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Synergy of Banana Starch and Moringa Leaves in Maintaining Hemoglobin Homeostasis and Body Weight in Dextran Sodium Sulfate-Induced Rats

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ABSTRACT

Background: *Colonic inflammation can be triggered by gastrointestinal infections and by chemical agents such as Dextran Sodium Sulfate (DSS). Elevated glucose levels increase insulin resistance and raise circulating insulin-like-growth factor-1 (IGF-1), which may contribute to cancer risk. One possible approach to modulating this condition is through prebiotics contained in kepok banana, because its inulin and resistant starch act as natural prebiotics.*

Research Purposes: *This study aimed to analyze the effect of kepok banana and moringa leaf-based prebiotics on random blood glucose levels in Wistar rats exposed to Dextran Sodium Sulfate (DSS).*

Research Methods: *This true experimental study used a Randomized Block Design (RBD) with five treatment groups and six rats per group. Blood glucose levels were analyzed using one-way ANOVA with a 95% confidence level. Data on feed intake and intake of the kepok banana–moringa starch were analyzed using the Kruskal-Wallis test at a 95% confidence level.*

Research Result: A*dministration of kepok banana–moringa starch in DSS-induced rats did not significantly affect feed intake or reduce blood glucose levels (p=0.121). The most favorable response was observed in P2 group 0.05 g/rat), which showed a tendency toward lower blood glucose levels in DSS-induced rats.*

Conclusion: *Kepok banana–moringa starch administration did not significantly reduce blood glucose levels in DSS-induced rats, although the P2 dose showed a tendency toward improvement.*

BACKGROUND

Colorectal cancer is one of the leading causes of cancer-related mortality worldwide and is closely linked to gastrointestinal immunity and environmental exposures. According to the World Health Organization (WHO), approximately 8.2 million people die from cancer each year, with breast (16.7%), cervical (9.3%), and colorectal (8.6%) cancers being among the most common types. In Indonesia, colorectal cancer ranks first among Southeast Asian countries in terms of incidence (Majid & Ariyanti, 2020). Colorectal cancer develops in the colon and rectum, which are the main segments of the large intestine. Untreated gastrointestinal infections can progress to chronic inflammation such as Inflammatory Bowel Disease (IBD), which markedly increases the risk of colorectal cancer (Hannah et al., 2023).

Environmental factors, including diet, stress, smoking, and exposure to gastrointestinal pathogens, play a major role in triggering IBD (Sudarmo et al., 2018). The gut microbiota is essential for maintaining mucosal integrity, digesting nutrients, and regulating the immune system. Dysbiosis can disrupt immune mechanisms and aggravate chronic inflammation, thereby facilitating the development of colorectal cancer. Blood glucose is also an important parameter for predicting disease progression in infectious and inflammatory

conditions. Stress-induced hyperglycemia can worsen clinical status, increase insulin resistance, and elevate IGF-1 levels, which support cancer cell growth (Hannah et al., 2023; Mansour et al., 2023).

Animal models using *Dextran Sodium Sulfate* (DSS) are widely applied to mimic colonic inflammation. DSS induces acute colitis by damaging the colonic epithelial barrier, allowing pro-inflammatory mediators and pathogens to penetrate the mucosa and cause systemic disturbances (Perše & Cerar, 2012). This inflammatory process leads to metabolic alterations, including impaired nutrient absorption and dysregulated blood glucose control. Over time, these changes can worsen gastrointestinal function and trigger systemic complications such as malnutrition or metabolic syndrome.

One strategy to attenuate intestinal inflammation and reduce colorectal cancer risk is the consumption of prebiotics. Prebiotics, such as fructo-oligosaccharides (FOS), galacto-oligocharides (GOS), inulin, and resistant starch, have been shown to promote the growth of beneficial microbiota and increase the production of *short -chain fatty acids* (SCFAs), which exert anti-inflammatory and anticarcinogenic effects (Nakamura & Omaye, 2012; Wangko, 2020). Kepok bananas contain high levels of resistant starch (27.7%) and have a low glycemic index (43), which contribute to improved blood glucose regulation (Diyah et al., 2018; Robertson, 2012). Moringa leaves (*Moringa oleifera*) are rich in flavonoids and antioxidants, which have demonstrated anticancer and anti-inflammatory properties (Rahmahani et al., 2013). The combination of kepok banana resistant starch and moringa leaves therefore has potential as a functional supplement to support gut health and prevent inflammatory complications.

