



## KEPOK–MORINGA PREBIOTIC IN A GASTROINTESTINAL DISORDER RAT MODEL

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### Abstact

Colorectal cancer is a prevalent global health concern, with millions of new cases reported worldwide. Colorectal cancer (CRC) incidence reached 34,189 cases (8.6%) in 2020, and its pathogenesis is closely linked to gastrointestinal immunity. When the protective mechanisms of the gut are compromised, it often leads to intestinal infections, with clinical manifestations such as weight loss, bleeding, and reduced microbiota levels caused by malabsorption and mucosal damage. Resistant starch from Kepok Banana extract is considered a potential preventive agent, while a diet rich in iron and vitamin C-particularly from moringa leaves-supports iron absorption and helps prevent chronic diseases. In preclinical research, Dextran Sodium Sulfate (DSS) induction is widely used to model intestinal inflammation similar to that seen in humans. This study evaluated the effect of a Kepok-Moringa Starch (KMS) prebiotic supplement on body weight and gut microbiota composition, focusing on *Lactobacillus* and *Shigella* spp., in female rats. The experiment used 30 female Wistar rats divided into five groups with varying KMS doses, preceded by one week of acclimatization, four weeks of treatment, and DSS induction for five days. The results showed that KMS supplementation in DSS-induced rats significantly increased body weight and altered gut flora composition, with the highest KMS dose producing an increase in beneficial bacteria and a reduction in pathogenic bacteria. These findings suggest that KMS has strong potential as a prebiotic candidate for further development in colorectal cancer prevention.

**Keywords:** Colon Cancer, Resistant Starch, Kepok Banana, Moringa, Prebiotic

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### INTRODUCTION

The pathogenesis of colorectal cancer (CRC) is intrinsically linked to gastrointestinal (GI) immunity, which is influenced by numerous factors, including the composition of the normal gut flora, mucosal integrity, and both humoral and cellular immunity. Disruption of any of these protective mechanisms can lead to gastrointestinal infections and inflammatory conditions such as colitis and enteritis. Clinical manifestations of these issues often include abdominal pain, fever, diarrhea, and potentially intestinal bleeding (Itani et al., 2021). When associated with colorectal infection, blood profiles

become important diagnostic indicators because they correlate with infection markers and symptoms such as bloody stools, abdominal pain, difficulty in defecation, and weight loss (Sanjaya et al., 2023).

Managing the dysbiosis and nutritional deficiencies that commonly occur in gastrointestinal infections requires a strategy that focuses on improving gut health and nutrient bioavailability. Resistant starch (RS), a significant component in bananas reportedly making up around 15% of total starch plays an essential role in this context. The nutritional quality of banana-based preparations can be further enhanced by combining them with moringa leaves, as this mixture provides considerable micronutrient content, including approximately 7.45 mg of elemental iron (Fe). Functionally, RS passes through the small intestine undigested and reaches the colon intact. In the colon, it undergoes fermentation by commensal bacteria such as Bifidobacteria and Lactobacilli, stimulating their growth and metabolic activity. This mechanism makes resistant starch a potent prebiotic. By definition, prebiotics are substrates that are selectively utilized by host microorganisms to provide health benefits. The targeted stimulation of beneficial microbial populations is vital for re-establishing a balanced gut environment, which is necessary to reduce pathogen load and support mucosal repair during infection.

When the gastrointestinal tract is compromised, the absorption of essential nutrients involved in red blood cell formation is impaired, which increases the risk of hemoglobin deficiency. Moreover, bleeding caused by gastric ulcers and intestinal infections contributes to a reduction in red blood cells (Gunawan, 2020). Gastrointestinal infections, including Inflammatory Bowel Disease (IBD), are often characterized by weight loss due to malabsorption in the affected digestive tract (Jabłońska & Mrowiec, 2023).

In experimental research, the Dextran Sodium Sulfate (DSS) model is widely used to replicate intestinal inflammation mechanisms. Administration of DSS damages the single layer of epithelial cells that line the colon, allowing pro-inflammatory intestinal contents such as bacteria and their products to penetrate the underlying tissue. As a result, DSS-induced colitis—often referred to as acute colitis—is established through continuous administration of 2–5% DSS over a short period of about 4–9 days (Perše & Cerar, 2012).

