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Effect of probiotic yeast *Pichia kudriavzevii* 2P10 and mannan-oligosaccharide on the intestine health of rat infected with *Salmonella* Typhimurium

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Abstract. *Wulan R, Astuti RI, Rukayadi Y, Estuningsih S, Meryandini A. 2025. Effect of probiotic yeast* Pichia kudriavzevii *2P10 and mannan-oligosaccharide on the intestine health of rat infected with* Salmonella *Typhimurium. Biodiversitas 26: 335-344.* Recent evidence suggests that probiotic yeast is efficacious against bacterial infections. This study evaluated the effects of dietary supplementation with the live yeast probiotic *Pichia kudriavzevii* 2P10, Mannan-Oligosaccharide (MOS), and their combination on the growth performance and intestinal health of male Sprague-Dawley (SD) rats infected with *Salmonella* Typhimurium ATCC 14028 (ST). Two groups were studied: one without ST infection, including *P. kudriavzevii* 2P10 (PRO), MOS, and their combination (PMOS), and one with ST infection, including CONTROL.ST, PRO.ST, MOS.ST, and PMOS.ST. A diet of 10^8 CFU/mL PRO and 5% MOS was administered orally for 15 days, followed by a challenge with 10^8 CFU/mL ST in the ST group. After three days of challenge, the parameters were observed. The findings of this research are that ST infection in rats can cause colonization of ST in the ileum, causing a decrease in white blood cells, necrosis of epithelial cells, and an increased villous-to-crypt ratio (VCR) as a response to inflammation. The administration of PRO, MOS, and their combinations prevents inflammation, as proven by the absence of necrosis and epithelial desquamation. PRO, MOS, and their combinations stimulate intestinal health by increasing villus height, width, and VCR. MOS was found to be the best for increasing lactic acid bacteria. During ST infection, *P. kudriavzevii* 2P10 can coagulate with ST in the ileum, with this yeast-bacteria binding mechanism allowing free *Salmonella* Typhimurium cells to bind more to *P. kudriavzevii* 2P10 cells than to intestinal epithelial cells so that bacterial infection can be prevented. In conclusion, *P. kudriavzevii* 2P10 administration was the best for stimulating growth performance and intestinal health and preventing severe ST infection in male SD rats, offering promising avenues for further research and potential practical applications in human and animal nutrition and health.

Keywords: Gastroenteritis, ileal health, non-*Saccharomyces* probiotic, prebiotic, rats

INTRODUCTION

Salmonella enterica subsp. *enterica* serovar Typhimurium or *Salmonella* Typhimurium, a non-typhoidal *Salmonella* (NTS), is a pathogen with a global impact, causing gastroenteritis-type salmonellosis (Liu et al. 2018; Madigan et al. 2022). Infection with *Salmonella* Typhimurium can lead to acute invasive inflammatory diarrhea, often necessitating hospitalization and posing a risk of death to susceptible patients worldwide. The zoonotic transmission of *Salmonella* Typhimurium, which contaminates animal food products such as milk, meat, and eggs, is a serious public health issue (Anderson and Kendall 2017; Madigan et al. 2022). This situation is exacerbated by poor environmental sanitation, especially in humid tropical environments such as Indonesia (Liu et al. 2018). Waturangi et al. (2019) found that a staggering 58% of drinks in circulation in Jakarta, Indonesia, tested positive for *Salmonella* spp., highlighting this topic's global relevance and importance.

The intracellular infection strategy of *Salmonella* Typhimurium, involving invasion of the intestinal epithelium and internalization in phagocytes, is a significant challenge. This virulence mechanism is regulated by five *Salmonella* Pathogenicity Islands (SPIs) on the *Salmonella* chromosome, each containing virulence genes crucial for pathogenesis. The pathogen also possesses other virulence traits, such as virulence plasmids, adhesins, flagella, and biofilm-associated proteins (Fàbrega and Vila 2013). Multidrug resistance of *Salmonella* Typhimurium is demonstrated by its ability to withstand various antibiotics, including chloramphenicol, ampicillin, streptomycin, sulfonamides, and tetracycline (Bakkeren et al. 2019; Wang et al. 2019) adds to this challenge. This makes *Salmonella* Typhimurium a potent pathogen that infects hosts and is difficult to control. One *S. enterica* serovar Typhimurium strain, *Salmonella* Typhimurium ATCC 14028, was more virulent in the BALB/c mouse model (García-Quintanilla and Casadesús 2011). To date, an effective vaccine to prevent gastroenteritis

caused by *Salmonella* Typhimurium remains elusive (Anderson and Kendall 2017).

Fluoroquinolone- and cephalosporin-based antibiotics can be administered to patients with acute gastroenteritis and low immunity (Fàbrega and Vila 2013). However, the use of antibiotics can cause dysbiosis or changes in the structure of the gut microbiota, creating pathogens that are resistant to antibiotics. Probiotics and prebiotics can be alternative solutions for preventing and treating various digestive tract infections. Clinical findings in patients with NTS suggest that administering synbiotics (a combination of probiotics bacteria and prebiotic) may be a treatment option. Piatek et al. (2019) reported that an NTS patient who was treated with a synbiotic treatment containing nine probiotic bacteria (*Streptococcus thermophilus* St-21 and *Lactobacillus helveticus* SP-27) and prebiotic Fructo-Oligosaccharides (FOS) was free of NTS symptoms, and feces no longer contained *Salmonella* Typhimurium on the 10th day of treatment.

Yeast probiotics have also been shown to improve the health of the digestive tract. The probiotic yeast that has been widely studied is *Saccharomyces cerevisiae* var. *boulardii*, which has been reported to treat intestinal infections caused by *Clostridium difficile* toxin (Carstensen et al. 2018). *Pichia kudriavzevii* 2P10 was reported to co-aggregate with *Salmonella* Typhimurium in vitro to block the attachment of the pathogen to the intestinal wall at an early stage of *Salmonella* Typhimurium infection. The glucomannan component of the yeast cell wall plays an essential role in co-aggregation ability. In addition, *P. kudriavzevii* 2P10, a probiotic, exhibits functional antioxidant properties (Wulan et al. 2021).

