

1 **Original Research Article**

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3 **Effect of chronic sweeteners consumption in**

4 **lymphocytes of Peyer's patches of two mice**

5 **strain**

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7

8 **ABSTRACT**

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**Aims:** To know the Effect of chronic sweeteners consumption in lymphocytes of Peyer's patches of two mice strain.

**Study design:** a prospective, longitudinal, comparative and experimental study.

**Place and Duration of Study:** The study was conducted in the Nutrition Research Laboratory of the Faculty of Medicine of Universidad Autónoma del Estado de México (UAEMéx) between August 2018 and May 2019 and was approved by the Bioethics committee.

**Material and methods:** We were used two groups of mice with different strains: 1) Balb/c and 2) CD1, both from 8 weeks old. The groups divided into 4 subgroups of non-nutritive sweeteners consumption: Control, Sucrose, Splenda and Svetia. The mice took the supplementation for 8 weeks. Were quantified glucose, percentage of lymphocytes, water and food consumption.

**Results:** Mice increased their body weight after 6 weeks of treatment. The animals of Control and Sucrose subgroups showed a significant gain of 5g of weight, compared with the Splenda and Svetia subgroups, which increased 4g. The same way the subgroup of Splenda

**Comment [S1]:** Stevia

**Comment [S2]:** This needs revision. Is it question sentence?

**Comment [S3]:** Stevia

significantly reduced blood glucose, Stevia and Control groups that consumed more water without sweetener. Food consumption was variety. By the end, the percentage of lymphocytes increased in the Sucrose subgroup, but decreased in other subgroups.

Comment [S4]: Stevia

**Conclusion:** It is a fact that sweeteners modify the lymphocyte population of Peyer 's patches and this variation depends on the frequency, the strain of the rodents and the type of sweetener.

Comment [S5]: It

10

11 *Keywords: sweeteners, Peyer's patches, lymphocytes, body weight, blood glucose, water*  
12 *consumption.*

13

14

## 15 **1. INTRODUCTION**

16 Sweeteners are chemical compounds that have the ability to produce a sensation of  
17 sweetness [1] and they have various effects on health [2, 3]. Sucrose (table sugar), is the  
18 oldest used sweetener and provides energy to the body [4]. The increase in chronic  
19 noncommunicable diseases and sedentary lifestyle are causing consumers to look for  
20 products that are reduced in energy and therefore in sugar, using more and more non-caloric  
21 substitutes [5]. These offer a sweet taste to food, but with a lower energy content [6, 7]. The  
22 preference for sweet taste varies according to genetics and age [8], it is fundamental in the  
23 nutritional status [9], therefore, there is a need to look for sugar substitutes, with a similar  
24 effect on taste, but with less energy [10]. Sweeteners are classified as natural and artificial  
25 [11]. Artificial as sucralose, are produced by chemical synthesis, have little or no energy  
26 supply, with power than sucrose sweetener [12]. Among the natural we found stevia, it's  
27 come from vegetable products, give energy power and they have a sweetening power  
28 inferior or similar to sucrose [13]. With the intention of improving the quality of food, sugars  
29 are partially or totally replaced by sweeteners, this is seen in the increase of commercial

30 products that contain them [14]. It is known that its use does not alter blood glucose  
31 concentrations [15], for which they are well accepted in diabetic patients [16], do not  
32 contribute to dental caries [17] and can be used in pregnant women [18].

33

#### 34 *Stevia*

35 Steviol glycosides, natural sweeteners isolated from the leaves of the plant, *Stevia*  
36 *Rebaudiana Bertoni*, contains a *Stevioside* and *Rebaudioside A* [19]. It is 300 times sweeter  
37 than sucrose [20]. Their metabolism begins in the intestine, they are broken down to steviol  
38 with help of the intestinal microbiota, mainly by *Bacteroides sp.*, they are absorbed by  
39 facilitated diffusion to the blood, finally, steviol is secreted in the urine as steviol glucuronide  
40 and in feces like free steviol [21, 22]. *Stevia* is safe when used as a sweetener, suitable for  
41 diabetic patients, with phenylketonuria, obese and for those who wish to avoid the  
42 consumption of sugar in the diet [23].

