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HEALTH SCIENCES

Fructooligosaccharides (FOS) intake modulates oxidative stress and restores gut morphology in *Giardia lamblia*-infected gerbils

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Abstract: The purpose of this study is to evaluate the effects of fructooligosaccharide (FOS) supplementation on the prevention of experimental giardiasis by analyzing morphological, biochemical, and parasitological parameters in a gerbil model. Gerbils were divided into four groups: uninfected control (CT), infected control (CTIN), uninfected and FOS-treated (FOS), and infected and FOS-treated (FOSIN). The FOS and FOSIN groups received 250 mg/day of FOS by gavage for 14 days. After this period, the CTIN and FOSIN groups were inoculated by gavage with 1 x 10^6 trophozoites/ml of *Giardia lamblia*. Fourteen days post-infection, all animals were euthanized, and blood, liver, small intestine, and feces were collected for analysis. FOS supplementation resulted in a 77% reduction in parasitic load. Additionally, FOS reduced infection-induced damage by preserving intestinal structure, improving metabolic parameters, and reducing hepatic oxidative stress. FOS was also effective in mitigating the effects of an established *G. lamblia* infection. These preliminary findings suggest that FOS has beneficial effects in the preventive treatment of *Giardia lamblia* infection.

Key words: Fructooligosaccharides, *Giardia lamblia*, *Meriones unguiculatus*, oxidative stress, metabolism.

INTRODUCTION

Giardiasis, the most common enteric parasitic infection in the United States, causes an estimated 1.2 million episodes of illness annually (Beer et al. 2017). It is the most prevalent enteric protozoal infection worldwide, affecting nearly 2% of adults and 8% of children in developed countries, and approximately 20–30% of individuals in developing countries (Dunn & Juergens 2024, Hajare et al. 2022). That prevalence is associated with poverty, poor sanitation, lack of access to clean and safe drinking water supply, and poor personal hygiene (Hajare et al. 2022).

The conventional treatment for this infection primarily involves nitroimidazoles (metronidazole, tinidazole) and benzimidazoles (albendazole, mebendazole), but treatment may fail mainly due to their side effects (Watkins & Eckmann 2014, Dunn & Juergens 2024). Both giardiasis and its treatment generate impacts on the gastrointestinal tract. However, advances in the development and identification of novel drugs and potential non-pharmacological alternatives have been studied. Such advances have the overall aim of identifying knowledge gaps and suggesting future directions for research (Lalle & Hanevik 2018).

Dysbiosis, defined as a disruption in the homeostasis of the intestinal microbiota, is strongly associated with both gastrointestinal and extraintestinal alterations (Domínguez et al.

2024). By modulating host immunity, mucosal cell function, and the intestinal microenvironment, the microbiome plays a key role in determining susceptibility or resistance to *Giardia* infection, as well as its severity and duration (Fekete et al. 2021).

Dietary intake of prebiotics, such as fructooligosaccharides, can modulate the gut microbiota, promoting the growth of beneficial intestinal bacteria and the production of metabolites that are potentially protective of gut functionality (Guarino et al. 2020). Prebiotics have been used to treat various conditions, such as Crohn's disease (Yamamoto et al. 2017), mucositis (Wang et al. 2016), and constipation in children (Closa-Monasterolo et al. 2017). However, there are no studies linking fructooligosaccharides to giardiasis.

Due to the high prevalence of giardiasis, reports of its side effects, and therapeutic failure of conventional treatment, there is an increasing interest in alternative therapeutic strategies, in which prebiotics may represent attractive options. This study aimed to evaluate the effects of fructooligosaccharides intake on the prevention of experimental giardiasis, analyzing morphological, biochemical, and parasitological features, using gerbils as a model.

MATERIALS AND METHODS

Animals and Experimental Design

The experiments were conducted with 32 male gerbils (*Meriones unguiculatus*), aged 4–6 weeks, previously treated with injectable ivermectin 1% to ensure that they were free of any parasites. The absence of enteroparasites was confirmed through standard microscopic examination of fecal samples, performed every two days prior to infection with *G. lamblia*, to detect cysts, trophozoites, and helminth eggs. Animals were maintained under standard laboratory conditions, with a 12:12 h light/dark cycle and controlled temperature (23 ± 3°C), and were provided *ad libitum* access to filtered water and a commercial diet (Nuvilab®).

All experimental protocols were approved by the Animal Experimentation Ethics Committee of the Federal University of Minas Gerais (CEUA/UFMG), protocol number 371/2017).

The animals were divided evenly based on their body weight into four groups (eight animals in each), assuring similar initial body weights for comparability: uninfected control (CT, 48.74 ± 3.23); infected control (CTIN, 50.18 ± 1.63); uninfected and FOS-treated (FOS, 49.86 ± 1.63), infected and FOS-treated (FOSIN, 50.51 ± 1.62).

