Probiotics, prebiotics and symbiotics attenuate chronic effects of passive smoking on physiological and biochemical parameters in rats: A randomized and controlled study

Probióticos, prebióticos e simbióticos atenuam os efeitos crônicos do tabagismo passivo sobre os parâmetros fisiológicos e bioquímicos em ratos: Um estudo randomizado e controlado

Los probióticos, prebióticos y simbióticos atenúan los efectos crónicos del tabaquismo pasivo sobre los parámetros fisiológicos y bioquímicos en ratas: Un estudio controlado aleatorizado

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Abstract
Passive and chronic exposure to tobacco smoke is a major cause of preventable diseases in humans and animals. The aim of this study was to evaluate the physiological and biochemical effects of functional foods: probiotics, prebiotics, and symbiotics in young rats exposed chronically to cigarette smoke. Ninety-six young male rats were randomly assigned to eight groups (n = 12): control (C); probiotic (Pro); prebiotic (Pre); symbiotic (Sym); smoking control (CS); smoking probiotic (ProbS); smoking prebiotic (PrebS); and smoking symbiotic (SymbS). After the experimental period of 189 days, the animals were anesthetized, blood samples were collected, and the animals were euthanized by exsanguination. The data were submitted to the Shapiro Wilk test, followed by the one-way ANOVA test, with contrasts by the Tukey method (P<0.05). The results demonstrated that chronic passive exposure to cigarette smoke had deleterious effects on the animals, and that these effects were attenuated in groups of animals supplemented with functional foods. In the supplemented groups there were significant improvements in weight gain, and mean values of liver enzymes (ALT, AST, GGT, and ALP), serum proteins (albumin and globulins), blood glucose, uremia, and creatininemia. We conclude that functional foods, probiotics, prebiotics, and symbiotics, attenuate the deleterious effects on the physiological and biochemical parameters of rats chronically exposed to cigarette smoke.
Keywords: Cigarette smoke; Functional foods; Liver; Kidney; Toxicology.

Resumo
A exposição passiva e crónica à fumaça do tabaco é uma das principais causas de doenças evitáveis em humanos e animais. O objetivo deste estudo foi avaliar os efeitos fisiológicos e bioquímicos de alimentos funcionais: probióticos, prebióticos e simbióticos em ratos jovens expostos cronicamente à fumaça do tabaco. Noventa e seis ratos machos jovens foram distribuídos aleatoriamente em oito grupos (n = 12): controle (C); probiótico (Pro); prebiótico (Pré); simbiótico (Sym); controle do tabagismo (CS); probiótico de fumar (ProbS); fumar prebiótico (PrebS); e fumar simbiótico (SymbS). Após o período experimental de 189 dias, os animais foram anestesiados, amostras de sangue foram coletadas e os animais foram eutanasiados por exsanguinação. Os dados foram submetidos ao teste de Shapiro-Wilk, seguido do teste ANOVA one-way, com contrastes pelo método de Tukey (P <0,05). Os resultados demonstraram que a exposição passiva crónica à fumaça do tabaco teve efeitos deletérios nos animais, e que esses efeitos foram atenuados em grupos de animais suplementados com alimentos funcionais. Nos grupos suplementados, houve melhorias significativas no ganho de peso e nos valores médios das enzimas hepáticas (ALT, AST, GGT e ALP), proteínas séricas (albumina e globulinas), glicemia, uremia e creatininemia. Concluímos que alimentos funcionais, probióticos, prebióticos e simbióticos atenuam os efeitos deletérios sobre os parâmetros fisiológicos e bioquímicos de ratos expostos cronicamente à fumaça do tabaco.

Palavras-chave: Fumaça de cigarro; Alimentos funcionais; Fígado; Rim; Toxicologia.

Resumen
La exposición pasiva y crónica al humo del tabaco es una de las principales causas de enfermedades prevenibles en humanos y animales. El objetivo de este estudio fue evaluar los efectos fisiológicos y bioquímicos de alimentos funcionales: probióticos, prebióticos y simbióticos en ratas jóvenes cronicamente expuestas al humo del tabaco. Se asignaron aleatoriamente noventa y seis ratas macho jóvenes a ocho grupos (n = 12): control (C); probiótico (Pro); prebiótico (Pre); simbiótico (Sym); control del tabaco (CS); probiótico para fumar (ProbS); tabaquismo prebiótico (PrebS); y tabaquismo simbiótico (SymbS). Después del período experimental de 189 días, los animales fueron anestesiados, se recolectaron muestras de sangre y los animales fueron sacrificados por exanguinación. Los datos fueron sometidos a la prueba de Shapiro Wilk, seguida de la prueba ANOVA de una vía, con contrastes por el método de Tukey (P <0,05). Los resultados demostraron que la exposición crónica pasiva al humo del tabaco tenía efectos deletéreos en los animales, y que estos efectos se atenuaron en grupos de animales suplementados con alimentos funcionales. En los grupos suplementados, hubo mejoras significativas en el aumento de peso y en los valores medios de enzimas hepáticas (ALT, AST, GGT y ALP), proteínas séricas (álbumina y globulinas), glucosa en sangre, uremia y creatininemia. Concluimos que los alimentos funcionales, probióticos, prebióticos y simbióticos atenuan los efectos deletéreos sobre los parámetros fisiológicos y bioquímicos de ratas expuestas cronicamente al humo del tabaco.

Palabras clave: Humo de cigarro; Alimentos funcionales; Hígado; Riñón; Toxicología.

1. Introduction

Passive smoking is considered to be the inhalation of smoke from tobacco derivatives by non-smokers (Yousuf et al., 2020). In European countries, restrictions on smoking in public places have significantly reduced the occurrence of passive smoking, however, these prohibitions have failed to reduce the situation in the homes of current smokers (Olivieri et al., 2019) where the population most exposed to tobacco smoke are low-income individuals and young people (Kaleta et al., 2016; Nazar et al., 2014).

