Effects of Agave Fructans, Inulin, and Starch on Metabolic Syndrome Aspects in Healthy Wistar Rats

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ABSTRACT: Healthy Wistar rats were supplemented during 20 weeks with commercial inulin (I) and *Agave tequilana* fructans (CAT), experimental fructans from *A. tequilana* (EAT) and *A. salmiana* (AS) mature stems, rice starch 10% (RS), and standard feed for rodents (C). Feed intake was kept steady, but with I, body weight and abdominal adipose tissue (6.01 g) decreased at the end. Glucose (mg/dL) (C, 120.52; I, 110.69; CAT, 105.75; EAT, 115.48; AS, 101.63; and RS, 121.82), total cholesterol (C, 89.89; I, 64.48; CAT, 68.04; EAT, 68.74; AS, 68.04; and RS, 82), and triglycerides (C, 84.03; I, 59.52; CAT, 68.56; EAT, 59.08; AS, 75.27; and RS, 81.8) kept being normal and without differences between fructans. At the end, there was a significant increase in lactic acid bacteria when the I and AS groups were compared to the C group (C, 9.18; I, 10.64; CAT, 10.34; EAT, 10.36; AS, 10.49; and RS, 9.62 log 10 CFU/g of feces). In addition, with fructans, there was an accelerated process in feces emptiness, Lieberkuhn crypts kept their morphology, and there was an increment of goblet cells.

1. INTRODUCTION

Functional food intake has been increased recently to improve metabolism, body composition, and gut microbiota. Probiotics stand out among many functional foods for being a substrate used by selective beneficial microorganisms to promote good health. The most used prebiotics are fermentable carbohydrates, such as fructooligosaccharides (FOS), galactooligosaccharides (GOS), resistant starch, and fructans obtained from the Jerusalem artichoke (*Helianthus tuberosus* L.), dahlias (*Dahlia* spp.), and mainly chicory (*Cichorium intybus* L.).

Chicory fructans are widespread used by the food industry due to their physical chemistry and functional traits. These fructans, routinely included in the diet, cause beneficial effects on health mainly as prebiotics since they decrease abdominal adipose tissue and plasmatic glucose and cholesterol concentration due to the improvement on fat and carbohydrate metabolism. Inulin and FOS that are obtained by partial hydrolysis are among many functional foods for being a substrate used by selective beneficial microorganisms to promote good health.

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use as an ingredient in diverse food products is an important competitive alternative to chicory fructans.12

Nowadays, commercial fructans from maguey are obtained from Agave tequilana F.A.C. Weber, but this species is also used to produce spirits; thus, it is important to explore other maguey species that are abundant and are under-utilized, such as A. salmiana Otto ex Salm-Dyck.17,19,20

Based on the precedent, the objective of the present study was to compare the effects of rice starch, commercial fructans (from chicory and from A. tequilana), and fructans from A. salmiana and A. tequilana stems with the OPM and similar extraction method on feed intake and body weight, abdominal adipose tissue, metabolism, feces pH and microbiology, and hepatic and colon histology in healthy Wistar rats.

2. RESULTS AND DISCUSSION

2.1. Feed Intake. Net feed intake is shown in Table 1; only AS and RS treatments showed significant differences ($p < 0.05$) during week seven to week nine. Inulin supplementation has been recognized with a metabolic satietogenic effect,10,23 and also chemical ones by modifying rheological properties of feeds.28,29 These effects in healthy mice had been attributed to fructan supplementation of A. tequilana,24 A. angustifolia, and with A. potatorum,24 as same as with obese mice supplemented with fructans of A. tequilana.31 However, in these studies, feed was offered ad libitum to the animals, but in the present study, feed was restricted to 25 g per day per rat.

