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RESEARCH ARTICLE

Inulin Effect on Intestinal Mucus-secreting Cells

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A B S T R A C T

Maintaining the normal functioning of mucin-secreting cells is essential for the health of gastrointestinal tract mucosa, with any modifications of the mucus barrier being followed by mucosal morpho-functional alterations (Petrou and Crouzier, 2018). One of the prebiotics known for its beneficial effects on the digestive system is inulin, a plant-derived fructan, widely used in complementary medicine. In this respect, our study aimed to evaluate the possible modifications to intestinal mucosa structure and functions induced by long-term oral inulin administration. During the 8-week study, groups of 8 Wistar rats (n = 4/sex) received normal saline solution (control), 625, and 1250 mg/kg/bw of inulin powder. Body weight and feed consumption were recorded daily. On day 29 an interim sacrifice was performed (n = 4/group). The remaining animals continued to receive treatment until day 56. Necropsy examination, histological (Goldner's trichrome staining), and histochemical (AB-PAS reaction) analysis of digestive system organs were performed on both interim and terminal sacrifices, for all the animals included in the study. The resulting histological and histochemical findings were consistent and confirmed that direct contact between the prebiotic and intestine mucosa did not cause any irritations, inflammations or functional alterations.

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INTRODUCTION

Any living organism is constantly in contact with a multitude of pathogens (viral, bacterial, fungal, parasitic, or other). To maintain homeostasis, animal organisms have developed various protective barriers against antigens, including surface ones. These surface barriers are composed of specialized cell epithelia that protect the body against external factors. The epithelium of the digestive tract is the greatest protective structure of this type (Peterson and Artis, 2014).

The integrity of the epithelial cells is ensured by the continuous presence of a mucus layer on their surface, which functions both as a physical and biochemical barrier (Peterson and Artis, 2014; Birchenough *et al*., 2015). The mucus layer is the first line of defense against pathogenic microorganisms while maintaining at the same time the balance between commensal bacteria and the host (Ahluwalia *et al*., 2017).

The cellular mucin secretion process takes place through two different pathways: constitutive and compound-regulated secretion. Among the elements that play a role in compound-regulated secretion, namely the production and discharge of gut mucus, are the microbiota and diet composition (Cornick *et al*., 2015; Paone and Cani, 2020). Prebiotics are known as "non-digestible dietary components that have a beneficial effect on the host's health, by influencing the microflora and its activity" (Simon *et al*., 2021).

By modifying the microflora composition, prebiotics can indirectly affect mucus production. The mechanism behind it is the production of short-chain fatty acids (acetate, propionate, and butyrate). Butyrate acts on mucus secretion through several mechanisms: 1) serves as a nutritional (carbon) source for colonocytes, thus influencing mucosa regeneration; 2) inhibits inflammatory processes by decreasing proinflammatory cytokines production (IFN-γ, IL-2) and regulates PGE1/PGE2 ratio (Willemsen *et al*., 2003); 3) causes an elevation in MUC2 mRNA levels and MUC2 expression (Burger-van Paassen *et al*., 2009; Cornick *et al*., 2015). Mucus secretion regulated by butyrate production occurs mainly through the exocytosis of mucus granules (Barcelo *et al*., 2000).

Based on their chemical structure, Khramtsov *et al*. (2018) classified prebiotics as follows: carbohydrates (monosaccharides, oligosaccharides, polysaccharides, and sugar alcohols), proteins (peptides) and others (lactobionic acid, polyphenols). Prebiotics can also be classified into: fructans, namely inulin and fructooligosaccharides (FOS), galactooligosaccharides (GOS), starch and glucose-derived oligosaccharides, other oligosaccharides and non-carbohydrate oligosaccharides (Davani-Davari *et al*., 2019).