Risk factors for digestive diseases such as IBD include a range of environmental influences: smoking, lifestyle and hygiene habits, drug exposure, appendectomy, dietary intake, and gastrointestinal pathogens. These factors have been linked to the increasing global incidence of IBD (Singh & Bernstein, 2022). One management strategy involves strengthening the body's natural defenses

through digestive system support, as the gut harbors the largest concentration of antibody-producing cells and antibodies in the human body.

Prebiotics derived from plant-based foods are a key focus in digestive health management. The plantain fruit (*Musa paradisiaca*) contains compounds such as inulin and fructooligosaccharides (FOS), which act as natural prebiotics. These compounds facilitate the proliferation of beneficial bacteria that convert lactose (milk sugar) into lactic acid (Aprila et al., 2023). Both inulin and resistant starch are classified as prebiotic fibers because they selectively stimulate the growth and activity of beneficial gut bacteria (Ruhdiana & Sandi, 2023).

A study by Hardisari & Amaliawati (2016) demonstrated that adding various concentrations of plantain (*Musa paradisiaca* forma typica) flour significantly affected the in vitro growth of *Lactobacillus casei*. The presence of plantain flour accounted for 85.9% of the observed effect on *Lactobacillus casei* count, leaving 14.1% attributed to other factors. This result supports the selection of plantain starch as a suitable prebiotic for promoting probiotic growth in the gut. *Lactobacillus casei* has been proven beneficial for gastrointestinal health and immune system modulation through its roles in phagocytosis, antibody production, and cytokine release, all of which contribute to the elimination of pathogenic bacteria (Hardisari & Amaliawati, 2016).

Research on the swelling power and resistant starch content of banana varieties has shown that the stone banana (*Musa textilis*) contains the highest resistant starch content (39.35%), followed by yellow plantain (27.70%) (Musita, 2012). Both animal and human studies have confirmed that resistant starch improves the absorption of essential minerals, including calcium, magnesium, zinc, iron, and copper, in the small intestine (Raigond et al., 2017).

In addition to resistant starch, other nutrients such as iron and vitamin C play crucial roles in maintaining health. Moringa leaves (*Moringa oleifera*) contain both, with an iron content up to 25 times greater than spinach per 100 grams (Riska Vidayana, 2020). Moringa also exhibits a variety of pharmacological activities, including antihypertensive, antispasmodic, anti-ulcer, anti-inflammatory, and antioxidant properties. The flavonoids and  $\beta$ -sitosterol in moringa contribute to reducing ulcers in the digestive tract, suggesting a protective role against tissue damage (Setiawan et al., 2018).

A study by Tangkas et al (2021) revealed that administering 1 cc of plantain peel extract to rats weighing 200–225g maintained hemoglobin and erythrocyte levels over 28 days. Female rats were used because irritable bowel syndrome (IBS) is more common in women and often presents with

severe symptoms accompanied by anxiety and depression. Research has shown significant gender differences in gut motility, transit time, visceral and somatic pain sensitivity, and psychological characteristics (Narayanan et al., 2021).

Despite the known health-promoting properties of plantain starch and moringa leaves, research exploring their combined prebiotic effects remains limited. Previous studies have focused separately on their ability to influence microbial balance, nutrient absorption, and inflammatory response, but not on their synergistic action in vivo. Given the increasing recognition of plant-derived food supplements as a potent approach to mitigating the mortality burden associated with CRC (Macharia et al., 2022), this gap highlights the need for a study investigating their combined role in gastrointestinal health.

The present study aims to investigate the effect of plantain–moringa starch prebiotic on body weight and gut microbiota count in rats afflicted with gastrointestinal infection. This includes examining changes in *Lactobacillus* and *Shigella* populations and evaluating the overall potential of the formulation to improve host health in DSS-induced inflammatory conditions.