Prebiotic MOS, considered a next-generation prebiotic, is a short chain of mannose that confers health benefits to the host (Shang 2022). The degradation of yeast cell wall mannans was derived from a commercial MOS (Kango et al. 2022). The mannose structure in MOS can bind to type 1 fimbriae pathogenic bacteria, blocking their attachment to the intestinal epithelial wall, thereby preventing *Salmonella* Typhimurium attachment during early infection (Zeiner et al. 2012). MOS consumption has also been proven to improve and maintain the structure of the gut microbiota, especially lactic acid bacteria (LAB), so that it can support the body's immune system to resist pathogens (Wang et al. 2018).

Until now, few reports have been published on the non-*Saccharomyces* yeast probiotic *P. kudriavzevii* for treating salmonellosis. The mechanism of the yeast probiotic *P. kudriavzevii* 2P10 in treating *Salmonella* infections and how it is combined with the prebiotic MOS has been less explored. This study aimed to evaluate the effects of dietary supplementation with live probiotic yeast (*P. kudriavzevii* 2P10) and prebiotic MOS on intestinal health, including total microbes (*Salmonella*, lactic acid bacteria, and yeast), hematological parameters, histomorphometry, and histopathology of male Sprague-Dawley rats infected with *Salmonella* Typhimurium ATCC 14028.

MATERIALS AND METHODS

Animals and treatments

The procedures and protocols for experimental animals in this study were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Institut Pertanian Bogor, Bogor, West Java, Indonesia (No. 46/KEH/SKE/XI/2021. Adult male Sprague-Dawley rats (180-230 g; 8-10 weeks old) were used in this study. They were purchased from iRATco Veterinary Laboratory Services (Bogor, Indonesia). Rats were reared in cages containing two rats in a temperature-controlled room temperature of 25-27°C, humidity of 60-70%, and 12 h light and 12 h darkness. The rats were acclimatized for two weeks, and ciprofloxacin (10 mg/kg) was administered to eliminate *Salmonella* in their intestines.

The sample size was calculated using Federer's formula [t (n−1)>15], where n is the number of replications and t is the number of treatment groups (Federer 1967). There were eight treatments, with five replicates each. The eight treatment groups comprised four patients without *Salmonella* Typhimurium ATCC14028 (ST) infection and four with ST infections (Table 1). For 15 days, the diet consisted of 1 mL of 10^8 CFU/mL *P. kudriavzevii* 2P10 and 5% commercial MOS (Bio-MOS Alltech, USA) administered to the rats in the treatment groups. Water was provided ad libitum throughout the study period. The rats were infected with 1 mL of 10^8 CFU/mL *Salmonella* Typhimurium ATCC 14028 on day 16th in a group with ST infection. On day 16th, the group without an ST infection was terminated. In contrast, the four groups with ST infection were administered ST infection and waited for three days after infection. On day 20th, the infected rats were sacrificed. Body Weight (BW) was measured at the beginning and end of treatment.

Cultivation of probiotic yeast and *Salmonella* Typhimurium ATCC 14028

The probiotic yeast *Pichia kudriavzevii* 2P10 was isolated from the cocoa (*Theobroma cacao*) fermentation process and has been well characterized as a probiotic yeast (Berutu et al. 2017; Wulan et al. 2021). The probiotic yeast *P. kudriavzevii* 2P10 was cultured in yeast extract peptone dextrose (YPD) broth for 15 h at 28°C under aerobic conditions. The pathogenic bacteria *Salmonella* Typhimurium ATCC 14028 was cultivated using Mueller-Hinton (MH) broth media for 18 h at 37ºC aerobically. Each culture was harvested separately by washing and resuspending in PBS twice by centrifugation at 4,000 rpm for 15 min. It was then dissolved in Phosphate-Buffered Saline (PBS) until a cell count of 10^8 CFU/mL was obtained. Cell counts were determined using the TPC method.

Collection of samples

The rats were euthanized using ketamine-xylazine (70:10 mg/kg BW). Blood was collected from the heart and placed in an Ethylenediamine Tetraacetic acid (EDTA) tube for blood collection for hematology analysis. Small intestinal (ileal) tissue samples were collected for histopathological analysis. The contents of the ileum, cecum, and feces were collected to measure the total lactic acid bacteria and yeast

P. kudriavzevii 2P10. The ileum content was also measured for *Salmonella* counts.

Hematological profile

Blood samples were collected from the heart of rats and placed in EDTA tubes. Hematological parameters were analyzed using a Hemavet HV950FS multispecies hematology analyzer (Mazkour et al. 2020). Hematological parameters included erythrocyte or Red Blood Cell (RBC) count, Platelet (PLT) count, Hemoglobin (Hb) concentration, Differential Count (DC), and Packed Cell Volume (PCV). Immune system parameters included white blood cell (WBC), lymphocyte, monocyte, and granulocyte counts.

Total *Salmonella*, yeast, and lactic acid bacteria

A total of 1 g of the contents of the ileum, cecum, and feces on days 15th and 20th days of treatment was aseptically collected, homogenized in 9 mL of sterile 0.85% NaCl solution, and then serially diluted. Serial dilutions were spread on SSA agar media for *Salmonella*, de Man Rogosa, and Sharpe (MRS)+ $CaCO_3$ 1% agar media for lactic acid bacteria (LAB), and YPD agar media for yeast. MRS+$CaCO_3$ 1% and SSA media were incubated at 37°C for 48 and 24 h, respectively. The YPD medium was incubated at 28°C for 24 h. The number of colonies that grew was calculated as CFU/g. *Salmonella* colonies on SSA media were transparent, with a black dot in the middle. Lactic acid bacteria formed a clear zone around the colony on MRS+$CaCO_3$ 1% agar, and the yeast was round and white.

Histological analysis of the small intestine

Small segments of the small intestine (ileum) were used for histological evaluation. The ileum (2 cm) was fixed in 10% neutral-buffered formalin for 24 h. The sections were then dehydrated and embedded in paraffin. The 5-µm paraffin ileum sections were prepared and stained with hematoxylin and eosin (HE). Histological micrographs were captured using an OptiLab Advance viewer and an Olympus CX23 Microscope device (Tokyo, JAPAN).