Prebiotic Fructooligosaccharide was obtained from a commercial lyophilized preparation (FOSVITA-FOS, Vitafor®). Gerbils in the FOS and FOSIN groups received 250 mg of FOS suspended in 250 µL of distilled water daily by oral gavage for 14 days prior to *G. lamblia* infection, and the treatment continued until the end of the experiment (28 days). Considering that gerbils consume approximately 4g of diet per day, 250 mg of FOS corresponds to 5% of the diet. This dosage was selected based on previous studies demonstrating both efficacy and safety of similar doses in rodent models (Le Bourgot et al. 2018, Mao et al. 2018, Matsumoto et al. 2017).

The infection with *G. lamblia* was carried out using trophozoites of the GS/M-clone H7 strain (ATCC 50581) isolated from a male diarrheal patient. The axenic strain was maintained in TYI-S-33 medium modified by Keister (1983) and subcultured weekly at 37 °C. On the 14th day of the experiment, the rodents from the CTIN and the FOSIN groups received 1 x 10^6 trophozoites of *Giardia lamblia* (strain Clone H7-ATCC 50581) in 0.8 ml of PBS by gavage. The experimental design is illustrated in Figure 1.

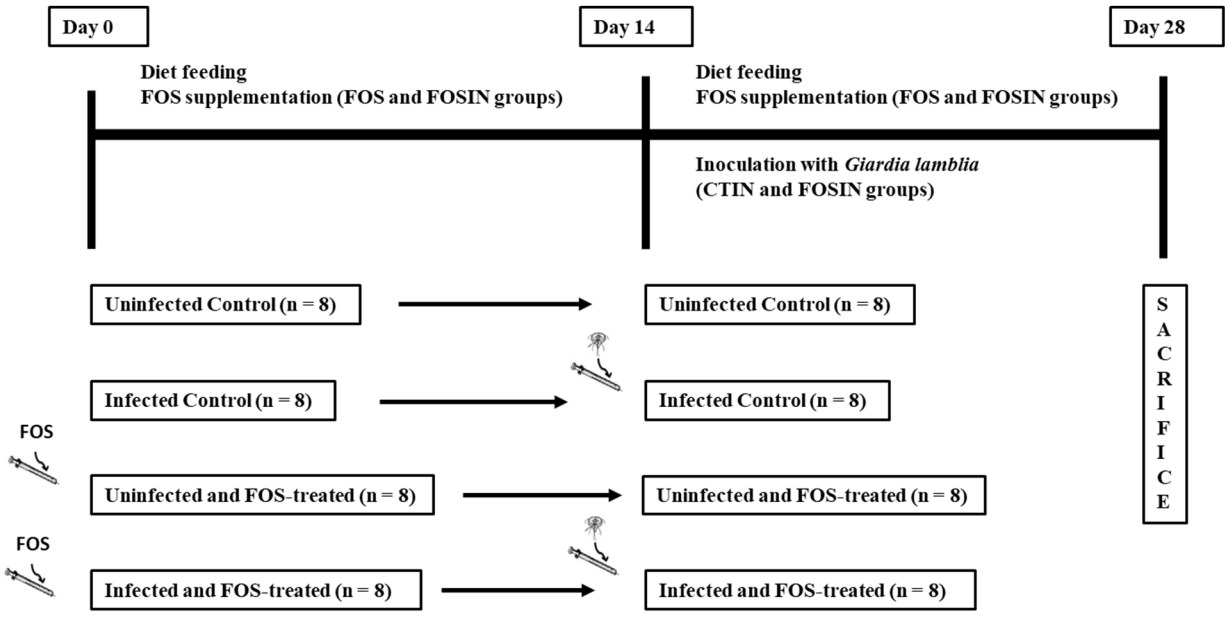

Figure 1. Experimental design. Gerbils were fed commercial diet and received 250 mg of Fructooligosaccharide daily (FOS and FOSIN groups) for 14 days, when they were inoculated with 1 x 10^6 trophozoites of *Giardia lamblia* (CTIN and FOSIN groups).

To confirm *Giardia* infection, the animal stools were diluted in water, stained with Lugol's solution, and examined under an Axiolab-Carl Zeiss microscope (Oberkochen, Germany) with a magnification of 40×.

After 28 days of the experiment, all the gerbils were anesthetized (100 mg/kg ketamine and 12 mg/kg xylazine), and blood was collected from the inferior vena cava, followed by cervical dislocation. The proximal portion of the small intestine was removed to produce histological sections; the liver was collected and weighed, and feces were collected for cyst counting. Their body weight was evaluated weekly and their food intake was checked daily.

Counting of *Giardia* cysts

Cysts were enumerated in fecal samples from the CTIN and FOSIN groups. Freshly passed feces were collected on alternate days post-infection. As the animals were housed together in the same cage, fecal material was pooled per cage to obtain a representative sample for each group at each time point. For each pooled sample, 0.1 g of feces was thoroughly homogenized with 1 mL of formal saline using a mortar and pestle. The suspension was then stained with Lugol's iodine, and *Giardia* cysts were counted using a hemocytometer under an Axiolab-Carl Zeiss microscope (Carl Zeiss, Oberkochen, Germany) with a magnification of 40×. Cyst counts were expressed as cysts/mL of the fecal suspension, corresponding to 0.1 g of feces homogenized in 1 mL of formal saline. The procedure followed the method described by Vinayak et al. (1979).

