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### The Effect of Giving Fermented Milk Starter *Lactococcus lactis* D4 Dadih on Obstructive Jaundice: An In Vivo Study

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#### ABSTRACT

**Background:** Every 1 person out of 3 people with obstructive jaundice will die from complications of obstructive jaundice. The absence of bile salts causes the loss of the protective layer of the intestinal mucosa, which causes an increase in the permeability of the intestinal wall, triggering the process of translocation of various bacteria from the intestinal tract to the blood circulation so that it can trigger septicemia and sepsis. Dadih or commonly known as ampiang dadih is a fermented buffalo milk product. Dadih contains lactic acid bacteria microbiota, which can function as probiotics fermented milk starter *Lactococcus lactis* D4 dadih to small intestine in obstructive jaundice in vivo. **Methods:** This study was an in vivo experimental study using 30 wistar rats, which were divided into 3 test groups. Observation of the condition of the small intestine was carried out histopathologically to assess the inflammatory process and the condition of the intestinal mucosa. Data analysis was performed using SPSS version 25 using univariate and bivariate methods. **Results:** Group 3 showed minimal histopathological inflammation compared to the control and was statistically different,  $p < 0.05$ . **Conclusion:** Giving fermented milk starter *Lactococcus lactis* D4 dadih was able to reduce the inflammatory response and repair the intestinal mucosa in conditions of obstructive jaundice in vivo studies.

#### 1. Introduction

Jaundice is a condition in which there is a disturbance in the distribution of bile salts to the intestine due to a blockage in the bile salt ducts. This bile duct obstruction causes the accumulation of bile salts and bilirubin products in the liver and bile. This causes the condition of jaundice, which causes a clinical appearance of yellowness in the skin and membranes. Obstructive jaundice is a serious problem in the world of health. Some epidemiological data show that this disorder is quite common, whereas in Saudi

Arabia, as many as 242 per 1000 cases of surgery are caused by obstructive jaundice. The United States shows that the incidence of obstructive jaundice occurs in 5 cases per 1000 population.<sup>1-5</sup>

Various studies show that obstructive jaundice has a fairly high mortality, which is around 33%. Every 1 person out of 3 people with obstructive jaundice will die from complications of obstructive jaundice. The absence of bile salts causes loss of the protective lining of the intestinal mucosa, which causes an increase in

the permeability of the intestinal wall. Increased permeability of the intestinal wall will trigger the process of translocation of various bacteria from the intestinal tract to blood circulation. It can trigger septicemia and sepsis. Various studies have shown that there is no optimal management or intervention in preventing disorders of the intestine due to the absence of bile salts.<sup>6-9</sup>

Efforts to explore new therapeutic modalities by utilizing the local wisdom of the people of West Sumatra are expected to be a solution to this problem. Minangkabau, West Sumatra has an original probiotic product called Dadih. Dadih or commonly known as ampiang dadih is a fermented buffalo milk product. Dadih contains lactic acid bacteria microbiota, which can function as probiotics. Dadih consists of lactic acid bacteria, which is the highest content. Lactic acid bacteria will dominate during the fermentation process and reduce pathogenic bacteria. Bacteriocins and LAB (*Lactococcus and Lactobacillus*), which predominate in curd, have an important role in suppressing potential pathogens in curd. The most intensive species to be studied in secreting bacteriocins so far is *Lactococcus lactis*. *Lactococcus lactis* has the broadest spectrum of bacteriocin activity among the other bacteriocins.<sup>10-13</sup>

This study aims to evaluate the potential of fermented milk starter *Lactococcus lactis D4* dadih to the small intestine in obstructive jaundice in vivo. Where in this study, an evaluation was carried out related to the Barthel spoil score, the thickness of the small intestine mucosa, and the diameter of the small intestine fermented milk starter *Lactococcus lactis D4* dadih to the small intestine in obstructive jaundice.

## 2. Methods

This study was an in vivo experimental study using the randomized control group posttest-only design approach. This study used Wistar rats (*Rattus norvegicus*) with inclusion criteria: male gender, 10-16 weeks of age, and 160-250 gram body weight. A total of 30 rats were included in this study. After acclimatization for 7 days, white rats were grouped into 3 groups, every 10 rats, randomly. Group 1:

sham-operated; Group 2: obstructive jaundice was induced; group 3: obstructive jaundice induction and fermented milk starter *Lactococcus lactis D4* dadih at a dose of 320 mg/200grBB per day for 7 days, intragastrically. This study was approved by the medical and health research ethics committee of the Faculty of Medicine, Universitas Andalas, Padang, Indonesia (No. 948/UN.16.2/KEP-FK/2022).

