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Original article

Single or combined use of intermittent fasting and probiotics reduce *Campylobacter* colonization in the murine gut

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Abstract

The objective of the present study was to investigate the impact of single or combined use of intermittent fasting and probiotics, which have been recommended as an alternative to antibiotics in recent years due to their proven efficacy, against *Campylobacter* colonization in mice. For this purpose, mice infected with *Campylobacter jejuni* were divided into groups and exposed to intermittent fasting and probiotics, alone and together. At the end of the experimental study, ileum and cecum contents were obtained for microbiological analyzes, and stomach and intestinal tissue samples were collected for histopathological analyzes. It was determined that the level of *C. jejuni* colonization in the ileum and cecum of mice in the positive control group (PC group) was significantly higher than in the other groups ($p<0.05$). It was also determined that the level of *C. jejuni* colonization in the ileum of mice in the probiotic and intermittent fasting group (PB +IF group) was lower than in the other groups and the difference was statistically significant ($p<0.05$). As a result of the histological analyzes, mild inflammatory reaction was observed to occur in the stomach and intestinal tissues of the animals in the experimental groups, and the severity of the inflammation was lower in the PB +IF group than in the other groups. The findings of this study indicate that single or combined use of intermittent fasting and probiotics may represent a safe and feasible strategy for the control of *Campylobacter* infections.

Keywords: *Campylobacter jejuni*, intermittent fasting, mice, probiotic

Introduction

Campylobacter is one of the most important foodborne pathogens with increasing prevalence all over the world (Liu et al. 2022). Campylobacter is found commensally in the intestines of many animals, especially poultry, and contaminate the environment with the feces of these animals (Ogden et al. 2009). People become infected by consuming contaminated food and water, especially poultry products that have not undergone adequate heat treatment (Sahane, 1992). *Campylobacter* infections are often overlooked or not given sufficient importance by routine laboratories because they cause self-limiting infections without the need for any treatment. However, Campylobacter that cause serious infections in individuals with suppressed immune systems should be treated or controlled with antibacterial drugs (Janssen et al. 2008). The indiscriminate use of antibiotics has emerged as a global concern over the last decade, with multi-resistant bacteria on the rise (Prestinaci et al. 2015). Scientists have investigated alternative treatment methods to overcome the drawback due to antibiotic resistance. In recent years, hypotheses have been put forward about whether anorexia occurring in infectious diseases is a natural defense mechanism of the body (Ayres and Schneider 2009, Janssen et al. 2023). Although no scientific research has been conducted to test this hypothesis so far, studies have been accelerated to investigate the role of food restriction or intermittent fasting in many diseases, such as infectious diseases, diabetes, obesity and cancer (Godínez-Victoria et al. 2014, Longo and Mattson 2014, Morales-Suarez-Varela et al. 2021). In contrast with the findings of earlier studies indicating that the immune system is suppressed in cases of hunger or anorexia (Katona and Katona-Apte 2008), recent studies have demonstrated that short-term food restrictions play a pivotal role in the fight against infections (Godínez-Victoria et al. 2014, Bhatti and Mindikoglu 2022, Grundler et al. 2023). One of the most striking studies on this subject was conducted by Wing and Young (1980) in mice exposed to acute starvation. The researchers observed a notable decrease in mortality rates following infection with an intravenous dose of *Listeria monocytogenes*, which is known to be lethal to mice fed without restriction, in mice that had undergone short-term starvation. In recent years, studies on bacteria that cause gastrointestinal tract infections such as *Salmonella* have revealed that both the intestinal colonization and the pathological damage caused by these agents in the intestines decreased in starved animals (Godínez-Victoria et al. 2014). However, food restriction can also lead to disruption and alteration of the intestinal flora. In a study conducted by Ali et al. (2021), while significant increases were reported in the number of some bacteria, including *Dorea, Klebsiella, Faecalibacterium, Sutterella* and *Parabacteroides*, decreases were noted in the number of other bacteria such as *Coprococcus*, *Clostridium_XlV* and *Lachnospiracea* in humans following intermittent fasting. Microorganisms that comprise the intestinal flora play a pivotal role in the fight against diseases, particularly gastrointestinal infections, in many different ways such as lowering the pH of the intestine, immunomodulation, reducing the colonization of pathogenic microorganisms or disrupting their metabolism (Pickard et al. 2017). Therefore, disruption of the intestinal flora during food restriction is likely to interfere with the body's fight against pathogens. For this reason, in case of food restriction, external probiotic supplementation is considered mandatory to restore the intestinal flora (Mazziotta et al. 2023). Many different probiotics, mainly comprising bacteria belonging to the genera *Lactobacillus*, *Bacillus*, *Bifidobacterium* and *Streptococcus* as well as yeasts of the genus *Saccharomyces*, are commercially available and frequently recommended to patients by physicians.