43

#### 44 *Sucralose*

45 Sweetener synthesized in 1976, is approximately 600 times sweeter than sucrose [24]. It is  
46 manufactured by selective halogenation of sucrose, is thermostable, resists a wide variety of  
47 pH, is not metabolized or stored in the body, and is excreted unchanged in urine and feces  
48 [25]. 85% of sucralose is not absorbed, the remaining 15% is absorbed by passive diffusion  
49 [26]. Baird, IM et.al, in 2000, published a study related to the tolerance of sucralose in  
50 humans, they confirm that it does not generate adverse effects on health [27].

51

#### 52 *Gut-associated with lymphoid tissue (GALT)*

53 The gut-associated with lymphoid tissue (GALT) is located in the mucosa of the  
54 gastrointestinal tract [28], contains the largest surface area of exposure to microorganisms,  
55 as it contains a diverse and dense microbiota that are not pathogenic to the host [29, 30].

56 The mucosa of the gastrointestinal tract is able to identify pathogenic and nonpathogenic

57 substances, and therefore discern between producing or not, an immune response [31]. The  
58 immunological defense in the intestine is carried out by the GALT lymphocytes, organized in  
59 compartments, the Peyer's patches (inductor site), the lamina propria (effector site) and the  
60 isolated lymphoid follicles [ILF] (32). The most important of these structures is that they  
61 contain a large number of cells, derived from a cellular precursor generated in the bone  
62 marrow [33]. In the small intestine, there are about 200 Peyer's patches (PP), each one  
63 consists in aggregates of B cells (lymphoid follicles), surrounded by rich areas in T cells and  
64 antigen-presenting cells (APCs) [34]. On its surface there are flattened epithelial cells with  
65 few villi and mucus-producing cells [35]. The PP can be considered as the immunological  
66 sensors of the intestine and are an initial contact site with the antigens [36]. When antigenic  
67 stimulation occurs in the PP, the lymphocytes migrate to the blood, proliferate and  
68 differentiate in the spleen before returning to the lamina propria and other areas of the  
69 mucosa [32].

#### 71 *Effect of sweeteners on the immune system*

72 The effect of sweeteners on the immune system is controversial and is not yet clear. It has  
73 been observed that the use of glucose, fructose and sucrose, cause reduction of phagocytic  
74 activity of peripheral blood neutrophils [37]. The effect of sucralose has been studied in  
75 lymphoid organs such as spleen and thymus [38], doses greater than 3000 mg/kg showed  
76 changes in the thymus [39] and reductions in peripheral white blood cells and lymphocyte  
77 count have been observed [40]. On the other hand, stevia administered at different doses  
78 increased phagocytic activity and proliferation of T cells [41]. In another study, they found  
79 that steviol has no effect on the release of TNF- $\alpha$ , and IL-1 $\beta$  in THP-1 human monocytic  
80 cells when stimulated by LPS [42]. In human colon carcinoma cell lines, the effect of  
81 stevioside on the release of IL-8 was studied, using TNF- $\alpha$  as a stimulator, they found that  
82 steviol reduces the expression of NF-kB [43]. With this previous context, and taking into  
83 account that Peyer's patches are the first immunological contact zone of sweeteners, the

Comment [S6]: Steviol glycosides

Comment [S7]: What is LPS

84 objective of this study was to compare the effect of chronic sweetener consumption on  
85 Peyer's patches lymphocytes from two strains of mice.

86

## 87 **2. MATERIAL AND METHODS**

### 88 **2.1 Study design**

89 A prospective, longitudinal, comparative and experimental study was carried out. Two  
90 different strains of mice were used: Balb/c and CD1, from 8 weeks old, weighing between  
91 19.5 g and 22.3 g. Both groups were fed normal standard food Rodent Laboratory Chow  
92 5001 from Purina and water ad libitum. They were kept in plastic cages in groups of 4 each,  
93 under pathogen-free conditions and with light/dark cycles of 12 hours. The study was  
94 conducted in the Nutrition Research Laboratory of the Faculty of Medicine of the Universidad  
95 Autónoma del Estado de México (UAEM) and was approved by the Bioethics Committee of  
96 the same faculty. The mice were managed based on NOM-062-ZOO-1999, Specifications  
97 for the production, care and use of laboratory animals [44].