2.2. Body Weight Changes. Results of evaluated body weight changes are shown in Table 2. There were differences ($p < 0.05$) for final weight, but starch had the highest value and inulin the lowest; the rest of the treatments were more similar. These results agree with those found in a study with rats supplemented with 10% inulin during 27 months.32 Regarding weight gain, rice starch had the highest value ($p > 0.05$), and the rest were similar ($p > 0.05$).

2.3. Abdominal Adipose Tissue Weight. Figure 1 shows the results of the effects of treatments on abdominal adipose tissue weight. There were differences ($p < 0.05$) on abdominal adipose tissue weight; the lowest value was for the chicory treatment, and the highest ($p < 0.05$) values were for the rice starch treatment. Treatments with agave fructans showed no differences ($p > 0.05$). Even though feed intake was constant during the whole experiment, treatments provoke significant differences in final body weight and accumulation of abdominal adipose tissue. Indeed, agave fructan supplementation in obese mice can improve metabolic disorders associated with overweight.33 In a study with chicory fructan supplementation (10%), it was found that such a treatment decreased feed intake and the amount of adipose tissue of the epididymis and increased the short-chain fatty acid production.34 In general, it has been found that consuming fructans promotes satiation by diminishing the appetite and the general energetic balance, which in turn reduces final body weight and adipose tissue in experimental animals.19,35–38

2.4. Effects in Serum Variables. At the beginning of the experiment, there were no differences ($p > 0.05$) for all the quantified serum variables (Table 3), and at the end of the experiment, there were no differences ($p > 0.05$) for glucose among the different treatments. There were differences ($p < 0.05$) for total cholesterol with the highest value for the control treatment, and the lowest value was for the inulin (I) treatment. The highest values for triglycerides were found for the control

### Table 1. Means of Feed Intake (g) in Healthy Wistar Rats during the Experimental Period

| Treatment | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|-----------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C         | 177.66 | 178.14 | 177.45 | 169.56 | 175.48 | 172.29 | 175.75 | 175.33 | 176.13 | 176.37 | 173.16 | 173.32 | 171.18 | 178.46 | 175.08 | 172.85 | 172.85 | 175.87 |
| I         | 173.08 | 176.22 | 171.16 | 173.61 | 172.82 | 171.51 | 169.22 | 169.13 | 167.22 | 167.22 | 169.13 | 167.62 | 169.13 | 167.62 | 169.13 | 167.62 | 169.13 | 167.62 | 169.13 |
| RS        | 177.52 | 176.22 | 175.02 | 174.51 | 174.31 | 173.93 | 173.93 | 173.93 | 173.93 | 173.93 | 173.93 | 173.93 | 173.93 | 173.93 | 173.93 | 173.93 | 173.93 | 173.93 | 173.93 |
| AS        | 171.22 | 172.94 | 177.32 | 175.87 | 175.87 | 175.87 | 175.87 | 175.87 | 175.87 | 175.87 | 175.87 | 175.87 | 175.87 | 175.87 | 175.87 | 175.87 | 175.87 | 175.87 | 175.87 |

The designation ab means that different letters between columns are statistically different ($p < 0.05$).
and rice starch treatments. Finally, inulin had the highest (p < 0.05) value for the high-density lipoproteins (Table 3).

Different explanations could be found on how fructans perform their metabolic action in the organism. It is mentioned that fructans modify the insulin and glucagon levels through the regulation of lipid and carbohydrate metabolism. Moreover, in a study with rats fed with fructooligosaccharides, low levels of cholesterol and serum triglycerides were found, which was attributed to a reduction in the de novo fatty acid synthesis in the liver and to the reabsorption of circulating bile acids.39

The supplementation with chicory and agave fructans given to the experimental animals kept the desirable glucose serum levels (98−152 mg/dL),40 total cholesterol (37−95 mg/dL),41,42 triglycerides (98−152 mg/dL), and HDL (30−64 mg/dL).43 It should be noted that commercial and experimental A. tequilana fructans create similar statistical results on all the serum variables. Healthy rats have been supplemented with A. salmiana fructans in optimal physiological maturity, without altering the normal concentration of serum metabolic variables at the end of a short-term experiment (5 weeks/dose of 12.5%),44 medium term (12 weeks/dose of 20%),45 and longer term (20 weeks/dose of 10%) like the present study.