This study is expected to provide scientific evidence supporting the development of plant-based prebiotic formulations that utilize local ingredients such as banana and moringa. The findings could contribute to functional food innovation, enhance understanding of the role of resistant starch and moringa in gut health, and promote strategies for reducing the incidence of gastrointestinal infections and colorectal cancer. Furthermore, such research supports sustainable nutrition approaches and the utilization of indigenous plant resources with both economic and health benefits.

## **METHODS**

### **Research Design and Animal Model**

This study employed a True Experimental Design utilizing a Completely Randomized Block Design (CRBD). The experiment consisted of five treatment groups: P0 (Standard feed only), P1 (Standard feed + DSS induction), P2 (Standard feed + low-dose plantain-moringa prebiotic + DSS), P3 (Standard feed + medium-dose plantain-moringa prebiotic + DSS), and P4 (Standard feed + high-dose plantain-moringa prebiotic + DSS).

The animal subjects were female Wistar rats aged 6–8 weeks with a body weight ranging from 80–150 grams. Each group contained 6 rats, resulting in a total sample size of 30 rats. Sample selection

was performed using simple random sampling, and the sample size was determined using Federer's formula. All rats underwent a 1-week acclimatization period before the 50-day intervention period commenced.

### **Research Location and Variables**

The study was conducted across several laboratories: the Chemistry, Pharmacology, and Culinary Laboratories at the Nutrition Department of Poltekkes Malang for the preparation of intervention materials; the Experimental Animal Development Laboratory (LPHC) at Brawijaya University (FKUB) for animal handling and treatment administration; and the Microbiology and Clinical Pathology Laboratories at FKUB for the examination of rat specimens.

The independent variable in this study was the administration of the prebiotic derived from plantain starch extract and moringa leaf powder. The dependent variables were hemoglobin levels and rat body weight.

### **Intervention and Procedures**

The research procedure began with the preparation of the plantain starch extract and the incorporation of the moringa leaf powder. Rats were housed in individual cages with adequate ventilation and provided with standard feed. Prebiotic intervention was administered orally via gavage (stomach tube) according to each group's specific dosage. The prebiotic doses, based on conversion from human to rat equivalents, were: 0.07 grams for the low dose (P2), 0.14 grams for the medium dose (P3), and 0.28 grams for the high dose (P4).

Specimen collection was performed at the end of the study, involving the collection of blood from the anesthetized rats' hearts, as well as the analysis of feces and cecum contents to observe the gut microbiota profile.

### **Data Collection and Statistical Analysis**

The data was collected, coded, and entered into a computer file using the SPSS for Windows Release 24.0 application. Data analysis utilized the Shapiro-Wilk test for normality and Independent sample t-tests for group comparisons. The One-Way ANOVA was employed to observe the general differences in the mean of every variable for the rats in all groups. Next, a post-doc (Tukey) test was conducted to find out which groups were different.

## Ethical Approval

This study received Ethical Approval from the Research Ethics Committee of the Health Polytechnic of the Ministry of Health, Malang (Poltekkes Kemenkes Malang), with registration number 811/KEPK-POLKESMA/2024, dated July 24, 2024. This ensures that all procedures involving the experimental animals adhere to established ethical principles and standards.

## RESULTS AND DISCUSSION

### Body Weight Dynamics

Based on (Figure 1), a trend of body weight changes was observed in female Wistar rats from the first to the eighth weekly measurement, with each treatment group displaying distinct patterns of weight gain and loss over time. The Positive Control group (P1), which was induced with DSS but received no prebiotic intervention, experienced a significant weight reduction after the third measurement, dropping from 165.32 grams to 163.57 grams. This loss reflects a typical physiological response to DSS-induced intestinal inflammation, which causes mucosal damage and microbiota imbalance, thereby impairing nutrient absorption and resulting in weight loss. Moreover, P1 exhibited the smallest net weight gain over the entire study period, totaling only 17.84 grams. Conversely, the Negative Control group (P0) showed stable body weight, indicating a normal physiological condition free from inflammatory disturbances.