Levels of total cholesterol, triglycerides, and plasma glucose

All measurements were taken using an enzymatic colorimetric assay (Labtest kit, Brazil) following the guidelines of the manufacturer's protocols. These biochemical parameters were measured only at the end of the experiment; therefore, pre-treatment (baseline) values were not available.

Lipid peroxidation and antioxidant enzyme analysis in the liver

First, liver tissues (100 mg) were homogenized in 1 mL of cold phosphate-buffered saline (pH 7.4) to prepare the samples. The homogenate was centrifuged at approximately 12,000 g (Neofuge 15R, Heal Force) for 10 minutes at 4 °C, and the supernatant was collected for analysis.

The concentration of thiobarbituric acid reactive substances (TBARS) was measured according to the protocol previously established by Buege & Aust (1978). Briefly, 200 µL of supernatant were mixed with a solution containing thiobarbituric acid (TBA 0.375%) in acid solution (15% trichloroacetic acid and 0.25 M hydrochloric acid), incubated in boiling water (95 °C) for 15 min and subsequently placed on ice for cooling. Samples were mixed with 600 µL of n-butanol and centrifuged at ~3,000 g for 10 min. Aliquots of the supernatant (150 µL) were transferred to a microplate (96 wells) and the absorbance was read at 535 nm on a microplate reader (Biotek, ELx800 absorbance microplate reader, VT, USA). The technique involved a step using n-butanol to extract interferents, followed by calibration with a standard curve generated from known concentrations of malondialdehyde (MDA). The results were expressed as micromoles of MDA/mg protein.

The hydroperoxide concentration was set according to Banerjee et al. (2002) protocol, and the results were expressed as micromoles of hydroperoxides per milligram of protein. The dosage of antioxidant enzyme superoxide dismutase (SOD) was based on its ability to clean the radical O_2^-, adapted from Dieterich et al. (2000). To calculate the result, it was considered that 1 unit (U) of SOD was able to autooxidize 50% of pirogallol of the standard at 570 nm. The result was then expressed as units of SOD per mg of protein. The measurement of catalase activity was based on monitoring the kinetics of hydrogen peroxide (H_2O_2) decomposition at 240 nm, as described by Nelson & Kiesow (1972). Readings were taken every 15 seconds over a 60-second period, and calculations were based on the difference between the initial and final readings divided by the sample volume (mL). This result was expressed as micromoles of H_2O_2/min/mg protein.

A commercial BCA assay kit (Pierce™ BCA Protein Assay Kit) from Thermo Fisher Scientific (IL, USA) evaluated the protein content.

Morphometric analysis

After euthanasia, a 4 cm segment from the proximal portion of the small intestine was collected and fixed in 10% buffered formaldehyde (pH 7.2). Transverse sections were prepared, embedded in paraffin, sectioned at a thickness of 4 µm, and stained with hematoxylin and eosin (H&E). Images were acquired at regular intervals using systematic random sampling, starting from a randomly selected field to minimize selection bias.

Thirty villi and intestinal crypts per animal were captured using a light microscope (Olympus, Miami, FL, USA) equipped with a 20× plan apochromatic objective. Images were digitized using the SPOT® Insight Color software (version 3.4.5; Diagnostic Instruments Inc., Sterling Heights, MI, USA). A digital camera mounted on an Olympus BX-40 microscope projected the images onto a monitor for analysis. The software was calibrated using a stage micrometer (1 division = 10 µm), and all measurements were performed at 40× magnification to ensure accuracy (Abdel-Salam et al. 2021)

Villus height was measured from the tip to the base, and crypt depth from the base of the villus to the mucosa. For each histological section, the mean villus height and crypt depth were calculated based on measurements from selected villi and crypts. The villus-to-crypt ratio

was determined by dividing villus height by the corresponding crypt depth. All morphometric data were expressed in micrometers (μm).

Statistical analysis

Giardia cyst counts were based on pooled fecal samples collected per group on seven alternate days post-infection. Because samples were pooled and individual animal data were not available, no statistical tests were applied to these values. The results are shown descriptively (mean ± standard deviation) in both the text and in Figure 2 to illustrate trends over time.

Grubbs' test was used to detect outliers, and the Kolmogorov–Smirnov test was applied to assess the normality of the variables. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for comparisons among groups. All statistical analyses were performed using GraphPad Prism version 9.5, with the significance level set at 5% ($p < 0.05$).

RESULTS

FOS treatment reduces *Giardia* lamblia infection

Oral supplementation of FOS resulted in a reduction of 77% of the parasite load of animals infected with *G. lamblia* (FOSIN group, $0.48\pm0.19\times10^6$ cysts/ml) compared to untreated infected animals (CTIN group, $2.11\pm0.81\times10^6$ cysts/ml). These values represent the mean ± standard error calculated from seven pooled samples per group, collected on alternate days post-infection (Days 2 to 14). (Figure 2).