It has been documented that when separate A. tequilana fructans according to their polymerization grade are used, physiological results are obtained depending of the complexity of the polysaccharide.25,27 In the present study, only whole fructans (A. salmiana and A. tequilana) were used without separation by the polymerization grade, and the recorded benefits (serum metabolic variables, body weight, and abdominal adipose tissue) were similar to those obtained with A. tequilana,24 A. angustifolia, A. potatorum,41 and A. fourcroydes fructans.46

### 2.5. Feces pH Values and Microbiology.

The recorded pH values in the colon (4.5 to 7.5) for all treatments are under the normal pH values for experimental animals.47 However, rice starch supplementation resulted in a significant acidity reduction, and fructan supplements only showed a consistent trend to produce higher acidity (Figure 2).

There were no differences (p > 0.05) in the enterobacteria concentrations among all treatments at the beginning and at the end of the experiment. The short-chain fatty acid production during fructan bacteria fermentation reduces only partially the pH average in the gut because gradually, these acids are generated, absorbed, and metabolized; while in in vitro studies, they stay in the medium and generate more acidity.48,49

Differences (p < 0.05) were observed in lactic acid bacteria CFU concentrations with the highest values for fructan supplementation, which agree with some published studies.25,45,50,51 The final increase in lactic acid bacteria caused by starch, statistically similar to the fructan supplementation,52,53 could be due to its thermal alteration when it was incorporated to the feed.24 These interesting results evidence the stimulation of an important target group of microorganisms (LAB); however, more details of the specific stimulation at the genus level are necessary since the MRS medium used allows the isolation of diverse genera.

With respect to the main bacteria genus, the control kept the original abundance of Lactobacillus; whereas, in the Agave fructan treatments, a decrease in Lactobacillus was observed along with bigger microbial diversity. It should be noted that CAT and EAT treatments increased the Faecalibaculum abundance. With analyzing the delta abundances (Figures 3 and 4), it can be seen that in AS and I, the Lactobacillus abundance was similar with each other but higher than CAT and EAT. The increase of Faecalibaculum in AS and I was lower than that in treatments CAT and EAT. The main-component analysis showed similar patterns between treatments, with the exception of AS by its higher diversity.

The microbiota is different if it is taken from the mucous or luminal portion.55−57 In addition, according to Li et al.,58 they saw the differences between starch and fructan treatments. Finally, it has been documented that when separate A. tequilana fructans according to their polymerization grade are used, physiological results are obtained depending of the complexity of the polysaccharides.25,27 In the present study, only whole fructans (A. salmiana and A. tequilana) were used without separation by the polymerization grade, and the recorded benefits (serum metabolic variables, body weight, and abdominal adipose tissue) were similar to those obtained with A. tequilana,24 A. angustifolia, A. potatorum,41 and A. fourcroydes fructans.46