The current decade is characterized by an increasing trend of prebiotic consumption as a part of preventive care, in both humans and production animals, with inulin being one of the most commonly used and studied prebiotics (Research and Markets, 2022). It is a polysaccharide composed of 2 to 140 beta-fructans units (Looijer-van Langen and Dieleman, 2009; Khramtsov *et al*., 2018) naturally found in chicory roots, Jerusalem artichokes tubers, *Inula helenium* roots, beetroots, leek, and other plants (Xie *et al*., 2020; Qiao *et al.*, 2022). Inulin can improve gut health by promoting the growth of Bifidobacterium and Lactobacillus spp., which in turn increase the produced quantity of short-chain fatty acids (SCFAs) (Akram *et al*., 2019) and suppress the proinflammatory cytokines production (Song *et al*., 2018; Qiao *et al*., 2022). The main source of natural prebiotic inulin distributed on the market are the roots of *Cichorium intybus*, a plant belonging to the family Asteraceae, genus *Cichorium*. Another plant species, from the same family, containing high quantities of inulin is *Inula helenium*, genus *Inula* L. Their roots are widely used in traditional medicine for the treatment of different disorders, including those affecting the digestive system: loss of appetite, colics, emesis, bloating, and diarrhea (Ghedira *et al*., 2011; Street *et al*., 2013).

Taking into consideration the annual increase in global inulin consumption and inconsistent findings regarding its beneficial effect on the intestinal mucosal barrier, in this study, we aimed to test the effect of the long-term oral administration of prebiotic inulin, on the rat gut epithelial and mucus barrier. Therefore, throughout the study, we monitored the daily changes in the body weight of animals and potential gastrointestinal side effects. The potential structural modifications of the intestinal mucosa, induced by inulin administration, were assessed using Goldner's Trichrome staining. Additionally, to identify the possible qualitative and quantitative changes in mucin-secreting cells, the histochemical Alcian blue (AB)–Periodic acid-Schiff (PAS) reaction was used.

MATERIALS AND METHODS

Fructan sample: Natural inulin powder was purchased from a licensed herbal store. Based on the product data sheet, inulin was extracted from the dried roots of *C. intybus*, originating from the Netherlands. The dry matter (DM) content of inulin powder ranged between 95-99%. From which at least 90% of DM was inulin, and the remaining 10% or less consisted of fructose, glucose, and sucrose, combined. The carbohydrate content was 97 g per 100 g of product, 90 g being represented by non-digestible carbohydrates, namely inulin. The product's physical characteristics were as follows: fine white powder, pH between 5.0 and 7.0, tapped density of 700±100 g/L, and an average chain length of 8 to 13 monomers.

Biological material: A total of 24 Wistar rats of both sexes, aged between 6 to 8 weeks and weighing 130±26 g, were used in this study, being purchased from the "Iuliu Haţieganu" University of Medicine and Pharmacy Laboratory Animal Facility, Cluj-Napoca, Romania. The animals were housed under standard environmental conditions and with ad libitum access to feed and tap water as per nutritional guidelines by Bülbül and Nawaz *et al*., 2020. Before experimental manipulations, for one week, rats were acclimated to the facility and working personnel. The study was conducted in accordance with the Declaration of Helsinki, and approved by the Bioethics Committee of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania (No.248/29.03.2021), and by the National Sanitary Veterinary and Food Safety Authority (ANSVSA) of Romania (No.258/13.05.2021).

Study design: For this research, we used the method described in the OECD Guidelines 407 and 452, with some modifications (OECD, 2008; OECD, 2018). The animals were divided into 3 groups (4 females and 4 males each), a control group (Group 1) that received a normal saline solution, and two tested groups (Group 2 and 3) that received inulin suspension, 625 mg/kg/bw, and 1250 mg/kg/bw respectively. Doses were calculated using an allometric scale, with the lower dose corresponding to the maximum recommended dose in humans. The suspension was administered by gavage, once a day, for 28 days, according to the animal's body weight. Behavior, physical appearance, body weight, feed, and water consumption were assessed daily throughout the experimental period. Weekly body weight gain (%) was calculated using the following formula:

$$\text{BWG}\,(\%) = \frac{(\text{final body weight (g)} - \text{initial body weight (g)})}{\text{initial body weight (g)}} \times 100\%$$

On the 29th day of the study, following an overnight fast (8 hours), 4 animals from each group (2 females and 2 males) were anesthetized and sacrificed by cervical dislocation (interim sacrifice). The remaining rats, 4 from each study group, continued to receive inulin until day 56 of the study. On the 57th day of the study, terminal sacrifice was performed, following the same protocol.