Scientific evidence has unequivocally established that exposure to tobacco smoke is a major cause of preventable diseases (Feldman & Anderson, 2013), and these can lead to disabilities and death (Olivieri et al., 2019). This exposure, when it occurs chronically, impairs gas exchange and causes a decrease in the supply of oxygen at the intestinal and systemic levels, leading to the growth of new blood vessels (angiogenesis), dysfunction of the gastrointestinal tract (GIT) epithelial barrier, nephrotoxicity, and hepatotoxicity (Fricker et al., 2018; Okamoto et al., 2018). The deleterious effects of exposure to cigarette smoke also cause alterations in physiological and nutritional patterns. These parameters include weight gain and/or loss (Harris et al., 2016; Miri-Moghadam et al., 2014). Some studies has also demonstrated alterations in biochemical profiles (Golli et al., 2016; Rupprecht et al., 2016) as total cholesterol, HDL, LDL, triglycerides (Malenica et al., 2017), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) (Alsalhen & Abdalsalam, 2014), total proteins (Phillips et al., 2017), glucose (López Zubizarreta et al., 2017), urea, and creatinine (Desai et al., 2016).
In murines and humans, due to the large number of numerous harmful agents in its composition, chronic exposure to cigarette smoke has been shown to cause oxidative stress. This effect has been mitigated by supplementation of functional foods (probiotics, prebiotics, and symbiotics) because of the anti-inflammatory, immunomodulator, and antioxidant roles of these compounds (Bezerra et al., 2015; George Kerry et al., 2018; Gibson et al., 2017; Hamed et al., 2018; Hill et al., 2014; Younan et al., 2018). From this perspective, it is suggested that supplementation in the diet with functional foods, in suitable quantities and periodicity can have beneficial systemic effects, however, there is little evidence regarding physiological and biochemical parameters, particularly using the pool of probiotic microorganisms: Lactobacillus acidophilus, Enterococcus faecium, Bifidobacterium thermophilum, and Bifidobacterium longum; the prebiotic mananoligosaccharide (MOS), and symbiotics resulting from the mixture of both. The current study aimed to evaluate the effects of supplementing the diet of young rats chronically exposed to cigarette smoke with functional foods (probiotics, prebiotics, and symbiotics), in relation to physiological and biochemical parameters.

2. Methodology

2.1 Animals and Care

The study was conducted in accordance with the ethical principles of the Universal Declaration of Animal Rights of the United Nations Educational, Scientific and Cultural Organization (UNESCO) and the study protocol was approved by the Ethics Committee on the Use of Animals, under Protocol 3575, from the University of Oeste Paulista, UNOESTE, Brazil.

Ninety-six young male Wistar rats (Rattus norvegicus albinus), 21 days of age, mean initial body mass of 43.1 ± 3.2 g, were housed in individual cages, with cycles of 12 hours of light/12 hours of dark at 23 ± 2°C, humidity 57 ± 9%, and mean air changes of 15 changes/hour. The diets and drinking water from the tap were provided at will, except during periods of exposure.

Allocation concealment, management strategy, and treatment of the groups of animals were performed to reduce bias in the study (Ma et al., 2017). The experiment lasted 189 days, with 5 days of adaptation and 184 days of experimental management strategy, exposure to cigarette smoke, and feeding with the control and experimental diets (Figure 1).

Figure 1 – Graphical representation of the experiment timeline.

Source: Authors.

2.2 Experimental Design and Diets

The animals were distributed, using a table of random number sequences generated by the program R (R
Development Core Team, 2013), into eight experimental groups (n=12). Half of the experimental groups were submitted to a protocol of exposure to cigarette smoke and fed with diets: control; probiotic; prebiotic; and symbiotic (respectively SC, ProbS, PrebS, and SymbS groups). The groups not submitted to the cigarette smoke exposure protocol and fed the control and experimental diets comprised groups C, Prob, Preb, and Symb. Control and experimental diets were formulated to meet the nutritional needs of the rats (National Research Council, 1995) and are shown in Table 1. The physiological parameters (initial body weight, final body weight, and weight gain) were measured weekly.

<table>
<thead>
<tr>
<th>Ingredients (g)</th>
<th>Standard</th>
<th>Prob</th>
<th>Preb</th>
<th>Symb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn bran</td>
<td>82.95</td>
<td>82.95</td>
<td>82.95</td>
<td>82.95</td>
</tr>
<tr>
<td>Soy oil</td>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.00</td>
<td>3.00</td>
<td>4.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamin mix*</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Mineral mix**</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
</tr>
<tr>
<td>Probiotic***</td>
<td>0.00</td>
<td>2.00</td>
<td>0.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Prebiotic****</td>
<td>0.00</td>
<td>0.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

* Vitamin mix/Kg: Nicotinic acid 30 mg; Pantothenate 15 mg; Pyridoxine 6 mg; Thiamine 5 mg; Riboflavin 6 mg; Folic acid 2 mg; Biotin 0.2 mg; Vitamin B12 25 mg; Vitamin E 75 IU; Vitamin A 4000 IU; Vitamin D3 1000 IU; Vitamin K 900 mg; Choline 1000 mg. ** Mineral mix mg/Kg: Calcium 5000; Phosphorus 1561; Potassium 3600; Sulfur 300; Sodium 1019; Choline 1574; Magnesium 507; Iron 35; Zinc 30; Manganese 10; Copper 6; Molybdenum 0.15; Selenium 0.15. *** Lactobacillus acidophilus, Enterococcus faecium, Bifidobacterium thermophilum, and Bifidobacterium longum (2–5 10^9 CFU each). **** Mannanoligosaccharide (MOS), composed of the active fractions α-1,3 and α-1,6, with 30% α-mannans and derived from yeast strain Saccharomyces cerevisiae. Source: Authors.