Fermented milk starter *Lactococcus lactis D4* dadih was obtained from the Faculty of Animal Husbandry, Universitas Andalas, Padang, Indonesia. This milk is rich in lactic acid bacteria. The process of inducing obstructive jaundice was carried out through a laparotomy process by carrying out a ligation process and cutting the common bile duct by first anesthetizing it with 0.1 mL/10 g BW biopentyl. After completion of treatment, the white rats were euthanized by injection of ketamine at a dose of 100 mg/kg BW. Next, the small intestine was evacuated from the white rats and put into a 10% Neutral Buffer Formalin (NBF) solution. Furthermore, the dehydration process is carried out by placing it in a 70% - 96% alcohol gradient solution and xylol I, II, and III solutions. Furthermore, the paraffinization process is carried out to obtain paraffin blocks. The paraffin block cut (5um thickness) was placed on a glass slide that had been coated with poly-L-Lysine, then deparaffinized by inserting the glass slide into xylol and alcohol solution. Next, stained with hematoxylin-eosin. Then a histopathological assessment process was carried out in which an assessment was carried out to assess the level of inflammation of the small intestine quantitatively by assessing submucosal edema, PMN infiltration in the lamina propria, goblet cells, epithelial integrity, thickness of the intestinal mucosa and diameter of the small intestine.

Data analysis was performed with the help of SPSS software version 25. Univariate analysis was performed to present the distribution of data frequencies for all test variables. Bivariate analysis was performed to compare the means between groups, with a p-value <0.05.

### 3. Results

Table 1 shows the mean spoiled Barthel scores between groups. The higher the spoiled Barthel score indicates, the more severe the degree of inflammation. Group 2 showed the highest score compared to other groups. Group 3 showed a Barthel spoiled score that

was relatively identical to the mean Barthel spoiled score in group 1. Statistically, there was a significant difference in the Barthel spoiled score between group 1 and group 2. There was no significant difference in the spoiled Barthel score between group 1 and group 3.

Table 1. Comparison of mean Barthel Manja score between groups.

Group	Mean Barthel Manja score $\pm$ SD	p-value
1	3.6 $\pm$ 0.97	-
2	5.5 $\pm$ 0.54	0.00*
3	3.8 $\pm$ 0.31	1.00*

\*Post-hoc Bonferroni VS group 1,  $p < 0.05$ .