Histopathology: Jejunum and colon samples were selected for this study based on existing data about their

permeability for inulin, under physiological conditions (Ma *et al*., 1991; Loh *et al*., 2006). Additionally, both gut segments are colonized by Lactobacillus and Bifidobacterium spp. (Shao *et al*., 2023), their population being positively influenced by inulin intake. Samples were collected during interim and terminal sacrifices, were fixed in 10% buffered formalin. After 7 days the samples were processed and embedded in paraffin wax. The wax blocks containing tissue samples were then sectioned (5 µm) using Leica RM 2125 microtome and mounted on microscope slides. The samples were stained using Goldner's Trichrome method for the determination of structural alterations of the examined tissue. For determination of potential functional alterations of mucin-secreting cells, jejunum and colon samples were stained using the histochemical Periodic acid-Schiff (PAS) – Alcian blue (AB) method, for neutral and acid mucins, respectively (Kiernan, 1999). Examination of the slides was performed using an Olympus BX41 light microscope with Olympus E-330 digital camera attached (Olympus, Japan). Adobe Photoshop 2021 version 22.0.1 software was utilized for image processing. ToupView software (AmScope, Irvine, CA, USA) was used for the quantification of acidic and mixed Goblet cells.

Statistical analysis: The weekly body weight gain (%) results were expressed as mean±standard deviation. The differences between control groups and groups that received inulin were analyzed using Welch's T-test. Statistical comparison between the number of acidic and mixed Goblet cells was performed using the One-Way ANOVA test. All the data were analysed in GraphPad Prism version 9.3.1 for Windows (GraphPad Software, San Diego, California USA). Differences were considered statistically significant at $p < 0.05$.

RESULTS

Clinical observation and body weight of rats: Animals treated orally with 625 and 1250 mg/kg/bw of inulin for 56 consecutive days did not display alterations in behavioral patterns, gastrointestinal system or signs of pain. During the first week of the study, the body weight gain of the female rats from inulin-treated groups was significantly greater compared to the control group (Fig. 1). However, in the following weeks, no significant difference between groups was observed, except for the group treated with 625 mg/kg/bw of inulin in course of week 7 (P≤0.05). The body weight gain of the male rats from the treated groups showed insignificant variations throughout the study compared to the control group (Fig. 2). Because no body-weight gain pattern was observed, its variations were not considered treatment or dose-related.

Histological and histochemical examination: At the level of the jejunum (Fig. 3), inulin administration for 28 and 56 days did not produce morphological modifications. The cells lining the intestinal villi, namely enterocytes and Goblet cells, had a normal appearance. Even the cells in the walls of the intestinal glands showed no detectable changes by optical microscopy. On microscopic examination of the lamina propria of treated and control groups was observed the presence of a few mononuclear inflammatory cells, which were not identified in the other structures of the intestinal mucosa. The presence of rare inflammatory cells in the lamina propria was not considered a sign of an inflammatory response to inulin administration. Since the infiltrate did not exceed the physiological limits, it was associated with the response to continuous exposure to antigens present in the chyme.

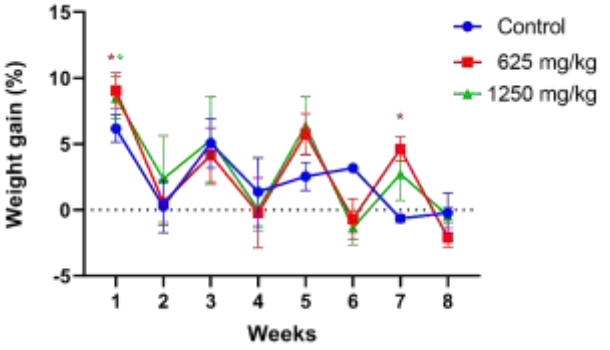

Fig. 1: Weekly body weight gain of female rats treated with various doses of inulin. Data with asterisks are significantly different compared with the control group (P≤0.05).