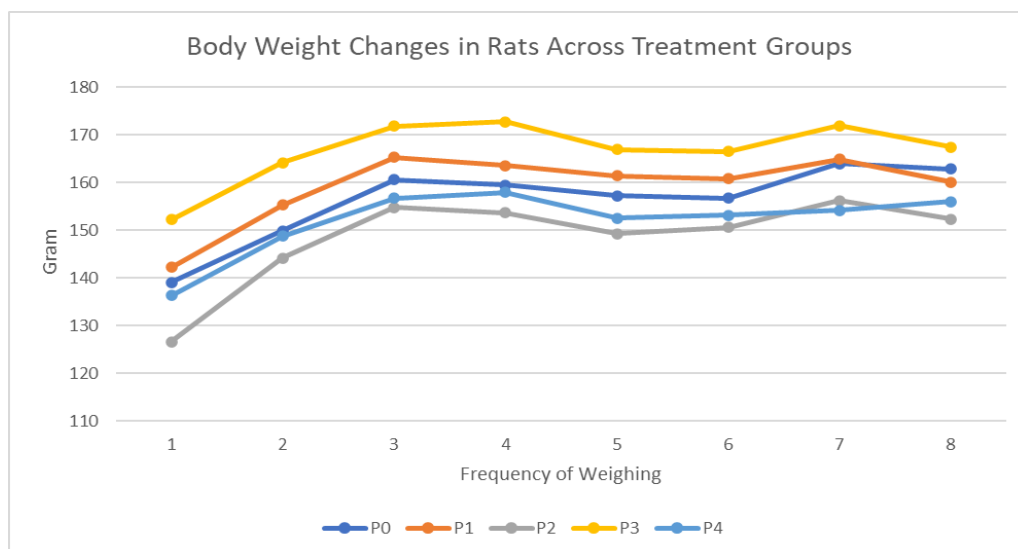


Figure 1. Body Weight Changes in Female Wistar Rats Across Treatment Groups

It is important to note that the variation in initial body weight among the rats may have influenced the outcomes and introduced potential bias. Therefore, utilizing animals with uniform weight characteristics is crucial to reduce confounding variables. This aligns with the findings of Chaka et al

(2022), who emphasize that uniformity in physical characteristics like body weight is essential for obtaining consistent metabolic responses to treatment.

Table 1. Body Weight Changes in Female Wistar Rats Across Treatment Groups

Groups	Body Weight (gram) Mean $\pm$ SD	p-value
P0	156.2188 $\pm$ 8.17190 <sup>ad</sup>	0.001
P1	159.2225 $\pm$ 7.54866 <sup>a</sup>	
P2	148.4650 $\pm$ 9.58446 <sup>be</sup>	
P3	166.7525 $\pm$ 6.62408 <sup>c</sup>	
P4	151.9663 $\pm$ 6.95760 <sup>de</sup>	

The average body weight of female Wistar rats across all treatment groups ranged between 148.47 grams and 166.75 grams during the study period. Table 1 provides a comprehensive breakdown of these average body weights for each treatment.

Table 1 illustrates the differences in mean body weight between the control and treatment groups, revealing noticeable variation in the response to the administration of plantain-moringa starch. Surprisingly, the Negative Control (P0) and Positive Control (P1) groups showed no statistically significant difference in average body weight. This suggests that the DSS induction in the P1 group did not cause a substantial drop in body weight compared to the normal condition. This outcome may be attributed to inter-individual variation among the experimental rats, as biological differences between animal models can lead to inconsistent results, including weight changes induced by stress or intervention.

### Effect of Plantain-Moringa Prebiotic

The Low-Dose group (P2) showed a lower final mean body weight than P1, indicating that this minimal dose was ineffective in promoting recovery and post-DSS weight restoration. This group exhibited a pattern similar to P1, with a decline in weight after reaching its peak, suggesting that the dosage was insufficient to support full recovery. Conversely, the Medium-Dose group (P3) achieved the highest average body weight (166.75 grams); however, this finding must be interpreted cautiously, as this group began with a notably higher initial body weight (152.23 grams) compared to the others, resulting in a relatively modest total gain of 15.24 grams. Meanwhile, the High-Dose group (P4), despite starting with a lower initial weight than P3, demonstrated the most stable and consistent weight gain throughout the study, with a total increase of 19.72 grams. This improvement closely approximated the pattern of the P1 control group, indicating a positive, though not entirely optimal, restorative effect on body weight.

