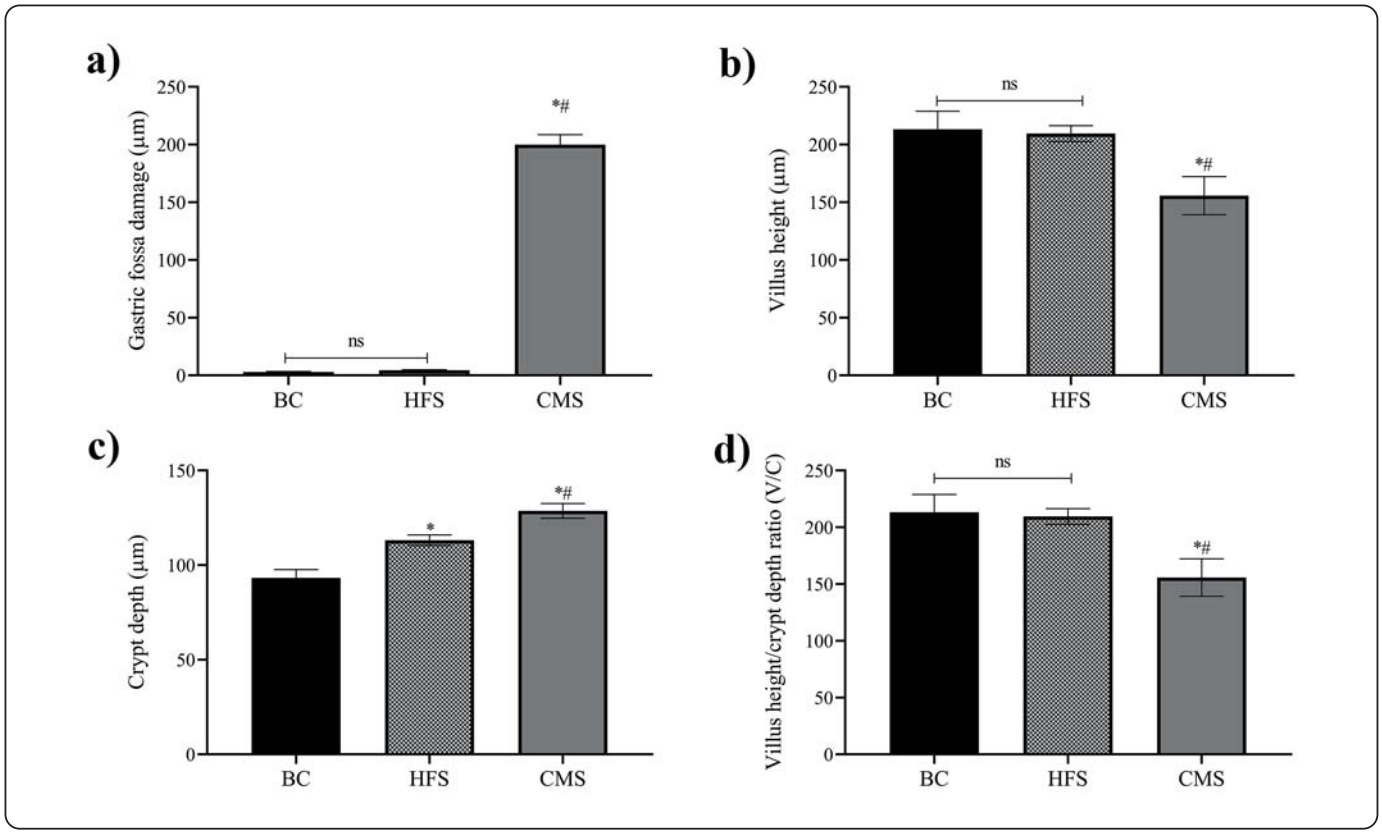

Figure 3. Analysis of gastric and small intestinal damage of the study groups. a) gastric fossa damage, b) villus height, c) crypt depth, and d) villus height and crypt depth ratio (V/C). BC: basal control group (administered with a standard diet, n= 6), HFS group: administered with high-fiber snack + standard diet (n= 7), and CMS group: administered with commercial corn-based snack + standard diet (n= 7), for a treatment time of 45 days. The data are expressed as a mean ± standard error. ANOVA with a Geisser-Greenhouse correction followed by a Tukey test. Bars denoting * indicate a statistically significant difference ($p \leq 0.05$) against BC and # ($p \leq 0.05$) against HFS.