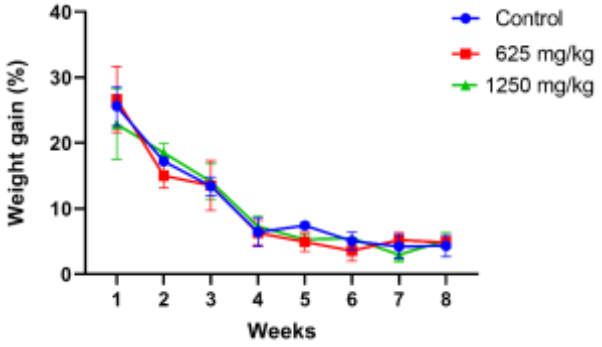

Fig. 2: Weekly body weight gain of male rats treated with various doses of inulin (P≤0.05).

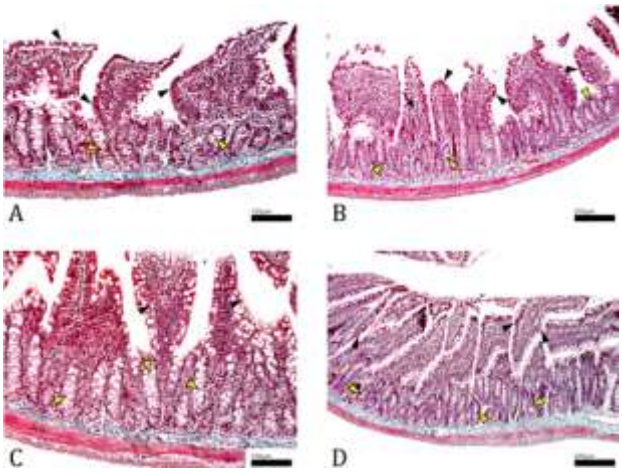

Fig. 3: Appearance of small intestine mucosa in the experimental groups (Wistar rats, GT coloration): A (group 1, control) - absence of histological lesions at the level of intestinal mucosa, enterocytes (black arrowhead) and wall of intestinal glands (yellow arrowhead); B (group 2, 28-days of treatment) - normal aspect of enterocytes (black arrowhead) and intestinal glands (yellow arrowhead), absence of quantitative and qualitative changes of the cell infiltration in the lamina propria (arrow); C (group 3, 28-days of treatment) - normal aspect of Goblet cells (black arrowhead) and intestinal glands (yellow arrowhead); D (group 3, 56-days of treatment) - normal aspect of enterocytes (black arrowhead) and intestinal glands (yellow arrowhead).

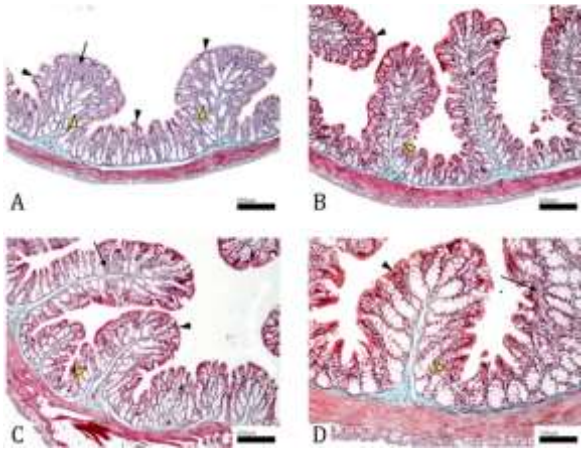

Fig. 4: Appearance of colonic mucosa in the experimental groups (Wistar rats, GT coloration) - absence of microscopic lesions at the level of surface epithelium (black arrowhead), glandular epithelium (yellow arrowhead) and normal levels of infiltrate in the lamina propria (arrow): A (group 1, control), B (group 2, 28-days of treatment), C (group 3, 28-days of treatment), D (group 3, 56-days of treatment)