2.3 Cigarettes and smoke generation

During the adaptation and experimental periods, exposure to cigarette smoke, commercial brand cigarettes (Tabacalera del Este S.A., Hernandarias, Paraguay), was used. The mean concentrations of tar, nicotine, and CO of 10.2 ± 0.1 mg/cigarette, 0.8 ± 0.0 mg/cigarette, and 10.1 ± 0.1 mg/cigarette were determined according to Renne et al., (2006). The conditioning to exposure and smoke generation were performed as described by Tsuji et al. (2013).

2.4 Characterization of the atmosphere of smoke exposure

The concentrations of wet total particulate matter (WTPM) and carbon monoxide (CO) were monitored via a real-time aerosol monitor (RAM; Microdust, Pro; Casella, Amherst, NH, USA) and CO monitor (TxiPro® - BioSystems Diagnostics Pvt. Ltd., USA). The coefficient of variation (% CV) of the exposure concentration (WTPM) was in the range of ± 10% by gravimetric analysis using the Cambridge glass fiber filter 47 mm (Performance Systematix Inc., Grand Rapids, MI, USA). The actual mean exposure concentrations were calculated from the mass collected in the filters and the total volume of air extracted by the filters (TSUJI et al., 2013). The temperature and humidity of the exposure atmosphere were measured daily.
using a humidity/temperature detector (Hygrotherm, Qualitäts-Erzeugnis, TFA, Germany).

2.5 Passive smoking exposure protocol

2.5.1 Adaptation and experimental period

For five days, the adaptation period, the animals of the CS, ProbS, PrebS, and SymbS groups were exposed for 10 minutes a day to cigarette smoke in a chamber, with a controlled temperature of 22ºC and the animals of the C, Prob, Preb, and Symb groups in received forced air ventilation in the same period. In the experimental period, 184 days, the animals of the CS, ProbS, PrebS, and SymbS groups were exposed to cigarette smoke for 60 minutes daily, 30 minutes in the morning (7:00 am) and 30 minutes in the evening (19:00h), five days a week. The mean concentration of cigarette smoke was adjusted to contain 350 parts per million (ppm) of carbon monoxide (CO) during the exposure (TxiPro® - BioSystems Diagnostics Pvt. Ltd., USA) (Kozma et al., 2014) and groups C, Prob, Preb, and Symb received forced air ventilation in the same period.

2.6 Euthanasia of the animals and collection of samples

At the end of the experimental period (rats 210 days old), all animals were weighed and anesthetized with a dose of 30 mg.Kg-1 live weight of Tiopental (Thiopentax®, Cristália - Produtos Químicos Farmacêuticos Ltda. - Brazil) intraperitoneally. After anesthesia, blood samples were taken by cardiac puncture and the rats were euthanized by exsanguination (Paiva et al., 2005).

2.7 Biochemical Analysis

After processing the blood samples, the serum and plasma were separated to carry out the evaluation of the enzymatic activity [aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT)] and serum levels of total proteins, albumin, globulin, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, urea, creatinine, and glucose. All dosages were performed using the BioPlus model Bio200F automatic analyzer (Bioplus Produtos para Laboratórios Ltda., Brazil) with LabTest brand reagents (Labtest Diagnóstica S.A., Brazil) (Spinelli et al., 2014).

2.8 Statistical Analysis

Physiological and biochemical parameters are presented as mean ± standard deviation and were subjected to the analysis of normality by the Shapiro Wilk test, then the one-way ANOVA test was used, with contrasts by the Tukey method. All analyses were performed using R program (R DEVELOPMENT CORE TEAM, 2016). The level of significance adopted was 5%.

3. Results

3.1 Physiological parameters

The atmosphere of exposure to cigarette smoke was well controlled in the study. There was no removal due to premature death or a condition that demonstrated suffering after chronic exposure to cigarette smoke or air ventilation. Immediately after exposure, animals in the CS group exhibited decreased locomotor activity, ataxic gait, irregular breathing, behavior indicative of nausea, and nasal noise. These signs were repeatedly observed during the experimental period, and the nasal noise remained until the next exposure period. The groups supplemented with functional foods and exposed to cigarette smoke, ProbS, PrebS, and SymbS, showed slight irregular breathing, decreased locomotor activity, ataxic gait, behavior
indicative of nausea, and nasal noise.

The mean initial weight of the rats did not differ significantly between the groups analyzed (P>0.05). At the end of the experiment, the mean body weight of the animals was reduced in groups exposed to cigarette smoke, when compared to those not exposed. In the groups exposed to cigarette smoke and supplemented with functional foods, this reduction was significantly lower (P<0.05) than the CS group. The mean weight of the Prob and Preb groups was lower (P<0.05) than the C and Symb groups (Figure 2).

**Figure 2** – Graph of the effect of different diets on the nutritional parameters of rats in the growth phase exposed or not chronically to cigarette smoke.

The columns and bars represent the mean ± standard deviation. Different letters indicate a significant difference in the means of the groups in the one-way ANOVA test, with contrasts by the Tukey method (P>0.05). (C): basal diet; (Prob): basal diet supplemented with 20 g Kg-1 of an association of probiotic microorganisms [Lactobacillus acidophilus, Enterococcus faecium, Bifidobacterium thermophilum, and Bifidobacterium longum (2–5 109 CFU each)]; (Preb): basal diet supplemented with 10 g Kg-1 of prebiotic [mannanoligosaccharide (MOS), composed of the active fractions α-1,3 and α-1,6, presenting 30% of α-mannans and derived from yeast strain Saccharomyces cerevisiae]; (Symb): basal diet supplemented with 20 g Kg-1 of an association of probiotic microorganisms and 10 g Kg-1 of prebiotic; (CS): basal diet + protocol for exposure to cigarette smoke; (ProbS): basal diet supplemented with 20 g Kg-1 of an association of probiotic microorganisms + protocol for exposure to cigarette smoke; (PrebS): basal diet supplemented with 10 g Kg-1 of prebiotic + protocol for exposure to cigarette smoke; and (SymbS): basal diet supplemented with 20 g Kg-1 of an association of probiotic microorganisms and 10 g Kg-1 of prebiotic + protocol for exposure to cigarette smoke. Source: Authors.