Based on this rationale, the present study aimed to analyze the effect of kepok banana starch (*Musa paradisiaca forma typica*) combined with moringa leaves (*Moringa oleifera L.*) on blood glucose levels in female Wistar mice induced with DSS. This study also evaluated feed intake and starch consumption as supporting parameters, and assessed the potential role of these prebiotics in metabolic recovery in DSS-induced intestinal inflammation.

MATERIAL AND METHODS

This study was a true experimental study using a completely randomized design with five treatment groups: P0 (negative control), P1 (positive control + DSS), P2 (DSS + low-dose prebiotics 0.05 g/rat), P3 (DSS + medium-dose prebiotics 0.10 g/rat), and P4 (DSS + high-dose prebiotics 0.20 g/rat). Thirty female Wistar rats aged 6–8 weeks and weighing of 80–150 grams were selected by simple random sampling, with six rats allocated to each group. The rats were acclimatized for 7 days, then given standard feed and prebiotic interventions for 50 days. The prebiotics formulation consisted of a combination of kepok banana resistant starch extract and moringa leaves, administered orally via gastric gavage. DSS (Dextran Sodium Sulfate) was administered at a concentration of 5% in drinking water for 5 days to induce colonic inflammation.

This study was conducted in several laboratories: The Chemistry and Culinary Laboratory of the Health Polytechnic of Malang (preparation of intervention materials), the Laboratory of Experimental Animal Development (LPHC) at the Faculty of Medicine, Universitas Brawijaya (animal maintenance and interventions), and the Microbiology and Clinical Pathology Laboratory of the same faculty (blood and fecal analysis). Materials included female Wistar rats, standard feed (COMFEED AD II), DSS, and resistant starch extracts from kepok bananas and moringa leaves. Starch processing involved drying, grinding, extraction, and precipitation, whereas moringa leaves were dried and milled before being incorporated into the prebiotic formulation.

Feed intake was recorded daily. At the end of the intervention blood samples were collected via intracardiac puncture and analyzed for blood glucose levels in the laboratory. Data were processed using SPSS for Windows. Normality was assessed using the Shapiro-Wilk test, and homogeneity using *the Levene's Test*. When data were normally distributed and homogeneous, One Way ANOVA was used to compare blood glucose levels between groups. When assumptions were not met, the Kruskal-Wallis was applied, followed by the Mann-Whitney post-hoc test if needed. The null hypothesis (H0) was that prebiotic administration has no effect on: blood glucose levels in rats; the alternative hypothesis (H1) was that prebiotic administration affects blood glucose levels in rats.

RESULTS

Feed intake data were obtained from standard feed (COMFEED AD II), which contains 51% carbohydrates, 15% crude protein, 7% crude fat, and 6% crude fiber. Each rat received 30 g of feed that had

been supplemented with different doses of kepok banana–moringa starch. Feed intake was calculated by weighing the remaining feed the following morning. The mean dietary intake for each group is shown in Figure 1 and Table 1.