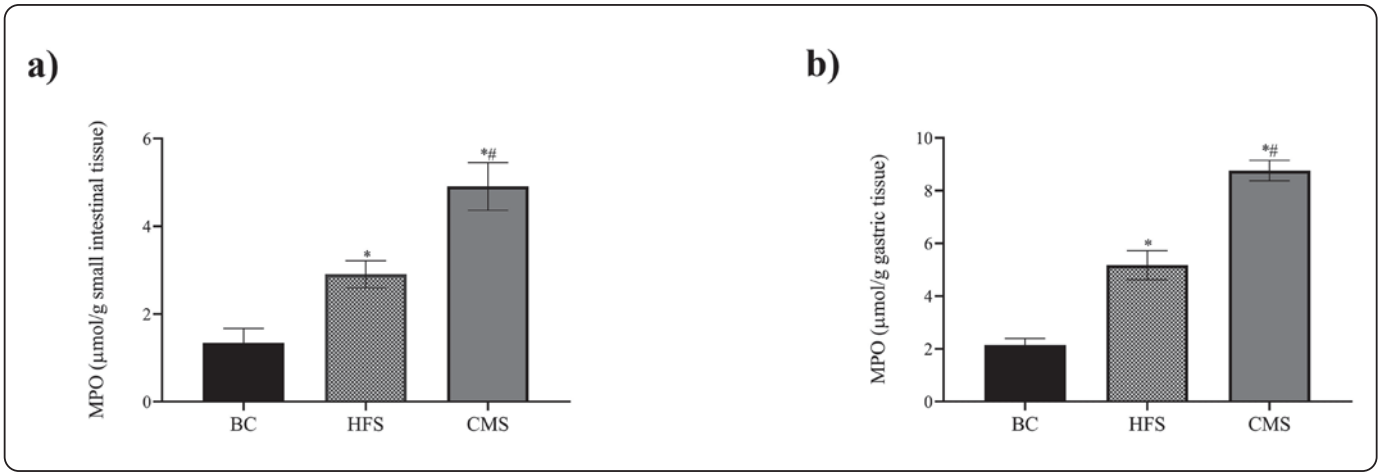

Figure 4. Comparison of myeloperoxidase levels (µmol/g tissue) in a) gastric tissue and b) intestinal tissue of the study groups. BC: baseline control group (administered with a standard diet, n= 6), HFS group: administered with high-fiber snack + standard diet (n= 7), and CMS group: administered with commercial corn-based snack + standard diet (n= 7), for a treatment time of 45 days. The data are expressed as a mean ± standard error. ANOVA with a Geisser-Greenhouse correction followed by a Tukey test. Bars denoting * indicate a statistically significant difference ($p \leq 0.05$) against BC and # $p \leq 0.05$ against HFS.

First, the effect of ingesting each diet on body weight was tested, observing no statistical difference between HFS, CMS, and BC treatment; however, in the weekly evaluation of feed intake and caloric ingest the CMS group had a higher value than HFS during the 45 days of administration. Similar results have been previously reported during the administration of healthy and unhealthy snacks in rats, where no significant differences between body weight values have been observed; however, there is a higher caloric intake and a progressive increase in brown and white adipose tissue in rats that consumed cafeteria diets (cookies, snacks, bacon, and sausages), attributing it mainly to the intake of fats from the unhealthy diet (Muntzel, Barclay & Ajasin, 2012; Martire, Holmes & Morris, 2013; Reynés, García-Ruiz, Díaz-Rúa, Palou & Oliver, 2014) because this macronutrient provides weak signals to control satiety (a process that determines the moment in which the act of eating is suspended) (Warrilow, Mellor, McKune & Pumpa, 2019).