### Table 2. Net Feed Intake and Change in Body Weight (g)

| treatment  | basal feed intake | final feed intake | basal body weight | final body weight | gain of body weight |
|------------|-------------------|-------------------|-------------------|-------------------|---------------------|
| C          | 177.66 ± 6.1      | 169.34 ± 5.7      | 247.89 ± 12.4     | 487.22 ± 36.8     | 239.33 ± 39.2       |
| I          | 173.08 ± 4.9      | 163.03 ± 5.2      | 250.33 ± 13.6     | 462.22 ± 30.6     | 211.89 ± 37.4       |
| CAT        | 178.16 ± 5.4      | 168.03 ± 5.8      | 254.33 ± 14.0     | 498.80 ± 32.0     | 244.47 ± 37.6       |
| EAT        | 173.83 ± 4.7      | 162.00 ± 6.1      | 265.78 ± 17.2     | 493.33 ± 35.9     | 227.55 ± 35.8       |
| AS         | 171.22 ± 4.2      | 169.49 ± 5.9      | 268.56 ± 18.6     | 484.44 ± 34.0     | 215.89 ± 31.2       |
| RS         | 177.52 ± 5.6      | 163.70 ± 5.4      | 260.10 ± 16.8     | 514.50 ± 38.4     | 254.40 ± 32.6       |
| SEM        | 1.75 ± 0.15       | 4.53 ± 0.75       | 8.44 ± 0.47       | 11.80 ± 0.44      | 10.74 ± 0.39        |
| p value    | 0.58 ± 0.04       | 0.75 ± 0.07       | 0.47 ± 0.03       | 0.048 ± 0.02      | 0.045 ± 0.01        |

The means ± SEM are shown (n = 9). Means with different letters per column are statistically different (p < 0.05). C, control; I, commercial inulin; CAT, commercial fructans from A. tequilana; EAT, experimental fructans from A. tequilana; AS, Fructans from A. salmiana; RS, rice starch.
found higher proportion of *Lactobacillus* and *Turicibacter* genus in the stomach and small intestine, while anaerobium like *Lachnospiraceae* and *Ruminococcaceae*, which ferment carbohydrates and aromatic vegetable compounds, constituted the higher proportion of colon microbiota.

With respect to the use of prebiotics to modulate gut microbiota, Everard et al.\(^59\) and Mao et al.\(^60\) showed through sequence techniques of the partial 16S rRNA gene that inulin consumption by obese mice encourages the abundance of bifidobacteria and lactobacillus, just as other bacteria families such as *Streptococcus*, *Clostridium*, *Enterococcus*, Olsenella, *Akkermansia*, and *Allobaculum*. Regarding the fructan supplementation with agave, Huazano-Garcia et al.\(^13\) showed effects on ceca microbiota of obese mice and found enriched genus of *Klebsiella* and *Citrobacter*.

2.6. Intestinal Length and Cecal and Colon Contents.

The colon being shortened has been considered as an inflammation sign,\(^61,62\) which could be the result of prolonged fructan consumption; moreover, the persistence of some agave fructan raffidia could cause mechanical harm in the intestine. With fructan supplementation, especially those of agave, there was a not statistically significant trend to produce colon lengthening; nevertheless, fructans in the small intestine tend to reduce the length, possibly due to a reduction of time of feed retention (Table 4). Based in the microscopic exploration, there was no harm in the intestinal tissue due to raffidia in the experimental animals. These results are in agreement with previous studies.\(^63\)

Results of cecal content evaluation are shown in Figure 5. The four treatments with fructan supplementation were similar \((p > 0.05)\) but higher \((p < 0.05)\) than the control and the starch treatment. The weight increase in cecal content was due to its higher water proportion and the lactic acid bacteria increment produced by the fructans since they constitute its natural substrate.\(^63,64\)

Control treatment (C) without supplementation had two-fold \((p < 0.05)\) colon content compared to the other treatments. Rice starch supplement had a more dry and compact content than that from fructans, which had more soft and wet consistency content. Indeed, even though the fructan supplement increases feces volume, when humidity and bacterial biomass retention increases, defecation facilitates and the retention time of the intestinal content decreases, which in turn prevents constipation and reduces the time of contact of harmful compounds in the intestine.\(^65\)

2.7. Histological Analysis.

The effects of the extended fructan supplementation on hepatocyte nucleus size and colon Lieberkühn crypts are shown in Table 4. None of the evaluated treatments produce a significant histological change in the liver; there was no lipid accumulation or intracellular bile pigments as a reaction of any pathology, which is in agreement with previous studies.\(^66\)