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99

### 100 **2.2. Distribution of groups and administration of sweeteners**

101 The mice were distributed into two groups: Group 1) Balb/c strain mice and Group 2) CD1  
102 strain mice. Each group were divided in 4 subgroups (n=8): A) Control Group (CL), without  
103 sweetener, B) Sucrose Group (Suc), C) Splenda Group (Spl), D) Svetia Group (Svt).

104 The solutions were prepared with sweeteners in ultrapure water, they were placed in the  
105 drinkers daily, for oral consumption during the 24 h 7 days of the week. The concentration  
106 used was 41.66 mg / mL of sucrose and 4.16 mg / mL of Splenda and Svetia. The treatment  
107 was administered for 6 weeks, starting on the 60th day old of the animals.

108

### 109 **2.3 Determination of body weight and blood glucose**

Comment [S8]: Stevia

110 Quantification of body weight was performed weekly, starting at week 8. Weight  
111 measurements were made with anesthetized mice (0.1 mL of 1% sodium pentobarbital).  
112 The concentration of peripheral blood glucose was quantified weekly with an Accu-Chek  
113 Perform glucometer. The sample was collected from the middle third of the tail.

114

#### 115 **2.4 Water consumption quantification**

116 The water consumption was done by placing 250 mL of water with or without sweetener in  
117 each drinker, at 24 h the volume of water consumed was measured and subtracted from the  
118 water that remained in the drinking fountain.

119

#### 120 **2.5 Obtaining samples**

121 After 6 weeks of treatment, the animals were anesthetized with 0.1 mL of 1% sodium  
122 pentobarbital and sacrificed by cervical dislocation. One millilitre of blood was obtained by  
123 direct cardiac puncture (using a syringe with 50 µl of heparin); from the millilitre of blood, the  
124 lymphocytes were purified by density gradient with Lymphoprep™ (Axis-Shield) (45). The  
125 small intestine was removed, and Peyer's patches were removed from it.

126 Once the Peyer's patches were removed, they were placed in Petri dishes with RPMI  
127 medium (3 mL), manually homogenized and filtered with nylon mesh (40-µm) to eliminate  
128 the remaining connective tissue. Centrifuged at 2500 rpm / 5 min, the cell button obtained  
129 from the Peyer's patches was placed in a hypotonic buffer solution (8.26 g/L of NH<sub>4</sub>Cl, 1 g/L  
130 of KHCO<sub>3</sub> and 0.037 g/L of EDTA-4Na, with a pH of 7.4) to lyse the erythrocytes. The cell  
131 suspension isolated from the Peyer's patches was washed with PBS. The cell viability of the  
132 isolated lymphocytes was immediately evaluated with a trypan blue assay. The lymphocytes  
133 were counted with Neubauer chamber to obtain the cellular percentage *per* mL of cell  
134 suspension.

135

136 **2.6 Statistic Analysis**

137 The statistical package SPSS version 19 for Windows was used to analyze the data. Tests  
138 were made of central tendency (mean), dispersion (standard deviation) and means were  
139 compared by means of one-way analysis of variance ANOVA, with Tukey's post hoc test to  
140 evaluate intra-group differences. Significance was considered with  $p < 0.05$ .

141

142 **3. RESULTS**

143 **3.1. Changes in body weight after consumption of sweeteners**

144 All mice in group 1 significantly increased their body weight after 6 weeks of treatment. The  
145 animals of Control and Sucrose subgroups showed a significant gain of 5 g of weight  
146 ( $p < 0.001$ ), compared with the Splenda and Svetia subgroups, which increased 4 g (Table 1).