**3.2 Liver profile**

Serum concentrations of ALT, AST, ALP, and GGT enzymes increased significantly (P<0.05) in the CS group, when compared to the other groups. In the ProbS, PrebS, and SymbS groups, the levels of these enzymes were significantly lower than in the CS group, and higher than in the C, Prob, Preb, and Symb groups, which did not differ from each other (P>0.05) (Table 2).
Liver profile and serum protein levels of Wistar rats, in the growth phase, fed diets supplemented with functional foods: probiotic, prebiotic, and symbiotic and exposed or not chronically to cigarette smoke.

### Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>Prob</th>
<th>Preb</th>
<th>Symb</th>
<th>SC</th>
<th>ProbS</th>
<th>PrebS</th>
<th>SymbS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU L⁻¹)</td>
<td>41.40 ± 4.31³</td>
<td>40.62 ± 3.54³</td>
<td>40.50 ± 6.10³</td>
<td>39.79 ± 2.07³</td>
<td>65.70 ± 6.92³</td>
<td>53.48 ± 4.20³</td>
<td>54.26 ± 5.03³</td>
<td>56.42 ± 4.62³</td>
</tr>
<tr>
<td>ALP (IU L⁻¹)</td>
<td>89.31 ± 3.56³</td>
<td>88.38 ± 4.74³</td>
<td>87.29 ± 6.87³</td>
<td>87.71 ± 5.46³</td>
<td>105.75 ± 5.75³</td>
<td>94.30 ± 3.74³</td>
<td>95.58 ± 7.36³</td>
<td>93.30 ± 7.52³</td>
</tr>
<tr>
<td>GGT (IU L⁻¹)</td>
<td>7.02 ± 0.28³</td>
<td>6.99 ± 0.26³</td>
<td>6.91 ± 0.19³</td>
<td>6.93 ± 0.27³</td>
<td>8.01 ± 0.19³</td>
<td>7.43 ± 0.15³</td>
<td>7.33 ± 0.27³</td>
<td>7.49 ± 0.34³</td>
</tr>
<tr>
<td>Total proteins</td>
<td>6.73 ± 0.26³</td>
<td>6.89 ± 0.26³</td>
<td>6.74 ± 0.19³</td>
<td>6.67 ± 0.27³</td>
<td>4.42 ± 0.17³</td>
<td>5.94 ± 0.26³</td>
<td>5.83 ± 0.15³</td>
<td>5.91 ± 0.16³</td>
</tr>
<tr>
<td>Albumin (g dL⁻¹)</td>
<td>3.71 ± 0.10³</td>
<td>4.08 ± 0.12³</td>
<td>3.96 ± 0.11³</td>
<td>3.94 ± 0.22³</td>
<td>2.49 ± 0.15³</td>
<td>3.41 ± 0.17³</td>
<td>3.37 ± 0.17³</td>
<td>3.46 ± 0.16³</td>
</tr>
<tr>
<td>Globulins (g dL⁻¹)</td>
<td>3.02 ± 0.19³</td>
<td>2.81 ± 0.19³</td>
<td>2.78 ± 0.12³</td>
<td>2.74 ± 0.19³</td>
<td>1.93 ± 0.16³</td>
<td>2.53 ± 0.18³</td>
<td>2.46 ± 0.14³</td>
<td>2.45 ± 0.18³</td>
</tr>
</tbody>
</table>

Mean values ± SD (n = 12). Different letters in the lines indicate a significant difference between the means of the groups, using the Tukey test (P< 0.05). Groups: (C): basal diet; (Prob): basal diet supplemented with 20 g Kg⁻¹ of an association of probiotic microorganisms [Lactobacillus acidophilus, Enterococcus faecium, Bifidobacterium thermophilum, and Bifidobacterium longum (25 10⁹ CFU each)]; (Preb): basal diet supplemented with 10 g Kg⁻¹ of prebiotic [mananoligosaccharide (MO₅), α,1,3 and α,1,6, presenting 30% of α-mannans and derived from yeast strain Saccharomyces cerevisiae]; (Symb): basal diet supplemented with 20 g Kg⁻¹ of an association of probiotic microorganisms and 10 g Kg⁻¹ of prebiotic; (SC): basal diet + protocol for exposure to cigarette smoke; (ProbS): basal diet supplemented with 10 g Kg⁻¹ of prebiotic + protocol for exposure to cigarette smoke; (PrebS): basal diet supplemented with 10 g Kg⁻¹ of prebiotic + protocol for exposure to cigarette smoke; and (SymbS): basal diet supplemented with 20 g Kg⁻¹ of an association of probiotic microorganisms and 10 g Kg⁻¹ of prebiotic + protocol for exposure to cigarette smoke.

### 3.3 Serum proteins

The mean values of serum protein concentrations (total proteins, albumin, and globulin) were lower in the CS group compared to the C group (P<0.05). The results revealed that in the groups exposed to cigarette smoke and supplemented with functional foods (ProbS, PrebS, and SymbS) the mean levels of these proteins were significantly higher than the CS group (P<0.05). Groups C, Prob, Preb, and Symb did not differ from each other (P>0.05) (Table 2).

### 3.4 Lipid profile

The cholesterol level in the CS group was significantly higher (P<0.05) than the other groups. The ProbS, PrebS, SymbS, C, and Prob groups did not differ from each other (P>0.05). The Prob, Preb, and Symb groups did not differ from each other (Table 2).

The mean level of HDL cholesterol was lower in the CS group and did not differ only from the ProbS group (P>0.05). Groups C, Prob, Preb, Symb, ProbS, PrebS, and SymbS did not differ among them (P>0.05).