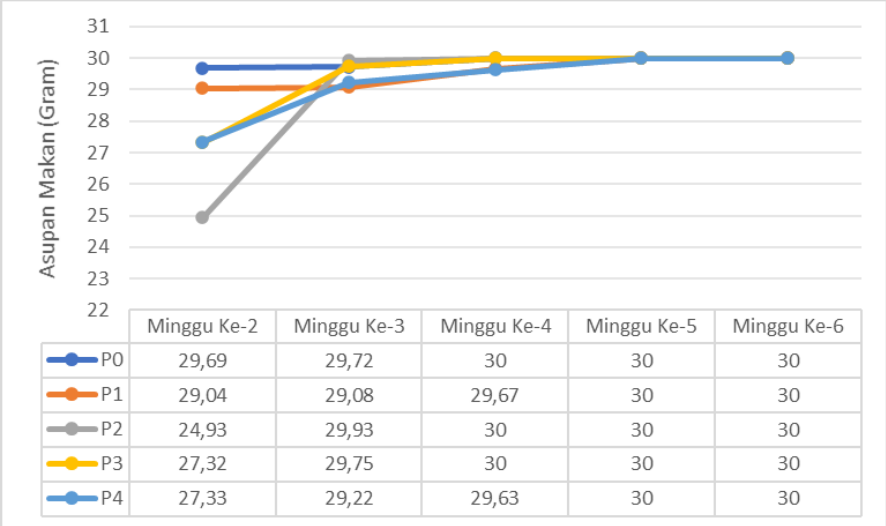

Figure 1. Graph of Average Rats' Dietary Intake for Each Treatment

Table 1. Dietary Intake of Rats by Treatment Group

Groups	Rats Feeding Intake Red ± SD	*ρ-value*
P0	29.8820 ± 0.16193	
P1	29.5580 ± 0.47436	
P2	28.9720 ± 2.25975	0.794
P3	29.4140 ± 1.17558	
P4	29.2360 ± 1.11307	

In table 1. shows changes in dietary intake between the control group and the treatment group. The results of the study showed that the average intake of rats in the positive control group was lower than that of the negative control group. This happened because the P1 group of the positive control group was only given DSS induction without being given the intervention of kepok banana-moringa starch. In the intervention treatment, the lowest average dietary intake was found in the 2nd treatment compared to P3 and P4, this occurred due to metabolic stress that was not acceptable to the rats so that appetite decreased. A significant inflammatory response can arise due to metabolic stress, such as increased levels of pro-inflammatory cytokines, which contribute to changes in appetite (Haningtyas et al., 2022).

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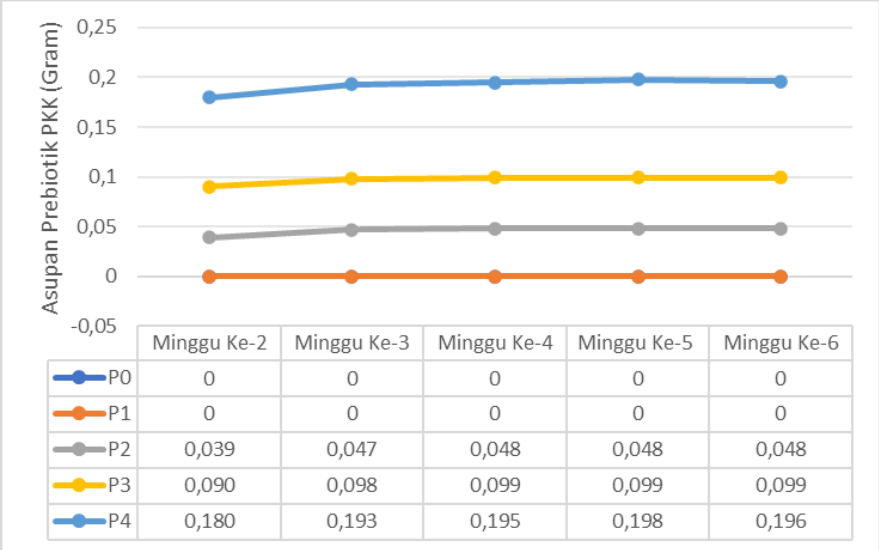

Figure 2. Graph of Average Intake of Kepok Moringa Starch in Rats per Treatment Per Week

In general, rats in the positive control group (P1) had lower mean feed intake than those in the negative control group (P0), likely because P1 received DSS induction without any prebiotic intervention. Among the intervention groups, the lowest mean feed intake was observed in P2, which may reflect metabolic stress and reduced appetite. Metabolic stress can provoke an inflammatory response and increase levels of pro-inflammatory cytokines, which are known to influence appetite regulation.

Table 2. Moringa Kepok Starch Intake Based on Treatment Group

Groups	**Rats Feeding Intake Red ± SD**	***ρ-value***
P0	0 ± 0a	
P1	0 ± 0a	
P2	0.046 ± 0.004b	0.000
P3	0.097 ± 0.004c	
P4	0.190 ± 0.006d	

Kepok banana–moringa starch intake increased over time in the P2, P3, and P4 groups, whereas it remained close to zero in the P0 and P1 groups, which did not receive the prebiotic formulation (Figure 2, Table 2). Mean prebiotic intake ranged from 0.046 to 0.190 g per rat across the treatment groups.