Histomorphometric analysis

Under a light microscope, the following intestinal histomorphometric parameters were evaluated in all H&E-stained sections: villus height and width, crypt depth, and crypt width. Histological data were visualized and measured using image J. The following parameters were measured: (i) villus height (vh), the height of the villi was defined from the tip to the base; (ii) villus width (vw), measured at the half-height point of the villus; (iii) crypt depth (cd), the distance from the top of the villus crypt to the muscularis mucosa; and (iv) crypt width (cw), measured at the crypt at the half-height point (see Figure 1). Calculations using villous height and width at half height yielded the villus surface area. The villi-to-crypt ratio (VCR) was calculated by dividing villus height by crypt depth (Seyyedin and Nazem 2017; Segu et al. 2022).

Data analysis

One-way ANOVA was used to compare the data, and statistical significance was set at $p<0.05$. Duncan's multiple range test was used to conduct post hoc analysis. Histological data were visualized and measured using image J.

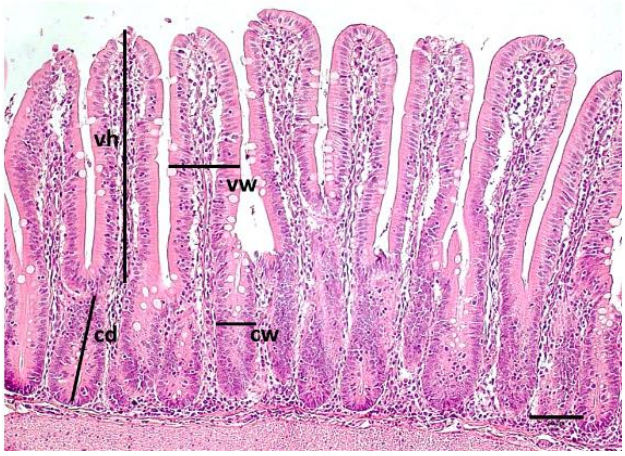

Figure 1. Measurement of the four parameters of villus height (vh), villus width (vw), crypt depth (cd), and crypt width (cw) on hematoxylin and eosin-stained, formalin-fixed cross-sections of the rat small intestine. Bar: 50 µm (private documentation)

Table 1. The experimental design of in vivo treatments in this study

Groups	Treatments			
	Day 1 to Day 15th	Day 16th	Day 16th to Day 19th	Day 20th
CONTROL	Aquadest	Termination	-	-
PRO	Probiotic Yeast	Termination	-	-
MOS	Prebiotic MOS	Termination	-	-
PMOS	Probiotic Yeast+ Prebiotic MOS	Termination	-	-
CONTROL.ST	Aquadest	ST Infection	Aquadest	Termination
PRO.ST	Probiotic Yeast	ST Infection	Probiotic Yeast	Termination
MOS.ST	Prebiotic MOS	ST Infection	Prebiotic MOS	Termination
PMOS.ST	Probiotic Yeast+ Prebiotic MOS	ST Infection	Probiotic Yeast + Prebiotic MOS	Termination

Notes: Probiotic (PRO): 1 mL 10^8 CFU/mL of *P. kudriavzevii* 2P10; Prebiotics: MOS 5%; ST: 10^8 CFU/mL of *Salmonella* Typhimurium ATCC 14028

RESULTS AND DISCUSSION

Body weight

The lowest to highest growth rates were observed in PMOS.ST, PMOS, MOS.ST, CONTROL, PRO, PRO.ST, MOS, and CONTROL.ST (Table 2). The body weight (BW) of the MOS and CONTROL.ST groups increased by 0.71% and 3.90% positive growth rates, respectively.

Total of *Salmonella* in small intestine

CONTROL.ST showed the highest percentage of *Salmonella*-positive rats in the small intestine (100%) (Table 3). All treatment groups had a lower percentage of *Salmonella*-infected rats than CONTROL.ST (100%). PMOS and PRO had lower rates than CONTROL (60%), which was not infected with *Salmonella* (20% and 40%, respectively).

Total of yeast probiotic

The probiotic yeast *P. kudriavzevii* 2P10 survived in the rat ileum, cecum, and feces of the PRO.ST and PMOS.ST treatment groups (Table 4). Notably, the probiotic *P. kudriavzevii* 2P10 showed robust survival in the cecum compared to that in the ileum, indicating the potential benefits of the treatment. Most probiotic yeasts survived in rats treated with PRO. ST.

Yeast was exclusively found in the cecum and feces of the PRO and PMOS groups, with no detection in the ileum. Notably, the groups that did not receive probiotic yeast treatment served as controls, with no yeast detected in the ileum, cecum, or feces.

Total of lactic acid bacteria

In this comprehensive study, we demonstrated that lactic acid bacteria are the most abundant in the cecum (average at 8.59), followed by the feces (average at 7.89) and ileum (average at 7.84), which have almost the same value (Table 5). In the ileum, cecum, and feces groups, the total BAL group with ST infection had a higher total BAL than those without ST infection. In the cecum, all treatments had a higher total BAL than CONTROL (8.42 $\log_{10}$ CFU/g) but were not significantly different, except for MOS.ST, which had the highest total BAL. In the ileum, all treatment groups with ST infection had higher numbers of lactic acid bacteria than those without ST infection. From highest to lowest, these were MOS.ST, CONTROL.ST, PRO.ST, PMOS.ST, CONTROL, PMOS, MOS, and PRO. The MOS.ST group had a total lactic acid bacterial count. In feces, the highest total LAB count was observed in the MOS.ST treatment. In CONTROL.ST, PRO.ST, and PMOS. In the ST group, the total BAL in the ileum and cecum decreased but was insignificant.

Table 2. The effect of yeast probiotics and mannan-oligosaccharide (MOS) on the growth rate of body weight

Groups	Initial weight	Final weight	Gain/loss	Growth rate (%)
CONTROL	186.20±15.68	182.40±20.72	Loss	-2.04
CONTROL.ST	184.00±21.62	191.17±24.52	Gain	3.90
PRO	197.00±8.86	193.20±8.11	Loss	-1.93
PRO.ST	211.17±29.67	210.83±25.04	Gain	-0.16
MOS	197.80±14.99	199.20±21.74	Gain	0.71
MOS.ST	222.83±24.60	214.67±20.34	Loss	-3.66
PMOS	217.20±21.25	207.40±25.27	Loss	-4.51
PMOS.ST	228.83±36.54	211.00±24.07	Loss	-7.79