The deficit in body weight relative to the Negative Control (P0) further clarified the efficacy: P2 showed the largest deficit (−7.734 grams), followed by P4 (−4.252 grams). This comparison suggests that the high dose (0.2 g/rat) was more effective than the low dose in restoring body weight closer to the normal, un-induced state (P0). This positive impact on weight gain aligns with the findings of Yusrina et al (2020), which indicated that the administration of banana-based ingredients, such as modified banana flour, can positively affect health parameters, including body weight improvement. This evidence indicates that the high dose of the plantain–moringa prebiotic provided the most significant effect in mitigating the negative impacts of DSS and supporting the restoration of intestinal function. Prebiotics such as resistant starch can improve glucose tolerance, lipid metabolism (International, 2018; Zhang et al., 2021). This weight improvement is presumably due to the plantain–moringa starch’s ability to repair intestinal conditions and enhance nutrient absorption, a finding supported by previous research (Mayang et al., 2019).

### **Microbiota Growth**

Table 2 reveals a statistically significant enhancement of Lactic Acid Bacteria (LAB) growth in the fecal samples of rats receiving the prebiotic supplement (P2, P3, and P4) compared to the DSS-only group (P1). During intestinal fermentation, LAB species such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus* multiply rapidly, reaching their highest concentration in the cecum, which serves as the primary fermentation site. The generally lower bacterial counts observed in the feces can be attributed to certain strains being unable to survive outside the cecum. The potent anti-inflammatory, immunostimulatory, and antibacterial activities of PKK are the proposed mechanisms responsible for this notable improvement in beneficial bacterial populations within the gut. Consistent with its restorative effects, a dose-dependent reduction was observed in the levels of pathogenic bacteria, including *Proteus* and *Shigella* species, present in both the intestines and feces as the PKK dose increased. *Lactobacillus* levels, which normally make up 1–5% of gut microbiota but rise significantly with high fiber and prebiotic intake (Boscaini et al., 2022; Yoo et al., 2024).

Subsequently, additional statistical analyses were conducted to determine the Effect Size (ES), a crucial measure used to quantify the magnitude of the treatment’s influence on outcome variables. ES is particularly important because it extends beyond the p-value to assess the practical significance and relevance of observed changes. This statistical indicator can be expressed through a correlation coefficient (the *r* family), such as Pearson’s *r* or Spearman’s *r*, or through a standardized or non-standardized difference (the *d* family), such as the mean or proportion difference. To calculate the effect size and compare the differences between two specific groups, typically following an independent t-test. Cohen’s *d* was employed in this study.