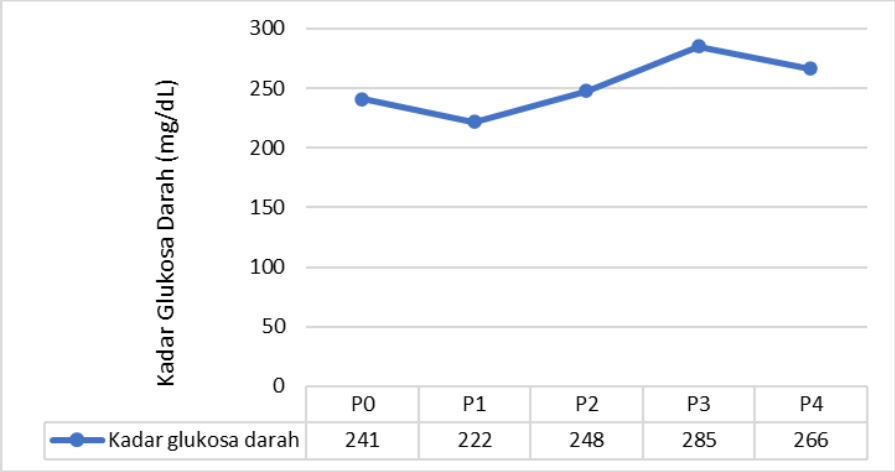

Figure 3. Graph of Average Blood Glucose Levels in Mice

Blood glucose data, obtained from terminal blood sampling and laboratory analysis, are presented in Figure 3 and Table 3. Mean blood glucose levels ranged from 221.83 to 285.17 mg/dL among the groups.

The negative control group (P0), which did not receive DSS, had a mean blood glucose level of 241.17 mg/dL, representing normal values in healthy rats. The positive control group (P1), which received DSS but no prebiotic, showed a decrease to 221.83 mg/dL, suggesting that DSS induction altered clinical and metabolic parameters in this colitis model.

Table 3. Blood Glucose Levels in Rats Based on Treatment Group

Groups	Rats Feeding Intake Red ± SD	*ρ-value*
P0	241.17± 57.21	
P1	221.83 ± 38.61	
P2	247.83 ± 32.15	0.121
P3	285.17 ± 37.21	
P4	266.33 ± 38.69	

The low dose group (P2) had a mean blood glucose level of 247.83 mg/dL, indicating that this dose was not sufficient to fully counteract the metabolic effects of DSS, although it may have provided a mild stabilizing effect. The medium dose group (P3) showed the highest mean blood glucose level (285.17 mg/dL), suggesting that this dose did not improve glycemic control and may have been associated with unfavorable changes in gut microbiota (dysbiosis). The high dose group (P4) exhibited a decrease in mean blood glucose (266.33 mg/dL) compared with P3, possibly reflecting a pharmacological effect at higher doses, although levels remained higher than in both control groups.

Overall, the smallest difference from the negative control was observed in the P2 group, followed by P4, implying that low-dose kepok banana–moringa starch (0.05 g/rat) may have the most favorable tendency toward restoring blood glucose levels.

Statistical analysis showed that blood glucose data were normally distributed and homogeneous. One-way ANOVA revealed no significant effect of kepok banana–moringa starch administration on blood glucose levels ($p = 0.121$). These findings suggest that, within the study duration, treatment-related changes in blood glucose were modest and may have been influenced by various physiological and environmental factors.

DISCUSSION

Table 3 shows the difference in blood glucose levels between the control group and the treatment group, the negative control group (P0) did not receive DSS induction showed a blood glucose level of 241.17 mg/dL, this value is the normal blood glucose level of healthy rats. Meanwhile, the positive control group (P1), induced by DSS without being given treatment, experienced a decrease in blood glucose levels to 221.83 mg/dL. This decrease indicates that DSS induction impacts changes in clinical and molecular parameters in the DSS colitis model, which can affect metabolism and blood glucose levels (Saputra et al., 2022).

The P2 group that received treatment with a low dose of kepok banana-moringa starch showed a blood glucose level of 247.83 mg/dL indicating that the low dose was not effective enough to neutralize the effects of DSS. Moringa starch administration is indigestible in the small intestine and is fermented by the microbiota in the colon, which produces short chain fatty acids (SCFAs) such as acetate, propionate and butyrate. This compound plays a role in increasing insulin sensitivity and lowering glucose by the liver, thus allowing for a decrease in blood glucose levels. It can be interpreted that moringa kepok starch provides a slight stabilizing effect, but it is not enough to significantly improve metabolic disorders due to DSS (Nair et al., 2015).