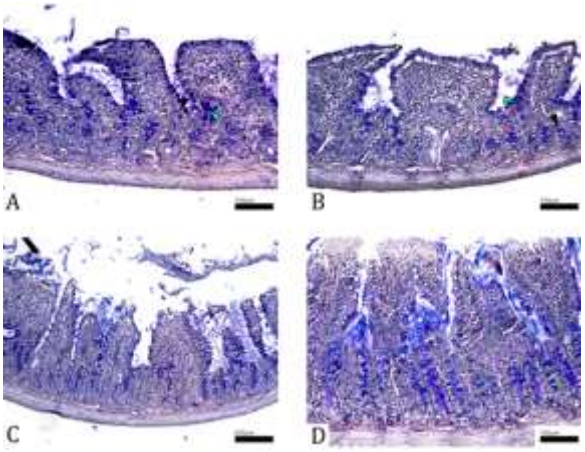

Fig. 5: Histochemical appearance of small intestine mucosa in the experimental groups of Wistar rats (PAS - alcian blue coloration) - normal functioning of Goblet cells secreting neutral mucins (blue arrowhead) and acid mucins (black arrowhead): A (group 1, control); B (group 2, 28-days of treatment); C (group 3, 28-days of treatment); D (group 3, 56-days of treatment).

Similar to jejunum, inulin did not induce any visible morphological alterations at the level of surface and glandular epithelium of the colon (Fig. 4). Additionally, the infiltrate present in the lamina propria showed no signs of inflammation. These findings were consistent and confirmed that direct contact between the inulin and intestinal mucosa did not cause any irritation or inflammation.

After noticing that inulin did not cause structural changes, we decided to conduct histochemical studies to determine whether this prebiotic may cause functional alterations in the mucin producing cells. Mucins are essential for the proper functioning of the digestive system, playing a significant role in the defense mechanisms of the gastric and intestinal mucosa. Using the Periodic acid-Schiff (PAS) – Alcian blue (AB) staining method, we determined the presence, arrangement and secretory activity of cells that synthesize both neutral and acid mucins.

In the jejunum (Fig. 5), cells that produce both neutral and acidic mucins, located in Lieberkühn glands and the lining of the villi, were identified. Their activity was appreciated as normal, without significant differences between cells, regardless of their location.

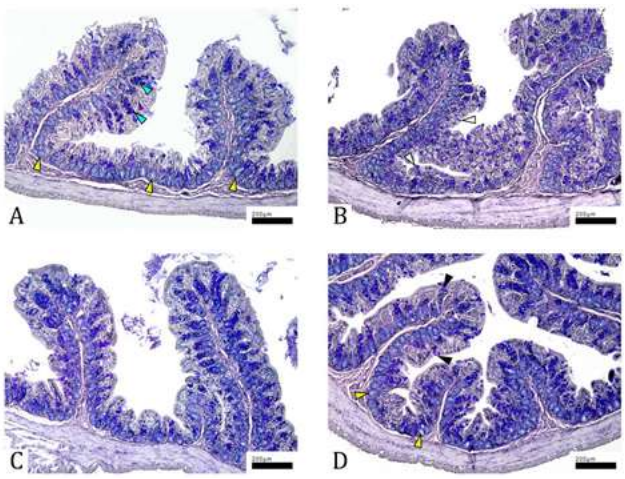

Fig. 6: Histochemical appearance of colonic mucosa in the experimental groups of Wistar rats (PAS - alcian blue coloration) - normal functioning of Goblet cells and DCS cells: A (group 1, control) - alcian blue positive cells (blue arrowhead) and DCS cells (yellow arrowhead); B (group 2, 28-days of treatment) - Goblet cells (white arrowhead); C (group 3, 28-days of treatment); D (group 3, 56-days of treatment) - PAS positive cells (black arrowhead) and DCS cells (yellow arrowhead)

In the colon (Fig. 6), two types of mucin-secreting cells were histochemically identified: Goblet cells and DCS cells (deep crypt secretory cells). Both types of cells have mixed secretion, both neutral and acid. according to the color density, it can be concluded that mucin production is higher in Goblet cells, compared to DCS cells, which is a normal finding. Furthermore, both mucin-producing cells showed no functional alteration, regardless of their localization.