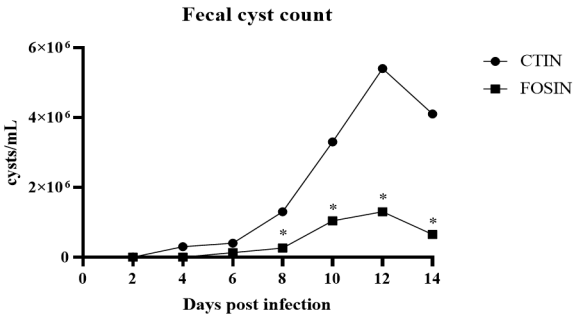

Figure 2. FOS treatment reduces *Giardia lamblia* infection. Each data point represents the cyst count from pooled fecal samples collected per group on seven alternate days post-infection. Statistical comparisons were not performed due to the pooled nature of the samples. The mean ± standard error of these seven pooled counts is reported in the text. n = 8.

Giardia lamblia infection changes glucose and lipid levels

According to the results presented in Table I, it was found that infection and early supplementation with FOS did not influence food consumption, body and relative liver weight, or high-density lipoprotein (HDL) levels. *Giardia lamblia* infection reduced serum cholesterol and triglyceride, and increased serum glucose levels, regardless of the treatment.

FOS treatment reduces hepatic oxidative stress in infected animals

Figure 3 displays oxidative stress in the liver. The hepatic levels of TBARS and hydroperoxides increased following infection with *G. lamblia*. Treatment with FOS in the presence or absence of infection had an opposite effect when compared to the infected group. The administration of FOS neutralized oxidative stress produced by the infection (Figures 3a and 3b). There was no difference in superoxide dismutase activity, but FOS supplementation increased catalase enzyme activity in infected gerbils compared to non-supplemented infected ones (Figure 3c and 3d).

FOS treatment improves histological parameters in infected animals

Giardia lamblia infection (CTIN group) reduced villus height and mucosal height and increased the crypt depth/villus ratio compared to the control group. Treatment with FOS with or

Table I. Food consumption, growth, and biochemical parameters.

Variables	CT	CTIN	FOS	FOSIN	*p*-value
Food intake (g/animal/day)	4.4 ± 0.2a	4.5 ± 0.4a	4.1 ± 0.3a	4.2 ± 0.3a	0.6412
Final body weight (g)	60.8 ± 2.2a	62.5 ± 1.0a	59.8 ± 1.3a	60.0 ± 2.0a	0.6768
Relative liver weight (%)	3.4 ± 0.1a	3.3 ± 0.1a	3.4 ± 0.1a	3.3 ± 0.1a	0.8078
Serum (mg/dL)					
Cholesterol	112.8 ± 17.2a	67.8 ± 4.6b	85.6 ± 7.3ab	48.6 ± 5.6b	0.0027
HDL	41.1± 2.6a	35.8 ± 5.2a	53.8 ± 14.8a	39.7 ± 11.2a	0.6068
Triglycerides	128.2 ± 19.9a	59.2 ± 10.8b	120.4 ± 10.8a	54.8 ± 8.0b	0.0003
Glucose	135.6 ± 5.7a	194.5 ± 14.8b	154.5 ± 8.2ab	192.2 ± 13.2b	0.0034

Different letters within the same row indicate statistically significant differences between groups, as determined by one-way ANOVA followed by Tukey's *post hoc* test ($p < 0.05$). Corresponding p-values are provided in the final column.

without infection (FOS and FOSIN groups) didn't change the intestinal parameters compared to the CT group. There was no change in crypt depth among the experimental groups (Figure 4).

Histological images from each experimental group (CT, CTIN, FOS, and FOSIN) were included (Figure 5), illustrating the results found.

DISCUSSION

Dietary prebiotics are defined as a non-digestible food ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota and benefit human health. Fructooligosaccharides (FOS) are oligosaccharides composed of linear chains of fructose units, linked by beta (2-1) bonds that have been increasingly included in food products and infant formulas due to their prebiotic effects, which stimulates the growth of nonpathogenic intestinal microflora (Sabater-Molina et al. 2009).

The present study assessed the impact of FOS on the metabolic and intestinal parameters of gerbils experimentally infected with *G. lamblia*. We evaluated body weight, food intake and relative liver weight, and found no differences among the groups, which suggests that oral supplementation of 250 mg of FOS in gerbils did not alter the dietary patterns of the rodents studied. We also found a great reduction (>70%) in cyst count after the preventive treatment with FOS. Shukla et al. (2016) observed that both prior and simultaneous supplementation with the prebiotic inulin, even in malnourished *Giardia*-infected gerbils, reduced both cyst and trophozoite counts and led to an increased fecal lactobacilli count. This is likely due to improved survival and colonization of lactobacilli in the gut, which may reduce the severity of giardiasis.