The objective of the present study was to investigate the impact of single or combined use of intermittent fasting and probiotics, which have been recommended as an alternative to antibiotics in recent years due to their proven efficacy, on *Campylobacter* colonization in mice.

Materials and Methods

Bacterial strain

A field strain of *C. jejuni* isolated from cattle feces in our laboratory was used for experimental infection of mice. This strain possessed *FlaA*, *RaJ*, *DnaJ*, *VirB* and *GyrA* genes and was resistant to ciprofloxacin. The field strain was inoculated on sheep Blood Agar (Oxoid, UK) and incubated at 37°C for 24-48 hours in a microaerobic environment. Four to five of growing colonies were transferred to *Brucella* broth medium (HiMedia, India) and incubated at 37°C with shaking at 125 rpm for one day. The bacterial culture was then subjected to centrifugation at 3500 g for 10 minutes at 4°C. The resulting pellet was diluted with Phosphate-Buffered Saline (PBS) to 10^7 colony-forming units (CFU) of bacteria per ml and administered to mice.

Experimental groups

A total of 40 female Balb/c mice (5-6 weeks old) weighing between 18 and 22 g were used in the experiments. The animals were housed in standard mouse

cages in rooms with a temperature of 21-24°C, 55% ± 10% humidity, 12 h of light and 12 h of darkness and, allowed unlimited access to sterile distilled water throughout the experimental study.

The mice were divided into five groups, each comprising eight animals: intermittent fasting group (IF group), probiotic group (PB group), probiotic and intermittent fasting group (PB + IF group), positive control group (PC group) infected with *C. jejuni* without any treatment, and negative control group (NC group) without experimental infection.

Experimental infection

This study was conducted with the approval of Bingöl University Animal Experiments Local Ethics Commission, Türkiye (06/11/2023-07-01).

Before starting the experimental study, one or two freshly emptied fecal pellets were collected from each animal in the groups and analyzed for the presence of *Campylobacter* species.

For intestinal microbiota dysbiosis prior to the experimental infection, mice were given an antibiotic mixture consisting of gentamicin (35 mg/ml), vancomycin (45 mg/ml), metronidazole (215 mg/ml), and colistin (850 U/ml) in drinking water for three days and then fed with normal sterile water for one day (Giallourou et al. 2018).

Fifteen minutes before the bacterial inoculation, a 5% sodium bicarbonate solution was administered via intragastric catheter to each mouse, resulting in the neutralization of the gastric pH. The experimental infection was performed in two different ways, namely, a single--dose and a double-dose approach. Single and double dose administration was preferred to determine whether there was a difference in the effects of probiotics and intermittent fasting on *C. jejuni* colonization. For this purpose, the animals in the groups were placed in separate cages with four mice in each group. For single-dose administration, mice were orally infected with 0.1 ml (10^7 CFU/ml) total volume of bacterial suspension via intragastric probe. The two-dose administration group was infected with *C. jejuni* again on the third day of the experimental study with the same method and dose. Mice in group PB infected with *C. jejuni* received 0.1 ml (containing between 10^7 and 10^8 CFU/ml) of a commercial probiotic (Probiogest, ZOOTECH) containing *Enterococcus faecium*, *Lactobacillus* spp., *Bacillus* spp., *Saccheramyces sereviciae* and *Pedicoccus* spp. orally one hour post-infection. On the following days of the experimental study, the probiotic was continued to be added to the drinking water of the animals. In the negative control group, 0.1 ml of sterile PBS was given in the same way. Following infection, animals in the IF and PB+IF groups were given mouse chow for 4 hours at 20-hour intervals and intermittent fasting was induced for a duration of seven days. Animals in the other groups were fed without restriction with mouse chow. The group that received a single dose of bacteria was euthanized under anesthesia after seven days and the group given two doses of bacteria after 14 days and, autopsies were performed. In order to determine *C. jejuni* colonization levels in the intestines, the ileum and cecum contents of the mice were placed in sterile sample containers and transported promptly to the laboratory. For histopathological analyzes, stomach and intestinal tissues of the animals were collected and transferred to formaldehyde solution.