Notes: CONTROL: Control without ST infection (negative control); CONTROL.ST: Control positive with ST infection; PRO: 1 mL of probiotics yeast *P. kudriavzevii* 2P10 10^8 CFU/mL; PRO.ST: 1 mL of probiotics yeast *P. kudriavzevii* 2P10 10^8 CFU/mL with ST infection; MOS: Mannan-oligosaccharide 5% in fed; MOS.ST: mannan-oligosaccharide 5% in fed with ST infection; PMOS: The combination of PRO and MOS; PMOS.ST: The combination of PRO and MOS with ST infection; ST. Infection with 1 mL of *Salmonella* Typhimurium ATCC 14028. Data are expressed as mean±standard deviation (SD) (n=5)

Table 3. Total of *Salmonella* in the small intestine (ileum) in all groups detected by *Salmonella* Shigella Agar

Groups	*Salmonella* in ileum ($\log_{10}$ CFU g^{-1})					Percentage of rat (%)	
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Negative *Salmonella*	Positive *Salmonella*
CONTROL	5.79	6.49	0.00	0.00	0.00	60.00	40.00
CONTROL.ST	7.93	6.54	7.15	7.56	6.85	0.00	100.00
PRO	5.23	6.90	0.00	0.00	0.00	60.00	40.00
PRO.ST	6.85	6.72	6.51	0.00	0.00	40.00	60.00
MOS	6.90	6.90	5.23	0.00	0.00	40.00	60.00
MOS.ST	6.52	7.29	7.90	7.93	0.00	20.00	80.00
PMOS	6.90	0.00	0.00	0.00	0.00	80.00	20.00
PMOS.ST	5.54	5.46	5.81	0.00	0.00	40.00	60.00

Notes: CONTROL: control without ST infection (negative control); CONTROL.ST: control positive with ST infection; PRO: 1 mL of probiotics yeast *P. kudriavzevii* 2P10 10^8 CFU/mL; PRO.ST: 1 mL of probiotics yeast *P. kudriavzevii* 2P10 10^8 CFU/mL with ST infection; MOS: mannan-oligosaccharide 5% in fed; MOS.ST: mannan-oligosaccharide 5% in fed with ST infection; PMOS: the combination of PRO and MOS; PMOS.ST: the combination of PRO and MOS with ST infection; ST. Infection with 1 mL of 10^8 CFU/mL *Salmonella* Typhimurium ATCC 14028

Hematology analysis

Hematological analysis included the number of erythrocytes or red blood cells (RBC), hemoglobin (Hb), thrombocytes or platelets, and hematocrit or packed volume cells (PVC). In all RBC treatments, hemoglobin, thrombocyte, and hematocrit levels were normal and did not show significant differences (Table 6). CONTROL.ST had a lower RBC count, Hb level, and PCV than CONTROL. The highest RBC count was observed in MOS and the lowest in MOST.ST. The highest hemoglobin level was observed in the MOS group, whereas the lowest was observed in the MOS.ST group. The number of thrombocytes was highest in PRO and lowest in PMOS. The highest hematocrit was found in MOS and the weakest in MOS.ST.

Table 4. Total of yeast in rat's gastrointestinal tract

Groups	Total of yeasts log_{10} CFU g^{-1}		
	Small intestine (Ileum)	Cecum	Feces
CONTROL	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
CONTROL.ST	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
PRO	0.00±0.00^{a}	4.50±0.45^{c}	5.51±0.88^{c}
PRO.ST	2.64±0.12^{b}	4.46±0.88^{c}	5.57±0.79^{c}
MOS	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
MOS.ST	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
PMOS	0.00±0.00^{a}	4.52±0.35^{c}	6.09±0.27^{c}
PMOS.ST	2.92±0.28^{c}	3.50±0.34^{b}	3.70±0.41^{b}

Notes: CONTROL: Control without ST infection (negative control); CONTROL.ST: control positive with ST infection; PRO: 1 mL of probiotics yeast *P. kudriavzevii* 2P10 10^{8} CFU/mL; PRO.ST: 1 mL of probiotics yeast *P. kudriavzevii* 2P10 10^{8} CFU/mL with ST infection; MOS: Mannan-oligosaccharide 5% in fed; MOS.ST: Mannan-oligosaccharide 5% in fed with ST infection; PMOS: The combination of PRO and MOS; PMOS.ST: The combination of PRO and MOS with ST infection; ST. Infection with 1 mL of 10^{8} CFU/mL *Salmonella* Typhimurium ATCC 14028. Data are expressed as mean± standard deviation (SD) (n=5). The numbers in the column followed by the same letters (a, b, and c) mean they do not differ significantly from Duncan's test results ($\alpha = 0.05$)

Table 5. Total of lactic acid bacteria in rat's gastrointestinal tract

Groups	Total of lactic acid bacteria (log_{10} CFU g^{-1})		
	Ileum	Cecum	Feces
CONTROL	7.87±0.19^{d}	8.42±0.34^{a}	7.47±0.21^{a}
CONTROL.ST	8.39±0.16^{de}	8.92±0.62^{ab}	8.35±0.40^{b}
PRO	6.95±0.26^{a}	8.51±0.34^{a}	7.16±0.26^{a}
PRO.ST	8.24±0.29^{d}	8.70±0.23^{a}	8.38±0.48^{b}
PMOS	7.59±0.33^{bc}	8.49±0.35^{a}	7.23±0.37^{a}
PMOS.ST	8.21±0.22^{d}	8.78±0.24^{ab}	8.59±0.38^{b}
MOS	7.45±0.24^{bc}	8.74±0.21^{ab}	7.19±0.29^{a}
MOS.ST	8.59±0.09^{e}	9.22±0.21^{b}	8.79±0.12^{b}

Notes: CONTROL: Control without ST infection (negative control); CONTROL.ST: Control positive with ST infection; PRO: 1 mL of probiotics yeast *P. kudriavzevii* 2P10 10^{8} CFU/mL; PRO.ST: 1 mL of probiotics yeast *P. kudriavzevii* 2P10 10^{8} CFU/mL with ST infection; MOS: Mannan-oligosaccharide 5% in fed; MOS.ST: mannan-oligosaccharide 5% in fed with ST infection; PMOS: The combination of PRO and MOS; PMOS.ST: The combination of PRO and MOS with ST infection; ST. Infection with 1 mL of 10^{8} CFU/mL *Salmonella* Typhimurium ATCC 14028. Data are expressed as mean± standard deviation (SD) (n=5). The numbers in the column followed by the same letters (a, b, and c) indicate that they do not differ significantly from Duncan's test results ($\alpha = 0.05$)