Some findings reveal that *G. lamblia* infection potentially impairs the metabolic status. Klimczak et al. (2024) described the impact of *G. lamblia* metabolites on the regulation of glucose and lipid metabolism, primarily through the inhibition of insulin action and the activation of protein kinase B (AKT) pathway. These alterations affect glucose and insulin levels in the host and influence lipid metabolism. In the present work, infected animals exhibited hyperglycemia and dyslipidemia, confirming that giardiasis may significantly impact carbohydrate, lipid, and hormonal metabolism. According to Khlaf & Shakir (2022), patients infected with *Giardia lamblia* exhibited a significant decrease in serum cholesterol and triglyceride levels, with no changes in HDL concentrations compared to

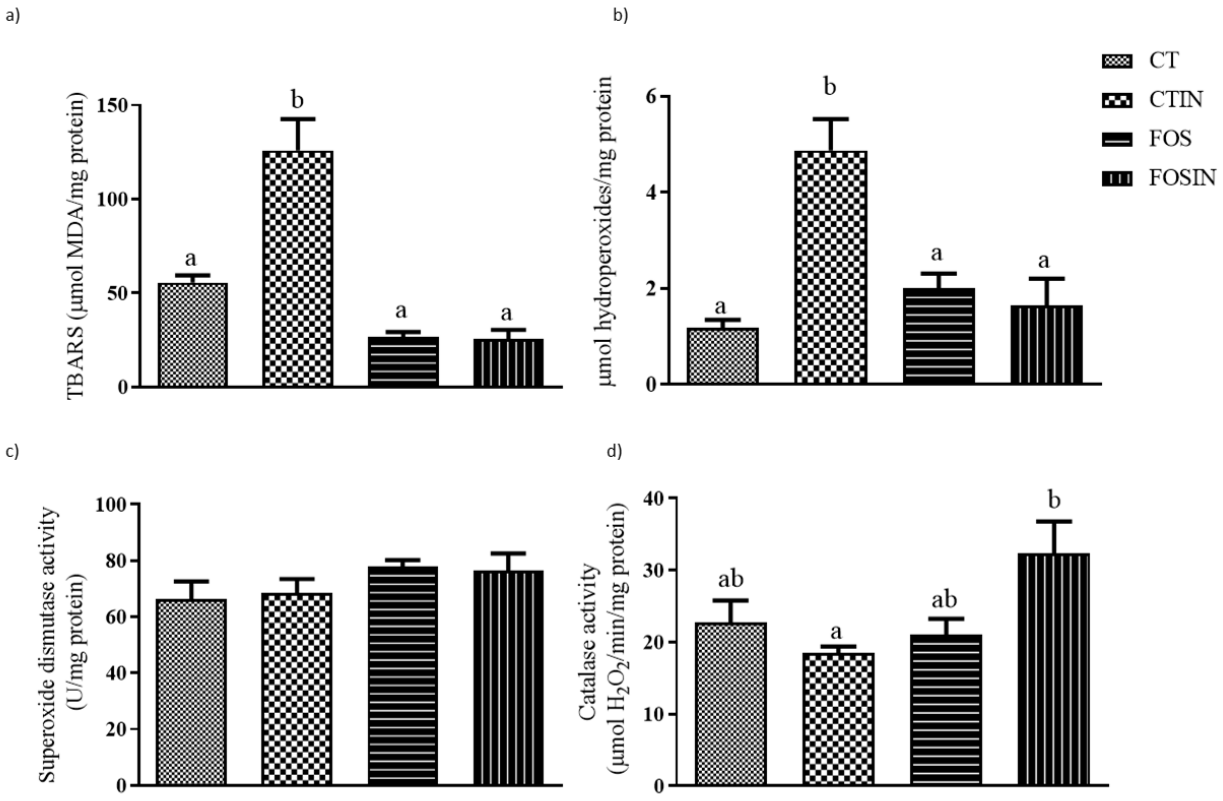

Figure 3. FOS treatment reduces hepatic oxidative stress in infected animals. (3a) TBARS $p < 0.0001$); (3b) Hydroperoxides ($p < 0.0001$); (3c) Superoxide dismutase enzyme activity ($p = 0.3418$); (3d) Catalase enzyme activity ($p = 0.0214$). Values are presented as mean ± standard error. Different letters identify statistical differences (ANOVA). n = 8.

the control group, corroborating the findings of the present study. These results may be related to the lipid requirements of *Giardia lamblia,* as the parasite acquires lipids and cholesterol from the external environment to survive, indicating active lipid metabolism at each stage of its development (Adam 2001). Hyperglycemia and dyslipidemia stimulate pro-inflammatory mechanisms, leading to insulin resistance and impaired insulin secretion, highlighting that giardiasis may significantly impact individuals with metabolic disorders (Klimczak et al. 2024). Although FOS supplementation did not alter metabolic parameters in infected animals in the present study, other authors have reported beneficial effects of prebiotics on lipid metabolism, including reduced absorption of cholesterol, bile acids, and triglycerides, leading to their increased excretion in feces (Pimentel et al. 2022, Kaewarsar et al. 2023).