Determination of bacterial colonization and molecular diagnosis

The ileum and cecum contents were diluted in 1 ml *Brucella* broth medium. The prepared suspension was diluted 10-fold with PBS and the colonization level was determined by colony counting method. For this purpose, each of the 10-fold diluted bacterial suspensions was inoculated (100µl) onto Charcoal Cefoperozone Deoxycholate agar (CCDA) (Oxoid) and incubated in a microaerobic environment at 37°C for 24-48 hours. Following incubation, the bacterial concentration was determined by counting the colonies that grew (Khattak et al. 2022). The colonies were identified by a polymerase chain reaction (PCR) using a pair of specific primers for confirmation of *C. jejuni* (Açik and Çetinkaya 2006).

Histopathology

Processing of tissue samples

At necropsy, the stomach, duodenum, ileum and cecum tissues resected from three randomly selected mice in each group were opened along their long axes and the digestive contents were cleaned with sterile saline. The tissues were then prepared according to the Swiss rolling technique and subjected to histopathologic examination (Bialkowska et al. 2016). The tissue samples were transferred to a 10% buffered formalin solution and fixed for 48 hours. After fixation, tissues were dehydrated by passing through a series of increasing concentrations of ethanol (50%, 70%, 80%, 96% and 100%) and, then cleared with xylene. Following paraffinization process, the cleared tissues were embedded in melted paraffin and paraffin blocks were prepared. 4 µm thick sections were taken from each paraffinized tissue block using a rotary microtome (Leica, 2125RT) and transferred to slides. Paraffin sections were stained with Hematoxylin-Eosin (H&E) after

Table 1. *Campylobacter jejuni* concentration levels in the ileum and cecum of mice.

Periods of time	Tissue	Groups					P*
		NC	PC	PB	IF	PB + IF	
7th day	Ileum	0.0 (0.0-0.0)e	11100 (7200-23000)[a]	24.5 (8.7-41)[c]	55.5 (35-130)[b]	4.25 (2.7-5.6)[d]	0.001
	Cecum	0,0 (0.0-0.0)[c]	48000 (8400-68000)[a]	6.35 (5.2-7.2)[b]	9.05 (1.4-23)[b]	3,0 (0.84-4.1)[b]	0.002
14th day	Ileum	0.0 (0.0-0.0)[c]	87000 (23000-180000)[a]	565 (220-860)[b]	8,75 (3.2-22)[c]	0.65 (0.16-1.3)[d]	0.001
	Cecum	0,0 (0.0-0.0)[c]	270000 (17000-540000)[a]	4.25 (1.2-5.3)[b]	5.65 (3.3-12)[b]	1.45 (0.18-2)[b]	0.002

Data are expressed as $x10^3$. Data are presented as Median (Minimum-Maximum) (n=4). *Kruskal-Wallis test was used. Dunn's multiple comparison test was used to determine the difference between groups. Different letters indicate statistically significant difference between the groups ($p<0.05$).

deparaffinization and rehydration (Bancroft and Gamble 2008). Finally, the slides were examined using an imaging system of adapted light microscope (Leica, DM2500/DFC295).