In addition, unhealthy snacks, considered obesogenic, tend to have a soft texture and a high energy content, which makes their consumption more light, avoiding a correct detection of the physiological responses that inform the intestine and brain about the presence of nutrients, and therefore, having a low response to satiation (García-Flores, Martínez-Moreno, Beltrán-Miranda, Zepeda-Salvador & Solano-Santos, 2017) which would explain the low feed consumption observed in BC and HFS, and a higher intake of CMS in this study, due to the standard rodent food (Purina, Mexico) and the high-fiber snack contained only 3.0 and 10.31 g/100 g of total fat, respectively, compared to 25.0 g/100 g presents in the commercial snack.

On the other hand, it has been observed that when a food has a crunchy texture with high protein content and an excellent source of dietary fiber, as in the case of HFS, it stays longer in the oral cavity, and changes the viscosity and volume of food in the gastrointestinal tract, particularly in the stomach, slowing down gastric emptying and increasing the response to satiation and satiety, thereby reducing energy intake (García-Flores, Martínez-Moreno, Beltrán-Miranda, Zepeda-Salvador & Solano-Santos, 2017; Ni, Gunness, Smyth & Gidley, 2021; Slavin & Green, 2007).

Likewise, when consuming food of plant origin, mainly legumes, considerable amounts of dietary fiber can be obtained because of their cellular structure, which positively influences the nutritional functionality of food through its effects on digestion and its fermentation by the gut microbiota in the gastrointestinal tract (Ni *et al.*, 2021).

Continuing with the effects produced by the consumption of the high-fiber snack, significant changes were observed in terms of lipid metabolism; there was particularly a decrease in triglycerides from day 15 of intake and up to 45 days of treatment; in contrast to the group that consumed the commercial snack, whose glucose values increased considerably at 30 days of intake and high values of cholesterol and triglycerides were observed.

Although there are no studies evaluating a product with the same formulation, it has been shown that incorporating chickpeas in the usual intake improves the intake of dietary fiber and polyunsaturated fatty acids associated with a reduction in total serum cholesterol (Murty, Pittaway & Ball, 2010) .

In the same way, it has been proven that the components of chia seed could be used as a new source of soluble fiber in foods due to their potential effect on reducing lipids, cholesterol, and glucose available to be absorbed in the small intestine (Tamargo, Martin Navarro del Hierro, Moreno-Arribas & Muñoz, 2020) while a high-fiber diet that included flaxseed, rice bran, and sesame reduced the total cholesterol and serum triglyceride level of 45 participants (Monk *et al.*, 2017).

According to several authors, these changes are mainly attributed to the presence of dietary fiber since when fermented by the intestinal microbiota, it increases the production of short-chain fatty acids, mainly propionate, and butyrate, which exert a beneficial effect on lipid metabolism, decreasing serum levels (Clayton *et al.*, 2019; Desai *et al*., 2016).

In addition, an increase in the viscosity of the chyme induced by the intake of dietary fiber reduces elevated concentrations of serum cholesterol by significantly decreasing the reuptake efficiency of bile in the distal ileum, causing it to be eliminated through the feces, provoking the hepatocytes to be able to stimulate expression of the LDL receptor, increasing the clearance of LDL cholesterol from the blood to synthesize more bile acids (cholesterol is a component of bile) and maintain enough bile for digestion, reducing LDL cholesterol without significantly affecting the concentration of HDL cholesterol (Lambeau & McRorie, 2017), proving again the advantages that regular fiber intake can offer.