We recognize the well-documented metabolic benefits of fructooligosaccharides (FOS) in various experimental models, particularly in animals with metabolic disturbances (Le Bourgot et al. 2018). However, to our knowledge, this is the first study evaluating the effects of FOS in Mongolian gerbils *(Meriones unguiculatus)* infected or not with *Giardia lamblia*. As such, the impact of FOS on glycemia and lipid metabolism in this specific host-parasite model remains poorly understood.

Our findings suggest that, in healthy gerbils, FOS supplementation does not significantly alter glucose or lipid profiles. This may reflect

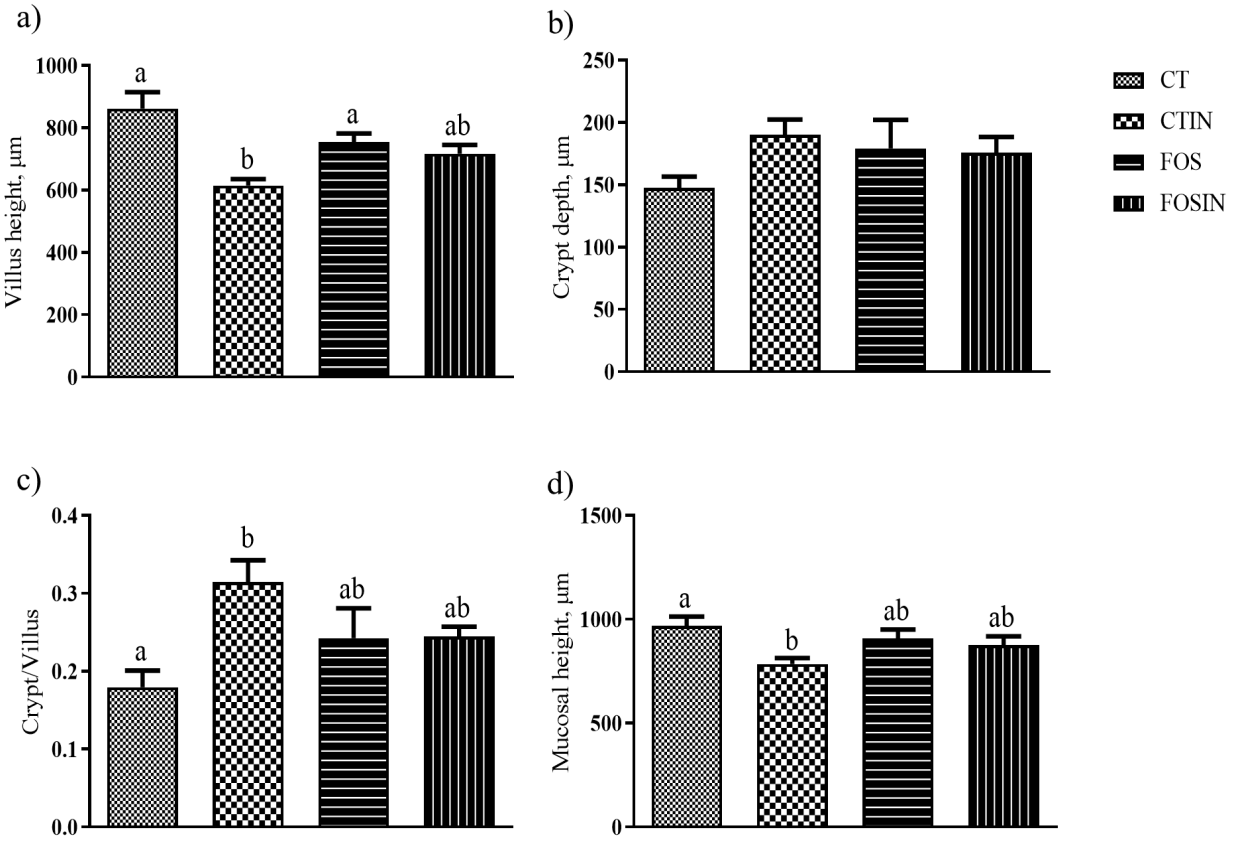

Figure 4. FOS treatment improves histological parameters in infected animals. (4a) Villus height; (4b) Crypt depth; (4c) Crypt /Villus ratio; (4d) Mucosal height. Values are presented as mean ± standard error. Different letters identify statistical differences ($p < 0.05$, ANOVA). n = 8.

species-specific differences, the physiological stability of the animals, or a context-dependent response in which the metabolic effects of FOS manifest only in the presence of underlying disturbances. Further studies are needed to clarify the mechanisms and conditions under which FOS exerts its metabolic effects in this species.