The moderate-dose P3 group showed an increase in blood glucose levels to 285.17 mg/dL, higher than the negative control group. These results suggest that moderate doses tend to be less effective in lowering blood glucose levels. There is a possibility of microbiota imbalance (dysbiosis) at this dose, where the bacteria that grow can actually trigger inflammation that worsens the control of blood glucose levels, such as an increase in bacteria related to insulin resistance (Smith et al., 2024). Meanwhile, in the P4 group given high doses, blood glucose levels decreased to 266.33 mg/dL. This decrease may indicate a pharmacological effect at higher doses. However, blood glucose levels were still higher than negative (P0) and positive (P1) controls, indicating that the treatment has not been successful in correcting metabolic dysfunction due to DSS. High doses can also contain mild toxic effects that stimulate physiological stress (Purba et al., 2020). Giving resistant starches in high doses allows gut bacteria to produce more SCFAs (*Short Chain Fatty Acids*) through the fermentation process. These SCFAs then help improve the composition of the gut microbiota, which plays an important role in stabilizing blood glucose levels (Aprianto, 2022).

Of the overall treatment groups, the average blood glucose level when compared to the negative group, the smallest difference was found in the P2 treatment group (-6.66) and also the P4 treatment group (-25.16). This shows the difference in the decrease in blood glucose levels given by moringa kepok banana starch in P0 negative control mice (normal rats), it is known from the difference in rat blood glucose levels that low doses of moringa kepok starch (0.05 g/head) can restore rat blood glucose levels, although it cannot recover optimally as in the negative control group (P0).

The results of statistical analysis showed that the data were distributed normally, then a variation test was carried out and homogeneous data was obtained, followed by a hypothesis test using One-way ANOVA and the results were obtained that there was no significant effect between the administration of kepok banana-moringa starch on blood glucose levels in rats with a value of $p= 0.121$ ($p>0.05$). Blood glucose levels showed no statistically significant differences, suggesting that between treatment groups could be linked through different physiological responses of mice. The response of blood glucose levels in animals can be influenced by many factors. In the study of Palupi et al. notes that the response to blood glucose levels in animals can be greatly influenced by environmental factors, sampling time, as well as the physiological status of the individual (Palupi & Nafilah, 2021).

Short-term changes in blood glucose levels may not have a significant effect on the results of direct blood glucose measurements, so they do not show statistically significant differences between groups. In line with research conducted by Afifah et al. (2020) on the effect of banana flour on blood glucose levels and physical performance in type 2 diabetic rats. The results showed that blood glucose levels in mice given banana flour decreased, although significant changes were not immediately seen in the initial phase of testing (Afifah et al., 2020).

Furthermore, a study by Paula et al. (2017) showed that the isolated protein of moringa leaves has hypoglycemic activity, but the effect is not directly related to a reduction in inflammation in the gut or a consistent long-term effect on blood glucose levels (C. Paula et al., 2017).

Thus, the effects of treatment on blood glucose levels do not always appear directly and take sufficient time to show significant differences, especially in short-term studies (Syafii et al., 2023). Short-term effect measurement requires additional parameters such as glycemic index testing and insulin tests to assess the effectiveness of test materials in viewing blood glucose levels (SUNITA, 2021). In addition, research by Kartadinata et al. stated that HbA1c measurement provides a clear picture of blood sugar levels in a span of 1-3 months (Kartadinata et al., 2023).

CONCLUSIONS

Based on this study, kepok banana starch (Musa paradisiaca forma typica) combined with moringa leaves (Moringa oleifera L.) did not significantly increase feed intake or lower blood glucose levels in DSS-induced Wistar rats. However, prebiotic intake differed significantly between control and treatment groups, indicating good compliance with the intervention. Blood glucose levels ranged from 221.83 to 285.17 mg/dL, and no statistically significant reduction was observed following kepok banana–moringa starch administration.

RECOMMENDATION

Low dose prebiotic administration (0.05 g/rat) showed the most promising tendency toward improved blood glucose levels, although the effect was not statistically significant. Future studies are recommended to further explore this dose, extend the intervention period, and include additional metabolic markers such as HbA1c, insulin levels, and glycemic index testing to obtain a more comprehensive picture of the impact of kepok banana–moringa starch on glucose regulation.

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