Table 6. The hematology of rat's blood

Groups	RBC (10^{6}/µL)	Hb (g/dL)	Thrombocyte (10^{3}/µL)	PVC (%)
CONTROL	7.73±0.37^{ab}	14.13±0.95^{a}	776.33±153.83^{ab}	42.07±1.52^{a}
CONTROL.ST	7.56±0.61^{ab}	13.50±1.65^{a}	925.00±138.11^{ab}	38.97±5.21^{a}
PRO	7.72±0.19^{ab}	14.15±0.52^{a}	1043.00±62.67^{b}	41.60±1.93^{a}
PRO.ST	7.46±0.12^{ab}	13.47±0.32^{a}	794.00±73.57^{ab}	38.67±0.67^{a}
PMOS	7.67±1.17^{ab}	13.30±2.07^{a}	570.33±181.32^{a}	39.30±5.40^{a}
PMOS.ST	7.68±0.65^{ab}	13.43±1.78^{a}	875.00±124.65^{ab}	39.60±4.92^{a}
MOS	8.78±0.95^{a}	15.20±2.21^{a}	908.33±531.24^{ab}	44.27±6.60^{a}
MOS.ST	7.18±1.48^{b}	13.07±2.68^{a}	686.33±339.32^{ab}	38.43±7.27^{a}

Notes: CONTROL: Control without ST infection (negative control); CONTROL.ST: Control positive with ST infection; PRO, 1 mL of probiotics yeast *P. kudriavzevii* 2P10 10^{8} CFU/mL; PRO.ST: 1 mL of probiotics yeast *P. kudriavzevii* 2P10 10^{8} CFU/mL with ST infection; MOS: Mannan-oligosaccharide 5% in fed; MOS.ST, mannan-oligosaccharide 5% in fed with ST infection; PMOS: The combination of PRO and MOS; PMOS.ST: The combination of PRO and MOS with ST infection; ST. Infection with 1 mL of 10^{8} CFU/mL *Salmonella* Typhimurium ATCC 14028. Data are expressed as mean± standard deviation (SD) (n=5). The numbers in the column followed by the same letters (a, b, and c) indicate that they do not differ significantly from Duncan's test results ($\alpha = 0.05$)

Hematological parameters are related to the immune system and include white blood cells (WBCs), lymphocytes, monocytes, and granulocytes. The results showed that the WBC count differed significantly between the groups based on these parameters. CONTROL, PRO, and PMOS had lower WBC counts in treatments without ST infection (Table 7). MOS.ST, CONTROL, PMOS, and PRO.ST had lower WBC counts than the other treatments. The highest leukocyte (WBC) counts were observed in the PMOS.ST (18.43) and CONTROL.ST (14.20) groups. The lymphocyte counts in all treatments were within the standard range; MOS.ST had lymphocyte values closest to those of CONTROL without ST infection (5.53), followed by PMOS (6.50) and MOS (6.67). In all treatments, monocytes were within the standard range, with PMOS.ST having the highest monocyte count. Additionally, Granulocyte values were not significantly different among all treatments; all treatments were in the standard range, 0.1-5.4 10^3/µL. PMOS.ST had the highest granulocyte count. PRO.ST and MOS.ST had almost the same granulocyte values as CONTROL.

Histomorphometric analysis

The results of the small intestine histomorphometric observations, including villus height, villus width, villus surface area, crypt depth, and crypt width, are shown in Table 8. All treatments resulted in significantly greater villus heights than those of CONTROL and CONTROL.ST. The order of villi height from highest to lowest was PMOS.ST, MOS.ST, PRO.ST, PMOS, PRO, MOS, CONTROL.ST, and CONTROL. PMOS.ST has the highest villus height, namely 129.90 µm, while CONTROL.ST has the lowest, namely 73.28 µm.

For villus surface area, all treatments also had a higher villus surface area than CONTROL (3009.10 µm^2) and CONTROL.ST (2209 µm^2), with the highest villus surface area in PMOS.ST (6947.35 µm^2) and PRO.ST (5672.89 µm^2). The crypt depths, from highest to lowest, were MOS.ST, PMOS, PRO.ST, PMOS.ST, CONTROL.ST, PRO, CONTROL, and MOS. However, the villus surface areas of PMOS, PRO.ST, PMOS.ST, CONTROL.ST, and PRO did not differ from those of CONTROL and CONTROL.ST. For Crypt width, all treatments had higher values than CONTROL (18.150 µm) and CONTROL.ST (16.12 µm). One of the essential parameters in histomorphometrics is the villus-to-crypt ratio (VCR); this study showed that the villus-to-crypt ratio showed that CONTROL had the lowest VCR value (1.58). All treatments had higher VCR values than CONTROL (1.58) without infection, except for MOS.ST (1.45) (Table 8).

Table 7. Hematological parameters related to the rat's immune system

Groups	WBC (10^3/µL)	Lymphocyte (10^3/µL)	Monocyte (10^3/µL)	Granulocyte (10^3/µL)
Normal standard	**2.10 - 19.50**	**2.00 - 14.10**	**0.00 - 0.98**	**0.10 - 5.40**
CONTROL	8.70±3.28^{a}	5.53±2.35^{a}	0.27±0.12^{a}	2.90±0.85
CONTROL.ST	14.20±2.95^{ab}	10.40±3.05^{ab}	0.37±0.05^{8a}	3.43±0.40
PRO	12.48±6.80^{ab}	8.25±4.34^{ab}	0.40±0.25^{b}	3.83±2.39
PRO.ST	10.83±1.62^{ab}	7.87±1.33^{ab}	0.30±0.00^{a}	2.67±0.90
PMOS	8.90±3.48^{a}	6.50±3.08^{a}	0.27±0.15^{a}	2.13±0.25
PMOS.ST	18.43±5.55^{b}	12.93±4.51^{b}	0.63±0.15^{b}	4.87±1.40
MOS	11.20±5.91^{ab}	6.67±2.56^{a}	0.33±0.15^{a}	4.20±3.46
MOS.ST	7.93±2.11^{a}	5.53±1.43^{a}	0.23±0.06^{a}	2.17±1.26

Notes: CONTROL: Control without ST infection; CONTROL.ST: Control with ST infection; PRO, 1 mL of probiotics yeast *P. kudriavzevii* 2P10 10^8 CFU/mL; PRO.ST: 1 mL of probiotics yeast *P. kudriavzevii* 2P10 10^8 CFU/mL with ST infection; MOS, mannan-oligosaccharide 5% in fed; MOS.ST: Mannan-oligosaccharide 5% in fed with ST infection; PMOS: The combination of PRO and MOS; PMOS.ST: The combination of PRO and MOS with ST infection; ST. Infection with 1 mL of 10^8 CFU/mL *Salmonella* Typhimurium ATCC 14028. Numbers in the same column followed by the same letters (a-b) indicate that they do not differ significantly from Duncan's test results ($\alpha = 0.05$).