Histopathologic evaluation

Histopathologic lesions in stomach, duodenum, ileum and cecum tissues were evaluated semi-quantitatively by averaging five randomly selected non-overlapping areas at 20x objective magnification using a light microscope. Gastric tissues were evaluated according to three parameters (presence of inflammation, neutrophil activity and presence of glandular atrophy) using a minor modification of the Updated Sydney System. Accordingly, each parameter was assigned a grade from 0 to 3 (0 = no lesion; 1 = mild; 2 = moderate; 3 = severe) and scored as the final mean of the sum of the relevant parameters (Eaton et al. 2007, Sipponen and Price 2011). Duodenum, ileum and cecum tissues were evaluated by considering the following parameters: epithelial damage- 0 (no lesion / normal), 1 (mild / superficial erosion), 2 (moderate / focal erosion), 3 (severe / diffuse erosion or ulceration); inflammatory cell infiltration- 0 (no lesion / normal), 1 (mild / focal increase in lamina propria), 2 (moderate / diffuse increase in lamina propria), 3 (severe / dense infiltration in lamina propria and submucosa); crypt abscess- 0 (no lesion / normal), 1 (present / neutrophil accumulation in the crypt lumen) and submucosal edema- 0 (no lesion / normal), 1 (present). The final average of the sum of the relevant parameters was then calculated (Chang and Miller 2006).

Statistical analyses

The statistical software package SPSS 22.0.0 for Windows (Release 22.0.0, SPSS Inc., The Apache Software Foundation, 1989-2022) was employed to determine the intestinal colonization levels of *C. jejuni* and to derive the statistical values of histopathological scores obtained from the examined tissues according to the groups. The conformity of the data to normal distribution was assessed with the Shapiro-Wilk test, and the homogeneity of variances was assessed with the Levene test. Data that did not meet parametric assumptions [presented as Median (Minimum-Maximum)] were compared with the Kruskal-Wallis and Dunn's multiple comparison tests. In all statistical analyses, the significance level was accepted as $p<0.05$ (Altman et al. 1983).

RESULTS

The Levels of Bacterial Colonization

The presence of *C. jejuni* was detected in the feces of the mice in the experimental groups from the third day following infection. It was determined that the level of *C. jejuni* colonization in the ileum and cecum of mice in the PC group, which received a single dose of bacteria, was significantly higher than the other groups ($p<0.05$). The level of *C. jejuni* colonization in the ileum of mice in the PB+IF group was significantly lower than in the PB and IF groups ($p<0.05$). It was also determined that the bacterial colonization level in the cecum of mice in the PB +IF group was similar to the PB and IF groups and the difference was not statistically significant. ($p>0.05$). Similar findings were obtained in mouse samples from the groups that received double doses and were euthanized on day 14. At the end of the 14th day, it was determined that the difference between the ileum bacterial colonization levels of the animals in the euthanized groups was statistically significant and the lowest bacterial colonization was noted in the PB+IF group (Table 1).