Comment [S9]: Stevia

147 In group 2 the increase in weight was similar, the mice of the Control and Sucrose  
148 subgroups increased on average 4 g of weight and the subgroups of Splenda and Svetia  
149 only 3 g ( $p < 0.014$ ). Svetia's group had the lowest weight gain (3 g), compared to Control  
150 ( $p < 0.028$ ), as shown in table 1. When comparing group 1 with group 2, significant  
151 differences were found ( $p < 0.001$ ), the weight of animals of group 1 was lower than those of  
152 group 2, although the behavior of weight gain was similar.

Comment [S10]: Stevia

Comment [S11]: Stevia

153 **Table 1.** Average weight of mice after 6 weeks of supplementation with sweeteners.

	Control	Sucrose	Splenda	Svetia	
Body Weight	Mean $\pm$ SD (g)	Mean $\pm$ SD (g)	Mean $\pm$ SD (g)	Mean $\pm$ SD (g)	$p$ Value
<b>Initial</b>					
Group 1	23.16 $\pm$ 0.956	23.98 $\pm$ 1.0	20.87 $\pm$ 0.587	20.58 $\pm$ 1.42	0.001*
Group 2	40.55 $\pm$ 0.597	37.85 $\pm$ 1.17	40.16 $\pm$ 3.49	37.5 $\pm$ 1.8	0.009*
<b>Final</b>					
Group 1	28.33 $\pm$ 1.05	28.81 $\pm$ 1.23	24.5 $\pm$ 0.609	24.92 $\pm$ 1.29	0.001*
Group 2	44.48 $\pm$ 0.448	41.45 $\pm$ 1.54	43.68 $\pm$ 4.22	40.67 $\pm$ 2.03	0.014*

Comment [S12]: Stevia

154 One-way ANOVA was performed to determine the differences between the subgroups, it was  
155 considered significant with  $p < 0.05$ . A Bonferroni *post hoc* test\* was performed to observe intra-group  
156 differences.

157

158

159 **3.2. Glycaemia**

160 The glucose in group 1 showed no significant differences ( $p < 0.122$ ) between the subgroups.  
161 In group 2, the blood glucose concentration was higher, the subgroup of Splenda  
162 significantly reduced blood glucose ( $p < 0.006$ ), compared with the Control, Sucrose and  
163 Splenda subgroups. When comparing the groups, differences were found between them  
164 ( $p < 0.001$ ), group 1 had lower glucose concentrations, even in the control groups (Table 2).

165

166 **Table 2.** Blood glucose after 6 weeks of treatment with sweeteners.

	Control	Sucrose	Splenda	Svetia	
Glucose	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	<i>p</i> value
Group 1	110.75 $\pm$ 13.9	100 $\pm$ 16.33	96.87 $\pm$ 10.88	108.5 $\pm$ 9.59	0.122**
Group 2	174.12 $\pm$ 33	201.62 $\pm$ 43.89	<b>133.25<math>\pm</math>40.73</b>	205.75 $\pm$ 47.33	0.010*

Comment [S13]: Stevia

167 One-way ANOVA was performed to determine the differences between the subgroups, it was  
168 considered significant with  $p < .001$ . A Bonferroni *post hoc* test\* was performed to observe intra-group  
169 differences.

170

171 **3.3. Water with and without sweetener**

172 Group 1 consumed more water with Sucrose and little water with Sucralose ( $p < 0.001$ ),  
173 compared with the Svetia and Control groups that consumed more water without sweetener  
174 (Table 3). In contrast, group 2 consumed more water with Svetia, without differences  
175 between water consumption with Sucrose, Sucralose and Control group, as shown in table  
176 3. When comparing the groups, it can be seen that group 1 consumed more water with  
177 sweetener than group 2, particularly in the sucrose subgroup ( $p < 0.004$ ), as shown in table 3.

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Comment [S16]: Sucralose or Splenda? Is splenda trade mark?

178

179 **Table 3.** Water consumption with and without of sweetener for 6 weeks of  
180 treatment.