Table 8. Histomorphometric of rat's small intestine

Groups	Villus height (µm)	Villus width (µm)	Villous surface area (µm^2)	Crypt depth (µm)	Crypt width (µm)	Villus-to-crypt ratio (VCR)
CONTROL	79.67±5.47^{ab}	37.82±4.04^{b}	3009.10±352.70^{b}	50.421±5.37^{b}	18.150±1.82^{b}	1.58
CONTROL.ST	73.28±11.21^{a}	30.80±5.10^{a}	2209.37±317.65^{a}	53.174±10.77^{b}	16.122±2.69^{a}	1.38
PRO	95.89±18.97^{c}	37.20±5.59^{b}	3535.25±612.19^{c}	51.458±7.99^{b}	19.391±2.56^{bc}	1.86
PRO.ST	109.41±11.91^{d}	52.15±8.40^{d}	5672.89±940.62^{e}	55.177±5.74^{b}	23.703±3.51^{d}	1.98
MOS	88.26±13.33^{bc}	40.05±7.63^{c}	3473.59±496.94^{c}	45.971±6.85a	19.331±3.25^{bc}	1.92
MOS.ST	115.88±25.07^{d}	35.40±5.79^{b}	4024.65±709.46^{d}	79.987±9.51^{c}	23.967±3.95^{d}	1.45
PMOS	107.09±13.28^{d}	40.87±7.44^{c}	4324.49±658.68^{d}	55.261±6.50^{b}	20.270±2.47^{c}	1.94
PMOS.ST	129.90±16.82^{e}	54.53±7.67^{d}	6947.35±761.34^{f}	53.967±5.50^{b}	22.799±2.87^{d}	2.41

Notes: CONTROL: Control without ST infection (negative control); CONTROL.ST: Control positive with ST infection; PRO: 1 mL of probiotics yeast *P. kudriavzevii* 2P10 10^8 CFU/mL; PRO.ST: 1 mL of probiotics yeast *P. kudriavzevii* 2P10 10^8 CFU/mL with ST infection; MOS: Mannan-oligosaccharide 5% in fed; MOS.ST: Mannan-oligosaccharide 5% in fed with ST infection; PMOS: The combination of PRO and MOS; PMOS.ST, the combination of PRO and MOS with ST infection; ST. Infection with 1 mL of 10^8 CFU/mL *Salmonella* Typhimurium ATCC 14028. Data are expressed as mean ± standard deviation (SD). Numbers in the same column followed by the same letters (a-f) indicate that they do not differ significantly from Duncan's test results ($\alpha = 0.05$)

Histopathological of small intestine

Histopathology revealed desquamation of the intestinal epithelium in the ileum of rats treated with the positive control (CONTROL.ST); however, this was not observed in the CONTROL, PRO, PRO.ST, PMOS, and PMOS.ST treatment groups (Figure 2). PMOS.ST showed very dense and good epithelial cells compared to the other MOS and MOS.ST treatments, with slight epithelial desquamation (ed) at the tips of the villi.

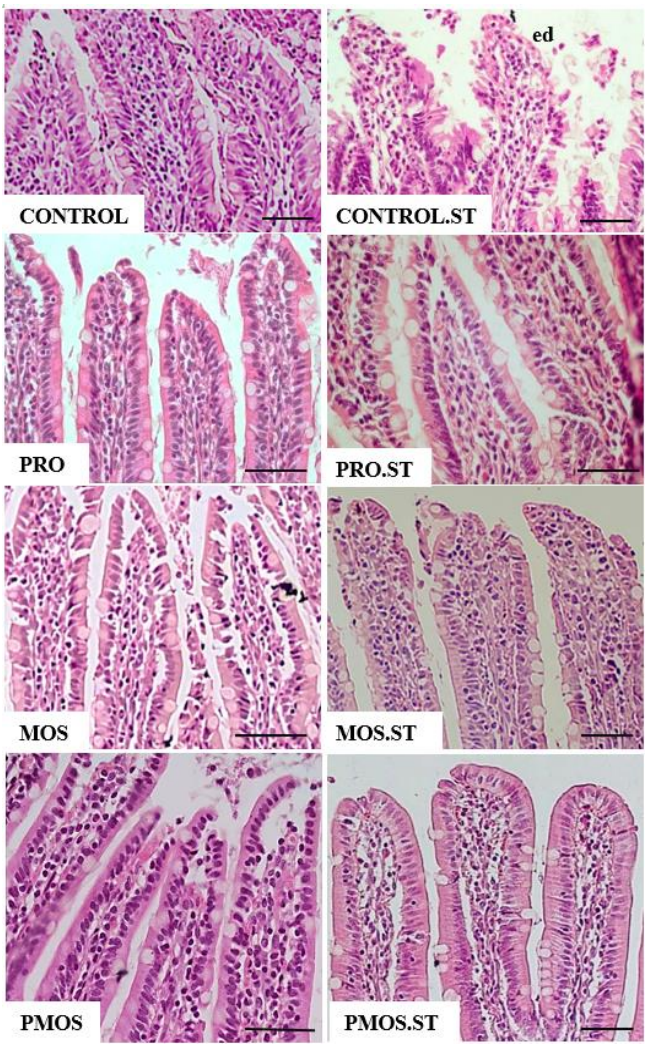

Figure 2. Histology of rat small intestine in all treatments with Hematoxylin-Eosin (HE) staining: CONTROL, CONTROL.ST: control with ST infection, PRO: Probiotic yeast *P. kudriavzevii* with no ST infection, PRO.ST: Probiotic yeast *P. kudriavzevii* with ST infection, MOS: 5% of prebiotic MOS with no ST infection; MOS.ST: 5% of prebiotic MOS with ST infection; PMOS=Combination of probiotic yeast and prebiotics with no ST infection; PMOS.ST: Combination of probiotic yeast and prebiotics with ST infection after the fourth day of *Salmonella* Typhimurium (ST) ATCC 14028 10^8 CFU/mL infection. ed= epithelial desquamation. Scale bar: 30 µm