The levels of LDL cholesterol and triglycerides in the CS group were higher when compared to group C (P<0.05). There were no significant differences in LDL between the smoking and supplemented groups, and the CS did not demonstrate a significant difference from the ProbS group, but did from the PrebS and SymbS. However, it is possible to state that the biochemical measurements showed a significant improvement in the lipid profile in the groups supplemented with functional foods, exposed or not to cigarette smoke, when compared to the control groups.
Table 3 – Lipid, renal, and serum glucose levels of Wistar rats, in the growth phase, fed diets supplemented with functional foods: probiotic, prebiotic, and symbiotic and exposed or not chronically to cigarette smoke.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups/treatments</th>
<th>C</th>
<th>Prob</th>
<th>Preb</th>
<th>Symb</th>
<th>SC</th>
<th>ProbS</th>
<th>PrebS</th>
<th>SymbS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg dL⁻¹)</td>
<td></td>
<td>81.89 ± 5.32B</td>
<td>79.21 ± 4.37AB</td>
<td>74.07 ± 4.46A</td>
<td>72.86 ± 6.01A</td>
<td>95.56 ± 5.44C</td>
<td>83.64 ± 3.74B</td>
<td>81.64 ± 3.64B</td>
<td>78.65 ± 6.43A</td>
</tr>
<tr>
<td>HDL (mg dL⁻¹)</td>
<td></td>
<td>50.42 ± 5.66B</td>
<td>55.82 ± 8.05B</td>
<td>57.18 ± 8.69B</td>
<td>54.57 ± 5.47B</td>
<td>37.03 ± 4.39A</td>
<td>45.14 ± 6.19AB</td>
<td>46.74 ± 6.67B</td>
<td>47.05 ± 9.95B</td>
</tr>
<tr>
<td>LDL (mg dL⁻¹)</td>
<td></td>
<td>27.98 5.44AB</td>
<td>25.01 ± 5.84A</td>
<td>24.67 ± 8.35A</td>
<td>25.18 ± 8.18A</td>
<td>41.58 ± 5.99C</td>
<td>33.48 ± 7.29ABC</td>
<td>35.57 ± 7.69BC</td>
<td>34.36 ± 7.82BC</td>
</tr>
<tr>
<td>Triglycerides (mg dL⁻¹)</td>
<td></td>
<td>58.64 ± 7.08AB</td>
<td>51.50 ± 6.28A</td>
<td>54.98 ± 4.46A</td>
<td>55.37 ± 5.12A</td>
<td>73.33 ± 4.19p</td>
<td>66.14 ± 5.90CD</td>
<td>63.97 ± 5.68BC</td>
<td>64.52 ± 6.17BC</td>
</tr>
<tr>
<td>Urea (mg dL⁻¹)</td>
<td></td>
<td>30.55 ± 4.01A</td>
<td>28.75 ± 3.24A</td>
<td>28.26 ± 3.99A</td>
<td>27.14 ± 2.89A</td>
<td>38.38 ± 5.44B</td>
<td>30.05 ± 3.05A</td>
<td>30.24 ± 3.52A</td>
<td>31.27 ± 4.78A</td>
</tr>
<tr>
<td>Creatinine (mg dL⁻¹)</td>
<td></td>
<td>0.75 ± 0.07AB</td>
<td>0.71 ± 0.09A</td>
<td>0.71 ± 0.10A</td>
<td>0.68 ± 0.05A</td>
<td>1.10 ± 0.08C</td>
<td>0.83 ± 0.09B</td>
<td>0.84 ± 0.10B</td>
<td>0.85 ± 0.10B</td>
</tr>
<tr>
<td>Glucose (mg dL⁻¹)</td>
<td></td>
<td>108.26 ± 5.08AB</td>
<td>102.39 ± 5.72A</td>
<td>98.61 ± 8.65AB</td>
<td>100.79 ± 7.52A</td>
<td>113.22 ± 5.72C</td>
<td>104.80 ± 8.42BC</td>
<td>106.91 ± 8.54BC</td>
<td>104.08 ± 11.53BC</td>
</tr>
</tbody>
</table>

Mean values ± SD (n = 12). Different letters in the lines indicate a significant difference between the means of the groups, using the Tukey test (P > 0.05). Groups: (C): basal diet; (Prob): basal diet supplemented with 20 g Kg⁻¹ of an association of probiotic microorganisms [Lactobacillus acidophilus, Enterococcus faecium, Bifidobacterium thermophilum, and Bifidobacterium longum (2–5 10⁹ CFU each)]; (Preb): basal diet supplemented with 10 g Kg⁻¹ of prebiotic [mananoligosaccharide (MOS), composed of the active fractions α-1,3 and α-1,6, presenting 30% of α-mannans and derived from yeast strain Saccharomyces cerevisiae]; (Symb): basal diet supplemented with 20 g Kg⁻¹ of an association of probiotic microorganisms and 10 g Kg⁻¹ of prebiotic; (CS): basal diet + protocol for exposure to cigarette smoke; (ProbS): basal diet supplemented with 20 g Kg⁻¹ of an association of probiotic microorganisms + protocol for exposure to cigarette smoke; (PrebS): basal diet supplemented with 10 g Kg⁻¹ of prebiotic + protocol for exposure to cigarette smoke; and (SymbS): basal diet supplemented with 20 g Kg⁻¹ of an association of probiotic microorganisms and 10 g Kg⁻¹ of prebiotic + protocol for exposure to cigarette smoke. Source: Authors.
3.5 Renal profile and plasma glucose level

The mean serum urea level was found to be significantly higher in the CS group compared to the other groups, whose means did not differ among them.

The mean serum creatinine level in the CS group was significantly elevated (P>0.05) when compared to the other groups. In the Prob, Preb, Symb, ProbS, PrebS, and SymbS groups, serum creatinine levels did not differ from group C. The Prob, Preb, and Symb groups differed from the ProbS, PrebS, and SymbS groups. The mean serum glucose level in the CS group was significantly higher (P>0.05) compared to group C.