	Control	Sucrose	Splenda	Svetia	
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	<i>p</i> value
	mL	mg/mL	mg/mL	mg/mL	
Water consumption with and without of sweetener					
<b>Initial</b>					
Group 1	47.68 $\pm$ 0.972	<b>101<math>\pm</math>1.32*</b>	<b>31.83<math>\pm</math>0.987*</b>	43.29 $\pm$ 0.896	0.001**

Comment [S17]: Stevia



Group 2	61.65±0.481	<b>65.95±0.481*</b>	62.95±1.87	60.1±1.17	0.001**
<b>Final</b>					
Group 1	43.29±1.0	<b>166.31±1.16*</b>	48.37±1.36	47.15±1.88	0.001**
Group 2	69.1±0.320	69.1±0.962	69.1±0.320	<b>72.3±0.641*</b>	0.001**

One-way ANOVA\*\* was performed to determine the differences between the subgroups, it was considered significant with  $p < 0.001$ . A Bonferroni *post hoc* test\* was performed to observe intra-group differences.

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184

### 3.4 Food consumption

The subgroups of sucrose and sucralose consumed less food ( $p < 0.001$ ), compared to the Control and **Svetia** subgroups. At the end of the 6 weeks of supplementation, the mice of group 1, subgroup of sucrose, further reduced their feed intake ( $p < 0.001$ ). In group 2, at the beginning they consumed less amount of food in the Sucrose subgroup, although the **Svetia** subgroup increased their food consumption. At the end of the treatment, the Splenda subgroup consumed more food ( $p < 0.001$ ). When comparing group 1 with group 2, it can be seen that there are no differences ( $p < 0.60$ ) between the groups regarding the amount of consumption, the differences observed are between the subgroups.

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**Table 4.** Consumption of food for 6 weeks of supplementation with sweetener.

	Control Mean ±SD (g)	Sucrose Mean ±SD (g)	Splenda Mean ±SD (g)	<b>Svetia</b> Mean ±SD (g)	<i>p</i> value
<b>Food consumption</b>					
<b>Initial</b>					
Group 1	32.08±0.02	<b>24.08±0.011*</b>	<b>25.68±0.03*</b>	29.92±0.034	0.001**
Group 2	27.1±0.32	<b>25.6±0.641*</b>	26.52±0.293	<b>29.7±0.641*</b>	0.001**
<b>Final</b>					
Group 1	32.9±0.755	<b>16.07±0.939*</b>	31.12±0.649	32.73±1.5	0.001**
Group 2	29.7±0.641	28±0.641	<b>30±2.77*</b>	<b>27.7±0.320*</b>	0.006**

Comment [S20]: Stevia

198

199 One-way ANOVA\*\* of one factor was performed to determine the differences between the subgroups,  
200 it was considered significant with  $p < 0.05$ . A Bonferroni *post hoc* test\* was performed to observe intra-  
201 group differences.  
202

203

### 204 3.5. Percentage of lymphocytes of Peyer's patches

205 In group 1, the percentage of lymphocytes increased in the Sucrose subgroup, but  
206 decreased in the Splenda and Svetia subgroups, although the differences are not significant  
207 ( $p < 0.077$ ). In group 2, a significant decrease can be seen in the subgroups that consumed  
208 sweeteners ( $p < 0.028$ ), particularly in the Sucrose subgroup ( $p < 0.022$ ), compared with the  
209 control subgroup. When comparing groups 1 and 2, differences in lymphocyte percentages  
210 can be appreciated, as well as the different behavior between strains.

211

212 **Table 5.** Percentage of Peyer patches lymphocytes in mice supplemented with  
213 sweeteners during 6 weeks.

	Control	Sucrose	Splenda	Svetia	
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	$\rho$ Value
	%	%	%	%	
Lymphocytes					
Group 1	28.66 $\pm$ 3.9	30 $\pm$ 4.8	26.1 $\pm$ 4.1	26.48 $\pm$ 4.3	0.238
Group 2	74.37 $\pm$ 4.3	<b>30.62<math>\pm</math>1.5*</b>	43.87 $\pm$ 2.2	49.12 $\pm$ 2.0	0.028**

214 ANOVA\*\* of one factor was performed to determine the differences between the subgroups, it was  
215 considered significant with  $p < 0.05$ . A Bonferroni *post hoc* test \* was performed to observe intra-group  
216 differences.  
217

218

## 219 4. Discussion

### 220 4.1. The preference for food and water intake, as well as changes in body 221 weight vary in each strain of mice.