Discussion

Salmonellosis-type gastroenteritis is a human disease caused by Gram-negative, non-typhoidal *Salmonella* (NTS) bacteria, namely *Salmonella* Typhimurium. *Salmonella* Typhimurium mostly comes from animal-based foods and can also be transmitted zoonotically. After eating food contaminated with *Salmonella* Typhimurium, *Salmonella* Typhimurium reaches and touches the intestinal mucosa, penetrates the intestinal epithelial barrier, and forms intracellular colonization in the form of *Salmonella* vacuole colonization. *Salmonella* Typhimurium invasion causes damage and inflammation to the intestinal epithelium and can then enter the bloodstream and spread to the spleen and liver, causing bacteremia (Ménard et al. 2022). The impact of *Salmonella* Typhimurium infection includes damage to intestinal morphology, necrosis, and inflammation caused by the entry of intestinal *Salmonella* into intestinal epithelial cells. Dysbiosis of the intestinal microbiota also affects hematology and can cause gastrointestinal cancer (Zha et al. 2019).

Salmonella Typhimurium is resistant to various drugs with a penta-resistant ACSSuT pattern (ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline). Because of its resistance properties, *Salmonella* Typhimurium is usually used as a resistance model (Wang et al. 2019; Newson et al. 2023). Yeast probiotics can be used as an alternative to antibiotics to treat virulent *Salmonella* Typhimurium infections. Preventive treatment with the probiotic yeast *Salmonella boulardii* significantly reduced the activation of signaling pathways owing to reduced inflammation, clinical manifestations, tissue damage, and intestinal death in mice. The binding of yeast bacteria may reduce the number of free bacteria reaching epithelial cells, thereby reducing the activation of proinflammatory signaling pathways that cause early intestinal damage. Alternative adhesion of these pathogenic bacteria to the probiotic surface, instead of their intestinal receptors, may partially explain the probiotic effect (Martins et al. 2013).

The study revealed that administration of a combination of the probiotic yeast *P. kudriavzevii* 2P10 and MOS (PMOS) and mannan-oligosaccharides (MOS) infected with *Salmonella* Typhimurium resulted in a reduction in body weight during treatment, as indicated by a decrease in growth rate (-7.79 and -3.66, respectively). Giving PRO without ST infection also reduced the growth rate by -1.93 (Table 2). Evidence strongly supports the beneficial effects of probiotic or synbiotic supplementation on weight loss, body mass, body fat reduction, and the metabolic profile of obese patients (Moszak et al. 2023). Dietary supplementation with the recombinant yeast *Saccharomyces boulardii* (10^9 yeast cells/mouse/day) in obese rats significantly reduced body weight; therefore, it has potential as an anti-obesity probiotic (Nayebhashemi et al. 2023). By promoting the growth of certain bacteria in the gut, such as *Bifidobacterium* and *Lactobacillus*, prebiotics can influence signals related to hunger and satiety to reduce body weight and total fat content (Wang et al. 2018; Geng et al. 2022).

Rats also have approximately 95% similarity with human DNA, and hence are more or less susceptible to diseases similar to humans and respond to similar treatments,

including salmonellosis (Delwatta et al. 2018). *Salmonella* binds to the ileal epithelium four hours after intragastric intubation. It preferentially colonizes the distal ileum (approximately 6 cm above the ileocecal junction), which is a niche enriched in formic acid metabolites. The relatively high concentration of formic acid metabolites in the distal ileum prevents other immune signals from suppressing *Salmonella* invasion (Chowdhury et al. 2023).

Salmonella Typhimurium could be caused to infect the rat's gastrointestinal three days after infection with a single dose of *Salmonella* Typhimurium ATCC 14028, the CONTROL.ST rat group was 100% positive for ST in the ileum. However, the PRO.ST and PMOS.ST groups showed reduced ST infection in SD rats (Table 3). The yeast cell walls, including those of *S. boulardii* and *P. kudriavzevii*, contain mannose as a cell wall component. Mannose-specific adhesins/receptors, such as fimbriae, on *Salmonella* bacterial cell walls can bind to mannose on yeast cell walls. Bacterial pathogens, including *Salmonella* species, have been reported to bind better to probiotic yeasts than to parabiotic or yeasts (dead cells), such as MOS from degraded yeast cells (Martins et al. 2010; Gut et al. 2018).

The probiotic yeast *P. kudriavzevii* 2P10 can live in the GI tract and colonize both the cecum and ileum. The probiotic yeast *P. kudriavzevii* 2P10 showed better survival in the cecum than in the ileum. The best yeast growth was observed in the cecum of PMOS at 4.52, ileum PMOS.ST at 2.92, and feces PMOS at 6.09 $\log_{10}$ CFU/g (Table 4). In the PRO.ST and PMOS.ST groups, yeast was found in the ileum, cecum, and feces. However, in PRO and PMOS treatments without ST infection, yeasts were not found in the ileum, but only in the cecum and feces of rats. In monogastric mammals, bacterial populations in the stomach and small intestine are usually less abundant than those in the large intestine (Gart et al. 2016). The yeast found in the cecum was also more significant than that in the ileum because the small intestine deals with pancreatic enzymes and bile to continue digestion. *P. kudriavzevii* 2P10 has characterized and could tolerate bile salt 0.5% on probiotic evaluation (Wulan et al. 2021). In addition, pancreatic and hydrolytic enzymes and secondary metabolic products of the gut microbiome and epithelial brush border in the small intestine can destroy microorganisms, including yeast (Alkalbani et al. 2022). The cecum does not contain digestive enzymes, which makes it safe for microbes. The microbial composition of the cecum is higher than that of the rat small intestine (Lee et al. 2018). PRO, MOS, and PMOS treatments also resulted in more lactic acid bacteria (LAB) in the cecum than in the ileum and feces. In the cecum, the most abundant carbohydrate fermentative site and lactic acid bacteria were found in the MOS.ST (Li et al. 2017; Sivixay et al. 2021).