For glucose, the serum levels of the Prob, Preb, and Symb groups did not differ from the C group. The mean glucose levels of the ProbS, PrebS, and SymbS groups differed from the Prob and Symb groups. The mean serum glucose levels in the ProbS, PrebS, and SymbS groups did not differ from the CS group.

4. Discussion

In this study, young rats in the growth phase were used as an experimental model, since in murines it is possible to investigate aspects of the nicotine dependence cycle, which is not easily achieved in humans at the beginning of use. In addition, during the growth phase of murines, the pharmacokinetics of nicotine are substantially affected, as well as the increase in plasma and brain levels of this compound and its metabolites (Casey & Jones, 2010; Craig et al., 2014).

The population of young humans is also more exposed to nicotine through passive smoking, especially in the domestic environment (Nazar et al., 2014; Yousuf et al., 2020), as well as being more vulnerable to the harm caused by inhaling tobacco smoke (Tucker et al., 2019). Some reasons for this include their immunological immaturity, narrow and short airways, and long periods indoors, such as at home (Öberg et al., 2011; Sigaud et al., 2016). In addition, babies and children inhale twice the amount of household dust compared to adults, and thus inhale more cigarette smoke (Thomson et al., 2006). In this population there is greater hand/object/mouth contact, which provides greater absorption of smoke through the digestive system, in addition to being passively inhaled through the respiratory system (Matt et al., 2004). In adolescence, contact with smoking causes longer reaching effects, leading to higher and longer lasting nicotine dependence rates, which have the consequences of physiological and brain alterations and cognitive problems (Ponzoni et al., 2015).

During the experiment, the toxic effects observed immediately after exposure to the cigarette smoke exposure protocol (locomotor, balance, and respiratory alterations, in addition to a significant decrease in body weight and weight gain) must be associated with the action of nicotine and other inhaled toxic compounds (Genchi et al., 2020; Wang et al., 2020), mainly through direct activation of brain nAChRs (Stolerman et al., 1997). This molecule can bind to nicotinic acetylcholine α3β4 receptors and induce the release of several neurotransmitters, such as catecholamines, serotonin, acetylcholine, and γ-aminobutyric acid, activating the pro-opiomelanocortin (POMC) and the transcript regulated by the cocaine-amphetamine (CAR), with consequent suppression of eating and increased metabolic rate (Mineur et al., 2011). In addition, nicotine induces lipolysis by peripheral sympathomimetic stimulation, increasing peripheral energy expenditure and thermogenesis, resulting in an increase in energy expenditure and consequent decreased weight gain (Andersson & Arner, 2001; Verhaegen & Van Gaal, 2017).

In groups of rats supplemented with functional foods, probiotics (Lactobacillus acidophilus, Enterococcus faecium, Bifidobacterium thermophilum, and Bifidobacterium longum), prebiotics (mananoligosaccharide), and symbiotics (association of probiotic and prebiotic) and exposed to cigarette smoke the results revealed a significant increase in weight gain and final weight in relation to the CS group, demonstrating the beneficial effect of these foods when added to the diet, due to
maintenance or beneficial alteration of the host microbiota (Moraes & Colla, 2006; Thomas & Greer, 2010).

Studies demonstrate that the microbiota plays a crucial role in human physiology and is involved in the pathophysiology of chronic diseases inside and outside the intestine, particularly in smokers (Clemente et al., 2012; Maftei, 2019; Shimauchi et al., 2008). Smoking in humans alters the composition of the intestinal microbiota, significantly increasing the levels of Bacteroidetes, with lower proportions of Firmicutes and Proteobacteria compared to individuals who have had active or passive contact with cigarette smoke (S. H. Lee et al., 2018). Harmful alterations in the microbiota are caused by the immunosuppressive nature of cigarette smoke, which increases oxidative stress, alters intestinal joints, and intestinal mucin composition, and leads to changes in acid-base balance (Savin et al., 2018; Vogtmann et al., 2015).

The significant increase in liver enzymes (ALT, AST, ALP, and GGT) in the CS group, reinforces the harmful effects of passive smoking (Adekomi et al., 2011; Avti et al., 2010; Vgbor et al., 2013). ALT and AST are recognized indicators of liver disorders, despite their non-specificity (Salunke et al., 2011; Zuo et al., 2014) and are the first enzymes to demonstrate plasma elevation in situations of hepatocyte injury (Adekomi et al., 2011; Avti et al., 2010). These effects are due to the phytochemical constituents of tobacco, including nicotine (Bo, 2005; Kerner, 2004; Salahshoor et al., 2016), that induce inflammation of hepatocytes, blockage of liver sinusoids (Vgbor et al., 2013), and formation of oxidants, which in turn induce oxidative stress and the oxidation of proteins and thiol groups (Barreiro et al., 2010; Wieczfinska et al., 2018), causing the leakage of ALT and AST from cell content, with consequent plasma elevation (Vgbor et al., 2013).

Passive smoking increases serum ALP levels, produced by bone and kidney, as well as by the liver in humans (Gordon, 1993; Jang et al., 2012) and animals (Vgbor et al., 2013), which reinforces the results observed in this study. Evidence attributes hepatocellular damage to the action of nicotine and its main metabolite, cotinine, which induce liver toxicity and hepatocellular apoptosis through direct, indirect, immunological, and oncogenic effects. The direct toxic effect of nicotine and cotinine is related to increased oxidative stress, lipid peroxidation, hepatocyte degradation, and DNA damage by free radicals generated by the rupture of the mitochondrial respiratory chain, and all cause hepatocellular damage (El-Sherbeeny et al., 2016; El-Zayadi, 2006; Husain et al., 2001; Ogenyi et al., 2015).