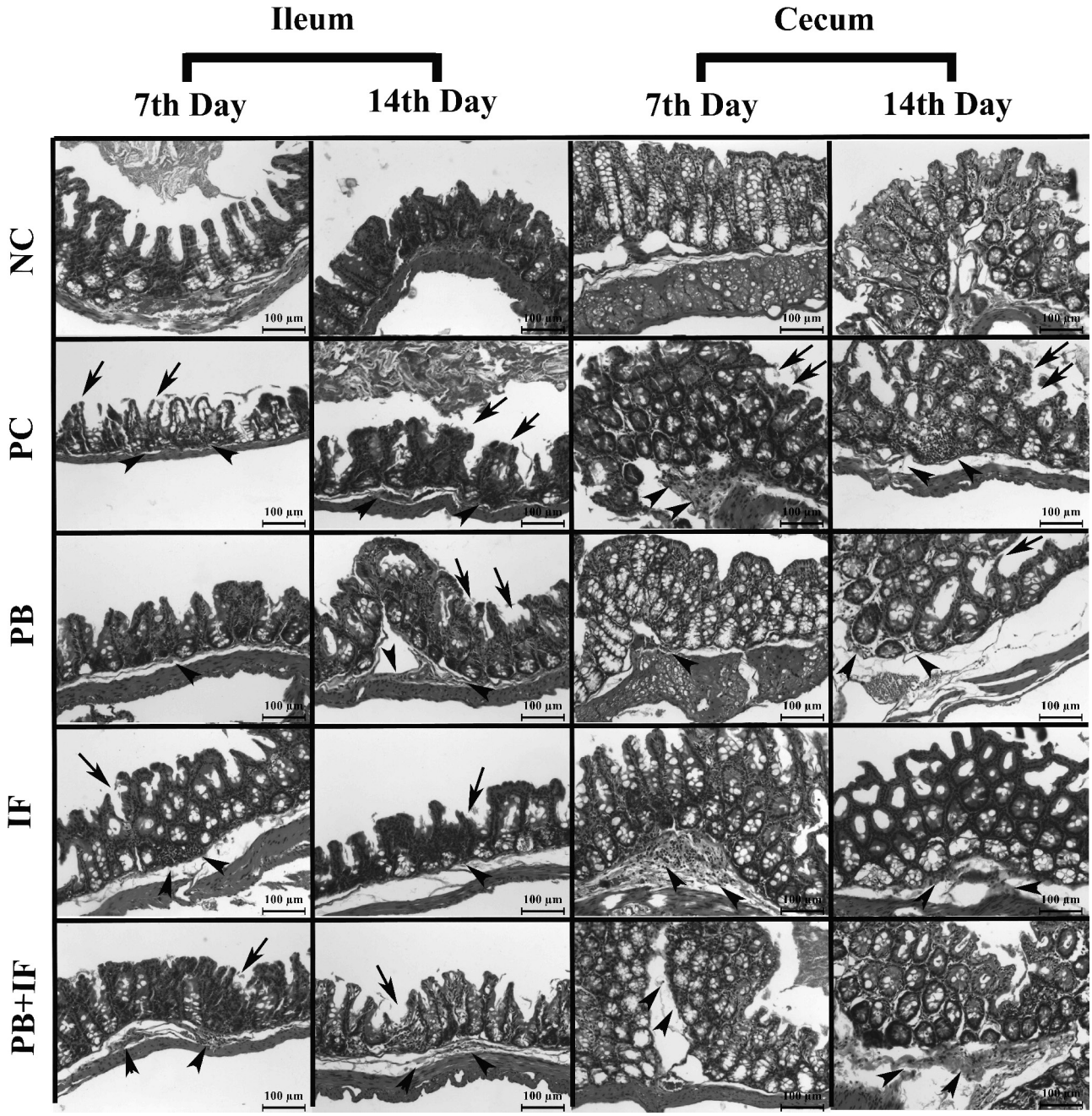

Fig. 1. Histopathology of ileum and cecum tissues. Epithelial cell damage (epithelial erosion and desquamation) (black arrows) and submucosal (lamina propria) inflammatory cell infiltrations (neutrophils and lymphocytes) (black arrowheads). H&E staining, x200 magnification, measuring bars 100 µm.

Histopathologic findings

At the end of the first and second week, normal histologic structure was observed in the stomach, duodenum, ileum and cecum tissues of the animals in NC group. In contrast, polymorphonuclear (neutrophils) and mononuclear (lymphocytes and plasma cells) inflammatory cells were detected in the submucosa (lamina propria) of stomach tissues of mice in the PB and PC groups. In the duodenum, ileum and cecum, the presence of villous atrophy, marked desquamation and erosion of epithelial cells was noted, accompanied by focal neutrophilic and lymphocytic inflammatory cell infiltrations within the lamina propria. In parallel with the increase in the severity of histopathologic lesions in ileum and cecum tissues, the increase in goblet cell loss was remarkable (Fig. 1). The histopathologic lesions were more widespread and severe in PC and PB groups, whereas they were somewhat lesser in IF and PB + IF groups. In general, inflammatory reaction in ileum tissues was more prominent compared to other tissues (Table 2).

Table 2. Histopathologic scoring of stomach and intestinal lesions in mice.