Goblet cell quantification: As observed in the histological images, the number of mixed Goblet cells was lower compared with the acidic ones, in both intestinal segments. The observation was available regardless of the experimental group or the duration of the intervention (*i.e.* Group 2 at 29 days - Small intestine - acidic 4.27% vs mixed 95.73% compared with Group 2 at 56 days acidic 5.74 % vs mixed 94.26%). Ordinary One-Way ANOVA test suggested that there are significant differences between the number of the two types of Goblet cells ($P<0.05$ for small intestine and $P<0.0001$ for large intestine), in all the groups and in both intestinal segments.

The experimental intervention duration did not influence the number of the cells in the case of mixed Goblet cells from both the small and large intestine, or in the case of acidic Goblet cells from the small intestine ($P>0.05$), while in the case of acidic Goblet cells from the large intestine, the differences are statistically significant (*, $P=0.0110$).

When comparing between groups the number of the same Goblet cell type/ intestinal segment, in most of the cases there were no significant differences. However, the Ordinary One-Way ANOVA test suggested that when comparing mixed Goblet cells from the small intestine at 29 days between the groups, the differences are statistically significant (*, $P=0.0217$). The same observation was available in the case of acidic Goblet cells from the small intestine at 29 days (*, $P=0.0103$) and for the acidic Goblet cells from the large intestine at 29 days (**, $P=0.0058$).

DISCUSSION

Inulin is known to improve gut health by two different pathways, direct and indirect. Its direct mechanisms of action are mainly represented by the prevention of lipid peroxidation in the stomach and consequently the reduction of oxidative stress (Kanner and Lapidot, 2001). Additionally, inulin exerts a direct anti-inflammatory effect by reducing the NF-κB protein levels (Farabegoli *et al*., 2023). The main impact of inulin on gut health is achieved through its indirect mechanisms of action. Inulin intake determines the increase of SCFAs, mainly acetate and lactate resulting from its direct fermentation by Lactobacilli and Bifidobacteria (Gopalakrishnan *et al*., 2012; Akram *et al*., 2019). In turn, acetate and lactate serve as energy sources for butyrate-producing bacteria (Faecalibacterium, Eubacterium, and Roseburia) (Holscher, 2017). As aforementioned, butyrate plays an important role in maintaining intestinal homeostasis by exhibiting anti-inflammatory activity, stimulating mucus secretion, and serving as a nutritional source for colonocytes. In dextran sodium sulfate (DSS)-treated mice, inulin manifests anti-inflammatory activity by decreasing the production of certain pro-inflammatory cytokines: 1) IL-1β, which plays a major role in IBD by increasing intestinal inflammation and permeability; 2) Toll-like receptor 4 (TLR4), that causes the activation of inflammatory cytokines; and 3) TNF-α, which disrupts intestinal barrier integrity and causes epithelial cells apoptosis via TNF-R1-dependent pathway (Al-Sadi *et al*., 2012; Li *et al*., 2013; Leppkes *et al*., 2014; Dai *et al*., 2022; Qiao *et al*., 2022). Additionally, inulin maintains intestinal barrier integrity and decreases its permeability by increasing the gene expression of tight junction proteins Claudin-1, Claudin-3 and Claudin-7 (Song *et al*., 2018; Qiao *et al*., 2022).