The colon grave crypt width was similar \((p > 0.05)\) for all treatments (Table 4); in addition, they present the same size, the morphology was intact, and the pericryptal space appears well-defined. However, the crypts corresponding to fructan treatments presented more vacuole structures with intracellular mucin in the calciform or Goblet cells (Figure 6), which is beneficial for the organism.\(^67\)

Finally, it is important to mention that the animals supplemented with agave fructans obtained in our laboratory (EAT and AS) did not show mechanical harm, which is evidence...
that the raffia present in the stems were separated and totally eliminated during the applied extraction process.68

3. CONCLUSIONS

Feed intake was the same for all evaluated treatments in the healthy animals. Chicory fructans (inulin) statistically reduced the final body weight. Chicory fructan supplementation decreased abdominal adipose tissue weight. Fructans kept the serum evaluated variables on desirable values. Starch supplementation produced an increase in feces pH value. Feces enterobacteria concentration had no significant changes in all treatments; however, fructan and starch supplementation increased lactic acid bacteria concentration. Agave and chicory fructan supplementation increased cecal content, helped in intestinal evacuation, and improved feces consistency. This supplementation resulted to be innocuous for the liver and for the intestinal tract, and an increase of intracellular mucin of grave crypts of colonocytes was observed. In general, whole-fructan supplementation of A. salmiana and A. tequilana to healthy animals produced similar beneficial effects to those found with inulin.

4. EXPERIMENTAL SECTION

4.1. Polysaccharide Tested. The commercial polysaccharides used were inulin or commercial inulin (C. intybus) (Orafti Synergy 1, Tienen, Belgium), commercial fructans from A. tequilana (Inufib, Jalisco, Mexico), and rice starch (Tres Estrellas, Toluca, Mexico). In addition, as polysaccharides

![Figure 2. Variation of wet feces pH during the experimental period. C, control; I, commercial inulin; ATC, commercial fructans of A. tequilana; ATE, experimental fructans of A. tequilana; AS, fructans of A. salmiana; RS, rice starch. Means ± SEM (n = 9).](image2)

![Figure 3. Relative abundance of significantly different ASVs in feces at the beginning (B) and at the end of the experiment (F) of each treatment: C, control; I, commercial inulin; CAT, commercial fructans from A. tequilana; EAT, experimental fructans from A. tequilana; AS, fructans from A. salmiana; RS, rice starch. Each taxon representing >1% of the average relative abundance in each treatment is indicated by a different color.](image3)
were obtained directly (no commercials), fructans from *A. salmiana* and *A. tequilana*, extracted from the stem from six OPM individuals from the region of Charcas, SLP and Arandas, Jalisco, respectively, and processed according to the method developed in our laboratory, were used.68

4.2. Animal Model. Fifty-four two-month-old male Wistar rats with an average body weight (BW) of 200–250 g were housed in individual polypropylene cages in a conditioned room at the Institute, with a temperature ranging between 20 and 25 °C, relative humidity between 30 and 60%, and circadian cycle adjusted to 12 h light and 12 h dark.

The study was conducted following the animal care guidelines specified.69,70 The protocol was approved by the ethics committee (CONBIOÉTICA-24-CEI-003-20160830). Animals had a one week period for adaptation with a ration of 25 g of standard feed for rodents (Chow 5008, Brentwood, MO, USA) and ad libitum water. Then, rats were randomly allocated in six treatments (n = 9): commercial feed (C); 90% commercial feed and 10% of the following polysaccharides: commercial inulin (I), commercial fructans from *A. tequilana* (CAT), experimental fructans from *A. tequilana* (EAT), or from *A. salmiana* (AS); and commercial rice starch (RS). The 25 g feed with or without supplementation was administered every day at the beginning of the dark period.