Oxidative stress is defined as the imbalance between the pro-oxidants and antioxidants, which play a significant role in the development and course of parasitic infections. This occurs when the concentration of antioxidants in the host decreases, while the concentration of oxidation products of cellular components increases (Pawłowska et al. 2023). Polyunsaturated fatty acids and other lipids are oxidized by free radicals, leading to the formation of conjugated dienes, malondialdehyde (MDA), and lipid hydroperoxides. Meanwhile, antioxidant factors, such as superoxide dismutase, catalase, and glutathione peroxidase, play a protective role against oxidative stress by scavenging reactive species (Demirci-Çekiç et al. 2022). In the present study, infection with *Giardia lamblia* increased hepatic lipid peroxidation, corroborating findings from previous human and experimental studies and providing further evidence that *Giardia* infections lead to elevated oxidative stress biomarkers (Masoori et al. 2024, Ismail et al. 2022). On the other hand, infected animals previously supplemented with FOS showed an improvement in antioxidant defenses, characterized by reduced levels

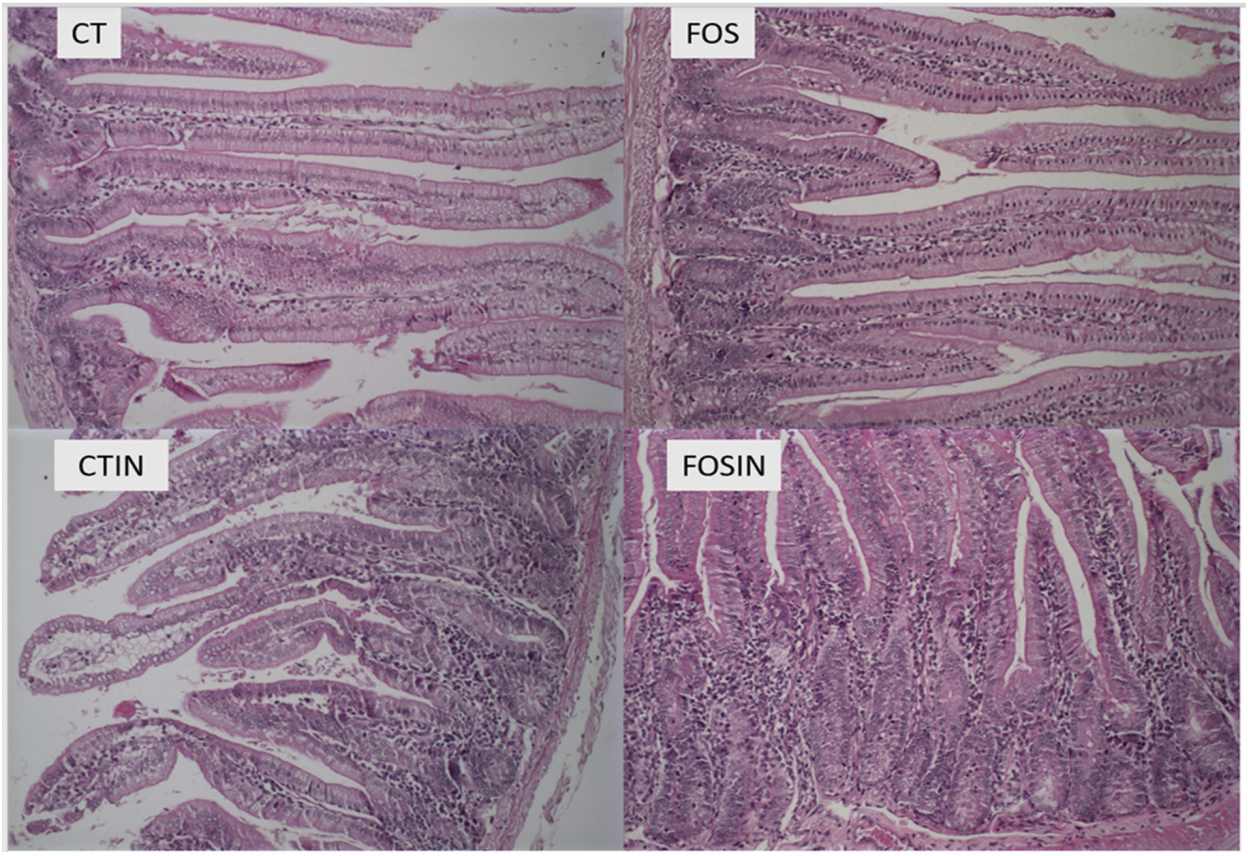

Figure 5. Representative histological sections of the small intestine from (a) uninfected control (CT), (b) infected control (CTIN), (c) uninfected and FOS-treated (FOS), and (d) infected and FOS-treated (FOSIN) gerbils. CT: Normal villus height, crypt depth, intact epithelial lining, and preserved mucosal architecture; CTIN: Villus atrophy, epithelial disruption, and signs of mucosal inflammation, consistent with *Giardia lamblia* infection; FOS: Intestinal morphology similar to CT, with well-preserved villi and crypts, indicating no adverse histological effects from FOS treatment alone; FOSIN: Villus and crypt morphology comparable to CT and FOS groups, with preserved epithelial integrity and mucosal structure, suggesting that FOS pre-treatment mitigated the histological damage typically caused by *Giardia* infection. 20×. Hematoxylin and eosin (H&E) staining.

of TBARS and hydroperoxides, along with increased catalase activity, without changes in SOD activity. This suggests a selective activation of specific antioxidant defense mechanisms, possibly reflecting a compensatory response more focused on the detoxification of hydrogen peroxide, a downstream product of SOD activity (Jomova et al. 2024).