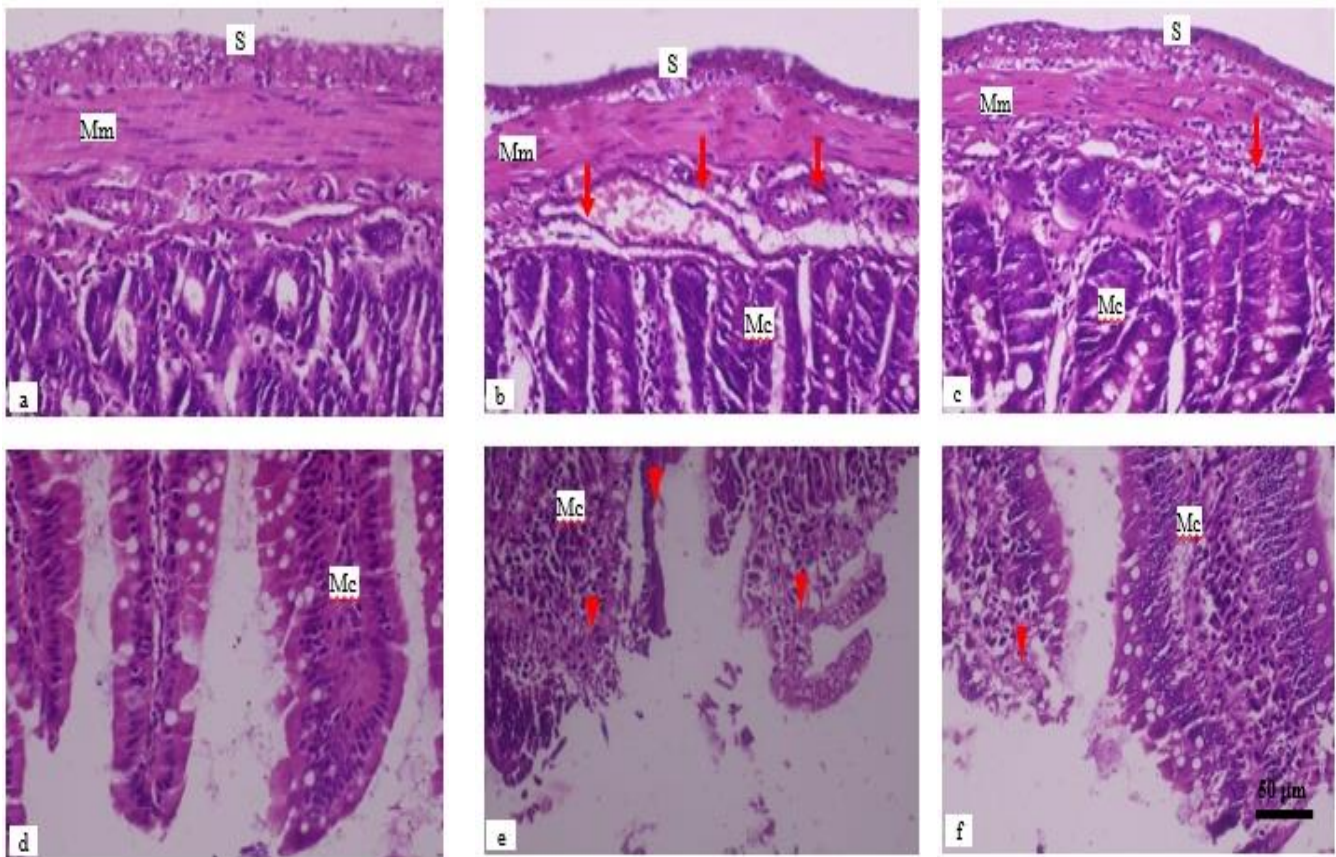


Figure 1. Histological assessment of intestinal mucosal damage based on the Barthel Manja score. Intestinal tissue showed layers of mucosa (Mc), submucosa (Sm), muscularis (Mm), and serosa (S) in experimental animals group 1 (a, d), group 2 (b,e), and group 3 (c,f). Hematoxylin eosin staining. Magnification 400x.

Table 2 shows the mean mucosal thickness between groups. Group 2 showed the lowest thickness compared to the other groups. Group 3 showed the thickest mucosal thickness compared to groups 1 and

2. Statistically, there was a significant difference in mucosal thickness between group 1 and group 2. Likewise, there was a significant difference in mucosal thickness between group 1 and group 3.

Table 2. Comparison of mean thickness intestinal mucosa between groups.

Group	Mean intestinal mucosa thickness (um) ±SD	p-value
1	388.5 ± 84.68	-
2	304.5 ± 67.44	0.04*
3	479.2 ± 66.95	0.02*

\*Post- hoc Bonferroni VS group 1, p<0.05.

Table 3 shows the mean intestinal diameters between groups. Group 2 showed the highest intestinal diameter compared to the other groups. Group 3 showed a relatively identical intestinal diameter to the mean intestinal diameter of group 1.

Statistically, there was a significant difference in intestinal diameter between group 1 and group 2. There was no significant difference in intestinal diameter between group 1 and group 3.

Table 3. Comparison of mean intestinal diameter between groups.

Group	Mean intestinal diameter (um) ±SD	p-value
1	2685.2 ± 510.36	-
2	3389.2 ± 303.98	0.00*
3	2626.4 ± 304.41	1.00*

\*Post-hoc Bonferroni VS group 1, p<0.05.

#### 4. Discussion

Obstructive jaundice can cause an increase in endotoxin, which stimulates an increase in intestinal permeability, disruption of tight junction cells, and the release of inflammatory cytokine mediators, tumor necrosis factor-alpha (TNF-α), interleukins -1, interleukin-6, interferon-gamma (INF-γ), nitric oxide and oxygen free radicals. Increased intestinal permeability results in edema of the mucosa and submucosa, and disruption of tight junction cells will result in disruption of epithelial integrity. Bile salts are thought to prevent intestinal endotoxin and bacterial translocation by binding to intraluminal and bacterial endotoxins directly and creating detergent-like complexes that are poorly absorbed. In addition, bile is essential for the maintenance of tight junction enterocyte integrity, regulating the expression of the essential tight junction-associated proteins occludin and ZO-1, thereby maintaining the intestinal

paracellular barrier. In the absence of bile salts in the digestive tract, it will disrupt the balance of the intestinal microflora with the overgrowth of gram-negative bacteria and also increase endotoxins in the intestinal lumen. This substance will cause damage to the small intestine by increasing intestinal permeability (mucosal expression decreases in TJ-associated protein ZO-1) and also atrophy of the intestinal mucosa (cell death than cell proliferation).<sup>14-19</sup>

Activation of various inflammatory mediators in obstructive jaundice will result in the activation of the death ligand, which in turn activates cell apoptosis in the small intestine. Cell death will cause the thickness of the intestine to decrease and cause the intestinal lumen to become wider.<sup>20</sup> The results of this study show that obstructive jaundice causes serious inflammation of the small intestine. Inflammatory conditions cause a decrease in the thickness of the

intestinal mucosa accompanied by an increase in intestinal diameter. The provision of fermented milk starter *Lactococcus lactis D4* dadih was able to reduce inflammation of the intestinal mucosa. The decrease in inflammation causes an increase in the thickness of the intestinal mucosa accompanied by a decrease in the diameter of the intestine.

## 5. Conclusion

Giving fermented milk starter *Lactococcus lactis D4* dadih can reduce the inflammatory response and repair the intestinal mucosa in obstructive jaundice in vivo studies.

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