Table 4. Nuclear Width (μm) of Hepatocytes and Lieberkühn Crypts

| treatment | hepatocytes | Lieberkühn crypts |
|-----------|-------------|-------------------|
| C         | 9.25 ± 0.41a| 40.29 ± 2.91a     |
| I         | 9.72 ± 0.60a| 43.63 ± 3.57a     |
| CAT       | 9.44 ± 0.91a| 41.60 ± 7.03a     |
| EAT       | 9.76 ± 0.62a| 41.82 ± 2.49a     |
| AS        | 9.19 ± 0.46a| 46.83 ± 4.73a     |
| RS        | 9.18 ± 1.00a| 41.15 ± 4.23a     |

aC, control; I, commercial inulin; CAT, commercial fructans from *A. tequilana*; EAT, experimental fructans from *A. tequilana*; AS, fructans from *A. salmiana*; RS, rice starch. Means ± SD. *Means with different letters in column are statistically different (p < 0.05).

Figure 4. Heat map of delta values for each bacterium and treatment, representing the percent of ASV at the beginning and at the end of the experiment. The red color means an increase while the blue color means a decrease; the numbers of each color correspond to the value of the difference.

Figure 5. Weight of wet cecal content (g). C, control; I, commercial inulin; CAT, commercial fructans from *A. tequilana*; EAT, experimental fructans from *A. tequilana*; AS, fructans from *A. salmiana*; RS, rice starch. Means ± SD (n = 9).
The experimental trial lasted 20 weeks; feed intake and body weight were recorded weekly. At the end of the experimental trial, animals were sacrificed with sodium pentobarbital (0.063 g/mL; SEDALPHARMA, Pet’s Pharma, Mexico) at a dose of 40 mg/kg BW by intraperitoneal injection; the abdominal adipose tissue was dried and weighed, and the intestinal gut was dried and samples were taken for histological analysis.

4.3. Biochemistry of Serum Samples. Blood samples were obtained by puncture in the caudal vein at the beginning and at the end of the experiment. After 12 h of fast, 600 μL of whole blood was extracted; the sample was set aside by 10 min and then centrifuged at 2500 rpm for 5 min at 20 °C (Centra CL3-R, Thermo IEC, San Antonio, TX, USA) to obtain the serum and stored at −20 °C until analysis. The measured variables were glucose, total cholesterol, HDL, and triglycerides, with enzymatic commercial kits (Bayer, Sees, France) using semi-automatic equipment for chemical analysis (Excel, Stanbio, USA).

4.4. pH Variation and CFU of Feces Microbiology. Monthly pH variation (Hanna Instruments pH 211, USA) of feces during the whole experimental trial was estimated in 1 g of fresh feces sample from each rat, which was placed in sterile tubes and homogenized with 9 mL of sterile saline solution (0.85%).

To quantify colony forming units (CFU) per g of feces, the Miles et al. modified method was used.71 Serial dilutions were made with a concentration of 10^9 to 10^11 and were then allocated 10 μL of each dilution in bacteriologic solid medium by triplicate. The lactic acid bacteria (LAB) group was prepared using Man Rogosa Sharpe medium (Difco, USA) and was incubated in anaerobic jars (Gas Pack) at 37 °C for 48 h. This group includes the Lactobacillales order, and some of the more representative genera are Lactobacillus, Streptococcus, and Leuconostoc. In those conditions, bacteria of the genus Bifidobacterium could also be isolated in the MRS medium. On the other hand, the genera of the family Enterobacteriaceae were isolated on MacConkey medium (Difco, USA) and were incubated at 37 °C for 48 h. Afterward, CFU/g count was performed for each medium.