Periods of time	Tissue		Groups					P*
			NC	PC	PB	IF	PB+IF	
7th day	Stomach	I	0(0:0)^{c}	2(2:2)^{a}	2(2:2)^{a}	1(1:1)^{b}	1(1:1)^{b}	0.007
		NA	0(0:0)	1(1:1)	1(1:1)	1(0:1)	1(0:1)	0.078
		GA	0(0:0)^{b}	1(1:1)^{a}	1(1:1)^{a}	1(1:1)^{a}	1(1:1)^{a}	0.007
	Duodenum	ED	0(0:0)^{b}	2(1:2)^{a}	1(1:2)^{a}	2(1:2)^{a}	1(1:1)^{a}	0.044
		ICI	0(0:0)^{b}	2(2:2)^{a}	2(1:2)^{ab}	2(1:2)^{ab}	1(1:2)^{ab}	0.047
		CA	0(0:0)	1(1:1)	1(1:1)	1(0:1)	0(0:1)	0.066
		SE	0(0:0)	0(0:1)	0(0:1)	0(0:1)	0(0:1)	0.866
	Ileum	ED	0(0:0)^{b}	3(2:3)^{a}	1(1:2)^{ab}	1(1:2)^{ab}	1(1:2)^{ab}	0.027
		ICI	0(0:0)	1(1:2)	1(1:2)	1(1:2)	1(1:2)	0.085
		CA	0(0:0)	0(0:1)	1(0:1)	1(1:1)	1(1:1)	0.066
		SE	0(0:0)	0(0:1)	0(0:1)	0(0:1)	0(0:1)	0.866
	Cecum	ED	0(0:0)^{b}	2(2:3)^{a}	1(1:1)^{a}	2(1:2)^{a}	2(1:2)^{a}	0.023
		ICI	0(0:0)^{c}	2(2:2)^{ab}	1(1:1)^{b}	2(2:2)^{a}	2(1:2)^{ab}	0.014
		CA	0(0:0)	1(1:1)	0(0:1)	1(1:1)	1(0:1)	0.066
		SE	0(0:0)	1(1:1)	0(0:1)	1(1:1)	1(0:1)	0.066
14th day	Stomach	I	0(0:0)^{c}	2(2:2)^{a}	2(2:2)^{a}	1(1:1)^{b}	2(2:2)^{a}	0.007
		NA	0(0:0)^{b}	1(1:1)^{ab}	1(1:1)^{a}	0(0:1)^{ab}	1(1:2)^{a}	0.03
		GA	0(0:0)^{b}	1(1:1)^{ab}	1(1:1)^{a}	1(0:1)^{ab}	1(1:1)^{a}	0.03
	Duodenum	ED	0(0:0)^{c}	2(2:2)^{a}	2(1:2)^{ab}	1(1:1)^{abc}	1(0:1)^{bc}	0.018
		ICI	0(0:0)^{c}	2(2:2)^{a}	2(2:2)^{a}	1(1:1)^{b}	1(1:1)^{b}	0.007
		CA	0(0:0)	1(1:1)	1(1:1)	1(0:1)	0(0:1)	0.066
		SE	0(0:0)	1(0:1)	0(0:1)	0(0:1)	0(0:1)	0.592
	Ileum	ED	0(0:0)^{b}	1(1:2)^{a}	1(1:1)^{a}	1(1:1)^{a}	1(1:1)^{a}	0.017
		ICI	0(0:0)	2(1:2)	1(1:2)	1(1:2)	1(1:2)	0.078
		CA	0(0:0)^{b}	1(1:1)^{ab}	1(0:1)^{ab}	1(1:1)^{b}	1(1:1)^{b}	0.03
		SE	0(0:0)	0(0:1)	0(0:1)	0(0:1)	0(0:1)	0.866
	Cecum	ED	0(0:0)^{b}	2(2:2)^{a}	1(1:1)^{b}	1(0:1)^{b}	1(0:1)^{b}	0,024
		ICI	0(0:0)^{c}	2(2:2)^{a}	1(1:1)^{b}	1(1:1)^{b}	1(1:1)^{b}	0.007
		CA	0(0:0)	1(1:1)	0(0:1)	0(0:1)	0(0:1)	0.183
		SE	0(0:0)	1(1:1)	0(0:1)	1(0:1)	1(0:1)	0.165