222 In recent years the consumption of products containing both natural and artificial sweeteners  
223 have acquired great demand for its low energy intake and its sweetness, which can be found  
224 in multiple products. In this study, mice of group 1 and 2 gained weight with Sucrose  
225 consumption, compared with the subgroups of Splenda and Svetia. In group 2, the Svetia

Comment [S21]: Stevia

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226 subgroup had lower weight gain compared to the Sucrose and Splenda subgroups. Group 2  
227 had greater weight gain, this may be due to the characteristics of the strain. In addition, mice  
228 of group 1 had a greater predilection for the consumption of sweeteners, particularly of  
229 Sucrose, and lower for Splenda. Group 2 had a greater predilection for the consumption of  
230 water with Svetia. This behavior is derived from the absence or low energy content of  
231 Sucralose and Stevia respectively [46, 47], therefore, there was no increase in weight in  
232 these groups, compared with the group of Sucrose. It is a fact that drinks with high Sucrose  
233 content promote weight gain [48], and is associated with other metabolic disorders that  
234 cause states of inflammation and some types of cancer, such as colon cancer [49]. This  
235 effect may be due to the fact that carbohydrates interact with receptors of the small intestine  
236 that cause secretion of satiety peptides such as the glucagon-like peptide 1 (GLP-1) [50], in  
237 addition to gastric distension caused by high water intake with sucrose.

238  
239 The preference for water with sucrose in rodents is documented [51, 52], and it has been  
240 linked to the discovery of sweet taste receptors T1R3 or gusducin in the intestine [53]. In  
241 contrast, in the study conducted by Bello and Hajnal in 2005 with rats, they showed that rats  
242 do not like drinks with Sucralose, since the consumption of water without sucralose was  
243 similar to the consumption of water with Sucralose [54]. The preference of rodents to  
244 sweeteners like Stevia was also studied and it was observed that it has better acceptance  
245 compared to other non-caloric sweeteners such as saccharin [55]. This shows that there is  
246 variation in the preference between different non-caloric sweeteners and even between  
247 species such as mice and rats. Preference also varies between genera; females have a  
248 better response to sweetness than males [56].

249  
250 In groups 1 and 2, Sucrose subgroups consumed less food, but in group 2, Splenda and  
251 Svetia increased food consumption. This situation can be attributed to the energy  
252 contribution of each sweetener, sucrose provides greater energy content, which causes a

253 satiety sensation in rodents and inhibits appetite. Groups of non-nutritive sweeteners, which  
254 contribute little or very few calories, could cause an increase in appetite [48].

255

256 **4.2 Blood glucose did not change in group 1, but its concentration was lower**  
257 **than in group 2.**

258 In group 2, sucralose showed a lower concentration compared to the other subgroups. In the  
259 Chang *et.al.* study, in 2010, they evaluated the proximal small bowel exposure to sucralose,  
260 applied an intraduodenal glucose infusion in ten healthy subjects, took blood samples at  
261 frequent intervals and determined that Sucralose does not modify the glycemic response  
262 rate [57]. In addition to Sucralose other artificial sweeteners report a glycemic index similar  
263 to Sucrose [58]. In another study conducted by Wang *et.al.* in 2011, they investigated the  
264 effect of steviol on insulin resistance and the pro-inflammatory status of adipose tissue in  
265 mice fed a high-fat diet; oral administration had no effect on body weight, basal insulin  
266 levels, glucose tolerance, and insulin sensitivity improved and decreased secretion of  
267 inflammatory cytokines in adipose tissue [59], concluded that the use of Stevia is beneficial  
268 and helps control blood glucose levels.

269

270 A study designed to evaluate the effects of stevia on blood glucose concentration and blood  
271 pressure (BP) with active treatment of steviol glucoside or placebo for 3 months. There were  
272 no changes in systolic/diastolic blood pressure, glucose concentration and glycosylated  
273 hemoglobin (HbA1c), therefore, oral stevia is well tolerated and has no pharmacological  
274 effect [19].