4.5. Metagenome Analysis. Total genomic DNA extraction of bacteria populations of rats’ feces was based on Tannock et al.’s72 methodology with some modifications. Therefore, partial sequences of the hypervariable region of the 16S rRNA gene fragment (V4 & V5) of the baseline and final stool sampling DNA were PCR-amplified using the primers 520 F (5′-AYTGGGYDTAAAGNG-3′) (30) and 907 R (5′-CCGTCAATTCMTTTRAGTTT-3′)73 with two PCR runs as recommended by Kaplan et al.74 Denoising quality, chimera check, and clustering to amplicon sequence variants (ASVs), a higher-resolution analogue of the traditional operational taxonomic unit, was conducted using the Quantitative Insights Into Microbial Ecology (QIIME2) (version 2018.4-2018.8, https://qiime2.org) plug-in DADA2.75 Sequences were assigned to taxonomy using a pre-trained Naive Bayes classifier trained on the database Silva 132.76 After taxonomic affiliation, mitochondrial and chloroplast sequences were filtered out. Microbial richness, which measures the number of taxa in every sample (abundance of microbes), was determined by calculating the number of observed ASVs. In likeness, the measurement of the relative number of taxa in samples (the frequency of each

Figure 6. Sample section of colon tissue (40×) stained by hematoxylin-eosin. C: Control, I: Commercial inulin, (c) CAT: Commercial fructans from A. tequilana, EAT: Experimental fructans from A. tequilana, AS: Fructans from A. salmiana, RS: Rice starch.
taxon was detected in a sample) was done using the Shannon and Simpson indices. Preparation of microbiome sequence data was carried out using R studio software 1.1.419, R packages Phylseq 1.2.2.37 and Vegan 2.4-6.76

4.6. Histological Analysis. At the end of the experiment, the whole intestine was dried, the length (Table 5) from the cecum to the anus was measured, and the colon was separated; then, cecum and colon contents were weighed; the colon was cut with sterile saline solution (0.85%), and then, it was cut longitudinally and allowed to sit on filter paper with the lumen placed toward the outside. In addition, a cubic liver fraction was placed toward the outside. In addition, a cubic liver fraction was removed, approximately 0.5 cm, and both tissues were set on alcohol-xilol gradients and were added with paraformaldehyde (Sigma-Aldrich, Milwaukee, USA) at 10% in phosphate buffer. After tissue fixation, samples were dehydrated with alcohol-xilol gradients and were added with paraffin, then were cut longitudinally at 6 μm, and dyed with hematoxilin and eosin. These tissues were analyzed by clear camp microscopy at 40X magnification (Olympus CellSens Entry, Olympus Corporation, Tokyo, Japan) in order to record possible histological changes due to the treatments. Measurements of mucous crypt (FC) grave diameter of the colon were made. In the hepatic tissue, nuclei were measured since macronucleosis is a reactive change that may be associated with toxicity.79

4.7. Experimental Design and Statistical Analysis. A complete random design was used with six treatments: feed supplementation (10%) with five polysaccharides (I, CAT, EAT, AS, and RS) and without supplementation (C), with nine repetitions. Data analyses were performed with SAS version 9.2 (SAS Institute, Inc., Cary, North Carolina, USA); normality tests, analysis of variance (ANOVA), and a Tukey mean test were performed. A repeated measurement test was performed during the 20 weeks. Significance was set at p < 0.05.

| Table 5. Intestinal Length (cm) of the Experimental Units
| treatment | large intestine | small intestine |
|-----------|----------------|-----------------|
| C         | 19.11 ± 3.44   | 119.17 ± 5.00   |
| I         | 19.50 ± 0.84   | 122.50 ± 6.81   |
| ATE       | 20.25 ± 1.91   | 111.00 ± 8.92   |
| ATC       | 20.39 ± 1.57   | 114.39 ± 11.12  |
| AS        | 20.63 ± 2.26   | 102.50 ± 14.46  |
| RS        | 19.11 ± 1.05   | 106.43 ± 14.05  |

“C, control; I, commercial inulin; ATC, commercial fructans from A. tequilana; ATE, experimental fructans from A. tequilana; AS, fructans from A. salmiana; RS, rice starch. Means ± SD. *Means with different letters per column are statistically different (p < 0.05).

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Notes
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