In the current study, inulin powder was administered orally to rats for 28 and 56 days. Throughout the study, animals were observed for the presence of adverse effects and clinical signs associated with treatment and weighed daily. The obtained data was used for the determination of weekly body weight gain (BWG). Statistical analysis of weekly BWG (%) showed no treatment or dose-related changes, although the BWG fluctuated throughout the study in all groups. Supporting our findings, no link between inulin administration and body weight was observed in several other studies (Cani *et al*., 2004; Li *et al*., 2021). Concurrently, inulin was found to decrease body weight, mainly in overweight rats or those receiving a high-fat diet (Cani *et al*., 2005). This is believed to be related to the ghrelin decrease, a hormone responsible for appetite control (Cani *et al*., 2005; Li *et al*., 2021).

In order to evaluate the inulin effect on the structural integrity of the intestinal mucosal barrier, necropsy and histopathology (GT staining) examinations were performed. Based on the necropsy and histopathology examination, it was observed that the 28 and 56-day oral administration of the inulin prebiotic did not alter the jejunum or colon morphology, at least not structural changes detectable by optical microscopy, at doses of 625 and 1250 mg/kg/bw.

The protection conferred by the mucus layer differs according to its anatomical structure. In the small intestine, the mucus layer is simple and not attached to the surface of the epithelium, facilitating intestinal absorption phenomena (Birchenough *et al*., 2015; Ahluwalia *et al*., 2017). Moreover, the continuous mucus secretion and its movement caused by peristaltic waves, make it difficult for pathogens to reach the epithelial surface (Pelaseyed *et al*., 2014). In the colon, there is a two-layered mucous system that serves as additional protection against increased bacterial load, the inner layer being attached to the Goblet cells and completely renewed within an hour (Johansson, 2012).

At the small intestine level, Goblet cells are the main structures involved in the mucus synthesis and secretion processes, with enterocytes providing the necessary bicarbonate supply (Birchenough *et al*., 2015). Unlike mucus-producing cells in the small intestine, Goblet cells in the epithelium of the large intestine (colon) can produce their own bicarbonate needs due to the presence of the necessary enzymatic equipment (Birchenough *et al*., 2015). In certain segments of the large intestine, especially in the colon, there is another type of mucus-producing cell called the "deep crypt secretory" cell (DCSc). As opposed to Goblet cells, which have the characteristic Goblet shape, DCSc is pyramidal in shape, with an ovoid nucleus located at the base of the cell and vacuoles in the cytoplasm (Altmann, 1983).

Evaluation of the inulin effects The Periodic acid-Schiff (PAS) technique detects neutral mucins, the aldehydes that result from the oxidation of 1,2 glycols being stained red or magenta. Alcian Blue (AB) with a pH of 2.5, is used for the detection of acidic mucins, staining them deep blue. Mixed types of mucins will stain different shades of purple, depending on their composition (Kiernan, 1999; Ghiurco *et al*., 2021). In our study, the histochemical examination revealed no changes in the function of neutral, acidic, and mixed mucin-secreting cells of the jejunum and colon for the inulin-treated animals, compared to the control group. Normal functioning of Goblet cells is essential for the health of gastrointestinal tract mucosa, reduction in the number or secretions of mucin-secreting cells causing alteration of the mucus barrier, followed by morphological lesions of digestive tract mucosa.

Statistical analysis of data obtained by quantifying the percentage of acidic and mixed Goblet cells from the total number of Goblet cells revealed a statistically significant decrease in the percentage of acidic cells in the group treated with 1250 mg/kg/bw of inulin for 28 days, in both jejunum and ileum. Even though the percentage of acidic Goblet cells from the total number of cells decreased in both groups treated with inulin for 28 days when counting the cells per 1000 μm^2 an increase in their number can be observed, in both small and large intestines (Table 1). Contrasting results have been reported by Mair *et al*. (2010), where a decrease in the number of acidic Goblet cells, at the level of jejunum, was reported in piglets that were treated with inulin for 28 days. It is thought that acidic mucins have a particular role in the protection against bacterial translocation, being less susceptible to bacterial glycosidases and host proteases (Deplancke and Gaskins, 2001). This comes in support of the fact that at the level of jejunum and colon, where the bacterial population of interest is higher than in other digestive segments, the number of acidic Goblet cells is predominantly (Deplancke and Gaskins, 2001). Moreover, the total number of Goblet cells in both the small and large intestines increased because of the short-term (28 days) inulin administration.