Also, it has been found that legumes are an excellent source of phytochemicals of which 85% of these belong to the group of phenolic compounds; furthermore, some other compounds such as phytosterols and lignans are also an important part of this food group (Tor-Roca, Garcia-Aloy, Mattivi, Llorach, Andres-Lacueva & Urpi-Sarda, 2020). These compounds exhibit functional properties for the benefit of human health, among which they present antioxidants, forming stable compounds in the body and other biological activities such as anti-inflammatory, anti-cancer, and anti-obesity (Luna-Guevara, Hernández-Carranza, Ruíz-Espinosa & Ochoa-Velasco, 2018).

In this sense, the phytosterols present in chickpeas have been reported to exhibit anti-ulcer, antibacterial, antifungal, antitumor, and anti-inflammatory properties, along with a lowering effect on cholesterol levels (Barman, Marak, Mitra-Barman & Sangma,

2019), while the lignans found in sesame seeds (sesamin) exert multiple functions, such as an antihypertensive effect, lipid reduction, and anticancer activity (Clayton *et al.*, 2018).

Besides, in past research, the consumption of chia has decreased blood cholesterol levels as well as body weight and waist circumference significantly in people with diabetes, in addition to promoting the improvement of the lipid profile (Toscano, Tavares, da Silva & Silva, 2015). Considering this, very important information since the possible reduction of triglycerides and the maintenance of cholesterol levels in rats that were fed with the snack rich in fiber may be due to, in addition to the presence of dietary fiber, the proper function of its phenolic compounds.

In this study, a concentration of 181.18 ± 8.08 mg GAE/100 g of sample was obtained in HFS, unlike the number of phenolic compounds found in the commercial snack (19.0 ± 0.43 mg GAE/100 g), observing that the concentration of phenolic compounds present in flours (83.0 ± 12.0 to 513.0 ± 41.0 mg GAE/100 g) remained viable even after a cooking process, which highlights the ability of these components to improve the nutritional characteristics of food products, which can generate a useful strategy in the development of nutritious or functional products that help in the prevention of overweight and obesity, in addition to the regulation of lipids (Ayaz, Akyol, Inan-Eroglu, Kabasakal, Samur & Akbiyik, 2017; Giaretta, Lima & Carpes, 2018).

Damage in gastric and intestinal tissue

As mentioned above, the consumption of low nutritional quality snacks, in addition to promoting poor nutrition, can cause hyperphagia and various diseases of the metabolic syndrome as well as diseases of the gastrointestinal tract (Fiorentino, 2019; Hess *et al.*, 2019; Tanaka, *et al.*, 2020).

This study evaluated the damage caused by the consumption of snacks in intestinal and gastric tissue in rats. No significant changes were observed in the gastric and intestinal tissue of the group that consumed the high-fiber snack (HFS), while the group administered with the commercial snack (CMS) presented a gastric wall with the absence of folds and damage to the structure of the cells of the superficial mucosa and gastric fossae, in addition to having affectations in the intestinal tissue, mainly in the structure and length of the villi as well as an increase in the depth of the Lieberkühn crypts.

Although gastric and intestinal tissue damage influenced by the consumption of healthy and unhealthy snacks and their effects on increased markers of inflammation have not been previously reported in rats, the results of this study can be compared with those obtained by other authors using similar diets.

For example, the administration of a high-fat diet in C57BL/6J mice caused a reduction of parietal cells as well as various morphological alterations of the foveolas or gastric fossae in the stomach, followed by glandular metaplasia (Arita, Kinoshita, Ushida, Enomoto & Inagaki-Ohara, 2016) and inflammation, similar to that observed in gastric tissue samples from the CMS group of this study.

Inagaki-Ohara, (2019) suggests that these changes are produced due to a greater expression of leptin in the gastric mucosa because this type of diet induces leptin signaling that accelerates a pro-tumorigenic gastric microenvironment, even independently of the body mass gain that animals may have when administered with this type of high-fat diets, unlike animals that have been administered with diets rich in dietary fiber, in which the amount of leptin is decreased (Mendoza-Herrera *et al.*, 2021).