275

276 **4.3. In group 1, the lymphocytes of the sucrose group were increased, but**  
277 **decreased in the subgroups of sucralose and stevia. In contrast, in group 2,**  
278 **lymphocytes decrease in the sucrose subgroup.**

279 Studies on the effect of sweeteners on the immune system of the small intestine and  
280 particularly Peyer's patches are still scarce. In the study by Sehar *et.al.*, in 2008, they report  
281 that Stevia can stimulate the proliferation of T and B cells, increasing humoral and cellular  
282 immunity [41], in lymphocytes from the spleen, in Balb/c mice of both sexes, evaluated  
283 viability by stimulating lymphocytes *in vitro* directly with stevioside and did not decrease  
284 viability. This study was carried out on lymphocytes purified from Peyer's patches, as a site  
285 of first contact with the ingested and absorption sweeteners. In addition, the response  
286 between strains was different, in Balb/c mice (group 1) sucrose increased the percentage of  
287 lymphocytes from Peyer's patches, and in group CD1 (group 2), sucrose reduced this  
288 percentage. Another possible explanation for the decrease is found in the type of study and  
289 sweetener used. In *in vitro* studies where the product used not for commercial use  
290 (Esvetia/Truvia) if not reactive grade, stevia was administered at different doses, some  
291 superior to those used in this work, without differences in the results [60]. These results  
292 could be extrapolated to the human being since the metabolism of Stevia is similar between  
293 rodents and humans. On the other hand, the consumption of sucrose has been related to a  
294 decrease in the phagocytic index in neutrophils [37], which means that the consumption of  
295 sucrose can alter the function of the cells and particularly in the Peyer's patches as the first  
296 contact site of the sweetener. The effect of Sucralose on the immune response of  
297 inflammatory bowel diseases has been observed [61], in chronic inflammatory processes as  
298 a consequence of an increase in intestinal permeability [62] which causes immunological  
299 reactions against diet antigens and components of the intestinal microbiota [63]. In the study  
300 carried out by Abou-Donia *et.al.*, in rats indicated that Splenda has adverse effects such as  
301 reduced microbiota, increased fecal pH, and over-expression of proteins that limit the  
302 bioavailability of drugs [64]. The cause of the inhibition of the bacteria of the intestine is  
303 related to the deterioration of the digestive proteases caused by the consumption of  
304 Sucralose [65] that increases the intestinal permeability that causes inflammation of the  
305 mucous membranes and that leads to the excessive activation of the lymphocytes, which

306 contributes to the pathogenesis of the Intestinal Inflammatory Disease and the Crohn's  
307 disease [66, 67].

308

#### 309 **4. CONCLUSION**

310 It is a fact that sweeteners modify in a greater or lesser proportion the lymphocyte population  
311 of Peyer's patches and this variation depends directly on the dose, the frequency, the strain  
312 of the rodents and the type of sweetener. In group 1, the **Svetia** subgroup had little weight  
313 gain compared to the subgroups of sucrose and sucralose. In contrast, Group 2 had a  
314 greater weight gain, perhaps due to the characteristics of the strain. In addition, mice of  
315 group 1 showed a greater predilection for the consumption of sweeteners, particularly of  
316 sucrose, and low for sucralose, but with a lower weight compared to group 2. Finally, in  
317 group 1, the lymphocytes of the sucrose subgroup increased, with decreased in the  
318 subgroups of sucralose and stevia. In contrast, in group 2, the lymphocytes decreased in the  
319 sucrose subgroup.

320

Comment [S25]: Stevia

321 **COMPETING INTERESTS**

322 Authors have declared that no competing interests exist.

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325 **ETHICAL APPROVAL**

326 All authors hereby declare that "Principles of laboratory animal care" (NOM-062-ZOO-1999)  
327 were followed, as well as specific national laws where applicable. All experiments have been  
328 examined and approved by the appropriate ethics committee.

329

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