Table 1: Number of acidic, mixed, and total Goblet cells quantified from jejunum and colon histological samples.

Day 28			Nr Goblet cells/1000 μm²		
	Group	Intestinal segment	Mixed	Acid	Total
	1	Jejunum	0.006	0.117	0.123
		Colon	0.012	0.089	0.101
	2	Jejunum	0.006	0.145	0.152
		Colon	0.017	0.145	0.161
	3	Jejunum	0.018	0.220	0.238
		Colon	0.025	0.134	0.158
Day 56			Nr Goblet cells/1000 μm²		
	Group	Intestinal segment	Mixed	Acid	Total
	1	Jejunum	0.007	0.141	0.147
		Colon	0.016	0.141	0.156
	2	Jejunum	0.015	0.241	0.255
		Colon	0.014	0.130	0.144
	3	Jejunum	0.003	0.121	0.124
		Colon	0.012	0.106	0.118

These findings are in agreement with the data reported by Corrêa *et al*. (2023) in a study on mice that received an inulin diet for 30 days. Additionally, in the study conducted by Trullàs *et al*. (2022) on red tilapia (*Oreochromis* spp.), the administration of *Helianthus tuberosus*, a representative of the family Asteraceae that contains inulin, caused a significant increase ($P<0.05$) of the number of AB, PAS, and AB-PAS-stained Goblet cells.

Data obtained from the groups that received the treatment for a longer period (56 days) revealed no statistical differences in the percentage of acidic or mixed Goblet cells. However, the total number of Goblet cells (Table 1), from the jejunum and colon, decreased in the group which was treated with 1250 mg/kg/bw for 56 days, and suffered little to no changes in the group that received 625 mg/kg/bw, when compared to the control group. Previously, unfavorable effects of higher doses of inulin have been mentioned by Song *et al*. (2018), with MUC2 gene expression and mucosal immune function being decreased when compared to lower inulin doses. regarding the small intestine mucus, the main molecule is gel-forming MUC2 mucin, a complex protein with a structural role (Johansson, 2012). In the large intestine, all the above-mentioned gel-forming mucins can be found. According to the study conducted by Xie *et al*. (2020), the administration of inulin to Wistar rats resulted in an increase in MUC2 production, which is believed to be caused by four immune-modulatory bacterial metabolites. Additionally, an increase in MUC3 mRNA level was induced by microbiota anti-inflammatory metabolites, resulting from inulin fermentation (Qiao *et al*., 2022). In the study conducted by Kim *et al*. (2020), the extract of Inulae Flos (flowers of *Inula japonica*), species of the genus *Inula*, inhibited gastric lesions in rats by increasing gastric wall mucus contents. In addition, inulin can reduce the penetrability of the attached colonic mucus layer, therefore impeding the pathogenic bacteria from reaching the colonic epithelium (Schroeder *et al*., 2018).

Conclusions: The study highlighted that the oral administration of 625 and 1250 mg/kg/bw inulin powder did not cause any pathological modifications in terms of morphology or mucin-producing cell function in either jejunum or colon, at least not detectable by optical microscopy, at this dose. Short-term (28-days) administration of inulin caused an increase in the number of goblet cells, both acidic and mixed, at both tested doses. However, in long-term (56-days) administration of the 1250 mg/kg/bw of inulin, a decrease in the total number of goblet cells was observed, which despite the lack of histological alterations of jejunum and colon could in time have a negative effect on gut homeostasis. Considering the above-mentioned, we can conclude that long-term administration of inulin at 625 mg/kg/bw does not affect the functionality of the mucin barrier in the digestive tract, and therefore it is safe for oral use. Although inulin is considered beneficial for the gut health prebiotic, more studies regarding its therapeutic index and long-term exposure effects should be conducted.

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