Data are presented as Median (Minimum-Maximum) (n=4). *Kruskal-Wallis test was used. Dunn's multiple comparison test was used to determine the difference between groups. Different letters indicate statistically significant difference between the groups ($p<0.05$). I – inflammation, NA – neutrophil activity, GA – glandular atrophy, ED – epithelial cell damage, ICI – inflammatory cell infiltration, CA – crypt abscess, SE – submucosal edema.

Discussion

A variety of methodologies have been successfully employed to establish experimental infections in mice with *Campylobacter*, including the administration of antibiotics that cause intestinal dysbiosis, the use of mice with limited microflora, and the introduction of abiotic or germ-free mice (Chang and Miller 2006, Giallourou et al. 2018, Mouchensavi et al. 2020). In the present study, intestinal dysbiosis was performed in mice using the method proposed by Giallourou et al. (2018) to establish experimental infection. However, unlike the study of Giallourou et al. (2018), none of the experimental groups in this study developed any clinical signs of disease including diarrhea and no visible macroscopic findings were observed at autopsy. Histopathologic analyzes revealed varying degrees of inflammatory cell infiltration, villus atrophy, desquamation and cell erosions, indicating mild to moderate inflammation, with notable differences between the groups. Similar findings were also obtained in previous studies using a mouse model for *C. jejuni* infection (Chang and

Miller 2006, O'Loughlin et al. 2015, Chen et al. 2024). The *Campylobacter* strain used in the experimental study, along with parameters such as virulence properties, breed and age are considered to have potential roles in the occurrence of different clinical and pathological findings in mice (Stahl et al. 2014, Chen et al. 2024). All these data suggest that mouse models with intestinal dysbiosis might be an ideal method to identify host responses to *C. jejuni* and to understand the pathogenesis of the disease, even if they do not produce obvious clinical signs and enteritis.

It has long been speculated that disease-induced starvation is a defense mechanism of the body (Ayres and Schneider 2009, Janssen et al. 2023). Given that the body requires a substantial amount of energy to combat infection and maintain the inflammatory response, it is intriguing that anorexia is a key feature of disease behavior. Although the role of anorexia in the control and prevention of disease has not been fully established, studies have shown that intermittent fasting, defined as a pattern of alternating between periods of eating and fasting, has potential health benefits (Sun et al. 2024). Intermittent fasting is induced by using many different methods such as zero-calorie alternate day fasting, fasting diet two days a week, time-restricted nutrition including fasting for 12-24 hours a day, and intermittent fasting including long fasting periods (Brogi et al. 2024, Elortegui Pascual et al. 2024). Studies indicate that time-restricted fasting can be applied for a long time without causing weight loss in animals and is healthier than the calorie restriction method (Jaramillo et al. 2023). In this study, a time-restricted intermittent fasting protocol of 20 hours fasting/4 hours feeding was applied. With this method, the animals were able to compensate for the caloric loss that occurs following prolonged fasting in a 4-hour period. This method was applied for two different periods of time, 7 and 14 days, and no difference was detected between the weight losses of the animals in the groups during the experimental study. Also, in previous studies that employed longer durations of intermittent fasting, no significant decrease in the body weight of the animals was observed (Cavalcante et al. 2021, Sun et al. 2024). It is of great importance that animals do not lose weight during intermittent fasting in order to avoid stress due to caloric needs.