The rats supplemented with functional foods in the diet and chronically exposed to cigarette smoke presented a significant decrease in the analyzed serum liver parameters (ALT, AST, ALP, and GGT), compared to the CS group, indicating an attenuation of the toxic effects on hepatocytes. Although the exact mechanisms of the beneficial effects of probiotics on the liver/intestine axis are not yet fully elucidated, the favorable therapeutic effects of probiotics may result from (1) modulation of the composition of the intestinal microbiome and the production of antibiotic factors; (2) modification of the permeability and function of the intestinal epithelium; and/or (3) modulation of the immune system at the local and systemic levels (Kirpich & McClain, 2012; Ng et al., 2009; Sherman et al., 2009).

The serum concentration of total proteins and the albumin and globulin fractions were lower in animals chronically exposed to cigarette smoke. Human studies have shown a reduction in total proteins (Roohi & Ashraf, 2017), albumin, and globulins, which include IgG, IgA, and IgM (Gonzalez-Quintela et al., 2008; Roohi & Ashraf, 2017), due to the deleterious
effects of cigarette smoke on the liver or lymphocytes (Pessione, 2001), 2001), as well as increased caspase 3 activity, leading to cell apoptosis (Mazzone et al., 2010; Minicucci et al., 2016). In addition, the chemical products in cigarette smoke can have a direct and indirect effect on the serum protein profile, as they directly alter the binding properties of albumin, resulting in its degradation in the liver and loss of albumin by the kidney (hypoalbuminuria) (Roohi & Ashraf, 2017). Studies report similar results in rats supplemented with probiotic microorganisms: Lactobacillus acidophilus LA 14 and Bifidobacterium longum BL 05 (Lollo et al., 2012), Lactobacillus acidophilus (NCFM®), and Bifidobacterium lactis (Bi-07) (Irwin et al., 2018) and Lactobacillus casei, Lactobacillus rhamnosus, Streptococcus thermophilus, Bifidobacterium, Lactobacillus acidophilus, Bifidobacterium longum, and Lactobacillus bulgaricus (Esilamparast et al., 2014); prebiotic, Larix occidentalis (Irwin et al., 2018), fructooligosaccharide, and also when supplemented concomitantly forming symbiotics (Esilamparast et al., 2014). The action of prebiotics on liver function is not yet clear, however, it is believed that prebiotics are able to regulate the action of liver enzymes (Schley & Field C.J., 2002).

Cigarette smoke can cause systemic imbalance in the lipid profile (Nelson, 2013) and these detrimental effects on serum levels of total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were seen in this study. The values of total cholesterol, triglycerides, and LDL cholesterol increased significantly in the group exposed to passive smoke and non-supplemented, compared with group C. The level of HDL cholesterol decreased. Cigarette smoke has several free radicals that, when introduced into the body, damage some vital macromolecules and modify the metabolism of proteins and lipids, which can lead to dyslipidemia, the main fuel of atherogenesis (Deaton & Marlin, 2003). Dyslipidemia: elevated triglycerides, LDL, and VLDL, together with decreased HDL, is associated with the occurrence of atherosclerotic lesions in the vascular endothelium (Nelson, 2013; Sonagra et al., 2017) and predisposes to an increased risk of cardiovascular disease in smokers (J. S. Lee et al., 2017).

The biological mechanisms that link smoking and atherogenesis are complex, but one assumed link is that nicotine stimulates the adrenal sympathetic system to secrete catecholamines. These catecholamines increase lipolysis and the concentration of free fatty acids in plasma, which in turn increases the hepatic synthesis of triglycerides and VLDL in the bloodstream (An et al., 2016; S.-S. S. Lee et al., 2011; Nazar et al., 2014; Oates et al., 1988). As triglycerides and serum VLDL increase, the likelihood of their encounter with HDL and the cholesterol ester transfer protein (CETP) increases. CETP is responsible for the exchange of triglycerides with cholesterol ester (CE) between VLDL and HDL particles. These HDL particles rich in triglycerides and poor in CE are less stable, so they disintegrate leading to decreases in the HDL level in the serum (Talayero & Sacks, 2011).

Thus, the increase in triglycerides and VLDL is associated with a decrease in the level of HDL in smokers. The effects of smoking on plasma lipoproteins are also attributed to decreased activity of lipoprotein lipase (LPL) and plasma activity of the enzyme lecithin-cholesterol acyltransferase (LCAT), and increased free fatty acids (FFA), while hepatic lipase (HL) is not influenced. Even in the absence of dyslipidemia, smoking impairs several stages of reverse cholesterol transport (Athyros et al., 2013), this mechanism of action justifies the low HDL level in the control group intoxicated with cigarette smoke in our work.

Probiotics have been shown to cause a decrease in serum cholesterol, through the ability of probiotic microbes to enzymatically decouple bile acid using bile salt hydrolase (BSH). In the conjugated form, bile acid will dissolve, mainly so that a small portion is absorbed in the intestine, in this way, the majority is excreted in the feces, the absorbed cholesterol will then be used to synthesize new acids from bile (which is a homeostatic response), resulting in decreased serum cholesterol level (Begley et al., 2006; Brashears et al., 1998; Nocianitri et al., 2017). Lactobacillus strains were able to produce intracellular cholesterol reductase, and extracellular cholesterol reductase was also detected, indicating that the bioconversion of cholesterol
to coprostanol could also occur outside the cell, and this could benefit the host by reducing blood cholesterol levels (Lye et al., 2010; Nocianitri et al., 2017). In addition, the results revealed the ability of probiotic microorganisms to remedy the deleterious effects of chromium present in cigarette smoke, increasing HDL levels and decreasing LDL (Younan et al., 2018).

The plasma decrease in triglycerides observed in animals supplemented with probiotics and exposed to cigarette smoke was also observed in mice (Salahuddin et al., 2013). The hypotriglyceridemic effect of probiotics may be related to the initiation of lipase activity (especially LPL), which is responsible for triglyceride metabolism, decreasing intestinal lipid absorption or increasing lipid catabolism and/or antioxidant activity (Harisa et al., 2009; Salaj et al., 2013).