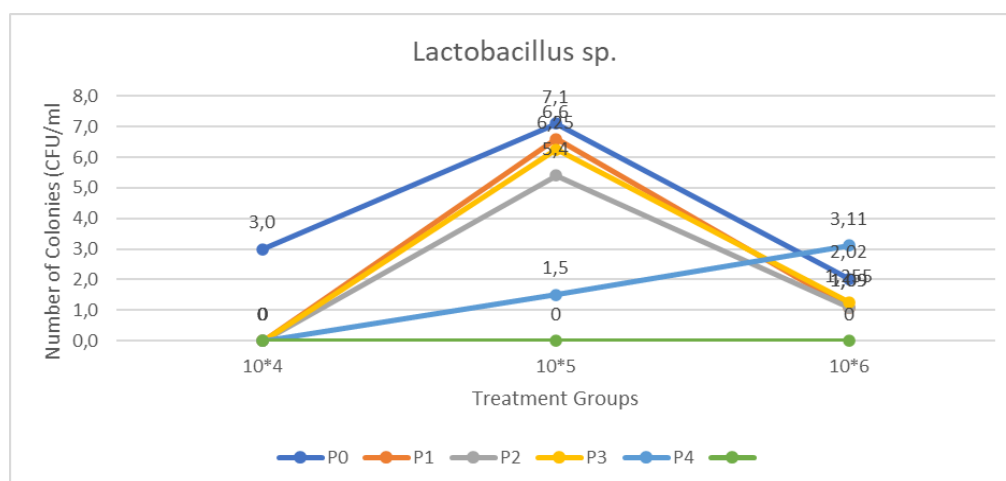


Figure 2. Lactobacillus sp. Counts Across Treatment Groups

Table 2. Lactobacillus sp. Counts Across Treatment Groups

Variables		Treatments (Mean±SD)				p-value
		P1	P2	P3	P4	
Pathogens(103CFU/g)	Feses	2.3±1.2	5.1±3.1	4.1±2.9	3.2±2.	0.50
	Intestine	4.3±3.3	3.9±2.1	4.7±2.7	7.2±3.9	0.03*
LAB(106CFU/g)	Feses	2.6±0.6	1.4±0.2	2.3±0.6	1.7±0.7	0.03*
	Intestine	1.7±0.6	2.0±0.8	2.3±0.6	2.0±0.7	0.72

### Statistical Findings

The statistical analysis indicated that the data did not follow a normal distribution ( $p < 0.05$ ), necessitating the use of non-parametric tests. The subsequent Kruskal–Wallis test revealed a significant difference ( $p = 0.001$ ), confirming that plantain–moringa starch administration had a significant overall effect on the body weight of female Wistar rats. The post hoc Mann–Whitney U test further identified specific significant differences between the Negative Control (P0) and groups P2 and P3; the Positive Control (P1) and groups P2, P3, and P4; as well as between groups P2 and P3, and P3 and P4. Collectively, these results indicate that the effect of the plantain–moringa prebiotic on body weight was dose-dependent. The medium dose (P3) exhibited the most statistically discernible effect, while the high dose (P4) remained effective though slightly less potent than P3, and the low dose (P2) showed no meaningful restorative impact.

To better quantify the treatment's influence on both body weight and microbiota composition, the Effect Size (ES) was calculated, aligning with the study's primary objective of determining the magnitude of Moringa–Banana Starch (PKK) effects on infection risk and beneficial bacterial growth. As shown in Table 3, PKK administration produced a strong overall influence, particularly in the medium-dose group (P3), which demonstrated a substantial body weight increase with a high ES

value of 1.20. Furthermore, the fecal growth of *Lactobacillus* also exhibited a significant enhancement, reflected by an ES of 0.95, indicating a robust prebiotic effect of PKK supplementation.

### **Optimal Dose and Mechanism of Action**

The 1 g PKK/rat/day (P3) dose is proven to have an optimal effect based on the *Lactobacillus* growth data found in the cecum post-intervention. This finding is consistent with the established mechanism of resistant starch (RS), a key component of PKK. RS is known to promote the growth of beneficial bacteria like *Lactobacillus* while inhibiting pathogenic species such as *Enterobacter*, *Salmonella*, and *Escherichia coli*.

This beneficial effect is directly linked to the prebiotic properties and subsequent fermentation processes in the colon. As a complex carbohydrate, RS bypasses digestion in the small intestine and is instead fermented by the colonic microbiota. This fermentation generates Short-Chain Fatty Acids (SCFAs) including butyric acid, acetate, and propionate—which specifically support the proliferation of *Lactobacillus* and *Bifidobacterium*. These beneficial bacteria, in turn, create an acidic environment that is unfavourable for the growth of pathogens like *Salmonella* and *E. coli*.

### **Mucosal Integrity and Antimicrobial Production**

Beyond nutrient provision for beneficial flora, the resistant starch in bananas is capable of increasing the production of mucosal proteins, such as MUC2, thereby enhancing the integrity of the intestinal epithelium. This strengthening of the gut barrier helps to prevent the colonization of pathogens and reduces inflammation triggered by infections from organisms like *Salmonella* or *E. coli*. Additionally, the beneficial bacteria that multiply due to RS fermentation produce antimicrobial metabolites, most notably bacteriocins, which are peptides that directly inhibit pathogen growth.

## **CONCLUSION**

PKK supplementation significantly improved weight and *Lactobacillus* growth, with notable effects in P3. Despite the limited duration, findings support PKK as a promising prebiotic for gut and metabolic health.

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