Moreover, the increase in TBARS and hydroperoxides in infected animals indicates elevated oxidative stress. It is possible that superoxide dismutase (SOD) activity was already sufficient to manage superoxide levels, or that its regulation is less sensitive to changes induced by FOS or infection in this context. In contrast, catalase activity may have been upregulated in response to increased hydrogen peroxide levels, which can result from both the infection itself and the action of SOD. A previous study by our group (Silva et al. 2022) also reported increased catalase activity while SOD activity remained stable, even when oxidative damage markers such as malondialdehyde (MDA) and hydroperoxides were elevated. This may be due to the higher binding affinity of catalase for certain compounds, enhancing its activity. Thus,

the unchanged SOD activity, alongside increased catalase activity and elevated oxidative damage markers, suggests a differential modulation of antioxidant enzymes, with catalase playing a more prominent role in the oxidative stress response under these conditions.

Luo et al. (2022) investigated the impact of pre-treatment with fructooligosaccharides (250 mg/kg) on growth performance, antioxidant status, and immune response in a piglet model infected with enterotoxigenic *Escherichia coli*. The authors observed an improvement in antioxidant capacity in both the intestine and serum, evidenced by reduced serum malondialdehyde levels and increased activities of serum catalase and glutathione peroxidase, with no changes in superoxide dismutase (SOD) activity. These effects were attributed to the upregulation of genes involved in the production of various antioxidant enzymes. A systematic review by Costa et al. (2022) further supported these findings, showing that FOS supplementation was consistently associated with anti-inflammatory and antioxidant effects, likely contributing to enhanced gut immune function. Collectively, our data show that previous treatment with FOS reduced oxidative stress in gerbils infected with *Giardia lamblia*.

Corroborating the findings of the present work, previous publications by our research group have shown that *Giardia lamblia* infection in gerbils worsens the morphological characteristics of the intestinal epithelium, which were reversed by treatment with the probiotic *Saccharomyces boulardii* (Ribeiro et al. 2018, 2021). Shukla et al. (2016) showed that supplementation with the prebiotic inulin restored weight gain and gut morphology in *Giardia duodenalis*-infected malnourished mice. The authors proposed that prebiotic fermentation promotes the multiplication of beneficial bacteria (lactobacilli and bifidobacteria) which produce short-chain fatty acids (SCFA), reducing intestinal pH, improving both the proliferation of gut epithelial cells and the integrity of intestinal cellular junctions, leading to a healthier colon. Kocot et al. (2022) reported that dietary supplementation with FOS, under inflammatory and non-inflammatory conditions in BALB/c mice, was effective in maintaining histological and morphometric parameters, as well as IgA production, thereby protecting the gut barrier. Among the benefits associated with higher levels of short-chain fatty acids (SCFAs) is their use as energy substrates by intestinal mucosal cells, which leads to suppressed inflammatory responses and improved intestinal epithelial function and microbiota composition. Liu et al. (2020) demonstrated these effects in a study examining the impact of a diet containing 250 mg/kg of fructooligosaccharides (FOS) on the intestinal health of weaned pigs exposed to enterotoxigenic *Escherichia coli*. The authors attributed the beneficial outcomes to the fermentation of FOS, which significantly increased the abundance of *Bifidobacterium* and *Bacillus* in the cecum and mitigated the disruption of intestinal epithelium caused by the pathogen. In this regard, although preliminary, our results support the use of FOS to mitigate the deleterious effects of *Giardia lamblia* infection on intestinal architecture.

This study has some limitations that should be acknowledged. First, the sample size was relatively small, with eight animals per group, which may limit the statistical power and generalizability of the findings. Additionally, important analyses such as gut microbiota profiling and measurement of short-chain fatty acids (SCFAs) were not performed. Although these parameters are highly relevant and could provide deeper insight into the mechanisms involved, they were beyond the scope of our current objectives. We used gerbils as the

experimental model and focused specifically on morphological, biochemical, and parasitological parameters. We highly recommend that future studies incorporate these analyses to better elucidate the complex interactions between FOS supplementation, the gut microbiota, and host metabolism.

CONCLUSIONS

Our results demonstrated the efficacy of FOS supplementation in preventing and attenuating damage caused by *G. lamblia* infection. FOS reduced the parasitic load, improved the metabolic profile, inhibited hepatic oxidative stress, and attenuated intestinal mucosal damage caused by the infection, thereby preserving its architecture. Our study was the first to demonstrate the benefits of prior FOS treatments in both infected and uninfected gerbils, paving the way for future research to address numerous questions regarding the mechanisms of FOS action in gerbils, including intestinal SCFA production, microbiota composition, and immune response.

Acknowledgments

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