The elevated plasma concentrations of creatinine and urea in animals in the group exposed to cigarette smoke and not supplemented with functional foods are in agreement with a study that demonstrated that exposure to cigarette smoke is able to increase the concentration of creatinine and urea of rats (Drummond et al., 2016; Okonkwo et al., 2013). Cigarette smoke contains nephrotoxic substances, including cadmium (Cd) and lead (Pb) (Cooper, 2006; Desai et al., 2016; Fadrowski, 2010; Golli et al., 2016), which cause alterations in proximal tubular function, leading to increased creatinine and serum urea. Furthermore, Jaimes et al. (2004) demonstrated that stable thiol-reactive compounds present in cigarette smoke can activate NADPH oxidase and increase the vascular production of reactive oxygen species (ROS), reducing NO bioactivity and causing endothelial dysfunction.

The analysis of the basic and classic biochemical parameters used to evaluate renal function showed significant alterations in the group of rats supplemented with probiotics, prebiotics, and symbiotics in the diet and chronically exposed to cigarette smoke. This reduction in harmful effects may be due to the beneficial modulation of these functional foods in bacterial growth in the colon, increasing the concentration of saccharolytic microorganisms and decreasing the concentration of proteolytic species, resulting in a reduction in the generation of toxins harmful to the kidney (Venkateswarlu et al., 2017).

Probiotics can prevent the binding of pathogenic enteric substances, making it difficult to bind to local receptors, causing an increase in the clearance of the pathogens from the gastrointestinal tract and thus preventing them from invading the host (Linden et al., 2008). Probiotics also stimulate the innate immunity of the signaling system for dendritic cells, which can translocate to the mesenteric lymph nodes, where they induce regulatory T cells (FoxP3 +) and the production of anti-inflammatory cytokines (interleukin-10 and transforming growth factor-β) (Dalmasso et al., 2006). In addition, they have the ability to promote the differentiation of B cells and increase the production of secretory IgA that adheres to the mucus layer which covers the intestinal epithelium and binds to pathogenic microorganisms, reducing its ability to gain access to endothelial cells (Koppe et al., 2015).

Supplementation of the prebiotics also reduced the biochemical levels of urea and creatinine, which may be due to the ability of this functional food to expand the growth of specific beneficial microbial populations, such as Lactobacillus and Bifidobacterium (Bindels et al., 2015) and in the group that was fed a symbiotic-supplemented diet, we found similar results, showing the same results as when the functional food is added separately to the feed.

Blood glucose measurements in animals in the CS group were significantly higher when compared to group C. This result may be due to the action of nicotine, which directly induces hyperglycemia by activating glycogenolysis and gluconeogenesis in liver cells (Jósê et al., 2009; Vu et al., 2014). It has also been linked to insulin resistance and decreased glucose tolerance, which could culminate in the development of type 2 diabetes (Wu et al., 2015). In the study by Adhami et al. (2016), 49% of the population of mice exposed to cigarette smoke developed symptoms of insulin resistance and metabolic syndrome, with increased fasting blood glucose and fasting serum insulin. In contrast, the study by Ebersbach-Silva et al. (2013), observed no alteration in fasting glucose after exposure to cigarette smoke.

The effects of nicotine are attributed to an increase in lipolysis in adipose tissue through the activation of AMPKα2.
Through the activation of AMPKα2 in white adipose tissue, nicotine impairs insulin signaling, regulates lipolysis, and increases circulating free fatty acid (FFA) concentrations, inducing insulin resistance. It also promotes lipid redistribution and deposition of ectopic fat, for example, hepatic steatosis, increasing hepatic and serum triglycerides.

Yadav et al., (2013) reported that the probiotic VSL#3 [four strains of lactobacilli (Lactobacillus casei, L. plantarum, L. acidophilus, and L. delbrueckii subsp. bulgaricus), three strains of bifidobacteria (Bifidobacterium longum, B. breve, and B. infantis), and Streptococcus salivarius subsp. thermophilus] led to modulation of the composition of the intestinal microbiota and an increase in the glucagon-like peptide hormone (GLP-1). This glucagon-like peptide is seen as a hormone produced in intestinal L cells, which act via circulation on satiety in the brain, intestinal motility, and insulin and glucagon secretion in the pancreatic islet. The beneficial effects of VSL#3 were associated with an increase in the levels of a short-chain fatty acid (SCFA): butyrate. Using a cell culture system, the authors also demonstrated that butyrate promoted the release of GLP-1 from intestinal L-cells. These results support the notion that the probiotic-intestinal flora-butyrate-GLP-1 axis promotes metabolic efficiency and protects against the deleterious effects of obesity induced by a high-fat diet and diabetes (Chapman et al., 2007).

Evidence supporting the use of functional foods: probiotics, prebiotics, and symbiotics is clearly increasing in humans and animal models, but several questions remain: (i) Which microorganism(s) can reduce the deleterious effects of cigarette smoke? (ii) What is the minimum dose or concentration required for each probiotic to see a benefit? And (iii) is a mix of probiotics synergistic compared to using a particular strain?

As understanding of the human microbiome grows, we deepen our appreciation of how individualized and complex the human microbial environment is. We ultimately imagine the need to customize a regime of care for the digestive tract for each human being after sampling the patient's unique microbiological “fingerprint”. Our results emphasize that in biologically relevant exposure regimes (cigarette smoke) it is essential to study functional foods that can reduce pathophysiological responses in animal and human models.

5. Conclusion

Supplementation of functional foods: probiotics (Lactobacillus acidophilus, Enterococcus faecium, Bifidobacterium thermophilum, and Bifidobacterium longum), prebiotics (mananoligosaccharide), and symbiotics (combination of probiotics and prebiotics) in the diet of rats, as an animal model, reduced the harmful toxic effects of chronic exposure to passive smoking, in relation to physiological (weight and weight gain) and biochemical parameters (profiles: hepatic; protein; lipid; renal; and glycemic).

References