Otherwise, when performing the microscopic analysis of the tissues, there was an increase in the depth of the crypts and a decrease in the villus height and crypts depth ratio (V/C) in the CMS group, which would indicate a lower digestion capacity and poor absorption of water, electrolytes, and nutrients, the main functions of the villi (Picut & Coleman, 2016). In addition to the fact that the increase of the depth of the crypts is characteristic of moderate hyperplasia and intestinal inflammation (Erben *et al.*, 2014) since the expression of anti-inflammatory cytokines is significantly decreased, also modifying the intestinal microbiota (Hamilton, Boudry, Lemay & Raybould, 2015).

In this study, the decrease in the expression of anti-inflammatory cytokines was observed with the increase of MPO in the gastric and small intestinal tissue of CMS, which presented significant differences with the HFS group, assuming a possible inflammation due to the metabolites produced by this enzyme that contribute with the production of pro-inflammatory cytokines (Hansberry, Shah, Agarwal & Agarwal, 2017).

This results in decreased epithelial integrity and increased intestinal permeability, as well as lower expression of tight-junction proteins resulting in ileal inflammation due to increased macrophage infiltration and expression of the TLR4 protein (involved with the production of inflammatory cytokines) and TNF-alpha (Shen *et al.*, 2014) thus following this type of diet where fiber is not included but there is an excess of fats and carbohydrates, such as those contained in commercial snacks, can lead to the deterioration of intestinal tissue, unlike the consumption of healthy snacks that include legumes and oilseeds in their formulation.

In this sense, the use of this food group in the diet has also been studied showing beneficial effects in the body, even improving intestinal permeability due to a greater expression of tight-junction proteins such as Zonula occludens-1 (ZO-1) and a decrease in the activation of nuclear factor-kB (NF-kB), one of the most important regulators of pro-inflammatory

gene expression (Monk *et al.*, 2019), highlighting once again, the importance that these components play in improving intestinal permeability and inflammation, and that part of the anti-inflammatory effects of the snack can also be attributed to its content in polyphenols that have antioxidant and anti-inflammatory effects (Yahfoufi, Alsadi, Jambi & Matar, 2018), as expressed above.

Conclusions

A snack rich in fiber was made from yellow corn, chickpeas, sesame, and chia flours, which sensorial had a good acceptance, in addition to having a high proportion of protein, as well as total dietary fiber and a high antioxidant capacity. The administration of this snack in a rat trial decreased triglyceride levels and inflammatory markers, unlike the effects caused by the consumption of a commercial low-fiber and high-fat corn snack which, in addition to increased glucose and cholesterol levels, also caused metaplasia in gastric tissue and a decrease in the villus height in the small intestinal tissue (ileum), while the gastric and intestinal tissue samples of the rats administered with the high-fiber diet were observed intact and without significant difference in comparison with the control group, which shows that the use of legume and oilseed flours in food matrices accessible to the population as snacks, can present anti-inflammatory effects and positive changes in the regulation of lipids in the body, in addition to being an alternative strategy aimed at modulating the healthy intestinal microbiota and thus preventing or reducing the symptoms of gastrointestinal diseases.

Acknowledgments

The authors thank Dr. María de Jesús Perea and Dr. Alberto Peña Barrientos from the Nanoscience and Nanotechnology Research Center for their support in obtaining the micrographs of the gastric tissues of the experimental animals. The authors thank the support provided by the National Council of Science and Technology (CONACYT project 285416) and the Secretariat of Research and Postgraduate Studies of the IPN, Mexico (SIP Project: 20221245). Sarahi Cruz Valderrama was a CONACYT fellow (registration number 739797). AEChP and RME thank the economic support of the SNI, COFAA, and EDI.

Conflict of interest

The authors declare no conflicts of interest.

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