The total lactic acid bacteria in the GI tract of rats are also affected by the presence of *Salmonella* Typhimurium. The total LAB count was higher in the group with ST infection than that in the group without ST infection. *Salmonella* Typhimurium competes with the microbiota for nutrients and overcomes colonization resistance to infection (Gart et al. 2016). The growth of lactic acid bacteria, a natural defense mechanism, can effectively prevent the development of enteric pathogens, such as *Salmonella*, by creating an acidic environment (Kim et al. 2013). The LAB population in the MOS.ST group had the highest values in the ileum, cecum, and feces. The administration of mannose-based oligosaccharides extracted from palm kernel cake (PKC) increases the cecal population of beneficial bacteria and decreases that of pathogenic bacteria in rats (Jahromi et al. 2017). Alpha-Mannose-Oligosaccharide (MOS) prebiotics are widely used in animal husbandry as immunomodulators to improve growth and gut health. Their mode of action is thought to be mediated by their impact on host microbial communities and their associated metabolism.

ST-infected rats showed hematological changes in several parameters. Regarding hematological parameters, RBC, Hb, and PVC decreased in the ST infection group, although they were still within standards and were not significantly different (Table 6). Decreased RBC, Hb, and PVC levels are associated with anemia. The hemolysin produced by *Salmonella* Typhimurium can lyse red blood cells. Hemolysis in *S. enterica* serovar Typhimurium can form pores that puncture erythrocyte membranes of red blood cells (erythrocytes) (Miki et al. 2004). Non-typhoidal salmonellosis (NTS) is also associated with a subsequent risk of hematological malignancies, especially in patients older than 60 years. Probiotic yeast treatment did not alter the rats' normal blood profile parameters; therefore, it was safe as a probiotic. MOS as a feed additive also did not affect normal hematological parameters and could increase the performance of male broiler chickens (Yulianto et al. 2024).

This study also studied hematological parameters related to the immune system, including WBC, lymphocytes, monocytes, and granulocytes. The increased presence of WBC is a response to infection or inflammation. In CONTROL, *Salmonella* infection resulted in increased WBC (Table 7), and this follows Mazkour et al. (2020), who reported that a group of rats infected with *Salmonella* Typhimurium also had increased WBC, mainly lymphocytes and neutrophils; however, PRO.ST treatment was able to reduce WBC, although not significantly. The probiotic *P. kudriavzevii* 2P10 can inhibit *Salmonella* Typhimurium infection in rats, thereby reducing WBC values. The number of lymphocytes in the PRO and MOS groups was also lower than that in the CONTROL group infected with ST (Table 7). Granulocytes did not differ significantly between treatments; they comprised a combination of neutrophils, eosinophils, and basophils.

Intestinal health can be observed from histomorphometric parameters, such as villus height and area and the villi-to-crypt ratio (VCR). Probiotic yeast, MOS, and their combination improved intestinal health, including the height and surface area of the ileum villi in rats during 15 days of administration. ST infection without treatment with probiotic yeast, MOS, or their combination can reduce ileal villus height in rats. Enterotoxigenic F18+ *Escherichia coli* infection increases crypt depth in pigs. Synbiotics, a combination of xylanase and *Bacillus* sp., can reduce crypt depth and increase villus height (Duarte 2020). The probiotic yeast *S. boulardii* plays a crucial role in preserving and

restoring intestinal barrier function in multiple disorders (Terciolo et al. 2019).

VCR is an essential parameter in histological studies of the digestive tract, especially in the ileum (small intestine), because of its association with the functional health of the ileum. Villi are the structures responsible for the absorption process, and their height indicates an increase in the absorption surface area. Crypts are sites of epithelial cell proliferation. The depth of the crypts suggests an increase in cellular turnover, often a response to disease or stress (Wilson et al. 2018). In the present study, the villus-to-crypt ratio showed that CONTROL had the lowest VCR value (1.58). Except for MOS, all treatments had higher VCR values than CONTROL (1.58) without infection and ST (1.45). *Salmonella* infections also support crypt hyperplasia in chickens (Xie et al. 2020).

Intestinal epithelial cells are located at the interface between the gut lumen and mucosal immune system and form the first layer of defense against the invasive enteric pathogen *S. enterica* serovar Typhimurium. The intestinal epithelium is the primary site of inflammation-mediated immune defenses (Crowley et al. 2020). In this study, *Salmonella* Typhimurium infection caused desquamation of the epithelium of the ileum; some cells were detached, and the surface was bumpy (Figure 2). Early infection (2 days post-infection) of *Salmonella* Typhimurium in pigs results in damaged mucosa with shortened villi and *Salmonella* infiltration, as well as damaged mucosa with complete erosion of villi and a high concentration of *Salmonella* in the ileum (Argüello et al. 2018).

The significant findings of this research can be summarized as follows: First, Infection of rats with a single dose of *Salmonella* Typhimurium ATCC 14028 can cause colonization of *Salmonella* Typhimurium bacteria in the ileum, causing necrosis of intestinal epithelial cells, decreased villi height and width, and increased VCR as a response to inflammation. In addition, *Salmonella* Typhimurium infection causes a decrease in white blood cell levels and the number of red blood cells. In response, the population of lactic acid bacteria in the ileum, cecum, and feces increases. Second, the administration of PRO, MOS, and their combinations can prevent inflammation caused by *Salmonella* Typhimurium infection, characterized by the absence of necrosis and desquamation of the epithelium in the ileum and no increase in white blood cells. PRO, MOS, and their combinations can also stimulate intestinal health, as indicated by an increase in villus height and width, a decrease in crypts, and an increase in VCR. Compared to other treatments, MOS is very effective in increasing the population of lactic acid bacteria in the digestive tract. Third, *P. kudriavzevii* 2P10 grew in the cecum and ileum. During ST infection, *P. kudriavzevii* 2P10 can coagulate with ST both in the ileum, with this yeast-binding bacteria mechanism allowing free *Salmonella* Typhimurium cells to bind more to *P. kudriavzevii* 2P10 cells than to intestinal epithelial cells so that infection can be prevented.

In conclusion, our study revealed that administering *P. kudriavzevii* 2P10 was the best way to stimulate growth performance in intestinal health and prevent severe *Salmonella* Typhimurium infection in male SD rats. Our study suggests that *P. kudriavzevii* 2P10 may be helpful as an alternative treatment for patients with salmonellosis. This possibility should be tested in well-controlled human clinical trials.

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