Although many studies have demonstrated the efficacy of intermittent fasting in the management of various diseases including cancer, metabolic, cardiac, neurodegenerative dysfunctions and obesity, there is a paucity of information on its potential role in the control of infectious diseases (Morales-Suarez-Varela et al. 2021, Tiwari et al. 2022). Godínez-Victoria et al. (2014) reported that prolonged intermittent fasting reduced the intestinal and systemic bacterial load and resulted in higher intestinal IgA levels after *Salmonella* Typhimurium infection compared to the fed without restriction group. In the present study, it was determined that the *C. jejuni* colonization level was significantly decreased in the time-restricted intermittent fasting groups compared to the fed without restriction group. In spite of the fact that the role of intermittent fasting in the treatment and control of infectious diseases is not fully understood, studies have shown that the low level of stress induced by intermittent fasting enhances the ability of cells to cope with stress and resist disease (Longo and Mattson 2014). Intermittent fasting has also been reported to support immunity by activating autophagy (Hannan et al. 2020). Considering the regulatory functions of intermittent fasting on autophagy and immunity, it is predicted that it may serve as a preventive strategy against infections. Despite the paucity of scientific studies, it is hypothesized that calorie restriction due to intermittent fasting contributes to the deceleration of the growth of gastrointestinal pathogenic bacteria and prevention of colonization. On the other hand, it is clear that Campylobacter, like other bacteria, require energy to colonize the intestine. Intermittent fasting reduces the amount of nutrients in the gut and is likely to force a competition for nutrients between *Campylobacter* and gut bacteria. Furthermore, a lack of sufficient nutrients is thought to reduce the colonization of the intestine by pathogens.

Nutrient restriction due to intermittent fasting prevents the growth of pathogens and is expected to have negative effects on intestinal microbiota. Indeed, studies have reported that intermittent fasting causes changes in intestinal microbiota (Ali et al. 2021, Popa et al. 2023). According to Hu et al. (2023), intermittent fasting has been observed to induce alterations in the microbiota by increasing the number of bacteria and parabacteria known to have beneficial effects on health. Khan et al. (2022) reported that intermittent fasting caused an increase in the number of intestinal bacteria such as *Bifidobacterium*, *Lactobacillus*. *Bifidobacteria* have been demonstrated to reduce TNF-α expression, produce acetate essential for butyrate and propionate synthesis, regulate immune cells and thus cause a decrease in inflammatory responses (Parada Venegas et al. 2019). In the current study, intestinal dysbiosis was performed with an antibiotic cocktail before infection to successfully induce *Campylobacter* colonization in mice. In addition, applying intermittent fasting to animals was expected to cause further changes in the intestinal microbiota. One of the experimental groups was supplemented with probiotics in order to reorganize the gut microbiota, which is likely to occur due to intermittent fasting. It was established that *C. jejuni*

colonization level in the ileum of mice in this group decreased at a higher rate than the groups that was administered intermittent fasting and probiotics alone. It was plausible to suggest that the decrease in *C. jejuni* due to intermittent fasting was further deepened with the effect of probiotic bacteria.

Studies have shown that probiotic intake during intermittent fasting has a positive effect on intestinal health and supports normal gut flora (Zmora et al. 2019). Teker et al. (2024) reported that the combined use of intermittent fasting and probiotics caused unique and potentially beneficial changes in the ileum and colon tissues of rats. Likewise, histopathological lesions in the stomach and intestinal tissues of mice infected with *C. jejuni* in the IF and PB +IF groups were less severe and widespread in comparison with the other groups in the present study. In addition, it has been reported that probiotics affect hormones associated with appetite and satiety, enabling individuals to adapt to fasting programs more easily and contribute to managing hunger (Cabral et al. 2021). Probiotic intake during intermittent fasting also contributes to weight loss and glycemic recovery and, therefore provides important psychological support to individuals (Tay et al. 2020). Therefore, it was concluded that it is of great importance to support the intestinal flora with external probiotics in intermittent fasting practices.

The inability to determine the immunologic response following infection in animals, the lack of determination of the host response and the absence of alterations in the gut microbiota in mice were considered as the most important limitations of this study due to the lack of adequate financial support.

The findings of this study indicate that single or combined use of intermittent fasting and probiotics may represent a safe and feasible strategy for the control of *Campylobacter* infections. Extensive studies using different intermittent fasting regimens are needed to confirm the findings of this study and to understand the immunological and molecular mechanisms underlying the decrease in *Campylobacter* colonization.

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